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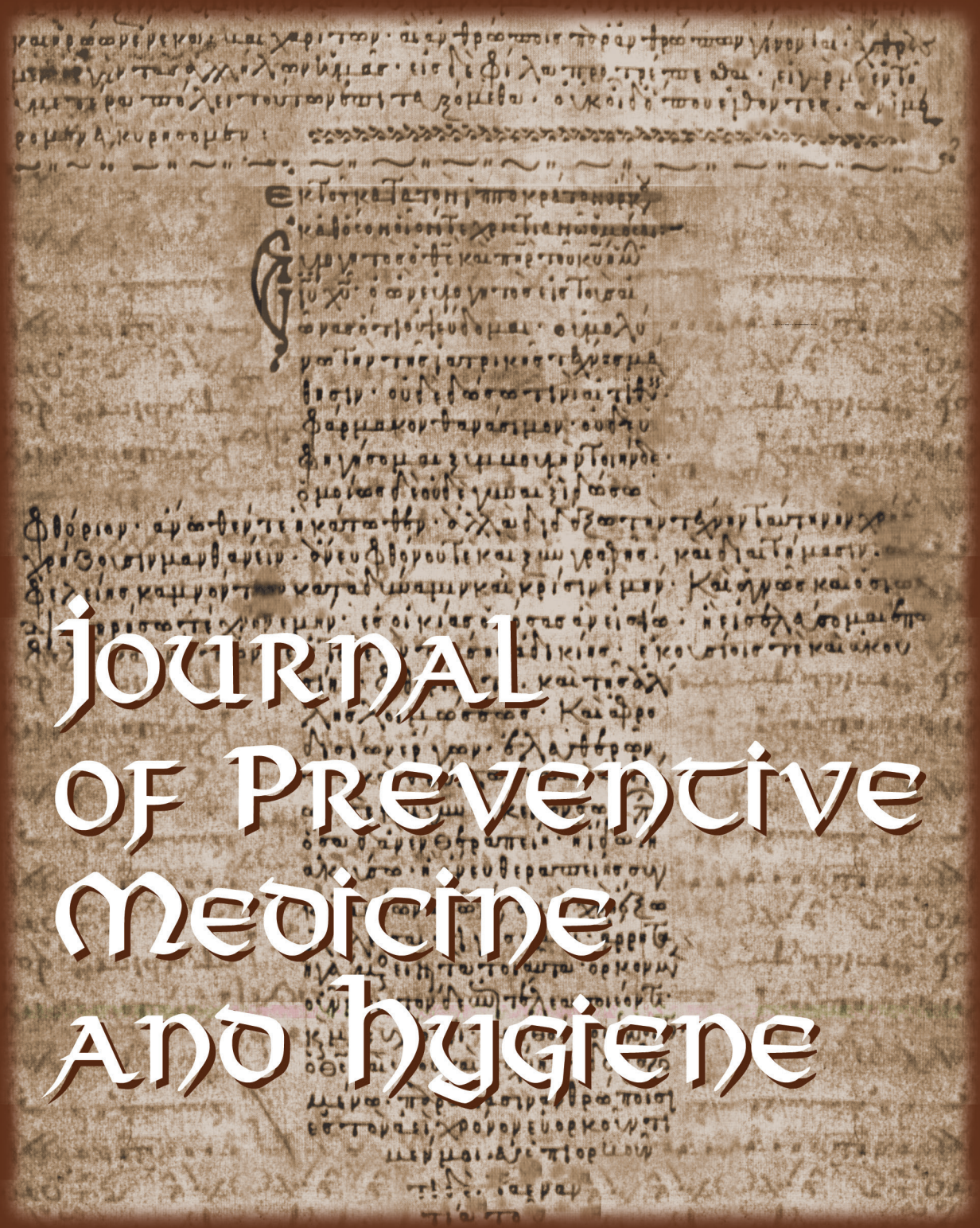

The original document of Hippocrates' oath

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CONTENTS

Case report

Detection of influenza A(H1N1)pdm09 virus in a patient travelling from Shanghai to Italy in July 2018: an uncommon clinical presentation in a non-seasonal period

F. BUTERA[1], S. SCHENONE[1], F. GRAMMATICO[1], V. TISA[1], G. BARISIONE[1], G. GUARONA[1], B. BRUZZONE[2], G. MURDACA[3], M. SETTI[3], A. ORSI[1,2]

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Keywords

Influenza • Surveillance • Travellers' infectious disease • Leukopenia

Summary

Influenza is one of the most common infectious diseases in travellers, especially in those returning from subtropical and tropical regions. In late June 2018 an influenza A(H1N1)pdm09 virus infection was diagnosed in a 36-years-old man, returned from a travel in Shanghai and hospitalized at the Ospedale Policlinico San Martino, Genoa, Italy, with a diagnosis of fever and an uncommon clinical presentation characterised by a persistent leukopenia. Phylogenetic analysis revealed a closeness with influenza A(H1N1)pdm09 strains circulating in the US in May-June 2018.

Prompt recognition of influenza infection led to a proper case management, demonstrating the crucial role of the continuous influenza surveillance programme.

Introduction

Travels are associated with an increased risk of infectious diseases, and influenza is one of the most frequent acquired infectious diseases in travellers [1].

Influenza is characterized by a seasonal pattern only in temperate climates, with a virus circulation usually observed during the cold season, coinciding with a period that lasts from November to March in the Northern Hemisphere, and from April to October in the Southern Hemisphere. In tropical areas, however, influenza virus circulates at low levels all the year round [2]. Trips by air, ship or train facilitate the viral spread, depending of the length of the trip (0-1 infections could occur during a 5 hours flight, 1-3 during an 11 hours flight and 2-5 during a 17 hours flight) and the number of passengers [3, 4]. The short incubation period and the high infectivity of influenza (basic reproduction number - R0 around 1.5) are the key reasons for the high frequency of influenza among travel-related diseases [1].

Fifty million people are estimated to travel to the tropics each year and approximately half of these presents a travel-related health problem [5-8]. The proportion of influenza in travellers returning from subtropical and tropical regions ranges between 5% and 15% as reported by different studies [3, 9-11].

Case report

A 36-years-old man, returned four days earlier from a travel in Shanghai lasted 2 weeks, accessed to the Emergency Department (ED) of Ospedale Policlinico San Martino, Genoa, Italy, on 28th June 2018 with a diagnosis of fever. The main clinical manifestation at the ED access was a hyperpyrexia started two days earlier (maximum body temperature: 39.5°C) responsive to paracetamol; associated symptoms were arthralgia, nausea and vomiting. The physical examination revealed a pharynx hyperaemia without tonsillar hypertrophy; no skin rash, headache or signs of nuchal rigidity were observed. His past medical history wasn't characterized by any disease of note. In the ED a symptomatic therapy with paracetamol (1 g/100 mL) was started.

In Table I the laboratory tests performed during patient's hospitalization are reported. The tests performed at the ED revealed leukopenia (White Blood Cell count: 3 x 10^9/L) and a modest increase in the levels of C-reactive protein (13.6 mg/L), creatinine (1.2 mg/dL), and a low decrease of potassium (3.3 mEq/L) and alkaline phosphatase (ALP, 36 IU/L). Detection of malaria antigens, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) serology, blood culture and urine culture were performed and revealed only a previous EBV infection and a Escherichia coli count in the urine of 106 CFU/

Tab. I. Laboratory tests performed during patient's hospitalization (Emergency Department and Clinical Immunology Unit, Ospedale Policlinico San Martino).

Variable	Reference range	Day 1 (ED)	Day 2 (ED)	Day 5	Day 6	Day 7	Day 8	Day 12
WBCs	4.50-9.80 x 109/L	**3**	**2.72**	**1.76**	**1.76**	**2.35**	**3.16**	4.4
RBCs	4.5-5.9 x 1012/L	5.2	5	4.9	4.6	4.5	4.5	4.9
Hemoglobin	135-175 g/L	144	141	139	**130**	**124**	131	143
Hematocrit	39-51%	44	43.1	41.3	**38.2**	**37.2**	**37.7**	42.1
MCV	80-100 fL	85.3	85.9	83.6	82.9	83.3	83	85.4
MCH	26-32 pg	27.9	28.1	28.1	28.3	27.8	28.9	29
MCHC	320-360 g/L	327	327	337	341	334	348	340
RDW	11.5-14.5%	12.8	12.8	12.2	11.8	11.9	11.8	12.6
Platelet	130-430 x 109/L	143	148	**113**	**114**	131	157	278
Neutrophils	1,80-7,80 x 109/L	2	**1.7**	**0.67**	**0.74**	**1.22**	**1.63**	2.7
Neutrophils	40,0-70,0%		62	**37.9**	41.9	51.8	51.7	61.8
Lymphocytes	1,10-4,80 x 109/L		**0.7**	**0.88**	**0.82**	**0.93**	1.22	1.4
Lymphocytes	19,0-48,0%		24.4	**49.9**	46.9	39.7	38.5	32.2
Monocytes	0,20-0,96 x 109/L		0.4	**0.07**	**0.07**	**0.08**	**0.09**	0.2
Monocytes	3,4-12,0%		**13.2**	4	3.8	**3.2**	**2.8**	4.5
Eosinophils	0,00-0,50 x 109/L		0	0.02	0.03	0.06	0.12	0.1
Eosinophils	0,0-8,0%		0.1	0.9	1.9	2.7	3.8	1.3
Basophils	0,00-0,20 x 109/L		0	0.02	0.03	0.02	0.02	0
Basophils	0,0-1,5%		0.3	1.1	**1.6**	0.7	0.7	0.2
LUC	0,0-4,0%			**6.2**	3.9	1.9	2.4	
Reticulocytes	0,7-1,7 x 100 RBCs				0.3	0.5	0.6	
Creatinine	0,67-1,17 mg/dL	**1.2**	**1.2**	1	1			
Prothrombin time	% (10-13 sec)	73	82	101	101			
Prothrombin time INR	0.80-1.20	**1.24**	1.15	0.99	0.99			
Activated partial thromboplastin time	28,0-40,0 sec	34.8	33.7	33.2	31.9			
Total bilirubin	0.20-1.20 mg/dL	0.46	0.35	0.30	0.27			
Sodium	135-150 mmol/L	136	137	141				
Potassium	3,5-5 mmol/L	**3.3**	3.5	4				
Chloride	90-120 mEq/L		99	102				
Calcium	8,5-11,0 mg/dL		**8.4**	9.1				
Phosphorus	2.5-4.5 mg/dL		3.7	3.8				
Magnesium	1.9-2.5 mg/dL		2.1	2.1				
Glucose	65-110 mg/dL	97	96					
Albumin	34-50 g/L		36.4		40.3			
C-Reactive Protein	0-5 mg/L	**13.6**	**9.6**		3.2	3.2		
ALP	50-116 U/L	**36**	**38**	**30**	**28**			
ALT	0-40 U/L	26	23	18	16			
gGT	11-50 U/L	13		19	16			

WBCs: White Blood Cells count. RBCs: Red Blood Cells count. MCV: Mean Corpuscular Volume. MCH: Mean Cell Hemoglobin. MCHC: Mean Corpuscular Hemoglobin Concentration. RDW: Red cell Distribution Width. LUC: Large Unsustained Cells. ALP: ALkaline Phosphatase. ALT: ALanine aminoTranspherase. gGT: gamma GlutamylTranspherase. INR: International Normalized Ratio.
Values out of range are in bold and underlined.

mL. The latter wasn't treated because asymptomatic. No alterations were visible on the chest X-ray performed on 28th June.

Upon admission at Clinical Immunology Unit, Department of Internal Medicine of Ospedale Policlinico San Martino, the patient was substantially asymptomatic except for a mild non-productive cough. In fact the fever, after the peak at 38.5°C on 28th June at ED, slowly decreased. Leukopenia was still present (from 28th June to the discharge at 10th July) and characterized by low levels of neutrophil granulocytes (0.67 x 10^9/L at the nadir on 2nd July) and also a reduced reticulocyte count was observed from 3rd July (0.3%). Only on-demand therapy with paracetamol and metoclopramide was introduced.

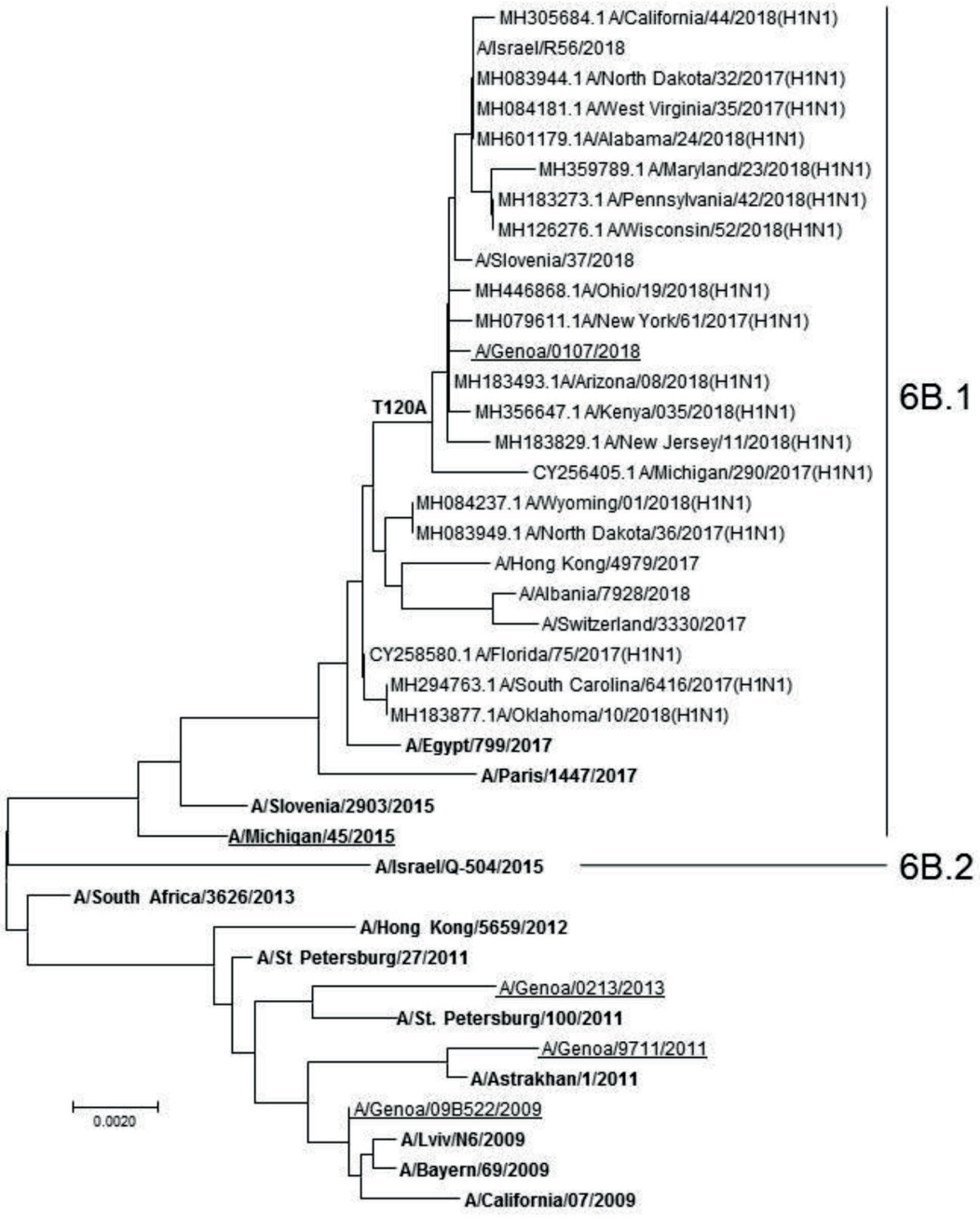
A series of laboratory tests were performed during hospitalization: blood cultures, sputum cultures, detection of Streptococcus pneumoniae and Legionella antigens in urine, parasites tests, detection of malaria antigens in blood samples, serological markers of infection by hepatitis A (HAV), B (HBV) and C (HCV), human immunodeficiency virus (HIV), Zika virus, Parvovirus, Salmonella typhi and paratyphi, Brucella, Proteus,

Schistosoma and Leishmania. Nasopharyngeal swab for respiratory viruses and bacteria was performed for the first time on 2nd July and resulted positive on 4th July, revealing the presence of influenza A(H1N1)pdm09 virus and Haemophilus influenzae.

Antiviral therapy with oseltamivir (150 mg/day) was started on 4th July and the patient was concurrently isolated. The general outcome was good and the patient was discharged at the end of antiviral therapy on 10th July, with a diagnosis of influenza A(H1N1)pdm09 infection and transient neutropenia probably due to infection. The patient received the recommendation to limit contact with people at greater risk (cardiopathic, pneumopathic, elderly in general, immunocompromised people, pregnant women, infants) and to wear the mask until the result of the nasopharyngeal swab performed at the discharge. This last resulted negative three days later, on July 13th.

Phylogenetic analysis of the virus strain were performed as previously described [12] between July and August 2018 and revealed a similarity with viruses isolated in the US in May and June 2018 and characterized by T120A mutation (Fig. 1).

Fig. 1. Phylogenetic analysis of the hemagglutinin (HA) nucleotide sequences from influenza A(H1N1)pdm09 viruses. The phylogeny tree was generated by the neighbor-joining method with 1000 bootstrap replicates. Ligurian strains (underlined), WHO recommended candidate vaccine for 2017/2018 and 2018/2019 Northern Hemisphere influenza season (bold, underlined) and reference strains (bold).



Discussion

Worldwide, in the period June-July 2018 seasonal influenza type A viruses accounted for the majority of detections. A(H1N1)pdm09 prevailed over A(H3N2) viruses. In temperate zone influenza activity was at inter-seasonal levels, whereas in tropical countries of Central America, South America, African region and Asia influenza activity remained low in the same period. In particular virological surveillance in China from week 18 to week 31 of 2018 revealed that influenza subtype A(H1N1)pdm09 virus was the most frequent detected [2]. However, no influenza virus isolation has been notified in Shanghai in the same period [13].

Influenza infection must be taken into account in differential diagnosis in every season and in any patients with acute respiratory disease coming from an intercontinental travel, even though with an atypical clinical presentation. In our case, the diagnosis of influenza infection settled diagnostic doubts, therefore providing the better patient management.

Continuous virological surveillance allows constant monitoring of circulating influenza viruses, representing a key tool for characterization and selection of influenza strains to be included in vaccine composition, as well as for potential detection of new viral strains, including those carrying a pandemic risk.

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Conflict of interest statement

None declared.

Authors' contributions

FB analysed data, wrote, drafted and revised the manuscript. SS, FG and VT advised on analysis, wrote, drafted and revised the manuscript. GB and GG performed virus detection and genotyping and revised the manuscript. BB coordinated laboratory activities. GM and MS coordinated clinical activities and revised the manuscript. AO supervised virological analysis and revised the manuscript. All the authors reviewed and approved the final manuscript.

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ORIGINAL ARTICLE

Impact assessment of an education course on vaccinations in a population of pregnant women: a pilot study

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Keywords

Vaccine • Pregnancy • Hesitancy • Education

Summary

Introduction. *Although the benefits of vaccinations have been extensively demonstrated, vaccination coverage remains unsatisfactory as result of many people's poor knowledge and negative perception of vaccination.*
We evaluated the impact of an education course on vaccinations in a population of pregnant women.
Methods. *A total of 214 pregnant women were invited to participate in this project, which was undertaken at the Obstetrics and Gynaecology Department of Careggi University Hospital in Florence (Italy). Anonymous questionnaires were administered to women before and after the intervention.*
A descriptive and statistical analysis was carried out in order to compare the responses obtained before and after the intervention.
Results. *Adherence to the initiative was good (98%): initially, the respondents were not hostile to vaccinations, though many (43%) were poorly or insufficiently informed. The educational intervention had a positive impact. After the intervention, the number of women who rated their level of knowledge of vaccinations as poor or insufficient had decreased by 30% and the number of "hesitant" respondents had decreased with respect to all aspects of the study, especially the decision to be vaccinated during pregnancy.*
Conclusions. *Hesitancy stems from a lack of accurate information. Healthcare professionals need to improve their communication skills. Appropriate education during pregnancy, when women are more receptive, may have a highly positive impact. These observations need to be considered in the planning of courses to prepare pregnant women for delivery also in other maternal-foetal centres in Italy.*

Introduction

The introduction of vaccination into clinical practice has significantly helped to reduce infectious diseases. However, paradoxically, vaccinations have become "victims of their own success". Indeed, following the disappearance of infectious diseases as a result of the effectiveness of immunization programmes, attention has turned to rare side-effects, which have been blown up and distorted to create alarming news bereft of any scientific validity. Today, these attitudes and fears are amplified through the web in an uncontrolled way [1, 2]. The unavoidable result has been a gradual decline in vaccination coverage for some infectious diseases that were under control for many years [3].
Nowadays, it is common to find (not only on the web) information with little or no scientific basis. Such information is usually in contrast with the principles of vaccinology. This misinformation has led to mistrust and scepticism towards vaccination, which demonstrates that the media play a key role in channelling health-related information and affect parents' decisions about having their children vaccinated. Health authorities should therefore broadcast evidence-based preventive medicine messages through the media [4, 5].
"Vaccine hesitancy" is defined as delayed administration or refusal of vaccination, despite the availability of vaccination services and effective vaccines [6-8].
The WHO Strategic Advisory Group of Experts (SAGE) on Immunization has demonstrated the negative impact that "vaccine hesitancy" has on achieving pre-established targets, which are mainly measured in terms of vaccination coverage [9]. In order to recognize, monitor and correctly address vaccine hesitancy, and to promptly respond to anti-vaccination lobbies in the case of misinformation, it is important to develop institutional procedures and health policy acts - such as those recently introduced in Italy - that impose mandatory vaccination for infants as a prerequisite to school attendance [10]. However, these strategies must be combined with correct information campaigns that have solid scientific foundations.
Pregnant women are particularly interested in obtaining information regarding the health of the unborn, and the prevention of infectious disease by means vaccines is of particular interest to them.
Two vaccinations are recommended during pregnancy: one against pertussis (with combined vaccines including diphtheria and tetanus - TDPa) and the other against influenza, in the case of pregnancy during the flu season. These two vaccinations are safe and protect the mother

(influenza vaccine) and her baby in the first six months after birth (TDPa) [11, 12]. All major scientific societies recommend these vaccinations during pregnancy [13-17]. Both during pregnancy and after the birth, the territorial healthcare service accompanies the expectant mother / couple from conception through the first year of life of the child, providing support and ensuring the continuity of care; information to encourage vaccination can be provided in this context, since health promotion interventions conducted "around" the time of birth are internationally recognized to be among the best in terms of efficiency and effectiveness.

The aims of the current pilot study were: to evaluate pregnant women's knowledge of and attitudes towards vaccination, their sources of information on vaccination, and the impact of an educational intervention carried out by experts on vaccination.

Methods

The study was approved by the Ethics Committee of the University of Florence.

Pregnant women at different gestational ages, who were referred to the Obstetrics and Gynaecology Department at the University of Florence from October 2017 to May 2018 in order to attend either childbirth preparation courses or prenatal diagnostic counselling on congenital defects, were invited to participate in the study.

Participants gave their informed consent to participate in the study. Each participant agreed to:

- fill in a "pre-intervention" questionnaire (supplementary file 1);
- listen to an informative and educational intervention on the prevention of infectious diseases carried out by highly qualified doctors of the Department of Health Sciences of the University of Florence;
- fill in a "post-intervention" questionnaire (supplementary file 2).

The "pre-intervention" questionnaire consisted of two sections. The first concerned the woman's knowledge of and attitudes toward vaccinations and, in detail, the Italian vaccination schedule [10, 18]. The second section included personal information (age, country of origin and educational qualification).

The "post-intervention" questionnaire was identical to the "pre-intervention" one, except for those items (e.g. personal information) which could not be influenced by the intervention.

The 30-minute interventions on vaccine prevention were conducted by experts on vaccinations. The interventions were supported by the use of a set of slides, the paper version of which was then distributed to each participant. The topics covered were: what a vaccine is; how a vaccine works; the "herd immunity" effect; vaccine contraindications and risks; the National Plan for Vaccine Prevention (PNPV) 2017–2019 (explained in detail); the success of vaccines; recent epidemics; false myths; vaccines during pregnancy; the law on obligatory vaccinations; and advice on how to obtain correct information.

A descriptive analysis of the sample was carried out in terms of the participants' socio-demographic characteristics, obstetric history, previous vaccination experience and sources of information. With regard to questions on the level of concern about infectious diseases, we dichotomized answers into two levels: no/low/moderate concern *vs* high/very high concern.

A statistical analysis of paired data was performed by means of Stata 12, and was applied only to those questionnaires filled in on both occasions (pre- and post-test). We evaluated the impact of the educational intervention on two sets of questions: the first contained questions on the women's intention to be vaccinated during pregnancy and to have their children vaccinated, and their opinion of mandatory vaccinations; the second included questions that investigated their opinion of the most frequent fake news about vaccines. To do this, we first assigned a variable score to each answer: "0" to answers against vaccines; "1" to neutral/hesitant answers; "3" to answers in favour of vaccines. The variable score "2" is not expected. We then inserted the scores of the individual replies into each of the two above-mentioned sets of questions in the pre- and post-questionnaires. We calculated the average of the pre- and post-scores for these two sets of questions and, to conclude, we performed a paired-sample t-Test to compare the two averages; subjects who answered only one of the two questionnaires were excluded from the analysis.

Results

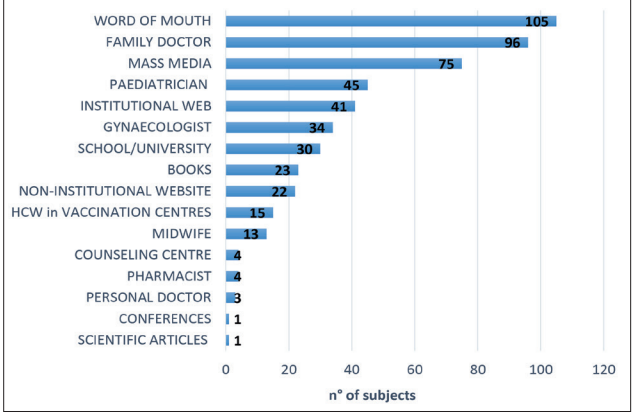
Of the 214 pregnant women invited to participate in the survey, 210 (98%) accepted and signed the informed consent form: 201 (96%) of these completed both pre- and post-questionnaires.

The sample consisted of 198 Italian women (94%) and 12 foreign women (6%). The average age of the participants was 34 years. Educational qualifications were specified by 163 women: 73 (45%) were graduates; 50 (31%) had a high school diploma; 29 (17%) had obtained post-graduate qualifications; 7 (4%) had a professional diploma; 3 (2%) a middle-school diploma; and 1 (1%) an elementary school diploma.

The questions regarding previous vaccination experiences and sources of information on vaccinations showed that 60 women (29%) knew which vaccinations they had had during their lifetime. Of the women who had already had children (44), 36 (82%) reported that their children had had all the vaccinations proposed, while eight stated that their children had only had some of these vaccinations.

The most common sources of information were word of mouth (friends, family, etc.) (50%), the family doctor (45.7%), and the traditional mass media (TV, radio, newspapers) (35.7%). Paediatricians and gynaecologists were sources of information in 21.4% and 16.2% of cases, respectively. Moreover, 19.5% of the participants consulted institutional websites to retrieve information on vaccinations (Fig. 1).

Fig. 1. Sources of information on vaccinations.



During the pre-intervention phase, the quality of the information received from health professionals was judged to be excellent (9%), good (31%), sufficient (31%), insufficient (17%), and scarce (11%).

The results of the educational intervention in terms of knowledge of the recommended/mandatory paediatric vaccinations are shown in Table I.

Table II reports the pre- and post-intervention percentages of women with the highest level of fear of vaccine-preventable diseases.

Table III reports the pre- and post-intervention answers to questions on the women's intention to have their neonates vaccinated and to receive the recommended vaccination during pregnancy, and on their opinion of mandatory vaccination.

The percentage of pregnant women who disagreed with the introduction of mandatory vaccinations for school attendance was not changed significantly by the educational intervention; however, the percentage of hesitant future mothers decreased from 19% to 9% (Tab. III).

The average score on the items concerning the women's intention to be vaccinated during pregnancy and to have their children vaccinated was 35.46 before the intervention (95% CI 33.62-37.30) and 42.57 (95% CI 41.31-43.82) after the intervention. The paired-sample t-Test showed significant differences between the mean pre-intervention and post-intervention scores ($t = 7.36$, $p < 0.001$).

The participants' self-assessment of their level of knowledge of vaccinations changed significantly with the educational intervention, as shown by the reduction (from 43% to 13%) in answers indicating a low level of knowledge (poor/insufficient level).

Table IV shows the effectiveness of the educational intervention in modifying attitudes towards some of the most frequent fake news regarding vaccination. The average score on the questions that investigated the women's opinion of the most frequent fake news about vaccines was 17.45 before the intervention (95%CI 16.51-18.39) and 22.47 (95%CI 21.63-23.32) after the intervention. The paired-sample t-Test showed a significant difference between the two averages ($t = 10.61$, $p < 0.001$).

Tab. I. Pre- and post-intervention knowledge of recommended/mandatory paediatric vaccinations.

Vaccination	**Pre (%) N = 210**	**Post (%) N = 201**	Post – Pre %
Diphtheria	103 (49)	179 (89)	**+ 40**
Tetanus	151 (72)	179 (89)	**+ 17**
Pertussis	142 (68)	180 (90)	**+ 22**
Poliomyelitis	116 (55)	157 (78)	**+ 23**
Hib	30 (14)	154 (77)	**+ 63**
Hepatitis B	99 (47)	174 (87)	**+ 40**
Hepatitis A	57 (27)	26 (13)	**- 14**
Measles	163 (78)	181 (90)	**+ 12**
Rubella	145 (69)	165 (82)	**+ 13**
Mumps	102 (49)	148 (74)	**+ 25**
Varicella	115 (55)	147 (73)	**+ 18**
Men B	98 (47)	150 (75)	**+ 28**
Men C	103 (49)	141 (70)	**+ 21**
Pneumococcus	58 (28)	123 (61)	**+ 33**
HPV	13 (6)	57 (28)	**+ 22**
Influenza	15 (7)	40 (20)	**+ 13**
Tuberculosis	35 (17)	24 (12)	**- 5**
Rotavirus	29 (14)	127 (63)	**+ 49**

The recommended/mandatory paediatric vaccinations according to Italian Vaccine Prevention Plan 2017-2019 are reported in bold.

Tab. II. Percentage of women highly concerned about vaccine-preventable diseases before and after the educational intervention.

Disease	High concern (%)	
	Pre N = 210	Post N = 201
Diphtheria	23	38
Tetanus	37	42
Pertussis	39	50
Poliomyelitis	37	40
Hepatitis B	43	47
Measles	32	47
Rubella	26	36
Mumps	20	36
Varicella	23	33
Hib meningitis	63	62
Men C meningitis	68	69
Men B meningitis	67	68
Pneumococcus meningitis	61	63
Rotavirus	21	29

Discussion

This study yielded information on pregnant women's knowledge about vaccination preventable infectious diseases and their attitude towards vaccinations. Moreover, it provided a measure of the impact and the effectiveness of an educational intervention held by health personnel. Indeed, this survey was conducted by administering pre- and post-intervention questionnaires. Questionnaires filled in before and after the educational intervention were subjected to a comparative assessment of the same answers.

Tab. III. Pre- and post-intervention answers concerning the intention to vaccinate the future neonate, to receive the recommended vaccinations during pregnancy and opinions on mandatory vaccination.

	Yes n° (%)		No n° (%)		Don't know n° (%)		No Response n° (%)	
	Pre N = 210	Post N = 201	Pre N = 210	Post N = 201	Pre N = 210	Post N = 201	Pre N = 210	Post N = 201
For which of the following diseases do you want to have your child vaccinated?								
Diphtheria	139 (66)	177 (88)	4 (2)	3 (1.5)	50 (24)	14 (7)	17(8)	7 (3.5)
Tetanus	163 (78)	184 (92)	2 (1)	2 (1)	29 (14)	9 (4)	16 (7.6)	6 (3)
Pertussis	146 (70)	183 (91)	7 (3)	1 (0.5)	40 (19)	11 (5)	17 (8)	6 (3)
Hepatitis B	160 (76)	187 (93)	1 (0.5)	0 (0)	33 (16)	7 (3)	16 (7.6)	7 (3.5)
Hib	110 (52)	174 (87)	12 (6)	2 (1)	71 (34)	17 (8)	17 (8)	8 (4)
Poliomyelitis	152 (72)	171 (85)	3 (1)	4 (2)	39 (19)	20 (10)	16 (7.6)	6 (3)
Measles	146 (70)	180 (90)	8 (4)	2 (1)	39 (19)	14 (7)	17 (8)	7 (3.5)
Mumps	136 (65)	174 (87)	8 (4)	1 (0.5)	48 (23)	21 (10)	18 (8.5)	7 (3.5)
Rubella	142 (68)	174 (87)	7 (3)	3 (1.5)	43 (20)	19 (9)	18 (8.5)	7 (3.5)
Varicella	134 (64)	163 (81)	14 (7)	6 (3)	44 (21)	24 (12)	18 (8.5)	8 (4)
Pneumococcus	142 (68)	178 (89)	2 (1)	2 (1)	49 (23)	14 (7)	17 (8)	7 (3.5)
Men B	155 (74)	185 (92)	1 (0.5)	0 (0)	37 (18)	11 (5)	17 (8)	5 (2.5)
Men C	162 (77)	138 (69)	1 (0.5)	12 (6)	30 (14)	44 (22)	17 (8)	7 (3.5)
Rotavirus	99 (47)	138 (69)	15 (7)	12 (6)	80 (38)	44 (22)	17 (8)	7 (3.5)
Pertussis and influenza vaccination are recommended during pregnancy. Would you be willing to be vaccinated?								
	72 (34)	130 (65)	67 (32)	33 (16)	68 (32)	37 (18)	3 (1.4)	1 (0.5)
Do you agree with the introduction of mandatory vaccines for school attendance?								
	142 (68)	160 (80)	24 (11)	21 (10)	40 (19)	19 (9)	4 (2)	1 (0.5)

Tab. IV. Attitudes of women towards the most frequent fake news about vaccinations.

	Yes		No		Don't know		No response	
	Pre*	Post°	Pre*	Post°	Pre*	Post°	Pre*	Post°
The effectiveness of the vaccines has been scientifically proven	174	190	6	5	30	4	/	2
Autism could be caused by vaccinations	25	9	92	169	90	21	3	2
The substances contained in the vaccines are dangerous for humans	24	12	118	169	68	17	/	3
Unvaccinated children are more resistant to infections	25	19	143	160	42	19	/	3
The administration of multiple vaccines at the same time may be harmful to my child's health	57	32	66	141	78	23	9	5
The side effects of the vaccines worry me	83	56	70	115	48	25	9	5
Vaccines are mainly a lucrative business for the pharmaceutical industries	34	17	111	148	56	32	9	4
I have seen / heard of severe side effects of the MPR vaccine	41	36	70	125	90	32	9	8
I have seen / heard of severe cases of measles	100	110	30	54	70	29	10	8
I prefer my child to catch measles naturally rather than to be vaccinated	15	18	142	162	44	16	9	5

Legend: Pre*= 210 women; Post°= 201 women.

Participation in the initiative was very good and "compliance" was excellent, in that the vast majority of participants answered all the questions. The level of education of the women enrolled ranged from medium to high. The most used sources of information about vaccinations were word of mouth and the mass media, while general practitioners and specialists (paediatricians and gynaecologists) were rarely consulted. These results are in line with census data on parents aged between 35 and 55 years [19].

The educational intervention carried out in our project had a positive impact: after the intervention, the percentage of women who considered their level of knowledge

of vaccines to be poor or insufficient had decreased significantly.
Prejudices against vaccinations during pregnancy, due to false beliefs, emerged from the comparative assessment of questions relating to the intention to be vaccinated during pregnancy. Indeed, the percentage of women favourable to vaccinations significantly increased, underlining the effectiveness of a qualified educational approach. Furthermore, before the educational intervention, answers to the question regarding experiences of post-vaccination side-effects revealed the existence of many false beliefs about vaccines. Indeed, 32 women (15%) claimed to have had direct or indirect personal experience of one or more post-vaccination side-effects, specifically, autism, pain, meningitis, measles, malaise, swelling, fever, vomiting, drowsiness, visual disturbances, deafness, fainting, arm stiffness, adenoid problems, psychomotor deficiency, diarrhoea, poliomyelitis, fulminant leukaemia, and arm infection. In the post-intervention questionnaire, however, the reported number of personal negative experiences, direct or indirect, following the MMR vaccination was seen to have decreased. In theory, this should not have been influenced by the intervention; the higher number of experiences reported in the pre-intervention questionnaire was therefore probably due to conditioning linked to false beliefs or "fake news" rather than to real personal experiences.
The safety of vaccinations during pregnancy has been clearly demonstrated by many scientific studies worldwide. Despite this evidence, vaccination hesitancy among pregnant women is still high, and stems from a lack of accurate information, probably as a result of the use of unqualified information sources. Indeed, regarding the taboo against vaccines in pregnancy, De Martino affirms that the recommendations of healthcare providers are the keystone of vaccination uptake [20]. Moreover, a study conducted in 2016 revealed that increasing vaccination coverage against pertussis among pregnant women depends not only on recommendation by physicians, but also on educational interventions and campaigns to promote maternal immunization [21]. Indeed, in line with the literature data, the percentage of pregnant women in our study who were positively oriented toward vaccination increased after the educational intervention.
Our respondents' answers to the question concerning mandatory vaccination for school attendance confirm that providing correct information reduces maternal vaccination hesitancy, encouraging subjects to adopt a pro-vaccination stance; however, it does not significantly influence the no-vaccine position [22, 23]. These findings are in line with the results of a 2018 multi-centre study, which demonstrated that it is necessary to strengthen the quality of information and confidence in health professionals in order to increase the acceptance of mandatory vaccines and reduce vaccine hesitancy [24].
Our sample consisted of women who were generally positively oriented toward vaccinations, but whose information was deficient; these gaps were significantly filled by the educational intervention, as was shown by the comparative evaluations of the pre- and post-intervention answers . The efficacy of our intervention is demonstrated by the fact that those women who were positively oriented toward vaccinations before the intervention did not subsequently change their position, while those who were hesitant became more confident.
Other studies have investigated knowledge and attitudes regarding vaccinations among parents of young children [25, 26] or among future parents [27]. On the basis of self-reported vaccination status, Giambi et al. classified parents in three categories: a) pro-vaccination; b) hesitant; and c) anti-vaccination. The three groups were compared in terms of attitudes, beliefs, and sources of information, in order to identify the profiles of these three categories. Vaccine safety was perceived as a concern by all parents, and more so by hesitant and anti-vaccination parents. Like pro-vaccination parents, hesitant parents considered vaccination an important preventive tool and trusted their family paediatricians, which suggests that they could benefit from appropriate communication interventions. Training health professionals and providing homogenous information on vaccinations, in line with national recommendations, are crucial, in order to respond to the concerns of these parents. It is therefore important to know the characteristics of the individual categories, as this knowledge can help to guide appropriate educational programmes. Indeed, health professionals need to identify what drives parents' decisions concerning the vaccination of their children, in order to communicate more effectively with them [28-31].
A limitation of the present study is that the timing of each questionnaire in relation to the stage of pregnancy was not recorded; this information would have allowed the researchers to evaluate the possible influence of gestational age on the respondents' replies.
Further studies will be performed in order to explore the socio-demographic characteristics of the study sample in greater depth and to evaluate the effectiveness of the intervention on specific population groups.

Conclusions

The present study not only provides an overview of the state of knowledge of future mothers, but also demonstrates that a qualified intervention is able to modify and reduce vaccine hesitancy. Educational interventions held during pregnancy, when women are more receptive, may have a highly positive impact on lifetime attitudes towards vaccination.
In order to increase the sample size and to confirm the results of this pilot study, it would be useful to involve other maternal-foetal centres in Italy, the ultimate aim being to improve the health of both women and children.

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Conflict of interest statement

None declared.

Authors' contributions

AB, SB, DA and DP conceived and designed the study. AM, FP, GS and VS collected data. AB, SB, AM, FP carried out the educational interventions. GS and AM performed statistical analysis and interpreted results. AB, SB, PB, AM and DP participated in drafting the article or revising it critically for important intellectual content. All authors gave their final approval of the manuscript.

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ORIGINAL ARTICLE

Vaccination coverage in healthcare workers: a multicenter cross-sectional study in Italy

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Keywords

Healthcare workers • Vaccination coverage • Vaccines

Summary

Introduction. *In recent years, a phenomenon known as "vaccine hesitancy" has spread throughout the world, even among health workers, determining a reduction in vaccination coverage (VC).*
A study aimed at evaluating VC among healthcare workers (HCWs) in 10 Italian cities (L'Aquila, Genoa, Milan, Palermo, Sassari, Catanzaro, Ferrara, Catania, Naples, Messina) was performed.
Materials and methods. *Annex 3 of the Presidential Decree n. 445 of 28 December 2000 was used to collect information on the vaccination status of HCWs. The mean and standard deviation (SD) were calculated with regard to the quantitative variable (age), while absolute and relative frequencies were obtained for categorical data (sex, professional profile, working sector, vaccination status). The connection between VC and the categorical variables was evaluated by chi-square method (statistical significance at $p < 0.05$). The statistical analyses were performed by SPSS and Stata software.*
Results. *A total of 3,454 HCWs participated in the project: 1,236 males and 2,218 females.*
The sample comprised: physicians (26.9%), trainee physicians (16.1%), nurses (17.2%) and other professional categories (9.8%). Low VC was generally recorded. Higher VC was found with regard to polio, hepatitis B, tetanus and diphtheria, while coverage was very low for measles, mumps, rubella, pertussis, chickenpox and influenza (20-30%).
Conclusions. *This study revealed low VC rates among HCWs for all the vaccinations. Measures to increase VC are therefore necessary in order to prevent HCWs from becoming a source of transmission of infections with high morbidity and/or mortality both within hospitals and outside.*

Introduction

Although vaccination is widely considered to be an efficacious and cost-effective health technology, the phenomenon known as 'vaccine hesitancy' is spreading, not only among citizens, but also among healthcare workers (HCWs), with a consequent steady reduction in vaccine coverage (VC) [1-8]. This is a serious health problem, as HCWs may spread infections to patients, colleagues and relatives. Indeed, low VC among HCWs can lead to dangerous outbreaks of disease, reduce productivity and increase absenteeism [9-11]. HCWs are therefore a priority target group for vaccinations [12-14].
Inadequate vaccination coverage among HCWs is a major concern for all national healthcare organizations. The World Health Organization (WHO) estimates that approximately 59 million HCWs worldwide are potentially exposed to hazardous biological agents daily [13]. In Europe, the average VC among HCWs is very low [3]. As recommended by the WHO Strategic Advisory Group of Experts (SAGE), vaccine hesitancy, and thus VC, need to be evaluated both globally and locally [1, 2].
The 2017-2019 Italian National Immunization Prevention Plan (INIPP) strongly recommends that HCWs be vaccinated [14]. However, although data are not systematically available, VC among HCWs is estimated to be very low. Indeed, only few studies have been conducted on this issue, and these have concerned a limited number of hospitals and are clearly not representative of the national scenario in Italy. One systematic review regarding Italy revealed suboptimal VC for hepatitis B and measles, and very low VC for varicella (3.6%) and influ-

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enza (10-25%) [15]. Similarly, a study from the Puglia region found tetanus-diphtheria vaccination uptake to be only15.5% [16]. Another study, published in 2015, reported an influenza vaccination uptake of 16% among HCWs [17].
In 2017, in accordance with the INIPP, a committee of Italian experts drafted "The Pisa Charter of Vaccinations", which identified the main pillars of a strategy to boost VC among HCWs [18].
Law 119/2017, concerning "Urgent provisions on vaccination prevention, infectious diseases and disputes related to the administration of drugs" [19], and Ministerial Circular 25233 of 16 August 2017 required that data be collected on the vaccination status of workers in schools, healthcare and social care. Indeed, as required by Presidential Decree 445 of 28 December 2000, teachers, social workers and HCWs must submit a report of their vaccination status to the institutions for which they work. However, despite these legal measures, vaccinations have not become mandatory for HCWs. Hence, healthcare professionals are under no legal obligation to be vaccinated [20-22].
The aim of our study was to investigate the VC of HCWs at several academic hospitals in Italy.

Materials and methods

A multicenter cross-sectional study was conducted from March to September 2018 in the following 10 cities: L'Aquila, Genoa, Milan, Palermo, Sassari, Catanzaro, Ferrara, Catania, Naples and Messina.
Ethical approval was obtained from the Ethics Committee of the University Hospital "AOU G. Martino di Messina". Written informed consent was obtained from all subjects before participation in the study.
Annex 3 of Presidential Decree n. 445 28 December 2000 was used to collect information on the vaccination status of HCWs [22]. Statistical analysis of the parameters considered relevant was performed. The mean and standard deviation (SD) were calculated with regard to the quantitative variable (age), while absolute and relative frequencies were obtained for categorical data (sex, professional profile, working sector, vaccination status). All possible associations between VC and the data collected (sex, professional profile, working sector, vaccination status were investigated; 2 x 2 contingency tables were constructed, and assumptions were tested by means of the chi-square method. Statistical significance threshold was set at $p = 0.050$; p-values of less than 0.050 on two-tailed tests were considered statistically significant. The summary and inferential statistics were analyzed by means of SPSS and Stata software.

Results

A total of 3,454 HCWs participated in the study: 1,236 males and 2,218 females.
The sample comprised physicians (26.9%), physicians in training (16.1%), nurses (17.2%) and other professional categories (9.8%). In 30% of cases, the respondents did not state their profession.
The mean age of the whole sample was 45.85 years (SD: 11.82; CI: 45.45-46.24; IQR: 21.8). The mean age of male subjects was 46.39 years (SD: 12.67; CI: 45.68-47.10; IQR: 24.6), and of females 45.55 years (SD: 11.32; CI: 45.08-46.02; IQR: 20.9). The sample was also stratified by age-group: 20-30 years (11.61%), 31-40 (24.2%), 41-50 (22.32%), 51-60 (28.31%), ≥ 61 (12.88%); 0.68% of the sample did not state their age.
The sample was divided into three working areas: clinical (32.7%), surgical (29.8%) and services (21.9%); 15.6% of the sample did not provide information on this item.
Regarding the provenance of the respondents: 6.5% were from the north of Italy, 1.1% from the central regions and 67.2% from the south; 873 subjects (25.3%) did not report their region of birth.
Evaluation of replies regarding immunization status revealed inadequate VC, i.e. below 95% for all vaccinations examined. Higher VC was found with regard to polio, hepatitis B, tetanus and diphtheria, while coverage was very low for measles, mumps, rubella, pertussis, chickenpox and influenza (20-30%); lower VC rates were found for vaccinations not specifically recommended for HCWs (i.e. herpes zoster, meningococcus). Many HCWs could not remember or did not report their immunization status (Tab. I).
The vaccinations that are not specifically recommended for HCWs were excluded from the next statistical analysis (*H. influenzae, Meningococcus C, Meningococcus B, Pneumococcus*, Hepatitis A, Papilloma virus, Herpes zoster, Tuberculosis).
Differences of coverage rates considering sex, age, working area and professional category were analyzed. For these analyses the category "not reported or not remember" was excluded.
Regarding to sex we did not find significant differences except to rubella, chickenpox and mumps. Men HCWs showed higher vaccine coverage than women.
Concerning age, we evaluated the difference of coverage rates for the following vaccines: polio, diphtheria, tetanus, hepatitis B, pertussis, measles, rubella, mumps, influenza considering five age-groups. Vaccination coverage by age is shown in Table II. Significant differences were found for poliomyelitis, tetanus, pertussis and influenza. 51-60 age-class showed higher VC for poliomyelitis, diphtheria and tetanus, while higher VC for influenza was detect in ≥ 61 years subjects.
Excluding HCWs who did not report their working sector, we evaluated the vaccination coverage for the following vaccines: polio, diphtheria, tetanus, hepatitis B, pertussis, measles, rubella, mumps, chickenpox and influenza (Tab. III). Significant differences were found only for hepatitis B vaccine.
Excluding HCWs who did not reported their professional profile, we evaluated the vaccination coverage for the following vaccines: polio, diphtheria, tetanus, hepatitis

Tab. I. Vaccination coverage and natural immunity reported by HCWs, broken down by type of vaccination.

	Not vaccinated (%, CI)	Vaccinated (%, CI)	Not reported (%, CI)	Natural immunity (%, CI)
Poliomyelitis	3.1 (2.55-3.71)	80.0 (78.69-81.36)	16.9 (15.55-18.04)	0.0
Diphtheria	6.2 (5.42-7.03)	72.4 (70.95-73.93)	21.3 (19.94-22.67)	0.0
Tetanus	5.9 (5.09-6.66)	76.8 (75.34-78.16)	17.3 (16.08-18.60)	0.0
Hepatitis B	9.6 (8.60-10.56)	77.3 (75.93-78.73)	12.5 (11.43-13.64)	0.6 (0.35-0.86)
Pertussis	33.9 (33.35-35.51)	29.5 (27.95-30.99)	31.6 (30.056-33.17)	5.0 (4.25-5.71)
Measles	27.5 (25.99-28.96)	30.3 (28.72-31.79)	27.4 (25.9-28.88)	14.8 (13.69-16.07)
Rubella	29.1 (27.61-30.64)	30.9 (29.35-32.43)	27.3 (25.79-28.76)	12.7 (11.60-13.82)
Chickenpox	32.6 (31.07-34.19)	16.4 (15.21-17.78)	28.5 (27.01-30.02)	22.5 (21.02-23.80)
Mumps	36.2 (34.59-37.79)	23.7 (22.26-25.10)	31.5 (29.98-33.08)	8.6 (7.66-9.53)
H. influenzae	56.1 (54.4-57.71)	4.1 (3.42-4.74)	39.7 (38.18-41.44)	0.1 (0.01-0.23)
Influenza	44.2 (42.53-45.84)	14.0 (12.87-15.18)	41.8 (40.14-43.43)	0.0
Meningococcus C	53.5 (51.81-55.14)	7.1 (6.24-7.95)	39.4 (37.8-41.06)	0.0
Meningococcus B	57.2 (55.59-58.89)	3.1 (2.49-3.64)	39.7 (38.06-41.32)	0.0
Pneumococcus	57.4 (55.73-59.03)	2.7 (2.18-3.26)	39.8 (38.18-41.44)	0.1 (0.01-0.23)
Hepatitis A	52.2 (50.50-53.84)	7.1 (6.31-8.03)	40.2 (38.56-41.83)	0.0
Papilloma virus	58.7 (57.00-60.29)	1.9 (1.53-2.46)	39.4 (37.79-41.05)	0.0
Herpes zoster	58.9 (57.30-60.58	0.7 (0.47-1.03)	40.0 (38.36-41.63)	0.4 (0.22-0.65)
Tuberculosis	41.5 (39.83-43.11)	24.5 (23.06-25.93)	33.8 (32.26-35.41)	0.3 (0.14-0.50

Tab. II. Vaccination coverage, by age.

	20-30 y (%, CI)	31-40 y (%, CI)	41-50 y (%, CI)	51-60 y (%, CI)	61+ y (%, CI)	p <
Poliomyelitis	77.89 (73.97-81.37)	78.51 (75.51-81.23	78.89 (75.94-81.56)	83.16 (80.68-85.38)	79.84 (75.45-83.61)	0.05
Diphtheria	70.04 (65.81-73.96)	72.69 (69.48-75.69)	71.23 (68.02-74.25)	74.64 (71.81-75.61)	71.24 (66.43-75.61)	N.S.
Tetanus	76.03 (72.02-79.63)	76.61 (73.53-79.43)	74.57 (71.45-77.45)	79.36 (76.70-81.79)	75.54 (70.91-79.64)	0.05
Hepatitis B	78.26 (74.36-81.72)	79.90 (76.96-82.55)	75.80 (72.73-78.63)	76.90 (74.15-79.44)	75.81 (71.19-79.89)	N.S.
Pertussis	23.14 (19.60-27.11)	29.37 (26.29-32.64)	32.47 (29.33-35.77)	29.98 (27.18-32.93)	30.91 (26.42-35.80)	0.01
Measles	30.17 (26.24-34.41)	32.24 (29.07-35.58)	29.01 (25.99-32.24)	29.98 (27.18-32.93)	30.65 (26.17-35.52)	N.S.
Rubella	28.51 (24.66-32.70)	31.23 (28.09-34.54)	32.10 (28.97-35.40)	30.80 (27.98-33.77)	31.45 (26.93-36.35)	N.S.
Mumps	21.28 (17.86-25.16)	24.78 (21.89-27.91)	23.46 (20.66-26.50)	24.23 (21.64-27.02)	23.92 (19.86-28.53)	N.S.
Influenza	8.26 (6.12-11.07)	14.16 (11.90-16.77)	14.94 (12.64-17.56)	15.30 (13.17-17.70)	15.59 (12.25-19.64)	0.001

Tab. III. Vaccination coverage, by working sector.

	Clinical sector (%, CI)	Surgical sector (%, CI)	Service sector (%, CI)	p <
Poliomyelitis	81.40 (79.02-83.56)	82.78 (80.35-84.97)	81.82 (78.91-84.40)	N.S.
Diphtheria	72.98 (70.32-75.50)	73.54 (70.76-76.15)	73.39 (70.12-76.41)	N.S.
Tetanus	75.38 (72.78-77.80)	76.75 (74.07-79.23)	77.34 (74.22-77.88)	N.S.
Hepatitis B	73.76 (71.11-76.25)	77.24 (74.57-79.70)	79.71 (76.70-82.42)	0.05
Pertussis	28.01 (25.47-30.71)	30.45 (27.71-33.33)	31.09 (27.90-34.48)	N.S.
Measles	27.81 (25.27-30.50)	28.99 (26.29-31.84)	28.72 (25.61-32.05)	N.S.
Rubella	28.61 (26.05-31.32)	31.03 (28.27-33.93)	30.57 (27.39-33.94)	N.S.
Mumps	21.97 (19.65-24.48)	24.32 (21.79-27.04)	22.00 (19.20-25.09)	N.S.
Chickenpox	18.60 (16.44-20.98)	17.61 (15.40-20.06)	18.18 (15.60-21.09)	N.S.
Influenza	17.54 (15.43-19.87)	13.62 (11.65-15.86)	13.70 (11.43-16.34)	N.S.

B, pertussis, measles, rubella, mumps, chickenpox and influenza (Tab. IV). Significant differences were found for polio, diphtheria, hepatitis B, rubella and chickenpox. Physicians showed significant higher coverage than nurses and other healthcare workers for hepatitis B and rubella.

Regarding to influenza vaccination the highest coverage was found in other professional categories respect to physicians and nurses.

Discussion and conclusions

This study revealed low VC rates among HCWs for all the vaccinations. Such coverage rates are totally inadequate in terms of preventing not only disease transmission by susceptible HCWs, but also nosocomial outbreaks, an example being the recent outbreaks of measles in Italy [23, 24] confirming data from previous studies at the national and international levels [3, 7, 8, 12, 16, 17, 25, 26].

Tab. IV. Vaccination coverage reported by different categories of HCWs.

	Physicians (%, CI)	Nurses (%, CI)	Other healthcare workers (%, CI)	p <
Poliomyelitis	78.38 (76.22-80.41)	81.96 (78.65-84.85)	82.60 (78.18-86.27)	0.05
Diphtheria	71.92 (69.58-74.15)	72.51 (68.78-75.96)	74.04 (69.11-78.43)	0.05
Tetanus	78.05 (75.87-80.08)	76.73 (73.15-79.96)	76.40 (71.58-80.62)	N.S.
Hepatitis B	79.45 (77.31-81.43)	76.56 (72.98-79.80)	72.57 (67.57-77.06)	0.01
Pertussis	28.08 (25.85-30.42)	31.20 (27.59-35.04)	29.59 (24.96-34.67)	N.S.
Measles	32.73 (30.39-35.16)	30.86 (27.27-34.70)	30.68 (26.00-35.80)	N.S.
Rubella	31.87 (29.53-34.27)	31.70 (28.08-35.56)	31.56 (26.83-36.71)	0.05
Mumps	24.58 (22.45-26.84)	23.10 (19.88-26.67)	25.96 (21.57-30.89)	N.S.
Chickenpox	13.94 (12.27-15.80)	19.93 (16.88-23.31)	16.81 (13.20-21.18)	0.001
Influenza	11.38 (9.86-13.10)	14.00 (11.43-17.03)	17.40 (13.73-21.82)	0.001

Higher coverage was observed among men than women; this is in contrast with the literature data, where the higher rates in women are due to the prevention of risks related to some infections in pregnant women [27, 28, 29]. Our finding may be linked to the high percentage of HCWs who filled "not remember" or did not reported their vaccination status.

As regards age, the youngest showed higher rates of vaccination against hepatitis B although no significance difference was found. The seasonal influenza vaccination coverage was higher in ≥ 61 years subjects ($p < 0.001$) although VC was very far from the minimum recommended level. These findings are in line with the literature [8, 9, 17, 30-40].

The international literature reports higher VC rates of physicians than other healthcare workers [33-35, 41, 42], however we find this only for hepatitis B and rubella. This result could be explained on the hand with high percentage of physicians who did not declared their vaccination status, on the other hand with the high percentage of HCWs who did not report their professional profile. The highest coverage rates were found in pediatric workers (data not shown) but the differences between these and other clinical groups were not statistically significant. Although international literature data on vaccination coverage among pediatricians are limited, higher coverage rates and more positive attitudes towards vaccinations have been reported [7, 12, 36, 43].

Limited differences were found based on the working sector, higher rates of VC were found among staff working in services than among workers in either surgical or medical departments only for hepatitis B.

The low vaccination coverage for all vaccines could be explained by the fear of adverse effects, despite the fact that many scientific studies, systematic reviews and meta-analyses of the literature have shown such fears to be groundless [44-47]. In order to combat "vaccine hesitancy" among HCWs, it is essential to promote clear and effective communication regarding vaccinations and to adopt innovative strategies (e.g. promoting vaccination via social networks and the mass media, training HCWs, providing vaccination in the workplace) [48, 49].

Vaccine-hesitant HCWs might also deter patients' vaccination uptake [49]. Another issue detected in our study was the high percentage of HCWs who declared not recalling what vaccinations they had received; this probably reflects a lax attitude and a lack of confidence in vaccination. HCWs should understand that all vaccines are safe and useful; they should regard vaccination both as a right and as a duty, in order to protect themselves and their patients [37].

A major strength of the present study is that it was a multicenter study involving several centers located in northern, central and southern regions of Italy and used an official form to collect data on vaccination status. By contrast, the fact that the data were self-reported constitutes a limitation. Indeed, many HCWs, especially in the older age-groups, may not have recalled which vaccinations they had received or might have declared that they did not remember, in order to avoid incurring legal action. Further, it was not possible evaluating the differences on propensity to vaccines between HCWs residing in North Centre and South Italy as about 30% of HCWs did not report their residence and about 40% were residents in the major islands of Italy (Sicily and Sardinia).

In conclusion, the question of vaccination among HCWs is challenging and fraught with ethical issues. Mandatory measures may be needed in order to achieve better coverage, such as those implemented by the regional Laws of Emilia Romagna, Marche and Puglia [50-52]. Mandatory policies are currently under debate in several countries, and high-quality studies would help policymakers and stake-holders to shape evidence-based initiatives and programs to improve VC and the control of infectious diseases through the correct application of guidelines on prevention [53, 54].

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Conflict of interest statement

None declared.

Authors' contributions

GC and RS conceived the study. GT verified the analytical methods. All authors contributed to data acquisition. GC, RS, RS, GI, DP, IA and MP contributed to the interpretation of the results. GC and RS wrote the manuscript, with input from all authors.

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ORIGINAL ARTICLE

Uncompleted Emergency Department Care (UEDC): a 5-year population-based study in the Veneto Region, Italy

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Keywords

Health care management • Emergency department • Quality of care

Summary

Introduction. *Uncompleted visits to emergency departments (UEDC) are a patient safety concern. The purpose of this study was to investigate risk factors for UEDC, describing not only the sociodemographic characteristics of patients who left against medical advice (AMA) and those who left without being seen (LWBS), but also the characteristics of their access to the emergency department (ED) and of the hospital structure.*
Methods. *This was a cross sectional study on anonymized administrative data in a population-based ED database.*
Results. *A total of 9,147,415 patients attended EDs in the Veneto Region from 2011 to 2015. The UEDC rate was 28.7‰, with a slightly higher rate of AMA than of LWBS (15.3‰ vs 13.4‰). Age, sex, citizenship, and residence were sociodemographic factors associated with UEDC, and so were certain characteristics of access, such as mode of admission, type of referral, emergency level, waiting time before being seen, and type of medical issue (trauma or other). Some characteristics of the hospital structure, such as the type of hospital and the volume of patients managed, could also be associated with UEDC.*
Conclusion. *Cases of UEDC, which may involve patients who leave AMA and those who LWBS, differ considerably from other cases managed at the ED. The present findings are important for the purpose of planning and staffing health services. Decision-makers should identify and target the factors associated with UEDC to minimize walkouts from public hospital EDs.*

Introduction

Emergency departments (EDs) are becoming increasingly overcrowded, with patients waiting longer to be seen and becoming more dissatisfied, and sometimes leaving the ED prematurely [1, 2].
Cases of uncompleted emergency department care (UEDC) are a patient safety concern. There are two types of UEDC, one involving *patients who leave without being seen* (LWBS) by a physician, and the other concerning patients who leave *against medical advice* (AMA).
There is a growing body of literature on patients who LWBS [3-8], possibly because such cases are more common than patients who leave AMA, and because LWBS events are associated with ED overcrowding [1, 9-11].
While it is commonly believed that patients who LWBS may have medical problems that are not really urgent, some studies have shown that they may actually require hospitalization and surgery on further consultation [3, 4, 12], and also that many patients who LWBS seek medical attention elsewhere [13]. As such cases may have severe clinical outcomes and subsequently require critical treatment, health systems may miss an opportunity to make contact with these patients. The rate of patients who LWBS has been judged one of the most important performance indicators for EDs [6, 14, 15].
Several studies from high-income countries with well-established primary health care systems have reported LWBS rates ranging from less than 1% to 20% of all arrivals at EDs [8, 12, 16-18].
Several factors have been found associated with cases of LWBS and AMA, such as low-acuity illness, young age, male sex, and long waiting times [5, 11, 13, 19, 20]. Triage times, previous ED visits, seasonal variations, accessibility of primary care, and ED overcrowding have also revealed a significant impact on LWBS rates [8, 21-25].
The purpose of this study was to further investigate the risk factors for UEDC, describing not only the sociodemographic characteristics of the patients (both those who left AMA and those who LWBS), but also how they accessed the emergency services, and the characteristics of the EDs and hospitals involved.

Methods

This was a cross-sectional study on anonymized administrative data in a population-based ED database [26].
All patients admitted to EDs at public and private hospitals in the Veneto Region, in north-east Italy, between 1 January 2011 and 31 December 2015 were included in the sample. During the period investigated, there were 52 EDs in the Veneto Region, 46 of them public and 6

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private. Healthcare facilities are connected within a regional hospital network comprising: a) 7 "hub" hospitals with highly-specialized services located in the main cities, 2 of which are university hospitals; b) 24 medium-sized "spoke" hospitals, each serving an average population of 250,000; and c) 21 small local hospitals.
The EDs were classified on the grounds of the annual number of admissions (< 25,000; 25,000-50,000; 50,000-75,000 and > 75,000).
Information on patients' age, sex, citizenship, and residence were extracted from the ED records for each episode of care. The mode of access to the EDs and the characteristics of the hospitals were also taken into account.
The triage codes assigned to patients at the check-in desk featured four emergency levels, based on the level of assistance required, and its urgency.
Finally, to compare the UEDC rates, the LWBS and AMA rates were calculated separately. These analyses give an extension of previous data evaluating only LWBS phenomena [27].
Odds ratios (ORs) and 95% confidence intervals (95%CI) were calculated to shed light on which factors most affected the probability of LWBS or AMA events.

Ethical issues

The study was conducted on data routinely collected by the health services in anonymized records with no chance of individuals being identified. The data analysis was performed on aggregated data. The data in the Local Health Authority registries are recorded with the patient's consent, and can be used as aggregated data for scientific studies without further authorization (*Garante per la protezione dei dati personali*, Resolution of 1 March 2012, n. 85). The study complies with the Declaration of Helsinki, and with the Italian Decree n. 196/2003 on the protection of personal data.

Results

A total of 9,147,415 patients attended the EDs of the Veneto Region from 2011 to 2015. The UEDC rate among them was 28.7‰, and there were slightly more patients who left AMA than those who LWBS (15.3‰ vs 13.4‰; OR 114; 95%CI 1.13-1.15; p < 0.05). There were more males than females among the cases of UEDC (OR 1.18; 95%CI 1.18-1.19; p < 0.05); and the average age was higher among the female patients (F 47.2 vs. M 43.5; p < 0.05).
Table I shows the sex and age distribution of the UEDC patients. The probability of self-discharge was higher for patients 15-24 years old (OR 1.06; 95%CI 1.05-1.07; p < 0.05), followed by the group 25-44 years old (taken for reference because it was the most represented, accounting for 25% of the whole sample). The AMA and LWBS risk distribution by age group was similar except for the very young and the very old. ED admissions involving newborn infants accounted for 2% of the sample and were associated with the highest risk of patients leaving AMA (OR 1.19; 95%CI 1.15-1.23; p < 0.05), as opposed to a distinctly low risk of their LWBS (OR 0.53; 95%CI 0.15-0.16; p < 0.05). Advanced age was clearly associated with a very low risk of UEDC. The majority of patients attended an ED at the Local Health Unit nearest their home (71%) and the risk of self-discharge was lower for people who lived in the area served by the same unit, while it increased with distance, becoming highest for patients who lived abroad (OR 2.59; 95%CI 2.54-2.64; p < 0.05).
Foreign citizenship was associated with UEDC: the risk of patients leaving AMA was almost twice among foreigners (OR 1.95; 95%CI 1.93-1.98; p < 0.05).
As shown in Table II, the vast majority of patients arrived at the ED at their own discretion (72%), and with their own means of transport (86%). ED admissions on the advice of a physician (OR 0.71; 95%CI 0.71-0.72; p < 0.05) or by ambulance (OR 0.58; 95%CI 0.58-0.59; p < 0.05) were major protective factors against self-discharge, particularly for LWBS events.
As expected, after stratifying the UEDC risk by underlying medical conditions and levels of urgency at the time of triage, there was an association between the severity of a patient's condition and how their visit to the ED concluded, both overall (p < 0.05), and for patients LWBS (p < 0.05). Another factor protecting against UEDC events, though more for AMA than for LWBS, was trauma as a reason for accessing the ED (OR 0.79; 95%CI 0.79-0.80; p < 0.05), which was the case for 30% of all patients accessing these services.
As regards waiting times, 77% of patients were examined within 1 hour of arrival, and 90% within 2 hours. It emerged that the waiting time was an important significant determinant of UEDC events. The statistical association was significant (p < 0.05), underscoring that having to wait for more than 4 hours was associated with a high risk of patients LWBS (OR 12.9; 95%CI 12.71-13.13; p < 0.05).
As shown in Table III, EDs with higher volumes of activity correlated with higher rates of UEDC (X2 trend: 283883,120; p < 0.05), both for AMA and LWBS events. The data regarding private hospitals reflected this trend (OR 0.62; 95%CI 0.61-0.63; p < 0.05): 3 of the 6 private hospitals included in our analysis reported fewer than 25,000 ED admissions a year, while the other 3 had between 25,000 and 50,000 ED admissions a year.
A similar trend emerged for the hospitals' role in the regional network: 5 of 7 hub hospitals always had more than 75,000 ED admissions a year, and it was these hospitals that reported the highest risk of self-discharge (OR 2.33; 95%CI 2.30-2.36; p < 0.05). Teaching hospitals also carried a higher risk of UEDC than other hospitals.

Discussion

Age, sex, citizenship, and residence are sociodemographic factors associated with UEDC. Some characteristics of access to ED services, such as mode of admission, type of referral, emergency level, waiting time, and type of medical issue (trauma vs other) also influence UEDC rates, and so certain features of the hospitals con-

Tab I. Uncompleted emergency department care by sociodemographic factors.

	ED contacts	p%	N° UEDC	p‰ UEDC	OR	95%CI	p	N° AMA	p‰ AMA	OR	95%CI	p	N° LWBS	p‰ LWBS	OR	95%CI	p
Gender																	
Female	4486308	49%	118028	26.3	1			54983	12.3	1			63045	14.1	1		
Male	4661107	51%	144524	31.0	1.18	1.18-1.19	p < 0.05	67736	14.5	1.19	1.18-1.20	p < 0.05	76788	16.5	1.18	1.16-1.19	p < 0.05
Age																	
0 y	188704	2%	6085	32.3	0.86	0.83-0.88	p < 0.05	4165	22.1	1.19	1.15-1.23	p < 0.05	1920	10.2	0.53	0.50-0.55	p < 0.05
01-05 y	643686	7%	19190	29.8	0.79	0.78-0.80	p < 0.05	12222	19.0	1.02	1.00-1.04	p > 0.05	6968	10.8	0.56	0.55-0.58	p < 0.05
06-14 y	606733	7%	16263	26.8	0.71	0.70-0.72	p < 0.05	9180	15.1	0.81	0.79-0.83	p < 0.05	7083	11.7	0.61	0.59-0.62	p < 0.05
15-24y	800309	9%	31962	39.9	1.06	1.05-1.07	p < 0.05	15682	19.6	1.05	1.03-1.07	p < 0.05	16280	20.3	1.07	1.05-1.09	p < 0.05
25-44y	2308377	25%	87138	37.8	1			43091	18.7	1			44047	19.1	1		
45-64 y	1994940	22%	59064	29.6	0.78	0.77-0.79	p < 0.05	30084	15.1	0.81	0.79-0.82	p < 0.05	28980	14.5	0.76	0.75-0.77	p < 0.05
65-74y	990328	11%	20859	21.1	0.56	0.55-0.56	p < 0.05	12134	12.3	0.65	0.64-0.67	p < 0.05	8725	8.8	0.46	0.45-0.47	p < 0.05
75-84y	1033817	11%	16282	15.8	0.41	0.41-0.42	p < 0.05	9737	9.4	0.5	0.49-0.51	p < 0.05	6545	6.3	0.33	0.32-0.34	p < 0.05
+85 y	580521	6%	5709	9.9	0.26	0.25-0.27	p < 0.05	3538	6.1	0.32	0.31-0.34	p < 0.05	2171	3.8	0.19	0.19-0.20	p < 0.05
Residence																	
Same LHU	6473783	71%	156482	24.2	1			81087	12.5	1			75395	11.7	1		
Veneto Region	2075420	23%	77225	37.2	1.56	1.55-1.57	p < 0.05	42376	20.4	1.64	1.62-1.66	p < 0.05	34849	16.8	1.45	1.43-1.47	p < 0.05
Other region	395526	4%	16635	42.1	1.77	1.74-1.80	p < 0.05	8742	22.1	1.78	1.74-1.82	p < 0.05	7893	20.0	1.73	1.69-1.77	p < 0.05
Abroad	202686	2%	12210	60.3	2.59	2.54-2.64	p < 0.05	7628	37.6	3.08	3.01-3.16	p < 0.05	4582	22.6	1.96	1.90-2.02	p < 0.05
Citizenship																	
Italian	7849343	86%	203012	25.9	1			106021	13.5	1			96991	12.4	1		
Foreign	1298072	14%	59540	45.9	1.81	1.79-1.83	p < 0.05	33812	26.1	1.95	1.93-1.98	p < 0.05	25728	19.8	1.61	1.59-1.74	
Exemption from co-payment																	
No	8402314	92%	255891	30.5	1			134838	16.1	1			121053	14.4	1		
Yes	745101	8%	6661	8.9	0.29	0.28-0.29	p < 0.05	4995	6.7	0.41	0.40-0.43	p < 0.05	1666	2.2	0.15	0.15-0.16	

cerned, including the type of facility, and the volume of patient admissions.

The rate of UEDC found in this study (28.7‰) is among the lowest to have been reported in the literature [8,12,16,17,18]. Unlike the trend reported in similar studies, the AMA rate was significantly higher than the LWBS rate, for both males and females [3-11]. Young adults were more likely to LWBS than to leave AMA, whereas the newborn were more likely to leave AMA. A possible explanation for this latter phenomenon lies in that such admissions often involve an important element of parents needing to be reassured [12]. The high rate of UEDC among foreigners could be explained by their going to an ED for primary care, bearing in mind that most LWBS cases are likely to be of low acuity. In fact, a previous study found that foreigners visiting the country, and those from high migration pressure countries were less likely than Italians to seek a primary care physician (family physicians, or doctors providing continuity of care), who should serve as the health system's gatekeepers and be consulted before seeking secondary healthcare services [28].

In line with other studies, higher-acuity visits (high triage priority, arrival by ambulance) were less likely to conclude with LWBS events [5, 12, 25]. This would again suggest that patients who LWBS have less urgent medical issues and may be at lower risk of complications. Research has shown a dose-response relationship between LWBS and triage level [29], with 0.1% of the highest-level patients and 15.2% of the lowest-level patients LWBS [8]. Another study found a 58.3 times higher risk of LWBS for non-urgent than for urgent triage levels [30]. In recent times, there has been a significant increase in ED attendance worldwide, relating largely to higher numbers of non-urgent cases. In Italy, for example, the

Tab. II. Uncompleted emergency department care by mode of access to EDs.

	N° access	%	N° UEDC	p‰ UEDC	OR	95%CI	p	N° AMA	p‰ AMA	OR	95%CI	p	N° LWBS	p‰ LWBS	OR	95%CI	p
Mode of admission																	
Ambulance	1259112	14%	22485	18.0	0.58	0.58-0.59	p < 0.05	14962	12.0	0.75	0.74-0.77	p < 0.05	7523	6.0	0.41	0.40-0.42	p < 0.05
By oneself	7888303	86%	240067	30.4	1			124871	15.8	1			115196	14.6	1		
Referral																	
Physician	2535103	28%	56646	22.4	0.71	0.71-0.72	p < 0.05	34199	13.5	0.84	0.83-0.85	p < 0.05	22447	8.9	0.58	0.57-0.59	p < 0.05
Own discretion	6612312	72%	205906	31.2	1			105634	16.0	1			100272	15.2	1		
Emergency level																	
Not reported	182132	2%	6265	34.4	0.8	0.78-0.82	p < 0.05	2027	11.1	0.5	0.48-0.53	p < 0.05	4238	23.3	1.12	1.08-1.15	p < 0.05
1. Very urgent	133891	1%	1776	14.0	0.32	0.30-0.33	p < 0.05	1607	12.7	0.57	0.54-0.60	p < 0.05	169	1.3	0.06	0.05-0.07	p < 0.05
2. Urgent	1562065	17%	18434	11.8	0.27	0.26-0.27	p < 0.05	14637	9.4	0.42	0.42-0.43	p < 0.05	3797	2.4	0.11	0.11-0.12	p < 0.05
3. Low acuity	4124388	45%	101448	24.6	0.56	0.56-0.57	p < 0.05	52710	12.8	0.58	0.57-0.59	p < 0.05	48738	11.8	0.56	0.55-0.57	p < 0.05
4. No acuity	3144939	34%	134629	42.8	1			68852	21.9	1			65777	20.9	1		
Waiting time																	
< 1 hours	7025159	77%	149798	21.4	1			89318	12.7	1			60480	8.6	1		
1–2 hours	1213763	13%	33738	27.8	1.31	1.30-1.33	p < 0.05	16959	14.0	1.1	1.08-1.12	p < 0.05	16779	13.8	1.61	1.58-1.64	p < 0.05
2–3 hours	483366	5%	24812	51.3	2.48	2.45-2.52	p < 0.05	10744	22.2	1.76	1.73-1.80	p < 0.05	14068	29.1	3.45	3.38-3.51	p < 0.05
3–4 hours	212532	2%	17568	82.7	4.13	4.06-4.20	p < 0.05	7638	35.9	2.89	2.82-2.96	p < 0.05	9930	46.7	5.64	5.52-5.76	p < 0.05
> 4 hours	212595	2%	36636	172.4	9.55	9.43-9.67	p < 0.05	15174	71.4	5.96	5.86-6.07	p < 0.05	21462	101.0	12.9	12.71-13.13	p < 0.05
Type of medical issue																	p < 0.05
Trauma	2733345	30%	66699	24.4	0.79	0.79-0.80	p < 0.05	30375	11.1	0.77	0.76-0.78	p < 0.05	36324	13.3	0.82	0.81-0.83	
No trauma	6414070	70%	195853	30.6	1			92344	14.4	1			103509	16.2	1		

Italian Society of Emergency Medicine (SIMEU) reported in 2010 that ED visits had risen by 5-6% a year over the previous 5 years, and this was partly as a consequence of inappropriate referrals by primary care physicians [31]. Strengthening primary healthcare can help to improve the equity, efficiency, effectiveness, and responsiveness of health systems [32-34] also reducing the inappropriate use of ED – especially by disadvantaged population groups [35].

Even if patients who LWBS have low-acuity conditions, many studies nevertheless report that approximately half of these patients will seek care elsewhere. On the other hand, an important proportion of patients may be sufficiently reassured by their triage assessment and no longer feel such an urgent need to seek medical advice. Although it would seem that care for patients triaged as non-urgent could be deferred, studies have found that such patients may still be genuinely ill [36]. It is notable, however, that 1% of the patients in our sample with the highest triage levels LWBS. As unexpected as this might seem, other studies also found that patients in the highest triage categories might still LWBS [36]. At the same time, the higher odds of LWBS events involving patients with non-traumatic conditions is to be expected given that most patients with injuries required acute attention, while those with a low acuity rating sought alternative medical care.

This study found a strong association between waiting time and the risk of UEDC, but waiting time did not appear to influence patients who left AMA as much as it did those who LWBS. The association between UEDC and waiting time, for LWBS events in particular, explains the high UEDC rates at hospitals with large volumes of ED admissions and consequent overcrowding, as amply described elsewhere [37-40]. Overcrowding is a well-known barrier affecting access to healthcare, and keeping ED waiting times short is fundamental to reducing the numbers of patients LWBS. These findings also highlight the importance of accurate triaging, as this clearly influences waiting times and the chances of a patient becoming a case of UEDC.

Other strategies could be implemented, however, to address the problem of UEDC. In fact, other studies found social issues fundamentally important, especially in such a sensitive environment as the ED,

Tab. III. Uncompleted emergency department care by characteristics of hospitals.

	N° access	%	N° UEDC	p‰ UEDC	OR	95%CI	p	N° AMA	p‰ AMA	OR	95%CI	p	N° LWBS	p‰ LWBS	OR	95%CI	p
Type of hospital																	
Private	659144	7%	12177	18.5	0.62	0.61-0.63	p < 0.05	5362	8.1	0.51	0.50-0.52	p < 0.05	6815	10.3	0.75	0.74-0.77	p < 0.05
Public	8488271	93%	250375	29.5	1			134471	15.9	1			115904	13.7	1		
Territory served																	
HUB	2979227	33%	124839	41.9	2.33	2.30-2.36	p < 0.05	65169	21.89	2.63	2.58-2.68	p < 0.05	59670	20.045	2.02	1.99-2.06	p < 0.05
Spoke	4510443	49%	107152	23.8	1.3	1.28-1.31		60690	13.47	1.61	1.58-1.64		46462	10.314	1.03	1.01-1.05	p < 0.05
Integrative of network	1657745	18%	30561	18.5	1			13974	8.436	1			16587	10.014	1		
University hospital																	
Yes	1242581	0,136	58588	47.2	1.87	1.85-1.89	p < 0.05	32415	26.1	1.94	1.92-1.97	p < 0.05	26173	21.1	1.74	1.72-1.76	p < 0.05
No	7904834	0,864	203964	25.8	1			107418	13.6	1			96546	12.2	1		
Annual volume of patients																	
< 25,000	1310776	14%	19701	15.0	1			8462	6.5	1			11239	8.6	1		
25,000-50,000	4053574	44%	115551	28.5	1.92	1.89-1.95	p < 0.05	67370	16.6	2.6	2.54-2.66	p < 0.05	48181	11.9	1.39	1.36-1.42	p < 0.05
50,000-75,000	1446234	16%	38608	26.7	1.8	1.77-1.83	p < 0.05	17345	12.0	1.86	1.82-1.91	p < 0.05	21263	14.7	1.73	1.69-1.77	p < 0.05
> 75,000	2336831	26%	88692	38.0	2.59	2.55-2.63	p < 0.05	46656	20.0	3.14	3.06-3.21	p < 0.05	42036	18.0	2.12	2.07-2.16	p < 0.05

where patients and those accompanying them are often in a state of physical pain and psychological distress. It is therefore worth considering architectural design features and other factors of the built environment in an effort to make waiting at the ED less stressful, and more comfortable [41, 42].

In conclusion, patients involved in UEDC, whether they leave AMA or LWBS, differ considerably from other patients admitted to EDs. It is important to bear these differences in mind when planning and staffing health services. Decision-makers should identify and target factors to minimize walkouts from public hospital EDs, taking a broad approach to the issues involved. Action could range from structural improvements to humanizing the services. For example, the Veneto Regional Authorities have introduced stewards (or assistants) to make attending the ED less stressful [43]: these assistants provide patients with information and advice, collect details from them, reporting them to the healthcare personnel if necessary, but mainly responding to the patient's need to have someone who will listen, understand, and provide information. This figure integrates, but does not replace the function of the healthcare personnel. It serves mainly to make contact with patients and prevent them from feeling abandoned.

Another approach involves reducing inappropriate uses of EDs, which can generate UEDC phenomena. A greater continuity of care between primary and secondary healthcare services is associated with a lower risk of avoidable ED admissions. Integrating health care and social care services can help too. For example, the Veneto Regional Authorities have created territorial centers that operate around the clock to ensure continuity of care. These centers have a central role in the healthcare network, and are intended for people with special needs and their families or caregivers, who are particularly in need of care, assistance and support in the case of illness. The territorial centers also provide a functional link between health, social and other care facilities, with a view to humanizing the care process and ensuring the centrality of the individual in the delivery of such services [44].

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Conflicts of interest statement

None declared.

Authors' contributions

VB conceptualized the study, coordinated all study phases, and approved the final manuscript as submitted. AB reviewed and revised the manuscript, and approved the final manuscript as submitted. MF wrote the paper. RF draft paper and approved the final manuscript as submitted. CB coordinated data collection and approved the

final manuscript as submitted. MS conducted analyses, collected data and approved the final manuscript as submitted.

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ORIGINAL ARTICLE

High prevalence of fluoroquinolone-resistant Escherichia coli strains isolated from urine clinical samples

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Keywords

Antimicrobial resistance • Quinolone resistance-determining region • Urinary tract infection • Mutations

Summary

Background. *Fluoroquinolone resistant Escherichia coli isolates have become an important challenge in healthcare settings in Iran. In this study, we have determined Fluoroquinolone resistant E. coli isolates (from both outpatients and inpatients) and evaluated mutations of gyrA and parC within the quinolone resistance-determining regions (QRDR) of these clinical isolates.*
Materials and methods. *Clinical isolates were recovered from the urine sample of patients with urinary tract infections admitted at Alzahra hospital, Iran, between September and February 2013. We assessed antimicrobial susceptibility of all isolates and determined mutations in QRDR of gyrA and parC genes from 13 fluoroquinolone-resistant isolates by DNA sequencing.*
Results. *A total of 135 E. coli strains were obtained from 135 patients (91 outpatients and 44 inpatients). The resistance rate of fluoroquinolones (Ciprofloxacin, Norfloxacin and Ofloxacin) among our strains was 45.2%. Two E. coli isolates were shown just a single mutation, but other isolates possessed 2-5 mutations in gyrA and parC genes. Mutations in the QRDR regions of gyrA were at positions Ser83 and Asp87 and parC at positions Ser80, Glu84, Gly78.*
Conclusions. *Ciprofloxacin is the most common antimicrobial agent used for treating urinary tract infections (UTIs) in healthcare settings in Iran. Accumulation of different substitutions in the QRDR regions of gyrA and parC confers high-level resistance of fluoroquinolones in clinical isolates.*

Introduction

Urinary tract infections (UTIs) are one of the most frequent bacterial infections around the world that almost occurs in the healthcare setting [1]. UTIs are the second most common type of infections in the human that poses a serious health problem because of the antibiotic resistance and high recurrence rates. The available data shows that 150 million cases of UTIs occur on a global basis per year, resulting in more than 6 billion dollars in treatment costs [2]. Uropathogenic *Escherichia coli* (UPEC) is the essential cause of UTIs, including both cystitis and pyelonephritis and are responsible for more than 80% of these infections [3, 4]. It is supposed to, the pathogenic potential of UPEC isolates is dependent on a multitude of virulence factors (VFs) located on chromosome regions, referred to "pathogenicity islands" (PAIs) [3]. These different virulence factors promote colonization and infection of urinary tract [2].
Due to the complications of urinary tract infections, well-timed treatment of these infections has special importance and treatment often accomplish based on the most prevalent pathogenic bacteria [5]. First, quinolones were introduced with nalidixic acid in 1962 for the treatment of UTIs. In five decades, different generations of quinolones have introduced for clinical use. Since it was specified that fluoroquinolones have more potency than older quinolones, therefore use of these expanding classes of antimicrobial agents increased significantly [6]. Fluoroquinolones are essential antimicrobial agents used to treat UTIs [7]. Ciprofloxacin is the most frequently used fluoroquinolone for the treatment of UTIs in healthcare settings, because of its availability in oral and intravenous formulations [8, 9].
Quinolones act via inhibition of DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death [10]. Clinical experience has shown diverse antibiotic resistance among uropathogens [11-13]. The increased use of fluoroquinolones has caused a remarkable emergence of resistance that varies by both organism and geographic region [6]. This resistance commonly is the consequence of mutations involving genes encoding *gyrA* and *parC* [14]. In *E. coli*, alternation at positions Ser-83 or Asp-87 in *gyrA* and Ser-80 and Glu-84 in *parC* are the most frequent mutations [15]. The other substitutions are rare in clinical isolates [16].
The aim of this study was to determine the patterns of antimicrobial resistance and the presence of mutations in quinolone resistance coding regions in *gyrA* and *parC* in clinical isolates of *E. coli* from a hospital in Isfahan, Iran.

Methods

Data collection

In order to describe the demographic and clinical characteristics of patients with urinary tract infections, admitted patients were selected. Due to lack of access to inpatients, a permission was reached to access the inpatient files. The final results were summarized after careful examination of the files.

Bacterial isolates

All clinical isolates were recovered from 135 consecutive and not repetitive urine specimens of patients (91 outpatients and 44 inpatients) with urinary tract infections admitted at Alzahra hospital, Isfahan, Iran, between September and February 2013. Diagnosis of *E. coli* isolates have done according to Bailey & Scott's diagnostic microbiological and biochemical methods, including appearance of bacterial colonies on culture medium, Gram staining, shape, motility, catalase, oxidase, MR, VP, oxidative/fermentative (OF), indole, citrate, urease, nitrate reduction, H2S, Gas, PYR, CAMP, gelatin, coagulase, bile solubility, DNase, fermentation of Fructose, Glucose and Lactose tests [17].

Susceptibility testing

Susceptibility testing was determined by disk diffusion technique as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines [18], using Mueller Hinton medium (Himedia Company). Antimicrobial disks used in this study (purchased from Himedia Company) were: Ciprofloxacin (5µg), Norfloxacin (10µg), Ofloxacin (5µg), Nalidixic acid (30µg), Amikacin (30µg), Ampicillin (10µg), Cefotaxime (30µg), Gentamicin (10µg), Nitrofurantoin (300µg), Trimethoprim/sulfamethoxazole (1.25/23.75µg), Cefoxitin (30µg), Meropenem (10µg), Cefepime (30µg), Ceftazidime (30µg), Cephalothin (30µg).

Escherichia coli ATCC25922 was used as a quality control strain. Then the data were entered into Whonet 5.6 (WHO, Geneva, Switzerland) software.

Preparation of bacterial DNA

Quinolone-resistant isolates (61 isolates) were cultured according to Baily & Scott's quantitative culture method [17] and cultured on Eosin Methylene Blue (EMB) and blood agar (BA) medium at the same time. DNA to be amplified was extracted from these isolates by boiling. In this method, cell pellets were transferred to 50µl of distilled water in an eppendorf tube and incubated at 100°C for 10 min. After centrifuging of the lysate at 6000×g for 10 min, the supernatant was stored at -20°C as a template DNA stock [19, 20].

PCR and DNA sequencing

Polymerase chain reaction (PCR) was performed by primer sequences designed for quinolone resistance-determining region (QRDR) of *gyrA* and *parC* genes of *E. coli* isolates. Oligonucleotide primers for the PCR amplification in this study have been shown in Table I.

DNA was amplified using an initial denaturation step of 5 min at 95°C, followed by 30 cycles consisting of 30 seconds at 94°C, 30 seconds at the annealing temperature of 55°C and 58°C (for *gyrA* and *parC,* respectively) and 45 seconds at 72°C, and a final extension step of 10 min at 72°C. PCR products were resolved by electrophoresis on 1.2% agarose gel containing ethidium bromide. Afterward, among fluoroquinolone resistant *E. coli* strains, 13 isolates randomly selected for genetic characterization of the QRDR of the *parC* and *gyrA* genes by sequencing process (Macrogene Company, Macrogen Inc., Seoul, Korea). *E. coli* ATCC25922 was used as a quality control for all PCR and sequencing reactions. After all, sequences were compared with the nucleotide sequence of the *gyrA* and *parC* genes in the GenBank database (accession numbers: FN554766.1 and CP003034.1respectively). These data were analyzed using MEGA4 and Gene Runner softwares.

Results

Distribution of fluoroquinolones resistant *E. coli* strains was determined in different age and gender groups. Most resistant isolates were observed among outpatient adult women (Tab. II). Based on the results of our study, meropenem, cefoxitin, amikacin, nitrofurantoin and gentamicin showed the best activity against *E. coli.* Percentage of resistance to antimicrobial agents is shown in Figure 1. No resistance has observed to meropenem while ampicillin has shown the least activity against *E. coli* isolates. Among the inpatients individuals, 50% had a history of catheter utilization, and 57% had a history of surgery.

A high resistance to three antibiotics ciprofloxacin, norfloxacin and ofloxacin was observed among strains (Fig. 1). Resistance rates to these three antibiotics were completely equal (45.2%). Out of the 135 *E.coli* isolates, 61 strains were resistant to fluoquinolones and all fluoquinolones- resistant strains include *gyrA* and *parC* genes (Fig. 2).

After sequencing process, two mutations were detected in the QRDR of *gyrA* gene, one at position 83

Tab. I. Oligonucleotide sequences of primer sets for PCR.

Primer	Sequence	PCR Product Size(bp)	Ref.
parC-F parC-R	5′- TTCAGCGCCGCATTGTGTAT -3′ 5′- GTTATGCGGTGGAATATCGGTC-3′	395	This study
gyrA-F gyrA-R	5′-TTACACCGGTCAACATTGAGG -3′ 5′- GACGACCGTTAATGATTGCC -3′	647	This study

Tab. II. Age category.

Patients	Sex	Adult	Pediatric	Newborn
Inpatient		12	1	1
Outpatient	Female	24	0	1
Inpatient	Male	10	1	0
Outpatient		11	0	0

(Ser_Leu) and another at position 87 (Asp_Asn). Between 13 isolates of fluoroquinolone-resistance strains, 11 isolates possessed these two mutations and 2 isolates showed a single mutation (Ser83Leu) in the *gyrA* gene. Also, five different mutations were detected in *parC* gene of *E. coli* isolates, encoding Ser80Ile, Ser80Val, Ser80Arg, Glu84Val, and Gly78Ser. One isolate showed three mutations in *parC*; three isolates showed two mutations and the rest (six isolates) showed a single mutation. On the other hand, three isolates showed no mutation in *parC* gene (Fig. 3). The mutations detected in the QRDR of the *gyrA* and *parC* genes are shown in Table III.

The demographics and clinical characteristics of inpatients are shown in Table IV. The number of inpatients in this study was 44, but due to a defect in the case of 4 patients, the information provided in this section is based on data from 40 inpatients.

Fig. 1. Antibiotic resistance pattern of *E. coli isolates* by CLSI disk diffusion methods.
CEP: Cephalothin, CTX: Cefotaxime, FOX: Cefoxitin, AMK: Amikacin, NAL: Nalidixic acid, NOR: Norfloxacin, STX: Trimethoprim/sulfamethoxazole, AMP: Ampicillin, CAZ: Ceftazidime, FEP: Cefepime, MEM: Meropenem, GEN: Gentamicin, CIP: Ciprofloxacin, OFX: Ofloxacin, NIT: Nitrofurantoin.

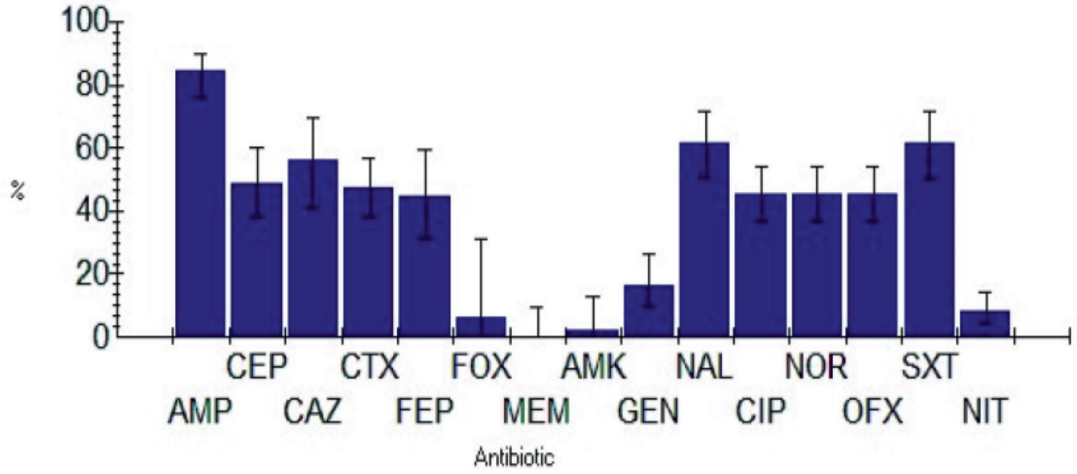


Fig. 2. a) PCR assay for the detection of *gyrA* gene. b) PCR assay for the detection *parC* gene.

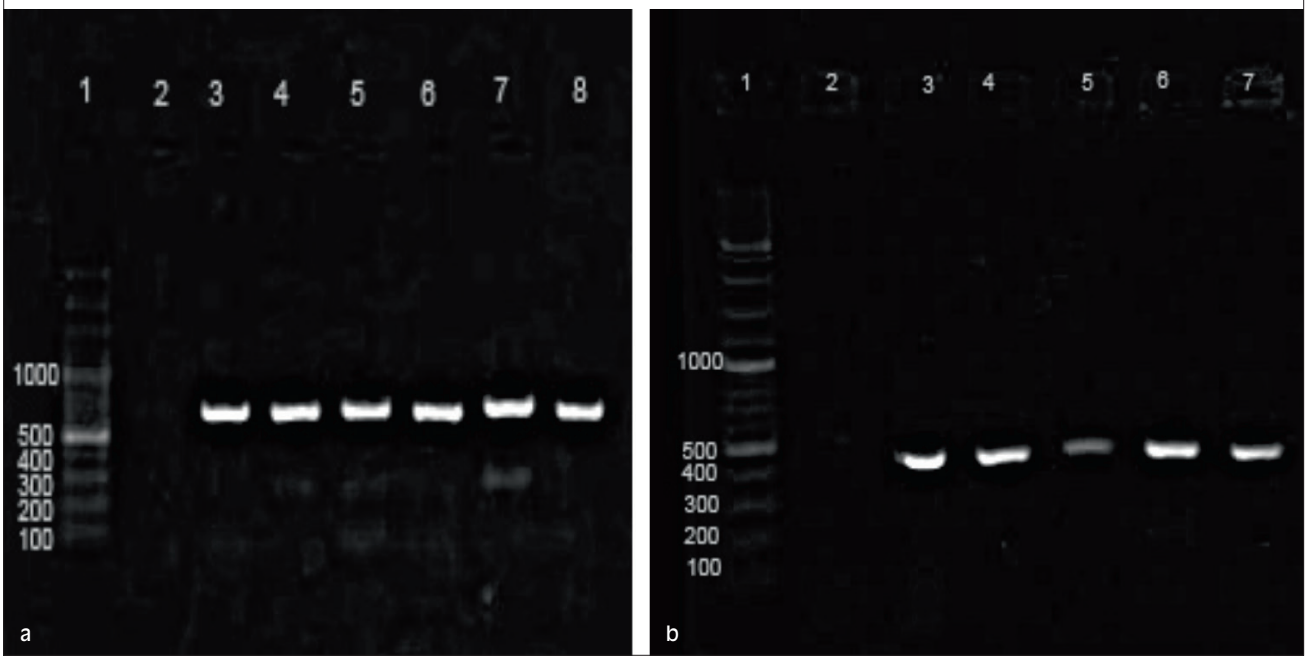
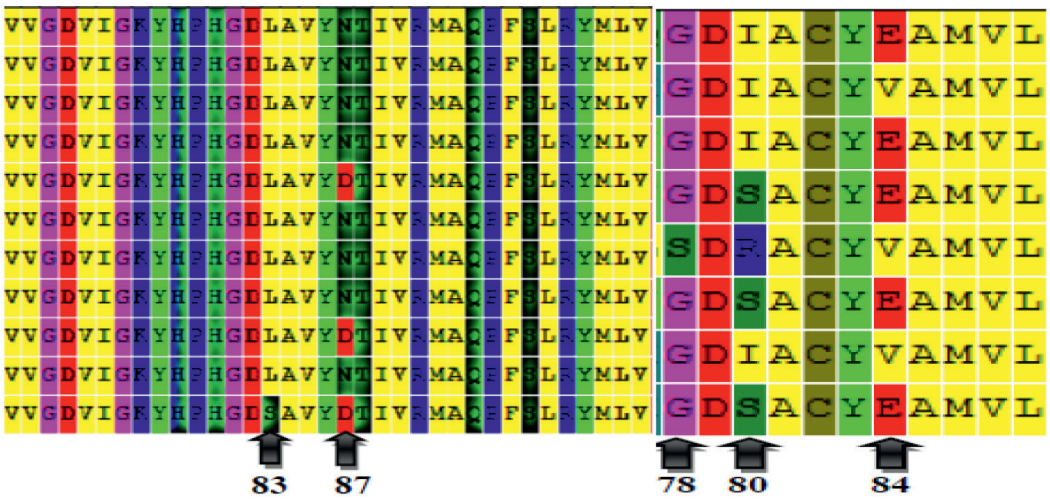


Fig. 3. Simultaneous alignment of partial *gyrA* and parC amino-acid sequences.



Tab. III. Location of mutations detected in *gyrA* and *parC* genes of *Escherichia coli* isolates.

Isolate	Mutation											
	gyrA					parC						
	83	84	85	86	87	78	79	80	81	82	83	84
	Ser	Ala	Val	Tyr	Asp	Gly	Asp	Ser	Ala	Cys	Tyr	Glu
E-1	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	.....
E-2	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	.....
E-3	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	Val
E-4	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	.....
E-5	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Val-	.....-	.....-	.....-	Val
E-6	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	.....
E-7	Leu-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....
E-8	Leu-	.....-	.....-	.....-	-Asn	Ser-	.....-	Arg-	.....-	.....-	.....-	Val
E-9	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	.....
E-10	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	.....
E-11	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	.....-	.....-	.....-	.....-	.....
E-12	Leu-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....
E-13	Leu-	.....-	.....-	.....-	-Asn	.....-	.....-	Ile-	.....-	.....-	.....-	Val
E-14	.....-	.....-	.....-	.....	.....-	.....-	.....-	.....-	.....-	.....-	.....-	.....

E-14: quality control (*Escherichia coli* ATCC25922).

Tab. IV. Demographic and clinical characteristics for urinary tract infection in inpatients (n = 40).

Characteristics	No of inpatient (%)
Female	21 (52.5)
Male	19 (47.5)
History of previous urinary tract infection	3 (7.5)
Antibiotic use in the last 3 months	16 (40)
Catheter	23 (57.5)
Catheter history	20 (50)
Surgical history	23 (57.5)
History of admission	13 (32.5)
History of surgery in the last 12 months	11 (27.5)
Prostate enlargement	3 (7.5)

Discussion

Mutations in the *gyrA* gene are the main cause of the resistance to fluoroquinolones. The most mutations have been shown to near the start region of the *gyrA* gene, known as the "QRDR". This region encodes amino acid residues 67 to 106 in *gyrA* and the most common alterations occur at positions 83 and 87 [21, 22]. Topoisomerase IV is a secondary, less sensitive target for fluoroquinolone action in *E. coli* [23]. On the other hand, alterations in the *parC* gene, correlate with reduced susceptibility to quinolones [24].

In the present study, two isolates of *E. coli* possessed a single mutation (in *gyrA* gene) and consequently they were susceptible to fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin), but were resistant to nali-

dixic acid. These findings are in agreement with other studies, indicating that nalidixic acid could be used as a good marker for a single mutation by use of the disk diffusion method [25-28].

Another 11 isolates showed different mutations in *gyrA* and *parC* genes and were resistant to fluoroquinolones. This confirms that multiple mutations are necessary to a great extent for the high level of quinolone resistance [26]. Our study has several limitations, including; first, the number of sequencing isolates were too small for a definitive evaluation. Second, we have examined just two genes of *E. coli* isolates. Several studies have shown a correlation between *gyrB* and/or *parE* and FQ resistance in *E. coli* [29]. Also efflux pump genes can be a cause of FQ resistance.

In the present study, we determined antimicrobial resistance pattern (by focus on fluoroquinolones) of *E. coli* isolates from a university medical center, Alzahra Hospital, Isfahan, Iran. Generally, empirical therapy of patients with UTIs begins with extended-spectrum antibiotics (it often consists of FQ, especially ciprofloxacin). These treatments before the final microbiological results lead to the increased resistance and emergence of resistant strains. According to the type and method of taking antibiotics in each country, there is a considerable difference in susceptibility and resistance to antimicrobial agents in *E.coli* causing urinary tract infections [30]. For the hospitalized patients with urinary tract infections, 57% used the catheter during admission, 50% had a history of using the catheter and 57% had a history of surgery. It is possible that these factors can increase the risk of urinary tract infections, if validated by proper risk factors analyses.

This study has shown a significant high resistance to fluoroquinolones in respect of other surveys in different regions of Iran and different countries in Europe. These findings serve as a warning that resistance to fluoroquinolone is increasing quickly. As fluoroquinolones are the most important used antimicrobial agent in the treatment of UTIs in Iran, increasing resistance to these agents has caused concern to relevant treatment of these infections. There are different resistance mechanisms to FQ. One of them is mutations that alter the drug targets. We observed different mutations in the QRDRs of grain and pores that cause a great effect on FQ resistance. By doing more research on the molecular basis of FQ resistance, new therapeutic strategies will create for FQ-resistant *E. coli*. With regard to the continuous changing in antibiotic sensitivity pattern, we recommend a guideline for physicians, which could determine bacterial sensitivity in populations yearly and choose the correct empirical treatment according to these patterns.

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Conflict of interest statement

None declared.

Authors' contributions

JF contributed to the conception and design of the work; the acquisition, analysis, and interpretation of data for the work. HK contributed to data collection and interpretation of data for the work. RD contributed to design of the work, data collection and final approval of the version to be published. MS contributed in data analysis, Drafting the work and revising it critically for important intellectual content. AZ and RD contributed in the revising the draft and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ORIGINAL ARTICLE

Improvement of hand hygiene compliance among health care workers in intensive care units

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Keywords

Compliance • Hand hygiene •HCWs

Summary

Aim. *Hand hygiene (HH) is an essential component in preventing healthcare associated infections. The purpose of this study was to evaluate HH compliance among health care workers (HCWs) in intensive care units at Beni-Suef university hospital, Egypt before and after an intervention educational program.*
Methods. *Data were collected by using the standardized WHO method for direct observation "Five moments for HH" approach. Observations were conducted in six ICUs before intervention (March to April 2017) and after the intervention (July to August 2017). The study included 608 opportunities (observations) among 177 HCWs collected before and 673 opportunities among 163 HCWs collected after the intervention.*
Results. *Overall HH compliance increased significantly from 30.9 (95% CI: 27.2-34.6%) before intervention to 69.5 (95% CI: 65.2-72.6%) post intervention; with the highest HH compliance rate among nurses compared to physicians and workers (P = 0.001). Significantly higher HH compliance rates were observed after body fluid exposure, before aseptic procedures, and after patient contact compared to before patient contact and after patient surrounding contact (P = 0.001). In binary logistic regression analyses a statistically significant difference was shown (P = 0.047) for HH compliance among events before and after patient contact (OR = 1.399, 95% CI: 1.004-1.948).*
Conclusions. *The interventional educational program improved the HH compliance among ICUs-HCWs at Beni-Suef university hospital. The hospital should conduct monthly observational monitoring for the ICUs units sharing the findings to spread best practices. Provision of sustained training programs to help efficient and effective HH for care delivery is mandatory.*

Introduction

Hand hygiene (HH) is the easiest, most influential, and economical method in reducing Hospital acquired infections (HAIs) [1, 2] which results in increased healthcare costs, length of hospitalization, use of drugs, and unnecessary laboratory investigations both in developed and developing countries, resulting in [3, 4] health care associated infections (HCAIs); HAIs known as nosocomial infections accounts for 5-10% and >15%% in developed and developing countries respectively [5]. Knowing that, compliance with HH alone essentially enhances patient safety; the reported compliance levels among healthcare workers (HCWs) remains suboptimal, with compliance rates being 30-75% [6-8].
In order to improve health care worker practices; the World Health Organization (WHO) standardized hand hygiene practice and recommended 100% compliance [5]. Effective measurement of HH adherence involves three concepts: indication, opportunity, and action; with Indications being the principal rationale for performing HH [9]. Both WHO and CDC guidelines recommend HCWs with a hand wash using soap and water when there is visible dirt. Alcohol-based hand hygiene is recommended for all other opportunities using Alcohol containing hand disinfection (AHD) which is an effective alternative to soap and water [5, 10].

Non compliance with HH protocols in hospitals, especially in ICUs, is a serious contributing yet preventable cause of HAIs. Most ICU endemic infections result from HCWs hands contamination with micro-organisms with frequent outbreaks due to cross transmission due to frequent invasive procedures for ICU patients [11-13]. The purpose of the current study is to measure the compliance with HH practices among HCWs in ICUs at Beni-Suef university hospital before and after an intervention program for HH based on WHO strategies.

Materials and methods

This study was conducted among 177 HCWs working in six different ICUs - Beni-Suef university hospitals, Egypt; between March and August 2017.
ICUs included in the study were: six ICUs were included in the study. The Critical Intensive Care Unit (CICU): 19 beds; the Surgery Care Unit (SCU): 12 beds; the Cardiothoracic Care Unit (CCU): 6 beds; Chest Care Unit: 8 beds; Neonatal Intensive Care Unit (NICU): 10 beds; and the Pediatric Care Unit (PICU): 10 beds. All of the ICUs followed local infection control policies and procedures. Alcohol-based hand rub dispensers are available for each ICU, and one dispenser per every two ICU beds within each unit.

Design

This is a prospective, Interventional study divided into three phases:

Phase 1: pre-intervention; from March to April 2017; 8 weeks. Baseline hand hygiene compliance rate was assessed.

Phase 2: interventional phase, from May to June 2017, 8 weeks. Interventional training and education were carried out by the infection control team for the study participants. The educational programs aimed at raising their awareness at all levels. The training was held at least on three different occasions for each ICU HCWs to ensure their active participation concerning HH knowledge and practice. Workplace posters and explanatory Leaflets depicting the 5 moments for hand hygiene, instructions on the techniques of hand Sanitizers and hand washing were posted to act as a reminder for them. In addition, active presentations, video show and training handouts were given to each participant.

Phase 3: post-intervention, from July to August 2017; 8 weeks. Hand hygiene compliance rate was assessed post-interventional training.

Hand hygiene compliance assessment in phase 1 and 3: An observation record form was used for an unscheduled direct observation by members of the infection control team for the 5 HH opportunities [14] among ICUs HCWs; (1) before patient contact, (2) before an aseptic task, (3) after exposure to bodily fluids, (4) after patient contact and (5) after contact with patient surroundings. The observations were carried out in a 20-30-min periods, several times a week. No more than two patients were observed at a time. HCWs did not know the schedule of the observation periods. The HH compliance rate was calculated. The HH compliance data were discussed regularly during the infection control committee (ICC) meeting and with the ICU staff. The data were reported in a composite unit by job category.

Study subjects

Post intervention observations were done for 163 HCWs; 106 nurses, 34 physicians, and 23 workers (radiographers, laboratory technicians, ECG technicians, physiotherapists and respiratory therapists). Distribution of study subjects shown in Table I revealed that 95%, 89.5% and 85% of nurses, physicians and workers were observed post-intervention.

Ethical considerations

To ensure privacy, dignity, and integrity, the used questionnaire was anonymous. All required permissions were obtained from the hospital administration and from the head of the infection control unit.

Statistical analysis

Data were analyzed using the software, Statistical Package for Social Science, (SPSS Inc. Released 2009 - PASW Statistics for Windows Version 18.0. Chicago: SPSS Inc.) Frequency distribution, percentage and descriptive statistics including mean and standard deviation were calculated. Mcnemar test was performed when indicated. A Binary logistic regression model was conducted. Odds ratio (OR) and antecedent 95% confidence intervals were used to identify potential determinants of HH compliance. P-value was considered significant if ≤ 0.05.

Results

This study involved observing 112 nurses (89% females & 11% males) 67 % of them were staff nurse and 33% were head nurse with a mean age of 32.41 years ± SD 11.26. Their mean work experience was 9.97 years ± SD 9.58. 38. Thirty-eight physicians were observed for HH compliance (45.7% males & 54.3% females), 33% were clinical residents, 58% were specialists and 10 % were consultants. Their mean age was 30.74 years ± SD 6.8 with a mean work experience of 5.74 +± SD 6.56. Workers constituted 15% of the study group (32% males & 68% females) with a mean age of 32.41 years ± SD 11.26 and a mean work experience of 9.97 years ± SD 9.58.

Study observations included 608 ICU opportunities, collected before the intervention program (March to April 2017), and 673 observations collected after the intervention program (July to August 2017).

A statistically significant improvement (P = 0.01) in the overall HH compliance rate from 30.9(95% CI: 27.2-34.6%) before the intervention to 69.5(95% CI: 65.2-72.6%) post intervention (P = 0.001) is shown in Table II.

Pre-intervention compliance rates were lower for the neonatal and cardiac ICUs. Table II also represents the difference between HCWs HH compliance rate pre and

Tab. I. Distribution of the study group among the 6 ICUs.

Type of ICU		Physician		Nurses		Workers	
		Pre	Post	Pre	Post	Pre	Post
1	Critical Intensive Care Unit	11	9	32	30	8	7
2	Surgery Care Unit	7	6	20	19	4	4
3	Cardiothoracic Care Unit	5	5	11	11	3	3
4	Chest Care Unit	4	4	15	14	5	4
5	Neonatal Intensive Care Unit	6	5	18	17	4	3
6	Pediatric Care Unit	5	5	16	15	3	3
Total		38	34	112	106	27	23

Tab. II. Pre- and post-intervention hand hygiene compliance rates.

Variable*	Compliance rate% (95% CI)	
	Pre-intervention	Post-intervention
ICUs		
Pediatric ICU	37.8 (27.9-47.8)	74.2 (66.5-81.9)
Chest ICU	32.3 (22.7-41.8)	71.1 (63.1-79.1)
Surgery ICU	35.4 (26.5-44.5)	68.8 (59.3-79.2)
Neonatal ICU	25.0 (10.9-39.0)	69 (59.0-79.0)
Critical ICU	30.4 (21.9-39.0)	71.1 (63.1-79.1)
CCU	24.3 (17.4-31.2)	61.0 (51.5-70.04)
Healthcare workers		
Nurses	37.9 (32.8-42.8)	71.7 (67.2-76.2)
Physicians	21.7 (15.8-27.6)	67.5 (61.4-73.6)
Others	17.5 (7.4-27.7)	62.5 (49.4-75.6)
Hand hygiene indication		
Before patient contact (Moment 1)	22.1 (15.8-28.4)	69.0 (61.7-69.2)
Before aseptic procedure (Moment 2)	40.5 (31.3-49.8)	73.3 (66.0-80.5)
After body fluid exposure (Moment 3)	55.4 (43.0-67.8)	75.7 (69.0-82.8)
After patient contact (Moment 4)	35.0 (27.6-42.4)	72.8 (66.6-79.0)
After patient surrounding contact (Moment 5)	12.4 (5.7-19.04)	58.5 (48.5-67.7)
Overall HH compliance rates	30.9 (27.2-34.6)	69.5 (65.2-72.6)

ICU: Intensive care unit; HH: Hand hygiene; Others: Radiographers, laboratory technicians, ECG technicians, physiotherapists and respiratory therapists

post intervention in the six ICUs for the *5 moments* with a significant improvement among all HCWs in the six ICUs (P = 0.001).HH compliance rates were highest among nurses in the pre-intervention phase, which increased for all HCWs after the interventional program (P = 0.001). Moments 1&5 had the lowest HH compliance rates pre-intervention and a significant difference was achieved post the interventional program for all 5 moments (P = 0.001).

Using binary logistic regression analysis model; we use the hand hygiene after the intervention (done or missed) as a dependent factor and HCWs type, Events of HH and ICUs type and predictors or independent variables for hand hygiene improvement after the intervention. It was illustrated that the type of HCWs, type of ICUs didn't not affect the compliance of HCW towards HH and the only positive predictor was the event or the indication for HH after touch patients and after an invasive procedure (OR = 1.399, 95% CI: 1.004–1.948) with P = 0.047 (Tab. III).

Discussion

Hand hygiene is an effective tool in the reduction of health care associated infection (HAIs) in healthcare facilities, especially in intensive care units (ICUs), and poor compliance for hand hygiene is associated with high rates of HAIs [15]. In the present study, the success of the interventions (educational) program carried out for ICU HCWs showed a significant improvement in the HH compliance rates evidenced by the increase in overall hand hygiene compliance rate in all ICUs from 30.9% before the intervention to 69.5% after the intervention (Tab. I). This finding is in agreement with similar Middle East studies from Saudi Arabia, Kuwait reporting improvement from 43-60.8% before intervention to 61.4-86.4% post-intervention [16, 17], and similar to the reported improvement post intervention from 23.1% to 64.5% in Argentina [18], and from 30.0% to 56.7% in Brazil [19] and from 51.0% to 67.2% in a multi-center Multi-national study including 55 departments in 43 hospitals in Costa Rica, Italy, Mali, Pakistan, and Saudi Arabia [4].

In the current study, HH compliance was highest for moments 2, 3 & 4 and lowest for moments 1&5 (P = 0.001). This observation was constant in the pre and post interventional phases.

Improvement of HH practice was observed among HCWs for the 5 moments post the interventional program. Moment 1 improved from 22.1% to 69% in agreement with similar European and Arabian studies reporting improvement from 35% and 52% [17, 20, 21], reflecting lesser concern of personal HCWs risk of contamination before patient's contact or representing a vector for pathogenic

Tab. III. Factors determining hand hygiene compliance in intensive care units.

Factors	P-value	OR	95% C.I. for OR	
			Lower	Upper
HCWs: nurses vs physicians and other HCWs	0.175	0.794	0.569	1.108
Event: after vs before patient contacts	0.047	1.399	1.004	1.948
ICUs: medical vs surgical	0.626	1.097	0.756	1.591
Constant	0.000	1.992		

CI: Confidant interval; OR: Odds ratio, HCWs: Health care workers; ICU: Intensive care unit

organisms transmission to others [22-24]. Other factors such as work overload and insufficient time could be the cause of this result.
Moment 2 improved from 40.5% to 73.3 % similar to the reported improvement from 51.0% to 67.2% in a multinational study conducted in six pilot sites [20].
As for a Moment 3, an observed higher compliance rate from 55.4% to 75.7%, higher than the reported percentages in an Indonesian study with an improvement from 22.2% to 33.3% [26] and similar to that reported on a Saudi Arabian study from 65.2% to 85.2% [16]. High compliance rate of HCWs is logical when hands are visibly dirty or sticky.
Similarly, results of higher compliance rate for a Moment 4 were observed from 35% to 72.8%, a finding which ranges consistent with similar reported improvement from (20.6-78.6% in pre-intervention to 34.1-89.7% in post-intervention) [16, 26].
Compliance with the WHO recommendation for HH practice after contact with patient surroundings (surfaces and objects) was poorly implemented by HCWs in the current study. This is shown by the lowest compliance rates of Moment 5 in the pre and post intervention phases in spite of the highest improvement rates from 12.4% to 58.5% (P = 0.001) yet did not reach a satisfactory percentage. Findings which are similar to the reported improvement percentages for Moment 5 in Indonesia and another study conducted in at six pilot sites [20]. Explanation of which might be due to HCWs belief that patient's surroundings harbor less risk for acquired infections. Therefore, convincing evidence should drive HCWs to practice effective HH to protect themselves [20, 26-28].
Hand hygiene compliance rate among nurses was significantly higher (P = 0.001) compared to the compliance of physicians and other HCWs in pre- and post-intervention phases. This is in concordance with other studies [16, 20-22, 24, 29]. In general, physicians were found to be poor compliant with infection control standards [30].

Conclusions

The HH compliance rate among HCWs improved with the Interventional, teaching program in the six ICUs in Beni-Suef university hospital. Nurses were found more compliant with the HH practice compared to physicians and other HCWs. HH compliance rates after Moments 2, 3 and 4 were significantly higher compared with Moments 1 and 5. Continuous professional performance improvement programs should be periodically implemented and audited to maintain an adequate, safe environment for the HCWs and the patients.

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Conflict of interest statement

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Authors' contributions

The authors have contributed substantially to conception of the study, analysis and interpretation of data, drafting of the article, and critical revision of the article. Both authors have given final approval to the article as submitted.

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ORIGINAL ARTICLE

Smoke-free environment policy in Vietnam: what did people see and how did they react when they visited various public places?

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Keywords

GATS (Global adults tobacco survey) • SHS (Secondhand smoke) • Smoke-free environment policy • Smoke-free regulations • MPOWER • Vietnam

Summary

Introduction. *Since Vietnam has signed WHO framework on tobacco control (FCTC) in 2003 and has issued tobacco control law in 2013, there has been little research concerning about what impacts smoke-free regulations have had on public compliance. The objective of this study was to assess public exposure to secondhand smoke and reaction toward smoke-free policy regulations in Vietnam and the associated factor.*

Methods. *Using the design of GATS (Global Adult Tobacco Survey), a nationally representative sample of 8,996 adults were approached for data collection. Logistic regression was used to examine the associated factor.*

Results. *The study revealed that the prevalence of respondents exposed to secondhand smoke was much higher in bars/café/tea shops (90.07%) and restaurants (81.81%) than in any other public places, universities (36.70%), government buildings (31.12%), public transport (20.04%), healthcare facilities (17.85%) and schools (15.84%). 13.23% of respondents saw smokers violate smoke-free regulations. Among those who saw them violate smoke-free regulations, just one-third cautioned them to stop smoking. Strikingly, a higher rate of cautioning smokers to stop smoking was observed among the older, married, and better educated respondents. Respondents who were married, better educated and in lower economic status were more likely to remind smokers to stop smoking.*

Conclusions. *The study has called for strengthening two of the six MPOWER (Monitor, Protect, Offer, Warn, Enforce and Raise) components of the tobacco free initiative introduced by WHO, Monitoring tobacco use and prevention policies and Protecting people from tobacco smoke.*

Introduction

Smoke-free policies are one of the most important initiatives to protect people from exposure to secondhand smoke, help smokers quit and reduce youth smoking [1]. Of the 195 members enrolling the WHO FCTC, 118 states (60%) have implemented the regulations of smoke-free environment policies from minimal to complete level [2]. In addition to raised tobacco taxation rates, smoke-free policies have been found as one of the most effective tobacco control measures [3-5]. Many studies have shared best practices in adopting smoke-free policies and proved health economic outcomes of these policies in the world despite the opposition and obstruction from public and tobacco industries [6]. The outstanding example is Turkey, the nation with the highest rate of adults smoking in Europe with 40.0% in 2006. After six years of policy release, it had achieved the rate of 13.4% by applying the MPOWER (*Monitor, Protect, Offering, Warn, Enforce, Raise*), especially the smoke-free environment regulations [7]. The Article 8 of WHO FCTC and the letter "P" in MPOWER encourage states and nations to take action to protect their people from exposure to secondhand smoke in their work places, public transport and indoor public places.

Although many countries have been trying different approaches to applying the smoke-free regulations on national scale, only 18% of the world's population is protected with comprehensive and national laws which ban tobacco smoke in workplaces and public places, such as restaurants and pubs [2, 8, 9]. However, many smoke-free regulations are still implemented successfully at local level and multiplied in different places - outdoor areas and in shared housing settings [6, 8, 10, 11]. In any circumstances, not only is it non-smokers who take benefits from smoke-free regulations by being protected from exposure, but also smokers who want to quit. It has been found in several industrialized countries that smoke-free policies in work places reduced total tobacco consumption among workers by an average of 29% [1].

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Vietnam is among the countries with the highest smoking rate and its government has early recognized the burden of tobacco use as well as the high need of tobacco control policies in which smoking bans indoor and outdoor locations are of top priorities [12]. Prior to the adoption of WHO FCTC, the Government of Vietnam had enacted the National Tobacco Control Policy 2000-2010 which prohibited smoking in theaters, offices, health facilities, schools and other public areas [13]. After having signed the WHO FCTC in 2003, the Prime Minister and the Government have shown a strong commitment to strictly ban smoking from indoor workplaces and public places through Directive 12/2007/CT-TTg on strengthening tobacco control activities and the Decision No. 1315/QĐ-TTg on the Ratification of the Plan for the Implementation of the Framework Convention on Tobacco Control [14, 15]. Since 2013, the National Assembly has ratified the Law on Prevention and Control of Tobacco Harms [16]. This is the official document with the highest legal validity on tobacco control in Vietnam. In article 11, public places where smoking are completely prohibited include health facilities; education facilities; child care facilities and entertainment area designated for children; areas with high risk of fire and explosion; workplaces; universities and colleges; academic institutes and public means of transport (automobiles; air planes; sky train/metro). Article 12 of the law indicates areas where indoor smoking is prohibited, but allowed for separate designated smoking areas, such as airports segregation areas (waiting areas before boarding the plane); bars, karaoke lounges, discos hotel and guesthouses; on the public means of vehicle that are ships and trains [16]. In 2013, the government of Vietnam also approved Decision No 229/QD-TTg on the National Strategy for Tobacco Control by 2020 [17]. Since the law has been approved, several related policies have been implemented including the establishment of smoke-free places, increase in the size of graphic health warning labels, restricting tobacco advertising, promotion and sponsorship, and establishment of tobacco control fund. However, little is known about how public are exposed to secondhand smoke and how institutions have followed these policy initiatives. The objective of this study was to assess public exposure to secondhand smoke and reaction toward smoke-free environment policy regulations in Vietnam and associated factors.

Methods

Design. This is a cross-sectional quantitative survey on a nationally representative sample using the design of GATS. The study protocol was well completed through the technical support from CDC and WHO, Vietnam Tobacco Control Fund - VINACOSH (formerly VINACOSH), Institution for Preventive Medicine and Public Health, Hanoi Medical University, and Social-Environmental Statistics Department, General Statistics Office of Vietnam, and WHO.

Sample and sampling. The survey was taken on a nationally representative sample of 8,996 adults, including all men and women age 15 years old or older, in conformity with the GATS design. This target population included all people whose country of residence is Vietnam. This included those individuals residing in Vietnam even though they may not be considered a citizen of Vietnam. The sampling did not comprise those who were visitors (e.g. tourists), institutionalized in hospitals, or residing in an assisted living facility/nursing home, on a military base, and others. To reach a complete sample, the General Statistics Office developed a master sample, which consisted of 15% of population-based 2009 Census. It was sampled with the stratified two-stage random systematic sampling method. The sample contained 25,500 enumeration areas (EAs) from 706/708 districts of Vietnam (2 island districts were excluded from the GSO master sample frame). The sample was eligible if it met 1) random selection which was used in each sampling stage so that every member of the target population had a non-zero chance of being selected into the sample, and 2) the probability of selection for every unit (household and person) selected at each stage of the design was known and retained on the final analytic files for the study.

Data collection was conducted during August to October, 2015 in all 63 provinces of Vietnam. The pre-test took place before the main survey. The pre-test showed that it was technically feasible for the main survey as it met the criteria of face validity and content validity. The main survey was then done by the General Statistics Office by using electronic data collection (tablet PC) involved by 20 data collection teams, consisting of a total of 100 interviewers. Each team consisted of one team leader and 4 interviewers to ensure close supervision and collection of high quality data. They were trained by lecturers/resource persons from GSO, WHO, CDC and Vinacosh. After 2 training courses, the Steering Committee built the survey schedule for each team in cooperation with the Professional Statistics Offices (PSOs). Team leaders carried lists of selected households for assigned EAs. All the selected households were contacted during the main survey, and no replacement were made if a selected household or individual was absent during data collection. There was a standard provision of at least three call backs. Each team interviewed about 500 households on average. The survey was rigorously supervised by checking interview procedure, observing methodological compliance and managing data before, during and after the survey.

Measures. Key variables measured how public were exposed with secondhand smoke. There were two kinds of questions. The first included 7 variables which derived from 7 yes/no questions asking respondents if they were exposed to and inhaled the smoke from anyone who smoked inside of any of the following sites: government buildings or government offices, healthcare facilities, restaurants, bars/cafés/tea shops, public transport, kindergartens/schools, and universities. The second kind of question asked if respondents saw anyone who was both smoking and violating smoke-free regulations during the past 30 days.

Dependent variable was respondents cautioning smokers to stop smoking when seeing them violate smoke-free environment policy regulations, which was measured with one "yes/no/refused" question "Did you remind these smokers to stop smoking when you saw they were violating smoke-free regulations during the past 30 days?" All cases with refused response were excluded from data analysis.

Predictor variables were selected from the available GATS Vietnam 2015 data. They were *Gender* (male or female), *Age* (15-24, 25-44, 45-64 or ≥ 65 years old), *Marital status* (single, currently married or separate/divorce/widow.), *Ethnicity* (the Kinh, the largest ethnic group in Vietnam or the ethnic group), *Educational levels* (completed primary or less, lower secondary, higher secondary school, and college or above degrees), *Residence* (urban or rural areas), *Main occupation* (unskilled, semiskilled, skilled, clerk, professional, or managerial), *Economic status* (5 quintiles from the poorest to the richest group), *Awareness of penalty policy regulations in ban places* (aware or not about the penalties for smoking in places where it is no longer allowed).

Data analysis. Descriptive statistics were undertaken to depict an overall picture of the current state of public exposure to secondhand smoke and reaction toward smoke-free environment policy regulations. χ^2 test was employed to compare dependent variables of interests by selected socio-demographical characteristics. Logistic regression was performed to assess the factors associated with respondents cautioning smokers to stop smoking when seeing them violate smoke-free regulations. The final model was determined using indices of model fit (p-values of the model coefficients < 0.05 and the Hosmer and Lemeshow test statistic > 0.05) [18].

Research ethics. This study was ethically approved by the Institutional Review Board, Hanoi Medical University, Vietnam. The survey was anonymous and voluntary.

Results

Selected socio-demographic characteristics of the study population

Of the 8,996 respondents who completed the interview, 48.6% were men and 51.4% were women. By age group, people age 25-44 made up the largest proportion (41.9%) and those 65 and above accounted for the smallest share (8.8%). Two-thirds of people aged 15 old and over were living in rural areas. The majority of the study population reported having lower secondary school education (52.5%) or primary or less education (26.0%). People with a college degree or above made up 7.2% of the study population. The main occupation of the study population was farmer (49.6%), followed by service/sales (19.2%) and production/driving (12.9%). Other occupations were manager/professional (6.6%), construction/mining (5.2%), office workers (2.0%), forestry/fishing (1.8%) and other (2.7%).

Public exposure to secondhand smoke in public places

Table I shows percentages of respondents who were exposed to and inhaled tobacco smoke when visiting various public places during the past 30 days. As seen, the rate of respondents exposed to and inhaled secondhand smoke was the highest in bars/café/tea shops and restaurants, followed in universities and government buildings. However, the percentage of respondents who saw any smokers both smoking and violating smoke-free regulations was quite lower (13.23%).

Tab. I. Percentage of respondents' exposure to secondhand smoke when visiting various public places during the past 30 days.

Variables (N)	% (95%CI)
Percentage of respondents exposed to secondhand smoke when visiting various public places in the past 30 days	
Government buildings (2,169)	31.12 (29.17-33.07)
Healthcare facilities (2,554)	17.85 (16.37-19.34)
Restaurants (3,326)	81.81 (80.50-83.12)
Bars/Cafes/Tea shops (2,739)	90.07 (88.95-91.19)
Public transport (1,357)	20.04 (17.91-22.18)
Schools (2,575)	15.84 (14.43-17.27)
Universities (376)	36.70 (31.81-41.60)
Percentage of respondents exposed to secondhand smoke when visiting from one to seven public places in the past 30 days (8,987)	
None public place	54.48 (53.45-55.51)
One public place	20.86 (20.04-21.71)
Two public places	17.48 (16.70-18.28)
Three or more public places	7.18 (6.66-7.73)
Percentage of respondents who saw any smokers violating smoke-free regulations (N = 6,985)	
	13.23 (12.43-14.02)

Public practice toward smoke-free environment policy in Vietnam

Table II compares, by selected socio-demographic characteristics, percentages of respondents who reminded any smokers to stop when they were violating smoke-free regulations during the past 30 days. Among the respondents who saw smokers violating smoke-free regulations, just 28.25% cautioned them to stop smoking. There were significant differences in the percentage of respondents reminding smokers to stop smoking among age groups, marital status and educational level. A higher rate of advising smokers to stop smoking was found among the respondents who were older, married, and better educated ($p < 0.01$).

Factors associated with public practice toward smoke-free policy regulations

Table III indicates the results of regression analysis of the factors associated with respondents cautioning smokers to stop smoking when they saw them violate smoke-free regulations. As shown, the final model (p-value of model coefficients < 0.05 and p-value of Hosmer and Lemeshow > 0.05) includes four factors, namely marital status, educational level, economic status and awareness of penalty policy regulations in ban places. The respondents who were married (AOR > 1, 95%CI ≠ 1), better educated (AOR > 1, 95%CI ≠ 1) and in lower economic

Tab. II. Percentages of respondents who reminded any smokers to stop smoking when they were violating smoke-free regulations by selected socio-demographic characteristics.

Variables (N=924)	% (95%CI)	*p*-value (χ^2)
Overall	28.25 (25.34-31.15)	NA
Gender		
Male	26.43 (22.34-30.98)	-
Female	29.64 (25.87-33.70)	
Age (years)		
15-24	18.99 (13.88-25.44)	***
25-44	24.37 (20.18-29.11)	
45-64	35.43 (30.22-41.00)	
65+	38.37 (28.67-49.10)	
Ethnicity		
Ethnic minority	26.44 (18.19-36.74)	-
Kinh	28.43 (25.47-31.59)	
Marital status		
Never married	15.87 (11.49-21.50)	***
Currently married	32.10 (28.62-35.80)	
Separate/Divorce/Widow	29.70 (19.73-42.04)	
Residence		
Urban	27.16 (23.68-30.94)	-
Rural	30.06 (25.45-35.11)	
Education Level		
Primary or less	29.05 (22.28-36.90)	**
Lower secondary	28.52 (23.56-34.06)	
Upper secondary	19.50 (14.57-25.60)	
College or above	33.56 (28.36-39.19)	
Occupation		
Unskilled	26.23 (21.58-31.47)	-
Semi-skilled	26.67 (15.70-41.53)	
Skilled	25.00 (9.37-51.81)	
Clerk	28.21 (19.28-39.25)	
Professional	33.55 (26.47-41.46)	
Managerial	37.93 (22.14-56.76)	
Economic status		
Poorest quintile	29.21 (20.67-39.53)	-
Second quintile	30.43 (23.80-38.00)	
Middle quintile	33.07 (25.41-41.74)	
Fourth quintile	27.71 (22.49-33.62)	
Richest quintile	25.17 (20.55-30.42)	

NA: Not applicable; CI: Confidence interval, "-": Not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Tab. III. Multivariable logistic regression of factors associated with respondents reminding any smokers to stop smoking when they saw them violate smoke-free regulations.

Predictors (N=923)	Reminding smokers to stop smoking	
	AOR	95%CI
Marital status		
Never married	1	
Currently married	2.51	1.64-3.84***
Separate/Divorce/Widow	2.25	1.14-4.42*
Education Level		
Primary or less	1	
Lower secondary	1.24	0.78-1.97
Upper secondary	0.80	0.47-1.36
College or above	1.70	1.03-2.79*
Economic status		
Poorest quintile	1	
Second quintile	0.87	0.48-1.53
Middle quintile	0.97	0.53-1.79
Fourth quintile	0.64	0.36-1.13
Richest quintile	0.52	0.29-0.93*
Awareness of penalty policy regulations in ban places		
No	1	
Yes	1.64	0.89-3.05
Model fit indicators		
p-value of model coefficients	0.000	
p-value [χ2(df) of Hosmer and Lemeshow]	0.190	
Nagelkerke's R2	3.9%	

AOR: adjusted odds ratio; CI: Confidence interval, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

status (AOR < 1, 95%CI ≠ 1) were more likely to persuade smokers to stop smoking. However, knowledge about penalty policy regulations was not strong enough to affect respondents to remind smokers to stop smoking (OR > 1, 95%CI = 1).

Discussion and conclusions

Public exposure to secondhand smoke in public places

Smoke-free environment regulations in Vietnam have been found remarkably effective in reducing smoking prevalence in some educational places, but minimally effective in the entertainment places. Compared to five years ago, the percentages of adults' exposure to secondhand smoke in university have declined remarkably from 54.3 to 17.6%, while the proportion of those who were exposed to secondhand smoke in bar/cafes/tea shop and restaurants was still high and has changed slightly from 92.6 to 90.07% and from 84.9 to 81.8% [19]. However, a positive result is that the percentage of adults exposed to secondhand smoke in public transport have declined considerably from 34.4 to 14.4% compared to five years ago [19]. According to Hyland et al. [20], such a decline in some countries can be attributed to the strict enforcement of the smoke-free policy. However, we hold that the strict enforcement in practice may not be equally made across different settings in Vietnam. The compliance would be strictly monitored in one or several areas, but not in the others. Also understandably, at the office areas such as government buildings and health care facilities, the proportion of exposure to secondhand smoke remained quite high and only decreased slightly from 34.8 to 31.1% and from 21.6 to 17.9%, respectively compared to 2010. This finding suggests that communication campaigns and awareness are not the only determinants of smoking behavior [19], but also the broader level of public health intervention is also needed to reduce people's exposure to secondhand smoke in public places.

According to the GATS China 2015 country report, the percentage of public exposure to secondhand smoke in public places in China was generally higher than that in Vietnam. The data in China showed that public exposure to secondhand smoke was 93.1% inside bars or night clubs, 76.3% in restaurants, 57.1% in households homes, 54.3% at working places, 38.1% in government buildings, 26.9% in health care facilities, 23.8% in universities, 17.2% in primary and high schools (both indoor and outdoor areas) and 16.4% in public transportation [21]. This finding implies that smoke-free environment policy regulations in Vietnam appear to be effective to some extent. However, smoking in, and/or exposure to secondhand smoke in, public places depends upon many factors, while tobacco control policies or laws are just part of the whole picture, but play an important role. From the policy perspective, we argue that the nature of regulations is also crucial. What is more important is to monitor and supervise the implementation of policies. Tobacco control programs and key stakeholders need to know how tobacco is used, how it is restricted, prohibited and fined as well as how people are protected. According to Vietnam National Assembly [16], if anyone violates smoking bans in the smoke-free areas, they are requested to stop smoking or to leave the facilities or to be refused to services and have to pay fines for violations. However, despite smoking ban signs posted everywhere in public areas, smokers still ignore in some places. This has been blamed for "not strict enough" punishments from authorities [22, 23]. Compared to Japan and Singapore, the penalty for violating smoke-free regulations is about 50 times higher in these countries (240-600 USD in Japan and 140-700 USD in Singapore) [24, 25]. The question is asked now if smoke-free regulations in Vietnam are appropriate and/or powerful enough to prevent and stop people smoking in public places.

Public practice towards smoke-free environment policy

Since the establishment of smoke-free regulations at work and in public places in Vietnam, there has been increasing attention and support from the majority of adults - both non-smokers and smokers. The Global Adult Tobacco Survey (GATS) in 2010 revealed that the proportion of Vietnamese adults who supported smoke-free environments was quite high, reaching up to 76.2% in bars and 95.3% in public transport [19, 26]. However, there is a gap between attitude and practice. Our study has shown that only 28.3% of respondents cautioning the others to stop smoking in smoke-free areas, which also means that more than 70% of respondents are tolerant to tobacco smoke in smoke-free areas. This result is similar to a study among hospitality venue owners and employees in 7 cities of Turkey where 71.3% of participants displayed a positive attitude towards the smoke-free law, but only 19.5% of participants reported requesting customers or employees to stop smoking in the venues [27]. The practice of intervening a smoker who is both smoking and violating smoke-free regulations plays an important role in tobacco control for several reasons. First, in resources-contrained settings such as in Vietnam, the government, tobacco control program and relevant stakeholders can not arrange people or teams to regularly and directly inspect or check people's compliance with smoke-free regulations in banned places. Second, we argue that the model of cautioning smokers to cease their smoking appears feasible as we can mobilize social and community participation in tobacco control. However, this intervention should be conducted in a friendly manner and actors should be equipped adequate skills to do such. The information on the effect of such a model is important to inform tobacco control initiatives. However, in the current design of GATS, there is no question or measure to assess the effect of respondents who cautioned people who were both smoking and violating the rules. We could know how many respondents who intervened a smoker violating the rules; yet, we do not know how many smokers requested by responents successfully stopped smoking or at least smoked in permitted areas. As there is a gap between attitude and practice, there should be barriers, reasons and factors that could help to explain this phenomenon. The study in China by Yang et al. [28] indicates that lack of comprehensive laws, inappropriate penalties and a combination of weak public health education are the barriers for success of smoke-free regulations. One more important factor are unmet public expectations, which, according to Yang et al., have motivated many governments to continue to work on their own smoke-free policies [28]. The study of Yang et al. emphasizes the importance of revising or strictly monitoring smoke-free regulations to meet the requirements of the WHO FCTC [28].

In this study, we found that the older, married and higher educated respondents were more likely to remind a smoker to stop smoking in prohibited areas than the remaining groups and the difference was significant. Similarly, a study by the Ministry of Health of Vietnam et al. in 2010 [19] also reported that the higher proportion of supporting for smoke-free law fell on the group of the older age, married and higher educational attainment. The reason is that these people are more knowledgeable about the health effects of secondhand smoke and show more positive attitudes towards smoke-free law [27, 29]. Interestingly, we found that people with lower economic status such as in the second and middle quintile were more likely to request smokers to stop smoking than those in the fourth and richest quintile. This result is not in concert with the study by An et al. [30] showing that people with higher income demonstrated more positive attitudes towards "no smoking at workplaces" and at "public places".

Factors associated with public practice towards smoke-free policy regulations

Four predictors associated with the response of the people to speak up to any smokers to stop smoking in smoke-free areas were detected. To begin, we found that the currently married respondents were more likely to remind smokers if they noticed them violate smoke-free areas. Other studies outside Vietnam such as those by

Cheng et al. [31] and by Kruger et al. [32] also found that people who were aged, male, married and highly educated tended to practice 100% smoke-free home rule in America. It seems that married people are more aware of the risk of smoking and more responsible for the health of their own family especially their kids, hence more likely to follow smoke-free regulations. The second factor is educational level. Some previous studies indicate that the educational attainment is one of the key elements for the success of smoke-free policies [1, 27, 33]. According to the Ministry of Health of Vietnam et al. [19] and Thrasher et al. [33], the group with higher education was more likely to support the smoke-free policy. In our current study, the respondents with higher educational level were also more likely to practice more the rights and responsibility to stop smokers in prohibited areas. This can be explained that educational attainment could contribute to better awareness of the health risk of smoking and the understanding of the national smoke-free environment regulations as well as the law on tobacco control.

In terms of economic status, contradictory to our expectations, the poorest was more likely to ask smokers to stop smoking than the richest. This finding suggests a gap between attitude and practice of the poor against the violation of smoke-free regulations. On the contrary to the study finding by An et al. [30], the poorer were less likely to support smoke-free policy. This difference could be explained by some hidden factors such as social norms as many scientists defined as "unwritten rules about behaviors" [5, 33-36]. Qualitative research in some developing countries has also revealed a number of social factors associated with tobacco control practices, including social norms [35].

Our study has some limitations. As a cross-sectional design, the direction of effects may not be determined. Further, because this is a quantitative study, it is difficult to identify the reasons behind the key findings as indicated by the quantitative study. Also, due to the limited scope of our study which focused largely on the variables included in the GATS questionnaire, we could not have an opportunity to explore other complex factors such as social norms and social contexts that also play an important role in explaining the phenomenon. It is expected, therefore, that future research use either longitudinal design using mixed methods, both quantitative and qualitative approaches.

Despite its limitations, as a large scale design using globally recognized systematic and standardized methods, this study can provide a strong case for tobacco control recommendations. To reduce smoking and public exposure to secondhand smoke, it is crucial to strengthen two of the six MPOWER components of WHO. Besides public awareness raising campaigns, it is important to continue monitoring tobacco use and smoke-free regulation adherence of the public to provide evidence for tobacco control initiatives. Another strategy is to find more innovative ways to protect people from tobacco smoke. A good example of this would be to use a peer model to motivate public to caution smokers to stop smoking. It is also recommended that the government continue to adapt or modify the existing smoke-free regulations to meet public expectations. People to caution tobacco control violators should be well-prepared such as mature and better educated as they have been believed to be self-confident to offer advice on stopping violation and smoking. It should be noted, however, that the process of change require continued commitment from the government as well as active participation of many related actors and stakeholders at all levels. As Vietnam has much in common with other developing countries in Southeast Asia, this research could provide evidence for useful policy and practice for tobacco control in similar countries.

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Conflict of interest statement

None declared.

Authors' contributions

HVN outlined the manuscript, analyzed the data, drafted and revised the manuscript. ADD and HTTD wrote some parts of the manuscript. ATMD advised and edited the manuscript. GBK, HTP, HTD, KNL, LTN, MVH, NQP and QTN designed research proposal and tools and managed data collection. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

A survey of social cognitive determinants of physical activity among Iranian women using path analysis method

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Keywords

Physical activity • Self efficacy • Self-regulation • Social support • Women

Summary

Introduction. *This cross-sectional study was carried out on 400 women selected from urban health centers in Isfahan through stratified sampling. The study was designed to evaluate the social cognitive theoretical model in explaining the determinants of physical activity among women using path analysis method.*
Methods. *In a hypothetical framework, the relationship between self-efficacy, outcome expectation, social support and self-regulation and physical activity were assessed using path analysis and indices of fitness. Furthermore, the predictive power of the model was evaluated.*
Results. *The social cognitive theoretical model had a good predictive power for physical activity. Confirmatory factor analysis revealed the suitability of the theoretical model; this model is able to cover 80% of the physical activity variance. Evaluation of the social cognitive theoretical model using path analysis showed that self-regulation was the strongest determinant of physical activity. Social support and outcome expectation had very weak effects on physical activity; nonetheless, their effect was enhanced by the presence of self-regulation. Self-efficacy had a weak effect on physical activity, however, as an intermediate variable, it reinforced the impact of social support and outcome expectation on physical activity.*
Conclusions. *The use of the present hypothetical model is suggested as an appropriate framework in research related to physical activity among women as well as to strengthening self-regulation skills in designing and implementing programs promoting physical activities.*

Introduction

Regular physical activity will be one of the most important indicators of health by 2020 [1]. Physical activity leads to a decrease in incidences of chronic diseases such as osteoporosis, type II diabetes, cardiovascular diseases, breast cancer, high blood pressure and cerebral stroke [2]. Despite the benefits of exercise for health, only 48% of women in high-income countries and 21% of women in low-income countries are involved in appropriate physical activities [3]. The national research findings of risk factors for non-communicable diseases showed that 48.6% of Iranian women had low physical activity levels [4]. Women's participation in physical activities is influenced by social cognitive factors. For this reason, several studies have been conducted to identify these factors [5, 6]. Researchers believe it is unlikely that age be correlated with physical activity and the appropriate social economic status (education, income level) may have a positive impact on physical activity [7-9]. Self-efficacy is one of the most important determinants of physical activity. Self-efficacy means one's confidence in his ability to perform a particular behavior [10, 11]. In most research studies, self-efficacy has been identified as one of the strongest predictors of physical activity that may affect physical activity directly or indirectly through other factors such as self-regulation or outcome expectations [8, 9, 12]. Outcome expectations are the positive and negative beliefs of individuals in achieving results and values associated with carrying out a behavior [10, 11]. According to researches, outcome expectation is one of the weak determinants of physical activity. It is probable that outcome expectation impacts physical activity indirectly through other factors such as self-efficacy or self-regulation [7, 13]. Self-regulation means managing and modifying individual behaviors through the process of setting goals, observing behaviors and modifying them [10, 11]; one of the important factors affecting women's participation in sports activities. Self-regulation can affect physical activity either directly or indirectly through other perceptual factors such as Social support [14-16]. Social support means getting help by communicating with others [10, 11]. There are very few studies that have examined the relationship between social support and physical activity [8, 17]. According to Bandura's theory, social support has a direct impact on physical activity [18]; nevertheless, Anderson's research showed that social support, through self-efficacy and self-regulation, had a better indirect impact on physical activity [16]. Several studies have shown that these four constructs (i.e. Self-efficacy, Outcome expectation, Self-regulation, and Social support) play an important role in promoting physical activity [5, 16, 17, 19]. Social cognitive theory is an appropriate model for inves-

tigating the relationship between these factors with each other on the one hand and with physical activity on the other [17]. Challenges and vague points existed, however, in the studies conducted to survey determinants of physical activity using the social cognitive theoretical model; perhaps a review of these relationships within the framework of a theoretical model can answer some of these questions. The results of the study conducted by Resnick on 201 elderlies showed that outcome expectation and self-regulation had direct and indirect effects on physical activity; nonetheless, the role of self-efficacy was not investigated [20]. Rovniak, in a study on 277 students, stated that social support influenced physical activity through self-efficacy, self-efficacy influenced physical activity through self-regulation and self-regulation influenced physical activity directly. In this study, the role of outcome expectation had not been attended to [21]. In the study by Anderson conducted on a sample of 299 men and women, self-regulation was recognized as the strongest predictor of physical activity; in contrast, the effect of self-efficacy on physical activity was weak and the role of outcome expectation was not clear [16]. Ayotte, in a study on a sample of 116 middle-aged and married individuals, stated that self-efficacy affected physical activity directly and indirectly through outcome expectation and self-regulation. In this study, the role of social support had been neglected [22]. Considering the important role of social cognitive determinants as well as ambiguous results of the studies, the present study was carried out with the aim of investigating the relationship between these factors and their impact on physical activity using social cognitive theory on a group of women living in Isfahan city carrying out the path analysis method. Based on the relationships established in previous studies, the relationships between the variables of the theoretical framework of the present study are defined in Figure 1. As seen in the proposed framework, self-efficacy affects physical activity directly and indirectly through self-regulation. Social support indirectly influences physical activity through self-efficacy, outcome expectation and self-regulation. Self-efficacy is a mediating factor between outcome expectation and physical activity. Self-regulation is a key factor that directly promotes physical activity. Moreover, these relationships are based on a review of the existing literature [16-18, 20-23] and the presuppositions of the present study. The present study, focusing on the role of outcome expectation, examines the following three hypotheses:

1. outcome expectation has a direct effect on physical activity;
2. outcome expectation influence physical activity indirectly through self-efficacy;
3. self-regulation is an intermediate variable between outcome expectation and physical activity.

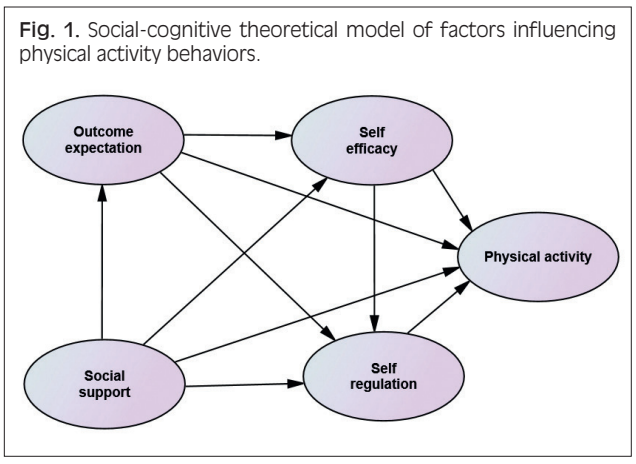


Fig. 1. Social-cognitive theoretical model of factors influencing physical activity behaviors.

Methods

The present cross-sectional study was conducted in Isfahan, Iran, from May to September 2016. Taking into account the 0.95 confidence level (1.96), power of 80% (0.84) and given that the correlation coefficient of self-efficacy and physical activity equal to 0.15% [9] as well as 15% chance of loss, a total of 400 subjects were chosen as the sample. At first, urban health centers in Isfahan were selected randomly and then 10 centers were selected from among 25 centers through cluster sampling. Furthermore, the number of samples from each center was determined proportionally to the population covered by each center. After being acquainted with the participants, explaining to them the research objectives and how to fill out the questionnaire, they completed the informed consent and completed the questionnaires. The inclusion criteria for this study were informed consent and the ability to respond to the questions. The exclusion criteria were physical and mental disability and unwilling to complete the questionnaires.

The instrument for collecting information consisted of three sections:

1. Demographic factors questionnaire consisting of 10 questions about age, education, marital status, employment status, and income.
2. Social cognitive factors questionnaire: after extensive library studies and reviewing numerous works, a number of questionnaire items were collected to measure the determinants of physical activity. Subsequently, the items were translated into Persian and necessary corrections were made for the cultural and linguistic adaptation of the questionnaire by a committee of five bilingual experts. A number of items were removed due to inappropriate and vagueness [24]. The Initial development questionnaire consisted of 39 items and four constructs: self-efficacy, outcome expectation, self-regulation and social support. To determine Content Validity Index (CVI) and Content Validity Ratio (CVR), the questionnaires were evaluated by a panel of 20 health education professors. According to the Lawshe table and the number of specialist participants, the CVR approval criterion for each item was considered to be equal to 0.42 or higher. Moreover, the acceptance criterion for CVI was considered to be 0.79 [24]. At

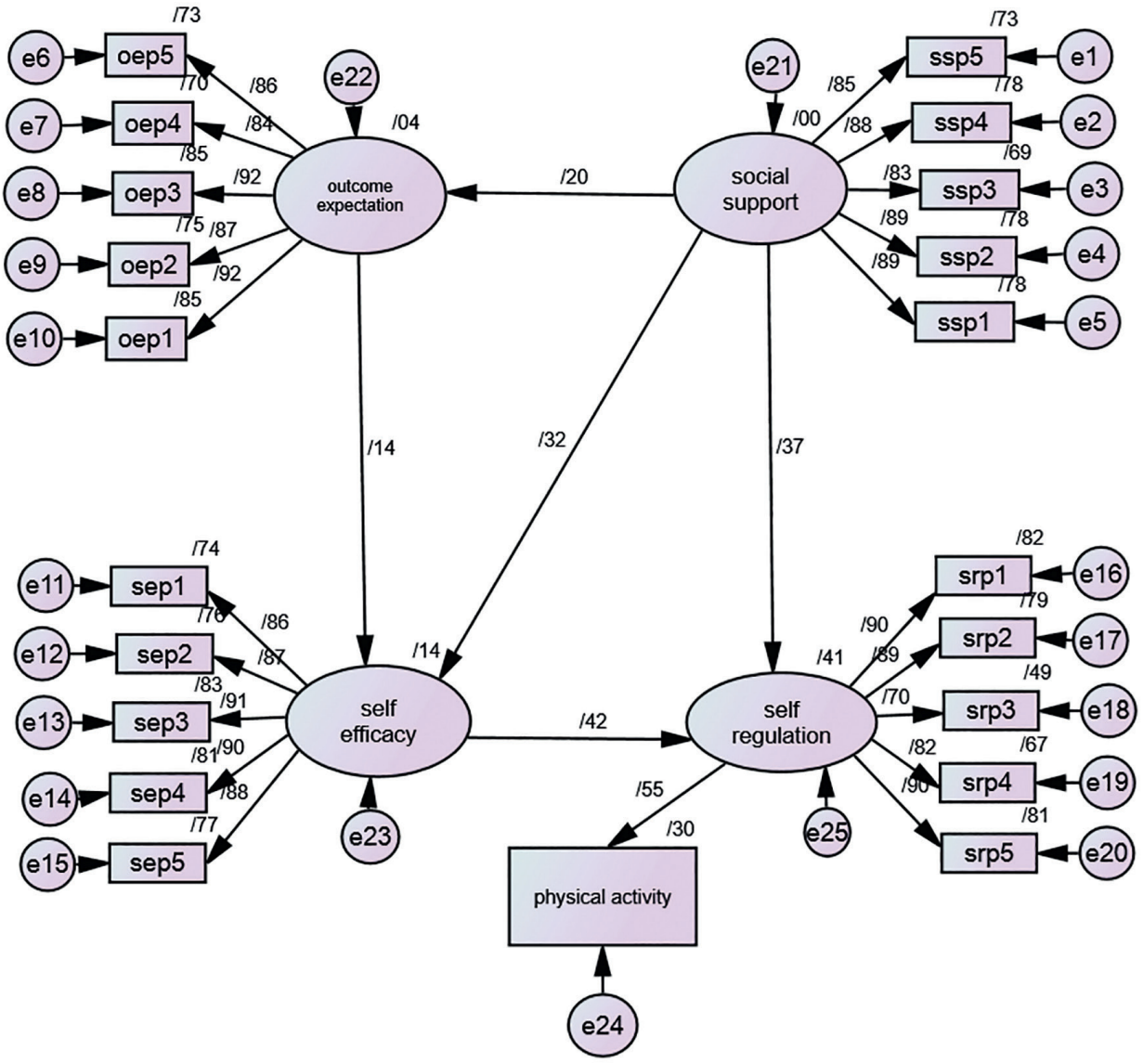
this stage, 19 items were deleted and 4 items were revised. Finally, 20 items were accepted of which five were considered for each construct. The score range was based on a 10-point Likert-type scaling (1 "strongly disagree" to 10 "strongly agree"). The lowest score was 20 and the highest was 200. Cronbach's alpha reliability of the questionnaire was 0.91. Also, Cronbach's alpha reliability of the questionnaire of self-efficacy was 0.94, 0.94 for outcome expectation, 0.93 for social support and 0.92 for self-regulation [25].

3. Tools to assess physical activity: Standard questionnaire of physical activity was used in this regard. The international physical activity questionnaire (IPAQ) was used to determine appropriate levels of physical activity among adults aged 15 to 69 years [26], and its validity and reliability have been reported [27, 28]. According to its instruction, people are classified into three groups in terms of physical activity: low activity (0-599 MET-min/week) of moderate activity (600-3000 MET-min/week) and intense activity (greater than 3000 MET-min/week) [26].

Data analysis

Statistical tests were performed using SPSS and Amos Graphic software. In order to evaluate the predictive power of the main variables, multiple regression analysis was performed using the Enter method. Spearman's correlation coefficient was used to analyze the relationship between predictor variables and physical activity with the significance level of less than 0.05. The predictive power of the social cognitive theoretic model was tested using the path analysis method (Fig. 2). Fitting indicators were selected to evaluate the social cognitive theoretical model from all three categories (absolute,

Fig. 2. Structural equation analysis of the final social-cognitive model of physical activity.



comparative and thrifty) and were calculated by using Confirmatory Factor Analysis (CFA). The CFA model, using the robust maximum likelihood, was used to estimate model parameters. The model was considered acceptable if Normed Chi-Square (CMIN/DF) was between 1 and 5, Comparative Fit Index (CFI) was greater than 0.8, Parsimonious Comparative Fit Index (PCFI) was more than 0.6, Tucker-Lewis Index (TLI) was more than.0.9, Root Mean Squared Error of Approximation (RMSEA) was < 0.05 good fit or between 0.05 and 0.08 adequate fit [29, 30].

Results

The number of participants in the study was 400 women, of which 40 were excluded from the study due to illness, disability or lack of willingness to complete the questionnaires. Finally, 360 participants remained. The mean of participants' age was 33.67 (M = 33.67, SD = 8.35). The lowest age was 14 and the highest was 50. The characteristics of the participants are presented in Table I.

The results of the physical activity of participants (Mean = 934.33, SD = 1051.598) based on the IPAC questionnaire and MET min / week criterion are shown in Table II.

Multiple regression test results, presented in Table III, showed that this model is able to explain 29% of the physical activity variance (R Square = 0.29, F Change = 36.42, pvalue < 0.001). The strongest predictor of physical activity was self-regulation (Beta = 0.47) and secondarily self-efficacy (Beta = 0.11). Social support and outcome expectations did not play a significant role in predicting physical activity.

The result of Spearman correlations showed that variables of the social cognitive theoretical model had a significant relationship with each other and with physical activity (Tab. IV). Furthermore, physical activity had the highest correlation with self-regulation. Therefore, we can examine the relationship between these factors in one theoretical model.

Tab. I. Characteristics of women who participated in study(n = 360).

Groups Variables	Frequency (n)	Percent (%)
Years of Education		
Illiterate	5	1.4
The ability to read and write	13	3.6
Primary school	36	10.0
Middle & high school	49	13.6
Diploma	145	40.3
Collegiate	112	31.1
Income Status		
Little	38	10.6
Moderate	205	56.9
Good	100	27.8
Excellent	17	4.7
Marriage Status		
Marriage	314	87.2
Single	36	10.0
Widow	6	1.7
Divorced	4	1.1
Job Status		
Employed status	68	18.9
Unemployed	292	81.1

Tab. II. The results of the physical activity of women who participated in study (n = 360).

Total physical activity	Frequency (n)	Percent (%)
Low	168	46.7
Intermediate	178	49.4
High	14	3.9

Structural equation model

In order to evaluate the structural relations between the predictors of physical activity, a model was drawn by the presence of all the constructs of self-efficacy, outcome expectations, social support and self-regulation. Then, the paths with weak relationships were eliminated from the model and the final model was analyzed using the path analysis method (Fig. 2). The CFA modl showed the suitability of the theoretical model; the model was able to predict 80% of the physical activity variance.

Direct, indirect and total effects

The evaluation of the social cognitive theoretical model using path analysis showed that self-regulation was the strongest determinant of physical activity ($\beta_{Direct} = 0.55$). The direct effect of social support on physical activity was very weak, but the indirect effect of social support on physical activity through outcome expectation, self-efficacy and self-regulation were 0.2, 0.32 and 0.37, respectively. Moreover, social support through these three variables affected physical activity, whose influence on physical activity through self-regulation was stronger than the others paths. The direct effect of the outcome expectation on the physical activity was very weak and outcome expectation had the greatest impact on physical activity through self-efficacy. The direct impact of self-efficacy of physical activity was very weak. Self-efficacy with an indirect effect on physical activity through self-regulation could affect physical activity, which has a meaningful effect. The strongest direct effect of physical activity was related to self-regulation and the strongest indirect effect on physical activity was related to self-efficacy and social support, which had the greatest impact on physical activity through self-regulation (Tab. V).

Discussion

The aim of this study was to investigate the correlation of social cognitive variables with physical activity in a group of women in Isfahan. For this purpose, the predictability power of social cognitive theory constructs was evaluated using path analysis method. The results are discussed based on the proposed framework and assumptions of the present study. The basic premise of this study was whether the proposed theoretical framework was able to explain the physical activity behavior. This

Tab. III. The results of multiple regression analysis of social cognitive variables in the prediction of physical activity.

Model	Beta	Std. Error	t	p_{value}
(Constant)	97.35	186.2	.52	.60
Self-efficacy	8.78	3.86	2.27	.024
Outcome expectation	-2.98	4.31	-.69	.48
Social support	1.31	3.99	.33	.74
Self-regulation	35.41	4.18	8.46	0.000

Tab. IV. Spearman's correlation coefficients between with the social cognitive factors and physical activity.

Structures	1	2	3	4	5
1. Self-efficacy	1				
2. Outcome expectation	0.19*	1			
3. Social support	0.33*	0.18*	1		
4. Self-regulation	0.49*	0.21*	0.46*	1	
5. Physical activity	0.34*	0.09*	0.27*	0.52*	1

Notes:*Correlation is significant at the 0.01 level (two-tailed).

Tab. V. Direct, indirect and total effects of variables in the social-cognitive model of physical activity.

Variable	Coefficients	Social support	Outcome expectation	Self-efficacy	Self-regulation	Physical Activity
Social support	Direct	-	0.2*	0.32*	0.37*	0
	Indirect	-	0	0.02	0.14	0.28
	Total	-	0.2	0.34	0.51	0.28
Outcome expectation	Direct	-	-	0.14	0	0
	Indirect	-	-	0	0.05	0.03
	Total	-	-	0.14	0.05	0.03
Self-efficacy	Direct	-	-	-	0.42*	0
	Indirect	-	-	-	0	0.23
	Total	-	-	-	0.42	0.23
Self-regulation	Direct	-	-	-	-	0.55*
	Indirect	-	-	-	-	0
	Total	-	-	-	-	0.55

Notes: * is significant at the $p < 0.001$.

assumption was evaluated using Amos software and path analysis, and the results showed that the proposed model has a proper predictive power and its fitting indicators are acceptable and the basic assumption of the present study is confirmed, which is similar to the result of the Ieverslandis & Rovniak studies [17, 21]. The analysis of of structural equations showed that the social cognitive theoretical model can predict 80% of the variance of physical activity and in similar studies the obtained variance was 46 and 71% [16, 22]. It is possible that this theory can be used as a framework of reference for designing physical activity programs in women. In the following discussion of the results, we evaluate the relationships within the proposed theoretical framework approved by path analysis. One of the hypotheses in this study was whether self-efficacy is effective on physical activity. Based on the results of Ayotte 's study, Self-efficacy affects physical activity directly [22]; however, the results of this study showed that the direct effect of self-efficacy of physical activity is weak and self-efficacy with an indirect effect on physical activity through self-regulation can affect physical activity. Moreover, this finding is similar to the result of the study performed by Rovniak that has introduced self-regulation as a mediator between self-efficacy and physical activity [21]. It is possible that self-regulation is an essential factor for physical activity in addition to self-efficacy. According to Bandura theory, although self-efficacy is a prerequisite factor for starting and maintaining physical activity, self-regulation is a key factor in achieving a healthy lifestyle [18, 23]. This can indicate the key role of self-regulation in promoting physical activity that should be considered as an important factor in interventions to promote physical activity. Another study investigated whether the self-regulation affects physical activity or not. The results of this study indicated that self-regulation is the strongest predictor of physical activity, which is similar to the results of Anderson and Wolfe's studies [16, 31]. Also, self-regulation is relevant to other structures in the social cognitive theoretical model, and other structures indirectly related to physical activity through self-regulation. In general, if one has the goal and proper planning, exercise leads to increased self-esteem. Furthermore, the individual looks at the positive results of exercise leading to im-

proved physical activity [23]. One of the hypotheses of the present study is that social support affects physical activity directly or indirectly (through influence on other variables such as outcome expectation, self-efficacy and self-regulation). According to the results of this study, social support has a very weak direct effect on physical activity, which is similar to the results of Wolfe and Anderson's studies [16, 31], but the result of Ieverslandis & Hsieh studies showed that social support affected physical activity [9, 17]. Also, the results of the present study showed that the effect of social support on physical activity through the variables of outcome expectation, self-efficacy and self-regulation are acceptable and significant, which can be confirmed by the results of studies conducted by Duncan and Rovniak [21, 32]. Based on the above results, it can be said that social support alone is insufficient for physical activity and other variables such as outcome expectation, self-efficacy and self-regulation are also necessary. Moreover, Bandura believes that a lack of social support is not a barrier to physical activity, but it can affect physical activity by affecting other variables [18, 23]. In this study, three hypotheses were proposed regarding the direct and indirect effects of outcome expectation on physical activity. The results showed that outcome expectation had no direct effect on physical activity, so the first proposed hypothesis of this study is not confirmed, which is similar to the results of the previous studies [21, 22, 31]. However, the second and third suggested hypotheses of the present study are confirmed with regard to the effect of outcome expectation on physical activity through self-efficacy and self-regulation, which is somewhat acceptable and appropriate. In explaining the results, it can be said that although the results related to exercise are quite obvious and proven; it alone does not lead to the participation of a person in physical activity and perhaps the presence of self-efficacy and self-regulation strengthen the effectiveness of outcome expectation on physical activity. The effect of outcome expectation on physical activity is more than the indirect effect. What is more, self-efficacy and self-regulation can enhance the impact of outcome expectation on physical activity, and it is recommended that more attention be paid to the effects of outcome expectation on physical activity through self-efficacy and self-regulation in interventions related to physical activity [18, 23]. As the results of this study showed, the proposed model has an appropriate predictive power for physical activity. Additionally, the internal assumptions of the model that are based on the relationships between variables, showed that their direct and indirect effects on physical activity are acceptable and the use of theoretical model is suggested as an appropriate framework for research on physical activity among women. The poor path of this model is related to the direct impact of self-efficacy, outcome expectation and social support on physical activity, which may be due to their incorrect position within the proposed framework. Also, in this study, the effect of self-efficacy on outcome expectation has not been investigated. Therefore, it is recommended that additional studies be carried out in this area in the future. The strength of this study is the use of social cognitive theory to investigate the determinants of physical activity and using a theoretical model to prove the hypothesis. The present study had some limitations, including completion of questionnaires which was a form of self-report, the short duration of research and failure to examine the relationship between demographic variables in a social cognitive theoretical model, all of which indicate that the results of the present study should be used with caution.

Conclusions

The results of this study were indications of the importance of simultaneous examination of the structures of social cognitive theory in a theoretical model to explain the behavior of physical activity. It is suggested that more attention be paid to self-regulation in designing and implementing programs for the purpose of promoting physical activity. In addition, and physical activity in women should be increased with training and strengthening their self-regulation skills.

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Conflict of interest statement

None declared.

Authors' contributions

AE developed the original idea, statistical analysis, and early manuscript revising. MN collected data, analyzed them and wrote the initial manuscript.

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ORIGINAL ARTICLE

Skin safety and health prevention: an overview of chemicals in cosmetic products

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Keywords

Health Prevention • Cosmetics • Cocktail Effect • Additive Effect

Summary

Introduction. *Cosmetic products contain a wide range of chemicals to which we are exposed every day. The aim of the study was to determine the presence of potential dangerous substances which can cause adverse health effects by examining product labels.*

Materials and methods. *A total of 283 products were collected from various shops in Lecce (Italy) and divided into 3 categories: rinse-off, leave-on and make-up. The label of every product was examined and a list including fragrances, preservatives and other chemicals of concern was created.*

Results. *Fragrances were present in 52.3% of the examined products, mostly limonene (76.9%) and linalool (64.6%) but also citronellol (34.1%), geraniol (31.5%), coumarin (30%) and hexyl cinnamal (29.2%). Preservatives showed a rate of 60% and the most frequently identified were phenoxyethanol (48.7%), sodium benzoate (35.6%), potassium sorbate (22%), methylparaben (15.2%) and MI/MCI (9.9%). The other chemicals of concern were detected in 58% of products; included PEGs (62.3%), acrylate copolymer (34%), petrolatum (17.2%), polysorbates (14,8%), BHT (14.7%), ethylhextyl methoxycinnamate (13.6%), benzophenone-1 (3.7%), benzophenone-3 (4.9%), BHA (1.6%), cocamide DEA and toluene (1.2%).*

Conclusions. *The use of many of these substances is allowed within certain limits, due to their toxicity at higher concentrations. Other important aspects should be considered as, for instance, the possibility of long-term effects. On the other hand, other substances may induce several acute adverse side-effects, i.e. contact dermatitis and allergic reactions. For these reasons, an enhancement of the criteria used for cosmetics formulation is required since many chemicals used singularly or combined are potentially unsafe.*

Introduction

In their everyday life people are exposed to a great range of chemicals most of which occur naturally in the environment, but others are derived from human activities, being present in foods, water and various daily use products. Because our skin is the largest surface of the body interacting with external environment, it is both involuntarily exposed to abiotic [1, 2] and biotic factors [3, 4], and voluntarily, due to personal care and cosmetic products use. Many of these are used or applied on a daily basis and in different ways, consequently, these products are assumed for enhancing our personal hygiene and appearance and they are reputed to be harmless for body's health.

In the light of the frequent and intimate nature of the contact on skin and mucosa with these products, it is important that they do not contain potentially dangerous substances.

As a matter of fact, all the ingredients used in cosmetic products meet certain regulatory requirements [5]. However, the use of many substances is allowed within certain limits, due to their toxicity at higher concentrations. Other important aspects should be considered as, for instance, the possibility of long-term effects [6, 7]. On the other hand, other substances may induce several acute adverse side-effects, i.e. contact dermatitis and allergic reactions [8]. Moreover, the everyday use and continuous exposition of humans to a wide range of personal care products and to different kinds of chemicals, derived from several sources, may cause the so-called "cocktail effect" due to the synergistic interaction of different substances and, also, the "additive effect" because of the presence of the same ingredient in many products [9, 10].

The purpose of the current study was to determine, among the ingredients listed on the label, the presence of substances with known adverse health effects in commonly used personal care and cosmetic products. We considered fragrances, preservatives and other substances known as skin sensitizers or potentially harmful on general health.

Materials and methods

Different kinds of beauty and hygiene products were selected between October and November 2017 from various shops in Lecce (Italy), mainly supermarkets with nationwide coverage, beauty shops, and pharmacies as well as online shops. Ingredient information from labels was collected by taking photos in the shops or downloading data sheets from webshops. Because of the lack

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of available data on sales rates of specific products to the public, as in other studies [11], a crude selection of products estimated to be sold in large volume was made, on the basis of information from shop assistants and the authors' own perceptions.

All products were divided into 3 categories: rinse-off products (shower gel, shampoo, toothpaste, liquid soap, intimate soap, shaving foam) leave-on products (body cream, face cream, hand cream, deodorant, sunscreen, aftershave) and make-up ones (lipstick, lipbalm, foundation, nail polish). Such a classification was based on the time of skin application: rinse-off products stay a very short time on it, as they are usually rapidly washed away (even if it would also be appropriate to consider the frequency of application); leave-on and make-up products stay longer on the skin, but the former are more usually used for skin care, in order to protect it, perfume it and keep it in good conditions (moisturising, nourishing, tonifying, etc.), the latter have an aesthetic purpose and are intended to improve someone's look.

Every group included also organic and children's products. The first were identified on the basis of organic and natural certifications disclosed on the brand's website and indicated on the label (Cosmos, Ecolabel UE, Ecocert, Icea, Natrue, etc.); the latter showed on the label the word "baby" or "kids".

Subsequently, the label of every product was examined and chemicals which could possibly affect human health were detected. The selection of substances was based on scientific evidence: for fragrances the list of 26 allergens which have been identified as skin sensitizer by the Scientific Committee on Consumer Safety (SCCS) and whose names should be listed on the label [12] was considered; for the other substances a literature's review was conducted [13-15] and only those reporting possible harmful effects on human heath were selected.

A list with fragrances, preservatives and other chemicals of concern, including some UV filters, antioxidants, emulsifiers, surfactants and other synthetic compounds, was created. Data were recorded in Microsoft® Excel and analysed by calculating rate, median and maximum of substances for every category. No chemical analyses were performed in the present study.

Results

A total of 283 products were examined: 112 rinse-off, 103 leave-on and 68 make-up (Tab. I). Fragrances individuated on the labels were 19, preservatives were 16 and other chemicals of concern were 11.

Fragrances

The 19 fragrances individuated (Tab. II) are all included in the list of 26 allergens redacted by SCCS, whereas the missing seven ones were: amylcinnamyl alcohol, anise alcohol, benzyl cinnamate, cinnamal, evernia furfuracea, evernia prunastri, methyl 2-octynoate. More than fifty-two per cent of the products contained at least one of the fragrances investigated, especially rinse-off products (61.6%). Generally, the most frequently identified fragrances were limonene (76.9%), linalool (64.6%), citronellol (34.1%), geraniol (31.5%), coumarin (30%) and hexyl cinnamal (29.2%). Moreover, limonene was more present in rinse-off (70.7%) and make-up products (73.3%), whereas linalool was more found in leave-on ones (87.7%).

In addition, the presence of fragrances was found in organic and children's products (Tab. III), respectively 56.3% and 18.6%. Limonene was the fragrance most listed on the labels for both kinds of products (respectively 84.4 and 83.3%), followed by linalool (65.6 and 33.3%) (Not in the table).

Preservatives

Sixty per cent of the selected products contained at least one of the preservatives investigated, above all among rinse-off products (75%). The most frequently identified preservatives (Tab. IV) were phenoxyethanol (48.7%), sodium benzoate (35.6%), potassium sorbate (22%), methylparaben (15.2%) and methylisothiazolinone/methylchloroisothiazolinone (MI/MCI) (9.9%). Sodium benzoate was the most common preservative in rinse-off products (57.6%) and phenoxyethanol in leave-on (70.1%) and make-up ones (58.6%).

Four different parabens were identified (methylparaben, ethylparaben, propylparaben, butylparaben) and almost 15% of the products contained one or more parabens, mostly leave-on products (face and hand cream, sunscreen, aftershave). The most detected was methylparaben, found in all of those products containing at least one paraben, followed by ethylparaben (55.2%) and propylparaben (51.7%). All four parabens were contained in six products (foundation, face cream, lipstick, aftershave, two sunscreens) and three parabens in three products (aftershave, two face creams).

Tab. I. Products divided into rinse-off, leave-on and make-up categories with frequency of occurrence and proportion of products containing fragrances, preservatives and other chemicals of concern and their distribution in term of median and maximum.

	Examined products	Products containing fragrances			Products containing preservatives			Products containing other chemicals of concern		
		N (%)	Median	Max	N (%)	Median	Max	N (%)	Median	Max
Rinse-off	112	69 (61.6)	2	10	84 (75)	2	6	65 (58)	2	5
Leave-on	103	61 (59.2)	6	15	64 (62.1)	2	7	55 (53.4)	2	7
Make-up	68	18 (26.5)	2.5	6	22 (32.4)	2	7	44 (64.7)	2	5
Total	283	148 (52.3)	3	15	170 (60)	2	7	164 (58)	2	7

Tab. II. Frequency of occurrence and percentage of fragrances identified on the label of selected products and referring to rinse-off, leave-on and make-up categories.

Fragrances	CAS no.	Rinse-off		Leave-on		Make-up		Total	
		(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Alpha-isomethyl ionone	127-51-5	6	10.3	22	38.6	4	26.7	32	24.2
Amyl cinnamal	122-40-7	3	3.2	3	5.3	1	6.7	7	5.4
Benzyl alcohol	100-51-6	25	29.4	25	32.5	5	17.2	55	28.8
Benzyl benzoate	120-51-4	3	3.2	9	15.8	4	26.7	16	12.1
Benzyl salicylate	118-58-1	12	20.7	20	35.1	1	6.7	33	25.4
Butylphenyl methylpropional	80-54-6	12	20.7	16	28.1	-	-	28	21.5
Cinnamyl alcohol	104-54-1	1	1.7	3	5.3	-	-	4	3.1
Citral	5392-40-5	2	3.4	26	45.6	4	26.7	32	24.2
Citronellol	106-22-9	11	19	33	56.9	1	6.7	45	34.1
Coumarin	91-64-5	11	19	26	45.6	2	13.3	39	30
Eugenol	97-53-0	10	17.2	11	19.3	1	6.7	22	16.9
Farnesol	4602-84-0	-	-	3	5.3	-	-	3	2.3
Geraniol	106-24-1	7	12.1	32	56.1	2	13.3	41	31.5
Hexyl cinnamal	101-86-0	18	31	18	31.6	2	13.3	38	29.2
Hydroxycitronellal	107-75-5	2	3.4	8	14	1	6.7	11	8.5
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	-	-	12	21.1	-	-	12	9.2
Isoeugenol	97-54-1	-	-	6	10, 5	-	-	6	4.4
Limonene	138-86-3	41	70.7	48	84.2	11	73.3	100	76.9
Linalool	78-70-6	26	44.8	50	87.7	8	53.3	84	64.6

Tab. III. Frequency of occurrence and percentage of substances identified on the label of children's products.

Substances	Products	n	%
Limonene	Shower gel, toothpaste, sunscreen, lipstick, lipbalm	16	83.3
Linalool	Toothpaste, sunscreen, lipstick, lipbalm	7	33.3
Citral	Lipbalm	1	5
Benzyl alcohol	Shower gel, sunscreen, nail polish	5	25
Eugenol	Toothpaste	1	5
Alpha-isomethyl ionone	Sunscreen	1	5
Citronellol	Sunscreen	1	5
Coumarin	Sunscreen	1	5
Potassium sorbate	Shower gel, intimate soap, toothpaste, liquid soap	4	12.5
Sodium benzoate	Shower gel, shampoo, intimate soap, toothpaste, liquid soap, body cream	12	37.5
Phenoxyethanol	Shower gel, shampoo, toothpaste, body cream, sunscreen, lipstick, nail polish	14	43.8
Chlorphenesin	Shampoo, body cream	2	6.3
Imidazolidinyl urea	Shampoo	1	3.1
Diazolidinyl Urea	Nail polish	1	3.1
Methylparaben	Lipstick	1	3.1
Propylparaben	Lipstick	1	3.1
Benzoic acid	Sunscreen	1	3.1
Cocamide DEA	Shampoo	1	4
BHA	Shampoo	1	4
BHT	Shower gel, shampoo, lipbalm	4	16
PEG's	Shower gel, shampoo, intimate soap, toothpaste, liquid soap, body cream, sunscreen	17	68
MI/MCI	Shampoo, liquid soap	2	8
Ethylhextyl methoxycinnamate	Lipbalm	3	12
Petrolatum	Body cream, lipstick, lipbalm	6	24
Acrylate copolymer	Shampoo, sunscreen, lipstick, nail polish	6	24
Polysorbate-80/-60/-20	Shampoo, body cream	3	12

Tab. IV. Frequency of occurrence and percentage of preservatives identified on the label of selected products and referring to rinse-off, leave-on and make-up categories.

Preservatives	CAS no.	Rinse-off		Leave-on		Make-up		Total	
		(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Methylparaben	99-76-3	8	9.4	16	20.8	5	17.2	29	15.2
Ethylparaben	120-47-8	2	2.4	11	14.3	3	10.3	16	8.4
Propylparaben	94-13-3	3	3.5	8	10.4	4	13.8	15	7.9
Butylparaben	94-26-8	-	-	4	5.2	2	6.9	6	3.1
Triclosan	3380-34-5	4	4.7	2	2.6	-	-	6	3.1
Imidazolidinyl Urea	39236-46-9	2	2.4	8	10.4	1	3.4	11	5.8
Diazolidinyl Urea	7849-02-8	-	-	-	-	1	3.4	1	0.5
5-bromo-5-nitro-1, 3 dioxane	30007-47-7	1	1.2	-	-	-	-	1	0.5
2-bromo-2-nitropropane-1, 3-diol	52-51-7	1	1.2	-	-	-	-	1	0.5
DMDM Hydantoin	6440-58-0	12	14.1	4	5.2	-	-	16	8.4
Phenoxyethanol	122-99-6	22	25.9	54	70.1	17	58.6	93	48.7
Methylisothiazolinone/ Methylchloroisothiazolinone	2682-20-4, 26172-55-4, 55965-84-9	19	22.4	-	-	-	-	19	9.9
Chlorphenesin	104-29-0	1	1.2	2	2.6	2	6.9	5	2.6
Benzoic acid	65-85-0	5	5.9	9	11.7	1	3.4	15	7.9
Sodium benzoate	1-23-235	49	57.6	17	22.1	2	6.9	68	35.6
Potassium sorbate	24634-61-5	23	27.1	15	19.5	4	13.8	42	22

Formaldehyde-releasers (imidazolidinyl urea, diazolidinyl urea, 5-bromo-5-nitro-1, 3 dioxane, 2-bromo-2-nitropropane-1, 3-diol, DMDM hydantoin) showed almost the same rate of parabens (15%) but they were more present in rinse-off products. Among the five formaldehyde-releasers, the most common were DMDM hydantoin (53.6%) and imidazolidinyl urea (39.3%), which were both found also in two body lotions.

MI/MCI was found in 9.9% of the examined products, especially in rinse-off ones. Six products contained triclosan (3.1%) (two deodorants, two intimate soaps, a liquid soap, a shaving foam).

As far as children's products are concerned, more than seventy-two per cent contained at least one of the preservatives among those considered, in particular the most present was phenoxyethanol (43.8%), followed by sodium benzoate (37.5%). Formaldehyde-releasers were found into two products (shampoo, nail polish), parabens in a lipstick, chlorphenesin in a body cream and a shampoo, MI/MCI in two rinse-off products (shampoo, liquid soap) (Tab. III).

Almost fifty-four per cent of organic products showed on the label at least one of the preservatives investigated, in particular the most common was sodium benzoate (50%) followed by potassium sorbate (47.2%). It is notable the presence of triclosan in an organic deodorant.

Other chemicals of concern

Fifty-eight per cent of the examined products contained at least one of the other chemicals of concern, especially make-up ones (64.7%). The substances most frequently identified in this group (Tab. V) were PEGs (polyethylene glycols) (62.3%) and acrylate copolymer (34%). The first were more common in rinse-off (81.5%) and leave-on products (69.1%), while make-up ones showed a high presence of acrylates (45.2%) and petrolatum (33.3%).

UV filters (ethylhextyl methoxycinnamate, benzophenone-1, benzophenone-3) were present in 19.1% of the products, especially in make-up ones (45.2%). BHT showed a rate of 14.7% and was found with BHA (butylated hydroxyanisole) in three products (a shampoo, two lipbalms). Noteworthy, two nail polishes which contained toluene.

Referring to children's products, almost fifty-eight per cent contained one or more of the aforementioned substances. Most of these (68%) showed PEGs on their label and the presence of ethylhextyl methoxycinnamate in three lipbalms is remarkable (Tab. III). Organic products contained this type of compounds for a rate of 10.9% and a nail polish contained benzophenone-3.

Discussion

In this study the presence of chemicals that can affect human health in consumer-available personal care and cosmetic products used by a large part of the population and frequently into contact with the body was examined. More attention should be given to leave-on and make-up products which stay longer on the skin. For this reason, dangerous substances could determine greater negative effects on human's health. Make-up products, in particular, are often applied close to mucosa and frequently used by more sensitive categories, such as teenagers.

Fragranced ingredients are widespread diffused in cosmetic products but many of these may cause sensitizations, allergies and skin irritations [11]. For this reason, the EU established limits to their utilization and the obligation to indicate their presence on products labels, when the concentration is higher than 0.01% in rinse-off products, and 0.001% in leave on products [5].

The most common fragrance identified in the present study was limonene (76.9%) which, together with citral

Tab. V. Frequency of occurrence and percentage of other chemicals of concern identified on the label of selected products and referring to rinse-off, leave-on and make-up categories.

Other chemicals	CAS no.	Rinse-off		Leave-on		Make-up		Total	
		(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
PEGs*	25322-68-3	53	81.5	38	69.1	10	23.8	101	62.3
Acrylate copolymer	25133-97-5	16	24.6	20	36.4	19	45.2	55	34
Petrolatum	8009-03-8	2	3.1	12	21.8	14	33.3	28	17.2
Polysorbate-80/-60/-20	9005-65-6, 9005-67-8, 9005-64-5	16	24.6	7	12.7	1	2.4	24	14.8
Ethylhextyl methoxycinnamate	5466-77-3	-	-	9	16.4	13	31	22	13.6
BHA	25013-16-5	1	1.2	-	-	2	6.9	3	1.6
BHT	128-37-0	3	3.5	14	18.2	11	37.9	28	4.7
Benzophenone-1	131-56-6	-	-	-	-	6	14.3	6	3.7
Benzophenone-3	131-57-7	-	-	3	5.5	5	11.9	8	4.9
Cocamide DEA	68603-42-9	2	3.1	-	-	-	-	2	1.2
Toluene	108-88-3	-	-	-	-	2	4.8	2	1.2

*we considered all ingredients indicated on labels as "PEG" or "-eth", followed by a number.

(24.2%), is classified as skin sensitizers (H317), according to the regulation on the classification, labelling and packaging of substances and mixtures (CLP) [16]. In addition, many fragrance ingredients were categorized as weak allergens [17]; since a large number of products contains a mixture of fragrances, the consumers are more likely to be exposed to mixtures of allergens. Bonefeld et al. [18] found that mixtures of fragrance allergens have an increased potency in sensitization and elicitation of contact allergy as compared with an isolated fragrance allergen.

Among preservatives, parabens are considered as a class of endocrine disruptors, especially propylparaben and butylparaben. Many studies observed that parabens were able to chemically imitate the oestrogenic activity leading to adverse health outcomes [19, 20]. Moreover, parabens could play a role in the development of human breast, ovary and testicles cancer [21, 22]. For these reasons, many countries have banned the use of some parabens in personal care products intended for newborns and children [23].

Formaldehyde-releasers are important sources of formaldehyde exposure and allergic Contact Dermatitis They are able to release formaldehyde that has the capability to cause hypersensitivity reactions [24]. For some time now, formaldehyde is considered carcinogenic to humans [25] and, even if concentrations of these kinds of preservatives added to cosmetics are very low, they are still present in a large number of products whose use occurs frequently and daily.

MCI and MI are preservatives whose use has recently increased in cosmetics, but there is a limit of concentration both for the single ingredient and for the MI/MCI mixture [5]. Many studies focused on contact allergies associated with the use of MI/MCI, even if the dose allowed is respected [26, 27]. The use of chlorphenesin is allowed in concentration lower than 0.3% [28]. At a higher concentration it may cause irritations and contact dermatitis, especially on sensitive skin [29]. Due to the possibility of collateral side-effects on children, in particular on the respiratory tract and the central nervous system, the Food and Drug Administration (FDA) advised against the use of products containing chlorphenesin for children and women while breastfeeding [30]. In this work we underlined the presence of clorphenesin in two children's products.

We found triclosan in few products, however, because of its relevance, it is important to focus attention on it. It is an antimicrobial additive considered potentially harmful for health as an endocrine disruptor, as a result of a prolonged use [31-33]. It may be found together with dioxin, formed during its synthesis process, which could also be formed by photodegradation of triclosan in the urban wastewater [34]. Moreover, the massive diffusion of this antimicrobial compound may determine an increase in the bacterial resistance to the most common antibiotics used in the medical field [35]. The widespread use of this substance is demonstrated by the detection of triclosan traces in fish's fatty tissues and, even worse, in maternal milk. That evidence confirms the continuous exposition to very low or minimal concentrations of triclosan may lead to living organisms to absorb that compound [36, 37]. For all these reasons, triclosan was banned in 2013 by the FDA [38]. Nevertheless, in Europe the use of triclosan is still allowed in cosmetic products.

Lastly other chemicals, considered in our investigation, were substances different from fragrances and preservatives. Benzophenone-1 and benzophenone-3 are chemical filters used for the protection from UV radiations, reputed endocrine disruptors. Exposure to these ingredients, although definitive studies are lacking, could cause negative effects on humans, as well as a neuronal delay and alterations in behavioural development, congenital malformations, fertility deficiency for men, etc. [39, 40]. In addition, the International Agency for Research on Cancer (IARC) classified benzophenone as a possibly carcinogenic to humans (2B group) [41]. These ingredients have good lipophilic properties and after only a few hours from their application on the skin, it is possible to detect them in biological fluids like maternal milk [42]. Also ethylhexyl methoxycinnamate is a UV filter added to cosmetics, and some studies show how it can affect and modify the regulation of the endocrine system [43].

Considering BHA and BHT, the Cosmetic Ingredient Review (CIR) Expert Panel established concentration limits for these substances (0.5% max) because of their uncertain toxicological profile and the potential irritating power on skin and mucosa [44, 45].
Cocamide DEA is a skin irritant [46], classified in 2B group by the IARC [47]. Moreover, in 2012 the California Office of Environmental Health Hazard Assessment added cocamide DEA to the list of chemical compounds that cause cancer [48].
PEGs are characterised by low cutaneous toxicity and generally they are weakly irritants. They come from the polymerization of ethylene oxide, a well-known carcinogenic agent [49]. These substances can contain residual impurities derived from the ethoxylation process: ethylene oxide, dioxane, polycyclic aromatic compounds, heavy metals like lead, iron, cobalt, nickel, cadmium, arsenic [50].
Petrolatum (indicated on labels as paraffinum liquidum/petrolatum/paraffin/vaseline/mineral oil) is widely used in cosmetics but there are some potential health risks linked to its utilization, especially for the possible presence of impurities. In 2011 a scientific study showed that hydrocarbons derived from petrolatum are the most present contaminants in human body and the contamination occurs, above all, through the inhalation of polluted air, the ingestion of contaminated food and cutaneous absorption. This study also underlined that cosmetics can represent one of the most significant source of these compounds [51].
Toluene exposure from nail polish application was assumed to occur through both dermal and inhalation routes: a high concentration can cause irritation on the mucosa and skin irritation. It was listed in 1991 under State of California Proposition 65, as a chemical known for causing reproductive toxicity and having adverse effects on the central nervous system [52].
Finally, acrylates and polysorbates are considered weakly irritants: the concern related to the first compounds is the possible presence of toxic residuals like acrylic and methacrylic acid which are characterized by allergenic activity [53]; while the second ones are less irritants, even if cases of contact dermatitis due to these substances have been proved [54].

Conclusions

As a result of what explained, it is evident how, through the use of cosmetics, most people are exposed worldwide to a variety of potentially harmful substances.
Although the amounts may be small, and their effects sometimes poorly understood, continuous exposure to a mix of these chemicals over long periods could have consequences for the health and well-being of people and society. Actually, the current legislation takes these risks into account and many substances are subject to a threshold concentration, but there is a potential "cocktail effect" due to the utilization of combined products during the daytime. In addition, the same substance can be found in more than one product and can derive from different sources ("additive effect"), in this way, the safety threshold established could be overcome. For example, we can think about formaldehyde which is found in a variety of consumer products: clothing, plastics, dry cleaning agents, paper, glue, drywall board, resins, wood panelling, etc.
It is necessary to improve the legislative approach, since there are chemicals whose use is not completely safe, but still allowed, so that it would be suitable to resort to the precautionary principle. Moreover, it would be appropriate to enhance cytotoxicity studies in order to assess the actual harmlessness of the formulations in vitro [55] and to prefer alternative substances [56] compared to those potentially dangerous used for the stability and the attractiveness of the products.

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Conflict of interest statement

None declared.

Authors' contributions

All authors made substantial contributions to the conception and design of the study and were involved in drafting and critically revising the manuscript in terms of intellectual content. In addition, AP conceived the study, performed the analysis and interpreted the results. FS contributed in the interpretation of results. FB contributed in the analysis and interpretation of data. TG was involved in the study design and methodology. AI and MDG were involved in collection and management of data. MG and MC were involved in planning and supervising the work. ADD was involved in study design, interpretation of results and supervised the findings of this work.

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ORIGINAL ARTICLE

Training to improve resilience and coping to monitor PTSD in rescue workers

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Keywords

Coping • Resilience • Earthquake • Rescue

Summary

Background. *Few studies focus on the role training has on rescue workers who are active as volunteers or actual workers in emergency situations such as an earthquake. In October 2016, a strong earthquake hit Central Italy and in particular the province of Macerata in the Marche region. Lots of rescuers were called to deal with the emergency. The aim of this study was, therefore, to examine their preparation, studying resilience and coping strategies, as these qualities can protect against complications brought on by traumatic situations (post traumatic stress disorder).*
Study design. *An observational study on 70 rescue workers who active in the area affected by the disaster within the province of Macerata was carried out.*
Materials and methods. *The questionnaire proposed by the Coping Inventory for Stressful situations (CISS) was used, while individual resilience levels were measured with the Resilience Scale. Both methods were employed in two separate interviews conducted before and after their intervention in the disaster area. To verify a possible difference between the resilience and coping values before and after the activity of rescue effort, the paired Student t test was applied.*
Results. *The sample showed medium to high levels of coping (91.6%) and resilience (89.6%) were present in both interviews and not significant statistical differences was observed for the resilience (among females t = 1.63, p-value = 0.179, and among males (t = -0.76; p-value = 0.487). In contrast, CISS scores before and after the rescue effort showed significant statistical differences both among females (t = 4.81; p-value = 0.009) and males (t = 10.06; p-value = 0.001).*
*Some areas relative to coping mechanisms, such as social avoidance and distraction are preferred by women, while men prefer avoidance and task-oriented activity**.** Results for resilience show a slight difference for perseverance in men.*
Conclusions. *The ability to use mechanisms of self-preservation like coping and resilience helps rescue workers to better respond in emergency situations. Surely one of the most appropriate ways to reach this result is provided by their preparation.*

Introduction

In October 2016, Central Italy was struck by a magnitude 6.5 earthquake affecting the Marche region, Abruzzo and Lazio. The extent of the event resulted in an unprecented migration of inhabitants from the mountainous areas towards the coast. Relief efforts were immediate and hundreds of rescue workers were mobilized by the Italian Civil Protection department. Rescue workers perform their role as volunteers or paid workers and offer out-of-hospital medical aid [1-4].
In view of this type of complex activity, rescue workers can be more exposed to stressful situations that can give rise to disturbances such as Post Traumatic Stress Sindrome (PTSD), which increase with the frequency of traumatic events [5-10]. Studies carried out in connection with separate disasters on people involved in rescue efforts suggest that the symptoms associated with post traumatic stress disorder are stronger depending on the magnitude of the event, exposure to corpses, failure to save survivors, identifying with victims, stress, fatigue and potential first-person exposure to danger [11, 12]. Some categories, such as maritime search and rescue workers, associate the stress of a particularly tough training period (although inevitable for that particular mission) with work-related stress. Factors which, when combined, can increase the frequency of PTSD and adjustment disorders (ADS) [13, 14].
Adjustment disorders, though more frequent, are generally not given the same importance as PTSD. Characterized by milder symptoms which are actually treacherous as they are more easily hidden or disguised, and perhaps not even fully understood by the rescue workers themselves, leading them to ignore their unease and aggravate existing problems [15].
Therefore, it is central to investigate methods which can best help rescue workers to identify and use individual, interpersonal, medical and environmental resources to reduce levels of stress and encourage adjustment in this high-risk group. For example, resilience and coping are important factors in the development of strategies to prevent and cure psychological distress (depression, anxiety and stress), allowing the person to overcome difficult situations.
Resilience, generally associated with low levels of psychological stress, should be taken into consideration when evaluating protection and risk factors. To face internal and external needs associated with the task at hand,

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the rescue worker has to be able to marshal and make use of all his/her resources [16, 17]. Given the above, training is fundamentally important for rescue workers and should increase their knowledge on subjects which are not limited to organisational management but include items such as Emergency Response Psychology, Public Healthcare, Hygiene and Preventive Medicine [18-24]. In the light of events connected to the latest earthquake in Central Italy and in view of the lack of knowledge on the relationship between specific training and resilience/coping strategies adopted by emergency rescue workers, we thought it would be interesting to take a closer look at their psychological well-being in relation to the sort of training they had received [25, 26].

Materials and methods

The study was carried out in the province of Macerata, in areas affected by the Central Italian earthquake of 2016, and was conducted in accordance with the latest version of the Declaration of Helsinki. The sample consisted of volunteers who came to the aid of victims, randomly selected from lists provided by voluntary associations that had taken part in the rescue effort, trying to include an equal sample of women and men. During the emergency phase, volunteers were interviewed after being informed about the study by specifically trained personnel under the supervision of psychologists. During the first phase of testing, before the rescue effort, a questionnaire was administered to measure general levels of resilience and coping, and the results were reported as percentage. This had previously been evaluated by submitting it to a sample of 100 volunteers who were involved in the earlier Aquila earthquake (2009), a fact which also allowed us to investigate the socio-demographic characteristics of the sample. Afterwards, in order to evaluate aspects related to resilience and coping levels in rescue workers prior to and after their participation in the rescue effort, two approved questionnaires were administered. The Coping Inventory for Stressful Situation (CISS) for adults was used to investigate various coping strategies such as task-oriented, emotion-oriented, avoidance-oriented, distraction and social diversion [27]. The questionnaire is composed of 48 questions to which responders choose an answer from 1 (not at all) to 5 (very much). The "standard score" is calculated (T scale) as well as the percentile rank (% ile). Results are "lower than average" to "higher than average" and the cut-off has been set at 50. Individual resilience was measured using the "Resilience Scale" (US English version of the Resilience Scale, di Gail M, Wagnild RN - Resilience Center Worden, Montana, USA) which assesses self reliance, meaning, equanimity, perseverance and existential aloneless [28]. The resilience scale is composed of 25 questions, to which the responder must give an answer ranging from 1 (strongly disagree) to 7 (strongly agree); results are interpreted on a total RS score and values are confronted with the following scores in the manual: 25-100 (very low degree of resilience), 101-115 (low degree of resilience), 116-130 (moderately low degree of resilience), 131-144 (moderate degree of resilience), 145-160 (high resilience degree), 161-175 (very high degree of resilience) [29]. A second interview was administered after their participation in the rescue effort to measure any changes in their coping and resilience levels. Data were gathered by first obtaining informed consent from the interviewee and asking the participant to sign it to signify willingness to take part in the study.

Statistical analysis

To verify a possible difference between the resilience and coping values before and after the activity of rescue effort, the paired Student t test was applied, processing the data with the XLStat software at a significant level (%): 5 [30].

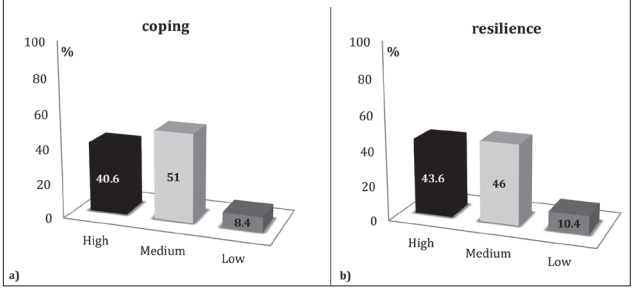
Results

A total of 70 questionnaires was handed out and 52 of these were completed, bringing compliance up to 74%. The percentage of males and females in the sample is almost equal. Most survey respondents are aged between 21 and 60 (90.4%) with the most common group aged 21-30 (48%) (Tab. I).

Tab. I. Sample characteristics.

Gender	n.	%
Males	24	46.00
Females	28	54.00
Total	**52**	
Age		
< = 20	0	0
21-30	25	48.00
31-40	8	15.40
41-50	6	11.50
51-60	8	15.40
> = 61	5	9.60
Total	**52**	
Level of education		
Primary school certificate	3	5.80
Middle school certificate	2	3.90
Secondary school certificate	29	55.80
Bachelor's degree	12	23.00
Master's degree	5	9.60
n.r.	1	1.90
Total	**52**	
Employment		
Student	10	19.20
Worker	20	38.50
Student and worker	12	23.00
Housewife	1	1.90
Unemployed	3	5.70
Pensioner	5	9.50
n.r.	1	1.90
Total	**52**	

Fig. 1. a) Coping level (%), b) Resilience level (%), calculated on the whole sample, during the first phase of testing.



Generally speaking, the sample shows a medium/high coping level (91.6%) and a low level of 8.4% (Fig. 1a). Regarding individual resilience, results are in line with values associated with coping, with 89.6% of the sample displaying high/medium levels of resilience; on the contrary, only 10.4% display low levels (Fig. 1b).

Results for the single items measured by the CISS obtained in the first and second interviews show levels above 50 %ile (the cut-off point between below average coping levels and above average coping levels) in all subscales, with the exception of the emotion-oriented scale. For task-oriented coping mechanisms, women are on the cut-off point, while men have higher scores (Fig. 2).

Avoidance seems most used by men, while distraction and social diversion is preferred by women.

Levels of resilience do not seem to have undergone significant changes between the first and second interview, though a higher score was noticed for men (total value of resilience). Considering the fact that scores for very low resilience go from 25-100, 101-115 for low resilience, 116-130 for moderately low resilience, 131-144 for moderate resilience, 145-160 for moderately high resilience, 161-175 for high resilience, both men and women show moderate levels (Fig. 3).

An analysis of the individual items that make up the Resilience Scale shows the only difference can be seen for Perseverance, that is to say a person's continued effort to reach his or her personal or family goals in spite of obstacles. More specifically, a slight increase was seen in the second interview which was conducted after the rescue effort.

Statistical analysis

No significant statistical differences was observed for the resilience before and after the rescue effort activity among females (t = 1.63; p-value = 0.179), and among males (t = -0.76; p-value = 0.487).

In contrast, the p-values computed for CISS before and after the rescue effort showed significant statistical dif-

Fig. 2. Coping Inventory for Stressful Situations (CISS) scores in percentile rank (% ile; CUT OFF = 50%ile) in males, females and total sample for Task: task-oriented coping factor, Emotion: emotion-oriented coping factor, Avoidance: avoidance-oriented coping factor in a) first interview and in b) second interview. (Student t test between the first and second interview among females t = 4.81; p-value = 0.009, and among males t = -10.06; p-value = 0.001).

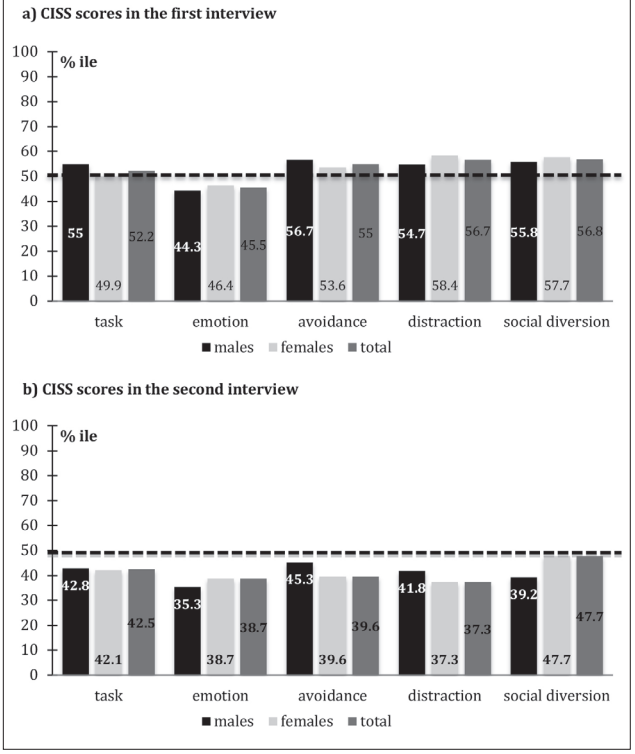
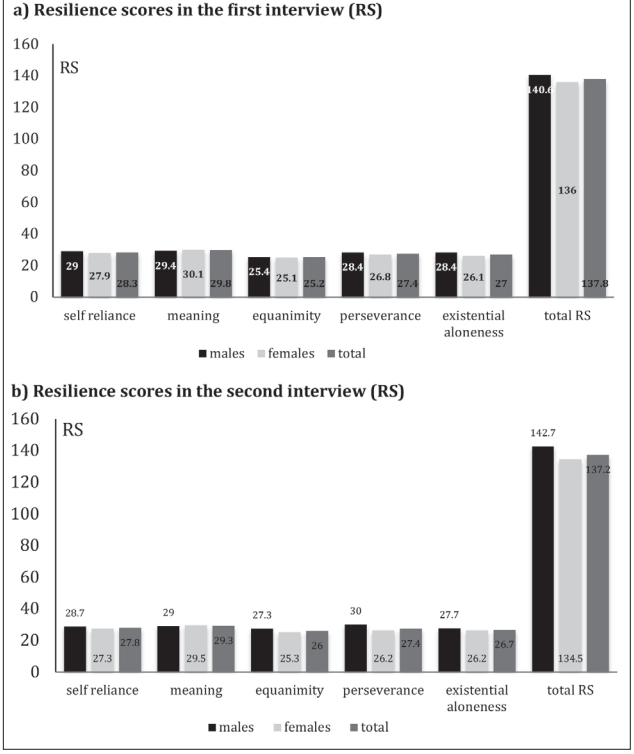


Fig. 3. Resilience Score (RS) in a) first interview, and b) second interview in males, females and total sample. Resilience Scale: 25-100 (very low resilience), 101-115 (low resilience), 116-130 (moderately low resilience), 131-144 (moderate resilience), 145-160 (moderately high resilience), 161-175 (high resilience) for self reliance, meaning, equanimity, perseverance and existential aloneness. (Student t test between the first and second interview among females t = 1.63; p-value = 0.179, and among males t = -0.76; p-value = 0.487).



ferences both among females (t = 4.81; p-value = 0.009) and males (t = 10.06; p-value = 0.001).

Discussion and conclusions

The current study has addressed the issue of emergency from a medical standpoint, focusing in particular on the psychological well-being of emergency medical responders who are faced with the problems and tragedies experienced by the victims of a catastrophe such as an earthquake. This study shows medium/high levels of coping and resilience in participants and identifies a link between coping strategies and individual resilience. This is probably due to the fact that the training and preparation received outside of the emergency situation is adequate.
Training plays a part in minimizing factors that lead to the development of problems and enables responders to adapt to highly stressful logistical and emotional situations. Rescue workers are thus able to help people in need in an appropriate manner. In relation to coping and resilience, the medium/high levels can be explained by the fact that the people who participated in the study belong to responder teams who are prepared from a practical point of view, but who are also made aware of their emotional setup; without a doubt, training contributes to being better equipped to face situations with the right degree of involvement.
More specifically, the CISS scores show some differences among females and males, before and after the rescue effort, confirmed by paired Student t test. In particular, scores for "Avoidance" might depend on the fact that women feel the need to share their experiences and build a wider network of interpersonal relationships.
Concerning resilience, the basic correlation between the two scores, measured before and after the rescue effort, could be caused by a number of elements such as: theoretical and practical training; turn-over (from 4 to 8 days); ability of individuals and community to promote and build resilience up all-round; support and sharing between peers and through one's organisation in support of the network provided by family and friends.
Even if not statically significant, a slight difference can be seen in the results of the Resilience Scale too, in particular between the first and second interviews relating to perseverance scores. This difference could depend on having processed the experience, reinforced by feelings of self-esteem. Results show that a majority of participants adopt mechanisms of self-preservation such as coping and resilience, which come to their aid and help them face emergency situations. In fact, it should be noted that trauma in volunteers is mainly due to their contact with victims who are in a state of shock; this intensifies when an emotional link with victims is present.
In this spirit, it appears essential to make sure that the training received by volunteers not be limited to organisational and emotional aspects, but be enriched by new types of knowledge which acknowledge our aging populations, and the likelihood of assisting people who are elderly, disabled or ill, and therefore depend on specific drug therapies or diets (diabetes, heart disease etc.) [31-36].
Training before a disaster is the first step towards guaranteeing survival, above all for the more vulnerable subgroups, allowing communities to prioritize disaster preparation. Training for emergency rescue workers and the development of support programmes for the community could provide important sources of assistance, especially as it becomes essential to change normal habits during evacuation and the post-disaster phase [37]. Everything contributes to avoid harmful lifestyle choices (change of diet, alcohol consumption, smoking etc.) which effect the individual's health [38-43].

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Participant consent

The completion of questionnaires was absolutely anonymous and voluntary. All participants provided written informed consent.

Conflict of interest statement

None declared.

Authors' contributions

SS conducted the statistical data analyses and wrote the manuscript. NTTC contributed to the study design and interpretation of results, PF participated in designing the study protocol and wrote the manuscript. GI designed the study, built the questionnaire, and made the critical review of the manuscript. All authors gave substantial contribution to manuscript revising and editing.

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Overview

The Spanish Influenza Pandemic: a lesson from history 100 years after 1918

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Keywords

History of Pandemic • Flu • Public Health • Mortality rate

Summary

In Europe in 1918, influenza spread through Spain, France, Great Britain and Italy, causing havoc with military operations during the First World War. The influenza pandemic of 1918 killed more than 50 million people worldwide. In addition, its socioeconomic consequences were huge.
"Spanish flu", as the infection was dubbed, hit different age-groups, displaying a so-called "W-trend", typically with two spikes in children and the elderly. However, healthy young adults were also affected.
In order to avoid alarming the public, several local health authorities refused to reveal the numbers of people affected and deaths. Consequently, it was very difficult to assess the impact of the disease at the time.
Although official communications issued by health authorities worldwide expressed certainty about the etiology of the infection, in laboratories it was not always possible to isolate the famous Pfeiffer's bacillus, which was, at that time, deemed to be the cause of influenza.
The first official preventive actions were implemented in August 1918; these included the obligatory notification of suspected cases and the surveillance of communities such as day-schools, boarding schools and barracks.
Identifying suspected cases through surveillance, and voluntary and/or mandatory quarantine or isolation, enabled the spread of Spanish flu to be curbed. At that time, these public health measures were the only effective weapons against the disease, as no vaccines or antivirals were available.
Virological and bacteriological analysis of preserved samples from infected soldiers and other young people who died during the pandemic period is a major step toward a better understanding of this pandemic and of how to prepare for future pandemics.

War and disease: the spread of the global influenza pandemic

On March 4, 1918, Albert Gitchel, a cook at Camp Fuston in Kansas, was afflicted by coughing, fever and headaches. His was one of the first established cases in the history of the so-called Spanish flu. Within three weeks, 1100 soldiers had been hospitalized, and thousands more were affected [1].
In Europe, the disease spread through France, Great Britain, Italy and Spain, causing havoc with First World War military operations. Three quarters of French troops and more than half of British troops fell ill in the spring of 1918. In May, the flu hit North Africa, and then Bombay in India; in June, the first cases were recorded in China, and in July in Australia.
This first wave is not universally regarded as influenza; the symptoms were similar to those of flu, but the illness was too mild and short-lasting, and mortality rates were similar to those seen in seasonal outbreaks of influenza [2].
In August, a deadly second wave of the Spanish pandemic ensued. This was probably caused by a mutated strain of the virus, which was carried from the port city of Plymouth in south-western England by ships bound for Freetown in Sierra Leone and Boston in the United States. From Boston and Freetown, and from Brest in France, it followed the movements of the armies.
This second wave lasted almost six weeks, spreading from North America to Central and South America, from Freetown to West Africa and South Africa in September, and reaching the Horn of Africa in November. By the end of September, the flu had spread to almost all Europe, including Poland and Russia. From Russia the epidemic spread throughout northern Asia, arrived in India in September, and in October it flared up again in China. In New York, the epidemic was declared to be over on 5th November, while in Europe it persisted, owing to the food and fuel shortages caused by the war. Most cases of illness and death due to the pandemic occurred during the second wave [3].
Deadly clusters of symptoms were recorded, including nasal hemorrhage, pneumonia, encephalitis, temperatures of up to 40°C, nephritis-like blood-streaked urine, and coma [4]. While the new virus struck military personnel, influencing war strategies, it did not spare those who lived in privileged conditions, one of the most famous cases being that of the King of Spain, Alfonso XIII, who was certainly not afflicted by the privations of the war.

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By December 1918, much of the world was once again flu-free, and in early 1919 Australia lifted its quarantine measures. However, in the austral summer of 1918-1919, more than 12,000 Australians were hit by the third wave of the disease. In the last week of January 1919, the third wave reached New York, and Paris was hit during the post-war peace negotiations. Overall, fewer people were affected by the disease during the final influenza wave. Nevertheless, mortality rates are believed to have been just as high as during the second wave [5]. In May 1919, this third pandemic was declared finished in the northern hemisphere. In Japan, however, the third epidemic broke out at the end of 1919 and ended in 1920.

Looking for the Spanish flu bacillus

Although official communications issued by health authorities worldwide expressed certainty about the etiology of the infection, in laboratories it was not always possible to isolate the famous Pfeiffer's bacillus, the *Haemophilus influenzae* bacterium first identified by the renowned German biologist in the nasal mucus of a patient in 1889, which, at the time, was considered to be the causal agent of influenza [6]. In October 1918, Nicolle and Lebailly, scientists at the Pasteur institute, first advanced the hypothesis that the pathogen responsible for the flu was an infectious agent of infinitesimal dimensions: a virus. Its immuno-pathological effects transiently increased susceptibility to ultimately lethal secondary bacterial pneumonia and other co-infections, such as measles and malaria, or co-morbidities such as malnutrition or obesity [7, 8].
The Spanish flu hit different age-groups, displaying a so-called "W-trend", with infections typically peaking in children and the elderly, with an intermediate spike in healthy young adults. In these last cases, lack of pre-existing virus-specific and/or cross-reactive antibodies and cellular immunity probably contributed to the high attack rate and rapid spread of the 1918 H1N1 virus, and to that "cytokine storm" which ultimately destroyed the lungs.
Only in 1930 was the flu pandemic rightly attributed to a virus, and in 1933 the first human influenza virus was isolated [9].

Public health measures to control the disease

There was no cure for the disease; it could only be fought with symptomatic treatments and improvised remedies. Moreover, the return of soldiers from the war fronts, the migration of refugees and the mobility of women engaged in extra-domestic activities had favored the rapid spread of the virus since the onset of the first pandemic wave. Preventive public health measures were therefore essential, in order to try to stem the spread of the disease [10].
The first official preventive measures were implemented in August 1918; these included the obligatory notification of suspected cases, and the surveillance of communities such as day-schools, boarding schools and barracks. In October 1918, local authorities in several European countries strengthened these general provisions by adding further measures, for instance the closure of public meeting places, such as theaters, and the suspension of public meetings. In addition, long church sermons were prohibited and Sunday instruction was to last no more than five minutes.
Street cleaning and the disinfection of public spaces, such as churches, cinemas, theaters and workshops, were considered to be cornerstones in controlling the spread of Spanish flu, in addition to banning crowds outside shops and limiting the number of passengers on public transport. However, they did not prove very effective.
Among public health interventions, local health departments distributed free soap and provided clean water for the less wealthy; services for the removal of human waste, the regulation of toilets, and the inspection of milk and other food products were organized; spitting in the street was forbidden, which determined the spread of pocket spittoons, and announcements in newspapers and leaflets advertised the therapeutic virtues of water.
To simplify mortuary police services, many administrations in the worst affected centers in Italy set up collection points for corpses and abolished all the rituals that accompanied death.
In addition, identifying cases of illness through surveillance, and voluntary and/or mandatory quarantine or isolation, also helped to curb the spread of Spanish flu, in a period in which no effective vaccines or antivirals were available.

The silence of the press: the censored Spanish flu

As Spain was neutral in the First World War, newspapers there were free to report the devastating effects that the 1918 pandemic virus was having in that country. Thus, it was generally perceived that the pandemic had originated in Spain, and the infection was incorrectly dubbed "Spanish flu" [2]. During the fall of 1918, the front pages of Spanish newspapers were filled with the names of those who had died of the pandemic in the country [2, 3]. In other European countries, however, the press refrained from reporting news of the spreading infection, in order to avoid alarming the general population, which was already suffering the privations caused by the First World War. On 22nd August 1918, the Italian Interior Minister denied the alarming reports of the spread of the flu pandemic, and in the following months, both national and international newspapers followed suit. Nor was censorship restricted to news of the spread of the fearsome infection; it also extended to information and comments that contrasted with the official versions of the nature of the disease.
In order to avoid public alarm, several local hygiene authorities refused to reveal the numbers of people affected and deaths [11]. Moreover, it was announced that

the average duration of the epidemic did not exceed two months. By the middle of October 1918, however, it had become impossible to verify this claim.
Some scientists believed that one of the causes of the epidemic was the poor quality of food, which was rationed at the time of the epidemic crisis. The extent to which the gravity of the pandemic was accentuated by malnutrition among war-tired populations is unclear. However, the fact that the disease, even in serious forms, spread through countries that were neutral or completely uninvolved in the war, such as Spain, seems to suggest that malnutrition was not a key factor.
Another thesis was that the disease had been triggered by a bacteriological war waged by the Austro-German enemy. On the one hand, newspapers were essential to publicizing emergency measures to contain the epidemic, such as closing cinemas and theaters or prohibiting other types of gathering, including funerals. On the other, any mention of the horror that was unfolding was to be avoided. Even sounding death bells was sometimes forbidden, to prevent their continual dismal tolling from revealing the extent of the tragedy that was to be hidden. The unseen enemy mainly attacked young people, causing major social upheaval; if Spanish flu did not take the lives of children, it made them orphans.

A tragic legacy: mortality worldwide

The influenza pandemic of 1918 killed more than 50 million people and caused more than 500 million infections worldwide. In the military camps and trenches during the First World War, the influenza pandemic struck millions of soldiers all over the world, causing the deaths of 100,000 troops. However, it is not clear whether it had an impact on the course of the war [12]. The highest morbidity rate was among the Americans in France, during the Meuse-Argonne offensive on the Western Front from September 15 to November 15, 1918, when over one million men of the US Army fell sick [12].
General understanding of the healthcare burden imposed by influenza infections was unclear. Several factors were suspected of increasing the risk of severe flu: length of service in the army, ethnicity, dirty dishes, flies, dust, overcrowding and the weather. In overcrowded camps, the risk of flu, and its principal complication, pneumonia, increased 10-fold [13]. Bacterial pneumonia secondary to influenza was the overwhelming cause of death, owing to increased susceptibility due to transient immuno-pathological effects and dysregulated, pathological cellular immune responses to infections [14, 15].
It is difficult to ascertain the mortality rate of the pandemic, as data on deaths were transmitted in incomplete form to the Central Statistical Office. In Italy, the "Albo d'oro" collected documentation on the number and demographic characteristics of the soldiers who died during the conflict, which enabled more accurate data to be obtained on deaths due to influenza among military personnel [16].
Military nurses and medical officers were intensively and repeatedly exposed to the influenza A (H1N1) pandemic strain in many areas. However, during the lethal second wave, nurses and medical officers of the Australian Army, and other groups of healthcare workers, displayed influenza-related illness rates similar to those of other occupational groups, and mortality rates that were actually lower. These findings suggest that the occupational group most intensively exposed to the pandemic strain had relatively low influenza-related pneumonia mortality rates [17, 18]. The dynamic relationship between the host and the influenza virus during infection, the unusual epidemiological features and the host-specific properties that contributed to the severity of the disease in the pandemic period still remain unknown [19, 20].

Conclusions

The 1918 pandemic influenza was a global health catastrophe, determining one of the highest mortality rates due to an infectious disease in history.
Virological analysis of preserved samples from infected soldiers and others who died during the pandemic period is a major step toward a better understanding of this pandemic. Such knowledge may contribute to the discovery of new drugs and the development of preventive strategies, including insights into the appropriate timing of the administration of antivirals and/or antibiotics, thereby providing indications on how to prepare for future pandemics.
The 1918-1919 pandemic led to enormous improvements in public health. Indeed, several strategies, such as health education, isolation, sanitation and surveillance, improved our knowledge of the transmission of influenza, and are still implemented today to stem the spread of a disease that has a heavy burden.

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Authors' contributions

MM and IB conceived the study, MM and IB drafted the manuscript, VG and NB revised the manuscript. IB, MM, VG and NB performed a search of the literature. All authors critically revised the manuscript. All authors have read and approved the latest version of the manuscript.

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Overview

Anti-rabies vaccination between the 18th and 19th centuries and its pioneer Eusebio Giacinto Valli (1755-1816)

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Summary

An eclectic, versatile Tuscan doctor, Eusebio Giacinto Valli (1755-1816) was a scholar of several branches of medicine, particularly public health, preventive medicine and epidemiology. His brilliant and wide-ranging education, and his intense passion for physics and chemistry, as applied to the human body, enabled him to conduct numerous studies in the field of vaccinology.
He travelled to the Middle East in order to study the epidemiology of the plague and carried out experiments aimed at discovering a cure and a prophylaxis for rabies, succeeding in attenuating the rabies virus by inoculating a mixture of saliva from rabid dogs and gastric juice from frogs.
Having travelled to Spain and then to Cuba, where he undertook the study of yellow fever, he died in Havana in September 1816, after injecting attenuated germs of the disease into his own body.
He was buried in the great Monumental Cemetery "Cristobal Colon", where his tomb bears the epigraph: "victima de su amor à la humanidad ("a victim of his love for humanity").

The life of Eusebio Giacinto Valli

Born in Casciana Alta di Lari (Pisa) on 16 December 1755, Eusebio Giacinto Valli was the son of a local doctor, Giuseppe Valli (from the family once known as Valle, Valla, della Valle, and today Valli) and Anna Maria Iacoponi, both of whom originally came from Ponsacco. He was a multifaceted, eclectic physician, whose interests included pathophysiology, internal medicine, public health and preventive medicine, epidemiology and vaccinology, though his greatest passions were physics and chemistry, especially as applied to the human body. In September 1816, he died in Havana, Cuba, where he is buried in the great Monumental Cemetery "Cristobal Colon", a "*victima de su amor à la humanidad*" [1].
After completing his high-school studies in Florence, where he studied classical languages and a few modern languages (English and French), and also dabbled in poetry, Valli took a degree in philosophy and medicine, after his elder brother, Jacopo, had graduated in canon and civil law.
In 1776, when Valli was in the second year of his degree course, his father died. The following year, his mother also died. Despite his restricted financial circumstances (he lodged at the house of one Domenico Cola in via Santa Maria, in the historical centre of Pisa), he managed to win a place at the Collegio della Sapienza di Pisa (which later became today's Scuola Normale di Pisa); his uncle Michelangelo Valli acted as his guarantor.
Having graduated in philosophy and medicine, he moved to Turkey (Izmir, Constantinople), and then to Greece and the Aegean islands (1783-1785). Here, he became friends with the Greek physician, scientist and theologian, Angelo Kalogerà (1699-1768), author of a "Collection of Scientific and Philological Pamphlets" [2, 3].
During his stay on the islands, Valli visited "*in 1784, the beautiful, picturesque, rocky Island of Chios, Homer's native land*", which was at that time in the grip of the plague. He probably wrote a brief tract on the epidemic that afflicted the Island of Chios, but this has, unfortunately, been lost to us.
He subsequently travelled to Paris (1785-1786), where he served as a doctor in the Cablys' regiment, then to Hindustan (today Pakistan, in 1786-1788), and finally to Egypt, to study the epidemiology of the plague, of smallpox, and of certain "malignant putrid fevers" (probably outbreaks of malaria).

Eusebio Valli's scientific discoveries

In 1781, he discovered the anti-fermentation action of the red precipitate in wine preparation.
During his stays in the Middle East and Asia Minor, Valli worked out a theory according to which the etiopathogenetic development of the plague required the so-called "principle of affinity", that is to say, a sort of predisposition to the disease. "*The forces that it deploys in the*

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various subjects depend more on the constitution of each individual than on the character of the miasma".
In France, he took part in the debate between the humoral theory and solidism, embracing the latter; solidism was expounded in the principal work, "*Elementa medicinae*", of the Scottish physician John Brown (1735-1788), which was published in 1780 and translated into Italian by the clinician Pietro Moscati (1739-1824) [4].
In his "*Discorso sopra il sangue considerato in stato di sanità e di malattia*"*(Discourse on blood in the state of health and of disease)*, Valli claimed that alterations in haematological parameters were due to the influence not of humoral agents, but of solid agents, or bodily organs. "*The blood is never altered by the germ of any disease whatever, nor by the forces of diseases themselves; on the contrary, it is the most resistant fluid, even to the action of poisons*" [5].
In the spring of 1789, at the outbreak of the French Revolution, he returned to Italy and settled in Pavia, where he met Francesco Volta. Despite the friendship and the excellent relations between the two, Valli took the side of Luigi Aloisio Galvani (1737-1798) in the dispute over the origin of animal electricity or bio-electricity, calling Volta's electricity of metals "*imaginary*" [6-8].
Valli repeated Volta's experiments meticulously, in an attempt to reproduce the results. In 1797, the German scientist and naturalist Alexander von Humboldt (1769-1859) would also try to reproduce these experiments [9].
Valli subsequently became the head of a hospital department in Mantua, and in 1802 returned to Turkey to experiment with the inoculation of smallpox vaccine to protect against the plague [10].
Indeed, over the years, Valli had elaborated the theory that infection by smallpox excluded infection by the plague, and vice versa. "*Those who have had smallpox either do not contract the plague or, if they do, they do not risk death. The plague becomes a benign disease, or fades out as soon as a smallpox epidemic arises*".
Thus, he held, there were two "poisonous pura", one produced by the plague and the other by smallpox, and contamination between the two would give rise to a "good pus", which was potentially curative. Valli's experimentation, which was dubbed "*hazardous but fascinating*" by Pietro Moscati, a doctor and minister of the Cisalpine Republic, received a sort of scientific endorsement from this latter. Indeed, on 31 May 1792, Valli became a corresponding member of the Academy of Sciences in Turin.
In 1799, in Livorno, Valli partly reproduced experiments conducted by the French abbot, mathematician and physician Robert Rimbaud Deidier (1670-1746). Since 1772, he had succeeded in immunising "*several animals by inoculating saliva taken from a hydrophobic dog. None of the animals inoculated with the saliva, to which gastric juice from frogs had been added, contracted rabies*". With this preparation, Valli succeeded in treating a certain Pisan lady named Rosermini and her maidservant, achieving a very good result [11].
In 1802 and in 1818-1819, these experiments would be repeated by the French military doctor René-Nicolas Dufriche Desgenettes (1762-1837) and by the Spanish doctor Serafin Sola in Tangier (Marocco), as reported by the Swedish consul Jacob Graf Graberg Hemsö [12].
Valli subsequently travelled to Dalmatia as a military doctor with the Franco-Italian army. There, he treated an officer's wife, who had been bitten by a rabid dog; she did not contract rabies.
In June 1809, he went to Spain to serve on a military medical commission [13].
Following his return to Italy, he was appointed in 1811 by the Italian government to examine the thermal waters of Monte Ortone, south-west of Padua, in the Euganei hills.
In 1815, he briefly stayed in Milan, but then returned to Mantua, a city he loved and regarded as his "*second home*".
Finally, he travelled to Cuba, arriving there from New York in September 1816. Accompanied by Dr. Antonio Mendoza, he visited San Juan de Dios Hospital, where he studied the epidemiology of yellow fever.
"*At that time, Havana had a sad appearance: the streets were mostly narrow, winding and unpaved, which, together with the lack of gutters, contributing to maintaining the dirt, and also the unwholesomeness, which was exacerbated by the nearby swamps; consequently, diseases were common, and yellow fever raged more vigorously than in other parts of America. In the months of August and September, mortality was very high, and during that time the death rate was 25 per day on a population of about 130,000 inhabitants*". While attempting to find a remedy for yellow fever, Valli died, struck down by the germs of the terrible disease, which he had inoculated into himself, a "*voluntary martyr to an overarching boldness in his art*" [14].

Eusebio Valli and his scientific legacy

Valli was criticised by several academics. For example, Professor Giovanni Pietro Frank (1745-1821) said of him: "How could this young man have been able to write about chronic diseases, when I myself would perhaps be scarcely able to do so after practising medicine for more than 40 years?".
Instead Prof. Ulrico di Aichelburg, who taught microbiology at the University of Turin, described some of Dr Valli's important professional characteristics, including his particular interest and competence in the field of vaccinology:
"And it must be remembered... that Eusebio Valli strove to prevent disease by inoculating an attenuated form of its infectious principle (which, please note, was unknown in those days: this was in the 18th century) [...].
By mixing gastric juice with pus from plague and smallpox lesions, and with saliva from rabid dogs, and injecting these relatively innocuous mixtures into healthy persons, Valli claimed to have elicited immunity to the plague, smallpox and rabies [...].
We may doubt the results of Valli's claims, but we cannot doubt that he had inferred that scientific principle which Pasteur would later apply successfully: the principle of vaccination" [15].

Valli's studies and his various manuscripts, including the original copy of his death certificate, written by the Holy Guardian of the Church of the Angel in Havana, are now conserved in a room of the Istituto di Storia della Medicina (Institute of the History of Medicine) in Rome.
In the year in which a relative, the lawyer Giuseppe Valli, authorised the biography of Eusebio, Louis Pasteur devoted himself to developing an anti-rabies vaccine. On 6 July 1885, after four years of study, Pasteur succeeded in treating Joseph Meister (1876-1940), who survived [16, 17].
In both Casciana and Ponsacco, streets and plaques have been dedicated to Valli.

Conclusions

Eusebio Valli was a great physician and a pioneer of modern vaccinology: a personage who deserves to stand alongside Edward Jenner, whose work Valli greatly contributed to publicising. He blended observation and experimentation, being well aware that, without these, even the most fascinating hypotheses and theories "*are worth nothing*". He possessed both courage and ambition: "*the man who is gnawed by the ambition of glory can overcome any obstacle*". Moreover, he had to fight against "*the charlatans... the physicians who are ignorant or in bad faith, who... have discredited the greatest discovery of the century, shamelessly preaching the heresy that vaccination does not prevent and cannot protect against smallpox*" and, in doing so, he did not hesitate to boldly try his own remedies on himself.
Valli can be regarded as one of the first physicians, or perhaps the very first, to take up and publicise Edward Jenner's discovery of an anti-smallpox vaccination. Indeed, he formulated the principle according to which immunity to a contagious disease could be elicited by injecting the same, appropriately attenuated, "material" responsible for the infection. In this way, a mild form of the disease would be caused, which would be memorised by the immune system, thereby eliciting protection against more severe forms.
Moreover, Valli was the first vaccinator to operate in several countries in the world. In a sense, therefore, alone and as far as was possible at that time, he anticipated the activity, and to some extent also the philosophy, of *Médecins sans Frontières* [10].

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None declared.

Authors' contributions

MM and NB conceived the study, MM, NB drafted the manuscript, MM and BC revised the manuscript. NB, MM and BC performed a search of the literature. MM and BC revised critically the manuscript. All authors read and approved the last version of the manuscript.

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