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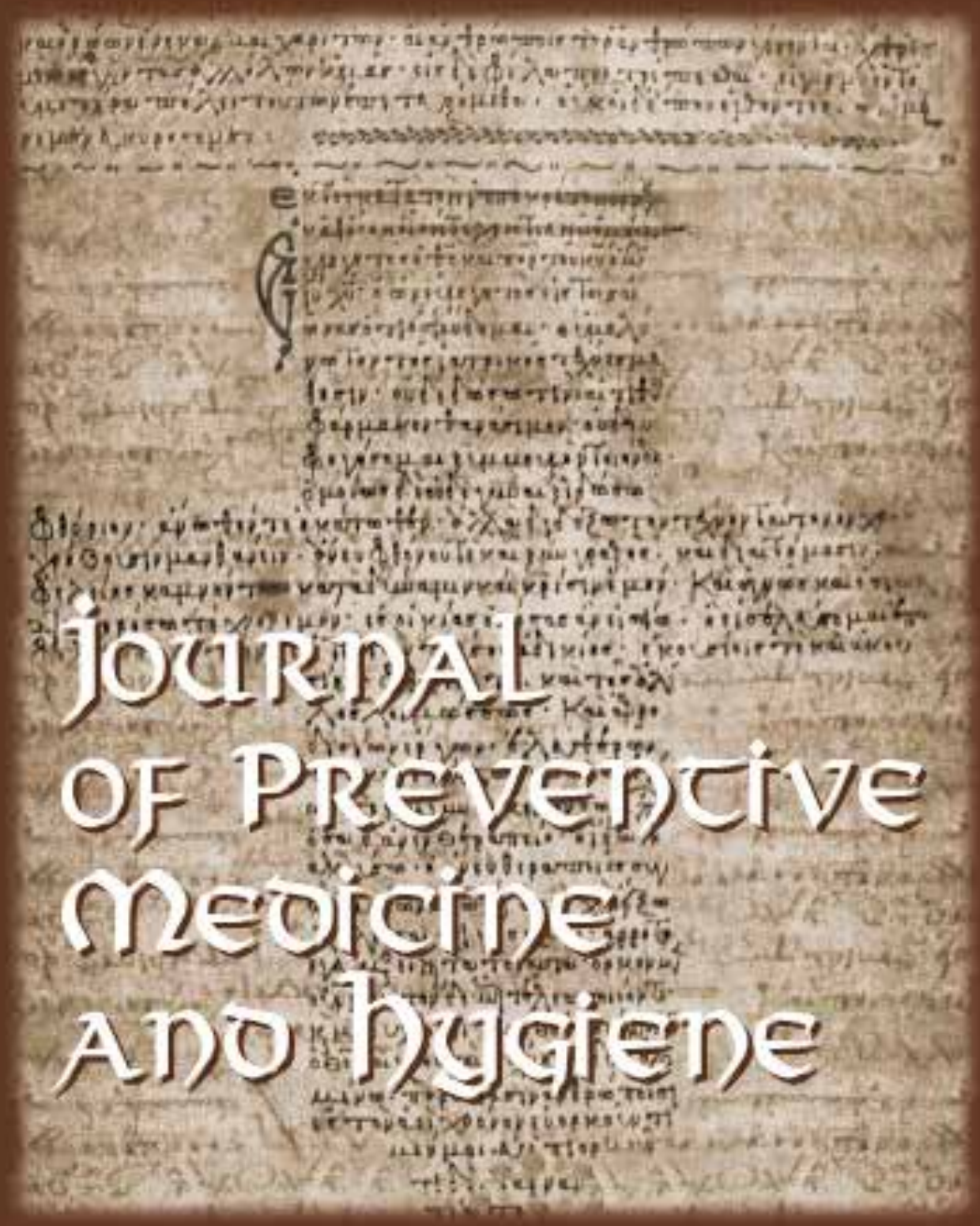

JOURNAL OF PREVENTIVE MEDICINE AND HYGIENE

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Review

Campylobacter: from microbiology to prevention

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Keywords

Campylobacter • Foodborne pathogens • Epidemiology • Prevention

Summary

In last years, Campylobacter spp has become one of the most important foodborne pathogens even in high-income countries. Particularly, in Europe, Campylobacteriosis is, since 2005, the foodborne disease most frequently notified and the second in USA, preceded by the infection due to Salmonella spp. Campylobacter spp is a commensal microorganism of the gastrointestinal tract of many wild animals (birds such as ducks and gulls), farm animals (cattle and pigs) and companion animals (such as dogs and cats) and it is responsible for zoonoses. The transmission occurs via the fecal-oral route through ingestion of contaminated food and water. The disease varied from a watery diarrhea to a severe inflammatory diarrhea with abdominal pain and fever and can be burdened by some complications. The main recognized sequelae are Guillain-Barré Syndrome (GBS), the Reactive Arthritis (REA) and irritable bowel syndrome (IBS). Recently, many cases of Campylobacter spp isolated from human infections, showed an important resistance to various antibiotics such as tetracyclines and fluoroquinolones. For these reasons, the prevention of this infection plays an essential role. Many preventive measures exist to limit the transmission of the pathogens and the subsequent disease such as the health surveillance, the vaccination of the poultry and the correct food hygiene throughout the entire production chain. A global surveillance of Campylobacteriosis is desirable and should include data from all countries, including notifications of cases and the microbiological data typing of strains isolated from both human and animal cases.

Introduction

The bacteria belonging to the genus *Campylobacter* have long been recognized among the most common responsible agents of enteritis and gastroenteritis in humans, both among adults and in pediatric patients [1-3]. In recent years, in high-income countries, cases of Campylobacteriosis have exceeded those caused by classic enteric bacteria. The micro-organism is isolated from patients with infections of the alimentary tract with a frequency of about 3-4 times higher than in *Salmonella* or *Escherichia coli* [4]. In low- and middle-income the data, although poor, suggests that the rate of infection by *Campylobacter* has increased in recent years [5]. It is often difficult to trace the sources of exposure to *Campylobacter*, this is due to the sporadic nature of the infection and the important role of cross-contamination. For these reasons, over the past decade, many countries have put in place a number of important preventive measures to avoid these food-borne infections [6]. In addition, recent scientific advances, such as the complete sequencing of the genome of the microorganism, the new findings on causes of the infection and the recognition of the role of immunity in protecting against *Campylobacter* infections [7], exploitable process for the development point of the appropriate vaccine have led to a better understanding of the pathogenesis [8] and have helped to guide the Assessment and Management Risk along the chain "farm-to-table".

Nevertheless, Campylobacteriosis remains a difficult disease to prevent and infection epidemiological trend continues to remain high throughout the world.

Microbiology

The taxonomy of the genus *Campylobacter* has been extensively revised; currently the immediate family is that of Campylobacteriaceae, which includes three distinct genera: *Campylobacter*, *Arcobacter* and *Helicobacter* [9]. The genus *Campylobacter* includes 22 species, of which the best known are *C. jejuni* and *C. coli*, the main responsible of gastroenteritis in humans, although other species such as *C. concisus*, *C. upsaliensis*, *C. ureolyticus, C. hyointestinalis* and *C. sputorum*, species now considered "emerging", have been associated with gastroenteritis and periodontitis [9, 10]. All these species normally colonize different apparatuses of domestic or wild animals and can be found in many foods of animal origin [10]. The genus *Campylobacter* comprises gram-negative microorganisms, non-sporeforming and with variable dimensions, with a length between 0.5 and 5 μm and a width comprised between 0.2 to 0.9 μm [11]. Most of the species is mobile and is characterized by a spiral movement caused by a polar flagellum present on one or both ends of the cell. The only exceptions are *Campylobacter gracilis*, which is motionless, and *Campylobacter showae* that has multiple flagella [12]. The

DNA is around 1.6-1.7 Mbps and is rich in adenine and thymine; GC ratio is, in fact, about 30% [13-15]. From a metabolic point of view it is of micro-aerofili bacteria that, therefore, survive and grow best in an environment characterized by a low oxygen tension (5% O_2, 10% CO_2, and 85% N_2) [9, 16].
All species, except *C. gracilis*, synthesize the oxidase enzyme. Not ferment nor oxidize carbohydrates but they get energy from amino acids or tricarboxylic acid [12]. *Campylobacter* species is able to grow at pH between 6.5 and 7.5 and at temperatures between 37° and 42° C. For this reason is defined, by some authors, "thermophilic". Levin has, however, proposed that these microorganisms are more correctly referred to as "thermotolerant" since they do not present a real thermophilic, being unable to grow at temperatures equal to or above 55° C [17]. They are also unable to grow at temperatures below 30° C, for the absence of the genes coding for the heat-shock-protein that play a role in the adaptation to low temperatures. Finally, it was shown that the growth does not occur in environments with water activity (aw) concentrations of less than 0.987 (sensitive to sodium chloride of greater than 2% w/v), while it is optimal if equal to 0.997 (about 0,5% w / v NaCl) [18].

Reservoirs and transmission

Campylobacter spp is a commensal germ of the gastrointestinal tract of many wild animals (birds such as ducks and gulls), farm animals (cattle and pigs) and companion animals (such as dogs and cats). It is, also, predominantly, in all avian species fit for human consumption [19-21]. They are micro-organisms responsible for zoonoses and the transmission occurs through the fecal-oral route through ingestion of contaminated food and water [22-24]. The main environmental niche is represented by the intestinal tract of all avian species, particularly poultry (ie broilers, laying hens, turkeys, ducks and ostriches) which is considered the main route of transmission [25-29]. The consumption of this meat, in fact, represents 50-70% of human cases of Campylobacteriosis [30]. However, even the consumption of raw milk, raw red meat, fruits and vegetables has been identified as a possible cause of transmission [31, 32]. Moore *et al.* have indicated that the prevalence of colonization by *Campylobacter spp* in cattle varies widely, even between 0-80% while it is around 20% in sheep [33].

Poultry

Eating or handling raw or undercooked meat of chicken would be the main risk factor for contracting campylobacteriosis [34,35,36,37]. It was seen, in fact, that the feces of infected poultry may contain up to 105-108 CFU/g. These high levels permit bacteria to spread easily in the environment, thus allowing the contamination [38]. Bull *et al.* has estimated that the chicken meat retail is contaminated with *C. jejuni* up to 98% of cases in the US and from 60% to 80% of cases in Europe [39]. Contamination occurs between the same farm animals, where transmission can be vertical in nature (*i.e.* from hen to chick via egg), quite rare event, or horizontally within the environment where the animals are bred [40, 41]. The infection can be contracted in the very first days of life, but the presence of the organism in stool samples is detected no earlier than two or three weeks old [42]. The reason for this lag phase is still unknown, but it could be due to the protective effect of maternal antibodies [43] or to the microbial flora of the animal itself. In the latter situation, the microbial flora residing in the chicken gastrointestinal apparatus could play a competitive role against *Campylobacter*, delaying the colonization [44]. During slaughter, however, the main critical points for contamination of carcasses were identified in plucking, evisceration and final washing. The treatment with water at temperatures above 60° C, causes a decrease of the bacterial load which, however, increases during the plucking operations causing a cross-contamination [45, 46]. The bacterial load also can further increase during the evisceration due to spill of intestinal content rich of *Campylobacter* [45, 47]. Moreover, the spread of the microorganism occurs through the shedding into the environment of wild bird feces [48]. Their presence in playgrounds has been recognized as an emerging environmental source of Campylobacteriosis, especially for children, who frequently put her hands to her mouth favoring the ingestion of germs [49, 50]. Many playgrounds are natural habitat for a variety of wildlife including birds, lizards, dogs and stray cats. New Zealand researchers have analyzed the bird fecal material collected in children's playgrounds and isolated *C. jejuni* in 12.5% of samples [49].

Milk

Unpasteurized cow's milk and dairy products are common vehicles for the transmission of *Campylobacter spp*; to identify them as a source of human Campylobacteriosis is already known since 1978, when four cases of infection by *C. fetus* have been identified in a hospital in Los Angeles County [51]. In 1996 Evans *et al.* has described an outbreak of Campylobacteriosis associated with the ingestion of raw milk occurs in U.K. [52].
Javid, later, led a study of cattle from a dairy, highlighting that 12% of samples of raw milk were contaminated with *C. jejuni* [53]. The likely causes of contamination of milk are possible contact with bovine feces, contaminated water or direct contamination due to bovine mastitis [54, 55].

Fruits and vegetables

Numerous studies have shown the presence of *C. jejuni* and *C. coli* in lettuce, spinach, radishes and peas [56-59]. It is likely that the contamination of vegetables to occur as a result of irrigation with contaminated water, use of natural fertilizers or through the same soil contaminated with droppings predominantly avian origin [60-62]. It is also possible to cross-contamination during the handling and packaging or through kitchen utensils used for cutting of other foods such as poultry [63]. Verhoeff-Bakkenes *et al.* have shown that the consumption of fruit and vegeta-

bles, especially packaged, is an important risk factor for Campylobacteriosis: on 5.640 samples of fruit and vegetables analyzed in their survey, 13 (0.23%) were positive to *Campylobacter*, with a higher percentage (0.36%) in packaged products compared to fresh ones (0.07%) [31]. Kirk *et al.* and Blaser *et al.*, in the past published two reports relating to two *Campylobacter* outbreaks caused, respectively, by the consumption of cucumbers served at a buffet [64] and the consumption of salad prepared by an employee of a soup kitchen from whose hands was isolated *Campylobacter* [65].

Water

European legislation provides that the natural mineral water obtained from springs and, occasionally, by drilling sources is free from parasites and pathogens. Unlike the water distributed through the taps, it cannot be subjected to any type of treatment that could alter its chemical composition [66]. A variety of organisms, including coliforms, can be found in mineral waters, in particular non-carbonated water supplied in plastic bottles and bottled by hand [67]; Gillespie *et al.*, reported a case in which the bottled water has been identified as a possible vehicle for *Campylobacter* infection [68].

Swine and cattle

It is important not to underestimate the role of cattle and pigs that are often colonized with *C. jejuni* and *C. coli* [69-74].
A study carried out by Taylor *et al.* in the US has revealed that 5% of outbreaks of Campylobacteriosis in the period from 1997 to 2008, was due to the consumption of contaminated meat pork, beef and game [75]. Multiple studies have also shown that *Campylobacter*, preferentially, colonize the lower gastrointestinal tract of cattle [72] but has also been found in the liver, gall bladder and bile juice [69, 73, 74]. Moreover, there is a higher prevalence of *Campylobacter* in cattle from intensive farming [68%] than in adult cattle grazing (7.3%) [71]. This could be explained by the greater density of animals that are constantly in contact with their own faeces and the sharing of areas including drinking water and food distribution [76, 77].
As for the pigs, these appear to be predominantly colonized by *C. coli* and, less frequently, by *C. jejuni* [74]; some studies have shown, however, the possible coexistence, in these animals, of both the microorganisms [78, 73]. As for cattle, colonization by *Campylobacter* in pigs was particularly notable among animals in intensive farming [69] than those reared in traditional agricultural systems [79].

Shellfish

Wilson and Moore have shown the presence of *Campylobacter* also in molluscs, colonization due, probably, to the contamination of the waters in which stalling and are collected [80]. In this study, have been isolated thermotolerant *Campylobacter spp* in 42% of samples analyzed. The majority of these (57%) were urease-positive thermophilic *Campylobacter* (UPTC) [81, 82], with a clear predominance of *C. lari* [80]. In particular *C. lari* colonizes the intestine of seagulls that contaminate the water with their feces [83].

Pets

A number of domestic animals have been identified as hosts for *Campylobacter spp* [84-86]. In European and Asian research many Authors have reported the isolation of *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. helveticus*, and *C. lari* from canine fecal samples [87-91]. In the United States, Stehr-Green *et al.* referred that 3% of all cases of salmonellosis and 15% of Campylobacteriosis can be attributed to contact with pets [92]. Chaban *et al.* reported the isolation of *C. jejuni* in 5 dogs feces of 70 (7%) at concentrations up to 10^6 CFU/g [86]. Seeing as the infective dose of *C. jejuni* is estimated at 500 microrganisms [93], these high concentrations present in feces represent a possible risk factor for environmental contamination and human infection by accidental exposure. Veterinarians assume that the animal contamination could occur following the ingestion of raw meat [94, 95].
The transmission mode of *Campylobacter* from animals to humans have not yet been described; however, pet the animals and manipulate objects came into contact with them could transfer pathogens from the fur or object contaminated to the human hands and lead to infection [96].

Flies

It has been shown that even the flies represent an important carrier for *Campylobacter* and they are, therefore, able to contaminate both humans that animals [97-99]. Gordon *et al.* have shown that some cases of diarrhea increased especially during the summer season when the larvae grow and mature by increasing the number of adult insects [100]. Some studies support this theory. Layton *et al.* and Neal *et al.* have reported the reduction of cases of diarrheal syndromes following the application of measures for fly control [101, 102]. They have assumed that the transmission of the disease occurs by direct contact of foods with the paws, proboscis and body fur of the insects that were contaminated with fecal or regurgitated material contaminated [100]. Contamination can occur at any stage of the food chain.

Epidemiology

United States

In the US, there is an active surveillance system called FoodNet, which constantly monitors the spread of foodborne diseases. In particular, the surveillance program is concerned of control of 7 bacterial infections confirmed in the laboratory (*Campylobacter, Listeria, Salmonella, Escherichia coli* O157 and non-O157 of shiga-like toxin producers [STEC], *Shigella, Vibrio* and *Yersinia*), 2 parasitic infections (*Cryptosporidium* and *Cyclospora*) and cases of HUS. FoodNet system to belong, currently, 10 states (Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, California, Colorado

and New York), which together make up 15% of the US population (48 million people in 2011) and is the result of a collaboration between CDC, the Departments of Health of the 10 Member States, the UFSA-FSIS (US Department of Agriculture's Food Safety and Inspection Service) and the FDA (Food and Drug Administration).
According to this network [103], in 2012 were 19,531 reported infections, 4,563 hospitalizations and 68 deaths associated with foodborne diseases. For most infections, the incidence was higher among children aged < 5 years, but the percentage of people hospitalized and died was highest among persons aged ≥ 65 years. In 2012, compared to the period 2006-2008, the overall incidence of infections was unchanged but increased cases of infections caused by *Campylobacter* and *Vibrio*. *Campylobacter*, in particular, ranked second, after *Salmonella*, as a cause of food-borne infections. The number of *Campylobacter* infections (incidence per 100.000 population) was 6.793 [14, 30] and, of them, were typed 2.318 (34%) isolates of wich 2.082 (90%) were *C. jejuni*, and 180 (8%) were *C. coli*. Estimated incidence of infection was higher in 2012, compared to the period 2006-2008 (up 14% CI: 7%-21%). The percentage of hospitalized subjects was 31% while the percentage of patients who died ranged from 0.2%.
The 2013 data confirm that the food-borne infections continue to be an important public health problem in the United States and emphasize the importance of preventive measures. In particular, infections due to *Campylobacter spp* accounted for 35% of the total, preceded by those due to *Salmonella spp* (38%).
In 2014, FoodNet has identified 19,542 cases of infection with 4,445 hospitalizations and 71 deaths. The crude number and incidence was 6,486/100.000.

Europe

All the data concerning the epidemiology of foodborne infections in the European Union (EU) are published, annually, by the European Food Safety Authority [EFSA]. EFSA's headquarters is located in the city of Parma (Emilia Romagna, Italy). The data shows that in Europe Campylobacteriosis is, since 2005, the foodborne disease most frequently notified with over 190,000 cases reported each year in humans. However, it believes that the actual number of cases to be about nine million/year. In addition, according to the EFSA, the Campylobacteriosis cost for health systems, in terms of lost productivity, is approximately 2,4 billion euro per year.
In 2011 Campylobacteriosis has established itself as the most frequently reported zoonotic disease in humans, with a continuous increase of the reported cases [104]. In particular, a total of 220,209 cases of infection were reported, 2.2% more than in 2010. The food where *Campylobacter* was most found was the chicken meat. Despite the significant decrease in recent years, salmonellosis was again the second reported zoonotic disease with a total of 95,548 cases. Altogether *Campylobacter* was the most frequently reported cause, it is mentioned less often as the cause of outbreaks of food-borne. The most common food sources of outbreaks were eggs and egg products, composite foods, fish and seafood products.
For the first time in five years, in 2012 human Campylobacteriosis decreased slightly, but is still the most commonly reported zoonotic disease, responsible for 214,268 cases of infection with a 4.3% decline compared to 2011 [105]. The notification rate was 55.49/100,000 inhabitants. Considering the high number of cases, the gravity (reported deaths) was low (0.03%). Overall, 23.6% of fresh chicken meat samples tested were positive for *Campylobacter spp*, less than in 2011 in which was positive for 31.3% of the samples.
Campylobacter spp in 2013 continued to be the most commonly reported gastrointestinal pathogen in the European Union (EU). The number of confirmed cases reported was 214,779, with an EU notification rate of 64.8/100,000, the same level of 2012 [106]. Mortality was low (0,05%). Overall, 31.4% of fresh chicken meat samples checked were positive for *Campylobacter spp*. In the period 2012-2013, this increase in *Campylobacter*-positive samples was mainly due to the placing of the data coming from Croatia, which reported results for the first time in 2013. *Campylobacter* it was also detected less frequently in the flesh of turkey and other foods. In 2013, moreover, they have been reported from 414 *Campylobacter* outbreaks. The sources of these outbreaks were, in order of importance, chicken meat and dairy products and other foods such as milk and mixed foods.

Italy

In Italy, the latest data available on Campylobacteriosis concern 2006 with 476 isolations of *Campylobacter spp* from clinical specimens that have been reported by the laboratories of the Enter-net network. In 73,9% of cases the laboratories carried out the identification of species. *C. jejuni* was the most frequently isolated species. 35,5% of strains were isolated from pediatric patients under the age of 6 years, especially in the summer months. The presence of antimicrobial-resistant strains is high in particular for quinolones and fluoroquinolones [107].

Pathogenesis and virulence factors

Colonization and intestinal epithelium adherence are the first and indispensable stages of the disease pathogenesis. For this reason, the characteristic motility of the bacterium by polar flagella that the cell possesses is fundamental [108]. The flagella determine a rotational propulsive movement of the cell body while the helical shape determines a typical movement like a corkscrew [109]. The intestinal epithelium colonization is secondary to a chemotaxis process in which the main chemoattractors are the mucins and glycoproteins constituting the intestinal mucus [110]. The main bacterial chemoceptors are represented by proteins called What A, B, R, W, Y and Z [111].
The subsequent bacterial adhesion to the intestinal epithelial surface is mediated by several adhesins placed

on the surface of the bacterium [112]. In particular, a key role is played by an external membrane protein that binds the fibronectin named CADF [113] and by a protein called CapA or protein of *Campylobacter* Adhesion [112]. The consequent cell damage is related to the production of various cytotoxins [114, 115]. The most studied Cytotoxin is the CDT or Cytolethal Distending Toxin [116]. This toxin has desoxyribonuclease activity and determines the cell cycle block in the G2 phase [116] and fragmentation of the nucleus resulting in cell death [117].

Clinical manifestations and related complications

The clinical spectrum of *Campylobacter* varied from a watery diarrhea without blood to a severe inflammatory diarrhea with abdominal pain and fever. The disease appears to be less severe in developing countries than in industrialized countries [118, 119]. In detail, in industrialized countries, the clinical picture is generally characterized by bloody stools, fever and abdominal pain and is often more severe than that caused by *Salmonella* and *Shigella spp*, in developing countries, instead, the symptoms are generally represented by watery stools with leukocytes, fever, abdominal pain, vomiting, dehydration [120, 121]. The *Campylobacter spp* infection can be burdened by some major complications. The main recognized sequelae are Guillain-Barré Syndrome (GBS), the Reactive Arthritis (REA) and irritable bowel syndrome. The Miller Fisher Syndrome, a variant of GBS, can also be associated with a previous *Campylobacter* infection. Evidence suggest a possible association with Inflammatory Bowel Disease (IBD), and there is evidence that other functional gastrointestinal disorders may be related to gastroenteritis in general (not specifically caused by *Campylobacter*). This aftermath, of course, they contribute significantly to the burden of disease [122].

Guillain-Barré Syndrome

The role of *Campylobacter spp* has now been extensively studied in triggering an autoimmune response that leads to damage of the peripheral nervous system and the development of GBS. The *Campylobacter*-induced GBS is now considered a real disease and it seems that the basis of its unleashing there is the phenomenon of molecular mimicry. There are quite comprehensive data on the incidence of GBS in Europe and North America [123, 124]. The disease has also been well studied in China [125] and Japan [126], but the population incidence data are still scarce. The data on the worldwide incidence of GBS are limited with regard to low-income countries standards. In Bangladesh a recent publication reports that the disease has a higher incidence, and the presence at a young age, compared to high-income countries [127]. The lack of a common definition of GBS hampers the comparability of data and uniformity in the notification. Recently, there has been proposed guidance for a standardized definition of the clinical case of GBS, the so-called "Brighton criteria" that are receiving broad international support [128]. Globally, around a third of cases of GBS have been attributed to a previous *Campylobacter spp* infection [129]. A link between reduced incidence of Campylobacteriosis and reduced incidence of GBS has been reported in New Zealand [130]. A link between reduced incidence of Campylobacteriosis and reduced incidence of GBS has been reported in New Zealand [130]. Some researchers have studied the clinical course of GBS and have shown that cases of GBS preceded by *Campylobacter spp* infection are more severe and are characterized by poorer therapeutic results with long-term possibility of disability [131, 132]. Treatment of the disease includes a general multidisciplinary assistance and specific treatment with plasmapheresis and/or intravenous immunoglobulin. Approximately 20% of patients is hospitalized in an intensive care unit to support ventilation and to monitor the autonomic dysfunction. Access to optimal treatment, however, varies greatly around the world, especially in less developed countries, where the GBS remains a serious and potentially fatal disease. The fatality rates vary widely and range between 3% and 10% in high-income countries while the lethality in countries developing is assumed to be higher. A recent meta-analysis concluded that as many as 31% of GBS cases could be attributed to *Campylobacter spp* [129]. This meta-analysis was based on studies conducted mainly in high-income countries and China and India, while it was only considered a study conducted in a country classified by the US as "less developed." A more recent study in Bangladesh showed that 57% of cases of GBS could be attributed to *Campylobacter spp* [133].

Reactive arthritis

Available data suggest that reactive arthritis occurs 1-5% of individuals infected with *Campylobacter spp*. The annual incidence of REA after *Campylobacter spp* infection is estimated at 4.3 per 100,000 inhabitants in high-income countries [134]. In a study, in 5% of subjects the resulting reactive arthritis to *Campylobacter spp* infection is found to be chronic or recurrent [135]. There is evidence that musculoskeletal disorders can be triggered by *Campylobacter* and other enteric infections. In a US study published in 2008 and conducted by Townes *et al.* [136] in Minnesota and Oregon, the individuals with positive stool culture for *Campylobacter spp*, *Salmonella spp*, *Shigella spp*, *Yersinia spp* and *Escherichia coli* O157 were followed for 8 weeks. In particular, they were monitored 6379 patients with a confirmed infection; of these, 70% have completed screening and 575 (13%) have developed reactive arthritis. Other studies have reported a long-term disabilities resulting reactive arthritis. Hannu *et al.* [137] have estimated that 25% of patients with reactive arthritis can develop a chronic spondylo-arthropathy, with different manifestations.

Irritable bowel syndrome [IBS]

The infectious gastroenteritis is one of the major predisposing factors for the development of IBS [138, 139].

Some studies have reported that up to 36% of individuals with acute Campylobacteriosis develop IBS within 1-2 years [140]. There seems to be a close correlation between risk of developing IBS and the severity of the acute illness. Following an outbreak of infection with *Campylobacter spp* and *Enteromorragic E. coli* (EHEC) caused by contaminated water, Marshall *et al.* have reported an increased risk of IBS among those who had had a greater length of diarrhea, dysentery and abdominal cramps during the acute phase of the disease [141]. The studies carried out on patients with IBS post-*Campylobacter* infection have shown an increase of intraepithelial lymphocytes and upregulation of cytokines in colon-rectal mucosa, typical of a persistent immune activation [142-145]. The intestinal inflammation and hyperplasia of enterochromaffin cells in IBS post-infection are also accompanied by an increase in intestinal permeability resulting in an increase in the antigenic load and further activation of the immune system [146].

Other functional gastrointestinal disorders related to *Campylobacter*

Scientific evidence linking infectious diarrheal syndromes with other functional gastrointestinal disorders such as functional dyspepsia. Mearin *et al.* [147] and Porter *et al.* [148] have reported an association between infectious diarrhea invasive, respectively *Salmonella spp* and from all causes, and post-infectious functional dyspepsia (OR 5,2 and 5,0, respectively). Similarly, Parry *et al.* showed an increase of 2,9 times of functional dyspepsia resulting in bacterial gastroenteritis (including *Campylobacter*) compared to non-exposed controls [149]. A further study also identified a link between acute enteric infection and functional dyspepsia in children [150]. It seems that there is lastly a relationship between the presence of *Campylobacter* and other functional gastrointestinal disorders such as diarrhea, functional constipation and gastro-oesophageal reflux disease [151, 152].

Inflammatory bowel disease (IBD)

In recent years, it has strengthened the hypothesis of an association between IBD and acute diarrheal infection caused by *Campylobacter*. The first studies that described the possible association between acute infection and inflammatory bowel disease date back to the '90s; Schumacher, for example, observed that cases of traveler's diarrhea were associated with a first attack of IBD in 62% of patients [153]. *Campylobacter* was isolated from 10% of cases of IBD relapsing [154]. Recent cohort studies have shown a higher risk of developing IBD following an acute infection with *Campylobacte* [155 156].
A study by Garcia-Rodriguez *et al.*, Published in 2006, showed that the risk of developing IBD has increased after a year by an episode of acute gastroenteritis, with an incidence of 60 cases per 100,000 inhabitants-year [157]. The pathogenesis of post-infectious IBD remains unclear. At the base there appears to be an enhanced host immune response to intestinal microbiome [158] due to an increased absorption of bacterial antigens secondary to increased intestinal permeability that residual, as damage, after the infectious episode [157].

Laboratory diagnosis

The conventional method for the isolation of *Campylobacter* species in stool is represented by seeding the sample on selective media followed by incubation at 42° C in microaerophilic environment (5% O_2, 10% CO_2, 85% N_2). Some species (*C. sputorum*, *C. concisus*, *C. mucosalis*, *C. curvus*, *C. rectus* and *C. hyointestinalis*) for isolation may require the additional presence of hydrogen [159]. Media used, made selective in order to suppress the competitive bacteria and promote the growth of *Campylobacter spp* may be added to blood or coal, both of which contain one or more antibiotics.
The most commonly used culture media between those that contain blood, are the selective medium of Butzler (sheep blood agar to 10% with bacitracin, novobiocin, colistin, cephalothin and actidione), the Blaser media (agar-blood sheep to 10% with vancomycin, trimethoprim, polymyxin B, cephalothin, and amphotericin B) and the Skirrow media (horse blood agar lysate to 7% with vancomycin, polymyxin B and trimethoprim). Among media with coal the most used is certainly the Preston medium, containing cefoperazone. Merino *et al.* [160] have shown that the latter is the best in the recovery of the higher number of germs from fecal material.
It recently launched a selective chromogenic medium for *Campylobacter* (CASA Agar), which greatly facilitates the isolation and detection of these bacteria. On CASA agar, there is a strong inhibition of the growth of competitive intestinal flora while the colonies of *Campylobacter spp* are red and easily recognized [161]. Often the various species of *Campylobacter* isolated from human samples are not easily identifiable. Only *C. jejuni* can be identified with the use of phenotypic markers such as the morphological appearance of the colonies, biochemical reactions and optimal growth temperature; the other species require a polyphasic approach, using a combination of phenotypic and molecular markers.
In most clinical laboratories, even the identification of *Campylobacter spp* is performed only at the level of genus. The MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) represents a new and interesting means of identification of *Campylobacter spp*, despite not provide information on bacterial resistance and lacks a computer system able to suggest other tests additional [162]. They were finally developed molecular assays using species-specific reactions or multiplexes, based on 16S rRNA gene sequences, or other species-specific gene sequences and identification systems based on microarray. All these systems can provide valuable support in official laboratories for Public Health and Food Safety [163, 164].

Antibiotic-resistance

In 2010 in the United States, only 1% of the strains of *C. jejuni* isolates from human infections were resistant to erythromycin, while 43% were resistant to tetracycline and 22% to ciprofloxacin [165]. In the same year, the FDA has reported almost overlapping data observed in strains of *C. jejuni* isolates from chicken meat [166].
In 2010 In the European Union 2% of *C. jejuni* from humans were resistant to erythromycin, 21% to tetracycline and 52% to fluoroquinolones; in strains isolated from chicken meat values were 2%, 22% and 50%, respectively. Both in the US than in Europe, the antibiotic resistance is greater in *C. coli* than in *C. jejuni* [167].
The cause of high resistance to fluoroquinolones appears to be the habitual use of veterinary antibiotics (enrofloxacin and danofloxacin) in the pharmacological treatment of poultry [168]. Because of this, in the United States the use of fluoroquinolones in poultry authorization was withdrawn in 2005 [169]. In Australia, where this use has not been approved, the resistance to these drugs is rare [168]. It was noted that infections resistant to fluoroquinolones are often associated with travel in both developed countries and in developing countries [170-172].
A recent study published in 2015 by Ghunaim *et al.* [173] shows an erythromycin resistance of *C. jejuni*, relatively low: only 8,6% of the isolates were resistant, while 63.2% were resistant to ciprofloxacin. A high rate of resistance to ciprofloxacin was also reported in the UAE, where 85,4% of the isolates were resistant [174]; in Poland they were published [175] lower rates of resistance (40%) and only 2% in Australia [176].

Prevention

There are numerous ways to prevent this infection, including vaccination and poultry control.

Health surveillance

According to Thacker S. the "Health Monitoring" is the systematic collection, analysis and interpretation of data on specific diseases within a determinate population in order to guide the actions and decisions in the field of Public Health [177]. A well-structured surveillance program for Campylobacteriosis can provide crucial information about the importance and the presence of the disease than other enteric infections contributing, also, to identify the most common routes of pathogen transmission. A complete surveillance of Campylobacteriosis should be carried out at national level, with data from all regions, including notifications of cases and the microbiological data typing of strains isolated from both human cases and from cases animals. Alternatively, they could be monitored specific sentinel sites, adequately resourced, and broadly representative of the whole country. In New Zealand, a hybrid approach unifies the national data of reported cases, and the epidemiological information related to supervision of sentinel sites [178].

Vaccination

The WHO recognizes a considerable potential in anti-*Campylobacter* vaccines for both humans and animals. In humans, in particular, this potential concerns the prevention not only of acute infection but also of sequelae, with a remarkably reduction of patients. It is unlikely that a vaccine can be used for preventive purposes on a large scale, but it could be important for those who are at greatest risk. However, you still need considerable research before this can be achieved. Currently there are still no approved vaccines against diarrhea associated with C*ampylobacter*. The development of effective vaccines against *C. jejuni* is limited by incomplete understanding of the pathogenic mechanisms of the disease and the strong association of this with GBS. Most strains of *C. jejuni* produces lipo-oligosaccharide (LOS) that contain sialic acid (Neu5Ac) with a structure very similar to human gangliosides. Antibodies directed against these molecules can cross-react with human peripheral nerves causing the establishment of GBS. This association between *C. jejuni* and GBS preclude many vaccination approaches. However, the recent discovery that *C. jejuni* (unlike other enteric pathogens) expresses a capsular polysaccharide (CPS) has favored the creation of a CPS-conjugate vaccine similar to those that have been developed for other pathogens [179]. Although few details are known of the molecular pathogenesis of the disease, the invasion of the intestinal epithelial cells appears to be a critical stage, and the PSC appears to play an important role in adherence to the cells. Thus, antibodies against the CPS may induce a protective immune response. Also, unlike the LOS, there is no evidence of molecular mimicry of the PSC with human gangliosides and, therefore, the antibodies should not trigger autoimmune reactions. There is no reason to think that vaccination could prevent, in the future, the development of *Campylobacter*-associated GBS. A common problem associated with capsular vaccines is the poor immunogenicity particularly in infants, a population to which many of these vaccines are directed. This occurs because polysaccharides are cells T-independent antigens and are able to stimulate only the mature B cells. However, the conjugation of polysaccharide with proteins transforms the immunological response from T-cell-independent to T-cell-dependent leading to the development of immunological memory both in children and in adults [180].

Control measures in poultry

All types of poultry can be colonized with *Campylobacter spp* [181]. Vertical transmission of the bacterium through eggs is an extremely rare event [182]. The vast majority of broiler chickens are free of *Campylobacter* in the day of egg hatching, which means that at the beginning, each new group of chickens is *Campylobacter*-free. Once the *Campylobacter* is introduced into a group, it spread with the faeces rapidly colonizing almost all animals (up to 10^8 *Campylobacter/*gr faeces). The colonization rate remains almost at the same level until the age of slaughter (42 days in conventional production systems).

Colonization does not determine the onset of clinical signs or a reduction of the life of infected individuals. The broiler chickens can be colonized with *C. jejuni* and *C. coli*. However, approximately in 6 weeks old chickens, most of the isolated strains is represented by *C. jejuni* while in older animals predominates *C. coli* [183, 184].
In literature there are many articles about the possible actions to be taken to control chickens contamination by *Campylobacter* both on farms than in slaughter and processing houses.
There are considerable differences in the production of poultry in different parts of the world as a type of establishment (indoor or outdoor), the equipment used for the supply of food and water, the type of litter used (new or reused between groups), the microclimate, the method of ventilation and, finally, breed of chickens used. These differences will have a weight on the effectiveness of preventive interventions and determine on which interventions should be emphasized in order to achieve the greatest risk reduction.
Although the *Campylobacter spp* contamination control is a global problem, each country must develop its own strategies for achieving it. The only intervention proved to be effective in preventing the introduction of *Campylobacter spp* in the establishments of production is the application of strict biosecurity measures [185, 186]. They include:

- a strict control of establishment access to minimize the entry of unauthorized persons, birds, rodents or other animals;
- an insect control (*e.g.*, flies and cockroaches) [187, 190];
- a workers' control (such as the introduction of hygiene barriers and the change of footwear before entering in the plant) [191];
- water purifying (chlorination) [191];
- control of litter and waste, avoiding the exchange between the groups [192, 193];
- other animals and rodents control [194];
- disinfection of cleaning tools [195];
- cleaning and disinfection of the whole plant and of all the equipments [190].

There are, moreover, a whole series of other interventions "pre-harvest" that have been successful in the field of research, but that have not yet proven effective when applying. They include the use of bacteriocins, bacteriophages, organic and inorganic acids in the feed or drinking water. However, the advantage of an intervention on the field can be lost if there is no simultaneous action in the transportation from the farm to processing establishments that reduce cross-contamination [195, 196].
Other important phases are selection of the animals, transport to the slaughterhouse, time spent in the slaughter facility [187]. The removal of the feed and water prior to the collection of animals has a significant impact on the *Campylobacter* load because may contaminate the animals during the transport and in the treatment plant. This is considered an integral part of the post-harvest control. Even at this stage, a good hygiene is crucial to the success of the control procedures [188-190]. Appropriate measures include cleaning, disinfection and drying of transport tools, cages and coops, a proper stocking density, sanitize surfaces and liquids used (heaters, coolers, *etc*.) that come in contact with each carcass in order to reduce cross-contamination, and the use of specific food safety protocols as the application of good hygiene practices. Carcass decontamination by physical or chemical means is the procedure with the greatest chance of success among all post-harvest interventions proposed [191, 192]. The methods include the use of large volumes of water to wash carcasses, countercurrent flow of water in the heaters and water coolers, freezing of carcasses, treatment of carcasses with heat (steam) or irradiation. The chemical decontamination includes the use of chlorine compounds, organic acids, ozone, peracetic acid, peracetic acid with hydrogen peroxide, trisodium phosphate, as well as some "natural" methods such as the use of bacteriophages and bacteriocins.
Finally, the meat is likely to be contaminated even when it comes on the market. At this time, it is essential that the control measures are extended to distributors, retailers and end-consumers. As with any raw product, good hygiene practices are required during the preparation, storage and distribution of food. These practices include washing hands before and after handling food; the handling raw and cooked food; it is important to keep raw meats separate from cooked or ready to eat food; avoid using the same utensils to handle raw meats and other foods (such as cutting boards and/or other surfaces, knives and dishes); wash and disinfect all surfaces and utensils that have been in contact with raw meat; do not wash with tap water the raw meat in order to avoid further spread of microorganisms in the working surfaces. Because, finally, *Campylobacter* is sensitive to cooking temperatures, cook the food at 70° C will minimize the risk of contracting the infection.

Conclusions

Evaluating the epidemiology of Campylobacteriosis we have revealed an increasingly important role for *Campylobacter* infection in Public Health. While global efforts to control the transmission of enteric pathogens have been effective at reducing the incidence of a number of major foodborne pathogens, the human *Campylobacter* infections have been increasing in the last decade with a prevalence of infection ever increasing across most developed nations. Many progresses have been made in diagnostics that are helping to refine estimates of the acute and long-term burden of disease associated with a broad range of *Campylobacter spp*. We assume that better and efficient applied assays are necessary for an improved understanding of the epidemiology of different *Campylobacter spp* and to allow vaccine development. We also believe that it is necessary, due to growing antimicrobial resistance, implement control strategies on their use. Furthermore, it is now well established that poultry and other domesticated animals, such as cattle and pigs, and environmental sources, such as contaminated water, also

play a vital role in the direct transmission of these organisms to humans. For this reason, it is very important the realization of standardized biocontrol methodologies in the poultry sector, a principal source mediating *Campylobacter* transmission to humans.

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Authors' contribution

PL and AD suggested the argument of the paper, supervised the work and edit the manuscript; AF, RR, EA and GV provided the bibliography and wrote the manuscript.

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ORIGINAL ARTICLE

Improvement in vaccination knowledge among health students following an integrated extra curricular intervention, an explorative study in the University of Palermo

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Keywords

Medical education • Vaccination • Educational intervention • Survey • Medical students

Summary

Introduction. *Vaccination coverages threaten to decrease because of false beliefs in their unsafety and inefficacy. Therefore formation of future health-care workers on this topic is fundamental to deal with any doubt and to promote active immunization among general population.*

Methods. *In order to assess health-care students' knowledge about vaccination before an integrated seminar on this topic, and to evaluate their improvement after the educational intervention, an integrated educational intervention was held by a multidisciplinary team. Before and after the seminar, 118 students of medicine and biology schools at Palermo University were asked to answer 10 multiple-choice questions regarding vaccine history, mechanism of action, side effects, composition, use and nowadays issues (hesitancy). Two more questions investigating possible changes on students' attitudes towards vaccination and the usefulness of the formative intervention, were added at the post-test phase of the survey.*

Results. *Eighty-one out of 118 students (68.6%) answered to both pre- and post-test questions. 97.6% and 81.5% of the participating group also completed the two additional questions about their improvement in knowledge (question 11) and attitudes (question 12) towards vaccinations. The post-test results showed a significant improvement for all questions administered, except for number 3 (about a specific immunological content), with an overall percentage of correct answers increasing from 38.8% to 77.6% ($p\copyright < 0.001$).*

Conclusions. *The present explorative study put the basis for future studies, stronger in the methodology, and highlights the importance of educating health-care professions students by integrated extra-curricular intervention to be held early in their degree curricula and in order to improve knowledge and attitudes towards vaccinations and to prepare them to promote vaccines among the general population.*

Introduction

Vaccinations are one of most powerful public health strategy, saving millions of human lives every year [1]. Despite this undeniable success, nowadays vaccination is perceived as unsafe and unnecessary by a growing segment of population. A huge number of attacks by anti-vaccination movements, concerning not evidence-based issues on vaccine safety, have spread all over the industrialized countries due to the general belief of considering the Internet as a self-acknowledgement item for solving health problems [2]. This wide-spectrum of doubtful attitudes towards vaccine immunization represent a serious danger to public health, and has been collectively called "hesitancy" [3]. Current events involving vaccines negatively impacted the public opinion, resulting in coverage rates decrease. As a consequence of that, vaccine-preventable disease outbreaks and epidemics increased [4-7].

Despite influenza vaccination of health-care workers was strongly recommended by International and National Public Health Authorities [8, 9], vaccination coverage reported in Italy are still lower than 75% expected [10, 12]. Considering this background, health-care workers (HCWs) must be well prepared to increase people knowledge about vaccine safety and efficacy. Therefore, educating future generations of HCWs early in their career is a critical point in the strategy of promoting vaccines among the population, especially parents of young children. Evidences demonstrated how multidisciplinary formative interventions [11] are the preferred strategy for Italian HCWs to improve their adherence, attitude and knowledge about vaccinations, comparing with the ones proposed by other countries (mandatory vaccination, incentives to vaccination, *etc*.) [11-13]. Moreover, the "key role" of HCWs for a proper vaccination counseling to the patients and the importance of an up-to-date training on preventive medicine was frequently reported [15-18].

The aim of this study was to evaluate the efficacy of an integrated curricular intervention to improve knowledge and attitude towards vaccinations of biomedical students attending a seminar at Palermo University, South of Italy.

Methods

At the University of Palermo, located in the South of Italy, six of the twenty existing departments are related to biomedical disciplines. The Department of "Science for Health Promotion and Mother-Child care" organized an integrated educational intervention for Palermo University students of health-related faculties (Medicine, Health Assistance, Health Biology, Pharmacy, Nursing, Biology, Pharmaceutical and Chemical Technology).
Students were divided into three subgroups according to the attended faculty, respectively, in order to simplify and improve data analysis:

1. Medicine;
2. Master of Sciences (Health Biology, Pharmacy, Pharmaceutical and Chemical Technology);
3. Basic Sciences (Health Assistance, Nursing, Biology).

The interdisciplinary intervention was held by university personnel belonging to Department for Prevention and public health physicians employed by the Local Health Units of the National Health Service. They were all experts in the fields of vaccination and immunization, and debated the following topics:

- history of vaccination (from the origins to the newest improvements);
- principal immunological aspects of vaccination;
- vaccination strategies of Sicilian Region;
- false myths about adverse effects of vaccines;
- communication on vaccination between health personnel and general population.

This pre-post test study contemplates information gathered through ten multiple choice questions survey, compiled both before and after lecturers' speeches. Questions were grouped two by two according to the five main topics of the educational intervention and were projected before and after the educational intervention in the meeting room screen for 1 minute to allow the response on a printed answers sheet. Pre and post-intervention results were analyzed separately. Students were informed about the protocol of the study, and they participated anonymously. Since the survey data did not influence students' privacy, and the issue being investigated is a matter of public record, ethical approval for the study was not required.

Statistical analysis

We entered all the information in a database created within Excel 5.0 software. Data analysis was performed using the EpiInfo 3.5.1 software. Absolute and relative frequencies were calculated for qualitative variables. Quantitative variables were normally distributed and summarized as means (standard deviation).
Socio-demographic, academic characteristics and the percentage difference between pre and post intervention test were evaluated by the Fisher Exact Test (dichotomous variables) or Chi-squared test (categorical variables). Differences in means were compared with the Student t-test for paired sample. The significance level chosen was $p < 0.05$ (two-tailed).

Results

Eighty-one out of 118 (68.6%) students actively participated to both pre and post educational intervention.
Socio-demographic data were reported in Table I. There were no significant difference between gender ($p = 0.63$), mean age (22.9 ± 4.6 and 23.1 ± 3.0 respectively, $p = 0.64$), attended courses ($p = 0.62$) and year of course ($p = 0.47$) among pre and post test groups. The majority of students were female (about the 65%), and attended Medicine course.
After the intervention, 97% of respondents stated their knowledge concerning vaccination had been improved at least partially and 81.5% of them admitted also an improvement of their attitude towards active immunization, thanks to the seminar.
Percentage of correct answers significantly improved after the seminar relating to historical, immunological aspects and vaccination strategy (number 1, 2, 4 to 8) ($p < 0.001$), and for questions on communication on vaccination between health personnel and general population (question 9 and 10) ($p < 0.05$ and $p < 0.01$, respectively). Question number 3 on specific immunological and pathogenetic mechanisms showed a not significant decrease of proper responses percentage (60.2 vs 56.8; $p = 0.80$) (Tab. II).
The mean value of correct answers was globally 38.8% in the pre-intervention survey, with the most trained students attending Medicine (44.2%) followed by students attending Basic Sciences (38.7%) and Master of Sciences courses (30.1).
The figures, as reported in Table III, increased to 77.6% after the seminar ($p < 0.001$), and significant improvements were attained by students of Medicine (81.3%), Master of Sciences courses (75.9%), and Basic Sciences courses (75.4%) ($p < 0.001$, in all cases).

Discussion

Vaccination has long been relegated as a secondary topic in Medicine *curricula* of most Italian universities, often with insufficient time dedicated to, or even entirely omitted [19], leaving it to spontaneous and autonomous diligence of students to deepen their own knowledge. Only in recent years Italian universities abandoned the traditional education pattern based on monographic courses adopting extracurricular interventions which may provide scholars a more complete view of each topic, thanks to a multidisciplinary approach. Our study was based on the evaluation of the efficacy of multidisciplinary formative course including different aspects

Tab. I. Socio-demographic and academic characteristics of study samples (pre and post intervention).

Determinant	Pre intervention questionnaries	Post intervention questionnaries	p-value
Number of respondents	118 (100)	81 (68.6)	
Age, mean in years ± SD	22.9 ± 4.6	23.1 ± 3.0	0.63
Gender, n (%)			
• Male	38 (32.5)	27 (34.2)	0.64
• Female	79 (67.5)	52 (65.8)	
Classes of degree, n (%)			
• Medicine	54 (45.7)	30 (37.0)	0.62
• Master of Sciences (Health Biology, Pharmacy, Pharmaceutical and Chemical Technologies)	31 (26.3)	24 (29.6)	
• Basic Sciences (Health Assistance, Nursing, Biology)	31 (26.3)	22 (27.2)	
• Missing	2 (0.02)	5 (0.06)	
Year of course , n(%)			
• I	31 (26.3)	18 (22.2)	0.40
• II	34 (28.8)	25 (30.9)	
• III	27 (22.9)	15 (18.5)	
• IV	7 (5.9)	3 (3.7)	
• V	9 (7.6)	7 (8.6)	
• VI or more	3 (2.5)	2 (2.5)	
• Missing	7 (5.9)	11 (13.6)	
Knowledge improvement after educational intervention, n (%)			
• Yes, fully		51 (63.0)	
• Yes, partially		28 (34.6)	
• No		0 (0.0)	
• Not answered		2 (2.5)	
Attitudes towards vaccination change after educational intervention, n° (%)			
• Yes, they will improve		66 (81.5)	
• Yes, they will worsen		0 (0.0)	
• No, they will remain unchanged (intervention not effective)		1 (1.2)	
• No, I was already aware of discussed topics		10 (12.3)	
• Not answered		4 (4.9)	

of vaccination that was held in one day, with a pre-post survey.

As stated in Table I, there were no significant differences between pre and post-test groups concerning demographic characteristics, degree or year of study course (attendance).

Efficacy of integrated intervention was self-reported by large majority of post-test group, since 97.6% admitted knowledge implementation and 81.5 % recognized attitude improvement towards vaccinations.

Objective success in strengthening vaccine learning was demonstrated by the radical increase in the percentage of correct answers after the seminar (Tab. II).

All the questions except number 1, 3 and 9 achieved statistical significant improvements ($p < 0.001$) after the seminar with more than 80% of correct answers (2, 5, 6, 7) and about 60% for the remaining ones (questions 4, 8 and 10).

On the other hand, the query concerning the reduction in pathogenicity obtained by the modification of a toxin into a toxoid (number 3), that resulted in the second better performance in the pre-test, was the only one to show a paradoxical worsening after the intervention, decreasing to 56.8% of appropriate responses, though not statistically significant ($p = 0.80$).

Finally, answers from question 9, highlighted that almost 84% of students were already aware that the main motivation to influenza vaccination for health-care workers was to prevent flu transmission to patients; the

Tab. II. Percentage of correct and incorrect/missing answers in pre and post intervention among study participants stratified for each question.

Questions	Before intervention (n = 118) Correct answer (%)	Post intervention (n = 81) Correct answer (%)	p-value
1) Who invented the term vaccination?	65 (55.1)	73 (90.1)	< 0.001
2) Protection against smallpox obtained by vaccine administration was an example of antigenic cross-reactivity?	27 (22.9)	66 (81.5)	< 0.001
3) Modification of a toxin into a toxoid reduces the pathogenicity of the toxin itself?	71 (60.2)	46 (56.8)	0.800
4) Salk and Sabine vaccines have the same efficacy?	33 (28.0)	50 (61.7)	< 0.001
5) What sex and age anti-papillomavirus vaccination is recommended in Sicily to?	22 (18.6)	75 (92.6)	< 0.001
6) What is the schedule of anti-pneumococcal vaccination in subjects with underlying health conditions?	32 (27.1)	69 (85.2)	< 0.001
7) Which vaccine was wrongfully related to autism onset by Wakefield study?	45 (38.1)	67 (82.7)	< 0.001
8) Which vaccine adjuvant was reported to alter neuro-psychological development in children?	19 (16.1)	48 (59.3)	< 0.001
9) What might be the main reason for influenza vaccination adhesion among health-care workers?	99 (83.9)	74 (91.4)	< 0.05
10) What is the estimate of parents that actually did not vaccinate their children because of hesitancy in Italy?	45 (38.1)	51 (63.0)	< 0.01

seminar managed to further increase this percentage to 91% ($p < 0.05$). This data seems to be different comparing what observed during the past years in other Italian study among medical resident and general practitioner trainees [12, 15, 19].

Stratifying the survey for study courses as reported in table 3, a substantial improvement was depicted for all classes of degrees with a significant p-value (< 0.001). The best results were attained in particular by Medicine and Health Assistance undergraduates, suggesting that they could represent a key subject for vaccines promotion among general population and themselves. Stratifying the survey for study courses as reported in Table III, a substantial improvement was depicted for most of them (in particular for Medicine, Health Assistance, Health Biology, Pharmacy, Pharmaceutical and Chemical Technology with a significant p-value < 0.001). The best results were attained by Medicine and Health Assistance undergraduates, suggesting that they could represent a key subject for vaccines promotion among general population and themselves. Anyway, the fundamental role of the other professional figures in the Health Care System should target on the need of substantial improvement of their education and motivation as well.

The limits of our survey are mainly two. Questionnaires anonymity represented a limit since matching of pre-intervention answers to post-intervention ones for each student was not possible. As a consequence of that, we could not exclude that leaving of less prepared scholars before completing the post-intervention phase contributed in increasing overall percentage of correct answers, thus biasing the survey.

On the other hand, it favored the achievement of higher response rates (far beyond the half of pre-phase interviewed students), since nominal questionnaires would have been considered by students as examinations.

Moreover, the absence of statistically significant differences between pre- and post-phase students socio-demographic distribution, along with the striking improvement in correct answers percentages (more than doubled for most of the queries), support the efficacy of our multidisciplinary seminar in an effective knowledge and attitudes enhancement.

Another important limitation of the study is the relatively small sample size and that it was conducted at an heterogeneous audience with different background. Moreover, it was an optional, not curricular intervention and, clearly, it was focused on a single university [11]. A national survey, that may reveal regional variations, should be structured in the future.

Synoptically, our data demonstrated a lack of knowledge of the history, the local schedules and organization of vaccination service in Sicilian territory and most importantly about the mechanism of action and appropriate indications of vaccines. These results suggest the need to incorporate multidisciplinary courses into biomedical curricula in order to clear any confusion and to overcome any doubt in future HCWs who are going to promote and supply immunization against preventable and potentially lethal infectious diseases [20].

Hopefully, such specific education should begin early in their formative training, in order to increase vaccination coverage rates among medical students during clinical training and medical residents [12].

Tab. III. Number and percentage of correct and incorrect/missing answers for all questionnaire in pre and post intervention among study participants, stratified by study course. (N.A. Not Assessable).

Classes of degree (Total n° of students)	Pre intervention (n = 118)		Post intervention (n = 81)		p-value
	N° of correct answers	% of correct answers	N° of correct answers	% of correct answers	
Overall (n = 199)	458	38.8	629	77.6	< 0.001
Medicine (n = 84)	239	44.2	244	81.3	< 0.001
Master of Sciences (n = 55)	93	30.1	181	75.4	< 0.001
Basic Sciences (n = 53)	120	38.7	167	75.9	< 0.001
Missing data (n = 7)	6	30.0	27	54.0	0.25

Conclusions

The present explorative study dealt with the first attempt to organize and fulfill an integrated course focused on vaccinations and oriented to undergraduates of biomedical faculties in the South of Italy, assessing their knowledge before and after the seminar. Multidisciplinary lectures should be included early in university curricula since they could improve students attitudes and strikingly increase their learning about the topics discussed [16, 21].
Education of HCWs on active immunization should therefore potentiate their preventive medicine skills, an essential step to promote vaccination practices among the general population [22]. To further confirm the success of these kind of initiatives, the correlation with vaccination coverages (against influenza for instance) among undergraduates might represent an helpful indicator and an interesting field for future research.

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Authors' contributions

FV and AC conceived, designed, coordinated and supervised the research project. CC, VR, FD, NC and CD performed the integrated lectures. CM and GV collected data. CM, DDR, CC, VR, and GV performed the data quality control, optimized the informatics database, performed the statistical analyses and evaluated the results. CM, DDR and GV wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Knowledge and behaviour of nursing students on the prevention of healthcare associated infections

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Keywords

healthcare associated infections, nursing students, hand hygiene, standard precautions

Summary

Introduction. *Hospital infections, or "healthcare associated infections" (HAI) represent the most common and serious complications of healthcare. Adoption of safe care practices able to prevent or control the transmission of infections, both in hospitals and in other healthcare settings is crucial. The aim of the study is to assess the awareness about the risk factors and the most effective measures of prevention of HAI in the University of Ferrara nursing school students, giving particular attention to the hand hygiene practices and the use of standard precautions.*
Methods. *339 students attending all the three years of course of the same academic year were enrolled. An anonymous questionnaire was administered in order to investigate the knowledge about three specific areas: infections associated with healthcare practices (HAI), standard precautions (SP) and hand hygiene (HH).*
Results. *A sufficient level of knowledge by all the three groups of students was observed only in the SP area. A barely sufficient score was reached only by the third year students with regard to the proper HH. The level of knowledge about HAI was inadequate.*
Conclusions. *A periodically check of nursing students' knowledge would be advisable in order to fill any gaps, improve training, reduce HAI and increase prevention measures compliance.*

Introduction

Healthcare associated infections (HAI) also referred to as "nosocomial" or "hospital" infections represent the most frequent and serious complications of healthcare [1, 2].
They are defined as infections, occurring in a patient 48 hours or more after admission to a hospital, that was not present or incubating at the time of admission. The infection can be acquired during the process of care in a hospital or other healthcare facility (primary care, nursing home care and outpatient services) [1].
HAI affect primarily patients and, less often, hospital staff, students and trainees [3]. Nurses and students that perform invasive procedures are particularly at risk. An Italian survey [4] showed a lower percentage of occupational injuries with biological risk in nursing students than in nurses (18.82% versus 81.18% respectively), that could be related to suitable and recent university training and proper use of personal protective equipment.
Around the 80% of HAI involves four main areas: the urinary tract (accounting for 35-40% of all HAI), surgical wounds, the respiratory system and systemic infections (sepsis, bacteremia) [3]. However, in the last fifteen years, a rising number of cases of bacteremia and pneumonia were observed and this can be the consequence of a progressive increase in specific risk factors, in particular, the frequent and or inappropriate use of antibiotics and vascular catheterizations. In 2011-2012, 29 EU member states and Croatia participated in a prevalence study on HAI and the use of antibiotics in acute care hospitals, coordinated by the European Centre for Disease Prevention and Control (ECDC) [5]. The prevalence of HAI varied from 4.8% in first-level hospitals to 7.2% in those of the third level. The patients of intensive care units were more affected, 19.5% of them having at least one infection than an average value of 5.2% of other clinical units.
The most frequently isolated microorganisms were *Escherichia coli* (15.9%), *Staphylococcus aureus* (12.3%), *Enterococcus spp* (9.6%) and *Pseudomonas aeruginosa* (8.9%). A considerable antibiotic resistance was observed.
The HAI prevalence in Italy (6.6%) was similar to the European average (6.0%, with values ranging from 2.3% to 10.8% in the different countries), while the incidence of some infections (for example, the infections related with vascular catheterizations) was higher than in other European countries. However, in Italy, the problem is even more dramatic because of the spread of multidrug resistant microorganisms [5]. According to a recent Italian survey on HAI [6], *Escherichia coli* was the most common pathogen isolated in microbiological samples, followed by *Klebsiella pneumoniae* and *Enterococcus faecalis*.
The World Health Organization (WHO) [7, 8] indicated some key factors for the management and control of HAI: the improvement of reporting and surveillance systems both at national and local level as part of a prevention program; the implementation of preventive measures, particularly adequate hand hygiene practices, principally at the bedside and during invasive procedures; the promotion of healthcare workers training and responsibility.

Hand hygiene is a critical issue in HAI prevention. This preventive procedure is strongly recommended by WHO that recently promoted a worldwide campaign in order to encourage hand washing [9]. Nonetheless the use of alcohol-based products for hand hygiene should be further promoted: a study on 49 Italian hospitals showed a consumption lower than 10 litres per 1,000 days of hospitalization (the lowest category in Europe), against an European average of 18.7 litres/1,000 with peaks of 40 in the Scandinavian countries [5].
Major consequences of HAI are the delay of patients' recovery, extended hospitalization and the need of subsequent outpatient controls (e.g. for surgical wound infections). According to the Centre for Disease Control (CDC) [10], the hospitalization is prolonged up to 4 days for urinary tract infection, up to 3 weeks for sepsis and up to a month for pneumonia.
In addition, HAI represent a cost, not only a financial burden for the utilization of healthcare service but also for human suffering of patients, families and personnel. About 450,000-700,000 infections are annually acquired in Italian hospitals (first of all urinary infections, followed by surgical wound infections, pneumonia and sepsis). Of these, about 30% of cases (135,000-210,000), and 1% of deaths (1,350-2,100 deaths/year) are potentially preventable, respectively [11].
The adoption of safe healthcare practices, able to prevent or control the transmission of infections both in hospitals and in other healthcare setting are strongly required. Despite new emerging microorganisms that the healthcare workers are facing (*e.g.*, hepatitis B and C viruses, human immunodeficiency virus, vancomycin-resistant enterococci [VRE], methicillin resistant *Staphylococcus aureus* [MRSA], avian or pandemic influenza viruses), the standard precautions still remain essential [12] and the nursing staff quality of training is a key element in the prevention and control of HAI [13].
As the potential infectious condition of a hospitalized patient is unknown, nursing students, as the healthcare workers, are at an elevated risk of exposure to biological agents [14]. It is therefore particularly important that not only the professionals but also the students early acquire knowledge of the possible risks of infection connected to healthcare and acquire skills regarding the use of main preventive measures.
The aim of the study was to assess the knowledge about the risk and the most effective measures of prevention of HAI in students of an Italian university nursing school during their 3 years of course, giving particular attention to hand hygiene practices and the use of standard prophylactic precautions. Moreover, potential areas of major criticisms were explored in order to offer useful guidance for the improvement of the training (both theoretical and practical activities).

Methods

The study was carried out by first, second and third year nursing students of the same academic year attending the University of Ferrara (Italy). All students have completed at least one training course (theoretical and or practical) learning information about HAI.
Preliminary information about the purpose, the protocol and the method of the study, including guarantee of anonymity was supplied (according to Legislative Decree no. 196/2003 "Code concerning the protection of personal data").
The research was carried on in accordance with the World Medical Association Declaration of Helsinki. It does not report any experiment on human or biological samples, nor research on identifiable human material and data because it is an observational survey conducted by an anonymous questionnaire among university students. Indeed, in order to protect the privacy of the subjects and confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity (stated in the article n. 23 of the above mentioned Helsinki declaration) the research was wholly conducted anonymously; thus no identifiable personal data are reported. Verbal informed consent was obtained from all subjects.

Characteristics of the questionnaire

An anonymous questionnaire was used in order to investigate students' knowledge about three specific areas: Healthcare Associated Infections (HAI), Standard Precautions (SP) and Hand Hygiene (HH). The questionnaire was developed on the model of that used by Tavolacci et al., 2008 [15], with slight modification in order to adapt to Italian epidemiological situation. The first part was dedicated to the demographic aspects (age, gender, year of attendance), followed by a question concerning the correct definition of HAI. The second section was composed of six questions, each of which contains statements that require a dichotomous response (true, false) for a total of 25 responses. Each area had a maximum of 10 points for an overall score of 30, excluding the first question on HAI definition that received no score. In detail, HAI area consisted of a single question with 5 dichotomous responses (2 points per response); SP consisted of 3 questions each with four answers (0.83 points per response) and HH was composed by 2 questions each with four answers (1.25 points per response). Acceptable score was arbitrarily set at ≥ 7, according to Tavolacci et al., 2008 [15].

Statistical analysis

The answers have been collected in a database using Microsoft Excel 2007. The obtained data were analysed by Statview® 5.0.1 software (Abacus Concepts, Berkeley, CA, USA). ANOVA was applied to reveal the difference in the average score of the three different areas, according to the year of attendance. The chi-square test was used to detect the differences in students' knowledge according to year of attendance for each question. A value of $p < 0.05$ was considered statistically significant.

Results

The study recruited 339 students attending a degree course in nursing (with a response rate of 51% considering the total number of students enrolled in the three years of course), of whom 203 (59.9%) at the first year, 88 (25.9%) at the second year and 48 (14.2%) at the third year, respectively. The 64.3% of the participants were aged less than 22 and 74.6% were female (Table I). The percentage of correct answers to HAI section is reported in Table II. The majority of the students of all the three years precisely identified age and invasive procedure as risk factors for HAI onset (with a statistically significant difference between the years of course of 0.0044 and 0.0117 respectively). The awareness of environment as source of infection increased with the continuation of the training ($p < 0.0001$). Surprisingly the third year students gave the correct answer to HAI associated mortality to a lesser extent than the students of first and second year ($p = 0.0130$). No difference was found about knowledge of HAI prevalence rate.

The features regarding the adoption of standard precautions and the risk of contamination with biological fluids (SP section) were well known by students of all three years of course with correct response rates very close or up to 100% (Table III). The indications for the use of

Tab. I. Characteristics of the sample of the 339 nursing students.

Socio-demographic characteristics	Frequency N (%)	First year N (%)	Second year N (%)	Third year N (%)
Age				
< 22 years	218 (64.3)	158 (77.8)	52 (59.1)	8 (16.7)
≥ 22 years	121 (35.7)	45 (22.2)	36 (40.9)	40 (83.3)
Gender				
Females	253 (74.6)	154 (75.9)	63 (71.5)	36 (75.0)
Males	86 (25.4)	49 (24.1)	25 (28.5)	12 (25.0)
Total	**339 (100)**	**203 (59.9)**	**88 (25.9)**	**48 (14.2)**

Tab. II. Percentages of correct answers to the healthcare associated infections section according to the year of course.

HAI Section	First year	Second year	Third year	p value
The environment is the major source of bacteria	20.2%	39.8%	45.8%	<0.0001
Advanced age or very young age increases the risk	70.4%	87.5%	85.4%	0.0044
Invasive procedures increase the risk	89.7%	96.6%	100%	0.0117
Nosocomial infection has a prevalence ranging between 5% to 10% in Italy	44.8%	47.7%	41.7%	0.7320
Nosocomial infections are responsible for approximately 5,000 deaths per year in Italy	50.7%	47.7%	27.1%	0.0130

Tab. III. Percentages of correct answers to the standard precautions section according to the year of course.

SP Section	First year	Second year	Third year	p value
Standard precautions				
Include recommendations to protect only the patients	93.6%	95.5%	100%	0.1790
Include recommendations to protect the patients and the healthcare workers	97.5%	98.9%	100%	0.4368
Apply for all the patients	95.1%	97.7%	35.4%	0.4297
Apply for only healthcare workers who have contact with body fluid	93.1%	94.3%	91.7%	0.8268
Use of gloves is recommended				
For each procedure	23.2%	61.4%	50%	<0.0001
When there is a risk of contact with the blood or body fluid	95.6%	95.5%	100%	0.3279
When there is a risk of a cut	82.3%	69.3%	75%	0.0527
When healthcare workers have a cutaneous lesion	94.1%	96.6%	95.8%	0.9040
When there is a risk of splashes or spray of blood and body fluids, the healthcare workers must wear				
Only mask	97.5%	95.5%	100%	0.2866
Only eye protection	97.5%	95.5%	97.9%	0.6974
Only a gown	95.6%	97.7%	100%	0.2423
Mask, goggles and gown	98%	98.9%	97.9%	0.8640

Tab. IV. Percentages of correct answers to the hand hygiene section according to the year of course.

HH Section	First year	Second year	Third year	p value
Recommendations for hand hygiene				
Before or after a contact with (or care of) a patient	87.2%	85.2%	93.8%	0.3476
Before and after a contact with (or care of) a patient	99.0%	100.0%	100.0%	0.5055
Between patient contacts	96.6%	97.7%	95.8%	0.8012
After the removal of gloves	85.2%	79.5%	87.5%	0.4019
Directions for use of alcoholic solutions				
instead of a traditional handwashing (30 s)	43.3%	62.5%	62.5%	0.0038
instead of a antiseptic handwashing (30 s)	49.8%	33.0%	47.9%	0.0407
instead of surgical handwashing (3 min)	30.5%	5.7%	12.5%	< 0.0001
A traditional handwashing must be done before handwashing with alcohol-based hand rub	42.9%	39.8%	62.5%	0.0247

Tab. V. Score for each area according to year of course.

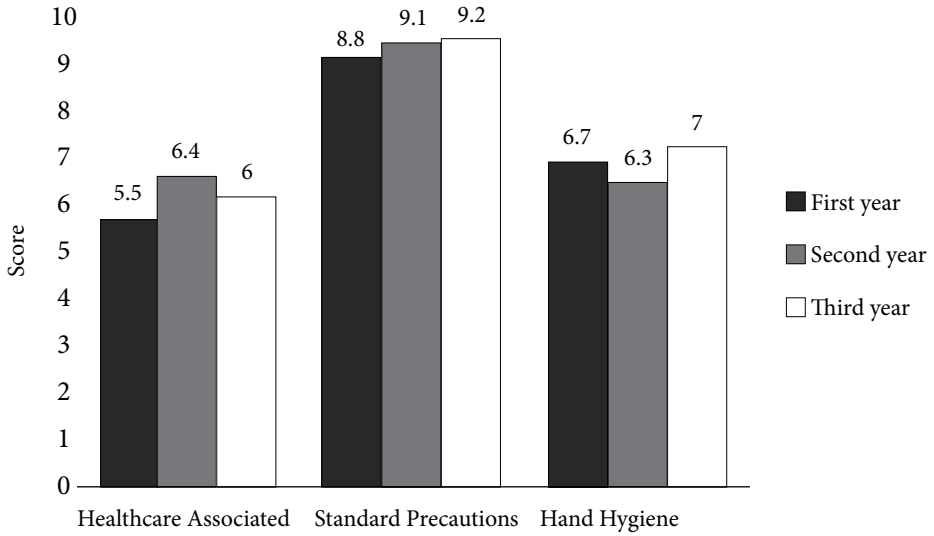
	HAI	SP	HH	Total Score
First year	5.5	8.8	6.7	21.0
Second Year	6.4	9.1	6.3	21.8
Third year	6.0	9.2	7.0	22.2
Total	**5.8**	**8.9**	**6.6**	**21.4**

gloves have been well known without any differences between the students attending all years of training, except for the recommendation on the use of gloves for all procedures that, although with an upward trend, was correctly indicated only by half of the third year students with a statistically significant difference between the three years of the course ($p < 0.0001$).

The recommendations for hand hygiene were correctly recognised by almost all the students without statistically significant difference between the years of attendance (Tab. IV). Low percentages of corrected answer were observed for all questions regarding the use of alcohol-based solutions. The notions about the substitution of traditional handwashing and that alcohol-based products do not require association with traditional handwashing increased with training progression, with a statistically significant difference of 0.0038 and 0.0247 respectively. The availability of substitute antiseptic handwashing with alcohol-based products was known by less than the half of students in all the three years of course ($p = 0.0407$). A very scarce knowledge about the possible substitution of surgical handwashing was observed with a statistically significant difference between the years of attendance ($p < 0.0001$).

According to Tavolacci et al. [15], the total score reached in the different areas was calculated considering accept-

Fig. 1. Scores obtained in the 3 investigated sections (HAI, SP, HH).



able a value greater or equal to 7 (Table V, Fig. 1). Overall score was sufficient for all investigated students but insufficient scores were achieved for HAI and HH areas. A satisfactory score was obtained only for SP area in all the years of course. The score in HAI area did not reach an adequate level not even at the third year, while knowledge about HH just reached the sufficiency at the last year of training.

Discussion

In agreement with the results obtained by Tavolacci et al. in 2008 [15], a sufficient level of knowledge, identified by a score ≥ 7, was reached by all three groups of students, only in the section regarding SP.
This finding implies a good level of preparation already from the first year of the course about this topic. A barely sufficient score was reached only by the third year students with regard to the proper HH. For all the other areas, the level of knowledge shown by the students belonging to the three years was inadequate. This is particularly true for the section regarding the HAI. This outcome is once more in agreement with Tavolacci et al. [15], which reported lower scores concerning the knowledge of HAI not only by the nursing school students, but also by the medical, physiotherapy and assistant radiologist students. Also another study, conducted in 2014 by Mitchell et al. [16] on nursing school students, showed a higher level of knowledge on SP, with a percentage of 88.9% of correct answers.
The worst knowledge involved mainly prevalence and mortality associated to HAI and the role of the setting in their onset, while the role of age and invasive procedure were more recognised. More efforts should be undertaken to emphasize the severity of HAI so that students do not underestimate the burden and importance of this topic.
An excellent level of knowledge emerges with regards to SP from all three years of the course; nevertheless an insufficient awareness of the necessity to wear gloves for all the patients was observed. A previous study conducted on nursing school students of the same University identified as main gap the belief that the SP (in particular the use of gloves) are necessary for a self-protection and not to prevent hospital infections [17].
As for the proper HH, all the students show lack of knowledge mainly regarding appropriate use of alcohol-based solutions, particularly in substitution of traditional and surgical handwashing. Nonetheless, the observed differences between the students are not due to different training programs because all the students attended the same university and healthcare settings. The better awareness about standards precautions than other issues can be attributed to the practical experience already started from the first year, while more theoretical topics, such as epidemiology, tend to be undersupplied. D'Alessandro et al. [18] evaluated, by means of a questionnaire, the knowledge of both nursing and medical students in the same areas. This study involved 607 medical students and 854 nursing school students, from 9 Italian universities and revealed better knowledge from the nursing school students than the medical students on the three examined topics and, among the same nursing school students, a better level of knowledge by the younger subjects (< 24 years of age). This finding is opposite to our result that showed better scores from the students at the last year of course, in agreement with Cheung et al. [19] that revealed a better compliance towards SP within the students of the second and third years of the course.

Conclusions

The nursing personnel play an important role in the prevention and management of HAI and the combination of the theoretical and practical training can improve the knowledge of the nurses and the quality of assistance. To date, literature data confirm how the awareness about HAI of future nursing school graduates is often already inadequate.
The information for the prevention of HAI should be reiterated to the students after the completing of each procedure with the aim of increasing the awareness of their own role in reducing HAI [20].
It would be advisable to periodically check the knowledge of the students to improve training and consequently reduce HAI, giving the students more responsibility in the area of healthcare and increasing their compliance with prevention, giving more scope to on the field training through the support of a tutor demonstrating in practice what they have learned in theory.

Limits of the study

The study was carried out on students attending the lessons of the degree course who were administered the questionnaire; the difference in the numbers of the three years is due to that the practical training gradually increases and the students attend the theoretical lessons to a lesser extent.
Nevertheless, the results obtained are in line with those reported in other studies in the literature.

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Authors' contributions

All Authors have made a substantial contribution to the conception, design, analysis and interpretation of data,

drafting the article and revising it critically for intellectual content; all Authors approve the final version submitted to the *Journal of Preventive Medicine and Hygiene*.

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ORIGINAL ARTICLE

Influence of physical activity and interest for food and sciences versus weight disorders in children aged 8 to 18 years

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Keywords

Physical activity • Weight disorders • Children • Adolescents

Summary

Introduction. *Being overweight and obesity are a growing problem and are often related to a lack of physical activity among younger people. This study aims to describe the prevalence of weight disorders in Belgian schoolchildren. Secondly, this study examines the association between physical activity, weight disorders and the interest in food and sciences.*
Methods. *We examined 525 children aged between 8 and 18 years old, who attended the Brussels Food Fair or the Belgian Science Day in 2013. They completed a standardized questionnaire about lifestyle and physical activity. Their weight, height, blood pressure and waist circumference were measured. The physical condition of participants was estimated using the Ruffier test.*
Results. *The average age of all participants was 11.2 years (95% CI: 8.7-13.7), the prevalence of being overweight and obesity was 16.3% and 5.4%, respectively. For all participants in the representative group the affiliation to a sports club was associated with a normal weight ($P < 0.05$). According to this study, the kind of transportation to school (foot/bike or car/bus) had no effect on their body mass index (BMI). Neither was there a significant relationship between the physical activity of the children and the result of their Ruffier test.*
Conclusions. *The prevalence of being overweight and obesity in children aged 8 to 18 years is alarming. Membership to a sports club was linked significantly to a normal weight and a lower prevalence of being overweight and obesity.*

Introduction

The prevalence of being overweight and obesity in children has increased worldwide and rapidly in recent years [1-4]. Despite all efforts from media and experts, the number of children with weight disorders is still increasing. Obesity is officially registered - since 1998 - as a chronic disease [5].
The rising prevalence of being overweight and obesity is a major public health problem in most industrialized countries. The consequences in the short and long term place a heavy burden on our public health system. An increase of cardiovascular diseases, diabetes, hypertension, various cancers, sleep disorders, musculoskeletal and psychosocial problems is expected [1-4]. The World Health Organization (WHO) considers being overweight and obesity, and its consequences, as avoidable diseases and therefore prevention has become a priority for public health [5]. A healthy diet and regular physical activity are the most important preventive measures recommended by WHO.
The WHO European Childhood Obesity Surveillance Initiative (COSI) was set up as a response to the need for harmonized and comparable data on overweight/obesity among primary-school children. Countries participating in COSI routinely measured trends in overweight and obesity in primary school children (6-9 years). Twenty-five countries participated, including Belgium [6]. Large between-country differences were found in the availability of food. BMI levels increased in all countries compared to the 2007 WHO levels.
The Health Behaviour in School-aged Children study (HBSC) is a collaborative cross-national study that examines the physical and mental health of young people aged 11, 13 and 15 years in 44 countries [7]. The findings highlight important health inequalities and contribute to a better understanding of the social determinants of health and well-being among young people.
A Belgian study shows that a lower educational level is associated with higher prevalence of indicators of an unhealthy lifestyle. The results are less straightforward for the relationship between socio-economic status and lifestyle behaviors [8].

Prevalence of weight disorders

The increase in the prevalence of being overweight and obesity in children is a global problem. The International Obesity Task Force (IOTF) reported a worldwide prevalence of 20% of overweight and obesity in children and adolescents in 2004 [9]. In the United States, the prevalence of obesity increased in children from 6.5% in the late Seventies up to 19% in 2003 [10]. In the United

Kingdom, the incidence of obesity among teenagers increased between 1995 and 2007 from 2.7% to 4.8% among boys, and from 4.7% to 6.1% among girls [11].
A 2012 study in seven European countries showed a prevalence for being overweight among schoolchildren between 19% in Norway, and 50% in Greece. Obesity was found in 4% in Norway, and 21% in Greece [12]. For Belgium overweightness was found in 21% and obesity in 6%. The prevalence of being overweight and obesity was higher in the countries where children watched more television and used their bike less, to go to school. In Belgium, the National Health Interview Survey (HIS) is a good instrument to monitor the evolution of overweightness in the general Belgian population. The prevalence of overweightness was 8.9%, 11%, 18%, 18% and 20% in 1997, 2001, 2004, 2008 and 2013, respectively. For obesity the prevalence was 4.4%, 4.3%, 6.0%, 5.0% and 7.1%, respectively [13].

Causes of weight disorders

Excess eating, a sedentary lifestyle and too little physical exercise, are a few of the main causes for overweightness, resulting in an energy intake which is higher than the energy consumption of the human body [13].
A healthy lifestyle is important for everyone. This includes a healthy diet and regular physical exercise. The concept of "health-related physical activity" was introduced in 1992 [14]. This concept refers to all movements of the body produced by striated muscles, which result in a significant increase in the energy consumption. It applies to all forms of physical activity, performed in leisure time, household activities, transfers and in the workplace.
Many studies have shown the beneficial effects of regular exercise on health. Even moderate physical activity such as gardening has a beneficial effect on health [15]. Also among adolescents physical activity showed health benefits [16]. Every individual should do almost every day of the week for at least 30 minutes some (moderate) exercise [17]. There is an international consensus that children and adolescents should have at least one hour of moderate to intense physical activity every day. Moreover, they need adapted exercises at least three times per week to improve muscle strength and bone quality [15, 16].
The aim of this study is to describe the prevalence of weight disorders in Belgian children and to find associations between the (lack of) physical activity and these weight disorders.

Material and methods

Study population

This research was conducted at the Brussels Food Fair from the 5th to 20th of October 2013 and at the National Science Day on November 24th, 2013. A total of 525 children were enrolled during these two occasions.
Three study groups were defined. The first study group was a representative sample of children between 8 and 18 years of age. They were recruited during class visits of schools who organized a trip to the Brussels Food Fair.
The second were children with special interest for food. They were recruited during the weekends among the children who visited the Brussels Food Fair with their parents.
The third were children with special interest in science, who were recruited during the National Science Day. During that yearly event, scientific institutes and universities demonstrate scientific experiments on different locations, to make children familiar with practical science.
The accompanying parents received a brief explanation about the design of the study and the investigated parameters at both occasions. For the three groups the parents had to give their informed consent prior to participation.
The assignment to the different subgroups was made on the basis of their presence at one of the activities. The first group were school children from a general population who did not chose to attend the food fair but their school organized the visit. This group was considered as the reference group. The children from the second group or at least their parents chose to visit the food fair. The children of the third group or their parents chose to visit the Science Day. The children themselves were not questioned about their interest in food or science.

Inclusion and exclusion criteria

The two inclusion criteria for this study were having an age between 8 and 18 years, and having an informed consent signed by one of the parents. The two exclusion criteria were pregnancy, or alcohol, drug or medication abuse reported by the participants.

Questionnaire

The study consisted in part of completing a standardized questionnaire, developed by the Netherlands Nutrition Centre, to identify the dietary habits of the participants (Appendix 1) [18]. This questionnaire was completed by the participants with (neutral) assistance of one of the researchers. The children were asked if they had some sweats or soft drinks the day before the fair, if they had breakfast, lunch and dinner, if they ate fruits, vegetables, meat and bread and if they drank milk.

Physical examination

Weight, height, waist circumference, and blood pressure were measured for all participants. For the measurements we used a weighing scale (Seca Sensa 804), a measuring stick (Seca Body Meter 206), a measuring tape, a sphygmomanometer (Welch Allyn DS54) together with a stethoscope (Littmann Classic II SE).
After the examination, the children received a personalized advice, based on the answers they gave to the questionnaire, their eating habits, and body mass index (BMI). The determination of normal BMI values was based on the Flemish Growth curves of the Vrije Universiteit Brussel [19]. On these curves, the calculated BMI values were plotted as a function of the sex and age of the children studied. If the BMI values were located on these curves in the gray area, above the P50, the diag-

nosis of overweightness was made. BMI values above this gray zone corresponded to the category of obesity. The upper and lower limits of that gray zone correspond to respective BMI values of 25 and 30, at the age of 18 years.

Diagnosis of weight disorders

Weight disorders are diagnosed from the age of 18 years by means of the BMI and waist circumference. Overweightness is diagnosed when having a BMI between 25 and 30 kg/m², and obesity from 30 kg/m². This definition cannot be used for children [20-22].

The International Obesity Task Force has published international BMI reference values in 2000, for children and adolescents [9]. These BMI-age charts are based on six major growth studies from the Netherlands, Brazil, The United Kingdom, Hong Kong, Singapore, and the United States.

In 2004, specific growth curves were published for Belgian boys and girls aged 2 to 20 years of age. They are based on a representative cross-sectional study of 7920 Flemish boys and 8176 Flemish girls [23].

These growth curves are also based on the calculation of the BMI for each age group, resulting in percentile lines. Overweightness is defined as having a BMI between the 85th and 97th percentile, and obesity for having a BMI above percentile 97. These percentile lines for BMI are different for boys and girls.

The definition of being overweight and obesity described by the IOTF, and those used in the Flemish growth curves, are basically identical because they use both the same BMI cut-off values. They differ only in the reference values used, which are, for what the Flemish growth curves are concerned, adapted to the Belgian population.

Ruffier test

The Ruffier test was used to evaluate the physical condition of the participants [24]. Participants were asked to make 30 knee bends in 45 seconds. Both heart rate and oxygen saturation in the blood were measured using a pulse oximeter (Meditech Fingertip Oximeter Oxyo) prior to exercise, right after and one minute, after the end of the effort. The Ruffier test was selected to measure the physical condition, because it takes only a few minutes, it is not expensive and it has a low chance for adverse events.

The 'degree of physical condition' was estimated with the formula IR = (p0 + p1 + p2 - 200) / 10 with IR = Ruffier Index, p0 = heart rate before exercise, p1 = heart rate just after the exercise and p2 = heart rate one minute after exercise. For the interpretation of the Ruffier index (IR), we used the following values: IR < 0 = very good, 0 < IR < 5 = good, 5 < IR < 10 = moderate, 10 < IR < 15 = poor, IR > 15 = very bad.

Approval ethical committee

For this study, the approval of the Ethics Committee of the University Hospital Brussels was obtained.

Statistical analyses

Statistical analyses were carried out using IBM® SPSS® Statistics 22. Patient data that were incomplete with regard to weight and height, were excluded for the analyses. Two by two tables were used to evaluate the relationship between two dichotomous variables, by means of a Chi-squared test. Two by three tables were used for three dichotomous variables. The Fisher's exact test was used when at least one expected value was below five. The T-test was used to compare the means of two independent samples. ANOVA was used to compare the means of three independent samples.

We also performed multivariable logistic regression to explore characteristics associated with overweight and obesity. The dependent variable compared participants with overweight or obesity with those with normal BMI. The logistic regression included age, sex, membership of a sports club, transportation to school and Ruffier test results as independent variables.

All study results were standardized for age and gender by means of a direct standardization. Comparisons were statistical significant for p-values below 0.05.

Results

Demographics

The records of 522 participants were eligible for our analysis: 287 (55%) in the "representative group", 194 (37%) in the "food group" and 41 (8%) in the "science group" (Tab. I). In all groups, there was an overrepresentation of the girls, except in the science group where more boys than girls participated. The mean age of all participants was 11.2 years of age (95% CI = 8.7-

Tab. I. Baseline characteristics of the participants for the total study population and for the three study groups.

	Total study population	Reference study group	Food study group	Science study group
Number	522	287	194	41
Girls (%)	301 (57,7%)	161 (56,1%)	123 (63,4%)	17 (41,5%)
Boys (%)	221 (42,3%)	126 (43,9%)	71 (36,6%)	24 (58,5%)
Mean age (SD)	11,2 (2,5)	10,7 (2)	12,1 (2,9)	10,2 (2,3)
Mean weight (SD)	41,5 (14)	40,9 (13,9)	43,6 (14,6)	36,4 (9,4)
Mean height (SD)	146,9 (13)	144,6 (12)	150,8 (13,9)	144,2 (11,7)
Mean BMI (SD)	18,6 (3,8)	18,9 (4)	18,5 (3,6)	17,1 (2,2)

Tab. II. Prevalence of weight disorder for the total study population and for the three study groups.

	Total study population (N = 522)	Reference study group (N = 287)	Food study group (N = 194)	Science study group (N = 41)	p(2)-value
Underweight	9.8%	7.0%	13.4%	12,2%	0.057
Normal weight	68.6%	65.5%	72.2%	73,2%	0.24
Overweight	16.3%	19.9%	11.3%	14,6%	0.043
Obesity	5.4%	7.7%	3,1%	0.0%	0.026
Overweight + obesity	21.6%	27.5%	14,4%	14,6%	0.002

13.7), the mean weight 41.5 kg (95% CI = 27.5-55,5) and the mean BMI 18.6 kg/m² (95% CI = 14.8-22.4). The mean age differed significantly between the three groups, with a significant higher age for the food group ($p(2) < 0.001$). The mean weight and the mean height were also higher for the food group with p(2) 0.003 and 0.005, respectively. The mean BMI differed also significantly between the three groups with a significant lower BMI for the science group $p(2) = 0.005$).

Prevalence of weight disorders

One third of the reference population has a weight disorder: 7% is underweight, 20% overweight and 8% obese (Tab. II). In the groups with special interest for food or science we found significantly less children with overweightness and obesity.

Influence of physical activity

About two thirds of all participants were member of a sports club (Tab. III). This was also the case in the reference group and subgroups. In the reference population, the membership to a sports club is associated with a normal weight, and children that are not member of a sports club have a higher risk for being overweight and obese. These associations however, were not observed in the food and sciences groups.

About one in two of the children went to school on foot or by bicycle (Tab. VI). Only in the food group we found

Tab. III. Prevalence of weight disorder according to membership to a sportsclub for the total study population and for the three study groups..

	Member of sportsclub	Non-member of sportsclub	p-value
Total study population			
Number (%)	339 (64.9%)	183 (35.1%)	<0.001
Underweight	9.1%	11.5%	0.40
Normal weight	71.7%	62.3%	0.029
Overweight	15.3%	17.5%	0.52
Obesity	3.9%	8.7%	0.020
Overweight + obesity	19.2%	26.2%	0.062
Reference study group			
Number (%)	178 (62.0%)	109 (38.0%)	<0.001
Underweight	6.7%	6.4%	0.92
Normal weight	70.8%	58.7%	0.036
Overweight	18.6%	22.1%	0.47
Obesity	3.9%	12.8%	0.005
Overweight + obesity	22.5%	34.9%	0.022
Food study group			
Number (%)	131 (67.5%)	63 (32.5%)	<0.001
Underweight	10.7%	19.1%	0.11
Normal weight	73.3%	69.8%	0.62
Overweight	13.0%	7.9%	0.30
Obesity	3.0%	3.2%	0.96
Overweight + obesity	16.0%	11.1%	0.36
Science study group			
Number (%)	29 (70.3%)	12 (29.7%)	<0.001
Underweight	13.8%	8.3%	0.63
Normal weight	75.9%	66.7%	0.55
Overweight	10.3%	25.0%	0.23
Obesity	0.0%	0.0%	-
Overweight + obesity	10.3%	25.0%	0.23

Tab. IV. Prevalence of weight disorder according to transportation to school for the total study population and for the three study groups.

	Walking or cycling to school (N = 289)	With car or bus to school (N = 233)	p-value
Total study population			
Number (%)	289 (55.4%)	233 (44.6%)	< 0.001
Underweight	9.0%	11.1%	0.60
Normal weight	71.3%	66.4%	0.24
Overweight	15.0%	17.3%	0.48
Obesity	4.7%	5.2%	0.06
Overweight + obesity	19.7%	22.5%	0.45
Reference study group			
Number (%)	142 (49.5%)	145 (50.5%)	0.80
Underweight	6.2%	7.0%	0.77
Normal weight	69.7%	62.1%	0.16
Overweight	16.5%	23.2%	0.62
Obesity	7.6%	7.7%	0.96
Overweight + obesity	24.1%	29.9%	0.19
Food study group			
Number (%)	126 (64.9%)	68 (35.1%)	< 0.001
Underweight	14.7%	12.7%	0.70
Normal weight	69.1%	73.8%	0.49
Overweight	14.7%	9.5%	0.28
Obesity	1.5%	5.0%	0.34
Overweight + obesity	16.2%	13.5%	0.61
Science study group			
Number (%)	20 (48.8%)	21 (51.2%)	0.83
Underweight	5.0%	19.0%	0.17
Normal weight	90%	57.2%	0.018
Overweight	5.0%	23.8%	0.09
Obesity	0.0%	0.0%	-
Overweight + obesity	5.0%	23.8%	0.09

a significant higher proportion of children going to school on foot or by bicycle. Taking the bus or going to school by car was not associated with weight disorders in the reference group. Only in the science group we found a significant association between going to school on foot or by bicycle, and a normal weight.

Physical condition

In the reference population, 60% had a good to moderate physical condition, according to the Ruffier test (Tab. V). Although the figures in the food group seemed a little better and in the science group a little worse, there was no significant difference between the groups, even not after dichotomizing the groups in good to moderate, and poor to bad (p = 0.41).

The results of the Ruffier test for the members of a sports club were not significantly better than for those who were not member of a sports club, with a good to moderate Ruffier test in 35% and 43%, respectively (p = 0.069). The Ruffier test was not better for those who went to school on foot or by bicycle, as compared to those who went to school by car or bus. Both groups showed good to moderate Ruffier test results in 38% and 37%, respectively (p = 0.73).

A logistic regression associated weight disorders with older age and a poor Ruffier test in the reference group. It could not determine an association in the other study groups (Tab. VI).

Tab. V. Distribution of the physical condition in four classes based on the Ruffier test for the total study population and for the three study groups.

	Total study population (N = 522)	Reference study group (N = 287)	Food study group (N = 194)	Science study group (N =41)
Good	10.4%	9.6%	12.2%	4,5%
Moderate	51.5%	50.3%	52.7%	51,3%
Insufficient	30.1%	32.1%	28.0%	32.7%
Bad	8.0%	8.0%	7.1%	11.5%

Tab. VI. Multivariable logistic regression for variables predicting overweight or obesity in the total study population and for the three study groups.

	Significance	Exp(B)	Lower 95% CI	Upper 95% CI
Total study population				
	No significant independent variables			
Reference study group				
Older age	0.046	1.138	1.002	1.292
Poor Ruffier test	0.038	1.406	1.018	1.940
Sports study group				
	No significant independent variables			
Science study group				
	No significant independent variables			
The logistic regression included age, sex, membership of a sports club, transportation to school and Ruffier test results as independent variables				

Discussion

Prevalence of weight disorders

There is no scientific doubt about the increased prevalence of overweightness and obesity among young people in recent decennia. According to the national HIS of 2013, not less than 20% of young people between 2 and 17 years of age were overweight, and 7% were obese [13]. Compared to the previous HIS, the rates have increased clearly.

This study shows a prevalence of overweightness and obesity of 20% and 8% respectively in an age group between 8 and 18 years of age. It is difficult to compare these figures with other studies, because of the difference in age groups between the studied populations. However, these percentages seem to be similar to the HIS of 2013.

The 2012 surveillance study in the seven European countries (see *supra*), also showed alarming figures, especially for the southern European countries [12]. Other studies in France, the UK and the US, show similar increases of overweightness.

The achieved figures show that the situation is worrying and requires an urgent intervention. This is especially important because childhood obesity eventually contributes to the epidemic of obesity in adults.

We initially hypothesized that we would find more persons with overweightness and obesity in the food group, and less in the science group. As the food fair is rather focused on the gastronomic aspects of food and less on healthy food, we assumed that children (and their parents) who attend the food fair would focus less on healthy food than children with an interest in science. We assumed that the latter would have a better knowledge about healthy food. Better nutritional knowledge was correlated with lower body weight in Mexican women with a low socioeconomic level [25]. In high-school students, a better nutritional knowledge increased their self-concept and self-efficacy in order to adopt healthy behaviours [26]. However, the proportion of children with overweightness and obesity was lower in the food and science groups, as compared to the reference group. Probably some selection bias is involved. For the reference group, almost all visiting schoolchildren participated in the study. For the food and science groups it was easier for the children to refuse participation. It is possible that more children with overweightness refused participation because of their potential awareness of the problem.

Age, weight and height were higher in the food group. Most likely, the higher weight and height are related to the higher age.

The causes for the obesity pandemic are well known: bad dietary habits, too little physical exercise, eating disorders, and heritability. Before the eighties, overweightness was not an issue, because most people grew up during or just after the Second World War. The lack of food resulted in a kind of "protective underweightness". But the generation of young people growing up in the eighties never knew the issue of lack of food. Since 1988, up until today, the proportion of people with overweightness has doubled.

However, the Second World War was not the only "factor" in this growing health problem. Since the eighties, the price of food decreased, the amount of food advertising increased, fast food restaurants appeared, more people had sedentary jobs, and more people owned computers and televisions, all resulting in less physical activity. Moreover, more households had two-person jobs and incomes, that resulted in an increased number of restaurant visits.

The problem of overweightness appeared together with the increased welfare, and is for that reason a lifestyle disease.

Effect of physical activity on overweightness

Not less than 64% of the participants in our study were member of a sports club. These children had significantly less weight disorders. Especially children with obesity were not member of a sports club. For those with overweightness, no significant difference was observed. We cannot conclude that membership of a sports club protects against obesity, but we can conclude that those who have the greatest benefit in physical activity are not subscribed in a sports club. Probably the activities in the sports club are not adapted to children with obesity. It would most likely be helpful should sports clubs make

more efforts to provide adapted programs for children (and adults) with obesity.
We found no association between weight disorders, and the transportation method to school. The distance between the homes of the children and the schools in Belgium is quite short on the whole, and probably not lengthy enough to have any effect on weight.
Neither did we find an association between the physical condition on one hand, and the membership to a sports club or the transportation method to school on the other hand. We are aware that the Ruffier test is not the most sophisticated test to detect small differences and association of this kind [27]. However, weigh disorders were associated with a poor Ruffier test and older age in the logistic regression.
The comparative study of the seven European countries showed that children from the countries with the highest proportion of overweightness and obesity (Greece and Hungary) had the lowest physical activity and watched more television than other children [12].
The amount of physical exercise is an important measurement for the prevention of overweightness and obesity, both in children and adults [28-30]. Physical activity improves physical fitness, weight and health in some studies but not in all [31]. However, it reduces the risk of various diseases (cardiovascular diseases, obesity, diabetes, osteoporosis) [1, 2]. Even in obese adolescents, a program promoting physical activity may help to reduce body weight [31].
There is an international consensus that children and adolescents must have at least one hour of daily moderate to intense physical activity. Moreover, they need more thorough exercise, at least three times a week, to improve bone quality and muscle strength [33].
A Cochrane meta-analysis of 55 studies evaluated the effectiveness of various preventive interventions for obesity in children. Regular physical activity was one of the most important actions to prevent it [15].
Some countries already started in the early nineties with prevention programs. The EPODE (Ensemble Prévenons l'Obésité des Enfants) project was launched by the university of Lille in 1992 [34]. The EPODE project consisted of various activities in the community, at school, or in the family, and is based on providing information about healthy food and exercises, involving parents, professionals and the villagers. In 2004, the project was evaluated favorable and implemented in several other countries such as Belgium, Spain, Greece and Australia. The JOGG project (Jongeren op Gezond Gewicht) was based on the French EPODE project [35].

Strengths and limitations of the research

The main strength of the study is the use of a representative sample of the young population in Belgium.
In other studies, attention was paid to the difference between girls and boys. In our research, we did not consider these differences as a research question.
To determine the physical condition of children, the Ruffier test was used. In the literature only few data are available on the reliability of this test [24]. However, this test is widely used and practical to estimate the physical condition. It has the advantage of being simple, fast, and reproducible. It requires no special material and can be used both for children and adults.
We did not identify what types of sporting activity was carried out, and at what frequency it was performed. This seems to be of great importance, because there exists a clear dose-response relationship between the intensity of physical activity, and health benefits [3, 4, 35, 36]. Some bias may exist, because some children were not (or no longer) member of a sports club, but they may practice regular physical activity for example by cycling, jogging or swimming outside the regime of a sports club.
The origin of the participants was not taken into account in our research. There was an important proportion of children of non-European origin (Moroccan, Turkish, South African...). Genetic and cultural differences, such as eating habits, play an important role in the development of overweightness and obesity in children. Using the Flemish growth curves is controversial in this population segment, because the prevalence of obesity among young non-European residents is almost twice as high as among the Flemish youth. In the Netherlands, specific growth curves are developed for children of Turkish and Moroccan origin [37].
We did not use the international growth curves, because the local ones are better adapted to the Belgian situation.

Conclusions

The prevalence of overweightness and obesity in children aged 8 to 18 years of age, remains high. Membership to a sports club was significantly linked to having a normal weight and a lower prevalence of overweightness and obesity. The mode of transportation to school was not associated with their BMI. There was also no significant association between the exercise habits of these children, and their physical condition. However, a poor Ruffier test was associated with an increased BMI.

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The authors declare no competing interest.

Authors' contributions

AF: conception, design, analysis and interpretation of data, drafting of the manuscript and final approval of the version submitted. EV: conception, design, analysis and interpretation of data, drafting of the manuscript and final approval of the version submitted. IVR: analysis and interpretation of data, drafting of the manuscript and final approval of the version submitted. DD: conception,

design, analysis and interpretation of data, drafting of the manuscript and final approval of the version submitted.

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Appendix 1

Questionnaire

What is your gender? O Man O Woman

What is your postal code? _____

What is your year of birth? _____

How would you rate your health on a scale from 1 to 100 where 1 corresponds to "very poor" and 100 "very good"? Put a cross on the line.

1----------100

Weight: _____ kg

Height: _____ cm

Abdominal circumference: _____ cm

Blood pressure: _____ /_____ mmHg

Are you affiliated with a sports club?	O Yes	O No	O Don't know
Did you have some sweats yesterday?	O Yes	O No	O Don't know
Did you have some soft drinks yesterday?	O Yes	O No	O Don't know
Did you have breakfast yesterday?	O Yes	O No	O Don't know
Did you have dinner yesterday?	O Yes	O No	O Don't know
Did you have supper yesterday?	O Yes	O No	O Don't know
Did you have some vegetables yesterday?	O Yes	O No	O Don't know
Did you have some fruit yesterday?	O Yes	O No	O Don't know
Did you have some meat yesterday?	O Yes	O No	O Don't know
Did you have some bread yesterday?	O Yes	O No	O Don't know
Did you have some milk or yoghurt yesterday?	O Yes	O No	O Don't know
How do you usually go to school?	O By bus or car		O On foot or by bicycle

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ORIGINAL ARTICLE

An overview of different health indicators used in the European Health Systems

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Keywords

Health systems • Europe • Health indicators • Health care performance

Summary

Introduction. *In the European Union three different health systems could be defined according to service delivery, financing, and economic policies: Beveridge, Bismarck and Mixed system. Although health systems are hardly to compare, various organizations are developing methods assessing performance. In the present work the performance of the three systems were evaluated using European Community Health Indicators according to Organization for Economic Cooperation and Development.*
Methods. *The study has been conducted among the 28 states of the European Union using the following indicators: Standardized death rate for diseases of the circulatory system, standardized death rate of malignant neoplasms, road traffic accidents with injury, life expectancy at birth, incidence of Human Immunodeficiency Virus (HIV), infant deaths, pure alcohol consumption, infants vaccinated against Diphtheria Tetanus Pertussis (DTP), public and total expenditure on health over the period 2001-2010.*
Results. *The variation of health indicators over the observational time shows similar trend of circulatory system diseases and malignant neoplasms death rates, road accidents with injury, infant deaths, life expectancy at birth, public and total health expenditure. Some differences in the trend of HIV incidence, alcohol intake and DTP vaccination rates arise among systems. Grouping countries by health system paradigm and geographical area, resulted in a relevant heterogeneity ($I^2 \geq 90\%$, Pvalue < 0.0001). No clear superiority of a given health delivery system was found with respect to other paradigms.*
Conclusions. *In accordance with the evidence of our study, it can be stated that best performances are more likely to be linked to country specific economic factors. In conclusion, it was not possible to identify the best health system model.*

Introduction

Health systems in the European Union are managed in a different way according to models of service delivery, financing, and economic policies. Three different systems could be defined. National health services (also referred to as "Beveridge" systems) are distinguished from social insurance systems (also referred to as "Bismarck" systems) with respect to the role of the state as financier and owner of facilities [1]. Different grading of mixed systems between the two is quite common worldwide [2-4].
The Beveridge model, first established in the United Kingdom in 1942, is financially granted by public taxes and the state directly finances structures. This model is also referred to as National Health System (NHS) and provides universal health coverage. In Europe this model is adopted by Cyprus, Denmark, Finland, Ireland, Italy, Latvia, Malta, Portugal, Spain, Sweden and United Kingdom [5, 6].
On the other hand, in Bismarck model (Germany, 1880), the financial funding of the health care system is granted through compulsory social security contributions by employers and employees. The management of the funds is exerted by no profit agencies. The State provides health care coverage to those who are not enrolled in the employment insurance fund. This model is also referred as Social Health Insurance System (SHIS) and is adopted in Belgium, Estonia, France, Germany, Lithuania, Luxembourg, Netherlands, Poland, Czech Republic, Romania, Slovakia, Slovenia and Hungary [5, 6].
In the Mixed model, private funding from voluntary insurance schemes or upfront payments is significant. This model is also referred as the Private Health Insurance System. European countries that have adopted this system are Austria, Bulgaria, Greece and Croatia [5, 6].
Health Systems are different and complex, therefore they are hardly to compare. Various Countries and international organizations studied methods for assessment of health system performance. The most interesting models of evaluation are developed by World Health Organization (WHO), Organization for Economic Cooperation and Development (OECD), European Community Health Indicators (ECHI) and Bloomberg L.P., a privately held financial software, data, and media com-

pany. The WHO index is built on following indicators: disability-adjusted life expectancy, responsiveness and fair financial contribution [7]. The OECD makes a correlation analysis between outcomes, resources and other determinants; it has not yet developed a real numerical index of evaluation but the individual determinants of health were assessed among its Member States [8]. The ECHI initiative started with the 1997-2002 EU Health Monitoring Program, pointed to get a harmonized picture of European health conditions [9]. Bloomberg L.P. propose an index where each country was ranked on three criteria: life expectancy, relative per capita cost of health care and absolute per capita cost of health care [10].
Nevertheless, every year different agencies define an official country ranking according to different health determinants, which of the three health care models is best performing is still under discussion. In the present work, the research group would evaluate and compare the performance of the three systems using European Community Health Indicators (ECHI) [9].

Methods

The research group conducted the study among the member states of the European Union (EU). The EU is a political-economic union of 28 countries established under its current name in 1993 by The Maastricht Treaty [11, 12].
To evaluate the three Health Systems, the following ten indicators were chosen from ECHI program [9]: standardized death rate for diseases of the circulatory system, standardized death rate for malignant neoplasms, road traffic accidents with injury, life expectancy at birth, incidence of Human Immunodeficiency Virus (HIV), infant deaths (health status indicators); pure alcohol consumption (health determinants indicator); infants vaccinated against Diphtheria Tetanus Pertussis (DTP), public expenditure on health, total health expenditure (health interventions and health service indicators).
The choice was based on the *usefulness rating of ECHI indicators* [13], an index obtained by a survey carried out among European policy makers, and on the availability in *WHO-Health For All database* [14] for the 28 EU member States at the time of research (September 2015). The health indicators have been collected for each country in a time frame of 10 years from 2001 to 2010.
Ethical approval was not required for this study.

Statistical methods

Indicators were described by country and by health system paradigm using median and interquartile range over the period 2001-2010. The logarithmic transformation was used for count variables while the arcsine transformation was used for rates when performing country-specific repeated measures models. Estimates by year were performed and used to remove linear trends and account for autocorrelation of the measures. Those values were meta-analysed by countries grouped in four main geographical areas defined as North-Baltic (Denmark, Estonia, Finland, Latvia, Lithuania and Sweden), Central-West (Austria, Belgium, France, Germany, Ireland, Luxembourg, Netherlands and United Kingdom), Central-East (Bulgaria, Czech Republic, Hungary, Poland, Romania and Slovakia) and South-Mediterranean (Croatia, Cyprus, Greece, Italy, Malta, Portugal, Slovenia and Spain). Overall heterogeneity given by country and geographical area was evaluated by health system paradigm using the Cochrane's Q test and the I^2 statistic. Source of heterogeneity were addressed using random effect meta-regression grouping countries by health system paradigm and geographical area. Thus, unrotated principal component based cluster analysis was performed using the Z-score value of the time de-trended variables during the period 2001-2010. Countries were plotted using the first two principal components scores and a dendrogram was used to report clustering among countries. Generalized Linear Models (GLM) of the first two principal components were interpolated with respect to health system paradigm, demographic, social and economic parameters and clusters previously identified.
All statistical evaluations were performed by SAS vers.9.3.

Results

The variation of health indicators over the observational time from linear regression analysis clearly shows a decreasing trend of cardiovascular diseases (CVD) and malignant neoplasms death rates, road accidents with injury, and infant deaths (Tab. I, Fig. 1). On the contrary, life expectancy at birth and health expenditure increases consistently despite health system paradigm. Some differences in the trend of HIV incidence, alcohol intake and DTP vaccination rates arise among country. Supplementary tables 1-10 reports health indicators changes over time by country.
Countries have been grouped by health system paradigm and geographical area and it has been reported the average and standard error over the observational period for all health indicators in Figures 2 and 3. According to these evaluations, it appears a relevant heterogeneity ($I^2 \geq 90\%$, P value < 0.0001) for all indicators with the exceptions of infant deaths and DTP vaccination rates, where differences by health system paradigm were not found according to paradigm and area random effect metaregression ($P_{Area} = 0.284$, $P_{System} = 0.806$).
Repeated measure analysis showed us the relation between determinants of the heterogeneity reported above and the interaction between factors, as geographical area, health system paradigm, and time. The relation between indicators and time was consistent with the exception of alcohol intake and infant vaccination against DTP. A significant interaction between CVD death rate, road traffic accidents with injury, life expectancy at birth, HIV incidence and both public and total health expenditure was found by within country analysis of variance. A statistical significant effect of the interaction between time and

Tab. I. Descriptive statistics of selected indicators by health system paradigm over the period 2001-2010.

	Bismarck		Mixed		Beveridge	
Indicator	Δ/year	Median (q1,q3)	Δ/year	Median (q1,q3)	Δ/year	Median (q1,q3)
SDR cardiovascular diseases x 100,000†	-12.31	325.8 (295.3, 354.8)	-12.88	374.4 (345.6, 413.2)	-9.65	235.7 (213.4, 259.3)
SDR malignant neoplasms x 100,000†	-2.07	190.9 (187.9, 197.2)	-0.60	173.5 (172.7, 173.9)	-2.56	163.2 (157.1, 168.6)
Road traffic accidents with injury x 100,000†	-3.80	193.8 (182.6, 200.3)	-2.69	229.2 (219.6, 231.2)	-6.94	214.3 (204.2, 238.8)
Life expectancy at birth (year)	0.33	76.48 (75.71, 77.24)	0.23	76.97 (76.35, 77.51)	0.32	78.93 (78.26, 79.70)
HIV incidence x 100,000†	0.17	3.23 (2.93, 3.76)	0.14	2.35 (1.96, 2.63)	-0.01	6.66 (6.27, 6.86)
Infant deaths x 1000†	-0.28	4.88 (4.13, 5.70)	-0.27	5.32 (5.01, 6.05)	-0.16	4.14 (3.79, 4.53)
Pure alcohol consumption litres x person	0.02	11.34 (11.27, 11.56)	-0.09	10.87 (10.68, 11.08)	-0.01	9.78 (9.47, 9.87)
DTP vaccination of infants (%)‡	0.11	97.6 (97.1, 97.7)	0.41	94.0 (92.7, 94.4)	-0.16	95.3 (94.8, 95.6)
Total health expenditure PPP$ x person	140.2	2255 (1934, 2612)	118.6	1993 (1711, 2379)	146.1	2451 (2111, 2856)
Public health expenditure PPP$ x person	98.1	1654 (1453, 1935)	94.2	1485 (1279, 1769)	121.6	1876 (1555, 2197)

Note: †log transformed and ‡arsin transformed to perform regression over time. Δ/year = variation by year performed by regression over time, Median (q1,q3) = median, first and third quartile of raw dataover the observational time (2001-2010). SDR = standardized death rate. HIV = human immunodeficiency virus. DTP = diphtheria tetanus pertussis. PPP = purchasing power parity

geographical area for circulatory system diseases death rate, alcohol intake and both public and total health expenditure was shown by within country analysis.

Multivariate clustering of countries

Principal component analysis of detrended variables over time resulted in two component corresponding to an overall 56.5% of explained variance: the first accounts for 33.2% of the variance and the second one for 23.3% (Fig. 4A).

The first component counterpoises CVD and cancer death rates having loadings of 0.42 and 0.35 respectively to health expenditure both total and public (factor loadings of 0.51 and 0.49 respectively); this component represent a general score ranking death prevention efficacy in terms of health expenditure.

The second component counterpoises CVD deaths and HIV incidence and infant deaths (with loadings of 0.31, 0.58 and 0.28 respectively) to cancer death rate, alcohol intake and life expectancy having loadings of 0.25, 0.24 and 0.56 respectively (Fig. 4B); this component seems to be related to a more specific score ranking countries according to early vs late deaths, with HIV incidence playing a role on the side of early death factors being probably related to behaviours at risk as a proxy.

According to those two components, the biplot (Fig. 4C) shows a clear cluster of countries with positive scores on the first component. Those countries having better health system performances (Ireland, Denmark, United Kingdom, Austria, Germany etc.) are opposed to less efficient countries (Bulgaria, Lithuania, Romania, Latvia, Estonia and Hungary) for which the health system performances are less brilliant. This pattern is graphically represented on the dendrogram reported on Figure 4D.

Discussion

Analysing the variation of health indicators over the considered period, it can be observed that some variables show the same temporal trend for the three systems. Although the cardiovascular diseases are the principal cause of death for EU28 member States (37.5% of all deaths) [15], standardized death rates were gradually decreased as the research group expected since the improving of specific treatments and therapies, and risk factors reduction (high blood pressure, high cholesterol level). Similar tendency is shown for standardized death rates malignant neoplasms, for early diagnosis thanks to secondary level prevention (*i.e.* screening tests). About HIV incidence, this indicator seems to be quite stable over the time. Therefore, according to the OECD Report [8] this result hides diverging trends across countries. For example, the newly diagnosis of HIV has nearly double in Greece and the other hand the rates have dramatically dropped in Estonia. All the countries have seen their alcohol consumption increase from 2001 to 2007 and then fall since 2008. Considering that the variation of this indicator reflects change in drinking habits and primary health care policies [8], the rise in unemployment caused by 2008 economic recession could have been associated with a decrease in alcohol intake [16]. All European countries have achieved remarkable progress in infant mortality rates, with an almost steady decline from 2001 to 2010. Infant mortality reflects socioeconomic conditions, health and individual lifestyles of mothers, as well as the quality and efficiency of the health system [17]. The reduction of infant death rate along with the reduction of mortality before the fifth birthday explain the significant overall increase of life expectancy at birth. Decreasing of death rates and rising of life expec-

Fig. 1. Trend over the observational time for the 10 indicators considered by health system delivery paradigm. Dotted line portray mobile mean interpolation (period 2).

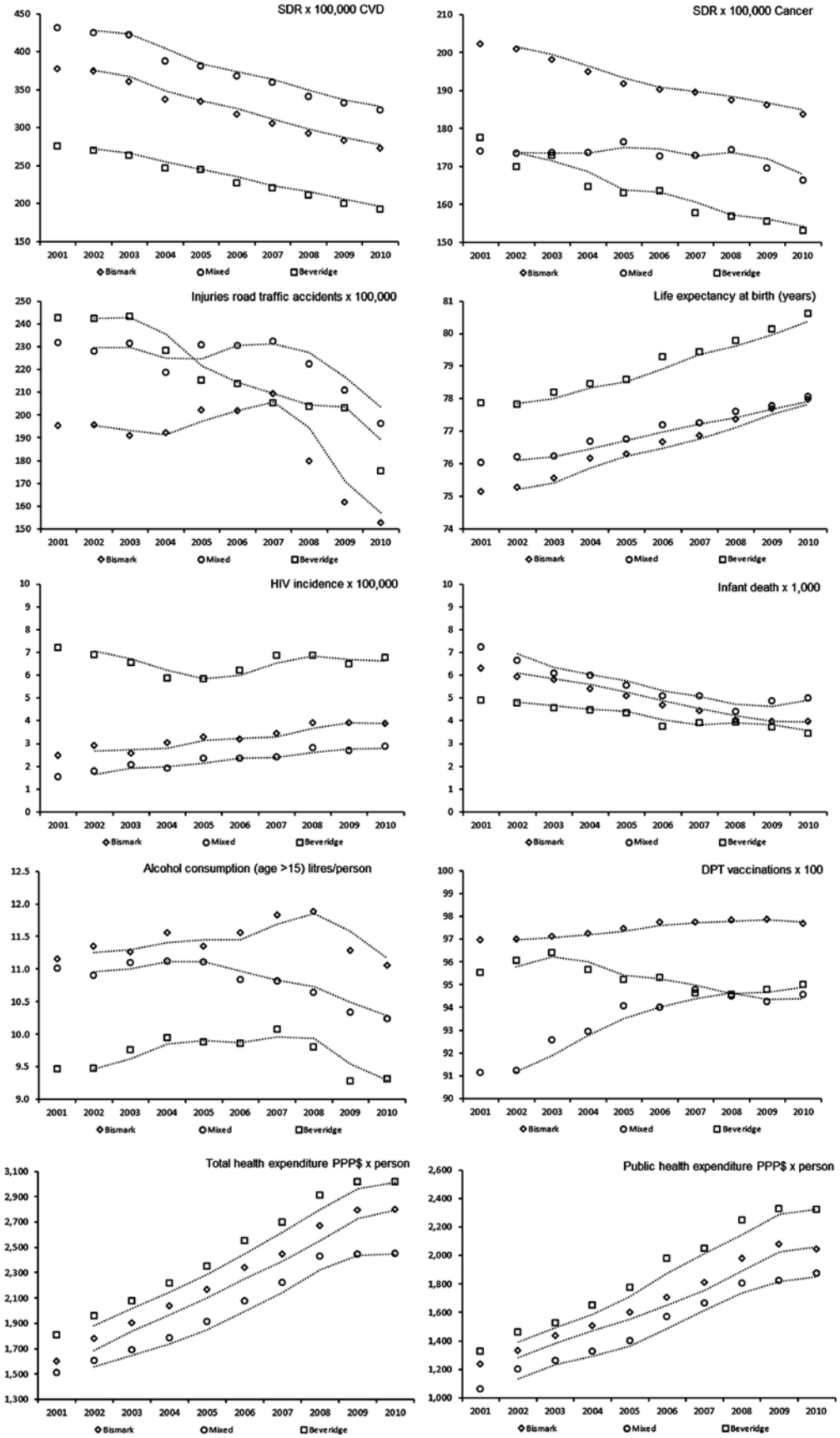


Fig. 2. Standardized Death Rate (SDR) for cardiovascular diseases (CVD) and cancer, Road traffic accidents (RTA) injuries, life expectancy and HIV incidence by health system paradigm over the period 2001-2010.

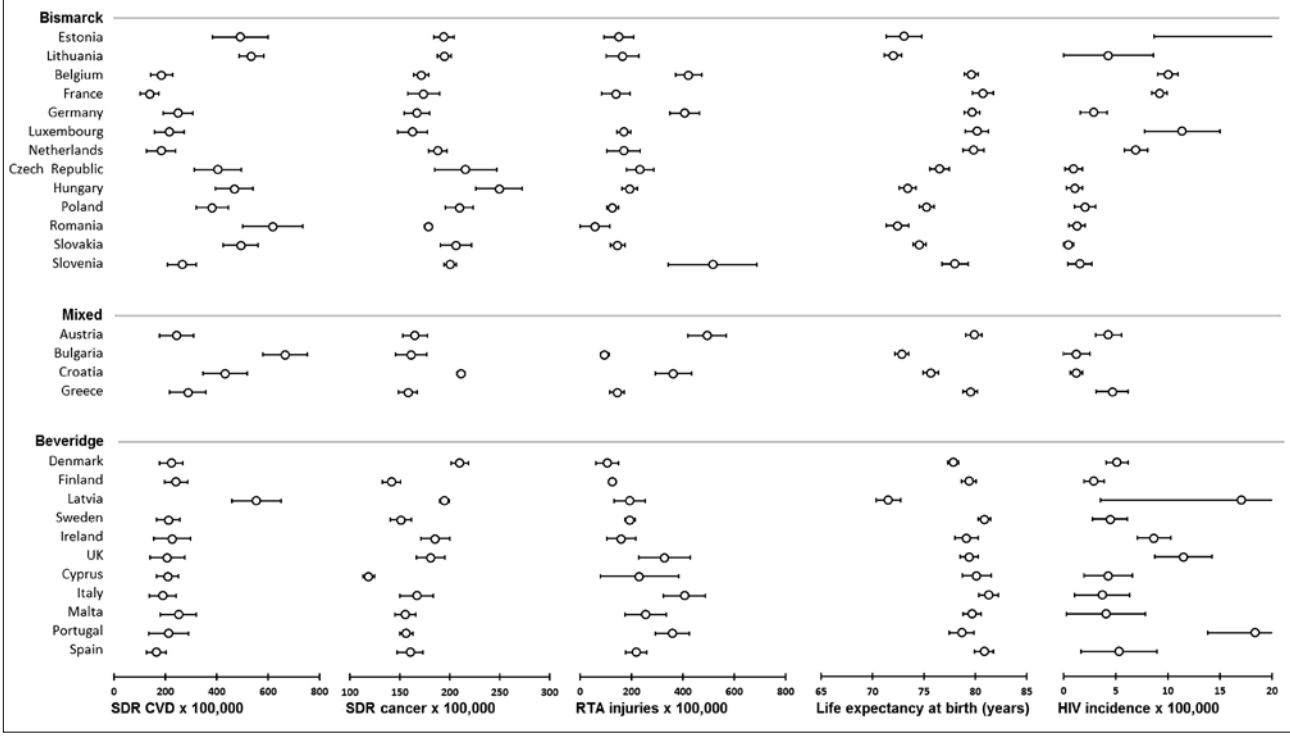
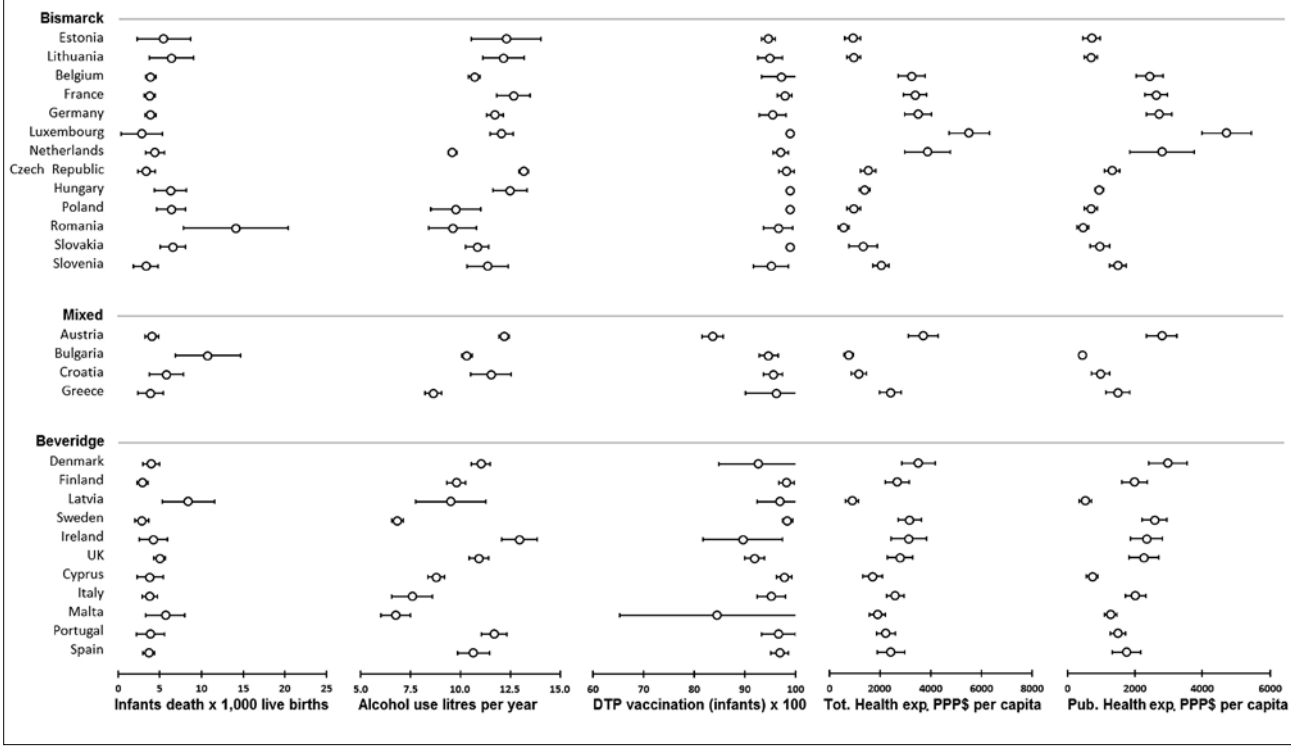


Fig. 3. Infant deaths, alcohol intake, DTP vaccination, and public health expenditure by health system paradigm over the period 2001-2010.

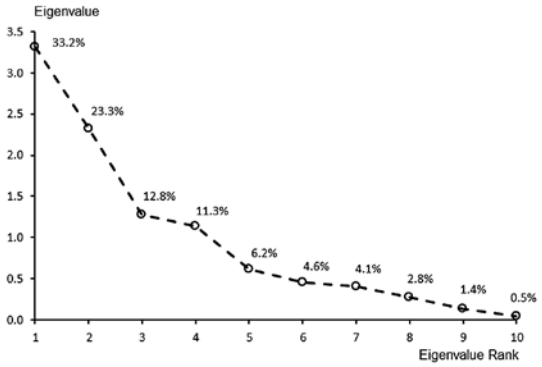


tancy have led to a substantial growth in the number of elderly people with chronic illness or disability. These conditions, together with advances in health care technologies could be considered as major determinants of the increasing in health expenditure [18] over the covering period.

With regard to road traffic accidents with injury and infants vaccinated against DTP, a high heterogeneity has

Fig. 4. Multivariate analysis of detrended indicators and country clustering.

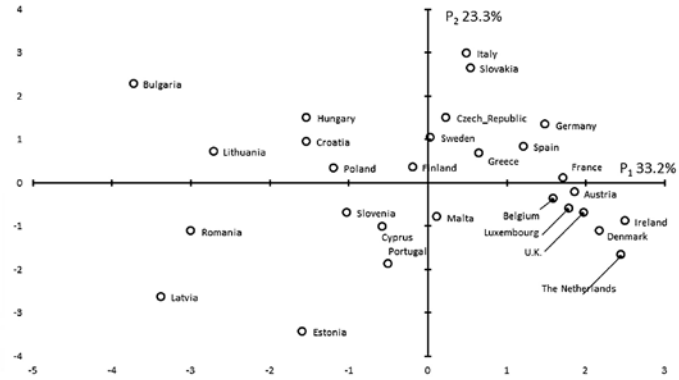
A) Eigenvalue plot. Eigenvalue explained variance by rank



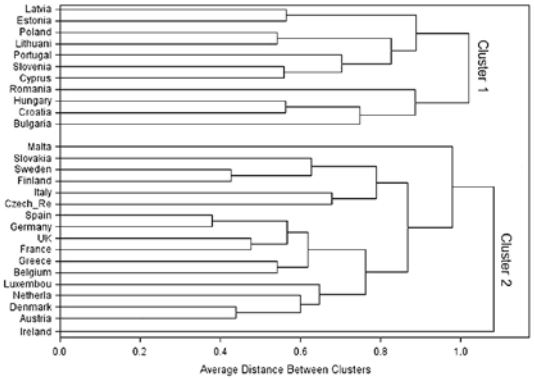
B) Variable loadings on the first three principal components

Indicator	L1	L2	L3
CVD deaths	-0.4244	0.3147	0.1167
Cancer deaths	-0.3544	-0.2507	0.0737
Road injuries	-0.0735	0.1573	-0.2193
Life exp	-0.0297	-0.5561	-0.2701
HIV incidence	-0.0569	0.5794	-0.0277
Infant death	0.3421	0.2788	0.3800
Alcohol use	-0.2016	-0.2430	0.4928
DTP vaccination	0.1567	0.1231	-0.6645
Tot health expenditure	0.5086	-0.0923	0.1283
Public health expenditure	0.4938	-0.0891	0.1177

C) Biplot of country scores over the first two components



D) Dendrogram of multivariate clustering



been observed in temporal variation intra and inter health system models. In presence of a large variety of vaccine offers, the vaccination schedules are programmed in different ways within Europe: DTP is mandatory in 9 of 28 EU state members (Bulgaria, Croatia, Czech Republic, Hungary, Latvia, Poland, Romania, Slovakia and Slovenia) and in four more countries (France, Greece, Italy, Malta) only Tetanus and Diphtheria. In the remaining 15 nations DTP is recommended [19, 20]. Although mandatory vaccination may be a way to improve the compliance with vaccination program, it is not possible to highlight a plain overlap between high coverage and country that oblige DTP vaccination. Indeed, many other factors, as the use of combined vaccines and informative and promoting campaigns, may play a role in vaccination coverage. Both cultural and historical background shall be accountable for such variabilities [19]. Despite in the last few decades the incidence of road traffic accidents with injury was decreased, this decline has certainly happened unevenly throughout Europe. Road traffic accidents are primarily affected by several factors, such as alcohol consumption, vehicles utilisation rateand economic status, resulting in the fluctuation emerged in our data.

Conclusions

As final remarks, it can be affirmed that health system performance is not due to health system paradigm and proxies of the economic status of a country should be taken into account. In fact, as shown here, geographical area has more impact on the variability of such indicators as death rates for circulatory system diseases, alcohol intake and total and public health expenditure.

However, a limit of this study could be the decision to use in the research only the indicators that were up to date for all countries in the time frame selected. This was due to the unavailability in the European Health for All Database, of data concerning specific indicators for some countries.

Furthermore, it would be useful to perform alternative analysis taking into account other possible factors, as Gross Domestic Product, the time length of the permanence in the European Union and other demographic or socioeconomic indicators.

Therefore, in accordance with the evidence of our study, it is not possible to identify the best performing Health System. Nevertheless, the multivariate clustering analysis points out that the best performing countries are those in which the health expenditure is higher in absolute terms, regardless of their health system. Hence,

the study confirms that, as expenditures are important, so total health expenditure is a crucial part for a good performing health system. How the health expenditure could be evaluated in relative terms and how this may influence the health system efficiency is still an open question.

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Authors' contributions

MG conceived of and planned the study, performed statistical analysis and participated in the interpretation of results. FC planned the study, participated in the interpretation of results, draft the manuscript. LC made English language revision. GMS planned the study, participated in the interpretation of results, draft the manuscript. LG planned the study, participated in the interpretation of results, draft the manuscript. GB and GP made critical revision of the manuscript. CR conceived of and planned the study, performed statistical analysis and participated in the interpretation of results.
All authors approved the final manuscript.

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ORIGINAL ARTICLE

Exploring patient safety culture in preventive medicine settings: an experience from Northern Italy

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Keywords

Patient safety • Staff culture • Territorial preventive care • Italy

Summary

Introduction. *Patient safety and quality in healthcare are inseparable. Examining patient safety culture in staff members contributes to further develop quality in healthcare. In Italy there has been some experience in assessing patient safety culture in staff working in hospital. In this pilot study we explored patient safety culture in public health staff working in Italian Local Health Authorities.*

Methods. *We carried out a descriptive cross sectional study in four Italian territorial Prevention facilities in Northern Italy. We administrated an adapted Italian version of the US Hospital Survey of Patient Safety Culture to all the staff within these facilities. The survey consisted of 10 dimensions based on 33 items, according to the results of a previous psychometric validation.*

Results. *Seventy per cent of the staff responded to the survey (N = 479). Overall, six out of the 10 dimensions exhibited composite scores of positive response frequency for patient safety culture below 50%. While "communication openness" (65%) was the most developed factor, "teamwork across Units" (37%) was the least developed. The work areas with the highest composite scores were Management and the Public Health Laboratory, while in terms of professional categories, Physicians had the highest scores. Patient safety culture in the staff participating in this study was lower than in hospital staff.*

Discussion. *Our descriptive cross sectional study is the first to be carried out in Preventive medicine settings in Italy. It has clearly indicated the need of improvement. Consequently, several interventions with this aim have been implemented.*

Introduction

Patient safety, defined as "the prevention of harm caused by errors of commission and omission", is a critical challenge of healthcare systems around the world [1]. Risk profile in healthcare settings depends on a lot of factors, of which users' characteristics and organizational variables are the most important [2]. Users' characteristics, such as age and current health condition, establish the access point to the healthcare system, but they cannot be largely influenced in order to increase patient safety [2]. For instance, objectively healthy people are served by preventive medicine facilities; community members with less serious conditions are in charge of primary care settings; people with serious acute conditions needing high diagnostic and therapeutic technologies make use of hospitals, while frail elderly people with chronic diseases and lower need of medical technologies are hosted in nursing homes. Conversely, organizational factors such as procedures, staff competence and skills, quality systems and organizational culture, which can be influenced, should be systematically assessed and improved to continually increase patient safety.

Quality of care and patient safety should be guaranteed in all access points to the healthcare system of a country, independently of the intensity of care needed by their health condition. Examining staff attitudes with regard to patient safety (safety culture) in each type of healthcare setting may contribute to the better understanding of performance variations across them in terms of quality and safety.

Several international surveys showed that differences in patient safety culture exist between primary care, hospital and nursing home staff [3-5]. Surveys of patient safety culture that include territorial Preventive medicine staff are scarce and no disaggregated/specific data are available [6].

Across Italy, 154 regional public agencies called Local Health Authorities (LHAs) manage healthcare services for subsets of the regional population in defined geographical areas (the average population served is 390.000 inhabitants) [7]. Within the LHAs in Northern Italy, the Department of Medical Prevention works ac-

cording to regional prevention programs to provide sanitary education, healthy life style promotion, vaccinations, screenings, safety and hygiene services for food, the environment, the workplace etc., infectious disease surveillance and public health lab analyses [8-11]. Staff work in multidisciplinary teams of public health professionals and workers, including doctors (*e.g.* specialists in Public Health, Preventive medicine, Infectious diseases, Environmental epidemiology, Toxicology), sanitary assistants (*e.g.* assistant medical officers, public health nurses), technicians (*e.g.* environmental health officers or public health inspectors), clerks and others (*e.g.* psychologists, dietitians and nutritionists, engineers, public health lawyers, sociologists) [12].

The Department of Medical Prevention closely collaborates with the Department of Veterinary Prevention, according to the "one health" pattern, based on a socio-ecological system perspective, in which several distinct service providers contribute to public health in their catchment area in a coordinated manner, each overseeing a different branch. While in some Italian LHAs medical and veterinary preventive activities are provided by separate departments (*e.g.* Lombardy region), in others these activities are provided by the same department, *i.e.* the Department of Prevention (*e.g.* Piedmont region).

The aim of this pilot study was to examine patient safety culture in Italian territorial Prevention facilities by investigating this in four different settings across Northern Italy. Assuming that patient safety culture in the staff members of an organization is a multidimensional concept, we applied the Agency for Healthcare Research and Quality (AHRQ) Hospital Survey on Patient Safety Culture (Hospital SOPS) [13], which was translated into Italian and adapted to our target settings, to find out which areas of patient safety were poor and needed improvement. We also examined differences across work areas and professional categories.

Methods

Study design and Settings

A descriptive cross-sectional study was carried out in four Italian territorial Prevention facilities: three Departments of Medical Prevention in the Lombardy region and one Department of Prevention in the Piedmont region. These settings voluntarily participated in an international project aimed at developing patient safety culture in Italian territorial Prevention facilities and Eastern European hospitals (*il progetto IRIDE: Italia - Romania - Repubblica Moldova in Rete: Imparando dagli errori verso una cultura della sicurezza dei pazienti/utenti*). They serve a population varying from about 200,000 to 1,100,000 inhabitants. Data were collected with an online form. The survey was administered from October 1st 2013 until the end of the month in two settings and between September 15th through to October 15th 2014 in the other two settings. Two reminders were sent in each setting before ending data collection, in order to increase the response rate.

Participants

The target population was represented by all Units and staff members in the target facilities.

Most Units existed in all four facilities at the time of the study (*e.g.* Hygiene and Public Health, Infectious Disease Prevention and Epidemiology, Community Preventive Medicine, Prevention and Safety in the Workplace, Plant engineering and Safety in the Workplace, Food & Nutrition Hygiene and Management). Some other Units (*e.g.* Public Health Laboratory, Environmental Medicine, Legal Medicine and Veterinary) did not exist in all facilities.

The main professional categories were represented by Technicians, followed by Physicians, Nurses/Sanitary assistants and Unit assistants/clerks/secretaries.

Data sources and measurement

Since its release in 2004, the AHRQ Hospital SOPS was translated in 31 languages and administered in 66 countries [14]. It showed acceptable psychometric properties in Europe [15-24], Asia and the Middle East [25-27]. It had been already translated into Italian (with the back translation method) [28] and applied in several Italian hospitals [28, 29, 30]. It was slightly adjusted for application in our settings and pre-tested on a small group of staff members from different professions. Psychometrics of the Italian version of the Hospital SOPS for territorial Prevention facilities were then explored. Among the 42 items of the12-factor original US survey, only 33 items based on a 10-factor model showed acceptable psychometrics for application in our target facilities [31]. Moreover, the survey assessed two output indicators and required additional information on work area, staff position, and whether they have direct or indirect interaction with patients. The survey also allowed for open comments to be written at the end.

Items were measured using a 5-point Likert-type scale and were then aggregated into 10 composites (factors). Most safety culture composites used the scale of response option in terms of agreement (*Strongly agree to Strongly disagree*) and three composites in terms of frequency (*Always to Never*). Patient safety grade (output indicator) was measured with a five-point scale ranging from "*Excellent*" to "*Failing*". Another output indicator was the number of reported adverse events in the last 12 months, assessed through five frequency categories. Participants were asked to respond to this item only if there had been an incident reporting system in their facility.

Anonymity was ensured throughout the study. To reduce respondents' fear of being identified, several methods were adopted. Units with very low number of staff were aggregated to Units with higher number of staff, within the same work area. Moreover, a work area called "*Not otherwise specified*" was added to the seven work areas obtained, in order to be ticked by the respondents who did not want to indicate his/her true Unit. The same was done for the professional categories. The pre-test participants were informed that they would not be further invited to complete the survey. A thorough quality check was carried out on the surveys received. Forms with less

than one entire section completed, with less than half the questions answered, and straight-lining forms (where responses to all items in Sections A, B, C, D and F were the same) were excluded.

Study size

Overall, 673 workers received the survey (staff census). After the quality check, the final dataset consisted of 479 respondents across four territorial prevention facilities.

Statistical methods

Analyses were performed using STATA. The percentage of missing data was very low and therefore it was not necessary to address this issue. Frequency distributions were computed for the demographic characteristics of the respondents, for the two output indicators of the survey, as well as for the responses to each one of the 33 items of the survey. Negatively worded items were reverse coded before calculating the 10 composites scores. Patient safety culture was measured overall, by work area,

Tab. I. General characteristics of the respondents (I) and output indicators (II).

Variable		Frequency	%
I. ACTUARIAL CHARACTERISTICS			
Your Work Area	A. Hygiene and Public Health	169	35
	B. Workplace Prevention	130	27
	C. Food & Nutrition Hygiene	71	15
	D. Public Health Laboratory	25	5
	E. Legal Medicine	21	4
	F. Veterinary Medicine	11	2
	G. Management	26	5
	H. Not otherwise specified	26	5
Work in the Department (years)	< 1	10	2
	1-5	59	12
	6-10	48	10
	11-15	83	17
	16-20	63	13
	21 or more	216	45
Work in the Unit (years)	< 1	15	3
	1-5	70	15
	6-10	67	14
	11-15	99	21
	16-20	73	15
	21 or more	155	32
Working hours in the Department per week	< 20	7	1
	20-38	355	74
	39-59	110	23
	60 or more	7	1
Staff position in the Department	Technician	206	43
	Physician	84	18
	Unit assistant/clerk/secretary	82	17
	Nurse/sanitary assistant	74	15
	Other (Chemist, Dietician, etc)	33	7
Direct interaction or contact with patients/users	Yes	430	90
	No	48	10
Experience in the profession (years)	< 1	7	1
	1-5	32	7
	6-10	44	9
	11-15	71	15
	16-20	65	14
	21 or more	259	54
II. OUTPUT INDICATORS			
Patient Safety Grade	Excellent	47	10
	Very Good	184	39
	Acceptable	223	47
	Poor	17	4
	Failing	4	1
Number of Events Reported	Non response	173	36
	None	256	53
	1-2	36	8
	3-5	8	2
	6-10	3	1
	11-20	0	0
	21 or more	3	1

Tab. II. Hospital SOPS adapted for Italian territorial Prevention facilities: response frequency and percentage of "positive" responses by survey item and composite, with 95% confidence intervals (CI).

Composite and survey item (N = 479 respondents)	Number of total responses	% "Positive" response[1]	95%CI
1. Teamwork Within Units[2]	**1905**	**59.0**	**56.7-61.2**
A1. People support one another in this Unit.	477	70.4	66.4-74.5
A3. When a lot of work needs to be done quickly, we work together as a team to get the work done.	477	56.6	52.2-61.1
A4. In this Unit people treat each other with respect.	477	56.2	51.7-60.6
A11. When one area in this Unit gets really busy others help out.	474	52.5	48.0-57.0
2. Supervisor/*Head*[5] Expectations & Actions Promoting Patient/*User* Safety[4]	**1425**	**58.6**	**56.0-61.2**
B1. My supervisor/manager says a good word when he/she sees a job done according to established patient/*user*[3] safety procedures.	476	52.1	48.6-56.6
B2. My supervisor/manager seriously considers staff suggestions for improving patient/*user* safety.	474	55.5	51.0-60.0
B4. My supervisor/manager overlooks patient/*user* safety problems that happen over and over.	475	68.2	64.0-72.4
3. Organizational Learning – Continuous Improvement	**1429**	**50.9**	**48.3-53.5**
A6. We are actively doing things to improve patient/*user* safety.	478	55.2	50.8-59.7
A9. Mistakes have led to positive changes here.	476	51.9	47.4-56.4
A13. After we make changes to improve patient/*user* safety, we evaluate their effectiveness.	475	45.5	41.0-50.0
4. Management Support for Patient/*User* Safety	**1421**	**43.8**	**41.3-46.4**
F1. *Department* management provides a work climate that promotes patient/user safety.	478	48.5	44.1-53.0
F8. The actions of *Department* management show that patient/user safety is a top priority.	472	42.4	37.9-46.8
F9r. *Department* management seems interested in patient/user safety only after an adverse event happens.	471	40.6	36.1-45.0
5. Feedback & Communication About Error	**1417**	**42.8**	**40.3-45.4**
C1. We are given feedback about changes put into place based on event reports.	471	34.4	30.1-38.7
C3. We are informed about errors that happen in this Unit.	474	41.4	36.9-45.8
C5. In this Unit we discuss ways to prevent errors from happening again.	472	52.8	48.3-57.3
6. Communication Openness	**1419**	**64.8**	**62.4-67.3**
C2. Staff will freely speak up if they see something that may negatively affect patient/*user assistance*.	473	70.8	66.7-74.9
C4. Staff feel free to question the decisions or actions of those with more authority.	473	58.8	54.3-63.2
C6r. Staff are afraid to ask questions when something does not seem right.	473	64.9	60.60-69.21
7. Frequency of Events Reported	**1412**	**48.4**	**45.8-51.1**
D1. When a mistake is made, but is caught and corrected before affecting the patient/*user*, how often is this reported?	471	54.4	49.9-58.9
D2. When a mistake is made, but has no potential to harm the patient/*user*, how often is this reported?	471	42.3	37.8-46.7
D3. When a mistake is made that could harm the patient/*users*, but does not, how often is this reported?	470	48.7	44.2-53.2
8. Teamwork Across Units	**1896**	**36.7**	**34.5-38.9**
F4. There is good cooperation among *Department* Units that need to work together.	474	36.5	32.2-40.8
F10. *Department* Units work well together to provide the best assistance for patients.	472	39.4	35.0-43.8
F2r. *Department* Units do not coordinate well with each other.	478	25.9	22.0-29.9
F6r. It is often unpleasant to work with staff from other *Department* Units.	472	45.1	40.6-49.6
9. Handoffs & Transitions	**1881**	**35.8**	**33.7-38.0**
F3r. Things "fall between the cracks" when transferring patients/*users* from one Unit to another.	470	34.0	29.8-38.3
F5r. Important patient/*user assistance* information is often lost during *handovers for absence due to training/vacation*.	468	42.7	38.3-47.2
F7r. Problems often occur in the exchange of information across *Department* Units.	470	23.2	19.4-27.0
F11r. *Handovers for absence due to training/vacation* are problematic for patients/users in this Department.	473	43.3	38.9-47.8
10. Non punitive Response to Errors	**1430**	**39.5**	**37.0-42.0**
A8r. Staff feel like their mistakes are held against them.	478	42.3	37.8-46.7
A12r. When an event is reported, it feels like the person is being written up, not the problem.	477	38.0	33.6-42.3
A16r. Staff worry that mistakes they make are kept in their personnel file.	475	38.3	34.0-42.7

Notes:
[1] According to the scale used for each item, "positive" response means "Agree"/"Strongly Agree" or "Most of the time"/"Always". For negatively worded (r) questions, "positive" response means "Strongly Disagree"/"Disagree" or "Never"/"Rarely".
[2] Composites are highlighted in Bold.
[3] Our changes to the original version of the Hospital SOPS, necessary to make it compatible with the activity of the staff working in the study facilities, are highlighted in Italic.
[4] The item "B3r. Whenever pressure builds up, my supervisor/manager wants us to work faster, even if it means taking shortcuts" was excluded during the psychometric validation process.

and by professional category. Overall composites were benchmarked with Italian and US hospitals and other outpatient settings from other countries [3, 4, 6, 28, 32]. In order to facilitate comparisons, 95% confidence intervals (CI) were computed.

Results

The response rate across the four territorial Prevention facilities varied from 67% to 73%, with an average of 71%. All items had good variability and the rates of missing responses ranged from 0% to 4% per item.

Table I shows respondents' demographics and response distribution concerning two output indicators.

Most respondents (35%) worked in the Hygiene and Public Health area, which was common to all four facilities, while the Veterinary Medicine area provided the least amount of respondents (2%), as it existed in one setting only. Half of the respondents experienced more than 21 years in the current profession (54%) and in the department (45%), but only a third of them (32%) in the current Unit, reflecting a job rotation process across the Units of the same department.

Most respondents (74%) usually worked between 20 to 38 hours a week. Almost half of the respondents were Prevention Technicians (43%). Ninety percent of the respondents worked in direct contact with patients/users.

Half of the respondents (49%) appreciated the "Patient Safety Grade" indicator as "excellent or very good". The most frequent response to the "*Number of events reported in the last 12 months*" indicator was "*No event reports*". Interestingly, 36% of the respondents did not answer it.

Table II shows the response frequency and the percentage of positive responses (with 95% CI) by survey item. Positive answers varied from 23% for the item "Problems often occur in the exchange of information across department Units", to 70% for the item "People support one another in this Unit". Composites scores varied from 36% for "handoffs and transitions" to 65% for "communication openness". Six out of 10 composites were poor (*i.e.* under the cut-off point of 50%). These were: "management support for patient/user safety", "feedback and communication about errors", "frequency of events reported", "non-punitive response to errors", "teamwork across Units", "handoffs and transitions".

Patient safety composites of positive responses (with 95% CI) by work area are shown in Table III and by professional group in Table IV. "*Communication openness*", "*Teamwork within Units*" and "*Supervisor/head expectations and actions promoting patient/user safety*" were sufficiently developed in all work areas and professional categories (scores >50%).

Compared to the overall results, significantly higher scores were found for all composites in the Management area (range: 64% for "*Teamwork across Units*" - 86% for "*Communication openness*") and for six composites in the Public Health Laboratory area .The poorest findings were in the Workplace Prevention area (range: 26% for

Tab. III. The AHRQ Hospital SOPS adapted for Italian territorial Prevention facilities: % of positive responses by composite and work area, with 95% confidence interval (CI).

Composite	% of positive responses with 95% CI							
	Hygiene and Public Health[1]	Workplace Prevention	Food & Nutrition Hygiene	Management	Public Health Laboratory	Legal Medicine	Other[2]	Overall
1. Teamwork Within Units	62.1 (58.6-66.0)	51.7 (47.4-56.1)	52.1 (46.3-57.9)	70.2 (61.4-79.0)	69.9 (59.6-77.8)	68.7 (58.7-78.6)	62.2 (54.4-70.0)	59.0 (56.7-61.2)
2. Supervisor/Head Expectations & Actions Promoting Patient/User Safety	60.1 (55.8-64.5)	50.5 (45.5-55.5)	50.5 (43.7-57.2)	84.4 (76.3-92.5)	73.3 (63.3-83.3)	66.7 (55.0-78.3)	60.7 (50.3-71.2)	56.6 (56.0-61.2)
3. Organizational Learning-Continuous Improvement	51.30 (47.2-56.0)	44.7 (39.8-49.7)	42.2 (35.5-48.8)	69.2 (59.0-79.5)	70.5 (58.9-79.8)	52.4 (40.1-64.7)	59.5 (50.3-68.6)	50.9 (48.3-53.5)
4. Management Support for Patient/User Safety	42.2 (38.0-46.7)	36.8 (33.9-41.6)	39.0 (32.4-45.5)	79.5 (70.5-88. 5)	39.0 (26.8-48.9)	50.8 (38.5-63.1)	59.6 (50.4-68.8)	43.8 (41.3-46.4)
5. Feedback & Communication About Error	40.6 (36.7-45.3)	33.3 (28.5-38.0)	39.0 (32.4-45.5)	70.1 (59.9-80.4)	70.5 (58.9-79.8)	50.8 (38.5-63.1)	50.5 (41.0-59.9)	42.8 (40.3-45.4)
6. Communication Openness	62.1 (58.1-66.6)	62.2 (57.3-67.1)	63.5 (57.0-70.0)	85.7 (77.9-93.5)	82.1 (72.5-90.2)	57.1 (44.9-69.4)	66.4 (57.5-75.2)	64.8 (62.4-67.3)
7. Frequency of Events Reported	49.9 (45.8-54.6)	33.6 (28.8-38.4)	51.7 (44.9-58.5)	79.5 (70.5-88.5)	75.6 (64.8-84.5)	44.4 (32.2-56.7)	47.7 (38.3-57.1)	48.4 (45.8-51.1)
8. Teamwork Across Units	37.8 (34.5-41.9)	29.8 (25.8-33.8)	27.8 (22.5-33.0)	64.4 (55.2-73.6)	44.3 (32.3-51.7)	32.1 (22.2-42.1)	50.4 (42.3-58.4)	36.7 (34.5-38.9)
9. Handoffs & Transitions	37.7 (34.4-41.7)	26.1 (22.3-30.0)	31.4 (26.0-36.9)	70.2 (61.4-79.0)	36.9 (25.0-43.7)	24.1 (14.9-33.3)	50.4 (42.3-58.4)	35.8 (33.7-38.0)
10. Non- punitive Response to Errors	37.6 (33.5-42.0)	33.4 (28.7-38.1)	36.5 (30.0-43.0)	65.4 (54.8-75.9)	62.8 (51.7-73.6)	17.5 (8.1-26.8)	53.2 (43.9-62.4)	39.5 (37.0-42.0)

[1] The work areas in Italic are common to all 4 territorial Prevention facilities participating in the study
2 This category includes "veterinary medicine" and "not otherwise specified" work areas

Tab. IV. The AHRQ Hospital SOPS adapted for Italian territorial Prevention facilities: % of positive responses by composite and professional category, with 95% confidence interval (CI).

Composite	% of positive responses with 95% CI					
	Physician	Nurse/ sanitary assistant	Technician	Unit assistant/ clerk/ secretary	Other	Overall
1. Teamwork Within Units	68.0 (63.0-73.0)	62.1 (56. 6-67.7)	53.2 (49.8-56.6)	55.3 (49.8-60.7)	74.2 (66.8-81.7)	59.0 (56.7-61.2)
2. Supervisor/Head Expectations & Actions Promoting Patient/User Safety	68.4 (62.6-74.2)	57.3 (50.7-63.8)	53.3 (49.4-57.3)	57.1 (50.9-63.3)	73.5 (64.7-82.2)	58.6 (56.0-61.2)
3. Organizational Learning-Continuous Improvement	57.1 (51.0-63.3)	51.6 (45.0-58.2)	46.7 (42.8-50.7)	46.9 (40.6-53.2)	68.7 (59.6-77.8)	50.9 (48.3-53.5)
4. Management Support for Patient/User Safety	50.4 (44.2-56.6)	33.3 (27.1-39.6)	39.2 (35.4-43.1)	46.3 (40.0-52.6)	73.5 (64.7-82.2)	43.8 (41.3-46.4)
5. Feedback & Communication About Error	55.7 (49.5-61.8)	38.3 (31.8-44.7)	35.9 (32.1-39.7)	42.1 (35.8-48.3)	65.7 (56.3-75.1)	42.8 (40.3-45.4)
6. Communication Openness	80.1 (75.1-85.0)	58.5 (52.0-65.1)	62.4 (58.6-66.3)	57.5 (51.3-63.8)	72.7 (64.0-81.5)	64.8 (62.4-67.3)
7. Frequency of Events Reported	63.6 (57.6-69.6)	56.3 (49.8-62.8)	40.0 (36.1-43.9)	42.2 (35.9-48.5)	59.6 (49.9-69.3)	48.4 (45.8-51.1)
8. Teamwork Across Units	46.6 (41.2-51.9)	31.0 (25.7-36.2)	30.7 (27.5-33.9)	39.3 (34.0-44.7)	55.3 (46.8-63.8)	36.7 (34.5-38.9)
9. Handoffs & Transitions	41.8 (36.5-47.1)	41.5 (35.9-47.1)	29.6 (26.5-32.8)	33.2 (28.1-38.4)	52.3 (43.7-60.9)	35.8 (33.7-38.0)
10. Non-punitive Response to Errors	50.4 (44.2-56.6)	38.0 (31.6-44.4)	36.7 (32.9-40.5)	30.6 (24.8-36.4)	54.6 (44.7-64.4)	39.5 (37.0-42.0)

Tab. V. The AHRQ Hospital SOPS adapted for Italian territorial Prevention facilities: an international comparison of % of positive responses [3 4, 6, 28, 32].

Composite	% of positive responses					
	Territorial Prevention facilities (Italy)	Hospital (Italy)	Hospital (US)	Health district (Spain)	Primary healthcare (Iran)	Primary healthcare (Turkey)
1. Teamwork Within Units	59	64	81	81	74	76
2. Supervisor/Head Expectations & Actions Promoting Patient/User Safety[1]	59	69	76	81	68	58
3. Organizational Learning-Continuous Improvement	51	74	73	72	72	47
4. Management Support for Patient/User Safety	44	28	72	57	75	43
5. Feedback & Communication About Error	43	60	67	60	44	50
6. Communication Openness	65	62	62	63	62	46
7. Frequency of Events Reported	48	59	66	49	50	12
8. Teamwork Across Units	37	30	61	62	77	58
9. Handoffs & Transitions	36	37	47	65	-	44
10. Non-punitive Response to Errors	40	35	44	42	17	18

[1] In the Italian version of the Hospital SOPS for territorial Prevention facilities this composite has only three of the four items of the original US version.

"*Handoffs and transitions*" - 62% for "*Communication openness*"), which exhibited significantly lower scores for six out of ten composites.

The Physician group exhibited significantly higher scores than the overall figure for six out of ten composites. Their composites ranged from 42% for "*Handoffs and transitions*" to 80% for "*Communication openness*". On the contrary, the Technician group showed the poorest results (range: 30% for "*Handoffs and transitions*" - 62% for "*Communication openness*"), with significantly lower composites than the overall figure for five composites. Significantly higher scores were found for staff belonging to other professional categories (*e.g.* engineers, dieticians, etc), ranging from 52% for "*Handoffs and transitions*" to 74% for "*Teamwork within Units*". However, they represented only 7% of the total number of respondents, so these results should be interpreted with caution.

Table V shows an international benchmark of composites scores. [3 ,4, 6, 28, 32]. The Italian experience pointed out that patient safety culture in Prevention facilities is less developed than in hospitals. While "*Teamwork within Units*" and "*Supervisor/head expectations & actions promoting patient/user' safety*" (range: 59%-81%) are the most developed safety culture aspects across the compared facilities, "*Non-punitive response to errors*" remain problematic in all settings (range: 17%-44%).

Discussion

This study represents the first examination of patient safety culture within the staff of territorial Prevention facilities within the Local Health Authorities of Northern Italy. Four facilities were included in the study. Since there was not a specific survey available to be used in these settings, after searching existing scientific literature, we selected the Hospital version of the AHRQ SOPS. Besides being one of the most popular surveys currently used at international level [15-27] and being already available in Italian [28], this survey explores most of the aspects of patient safety culture which we were interested in. Moreover, several research groups around the world found the AHRQ Hospital survey useful to explore patient safety culture in non-hospital settings [3-6]. Thus, the original survey was slightly adjusted for use in our facilities and pre-tested on a few staff members. The psychometrics were checked thereafter. Results of the psychometric validation pointed out that 10 factors and 33 items of the original US survey (based on 12 factors with 42 items) were satisfactory for use in our facilities [31].

The Italian experience indicates that patient safety culture is less developed in territorial Prevention facilities than in hospitals [28-30]. Interestingly, the latter showed composites lower than US hospitals [32]. Our results are consistent with results from other studies carried out in facilities for outpatients, such as primary healthcare services, characterised by a lower potential of life-threatening medical errors and procedures [3, 4, 6]. Nonetheless, it raises serious concern from a public health point of view, as prevention facilities deal with entire communities and/or sub-groups of the population and most of the individuals interacting with our territorial Prevention facilities are objectively healthy.

Overall, "*Communication openness*", "*Teamwork within Units*" and "*Supervisor/head expectations and actions promoting patient/user safety*" were the most developed aspects of the culture. Staff help each other, supervisors promote user safety and communication barriers between them are minimal, which suggests that some important basis for further developing user safety already exists. Conversely, "*Teamwork across Units*", "*Handoffs and transitions*" as well as "*Non-punitive response to errors*" are the least developed aspects of the culture, requiring prompt intervention. Many other studies have pointed out the same strengths and weakness of patient safety culture [3-6, 29].

Voluntary error reporting is a critical mechanism for identifying patient safety issues and improving quality in an organization [33]. Patients' safety culture enables providers to report mistakes and near misses [33]. In our facilities, a low frequency of events reported suggests the persistence of blame culture and under-reporting of incidents, as pointed out by other Italian studies [33, 34]. Respondents in the study only had to respond to the question about incident reporting if an incident reporting system was in place in their facility. The high proportion of non-response (36%) suggested that several staff members were not aware of the existence of the incident reporting system, which had been in place for several years. This is likely to be another cause of the under-reporting of incidents in the settings participating in the study.

We found a great variability of the positive responses among work areas and the profession of the respondents. The highest composites were exhibited by the Management area. Since it is the first recipient of the institutional strategic safety policies and has to account for their implementation into practice at each Unit level, we could consider this area highly auto-referential. Similar results have been observed in other studies [4, 32]. Our results also pointed out higher scores in the Laboratory of Public Health. This suggests that a strong leadership for quality, thorough external certification and accreditation processes, along with continuous internal auto-control, are important contributors to the development of good patient safety culture within staff. Physicians working in territorial Prevention facilities showed higher composite scores of positive responses for patient safety than other professionals (nurses, technicians, clerks). A recent study carried out by Nguyen *et a*l. [35] in two Italian hospitals supports our findings, showing that professional profile contributed significantly to differences in safety attitudes and teamwork climate, which were more developed in physicians that in nurses.

Our study has several limitations. Firstly, all our facilities consisted of Units and healthcare professions that are quite different from those existing in hospitals, for which the survey we used was originally elaborated. For instance, physicians and nurses represented only one third of all the staff surveyed. Secondly, the study was not based on a random sample with a selection in numerous Italian regions, but only on four voluntary facilities, located in two northern regions. Thirdly, the organisational heterogeneity of the four facilities included in the study could also have introduced some bias. In fact, contrarily to the three Departments of Medical Prevention in the Lombardy Region, the Department of Prevention in the Piedmont Region covers a small territory and population, has closer collaboration with the hospitals in its activities, and runs not only human but also veterinary preventive activities to preserve public health. It also has a larger proportion of staff members with shorter experience in the department/Unit/profession and with more than 38 working hours a week. These distinct characteristics contributed to different awareness levels about risk of error/adverse events with respect to the other departments (which were

more homogeneous), leading to the better development of some dimensions of patient safety culture.
Finally, some Units were so small that despite our effort to preserve anonymity, opportunistic staff attitudes due to fear of being identified were still possible.
For these reasons our results are not representative for all the facilities similar to ours in Italy and further application of the survey in other territorial Prevention facilities would be necessary to confirm our results. Although it might seem appealing, international comparisons of results are to be considered very cautiously.
The study has some important strengths as well. Firstly, we psychometrically validated the survey that we applied to measure patient safety culture [31]. Secondly, the overall response rate (71%) was satisfactory. Thirdly, we described patient safety culture through a multidimensional tool in territorial Prevention facilities for the first time in our country. Finally, based on the results of this study, several actions for improvement were set up: a) courses on risk management have been organized for all work areas and professions, with priority given to the areas with the poorest results; b) thorough revision of the existing incident reporting system, including major advertising and ensuring wide-spreading accessibility and feedback; c) application of pro-active risk management tools such as Failure Mode and Effect Analysis to some key processes; d) intense exchange of information regarding best practices among the four departments participating in the study. Thus, the results of this study constitute not only an opportunity to explore and understand staff perception of user safety in the Prevention field, they can also be used as a baseline for improvement interventions and future assessments of the efficacy of specific targeted interventions.

Conclusions

A voluntary and anonymous qualitative cross-sectional study was carried out for the first time in Italian territorial Prevention facilities using a psychometrically validated version of the US Hospital Survey of Patient Safety Culture. "*Communication openness*", "*Teamwork within Units*", "*Supervisor/head expectations and actions promoting patient/user safety*" and "*Organizational learning-continuous improvement*" were the most developed factors of patient safety culture, while the other six factors evaluated were quite poor. Management scored highest across work areas, and Physicians scored highest across professional categories. However, overall results were poorer than in Italian hospitals. To confirm the results of this pilot study, the survey should be further expanded to other Italian territorial Prevention facilities; post-intervention application in the same facilities could help monitor efficacy of improvement actions. In this study, intra-country comparisons provided some interesting information, which could be useful to prevent auto-referentiality. Inter-country comparisons might be influenced by cultural and geographical differences and therefore they should be considered with caution.

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The authors declare no conflict of interest.

Authors' contributions

CT, BP, LCS, RC, MA, GGB decided the study design and organised the study. CT, GM, FS prepared the Web version of the survey. CT, MV, CP, DB were responsible for the data collection. GS carried out statistical analyses. CT and GSal drafted the paper. All authors contributed to the interpretation of the results of the study and revised the paper. GSal also checked the English.

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ORIGINAL ARTICLE

Brain doping: stimulants use and misuse among a sample of Italian college students

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Keywords

Stimulants • Doping • College students

Summary

Introduction. *The non-medical use of prescription stimulants (NMUPS) has become the subject of great interest for its diffusion among university students, who abuse these substances to cope with the increasing load of academic stress. NMUPS has been widely investigated in the U.S. due to its increasing trend; this behavior, however, has also been reported in Europe. The aim of this cross-sectional study was to examine stimulants misuse in a Northern Italian geographic area, identifying possible developments of the phenomenon in Italy.*
Methods. *To evaluate academic and extra-academic NMUPS (Methylphenidate and Amphetamines), an anonymous multiple-choice questionnaire was administrated to a sample of Bachelor's and Master's degrees students attending a University North East of Italy. Data elaboration and CI 95% were performed with Excel software 2013. Fisher's exact tests were performed using GraphPad INSTAT software.*
Results. *Data from 899 correctly completed questionnaires were analyzed in this study. 11.3% of students reported NMUPS, with an apparent greater use by students aged 18-22 years (73.5%) and without any statistically significant gender predominance. Fifty-seven point eight percent of students used stimulants at most five times in six months, and the most frequent academic and extra-academic reasons to use them were respectively to improve concentration while studying (51.0%) and sports performance (25.5%). NMUPS was higher among working students than non-working ones ($p < 0.05$), suggesting a use of stimulants to cope with stress by the first ones.*
Conclusions. *These exploratory and preliminary data suggest that NMUPS is quite relevant in Northern Italy, suggesting a need for preventive and monitoring measures, as well as future analysis via a longitudinal multicenter study.*

Introduction

Pharmacological "cognitive enhancement" (CE) is defined as the use of any psychoactive drug by healthy individuals with the purpose of enhancing cognition by improving attention, vigilance, concentration, memory or mood [1, 2]. CE is also referred to as "Pharmacological Neuroenhancement", "Cosmetic Neurology", "Academic Performance Enhancement", "Academic Doping" or even "Brain Doping" [1, 3-8].
In this study, CE and "Brain Doping" are defined as the assumption of Methylphenidate (MPH) and Amphetamines (AMPH), either detected illegally or used off-label, by healthy students to improve their academic performances [1, 2, 9, 10, 11]. According to the literature [12-14], the terms "illicit use" and "non-medical use of prescription stimulants" (respectively IUPS and NMUPS) refer to the assumption of a psychotherapeutic stimulant medication, with or without a medical prescription, in larger amounts or for a period longer than the prescribed one. These psychoactive substances, also called "smart drugs", can increase brain functions in people with ADHD, but whether they can push a neurologically healthy individual onto a higher cognitive level is not yet clear [9].
University students are a population at risk for CE drugs misuse. These drugs are used to achieve a better academic performance or personal success. However, students are not aware ore deliberately ignore the fact that drug misuse can lead to addiction.
In the various studies conducted in the American continent, the prevalence rate of NMUPS in University students ranges from 8.1% (Midwestern University, U.S.) [12] to 27.6% (Puerto Rico) [17]. The prevalence of NMUPS (Methylphenidate and Amphetamines) has been found to range from 5% to 35% in surveys among young adult and adolescent populations of North America [18] and 25.6% and 8.3% in college students respectively in an University of the North West of Pacific [19] and in a University in the Midwest [20]. Other studies in in various areas of the United States showed similar results [13, 18, 21, 22].
NMUPS ranges among European university students from 0.78% (in Germany) [23] to 6.2% (in Switzerland) [24].
The most commonly reported reasons for NMUPS were to improve concentration (65.2%), studying performances (59.8%) and alertness (47.5%) [20]. Moreover, NMUPS is more frequently associated with male gender and linked to having experience with drugs. The most

common reasons reported to use stimulants are "to learn faster" and "to finish more work in less time" [24]. Other motivations included "getting high" (31.0%) and experimentation (29.9%) [20]. Additional relatively common motivation for use is weight loss [25].
Intrapersonal risk factors associated with NMUPS are Caucasian ethnicity, poor school performance, diagnosis of ADHD, and low self-esteem; interpersonal factors are off-campus residence, various sports participation, NMUPS perception as socializing agents. In addition, exposure to advertisements related to prescription drugs, knowledge of prescribed stimulants, and positive attitudes towards prescribed stimulants are environmental risk factors [19]. High levels of stress are also significantly correlated to high NMUPS as a strategy to cope with stress [17].
Adverse health effects associated to use or abuse of MPH are related to the cardiovascular system (*e.g.* angina, tachycardia, arrhythmia), the central nervous system (*e.g.* aggressiveness, agitation, confusion, headache, tremors and mood swings) and the gastrointestinal system (abdominal pain, loss of appetite, anorexia and nausea) [26]. Toxic manifestations of MPH are hyperthermia, euphoria, delirium, hallucinations and seizures [27]. It is interesting to note that in MPH-naive subjects, a toxic dose may be very close to the therapeutic dose when compared to patients undergoing long-term treatment [26]. Consumption of drugs containing amphetamines can cause similar side effects such as hyperactivity, hyperthermia, tachycardia, tachypnea, mydriasis, tremors and seizures [28].
Given that stimulant use among students in foreign countries is an established factor, we conducted a cross-sectional study to evaluate the phenomenon among students of a University North East of Italy and its correlation with academic stress.
In particular, the aim of this preliminary and exploratory study was to investigate the frequency of and the reasons for stimulants use and their effects on health.

Methods

This cross-sectional study was conducted during the 2014-2015 academic year and involved students from Bachelor's (three years of course) and Master's (six years of course) degrees of a University North East of Italy.
Bachelor's degrees sampled were Biomedical Laboratory Techniques, Cardiovascular Perfusion Techniques, Dental Hygiene, Imaging and Radiotherapy Techniques, Midwifery, Nursing, Physiotherapy, Psychiatric Rehabilitation Techniques, Speech and Language Therapy, while Master's degrees sampled were Medicine and Dentistry.
Data were collected through an anonymous multiple-choice questionnaire administrated to students attending the first and the third year of Bachelor's and Master's degrees as well as the fifth year of Master's degrees. Third year Physiotherapy, Dental Hygiene and Psychiatric Rehabilitation Techniques students were not sampled because during the period of our study their degree courses did not take place.
The validated questionnaire assessed information about personal socio-demographic characteristics such as age, sex, course degree, occupation (working student vs. non-working/unemployed student), nationality, Italian area of residence (Northern, Central-Southern).
Moreover, this survey investigated characteristics of the students' parents, such as current age, educational level (primary school, secondary school, high school, university) and occupation (self-employed, employee, unemployed, retiree, other). In order to detect the family's level of education, the highest educational level achieved by at least one parent was considered. Levels of education achieved were defined as "Low" for primary or secondary school or "High" for high school or university.
The final questions in the survey asked for information regarding the students' university track and their use of stimulants (Methylphenidate and Amphetamines).
Information on stimulant use was collected in the form of frequency of use (never, 1-5, 6-15, ≥ 16 times) within the 6 months prior to administration of the survey.
Psychophysical state post-use (condition unchanged, fatigue, satisfaction, guilt, depression, other) and side effects of stimulants (no side effects, insomnia, stomach pain, development of tics, loss of appetite, headache, else) were also investigated.
Some of the analysed reasons of stimulants use were related to academic field, such as improving in-classroom concentration, concentration while studying, exam performance, mental stamina for studying, self-confidence while studying, better grades than peers and better grades overall.
A positive response for at least one of the above reasons constituted an indicator of stimulants use to enhance cognitive performance (CE).
Extra-academics reasons were investigated as well: losing weight, improving social skills, driving, sports and working performances.
Finally, we added questions concerning whether stimulant were consumed alone or in combination with alcohol, illegal substances, or other medical drugs.
The data from this study were collected and processed in compliance with the national law on privacy (Law N. 196/2003).
All students were over 18 years old (legal age in Italy) and they were assured of the confidentiality of their responses. They were adequately informed about the survey and their participation only takes place if voluntary.
To ensure a high response rate, the survey was brief, easy to complete (requiring less than 15 minutes) and administered at the beginning of a lecture. All questionnaires were immediately collected and their information was entered in a database.

Statistical and data analysis

The elaboration of results, the Confidence Interval (95%) of stimulants users, and the multiple logistic re-

gression were calculated using Excel software 2013 and STATA 13.

Statistical analyses were carried out using Fisher's exact test or Chi-squared test with Yates correction, using GraphPad INSTAT software with significance of $p < 0.05$.

Bonferroni's correction was implemented for the Chi-squared with Yates, and Fisher's exact test performed to compare the trends of stimulants use in the various years of the different degree courses to improve concentration while studying and practicing sports.

A multiple logistic regression was performed to evaluate the following variables as possible predictors of stimulants use by students: male gender, being a Bachelor's degree student, age, being a working student, coming from the North of Italy and parents' high educational level.

The prevalence rates of the socio-demographic and academic features in the different groups examined were calculated differently (non-recalculated prevalence rates, prevalence rates calculated on the total sample and prevalence rates calculated on stimulants users) as reported in text and tables.

Results

A total of 1107 questionnaires were administered and the response rate was 89.4%. Of the 990 questionnaires completed, 899 were properly compiled and analyzed.

Table 1 describes the distribution of the enrolled subjects in the study, evaluating the total sample and subgroups of working (21.9 %) and non-working (75.4%) students in relation to stimulant use, gender, age classes, residence and university track attended. Only 2.0 % of stimulants users did not report their employment status.

Our survey showed that 11.3% (102/899; CI 95% 9.3%-13.6%) of all students consumed stimulants. Among them 2.3% and 9.0% used stimulants with and without a medical prescription respectively, showing that a large majority or users (79.4%) used stimulants without medical prescription.

Tab. I. Socio-demographic features and description of stimulants users of the total sample and of its subpopulations (working and non-working students. (Prevalence rate).

	Total sample		Non working students			Working students		
Variables	Pop. (N = 899)	Stimulants users (N = 102)	Pop. (N = 678)	Stimulants users (N = 69)**		Pop. (N = 197)	Stimulants users (N = 31)**	
	A (%)	B (%)	B (%)	B (%)	C (%)	B (%)	B (%)	C (%)
Gender								
Female	68.9 (619/899)	11.0 (68/619)	75.4 (467/619)	6.8 (42/619)	61.8 (42/68)	22.1 (137/619)	3.9 (24/619)	35.3 (24/68)
Male	30.3 (272/899)	11.8 (32/272)	76.5 (208/272)	9.2 (25/272)	78.1 (25/32)	22.1 (60/272)	2.6 (7/272)	21.9 (7/32)
Non responders	0.9 (8/899)	25.0 (2/8)	37.5 (3/8)	25.0 (2/8)	100.0 (2/2)	0.0 (0/8)	0.0 (0/8)	0.0 (0/2)
Age (years)*								
18-22	74.9 (673/899)	73.5 (75/102)	78.0 (529/678)	73.9 (51/69)	73.9 (51/69)	66.0 (130/197)	71.0 (22/31)	71.0 (22/31)
23-27	17.7 (159/899)	21.6 (22/102)	16.1 (109/678)	23.2 (16/69)	23.2 (16/69)	23.9 (47/197)	19.4 (6/31)	19.4 (6 /31)
≥ 28	4.8 (43/899)	2.0 (2/102)	3.8 (26/678)	0.0 (0/69)	0.0 (0/69)	8.1 (16/197)	6.5 (2/31)	6.5 (2/31)
Non responders	2.7 (24/899)	2.9 (3/102)	2.1 (14/678)	2.9 (2/69)	2.9 (2/69)	2.0 (4/197)	3.2 (1/31)	3.2 (1/31)
Residence								
North	84.4 (759/899)	11.9 (90/759)	75.9 (576/759)	8.3 (63/759)	70.0 (63/90)	22.9^ (174/759)	3.4 (26/759)	28.9 (26/90)
Central-South	10.5 (94/899)	4.3 (4/94)	83.0^ (78/94)	4.3 (4/94)	100.0 (4/4)	12.8 (12/94)	0.0 (0/94)	0.0 (0/4)
Non responders	5.1 (46/899)	17.4 (8/46)	52.2 (24/46)	4.3 (2/46)	25.0 (2/8)	23.9 (11/46)	10.9 (5/46)	62.5 (5/8)
Courses								
Medicine	23.4 (210/899)	6.7 (14/210)	83.8 (176/210)	5.7 (12/210)	85.7 (12/14)	15.7 (33/210)	6.1 (2/33)	14.3 (2/14)
Dentistry	5.9 (53/899)	7.5 (4/53)	81.1 (43/53)	3.8 (2/53)	50.0 (2/4)	13.2 (7/53)	14.3 (1/7)	25.0 (1/4)
Nursing	47.3 (425/899)	14.8 (63/425)	70.8 (301/425)	9.2 (39/425)	61.9 (39/63)	24.9 (106/425)	5.4 (23/425)	36.5 (23/ 63)
B.L.T.	5.2 (47/899)	19.1 (9/47)	85.1 (40/47)	17.0 (8/47)	88.9 (8/9)	14.9 (7/47)	2.1 (1/47)	11.1 (1/9)
S.L.T.	4.2 (38/899)	2.6 (1/38)	73.7 (28/38)	2.6 (1/38)	100.0 (1/1)	23.7 (9/38)	0.0 (0/38)	0.0 (0/1)
Midwifery	4.2 (38/899)	2.6 (1/38)	78.9 (30/38)	0.0 (0/38)	0.0 (0/1)	21.1 (8/38)	2.6 (1/38)	100.0 (1/1)
I.R.T.	3.8 (34/899)	5.9 2/34)	67.6 (23/34)	5.9 (2/34)	100.0 (2/2)	32.4 (11/34)	0.0 (0/34)	0.0 (0 /2)
Physiotherapy	3.0 (27/899)	14.8 (4/27)	63.0 (17/27)	7.4 (2/27)	50.0 (2/4)	37.0 (10/27)	7.4 (2/27)	50.0 (2/4)
C.P.T.	1.3 (12/899)	8.3 (1/12)	100.0 (12/12)	8.3 (1/12)	100.0 (1/1)	0.0 (0/12)	0.0 (0/12)	0.0 (0/1)
Dental Hygiene	1.1 (10/899)	20.0 (2/10)	60.0 (6/10)	20.0 (2/10)	100.0 (2/2)	30.0 (3/10)	0.0 (0/10)	0.0 (0/2)
P.R.T.	0.6 (5/899)	20.0 (1/5)	40.0 (2/5)	0.0 (0/5)	0.0 (0/1)	60.0 (3/5)	20.0 (1/5)	100.0 (1/1)

* In every column age is reported as non-recalculated prevalence rate; POP. = population **,^ $p < 0,05$ (Chi-square with Yates' correction)

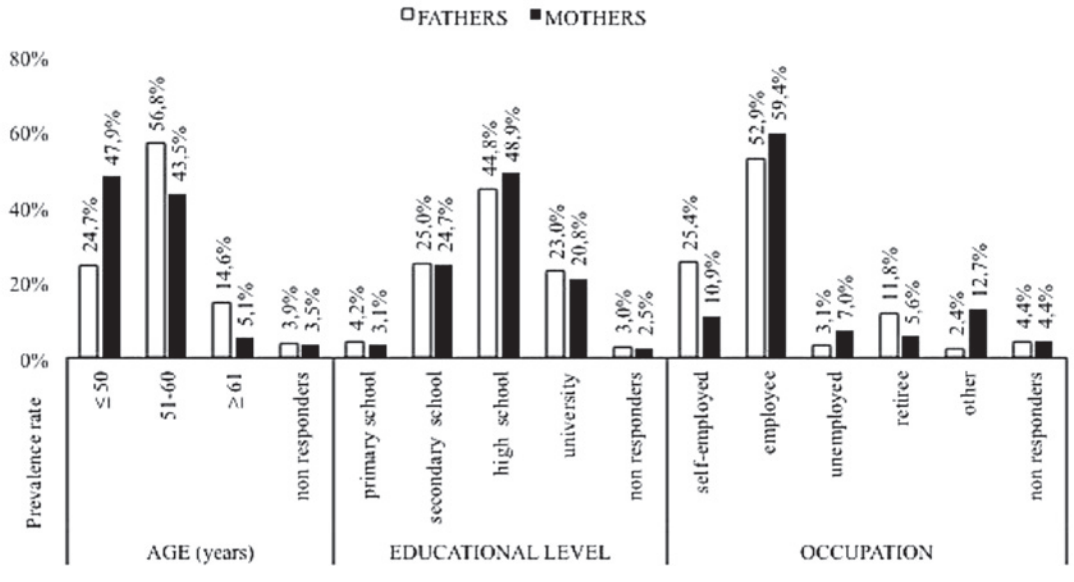
A= Non recalculated prevalence rate; B = Prevalence rate calculated on total sample; C = Prevalence rate calculated on stimulants users population

B.L.T.= Biomedical Laboratory Techniques; S.L.T.=Speech and Language Therapy; I.R.T.= Imaging and Radiotherapy Techniques;

C.P.T.= Cardiovascular Perfusion Techniques; P.R.T.=Psychiatric Rehabilitation Techniques

Working and non-working groups were homogeneous for gender composition ($p > 0.05$, Chi-squared with Yates' correction = 0.9936).
Stimulant users appeared to be homogeneous in terms gender composition (11.0% women and 11.8% men, $p > 0.05$, Chi-squared with Yates correction = 0.8227). Most of them were aged between 18 and 22 (73.5%), both in non-working students group (78.0%) and in working students (66.0%) group, and came from Northern Italy (11.9%).
Among working and non-working students the prevalence rate of stimulant use was respectively of 15.7% (31/197) and of 10.2% (69/678).
30.4% (31/102) of stimulants users were working students. Among all working students the prevalence rate of stimulants use was 15.7% (31/197).
67.6% (69/102) of stimulants users in our sample were non-working students and among all non-working students of the sample, the prevalence rate of stimulants use was 10.2% (69/678).
The comparison among stimulants users between working (15.7%) and non-working (10.2%) students showed a statistically significant difference ($p < 0.05$, Chi-squared with Yates correction).
The highest consumptions of stimulants were detected in younger students. At 19 years old: 25.5% in Bachelor's degrees *vs.* 3.9% in Master's degrees; at 20 years old: 15.7% in Bachelor's degrees *vs.* 2.0% in Master's degrees; at 21 years old: 13.7% in Bachelor's degrees *vs*. 4.9% in Master's degrees.
The lowest consumptions were detected at 27 years, 28 years and 39 years old, when the stimulants users were 1.0% in Bachelor's degrees and 0.0% in Master's degrees. This trend of reduction in stimulants use related to increasing age is not statistically significant when evaluated among students of 18-23 years and those aged ≥ 24 years ($p > 0.05$, Chi-squared with Yates' correction = 0.9031).
The majority of non-working students came from Central-Southern Italy (83.0%), while working students came principally from Northern Italy (22.9%), showing a statistically significant difference ($p < 0.05$, Chi-squared with Yates' correction).
The highest prevalence of students consuming stimulants was found in Dentistry (7.5%) among Master degree courses and in Dental Hygiene (20.0%) and Psychiatric Rehabilitation Techniques (20.0%) among Bachelor's degree courses.
Among non-working students the higher prevalence of stimulant users was found in Medicine (83.8%), Cardiovascular Perfusion Techniques (100.0%) and Psychiatric Rehabilitation Techniques (60.0%), and among working students was found in Psychiatric Rehabilitation Techniques (60.0%), Physiotherapy (37.0%), Imaging and Radiotherapy Techniques (32.4%) and Nursing (24.9%).
In each age group considered, the use of stimulants was significantly higher among students of Bachelor's degrees than in those of Master's degrees ($p < 0.05$, Chi-squared with Yates' correction).
On the total sample (N = 899), the prevalence rates of stimulants use to enhance cognitive performance with and without medical prescription were 1.9% *vs*. 6.2% respectively ($p > 0.05$, Fisher's exact test = 0.4167), while the prevalence rates of stimulants use for extra academic reasons with and without medical prescription were 0.8% and 4.6% ($p > 0.05$, Chi-squared with Yates' correction = 0.2425).
2% of total sample had used stimulants, for medical or personal reasons, only before the six months surveyed by our study.
The socio-demographic features of the 1798 students' parents are shown in Figure 1. Not every student who completed the questionnaire reported information on

Fig. 1. Socio-demographic characteristics of sample's parents. (Prevalence rate).



Tab. II. Stimulants use by associations, reasons (academic and extra-academic), psychophysical state, side effects and predictors of use. (Prevalence rate).

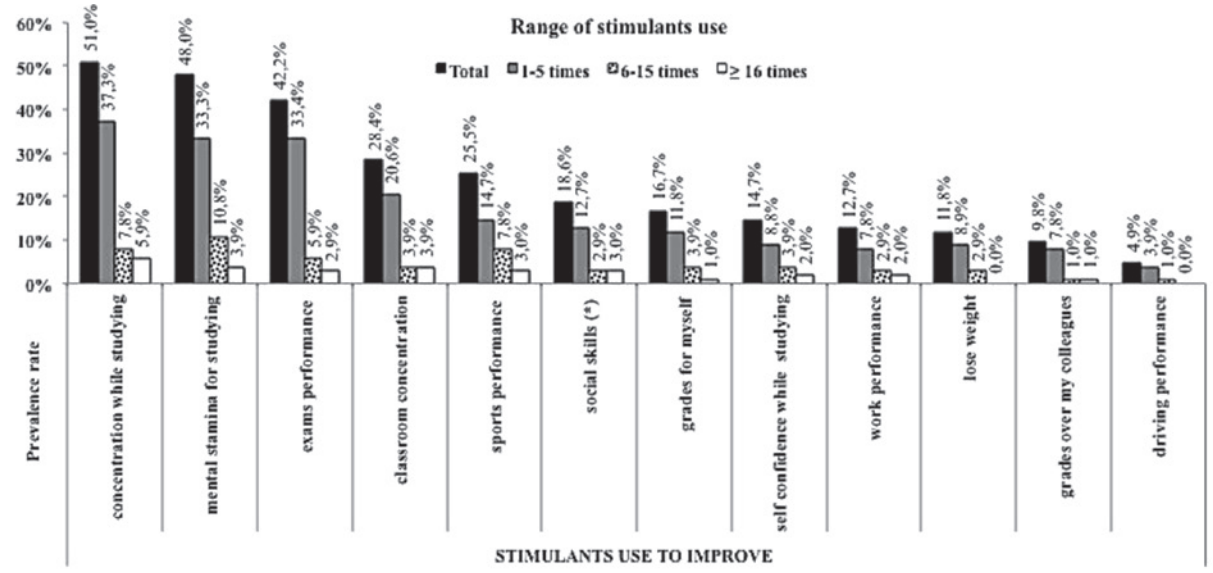
Variables	Prevalence rate
Associations	
No (stimulants alone)	51.0% (52/102)
Alcohol	12.7% (13/102)
Other medical drugs	5.9% (6/102)
Other drugs	2.9% (3/102)
Non responders	27.5% (28/102)
Academic reasons for use	
To improve concentration while studying	51.0% (52/102)
To improve mental stamina for studying	48.0% (49/102)
To improve exams performance	42.2% (43/102)
To improve classroom concentration	28.4% (29/102)
To get better grades for themselves	16.7% (17/102)
To improve self-confidence while studying	14.7% (15/102)
To get higher grades than peers	9.8% (10/102)
Extra-academic reasons for use	
To improve sports performance	25.5% (26/102)
To improve social skills (*)	18.6% (19/102)
To improve work performance	12.7% (13/102)
To lose weight	11.8% (12/102)
To improve driving performance	4.9% (5/102)
Psychophysical state post-use	
Condition unchanged	39.2% (40/102)
Fatigue	12.7% (13/102)
Satisfaction	6.9% (7/102)
Other	2.9% (3/102)
Guilt	2.0% (2/102)
Depression	1.0% (1/102)
Non responders	35.3% (36/102)
Side effects	
No side effects	55.9% (57/102)
Insomnia	6.9% (7/102)
Stomach pain	5.9% (6/102)
Else	2.0% (2/102)
Development of tics	2.0% (2/102)
Loss of appetite	2.0% (2/102)
Headache	1.0% (1/102)
Non responders	24.5% (25/102)

Model for stimulant's use predictors (Pseudo R2 = 0.0399, $p < 0.05$)	Odds ratio (95% CI)
Male gender	1.3 (0.8-2.1), p = 0.336
Being a Bachalor's Degree student	2.4 (1.3-4.4), p = 0.004
Student's age	0.9 (0.9-1.0), p = 0.124
Being a working student	1.3 (0.8-2.2), p = 0.273
Coming from the Northern of Italy	3.1 (1.1-9.0), p = 0.034
Parents' high educational level	0.6 (0.3-1.0) p = 0.059
* To improve alcohol or other substances effects, helping to socialize.	

both parents. The majority of them (50.2%) resulted in age class 51-60 years with a higher prevalence of high school level (46.9%) and of employment (74.3%). A statistically significant association between a low education level of family and stimulants use by students was found ($p < 0.05$, Chi-squared with Yates' correction).

Tab. II shows prevalence rates of stimulants use or misuse (alone or combined with other substances), academic and non-academic reasons for use, psychophysical state post-use and stimulants side effects referred among stimulants users (N = 102).

Fig. 2. Reasons for stimulants use (in academic and extra-academic fields) among stimulants users (N=102) by range of use. (Prevalence rate).



The frequency rates of use in the previous six months were: 7.8% 1 to 5 times, 2.9% 6 to 15 times, 2.0% ≥ 16 times.

The combination of stimulants and alcohol (12.7%) was the most common. However, the majority of respondents (51.0%) used stimulants alone.

Only a minority of subjects used stimulants combined with "medical drugs" (5.9%) with a frequency rate of 1 to 5 times (4.9%) and ≥ 16 times (1.0%), or with "other drugs" (2.9%, all of them with a frequency rate of 6 to 15 times).

Among stimulants users, stimulants consumption for at least one academic reason (72.5% = 74/102) was higher than the one for at least one extra-academic motive (47.1% = 48/102).

The questionnaire showed that 29.4% of consumers had at least one health consequence resulting from stimulants use, such as depression, loss of appetite, headaches, nervous tics development, fatigue, stomach pain, insomnia, and other not specified symptoms.

Table II includes the variables tested as predictors for stimulants use: male gender, being a Bachelor's degree student, age, being a working student, being from the North of Italy and parents' high educational level. According to multiple logistic regression, only the variables of being a Bachelor's degree student (OR 2.4, $p < 0.05$) and coming from Northern Italy (O.R 3.1, $p < 0.05$) were significantly positively associated to stimulants use.

Figure 2 shows the frequency rates of reasons for stimulant use (in academic and extra-academic fields) in the six months preceding this survey. The 57.8% of students used stimulants at most 5 times in 6 months and, among them, the most frequent reason for stimulant use was to improve concentration while studying (47.5%) and the less frequent one was to improve driving performance (1.7%), reflecting results detected among all stimulants users (considering all the frequencies of use). For each reason of stimulant use (academic and non-academic) the most recurrent frequency rate of use during the six previous months was 1 to 5 times.

The most common academic reason for stimulants use was "to obtain an extra improve concentration while studying" (51.0%), while the most non-academic reason was "to improve sports performance" (25.5%).

Table III shows the socio-demographic characteristics of stimulants users who reported the higher assumption respectively for academic and non-academic reasons (to improve concentration while studying and to improve sports performance).

Both among stimulant users to improve studying concentration and sport performances, a higher prevalence rate was found in younger age class, residence in North of Italy, and in Medicine, Dentistry, Nursing, Biomedical Laboratory Techniques, Physiotherapy and Dental Hygiene.

Females showed a higher prevalence rate for studying motivation (6.1% for females *vs*. 4.8% for males, $p > 0.05$, Chi-squared with Yates' correction = 0.571), while males mostly reported consumption to obtain better results in sports (4.0% for males *vs*. 2.4% for females, $p > 0.05$, Chi-squared with Yates' correction = 0.268).

Figure 3 shows the stimulants consumer trends in the various years of the different degree courses in order to increase the concentration in the study and to improve sports performance. The prevalence rates are obtained from the total sample (N = 899).

Stimulants use to improve concentration while studying:

- Among Medicine students, the stimulants use increased from the first academic year (2.9%) to the third (6.5%), and then decreased during the fifth year

Tab. III. Socio-demographic characteristics of stimulants users to improve concentration while studying (N=52) and sports performance (N = 26). (Prevalence rate).

Variables	Stimulants users to improve concentration while studying (N = 52)		Stimulants users to improve sports performance (N = 26)	
	A (%)	B (%)	A (%)	B (%)
Gender				
Female	6.1 (38/619)	55.9 (38/68)	2.4 (15/619)	22.1 (15/68)
Male	4.8 (13/272)	40.6 (13/32)	4.0 (11/272)	34.4 (11/32)
Non responders	12.5 (1/8)	50.0 (1/2)	0.0 (0/8)	0.0 (0/2)
Age (years)*				
18-22	71.2 (37/52)	71.2 (37/52)	73.1 (19/26)	73.1 (19/26)
23-27	21.2 (11/52)	21.2 (11/52)	23.1 (6/26)	23.1 (6/26)
≥ 28	1.9 (1/52)	1.9 (1/52)	3.8 (1/26)	3.8 (1/26)
Non responders	5.8 (3/52)	5.8 (3/52)	0.0 (0/26)	0.0 (0/26)
Residence				
North	6.1 (46/759)	51.1 (46/90)	3.2 (24/759)	26.7 (24/90)
Central-South	1.1 (1/94)	25.0 (1/4)	1.1 (1/94)	25.0 (1/4)
Non responders	10.9 (5/46)	62.5 (5/8)	2.2 (1/46)	12.5 (1/8)
Courses				
Medicine	3.3 (7/210)	50.0 (7/14)	2.4 (5/210)	35.7 (5/14)
Dentistry	5.7 (3/53)	75.0 (3/4)	1.9 (1/53)	25.0 (1/4)
Nursing	8.0 (34/425)	54.0 (34/63)	3.5 (15/425)	23.8 (15/63)
Biomedical Laboratory Techniques	10.6 (5/47)	55.6 (5/9)	4.3 (2/47)	22.2 (2/9)
Speech and Language Therapy	0.0 (0/38)	0.0 (0/1)	0.0 (0/38)	0.0 (0/1)
Midwifery	0.0 (0/38)	0.0 (0/1)	0.0 (0/38)	0.0 (0/1)
Imaging and Radiotherapy Techniques	0.0 (0/34)	0.0 (0 /2)	0.0 (0/34)	0.0 (0/2)
Physiotherapy	7.4 (2/27)	50.0 (2/4)	7.4 (2/27)	50.0 (2/4)
Cardiovascular Perfusion Techniques	0.0 (0/12)	0.0 (0/1)	0.0 (0/12)	0.0 (0/1)
Dental Hygiene	10.0 (1/10)	50.0 (1/2)	10.0 (1/10)	50.0 (1/2)
Psychiatric Rehabilitation Techniques	0.0 (0/5)	0.0 (0/1)	0.0 (0/5)	0.0 (0/1)

* In every column age is reported as non-recalculated prevalence rate
A= Prevalence rate calculated on total sample
B = Prevalence rate calculated on stimulants users population

(1.7%), although the differences between the various years were not statistically significant ($p > 0.05$, Fisher's exact test).

- Among Dentistry students, the stimulants use decreased from the first (4.0%) to the third year (0.0%) and then increased up to the fifth (16.7%), without a statistically significant difference across years ($p > 0.05$, Fisher's exact test).
- Among Bachelor's degree students the stimulant appears to be higher during the first year for Dental hygiene (10.0%), Physiotherapy (7.4%) and Nursing (9.7%), whereas for Biomedical Laboratory Techniques a rise in use is found during the third year (15.4%) without a significant difference from the first year (4.8%) of this degree course. There were no statistically significant differences between the various courses ($p > 0.05$, Fisher's exact test), except between the first (9.7%) and the third (6.4%) year of Nursing ($p < 0.05$).

Stimulants use to improve sports performance:

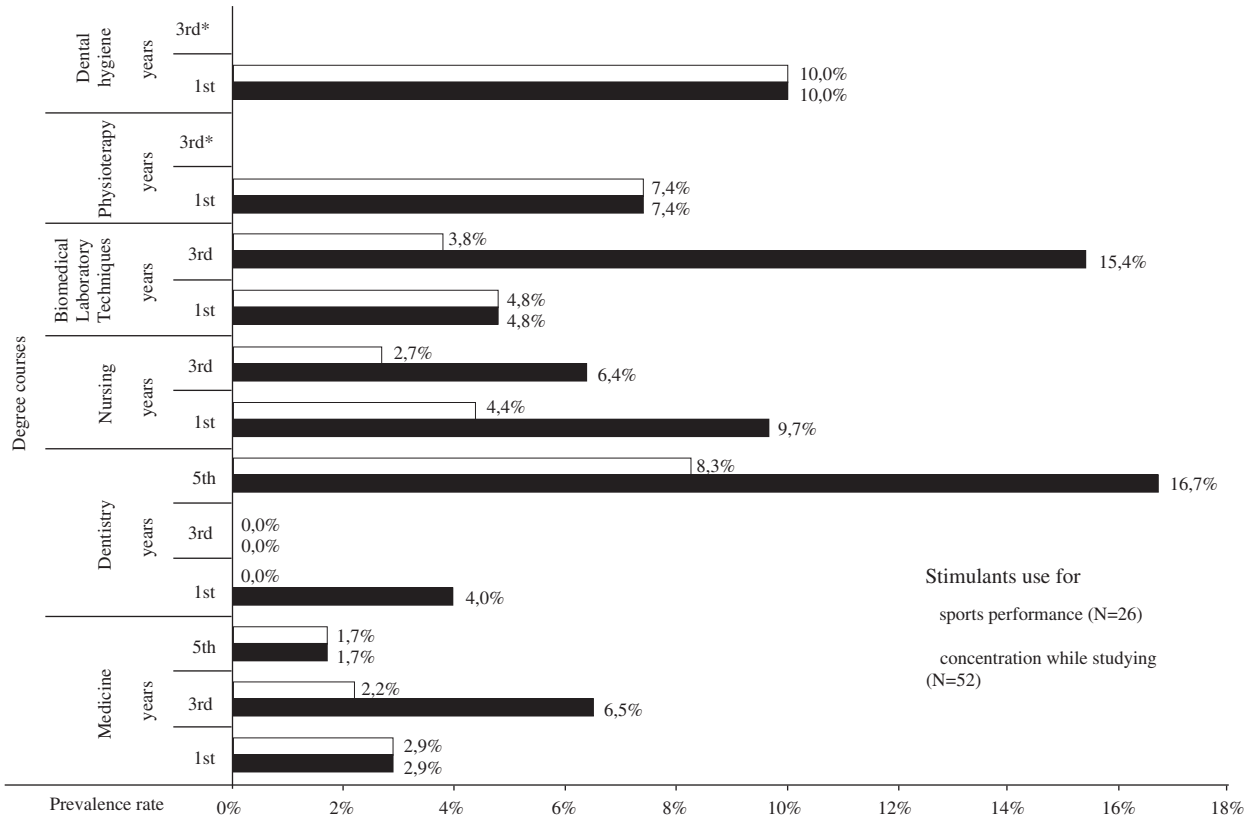
- Among students of Master's degree courses, from the first to the fifth year it was observed a decreasing trend in Medicine students and an increasing trend in Dentistry students, without a statistically significant difference across years ($p > 0.05$, Fisher's exact test).
- Among Bachelor degree students' stimulants use is greater during or even limited to their first academic year.

Overall, the difference in prevalence rates of stimulants use during each year of each degree course considered never showed a significant difference ($p>0.05$, Fisher's exact test and Chi-squared with Yates correction, with Bonferroni's correction).

Discussion

To our knowledge, this is the first report that has attempted to point out the prevalence rate of stimulants use among university students in the North-East of Italy. This cross-sectional study shows that a non-negligible portion (11.3%) of 899 sampled university students used stimulants – currently and/or in the past six months – and that a smaller proportion (2.0%) of the total sample had used stimulants in their lifetime (exclusively before the surveyed period).

Fig. 3. Prevalence rate of stimulants use for boosting concentration while studying and sports performance by year of degree courses.



* These years of courses were not surveyed; ** p < 0,05 (Fisher's exact test)

Teter *et al*. conducted a survey on 4580 college students and they found a prevalence rate of 5.9% for illicit use of prescription stimulants in the year before their survey [20].

The population of stimulant users of our survey was homogeneous for gender composition and the majority (73.5%) was aged between 18 and 22, not confirming the trends of a larger consumption of stimulants among males [13, 29-31] and older subjects [32-34] found in other studies. A statistically significant difference in stimulants use among students of 18-23 years old and older ones was not found.

We were able to detect a significant correlation between lower family educational level and stimulants use with Fisher's exact test; according to the multiple logistic regression, however, the family educational level was not significant as a predictor of stimulants use. Another European study [35] suggests an association between higher educational family level and the consumption of some type of drugs (alcohol, tobacco, cannabis) but not with prescription stimulants.

With regard to extra-academic benefits on NMUPS, the most frequent reason for stimulants use was to improve sports performance (25.5%). The second one was "to improve alcohol or other substances effects in helping to socialize" (18.6%) and, among all possible associations, the most common one was with alcohol (12.7%). Moreover, we found a prevalence rate of stimulants use to lose weight (11.8%) comparable to one detected in a study among US university students (11.1%) [19]. The use of stimulant drugs for dieting is reportedly also increasing in Asia [36].

These data are worthy of attention, suggesting an association between stimulants use and other harmful behaviors, such as alcohol abuse [37] and self-induced vomiting, use of laxatives, diuretics, and other pills for weight loss [25].

With regard to academic reasons for stimulants use, it is interesting to compare our results to those of an American study [19], which detected a prevalence of NMUPS of 25.6% during college and found that 26.4% of students engaging in NMUPS had a prescription for the drug. According to this data, NMUPS might be taking place among stimulants users with a medical prescription (2.3%), although it was not clearly declared. Moreover, the top four reasons for NMUPS detected in the US survey [19] were academic-related (to improve focus, to improve concentration, to stay awake and to make studying more enjoyable), resembling our identified primary reasons to improve concentration in studying (51.0%), mental stamina for studying (48.0%), exams performance (42.2%) and classroom concentration (28.4%).

Overall, we detected a prevalence of NUMPS for CE of 6.2%, which is greater than the one found by a study on 512 German university students (0.78%) [23] and comparable to two other surveys conducted on university students in Switzerland (6.2%) [24] and in Germany (5%) [28].
In New Zealand, the prevalence of use of NUMPS for CE in a population of university students is comparable to our results (6.6%) [38].
Our findings agree with the NMUPS for CE revealed by recent similar European studies [24, 33] and confirm that prevalence rates of stimulants use to practice CE are higher among American university students than European ones [1, 19, 23, 24, 33]. Easier access to stimulants, such as by using friends as a source or by simulating ADHD symptoms to obtain a false diagnosis [19, 39, 40], and an increasingly competitive and high pressure environment in the U.S. with fewer job opportunities after graduation [41] could explain the higher NMUPS among American students than European ones.
The use of a stimulant such as MPH, however, which has been available in the European Union (EU) since the 1950s, has markedly increased in Europe over the past decade [42], probably as a consequence of the modifications of the European lifestyle, with an increased load of stress and economic difficulties. We therefore cannot exclude that in the future the prevalence rates of stimulants use among European students will be comparable to those of American ones.
We found a statistically significant difference between stimulants use and being a working *vs.* a non-working student, probably due to the higher load of stress experienced by those who both work and study. Stress was in fact often associated with consumption of stimulants [17, 39-41] among medicine students [39], and several studies have suggested that NMUPS can be a strategy to cope with stress [17] and to try to reduce the negative effects of stress-related affective disorders [40, 41].
After calculating a multiple logistic regression, however, the variable of "being a working student" was not a significant predictor for stimulants use. Very serious side effects related to stimulant use have been reported in the literature [26], and in our survey almost one third (29.4%) of consumers had relatively serious health consequences. Side effects are important not only for acute and long-term health consequences, but also for their economic and social burden. Indeed, rising trends in emergency department admissions involving non-medical use of stimulants and adverse reactions to stimulants highlight the growing impact of NMUPS on public health and, in particular, on the health of young adults both males and females [43].
In the literature, suicidal ideation is described as a possible consequence of consumption of medications for ADHD in young populations [44, 45]; other potential side effects on the cardiovascular system or nervous system have been linked to use of stimulants [28].
Stimulant use is also related to important long-term problems such as dependence and cognitive dysfunction. An abrupt cessation after periods of regular use leads to psychiatric withdrawal symptoms, including depression, anxiety and cravings [46]. Pharmacotherapies aimed at treating addiction to stimulants are at in the early stages of development and testing [46], and together with psychotherapy they might lead to an increasing therapeutic success, improving the healthcare system. Furthermore, ADHD patients and relative prescriptions among school-aged students have continuously increased [47, 48] in the past few years, due to the improved ability of ADHD diagnosis [48]. However the possibility of over diagnosis or of misdiagnosis, implying the prescription of unnecessary medication or the absence of adequate ones, could lead to an increase in the need of medical services. This could cause an increase in social costs.
An important strength of this study is the large number of participants surveyed.
The present study shows some particularities in the population sampled: there were more women than men, reflecting the greater female presence in the Italian medical universities, and most of the surveyed people, because of the university location, came from the North of Italy. The results may therefore not be generalizable to university students in the rest of Italy. Moreover, collecting data at a single point in time through a longitudinal study would be a better design to support the study findings. For these reasons, it could be interesting to deepen the problem with further investigations, and future researches should examine stimulants use in a larger sample with a multicenter study.
Our study shows that abuse of stimulants in our geographic area is of some importance, and the higher consumption of stimulants among students from other countries might suggest a future increase in our country.
For these reasons, an educational health program should be planned and implemented to prevent stimulant abuse throughout the country and across all ages. Informing people by educating them as early as while they are attending elementary school (and by involving the children's parents) through the local health services would raise awareness about the phenomenon and would hopefully reverse it.

Conclusions

Stimulants use was relatively prevalent in this population (11.3%), with an apparent greater use by students aged 18-22 years (73.5%) and without any gender predominance. 57.8% of stimulants users consumed stimulants five times in six months at most, and 47.5% of them justified such use in order to improve concentration while studying.

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The authors declare no competing interest.

Authors' contributions

SM: design, organization and supervision of the study. Drafting of the text. DG: data analysis, participation in the drafting of the article, statistical analysis. SP: data

analysis, participation in the drafting of the article, statistical analysis. JP: data analysis, participation in the drafting of the article. AS: preparation and validation of the questionnaire, distribution of the questionnaire, data collection and data entry in the database. SF: preparation and validation of the questionnaire, distribution of the questionnaire, data collection and data entry in the database. EC: preparation and validation of the questionnaire.

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ORIGINAL ARTICLE

Knowledge, attitudes, beliefs and practices of Occupational Physicians towards seasonal influenza vaccination: a cross-sectional study from North-Eastern Italy

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Keywords

Healthcare workers • Influenza vaccine • Occupational physicians

Summary

Introduction. *The present study aims to characterize personal attitudes and knowledge of a sample of Italian Occupational Physicians (OPh) towards Seasonal Influenza Vaccine (SIV) in healthcare workers (HCWs).*
Methods. *In total, 92 OPh (42.4% males, 57.6% females, mean age of 47.3 ± 10.4 years, 50 specialists in Occupational Medicine, 42 specialists in Hygiene and Public Health) were asked about their attitudes towards influenza vaccine, their general knowledge of vaccine practice, their propensity towards vaccines and, eventually, their risk perception about the influenza and influenza vaccine was investigated. A regression analysis was then performed in order to better characterize predictive factors for vaccine propensity.*
Results. *Influenza was recognized as a vaccination recommended for HCWs in 89/92 of the sampled OPh (96.7%). However, prevalence of misconceptions about vaccines was relatively high, with 26/92 (28.3%) and 24/92 (26.1%) referring vaccinations as eliciting allergic and autoimmune diseases, respectively and identifying lethargic encephalitis (18/92, 19.6%), autism (17/92, 18.5%), diabetes mellitus (15/92, 16.3%) and multiple sclerosis (13/92, 14.1%) as causatively vaccine-related. Propensity towards influenza vaccination found a significant predictor in the general knowledge (beta coefficient 0.213, p value = 0.043), risk perception (beta coefficient 0.252, p value = 0.018) and general propensity towards vaccinations (beta coefficient 0.384, p value = 0.002).*
Discussion. *In spite of a diffuse propensity towards SIV, adherence of OPh was still < 50% of the sample. Moreover, sharing of misbeliefs and misconceptions was significant. As knowledge and risk perceptions were identified as significant predictors of vaccine propensity, our results suggest that information and training programs for OPh should be appropriately designed.*

Introduction

Seasonal influenza (SI) is a highly contagious vaccine preventable infectious disease (VPD), which can result in debilitating illness and potentially fatal complications in subjects at risk, representing a major public health problem with a heavy impact on National Healthcare Systems [1, 2]. Because of theirs professional duties, healthcare workers (HCWs) not only are at high risk of contracting SI, but also represent a significant source of transmission and circulation of the viruses in the community [3, 4]. SI vaccine (SIV) is safe and usually well-tolerated [5-8], and evidence suggests that policies involving immunization of HCWs may cost-effectively decrease employee absenteeism caused by SI. Moreover, by preventing its transmission between HCWs and patients, SIV would ultimately improve patient safety and decrease influenza-related morbidity and mortality [2, 9]. Since 1981, the United States Centers for Disease Control and Prevention (CDC) have therefore advised that HCWs will receive SIV [3, 10, 11], and in 2002 also the World Health Organization (WHO) began encouraging annual immunization where supported by national data and capacities, furtherly strengthening its recommendations during the 2009 H1N1 influenza pandemic [2, 5-8]. Nowadays, several European Public Health Authorities, such as the Italian National Health Service (in Italian: *Servizio Sanitario Nazionale*, SSN), have implemented SIV in HCWs through official recommendations (in Italy: National Immunization Prevention Plan / *Piano Nazionale di Prevenzione Vaccinale* or PNPV) [3, 7, 12-19]. However, vaccination coverage remains heterogeneous and usually unsatisfactory, with rates well below the minimum target of 75% required by the European Commission, as still ranging from about 15% to 50% in different countries [4, 15, 20-23]. Although Italian data on vaccination coverage among

HCWs are not routinely available, recent studied have confirmed an inadequate compliance, suggesting that vaccination rates would have significantly declined since 2009 H1N1 pandemic, being presumptively well below 20% [7, 12 15, 19, 24, 25].

A number of studied have examined specific factors influencing SIV uptake by HCWs, identifying major barriers in system failures (*e.g.* stock-outs, limited availability of vaccination services in terms of time, places, etc.) and in individual factors such as: doubts regarding the preventive usefulness of vaccines and the rationale for vaccination, lack of knowledge regarding natural infection (*i.e.* actual risk for HCWs) and its potential consequences, misbelieves about vaccine-related risks and vaccine safety, as well as a diffuse lack of trust in the health policies and in the health authorities that promote them [2, 16, 26, 27]. In facts, a significant share of HCWs still understand SI as a mild illness not requiring a specific prophylaxis, and that contracting the disease is somehow safer than getting the vaccine [2, 16, 18, 25-31]. Collectively, aforementioned factors concur to the definition of vaccine hesitancy (VE), *i.e.* the continuum between full acceptance of vaccines with no doubts and the complete refusal with no doubts [28-31], and VE would in turn impair proactive behaviors, ultimately contributing to low vaccination rates [2, 16, 18, 25-31].

Occupational Physicians (OPh) are the medical professionals responsible for health promotion on the workplaces [32], and may actively contribute to overcome false attitudes and misconceptions supporting VE. Moreover, OPh inform the workers about the pros and cons of recommended vaccinations, and may therefore undermine or even remove the mutual misunderstanding between public health professionals and vaccine hesitant individuals, eventually maximizing the consent for vaccination programs [32]. Unfortunately, although numerous studies have assessed knowledge, attitudes and practices (KAP) of specific occupational groups regarding vaccinations, and such interventions have been proven as quite efficient in designing appropriate vaccination campaigns, ultimately improving immunization rates [20, 23, 26, 33-35], KAP of OPh about influenza vaccine have been scarcely investigated [32, 36, 37]. Moreover, as determinants of VE are vaccine-, VPD- and context-specific [28, 29], available evidence from general studies about vaccine acceptance in HCWs and more specifically in OPh are of limited generalizability [36, 37].

The aim of this study, therefore, to assess KAP of OPh about SIV and vaccination policies, including both general and specific recommendations for HCWs, and how attitudes and knowledge relate to these recommendations. Eventually, we attempted to identify areas that may be targeted for improvement through specific informative and educative campaigns dedicated to OPh.

Materials and methods

Study design

A cross-sectional questionnaire-based study was performed in the second half of 2015, involving OPh operating in the Autonomous Province (AP) of Trento (North-Eastern Italy). Participants were inquired about their KAP towards vaccinations, and more specifically on the SIV. Sampling was performed through convenience, as the initial population included all OPh participating to a seminar on occupational health that took place in the AP of Trento in October 2015 and assisting at least one healthcare provider in the AP of Trento (n = 105, 43.9% of 239 OPh usually operating in the AP of Trento). All participants giving their preventive agreement in the following weeks received a telephonic interview assessing knowledge and attitudes towards SI and SIV in HCWs.

Questionnaire

Two specifically formed researchers compiled a structured questionnaire through a telephonic interview. The questionnaire was formulated in Italian (an English translation is presented as the Annex 1), and its test-retest reliability was preventively assessed through a survey on 10 health professionals completing the questionnaire at two different points in time. All questions were self-reported, and not externally validated.

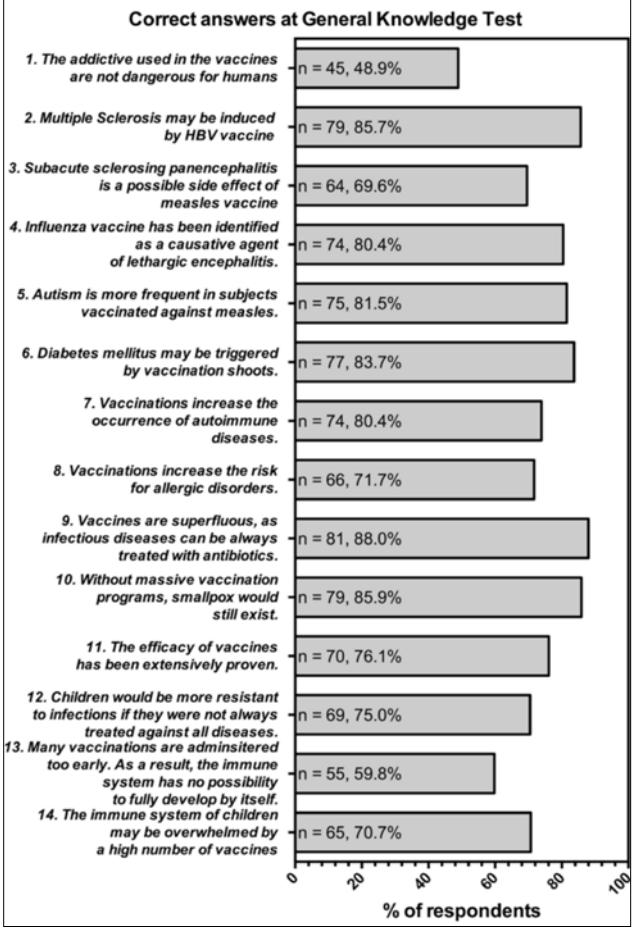
The final questionnaire comprised general demographic information (*i.e.* age, sex, country of origin) and the following areas of inquiry:

(1) Demographic data. Included: age, sex, country of origin (*i.e.* Italian born-people vs. foreign-born people), and medical specialization (*i.e.* in Italy, qualification as OPh is primarily obtained through specialization in occupational medicine, but also specialists in Hygiene and Public Health and in Legal/Forensic Medicine are legally authorized to work as OPh, as well as all physicians who were working as OPh before 1991) [38, 39].

(2) General knowledge. The questionnaire included a general knowledge test originally developed by Zingg and Siegrist [40], and containing a set of true-false statements such as "*vaccinations increase the occurrence of allergies*" (false) that cover some typical misconceptions on vaccination. General knowledge test was able to successfully predict risk perceptions and vaccination intentions in previous studies [32, 40]. A total of 14 statements (Fig. 1) were eventually presented, including the 9 original items from Zingg's questionnaire and 5 further items about vaccine misconceptions designed to better fit Italian settings (*e.g.* causative association between HBV vaccine and multiple sclerosis, influenza and lethargic encephalitis *etc.*). A General Knowledge Score (GKS) was then calculated as the sum of correctly and incorrectly marked recommendations: when the occupational physicians correctly answered, +1 was added to a sum score, whereas a wrong indication or a missing/"don't know" answer added -1 to the sum score.

(3) Risk perception. Perceived risk has been defined as a function of the perceived probability of an event and its expected consequences, and therefore assessed as the mathematical product of subjective probability and disease severity [32, 41]. We inquired the risk perception of OPh about influenza by asking the OPh about: the probability that HCWs get influenza infection, the frequency of vaccine-related adverse effects, and whether

Fig. 1. General knowledge test. The original knowledge test [19, 23] was modified including 5 additional items (n. 2 to 6); as disorders cited in items n. 2-6 were previously presented in the original items 2-3, items 7-9 were subsequently modified.



they perceived the severity of the natural infections and vaccine-related adverse effects. In order to summarize the results, we used a fully labeled 7-point scale (*i.e.* 1, "*almost zero*"; 2, "*low*"; 3, "*rather low*"; 4, "*moderate*"; 5, "*rather high*"; 6, "*high*"; 7, "*very high*"). A Risk Perception Score (RPS) was eventually calculated as cumulative score through the formula:

$$\text{Risk perception} = I^{INF} * C^{INF} - I^{VAC} * C^{VAC}$$

where:

I^{INF} = perceived probability of infection in HCWs

C^{INF} = perceived severity of natural infection in HCW

I^{VAC} =perceived probability of vaccine-related adverse effects

C^{VAC} = perceived severity of vaccine-related adverse effects

(4) Attitudes and Practices. Initially, participants rated their general attitudes towards vaccinations, and the answer was a 7-point Likert scale (*i.e.* 1, "*absolutely against vaccinations*"; 2, "*strongly against vaccinations*"; 3, "*somewhat against vaccinations*"; 4, "*neutral*"; 5, "*somewhat in favor of vaccinations*"; 6, "*strongly in favor of vaccinations*"; 7, "*absolutely in favor of vaccinations*"). The OPh were then asked to rate their attitudes towards influenza vaccine through a similar fully labeled 7-point Likert scale. A cumulative score (*i.e.* "*propensity score*") was calculated, both in general (G-PS) and for influenza vaccine (IV-PS).

Eventually, participants were asked whether they had received SIV during 2014-2015 winter season. Subjects self-assessed as "*not vaccinated*" fulfilled a subsequent set of items exploring the reasons for not having been previously vaccinated. In particular, participants were asked whether: (1) they had organization problems (*i.e.* "*not enough time*"); (2) they felts themselves as already immunized by previous vaccination campaigns; (3) would prefer recur to alternative countermeasures; (4) are not convinced that IV is useful; (5) have fear of injections or (6) of side effects; (7) they understand vaccination as a mild disease, making therefore useless the vaccine and eventually (8) whether IV is contrary to their personal / religious beliefs.

Ethical considerations

Before they give their consent, participants were informed that all information would be gathered anonymous and handled confidentially. Participation was voluntary, and the questionnaire was collected only in subjects who expressed consent for study participation. As individual participants cannot be identified based on the presented material, this study caused no plausible harm or stigma to participating individuals.

As the study design assured an adequate protection of study participants, and neither include clinical data about patients nor configure itself as a clinical trial, a preliminary evaluation by the Ethical Committee of the Provincial Agency for Health Services (in Italian: *Azienda Provinciale per i Servizi Sanitari,* APSS) was statutorily not required.

Data analysis

Two independent researchers, one of whom read the responses from each questionnaire while the other researcher reviewed the entered data, ensured the accuracy of data entry. The primary investigator examined unclear responses to determine the correct answer. We calculated the described indices for general knowledge (GKS), risk perception (RPS) and vaccine propensity (G-PS and IV-PS).

Continuous variables (*i.e.* age, GKS, RPS, G-PS, IV-PS) were expressed as mean ± standard deviation. Categorical variables were reported as percent values. Univariate confrontation between continuous variables was performed through Student's t test for unpaired data, whereas proportions were evaluated through Chi-squared test (with continuity correction). Association of

dichotomous variables was assessed in univariate analysis through calculation of respective Odds Ratios (OR) with their respective 95% Confidence Intervals (95% CI). Relations between the continuous variables were explored through the calculation of the Pearson product-moment correlation coefficient (*i.e.* Pearson's r). A logistic regression analysis (SPSS 23) was performed in order assessed the relative influence of personal attitudes and general knowledge on personal propensity to vaccinate. The analyses were controlled for age, sex, qualification. Odds Ratios similarly adjusted for age, sex, country of origin, and qualification (adjOR) were calculated through a binary logistic regression analysis for factors that in univariate analysis were associated with dichotomized propensity ("*somehow favorable*"/"*somehow against*" influenza vaccination) and previous SIV at $p < 0.150$. Significance level was 5%.

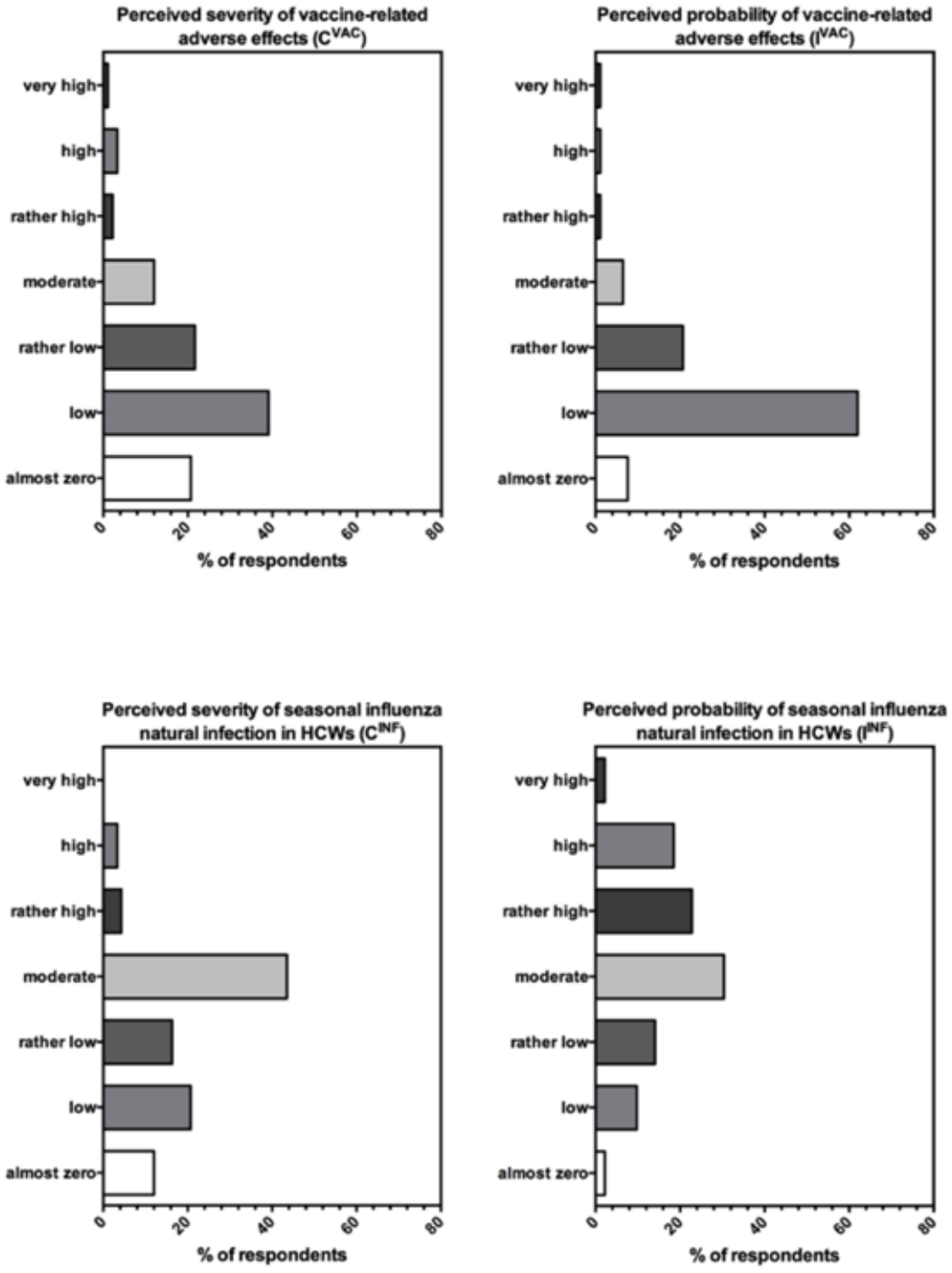
Results

(1) Demographic data. Overall, 95/105 participants (90.5%) gave their consent to the inquiry and 92/105 compiled the questionnaire regarding IV/SIV (87.6%, *i.e.* the 38.5% of all OPh operating in the AP of Trento): as shown in Table I, 39 (42.4%) were males, and 53 (57.6%) females, with a mean age of 47.3 ± 10.4 years (50.4 ± 9.3 in males vs. 49.4 ± 8.1 in females, $p = 0.582$), and 55.4% of the participants (51/92) were > 50 year-old. Among the sampled subjects, 50 (54.3%) were specialists in Occupational Medicine, whereas 42 (45.7%) were qualified as specialist in Hygiene and Public Health.
(2) General knowledge. Overall, 89/92 of the sampled OPh (96.7%) correctly recalled SIV as recommended by PNPV 2012-2014 in HCWs. Focusing on general knowledge test (Fig. 1), despite a potential range of -14 to +14, the actual mean score was 5.9 ± 4.5, and no one among sampled subject reached the maximum score of 14 (actual range: -9 to +11). With the exception of the claims about the safety of vaccine additives, as 47/92 (51.1%) failed to identify them as not dangerous, the majority of participants correctly identified the presented statements. In particular, most of participants were aware that infectious diseases cannot be always treated with antibiotics (88.0%, 81/92), and that without massive vaccination programs, infectious diseases such smallpox would still exist (85.9% of correct answers, 79/92). However, 23.9% of the sample (22/92) questioned the efficiency of vaccines, and 40.2% (37/92) exhibited the misconception that too many vaccinations are administered too early, whereas 29.3% (27/92) claimed that the immune system may be overwhelmed by a high number of vaccines. Eventually, around a fourth of sample erroneously stated that children would be more resistant to infections if they were not always treated against all diseases (23/92, 25.0%), and that vaccines may be causatively related with allergic disorders (28.3%, 26/92) and autoimmune diseases (26.1%, 24/92). More specifically, vaccines were associated with disorders of the immune systems such as diabetes mellitus (15/92, 16.3%) and multiple sclerosis (13/92, 14.1%), but also with neurological diseases such as subacute sclerosing panencephalitis (28/92, 30.4%), lethargic encephalitis (18/92, 19.6%), and even autism (17/92, 18.5%).
(3) Assessment of the risk perception. Despite a potential range -49 to +51, RPS was estimated in 7.5 ± 8.6 (actual range -26 to +27). As shown in Figure 2, not only the majority of participants perceived the potential severity (C^{INF}) of influenza natural infection as "*almost zero*" to "*rather low*" (59.8%, 55/92; mean score 3.2 ± 1.3), but also no one among the sample identified influenza as a VPD with a potentially "*very high*" severity, whereas 26.1% of participants similarly defined potential probability of influenza natural infection in HCWs (I^{INF}) as "*almost zero*" to "*rather low*", with a further 30.4% acknowledging a "*moderate*" probability of natural infection (mean score 4.3 ± 1.4). Regarding the adverse effects, around 90.2% of participants referred to perceive their probability (I^{VAC}) as "*almost zero*" to "*rather low*" (mean score 2.4 ± 0.9), and similarly 81.5% of the participants defined their potential severity as mild (i.e. "*almost zero*" to "*rather low*"; C^{VAC}, mean score 2.5 ± 1.3).
(4) Attitudes and Practices. Mean G-PS was 5.8 ± 1.1, with an actual range of 3 to 7. More specifically, 88/92 (95.6%) identified themselves as somehow favorable to vaccinations. Focusing on IV-PS, a mean score of 3.1 ± 1.8 was identified (actual range: 1 to 7), as 63/92 (68.5%) were somehow favorable to influenza vaccine.
Overall, 46.7% of the participants (43/92) referred to have been vaccinated against seasonal influenza in the previous year, and 49 subjects fulfilled the questionnaire's section exploring the reasons for refusing SIV (Fig. 3). The most frequently referred reason was the lack of time (23/49, 46.9%), followed by the belief to be "*already immune because of previous vaccinations*" (13/49, 26.5%), whereas 9/49 (18.4%) reported the preferential use of "*alternative countermeasures*", and 8/49

Tab. I. Demographic characteristics and qualification of sampled OPh (n = 95).

Characteristics	N (%)
Gender	
Males	39 (42.4%)
Females	53 (57.6%)
Age (years)	
≤ 29	3 (3.3%)
30 – 39	8 (8.7%)
40 – 49	30 (32.6%)
50 – 59	44 (47.8%)
≥ 60	7 (7.6%)
Country of origin	
Italian-born people	86 (93.5%)
Foreign-born people	6 (6.5%)
Medical Specialization	
Occupational Medicine	50 (54.3%)
Hygiene and Public Health	42 (45.7%)
Legal / Forensic Medicine	-
Other	-

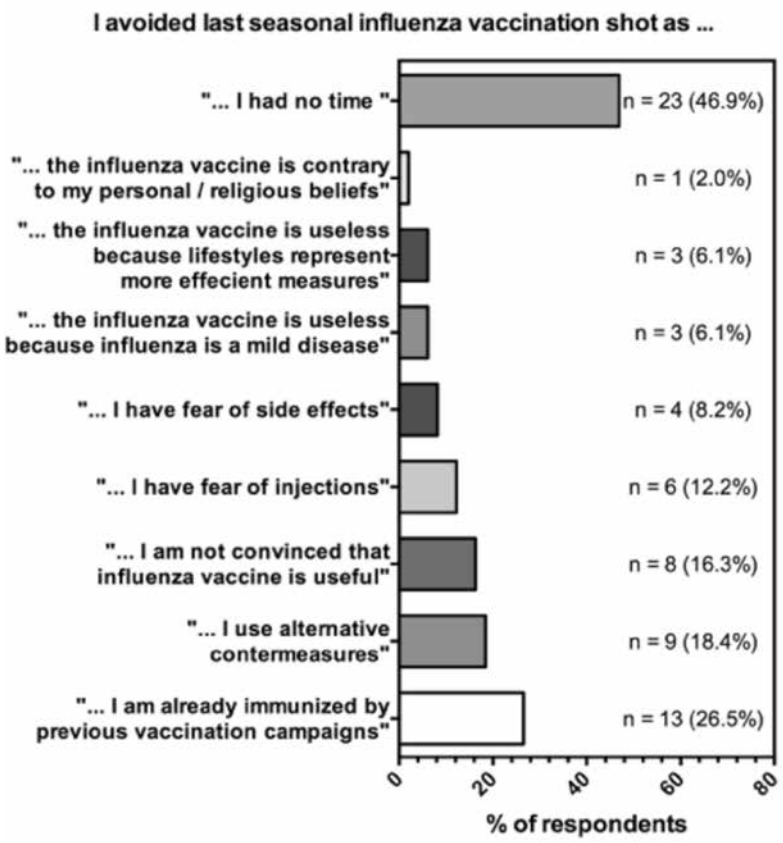
Fig. 2. Components of the Risk Perception Score in 92 Occupational Physicians participating to the survey (HCWs = health care workers).



(16.3%) were "*not convinced that influenza vaccine is useful*", and 4/49 (8.2%) referred "*fear of side effects*".

(5) Univariate analysis. In univariate analysis, although a greater share of participants younger than 50 years exhibited a somehow favorable attitude towards influenza vaccine (78.0% vs. 60.8% in subjects older than 50 years, p = 0.122; OR 2.294 95% CI 0.906-5.808), no significant association was found between demographic factors (*i.e.* gender, age group and country of origin), and vaccination status on the one hand and personal attitude towards seasonal influenza vaccine (Table II). However, focusing on the referred medical specialization, participants having a qualification in Occupational Medicine were associated with a significantly greater share of positive attitude towards seasonal influenza vaccine (90.0% *vs*. 42.9%, p < 0.001; OR 12.000 95% CI 3.964-36.331). Eventually, subjects referring a positive vaccination status more frequently exhibited an attitude somehow

Fig. 3. Reasons advocated by subjects not vaccinated against seasonal influenza during winter season 2014-2015. Because more than a single choice was possible, sum of percentages is not equal to 100%.



against influenza vaccination than participants exhibiting a negative vaccination status (82.9% *vs*. 56.9%, respectively; p = 0.012, OR 3.816 OR 1.422-10.239).

As shown in Table III, subjects correctly identifying as "*true*" the statement that "*the efficacy of vaccines has been extensively proven*" were significantly associated with the recalling of a positive seasonal influenza vaccine status (OR 3.600, 95% CI 1.169-10.83) and a positive attitude towards influenza vaccine (OR 3.741, 95% CI 1.374-10.19).

A positive attitude towards SIV was also significantly associated with the denying of misconceptions as the association between vaccines and autism (p < 0.001; OR 8.188 95%CI 2.528-26.52), the greater resistance to infections of children achieving a "natural" immunity (p = 0.007; OR 4.306; 95% CI 1.590-11.663), and the increased occurrence of auto-immune diseases after receiving vaccinations (p = 0.044; OR 3.000 95% CI 1.137-7.918).

On the contrary, as shown in Table IV, previous vaccination against SI and a favorable attitude towards SIV were not associated with a significantly higher share of the factors included in the assessment of risk perception.

As shown in Table V, both GKS and RPS were significantly higher in subjects younger than 50 y.o. (7.1 ± 3.1 vs. 4.9 ± 4.1, p = 0.009; and 9.6 ± 7.7 *vs*. 5.9 ± 8.9, p = 0.033, respectively), in foreign-born participants than in Italian-born ones (8.7 ± 2.7 *vs*. 5.7 ± 4.3, p = 0.013; and 11.7 ± 10.4 *vs*. 7.2 ± 8.4, p = 0.023, respectively), and eventually in participants referring a specialization in Occupational Medicine than in participants specialists in Hygiene and Public Health (7.0 ± 3.0 *vs*. 4.6 ± 5.1, p = 0.008; and 9.6 ± 7.4 *vs*. 5.1 ± 9.3, p = 0.011, respectively). No significant differences were identified in G-PS throughout assessed demographic factors (all comparison p > 0.05), whereas focusing on IV-PS, subjects younger than 50 y.o. had a significantly higher score than older participants (4.1 ± 1.0 *vs*. 3.5 ± 1.4, p = 0.020).

Bivariate Pearson's correlations among GKS, RPS, G-PS and IV-PS are shown in Table VI. More specifically, GKS was positively correlated with RPS (r =0.317, p = 0.002) and propensity scores, (r = 0.315, p = 0.022 and r = 0.492, p < 0.001 for G-PS and IV-PS, respectively). Focusing on the two propensity scores, G-PS and IV-PS were positively correlated (r = 0.451, p < 0.001). Whereas IV-PS was in turn correlated with RPS (r = 0.280, p = 0.007), G-PS was not (r = 0.203, p = 0.053).

6. Multivariate analysis. Binary logistic regression confirmed that subjects somehow favorable to influenza vaccination more frequently had a positive SIV status (adjOR 5.806 95% CI 1.242-27.15), and correctly identified as misconceptions the association between vaccines and autism (adjOR 25.05 95% CI 2.538-247.3), and that children would be more resistant to infections if they were not always vaccinated against all diseases

Tab. II. Univariate association of influenza vaccination practice and attitude regarding influenza vaccine, with recalled demographic factors in 92 OPh participating to the study (OR = Odds Ratio; 95% CI = 95% Confidence Interval).

	Previously vaccinated against seasonal influenza			Somewhat favorable to influenza vaccination		
	N (%)	P value	OR (95% CI)	N (%)	P value	OR (95% CI)
Gender						
Males	20 (51.3%)	0.368	1.604	30 (76.9%)	0.205	2.020
Females	21 (39.6%)		(0.696 – 3.697)	33 (62.3%)		(0.798 – 5.116)
Age group						
< 50 y.o.	16 (39.0%)	0.455	0.666	32 (78.0%)	0.122	2.294
≥ 50 y.o.	25 (49.0%)		(0.289 - 1.532)	31 (60.8%)		(0.906 – 5.808)
Country of origin						
Italian-born people	39 (45.3%)	0.883	1.660	58 (67.4%)	0.722	0.414
Foreign-born people	2 (33.3%)		(0.288 – 9.547)	5 (83.3%)		(0.046 – 3.716)
Specialization						
Occupational Medicine	25 (50.0%)	0.350	1.625	45 (90.0%)	< 0.001	12.000
Hygiene and Public Health	16 (38.1%)		(0.706 – 3.741)	18 (42.9%)		(3.964 – 36.33)
Vaccination status towards seasonal influenza						
Previously vaccinated	-	-	-	34 (82.9%)	0.014	3.685
Not vaccinated	-	-	-	29 (56.9%)		(1.377 – 9.860)

Tab. III. Univariate analysis of the association between the correct answer to the statements included in the general knowledge test, positive vaccination status and attitude towards seasonal influenza vaccine.

Statement	Previously vaccinated against influenza vaccine (n = 41)		Somehow favorable attitude towards influenza vaccine (n = 63)	
	Unadjusted OR (95% CI)	P value	Unadjusted OR (95% CI)	P value
1. The addictive used in the vaccines are not dangerous for humans (TRUE)	0.830 (0.364 – 1.892)	0.816	1.038 (0.430 – 2.503)	0.888
2. Multiple Sclerosis may be induced by HBV vaccine (FALSE)	1.544 (0.475 – 5.014)	0.671	0.960 (2.280 – 3.418)	0.796
3. Subacute sclerosing panencephalitis is a possible side effect of measles vaccine (FALSE)	0.905 (0.370 – 2.217)	1.000	0.316 (0.514 – 3.372)	0.742
4. Influenza vaccine has been identified as causative agent of lethargic encephalitis (FALSE)	1.734 (0.614 – 4.896)	0.434	2.700 (0.938 – 7.770)	0.110
5. Autism is more frequent in subjects vaccinated against measles (FALSE)	0.451 (0.145 – 1.408)	0.262	8.188 (2.528 – 26.52)	< 0.001
6. Diabetes mellitus may be triggered by vaccinations shoot (FALSE)	0.800 (0.259 – 2.467)	0.916	2.188 (0.708 – 6.763)	0.282
7. Vaccinations increase the occurrence of auto-immune diseases (FALSE)	1.072 (0.421 – 2.730)	1.000	3.000 (1.137 – 7.918)	0.044
8. Vaccinations increase the risk for allergic disorders (FALSE)	1.357 (0.546 – 3.374)	0.671	2.471 (0.957 – 6.378)	0.099
9. Vaccine are superfluous, as infectious diseases can be always treated with antibiotics (FALSE)	0.424 (0.105 – 1.715)	0.365	3.026 (0.840 – 10.90)	0.160
10. Without massive vaccination programs, smallpox would still exist (TRUE)	0.928 (0.286 – 3.012)	1.000	1.856 (0.459 – 7.498)	0.616
11. The efficacy of vaccines has been extensively proven (TRUE)	3.600 (1.169 – 10.83)	0.034	3.741 (1.374 – 10.19)	0.016
12. Children would be more resistant to infections if they were not always treated against all diseases (FALSE)	0.943 (0.364 – 2.441)	1.000	4.306 (1.590 – 11.66)	0.007
13. Many vaccinations are administered too early. As results, the immune system has no possibility to fully develop by itself (FALSE)	0.914 (0.395 – 2.117)	1.000	1.997 (0.817 – 4.882)	0.194
14. The immune system of children may be overwhelmed by a high number of vaccines (FALSE)	1.227 (0.499 – 3.020)	0.830	1.125 (0.432 – 2.993)	0.996

Tab. IV. Univariate analysis of the association between participants' risk perception about influenza and influenza vaccine, positive vaccination status and attitude towards seasonal influenza vaccine.

Variable	Previously vaccinated against influenza vaccine (n = 41)		Somehow favorable attitude towards influenza vaccine (n = 63)	
	Unadjusted OR (95% CI)	P value	Unadjusted OR (95% CI)	P value
Perceived severity of vaccine related adverse effects (C^{VAC}) as rather high to very high	1.263 (0.241 – 6.616)	1.000	0.205 (0.035 – 1.191)	0.144
Perceived probability of vaccine related adverse effects (I^{VAC}) as rather high to very high	0.613 (0.054 – 7.003)	1.000	0.218 (0.019 – 2.505)	0.484
Perceived severity of seasonal influenza natural infection in HCWs (C^{INF}) as rather high to very high	1.730 (0.365 – 8.208)	0.763	0.588 (0.123 – 2.814)	0.804
Perceived probability of seasonal influenza natural infection in HCWs (I^{INF}) as rather high to very high	2.123 (0.916 – 4.920)	0.120	1.396 (0.568 – 3.429)	0.616

Tab. V. Univariate comparison of General Knowledge Score (GKS), Risk Perception Score (RPS), Propensity Score towards vaccines in general (G-PS) and influenza vaccination (IV-PS) by recalled demographic factors in 92 OPh participating to the study.

	GKS		RPS		G-PS		IV-PS	
	Mean ± S.D.	P value	Mean ± S.D.	P value	Mean ± S.D.	P value	Mean ± S.D.	P value
Gender								
Males	6.3 ± 3.4	0.397	9.4 ± 8.3	0.079	5.8 ± 1.1	0.639	4.1 ± 1.1	0.055
Females	5.6 ± 4.9		6.2 ± 8.6		5.7 ± 1.1		3.6 ± 1.4	
Age group								
< 50 y.o.	7.1 ± 3.1	0.009	9.6 ± 7.7	0.033	5.9 ± 1.1	0.256	4.1 ± 1.0	0.020
≥ 50 y.o.	4.9 ± 4.8		5.9 ± 8.9		5.7 ± 1.1		3.5 ± 1.4	
Country of origin								
Italian-born people	5.7 ± 4.3	0.013	7.2 ± 8.4	0.023	5.8 ± 1.1	0.365	3.8 ± 1.3	0.672
Foreign-born people	8.7 ± 2.7		11.7 ± 10.4		6.2 ± 1.0		4.0 ± 1.1	
Specialization								
Occupational medicine	7.0 ± 3.0	0.008	9.6 ± 7.4	0.011	5.9 ± 0.9	0.377	4.4 ± 0.8	< 0.001
Hygiene and Public Health	4.6 ± 5.1		5.1 ± 9.3		5.7 ± 1.3		3.1 ± 1.4	

Tab. VI. Bivariate Pearson's correlation among General Knowledge Score (GKS), Risk Perception Score (RPS), Propensity Score towards vaccines in general (G-PS) and influenza vaccination (IV-PS).

	GKS	RPS	G-PS	IV-PS
GKS	-	r = 0.317 p = 0.002	r = 0.315 p = 0.022	r = 0.492 p < 0.001
RPS	r = 0.317 p = 0.002	-	r = 0.203 p = 0.053	r = 0.280 p = 0.007
G-PS	r = 0.315 p = 0.022	r = 0.203 p = 0.053	-	r = 0.451 p < 0.001
IV-PS	r = 0.492 p < 0.001	r = 0.280 p = 0.007	r = 0.451 p < 0.001	-

(adjOR 15.77 95% CI 2.364-105.2). Similarly, participants acknowledging that the efficacy of vaccines has been extensively proven had significant positive association with positive immunization status towards seasonal influenza (adjOR 3.999 95% CI 1.245-12.84).

Linear regression model included IV-PS dependent variable, GKS, RPS and G-PS as independent variables, and age, sex, medical specialization and immunization status as covariates. Eventually, GKS (beta coefficient 0.213, p value = 0.043), RPS (beta coefficient 0.252, p value = 0.018) and G-PS (beta coefficient 0.384, p value = 0.002) were identified as significant predictors of IV-PS.

Discussion

While in European Countries vaccination rates of HCWs against SI remain far below the target objective of 75%, still ranging between unsatisfactory rates of 14% and 50% [1, 42, 43], a growing number of authorities have developed initiatives aimed to increase SIV uptake among HCWs [2]. Addressing the factors that explain

Tab. VII. Multivariate analysis. The binary logistic regression analysis model evaluated variables that in univariate analysis were associated with vaccination status and favorable attitude towards influenza vaccine having a p value < 0.150, and included age, sex, medical specialization as covariates. Moreover, positive vaccination status was included as a covariate in the multivariate analysis about attitude towards influenza vaccine.

Statement	Previously vaccinated against influenza vaccine (n = 41)		Somehow favorable attitude towards influenza vaccine (n = 63)	
	Adjusted OR (95% CI)	P value	adjusted OR (95% CI)	P value
Previous vaccination against seasonal influenza vaccine	-	-	5.806 (1.242 – 27.15)	0.025
4. Influenza vaccine has been identified as causative agent of lethargic encephalitis (FALSE)	-	-	3.578 (0.760 – 16.86)	0.107
5. Autism is more frequent in subjects vaccinated against measles (FALSE)	-	-	25.05 (2.538 –247.3)	0.006
7. Vaccinations increase the occurrence of auto-immune diseases (FALSE)	-	-	3.810 (0.641 – 22.64)	0.141
8. Vaccinations increase the risk for allergic disorders (FALSE)	-	-	1.363 (0.370 – 6.378)	0.222
11. The efficacy of vaccines has been extensively proven (TRUE)	3.999 (1.245 – 12.84)	0.034	0.433 (0.078 – 2.412)	0.339
12. Children would be more resistant to infections if they were not always treated against all diseases (FALSE)	-	-	15.77 (2.364 – 105.2)	0.004
Perceived severity of vaccine related adverse effects (C^{VAC}) as rather high to very high	-	-	0.117 (0.008 – 1.681)	0.115
Perceived probability of seasonal influenza natural infection in HCWs (I^{INF}) as rather high to very high	2.380 (0.439 – 12.91)	0.120	-	-

insufficient adherence of HCWs to official recommendation about SIV has consequently become a growing focus of attention [15, 18, 25, 44-46]. Sound evidences do suggest that HCWs may share with the general population significant fears of side effects, misconceptions about vaccine safety, and even poor knowledge of vaccine's benefits, ultimately leading them to lower vaccination rates [16, 18, 47]. Moreover, a significant share of HCWs would underestimate not only the actual severity of seasonal influenza natural infection, but also their potential role in transmitting VPDs to the patients [3, 4, 10, 18, 23, 26, 48-51].

Despite the growing number of studies performed in recent years, at our knowledge few researches specifically evaluated KAP of OPh: overall, their knowledge of vaccines and vaccine recommendations were not consistently satisfactory [32,36, 37]. Also in our study, OPh were affected by a relatively high prevalence of misconceptions about vaccines [9]: interestingly enough, participants OPh shared false beliefs and misunderstandings about presumptive association between vaccines and autoimmune diseases (*i.e.* multiple sclerosis, diabetes), and also between certain immunizations and disorders such as autism, subacute sclerosing panencephalitis, and lethargic encephalitis. Worries about such associations were actually raised in the previous decades being then criticized or even largely disproved in the following years [52-54]. Although a significant base of evidence ultimately denies a causality between vaccinations, autoimmune and neuropsychiatric disorders, aforementioned warnings still receive diffuse emphasis on conventional media, remaining very influential on the "new media" (*i.e.* social media, personal blogs, *etc*.) [14, 15, 30, 31, 55, 56]. Interestingly enough, a greater share of false beliefs and misconceptions was identified in older subjects: we could tantalizingly suppose that such information gaps may be understood as a consequence of an insufficient continuous medical education and, as risk perception follows the acquisition of the knowledge [32, 60-62]. Such information gaps may in turn explain why the majority of sampled OPh identified influenza as a substantially indolent disease, and similarly around a quarter of the sample underestimated the probability for HCWs to develop seasonal influenza natural infection (26.1%). Actually, some international reports suggest that HCWs may avoid SIV as they understood its potential adverse effects as more severe and frequent than the avoided consequences of the natural infection [3, 18,20, 43].

Although in our sample doubts inherent vaccine safety were somehow reduced, as 90.2% of participants perceived probability of adverse effects as "*almost zero*" to "*rather low*", and the main reason referred by the participants to have not been vaccinated against SI was the lack of time, OPh with a better trust on the proven efficacy of vaccines, as defined by general knowledge test, more frequently reported vaccination against seasonal influenza (adjOR 3.999 95% CI 1.245-12.84), whereas no significant effect was found on individual vaccine propensity. Regarding the organization issues referred by participants, it should be recalled that Italian OPh are HCWs that usually work as private practitioner: in other words, their adherence to official recommendation towards SIV could have been significantly impaired by factors other than personal beliefs and misconceptions, as the limited availability of vaccination services [4, 11, 14-19].

Similarly, we found a significant correlation between GKS and RPS (r = 0.317, p = 0.002), and such correlation was not unexpected [32, 36], as well as that of both cumulative score with propensity towards SIV (r = 0.492, p < 0.001 and r = 0.280, p = 0.007, respectively). Consistently with previous researches in KAP in HCWs towards vaccinations [4, 11, 14-18, 32, 36], all factors presumptively involved in the building up of personal attitudes (*i.e.* GKS, G-PS, RPS) were then identified as significant predictors of the propensity towards SIV. In other words, a greater knowledge (*i.e.* less misconceptions and/or less personal attitudes guiding the vaccine decisions) of vaccine and vaccine-related disorders on the one hand, and a more accurate risk perception of SI on the other hand were associated with a better attitude towards SIV. In effect, there is a considerable evidence that a better awareness and a greater trust in vaccines increase the individuals' propensity to be vaccinated, and in OPh the latter would be in turn associated with a greater propensity to perform and promote vaccinations on the workplaces [14-16, 32, 67, 68]: in other words, any information gap in OPh would ultimately lead to diffusely hold and diffuse doubts or false beliefs about vaccines rejection of some vaccines [26, 56-59], being significant drivers of a more extended VE in HCWs and in turn in the general population with devastating consequences [32, 36].

However, it should be stressed that several factors not necessarily included in the knowledge and risk perception assessment contribute to building up vaccine confidence (and conversely VE) [63]: although adhesion to the official recommendations is usually characterized as weak driving factors [18, 25, 28, 29], attitudes of OPh may be significantly influenced by concerns about potential legal consequences of their actual implementation. In other words, participants may have reported behaviors unrelated with actual knowledge and risk perception, exhibiting a sort of "*social desirability bias*", *i.e.* the tendency of research subjects to give socially desirable responses instead of choosing responses that are reflective of their true feelings [64, 65]. Also the higher propensity towards SIV in specialists in Occupational Medicine than in specialists in Hygiene and Public Health, the latter assed as a dichotomous attitude (OR 12.000 95% CI, 3.964-36.33) and as a cumulative score as well (IV-PS, 4.4 ± 0.8 *vs*. 3.1 ± 1.4, p < 0.001), and better performances in both the general knowledge tests (7.0 ± 3.0 *vs*. 4.6 ± 5.1, p = 0.008) and in the assessment of the risk perception (9.6 ± 7.4 *vs*. 5.1 ± 9.3, p = 0.011) may be similarly explained. These results were otherwise unexpected, as vaccinology represents a cornerstone of the core curriculum of specialization courses in Hygiene and Public Health, and a significantly higher share of positive attitudes and appropriate knowledge was previously reported in residents in Hygiene and Public Health [66].

As risk perception may be understood as an intermediate step between knowledge and the developing of an attitude [32, 60-62], a self-reported positive vaccination status was unsurprisingly associated with a positive attitude towards vaccination (adjOR 5.806 95% CI 1.242-27.15). In this regard, although vaccination rate was well below 50% (43/92, 46.7%), our survey is consistent with previous studies on HCWs: despite data on European HCWs clearly show a very low compliance towards SIV, physicians have been usually described as more receptive to influenza vaccination than other HCWs, ultimately exhibiting similar vaccination rates [9, 18].

Limits of the study

Several major limitations of the study have to be addressed. For instance, we assessed a sample of relatively small size, gathered through convenience sampling and a regional basis. As Italy is highly heterogeneous in term of vaccination rates and vaccine acceptance, our sample may therefore not represent the whole Italian OPh populations [12]. Second, our sample was drawn from a very selected population that presumptively included OPh more sensitive to medical education themes (*i.e.* subjects participating to a Continuous Medical Education course): a significant selection bias cannot therefore be ruled out, ultimately suggesting that our sample overestimated actual vaccine acceptance of the parent occupational group. Moreover, as our questionnaire did not investigated the information sources from which assessed knowledge and elements of risk perceptions were drawn, we are unable to evaluate whether these results are a serendipitous association in the context of a small sample, or rather the actual consequence of a different post-graduate formation, and this may be acknowledged as another weakness of this study. Generalization of our results may be furtherly compromised by the very same design of the survey. In other words, not only participants may have overrated their actual vaccine propensity and similarly assessed the items of the general knowledge test in terms of "social desirability", but we cannot rule out a substantial lack of specificity in the recalling of vaccination status [9].

Conclusions

In conclusion, our results are consistent with previous reports on HCWs and with the limited available evidence on OPh. More specifically, the majority of OPh were somehow favorable to SIV, but a significant share of misbeliefs and false knowledge were also identified. As knowledge and risk perception were identified as significant predictors of vaccine propensity, our results suggest that that filling information gaps may significantly improve vaccine propensity of OPh, and possibly increase the vaccination rates in HCWs and, in turn, in the general population. Moreover, our results suggest that a significant share of HCWs may benefit from more flexibility, in term of time and accessibility, by healthcare providers performing SIV, and in particular with vaccination services. Their better interaction with OPh would be also useful in order to address personal misconceptions and target false beliefs, ultimately increas-

ing the awareness of the potential of SIV, in the HCWs and, subsequently, in the general population.

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Authors' contributions

MR was responsible of study design and data analysis, with the contribution of CS. FC was the main responsible of data collection, with the contribution of GG. Both GG and CS contributed to data analysis and manuscript preparation.

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ANNEX 1. Author's translation of the Questionnaire.

V.01	**Knowledge, attitudes and practices of Healthcare Workers towards vaccines – Section 1: seasonal influenza vaccine** References: Loulergue et al, Vaccine 2009;27:4240-4243 Little et al, Public Health 2015;129:755-762 Betsch e Wicker, Vaccine 2014;32:4478-4484	22.10.2015

Q01. Age: …… **Q02**. Sex: [M] [F] **Q03** Country of Origin: [ITA] [NO ITA]

Q04. Professional qualification:

[ ] Occupational Physician, with medical specialization in: [ ] Occupational Medicine
[ ] Hygiene and Public Health
[ ] Forensic medicine
[ ] other (D.Lgs 277/91)

[ ] General Practitioner [ ] other _____

Q05. Year of medical qualification: ……..

Q06. At you knowledge, seasonal vaccine influenza is a recommended one among healthcare workers?
[ ] yes [ ] no

Q07. Have you been vaccinated against influenza during last winter season (2014-2015)?
[ ] yes [ ] no

Q08. Whether during last winter season you have not received seasonal influenza vaccination, the reason was …

"I am already immunized by previous vaccination campaigns" [ ]
"I use alternative countermeasures" [ ]
"I am not convinced that influenza vaccine is useful" [ ]
"I have fear of injections" [ ]
"I have fear of side effects" [ ]
"The influenza vaccine is useless because influenza is a mild disease" [ ]
"The influenza vaccine is useless because lifestyles are more efficient measures" [ ]

Q09. Please rate from 1 (almost zero) a 7 (very high) how you perceive …

	Almost zero	**Low**	**Rather low**	**Moderate**	**Rather high**	**High**	**Very high**
… the probability of adverse effects after influenza vaccination	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
… the probability for influenza natural infection in HCWs	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
… the severity of adverse effects after influenza vaccination	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
… the severity of the influenza natural infection in HCWs	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Q10. Please self-rate your attitude towards vaccinations (in general):
[ ] absolutely against [ ] strongly against [ ] somewhat against [ ] neutral
[ ] somewhat favorable [ ] strongly favorable [ ] absolutely favorable

Q11. Please self-rate your attitude towards influenza vaccine
[ ] absolutely against [ ] strongly against [ ] somewhat against [ ] neutral
[ ] somewhat favorable [ ] strongly favorable [ ] absolutely favorable

Q12. At your knowledge …

	True	**False**	**Don't know**
The addictive used in the vaccines are not dangerous for humans	[ ]	[ ]	[ ]
Multiple Sclerosis may be induced by HBV vaccine	[ ]	[ ]	[ ]
Subacute sclerosing panencephalitis is a possible side effect of measles vaccine	[ ]	[ ]	[ ]
Influenza vaccine has been identified as causative agent of lethargic encephalitis	[ ]	[ ]	[ ]
Autism is more frequent in subjects vaccinated against measles	[ ]	[ ]	[ ]
Diabetes mellitus may be triggered by vaccination shoots	[ ]	[ ]	[ ]
Vaccinations increase the occurrence of auto-immune diseases	[ ]	[ ]	[ ]
Vaccinations increase the risk for allergic disorders	[ ]	[ ]	[ ]
Vaccine are superfluous, as infectious diseases can be always treated with antibiotics	[ ]	[ ]	[ ]
Without massive vaccination programs, smallpox would still exist	[ ]	[ ]	[ ]
The efficacy of vaccines has been extensively proven	[ ]	[ ]	[ ]
Children would be more resistant to infections if they were not always treated against all diseases	[ ]	[ ]	[ ]
Many vaccinations are administered too early. As results, the immune system has no possibility to fully develop by itself	[ ]	[ ]	[ ]
The immune system of children may be overwhelmed by a high number of vaccines	[ ]	[ ]	[ ]

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ORIGINAL ARTICLE

Distribution of *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes among ESBL-producing *P. aeruginosa* isolated from Qazvin and Tehran hospitals, Iran

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Keywords

Pseudomonas aeruginosa • ESBL • *bla*$_{TEM}$ • *bla*$_{SHV}$ • *bla*$_{CTX-M}$

Summary

Introduction. *Pseudomonas aeruginosa is as an important opportunistic human pathogen, which is associated with several clinical infections that are usually difficult to treat because of resistance to multiple antimicrobials. The production of extended-spectrum ß-lactamases (ESBLs) is an important mechanism of ß-lactam resistance. The aims of this study were to determine the prevalence of ESBLs, antimicrobial susceptibility, and to detect the bla$_{TEM}$, bla$_{SHV}$, and bla$_{CTX-M}$ genes.*

Methods. *In this study, carried out from March 2013 to December 2014, 266 P. aeruginosa isolates were collected from patients admitted to teaching hospitals of Qazvin and Tehran, Iran. All isolates were initially screened for ESBL production by disk diffusion method and were further confirmed using a combined disk method. Antimicrobial susceptibility of ESBL-producing isolates was determined by standard disk diffusion method. Polymerase Chain Reaction (PCR) and sequencing techniques were employed for detection of bla$_{TEM}$, bla$_{SHV}$, and bla$_{CTX-M}$ genes.*

Results. *In total, 262 (98.5%) P. aeruginosa isolates were non-susceptible to the used extended spectrum cephalosporins, and, among these, 75 (28.6%) isolates were ESBL producers. Fifty-nine (78.7%) of ESBL-producing isolates showed multidrug-resistance pattern. Of 75 ESBL-positive isolates, the bla$_{TEM-1}$ (26.7%) was the most common gene, followed by bla$_{CTX-M-15}$ (17.3%), bla$_{SHV-1}$ (6.7%), and bla$_{SHV-12}$ (4%), either alone or in combination.*

Conclusions. *The results of this study showed the notable prevalence of ESBLs among the clinical isolates of P. aeruginosa in Iran, indicating the urgency for the implementation of appropriate follow-up measures for infection control and proper administration of antimicrobial agents in our medical settings.*

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is an important opportunistic clinical pathogen, causing a variety of healthcare-associated infections, such as pneumonia, sepsis, wounds, and urinary tract infections [1, 2]. This organism is an important cause of septic mortality in burn patients [3]. *P. aeruginosa* is a major cause of chronic lung infections in children and young adults with cystic fibrosis and can be especially severe in neutropenic or cancer patients [4]. Infections caused by *P. aeruginosa* are often difficult to treat because of its intrinsic and acquired resistance to many commonly prescribed antimicrobial agents, eventually leading to the emergence of multidrug-resistant *P. aeruginosa* (MDRPA) strain [5]. *P. aeruginosa* is physiologically resistant to many antibacterial agents, especially to the extended-spectrum cephalosporins, due to the production of different classes of extended spectrum ß-lactamases (ESBLs), overproduction of chromosomal AmpC cephalosporinase, and non-enzymatic mechanisms, such as efflux pumps and outer membrane impermeability [6], among others. Nosocomial infections due to MDRPA strains are increasingly recognized throughout the world and are associated with increased morbidity, mortality, and cost of therapy [7, 8].

ESBLs are one of the main leading causes of resistance to ß-lactam antibiotics among Gram-negative bacteria [6]. These enzymes are plasmid-encoded β-lactamases that mediate resistance to penicillins, first-, second- and third- generation cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime [6, 9]. TEM, SHV, and CTX-M are the major genetic groups of ESBLs amongst clinically important Gram-negative bacteria [9, 10].

These enzymes are most commonly found in *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) and are also observed in other clinical isolates of Enterobacteriaceae and *Pseudomonas* [11, 12]. The first TEM-type β-lactamase, produced by a clinical *E. coli* strain, was reported in 1965. The TEM-type ESBLs are derivatives of TEM-1 and TEM-2 [9]. The SHV-type ESBLs may be found in clinical isolates more frequently than any other types of ESBLs and have been reported from several countries in Europe, such as Austria, France, Italy, and Greece, as well as in the United States and Australia [6, 9, 10]. The CTX-M-type ESBLs developed from TEM and SHV and can be divided into five subgroups according to their amino acid sequence simi-

larities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V [13, 14]. Detection of ESBLs is important for the surveillance and epidemiological studies of their transmission in medical settings [15]. One major concern regarding the spread of ESBL-producing *P. aeruginosa* within hospital settings is the treatment failure to infections caused by this organism due to the limitations in therapeutic choices [9, 16]. There are few reports describing the prevalence of TEM- or SHV-type ESBLs in *P. aeruginosa* in Iran. The aims of this study were to determine the prevalence of ESBLs and to detect the bla_{TEM}, bla_{SHV} and bla_{CTX-M}-types ESBL genes among clinical isolates of *P. aeruginosa* collected from hospitals of Tehran and Qazvin, two central provinces of Iran.

Methods

Bacterial isolates

The clinical isolates of *P. aeruginosa* (one isolate per patient) were collected from hospitalized patients of Tehran and Qazvin provinces from March 2013 to December 2014. The bacterial isolates were collected from different clinical specimens, including urine, wounds, sputum, broncoalveolar lavage (BAL), trachea, and blood. These isolates were obtained from patients admitted to intensive care units (ICUs), internal medicine, general surgery, neurology, neurosurgery, and infectious disease wards. Specimens of these patients were sent to the microbiology laboratory of the hospitals under study. The study was approved by the ethics committee of Qazvin University of Medical Sciences (code IR.QUMS.REC.1394.147). Written informed consent was obtained from all subjects enrolled in this study. All isolates were identified as *P. aeruginosa* using standard microbiological and biochemical tests [17]. The isolates were stored at -70°C in trypticase soy broth (TSB) containing 20% glycerol and subcultured twice prior to testing.

ESBL screening

All isolates were initially screened for ESBLs production using the standard disc diffusion method, using ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefpodoxim (30µg), and aztreonam (30µg). Isolates which were non-susceptible to any of third generation cephalosporins were selected for ESBLs detection phenotypically. The antibiotic disks were purchased from Mast (Mast Diagnostics Group Ltd). *P. aeruginosa* ATCC 27853 was used as a control strain in antimicrobial suscpetibility testings.

Confirmation of ESBL production

Phenotypic confirmatory tests [18], which were designed for detecting ESBLs in *K. pneumoniae* and *E. coli*, were also performed by comparing the inhibition zone of disks containing cefotaxime or ceftazidime with and without clavulanic acid. *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used as the quality control strains in antimicrobial susceptibility testing.

Molecular detection of ESBL-encoding genes

ESBL-producing isolates were subjected to Polymerase Chain Reaction (PCR) targeting bla_{TEM}, bla_{SHV}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-8,}$ $bla_{CTX-M-9}$, and $bla_{CTX-M-25}$ genes using the specific primers listed in Table I. Plasmid DNA was extracted using plasmid mini extraction kit (Bioneer Company, Korea). The PCR amplifications were performed in a thermocycler (Applied Biosystems, USA) as follows: 96º C for 5 min and 35 cycles of 1 min at 96º C, 1 min at a specific temperature for each primer and 1 min at 72º C and a final extension step of 10 min at 72º C. Amplification reactions were prepared in a total volume of 25 µl (24 µl of PCR master mix plus 1 µl of

Tab. I. Primers used in this study for detecting ESBL-encoding genes.

Targets	Primer Sequences (5'-3')	Annealing Temperature (°C)	References
TEM	ATGAGTATTCAACATTTCCG GACAGTTACCAATGCTTAATCA	50	19
TEM (Sequencing)	TAACCATGAGTGATAACACT CCGATCGTTGTCAGAAGTAA	50	19
SHV	CTTTACTCGCCTTTATCG TCCCGCAGATAAATCACCA	50	19
SHV (Sequencing)	ACTGCCTTTTTGCGCCAGAT CAGTTCCGTTTCCCAGCGGT	56	19
CTX-M-1 group	ATGGTTAAAAAATCACTGCGTC TTGGTGACGATTTTAGCCGC	55	20
CTX-M-2 group	ATGATGACTCAGAGCATTCG TGGGTTACGATTTTCGCCGC	55	20
CTX-M-8 group	ACTTCAGCCACACGGATTCA CGAGTACGTCACGACGACTT	55	20
CTX-M-9 group	ATGGTGACAAAGAGAGTGCA CCCTTCGGCGATGATTCTC	55	20
CTX-M-25	CACACGAATTGAATGTTCAG TCACTCCACATGGTGAGT	50	21

Tab. II. Antimicrobial susceptibility of ESBL-producing *P. aeruginosa* isolated from hospitals of Qazvin and Tehran (n = 75).

Antibiotics	S (%)	I (%)	R (%)
Amikacin	44 (58.7)	10 (13.3)	21 (28)
Piperacillin-tazobactam	40 (53.3)	9 (12)	26 (34.7)
Imipenem	35 (46.7)	9 (12)	31 (41.3)
Meropenem	34 (45.3)	7 (9.3)	34 (45.3)
Cefepime	30 (40)	1 (1.3)	44 (58.7)
Piperacillin	26 (34.7)	8 (10.7)	41 (54.7)
Ciprofloxacin	24 (32)	1 (1.3)	50 (66.7)
Gentamicin	22 (29.3)	-	53 (70.7)
Ceftazidime	22 (29.3)	4 (5.3)	49 (65.3)
Tobramycin	22 (29.3)	3 (4)	50 (66.7)
Ofloxacine	21 (28)	2 (2.7)	52 (69.3)
Levofloxacin	18 (24)	2 (2.7)	55 (73.3)
Ticarcillin	17 (22.7)	1 (1.3)	57 (76)
Aztreonam	14 (18.7)	10 (13.3)	51 (68)
Carbenicilin	14 (18.7)	3 (4)	58 (77.3)
Ceftriaxone	10 (13.3)	7 (9.3)	58 (77.3)
Cefotaxime	5 (6.7)	8 (10.7)	62 (82.7)

S: Sensitive, I: Intermediate, R: Resistant.

template DNA) including 5 ng of genomic DNA, 2.0 U of Taq DNA polymerase, 10 mM dNTP mix at a final concentration of 0.2 mM, 50 mM $MgCl_2$ at a final concentration of 1.5 mM, 1 µM of each primer, and 1X PCR buffer (final concentration). PCR products were electrophoresed on a 1% agarose gel at 100 volts and then were stained with ethidium bromide solution and finally visualized in gel documentation system (UVtec, UK). The purified PCR products were sequenced by the Macrogen Company (Seoul, Korea). The sequence alignment and bioinformatics analyses were performed using the Basic Local Alignment Search Tool (BLAST) online program of the National Center for Biotechnology Information (freely available at http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis was performed for descriptive statistics, including frequencies, cross-tabulation of microbiological, clinical, and demographic characteristics, using the commercial software Statistical analysis software package (version 16, IBM SPSS Corporation, Armonk, NY, USA).

Results

During the study period from March 2013 to December 2014, a total of 266 isolates of *P. aeruginosa* were collected from different clinical specimens including blood (94 isolates; 35.3%), urine (80 isolates; 30.1%), wounds (29 isolates; 10.9%), trachea (26 isolates; 9.8%), sputum (19 isolates; 7.1%), and BAL (18 isolates; 6.8%). Isolates were obtained from patients admitted to intensive care units (99-37.2%), internal medicine (64-24.1%), infectious diseases (61-22.9%), general surgery (26-9.8%), neurosurgery (12-4.5%), and neurology (4-1.5%) wards. One hundred and twenty-five (47%) were male and 141 (53%) were female. Out of the 266 *P. aeroginosa* isolates, 262 (98.5%) isolates were non-susceptible to at least one of the antibiotics used in the screening test and, among these, 75 (28.6%) isolates were identified as potential ESBL producers using combined disk method. Fifty-nine (78.7%) of ESBL-producing isolates were found to be multidrug-resistant (MDR), i.e. showed intermediate or fully resistance to at least three different classes of antimicrobial agents including β-lactams, aminoglycosides, and fluoroquinolones. Amikacin (58.7%) and piperacillin-tazobactam (53.3%) showed the highest rates of susceptibility among antimicrobials tested in this study, respectively (Table II). Further, 41 (54.7%) and 40 (53.5%) ESBL-producing isolates were fully or intermediate resistant to meropenem and imipenem, respectively. ESBL-producing isolates were mainly recovered from urine (30.2%), followed by wound (28.3%) samples. The patients affected were mainly those admitted in ICU (54.7%) and the internal wards (45.1%), respectively (Table III). Of the 75 *P. aeruginosa* isolates with ESBL phenotype, $bla_{\text{TEM-1}}$ (20-26.7%) was the most common gene, followed by $bla_{\text{CTX-M-15}}$ (13- 17.3%), $bla_{\text{SHV-1}}$ (5-6.7%), and $bla_{\text{SHV-12}}$ (3-4%), either alone or in combination (Table IV). In this study, isolates were, instead, negative for $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, and $bla_{\text{CTX-M-9}}$-group genes, as well as for $bla_{\text{CTX-M-25}}$.

Discussion

P. aeruginosa has recently emerged as a major cause of healthcare-associated infections, especially in immunocompromised people and burn patients [22, 23]. The treatment of *P. aeruginosa* infections is increasingly

Tab. III. Frequency of ESBL-producing isolates broken down by hospitals ward and source of clinical specimen.

Hospital wards	n (%)
ICU	33 (44)
Internal medicine	19 (25.3)
Infectious disease	12 (16)
Surgery	8 (10.7)
Neurosurgery	2 (2.7)
Neurology	1 (1.3)
Clinical specimens	(n/%)
Blood	24 (32)
Urine	17 (22.7)
Trachea	13 (17.3)
Wound	9 (12)
BAL	6 (8)
Sputum	6 (8)

ICU: intensive care unit, BAL: bronchoalveolar lavage

Tab. IV. Distribution of bla_{SHV}, bla_{TEM}, and bla_{CTX-M} genes in ESBL-producing *P. aeruginosa* isolates.

Resistance genes	n (%)
blaSHV-1	4 (5.3)
blaSHV-12	3 (4)
blaTEM-1	12 (16)
blaCTX-M-15	4 (5.3)
blaSHV-1 and blaCTX-M-15	1 (1.3)
blaTEM-1 and blaCTX-M15	8 (10.7)
No blaTEM-1, blaSHV-1 and blaCTX-M-15	43 (57.3)

complicated due to both intrinsic and acquired resistance to the most commonly prescribed antibiotics in hospital settings [2, 24]. The emergence of ESBL has become a matter of serious concern for the treatment of patients in Iran. ESBL detection is not routinely tested in most laboratories in Iran. There are only few reports on prevalence of ESBL among *P. aeruginosa* isolates in our country.

In the present study, 262 (98.5%) *P. aeruginosa* isolates were non-susceptible to at least one of the antibiotics used in the ESBL screening test and, among these, 75 (28.6%) isolates were ESBL positive. The prevalence rate of ESBL in our study is lower than the rate reported by Mirsalehian et al. (39.4%) [25] and Shakibaie et al. (34%) [26] from two burn centers in Iran, Begum et al. from Bangladesh (37.8%) [27], and Senthamaria et al. from India (42.3%) [28], but higher than the rate found by Zafar et al. from Egypt (7.4%) [29], Umadevi et al. from India (19.4%) [30] and Woodford et al. from United Kingdom (3.7%) [31]. In our study, 78.7% of ESBL-producing isolates were found to be MDR and showed relatively higher resistance rates to most antibiotics tested. These results are in accordance with those of other studies, such as those conducted by Fallah et al. and Hakemi Vala et al. in Iran [32, 33]. Our findings demonstrated that amikacin (58.7%) and piperacillin-tazobactam (53.3%) showed the highest rates of susceptibility among the antimicrobials tested, whereas cefotaxime and ceftriaxone revealed low suscepibility rates of 6.7% and 13.3%, respectively. The inappropriate management of infections through unnecessary and widespread administration of antibacterial agents is likely to be the main predisposing factor leading to the emergence of resistant bacteria in our medical centers. Moreover, the high resistance rate of ESBLs found among the isolates in this study emphasizes the need for a local and national antimicrobial resistance surveillance system for monitoring the administration of antimicrobials in our hospital settings.

It should be noted that, in this study, 71.5% of extended spectrum cephalosporin non-susceptible isolates were ESBL negative, which can be associated with other resistance mechanisms such as overproduction of chromosomal cephalosporinase (AmpC), up-regulation of efflux systems or decreased outer-membrane permeability. Additionally, our data showed that 54.7% and 53.5% of ESBL-producing isolates were non-susceptible to meropenem and imipenem, respectively. Since in hospital setting the appropriate treatment of infections caused by MDR Gram-negative bacteria is generally achieved by the administration of carbapenems, this finding indicated that the available therapeutic choices are currently limited. This has a relevant clinical impact, especially in the future in case these strains should become more prevalent. This study, in line with previous studies [34, 35], showed that ESBL-producing isolates were mostly collected from the patients admitted to ICUs. It seems that prolonged period of ICU stay, exposure to broad spectrum antibiotics, chronic underlying conditions, and the use of invasive techniques and devices predispose patients to infection caused by these resistant isolates.

In the present study, 26.7%, 17.3%, 6.7%, and 4% of ESBL-producing *P. aeruginosa* isolates carried bla_{TEM-1}, $bla_{CTX-M-15}$, bla_{SHV-1}, and bla_{SHV-12} genes alone or in combination, respectively. In the literature, there have been only rare reports of bla_{SHV-12} and $bla_{CTX-M-15}$-types ESBLs among *P. aeruginosa* worldwide. We believe that this is the first report of $bla_{CTX-M-15}$ and bla_{SHV-12}-related ESBL genes among *P. aeruginosa* isolates collected from Qazvin and Tehran hospitals. Shakibaie et al. reported that 6.6%, 4.1%, and 2.5% of ESBL-producing *P. aeruginosa* isolates carried bla_{SHV}, bla_{PER}, and bla_{TEM} family genes, respectively [26]. In another study from Iran, Shahcheraghi et al. reported that 24%, 22%, 17%, and 9% of MDR isolates of *P. aeruginosa* harbored bla_{VEB}, bla_{SHV}, bla_{PER}, and bla_{TEM} genes, respectively [36]. In Japan, Uemura et al. showed the presence of bla_{SHV-12} among *P. aeruginosa* isolates collected from burn patients [37]. Polotto et al. reported that the $bla_{CTX-M-2}$ (19.6%) gene was the most prevalent ESBL gene in Brazil [38]. Together, these data indicate successful spread of the ESBL-encoding genes around the world.

Conclusions

Findings of the our study show a high prevalence of ESBL-producing *P. aeruginosa* isolates carrying $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$ genes in Iran. The presence of ESBL-producing bacteria within the healthcare setting in Iran should be considered a public health concern both therapeutically and epidemiologically. As such, the identification, treatment, and infection control and management of patients infected with these organisms is of prime necessity.

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The authors declare no conflict of interest

Authors' contributions

AP initiated the study design, project and protocol development, and data analysis; TNF performed the data quality control and manuscript writing; EZ collected data and performed microbiological and molecular experiments; KHA was involved in the editing of the manuscript. All authors read and approved the final draft of the manuscript.

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ORIGINAL ARTICLE

Humidifiers for oxygen therapy: what risk for reusable and disposable devices?

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Keywords

Oxygen humidifiers • Microbiological contamination • Nosocomial pneumonia

Summary

Introduction. *Nosocomial pneumonia accounts for the vast majority of healthcare-associated infections (HAI). Although numerous medical devices have been discussed as potential vehicles for microorganisms, very little is known about the role played by oxygen humidifiers as potential sources of nosocomial pathogens. The purpose of this research was to evaluate the safety of the reuse of humidifiers by analysing the rate of microbial contamination in reusable and disposable oxygen humidifiers used during therapy, and then discuss their potential role in the transmission of respiratory pathogens.*

Methods. *Water samples from reusable and disposable oxygen humidifiers were collected from different wards of the University Hospital of Messina, Italy, where nosocomial pneumonia has a higher incidence rate due to the "critical" clinical conditions of inpatients. In particular, we monitored the Internal Medicine and Pulmonology wards for the medical area; the General Surgery and Thoracic and Cardiovascular Surgery wards for the surgical area and the Intensive Care Unit and Neonatal Intensive Care Unit for the emergency area. The samples were always collected after a period of 5 days from initial use for both types of humidifiers. Samples were processed using standard bacteriological techniques and microbial colonies were identified using manual and automated methods.*

Results. *High rates of microbial contamination were observed in samples from reusable oxygen humidifiers employed in medical (83%), surgical (77%) and emergency (50%) areas. The most relevant pathogens were Pseudomonas aeruginosa, amongst the Gram-negative bacteria, and Staphylococcus aureus, amongst the Gram-positive bacteria. Other pathogens were detected in lower percentage. The disposable oxygen humidifier samples showed no contamination.*

Conclusions. *This research presents evidence of the high rate and type of microbial contamination of reusable humidifiers employed for oxygen therapy. These devices may thus be involved in the transmission of potential pathogens. It could be important, for the prevention of nosocomial pneumonia, to replace them with single-use humidifiers for which the absence of microbial contamination has been confirmed.*

Introduction

Healthcare-associated infections (HAI) are some of the most frequent medical complications during hospitalization. A recent study conducted in 183 U.S. hospitals on a sample of 11,282 inpatients showed that 452 (4%) inpatients contracted one of more hospital-acquired infections. Of these, pneumonia and surgical wound infections accounted for 21.8% of cases, gastrointestinal infections accounted for 17.1%, and urinary tract infections for 12.9% [1]. Regarding the transmission of infections in hospitals, there are many studies about the role of inanimate surfaces, considered a probable source of nosocomial pathogens [2-6]. Respiratory care equipment that includes ventilators, oxygen humidifiers, and nebulisers has been identified as a potential vehicle causing major nosocomial infections if colonised by fungi or bacteria [7]. For this reason, because very little is known about the role played by oxygen humidifiers as potential sources of nosocomial pathogens, we focused our attention on these devices.

Respiratory infections are one of the major nosocomial infections. The etiology of nosocomial pneumonia is determined by the length of hospitalization [8]. Early-onset nosocomial pneumonia, which usually occurs within the first 4-5 days of hospitalization, is generally caused by community-acquired pathogens, such as *Streptococcus pneumoniae*, methicillin-sensitive *S. aureus*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Conversely, late-onset nosocomial pneumonia (usually after 5-6 days of hospitalization) is generally caused by *P. aeruginosa*, *Acinetobacter spp*., methicillin-resistant *S. aureus*, etc. *P. aeruginosa* and the Enterobacteriaceae are the most common pathogens that cause nosocomial pneumonia after 10 or more days of hospitalization [7]. In order to reduce the risk of infection for patients, it is necessary to eliminate all potential sources of respiratory pathogens.
Oxygen humidifiers are commonly utilized in hospitals, because the oxygen used is a dry and irritating gas that, if poorly humidified, causes lesions of the respiratory mucosa [9]. In past years, some studies have been concerned with the role of humidifiers used for oxygen therapy as sources of nosocomial pathogens causing respira-

tory infections [10-12]. Moiraghi et al. reported 5 cases of fatal pneumonia caused by *Legionella pneumophila*, as a result of nebuliser therapy that was administered using contaminated humidifiers [13]. Bacteria may derive from the oral cavity and distal airways of a patient using the device, or from the contamination of the water involved. On this last point, extensive evidence was given that some potentially pathogenic microorganisms, such as *P. aeruginosa*, are able to grow in distilled water [14]. Other potential sources of device contamination are associated with the inappropriate maintenance of medical devices by healthcare workers. In order to avoid this, the Centers for Disease Control and Prevention (CDC) published a set of guidelines for the prevention of nosocomial pneumonia. The CDC highly recommends the diligent use and careful maintenance of reusable wall-mounted oxygen humidifiers [15, 16]. However, meticulous daily maintenance of medical devices is a time-consuming and laborious task to fulfill in the clinical environment. A possible solution to this problem might be to use sterile disposable oxygen humidifiers. Some studies have demonstrated that disposable humidifiers are safer for patients than the commonly-used reusable ones [17, 18]. Indeed, evidence has been presented that disposable humidifiers can be safely used for 30 days [19, 20]. Another study conducted by Meehan et al. has shown that disposable humidifiers could remain sterile for up to 77 days [21]. The clinical and economic impact of nosocomial pneumonia is an especially important topic for Public Health policies and for the prevention of hospital-acquired respiratory infections.

The purpose of this research was to evaluate the safety of the reuse of humidifiers after their disinfection by determining the rate of microbiological contamination of reusable versus disposable oxygen humidifiers used in medical, surgical and emergency care units at 'G. Martino' University Hospital, Messina and to show their potential role in the transmission of important nosocomial pathogens that cause hospital-acquired pneumonia.

Materials and methods

The study was carried out over a six-month period (January-June 2015) in the most at-risk medical, surgical and emergency care units of the 'G. Martino' University Hospital, Messina. We focused our attention on wards where the incidence of HAI was more frequent than in others wards. Specifically, we monitored the Internal Medicine and Pulmonology wards for the medical area; General Surgery and Thoracic and Cardiovascular Surgery wards for the surgical area and Intensive Care Unit and Neonatal Intensive Care Unit for the emergency area. In the first step of the study, water samples from reusable wall-mounted oxygen humidifiers were randomly collected after 5 days of use: 100 samples were obtained from the medical area (50 from Internal Medicine and 50 from Pulmonology), 100 from the surgical area (50 from General Surgery and 50 from Thoracic and Cardiovascular Surgery), and 100 from the emergency area (50 from Intensive Care Unit and 50 from Neonatal Intensive Care Unit). In the second step, water samples from sterile disposable oxygen humidifiers were collected after the same amount of time used for reusable humidifiers. Water samples from 50 disposable oxygen humidifiers were gathered from each ward following the same method that was used in the first step of the research.

In order to obtain the samples, 3 ml of water were collected from the humidifiers using a sterile Pasteur pipette and transferring it to a sterile test tube containing nutrient broth (BBL Nutrient Broth, BD). Immediately after, water samples were transported to the laboratory and incubated at 37°C for 24-48 hours. We considered contaminated each sample that showed bacterial growth. From positive samples, we prepared further subcultures on different growth media: Blood Agar (bioMérieux) was used as a universal medium; Mannitol Salt Agar (Oxoid) was used for the isolation of *Staphylococcus spp*; MacConkey Agar (bioMérieux) was used for the isolation of Gram-negative bacteria; Enterococcosel Agar (bioMérieux) for *Enterococcus spp*; Sabouraud Agar (bioMérieux) for mycetes. Following that, the identification of microorganisms grown in subcultures was carried out using manual biochemical methods (API Identification System, bioMérieux: API STAPH for *Staphylococcus spp*; API 20 NE for non-Enterobacteriaceae Gram-negative bacteria; and API 20 E for Enterobacteriaceae Gram-negative bacteria) and automated biochemical ones (VITEK, bioMérieux).

Results

In the Internal Medicine ward, 41 out of 50 (82%) samples from reusable wall-mounted oxygen humidifiers showed evidence of microbiological contamination. Similarly, in the Pulmonology ward, 42 out of 50 (84%) samples were contaminated. The total positivity of the medical area was 83% (83 out of 100). In the General Surgery ward, 40 out of 50 (80%) samples provided evidence of microbial contamination and in the Thoracic and Cardiovascular Surgery ward, 37 out of 50 (74%) samples were contaminated. The total positivity of the surgical area was 77% (77 out of 100). Finally, in the Intensive Care Unit and Neonatal Intensive Care Unit, 20 out of 50 (40%) and 30 out of 50 (60%) samples showed evidence of microbiological contamination, respectively. The total positivity of the emergency area was 50% (50 out of 100). The microorganisms detected in the contaminated samples were: *P. aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Serratia marcescens*, *Serratia liquefaciens*, *Proteus mirabilis*, *Citrobacter freundii*, *Stenotrophomonas maltophilia*, *Chryseobacterium indologenes*, *Vibrio vulnificus* and *Ochrobactrum antropii* amongst the Gram-negative bacteria; *S. aureus*, coagulase-negative staphylococci, *Enterococcus spp*

Tab. I. Percentage and number of microorganisms detected in the various wards.

	MEDICAL AREA		SURGICAL AREA		EMERGENCY AREA	
	Internal Medicine	Pneumology	General Surgery	Thoracic-Vascular Surgery	Intensive Care Unit	Neonatal Intensive Care Unit
GRAM-negatives						
Pseudomonas aeruginosa	56% (28)	50% (25)	58% (29)	26% (13)	10% (5)	20% (10)
Acinetobacter baumannii	2% (1)	2% (1)	6% (3)	8% (4)	8% (4)	6% (3)
Klebsiella pneumoniae	4% (2)	14% (7)	0	0	4% (2)	0
Serratia marcescens	14% (7)	14% (7)	4% (2)	0	4% (2)	0
Serratia liquefaciens	2% (1)	12% (6)	0	0	0	0
Proteus mirabilis	12% (6)	6% (3)	26% (13)	0	0	0
Citrobacter freundii	6% (3)	2% (1)	10% (5)	4% (2)	0	0
Chryseobacterium indologenes	2% (1)	2% (1)	0	4% (2)	0	0
Vibrio vulnifucus	0	0	0	0	6% (3)	0
Ochrobactrum anthropii	0	0	0	0	0	6% (3)
Stenotrophomonas maltophylia	0	2% (1)	0	0	0	0
GRAM-positives						
Staphylococcus aureus	14% (7)	4% (2)	12% (6)	18% (9)	6% (3)	6% (3)
Coagulase-negative staphylococci	18% (9)	10% (5)	6% (3)	8% (4)	8% (4)	14% (7)
Enterococcus spp	18% (9)	10% (5)	10% (5)	28% (14)	6% (3)	6% (3)
Bacillus spp	6% (3)	6% (3)	0	0	4% (2)	0
Candida albicans	14% (7)	2% (1)	0	4% (2)	6% (3)	6% (3)
Negative samples	**16% (8)**	**18% (9)**	**20% (10)**	**26% (13)**	**60% (30)**	**40% (20)**

* The sum exceeds the actual total of samples because of the co-presence of multiple germs

and *Bacillus spp* amongst the Gram-positive bacteria. Tab. 1 shows the percentages of samples that resulted positive for each microorganism. Sometimes, various microorganisms have been found simultaneously on the same sample, so that positivity for a single microorganism is greater than the positivity of single samples. Conversely, samples from disposable oxygen humidifiers, which were collected in the second stage of the research, showed no evidence of any form of contamination.

Discussion and conclusions

Initially, the study was conducted using a large number of water samples from reusable wall-mounted oxygen humidifiers obtained from different wards. Special attention was given to those samples collected from wards where oxygen therapy is often delivered to patients with various pathologies (*e.g.* COPD, asthma, cardio-vascular diseases, *etc*.). The highest percentage of humidifiers that yielded positive results for the presence of one or more bacterial pathogens was recorded in medical areas with similar positivity percentage in the two wards assessed (Internal Medicine and Pulmonology). The surgical area showed a slightly lower rate of contamination, while the emergency area was the least contaminated. Looking at the individual departments, the highest percentage of positivity was found in the Pulmonology ward, while the lowest one was found to be in the Anaesthesia and Intensive Care ward. These results have been shown despite the existence of a hospital procedure that provides for proper maintenance of these devices including: their handling by healthcare workers with disposable gloves, disinfection with sodium hypochlorite 5% for 30 minutes, rinsing with sterile water and drying with a sterile cloth.

These results are probably due to several factors, particularly a larger number of patients are generally hospitalized in medical and surgical care units than are in emergency care ones. For this reason, the same reusable oxygen humidifiers were used more frequently. Secondly, high rates of microbiological contamination are probably associated with the inappropriate maintenance

of medical devices for an incorrect application of the sanification procedure.

In our research, Gram-negative bacteria account for the vast majority of detected microorganisms, with *P. aeruginosa* being the most commonly-isolated microorganism. This may be due to the ability of *P. aeruginosa* to grow and multiply in water. Other Gram-negative rods, such as *A. baumannii* and *K. pneumoniae*, which are known to cause nosocomial pneumonia [22], were isolated with lower frequency. Amongst Gram-positive bacteria, *Staphylococcus spp* were the most commonly-isolated species, followed by the others species.

These results confirm the idea that reusable oxygen humidifiers may be easily contaminated, and that they may play a role in the transmission of potential nosocomial pathogens, especially in those units that provide assistance and care to patients in critical clinical conditions (e.g. immunocompromised individuals, patients with chronic diseases, etc.). Aerosol particles that generate when small water particles mix with oxygen may serve as excellent vehicles in the transmission of microorganisms, for they reach the deep lung immediately after inhalation. Conversely, all sterile disposable oxygen humidifier samples, which were collected in the second stage of the research, confirm that these devices are safe for patients and that it is necessary to substitute reusable oxygen humidifiers with disposable ones. This could be a preventive way to reduce the risk of transmission of potentially pathogenic microorganisms and to minimize the possible development of nosocomial pneumonia.

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Ethical approval: not required (this study is not a clinical trial and did not involve human subjects).

Authors' contributions

VLF, GBC, RS conceived, designed and coordinated the research. AF, RR and AC, contributed to the acquisition, analysis and interpretation of data. VLF, AF, RR and GBC evaluated the results. VLF, AF and RR wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Five-year microbiological monitoring of wards and operating theatres in southern Italy

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Keywords

Microbiological surveillance • HCAI • Operating rooms • Wards

Summary

Introduction. *Nosocomial infections are one of the greatest problems in public health. Several studies have highlighted the role played by the hospital environment as a possible source of transmission of nosocomial pathogens.*
Methods. *A five-year monitoring of bacterial contamination on healthcare workers hands, surfaces most closely in contact with inpatient wards, operating theatres and "at rest" and "in use" operating theatre air samples. For the samples, we used sterile swabs, contact slides, manual API, and automated VITEK systems for identification.*
Results. *In the five-year period, a total of 9396 samples were collected and analysed. In ward patients, 4398 samplings were carried out with 4.7%, 9.4%, 7%, 10.8% and 7.9% positive results respectively from 2010 to 2014. For hands, 648 samplings were carried out, with a positivity of 40.74%. In operating theatres, 4188 samples were taken, with a positivity of 11.9%. Regarding air in empty and full theatres, 1962 samplings were carried out with a positivity rate equal to 31.9%. The monitoring showed a low rate of contamination with a progressive decrease in the five-year period on operating theatres surfaces and hands, while there was an increase in the surgical site wards and in the air of operating rooms.*
Conclusions. *Our investigation has revealed the presence of pathogens on the assessed surfaces and the need for environmental monitoring, which can be a valuable tool for reducing contamination.*

Introduction

Hospital infections are, even today, one of the main problems of public health [1]. Much importance was given, in recent years, to the contamination of the hospital environment in the onset of these infections. One of the most controversial and debated issues is the qualitative and quantitative role of the environment in the process of patient contamination, in particular the role of adjacent surfaces and furniture. It is known that these surfaces act as reservoirs for microorganisms, increasing the risk of cross-contamination through direct and/or indirect contact with the patient [2-5]. Recent studies have focused on the role of hospital environment sanitation processes, establishing a correlation between microbiological contamination of surfaces in direct contact with the patient and Healthcare Associated Infections (HCAI) [6]. The spread of microorganisms is undoubtedly related to the presence of the patients themselves, the latter being the first source of contamination of the environment and especially of all those sites that are closely associated with them, such as the bed, the bedside table, the power supply carriage etc., which are frequently touched ("high-touch surfaces") and easily contaminated [7]. For many infections of the surgical site, in addition to the patient's endogenous flora, the main source of infection is the contamination of the surgical site with desquamative cells [8]. Appropriate clothing and appropriate behaviour on the part of the health workers, along with a controlled ventilation system (CVS) are indispensable measures to reduce microbial air contamination. It was also demonstrated that many important nosocomial pathogens, such as the methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), are frequently found in the areas around the patients infected with these pathogens [9-12]. Several studies have also shown that microorganisms can pass directly from the contaminated surfaces to the hands of healthcare workers, in the absence of direct contact with patients [13, 14]. They also possess an ability to survive for a long time on dry surfaces. MRSA, VRE and *Acinetobacter spp*, under certain conditions, can survive for 4-5 months [15]. It is also important to underline the fact that, for many pathogens, the infective dose appears to be very low and therefore a slight contamination of the environment is sufficient to cause the onset of infection. For example, it has been shown that less than 15 cells of *S. aureus* are sufficient to cause infection in experimental lesions [16]. For many infections of the surgical site, in addition to the patient's endogenous flora, the main source of infection is the contamination of the surgical site's air by desquamative cells.
From all this we understand the importance of a continuous monitoring of hospital environments in order to minimize the contamination of the surfaces and, consequently, the possibility of the occurrence of infections.

Dancer has proposed the introduction of routine microbiological checks of surfaces in hospitals although this practice is not recommended by the CDC [17, 18].

Materials and methods

The purpose of our research was to detect, through five-year monitoring (January 2010-December 2014) in Messina's University Hospital, the presence of bacterial contamination of the surfaces of operating theatres and hospital wards. The presence of microorganisms on the hands of healthcare workers, which today represent one of the main vehicles of transmission of pathogens, particularly in the hospital setting, was also evaluated [19].
The wards and operating theatres included in the study were part of Medical (Internal Medicine and Paediatrics Operating Unit), Surgical (General Surgery, Orthopaedics, Neurosurgery, Maxillofacial Surgery, Thoracic-Vascular Surgery, Gynaecology and Obstetrics, Paediatric Surgery, Plastic Surgery Operating Units) and Emergency (Intensive Care, Neonatal Intensive Care Unit) areas.
All operating theatres (General Surgery, Otolaryngology, Ophthalmology, Plastic Surgery, Orthopaedics, Neurosurgery, Maxillofacial Surgery, Thoracic-Vascular Surgery, Gynaecology and Obstetrics and Paediatric Surgery Operating Units) were also included in the study. Surfaces considered to be the most immediately in contact with the patients were identified and, along with the hands of healthcare workers, were then sampled: bed bars and header, bedside table, taps, and handles in wards; surgical carts, light and tables in surgical theatre areas.
Random sampling on the hands of healthcare workers, both medics and nurses, who performed diagnostic and/or therapeutic procedures on the patient were also carried out. Contact slides (manufacturer: PBI) containing various culture media were used for sampling: PCA for the bacterial load, Vogel-Johnson agar for *Staphylococcus spp*, Cetrimide agar for *Pseudomonas spp*, Rose Bengal agar + CAF for yeast and mould, VRBG agar for Enterobacteriaceae and Bile-Esculin agar for *Enterococcus spp*. Each slide was placed in direct contact with the surfaces for 10 seconds. At the same time, sterile cotton swabs swiped in all directions (horizontal, vertical and diagonal) were used within a sterile disposable mask (10x10 cm^2) that was placed on the analysed surface. The swabs were immediately placed in a 5 ml tube containing an enrichment broth (brain-heart infusion broth) and, only subsequently, incubated at 37° C for 24 h.
For the evaluation of microbial contamination in operating theatre air, expressed as CFU/m^3, the sampling was carried out with a semi-automatic sampler (SAS Super100, Sampler Air System, PBI) containing a Plate Count agar (PCA) that aspired a volume of 180 l/min for 200 seconds. In particular, a sampling was performed in the "at rest" operating theatre and one hour after surgery commencement in the "in use" operating theatre.
All samples were taken immediately to the laboratory. The contact slides were incubated at a temperature of 37°C for 24-72 h and the PCA plates for the measurement of air bacteria were incubated at 37°C for 48h. Samples from the operating theatres were considered positive according to the parameters suggested by the ISPESL "guidelines on standards of security and occupational health in the operating department" [20]. Samples from wards and healthcare workers' hands were considered positive according to the manufacturer's instructions of the contact slides (> 14 colonies on slide corresponding to 117 CFU/100 cm^2). From the samples resulted positive, sub-cultures on various agar culture media were set up: mannitol salt agar (Oxoid) for the isolation of *Staphylococcus spp*, MacConkey agar (bioMérieux) for the Gram-negative, Enterococcosel agar (bioMérieux) for *Enterococcus spp* and Sabouraud agar (bioMérieux) for yeast and mould. The isolated microorganisms were then identified by manual (API Identification System, bioMérieux) and automatic (VITEK, bioMérieux) biochemical methods.

Results

During the five years in question a total of 9396 microbiological samples from a wide range of surfaces (bed bars and header, bedside table, taps, handles for wards; surgical carts, lights and tables in surgical theatres) were collected and analysed. Below we analyse the results concerning microbiological contamination expressed in percentage of positivity by area, for single microorganism and, for the latter, for each individual year.

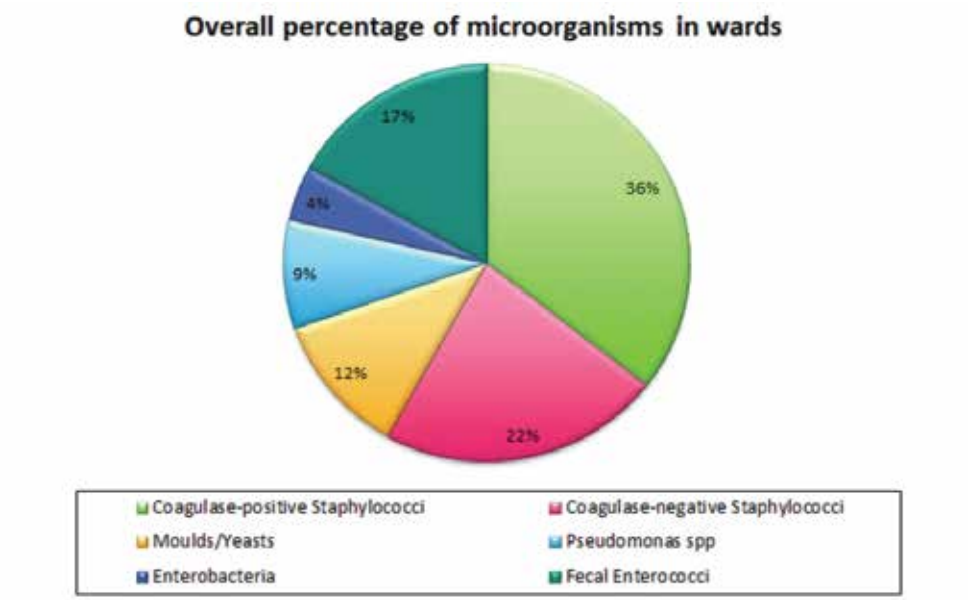
Wards

Of a total of 4398 microbiological samples carried out, positivity for wards was 4.7%, 9.4%, 7.0%, 10.8% and 7.9% from 2010 to 2014 respectively.
By evaluating the positivity for each individual organism isolated, a greater presence of *Staphylococcus spp* and, specifically, mostly for *Staphylococcus aureus* was shown (Fig. 1).
The evaluation of the slides carried out on ward surfaces highlighted the presence of microorganisms in 35.4% of cases (1557/4398) on all samples; analysing them for years, there has been a constant presence albeit in a different proportion of enterobacteriaceae, yeasts and moulds and coagulase-negative Staphylococci (CoNS), a substantial increase for *Staphylococcus aureus*, and less marked, with fluctuating values, for *Pseudomonas* and *Enterococcus faecalis* (Tab. I).
Comparing the trend over time for each individual operating unit (OU) considered, a decrease of positivity only for intensive care (ICU) and the neonatal intensive care unit (NICU) was observed (Fig. 2).

Healthcare workers' hands

Microbiological tests on the hands of 648 healthcare workers present in wards were carried out, of which 40.74% (264/648) tested positive. The results obtained highlight a decrease in the percentage of positivity equal

Fig. 1. Percentage of microorganisms by type in the 5 years in the wards.



Tab. I. Positive microorganisms per year in hospitalization.

	S. aureus	CoNS	Moulds/yeasts	Pseudomonas spp	Enterobacteria	Enterococcus faecalis
2010	27.4%	23.2%	15.8%	9.5%	5.3%	18.9%
2011	33.3%	6.7%	6.7%	10%	6.7%	36.7%
2012	32.6%	28.5%	13.0%	8.3%	5.2%	12.4%
2013	32.2%	31.7%	9.4%	8.3%	3.9%	14.4%
2014	47.3%	8.3%	10.7%	9.5%	4.1%	20.1%

Fig. 2. Temporal trend in the five years of positivity for operating units.

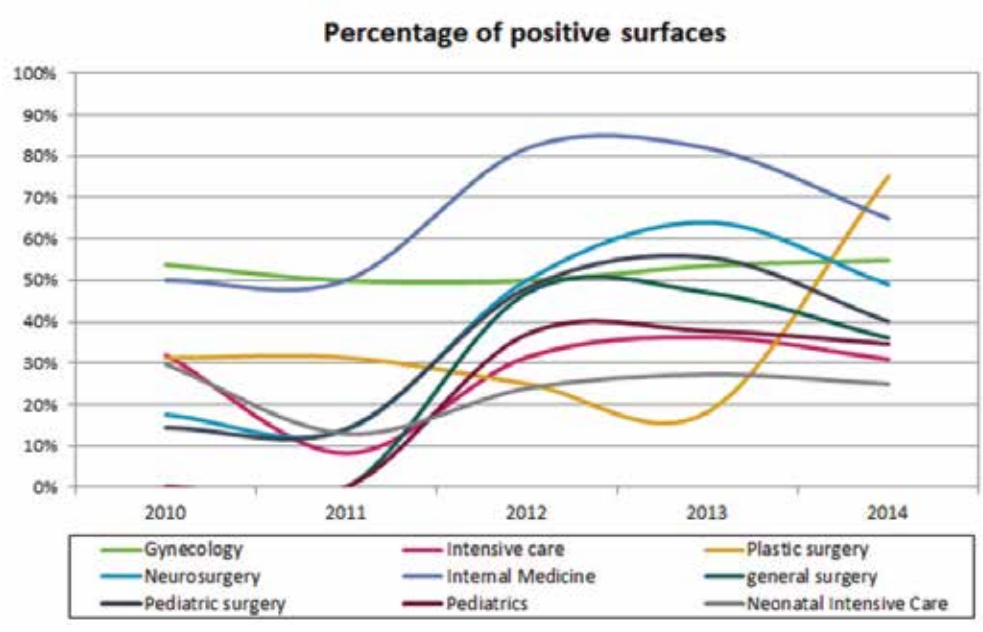
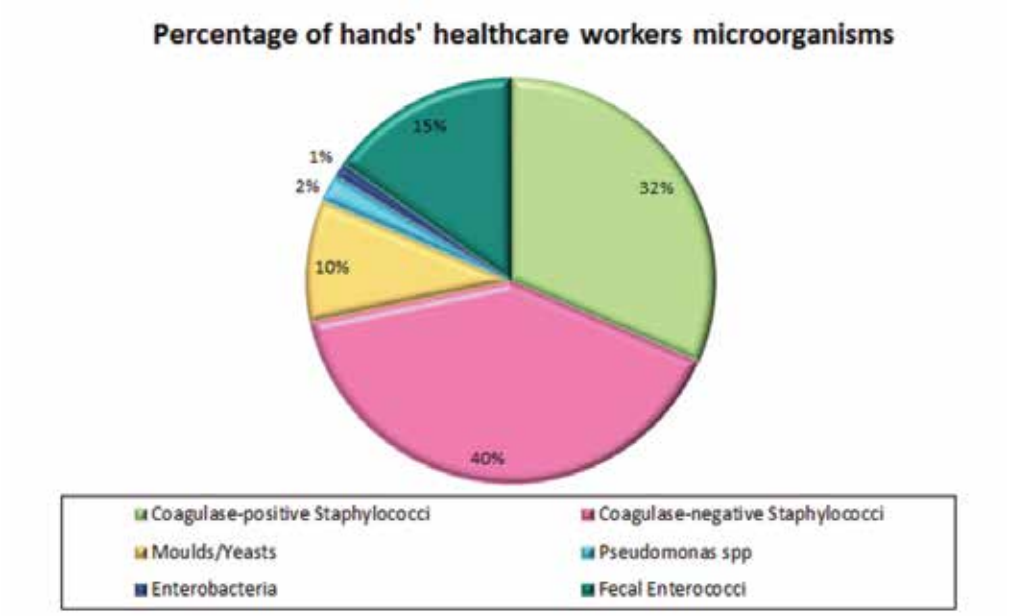


Fig. 3. Percentage of microorganisms by type in the period on the hands of health care workers.



to 9.5%, 8.0%, 5.5%, 6.9% and 5.1% from 2010 to 2014 respectively.

On the total sampling conducted from 2010 to 2014, performing a single microorganism evaluation it was also found, as for the wards, a higher positivity in contamination by *Staphylococcus spp*, although in this case the majority are coagulase-negative staphylococci (Fig. 3).

Comparing the isolated microorganisms per year there is always a growing trend for microbiological contamination of hands by *Staphylococcus aureus*, a decrease for coagulase-negative staphylococci, yeasts, and moulds; an up and down trend for *Enterococcus faecalis*, an initial increase (2011 and 2012) with an overall reduction from 2013 for *Pseudomonas* while *Enterobacteriaceae* already appear absent since 2011 (Tab. II).

Operating theatres

The microbiological assessment carried out on operating theatre surfaces has shown the presence of microorganisms in 11.9% of cases (498/4188) of the total number of samples taken in the five-year period, equal to 4188.

The percentage of total microbial positivity has been decreasing steadily over the years, going from 2.8% to 2.5%, 2.3%, 2.3% and 1.9% respectively from 2010 to 2014.

A higher positivity for *Staphylococcus spp* was noted (Fig. 4).

If we observe the trend by year for an isolated microorganism, as done previously for wards, there is a definite correspondence as regards the behaviour of *Staphylococcus aureus*; in fact, in both cases, there is a substantial increase of positivity percentages. A different behaviour of CONS, yeasts, moulds, and *Pseudomonas* is also observed in operating theatres; as regards enterobacteria there is a net improvement with absence of contamination in the year 2014 (Tab. III).

Evaluating the trend over time, for the operating theatres taken into account, a decrease of positivity in a majority of theatres was found, except for one surgical complex (maxillofacial surgery, plastic surgery and orthopaedics) (Fig. 5).

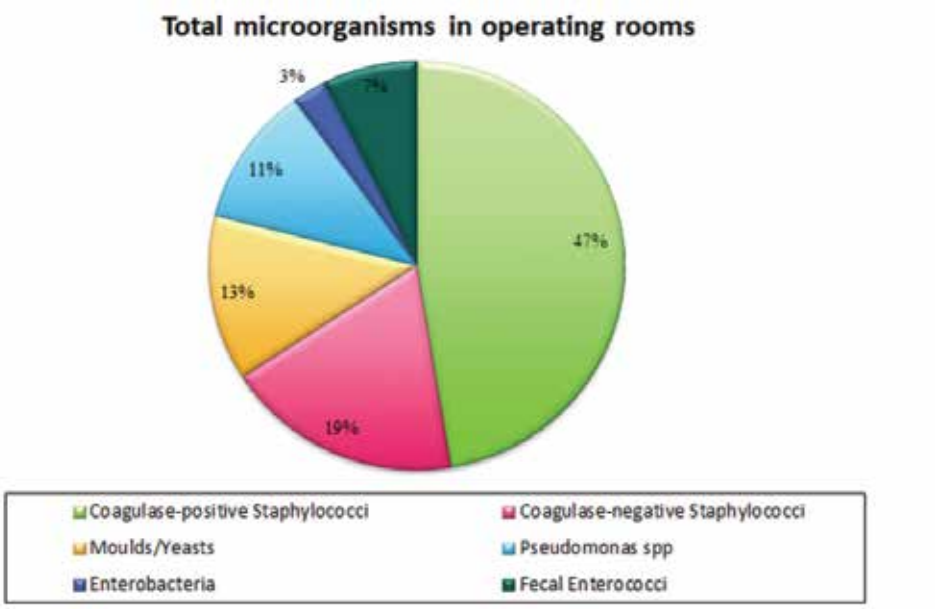
Air

Air samples were taken for each operating empty "at rest" and full "in use" theatre for a total, over five years, of 1962 samples with a positivity rate of 31.9% (627/1962).

Tab. II. Positive microorganisms per year on the hands of healthcare workers.

	S. aureus	CoNS	Moulds/yeasts	Pseudomonas spp	Enterobacteria	Enterococcus faecalis
2010	31%	42%	11%	0%	3%	14%
2011	18%	42%	9%	3%	0%	27%
2012	38%	44%	13%	6%	0%	0%
2013	50%	17%	0%	0%	0%	33%
2014	86%	14%	0%	0%	0%	0%

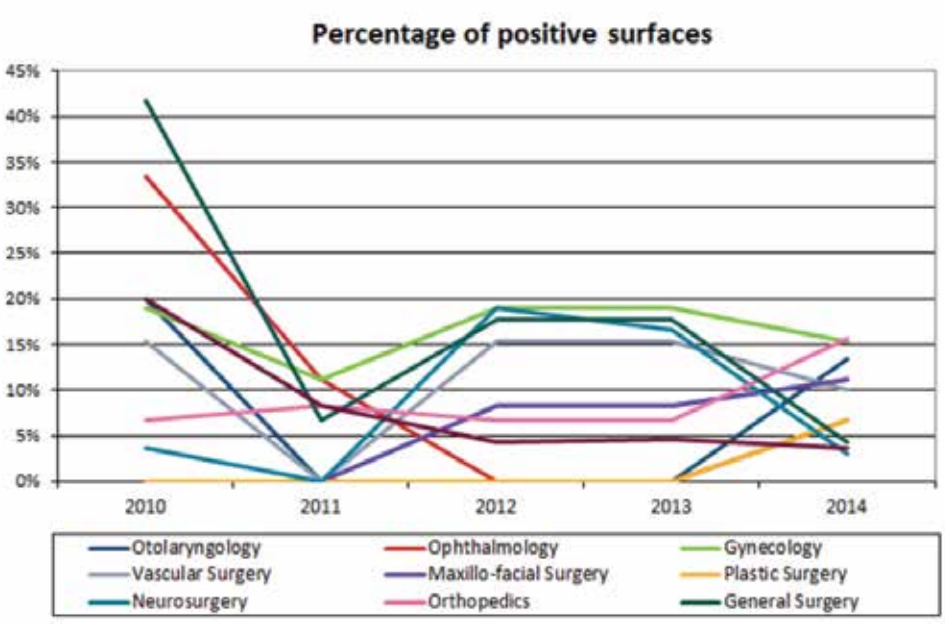
Fig. 4. Percentage of microorganisms by type in the five years in operating rooms.



Tab. III. Positivity of microorganisms per year in the operating rooms.

	S. aureus	CoNS	Moulds/yeasts	Pseudomonas spp	Enterobacteria	Enterococcus faecalis
2010	26.3%	31.6%	15.8%	18.4%	2.6%	5.3%
2011	44.4%	0.0%	33.3%	0.0%	0.0%	22.2%
2012	48.3%	16.7%	15.0%	11.7%	3.3%	5.0%
2013	50.0%	15.5%	13.8%	12.1%	3.4%	5.2%
2014	65.5%	13.8%	0.0%	3.4%	0.0%	17.2%

Fig. 5. Time course in the five years of positive operating rooms.



In 2010, empty theatre compliance was 64.0%, while when full it was 80.0%. In 2011, there was an increase in empty theatre compliance of 69.0%, while in full theatres the samples showed a compliance of 85.0%. There has been, on the other hand, a decrease in air compliance in operating theatres in the following three years: in particular, in 2012, 59.3% of empty theatres and 79.0% of the theatres in use were compliant. In 2013, however, 57.7% of empty theatres and 58.2% of theatres in use were compliant. In 2014 empty theatre compliance was 58.2% and full theatre 41.8%.

Discussions and conclusions

This work has highlighted the presence of pathogens that are potential cause of nosocomial infections on the surfaces we assessed with a percentage of positivity varying between 1.9% to 10.8%.

For inpatient care contamination levels increased progressively over the five years, especially for some of the surgical sites: in Plastic Surgery, positivity increased from 31.0% to 75.0%, in Neurosurgery from 18.0% to 49.0%, in Pediatric Surgery from 14.0% to 40.0%. Instead, in the Department of Internal Medicine, positivity remained high although with mixed values (50.0% in 2010-2011, an 82.0% spike in 2012-2013 biennium and 65.0% in 2014). There are various risk factors that are evident. The first to consider is that wards are often overcrowded with a high turn-over of both hospitalized patients and visitors, and this promotes the continuous re-contamination of the environments [21]. Another determining element is lesser attention to the sanitation of hospital stays compared to other areas. Indeed, we found a decrease of contamination in intensive care units where the sanitization procedures are more frequent and effective, probably because of the special attention given to these departments where patients are hospitalized in critical conditions and where the influx of visitors is regulated. In all departments, however, the most common microorganism is *S. aureus*, known agent of nosocomial infections.

As for the hands, finally, the monitoring has highlighted a low rate of contamination with a progressive decrease in the five-year period. This could be the result of greater awareness and attention in the cleaning and disinfection of the hands of healthcare workers, thanks also to the audit campaigns carried out by us constantly, and the presence of hydro-alcoholic gel dispensers in the various departments.

A good result was also obtained for the surfaces of operating theatres where, in five years, there has been a decrease of positivity.

The same does not apply, however, to the air, because contamination has increased in "at rest" operating theatres, especially in the last three years (2012-2014) and increased significantly in the "in use" ones in the 2013-2014 biennium. This result may be due to various factors that coexist with each other such as poor adherence by healthcare workers to ministerial recommendations [22]. We did in fact observe an inconstant application of these rules, which provide for the closing of doors throughout the duration of surgery, using the lowest possible number of operators in attendance, and proper surgical attire. Another key element to consider is the correct operation and powering of the ventilation system (VCCC) with proper maintenance of the operating theatre filters.

During the final study period we, regularly, conducted audits and educational meetings to inform the healthcare workers about the importance of adopting correct behaviours to the aim of avoiding microbiological contamination of hospital environment and so the transmission to patients of nosocomial pathogens potentially cause of nosocomial infections.

The strength of our research is to show the importance of a constant environmental microbiological monitoring that appears as one of the main tools for reducing environmental microbial load as a whole, through the evaluation and assessment of the following parameters: proper operation of VCCC systems and effectiveness of sanitation procedures put into place. Indeed, as a result of our control, many important policies were adopted in the hospital, especially the increased control and cleaning of air filters in the operating rooms, the greater attention to the sanitation of the hospital surfaces and the increased number of sanitizer dispenser for hands in hospital.

However, microbiological monitoring alone is not sufficient to improve the fight to the nosocomial infections. It is, also, necessary conducting periodic informative meetings to healthcare workers, ensuring correct application of the rules of conduct to be adopted by healthcare staff, performing periodic audits to regulate the influx of visitors and informing them to follow a few simple hygienic-health rules is also necessary, in order to decrease environmental recontamination in the hospital. Moreover, it would be desirable improve the cleaning of the hospital environment combining the classic cleaning methods with those recent such as ultraviolet devices, hydrogen peroxide systems, self-disinfecting surfaces and use of an innovative sanitization procedure using probiotic bacteria based on the principle of biological competition [23-25].

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Conflicts of interest: the authors declare that there are no conflicts of interest.

Ethical approval: not required. (This study is not a clinical trial and not involved human subjects).

Authors' contributions

VLF and RS conceived, designed and coordinated the research. VLF, RS, AF, CG and MARP contributed to

the acquisition, analysis and interpretation of data. VLF, AF, CG and MARP evaluated the results. VLF, RS and MARP wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript

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ORIGINAL ARTICLE

Risk assessment of legionellosis in cardiology units

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Keywords

Infective endocarditis • Legionella • Cardiac valve prosthesis • Environment

Summary

Infective Endocarditis (IE) is a disease with high morbidity and mortality. Nowadays, in addition to classic pathogens were isolated exigent Gram negative bacteria as A. baumannii, A. lwoffii, C. burnetii, Bartonella, Chlamydia and Legionella. We present our experience of Legionella isolations in environmental sample (water and air) collected from the Cardiology units belonging to two hospitals in Messina (Italy). A total of 80 samples were carried out, 30 and 50, respectively in the first and in the second structure: 55 of water and 25 of aerosol.
The positivity of 30% of the water samples analyzed and 15% of those aerosol strengthens the conviction of the need for greater environmental monitoring, especially in the wards at high risk.

Introduction

The continuous alert of legionellosis disease occurred in hospitals around the world and the clusters observed in major public places mean that the *"Legionella"* phenomenon became of primary importance for Public Health [1, 2]. The risk of illness increases dramatically if the germ is found in certain wards such as intensive care, cardiology, pulmonology for critical conditions of their hospitalized patients [3-8]. As reported in many articles in the scientific literature, we would like to emphasize as *Legionella* may be potentially related to endocarditis when it is found in the hospital environment.
The Infective Endocarditis (IE) is an infection of the endocardial surface of the heart, which may involve one or more heart valves, the mural endocardium, or a septal defect. If left untreated, IE is generally fatal. IE is in continuous evolution: in the face of an increasing role of degenerative valvular disease and iatrogenic factors such as the presence of prosthetic and intracardiac devices, factors like rheumatic disease have become secondary. The average age of patients has increased dramatically and with it the comorbidity. The frequency at which a particular organism causes endocarditis depends on how frequently can gain access to the circulatory system and its ability to survive in the bloodstream and stick to the components of NBTE (non-bacterial thrombotic endocarditis), to subendothelial structures exposed or to the endothelial surface. Over the past few years, despite the improvement recorded in both clinical-diagnostic and therapeutic circle, the incidence and mortality of infective endocarditis have not been substantially reduced [9, 10].
From an etiological point of view there are significant changes: in addition to classic pathogens (*S. aureus*, *Streptococcus* sp, *Enterococcus* sp) Gram negative bacteria are isolated, for example those belonging to the group HACEK (*Haemophilus* sp, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae*). Moreover, there are studies reporting cases of endocarditis caused *by A. baumannii, A. lwoffii, C. burnetii, Bartonella, Chlamydia* and *Legionella* spp as the responsible of associated forms with negative blood culture [11-16]. Concerning *Legionella*, in the literature are describes cases which *L. micdadei*, *L. bozemanii*, *L. anisa*, *L. dumoffi* and *L. cardiac*, in addition to *L. pneumophila*, are implicated in the genesis of cardiovascular disease, with involvement of valvular structures, both prosthetic that native [17-28].
In hospital and other health care facilities, waterborne diseases may originate from the bacterial colonization of water pipes, taps, cooling towers, showers and water supplies [29-35]. For hospitalized subjects, risk assessment on the basis of levels of exposure to contaminated water pipes should be calculated following constant environmental monitoring, and critically with strict clinical surveillance. As is known, *Legionella* is able to survive for long periods in water and even to replicate in the presence of chlorine, if it manages to create suitable conditions (biofilm, parasitism of amoebas and protozoic cysts, etc.) [36, 37].
The Italian National Institute of Health (in italian, Istituto Superiore di Sanità) reported that during 2015 in Italy nosocomial cases of legionellosis were 82 (5.3%) of total cases reported, of which 33 (40%) were of certain nosocomial origin, and 49 (60%) possibly of nosocomial origin [38].
It follows, therefore, that environmental surveillance of *Legionella* spp. is needed for risk assessment and prevention of hospital-acquired legionellosis.

The objectives of the present investigation were to carry out the risk assessment of Legionellosis in Cardiology Units, verifying the presence and distribution of *Legionella* in water and air samples, in order to optimize the prevention program in higher risk areas.

Materials and methods

Ethical Approval was not required as this research did not involve human beings and/or animals.
From October 2015 to September 2016, 55 samples drawn from the water distribution system of Cardiology wards belonging a two hospitals sited in Messina (Italy) were examined for *Legionella* spp.
To recover *Legionella* spp. from water samples the standard procedures reported in the Italian Guidelines for the prevention and control of legionellosis, approved State-Regions Conference, in its meeting of May 7, 2015, were used [39].
In addition to water samples, *Legionella* has also been searched in 25 samples collected from the aerosol formed around the faucet when the water flows. In this case two Petri dish with a diameter of 9 cm were placed on the sites of the tap. Simulating handwashing, the health-care worker responsible for sampling has caused the formation of aerosol. The plates were inoculated only for the passive fall of the aerosol and this imply an even greater risk for patients who use the washbasins [40-43]. The result was an average of values measured on 2 plates/1 h and expressed as CFU/dm^2/h.
The isolates were further identified as *L. pneumophila* serogroups using the microagglutination kit '*Legionella pneumophila antisera set 1 and 2' and Legionella* antisera for several *Legionella* species as *L. dumoffii, bozemani, micdadei*, etc (Biogenetics, Denka Seiken co., Ltd, Tokio, Japan).

Results

A total of 80 samples were carried out, 30 and 50, respectively in the first and in the second structure: 55 of water and 25 of aerosol. Of 55 water samples analyzed (20 in the Hospital 1 and 35 in the Hospital 2), 18 (33%) were positive for the presence of *L. pneumophila*, 7 (35%) in the first and 11 (31%) in the second.
With regard to samples of air, of 25 samples taken, in 8 (32%) *L. pneumophila* was detected. Besides *L. pneumophila* 1, the identified serogroups were 3 and 6, the latter only in the second hospital. The mean concentration of *Legionella* did not show significant differences between the two structures ($p>0.05$). As expected, the same serogroups isolated from water were found, in much smaller quantities, in respective aerosol samples.
The results are summarized in Table 1.

Discussion and conclusions

The positivity of more than 30% of the water and air samples analyzed, strengthens the conviction of the need for greater and continuous environmental monitoring, especially in the wards at high risk.
The relevance of this alert is focused on the fact that IE sustained by *Legionella* genus is not a frequent but always a possible cause of sudden death in patients undergoing cardiac surgery, especially for prosthesis implantation [44]. Despite the analytical results discovered, strongly indicative of a risk situation, in cardiology wards considered, no cases of Legionnaires' disease had been reported among patients.
Despite the amount of Legionella found in the aerosol is considerably low, an efficient air sampling combined with water surveillance is beneficial for preventing legionellosis. Monitoring the air around aerosol producing devices may assist in tracking the greatest potential for *Legionella* spp. aerosolization, identifying plausible infection sources, and assessing the distance that Legionella has spread.
Attention should be given in increasing environmental surveillance in higher risk areas, otherwise you could apply to new methods of estimation of the spread of germs, such as Geostatistics [45]. Environmental monitoring, carried out for nearly a year, has certainly been useful to underline the problem, optimizing corrective measures, some of which are already active at the time of sampling, but clearly not sufficient to contain the risk

Tab. I. Distribution of water and air samples taken on Cardiology wards, positivity (%) and serogroups identified.

	total number of samples	samples of water	samples of water (% POS)	Serogroups (CFU/l min-max)	samples of air	samples of air (% POS)	Serogroups (CFU/dm²/h min-max)
Hospital 1	30	20	7 (35)	L. pneumophila 1 (100-4000e+1) L. pneumophila 3 (100-2000e+1)	10	3 (30)	L. pneumophila 1 (0,78-5,5) L. pneumophila 3 (0,78-3,14)
Hospital 2	50	35	11 (31)	L. pneumophila 1 (200-1000e+2) L. pneumophila 3 (100-1000e+1) L. pneumophila 6 (100-2000e+1)	15	5 (33)	L. pneumophila 1 (0,78-6,28) L. pneumophila 3 (0,78-3,93) L. pneumophila 6 (0,78-2,36)
Total	80	55	18 (33)		25	8 (32)	

(as de-calcification, sanitization with chlorine agents, etc.), implementing a more accurate assessment of the state of the water system, considering the replace of some of its components (valves, end portion of the water pipe system, sanitation in recirculation water pipes in the interested departments concerned, etc.), as well as an increase of the level of active epidemiological surveillance on patients and staff of the departments concerned.

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Authors' contributions

PL and SD carried out: study design, laboratory analysis, data interpretation. EA and MC provided the bibliography and wrote the manuscript. GD performed manuscript revision and handled cardiological aspects.

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ORIGINAL ARTICLE

Effectiveness of ATP bioluminescence to assess hospital cleaning: a review

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Keywords

ATP Bioluminescence • Hospital Surfaces • Healthcare-Associated Infections

Summary

Introduction. *Contamination of hospital surfaces plays an important role in the transmission of several healthcare-associated microorganisms, therefore methods for evaluating hospital surfaces' cleaning gain particular importance. Among these, there are visual inspection, quantitative microbiology, fluorescent markers and adenosine triphosphate (ATP) bioluminescence. The latter seems to provide interesting features, detecting the presence of ATP on surface (as Relative Light Units, RLU), a proxy of organic matter and microbial contamination. Several studies have investigated the effectiveness of this technology; with this research, we aim to summarize the most significant results.*
Methods. *A systematic review was conducted. The keywords (namely, "ATP", "bioluminescence", "hospital" and "surfaces") were searched in PubMed/MEDLINE and Scopus databases, in order to find relevant data, from January 2000 to October 2014. After the selection, we globally considered 27 articles.*
Results. *Most of the studies were conducted in United Kingdom and in USA. Different threshold RLU benchmark values were identified by analyzed studies. Fourteen of these researches compared the ATP bioluminescence with microbiological methods, 11 identified a significant correlation between the two methods, although poor or not complete for 5.*
Discussion. *ATP bioluminescence is not a standardized methodology: each tool has different benchmark values, not always clearly defined. At the moment, we can say that the technique could be used to assess, in real time, hospital surfaces where cleanliness is required, but not sterility.*

Introduction

Healthcare-associated infections (HAIs) represent an important and widespread cause of morbidity and mortality among patients. Over the past decades, various scientific evidences have accumulated, indicating that contamination of hospital surfaces plays an important role in the transmission and diffusion of several healthcare-associated microorganisms [1, 2]. In particular, the hospital environment contributes to the transmission of several nosocomial pathogens, such as *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) [2, 3]. These bacteria could survive in this setting for a variable period, from hours to days and, in some cases, even months, and could contaminate the surfaces or the medical devices [4]. Consequently, pathogens could infect patients or contaminate the hands of healthcare staff and, then, patients [2].
Within this perspective, methods to assess hospital environments cleaning can be considered an integral part of infections prevention and control programs. Among these, the most known and used are: visual inspection, microbial methods, fluorescent markers and adenosine triphosphate (ATP) bioluminescence. The latter measures the presence of ATP on surfaces. The ATP bioluminescence consists in a swab, used to sample a standardized area, which, subsequently, is placed in a tool that uses the firefly enzyme "luciferase" to catalyze the conversion of ATP in Adenosine Monophosphate (AMP): this reaction results into an emission of light which is detected by the bioluminometer and quantified in Relative Light Unit (RLU). The presence of ATP on surface, obviously, is a proxy of organic matter and, consequently, of microbial contamination. This method has been used in food industries since over 30 years. Its use in the health care environment is growing, but it is still controversial, in that different tools consider different threshold values, and, therefore, this technique seems not to be standardized.
Several studies investigated the effectiveness and also evaluated the practical application of this technique in this setting. The aim of this study was to qualitatively synthesize and discuss the most significant results and implications of the applications of ATP bioluminescence in healthcare settings, reviewing the most recent scholarly literature.

Methods

We conducted a systematic review according to the "Preferred Reporting Items for Systematic Reviews and Meta-analyses" (PRISMA) guidelines [5]. The search

Tab. I. Search strategy utilized in the current review.

Search strategy item	Details
Keywords	Adenosine triphosphate, ATP, bioluminescence, bioluminometer, surfaces, hospital
Databases	PubMed/MEDLINE, Scopus
Inclusion criteria	Studies investigating the applications and effectiveness of ATP bioluminescence Studies conducted in healthcare settings
Exclusion criteria	Studies not carried out in healthcare settings Studies lacking sufficient details Studies not pertinent with the aim of this review Study design: overview/review articles
Time filter	January 2000-October 2014
Language filter	Only articles written in English
Target journals	American Journal of Infection Control; British Journal of Infection Control; Healthcare Infection; Infection control and hospital epidemiology: the official journal of the Society of Hospital Epidemiologists of America; International journal of hygiene and environmental health; Journal of Infection Control; Journal of Occupational and Environmental Hygiene

strategy consisted in a string of keywords such as ATP, bioluminescence, bioluminometer, hospital, healthcare setting, surfaces, connected by proper Boolean operators. For this scope, the keywords were searched in PubMed/MEDLINE and Scopus databases, in order to find relevant data. Time filter was applied and only articles from January 2000 to October 2014 were considered. Only articles written in English or for which an English translated text was available were included. Using Scopus we searched the keywords, selecting the option "all fields", whilst for PubMed/MEDLINE medical subject headings (MeSH) terms were used. All the searches as well as the screening were made by two researches EC and GM independently. Any disagreement was discussed until consensus was reached.

We included all types of studies, except: i) not peer-reviewed scholarly articles; ii) overview/review articles, which were excluded but scanned for including further potentially relevant studies, iii) articles not written in English language, iv) all laboratory studies, not conducted in health care settings, and v) articles lacking sufficient details or not pertinent with the aim of our review. Further, selected target journals were hand-searched for increasing the chance of getting relevant articles. For ensuring a high-quality of the included studies, we did not search in the grey literature.

Table I reports our search strategy.

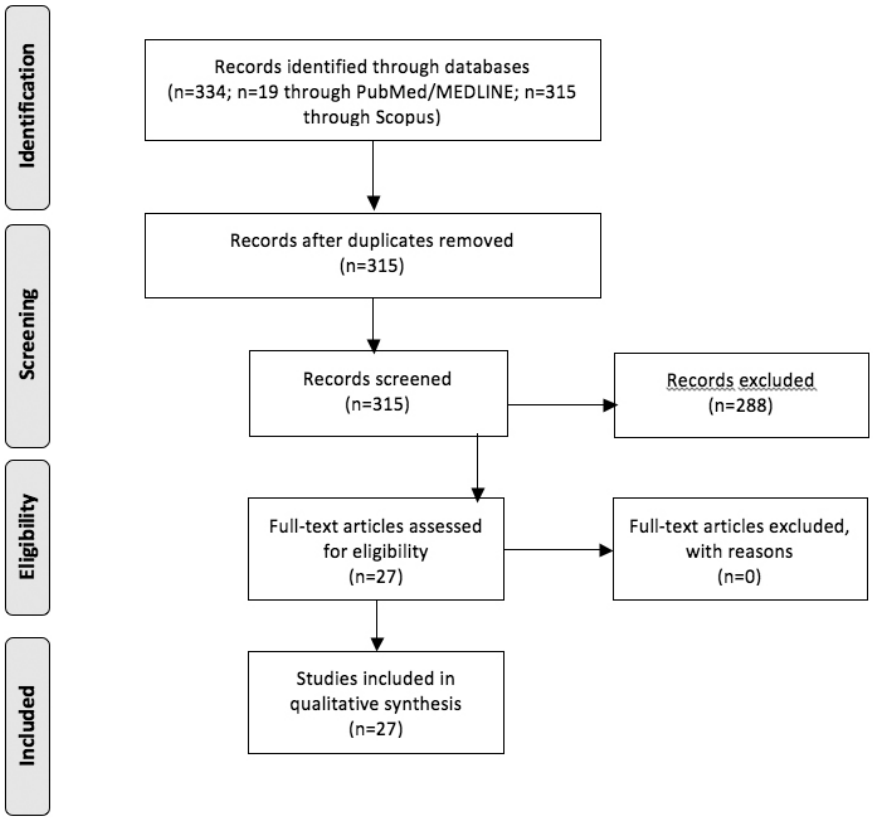
From each included study, we collected information about: i) the surname of the first author of the article and the year of publication; ii) the country where the research was carried out, iii) the setting in which the investigation was performed; iv) the samples used in the investigation; v) the RLU benchmark value discerning between clean and dirty surfaces (when investigated and reported), vi) the type of bioluminometers used, and vii) whether an eventual correlation between bioluminescence and microbiological methods existed (in terms of correlation coefficients, such as Pearson's coefficient, Spearman's rank coefficient, concordance $\varkappa$ coefficient or R^2). The effect size of the correlation coefficient, when reported, was interpreted using the following rule of thumb: very high positive (or negative) if ranging from 0.90 to 1.00 (or ranging from -0.90 to -1.00): high positive (or negative) if ranging from 0.70 to 0.90 (or ranging from -0.70 to -0.90), moderate positive (or negative) if ranging from 0.50 to 0.70 (or ranging from -0.50 to -0.70), low/poor positive (or negative) if ranging from 0.30 to 0.50 (or ranging from -0.30 to -0.50), and little/no correlation if ranging from 0.00 to 0.30 (or ranging from 0.00 to -0.30). In the case the exact correlation coefficient was not indicated, we reported whether the correlation was statistically significant or not, on the basis of the p-value. Data extraction was carried out by two reviewers independently. In case of discrepancy, any disagreement was solved by discussion until consensus was reached or a third reviewer was involved.

Results

Using PubMed/MEDLINE we found 19 studies, 4 of which were judged not relevant for our investigation and one was a review, examining 12 papers, already found by our research. The final number of studies considered using PubMed/MEDLINE was 14. Therefore, in Scopus we found 315 papers, and only 27 were useful for the review. As such, the final number of studied included in the review was 27 (Fig. 1).

Concerning the data extracted, all studies were conducted in health care settings. Most of them were carried out in United Kingdom (UK) (10/27) [6-15]; 9 of them were performed in USA [16-24], 1 in Italy [25], Turkey [26], Japan [27], Chile [28], Canada [3], Norway [29], Australia [30] and Brazil [31]. Eleven researches monitored the surfaces after cleaning [3, 7, 9-11, 15, 19, 20, 25, 27, 31], one before [23], 13 both pre and post cleaning [6, 8, 12-14, 16-18, 22, 24, 28-30] and in the remaining two studies this information

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flowchart - Studies selection.



was not reported [21, 26]. Twenty-three studies (85.2%) identified a RLU benchmark value, discerning between clean and dirty surfaces. This value corresponded to 250 for 10 studies [6, 10, 12, 13, 16-18 21, 23, 24], to 500 for 7 researches [8, 9, 11, 12, 14, 29, 31], to 100 for 4 studies [7, 15, 25, 29], to 300 for 2 studies [15, 19]; 127 [27], 1000 [3] and to 45 [20] for one study, respectively. Moore et al. [12] identified two threshold values: 250 and 500 RLU. Andersen et al. [29] used two different tools and, so, considered two different values: 500 RLU for ATP Biotrace Cleantrace system and 100 for Hygiena System. Another paper considered as cutoff 100 RLU for several patient rooms surfaces and 300 RLU for floors [15]. Details about RLU threshold values for each type of bioluminometers are shown in Figure 2. Figure 3 shows the benchmark values according to geographical provenance of studies.

Fourteen papers (51.8%) [8, 10, 11, 13, 15, 17, 18, 22-27, 31] compared the effectiveness of ATP bioluminescence to assess hospital surfaces' cleaning with microbiological methods; in particular, these studies evaluated the correlation between RLU and Aerobic Colony Counts (ACC). Three of these studies have shown no correlation between the two compared methodologies [18, 22 31], whilst the remaining 11 have highlighted a correlation [8, 10, 11, 13, 15, 17, 23-27], although it is poor/moderate according to 4 studies [15, 17, 25, 27] and one found only a pre-cleaning correlation [24].

Discussion

Contamination of hospital surfaces plays an important role in the transmission of several healthcare-associated microorganisms. In this perspective, methods for evaluating hospital surfaces' cleaning gain importance. Each of these methods show advantages and disadvantages, and directives indicating the most appropriate method to use in different health care settings do not exist. The ATP bioluminescence seems to provide interesting perspectives, detecting the presence of ATP on surfaces (as Relative Light Units, RLU), a proxy of organic matter and microbial contamination. The present review showed the ATP bioluminescence is not a standardized method for assessing cleanliness; each tool had different benchmark values, ranging from 45 RLU to 1000 RLU. The most used values were 250 and 500 RLU. It is also interesting to note that for the same brand of bioluminometer different threshold values were considered.

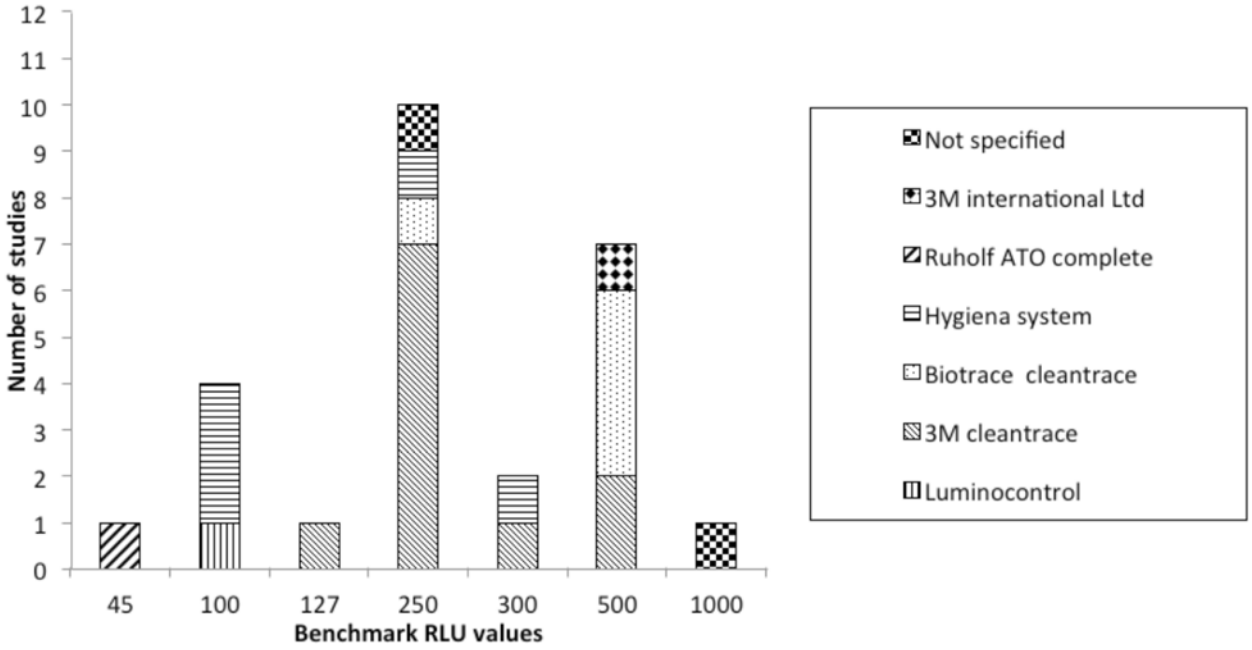
The country where the studies were conducted may have influenced the choice of the RLU cutoff values, for example in the U.S. the most often used value corresponded to 500 RLU. The tool used in most studies was 3M Clean-Trace ATP System, following by Hygiena system and Biotrace Cleantrace System.

These differences among the benchmark values make difficult the comparisons between measurements carried out with different tools [15].

Tab. II. General features of the studies.

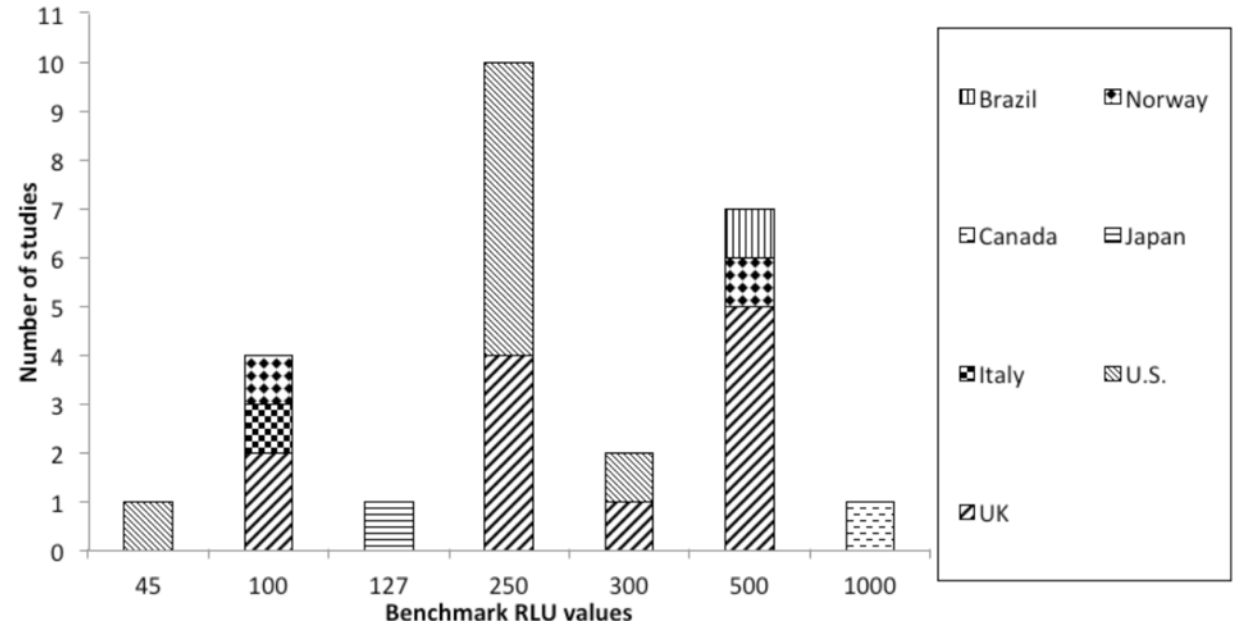
Author/year	Country	Setting	Sample	RLU benchmark	Correlation RLU/CFU	Brand bioluminometer
Ali et al., 2012 [6]	UK	Hospital	60 samples pre and 60 post cleaning	250	Not investigated	Not reported
Amin et al., 2014 [16]	U.S.	Clinic ophtalmology ward	396 samples pre and 396 post cleaning	250	Not investigated	3M Clean-Trace ATP System
Amodio et al., 2013 [25]	Italy	Teaching hospital	193 surfaces postcleaning	100	Poor (R2=0.29)	Luminocontrol II (PBI internarnational, Milano)
Andersen et al., 2008 [29]	Norway	Teaching hospital	96 samples pre and 96 post cleaning	100, 500	Not investigated	ATP Biotrace Clean-Trace system; Hygiena System
Anderson et al., 2011 [7]	UK	Hospital surgical ward	44 samples post-cleaning	100	Not investigated	SystemSure Plus system (Hygiena Int. Ltd)
Aycicek et al., 2015 [26]	Turkey	Hospital kitchen	280 from 14 surfaces	Not reported	Significant ($\varkappa$ = 0.249)	Pd-10 kikkoman Co, Japan
Boyce et al., 2009 [17]	U.S.	Teaching hospital	510 samples pre and 503 post cleaning	250	From poor to moderate (r from 0.356 to 0.649)	3M Clean-Trace ATP System
Boyce et al., 2011 [18]	U.S.	Teaching hospital	500 samples pre and 500 post cleaning	250	Not significant	3M Clean-Trace ATP System
Branch-Elliman et al., 2014 [19]	U.S.	Hospital	820 samples post clearing	300	Not investigated	3M Clean-Trace NG Luminometer
Cooper et al., 2007 [8]	UK	4 hospital	552 samples pre and 547 post cleaning	500	Significant	Biotrace Cleantrace system
Ferreira et al., 2011 [31]	Brazil	Hospital	100 samples post clearing	500	Not significant	3M Clean-Trace ATP System
Gillespie et al., 2012 [30]	Australia	Hospital	50 samples pre and 50 post cleaning	Not reported	Not investigated	Not reported
Gold et al., 2013 [20]	U.S.	Intensive Care Unit (ICU)	Postcleaning. Number of surfaces not specified	45	Not investigated	Ruholf ATO Complete contamination Monitoring System
Gordon et al., 2014[3]	Canada	Teaching hospital	15 HTOs in 36 patients rooms (first day) and in 37 patients room (second day)	1000	Not investigated	Not reported
Griffith et al., 2008 [9]	UK	Hospital	31 sites postcleaning	500	Not reported	Biotrace Clean-Trace system
Havill et al., 2011 [21]	U.S.	Teaching hospital	300 samples from 101 rolling blood pressure unit	250	Not investigated	3M Clean-Trace ATP System
Lewis et al., 2008 [10]	UK	Teaching hospital	180 samples post cleaning	250	Significant	Biotrace International, Ltd, Brigend, UK
Luick et al., 2013 [22]	U.S.	Teaching hospital	250 surfaces pre and post cleaning	Not reported	Not significant	Accupoint HC (Neogen)
Malik et al., 2003 [11]	UK	4 hospitals	non specified the samples number. Sampling done postcleaning	500	Significant	Biotrace Cleantrace system
Mulvey et al., 2011 [13]	UK	Teaching hospital	90 samples pre and post cleaning	250	Significant	Hygiena system
Moore et al., 2010 [12]	UK	Teaching hospital	90 samples pre and 90 post cleaning	250, 500	Not investigated	3M Clean-Trace Clinical Hygiene Monitoring System
Sherlock et al., 2009 [14]	UK	Two hospital wards	120 sample pre and 120 post cleaning	500	Not investigated	3M International Ltd, Brigend, UK
Smith et al., 2013 [23]	U.S.	Hospital	10 samples in 10 rooms pre and post-cleaning	250	Significant only precleaning	3M Clean-Trace ATP System
Smith et al., 2013 [24]	U.S.	Hospital	18 samples pre-cleaning in 10 rooms	250	Significant	3M Clean-Trace ATP System
Watanabe et al., 2014 [27]	Japan	Three hospital of different sizes	752 samples post cleaning	127	Poor (r = 0.287)	3M Clean-Trace ATP System
Willis et al., 2007 [15]	UK	Hospital	108 samples post cleaning	100(surfaces), 300 (floor)	Poor (r = 0.15)	Hygiena system
Zambrano et al., 2014 [28]	Chile	Teaching hospital	198 samples pre and post cleaning	Not reported	Not investigated	Lightning MVPTM (Arquimed)

Fig. 2. The benchmark Relative Light Units (RLU) values according to the different brands of bioluminometers used in the studies included in the current review.



*In three studies [22, 26, 28] other types of bioluminometers were used and the RLU benchmark values were not indicated

Fig. 3. The benchmark Relative Light Units (RLU) values according to geographical provenance of studies.



A large majority of studies was conducted in UK and in USA, probably because in these Countries there is a growing interest about environmental hospital hygiene and methods to assess it. Furthermore, it should be noted that among 14 studies investigating the correlation between ATP bioluminescence and microbiological methods, 11 have found a significant correlation, although poor, or partial, for 5 papers. ATP bioluminescence would not seem to be a methodology very accurate in detecting bacteria. In addition, it provides a quantifica-

tion of all organic material, including bacteria, but it also identifies others organic matters such as urine, milk and blood, which is a limiting aspect of the methods and is rather difficult to overcome [10].
Another limitation of this technique could be the residues of detergent or disinfectants on the surfaces [32], which may require rinsing of these surfaces before the use [33].
Despite of these considerations, some advantages of this technique can be listed, such as the possibility to provide real-time results (within 20 seconds of sampling), its simplicity of use (which makes possible the adoption of the method not only by trained healthcare staff), and the quantitative results. The latter allows comparisons between pre- and post-cleaning or between different surfaces.
Our study has a number of shortcoming that should be properly recognized. Despite the broad and systematic search, the main limitations of this study could be found in the selection of papers written only in English language and, therefore, in the possibility of having missed some relevant papers, which, considering the novelty of the technique, could be found, instead, in the "grey literature". On the other hand, it should be expected that the quality of the latter papers would be inferior to those present in the scholarly peer-reviewed indexed literature.
Concerning the main implications of the current study, we can conclude that the use of this technique could produce better results after a proper validation/standardization. To achieve this aim a multi-phase approach should be followed: i) to clarify the methods of sanitizing or disinfecting performed on each surfaces before the controls made with the bioluminometer; ii) to select the most appropriate surfaces where the analysis have to be conducted (for example "high risk objects" such as toilet seats, basins, door handles); iii) to choose the bioluminometer among the different brands available; iv) to calibrate the instruments studying the correlation between CFU and RLU, identifying the best threshold value (higher sensitivity and specificity) [33] which discriminates between clean and dirt surfaces.

Conclusions

In conclusion, the ATP bioluminescence could be considered a practical, useful method to assess hospital hygiene, performing better than visual inspection (namely, Bacharach scale, bassoumeter, and glossmeter), if properly adopted, also being aware of its possible limits.

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Authors' contributions

NN conceived the research. EC and GM researched and analyzed the study using for review and evaluated the results. EC, GM and DL performed the data quality control. EC, GM and PM wrote the manuscript. PM supervised the research. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Resource allocation criteria in a hospital

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Keywords

Resources • Allocation • Hospital

Summary

Introduction. *Allocate fixed resources among competing users is a challenge in terms of hospital management in order to obtain the best performance considering strategic objectives. In order to address this need, a system of evaluation in an important research and teaching hospital was designed. This study describes resource allocation criteria in a hospital focusing on the evaluation system and its developed application methodology.*
Methods. *The indicator system allows the strategic management to rapidly detect the priorities in the evaluations of the Strategic, Organizational, Managerial, Economic, Research and Qualitative conditions of each unit. The chosen indicators are expressed with three numerical values, (1 indicating critical status, 2 acceptable conditions and 3 a good operational situation).*
Results and discussion. *The adopted evaluation system considered different thematic areas: Strategic, Organizational, Managerial, Economic, Research and Qualitative. In order to define each area, 3 fields of evaluation have been chosen. The indicators have been structured according to a pyramid system allowing creating a single indicator for each area for each unit. Furthermore, a single indicator has been fixed in order to facilitate a first consideration on whether to carry out or not closer examinations of the most critical units. This manuscript describes an attempt to define objective criteria for the allocation of scarce resources in order to achieve the hospital's strategic objectives. The indicators identified allow to obtain an overall score for each unit, which allows the management to prioritize the needs.*

Introduction

The allocation process concerning the more and more limited health resources presents several criticisms at different levels: macro (national/regional), meso (institutional/territory-hospital) and micro (at patient bedside). In particular the meso level plays a fundamental role in the resources management by representing the point of reference in the health services supply [1].
The health sector and, more specifically, the hospitals have been asked, over the last years, to manage fixed resources with respect to the increasing activity levels [2], due to the ever-increasing number of needs from a population which is older and older and who bears many co-pathologies that have to be treated with territorial out-of-hospital care services which are not always effective. Therefore, it is necessary defining the hospital priorities and criteria of resources allocation.
The different models, that have been studied for the strategic planning of healthcare [1, 3, 4] and, in particular of hospitals, take into account different aspects: the context in which the organization operates, the involvement of stakeholders, the definition of those processes that shall be managed and of those criteria for the resources allocation testifying the work done and certifying its validity [1]. The resources allocation models also in the healthcare sector, are based on economic efficiency, justice, equity, sharing and optimization of the resources themselves [4].
This manuscript describes the evaluation system through indicators both from a conceptual and illustrative point of view explaining the principles of the system and the developed application methodology. The objective is evaluating the strategic positioning, the operating activities, the economic results, the research, organization and the qualities of all the hospital unit. The model was built for an hospital offering third level activities, first level post-university centre, a research centre benefiting from both national and international funding, a place of reference for over 250 rare diseases, three emergency departments and more than ten block.

Methods

The developed model identified 6 thematic areas that can represent the managerial condition of a complex hospital. They can be named as follows: Strategic, Operating, Research, Economic, Organizational, Quality. Within these areas, some qualitative and quantitative evaluation elements have been chosen; they are considered as most significant.
Each thematic area has been given three indicators that were considered as adequately representative to evaluate the position of a specific unit in that area. The indicators

selected to evaluated each area were chosen for some reasons:

- they were the same identified in other similar evaluations;
- the hospital has data to calculate them;
- the hospital management board wants comparing units in order to develop strategic activities devoted to research and teaching, but according to the medical and surgical activities that the hospital has to perform.

Some indicators are referred to the activity of doctors operating in the different units. In order to obtain a datum that reflects as much as possible their actual commitment. The concept of "Equivalent Doctor" (d_e), was adopted with the aims at reporting the real time that the physician dedicates to the clinical activity. Conventionally, as time of the d_e it is possible to use 1500 h/year. The indicators have been structured according to a pyramid system allowing to create a single indicator for each unit.

The purpose of the single indicator is to immediately highlight, within the same area, what is the positioning of the unit under consideration.

The indicator system allows the strategic management to rapidly detect the priorities in the evaluations of the Strategic, Organizational, Managerial, Economic, Research and Qualitative conditions of each unit, within the ambit of its own relevant area.

For each thematic area, the chosen indicators are expressed with three numerical values, attributing:

- value 1 to the values belonging to the segment lower than the average value, indicating the appropriateness of checking the insufficiency status of the unit taken into consideration (critical status);
- value 2 to the segment of approximately +/- 20% near the average value, indicating that the unit taken into consideration is among those to be considered in acceptable conditions;
- value 3 to those located in the segment higher than the average value, indicating, in this case, a good or satisfactory operational situation.

No ethical approval was requested to carry out this study.

Results

The model with its six areas and for each area the three fields of evaluation was presented.

A - Strategic Area

A1 - The Importance of the Hospital: at local, regional and national level

This evaluation takes into consideration the level of importance attributed to the activity carried out.

A2 - The Importance of the potential for development: at local, regional and national level

This evaluation plays a fundamental role in the decision-making process to accept or reject the request for new resources with regard to the hospital development programmes.

The top management on the strength of the objectives that it wants to achieve will therefore decide whether to encourage the development and the professional growth of a specific unit or not.

A3 - Necessary professional specificities even in the presence of enough personnel

This evaluation takes into consideration the professional level that in a specific unit has to be maintained or increased.

It is obvious that, unlike the indicators of the other areas, these evaluations are made on a qualitative basis.

The evaluations of this thematic area are also assigned a three-level score (1, 2, 3) in order to participate in the creation of the single indicator.

B - Operating Area

This area takes into account the medical staff, and it is useful to assess whether their number is adequate to the activity performed in a specific unit or not. The number of doctors can meet the accreditation requirements but it has to be considered the fact that these are minimum requirements therefore they can be insufficient for the type of work carried out in the individual unit.

B1 - Institutional clinical activity

For all the units it has been taken into consideration: the number of equivalent doctors and the number of yearly clinical performance for ordinary admissions to hospital (OA), for day hospital admissions (DH) and for the outpatient examinations (OE).

In the case of OA the number of performance has been adjusted multiplying it by the rate of case mix, in order to take into consideration the objective difference of clinical complexity, and by the number of the average days of hospitalization in order to consider the more time that the doctor has to spend performing this kind of clinical performance.

For each of these clinical activities the number of individual performance has then been compared with the average value of all the analogous performance carried out by the units belonging to the same area, assigning this way the three sub-indicators characterizing the degree of efficiency of the single clinical activities (OA-DH-OE) performed by every single unit. This assessment was performed as follows.

Having taken the average value as reference, a segment equal to +/- 20% was set, the coefficient 2 was included among its values. This number indicates that the activity referred to in the assessment can be considered as sufficiently in conformity with the value expected in a correct management, and therefore not in need of human resources allocation since it is balanced with the resources at disposal.

If there is no clinical activity it is given the 0 value; if it is lower than the average segment the value is 1 and if it is higher than the average segment the value is 3.

Then a single indicator of the institutional clinical activity has been established by transforming these three indicators into a single index named "clinical efficiency index", that in line with what has been previously stated,

allows to be transformed into the "institutional clinical activity" indicator.

B2 - Clinical activity performed for other units- Emergency medical service shifts

This evaluation element indicates the importance of the treatment activity that the examined unit carries out on behalf of the other units of the hospital that is to say that some working days have been subtracted to the clinical activity in the unit of belonging.

Since this service falls within the type of organization that the hospital decided to follow, it represents a commitment that all the units have to fulfil, we thought to compare the number of emergency medical service shifts performed by each unit divided by the number of doctors on the roll, with the average obtained by all the equivalent doctors of the other units belonging to the same area, to assess its position compared to the one that can be considered as an average obligation, and therefore in line with the standard procedures.

Also in this case the so obtained values coming within the +/- 20% segment have been given number 2, to indicate that the commitment to the service of the other units is in line with the standards and, therefore, this is not a critical situation. The values below this segment have been given number 1, to indicate that the commitment of the emergency medical services does not significantly vary the work performed in that unit being below the average, that is to say below the standard procedures.

In the opposite case, the values above the average segment, have been given number 3, which means a load of emergency medical services more demanding than what is usually expected.

Finally when the emergency medical service is not performed we give 0.

B3 - Doctors of an unit/total employed staff ratio

It represents the percentage of doctors in the total employed staff. If the indicator is below the average or percentages of the operating units belonging to the same area, the indicator is given number 1, which means a possible lack of doctors; if it is above the average it is given number 3 to indicate that the percentage of doctors is above the average and, therefore it points out a possible superabundance of doctors. Number 2 indicates that the unit comes within the +/- 20% segment near the average value and also that the unit is in a state of balance.

C - Research Area

Considering that the research is an integral part of the clinical activity of every doctor who works in a research hospital, with this thematic area we wanted to highlight the commitment of every doctor for research.

C1 - Impact Factor

As known, the impact factor (IF) gives an official representation of the research activity carried out by the researchers of an unit.

It is the significant official recognition of the research carried out, but it is not exhaustive since a part of the research performed is not mentioned in the official publications and, therefore, the IF cannot be considered as a recognition of the whole research carried out by an unit.

Considering that the research volume is presumably higher where the number of doctors is higher, we thought it proper to compare the IF to the number of equivalent doctors existing in the different units.

This benchmark is given a score from 0 to 3. 0 when there is no research activity, 1 when the activity is located below the average value decreased by 20%, 2 when the activity is placed in the segment defined by +/- 20% of the average value, 3 for the activity placed above the average value increased by 20%.

The benchmark reading therefore points out if the doctors' productivity is satisfactory, on average adequate or even critical, requesting close substantive analyses.

C2 - Extra financing for current research

This index aims at highlighting the production capacity that a specific unit has in obtaining financing not coming from the ministerial source.

This depends not only on the promotional ability that the chief doctor can exercise c/o the non-governmental and private bodies in order to obtain financing for the research, but also on the reputation that the unit has in the scientific world, and on its knowledge and experiences in specific areas.

The indicator was created starting from the financing given to each unit divided by the corresponding number of equivalent doctors considered as devoted to research, and comparing the result with the average value obtained by the same reports of the other units belonging to the same area.

As in the above indicators, its value is assessed according to its positioning whether inside or outside a segment of values included between +/- 20% of the average value.

Number 0 indicates that the unit does not raise funds besides the ones for the current research. Number 1, given to those values included below the average value decreased by 20%, indicates an unsatisfactory financing attracting capacity compared to the average of the other units. On the contrary, number 3 indicates a satisfactory situation.

C3 - Temporary staff devoted to research

This index represents the significance of the staff hired temporarily, such as for example the holders of a scholarship and contract researchers, employed in the research, compared to the number of doctors who devote to research and when such significance is relatively high it means that the research activity is substantial.

The ability to attract this kind of staff is connected to the ability to attract non-ministerial funds and it indicates a good functioning of the unit, which does not need any corrective action to be performed toward the employees, unless in case of specific strategic reasons such as to ensure the preservation of the know-how acquired through the research.

In order to assess the indicator, the number of this temporary staff shall be expressed as a percentage compared to the number of equivalent doctors. The average value of all percentages of the units belonging to the same area has to be considered as a reference to define the indicator, obtained with the same criteria repeatedly explained

in the other indicators, in the scale of reference, from 0 to 3. That is to say 0 when there are no holders of a scholarship and contract researchers, 1 when the percentage of this type of temporary employees together with the permanent employees is below the value reported by the average segment, 3 when it is above it.

D - Economic Area

D1 - Total revenues from clinical activity

The revenues from OA, DH and OE have been taken into account, obtaining this way the annual revenue of each unit.

The total revenues have been attributed to the number of doctors belonging to the considered unit, in order to quantify the production efficiency of each doctor. The deriving indicator has a value equal to 1 or 2 or 3 depending on the fact that such efficiency is lower than the average, on average or higher than the average respectively.

D2 - Operating margins

From the total annual revenue indicated above variable costs and the cost of personnel have been deducted obtaining, therefore, the Gross Operating Margin of each unit.

Among the variable costs we have taken into consideration the consumption of healthcare material, of office tools and supplies, the laboratory and radiology costs.

The indicator assessment is analogous to the one reported in section D1.

D3 - Other revenues

It mainly refers to grants related to income obtained by the unit thanks to special activities which cannot be identified through the DRGs, the range of fees of outpatient treatments and/or the research activity. The revenues from non-clinical activities, like the sale of plasma bags, also belong to this category.

The calculation methods for this indicator are equal to those indicated in the previous D1 and D2 sections.

E - Organizational Area

In this area the number of doctors are always evaluated taking into account the accreditations rules of the Lombardia Region.

E1 - Logistics: operating divisions

The location in which the activity of a specific unit is carried out is taken into consideration in order to evaluate whether the logistic conditions in which the work has been performed are optimum or not.

Where the arrangement of the working areas is not functional and coherent within the same block shared with the other units of the same Department, or in the presence of a chaotic arrangement or, worst, of a division into more blocks, obviously the work efficiency cannot be satisfactory.

In order to evaluate the effect on the work efficiency of such possible different situations a different importance has been attributed to the type of logistic arrangement in which every single unit operates, that is to say the number of floors or of blocks, and the sum of such values has been divided by the number of employed doctors who, in theory, are spread in them. According to the other scores value 1 means that in that specific unit doctors work in an environment which is more fragmented from a logistic point of view compared to the average, value 2 means that the situation is normal, and value 3 means that the presence of doctors in the departments is above the average.

E2- Event organization

This indicator refers to the organizational skills of the personnel. It examines the number of cultural events: conferences, professional refresher courses, on-the-job training, which have been organized during the examined period. The indicator depending on whether the number of events promoted by the doctors of the examined unit is below or above the average with 3 refers to the superior ability that doctors have shown.

E3- Outpatient clinic Logistics – Laboratories

With reference to every single unit, this index aims at pointing out the managerial complexity connected to the number of outpatient clinics and laboratories. A high number of outpatient clinics compared to the arithmetic mean of those existing in the other units of the same area could indicate a heavy functional organization.

As in E1 the indicator takes into account the number of doctors operating in the outpatient clinics/laboratories and it is expressed by means of number 1-2-3. In this case index 3 means that the number of doctors using the outpatient clinics and/or laboratories is higher than the average, and, therefore, there is an evidence that the use is high, while index 1 means that their number is below the average, and this could indicate that there can be a poorly targeted use of outpatient clinics and/or laboratories. Number 2, as usual, indicates an average use of outpatient clinics and/or laboratories as regards the units of the same area to which they belong.

F - Quality Area

From a strategic point of view, checking this aspect is fundamental, since it gives indications on the market positioning of the unit within the healthcare offer that is to say on the "reputation" (or "success") built through the quality of the service performed.

Quality indications can mix, obviously together with the other managerial and operating evaluations, proper and accurate corrective and/or improvement actions.

F1 - Commendations to personnel

Within the ambit of the Quality Area the indicator "Commendations to personnel" was selected as a proportion between the number of commendations praised on an unit, obtainable from the remarks that reached the offices in charge of the relations with the public, and the number of doctors operating in the unit itself.

If the proportion obtained is above the average of reports examined for all the units belonging to the same area, it can be presumed that, according to the user's opinion, the medical personnel is able to operate satisfactorily.

On the contrary, if the proportion mentioned above is below the average, it can be presumed that the users' satisfaction with the quality of healthcare performance is

not highly satisfied. The value of the indicator therefore allows to report a critical situation or, at least, susceptible of improvement.
Similarly to what is reported above, the indicators are expressed by increasing values 1, 2, 3 from a condition below the average to one above it.
F2 - Claims against clinical activity
It provides indications about the organizational and procedural activities of an unit, through the assessment of claims against it.
The *modus operandi* for the assessment of the indicator is always the same. However in this case index 3 means that in the unit claims against doctors are lower than the average and index 1 that claims against doctors are above the average.
F3 - Litigation
It indicates the actions brought against the hospital by patients or by their families subsequently to derelictions of duty and/or malpractice by the healthcare personnel, to diagnostic and/or therapeutic errors, to negative outcome after health interventions, etc. In general, considering the fact that the litigation is presumably more significant where the concentration of activities is higher, this is another example in which the litigation is referred to the number of doctors of the unit.
Also in this event the values go from 1 to 3, where 1 means a relatively high number of legal actions, 2 an average situation and 3 a low number of legal actions.

G - Single Indicator

After having fixed the indicators concerning the six thematic areas, A B C D E F, in order to facilitate a first consideration on whether to carry out or not closer examinations of the most critical units, a single indicator has been fixed. This is able to provide a macro insight of how the single unit of a same area is placed compared to the others.
This gives an immediate indication on the opportunity to evaluate more deeply and more in detail the most critical units through the analysis of the thematic areas indicators.
The single indicator is obtained through the sum of the indicators assigned to every single unit and through the division of the sum by the number of the units of the same rea, this way the average value of the single indicator can be achieved. The examination of the unit positioning compared to such average will enable a quick assessment of the criticality status, acceptable standard and satisfactory situation.
As for the other indicators, the segment included between +/- 20% of the average value is considered as an average standard working situation.
To each thematic area examined has been given a different importance, variable from 1 to 3, in order to take into account the different importance given to them during the data analysis. In particular, importance 3 has been given to the operating area, 2 to Research and 1 to the remaining areas.

Discussion

The presented method is not limitless. First of all the health outcomes are not examined, but only their proxies [5]. Then the development of the analysis technology concerns only one part of the strategic planning process, which refers to the definition of objectives, their sharing with all the interested parties and their operationalization. This last step consists in transferring the hospital strategy to specific objectives for every single unit [1].
This work illustrates a proposal for a practical method aimed at allowing the strategic management of a complex hospital to have a sort of "Dashboard" able to provide it with an overview on the conditions of the duly aggregated units for the decision making process purpose.
The "Dashboar" takes into consideration the assessment of the activity conditions and of the units positioning, such units are aggregated by "areas", by means of a multidimensional approach fixed on six thematic areas: Strategic, Operating, Research, Economic, Organizational, Quality.
Such thematic areas can be given different scores from 0 to 1 depending on the importance attributed.
Each area is characterized by a set of three quantitative indicators, except the Strategic Area which consists of qualitative indicators, to which are given conventional scores equal to 1, 2, 3 depending on their positioning compared to the average value of the values obtained by the other units belonging to the same area.
On the basis of such data it is possible to obtain a total score for each unit, by means of which significant comparisons can be carried out among the different units.
The comparison makes possible the fast detection of points of strength, criticality or weakness, such information can be followed by possible accurate deep investigations.
The feasibility and completeness of the method are based on the availability and accessibility of data concerning the activities identified by each thematic area. Such data shall be periodically produced and validated in order to gather all information to use for the assessment of indicators. For a good use of this observation and control method employed for the managerial activity of the different units it is necessary that the data collection from the competent sources, and the management of the "Dashboard" are performed in an organized and systematic way.
This method was yet not implemented because the strategic management board of the hospital finished its agency and they were not renewed. The new named strategic management board is evaluating it.
On the other hand it is possible to adopt, where present, an automatic data processing by means of a simple application software able to process the basic data by extrapolating them directly from the software of the hospital information system.
The possibility to make decisions on the basis of the information obtained through the indicators helps to avoid the impulsive decisions which are often objectively unfounded [6].

Conclusions

The developed instrument should allow the Management to have an overall view on the conditions of the clinical units, by means of a group of significant indicators.
The priority principle in the development of this studied model is the one to use a limited number of indicators in order to allow their easy and immediate use, without requiring a high waste of time by the user for their evaluation. Those data which are necessary to constitute the indicator can be easily acquired from the routine data flows of an hospital.
The allocation of fixed resources (i.e. personnel, services, etc.) among the users/suppliers in competition with one another, from a managerial point of view it represents a challenge aimed at obtaining the best hospital performance in full obedience of the shared strategic objectives.

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The authors declare that they have no competing interests.

Authors' contributions

SC conceived, designed and coordinated the research. AB collected data. SC and AB optimized the informatics database. SC, AB and AP evaluated the results. SC and AB wrote the manuscript. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Developing a Stoma Acceptance Questionnaire to improve motivation to adhere to enterostoma self-care

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Keywords

Acceptance • Stoma self-care • Educational needs • Motivation • Educational diagnosis form • Mokken scaling

Summary

Introduction. *In stoma care, patient education is often weak in terms of improving patients' level of acceptance of living with a stoma. Self-care educational interventions in enterostomal patients, which according to Orem's Theory should take into account these patients' specific needs, require instruments that measure patients' stoma acceptance to improve motivation based on the resumption of activities they used to carry out before having a stoma.*
The aim of the study was to develop an instrument that measures the level of stoma acceptance to improve motivation to adhere to enterostoma self-care.
Methods. *Aspects that improve stoma acceptance and consequently motivation to adhere to enterostoma self-care were identified through 10 focus groups. In the focus groups, the motivation indicators were grouped, categorised and results entered into a Stoma Acceptance Questionnaire (SAQ). The SAQ was then piloted with 104 enterostomal patients from three general hospitals.*
To assess the construct validity of the SAQ, Mokken Scaling was used to explore the latent structure of the SAQ. Mokken scaling is a non-parametric method that falls under the umbrella of methods described as item response theories (IRT).
Results. *The theme "Living with a stoma"; "Autonomy"; "Support"; "Ability to deal with stoma", plus a common underlying theme: "Stoma acceptance" were dissussed by the Focus Groups. The experts identified the items of the (SAQ) through these themes. Mokken Scaling identified the "resumption of enterostomal patients' normal activities" as a measure of stoma acceptance, thus confirming the construct validity of the SAQ.*
Conclusions. *The tool proposed affords a pioneering example of how this gap can be bridged. Indeed, the SAQ could enable nurses adopting a standardized approach for the assessment of enterostomal patients' motivation to resume their normal activities and identify needs linked to this. The SAQ could also be used to measure the effectiveness of psychosocial and educational interventions aimed at improving stoma acceptance.*

Introduction

It is known that quality of life of stoma patients is negatively influenced by stoma, and the patients' positive adjustment of their perception is possible only through acceptance of the stoma [1]. However, there is evidence that nursing documentation is often weak, and in particular it lacks a consistent and standardized approach to assessing the educational needs of the enterostomal patient linked to stoma acceptance [2-4]. The documentation of enterostomal patients' educational needs could be improved by using a structured approach employing appropriate instruments. Several tools are available, such as the Ostomy Adjustment Inventory - 23 (OAI-23) [5], but these mainly measure the patient's social and psychological adjustment to stoma surgery. Instead, it would be important to measure the stoma patient's educational needs to improve motivation to adhere to enterostoma self-care.
The literature shows how greater acceptance and understanding of personal clinical conditions appear to have a positive impact on patient quality of life and reduce setbacks [6, 7]. A case in point is bowel cancer surgery, often resulting in temporary or permanent digestive stoma. Enterostomal patients need to learn to adjust to and live with altered bodily conditions and intestinal functions. Lifestyle adjustments are therefore needed, and stoma therapy nursing becomes important at this point in the patient's journey towards acceptance [8]. Stoma surgery has a negative impact on quality of life in several ways, such as altered body image, sexual activity, social life, sports and leisure activities [1, 4] and practical, pre-operative stoma training and motivational support [9] are crucial to promote patient recovery and shorter hospital stays [10]. Stoma implies acquiring awareness of and becoming accustomed to changes and restrictions in everyday life [11]. The aim of the study was to develop and validate a "Stoma Acceptance Instrument" that will offer nurses a standard tool to conduct a more uniform and comparable assessment of the needs of enterostomal patients that impact on the acceptance of their new condition, and improve their motivation to participate in their own care. This study is based on Dorothea Orem's Self-Care Theory [12] according to which successful psychological adjustment to self-care and rehabilitation can be achieved through a well-structured, thorough assessment of patient, family or caregiver needs.

Methods

A Stoma Acceptance Questionnaire was developed (Fig. 1). The development of the Stoma Acceptance Questionnaire (SAQ) involved three phases:

1) Conducting ten focus groups to define a set of indicators that improve stoma acceptance, which were used to draw up the items of the questionnaire.
2) Piloting the SAQ questionnaire with 104 patients.
3) Investigation of the aspects of SAQ construct validity.

Focus groups

The SAQ was developed through the content analysis of data collected through the focus group methodology. A total of 350 stoma care nurses were involved in a series of 10 focus groups moderated by members of a transnational panel of stoma care experts during the parallel sessions of the Italian national congress of Stoma Care Nurses in October 2010. All the focus groups were audio-recorded for transcription purposes. Each focus group lasted for approximately 1 hour to provide sufficient time for discussions on all topics in the guide.

Due to the large number of participants, data saturation was achieved in two days. The transcripts were independently analysed using NVivo, a qualitative analysis software. Glaser and Strauss's (1967) grounded theory approach, by which empirical data are thematically categorized by induction, was adopted. The themes that emerged from the 10 Focus Groups were discussed by the transnational panel of stoma care experts, who then identified the indicators, which were then used to draw up the items for the SAQ.

Piloting of the SAQ

Tool piloting was conducted in three general hospitals by a group of researchers previously trained on how to use the new assessment tool. Patient inclusion criteria were: no cognitive disorders; a good grasp of spoken Italian; age > 18.

Construct validity of the SAQ

In order to explore the construct validity of the SAQ, we used the Mokken Scale method [10]. Mokken Scaling is a method for detecting unidimensional scales within multivariate datasets; unlike factor analysis, Mokken Scaling – which is a non-parametric form of item response theory – detects sets of items that are related in a hierarchical manner and the software available for analysing Mokken Scales provides a range of parameters whereby the strength of the scale, its reliability and ordering of items can be gauged.

In particular, Mokken Scaling is a non-parametric method that falls under the umbrella of methods described as item response theories (IRT). IRT methods provide a range of parameters that help to identify congruent clusters of items – known as scales. In this sense, they are similar to classical test theory methods such as factor analysis but have the added feature that they seek clusters of items which are hierarchical; the items are ordered in terms of "difficulty" – the extent to which they are endorsed by respondents. For example, if the two items "I feel sad" and "I feel like ending my life" are presented to a group of respondents, we would assume that fewer people would endorse the latter item and this would be the more difficult of the two. Items in Mokken Scales are ordered by their mean score and Mokken Scaling allows us to see how consistently items

Fig. 1. The Stoma Acceptance Questionnaire.

ASSESSING IMPORTANCE

In your opinion, how important is privacy for the family members?

Very much *Fairly* *A little* *Not at all*

How important is it for you to resume your previous sexual activity?
How important is it for you to maintain your level of autonomy?
How important is physical activity for you?
How important is clothing for you?
How important is it for you to not be a burden for the family members?
How important is it for you to have a fixed reference point (i.e. outpatients clinic)?
How important is it for you to resume working?

ASSESSING TRUST

How much do you trust in your ability to manage your stoma autonomously?
How much do you trust in your ability to manage embarrassing situations?
How much do you trust in the support of the family members?
How much do you trust in your ability to follow an adequate diet?
How much do you trust in other people's support?
How much will you be able to accept your stoma?

PROMOTING SELF-DETERMINATION AND RESPONSIBILITY

Do you feel the need to talk with other people who have the same problem?
Now that you are aware of the various types of devices, do you think you will be able to choose/decide which is the most appropriate one for you?
Do you think you are able to manage your life habits now that you have a stoma?

scale – i.e. they are ordered consistently – and this is measured by scalability using Loevinger's coefficient H. The minimum acceptable H is 0.30 for a Mokken Scale to be present.
As reported in the results below, other parameters in Mokken Scaling assist us with decisions about the quality of scales and individual items. Data in an SPSS file were converted to a form suitable for Mokken Scaling analysis using the public domain statistical software R (http://www.r-project.org/) and package "foreign". The automated item selection procedure in the R package "mokken" was used to analyse the data for strength of scale (H), reliability (Rho) and invariant item ordering (HT). The confidence intervals of the items were also examined.

Ethics
Approval was obtained by the ethics committees of all the three public hospitals in Genoa involved in this study. All participants signed an informed consent form. Anonymity, safety and confidentiality of patient data were guaranteed and patient comfort assured.
The study period was from October to December 2010.

Results

Focus groups

The themes that emerged from the 10 Focus Groups were: "Living with a stoma"; "Autonomy"; "Support"; "Ability to deal with stoma".
For each theme, the experts identified the following indicators:

1. Living with a stoma: privacy; sexual activity; clothing; embarrassing situations; dealing with daily life.
2. Autonomy: not being a burden for others; resuming same activities before the stoma; trust in oneself; dealing with own stoma/health conditions.
3. Support: support from family members and other people, having a fixed reference point; talking with others who have the same problem.
4. Ability to deal with stoma: know how to choose and use devices; follow an adequate diet; handling sensitive issues.

These indicators were then used to build the 17 items of the SAQ (Fig. 1). A common underlying theme across all these indicators was "Stoma acceptance".

SAQ Piloting

A total of 104 patients completed the SAQ questionnaire. While completing the questionnaire they were cognitively interviewed by our group of trained researchers to identify if there were any points that needed to be clarified. 75% of all the questionnaires were considered clear and completed.

Mokken Scaling

Results of the Mokken scaling are reported in Table I. Two items did not scale and were excluded from the analysis by the automated item selection procedure; two scales were formed, one with 11 items and one with four items; only the first scale, considered here as the one with four items, was not considered useful. A moderately strong Mokken scale (scalability coefficient H > 0.40) with a moderately strong invariant item ordering (HT > 0.40) was derived. Item pair plots were generated and showed minimal intersection between items. A sensible hierarchy of items, ordered by a mean score on items, was obtained ranging from "not wishing to be a burden on the family" (item 6), most strongly endorsed, through items relating to choice and autonomy to the least endorsed items concerning "accepting stoma" and "the importance of physical activity". As the sample size was quite small for Mokken Scaling, based on some very recent work by Kuijpers [11] using marginal models to establish the utility of standard errors and confidence intervals for scalability coefficients, we used the standard errors of the item scalability coefficients (Hi) and the standard errors of the scalability coefficients for item

Tab. I. Mokken scaling of the Stoma Acceptance Questionnaire (n = 104).

Item	Label	Mean	Hi	SE
4	How important is physical activity for you?	2.51	0.48	0.054
14	How much will you be able to accept your stoma?	2.49	0.43	0.058
2	How important is it for you to resume your previous sexual activity?	2.44	0.43	0.062
10	How good do you think you are at handling sensitive/embarrassing situations?	2.43	0.52 0.051	
8	How important is it for you to resume working?	2.25	0.49* 0.055	
9	How good do you think you are at dealing with your stoma / condition autonomously?	2.22	0.55	0.046
17	Do you think you can deal with daily life now that you have a stoma?	2.12	0.50	0.059
12	How good do you think you are at handling sensitive/embarrassing situations?	2.04	0.47	0.055
16	Do you think you will be able to choose the most appropriate device for you?	2.01	0.34	0.079†
3	How important is it for you to maintain your level of autonomy?	1.48	0.47	0.065
6	How important is it for you not to be a burden for the family members?	1.31	0.42	0.079†

H = 0.47; Rho = 0.88; HT=0.41; *-item does not show IIO; SE = standard error; †-confidence interval does not include 0.30

pairs (Hij) to calculate the 95% confidence intervals for each. Since the lower-bound level of scalability is set at 0.3, the confidence interval for items should contain 0.3, whereas for item pairs, the confidence intervals should not contain 0. There was found to be insufficient evidence to support the scalability of items 6 and 16, and that of item pairs 4, 6, and 14 with item 16.

Discussion

Mokken Scaling yielded one interesting and useful scale, again providing new insight into the structure of the SAQ. The item difficulty – the extent to which items were endorsed as shown by their mean scores [10] – ran from items related to not being a burden, autonomy and choice as the most highly endorsed with items related to resuming previous activities and acceptance of the stoma being the least endorsed. This suggests that "not being a burden" and "being able to look after oneself" is most important to people with a stoma, whereas, they are less likely to accept the stoma and find it hard envisaging resuming normal activities. It is conventional to name such hierarchical scales according to the most difficult items and as such, this new scale, which has "acceptance and resumption of normal activities" at the most difficult end of the scale, could be used to measure "acceptance of a stoma". The more respondents endorse items towards the difficult end of the hierarchy, the more they are likely to be accepting the stoma and the scale has potential as a measure of psychosocial and educational interventions to help people accept their stoma. The sample size here was probably at the lower-bound limit for Mokken Scaling [12]; some items had wide confidence intervals and one did not show invariant item ordering (IIO). Unpublished work suggests that these aspects of items in a Mokken Scale are related to sample size, therefore further work with a larger sample size is recommended to test the robustness of the Mokken Scale identified here.
Some limits of this study are present as the small sample size and the need to identify additional items to add to the two smaller factors: Autonomy and normality and Trust and burden. This could increase the accuracy of the SAQ. Further another limitation could be the selection of focus group targeted exclusively to nursing workers, and not for example to patients and category associations.

Conclusions

Due to the ageing of the population and the increasing numbers of people with chronic illnesses, the focus of health systems is rapidly shifting from hospitals to primary care prevalently provided in the community [13]. However, in many countries, like Italy, nurses are still not sufficiently prepared and do not have all the tools they need to provide effective primary care in the community [14]. In order to bridge this gap, Italian academic schools need to prepare nurses for advanced practice roles by including clinical modules into their nursing master's degree programs [15]. However, in addition to being prepared, there is a lack of appropriate tools that would enable nurses to meet the chronic needs of today's ageing population [14]. The SAQ proposed in this study, can be considered a pioneering example of how this gap can be bridged.
The method we adopted to develop an instrument based on the assessment of "stoma acceptance in enterostomal patients" based on meeting the need to resume previous activities yielded new insight into the important connection between 'resuming previous activities' and 'acceptance of the stoma'. Therefore, educational and psychosocial interventions that contribute to the resumption of pre-stoma activities will also be effective in enhancing the level of acceptance of the stoma.
Therefore, the next phase of this research will be to address the limits of this study in terms of increasing the sample size and identifying additional items to add the two smaller factors (Autonomy and normality; and Trust and burden), and subsequently increase the accuracy of the SAQ that will enable to develop more effective educational and psychosocial interventions aimed at improving stoma acceptance, which positively affects adherence to self-care. In this way, it will be easier to measure not only "acceptance of stoma" but also "self-with-a-stoma" [16].

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The authors declare that they have no conflicts of interest.

Authors' contributions

AB, RW and LS conceived, designed and coordinated the research. AB, MZ and GC collected data. RW and GC performed the data quality control. RW conducted the Mokken Scaling analysis. RW evaluated the results. RW and GA wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Knowledge and training needs on built environment and indoor health of Italian public health residents: a national survey

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Keywords

Residency • Public health • Post-graduate medical education • Building hygiene • Indoor environment

Summary

Introduction. *The Working Group for Hygiene of Built Environment and the National Council of Residents of the Italian Society of Hygiene, Preventive Medicine and Public Health conducted a nation-wide survey to evaluate the knowledge and training needs on Built Environment and Indoor Health of Italian public health residents.*

Results. *The compliance was very high (52,4%), covering the totality of Italian postgraduate schools. The results underline a severe lack of theoretical formation and practical training, but also diffuse discrepancies across the country, and show a strong interest of residents on this topic.*

Conclusions. *The authors propose to adopt a national core curriculum, and suggest some strategies to improve learning.*

Introduction

In Italy, the physicians who intend to become Public Health Officers in the different fields (Environment, Infectious and chronic diseases, Nutrition, etc.) must attend a post-graduate school (residency) named "School of Hygiene and Preventive Medicine", at the end of which they can participate to public competitions to enter Public Health positions [1]. After a 6-year School of Medicine, they must pass a nation-wide selection and choose among one of the 32 schools, which are open within the Schools of Medicine (only 4 Medical Schools in Italy do not offer this postgraduate curriculum).

Each school of Hygiene is free to organize and manage classes and internships following a general guideline delivered jointly by the MIUR (Ministry of Education, University and Research) and the Ministry of Health. The activities are to be allocated in 5 academic years [1].

According to international studies, in the most economically developed countries nowadays people spend up to 90% of their lifetime in indoor environment, making it one of the major (positive or negative) determinants of health worldwide. [2-5] The occurrence and re-occurrence of pathologies related to the quality of built environment, exacerbated by the severe current socio-economic crisis, upholds once more the ultimate importance of domestic environment as primary living space [2, 7-9]. It appears therefore crucial to appropriately train highly educated and evidence-based health professionals, with regards to human-built environment and, in particular, residences [3, 10, 11].

The Working Group (WG) for Hygiene of Built Environment (Igiene Ambiente Costruito - IAC), established by the Italian Society of Hygiene, Preventive Medicine and Public Health (Società Italiana di Igiene, Medicina Preventiva e Sanità Pubblica – S.It.I.) developed a survey to evaluate the possible educational discrepancies between the Schools of Hygiene of the country. The present study is the result of the cooperation between the IAC-WG and the S.It.I. National Council of Residents. This research aimed at comparing the training needs of Italian residents with the educational courses on offer in the different Italian schools of Hygiene, in order to propose new approaches and solutions for the harmonization of the training in the field of indoor environment.

Methods

The research was designed as a cross-sectional study, and was addressed to the residents of all Italian postgraduate Schools of Hygiene and Preventive Medicine.

Using Google Docs, a short questionnaire was prepared. The study was approved by the Executive Scientific Council of the Italian Society of Hygiene, Preventive Medicine and Public Health in January 2014. The questionnaire, anonymously administered and available for online completion, consisted of section:

- demographic and academic information;
- residents' curriculum regarding building hygiene and indoor environment, and their attitude towards the topic;
- five multiple choice questions, aimed at investigating residents' knowledge about building hygiene and indoor environment, focused on dwellings as main living space [2, 3, 7, 12].

Among the questions, elaborated by the IAC-WG scientific board, [13] issues that are relevant for indoor health were explored, such as natural lighting and insulation, indoor spread of infectious diseases [8, 14-17], but also practical questions to evaluate the familiarity with laws and regulations currently in force in Italy, and others which may be enforced in a near future, European Union Directives included.

The survey was publicized through the National Mailing List and Facebook page of the National Council of Residents.

Filled questionnaires were collected at the Department of Public Health, Experimental and Forensic Medicine of the University of Pavia, and analysed using Excel 2010. To calculate a denominator, the research team esteemed the number of residents on the basis of the five Ministerial Decrees that every year set the number of residents for each university. [18-22] This number is probably overrated, due to dropouts and parental leaves. The overall proportion of right answers was calculated; it was also stratified by knowledge questions. Chi-squared was used to compare the proportions of correct answers by each question among residents who were trained or not trained in built environment hygiene.

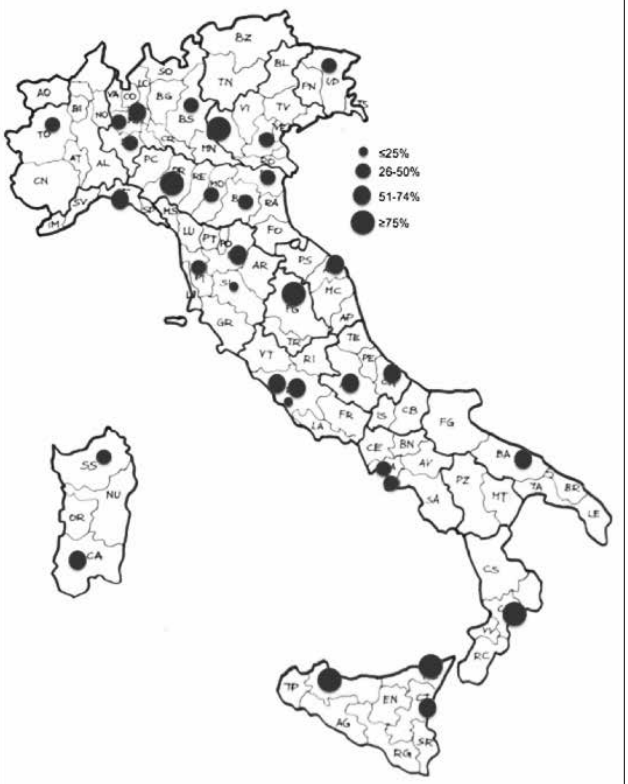
Results

All the 704 residents of the 32 Italian postgraduate Schools of Hygiene and Preventive Medicine were enrolled and received the questionnaire, and 369 (52.4%) chose to participate; participation rate of the different Schools ranged from a minimum of 23.5% to a maximum of 100% (Fig. 1).

Female gender was prevalent, accounting for 66.9% of responders. Table I shows the residents' characteristics.

Among the responders, 281 (76.1%) consider the training on "building hygiene" a very relevant issue, while others are little (83 subjects: 22.5%) or not at all (5 subjects: 1.4%) interested. With regards to the training on "building hygiene", 106 (28.7%) report a specific course (frontal teaching) in their curriculum, 101 residents (27.4%) had some lessons within a broader course, and 162 (43.9%) report that these themes were not included at all in their curriculum.

Fig. 1. Participation rates by Universities.



Tab. I. Characteristics of the respondent residents.

Gender	#	%
Male	122	33.1
Female	247	66.9
Year of course		
I year	63	17.1
II year	74	20.0
III year	96	26.0
IV year	73	19.8
V year	63	17.1
Frontal teaching in building hygiene		
In a specific course	106	28.7
Some lessons within a broader course	101	27.4
None	162	43.9
Internship in building hygiene		
I did an internship	82	22.2
No yet, but I will	70	19.0
I will not do an internship	217	58.8
Interest toward building hygiene		
Much	281	76.1
Little	83	22.5
None	5	1.4
Total	369	100.0

In terms of internship, only 152 residents (41,2%) report they did or will do an internship in this field during the post-graduated school, while 217 (58.8%) think they will never do that.
Table II summarizes the theoretical and practical curriculum of residents. 126 residents (34.1%) will never receive any kind of training in building hygiene.
Table III shows the percentage of correct answers provided for each question by the 369 responders. The lowest percentages of correct answers are those regarding the questions on normative topics, while health risks related to housing seem to be better known. The average proportion of correct answers is 3.78/5 (75.6%).
Considering that 82/369 (22.2%) residents have already had an internship in "Building Hygiene", using Chi-squared, the proportion of correct answers were compared with those of residents that had not followed any kind of training (lessons or internships) on this topic before the survey (141 residents), and this to verify if there were differences between the two groups.
Table IV shows the percentages of correct answers per each question in both groups; the overall proportions of correct answers are significantly different (78,2% vs. 71,3%; p = 0.010); more in depth, the significant differences regard overcrowding (97,6% vs. 87,2%; p = 0.007) and housing regulation (75,6% vs. 62,4%; p = 0.042).

Discussion

The nation-wide response is relatively good (52.4%), but it is not possible to affirm that this research provides a reliable result for all schools, due to the wide heterogeneity in schools coverage. In fact, response rate was very inhomogeneous, and in some universities the answer rate was really low (Fig. 1).
43.9% of respondent residents are not provided with classes on building hygiene and indoor health, both *ad hoc* or within a wider course (e.g. environmental health). We also observed that only 82 residents (22%) attended an internship and, among the remaining 288, only 70 will attend it in the future. These two data are quite worrying, because on a national scale almost 58.8% of the residents are not gaining any practical experience in building hy-

Tab. II. Curriculum in building hygiene reported by residents.

	Internship in building hygiene			
Frontal teaching of building hygiene	Never	Yes, but not yet	Yes, already done	Total
Yes, with a specific course	35	30	41	106
Yes, some lessons in a broader course	56	25	20	101
None	126	15	21	162
Total	217	70	82	369

Tab. III. Correct answers to questions about residents' knowledge.

	Total	
Questions	N.	%
1. Minimal surface requirement prescribed in Italian laws for habitable rooms	188	50.9
2. Which infection is related to overcrowding	342	92.7
3. What factors are related to indoor dampness	352	95.4
4. Requirement prescribed in Italian laws about natural lighting in habitable rooms	261	70.7
5. What are housing regulation among those proposed in the question	252	68.3
Total correct answers	1395	75.6

Tab. IV. Correct answers per each question and previous internship in "Building Hygiene".

	Internship (82)		No training (141)		
Questions	N.	%	N.	%	p
1. Minimal surface requirement	40	48,8	66	46,8	0.776
2. Overcrowding	80	97,6	123	87,2	0.007
3. Indoor dampness	77	93,9	134	95,0	0.763
4. Natural lighting	62	75,6	92	65,2	0.106
5. Housing regulation	62	75,6	88	62,4	0.042
Total correct answers	321	78,3	503	71,3	0.010

giene, and almost half of them (34.1% of residents) are not receiving any theoretical grounding in the field. On the other hand, over three quarters of the responders declared to be greatly interested on these topics, underlining their consciousness of the relevance of the themes.
Analysing the answers that dealt with theoretical knowledge, it was determined that the overall proportion of correct answers is 75.6%. It is necessary to underline that questions were very easy, investigating only basic knowledge. Biological aspects are better known than regulatory ones: in particular, respectively 92.7% and 95.4% of the residents identified (a) overcrowding as a risk factor for tuberculosis and (b) that indoor dampness influences both moulds proliferation and temperature perception. Poorer results were obtained in the other questions: 70.7% correctly indicate that natural lighting must be present in a living area, 68.3% identify the Ministerial Decree that deals with health requirements of dwellings, and only 50.9% of respondents are aware of the minimum area prescribed by law for habitable rooms (9 square metres). With regards to overcrowding and housing regulation, the researchers found a significant difference in the number of correct answers between the residents who attended an internship and those who did not, highlighting that a daily application of norms and a hands-on experience help to memorize regulatory frameworks more than just frontal teaching. This is a key point, because regulations for building hygiene are the major instrument for environmental practitioners to guarantee the highest standards in Public Health safeguard. [23-26].
The major limitations affecting the present study are essentially two. Firstly, the respondents included in the study were about half of the entire population, so the results may be not fully representative. Moreover, the low response rate (< 25%) observed in some Schools could be indicative of a selection bias, since respondents with positive personal attitude may have predominantly responded to the survey.
Finally, although all the data used in our study were collected anonymously, a potential residual social desirability bias cannot be ruled out. However, the consistency between results and internship in relation with correct answers lead us to conclude that these possible limitations only marginally affect the results of the study.
Despite the possible limitations, this is the first national study examining knowledge and training needs on built environment and indoor health among medical residents operating in one of the most populated European Country.

Conclusions

Data highlight the contribution of internship on residents' knowledge, underlining that it should be implemented on a nation-wide scale. This would be a proposal of IAC-WG, alongside with the definition of a core curriculum on building hygiene and indoor health for the post-graduate Schools of Hygiene and Public Health. The IAC-WG is elaborating a training package for residents taking into account the general considerations already expressed by our scientific society in other fields. [27, 28] The learning methods should be mainly non-formal, including problem solving strategies and cooperative learning. Priority ought to be given to:

- the analysis of real or simulated cases;
- participation to the totality of the activities within the Local Health Units (LHU), addressed to the understanding and adoption of operational procedures and regulation;
- raising the knowledge in the field of identification and reading/interpretation of the sources (scientific literature, regulations, guidelines, procedures).

Considering the role of living conditions as a major determinant of health and the current socio-economic situation, the training of Public Health Practitioners will become even more relevant in the coming years. Therefore, researchers reckon that both Ministry of Education and post-graduate Schools of Public Health need to take these issues into account.

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Author contributions

LC and DD designed the research. FC and CC acquired the data. FC, CC, MG and DD analysed the data. LC, MG, SC and DD interpreted the data. LC and DD drafted the work. MA, CC and SC revised the work.

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