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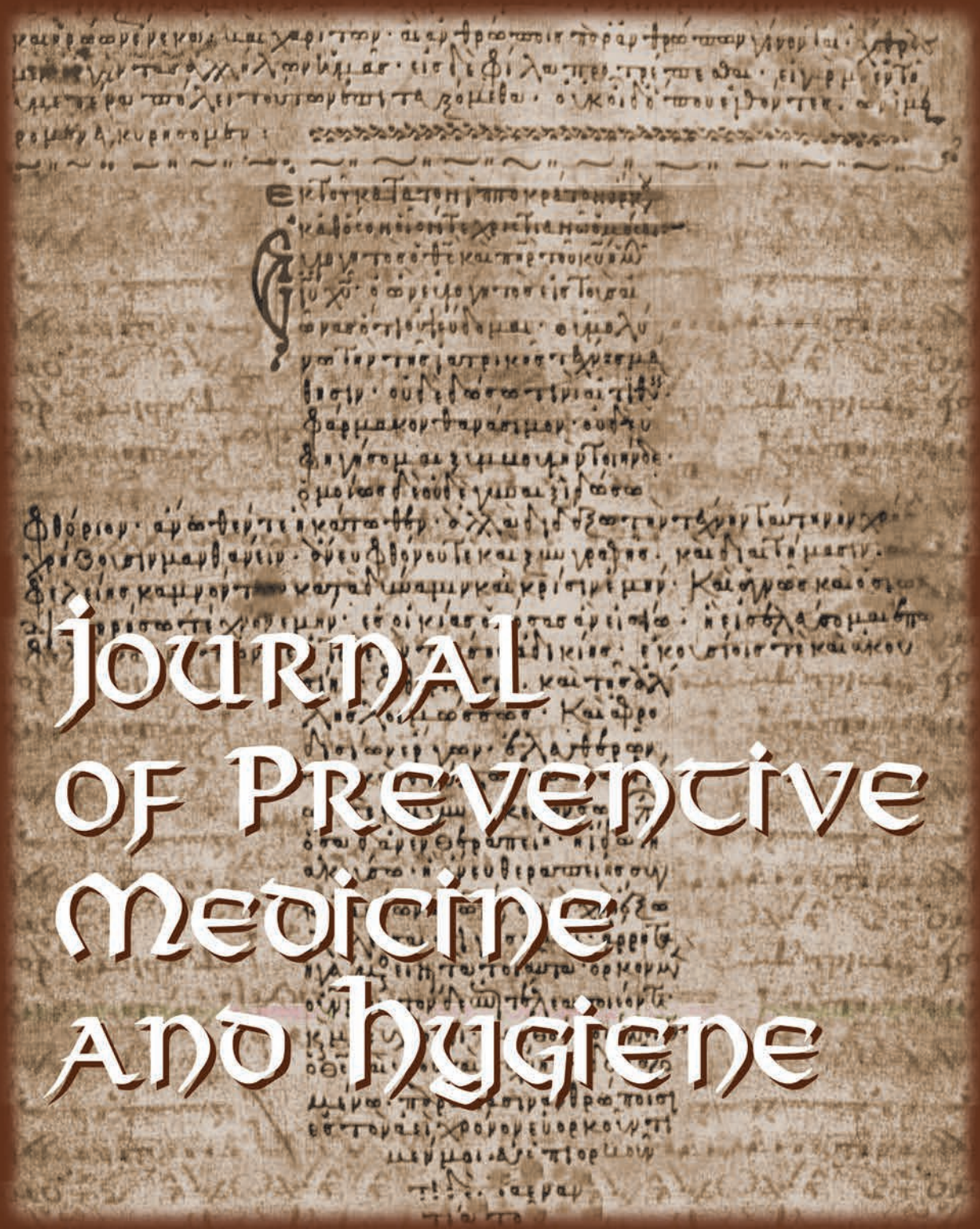

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ORIGINAL ARTICLE

Human *Trichinellosis* in Italy: an epidemiological review since 1989

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Keywords

Trichinellosis • Epidemiology • Italy

Summary

Objectives. *Trichinellosis is a worldwide zooantroponosis caused by a nematode of the genus Trichinella that can pose a risk to human health. Among the species of Trichinella, T. Spiralis is the most common represented. The main source of human infection is the consumption of raw or undercooked meat (especially from pigs, wild boars and horses). Infection with Trichinella was one of the most frequent parasitic diseases in Italy until 1959 when obligatory screening for these parasites in slaughtered swines was introduced. As the last review on this topic was performed in 1989, the aim of our study was to describe the epidemiology of Trichinellosis in Italy from 1989 to 2017.*
Study design. *We performed a systematic research in Pubmed (MEDLINE).*
Methods. *We included in our review studies that were published in the peer reviewed literature using the MESH terms "Trichinellosis" and "Italy". The only restrictions were the language (articles should be in English, Italian, Spanish or French) and the date of publication: from 1989 to March 2017. We excluded all the articles which referred to trichinellosis in the animals or which focused only on molecular biology of trichinella or on diagnostic techniques.*
Results. *We found 56 studies, but only 8 were considered eligible. During the study period, 764 cases of Trichinellosis occurred in Italy: 13.7% caused by T. Britovi and 84.4% by T. spiralis; in 14 cases the identification of the parasite was not performed. The outbreaks occurred in Umbria, Piedmont, Apulia (500 cases in 1990, by T. spiralis), Basilicata, Tuscany, Abruzzo, Emilia Romagna, Sardinia. In 2001 and in 2008 two outbreaks occurred in Lazio and Veneto respectively, but imported from abroad. The most important sources of infections were: horse meat (82.2%); wild boar meat (11.9%); pig meat (5.9%).*
Conclusions. *Trichinellosis is still present in Italy, but often forgotten by general practitioners and infectious diseases specialists. It's pivotal to improve awareness about this parasitic disease in Physicians and veterinarians. A strict surveillance, especially on meat products from endemic countries or from wild animals is necessary to considerably reduce the risk of acquiring the infection.*

Introduction

Trichinellosis is a worldwide antropozoonosis [1-3]: it is spread in a lot of Countries of Europe, South-East Asia, North and South America, New Zealand and North Africa. Outbreaks have been reported in 55 countries with an annual global average of 5,751 cases and five deaths [4]. According to the epidemiological data of the European Centre for Disease Prevention and Control (ECDC), *Trichinellosis* is most prevalent in eastern Europe but also in Italy and Spain where outbreaks have been reported in the past 10 years [5]. The parasite has a wide host spectrum due to its ability to virtually infect all mammals, birds and reptiles, depending on the involved *Trichinella* species [6]. *Trichinella spiralis* is the species that most adapted to domestic and wild swine but its life cycle could also include synanthropic rats. *T. spiralis* exhibits a wide and global distribution [7]. *Trichinella britovi* is the most widely distributed species within sylvatic life cycles of Europe, Asia, and Northern and Western Africa [8]. *T. spiralis* and *T. britovi* can also affect domestic pig populations mainly via extensive grazing systems or feed with scraps or carrion originating from sylvatic carnivores. Zoonotically, *T. britovi* is the second-most common species of *Trichinella* that may affect human health [7].
The incubation period for *Trichinellosis* symptoms is 7-21 days. Although an infected person may be asymptomatic, ingestion of a higher parasite load usually correlates with a shorter incubation period and with more severe symptoms. If clinical symptoms appear, they usually begin with several days of mild, non-bloody diarrhea, nausea, vomiting and abdominal discomfort. From 2 to 8 weeks later, host's immunologic reaction to larval migration into tissues can result in persistent fever, sweating, chills, periorbital edema, urticarial rash and conjunctival or sub-nails hemorrhages. Long-term effects depend on parasite load and site of infection. Myalgia is often present, and cardiac manifestations (e.g. myocarditis) may rarely occur later especially in moderate and severe cases. Eosinophilia is often substantial and early appears in the infection [9].
The main source of human infection is the consumption of raw or undercooked meat, especially from pig,

wild boar and horse [10, 11]. However, recently published studies described possible cases of human *Trichinellosis* linked also to other kinds of meat, such as beaver meat [12]. Infection with *Trichinella* species was one of the most frequent parasitic diseases in Italy until 1959, when obligatory screening for these parasites in slaughtered swine was introduced [13]. The last Guidelines for the surveillance, management, prevention and control of *Trichinellosis* reported (in the section related to the epidemiology in Italy) that only the sylvatic cycle (*T. britovi*) occurs among wildlife (e.g. red fox [*Vulpes vulpes*], wolf [*Canis lupus*], badger [*Meles meles*], marten [*Martes martes*], wild boar [*Sus scrofa*]) and the parasite is seldom transmitted to backyard or free-ranging pigs [14]. In the last 58 years, 13 backyard pigs were detected positive at the abattoir or were the source of infection for humans. *Trichinella* pseudospiralis has been documented once in two birds. Infections in humans have been documented from the consumption of pork from wild boar (*Sus scrofa*) or backyard and free-ranging pigs and meat from the red fox (*Vulpes vulpes*); however, the most important source of infection was horse meat imported from abroad [14]. The aim of our review was to describe the epidemiology of *Trichinellosis* in Italy, in order to continue the last review published in 1989 by De Carneri et al. [15].

Methods

In January-March 2017, we performed a systematic search for original peer-reviewed papers in the electronic database PubMed (MEDLINE). The key search Mesh terms were: "*trichinellosis*" AND "Italy". Abstracts and full-text papers were reviewed. The inclusion criteria considered to include articles in the review were:

- Type of article: original articles, but also letters to the editor if containing original data.
- Language: articles should be written in English, Italian, Spanish or French.
- Publication date: articles should be published in the period 1989-2017 (because the last review was written in 1989).

We excluded all the articles which referred to *Trichinellosis* in animals or which focused only on molecular biology of *Trichinella* or on diagnostic techniques.

When available, we collected data on: 1) laboratory methods used to identify the cases and the *Trichinella* species; 2) number of confirmed cases; 3) *Trichinella* species; 4) source of infection.

Studies were selected in a 2-stage process. Titles and abstracts from electronic searches were scrutinised by 2 reviewers independently (G.T. and N.N.) and full manuscripts and their citations list were analysed to retrieve missing articles and to select the eligible manuscripts according to the inclusion criteria. The level of agreement between the reviewers was high. Disagreements were resolved by consensus. Then, each article was further reviewed to identify the manuscripts suitable for our systematic review. The authors then extracted the data from included studies and collected them in a database for a unique analysis.

Results

The literature search yielded 56 publications. The titles, the abstracts and the full texts of these manuscripts were screened, resulting in 48 studies excluded because they didn't satisfy the inclusion criteria (28 because they reported data before 1989, 19 because they described animal cases of trichinellosis or were molecular biology studies about innovative techniques for the identification of the parasite; one was excluded because it was in Polish). Finally, we identified 8 manuscripts [10, 16-22] (Fig. 1).

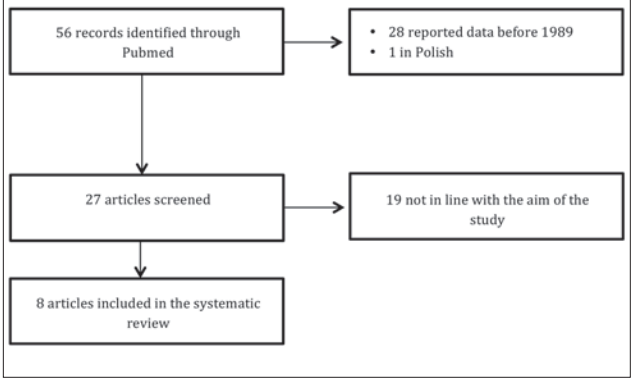
All the manuscripts reported data about human cases of trichinellosis, the geographical region and the year of the outbreak, and the source of infection. Two articles expressly reported the study design; 6 expressly reported the laboratory methods used to detect the parasite (ELISA or PCR).

The data collected from the 8 reviewed articles are shown in Table I (some original articles reported multiple data that we decided to report separately).

The total number of cases of human *Trichinellosis* in the last 25 years in Italy was 764 (Fig. 2).

T. spiralis was responsible for 84.4% of the cases; *T. Britovi* was responsible for 13.7% of the cases, in 1.83% the laboratory identification of the parasite was not performed. The major sources of infections were: horsemeat (82.2%); wild boar fresh sausages (11.9%), meat from pigs slaughtered without any veterinary control (5.9%). The outbreaks occurred in Umbria, Piedmont, Apulia (500 cases in 1990, by T. spiralis), Basilicata, Tuscany, Abruzzo, Emilia Romagna, Sardinia. In 2001 and in 2008 two small epidemics happened in Lazio (N = 8) and Veneto (N = 4) respectively, but imported from abroad (Fig. 3).

Fig. 1. Flow diagram for identifying studies included in our review.



Tab. I. Selected characteristics of the 8 studies included in the systematic review.

Period of outbreak	Place of outbreak	Diagnosis technique	Identification of *Trichinella* spp.	Source of infection	Infected toll (number of confirmed cases)	Source
1990	Piedmont	n/s	Spiralis	Wild Boar	11	[16]
1990	Apulia	n/s	Spiralis	Horse (imported)	500	[16]
1991	Basilicata	n/s	Britovi	Pig	6	[16]
1993	Tuscany	n/s	Britovi	Pig	4	[16]
1995	Abruzzo	n/s	Britovi	Wild Boar	23	[16]
1996	Abruzzo	n/s	Britovi	Wild Boar	10	[16]
1996	Basilicata	n/s	Britovi	Pig	3	[16]
1998	Emilia Romagna	n/s	Spiralis	Horse (imported)	92	[16]
2000	Apulia	n/s	Spiralis	Horse (imported)	36	[16]
2001	Lazio (imported)	ELISA	--	Pig	7+1	[21]
2002	Abruzzo	n/s	--	Wild Boar	2	[17]*
2005-2007	Sardinia	PCR/ELISA	Britovi	Pig	20	[18, 20]
2008	Veneto (Imported)	ELISA	--	Pig	4	[10]
2008	Piedmont	PCR/ELISA	Spiralis	Wild Boar	6	[19]
2012	Tuscany	PCR/ELISA	Britovi	Wild Boar	34	[17]
2016	Apulia	PCR/ELISA	Britovi	Wild Boar	5	[22]

n/s: not specified; *: unpublished data of Pozio et al. reported in the article.

Fig. 2. Number of confirmed trichinellosis cases in Italy reported from 1990 to 2016.

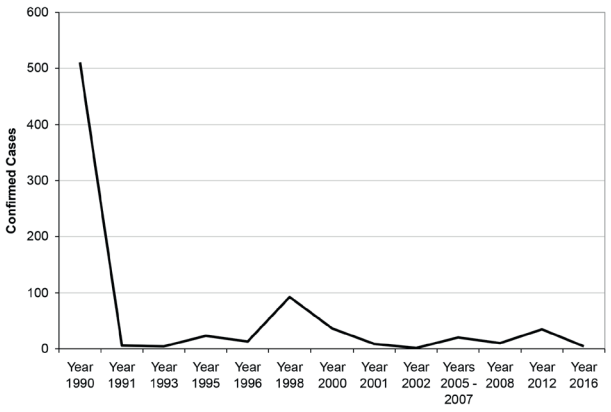


Fig. 3. Choropleth map of *Trichinellosis* cases in Italy occurred from 1990 to 2016.

white: no cases; light grey: 1-10 cases; grey: 11-50 cases; dark grey: 51-100 cases; black: more than 100 cases

Discussion

Our systematic review included 8 articles integrating all the available Italian data since 1990 till 2016 (the last published article on this topic, in 2017, reported data on an outbreak occurred in 2016) [22]. One of the most important limit of our review was the partially overlap between our results and those reported by Pozio et al. [16] that however was not a systematic review of the literature. According to our results, the number of reported cases of human *Trichinellosis* in the last 25 years in Italy was 764, but we can note a peak of incidence due to the outbreak in Apulia in 1990 (500 cases ascribed to horsemeat). *T. spiralis* was responsible for the 84.4% of the cases (but excluding the big epidemic occurred in 1990 this percentage dramatically falls), *T. Britovi* was responsible for 13.7% of the cases, in 1.8% the laboratory identification of the parasite was not performed. Scientific literature describes *T. britovi* as the most prevalent species, although cases from *T. spiralis* have also been reported [23]. In Italy, the most common etiological agent of infection is *T. Britovi*, which is maintained in nature by a sylvatic cycle in which the red fox (*Vulpes vulpes*) is the main reservoir [24].

The detection of specific anti-*Trichinella* antibodies in blood serum is of great diagnostic value and ELISA is the most commonly used approach for the detection of *Trichinella* infection in humans [7] whereas Real-time PCR is the most important and reliable assay for the identification of *Trichinella* species in muscle samples [25]. Only in some studies included in the review authors identified *Trichinella* species involved in the outbreak: this could be a limit for a complete epidemiological analysis of human *Trichinellosis* in Italy. It is known that cultural traditional habits of eating such foods play a key role in the spread of the disease and were responsible for past outbreaks [16]. Moreover, political and economic changes, could be responsible of increased prevalence and incidence (as described in

many former eastern European countries) especially because of a reduced efficacy of the veterinary control on susceptible production animals [7].
Our review has shown that the most important sources of infections were horsemeat (82.2%), wild boar fresh sausages (11.9%) and meat from pigs slaughtered without any veterinary control (5.9%), differently from that reported in the literature where the major source of infection is represented by insufficiently cooked pork products [26-31]. We have to consider that in our study 500 cases out of 764 was involved in Apulia-outbreak that was caused by horsemeat.
Our systematic review, therefore, adds some important information to the review of De Carneri et al. in 1989 [15]: before 1989, in Italy, the human infections were mostly caused by wild boar meat (11% of all cases) and by imported horse meat (43%), whereas the consumption of pork did not cause infection in men, as a result of a considerable reduction in foraging swine. Moreover *T. spiralis* was absent in Italy: only *Trichinella* species 3 (less pathogenic in men) was detected.
Our review demonstrated that after 1989, cases of pork-related *Trichinellosis* were identified, and that *T. Spiralis* resulted to be present in Italy (as demonstrated by the outbreaks occurred in 2008 [19], and in 1990 [16]).
The outbreaks occurred in Umbria, Piedmont, Apulia, Basilicata, Tuscany, Abruzzo, Emilia Romagna, Sardinia. In 2001 and in 2008 two small epidemics were described in Lazio and Veneto respectively, but imported from abroad. In 2001 the epidemics involved 7 immigrants from Eastern Europe who received a package containing smoked pork sausages as a present from their relatives. This unfortunate episode was similar to another one occurred in London among immigrants of another eastern European country after the consumption of infected sausages imported from their country of origin in December 1999 [21]. In 2008 a Romanian family living in Italy, during a visit to relatives and friends in Romania, ate ham produced from a pig slaughtered without any veterinary control [10]. As reported by R. Neghina in 2010, pork is the most frequent source of human *Trichinellosis* in Romania. "Pig's alms," a specific custom representing the thanksgiving meal offered to relatives, friends, or neighbors who participated in the slaughtering process may be a very good source of infection with *Trichinella* parasites, leading to unfortunate consequences, especially when animals are not veterinary tested [32].
Health education of the general population is one of the most important way to prevent *Trichinella* infection [33, 34]. It is important to remember that the necessary temperature to kill the larvae is 77°C and it is achieved when the meat is no longer pink. Freezing temperatures of – 15°C for 20 to 30 days, – 23°C for 10 to 20 days, and – 29°C for 6 to 12 days are also effective, except for *T. nativa* which can infect for several days at these temperatures [35]. Non-commercial sources of pork, as from wild animals and small rural farms not using modern hog management practices, still represent a significant health problem. In all the articles inserted in our review, in fact, the meat that caused the infection was not controlled by a vet, but in the study conducted by Fichi et al. [17], the pigs used to prepare the sausages have been slaughtered in an official abattoir and resulted negative for *Trichinella* by artificial digestion: in this case the wild boar meat (without a veterinary control) shuffled with pig meat to prepare the sausages was the real cause of infection. Our review demonstrates that the circulation of *Trichinella* parasite is not an extinguished problem and that it is impossible to define a region with a negligible risk of acquiring the infection. Health personnel and veterinarians should be regularly trained about this parasitic disease often forgotten by general practitioners and infectious diseases specialists. Differential diagnosis of *Trichinellosis* is especially difficult for isolated cases and atypical clinical courses, and physicians practicing in non-endemic countries are usually unfamiliar with the disease and may thus experience problems in diagnosing *Trichinellosis* [7].

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Conflict of interest statement

None declared.

Authors' contributions

GT had the idea of the study, collected data and wrote the article, NN provided support and suggestions.

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Original article

Impact of training conferences on high-school students' knowledge of sexually transmitted infections (STIs)

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Keywords

Adolescent health • Sexual health • Sexually transmitted infections • Intervention strategy

Summary

This study, part of the health promotion program of a high school in Milan (Italy), was aimed at evaluating the impact of training conferences on the awareness of STIs among adolescents aged 16-17.
Students attending the 3rd class of a Scientific and Linguistic High School in Milan (Italy) participated in this study in November 2017.
All students gave their anonymous answers on a voluntary basis in a pre-test survey, designed by psychologists and infectious diseases specialists, to test their basic knowledge, accuracy, and awareness of STIs. After a two-hour interactive conference, the students were asked to answer the post-test survey. A higher awareness of the spread and the mode of transmission of STIs, of high risk sexual and behavioural practices and prevention methods was observed in the post-test compared to the pre-test.
These findings outline both the need for sexual-health communication campaigns targeted at adolescents, who are at great risk of exposure and mostly unaware of STIs other than HIV/AIDS, and the short-term efficacy of a direct approach to the problem, guided by experts in infectious diseases and psychology. A long-term assessment of the effects of training conferences needs to be evaluated.

Introduction

Sexually transmitted infections (STIs) are a significant hazard for individual and public health. The type of problems related to STIs ranges from acute and chronic diseases to infertility, cancer, pregnancy complications, vertical transmission with foetal disease, death. From the social and economic point of view, moreover, problems are related to loss of working ability, stigma, and individual and social economic burden [1].
Adolescents are at high risk of STIs in the industrialized world. The high prevalence of STIs in young people has been attributed to increased risky behaviours, earlier sexual debut in the last decades, multiple sexual partners, unawareness of preventive methods [2, 3]. In a report from CENSIS (2017) on the knowledge and prevention of the HPV and sexually transmitted diseases among 1000 "millennials" aged 12-24 in Italy (2017) only 15.3% admit to being highly informed about STIs [4]. The interviewed millennials seem to pay attention more to avoiding pregnancies (92.9%), rather than to protecting themselves from infections (74.5%) during sexual intercourse; frequently a misunderstanding about prevention of infections and contraception was revealed. Moreover, the lack of knowledge of STIs, of the ways in which they are transmitted, of their symptoms, associated to fear of social stigma, to low perceived risk, to partner trust, and to confidentiality concerns, all cause delay in seeking medical services and advice for diagnosis and treatment. Consequently, there is an increased likelihood of secondary transmission and the risk of a worse outcome [5, 6].
The knowledge of risks is necessary, even if not sufficient, for safe sexual practices and sexual and reproductive health; this underscores the need to find public health intervention for the primary prevention of STI's in young and adolescent people. At the same time, this intervention must inform correctly and must be able to change attitudes, promoting risk-reduction behaviours [7, 8].
To understand the level of STIs knowledge, sexual behaviours, and the impact of interactive conferences on STIs awareness on adolescent students, we conducted a 3-step intervention study among students attending the 3rd class of a high school in Milan (Italy).

Methods

This project was included in the health promotion program of the high school "G Marconi" in Milan (Italy) whose purposes are the information and promotion of physical and psychological health, the increase of friendship and cooperation among the different components of the school community, the promotion of self-awareness, responsibility, and social involvement, the identification and knowledge of the public health structures and opportunities.

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Within the health promotion project, the Teachers' Board and School Board approved a three-step intervention to evaluate the impact of interactive conferences on knowledge and behaviour among students aged 16-17 attending the 3rd class of the school, in order to prevent STI's. The choice of this age was due to the fact that the average age of sexual debut among Italian adolescents has been reported to be 16 [9, 10].
Specialists in infectious diseases and psychologists designed the pre-test survey which was administered on an anonymous and voluntary basis in November 2017 to all the students attending the 3rd classes of the school by the teachers adhering to the project.
The survey was designated by Infectious Diseases specialists and psychologists and tested in previous one-shot surveys administered to adolescents and persons living with HIV/AIDS [11, 12]. The overall number of questions have been reduced, compared to the previous studies, to allow to be filled-in during a one-hour lesson time. Items were simple, short, and written in language familiar to the target people.
The survey consisted of nine multiple-choice and true-false close-ended items or open-ended items designated to test the key aspects of this study: the diffusion of STIs, the self-evaluated knowledge of STIs, the behaviours at risk, the prevention methods, the HPV vaccination, the HIV/AIDS, and, lastly, the preferential reference person(s) in case of sexual health problems.
The second step consisted of a 2-hour interactive conference held by a specialist in Infectious Diseases and by a Psychologist. Issues dealt with were: the spread, the risk, and the means of transmission of STIs, the types of behaviours at risk, the primary and secondary prevention methods, the psychological attitudes towards risky behaviour during adolescence, and lastly, the indications for responsible sexual behaviours. The conference was designed for a maximum of 40-50 people and was thus repeated three times to reach all the target students of the project.
The 3rd step consisted of a new anonymous survey, to be completed within one week of the conference, with the same range of closed and open questions included in the pre-test, and other questions aimed at evaluating changes in the students' behavioural attitudes, changes in preferential information sources and the psychological and behavioural impact of the conference.
Finally, the students completed a post-event feedback survey to evaluate the entire project.
Results are described as descriptive statistics for the pre- and post-test data using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. We used a two-tailed Chi-square or Fisher's exact tests according to the sample size and odds ratios were performed to test for dichotomous variables between pre- and post-test answers. The D'Agostino & Pearson omnibus normality test was used to test if the values come from a Gaussian distribution. The Mann-Whitney test was used to compare the distribution of unmatched groups of non-parametric variables.

Results

The survey was administered to all the students attending the 3rd classes of the school (8 classes for a total of 178 students; 66 males and 112 females, median age 16.5 years). The response rate was 78.1% with a total of 139 pre-test cards evaluable.
All the students actively participated in the conference, asking questions, and sharing personal experiences and knowledge.
The post-test survey was completed by 100 students (70.5% of the pre-test participants). A 6 items feedback form was filled in after the end of the survey by 119/178 students (66.85%).

Pre-test results

An overall 97.84% of the participants (136/139) who completed the pre-test survey declared that they were informed about STIs, and 85.1% of them (119/139) answered that STIs were very widespread in the world (questions 1 and 2). However, only a 47.5% (66/139) of them were able to complete the third question which asked the students to mention at least three STIs. Moreover, not all the diseases indicated as sexually acquired were correct (Fig. 1).
Knowledge of STIs was mostly limited to the HIV and AIDS infection/disease which, considered together, represented 41.73% of the STIs indicated by the students; every other STI was mentioned in less than 7% of the pre-test cards.
Question 4 *(according to you, which of the following methods are useful in the prevention of sexually transmitted infections?)* was made up of a closed question with several correct and incorrect possible answers to choose from, and an open part, leaving the possibility to add some other options. The use of a condom was indicated by 92.09% of participants as a reliable method for the prevention of STIs; vaccines were indicated by 63.31% of the participants as methods useful for STI prevention. However, among the preventive methods to avoid STIs, also the contraceptive pill (24.46%) the morning-after pill (20.14%), the diaphragm (11.51%) and the coitus interruptus (10.79%) (Fig. 2) were wrongly indicated.
Only 42.45% of the students interviewed indicated the number of sexual partners as an important risk factor for disease transmission. Intimate hygiene, probability of infection in the sexual partner, and contagious properties of the diseases were the most important factors implicated in STI transmission, according to the answers to Question 5 (Fig. 3).
Almost all the students declared that they were informed about HIV/AIDS, and the awareness of an HPV vaccine was slightly lower (87.77%) (Fig. 4).
The answers to the open question 8 (*List at least three sexual behaviours at risk for STIs)* show that a high proportion of adolescents (70.5%) are aware that sexual intercourse without condom protection can be considered at risk, but only a very low proportion of them indicated other risky sexual practices (Fig. 5).
Lastly, we asked students to list, in order of priority, who they would turn to if they suspected a sexually transmit-

Fig. 1. Sexually transmitted infections mentioned in the pre-test survey by 139 students for the question: *Mention at least 3 STIs*. Incorrect answers in red.

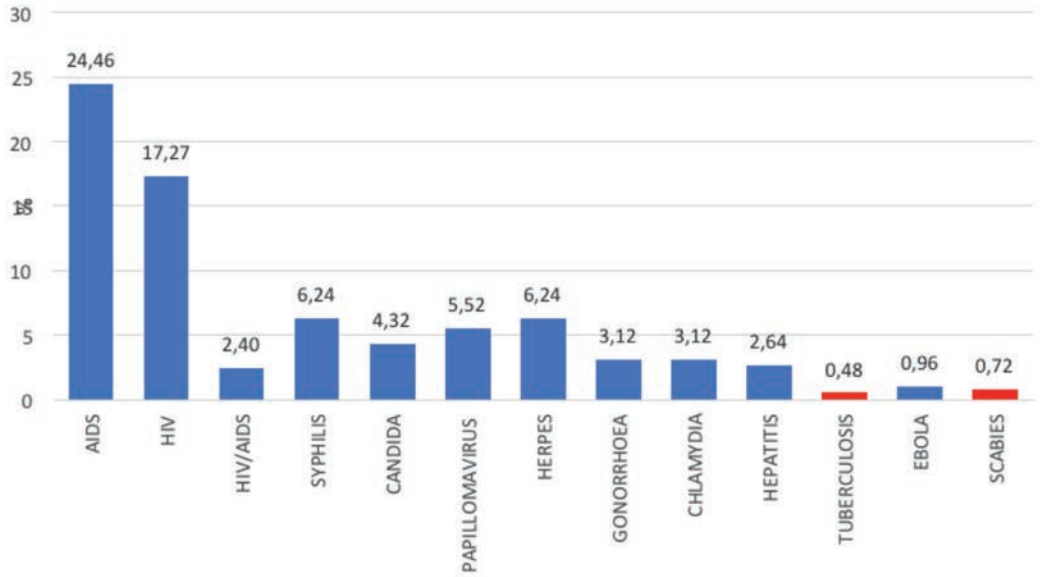
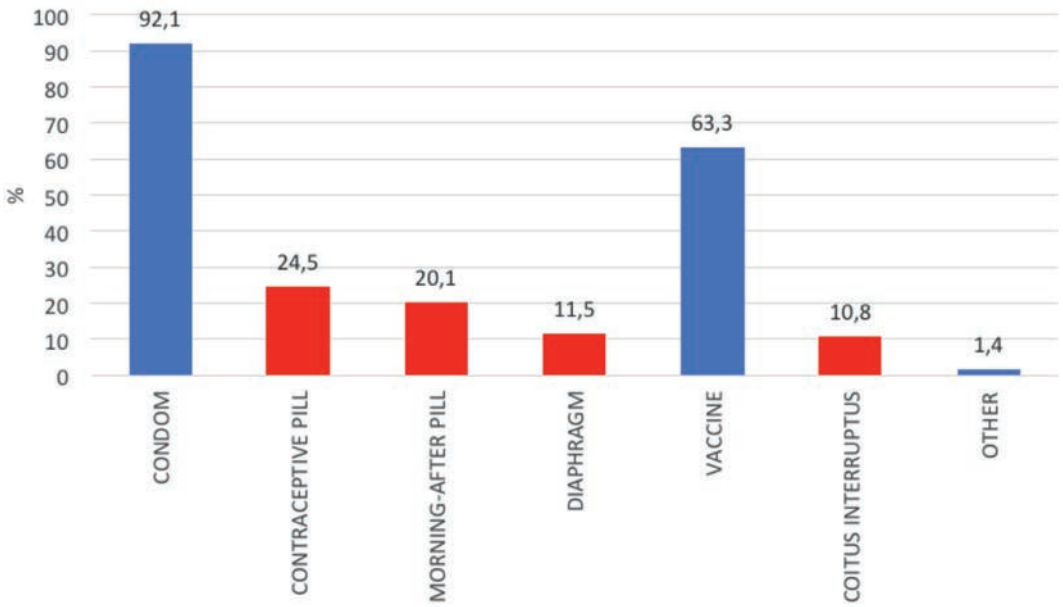


Fig. 2. STI prevention methods indicated in the pre-test survey for the question: *According to you, which of the following methods are useful for the prevention of the sexually transmitted infections?* Incorrect answers in red.



ted disease. We assigned a score according to the order of preference: 1 to the first choice and 7 to the last choice. In the pre-test, the main reference for adolescents who suspect a STI were the parents (score 2.0) followed by the family doctor (score 2.6), friends (score 3.6) or hospital (score 3.6) and local health facilities (ASL) (score 4.2); web-based information, such as internet sites or chats are the last options with scores of 4.6 and 6.1 respectively.

Comparison between pre-and post-test surveys

The proportion of students who declared that they were informed about STIs raised from 97.84% (136/139 students) of the pre-test to 99% (99/100 students) in the post-test survey but the difference was not significant (χ^2 test $p = 0.50$). More evident is the increase in the proportion of students who become aware of the wide diffusion and high prevalence of STIs in the world from 85.1% (119/139 students) to 98% (98/100 students) (χ^2 test $p = 0.001$).

The ability to list 3 STIs increased from 48.2% (67/139) to 88% (88/100) (χ^2 test $p < 0.0001$) with a change in the type of STIs listed. The range of STIs widened, with a shift toward non-HIV STIs (Fig. 6).

To calculate the proportion of STIs appropriately identified as sexually transmitted, we considered as denominator the number of possible correct answers (3 for each form filled): while in the pre-test survey we found sev-

Fig. 3. Conditions involved in STI transmission indicated in the pre-test survey for the question*: Which of the following conditions are involved in STI transmission?*

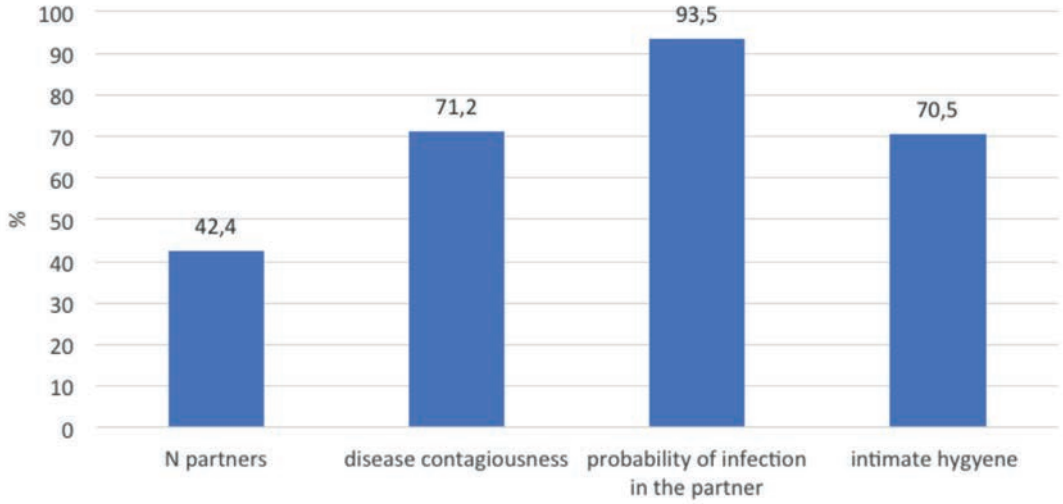


Fig. 4. Proportion of students who declared knowledge of HIV/AIDS and HPV vaccine answering the questions: *Have you ever heard about HPV vaccine and AIDS or HIV?*

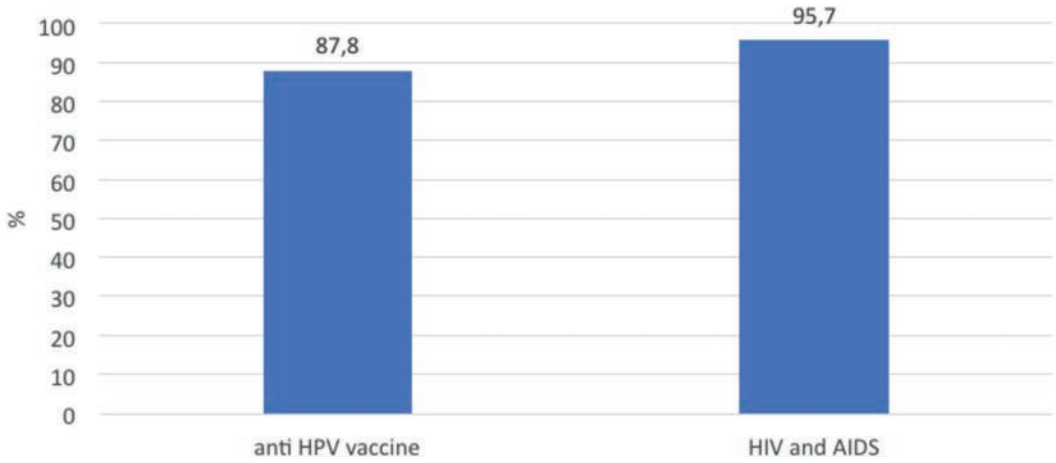


Fig. 5. Pre-test survey results for the question: *List at least 3 sexual behaviours at risk for STIs.*

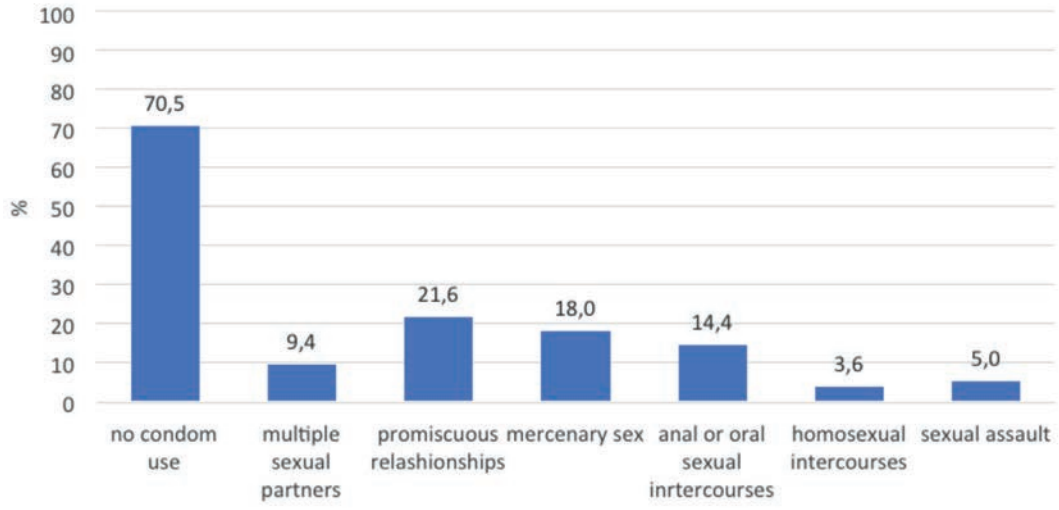
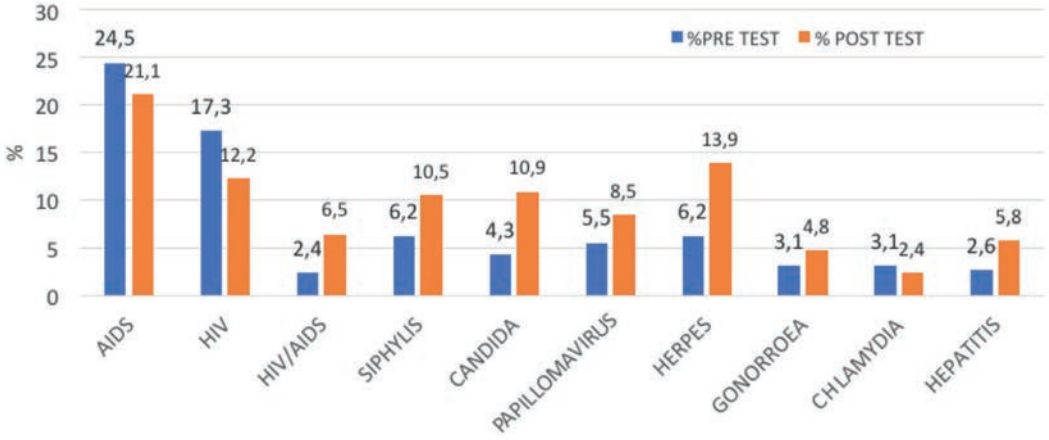


Fig. 6. Comparison between pre- and post-test answers to the question: *Mention at least 3 STIs.*



Tab. I. Comparison of the pre-test answers (available for 139 students) and post-test answers (available for 100 students) related to preventive measures, risk factors and risky behaviours for STIs.

	Pre-test N - (%)	Post-test N - (%)	χ^2 test	Odds ratio* (95%CI)
Preventive measures				
Condom	128 (92.1)	95 (95.0)	0.37	1.63 (0.55-4.86)
Vaccines	**88 (63.3)**	**87 (87.0)**	**< 0.0001**	**3.88 (1.97-7.64)**
Contraceptive pill	**34 (24.5)**	**14 (14.0)**	**0.046**	**0.5 (0.25-0.99)**
Morning after pill	**28 (20.1)**	**5 (5.0)**	**0.0008**	**0.21 (0.08-0.56)**
Diaphragm	16 (11.5)	7 (7.0)	0.24	0.58 (0.23-1.46)
Coitus interruptus	15 (10.8)	8 (8.0)	0.19	0.47 (0.72-1.77)
Others	2 (1.4)	1 (1.0)	/	/
Risk factors for transmission				
N of partners	**59 (42.4)**	**96 (96.0)**	**< 0.0001**	**32.54 (11.3-93.5)**
Disease contagiousness	**99 (71.2)**	**92 (92.0)**	**< 0.0001**	**4.65 (2.07-10.45)**
Probability of infection in the partner	**130 (93.5)**	**99 (99.0)**	**0.04**	6.85 (0.85-55.0)
Intimate hygiene	98 (70.5)	62 (62.0)	0.17	0.68 (0.39-1.18)
Risky sexual behaviours				
No condom use	98 (70.5)	72 (72.0)	0.80	1.08 (0.61-1.9)
Multiple sexual partners	**13 (9.4)**	**37 (37.0)**	**< 0.0001**	**5.69 (2.82-11.47)**
Promiscuous relationships	30 (21.6)	29 (29.0)	0.19	1.48 (0.82-2.68)
Mercenary sex	**25 (18.0)**	**6 (6.0)**	**0.0065**	**0.29 (0.11-0.74)**
Anal or oral sexual intercourses	20 (14.4)	10 (10.0)	0.31	0.66 (0.29-1.48)
Homosexual intercourses	5 (3.6)	2 (2.0)	0.47	0.55 (0.10-2.88)
Sexual assault	**7 (5.0)**	**0**	**0.022**	0.09 (0.00-1.56)

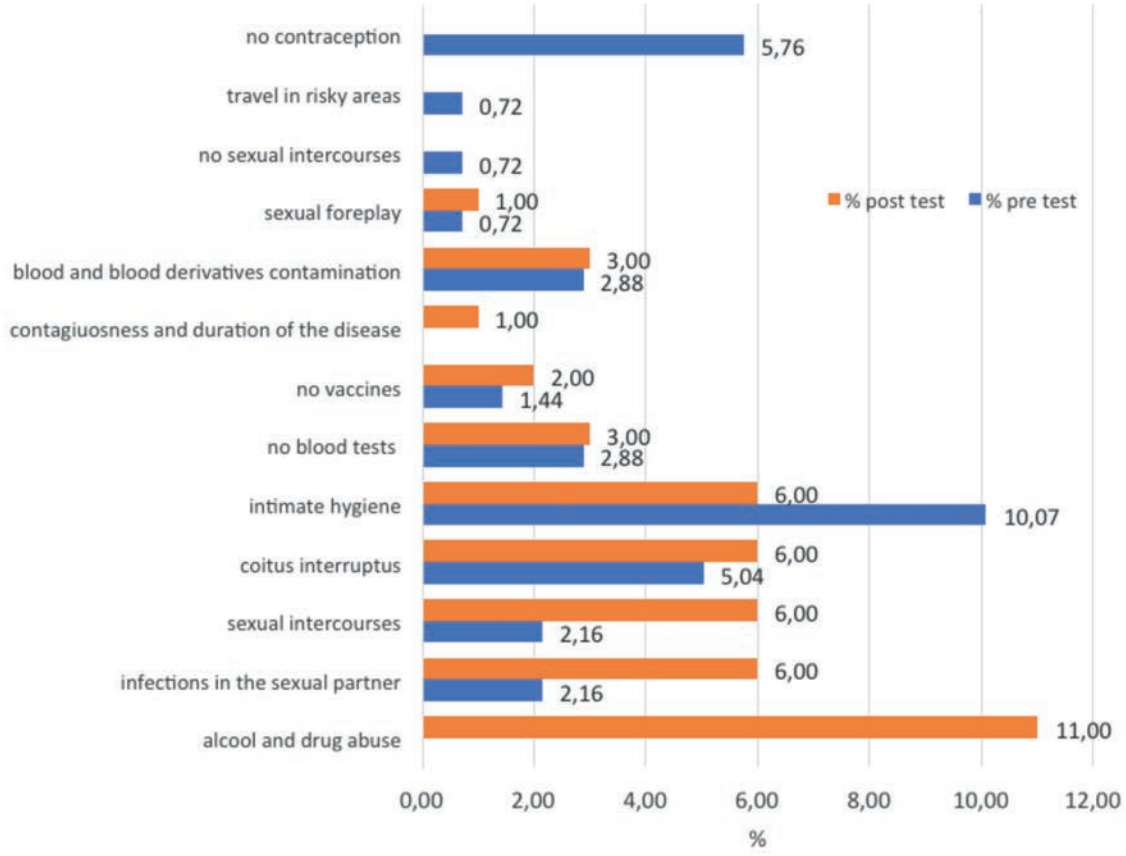
* Odds ratio with 95% Confidence interval in the post-test compared to the pre-test answers. In bold statistically significative differences between the two tests.

eral diseases wrongly qualified as sexually transmitted, the proportion of STIs appropriately identified as sexually transmitted rose in the post-test survey from 76.26% (318/417) to 97.96% (288/294) (χ^2 test $p < 0.0001$).

The effect of the training conference on the knowledge of the preventive methods to decrease the risk of STI acquisition is controversial. We found a significant increase in the awareness of the preventive effect of some vaccines against STIs (from 63.3% to 87%) but the idea that the contraceptive pill, the morning-after pill, the diaphragm, and the coitus interruptus could have some preventive properties, even if significantly reduced, still persists in the post-test survey (Tab. I).

We found a statistically significative increased consciousness of the importance of the number of sexual partners in infection transmission (χ^2 test $p < 0.0001$). The identification of this parameter as risk factor for STIs transmission increased by 32-fold in the post test. The role of disease contagiousness (which is the sum of intrinsic disease characteristics, time of diagnosis and treatment, efficacy of treatment) is better understood, given the increase of this factor from 71.22% to 92% in the pre- and post-test survey respectively (χ^2 test $p < 0.0001$, OR 4.65). Among the risk factors for STIs transmission, a slight increase of the probability of infection in the partner was observed, while the role attributed to the intimate hygiene decreased in the post-test. Although we found better knowledge of STIs, of preventive measures, of means of STI transmission, we found a stable attitude in considering sexual behaviours at risk.

Fig. 7. Sexual risky behaviour indicated in the open question in pre- and post-test.



The answers to the question on risky sexual behaviours reported in Table I continue to attribute the highest risk to sexual intercourses without condom protection.

A high risk was attributed in the post-test also to multiple sexual partner which rose from 9.35% to 37% and a lower risk was attributed to mercenary sex which decreased from 18% to 6%.

No significative differences were observed in the consciousness of the disease called HIV/AIDS and of the anti HPV vaccine already high in the pre-test survey.

The analysis of the answers given in the open section of the question on risky behaviours (Fig. 7) shows several misconceptions in the pre-test: no use of contraception, travel in "risky areas", no sexual intercourses are reported as sexual risky behaviours. In the post-test survey, 11% of participants recognized alcohol and drug abuse as risky factors for a reduced attention to safe sex practices never mentioned in the pre-test.

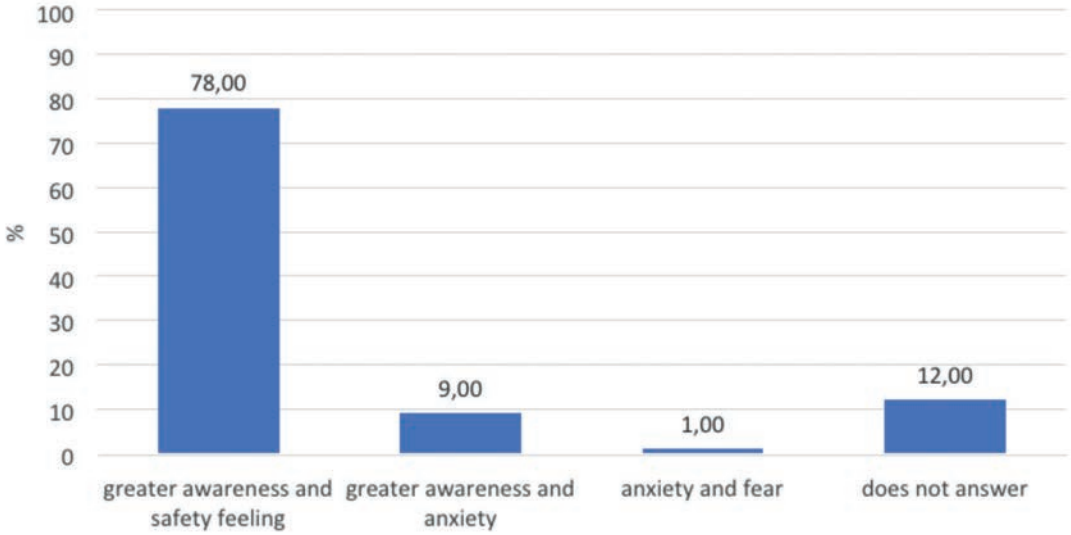
Parents, who were the main reference in the case of a suspected STI, fell in second place in the post-test (Mann-Whitney t test between pre and post-test score $p = 0.0002$). After the conference, the main reference for a suspected STI became the family doctor (Mann-Whitney t test between pre-test score and post test score $p = 0.04$) (Tab. II). In the post-test survey, we also asked students their opinion on who should give information

Tab. II. Order of reference participants would turn to in case of suspected sexually transmitted infection in the pre- and post-test survey **(a)**, and ranking of people and groups who are expected to be able to give information on STIs **(b)**.

a	Order of reference in case of suspected STI						
	1°	2°	3°	4°	5°	6°	7°
Pre-test	Parents (2.0)	Family doctor (2.6)	Hospital (3.6)	Friends (3.6)	Primary health care services (4.2)	Web based information (4.6)	Chat (6.1)
Post-test	Family doctor (2.2)	Parents (2.8)	Hospital (3.5)	Primary health care services (3.7)	Friends (4.2)	Web based information (5.1)	Chat (6.0)
b	Ranking of people able to give informations on STIs						
	1°	2°	3°	4°	5°	6°	
Post-test	Experts in STIs (2.0)	Teachers (3.1)	Parents (3.2)	Media (3.2)	Partner (4.4)	Friends (4.6)	

Personal feelings, suggestions, and general feedback.

Fig. 8. Personal feeling on the whole conference experience.



Tab. III. Score obtained in the feedback survey filled in by 119 participants in a scale from 1 to 5 (with 5 as the best score).

Question	Score value (mean)
Were these topics interesting to you?	3.95
Did the speakers explain these topics clearly?	3.96
Were these topics treated exhaustively?	3.84
Did you have time and opportunity to ask information and to say your opinions and doubts?	3.96
Was the organization of the meeting suitable both regarding the presentation and the instruments used?	3.73

on STIs; we assigned a score according to the order of preference: 1 to the first choice and 6 to the last choice. The ranking result is reported in Table II.

Lastly, we analysed the comments on the training conference, the free suggestions left and the feedback form. Greater consciousness and awareness of STIs, associated with a feeling of greater protection against infections is perceived by 78% of participants (78 students); 9% of them (9 students), however, reported consequent anxiety and discomfort (Fig. 8).

Suggestions were made by 11% (11 students) of the participants in the post-test survey. In 8/11 cases (72.7%) a positive comment was made, in 2/11 cases (18.2%) a more detailed discussion on the topic was claimed. In 1/11 case (9.1%) we found an appeal to reassure adolescents in order to avoid transmitting fear rather than awareness.

The scores, scaled from 1 to 5 in the feedback survey, filled-in by 119 individuals, are reported in Table III.

Discussion and conclusions

This is an interventional study which focused on the evaluation of the potential impact of training conferences on the knowledge and awareness of STIs in adolescent students. It was performed among students aged 16-17 attending a high school in Milan, Italy as part of a health promotion program.

Results outline the scarce basic knowledge of STIs among students. There is a high level of awareness of HIV/AIDS, but a low level of awareness of other STIs, their diffusion, means of transmission, prevention methods, behaviours at risk. These findings agree with other reports in literature about student's STI knowledge and awareness [9, 13-15] and suggest the need for targeted information programs.

A two-hour interactive conference held by Infectious Diseases and Psychology specialists seem to improve STI knowledge significantly in the short-term, even if several misconceptions persist.

It is noteworthy that only a very low proportion of participants declared that the conference led to anxiety or fear. This could be due to the fact that psychological consideration of behaviour at risk in adolescence was discussed during the conference: its positive value for the process of personal growth, and the importance of dealing with risk in a controlled and conscious way.

Although this study adds some insights into the sexual health knowledge and STI awareness in adolescent students and on the possible educational role of interactive specifically targeted conferences on STIs, there are several limits to be noted. First, the relatively low number of adolescents in a single high-school included in the analysis could be not representative of the entire population aged 16-17 years. Secondly, the anonymous participation to the survey, prevented us to perform a matched comparison of answers in the pre- and post-test survey.

Moreover, we did not perform a second control survey to verify the long-term effectiveness of the intervention on knowledge and awareness.
Lastly, we did not test how and to what extent this intervention could be translated into behaviour changes.
The adolescents' sexual health education plays a key role in STI prevention; school is the primary place to reach a large part of them. Collaboration between health specialists and teachers can prove to be extremely important in order to obtain successful behaviour change interventions and STIs control.

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Conflict of interest statement

None declared.

Authors' contributions

GO conceived and coordinate the study, designed the pretest and post-test surveys, held the interactive conferences, evaluated the results, and wrote the manuscript. MC contributed substantially to the conception and the design of the study, participated to the design of the pre- and post-test surveys, held the interactive conferences, and contributed substantially to the manuscript writing. SI organized and coordinated the whole intervention within the health promotion project of the school. GPJ revised the manuscript and provided English language editing.

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ORIGINAL ARTICLE

Knowledge of sexually transmitted infections and risky behaviours: a survey among high school and university students

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Keywords

Sexually transmitted infections • Students • Risky behaviours • Sex education

Summary

Introduction. *STIs are a serious public health problem. Worldwide, 500 million people a year acquire a STI, and young are the most affected.*

Methods. *This study was conducted administering an anonymous questionnaire to 1228 Sicilian students of high school and university.*

Results. *The students had variable understanding of STIs and their complications. The results demonstrate an extreme variability in the knowledge of STIs. Multiple linear regression showed that sexual health knowledge was associated with age and sexual orientation.*

Conclusions. *Our results show that knowledge of STIs is poor and inadequate. This finding can put students at risk of STIs.*

Introduction

Sexually transmitted infections (STIs) are one of the most serious public health problems around the world, both in industrialized and in developing countries. According to WHO estimates, STIs have an annual incidence of 357 million cases among which chlamydia (131 million), gonorrhoea (78 million), syphilis (5.6 million) and trichomoniasis (143 million) [1]. Worldwide, almost one million people contract an STI every day, and most of them are unaware of their infection status, representing thus a source of infection for others [1]. From these data should be ruled out HIV, the incidence and effects of which on the health and socio-economic status of entire countries [2-4] especially African those, have been considered, since long time, a real emergency [5]. Since the mid-1990s, an increase in the frequency of diagnosed sexually transmitted infections, including syphilis, gonorrhoea and chlamydia, has been reported in several European countries, especially among adolescents between 16 and 19 years of age [6].

The most at-risk age group is adolescents, as they represent half of newly acquired STIs cases; in Italy, 19.5% of all new cases of STIs are diagnosed in young people (15–24 years old) [7, 8]. The risk is high in this age group because young people have a low level of awareness of the risks related to their sexual behaviour [9]. The reduction of age at first sexual intercourse is a possible explanation for the increase in the number of sexually transmitted diseases [10]. Many studies have shown that adolescents who begin sexual activity before the age 16 are more likely to take risks [11] as most of these sexual encounters are not adequately protected by the use of condoms [12-14]. Also Italian adolescents have their first sexual experience at a very early age (15.6 ± 1.6 years old), often without protection against STIs [15]. Furthermore, a large percentage has multiple partners [16].

The reluctance of adolescents to use condoms is another possible explanation. Some surveys showed that, among youth, the idea that using condoms greatly reduces sexual pleasure is widespread [17, 18].

Furthermore, among adolescents, condom use is not considered as protection from sexually transmitted infections, but rather as a way to avoid a possible unwanted pregnancy [19]; therefore, condom use becomes irregular when other contraceptives are used [20, 21].

Among the sexually transmitted infections, HIV is certainly the best known; nevertheless, on this subject, the degree of awareness about modes of transmission is still poor [19]. Because the sexual transmission of infections continues to represent a serious public health problem, there is a strong need to minimize behaviours that place people at risk of contracting STIs by promoting safe sexual behaviours through consistent and correct use of condoms and by advising people to avoid promiscuous relationships [22].

Based on these considerations, we administered to adolescents and young people attening their last year of high school and the first courses of university in the city of Messina, a questionnaire on their sexual behaviour and on the adoption of prevention methods, in order to as-

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sess their risk perception. The questionnaire also served to understand their level of general knowledge on STIs and, in particular, that related to HIV/AIDS.

Methods

The study was conducted in Messina between January and June 2017, to analyse sexual behaviour among students and their knowledge about STIs. Particularly, we used a questionnaire reporting some questions about this topic that was specifically designed by us for this study and it was perfected and refined after having tested it on a small sample of students (20 subjects). After obtaining authorization from the headmasters, we organized a first meeting to explain the study and distribute the informed consent forms to 1,261 students. In a further meeting, a member of the research team distributed the questionnaires to students presenting the filled informed consent form accounted for 1,228 (participation rate = 98.3%). For students under 18 years of age, parental consent was obtained. Participation was voluntary, all questionnaires were anonymous, and the confidentiality of the information provided was strictly maintained. The only considered exclusion criterion was the absence of the parental informed consent for underage students.

Of 1,228 enrolled students, 832 were in their last year of high school while the university students who attended the first year of humanistic and scientific courses were 128 and 268 respectively.

Overall, the questionnaire comprised 22 questions divided into three sections concerning personal information of each student (personal details, nationality, sexual orientation) (Section 1), their knowledge about STIs, involving also questions to evaluate a highly specific scientific knowledge (Section 2), and the socio-educational aspects (adolescent's behaviours) (Section 3). Section 2 included questions with multiple answers of which only one was correct except for the question 12 that could have 6 possible correct answers. Then, for each correct answer, one point was awarded, while zero points were awarded for incorrect or missing answers. Total knowledge scores ranged between 0 and 18. Particularly, knowledge scores ranging between 0 to 6 were considered as low knowledge, 7 to 12 as mean knowledge, and > 12 as high knowledge.

Statistical analysis

Pearson correlation test were used to determine the predictors of sexual health knowledge. Moreover, multivariate analysis was conducted to estimate the proportion of variance in sexual health knowledge that can be accounted for by socio-demographic factors and current educational status. Analysis was performed by using the a priori model (i.e. considering as covariates all variables, regardless of *P* values to Pearson test) of multiple regression. The level of significance was set at 0.05. All analyses were performed using StatSoft software (StatSoft®, version 10).

Results

Table I shows the composition of the students who participated in the study by gender, age, national origin and sexual orientation, divided by field of study.

Considering sexual orientation, the number of individuals who claimed to be homosexual or bisexual increased in universities compared with high school (χ^2 28.76, $P < 0.01$). In fact, while in high schools the percentage was 8.2%, in scientific and humanistic university courses it reached 17.9% and 25%, respectively.

The Section 2 of the questionnaire investigated the actual knowledge of the students about STIs, prevention, transmission and clinical disease, in particular, on HIV/AIDS. All results obtained in the second section are shown in Table II.

The percentages of correct answers for each question demonstrate an extreme variability in the knowledge of STIs in terms of prevention, transmission and clinical disease. The average percentages of correct answers were 51.8% in high schools, while in humanities and scientific university courses, they reached 55.7% and 60.8%, respectively. Answers to the question about the methods of STIs prevention showed a good understanding of the methods to have be used; indeed, the majority of students answered

Tab. I. Socio-demographic characteristics of respondents (%).

	Secondary school	Humanistic university	Scientific university	Totals
N. students	832 (67.8)	128 (10.4)	268 (21.8)	1228
Gender				
Women	418 (50.2)	58 (45.3)	116 (43.3)	592 (48.2)
Men	414 (49.8)	70 (54.7)	152 (56.7)	636 (51.8)
Age groups				
17-19	743 (89.3)	50 (39.1)	61 (22.8)	854 (69.6)
20-22	89 (10.7)	62 (48.4)	171 (63.8)	322 (26.2)
> 22	0 (0.0)	16 (12.5)	36 (13.4)	52 (4.2)
Nationality				
Italian	816 (98.1)	122 (95.3)	265 (98.9)	1203 (98.0)
Foreigners	16 (1.9)	6 (4.7)	3 (1.1)	25 (2.0)
Sexual orientation				
Heterosexual	752 (90.4)	94 (73.4)	218 (81.3)	1064 (86.6)
Homosexual	31 (3.7)	10 (7.8)	26 (9.7)	67 (5.5)
Bisexual	37 (4.5)	22 (17.2)	22 (8.2)	81 (6.6)
Not declared	12 (1.4)	2 (1.6)	2 (0.8)	16 (1.3)

'condom'. Nevertheless, it must be emphasized that around 26% of students believe that the contraceptive pill is a means of prevention of STIs.

The most important result in this section concerns correct knowledge of the infections that could be sexually transmitted. The students were asked to select from

Tab. II. STIs knowledge of respondents (bolded the correct answers).

Question		Answers (%)				
			SS	HU	SU	Mean
Q1	Which of the following means are used to prevent sexually transmitted infections?	Birth control pill	2.2	0	3.2	
		Condom	73.2	64.1	74.3	**70.5**
		Both condom and birth control pill	22.6	31.4	20.6	
		None of the above means	2.0	4.3	1.1	
Q2	By what way you do not transmit HIV?	Blood and body fluids	9.2	5.2	7.4	
		Blood and contaminated needles	6.3	10.6	16.3	
		Saliva, urine	61.7	57.8	63.8	**61.1**
		Vertical (mother-child)	22.4	26.2	12.0	
Q3	What is the genetic material present in HIV?	DNA	28.7	31.4	22.2	
		DNA and RNA	29.3	28.1	13.5	
		RNA	30.7	34.4	57.1	**40.7**
Q4	Locate the correct timeline	Dangerous behaviour → AIDS → HIV → antiretroviral therapy	46.3	59.1	25.8	
		Dangerous behaviour → antiretroviral therapy → HIV → AIDS	15.1	14.3	11.0	
		Dangerous behaviour → HIV → antiretroviral therapy → AIDS	25.4	20.3	41.4	**29.0**
		HIV → AIDS → antiretroviral therapy → Dangerous behaviour	5.0	2.2	9.4	
Q5	What are, in your opinion, the major risk factors for transmission of HIV?	Poor hygiene of the classrooms desk	3.4	6.0	6.3	
		Blood and objects (sharp objects, needles etc.) contaminated with blood derivatives	79.6	84.4	80.6	**81.5**
		Through tears, sweat, saliva, urine, faeces, nasal secretions	11.6	7.8	7.7	
		Mosquito bites	4.2	0	3.3	
Q6	Can a person with AIDS heal?	Yes	9.1	5.1	10.1	
		No	52.8	48.4	58.2	**53.1**
		Yes, with right therapy	27.6	37.9	22.4	
		Yes, with stamina cells	7.8	8.5	6.8	
Q7	Do you think there might be a vaccine against HIV?	It exists, but I am not informed	25.4	27.0	19.0	
		It exists, but I'm afraid of the side effects of the vaccine	7.9	26.5	9.3	
		No, there is not	23.9	37.5	40.3	**33.9**
		I don't know, I was never interested	42.4	8.5	24.8	
Q8	A person seropositive for HIV who is treated, can completely eliminate the virus from his body?	Yes	10.3	2.4	13.9	
		Sometimes	31.4	27.6	14.4	
		No	30.8	32.8	48.9	**37.5**
		It depends on the person's immune response	26.1	35.8	17.6	
Q9	The HIV virus is transmitted through kissing, shaking hands or sharing public places with a HIV positive subject:	True	8.5	3.3	5.9	
		False	64.4	45.3	65.3	**58.3**
		Sometimes	19.2	23.3	16.7	
		It depends on the person's immune response	7.0	28.1	10.0	
Q10	Do you know what "seropositivity" means?	In the blood are found eosinophils that attack the virus	26.1	22.4	23.8	
		In the blood, there are not found antibodies	24.7	23.1	21.3	
		In the blood are found antibodies against the virus	21.5	37.5	36.2	**31.7**
		In the blood are found increased platelets	19.7	16.4	8.7	
Q11	Do you know which system hits the HIV virus?	Nervous system (e.g. neurons)	6.1	6.3	12.1	
		Endocrine system (e.g. thyroid follicular cells)	8.8	4.9	11.3	
		Hematopoietic system (e.g. Red blood cells and platelets)	42.3	47.8	22.7	
		Immune system (e.g. CD4 + lymphocytes)	38.8	39.1	51.5	**43.1**

continues

Tab. II. *follows.*

Question		Answers (%)				
			SS	HU	SU	Mean
Q12	Which of the following infectious diseases are transmitted primarily by sexual intercourse?	**HIV/AIDS**	99.3	100	97.4	**98.9**
		Syphilis	67.9	60.9	76.1	**68.3**
		Hepatitis A	52.8	70.3	62.7	**61.9**
		Hepatitis C	55.9	67.2	63.8	**62.3**
		HPV	23.0	40.6	41.4	**35.0**
		Meningitis	70.8	89.1	79.1	**79.6**
Q13	Which of these infectious diseases can be prevented by vaccination?	AIDS	4.1	2.0	10.3	
		Syphilis	12.3	9.4	8.9	
		Hepatitis B	41.2	46.9	54.5	**47.5**
		Scabies	24.6	35.8	17.6	
	Mean of correct answers		**51.8**	**55.7**	**60.8**	

SS: secondary school; HU: humanistic university; SU: scientific university.

Tab. III. Relationship between socio-demographic characteristics, current educational status and sexual health knowledge expressed as "Mean Score" (dependent variable).

Variable	Group	Mean score	Test	P value
Gender	Women Men	10.2 10.3	r: 0.016	0.594
Age	17-19 20-22 > 22	9.4 10.3 11.5	r: 0.129	< 0.001*
Sexual orientation	Heterosexual Homosexual Bisexual	10.6 8.5 8.1	r: -0.212	< 0.001*
Current educational status	Secondary school Humanistic university Scientific university	9.8 10.6 11.5	r: 0.209	< 0.001*

* Level of significance (p < 0.05).

Tab. IV. Multiple regression analysis showing factors associated with sexual health knowledge (Adjusted R^2 = 0.1006).

Covariates	B value	P value
Age	0.058	0.038
Gender	-0.003	0.896
Sexual orientation	-0.243	< 0.001
Current educational status	0.216	< 0.001

among a list of infections (including HIV, syphilis, hepatitis A, hepatitis C, HPV infection and meningitis) the ones they believed to be sexually transmitted. HIV/AIDS was the best known among the STIs proposed, while HPV was the less known. In particular, HIV/AIDS was correctly recognized as an STI by 98.9% of students (95% CI: 95.5-102.2), while 68.3% (95% CI: 49.5-87.2) of the respondents correctly recognized syphilis. Only 35% (95%CI: 9.1-60.9) of the students identified HPV infection as an STI.

The mean total score for knowledge, which could range from 0 to 18, was 10.3 ± 3.4 (95% CI: 10.1-10.5).

Table III shows the mean score by field of study, highlighting a growing trend in university students, especially scientific university students. The same table shows the correlations between knowledge scores versus biological variables and current educational status.

The knowledge score was positively correlated with age ($P < 0.001$); moreover, it was higher among heterosexuals compared with homosexual and bisexual subjects ($P < 0.001$). The score was also higher among Italians than foreign students ($P = 0.0048$). Moreover, university students, especially those with scientific orientation, have better knowledge of STIs ($P < 0.001$). Only 22.1% of high school students reached a knowledge score > 12, whereas the percentages increased to 28.1% and 40.7% among university humanities and scientific students, respectively.

Multivariate analysis using the a priori model of multiple regression was conducted to estimate the prediction of knowledge by socio-demographic factors and current educational status. Multiple linear regression analysis was performed by using as reference groups for each variable the younger subjects, males, and heterosexual respectively. In particular, the age was categorized in three groups and, regarding sexual orientation, we considered only heterosexual, homosexual and bisexual, ruling out the subjects that did not declare their behaviour. The results showed that sexual health knowledge was very significantly related to current educational status and, above all, to sexual orientation, while the role of age was more negligible ($P < 0.05$) and the one of gender was absent. As reported in Table IV, overall nearly 46% of the observed variability in sexual health knowledge was due to these factors.

The Section 3 of the questionnaire was related to questions on sexual behaviour and the sources of information received on the subject of STIs (Tab. V).

Many students have sex with casual partners and very often without protection. Thirty-two percent of high

Tab. V. Socio-educational aspects: sexual risk behaviour.

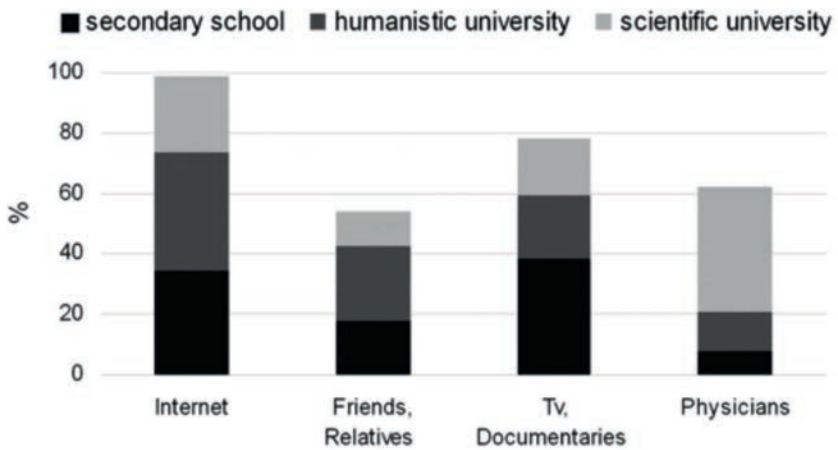
Question	Answers (%)				
		SS	HU	SU	Mean
Over the last 12 months, did you happen to have a sexual intercourse with a casual partner?	Yes	32.1	46.9	57.5	45.5
	No	67.0	51.6	40.7	53.1
If yes, did you use a condom?	Never	26.1	12.9	20.0	19.7
	Always	27.3	50.1	48.7	42.0
	Less than half the time	44.4	33.7	26.2	34.8
	I don't know the right way to use one	2.2	3.3	5.1	3.5
If you knew you were HIV positive	You would not tell anyone	15.9	29.7	41.8	29.1
	You would tell only your best friend	12.5	9.4	9.7	10.5
	You would tell only your partner	44.0	46.9	30.2	40.4
	You would tell everyone	24.0	12.5	14.2	16.9
From which of the following sources did you get more information about sexually transmitted infections?	Internet	34.3	39.1	25.4	32.9
	Friends, relatives	17.6	25.0	11.6	18.1
	TV, documentaries	38.6	20.3	19.0	26.0
	Physicians	7.9	12.5	41.1	20.5
Do you think that the informations on sexually transmitted infections are more reliable if provided	By school educators	26.6	7.8	16.4	16.9
	By mass-media (tv, internet)	7.6	4.7	7.5	6.6
	With information projects implemented by physicians	62.0	79.7	67.5	69.7
	With books and information brochure	2.5	4.7	6.0	4.5

SS: secondary school; HU: humanistic university; SU scientific university

school students claim to have had sexual intercourses with a casual partner, and of these, 27% said they had used a condom. The situation improves slightly among university students. In fact, among the 52% of students who said they had had sexual intercourse with a casual partner, about 50% had used a condom. In addition, an inverse relationship was observed between knowledge scores and sexual behaviour since students who reported engaging in high-risk behaviour have a lower score than those who did not. Particularly, students who had sex with casual partners without condom had a knowledge score of 8.7 (95% CI: 7.9-9.5) whereas students who do not have sex with casual partners had a knowledge score of 10.8 (95% CI: 10.5-11.0) ($P < 0.001$).

Figure 1 shows the main source of information on STIs and it demonstrates that the most frequent were Internet and TV for high school students, while university students claimed to have received more information from physician. On average, only approximately 18% of students declared that they had been informed about STIs by family or friends. Moreover, we found an awareness of the lack of knowledge about STIs among the young surveyed people. In fact, 62%, 80% and 68% of high school, humanities university and scientific university students, respectively, complained about the need to receive more information by qualified staff, i.e. through information projects implemented by physicians and/or health personnel.

Fig. 1. Results obtained from the Section 3 of the questionnaire. Sexual information sources and communication with parents, reported by high school students and humanistic or scientific university students.



Discussion

Increasing the awareness of sexually transmitted infections starting from school age may help young people to understand the correct behaviours to adopt for living a healthier life in their own interests and that of the entire society.

Our study showed a lack of accurate knowledge and misconceptions about sexually transmitted diseases, especially in younger students. In fact, older students had higher mean knowledge scores than their younger counterparts. As individuals grow older, their sexual curiosity and development lead them to seek for more information relating to sexual issues. These findings were consistent with those of other similar studies on university students [23, 24]. Furthermore, the results of the present study showed that students in science faculties had higher levels of sexual knowledge than other students; this can be explained by the fact that these university courses cover subjects on this topic. The obtained data show that not only older students have a better knowledge, but they also have a better understanding of their own sexuality and, at the same time, less difficulties in declaring it, as evidenced by the highest percentage of homosexual and bisexual individuals being found among university students.

The highest score obtained by heterosexual compared with homosexual and bisexual students emphasizes a critical point because it shows that the most vulnerable categories are the least informed about STIs. The highest vulnerability is linked to multiple reasons: the act of unprotected anal intercourse is associated with a higher risk of transmission of STIs than vaginal and oral intercourse [25-27]. Other high-risk sexual behaviours, such as multiple sexual partnerships and rougher sex, are all linked to increased rates of transmission [28, 29].

Secondly, homosexuality remains highly stigmatized in many countries; this often means that sexual behaviour is hidden, and the homosexuals often feel uncomfortable or unable to access the sexual health services they need or to disclose their behaviour to healthcare workers for fear of discrimination [30]. All these factors stress the importance of carrying out innovative and effective communication strategies to promote a lower risk behaviour culture to hard-to-reach group such as homosexuals and bisexuals.

Only 7.9% of students were able to correctly identify all STIs from a list of diseases. In this list we included also Hepatitis A that, listing only the STIs for which sexual way is the most "known" and the principal transmission route, we did not consider it as a "true" STI despite, in recent times, there was a spread of outbreaks due to fecal-oral pathogens following sexual contacts [31-34] This is particularly troubling because it indicates how unconscious are the young about STIs and that the latter can have serious consequences to their health and the health of others. The concern is also supported by the fact that 30% of the interviewed students were not able to properly distinguish between preventive methods and contraceptive methods; indeed, among the preventive measures against STIs, the students incorrectly indicated the birth control pill.

HIV/AIDS was the best-known STI among those listed; nevertheless, the question with the highest percentage of wrong answers regarded HIV/AIDS and, in particular, it emerges from our survey that they do not know the difference between HIV and AIDS and consequently, do not know the term seropositive (correlating the two questions, Q4 and Q10, the trend is statistically significant, data not shown).

The current results are in agreement with those of a previous study, which underlined the lack of knowledge of the difference between the term HIV infection and the meaning of the acronym AIDS [19]. This rather superficial knowledge of students on the subject is very alarming, considering that in Italy, there have been thousands of new cases of HIV infection since 1982, and the annual incidence is still approximately equal to 6 cases per 100,000 inhabitants. In addition, in 2015, of 3444 new cases, 85.5% were attributable to unprotected sex [35]. Therefore, it is important to give young people more information and teach them that there is currently no cure for AIDS. Despite the remarkable goals achieved by the management of HIV infection, the awareness and the risk perception is not improved compared to the past. Indeed, in the 90's, Buysse demonstrated that young people were particularly confused about issues concerning how HIV is not transmitted [36]. Moreover, Memon also found that although young people knew the main routes of transmission of HIV they also showed some uncertainty regarding the actual mechanisms and prevention of the virus [37]. In terms of perceived educational needs, the majority of respondents in 1990 agreed that they should be taught how to protect themselves from HIV/AIDS [38] More recently, Prati et al. showed that a greater knowledge and lower risk perception are associated with a higher socioeconomic status and, in addition, in contexts where effective antiretroviral treatments have led to longer survival rates, it seems plausible that people underestimate the risk of HIV/AIDS [39].

The students we interviewed had inferior knowledge of HPV compared with their knowledge of other sexual infections. It is very important for young people to be aware of the risks of HPV infection [40-42] given that they represent the age group most at risk. Panatto et al. demonstrated that in cervical swabs with normal cytology, positivity for at least one HPV type was found in 48.1% of women aged 16-17 years, in 15.4% of women aged 18-20 years, in 21.9% of women aged 21-23 years and in 15.5% of women aged 24 to 26 years [43]. For all these reasons, it is appropriate to encourage information campaigns about this important public health issue focusing mainly on vaccinations in general and anti-HPV in particular in order to improve coverages and avoid epidemics of vaccination-preventable infections that have recently affected our territory [44, 45].

Our results showed that the most frequently referred sources of sexual health information were the internet and TV or medical and paramedical staff; these results are in line with those of two similar studies among uni-

versity students [46, 47]. Is important to emphasize that parents are important sources of information on sexual matters but were not recognized by most students as a source of information; indeed, in our study, the least frequent sources were parents and friends. This finding is consistent with the results of a previous study on awareness of school students related to sexually transmitted infections, in which families were found to be the least common sources of information on sexual matters [48]; a good relationship between parents and children is associated with lower levels of unprotected sex, unwanted pregnancies and STIs in adolescents [49, 50]. Different studies from other Mediterranean Catholic countries have indicated that parent-adolescent communication is crucial for adolescent health indicators [51-53].
In our survey, we highlight the lack of parent-child communication. A possible explanation could be the fact that talking about sex in a family is considered as a taboo. Probably, students speak little with their parents about sexual issues because of the fear that their parents may think that they are engaging in sexual activity [49].
Our investigation, in agreement with other Italian studies [54, 55], has shown that many students have casual sexual relationships, even without condoms, a risk behaviour that underscores a superficiality among students, probably attributable to poor sexual health knowledge as evidenced by the significant correlation between risk behaviour and the score, and to current sexual habits that, overtime, have become easier than the past [56, 57]. Because the knowledge about diseases and their complications are poor and students are vulnerable to unprotected sex, there is an urgent need to introduce sex education as a proper subject in Italian schools in order to promote sexual relationships practiced in a safe and responsible manner and to implement the awareness of the risk of contracting and transmitting STIs.
The European Union stressed that sex education in Italy has been and is still lacking, putting emphasis on political and religious constraints. The European Union hopes that sex education is introduced soon in Italian schools, as it has been for many years in countries such as Denmark, Sweden, France and Germany [58], countries where there has been a decrease in teenage pregnancies, abortions and sexually transmitted infections. Italian authorities have long neglected this need, the first legislative proposal for the introduction of sex education in schools began in the early 1900s, but it was not successful. The latest legislative proposal in Italy dates to November 1992. Since then, the debate continues, but not much has been done in practice [54].

Conclusions

The finding of the current study that, due to the high participation rate (98.3%), would exclude bias due to the lack of participation of the students less motivated and/or with less knowledge, showed that the level of sexual health knowledge is quite poor and there are still some misconceptions about sexual issues. Inadequate knowledge may place students at risk of sexually transmitted infections, therefore, we believe that all high schools should introduce a course of sex education in the curriculum. Medical staff specialized in sexually transmitted infections, having both theoretical knowledge and practical experience in these topics, would be the most appropriate to instruct teachers and students. An exam at the end of the course would be useful to verify that students have acquired adequate knowledge, as is done in other subjects.

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Conflict of interest statement

None declared.

Authors' contributions

IP, AD and GV conceived, designed and coordinated the research; BC, FM and MPB administered questionnaires and collected data; GV, AF, MC, GFP, PS and GN contributed to the acquisition, interpretation of data, identified the endpoints analysed and prepared the figures and tables; AD, IP and GV wrote the paper.
All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Association of mother's handwashing practices and pediatric diarrhea: evidence from a multi-country study on community oriented interventions

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Keywords

Handwashing • Pediatric diarrhea • Community health workers • Health promotion • Social accountability

Summary

Introduction. *Improved hand hygiene in contexts with high levels of diarrheal diseases has shown to reduce diarrheal episodes in children under five years. A quasi-experimental multi-country study with matched comparisons was conducted in four rural districts/sub districts in Cambodia, Guatemala, Kenya and Zambia.*
Methods. *Community oriented interventions including health promotion for appropriate hand washing was implemented in the intervention sites, through community health workers (CHW) and social accountability mechanisms. Community councils were strengthened/established in all study sites. Using household surveys, information on mother's handwashing practices and diarrhea incidence of children 2 weeks preceding the study was obtained.*
Results and Conclusion. *Access to safe drinking water was reportedly higher for communities in Guatemala and Zambia (> 80%), than those in Cambodia and Kenya (< 63%), with significantly higher levels in intervention sites for Guatemala and Kenya. Improved sanitation was low (< 10%), for Kenya and Zambia, compared to Cambodia and Guatemala (> 40%); intervention sites reporting significantly higher levels, except for Zambia. Hand washing index; hand washing before food preparation, after defecation, attending to a child after defecation, and before feeding children was significantly higher for intervention sites in Cambodia, Guatemala and Kenya (Cambodia, 2.4 vs 2.2, p < 0.001, Guatemala, 3.0 vs 2.5, p < 0.001, Kenya, 2.6 vs 2.3, p < 0.001). Factors significantly associated with lower odds of diarrhea were; mother's marital status, higher educational status, one or more handwashing practices, wealthier quintiles, older (> 24 m), and female children. The findings suggest that caretaker handwashing with soap or ash has a protective effect on prevalence of diarrhea in children.*

Introduction

Diarrheal diseases continue to contribute to the major disease burden in children from low and middle- income economies, as the second leading cause of death [1]. Globally, 1.7 billion cases of childhood diarrheal cases are reported every year, resulting in an estimated 525,000 deaths of children under five years [2]. Diarrhea is also a leading cause of malnutrition in children under five [3]. Despite the impressive achievements in reducing child mortality by 69%, in 2015, governments and the development community have far to go in averting the 4.4 million deaths that have been projected for 2030 [4].
A recent systematic review on the evidence of handwashing and diarrhea prevention, reported that in Low and Middle-Income Countries (LMICs), handwashing promotion in communities prevents one quarter of diarrhea episodes with a higher effect size when soap was provided free of cost [5]. The standard practices for hand washing include before feeding a child, after defecating, or handling a child who has defecated and before cooking [6]. Specific times of hand washing have shown different outcomes of diarrhea episodes, as in a trial in rural Bangladesh where hand washing after defecation with and without soap had significantly higher odds of less diarrhea, but not before feeding a child, or after cleaning a child who had defecated [7]. Another study in rural Guatemala, reported no significant differences between intervention and control sites in self-reported hand washing practices, hygiene standards, prevalence of diarrhea and child growth following a three year water treatment and hand washing campaign [8].
Handwashing with soap, in low income economies has been challenged, primarily due to the constraints of time when mothers are busy, and the price of soap for all family members to consistently practice the behavior at all expected times [7, 9-10]. Only 17% of mothers with young children reported washing hands with soap after using the toilet, and 45% with water alone, in a 11-country review [11]. The objective of this study was to examine the association of mother's reported handwashing practices following community-oriented health promotion interventions on diarrhea in children under five years.

Methods

A multi-country assessment was conducted in Cambodia, Guatemala, Kenya and Zambia as part of a collaborative research study by the Johns Hopkins University, the National Institute of Public Health in Cambodia, Institute of Nutrition of Central America and Panama in Guatemala, Moi University School of Public Health in Kenya, and the Institute of Economic and Social Research at the University of Zambia.

In each country four districts or sub districts were selected from the World Vision Area Development Programs (ADP), 2 of the ADPs were assigned to the intervention and two matched ADP's to the comparison arms of the study (Tab. I). Matching was based on select socio-demographic and health profiles of the ADPs. An ADP is a defined geographic area, with a population ranging from 19,000 to 25,000, where World Vision implements a range of integrated health and developmental activities for 15-20 years. These may include health, nutrition, water and sanitation, food security, education, and child sponsorship/protection. Communities in the intervention sites, received a package of interventions including; a) household level health promotion by Community Health Workers (CHWs) focusing on timed and targeted counseling during pregnancy and early childhood and b) institution and or/support of social accountability mechanisms using Community Voice and Action and Community Scorecards, designed to strengthen community to health facility linkages, enhance community knowledge of health facility entitlements and support facility performance [12]. Facility and community management councils were established or strengthened through training and supportive supervision based on the Global Fund's Community Systems Strengthening Framework in all ADP sites [13]. Communities in the comparison ADPs also received routine services from the local district, World Vision and other Non-Governmental Organizations (NGOs), including Community Health Worker (CHW) services.

Household listing of all the communities in the 4 ADPS in each country was obtained. Required sample size was calculated to detect a significant difference in differences in the decline in severe malnutrition in children under-five, with a two-sided alpha of .05 ($\alpha = 0.05$) and power of 80% ($1-\beta = 0.80$), factoring a non-response rate of 5% and design effect of 1.2. Using a multi-stage sampling procedure, communities were sampled in proportion to population size. Eligible households with children under five or women who had a delivery in the previous two years, were selected from each sampling unit. Standardized household survey instruments used by the Demographic Health Surveys, were modified for the study [14]. Interviews were conducted with heads of households to obtain information on socio-demographic characteristics, main source of drinking water and type of sanitation facility and food security. Subsequently, women, aged 15-49 years, who had delivered in the previous two years, or had children or were caretakers of children under five were interviewed to elicit information on reproductive history, handwashing with soap or ash and illness and preventive care-seeking practices for maternal, newborn and child health. In each household one child under the age of five years was randomly selected for the study. The sample for this study only included women who had delivered in the previous two years with a biological child under five years. Since there were variations in the sampling of children at baseline, this paper examines results from the final evaluation conducted between 2016-2017.

The survey teams received training on conduct of field surveys, informed consent and confidentiality procedures for ensuring ethical standards. Household survey instruments were field tested in a neighboring community that was not part of the study site. Informed consent was obtained from all study participants, ensuring confidentiality and disclosures of risks and benefits. Data security was ensured and all standard procedures for field quality control and data management was followed during the conduct of the study.

Diarrhea was defined as the mother's report of a diarrhea episode in children under five, two weeks preceding the survey. Four hand washing practices were included; after defecation, after attending to a child who had defecated, before food preparation and before feeding the child. Handwashing with soap or ash was specified. For the regression analysis, to determine the association of hand washing and diarrhea, we included key demographic, caretaker and child characteristics. Handwashing index was computed for 4 key handwashing practices; handwashing before cooking, after defecation, after attending to a child who had defecated and before feeding the child. All handwashing practices were weighted equally with a value of 1, with an index range of 0-4.

Standard quality control procedures were followed for data cleaning, verification and analysis, using STATA V14.2 [15]. Deidentified data was used for analysis. We first performed a descriptive analysis of socio-demographic factors, followed by reported incidence diarrhea in children and handwashing practices. Bivariate and multivariate logistic regression models were created to determine the association between reported diarrheal

Tab. I. Selected study sites in each country – districts/sub districts.

Study sites	Intervention sites		Comparison sites	
Cambodia	Chulkiri	Comapa	Prasath Balang	Tbeng Meanchey
Guatemala	Comapa	Nuevo Amanecer	Apas	Tinamit Junam
Kenya	Karemo	Katito	Kegonga-Ntimaru	Magunga
Zambia	Luampa	Magoye	Choongo	Nyimba

illness and known predictors including handwashing, access to safe water and sanitation. Cases with missing data for the independent variables were not included in the regression models. Wealth quintiles were constructed employing a principle component analysis with a combination of 10 household assets (television, radio, bicycle, etc.) and household type (roofing). We excluded safe water and sanitation from the wealth quintiles. The index was categorized into five categories of poorest, poor, middle, richer, and richest. Testing for collinearity was performed for the independent variables.

Ethical approval for the study was obtained from the Johns Hopkins Bloomberg School of Public Health's Institutional Review Board (IRB # 00004986) and the ethical and research review boards of the research institutions in each country.

Results

The final sample included 2,995 mother and child pairs in Cambodia, 1,992 in Guatemala, 2,581 from Kenya and 1,057 from Zambia. About 90% of the households were headed by males in Cambodia, Guatemala and Kenya, and more than 30% were headed by females in Zambia (Tab. II). Most women were between the ages of 20-36 years, 98% of the women were married in Cambodia, 55-65% in Guatemala, 84-93% in Kenya and 69-74% in Zambia. Except for Cambodia, where 40% were primiparas, most mothers included in the sample were multiparous. Fifty to sixty percent of the women in the sample had a primary school education. Seventy percent of the children were above six months of age.

Source of drinking water varied between countries ranging from piped water, ground water, well or spring wa-

Tab. II. Selected sociodemographic characteristics of study participants.

Characteristics	Cambodia N = 2,995			Guatemala N = 1,992			Kenya N = 2,581			Zambia N = 1,057		
	I N = 1,254	C N = 1,741	p-value	I N = 898	C N = 1,094	p-value	I N = 1,485	C N = 1,096	p-value	I N = 588	C N = 469	p-value
	%	%		%	%		%	%		%	%	
Male head of household	89.9	89.5	0.759	90.6	92.6	0.120	86.4	89.6	0.014	45.2	68.8	0.000
Mother age												
15-19 y	3.9	4.7	0.317	7.7	8.7	0.417	8.7	7.2	0.167	15.6	16.4	0.735
20-36 y	88.3	86.6	0.174	79.2	79.2	0.993	84.2	86.4	0.112	71.3	72.9	0.549
37-49 y	7.8	8.7	0.368	13.1	12.2	0.512	7.1	6.4	0.451	13.1	10.7	0.222
Mother marital status												
Married	98.2	98.2	0.865	65.3	54.6	0.000	83.5	92.7	0.000	68.7	74.0	0.059
Single/ divorcee/ widow	1.8	1.8	0.865	34.7	45.4	0.000	16.5	7.3	0.000	31.3	26.0	0.059
Mother parity												
1st pregnancy	40.2	38.6	0.379	-	-	-	4.8	3.7	0.191	9.4	20.5	0.000
2 or more	59.8	61.4	0.379	100.0	100.0	-	95.2	96.3	0.191	90.6	79.5	0.000
Mother education												
No education	15.5	25.2	0.000	27.9	27.9	0.990	1.2	5.1	0.000	7.9	16.4	0.000
Primary	54.3	43.8	0.000	56.5	59.0	0.259	69.2	72.0	0.142	50.2	51.7	0.661
Secondary or more	30.3	31.0	0.679	15.6	13.1	0.109	29.6	22.9	0.000	41.9	31.9	0.002
Child gender												
Male	50.3	50.9	0.734	53.0	51.4	0.480	52.3	52.9	0.781	53.1	55.3	0.468
Female	49.7	49.1	0.734	47.0	48.6	0.480	47.7	47.1	0.781	46.9	44.7	0.468
Child age												
0-6 m	26.8	25.8	0.562	22.8	19.1	0.043	22.4	18.4	0.012	27.2	28.4	0.680
7-23 m	65.3	64.7	0.719	52.0	40.6	0.000	58.8	52.4	0.001	54.4	54.8	0.903
24-59 m	7.9	9.5	0.127	25.2	40.3	0.000	18.8	29.2	0.000	18.4	16.8	0.518
Wealth quintile												
1st	21.1	47.5	0.000	31.2	22.0	0.000	17.0	29.5	0.000	26.7	17.1	0.000
2nd	10.8	10.7	0.943	20.8	25.3	0.017	24.3	33.1	0.000	16.3	22.8	0.009
3rd	16.1	17.7	0.236	13.6	12.9	0.648	12.7	4.5	0.000	17.7	19.4	0.477
4th	25.3	13.6	0.000	16.8	22.3	0.002	20.6	18.7	0.228	25.9	24.3	0.565
5th	26.8	10.5	0.000	17.6	17.5	0.937	25.3	14.2	0.000	13.4	16.4	0.179

I: Intervention; C: Comparison.

ter, water provided by tankers or vendors, surface or rain water. Reported access to piped water was 90% in the intervention sites, and 57.7% in the comparison sites for Guatemala, the highest amongst all the countries. Intervention sites in Kenya also reported significantly higher access (21%) to piped water, compared to the comparison sites (6.3%). Access to safe water, classified based on Demographic Health Survey (DHS) definitions, was relatively high in both intervention and comparison sites for Guatemala and Zambia (83-99%), and <65% for Cambodia and Kenya (Tab. III). Ninety percent of the women in Cambodia and Guatemala reported 30 minutes or less to obtain water; the proportion for Kenya (< 54%), and Zambia (< 73%) were much lower. Intervention sites reported significantly higher access to safe water in Guatemala and Kenya ($p < 0.001$), and comparison sites were significantly higher for Cambodia and Zambia. Types of toilet facilities reported were shared or private or shared flush (40-60% in Cambodia and Guatemala), and traditional pit (85-98% in Kenya and Zambia). Open defecation outside the home was relatively common in Cambodia (40-48%) and Guatemala (11-19%).

Handwashing with soap or ash before food preparation was above 75% for Cambodia and 95% for Guatemala, with much lower levels for Kenya (60%) and Zambia (45%). More than 60% of women reported handwashing after defecation in all four countries, however, handwashing after attending to a child who had defecated was much lower for Cambodia and Zambia (29-45%). In Cambodia and Guatemala, hand-washing with soap or ash was much higher before food preparation (Cambodia 78-81%, Guatemala 95-96%) than after defecation (Cambodia 59-61% Guatemala 59- 70%). The trends were reversed for Kenya and Zambia where higher proportions reported handwashing after defecation than before food preparation. The results showed highly significant differences between intervention and comparisons sites for women in Guatemala, for both handwashing after defecation and attending to a child after defecation (after defecation 69.6% vs 59.4%, $p < 0.001$, after attending to a child who had defecated 70.7% vs 42.7%).

Reported handwashing with soap or ash before feeding children was significantly higher for mothers in Cambodia, Guatemala and Kenya ($p < 0.001$) for the intervention sites. Except for Zambia, where the comparison sites performed better, for all the other countries the handwashing index was significantly higher in the intervention sites (Cambodia, 2.4 vs 2.2, $p < 0.001$, Guatemala 3.0 vs 2.5, $p < 0.001$, Kenya 2.2 vs 2.3, $p < 0.001$, Zambia 1.8 vs 2.0, $p < 0.001$). Almost 90% of the women in the intervention and comparison sites from all countries reported at least one handwashing practice.

Based on mother's recall, report of childhood diarrhea two weeks preceding the survey was 11-12% for Cambodia, 9-10% for Guatemala, 6-9% for Kenya, and 16-20% for Zambia. Children who were below six months had a lower prevalence in all four countries. Except for Kenya (Intervention 9.9%, Comparison 6.1%, $p < 0.001$), there were no significant differences in diarrhea based on mother's reports, between the intervention and comparison sites in Cambodia, Guatemala and Zambia.

In the univariate regression model, gender of household head, age of mother, parity of mother, and type of sanitation facility were not significantly associated with lower diarrhea incidence (Tab. IV). Variables that were significant were included in the multivariate regression model (Tab. V). Children whose mothers were married had a lower odds of diarrhea incidence, for Cambodia and Kenya (Cambodia OR 0.469 [0.226-0.974], $p < 0.05$, Kenya OR 0.566 [0.358-0.897, $p < 0.05$]), Mothers with higher education, secondary school or more (OR 0.361 [0.182-0.716, $p < 0.01$]) for Guatemala, those from the 4^{th} or 5^{th} wealth quintile, for Cambodia, and Zambia, 4^{th} quintile for Guatemala and those reporting less than 30 minutes to drinking water source for Guatemala, reported lower incidence of diarrhea children. One or more handwashing practices for Cambodia and Guatemala, was associated with significantly less diarrhea in children. Male children in Zambia had a higher odds of diarrhea incidence and children younger than 2 years in Cambodia and Guatemala, had a higher odds of diarrhea incidence. Safe water source and sanitation did not show any significant differences in the odds of diarrhea illness in children. Women in the intervention sites in Kenya reported significantly higher incidence in diarrhea for children (OR 1.783, [1.215-2.616], $p < 0.01$).

Investments in community-oriented healthcare include strategies to ensure safe water sources, and appropriate sanitation facilities to prevent diarrhea in young children under five years. These water and sanitation interventions typically include health promotion at the household and community level for improving handwashing practices, as it has been evidenced to prevent one fourth of diarrhea episodes [5]. The results of the study provide some evidence of the community interventions to improve handwashing practices and reducing the incidence of reported diarrhea.

Reported access to safe water in the ADP, was much higher than the recent DHS 2014 figures for all countries except Cambodia (Guatemala DHS 66.1%, Intervention, 98.8%, Comparison, 83.2%, Kenya DHS 59.1%, Intervention, 62.9%, Comparison, 45.4%, Zambia DHS 46.6%, Intervention, 88.7%, Comparison, 97.8%). In comparison to recent DHS estimates, incidence of diarrhea for children under five was lower for all countries, the lowest reported for Guatemala, which could be partly attributed to the various community level interventions including community and household health promotion efforts, installation of water pumps etc. instituted by the program in these rural areas (Cambodia DHS 12.8%, Intervention, 8.4%, Comparison, 10%, Guatemala DHS 19.2%, Intervention, 5.9%, Comparison, 4.3%, Kenya DHS 15.2%, Intervention, 8.2%, Comparison, 7.5%, Zambia DHS 16.1%, Intervention, 9.2%, Comparison, 7.7%). Despite the bias in observational studies, as study participants are aware of being watched, when field workers observed food preparers wash hands before preparing the food in Bangladesh, it showed a reduction in the incidence of diarrhea in children under five years [7].

Tab. III. Water source, sanitation, hand washing practices and diarrhea episode for children under 5 years.

Characteristics	Cambodia N = 2,995			Guatemala N = 1,992			Kenya N = 2,581			Zambia N = 1,057		
	I N = 1,254	C N = 1,741	p-value	I N = 898	C N = 1,094	p-value	I N = 1,485	C N = 1,096	p-value	I N = 588	C N = 469	p-value
	%/ mean	%/ mean		%/ mean	%/ mean		%/ mean	%/ mean		%/ mean	%/ mean	
Water source												
Piped	10.3	7.1	0.003	90.0	57.7	0.000	21.0	6.3	0.000	7.7	2.2	0.000
Ground	16.7	30.7	0.000	-	0.4	a	21.0	7.0	0.000	75.0	94.9	0.000
Well	19.3	42.2	0.000	8.7	30.6	0.000	20.7	41.1	0.000	11.8	1.6	0.000
Spring	0.3	5.0	0.000	0.8	3.0	0.000	28.2	30.8	0.154	1.1	0.9	a
Tank/tanker	6.2	0.5	0.000	0.2	5.2	0.000	0.7	0.5	a	-	-	.
Surface	34.1	10.1	0.000	0.1	0.6	a	8.0	14.2	0.000	4.4	0.4	a
Rain	4.8	0.7	0.000	0.1	0.1	a	0.1	0.2	a	-	-	.
Purified	-	-	.	0.1	0.3	a	-	-	.	-	-	.
Vendors	8.2	3.6	0.000	-	2.1	a	0.2	-	a	-	-	.
Safe source[1]	47.2	53.7	0.000	98.8	83.2	0.000	62.9	45.4	0.000	88.7	97.8	0.000
Minutes to get water[2]												
< 30 m	-	-	-	96.6	93.8	0.004	53.0	51.1	0.368	71.8	72.6	0.779
≥ 30 m	-	-	-	3.4	6.2	0.004	47.0	48.9	0.368	28.2	27.4	0.779
Toilet facility												
Private flush	48.1	37.9	0.000	41.8	31.8	0.000	1.6	0.6	a	0.5	-	a
Shared flush	11.2	13.7	0.046	4.6	8.4	0.000	2.6	1.8	0.176	-	0.6	a
Traditional pit	0.2	0.5	a	36.6	36.2	0.830	86.1	92.7	0.000	94.5	98.5	0.000
Open backed	-	0.2	a	5.5	4.4	0.279	6.5	1.1	0.000	0.5	-	a
Defecation outside home	40.5	47.7	0.000	11.4	19.2	0.000	3.2	3.8	0.411	4.4	0.9	a
Other	-	0.1	a	0.2	-	a	-	-	.	-	-	.
Improved Sanitation[3]	59.3	51.5	0.000	46.3	40.2	0.006	4.2	2.4	0.008	0.5	0.6	a
HW practice												
Before food preparation	77.9	81.4	0.020	96.0	94.9	0.235	66.1	61.4	0.015	44.0	44.8	0.813
After defecation	61.3	59.0	0.209	69.6	59.4	0.000	79.9	63.1	0.000	67.5	72.3	0.093
After attending child defecation	39.2	41.9	0.147	70.7	42.7	0.000	62.0	65.0	0.124	28.7	45.0	0.000
Before feeding children	61.0	41.0	0.000	64.5	51.0	0.000	54.8	40.3	0.000	35.0	34.3	0.811
HW index[4]	2.4	2.2	0.000	3.0	2.5	0.000	2.6	2.3	0.000	1.8	2.0	0.001
1 or more practice	96.7	96.1	0.352	99.6	98.6	0.026	97.1	93.0	0.000	87.2	97.0	0.000
Diarrhea past 2 w (0-5 y)	11.4	12.1	0.547	10.3	9.5	0.548	9.9	6.1	0.000	16.2	20.3	0.089
Children 0-6 m	26.8	25.8	0.562	22.8	19.1	0.043	22.4	18.4	0.012	27.2	28.4	0.680
Diarrhea in past 2 w (0-6 m)	8.4	10.0	0.425	5.9	4.3	a	8.2	7.5	0.772	9.2	7.7	a

I: Intervention; C: Comparison; HW: Handwashing. [1] Safe water: piped water, (public) hand pump, covered well, protected spring, rain water and purified water for Cambodia, Kenya, Guatemala, and Zambia, and water from vendors for Cambodia, Kenya, and Guatemala. Unsafe water: open well, unprotected spring, tank/tanker water and surface water for Cambodia, Zambia, Guatemala, and Kenya, and water from vendors for Zambia. [2] 0 minute if water source is within the house/plot.[3] Improved toilet facilities: private flush and shared flush. Not improved toilet facilities: traditional/modern pit, open backed and defecation in field/outside house. [4] Hand wash index (0-4): before food preparation, after defecation, after attending to a child defecation, and before feeding children. [a] p-value not computed due to low sample size.

In our study we found variations of practice based on healthcare context. For Cambodia and Guatemala, a higher proportion of women reported handwashing before food preparation than after defecation or attending to a child after defecation. However, for Kenya and Zambia the trends were reversed where a higher proportion reported handwashing after defecation. This may be due to the types of hygiene prevention interventions,

Tab. IV. Bivariate logistic model of mother's handwashing practice and reported diarrhea in children under 5 years.

Variables	Cambodia	Guatemala	Kenya	Zambia
	OR [CI]	OR [CI]	OR [CI]	OR [CI]
Gender of household head (ref. female)	1.000	1.000	1.000	1.000
Male household head	1.021 [0.983-1.060]	0.982 [0.936-1.030]	0.984 [0.952-1.016]	1.016 [0.969-1.066]
Age of mother (ref. age < 24 years old)	1.000	1.000	1.000	1.000
Age 24-49	1.000 [0.974-1.027]	0.990 [0.962-1.018]	0.981 [0.959-1.003]	1.045 [0.997-1.096]
Parity of mother (ref. multiparity)	1.000	1.000	1.000	1.000
First pregnancy	1.014 [0.991-1.039]	1.000 [1.000-1.000]	1.050 [0.996-1.107]	0.940 [0.879-1.005]
Marital status (ref. Not married)	1.000	1.000	1.000	1.000
Currently married	0.934 [0.856-1.019]	1.018 [0.991-1.045]	0.947*** [0.917-0.978]	1.046 [0.993-1.102]
Mother's education (ref. none)	1.000	1.000	1.000	1.000
Primary	1.023 [0.993-1.055]	0.972 [0.943-1.002]	1.026 [0.958-1.099]	0.921 [0.847-1.001]
Secondary and more	1.027 [0.994-1.062]	0.935** [0.896-0.976]	1.012 [0.943-1.086]	0.921 [0.845-1.004]
Wealth quintile (ref. poorest)	1.000	1.000	1.000	1.000
2nd (poor)	1.020 [0.980-1.062]	0.999 [0.962-1.037]	0.988 [0.958-1.018]	0.979 [0.911-1.053]
3rd (middle)	0.962* [0.930-,0.996]	1.011 [0.967-1.057]	0.991 [0.950-1.033]	0.930 [0.864-1.001]
4th (richer)	0.969 [0.937-1.001]	1.046* [1.006-1.088]	1.006 [0.973-1.040]	0.967 [0.903-1.035]
5th (richest)	0.976 [0.943-1.009]	0.984 [0.945-1.025]	0.986 [0.955-1.019]	0.886** [0.819-0.957]
Child age (ref. 0-23 m)	1.000	1.000	1.000	1.000
Child 24 m-59 m	0.927*** [0.888-0.968]	0.944*** [0.914-0.974]	0.997 [0.971-1.024]	0.988 [0.922-1.058]
Child gender (ref. female child)	1.000	1.000	1.000	1.000
Male child	1.020 [0.996-1.044]	1.009 [0.982-1.035]	0.986 [0.965-1.007]	1.065** [1.016-1.117]
Intervention (ref. comparison)	1.000	1.000	1.000	1.000
Intervention group	0.993 [0.970-1.016]	1.008 [0.982-1.035]	1.038*** [1.016-1.061]	0.960 [0.915-1.006]
Safety of water source (ref. unsafe water)	1.000	1.000	1.000	1.000
Safe water source	0.999 [0.976-1.023]	0.957 [0.916-1.000]	1.011 [0.989-1.033]	1.020 [0.931-1.117]
Minutes to get water (ref. < 30 m)		1.000	1.000	1.000
≥ 30 minutes		1.064 [0.999-1.134]	1.020 [0.998-1.043]	1.023 [0.967-1.083]
Toilet facility (ref. not improved)	1.000	1.000	1.000	1.000
Improved facility	0.992 [0.969-1.015]	1.019 [0.992-1.046]	0.950 [0.896-1.007]	0.986 [0.724-1.344]
Number of HW practices (Ref. none)	1.000	1.000	1.000	1.000
1	0.968 [0.906-1.033]	0.817** [0.708-0.944]	0.987 [0.933-1.044]	0.963 [0.880-1.054]
2	0.929* [0.871-0.990]	0.811** [0.702-0.938]	0.953 [0.903-1.006]	1.032 [0.943-1.129]
3	0.906** [0.849-0.966]	0.861* [0.743-0.998]	0.975 [0.921-1.031]	1.027 [0.927-1.137]
4	0.959 [0.896-1.026]	0.818** [0.709-0.944]	0.943* [0.893-0.995]	1.052 [0.943-1.174]

OR: Odds Ratio CI: 95% Confidence Interval; HW Handwashing ; * p < 0.059; ** p < 0.01; *** p < 0.001.

where handwashing stations were constructed beside the latrines to foster appropriate behaviors in some of the ADP sites.

Caretakers in intervention sites received targeted counseling from CHWs, about the importance of handwashing practices as part of the maternal newborn and child health package of interventions. Other community oriented complimentary activities were included in the intervention package with community voice and action and community councils to improve hygiene and handwashing practices. The comparison sites also received the routine CHW services and were supported through community council activities. In a majority of World Vision ADPs, handwashing promotion is integrated especially if there are focused efforts on improving the infrastructure for safe water and sanitation facilities with handwashing stations. Community and facility councils were engaged in promoting appropriate hygiene behaviors and facilitated the construction of water pumps and shared toilet facilities in some ADPs. Hence this may have resulted in improved handwashing and other behaviors in comparison sites. Controlling for confounding factors, our study did not show a difference in the odds of diarrhea between intervention and comparison sites, except for Kenya, where children in the intervention sites showed higher odds of diarrhea. A study in rural Guatemala, which deployed community health promoters to improve water treatment and hand washing found no significant differences between the intervention and comparison sites for reported hand washing practices, hygiene standards, prevalence of diarrhea and child growth [8]. Interestingly another study in Kenya, which evaluated the effect of CHW and commu-

Tab. V. Multivariate logistic model of mother's handwashing practice and reported diarrhea in children under 5 years.

	Cambodia	Guatemala	Kenya	Zambia
	OR [CI]	**OR [CI]**	**OR [CI]**	**OR [CI]**
Marital status (ref. Not married)	1.000	1.000	1.000	1.000
Currently married	0.469* [0.226-0.974]	1.116 [0.782-1.592]	0.566* [0.358-0.897]	1.590 [0.967-2.614]
Mother's education (ref.none)	1.000	1.000	1.000	1.000
Primary	1.082 [0.764-1.532]	0.690 [0.474-1.004]	0.960 [0.330-2.792]	0.759 [0.402-1.434]
Secondary and more	1.435 [0.980-2.102]	0.361** [0.182-0.716]	0.844 [0.278-2.564]	0.826 [0.410-1.664]
Wealth Quintile (ref. poorest)	1.000	1.000	1.000	1.000
2nd (poor)	1.290 [0.867-1.919]	1.129 [0.680-1.874]	0.750 [0.462-1.217]	0.653 [0.360-1.183]
3rd (middle)	0.667 [0.444-1.001]	1.221 [0.680-2.193]	0.809 [0.418-1.566]	0.304*** [0.150-0.614]
4th (richer)	0.652* [0.429-0.992]	2.051** [1.248-3.372]	1.091 [0.664-1.792]	0.470* [0.251-0.878]
5th (richest)	0.699 [0.457-1.071]	0.939 [0.507-1.740]	0.933 [0.541-1.608]	0.255** [0.106-0.618]
Child age (ref. 0-23 m)	1.000	1.000	1.000	1.000
Child 24 m-59 m	0.445** [0.263-0.753]	0.478*** [0.328-0.696]	1.012 [0.690-1.484]	0.717 [0.417-1.233]
Child gender (ref. female child)	1.000	1.000	1.000	1.000
Male child	1.126 [0.871-1.454]	1.011 [0.723-1.415]	0.931 [0.663-1.308]	1.788** [1.150-2.780]
Intervention (ref. comparison)	1.000	1.000	1.000	1.000
Intervention group	1.034 [0.781-1.368]	1.123 [0.778-1.621]	1.783** [1.215-2.616]	0.706 [0.450-1.108]
Safety of water source (ref. unsafe water)	1.000	1.000	1.000	1.000
Safe water source	0.912 [0.704-1.181]	0.780 [0.422-1.442]	1.187 [0.834-1.691]	1.122 [0.463-2.715]
Minutes to get water (ref. < 30 minutes)	-	1.000	1.000	1.000
≥ 30 minutes	-	2.009* [1.046-3.862]	1.150 [0.813-1.626]	1.204 [0.755-1.920]
Toilet facility (ref. not improved)	1.000	1.000	1.000	1.000
Improved facility	1.058 [0.798-1.404]	1.189 [0.828-1.707]	0.396 [0.094-1.674]	1.000 [1.000-1.000]
Number of HW practices (ref. none)	1.000	1.000	1.000	1.000
1	0.650 [0.352-1.203]	0.197* [0.053-0.725]	0.933 [0.408-2.133]	0.707 [0.293-1.708]
2	0.541* [0.295-0.991]	0.189* [0.050-0.711]	0.478 [0.210-1.088]	1.710 [0.732-3.991]
3	0.414** [0.222-0.771]	0.327 [0.086-1.251]	0.757 [0.327-1.753]	1.305 [0.511-3.334]
4	0.763 [0.403-1.443]	0.222* [0.061-0.807]	0.485 [0.209-1.124]	1.140 [0.397-3.275]
N	2179	1452	1739	539

OR: Odds Ratio; CI: 95% Confidence Interval; HW: Handwashing ; * p < 0.059; ** p < 0.01; *** p < 0.001.

nity unit interventions in diarrhea prevention, water and sanitation showed that there was a higher odds of diarrhea prevalence in areas where CHW performance was high [16]. Apparently CHW and community unit interventions were focused more on high diarrhea prevalence sites. As in the Kenya study, our results suggest that the CHW and community promotion strategies improved hand-washing, but did not decrease diarrhea incidence when controlled for other factors.The study from Ethiopia showed a 35% reduction in diarrheal diseases in children under five for households who received health promotion messages given continuously for six months with the distribution of soap [4].

A previous study showed decreased diarrhea incidence in these rural areas for Cambodia, Guatemala and Kenya (comparison sites only), and Zambia (intervention sites only) following community oriented interventions [17], but did not include the effect of handwashing practices, and the sample included all children, not only those with biological mothers. It must be noted that overall hygiene in the household environment is also a critical contributor to diarrhea incidence aside from mother's handwashing practices. Recommendations for handwashing at all four times, may not be feasible as reported in other studies [7], but health promotion messages could emphasize washing hands before food preparation and after defecation or attending to a child who has defecated. To enhance the effectiveness of handwashing interventions, washing hands only at the most critical times, to interrupt pathogen transmission, and promoting handwashing with water alone has been recommended [7]. Similar to another study in Bangladesh, where women were questioned about handwashing with soap or ash, in our study a higher proportion of women in Kenya and Zambia reported handwashing after defecation, than before preparing food [7]. This practice was the reverse for Cambodia and Guatemala, where a high percentage reported handwashing before food preparation than after defecation. A dose effect was observed with the number of reported handwashing practices and diarrhea incidence for Cambodia and Guatemala as in the Bangladesh study [7].

Sources of water for drinking differed between countries, and there was also a wide variation in use of safe sanita-

tion facilities. A binary variable of safe and unsafe water and sanitation source was computed in the multivariate regression model and showed no significant differences in the odds of diarrhea illness in children. Another study from Guatemala, also reported no significant findings of home water treatment and diarrhea incidence but reported a reduction in diarrhea incidence of 20-22% when household members reported consumption of bottled water for drinking [18]. Diarrhea incidence among children without access to piped water was reported at 32.2% in the same study. Our findings showed that mothers from both intervention (90%) and comparison (57.7%) sites reported very high levels of piped water in Guatemala, compared to other countries. This may have resulted in reduced diarrhea incidence as reported in other studies [19, 20]. Mothers in Guatemala who reported 30 or more minutes to fetch water, had twice the odds of diarrheal episodes, but this was not significant for other countries, though higher levels were reported. Minutes to fetch water was reported to be a key factor associated with diarrheal incidence in children in a study in Ethiopia [21].

Diarrhea incidence in households with sanitation infrastructure was reportedly lower than those with no infrastructure in the Guatemala study [18]. In this study, Private flush facilities, was reported by 42% of the mothers in the intervention and by 32% in the comparison sites, which could have also reduced the illness incidence. However, the multivariate model showed no significant differences in the odds of lower diarrhea incidence when mothers reported improved sanitation.

Relative to other studies, showing a decline in diarrheal incidence in older children [7, 16], this study showed children under 2 years had higher odds of diarrhea.

Though a significant effect was only evident for Zambia when controlled for other factors, male children had higher diarrhea incidence than female children. This observation was also reported in other studies in Guatemala, Kenya and Ethiopia, hypothesizing that male children have greater environmental exposure, than female children [16, 18, 21].

Unlike other studies reported in the literature on hand washing, we did not perform structured observations of hand washing practices [7], as biases can be introduced due to direct observations. Water storage and water treatment practices have been shown to be predictors of diarrheal illness, but these measures were not included in our study [8, 21]. The cross-sectional study design could be another potential limitation, as causal relationships cannot be determined. However, a study in Burkina Faso reported consistent handwashing practices of mothers whose children had defecated, following repeated measures on different days [22]. The data only included biological mothers and child pairs, and patterns of diarrhea incidence could have been different for children who did not have biological mothers. It must be noted that child feeding especially for children under two years is performed by other members of the family and not just the mothers. Hence these results suggest that health promotion interventions need to be directed to the broader community and other family members.

Child's diarrhea episode was based on mother's report and not defined by three or more loose or watery stools in 24 hours or blood in stools. The recall period of 2 weeks may have introduced another source of bias, unlike the study in Guatemala where the recall period was 48 hours [8]. Although not feasible, given the minor variations in protocols, a difference in difference analysis, controlling for baseline values would have provided true effects of the intervention. We did not control for malnutrition, which has been shown to exacerbate diarrhea episodes [23] nor was the analysis controlled for breastfeeding practices which has been shown to be a protective factor in some studies [24-27]. Household and personal hygiene, and water storage facilities were also not included in the assessments. We focused on handwashing with soap/ash similar to the study from Ethiopia [21]. We did not enquire about handwashing alone, without soap/ash, which may have provided additional evidence on handwashing with water, as soap is an expensive commodity for most rural communities in low and middle-income economies, though ash may be more readily available. Point-of-use water treatment has shown significant reduction in diarrhea incidence in some studies, which may be another limitation in the safe water construct used in this study [28, 29].

Conclusions

Based on the 2010 World Health Organization (WHO) and United Nations Children's Fund (UNICEF) report, it is estimated that 900 million people worldwide lack access to improved drinking water [30]. To compliment the health intervention strategies to improve service utilization for basic maternal neonatal and child health, World Vision embraces the sustainability development goals for comprehensive development and provides a platform for multidimensional development strategies, including the construction of water pumps, safe sanitation facilities, promoting hand washing and hygiene practices to enable healthy behaviors, and mitigate the burden from preventable illnesses such as diarrhea in rural communities. Community oriented strategies through CHW and social accountability mechanisms foster healthy behaviors, as the study showed significantly higher handwashing index in all intervention sites, except for Zambia.

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Conflict of interest statement

The study was conducted as a Third-Party Evaluation by Johns Hopkins University, National Institute of Nutrition, Cambodia, Institute of Economic and Social Research, Zambia, Institute of Nutrition for Central America and Panama, Guatemala, and Moi University, Kenya. Research teams from each of these institutions were involved in the study design, data acquisition, and analysis through the contract. AE wrote and made the interpretations of the findings. AE, YJ, and CC were partially funded by the grant for conducting the research. AG and JC were employed by World Vision.

Authors' contributions

AE wrote the manuscript, YJ performed the analysis of the data and prepared the tables. CC conducted the field work. AG and JC were involved in the conceptualization of the research interventions. AE and CC were engaged in the execution of the research study. AG, YJ, CC and JC reviewed and edited the final version.

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ORIGINAL ARTICLE

Detection of bacterial pathogens in the hands of rural school children across different age groups and emphasizing the importance of hand wash

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Keywords

Hand hygiene • Bacteria • Children

Summary

Introduction. *Contaminated hands remain the mainstay cause of infection in children. Infections like diarrhoea and pneumonia were found to be common among children who have limited knowledge on the importance of hand wash. The present study was aimed to assess the relationship between the bacterial load sampled from the hands of school children and their routine hand wash practice methods.*
Methods. *Samples were collected from both the hands of 200 rural school children. Bacterial colonies isolated from the swabs were identified by standard microbiological procedures. Questionnaire was provided to gather matrix of routine hand wash practice from the subjects. Proper handwashing technique was demonstrated to children.*
Results. *More than 95% of the children harbored commensal like CoNS and Aerobic spore formers. Other pathogenic bacteria isolated include Acinetobacter species (36.5%), Pseudomonas species 4% (15), Enterococcus species (2%), Klebsiella species (3.5%), Flavobacterium species (1.7%), Escherichia coli (2%), and Enterobacter species (0.75%). It was found that the male children harbored more bacteria in their hands when compared to female population. Bacterial population like Pseudomonas species, Klebsiella species and Enterococcus species were predominant in the hands of children belonging to 7-10 years of age whereas Acinetobacter species, Escherichia coli and Flavobacterium species were slightly higher among 11-15 years of age. This information corresponds to the poor hand washing practices among the children.*
Conclusions. *It can thus be concluded from our study that simple handwashing practices can efficiently reduce the transmission of pathogenic bacteria from our hands and greatly reduce the transmission of infection.*

Introduction

Contaminated hands remain as one of the main sources of potentially pathogenic microorganisms causing respiratory and gastrointestinal tract infections in children [1]. The transmission of pathogenic organisms through feco-oral route was found to be common among children who fail to wash their hands properly. Transmission of these organisms can be prevented by simple handwashing with soap and water [2, 3]. Transient flora like *Escherichia coli, Salmonella species, Shigella species, Klebsiella pneumoniae*, etc., is transmitted through faeco-oral route. Other bacteria in contaminated hands include *Staphylococcus species, Streptococcus species, Pseudomonas species and Bacteroide*s *species* which can also remain as a source of infection in children. Resident commensal flora in the hand usually does not produce any infection and are a part of the normal flora of the hand. A few of the commensal bacteria include *Coagulase Negative Staphylococcus species, Bacillus species, Micrococcus*, *Aerococcus*, *Clostridium species* and *Streptococcus species* [4].
The present study was aimed to assess the relationship between the bacterial load sampled from the hands of school children and their routine hand wash practice methods. The importance of hand wash and the different steps involved in hand wash were also emphasized among the school children.

Materials and methods

This observational study was carried out in the Department of Microbiology, Chettinad Hospital and Research Institute, Kelambakkam for a period of three months. Samples were collected from both the hands of 200 rural school children using sterile saline dipped cotton swabs. The school children were split into two different age groups belonging to 7-10 and 11-15 years of age. Inclusion criteria: Male and female school children of age 7-15. Children with open wound on their hands, nail infection and children with congenital loss of motor activity in their hands were excluded from the study.
Clearance for the study was obtained from the Institutional Human Ethics Committee (IHEC) before beginning the study. Consent was obtained from school authorities and from the parents before collecting the samples from children. Assent from the children was also obtained before collecting the sample. The samples were collected before the lunch time recess of the school.
Samples were collected from both the hands of 200 rural school children (began with the flexor aspect of the wrist which including the palms, thumbs, creases, nail beds and ending with the dorsal aspect) using sterile saline dipped cotton swabs adhering to aseptic procedures and transferred to Microbiology laboratory in Amie's transport medium [1]. Each sample was provided with proper iden-

tification number and other details like age and sex of the child were also noted.

Swabs in the Amie's transport medium transported to the Microbiology laboratory within 1-2 hours were inoculated onto MacConkey agar and blood agar using standard streak plate procedure. The plates were incubated at 37°C overnight and inspected for bacterial colonies and potential bacterial strains were identified by standard microbiological procedures.

The standard microbiological procedures carried out for the identification of these bacterial strains included Gram stain and routine biochemical tests such as catalase test, oxidase tests, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple sugar iron agar reaction, Mannitol fermentation and motility test, Urease reaction etc. [5].

Questionnaire was provided to gather matrix of routine hand wash practice from the subjects [6]. The Questionnaire was translated in the vernacular language for better understanding of the children. The questionnaire was later analysed and tabulated.

The students were given a handwashing kit which also contained a pamphlet showing the various steps in hand washing in the vernacular language. A power point presentation and a short film were shown to the students to make them understand the importance of handwash. Students were randomly selected and were asked to demonstrate the steps in hand washing as per the WHO guidelines (modified) [7]. Posters which implied the importance of hand washing and the steps in hand washing were put up in each classroom.

Statistical analysis

The results were analyzed using IBM SPSS (version 21.0) software. Mann Whitney U test was used to analyze the prevalence of organisms between gender, two age groups and right and left hands.

Results

The hands of 200 school children both right and left were sampled. These children belonged to two age groups 7 to 10 and 11 to 15 years. About 47% of the children were male and 53% of them were female (Fig. 1). A total of 58% of the children belonged to the age group of 7-10 years and 42% of them belonged to 11-15 years (Fig. 2).

Organisms isolated from both hands of all of the school children include *Aerobic spore former(ASF)* 99% (397), *Coagulase negative Staphylococcus species* (CoNS) 95.5 % (382), *Acinetobacter species* 36.5 % (146), *Pseudomonas species* 4% (15), *Enterococcus species* 2% (8), *Klebsiella species* 3.5% (14), *Flavobacterium species* 1.7% (7) and *Escherichia coli* 2% (8), *Enterobacter species* 0.75% (3) (Fig. 3).

Most of the organisms isolated from school children were equally distributed in both the hands except for *Acinetobacter species* that was found to be higher in the left hand. Other Gram negative organisms like *Pseudomonas species, Flavobacterium species, Escherichia coli* and *Klebsiella species* were found to be slightly higher in right hand (Fig. 4).

Commensal bacteria like aerobic spore former and CoNS were found to be significantly higher in the hands of female children ($p = 0.32$) whereas *Acinetobacter species*, *Pseudomonas species*, *Enterococcus species*, *Escherichia coli* and *Flavobacterium species* were found to comparatively higher in the hands of male children ($p < 0.05$) (Fig. 5).

Pseudomonas species, Klebsiella species, Enterococcus species were found be significantly higher in the hands of children belonging to 7-10 years of age ($p = 0.01$). Other organisms like *Acinetobacter species* ($p = 0.00$), *Enterobacter species* ($p = 0.039$), *Escherichia coli* ($p = 0.001$) *and Flavobacterium species* was found be predominant in the hands of children belonging to 11-15 years of age. Commensal bacteria like *CoNS* and *Aerobic spore former* were equally predominant in the hands of children belonging to both age groups (Fig. 6).

In the age group of 7-10 years, 36% of the students responded that they wash their hands 1-5 times a day and about 63% of the students responded that they wash their hands 6-10 times a day. Only one student has responded as never. About 89% of the children have given the rea-

Fig. 1. % of male and female rural school children participated in the study.

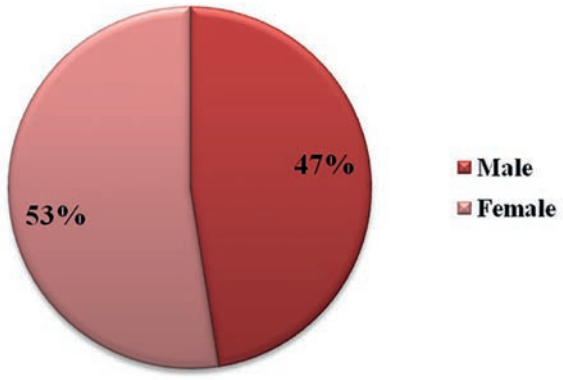


Fig. 2. % of children with two different age groups participated in the study.

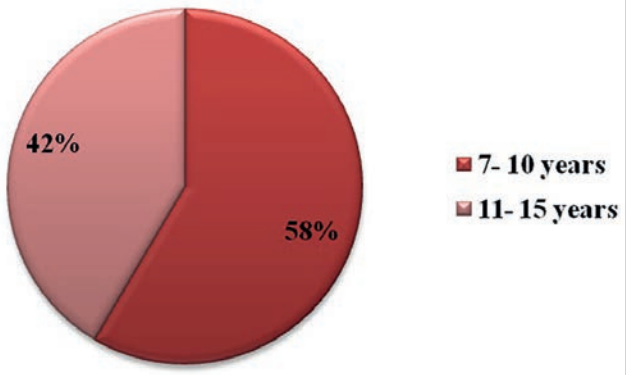


Fig. 3. Total organisms isolated from all children participated in the study.

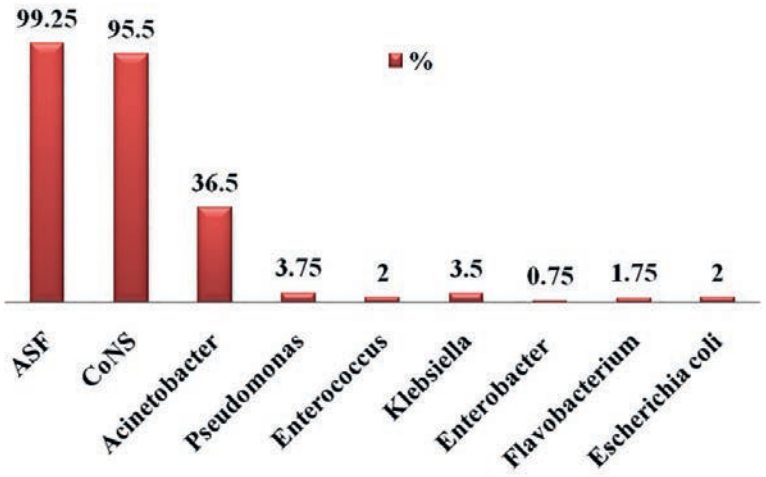
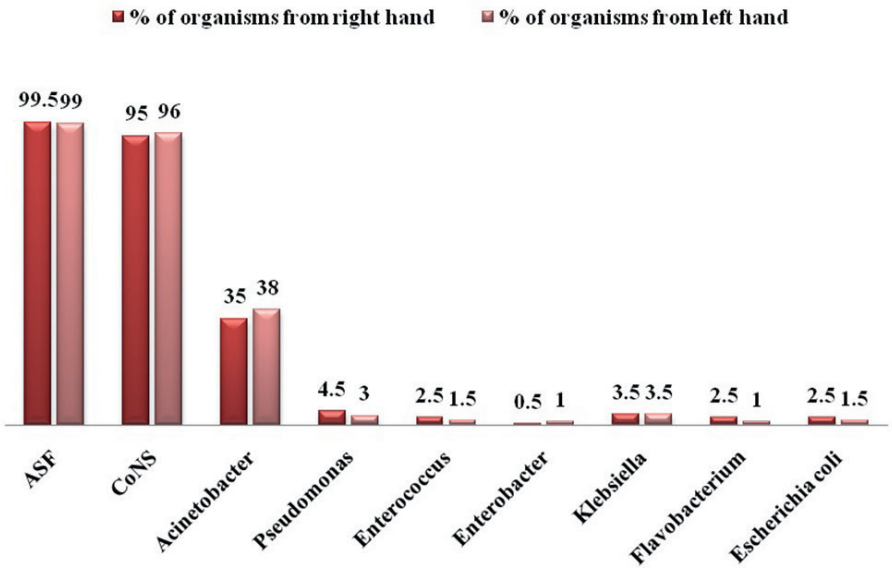


Fig. 4. Total organisms isolated from both hands.

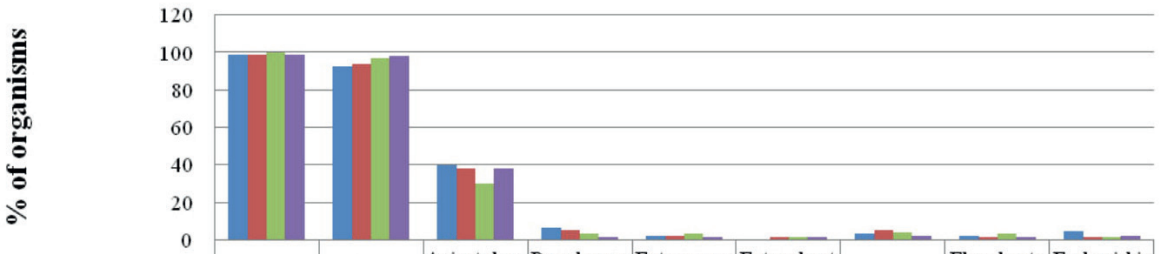


son for skipping handwashing as the wash sinks in the schools are placed in a far away location with no easy accessibility. About 72% of the children were aware that improper handwashing can result in the transmission of disease and 28% of the children were unaware of the fact that improper hand washing can cause disease (Tab. I).
In the age group of 11-15 years, 18% of the students responded that they wash their hands 1-5 times a day and about 82% of the students responded that they wash their hands 6-10 times a day. About 92% of the children have given the reason for skipping handwashing as the wash sinks in the schools are placed in a far away location with no easy accessibility. About 99% of the children were aware that improper handwashing can result in the transmission of disease and 1% of the children were unaware of the fact that improper hand washing can cause disease (Tab. II).

Discussion

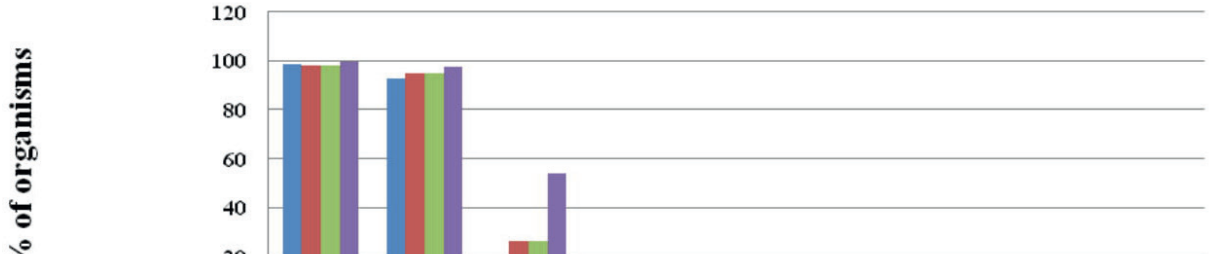
In the present study most of the rural school children harboured commensal flora like *Aerobic spore formers* and *Coagulase negative Staphylococcus species (CoNS)*. This was consistent with many studies. However, 2% to 36% of the children harboured pathogenic bacteria like *Acinetobacter species*, *Pseudomonas species*, *Klebsiella species*, *Flavobacterium species* and *Enterobacter species*. These bacteria can occur as opportunistic pathogens and can cause community acquired infections.
In this study, male children were colonized with relatively higher bacterial population when compared to female children. But the distribution of bacterial flora in right and left hand was found to be inconsistent. Organisms like *Coagulase negative Staphylococcus species (CONS)*, *Acinetobacter species* and *Enterobacter species* were found to be comparatively high in left hand.

Fig. 5. Organisms isolated from right and left hands of male and female children.



	ASF	CoNS	Acinetobacter	Pseudomonas	Enterococcus	Enterobacter	Klebsiella	Flavobacterium	Escherichia coli
Male Right hand	98.95	92.63	40	6.32	2.11	0	3.16	2.11	4.21
Male Left hand	98.95	93.68	37.89	5.26	2.11	1	5.26	1.05	1.05
Female Right hand	100	97	30	2.8	2.8	1	3.8	2.8	0.95
Female Left hand	99	98	38	0.95	0.95	1	1.9	0.95	1.9

Fig. 6. Organisms isolated from right and left hands of children belonging to two age group.



	ASF	CoNS	Acinetobacter	Pseudomonas	Enterococcus	Enterobacter	Klebsiella	Flavobacterium	Escherichia coli
7-10 years Right hand	99	93	18	7	4	0	4	0	0
7-10 years Left hand	98	94.8	26.5	5	2.5	0	5	0	0
11-15 years Right hand	98	94.8	26.5	5	2.5	1.2	5	3	0
11-15 years Left hand	100	97.6	54	0	0	2.4	1.2	1.7	3.6

Bacterial population like *Pseudomonas species, Klebsiella species and Enterococcus species* were predominant in the hands of children belonging to 7-10 years of age whereas *Acinetobacter species, Flavobacterium species* and *Escherichia coli* were predominant among 11-15 years of age. This was concordant with the study report of Ghimire et al. where maximum isolates were from children belonging to 10-12 years of age [6].

The prevalence of *Acinetobacter species* was high when compared to the study by Ghimire et al. who report *Acinetobacter species* isolation rate of only 2.5% [6]. According to the report given by Matthieu Eveillard et al., *Acinetobacter species* remains as an important cause of pneumonia in the community and also associated with wound infections and cause life-threatening infections in immunocompromised individuals as emergence of multidrug resistance is very common among these organisms [8].

Other Gram negative bacilli isolated in our study were *Pseudomonas species* 4% (15) *Klebsiella species* 3.5% (14), *Flavobacterium species* 1.7% (7) and *Escherichia coli* 2% (8) and *Enterobacter species* 0.75% (3). The colonization rate of these organisms was found to be comparatively low when compared to the study reports of Ghimire and Ray et al. [1, 6].

In this study about 1.7% of the children harboured *Flavobacterium* in their hands. This bacterium is ubiquitous and is commonly present in moist environments and tap water which can remain as a common source of contamination [9]. In our study *Escherichia coli* was present in 2% of the children. *Enterotoxigenic E. coli* remains as a major cause of diarrhea in young

Tab. I. Response to questionnaire by children belonging to 7-10 years of age.

S. No	Questions	Always (%)	Sometimes (%)	Never (%)
1	I wash my hands before meals	84	8	8
2	I wash my hands after meals	85	7	8
3	I wash my hands after using the restroom	85	8	7
4	I wash my hands before going to bed	26	54	20
5	I wash my hands after using public transportation	30	60	10
6	I wash my hands after waking up in the morning	75	10	15
7	I wash my hands after touching animals	76	11	14
8	I wash my hands only if they are soiled	78	11	11
9	I wash my hands after blowing my nose/sneezing	75	9	16
10	I wash my hands after touching the garbage	79	9	12
11	I wash my hands before touching sick people	70	13	17
12	I wash my hands after touching sick people	78	8	14
13	Do you use any soaps/ disinfection for hand washing?	71	11	18
14	Is there any wiping material available in restrooms?	72	6	22
15	Is there always soap and water available to wash your hands?	79	9	12

Tab. II. Response to Questionnaire by children belonging to 11-15 years of age.

S. No	Questions	Always (%)	Sometimes (%)	Never (%)
1	I wash my hands before meals	87	10	3
2	I wash my hands after meals	95	4	1
3	I wash my hands after using the restroom	93	6	1
4	I wash my hands before going to bed	35	60	5
5	I wash my hands after using public transportation	37	60	3
6	I wash my hands after waking up in the morning	85	10	5
7	I wash my hands after touching animals	90	6	4
8	I wash my hands only if they are soiled	91	7	2
9	I wash my hands after blowing my nose/sneezing	85	9	6
10	I wash my hands after touching the garbage	92	4	4
11	I wash my hands before touching sick people	85	7	8
12	I wash my hands after touching sick people	90	8	2
13	Do you use any soaps/ disinfection for hand washing?	91	8	1
14	Is there any wiping material available in restrooms?	80	12	8
15	Is there always soap and water available to wash your hands?	82	16	2

children. About 4% and 3.5% of the isolates of the children harbored *Pseudomonas species* and *Klebsiella species* which could serve as a source of lower respiratory infections like pneumonia [10]. Thus contaminated hands of children can be a major source of infection for individuals harbouring some underlying medical ailments.

Children belonging to 11-15 years of age had more awareness about handwashing when compared to children belonging to 7-10 years of age. Majority of the children from both the age groups skipped handwashing as the wash sinks were placed in a far away location.

In our study, we gathered a matrix to assess the handwashing practice among children and of the school children have better awareness about the importance of handwashing. But a study by Garg et al., promoted the importance of handwashing among children and concluded with pre and post intervention tests to assess the knowledge of the students about hand washing [11].

Conclusions

The present study shows that the hands of school children harbored a variety of pathogenic organisms which can cause serious diseases like respiratory tract infections and gastro intestinal infections which are possibly fatal if not attended to. Lack of a proper hand washing model, hand washing material like clean water, soap, wiping material and the presence of hand wash sinks at inaccessible locations were a few of the reasons for failure of handwashing practice in schools. Provision of these materials can bridge the gap between proper hand washing techniques and the prevention of spread of infection. It was thus very clear from our study that proper hand washing practices can prevent the spread of diseases. It is encouraged to educate the children on the importance of hand wash, as simple handwash procedure can help in controlling a variety of diseases and they should see them as an important action in their daily life rather than viewing it as a normal activity. Educating the students should not only stop with the schools. We

must also make sure that the information taught to the students is not only followed in schools but is also followed at home and thus can ultimately reach the society to understand the true benefits of hand washing.

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Conflict of interest statement

None declared.

Authors' contributions

RV, APS and PS designed the study. RV and APS collected and processed the samples and analyzed the results. RV, APS and PS participated in article revision. All the authors of this manuscript gave approval.

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ORIGINAL ARTICLE

Assessment of oral hygiene trends among dental patients in relation to chronic medical conditions by dental students. A cross-sectional study

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Keywords

Oral health awareness • General health • Dental education

Summary

Introduction. *There is a lack of information about knowledge, attitude and behavior regarding oral hygiene and dietary habits of adult patients attending dental clinical settings. Hypertension, diabetes, obesity and caries are increasing among different populations, resulting in the deterioration of the quality of life related to oral and general health.*
Objectives. *To involve second year dental students in assessing oral health knowledge, attitude, behavior, dietary habits and general health of dental patients, and screening for blood pressure, blood glucose level and obesity.*
Participants and methods. *A convenient sample of 652 adult subjects participated in the study. They were screened, and involved in an interviewer - led questionnaire, led by the students over the period of two successive academic years (2013-2014 and 2014-2015).*
Results. *Subjects with high blood pressure and /or blood glucose levels were more likely to disagree regarding the presence of a relation between general health and dental health [(correlation coefficient (r) = – 0.159, probability value P < 0.001) (correlation coefficient (r) = – 0.257, probability value P < 0.001) respectively]. Subjects with high BMI were less aware of the importance of using the tooth brush in prevention of gingival inflammation. The overall students' satisfaction score was 71.5%. The practical part of the research had the highest satisfaction score (83.7%).*
Conclusions. *There is scarcity of data regarding dental healthcare knowledge and attitudes in dental clinical settings. Additional clinical training for dental students would increase their willingness to play a preventive and educational role in the oral care of patients.*

Introduction

Health is a valuable asset for individuals and nations. Oral and dental health is now recognized as equally important as general health [1]. However, there is a lack of information about knowledge, attitude and behavior regarding oral hygiene and dietary habits in relation to general health among patients attending dental clinical settings. Knowledge, attitude and behavior of individuals are used to evaluate and indicate their understanding [2], reflect their own experiences, cultural perceptions, familial beliefs and they strongly influence the oral health behavior [3, 4]. The precise nature of the relationship between health-related attitudes, beliefs and behaviors is complex [1]. Insufficient oral health knowledge contributes to patients' undergoing low levels of oral self-care practices which increase the risks of oral and dental complications [5]. In fact, misconceptions about oral hygiene may lead to harmful behaviors especially in dental patients suffering from systemic chronic conditions [6].
Chronic diseases such as hypertension, diabetes, obesity and caries are increasing in developing countries like Egypt, resulting in the deterioration of the quality of life related to oral, as well as general health [7]. A strong relationship has been suggested to exist between hypertension, diabetes, obesity and oral health. However, most patients are still unaware that oral health influence general health [8]. The high prevalence of oral diseases reported amongst people with special health needs highlights the need for an increased intervention in providing preventative, emergency, definitive and long-term oral health care. Therefore, it is imperative for dental students and dental professionals to be adequately trained and acquire a strong basis in delivery of care to those patients to alleviate the increased oral disease burden and unmet treatment needs for such patients [9].
Hypertension is a known risk factor for atherosclerotic vascular diseases [10]. A possible pathogenetic mechanism connecting periodontitis with elevated blood pressure has been suggested by several authors [11, 12]. Hence, medical examination is required to prevent complications during dental treatment [13]. The dentist plays a cardinal role in diagnosis of hypertensive patients, and their referral to medical practitioners for further diagnosis and treatment [14, 15].
Diabetes mellitus is a chronic metabolic disease with serious oral health implications [16]. Globally, diabetes is expected to be the 7th leading cause of death in 2030 [17-19]. Oral complications and manifestations are in the form of gingivitis, periodontitis, xerostomia, opportunistic infections, greater accumulation of plaque, oral paresthesia and altered taste. Hence, both patients and dentists should be educated or made aware of the dental consequences of such disease [20-25].

Literature shows that most diabetic patients knew about general health complications of diabetes, but not oral health complications [16, 26]. On the other hand, patients felt they would be more careful about oral hygiene if they were informed [27, 28].

Obesity is an inflammatory condition associated with an increased presence of adipose tissue which is a source of inflammatory mediators [23]. Obesity and in particular abdominal obesity is commonly accompanied by elements of the metabolic syndrome, including insulin resistance, hypertension, and dyslipidemia [29]. In addition, several studies have demonstrated that obesity is associated with oral and dental problems such as chronic periodontitis [30, 31]. It was reported that both dental caries and obesity are diseases with multifactor etiology related to dietary habits that are closely correlated with sociodemographic background of the individuals [29, 32].

Similarly, diet has a direct, local effect on oral health, on the integrity of the teeth, and on the pH and composition of saliva and plaque [33]. Several studies have concluded that particular diets, those highly containing saturated fatty acids, non-milk extrinsic sugars and low in poly saturates, fibers and vitamins represent common risk factors for diseases such as diabetes, coronary heart diseases, obesity, and dental caries [34-36].

It is quite clear that oral health and general health are highly related and that patients may not be aware that they have medical problems that might be reflected on and/or affected by their oral health. Lack of training and experience of dental professionals as early as the undergraduate level are reported barriers to the provision of care for these groups of patients [37, 38]. Accordingly the present study aimed to involve second year dental students in assessing oral health knowledge, attitude, behavior, dietary habits and general health of dental patients, and screening for blood pressure, blood glucose level and obesity. It also aimed to assess the students' perception and satisfaction with education in this field.

Pilot study: three public health professors conducted content validity of the questionnaire before conducting the pilot study. The pilot study was conducted on 163 random attendees of the Misr International University (MIU) in Egypt dental clinics. Face validity was assessed during the pilot study to assess understanding and completion time of the questionnaire. Based upon the results of the pilot study, amendments were done to the questionnaire where some questions were modified. Results of the pilot study were not included in the final results.

Sample size: based upon the pilot study results, the predicted minimum sample size (n) was 450 subjects. The sample size was increased to 652 subjects to ensure participation of every second year dental student in the study. Sample size calculation was performed using IBM® SPSS® Sample Power® Release 3.0.1

Methods

The present non-randomized cross sectional study was performed by second year dental students and targeted a convenient sample of 652 adult subjects; 399 females and 253 males, attending the (MIU) dental clinics for dental examination and diagnosis over the period of two successive academic years (2013-2014 and 2014-2015). The participants participated in a detailed interviewer - led questionnaire and were screened for blood pressure, blood glucose level and obesity.

Inclusion criteria: all patients aged eighteen and above were included in the study.

Exclusion criteria: pregnant females were excluded as they might have increased blood glucose level, blood pressure and body mass index that are related to pregnancy.

Ethical approval

The present study was approved by the Institutional Review Board. (IRB number: 1617-032).

Before leading the questionnaire, the students received a training session to ensure the standardization of the questionnaire interviews. Each student provided each participant with a detailed verbal explanation of the different parts of the study, explaining the aim of performing the study, as well as informing the participants that they will be screened for blood pressure, blood glucose level and obesity; verbal approval, followed by signing a written approval for participation, was taken before proceeding with the study. Subjects who refused to participate in the study, or those who desired to quit at any point of the study were excluded, and were referred to the University clinics to receive their regular treatment.

An interviewer-led, structured questionnaire was prepared by the main investigators. The questionnaire included six sections each of them was concerned with one of the main items of the study.

Demographic data

This part included data about the participants' age, gender, education, residence, occupation and social conditions.

General health

This part included eight questions scored on a nominal scale about health conditions including hypertension and/or diabetes, intake of medications, previous history of surgical operations, as well as attitudes towards regular measurement of blood pressure and blood glucose level.

Oral health knowledge and attitude

This part included eleven questions scored on a 5-Point Likert scale about the importance of tooth brushing to gingival health, effect of fluorides in tooth pastes on teeth, knowledge about the effect of diet on general health, whether or not patients believed that teeth loss is a normal part of growing old, the importance of dental treatment, and the impact of oral health on general health.

Dietary habits

This part included two questions scored on a nominal scale about the number of daily consumption of sugary food, type of daily consumed food in different meals.

Oral health behavior

This part included seven questions scored on a nominal scale on the frequency and time of tooth brushing, brushing method, and the use of other oral hygiene aids, as well as reasons for visiting a dentist, and frequency of dental visits.

For screening and measurements

Training of the students was done by six resident physicians who were calibrated, in orientation sessions before beginning the research. Examiners' reliability was statistically assessed using Kappa scores that were (Cronbach's alpha = 0.746, ICC = 0.595) for systolic blood pressure, and (Cronbach's alpha = 0.692, ICC = 0.529) for diastolic blood pressure.

Recorded measurements

All measurements were done by the students, under complete supervision of the main investigators, and in the presence of the resident physicians who were available for any emergencies and/ or complications.

Blood pressure

Blood pressure was measured from the right upper arm using a blood pressure monitor with mercurial sphygmomanometers and stethoscope set (Shanghai Medical Instruments impt.& expt. Co. Limited). The subject was asked to sit down for at least five minutes before blood pressure was measured. A person was screened as a possible suspect for hypertension if blood pressure level is ≥ 140/90 mmHg. The measurement was repeated after two minutes by the resident physicians to confirm the previous recorded reading [10].

Blood glucose screening

The finger-tip blood sample Accu-Chek Performa Blood Glucose meter (Roche Diabetes Care, Inc. Switzerland) was used for assessing blood glucose levels. The reference range was a random plasma glucose ≥ 200 mg/dl (at any time of the day regardless of last meal time). Nurses were assigned to do the needle prick using disposable needles, while students were assigned to record the reading [23].

Body Mass Index

The height and weight of the participants were measured by the students. The subjects were weighed to the nearest 0.1 kg and height was measured in centimeters using digital weight and height floor type balance (MC, China). Body Mass Index (BMI) was calculated by dividing the body weight (in kilograms) by the square of the height (in meters). The BMI was divided into 4 groups according to the BMI ranges for adults related to standard weight status categories [31].

Waist circumference

Waist circumference was measured in cm with plastic measuring tapes, above the level of the iliac crest while the subject was at minimal respiration. According to the American Heart Association, women with more than 88 cm or men with more than 102 cm waist measurement are considered to have abdominal obesity [29].

Dealing with critical blood pressure and blood glucose levels

Dental treatment was postponed for patients with suspected problems in their blood pressure level, and /or uncontrolled blood glucose level. The patients were informed about the condition, and referred to the resident physicians who then referred them to receive the required medical care. Those patients were asked to bring a written medical report before proceeding with their dental treatment.

Students' satisfaction

A specially designed self- administered 5-Point Likert Scale questionnaire was prepared by the main investigators. The questionnaire aimed at assessing the degree of students' satisfaction with the usefulness of the practical part, and the effect of participation in such a study on motivating them to participate in upcoming researches. Finally, they were asked about their suggestions to improve future dental students' research projects.

Statistical analysis

Numerical data were presented as mean, median, standard deviation (SD), minimum, maximum and 95% Confidence Interval (95% CI) for the mean values. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Multiple Linear Regression analysis was performed to study the association between oral hygiene knowledge score, blood pressure, blood glucose level, BMI and waist circumference as well as to determine the possible confounding effect of socio-demographic variables on the relationship of obesity, hypertension and diabetes with dietary and oral hygiene habits. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics Version 20 for Windows.

Results

Demographics

The present study was conducted on 652 subjects; 399 females (61.2%) and 253 males (38.8%). The mean ± standard deviation values for age were 42.4 ± 11.9 years with a minimum of 18 and a maximum of 76 years old-with a 95% CI (41.5-43.3) years old. Education and occupation distribution is presented in Figure 1.

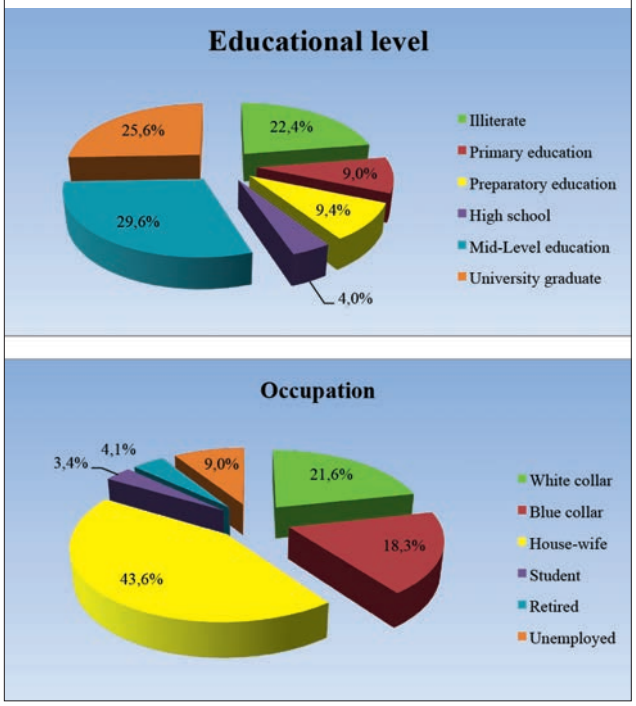
Measurements

Measurements are presented in Table I.

Reliability of measurements

There was good agreement between blood pressure measurements done by dental students and the medi-

Fig. 1. Pie charts representing distribution of educational levels and occupations.



cal seniors (Cronbach's alpha = 0.746, ICC = 0.595 for systolic blood pressure) and (Cronbach's alpha = 0.692, ICC = 0.529 for diastolic blood pressure).

General health

Responses to questions regarding general health are presented in Table II. The results showed that 85.4% and 77.8% of the participants reported they don't suffer from diabetes and blood pressure problems, respectively. Only 31.4% of the participants assessed their blood glucose level and/or blood pressure regularly. However, it was found that 3.7% of those who reported being non-diabetics had elevated blood glucose levels [Mean (SD) = 201.5 (75.2) g/dL] and 11.7% of those who reported not having blood pressure problems have elevated blood pressure measurements [Mean (SD) = 163.2/94.2 (10.6/12.8) mmHg].

Oral health knowledge, attitudes and behaviors

Responses to questions regarding oral health knowledge, attitudes and behaviors are presented in Table III. The results showed that 31.4% of the participants strongly disagreed that general health is correlated to oral health. Only 6.9% of the subjects strongly agreed that fluoridated toothpaste prevents tooth decay, 20.1% strongly agreed that eating sweets frequently lead to tooth decay, 44.3% of participants strongly disagreed that dental treatment is equally important to other body parts. As regards tooth brushing, 79.1% of the subjects mentioned that they use tooth brush to clean their teeth, and 76.9% reported that they use fluoridated tooth paste besides the tooth brush to clean their teeth. However, 77.8% of the subjects go to the dentist only when they feel pain.

General health knowledge and dietary habits

Responses to questions regarding general health knowledge and dietary habits are presented in Table IV. The results showed that 70.9% of participants reported that the patient is responsible for preventing and/or caring with diabetes, 87.9% of participants don't eat sweets daily. The most prevalent nature of daily food was home food (84.7%) followed by fruits and vegetables (39.3%) while only 14.9% of the participants reported fast food as their daily food nature and finally 43.4% of the participants don't care about their daily food nature.

Association between oral health knowledge, attitude and behavior of the participants and the recorded measurements

Multiple Linear Regression analysis models were constructed to find out the relation between blood pressure, blood glucose level and BMI, and oral health knowledge, attitude and behavior. The models also assessed the effect of socio-demographic variables as confounders. Education was found to be a confounder in the relation between oral health knowledge, blood pressure, blood glucose and BMI. The adjusted results revealed that subjects with recorded high blood pressure and/or blood glucose levels were more likely to disagree with or have a neutral opinion regarding the presence of a relation between general health and dental health

Tab. I. Descriptive statistics for the different measurements done by second year dental students for a group of patients attending MIU dental clinics.

Measurement	Mean	SD	Median	Range	95% CI	
					Lower bound	Upper bound
Height (cm)	166.2	9.9	166	143-195	165.4	167
Weight (Kg)	82.7	15.5	81.9	42.3-144	81.4	83.9
BMI (Kg/m^2)	30.1	5.8	29.6	14.3-51.7	29.6	30.5
Waist circumference (cm)	105	13.6	105	73-138	103.9	106.1
Blood pressure (mmHg)	123.4/81	13.5/10.9	120/80	90/40-182/120	122.3/80.2	124.5/81.9
Blood glucose level (g/dL)	112.6	43.9	101	45-406	109.2	116

Tab. II. Frequencies (n) and percentages (%) of responses to general health questions.

Question	n	%
Are you diabetic?		
• Yes	78/652	12
• No	557/652	85.4
• Don't know	17/652	2.6
If yes; what is the type of medication you use?		
• Tablets	20/78	25.6
• Insulin injection	11/78	14.1
• Others	33/78	42.3
• Don't know	14/78	17.9
If yes; do you use your medication regularly?	41/78	52.6
Do you suffer from any blood pressure problems?		
• Hypertension	124/652	19
• Hypotension	3/652	0.5
• No	507/652	77.8
• Don't know	18/652	2.8
If yes; do you use your medication regularly?	31/127	24.4
Do you assess your blood pressure and/or blood glucose level regularly?		
• Yes	205/652	31.4
• No	447/652	68.6
Do you use any regular medication?		
• Yes	42/652	6.4
• No	610/652	93.6
Do you suffer any kidney or liver problems?		
• Yes	57/652	8.7
• No	558/652	85.7
• Don't know	37/652	5.6
Have you undergone any surgery before?		
• Yes	347/652	53.2
• No	305/652	46.8

[($r = – 0.159$, P-value < 0.001) and ($r = – 0.257$, P-value < 0.001) for blood pressure and blood glucose level, respectively]. They also strongly agreed that losing teeth is a natural aging process [($r = 0.123$, P-value = 0.005) and ($r = 0.165$, P-value < 0.001) for blood pressure and blood glucose level, respectively]. Those participants visit the dentist more frequently when feeling pain than others with normal blood pressure levels [($r = 0.215$, P-value < 0.001) and ($r = 0.224$, P-value < 0.001) for blood pressure and blood glucose level, respectively].

Subjects with high BMI were less aware of the importance of using the tooth brush in prevention of gingival inflammation ($r = – 0.198$, P-value < 0.001). Subjects with small waist circumference were more aware about the relation between eating sweets and its relation to being diabetic ($r = – 0.318$, P-value < 0.001). They also showed a higher prevalence of using tooth brush regularly than subjects with larger waist circumference records ($r = – 0.177$, P-value < 0.001).

Subjects who believe that eating sweets lead to caries and that food type might prevent diabetes, hypertension and obesity showed lower frequency of eating sweets [($r = – 0.182$, P-value < 0.001) and ($r = – 0.210$, P-value < 0.001) for eating sweets question and food type question, respectively]. They were more likely to eat homemade food than those who believed that the type of food is not related to general and oral health ($r = 0.125$, P-value = 0.005).

Students' satisfaction with the research

Results are presented in Figure 2. The practical part of the research had the highest satisfaction (83.7%). The overall satisfaction score was 71.5%.

Discussion

There is scarcity of data regarding oral hygiene trends in relation to chronic medical conditions among patients in clinical dental settings. To develop a sound strategy for improving dental and oral health of any population, more representative data base should be made available. The current study sought to involve dental students in their early academic years in data collection and screening of patients as a main part of their under graduate curriculum in order to not only help in their clinical training and valuable educational experiences, but also to help in developing such missing data base which can consequently contribute to fulfill the basic dental care needs of the patients.

Regarding the responses to the general health questions, although the majority of the subjects reported that they were not suffering from blood pressure problems or elevated blood glucose level, respectively. However, the findings of the present study showed that 11.7%, and 3.7% of these subjects had blood pressure problems and high blood glucose level, respectively. Of all the exam-

ined subjects, only 31.4% assessed their blood pressure and/ or blood glucose level regularly. The previous findings confirm that several dental patients are unaware of their general health condition, and that the dentist might be the one who discovers that a patient is diabetic or suspected hypertensive patient. Several authors reported similar results and correlated patients' general condition to their oral health [13-17]. Accordingly, the authors of the present study recommend that it should be a routine in governmental and private dental practice to screen dental patients for blood pressure and blood glucose level.

Interesting and surprising results were reported in responses to questions regarding oral health knowledge, attitude and behavior. The highest percentage of subjects 33.1% strongly disagreed that tooth brushing helps prevent gingivitis. Similarly, the majority of the subjects 31.4% strongly disagreed that general health is correlated to oral health. The majority of respondents were not aware of the benefits of fluorides for prevention of

Tab. III. Frequencies (n) and percentages (%) of responses to oral health knowledge, attitude and behavior questions.

Knowledge and attitude questions	Strongly agree		Agree		Neutral		Disagree		Strongly disagree		No answer	
	n	%	n	%	n	%	n	%	n	%	n	%
Tooth brushing helps preventing gingival inflammation	110/652	16.9	103/652	15.8	59/652	9	149/652	22.9	216/652	33.1	15/652	2.3
General health is correlated with oral health	121/652	18.6	110/652	16.9	66/652	10.1	134/652	20.6	205/652	31.4	16/652	2.5
Fluoridated toothpaste prevents tooth decay	45/652	6.9	86/652	13.2	256/652	39.3	137/652	21	109/652	16.7	19/652	2.9
Eating sweets is related with diabetes	107/652	16.4	107/652	16.4	72/652	11	145/652	22.2	193/652	29.6	28/652	4.3
Eating sweets lead to tooth decay	131/652	20.1	81/652	12.4	20/652	3.1	105/652	16.1	299/652	45.9	16/652	2.5
Loss of teeth is a normal aging process	92/652	14.1	174/652	26.7	45/652	6.9	184/652	28.2	141/652	21.6	16/652	2.5
Natural teeth problems are more prevalent than artificial teeth problems	91/652	14	123/652	18.9	169/652	25.9	142/652	21.8	100/652	15.3	27/652	4.1
There are people with good health regardless of what they do and people with bad health regardless of what they do	95/652	14.6	143/652	21.9	87/652	13.3	159/652	24.4	139/652	21.3	29/652	4.4
Do you believe that dental treatment is equally important to other body parts?	136/652	20.9	91/652	14	16/652	2.5	101/652	15.5	289/652	44.3	19/652	2.9
Do you believe that reducing salt intake prevents hypertension?	88/652	13.5	115/652	17.6	98/652	15	126/652	19.3	210/652	32.2	15/652	2.3
Taking care of food type prevents against diabetes, hypertension or obesity	117/652	17.9	86/652	13.2	42/652	6.4	150/652	242/652	37.1	15/652	2.3	

continues

Tab. III. *follows.*

Behavior questions	n	%
How do you clean your teeth?		
• Tooth brush	516/652	79.1
• Dental floss or tooth picks	63/652	9.7
• Miswak	80/652	12.3
• Mouth wash	114/652	17.5
• I don't brush my teeth	64/652	9.8
What is the type of your tooth brush?		
• Hard	69/516	13.4
• Medium	217/516	42.1
• Soft	70/516	13.6
• I don't know	160/516	31
How often do you brush your teeth?		
• Less than one time/month	9/588	1.5
• One-two times/month	27/588	4.6
• One-two times/week	98/588	16.7
• Once daily	214/588	36.4
• Two or more times/day	200/588	34
• Never use the brush	40/588	6.8
How do you clean your partial or complete denture?		
• I don't have a denture	457/652	70.1
• Water	41/652	6.3
• Cleansing tablets	1/652	0.2
• Water and salt	21/652	3.2
• Using tooth brush	61/652	9.4
• I don't clean it	30/652	4.6
• I don't know it needs cleaning	41/652	6.3
What do you use to clean your teeth besides the tooth brush?		
• Fluoridated tooth paste	452/588	76.9
• Cleansing powder	25/588	4.2
• Sodium Bicarbonate	17/588	2.9
• I don't use any cleanser	94/588	16
When do you visit the dentist?		
• Once/Year	24/652	3.7
• Twice/Year	33/652	5.1
• When I feel pain	507/652	77.8
• I don't visit the dentist	88/652	13.5
What is the cause of your current dental visit?		
• Pain	243/652	37.3
• Continuing treatment	228/652	35
• Regular check-up	9/652	1.4
• Advice from physician	0/652	0
• Diagnosis	49/652	7.5
• Other	123/652	18.9

dental caries, only 6.9% of the subjects strongly agreed that fluoridated toothpaste prevents tooth decay, and only 20.1% strongly agreed that eating sweets frequently leads to tooth decay. Unfortunately, 44.3% of the subjects strongly disagreed that dental treatment is equally important to other body parts.

Yet positive attitudes towards the importance of tooth brushing were widespread, as 79.1% of the subjects mentioned that they use toothbrush to clean their teeth, and 76.9% reported that they use fluoridated tooth paste besides the toothbrush to clean their teeth. However, 77.8% of the subjects did not visit the dentist except when they felt pain.

Highly positive trends were recorded in the responses to the dietary habits questions, where 87.9% of the subjects revealed that they do not eat sweets daily, and 84.7% reported that their daily food nature is homemade food.

The reported agreement level between measurements recorded by the students and that recorded by the resident physicians was considered of a good level because Cronbach's alpha values greater than 0.6 are indicative for good agreement [39]. Additionally, this agreement helped in the evaluation of the students and gave an indication about the efficient training the students had before proceeding with the practical part of the study.

Out of all the measured socio-demographic variables, only educational levels of the participants were found to affect the relation between general and oral health, where participants of higher educational levels showed higher levels of general and oral health knowledge which goes in agreement with several previous studies that recorded similar findings [7].

Surprisingly, subjects with recorded high blood pressure and /or blood glucose levels were more likely to disagree

Tab. IV. Frequencies (n) and percentages (%) of responses to general health knowledge and dietary habits Questions.

General health knowledge and dietary habits Questions	n	%
Who is responsible for preventing diabetes and/or caring with diabetic patient?		
• The patient	462/652	70.9
• The physician	112/652	17.2
• Family members	89/652	13.7
• I don't know	59/652	9
Factors associated with blood pressure are:		
• Heredity	176/652	27
• Nervous tension	500/652	76.7
• Obesity	205/652	31.4
• Smoking	154/652	23.6
• Food type	143/652	21.9
• Aging	100/652	15.3
• I don't know	36/652	5.5
How many times do you eat sweets per day?		
• Once/daily	35/652	5.4
• Twice/daily	16/652	2.5
• More than two times/day	28/652	4.3
• I don't eat sweets daily	573/652	87.9
What is your daily food nature?		
• Home food	552/652	84.7
• Fruits and/or vegetables	256/652	39.3
• Fast food	97/652	14.9
• I don't care	283/652	43.4

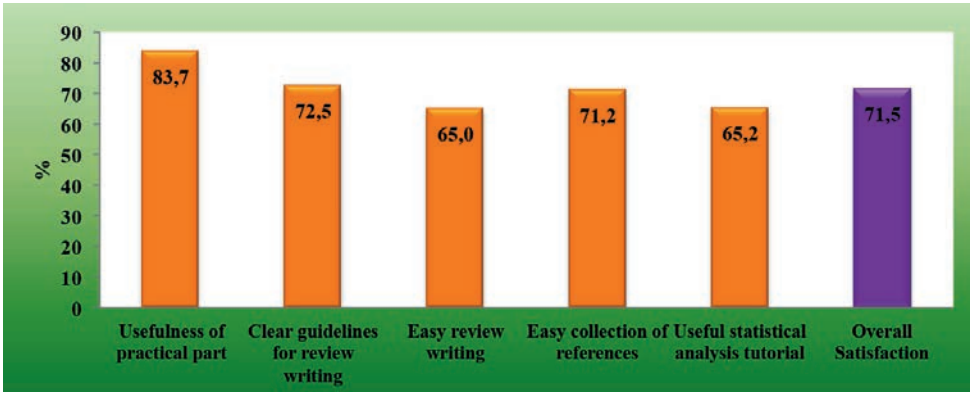
with or have a neutral opinion regarding the presence of a relation between general health and dental health. They also strongly agreed that losing teeth is a natural aging process. These results confirm the extreme lack of health literacy among such group of patients and emphasize the importance of raising the awareness and health education level of such groups of people. Similar low levels of dental and general health literacy were reported by other authors who suggested that the link between general health and oral health is not well known to patients, and that the financial obstacles to care are higher because of lower income and because dental care is given a low priority in expenditures [16, 17, 20].

On the other hand, our results showed that subjects with diagnosed high blood pressure levels visit the dentist more frequently when feeling pain than others with normal blood pressure levels.

As for BMI and its relation to knowledge, attitude and behavior, it was found that subjects with high BMI are less aware of the importance of using the toothbrush in prevention of gingival inflammation. Conversely, subjects with low waist circumference records were more aware about the relation between eating sweets and being diabetic. They also showed a higher prevalence of using toothbrush regularly than subjects with higher waist circumference records.

Regarding dietary habits, beliefs and their impact on type and amount of food intake, the current study reported that subjects with the belief that eating sweets leads to caries and that food type might prevent being diabetic, hypertensive and obese, showed lower frequency of eating sweets, and were more likely to eat homemade food than those who believed that the type of food has nothing to do with general and oral health.

Fig. 2. Bar chart representing students' satisfaction scores with different parts of the research.



One of the most important findings of the present study was the 71.5% students' satisfaction score, and the students' suggestions to have more opportunities to make contact and receive training to deal with patients in their early academic years. This conveys the benefits for the students to make contact with real-life patients, and get exposed to a greater variety of conditions and procedures. Many students valued the opportunity of accurately measuring blood pressure and glucose level. Usefulness of the practical part of the research was found satisfactory by 83.7% of the students. This goes in agreement with several previous studies [9, 37, 38] that reported the importance of undergraduate students training and the high level of satisfaction the students report when they receive such educational experiences during their early academic years. Furthermore, early contact with the patients might aid dental students developing their practical and team working skills.

Study limitations

The study is a cross-sectional study which has the limitation of not providing causal relationship. Convenient sampling method was chosen for easier enrollment of participants; however, it doesn't reflect the entire population. Accordingly, generalization of results is limited. It is also worth noting that as the results of the present study related to oral health knowledge, attitude and behavior rely on self-reported data, the rates of oral health behavior may be biased through over- and under-reporting due to social desirability.

Conclusions

Within the limitations of the present study it could be concluded that there is an urgent need for developing educational programs and establishing primary oral health services in order to meet the basic dental care needs of people with chronic health conditions.
It is also worth noting that earlier exposure of dental students to clinical training will provide them with valuable educational experiences which can subsequently contribute to teaching and training of the future generation of dentists.

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Conflict of interest statement

None declared.

Authors' contributions

MF developed the idea, organized the settings and participated in manuscript writing. KK analyzed the data and reported it. AB participated in data collection and wrote the initial manuscript.

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ORIGINAL ARTICLE

Effectiveness of a training course on smoking cessation knowledge and behaviour for health profession students: the SISMA project

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Keywords

Healthcare students • Training smoking cessation • Health education • Online course

Summary

Introduction. *University students are at risk of starting smoking or continuing and increasing the consumption of tobacco products. The aim of the study was to assess the impact of the training course, Sisma Project, about smoking in healthcare degree courses, in terms of knowledge, behaviour and to evaluate the course.*
Methods. *SISMA project was a pre- post study about an intervention delivered to healthcare profession students about smoking and smoking cessation. It had a before-after design and was an online optional course available on the eLearning platform Moodle 2. The course was structured in four lessons of sixty minutes, a debate among experts and a final test of evaluation. The McNemar test was used to measure the effectiveness of Sisma on smoking behaviour of students after the intervention. Students rated the course assigning a score from one to ten, and expressed free comments about point of strength and weakness of Sisma project.*
Results. *The participants were 365 students, 28.5% males and 71.5% females, most were nursing 194 (53.2%) and dental hygienists students 105 (28.8%). Current smokers were 161 (44.1%) before and 142 (38.9%) after the course, there was statistical significant difference in smoking status after attending the course ($p < 0.001$). Students evaluated the course giving a high score with a mean of 8.13 (SD: 1.1); the main points of strength were the content (33.2%), the structure (15.6%) and knowledge given by the course (12.6%). The main point of weakness were the online structure 62 (37%), problem related to length and time 17 (10%) and the final test 15 (9%).*
Discussion. *Given the central role health professionals play in patient care, students need to be aware and trained in tobacco cessation techniques. Our results indicate that smoking behaviour significantly changed after attending a university course for smoking cessation and students appreciated its contents and structure.*

Introduction

Young adults between the ages of 18 and 25 are at high risk of consuming cigarettes and becoming current smokers. Center for Diseases Control and Prevention (CDC) defines the "current smoker" an adult who has smoked at least 100 cigarettes in his lifetime and who currently smoke every day or somedays.
In Italy, the prevalence of smoking is 16.2% in the age group 15-24 years, and even higher among 25-44 years old (28.9% of men and 27.1% of women) [1].
Tobacco use starts primarily during adolescence and the average age of smoking initiation is about 18 years (17.6 years for men and 18.8 for women) according to CDC statistics [2]. Most smokers (82.6%) starts smoking between 15 and 20 years.
University students are at risk of smoking initiation [3], as well as continuing and increasing the consumption of tobacco products [4]. Surprisingly, tobacco has a high percentage of use among biomedical students. The prevalence of smoking among European medical students was 29.3% (95%CI: 28.1 to 34.7), with percentages ranging from 28% in Germany to 31.3% in Italy [5]. In the observational study of Ferrante [6] the percentage of current smokers among health professional students was 38.2%, with 94.3% of the total sample believing as important to receive specific training to quit smoking. Among physicians specializing in public health in 24 Italian universities, the prevalence of current smokers was reported as 20.9%. Even if 79.6% considered health professionals as behavioural models for patients, only 17% received specific smoking cessation training [7].
La Torre et al. [5] found almost a prevalence of 30%, although health professionals are expected to be trained and aware about of consequences of tobacco. So, special attention should be given to train healthcare profession students on smoking cessation considering the role they play both in healthcare and in prevention and as role model for patients.
In a previous experience of our group, a pilot school-based intervention seemed to be effective in reducing the prevalence of smoking among healthcare students [8]. After this experience, we standardized the methodology and considered a larger sample. So, the main objectives of this study were:

a) to evaluate the effectiveness of the course to change smoking status of students attending the course;

b) to do a quantitative and qualitative analysis of the course through the opinion and comments that students expressed about it.

Materials and methods

Study design, setting and participants

SISMA (Students Intervention on Smoking Attitude) project was an intervention delivered to health profession students of the Faculties of Medicine at Sapienza University of Rome, for all three years of their program, the participation to the course was voluntary. The monographic course was delivered on the online platform Moodle2.
SISMA was an experimental study, a pre-post design with a single group. The aim of the course was to give knowledge about tobacco and to help smokers to stop smoking. The smoking status was the main outcome, measured and compared before and after the course.
The students of healthcare professions courses were future nurses, dental hygienists, medical radiology technicians, prevention technicians. In order to attend the course students had to fill a pre-course assessment with related privacy information and informed consent.

Structure of course

The course was delivered over five days: the first four days were dedicated to online lectures of 60 minutes each, the students had to follow each online lesson which remained available only one day and was preparatory to the next one. The last day there was a short debate among experts about topics of the course. The online debate involved oncologist, pulmonologist, cardiologist, hygienist and pharmacologist about the severity of the damage to health caused by tobacco, other problems that smoking causes to the modern society.
The contents of the four lessons were as follows:

1. First lesson: epidemiology of smoking-related diseases, chronic obstructive pulmonary disease (COPD), lung cancer and cardiovascular diseases.
2. Second lesson: motivations to start tobacco smoking. Effects of nicotine. Electronic cigarettes.
3. Third lesson: nicotine addiction and motivation to quit. Smoking-related diseases.
4. Fourth lesson: Ask Advise Refer. Pharmacological and not pharmacological treatment of nicotine addiction.

Pre-course assessment

Students were asked if they knew about consequences of smoke as dependence and preventable illness and of damage caused by second-hand smoke. Moreover, their awareness about Italian law against smoking was investigated.
Smoking status was assessed by asking the students: "did you smoke in the last thirty days" (yes/no). Students who responded "yes" were classified as current smokers in the analysis while others as non- smokers.

Post-course assessment

The knowledge acquired after attending the course was assessed with a multiple choice test. The evaluation was carried out in thirtieths with the sufficiency established with a minimum of eighteen points.
Smoking status was assessed after the course asking students: "did you smoke in the last thirty days" (yes/no). Smoking behaviour was measured again after four months of follow up with a telephone interview using the following question: "which was your smoking status in the last four months" (I stopped/ I smoked as usual/I tried to stop/I increased/I reduced smoking/I restarted).
The meaning of the question has been explained orally during the telephone interview. In particular, the answer "I tried to stop" is referred to the students' attempt to stop smoking at least one time after the course, "I reduced smoking" was related to the decrease at least one third of the total cigarette consumption, as well "I increased" was of one third of total consumption.
Moreover, students filled out a questionnaire about their satisfaction of the course giving an evaluation in a linear numeric scale from 1 to 10. Students expressed their opinion concerning strength and weakness: this data was used for the qualitative analysis of the course.

Statistical analysis

A descriptive analysis of the sociodemographic characteristics of the study participants was carried out. Descriptive statistics were performed using absolute frequencies and percentages for categorical variables, while mean and standard deviation (SD) for quantitative variables. The associations between socio-demographic characteristics, attitude and knowledge, and smoking status were evaluated. The differences between groups with respect to the categorical variables were analyzed using the Chi-square test. The McNemar test was used to evaluate the effectiveness of the interventions on the variation of current smoking behaviour in the two periods. Data were analyzed with the software SPSS 25.0 for Windows. The statistical significance was set at $p < 0,05$.

Results

The characteristics of the sample concerning knowledge, attitude and behaviour on smoking are reported in Table I.

Characteristics of the sample

A total of 365 students filled out the online questionnaire (28.5% male and 71.5% female). The majority of students were between ages 19 and 24 years (73.2%). Most students were nonsmokers and there were not significant differences in smoking status among male and females, and among different age categories.
Out of 365 students, 71 (19.5%) attended the first year while 133 (36.4%) and 159 (43.6%) attended the second and third year of course. The majority of students attended nursing courses (53.2%), followed by dental technicians (28.8%) medical radiology technicians and

Tab. I. Descriptive and univariate analysis of health profession students.

		N (%)	Smoking status before course		
			Smokers (%)	No smoker (%)	p
Sample		365 (100)	161(44)	204 (56)	
Age	19-24	267 (73.2)	122 (76)	145 (71)	0.278*
	25-29	63 (17.3)	28 (23)	35 (17)	
	> 30	35 (9.6)	11 (1)	24 (12)	
Sex	M	104 (28.5)	53 (33)	51 (25)	0.096*
	F	261 (71.5	108 (67)	153 (75)	
Year of course	1st	71 (19.5)	27 (17)	44 (21)	0.286*
	2nd	133 (36.4)	61 (38)	72 (35)	
	3rd	159 (43.6)	71 (45)	88 (43)	
Type of course	Nursing	194 (53.2)	106 (66)	88 (43)	0.005*
	Dental hygienists	105 (28.8)	36 (22)	69 (34)	
	[1]MRT and P	48 (13.2)	15 (10)	33 (16)	
	[2]MDS	18 (4.9)	3 (2)	13 (6)	
Do you think that smoke create dependence?	Yes	364 (99.7)	160 (99)	204 (100)	0.26*
	No	1 (0.3)	1 (1)	0	
Do you think that smoke is the main cause of preventable death in Italy?	No	65 (17.8)	31 (19)	34 (16)	0.178*
	I do not know	108 (29.6)	76 (47)	116 (57)	
	Yes	192 (52.6)	54 (33)	54 (26)	
Do you know that there is a law about smoking ban in public places?	No	22 (6)	9 (5)	13 (6)	0.755*
	Yes	343 (94)	152 (95)	191 (94)	
Do you think that second hand smoking is harmful?	No	1 (0.3)	0 (0)	1 (1)	0.181*
	I do not know	5 (1.4)	4 (2)	1 (1)	
	Yes	359 (98.4)	157 (97)	202 (98)	
Smoking status after course	Yes	142 (38.9)	136 (84)	6 (3)	0,01**
	No	223 (61.1)	25 (15)	198 (97)	
Post course evaluation	< 18	25 (6.8)	12 (9)	13 (7)	0.817
	18-22	53 (14.5)	20 (15)	33 (25)	
	23-26	153 (41.9)	65 (47)	88 (66)	
	27-30	90 (24.7)	40 (29)	50 (37)	
Follow up of smokers after four months. How was your smoking status in the last four months?	I stopped	19 (11,8)			
	I smoked as usual	64 (39,8)			
	I tried to stop	12 (7.5)			
	I increased	10 (6.2)			
	I reduced smoking	47 (29.2)			
	I restarted	3 (1.9)			

* chi square test; ** McNemar test; 1 MRT and P: Medical Radiology Technicians and prevention technicians; 2 MDS: Master Degree in Sciences of the health professions and other.

prevention technicians (13.8%), students attending master's degree in sciences of the health professions (4.9%).
Overall, 161 (44.1%) students were classified as smokers, 53 (33.0%) of initial smokers were males. After the course, the number of smokers decreased to 142 (38.9%).
Data of follow up showed that: 11.8% stopped smoking and 7.5% tried to stop smoking after following the course, 29.2% reduced cigarette consumption. Instead, 6.2% increased cigarette smoked and 1.9% restarted smoking.
Most of the students passed the final test 296 (81.1%) and 90 (24.7%) got the highest grade. A small proportion (6.8%) failed the test.

Knowledge and attitude about smoking

Almost all students acknowledged that smoking leads to dependence (99.7%), and 52.6% believed that smoking is one of the main causes of preventable death 192 (52.6%). With respect to secondhand smoking, 94% of the sample was aware of the ban on smoking in public places and 98.4% were aware of the harmful effects of secondhand smoke.

Univariate analysis

Results indicate a significant difference between smokers and non-smokers by major, with nursing students

smoking at a higher rate. Significant differences were found concerning smoking status after following the course ($p < 0.01$) showing that students attending the course were more likely to stop smoking.

Qualitative and quantitative evaluation of the course

Students positively rated the course with a mean score of 8.13 (SD: 1.1).

Most students expressed as a point of strength the contents of the course (33,2%): epidemiology of smoking-related diseases, nicotine addiction and motivation to quit, treatment and technique to stop smoking. Students thought the course was clear simple and essential (14,5%), liked topic as risk and harms associated to smoke (12.2%), appreciated practical experience faced in the course (13.7%).

On the other hand, some examples of weaknesses underlined were: the lack of a live teacher (4.4%), lack of live debate (5.8%), the course online (7.9%), length of lessons (5.7%).

The main point of weakness that were underlined were the online structure of the course 62 (37%), problem related to length and time 17 (10%) and the final test 15 (9%).

Results about evaluation are reported in Table II and Table III.

Tab. II. Satisfaction evaluation of the course (qualitative analysis).

		N (%)
Point of strength of the course	Contents of the course	121 (33.2)
	Structure of the course	57 (15.6)
	Knowledge about danger caused by smoking	46 (12.6)
	The implementation of course in university	37 (10.1)
	Qualities of the course: clear, simple, essential	53 (14.5)
	Practical knowledge given by the course	50 (13.7)
	Online structure of the course	32 (8.8)
Point of weakness	Not useful	10 (6)
	Problem related to lenght and time	17 (10)
	Lack of material	7 (4)
	Online structure of the course	62 (37)
	Repeated content	8 (5)
	Content non clear for different reasons	7 (4)
	Long lesson	5 (3)
	Short lesson	5 (3)
	Lack of debate	5 (3)
	Nothing	10 (6)
	Difficult test or not clear	15 (9)
	Lesson not clear or to change	14 (8)
	Need to be advertised	2 (2)

Tab. III. Satisfaction evaluation of the course (quantitative analysis).

Score given by students	Score	N (%)
	5	7 (1.9)
	6	8 (2.2)
	7	69 (18.9)
	8	156 (42.7)
	9	66 (18.1)
	10	42 (11.5)

Discussion

The major objective of the study was to evaluate the training course delivered to healthcare profession students and examine the change in smoking status from pre to post course. Our study found several important results. First, we found that the prevalence of past 30-day smoking was 44.1% on a pool of 365 students of healthcare profession courses in Rome. Secondly, the study showed that the smoking status varied significantly after attending the smoking cessation course and at the follow up period. Considering that overall 21.4% of Italians are current smokers [9], the percentage of smokers found in this study is consequently far from what can be considered acceptable. Many cross-sectional studies found that there is a worldwide increasing trend of smoking during university [10]. Our findings suggest that health professional students could need training about nicotine addiction and tobacco cessation in their core university curriculum to help them stop smoking.

The university environment represents an important place where students shape their personal and professional behaviour. Moreover, because future healthcare professionals may influence the smoking behaviour of patients, their lifestyle should represent healthy behaviours and not ones like smoking [6]. Interestingly the past 30-day smoking was higher among nurses compared to other health profession students and medical students. Nurses play a central role in the field of health promotion compared to other people and health professions [11].

They have more interaction with patients and answer more health-related questions, both therapeutic and preventive. It is important for them to model healthy and positive behaviours [12].

Before the conduction of the course most students seemed to be aware of consequences of smoking as dependence, tobacco related diseases, danger caused by second hand smoke. Although smoking rate was considerably high. This showed the need for a course to help students quit smoking; the course was also able to change smoking status and was appreciated by students. Moreover, most students passed the final exam, the course seemed to be effective in conveying knowledge. Students underlined different point of strengths as the presence of an online course to be a convenient training and learning solution, compatible with university commitments. Although other students considered it a point of weakness, because of the lack of a talk with a teacher

and colleagues for a live debate. Contents of the course and the quality as essentiality and simplicity were still appreciated. Some students complained about problem related to length of lessons or timing problems.
Overall, greetings and support for the course suggested the necessity to spread the message and training against tobacco to the whole healthcare educational system and to other faculties.
The study presents several limitations which include the sample size which was not very big and the questionnaire which was not able to collect free comments from all students. In addition, the follow-up survey was held after four months and there was not a further evaluation. Nevertheless, the course was usable and widespread as it was online, had a clear and structured organization. The possibility to obtain the comments and evaluations of the students attending the course is another important aspect of value. Feedbacks from students can help to improve the characteristics and quality of the course.

Conclusions

Healthcare profession students need adequate training about nicotine dependence and tobacco cessation techniques in the core curriculum of their programs. This study showed that Sisma Project, a prototype online course on smoking cessation, was able to change smoking status of healthcare profession students and was appreciated.

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Conflict of interest statement

None declared.

Authors' contributions

Conceptualization: GLT; Investigation: RB and GLT; Methodology: AM, MCG, GLT; Statistical analysis: AM, GLT, RP; Writing, review and editing: VD, MC, GLT, GDV; Methodology: VD, GLT.

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ORIGINAL ARTICLE

Psychoactive drug consumption among truck-drivers: a systematic review of the literature with meta-analysis and meta-regression

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Keywords

Truck-drivers • Psychoactive drug consumption • Systematic review • Meta-analysis and meta-regression • Occupational health and wellbeing • Road safety

Summary

Few studies have assessed the extent of psychoactive drug consumption in the occupational setting. The trucking sector, in particular, is an important cause for concern, since psychoactive substance use has a relevant impact on the drivers' health and safety, increasing the risk of injuries and traffic accidents, potentially affecting the general public health as well. A systematic review of the literature and meta-analysis was performed in order to provide Occupational Health Professionals and policy-makers with an updated epidemiological perspective regarding this important issue. The results showed a prevalence of overall drug consumption of 27.6% [95%CI 17.8-40.1], particularly high considering illicit CNS-stimulants (amphetamine consumption of 21.3% [95%CI 15.7-28.1], and cocaine consumption of 2.2% [95%CI 1.2-4.1]). It appears that truck-drivers choose stimulant substances as a form of performance enhancing drug, in order to increase productivity. However, chronic and high dose consumption has been shown to decrease driving skills, placing these professional drivers at risk for health and road safety. Further research is required, particularly in Europe, in order to fill the knowledge gap and improve the strength of evidence.

Introduction

Illicit drug and psychoactive substance misuse is an important contributor to the global burden of disease. According to data presented in the World Drug Review 2018 published by the *United Nations Office on Drugs and Crime* (UNODC), 5.6% of the global population aged 15-64 years has used drugs in the previous year. Moreover, medical and non-medical prescription drug abuse, especially opioids, is reaching epidemic proportions in many parts of the world [1].

These figures represent an increasing trend over the years, especially in developed countries. In its European Drug Report 2018, the *European Monitoring Centre for Drugs and Drug Addiction* (EMCDDA) has reported an annual prevalence of drug users equal to 7.4%, and drug-induced mortality surpassing 5 thousands deaths in EU countries [2]. Since working age population makes up most of the overall population, substance use among workers is of primary interest from an occupational perspective. Indeed, it can cause loss of productivity, workplace injuries, absenteeism and increased illness [3].

Few international and national surveys using a systematic approach have studied drug use in the workplace: the *Center for Behavioral Health Statistics and Quality (CBHSQ) report regarding substance use and substance use disorder by industry* published by the *Substance Abuse and Mental Health Services Administration* (SAMHSA) reported an annual prevalence of illicit drug use of 9.5% among workers in the USA between the years 2008-2012 [3]. In Europe, there is a lack of up-to-date and high quality epidemiological data about prevalence of drug use in the workplace. The trucking sector, in particular, is an important cause for concern: truck-drivers are a vulnerable working population due to a wide variety of hazards [4-6] including physical and ergonomic ones with the risk of developing musculoskeletal disorders [7], hypertension [8], obstructive sleep apnea (OSA) and sleep deprivation [9, 10], exposure to diesel exhaust and risk of developing lung cancer [11]. Stressful conditions due to irregular working schedules, night shifts, being distant from families for long periods [12], the need for constant mental alertness, and high productivity demands [13] increase risky behaviors such as smoking, drinking, using psychoactive substances, and having casual sexual contacts [14]. In particular, psychoactive drugs affect the functioning of the brain by delaying cognitive and executive functions, which may lead to impaired driving [15]. These can have a relevant impact on truck-drivers' health, as well as on work safety, increasing the risk of injuries and traffic accidents [16, 17], often fatal: 21% of all lethal injuries occurred among "transportation and warehousing" workers in Iowa in 2005-2009 had a positive toxicology test for substance use [18]. Indeed, while the effects of ethanol on driving have been thoroughly studied by the literature, as shown in the previously published article by the Authors [19], the impact of other substances on driving is not as clear. The vast variety of substance classes,

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each with specific effects on physical and mental health, requires a detailed understanding of the interaction and effect on work specific tasks. Italian law has identified several occupational categories as at risk of harm to their and others' health and safety, to which it prohibits drug use, even occasional [20]. Nevertheless, not much is known regarding on site health surveillance and drug testing in this occupational sector.

A comprehensive analysis of this issue can adequately inform policy-makers in order to address legislative shortcomings and implement preventive measures in the workplace, reducing in turn the contribution of work-related drug health problems arising from working conditions to the general public.

The aim of the present systematic review and meta-analysis is to provide Occupational Health Professionals and policy-makers with updated epidemiological data regarding drug consumption among truck-drivers, in order to reduce the knowledge gap, and in turn to allow the implementation of effective countermeasures taking place in the workplace. The reduction of drug-related health problems induced by working conditions will also beneficially contribute to public health. This study adds to the findings regarding alcohol consumption in this occupational category, presented in our previously published article, and significantly updates and expands, through a rigorous quantitative analysis, the work performed by Girotto and colleagues [4].

Materials and methods

Systematic review

The current systematic review of the literature with meta-analysis and meta-regressions is reported according to the *Preferred Reporting Items for Systematic reviews and Meta-Analyses* (PRISMA) guidelines [21]. The study protocol was registered in the "International database of prospectively registered systematic reviews in health and social care" (PROSPERO database [22]; registration code CRD42016037077) [23]. The results of the study are reported in line with the PRISMA guidelines [24].

Briefly, a comprehensive pool of scholarly databases (namely, PubMed/MEDLINE (NLM), Scopus, SciVerse ScienceDirect, Science Citation Index Expanded (SCIE) and Social Sciences Citation Index from ISI/Web of Science, ProQuest Research Library, ABI/INFORM, CBCA), via the UNO per TUTTI Primo Central (Ex Libris) platform) was searched from inception using the following string of keywords: (truckers OR truck-drivers OR lorry OR commercial vehicles OR large good vehicles OR large vehicles OR heavy vehicles OR long vehicles OR trucking industry OR haul transport) AND (drugs OR psychostimulants OR psychoactive substances OR amphetamine OR benzodiazepines OR cocaine OR heroin OR opioids OR cannabis OR cannabinoids). Medical Subject Headings (MeSH) terms and wild-card option (truncated words) were used when necessary. Last search was carried out on 3rd December 2018. No language restriction or time filter were applied. Gray literature was consulted via Google Scholar. Further details of the search strategy are reported in Table I.

Literature search was performed by 2 researchers independently (NLB and AR). In case of disagreement, consensus was reached through discussion and consultation. Based on the PECO criteria, articles were included if: 1) focused on truck-drivers (P = truck-drivers); 2) investigating drug consumption (E = exposure to abuse substances); 3) stratifying according to parameters such as age, gender, marital status, experience years, mean distance travelled (per trip), work-load, night-shift or educational level, in terms of primary schooling level (C = any comparison); and 4) reporting prevalence rate of drug consumption (O = drug consumption rate). Concerning the study design, articles were selected if devised as prevalence studies. Articles were excluded if not meeting with the above-stated PECO criteria and if designed as letter to editor, editorial, commentary, expert opinion, review article (of any type).

Reviews were, anyways, scanned for reducing the chance of missing potentially relevant articles. Relevant information was extracted from each included article by two researchers independently (NLB and AR). In case of disagreement, a third researcher (GD) acted as final referee. For data extraction, an ad hoc Excel spreadsheet was designed and utilized. Besides tables, relevant information was summarized by means of a narrative review.

Methodological appraisal of studies quality

Study quality was assessed utilizing the "Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews-Checklist for Prevalence Studies". This tool explores different domains of quality: namely, 1) the appropriateness of the sample frame to address the target population; 2) the participants sampling technique; 3) the adequateness of the sample size; 4) the completeness of the description and details concerning the study subjects and the setting; 5) the coverage of the sample; 6) the validity of the methods; and 7) their reliability; 8) the appropriateness of the statistical analyses; and, finally, 9) the adequateness of the response rate. Based on the JBI tool, studies were deemed of high, medium and low quality, respectively.

Meta-analysis

For each outcome (amphetamine, benzodiazepines, cannabis, cocaine, heroin, opioid, OTC stimulants, overall drug consumption and poliabuse rates), effect size (ES) was computed pooling together the various prevalence rates, using the logit transformation approach.

Heterogeneity among studies was quantitatively assessed computing the I^2 statistics. An amount greater than 50% was considered statistically significant [25, 26]. Based on the amount of heterogeneity, a fixed- or a random-effect model was chosen.

Publication bias was studied both by visually inspecting the funnel plot in terms of asymmetry and by computing the Egger's regression test [27] and the Duval and Tweedie's trim-and-fill analysis [28]. Sensitivity analyses and cumu-

Tab. I. Pooled drug consumption rates and respective ranges stratified according to the type of study (studies based on questionnaires, urine or saliva samples).

Drug	Drug consumption		
	Questionnaire	Urine sample	Saliva sample
Amphetamine	21.3% [95%CI 15.7-28.1]	3.8% [95%CI 1.7-8.2]	1.3% [95%CI 0.7-2.4]
Benzodiazepines	1.0% [95%CI 0.1-6.1]	0.4% [95%CI 0.2-0.6]	NA
Cannabis	5.9% [95%CI 3.5-9.8]	2.1% [95%CI 1.0-4.3]	0.5% [95% 0.3-1.0%]
Cocaine	2.2% [95%CI 1.2-4.1]	1.1% [95%CI 0.7-2.0]	1.1% [95%CI 0.4-3.1]
Opioid	4.3% [95%CI 0.6-26.4]	2.0% [95%CI 0.6-6.6]	NA
OTC stimulants	4.1% [95%CI 2.7-6.2]	9.0% [95%CI 4.3-18.0]	NA
Overall drug consumption	27.6% [95%CI 17.8-40.1]	6.1% [95%CI 2.9-12.4]	4.1% [95%CI 1.2-13.1]
Poliabuse	2.7% [95%CI 0.2-25.6]	0.6% [95%CI 0.1-4.8]	0.3% [95%CI 0.1-0.7]

NA: not available; OTC: over-the-counter.

lative meta-analyses were further performed, in order to verify the reliability and the consistency of the findings.

All analyses were carried out with the commercial software *Comprehensive Meta-Analysis* (CMA version 3.0, for Windows).

For further details, the reader is referred to our previous publication [19].

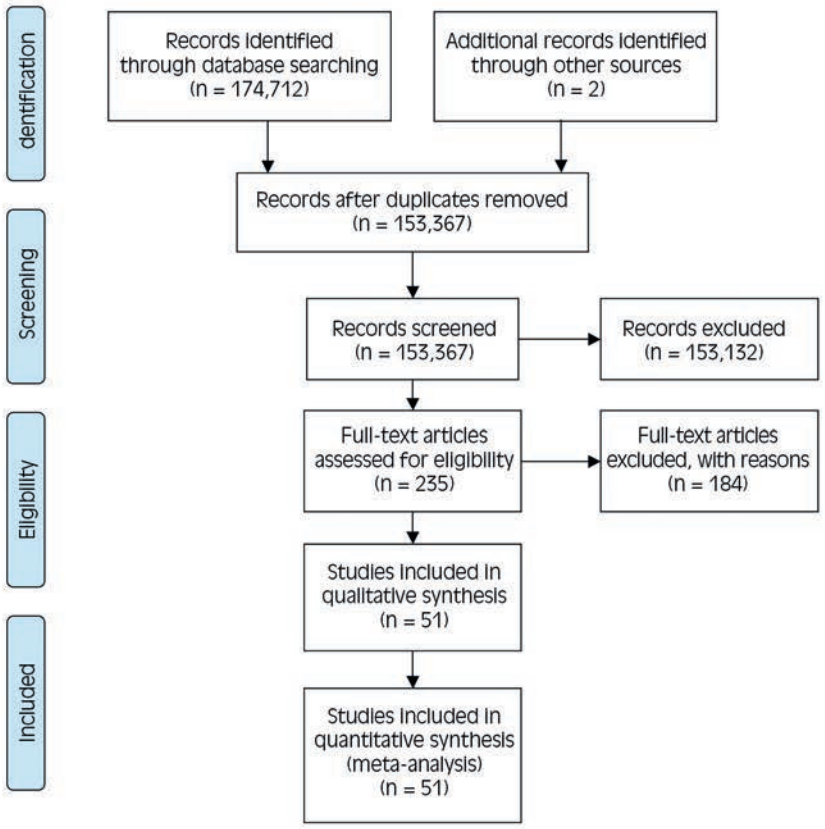
Results

Systematic review

The initial search resulted in a pool of 174,712 articles. After deleting duplicates, a set of 152,367 unique items was obtained. Screening titles and/or abstracts led to the exclusion of 153,132 items. A pool of articles was retrieved and accessed in full-text. Finally, 51 studies were included (Fig. 1).

Investigations were carried out between 1983 and 2018. Sample sizes ranged from 30 to 11,242 subjects, with a total of 43,673 participants. 31 studies investigated drug consumption among truck-drivers utilizing questionnaires, whilst 14 and 6 studies utilized urine and saliva samples, respectively. 35 studies were performed in the Americas (1 in Canada, 6 in the USA, and 28 in Brazil), 5 in Asia (3 in Thailand, 1 in Pakistan, and 1 in Iran), 5 in Europe (3 in Italy, 1 in France and 1 in Norway), 4 in Australia and 2 in Africa (1 in Morocco and 1 in Nigeria).

Fig. 1. PRISMA 2009, flow diagram. The process of retrieving and selecting articles adopted in the present systematic review and meta-analysis.



Age went from 33.5 to 43.85 years, with male percentage varying in the range 90.6-100.0%. Concerning the marital status, percentage of married subjects ranged from 62.0% to 94.9%. Percentage of truck-drivers with at least primary education varied between 35.2% and 100.0%, with schooling years going from 4.6 to 8.7 years. Work-load ranged from 7.8 to 14.8 hours, with mean distance travelled varying between 270 km and 1,159.7 km. Percentage of truck-drivers working for companies was highly variable, in the range 0-76%. Experience years went from 10 to 18.5 hours. Finally, percentage of truck-drivers doing night-shifts ranged from 10.7% to 33.0%.

Concerning the outcomes, amphetamine consumption ranged from 0.0% to 82.5%, whereas cannabis and cocaine use went from 0.0% to 29.9%, and from 0.1% to 8.9%, respectively. Heroin consumption varied between 0.1% and 0.9%. Opioid use ranged from 0.2% to 33.0%, while benzodiazepines consumption went from 0.0% to 2.1%. OTC stimulant use ranged from 4% to 13%, while poliabuse prevalence was more variable (0.0-8.9%). Finally, overall drug use was in the range 1.3-80.4%.

Amphetamine consumption

Based on questionnaires, the overall amphetamine consumption rate was 21.3% ([95%CI 15.7-28.1], z = – 6.94, p < 0.0001, k = 22) (Supplementary Fig. 1A). Due to the high statistically significant heterogeneity (I^2 = 97.15%), a random-effect model was applied (I^2 = 64.91%). No evidence of publication bias could be found. At the meta-regression analyses, significant moderators were found to be country (Q = 39.20, p < 0.0001, with the highest ES in Brazil, and the lowest ES in Nigeria) (Supplementary Fig. 2), age (coefficient = – 0.24, SE = 0.06 [95%CI – 0.36 to – 0.12], z = – 3.82, p = 0.0001) (Supplementary Fig. 3), marriage (coefficient = – 0.02, SE = 0.01 [95%CI – 0.04 to 0.00], z = – 1.99, p = 0.0470), experience years (coefficient = – 0.34, SE = 0.10 [95%CI – 0.54 to – 0.14], z = – 3.37, p = 0.0008) (Supplementary Fig. 4), working for companies (coefficient = – 0.04, SE = 0.01 [95%CI – 0.06 to – 0.01], z = – 2.72, p = 0.0065) and primary schooling level (coefficient = – 0.02, SE = 0.01 [95%CI – 0.03 to – 0.01], z = – 2.69, p = 0.0071). Male (p = 0.1040), mean distance (p = 0.7928) and work-load (p = 0.3804) were not statistically significant. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.

Based on studies utilizing urine samples, the overall amphetamine consumption rate was 3.8% ([95%CI 1.7-8.2], z = – 7.70, p < 0.0001, k = 13) (Supplementary Fig. 5A). Due to the high statistically significant heterogeneity (I^2 = 97.36%), a random-effect model was carried out (I^2 = 42.38%). No evidence of publication bias could be detected. At the meta-regression analyses, only country (Q = 23.64, p < 0.0001, with the highest ES in Thailand and the lowest ES in France) resulted a statistically significant moderator. Age (p = 0.1673), male (p = 0.0511) were not statistically significant moderators. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.

Based on studies utilizing saliva samples, the overall amphetamine consumption rate was 1.3% ([95%CI 0.7-2.4], z = – 13.63, p < 0.0001, k = 5) (Supplementary Fig. 6A). Due to the high statistically significant heterogeneity (I^2 = 83.68%) a random-effect model was applied (I^2 = 47.67%). No evidence of publication bias could be found. For all the moderators, meta-regression analyses could not be run due to insufficient number of studies.

Cannabis consumption

Based on questionnaires, the overall cannabis consumption rate was 5.9% ([95%CI 3.5-9.8], z = – 9.88, p < 0.0001, k = 10) (Supplementary Fig. 1B). Due to the high statistically significant heterogeneity, (I^2 = 96.81%), a random-effect model was carried out (I^2 = 62.28%). No evidence of publication bias could be found. At the meta-regression analyses, a significant moderator was found to be only country (Q = 15.85, p = 0.0146, with the highest ES in Pakistan, and the lowest ES in Iran). Age (p = 0.1044), male (p = 0.5799), marriage (p = 0.5939), mean distance (p = 0.4235), experience years (p = 0.7688), working for companies (p = 0.2192), and primary schooling level (p = 0.3200) were not statistically significant. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.

Based on studies utilizing urine samples, the overall cannabis consumption rate was 2.1% ([95%CI 1.0-4.3], z = – 9.97, p < 0.0001, k = 11) (Supplementary Fig. 5B). Due to the high statistically significant heterogeneity (I^2 = 97.62%), a random-effect model was chosen (I^2 = 4.52). At the meta-regression analyses, only age (coefficient = – 0.50, SE = 0.09 [95%CI – 0.68 to – 0.33], z = – 5.56, p < 0.0001) resulted a statistically significant moderator. Country (Q = 3.97, p = 0.5537), male (p = 0.2427) were not statistically significant moderators. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.

Based on studies utilizing saliva samples, the overall cannabis consumption rate resulted 0.5% ([95% 0.3-1.0%], z = – 15.69, p < 0.0001, k = 4) (Supplementary Fig. 6B). Due to the high statistically significant heterogeneity (I^2 = 68.12%), a random-effect model was applied (I^2 = 0.00%). The visual inspection of the funnel plot showed evidence of publication bias. At the Duval and Tweedie's trim-and-fill analysis, 2 studies were censored, resulting in a "real" ES of 0.3% ([95%CI 0.1-0.7], Q = 33.21) (Supplementary Fig. 7). For all the moderators, meta-regression analyses could not be run due to insufficient number of studies.

Cocaine consumption

Based on questionnaires, the overall cocaine consumption rate was 2.2% ([95%CI 1.2-4.1], z = – 11.75, p < 0.0001, k = 9) (Supplementary Fig. 1C). Due to the high statistically significant heterogeneity (I^2 = 91.66%), a random-effect model was applied (I^2 = 50.78%). No evidence of publication bias could be found. Country (Q = 6.06, p = 0.1951), age (p = 0.2460), male (p = 0.2433), marriage (p = 0.0541), mean distance

(p = 0.8952), experience years (p = 0.2604), working for companies (p = 0.3851), primary schooling level (p = 0.5713) were not statistically significant moderators. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.
Based on studies utilizing urine samples, the overall cocaine consumption rate was 1.1% ([95%CI 0.7-2.0], z = – 15.59, p < 0.0001, k = 10) (Supplementary Fig. 5C). Due to the high statistically significant heterogeneity (I^2 = 88.74%), a random-effect model was carried out (I^2 = 19.33%). No evidence of bias was found. At the meta-regression analyses, no statistically significant moderators could be found. Country (Q = 6.47, p = 0.1668), age (p = 0.5273), male (p = 0.3568) were not statistically significant moderators. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.
Based on studies utilizing saliva samples, the overall cocaine consumption rate resulted 1.1% ([95%CI 0.4-3.1], z = – 8.29, p < 0.0001, k = 3) (Supplementary Fig. 6C). Due to the high statistically significant heterogeneity (I^2 = 88.18%), a random-effect model was chosen (I^2 = 0.00%). No evidence of bias publication could be found.

Opioid consumption

Based on questionnaires, the overall opioid consumption rate was 4.3% ([95%CI 0.6-26.4], z = – 2.92, p = 0.003, k = 4) (Supplementary Fig. 1D). Due to the high statistically significant heterogeneity (I^2 = 98.12%), a random-effect model was performed (I^2 = 0.00%). No evidence of publication bias could be found. At the meta-regression analyses, only age (coefficient = – 0.49, SE = 0.13 [95%CI – 0.75 to – 0.23], z = – 3.70, p = 0.0002) resulted a statistically significant moderator. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.
Based on studies utilizing urine samples, the overall opioid consumption rate was 2.0% ([95%CI 0.6-6.6], z = – 6.13, p < 0.0001, k = 4) (Supplementary Fig. 5D). Due to the high statistically significant heterogeneity (I^2 = 95.97%), a random-effect model was chosen (I^2 = 0.00%). No evidence of publication bias was found. It was not possible to compute meta-regressions due to insufficient number of studies/missing information.

Benzodiazepines consumption

Based on questionnaires, the overall benzodiazepines consumption rate was 1.0% ([95%CI 0.1-6.1], z = – 4.81, p < 0.0001, k = 2). Due to the high statistically significant heterogeneity (I^2 = 70.57%), a random-effect model was carried out (I^2 = 0.00%). Since there were only 2 studies, it was not possible to conduct a publication bias analysis and meta-regressions.
Based on studies utilizing urine samples, the overall benzodiazepines consumption rate was 0.4% [95%CI 0.2-0.6], z = – 21.71, p < 0.0001, k = 4). Due to the absence of heterogeneity (I^2 = 0.00%), a fixed-effect model was applied. There was no evidence of publication bias. For all the moderators, meta-regression analyses could not be run due to insufficient number of studies.

Over-the-counter stimulant consumption

Based on questionnaires, the overall OTC stimulant consumption rate was 4.1% ([95%CI 2.7-6.2], z = – 14.09, p < 0.0001, k = 3). Due to the high statistically significant heterogeneity (I^2 = 76.18%), a random-effect model was conducted (I^2 = 28.35%). The visual inspection of the funnel plot gave evidence of publication bias. At the Duval and Tweedie's trim-and-fill analysis, one study was censored, with a "real" ES of 3.5% ([95%CI 2.3-5.3], Q = 15.46) (Supplementary Fig. 8). For all moderators, meta-regression analyses could not be run due to insufficient number of studies.
Based on studies utilizing urine samples, the overall OTC stimulant consumption rate was 9.0% ([95%CI 4.3-18.0], k = 2). Due to the high statistically significant heterogeneity (I^2 = 92.41%), a random-effect model was chosen (I^2 = 0.00%). Since there were only 2 studies, it was not possible to conduct a publication bias analysis and meta-regressions.

Poliabuse rate

Based on questionnaires, the overall poliabuse rate was 2.7% ([95%CI 0.2-25.6], z = – 2.80, p = 0.005, k = 2). Due to the high statistically significant heterogeneity (I^2 = 96.14%), a random-effect model was applied (I^2 = 0.00%). Since there were only 2 studies, it was not possible to conduct a publication bias analysis and meta-regressions.
Based on studies utilizing urine samples, the overall poliabuse rate was 0.6% ([95%CI 0.1-4.8], z = – 4.77, k = 5). Due to the high statistically significant heterogeneity (I^2 = 90.76%), a random-effect model was carried out (I^2 = 0.00%). No evidence of publication bias could be found. At the meta-regression analyses, only country (Q = 17.45, p = 0.0002, with the highest ES in the USA and the lowest ES in Italy) resulted a statistically significant moderator. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.
Based on studies utilizing saliva samples, the overall poliabuse rate was 0.3% ([95%CI 0.1-0.7], z = – 12.98, p < 0.0001, k = 3). Due to the statistically significant heterogeneity (I^2 = 61.68%), a random-effect model was performed (I^2 = 0.00%). No evidence of publication bias could be found. For all moderators, meta-regression analyses could not be conducted due to insufficient number of studies.

Overall drug consumption

Based on questionnaires, the pooled overall drug consumption rate was 27.6% ([95%CI 17.8-40.1], z = – 3.36, p = 0.001, k = 14) (Supplementary Fig. 1E). Due to the high statistically significant heterogeneity (I^2 = 99.04%), a random-effect model was applied (I^2 = 21.28%). There was no evidence of publication bias. At the meta-regression analyses, only age (coefficient = – 0.22, SE = 0.07 [95%CI – 0.36 to – 0.08], z = – 3.13, p = 0.0018) resulted a statistically significant moderator. On the contrary, country (Q = 1.47, p = 0.8326), male (p = 0.9460), marriage (p = 0.3583), mean distance (p = 0.9759), experience years (p = 0.1128), work-load (p = 0.9902), working

for companies (p = 0.8486), and primary schooling level (p = 0.3112) were not statistically significant moderators. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.

Based on studies utilizing urine samples, the pooled overall drug consumption rate was 6.1% ([95%CI 2.9-12.4], p < 0.0001, k = 8) (Supplementary Fig. 5E). Due to the high statistically significant heterogeneity (I^2 = 98.44%), a random-effect model was conducted (I^2 = 0.00%). The visual inspection of the funnel plot showed evidence of publication bias. At the Duval and Tweedie's trim-and-fill analysis, 1 study was censored, resulting in a "real" ES of 5.1% ([95% 2.4-10.1], Q = 543.42) (Supplementary Fig. 9). At the meta-regression analyses, only age (coefficient = – 0.36, SE = 0.09 [95%CI – 0.53 to – 0.19], z = – 4.13, p < 0.0001) resulted a statistically significant moderator. Country (Q = 1.65, p = 8003), and male (p = 0.8430) were not statistically significant moderators. For the other moderators, meta-regression analyses could not be run due to insufficient number of studies.

Based on studies utilizing saliva samples, the pooled overall drug consumption rate was 4.1% ([95%CI 1.2-13.1], z = – 4.90, p < 0.0001, k = 5) (Supplementary Fig. 6D). Due to the high statistically significant heterogeneity (I^2 = 98.89%), a random-effect model was conducted (I^2 = 0.00%). For all the moderators, meta-regression analyses could not be run due to insufficient number of studies.

Pooled drug consumption rates stratified according to the type of study are summarized in Table I.

Study quality

Findings of the critical appraisal of included studies are shown in Table II.

Discussion

To the best of our knowledge, this is the first systematic review with meta-analysis and meta-regressions on drug consumption rate among truck-drivers. Considering the meta-analysis performed on data extracted from questionnaires, the findings show an increased prevalence of drug use among truck-drivers, especially central nervous system (CNS) stimulants, compared to the general population. In particular, the overall annual prevalence of amphetamine use among truck-drivers of 21.3%, compared to the estimated global prevalence of consumption in the general population of 0.7% [1], shows an almost 30-fold higher rate.

Similarly, but to a lesser degree, the results regarding cocaine use showed a higher prevalence (2.2%) compared to the general population (0.37%) [1]. In previous studies, stimulant consumption among truck-drivers has been associated with night shifts, length of travel and younger age [17, 75, 76]. Other authors have suggested that also external factors play a role, such as productivity-based payments [73]. In the present analysis, being younger and having less professional experience showed the most significant correlations with stimulant use.

Drivers often take stimulants as a form of Performance Enhancing Drugs (PEDs), in order to sustain ever increasing work-loads and busy work schedules. Several studies performed in controlled clinical settings have suggested that low dose amphetamines could improve psychomotor skills, such as driving ability, even in fatigued subjects [77]. However, chronic and high dose users, taken in real life settings, showed poorer compliance with traffic rules and working hours regulations [78], with an increased risk of traffic accidents [79], mainly as a consequence of after effects such as hypersomnolence and fatigue [80, 81]. Some authors have suggested that blood concentration above 0.27-0.53 mg/l is associated with psychomotor impairment [79]. Similar considerations have been made regarding cocaine use and its effects on psychomotor skills [82-85]. Amphetamine use has been estimated to increase the risk of fatal accidents by 5-times, causing in 2013 around half of all road traffic deaths caused by illicit drug consumption worldwide, resulting in around 20 thousand deaths [15].

The *European Agency for Safety and Health at Work* (EU-OSHA) has acknowledged the spread and normalization

Tab. II. Critical appraisal of studies included in the present systematic review and meta-analysis.

Study	Domain I	Domain II	Domain III	Domain IV	Domain V	Domain VI	Domain VII	Domain VIII	Domain IX
Bombana et al. 2017 [29]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cerqueira et al. 2011 [30]	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Couper et al. 2002 [31]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
De Oliveira et al. 2015 [32]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
De Oliveira et al. 2016 [33]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Drummer et al. 2007 [34]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Girotto et al. 2015 [17]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gjerde et al. 2012 [35]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gjerde et al. 2014 [36]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

continues

Tab. II. *follows.*

Study	Domain I	Domain II	Domain III	Domain IV	Domain V	Domain VI	Domain VII	Domain VIII	Domain IX
Guinn et al. 1983 [37]	No	No	No	No	Yes	No	No	Yes	Yes
Ingsathit et al. 2009 [38]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Knauth et al. 2011 [39]	Yes	No	Yes	No	Yes	No	No	Yes	Yes
Korelitz et al. 1993 [16]	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Labat et al. 2008 [40]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Laraqui et al. 2011 [41]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Lemire et al. 2002 [42]	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Leopoldo et al. 2015 [43]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Leyton et al. 2012 [44]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lund et al. 1988 [45]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Maarefvand et al. 2016 [46]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mabbott and Hartley 1999 [47]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Mansur Ade et al. 2015 [48]	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Masson, Monteiro 2010 [49]	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Mieczkowski 2010 [50]	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Mir et al. 2012 [51]	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes
Mongkolsirichaikul et al. 1988 [52]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Moreira, Gadani 2009 [53]	No	No	No	No	Yes	No	No	Yes	Yes
Nascimento et al. 2007 [54]	Yes	No	No	No	Yes	No	No	Yes	Yes
Okpataku 2016 [55]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Peixe et al. 2014 [56]	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No
Penteado et al. 2008 [57]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Pereira et al. 2014 [58]	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Pidetcha et al. 1995 [59]	No	No	No	No	Yes	Yes	Yes	Yes	Yes
Pinheiro et al. 2015 [60]	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Pinho 2005 [61]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Remor et al. 2015 [62]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Riva et al. 2010 [63]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Riva et al. 2018 [64]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Sangaleti et al. 2014 [65]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Silva et al. 2003 [66]	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

continues

Tab. II. *follows.*

Study	Domain I	Domain II	Domain III	Domain IV	Domain V	Domain VI	Domain VII	Domain VIII	Domain IX
Sinagawa et al. 2014 [67]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Souza et al. 2005 [68]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Starmer et al. 1997 [69]	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Takitane et al. 2012 [70]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Teles et al. 2008 [71]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Valway et al. 2009 [72]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Williamson 2007 [73]	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
Yonamine et al. 2012 [74]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

of stimulant use among certain groups of workers, particularly transportation workers, shift workers, and workers in high pressure, competitive or bullying work cultures [86]. It highlighted the need to evaluate the potential effect of non-medical drug used as cognitive enhancers in the workplace, and to consider the work environment and the interaction between workers and their working conditions as important factors in the use of PEDs.

Opiates, opioids, and CNS depressants, such as cannabinoids and benzodiazepines, showed a lower prevalence in the study population, and a smaller difference compared to the prevalence in the general population. In particular regarding cannabinoid use, the relatively high prevalence shown (5.9%), with the highest rates extracted from North American and European studies, follows the overall increasing rate of recreational and medical use of this type of drug in the general population: while an estimated 3.9% of the global population has used cannabinoids in the previous year, in North America the prevalence was higher at 13% [1], reaching over 14% in the European Union with an increasing trend of high-risk use, meaning consuming cannabinoids 20 or more days in the past month [2]. Many CNS depressant have been found to reduce lane control by increasing the standard deviation of lateral position (SDLP) [87-93]. Cannabis has been shown to impair cognitive, particularly affecting working memory and divided attention, and psychomotor performances, increasing the chance of road accidents [94]: according to systematic review and meta-analysis performed by Asbridge and collaborators, acute cannabis consumption among drivers doubles the risk of crashes compared to non-user [95].

Authors have suggested that tetrahydrocannabinol (THC) impairs mainly lateral control of the vehicle, while not affecting longitudinal control [96, 97]. Moreover, interaction between cannabis and alcohol has been shown to have an additive effect on driving performance [91, 94, 98, 99] causing an increased risk of road accidents [100]. Indeed, cannabinoids are estimated to cause one fifth of all road traffic deaths caused by illicit drug consumption [15]. The importance of cannabis use in the workplace may grow further as countries reform medicinal and recreational cannabinoid laws enabling an increase in the rate of consumption [101].

Concerning the results of opiate/opioid use among truck-drivers, the present analysis shows a prevalence of 4.3%, significantly higher compared to the rate of persons who use opiates and persons who use prescription opioids for non-medical purposes among the general population equal to 0.7% worldwide [1], and 0.4% in Europe [2].

However, similarly to cannabis, data show that there is a growing trend in the use of prescription drugs such as opioids and sedatives, for medical and non-medical reasons, reaching epidemic proportions in some Western countries. In particular, past-year users of opioids in North America have reached a prevalence of 4.2% [1], similar to the results found in the present study. Moreover, opioids cause most of the negative health impact of drug use, accounting for three quarters of deaths from drug use disorders in 2015 [1]. Although the role of opiates and opioid use in impairing driving ability is still unclear [102-106], there is suggestive evidence that opioids can cause an increased risk of vehicle collisions [1, 107].

Although the consumption of benzodiazepine was not found to be as common among truck-drivers, it must not be underestimated, as there is ample evidence of their impairing effect on driving skills, particularly regarding long-term benzodiazepines [108-110]. Moreover, non-medical use of benzodiazepines is the most common type of misuse of prescription drugs in the world [1].

Concerning drug testing for recent use, the results obtained through saliva sampling showed generally lower rates than those found on urine. This might be explained by the fact that urine drug testing can detect consumption occurred days or weeks before the sampling, resulting in low specificity for recent substance use. Research has suggested that saliva sampling has a stronger correlation with blood concentrations compared to urine, being also easier and faster to analyze and less intrusive to drivers [111-113]. However, there is a lack of conclusive evidence, with other authors considering urine testing as a more accurate method for identification of substance use and disorders in the workplace [114]. Based on the prevalence of overall drug use obtained through biological sampling, around 1 every 20 workers was driving under the influence of drugs. It is

worth noting that the country of origin of the driver was an often found association with drug use. Indeed, considering the data obtained from the included studies, there appears to be a pattern of consumption of specific substances in different areas, such as prevalent stimulant use in South America, cannabis use in North America and Europe, and opioid use in parts of Asia, likely because of availability, as well as historic and cultural reasons.

Overall, the findings of the present study, adding to the results of the previously published systematic review and meta-analysis concerning at-risk drinking, show that substance use is widespread among truck-drivers globally, putting workers and the general public at an increased risk of harm. The *EU Action Plan on Drugs 2017-2020* states that, in order to reduce and prevent drug use, effective evidence-based prevention measures must take into consideration situational factors, including workplace conditions [115]. There is indeed an urgent necessity for updated epidemiological data and research studying effective Occupational Health Promotion programs, particularly in Europe, required in order to make and enforce effective policies, putting in place countermeasures such as regular worksite drug testing, which has been shown to deter drug use among workers [116], as well as assessing working conditions that facilitate drug consumption, such as excessive workloads demanded by companies.

Strengths and limitations

The strengths of our study consist in its methodological rigor, reproducibility and transparency. We deposited a priori study protocol, which further corroborates our meta-analysis. However, despite its novelty and its methodological robustness, our study is not without limitations, which should be properly acknowledged.

The high statistically significant heterogeneity may affect the generalization of the findings and calls up for caution in their interpretation. For some outcomes, few studies were available. In some cases, such paucity limited the possibility of conducting a full extensive e series of analyses, including publication bias analysis and meta-regressions.

Conclusions

The present systematic review of the literature with meta-analysis and meta-regressions showed a relevant drug consumption rate among truck-drivers. As such, this study has practical implications for Occupational Physicians dealing with the health and wellbeing of truck-drivers. In particular, it appears that truck-drivers choose mainly stimulant substances as a form of performance enhancing drug, in order to increase productivity. However, chronic and high dose consumption has been shown to decrease driving skills, placing these professional drivers, as well as the general public, at risk.

Current literature is lacking in updated and reliable epidemiological data, especially in Europe. Therefore, further research in the field is urgently needed in order to provide Occupational Health Professionals with up-to-date data, necessary for the implementation of preventive programs and effective workplace measures. Moreover, this can be useful for decision and policy-makers in order to fill the gaps and shortcomings in the regulations.

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Conflict of interest statement

None declared.

Authors' contributions

GD, NLB and PD conceived the study, AM and AR performed a search of the literature, extracted and collected data, AM and AR critically appraised the literature, GD and NLB analyzed data, GD, NLB, AM, AR and PD drafted and revised the manuscript. All authors have read and approved the latest version of the manuscript.

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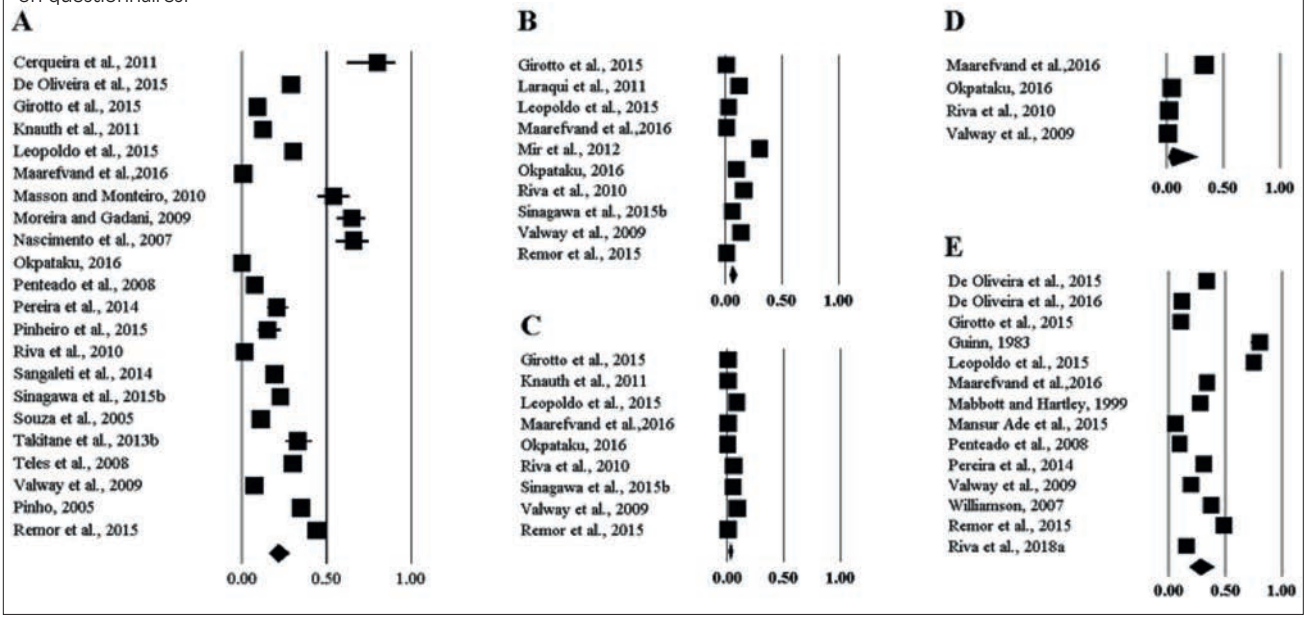
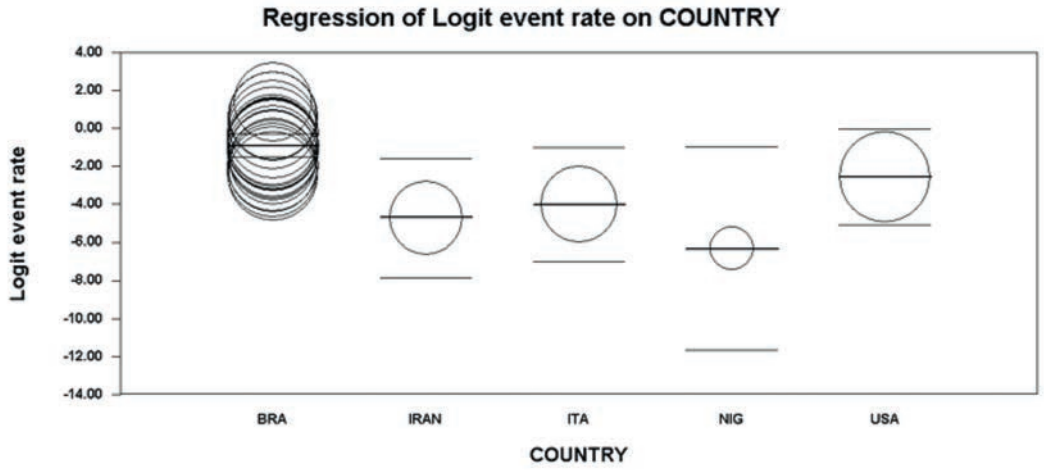
Supplementary material

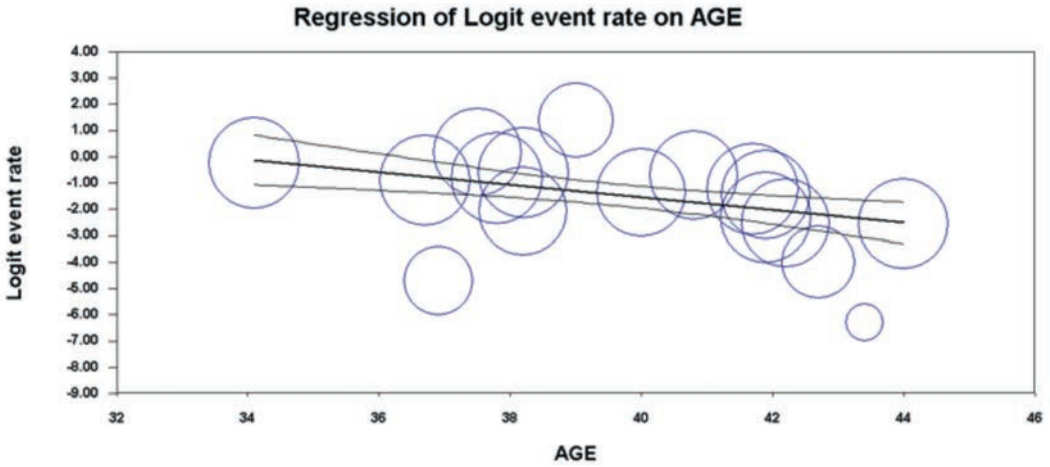
Supplementary Fig. 1. Forest plots of amphetamine **(A)**, cannabis **(B)**, cocaine **(C)**, opioids **(D)** and overall drug **(E)** use prevalence based on questionnaires.



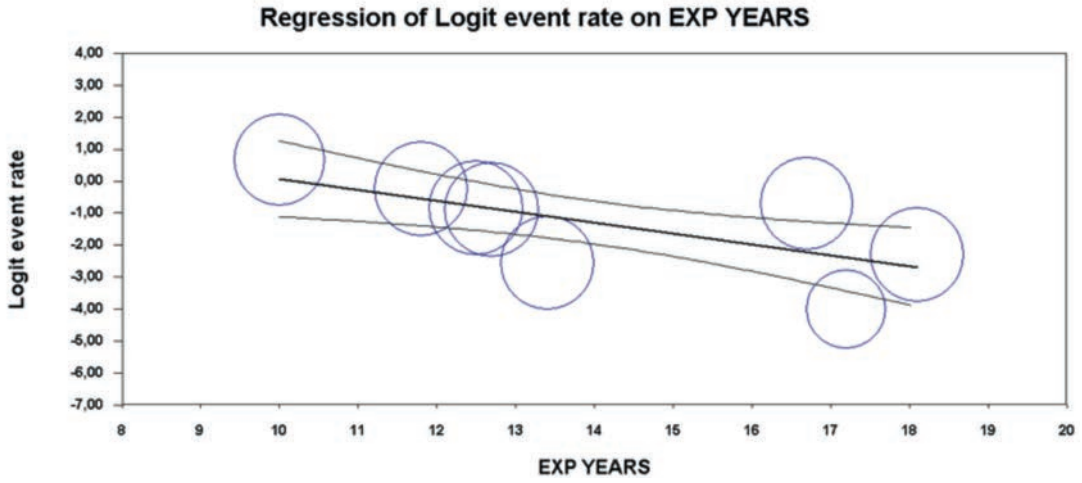
Supplementary Fig. 2. Meta-regression analysis of amphetamine use prevalence based on questionnaire, conducted for country as parameter.



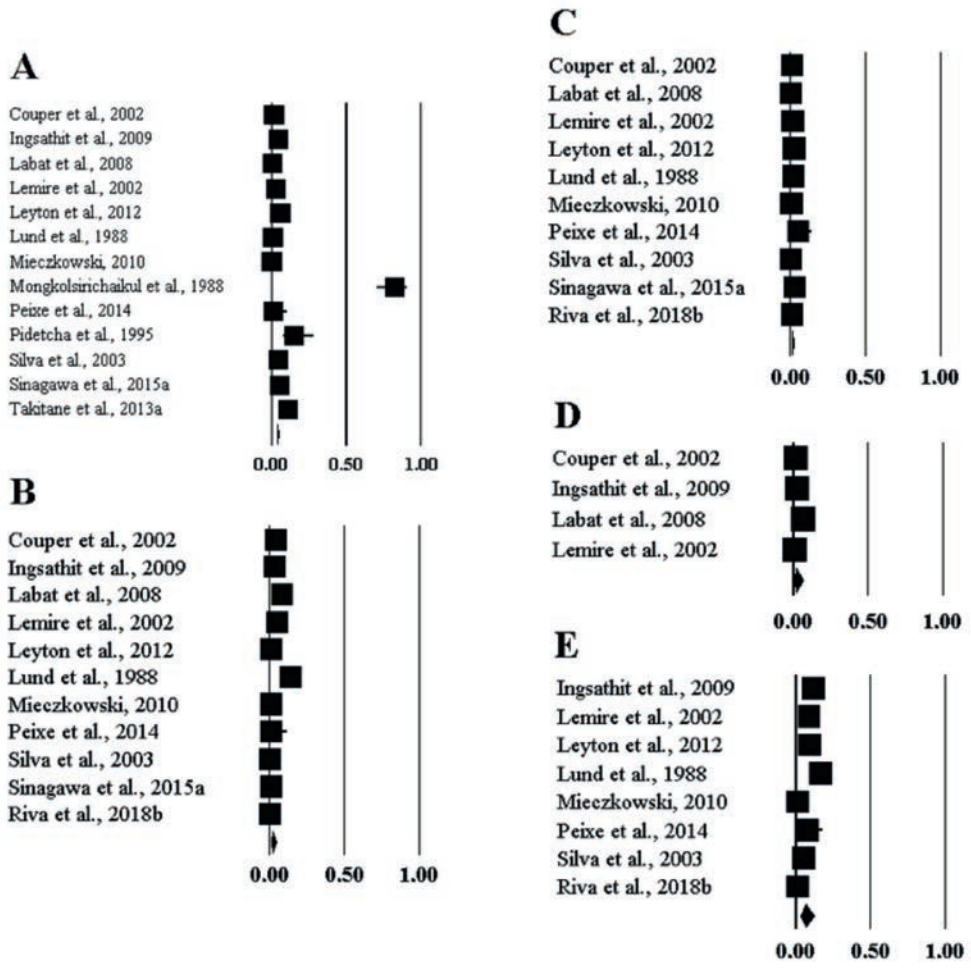
Supplementary Fig. 3. Meta-regression analysis of amphetamine use prevalence based on questionnaire, conducted for age as parameter.



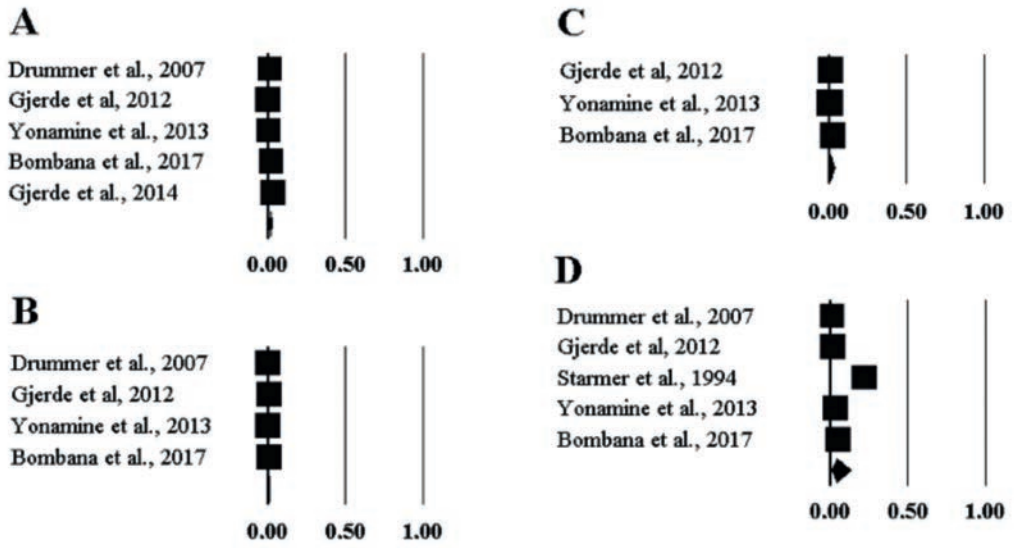
Supplementary Fig. 4. Meta-regression analysis of amphetamine use prevalence based on questionnaire, conducted for experience (in years) as parameter.



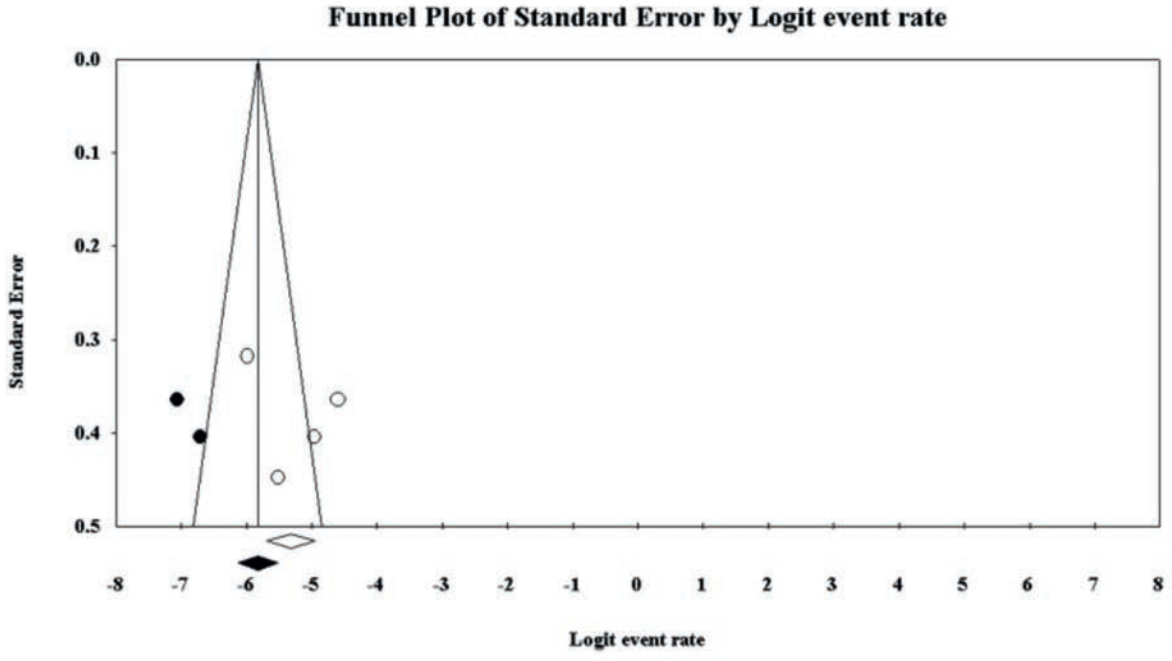
Supplementary Fig. 5. Forest plots of amphetamine **(A)**, cannabis **(B)**, cocaine **(C)**, opioids **(D)** and overall drug **(E)** use prevalence based on urine samples.



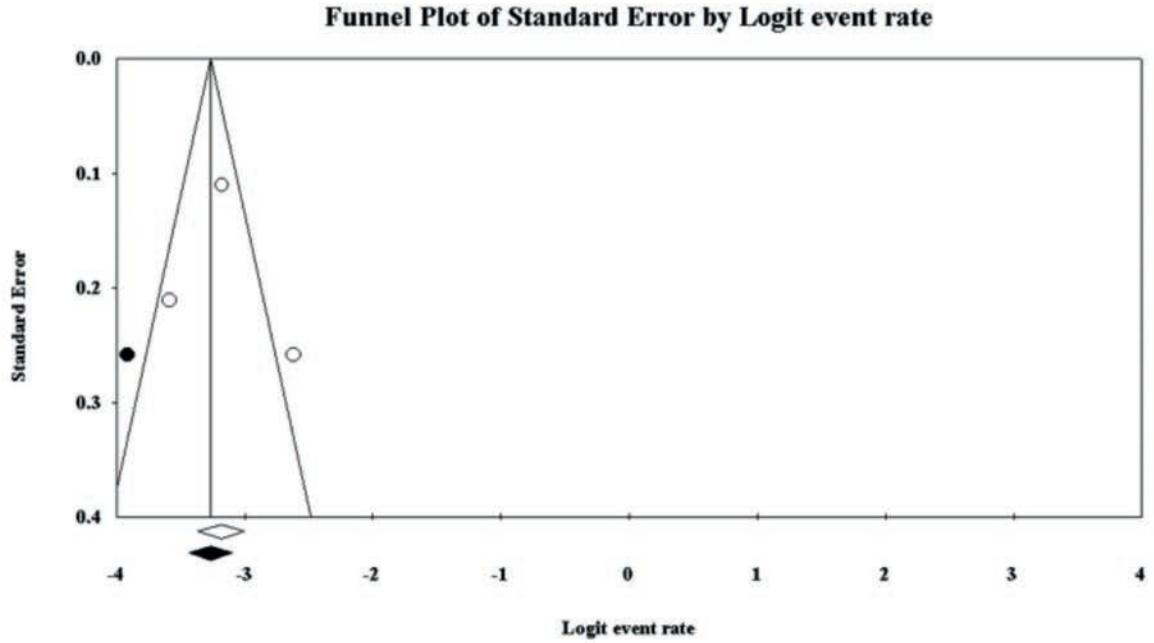
Supplementary Fig. 6. Forest plots of amphetamine **(A)**, cannabis **(B)**, cocaine **(C)** and overall drug **(D)** use prevalence based on saliva samples.



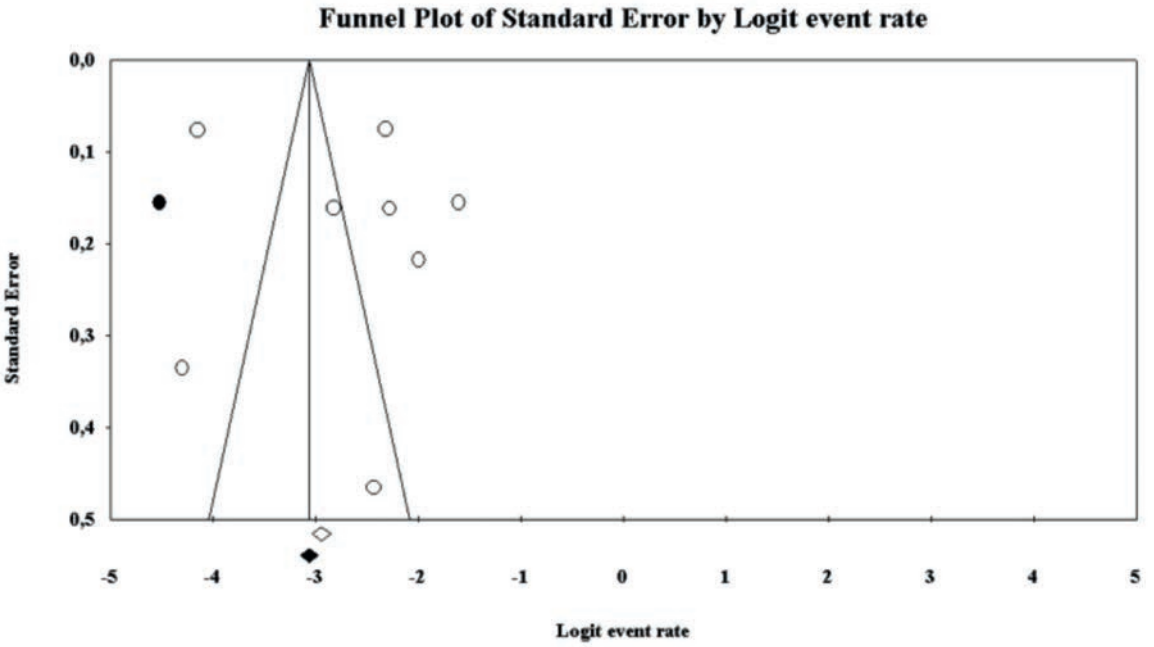
Supplementary Fig. 7. Funnel plot of cannabis use prevalence based on saliva samples. showing evidence of publication bias. In white observed effect sizes, in black imputed effect sizes.



Supplementary Fig. 8. Funnel plot of over-the-counter stimulant use prevalence based on questionnaire, showing evidence of publication bias. In white observed effect sizes, in black imputed effect sizes.



Supplementary Fig. 9. Funnel plot of overall drug use prevalence based on urine samples, showing evidence of publication bias. In white observed effect sizes, in black imputed effect sizes.



ORIGINAL ARTICLE

Evaluation of the total adenylate (ATP + ADP + AMP) test for cleaning verification in healthcare settings

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Keywords

ATP + ADP + AMP • Hygiene monitoring • Healthcare settings • High touch surface • Endoscope

Summary

Introduction. *Evaluation of cleaning methods is the first step in the prevention of healthcare-associated infections. ATP hygiene monitoring tests are widely used for assessing the effectiveness of cleaning procedures. The test is easy to use and gives immediate results, however, ATP can be metabolized and degraded to ADP and AMP. Recently, a total adenylate [ATP + ADP + AMP(A3)] monitoring test has been developed. Our objective was to evaluate the usefulness of the A3 test for cleaning verification in healthcare settings.*
Methods. *The detection sensitivities of the ATP and the A3 tests were compared using blood, and debris derived from gloved-hand method and endoscopes immediately after endoscopic examination. The performance of the A3 test in monitoring cleanliness of high touch surfaces in the hospital and endoscopes at each cleaning step was also evaluated.*
Results. *For the hemolysate, the measurement values of the A3 test were stable, although ATP was promptly degraded. In debris from hands, the amount of A3 was 20 times higher than that of ATP. The detection sensitivities of the A3 test on residues derived from gastroscopes and colonoscopes were 3 and 8 times higher, respectively, than those from the ATP test. A field study indicated that a large number of microorganisms tend to show high A3 values on high touch surfaces in the hospital and on endoscopes.*
Conclusions. *The A3 test showed higher detection sensitivities than the conventional ATP test for organic debris associated with healthcare settings.*

Introduction

In the last decade, much effort has been focused on the prevention of healthcare-associated infections (HAI). There are reports that contaminated surfaces, surgical instruments, and endoscopes cause the transmission of hospital pathogens [1-3]. Cleaning, disinfection, and sterilization of environmental surfaces, instruments, and hands are fundamental steps in reducing their potential contribution to the incidence of HAI. It is widely accepted that thorough cleaning is a prerequisite to effective disinfection/sterilization. According to the guideline for disinfection and sterilization in healthcare facilities from the Centers for Disease Control and Prevention (CDC), organic material that remains on the surfaces could interfere with the disinfection procedures [4]. First, some organic matter can interfere with the antimicrobial activity of disinfectants [4, 5]. This interference occurs due to a chemical reaction between the germicide and the organic matter, resulting in a complex that is less germicidal, reducing the active germicide available for disinfection/sterilization or totally eliminating germicidal activity. In addition, the organic material can protect microorganisms from the germicide by acting as a physical barrier [4, 5]. Therefore, rapid and easy evaluation methods for cleanliness and cleaning methods are in demand for use in healthcare settings.
Traditionally, visual inspection was the method used to assess the level of cleanliness, but visual acuity of inspectors affects the results. Furthermore, some specific structures, such as the inner surfaces of endoscopes are difficult to inspect. Measuring viable bacterial counts (VBC), using agar plates, has also been used; however, it takes 24-48 h to obtain results. Determination of residual protein contamination is also a widely used method [6-8]. However, since it involves laborious measurement procedures, immediate results are impossible. DNA-based techniques, including real-time polymerase chain reaction (PCR) test is highly sensitive to detect specific bacteria [9], but it is laboratory-use test and a wide range of the biomass can't be detected. Though the adenosine triphosphate (ATP) swabbing tests are not feasible to assay the bacteria accurately or specify the bacteria, they have attracted a lot of attention because they enable on site, rapid verification of sanitation procedures for the prevention of HAI [7, 8, 10-12].
ATP is found in all living beings where they produce effects both by intracellular and extracellular mechanisms. Intracellular ATP is primarily utilized to drive energy-requiring processes such as active transport, cell motility and biosynthesis, whereas extracellular ATP is considered a powerful signaling molecule [13]. Therefore, ATP is a more versatile molecule than a supplier of energy in both prokaryotic and eukaryotic organisms, and the presence of ATP on surfaces indicates improper cleaning and the presence of contamination, including organic debris and bacteria. Although the existence of ATP does not always mean the presence of living cells, the high levels of ATP after insufficient washing generally repre-

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sent a higher bacteria hazard by comparison with no detectable ATP or low levels of ATP after complete washing. The latest recommendation from the Healthcare Infection Control Practices Advisory Committee and CDC concerning environmental control in healthcare facilities states a category II recommendation to disinfect and clean high-touch surfaces (eg. doorknobs, bed rails, light switches) on a more frequent basis than minimal-touch surfaces [14]. CDC listed the ATP test as one of the methods that can be employed to rapidly evaluate the effectiveness of environmental cleaning [15].

Conventional ATP test systems, however, have a limitation in that ATP is degraded to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) by heat, acid/alkali, and enzymes [16, 17]. Because conventional ATP tests cannot detect these degradation products, they can miss insufficient cleaning and sterilization. Recently, a novel hygiene monitoring system to measure total the adenylate content [ATP + ADP + AMP, (A3)] was developed based on the luciferin-luciferase assay, with the combination of two enzymes, pyruvate kinase and pyruvate phosphate dikinase, which can convert ADP into ATP and AMP into ATP, respectively [17]. The newly developed A3 assay system afforded stable bioluminescence signals for ATP, ADP, and AMP, simultaneously. The evaluation of the A3 test for hygiene monitoring in healthcare settings has been demanded.

In this study, the amounts of ATP, ADP, and AMP in hemolysate, debris derived from gloved-hand method and endoscopes immediately after endoscopic examination, were assayed. Field tests for monitoring cleanliness of high touch surfaces in the hospital and endoscopes at each cleaning step were also carried out.

Methods

Reagents

Analytical grade ATP·2Na, ADP·K, and AMP·2Na were purchased from Oriental Yeast (Tokyo, Japan).

Measurement of the ratios of ATP, ADP and AMP

The amount of ATP, ATP + AMP, and A3 were assayed using commercially available test kits, the LuciPac II/Lumitester C-110, LuciPac Pen/Lumitester PD-30, and LuciPac A3 Surface/Lumitester PD-30 (Kikkoman Biochemifa, Tokyo, Japan), based on the luciferin-luciferase assay [17]. The sample collection swabs and testing devices, which contain the reagent and the extraction buffer are integrated in these kits. After a swab was removed from the tube, the sample solution was pipetted onto the swabs. The swab stick was then returned to the main tube and inserted completely. The tube was shaken to mix the sample, extraction solution, and reagent thoroughly. The tube was then inserted immediately into the luminometer, and the resulting luminescence was measured. All measurements were performed at 23°C, and data were recorded electronically. The measurement output was relative light units (RLU). According to the calibration curve study using reagent-grade adenylates in a previous report, it was verified that the A3 test afforded equivalent linear curves for amounts of ATP, ADP, and AMP in double logarithmic charts. Moreover, a given amount of ATP gave almost the same RLU value in the ATP, ATP + AMP, and A3 tests [17]. Therefore, the ratios of the three adenylates in any sample could be estimated by comparing their RLU values. RLUs derived from ADP and AMP were calculated by the values of A3-(ATP + AMP) and (ATP + AMP)-ATP, respectively. The ratios of ATP + AMP and A3 to ATP could be expressed as relative values, with the values of ATP being normalized to 1. Then, the ratios of ATP:ADP:AMP could be calculated as 1:(A3/ATP)-[(ATP + AMP)/ATP]:[(ATP + AMP)/ATP]-1.

Monitoring degradation of adenylates in hemolysate

Hemolysate was prepared by 10-fold dilution of blood (Nippon Bio-Test Laboratories, Saitama, Japan) with nuclease-free water and incubating at 35°C for 2 h. A 10 µl sample was applied onto the moistened swab with 100 µl nuclease-free water and ATP, ATP + AMP, and A3 tests were carried out over a time-course. The measurements were repeated 5 times, and the means were reported. The abundance ratios were expressed as relative values, normalized to RLU values before incubation, which were considered 100%.

Measurement of adenylates in gloved-hand sample

The gloved-hand samples were prepared in the following method [18]. Powder-free, nitrile gum gloves (AS ONE, Osaka, Japan) were worn by the subjects (n = 10) on one hand for 3 h. Then, 5 ml of nuclease-free water was introduced into the glove and the liquid was recovered. A blank sample was also prepared, as described above, using unused gloves. The ATP, ATP + AMP, and A3 tests were carried out using 100 µl of each sample. The resulting RLUs were obtained by subtracting the blank values. (The blank RLUs of ATP, ATP + AMP, and A3 inside the gloves were 3, 41 and 51, respectively.) The measurements were repeated 3 times, and the means for individuals were found. The ratios of ATP + AMP and A3 to ATP for individuals were calculated, then their means were obtained.

The inhibitory effects of disinfectant and cleaning agent on the A3 assay

Hydrogen peroxide (30%, Wako Pure Chemical, Osaka, Japan) was diluted with nuclease-free water to 3%. Enzyme-based immersion cleaning agent (Power Quick, Saraya, Osaka, Japan) was diluted 100-fold with nuclease-free water to prepare a 1.0% dilution according to manufacturer's instructions. Ten microliters of these solutions or water and 10 µL aliquots of 5 x 10^{-7} M adenylate solutions were pipetted onto swabs moistened with 80 µL of water, then the measurements were carried out. The RLU were expressed as relative values, with RLU values without chemicals being considered 100%.

The experiment was repeated 5 times for each aliquot, and the means are reported.

Environmental sampling and testing procedures in the hospital

High touch surfaces in the hospital [14] were swabbed using the swabs of LuciPac A3 Surface moistened with tap water before and after cleaning using moistened microfiber cleaning cloths (Toraysee™, Toray Industries, Tokyo, Japan). The items (n = 35) tested were telephone receivers (n = 2), PC mice (n = 2), desks (n = 2), carts [top boards (n = 2) and handles (n = 2)], stethoscopes (n = 2), blood pressure meter pumps (n = 2) in the nurses' station, door handles (n = 2), nurse call buttons (n = 2), bedside tables (n = 2), bed rails (n = 2), light switches (n = 2), refrigerator door handles (n = 2) in the hospital room, corridor handrails (n = 2), stretcher bed (n = 1), wheelchair handle (n = 1), cart handle in the ward (n = 1), infant incubators (n = 2) in the newborn nursery, door handles of the treatment room (n = 1) and the communication room (n = 1). Duplicate samples were different. These surfaces (10 cm x 10 cm areas) were sampled, using vertical and horizontal swabbing, carried out 10 times.

Each swab was immersed and washed in 5% glucose solution (1 ml, Otsuka Pharmaceutical Co., Tokyo, Japan) in 1.5 ml microcentrifuge tubes to prepare the analytical samples. Because the luciferase reaction is inhibited by salt [19], the 5% glucose solution without adenylates was used for protecting bacteria from osmotic pressure (data not shown). The samples were immediately cooled in a styrene foam box with a frozen gel pack and a lid. The blank sample was also prepared without surface swabbing. (The blank RLUs of ATP, ATP + AMP, and A3 were 3, 5, and 7 respectively.) The ATP, ATP + AMP, A3 tests and VBC were carried out using 100 µl of each sample. VBC was tested using tryptone soya agar (TSA) with incubation at 35°C for 1 day. The resulting RLU and colony-forming units (cfu) were multiplied by 10 because the swab can hold 100 µl liquid and the sample can be considered to be ca. 10-fold diluted with the 1 ml 5% glucose solution. The ratios of ATP + AMP and A3 to ATP for each point were calculated using the measurement values, and their means were obtained.

Sampling for gastrointestinal endoscopy and testing procedures

A 400 mm long stem swab (with a 2.8 mm/3.2 mm diameter cotton bud, LuciSwab, Kikkoman Biochemifa, Tokyo, Japan) was moistened with 5% glucose solution and inserted into the inner lumen of gastroscopes (2.8 mm working channel, n = 19, Olympus, Tokyo, Japan) and colonoscopes (3.2 mm working channel, n = 6, Olympus, Tokyo, Japan) from the distal tip to as far as the length of the swab permitted. Then the swab was pulled up slowly and immersed and washed in 5% glucose solution (1 ml) in 1.5 ml microcentrifuge tubes to prepare the analytical samples. The samples were immediately cooled in a styrene foam box with a frozen gel pack and a lid. The blank sample was also prepared without swabbing. ATP, ATP + AMP, and A3 tests; Bradford protein assay (Standard: Bovine serum albumin, Thermo Scientific, Waltham, MA, USA); and VBC were carried out after 1) removal, 2) manual cleaning, and 3) automated reprocessing (before alcohol flushing, Endoclens-D or -S, Johnson & Johnson, New Brunswick, NZ, USA). VBC was tested using TSA with incubation at 35°C for 1 day under aerobic conditions. The ratios of ATP + AMP and A3 to ATP for each instrument, just after endoscopic examination, were calculated using the measurement values, and their means were obtained.

Results

Monitoring of the change of adenylates in hemolysate

The ATP, ATP + AMP, and A3 tests showed 21035, 30141, and 36825 RLU for the hemolysate that was prepared by 10-fold dilution of blood with water. The (ATP + AMP)/ATP and A3/ATP ratios were 1.4 and 1.7, respectively, and the ratio of ATP:ADP:AMP was 1:0.3:0.4 (Tab. I). In the fresh hemolysate, ATP was the major adenylate. Successively, time-dependent monitoring of adenylates after incubation of the sample at 35°C for 2 h was also performed (Fig. 1). The results of the ATP, ATP + AMP, and A3 tests were 3882, 34733, and 39314 RLU after 1 h, and 226, 33616, and 33996 RLU after 2 h, respectively. The ratios of (ATP + AMP)/ATP and A3/ATP after 2 h were 148.7 and 150.4, and the ratio of ATP:ADP:AMP was 1:1.7:147.7 (Tab. I). These results demonstrate that ATP was decreased to below 20% after 1 h and almost completely decomposed to AMP after 2 h. This indicates that ATP is promptly degraded to AMP in hemolysates and the conventional ATP method may miss contamination from blood. The risk of missing blood contamination is likely decreased by adopting the ATP + AMP method. However, the measurement value was unstable, i.e. the amount of ATP + AMP was temporarily reduced by 7% after 15 min and eventually to around 110% after 30 min (Fig. 1). Since ADP in the hemolysate is estimated to be 18% of the A3 before incubation (Tab. I), ATP and ADP were likely decomposed and accumulated as AMP, around 110% for ATP + AMP appears to be reasonable. On the other hand, A3 was stable within a range of ± 8% (Fig. 1). Therefore, the A3 test is an effective tool for the rapid verification of cleaning procedures for surgical instruments contaminated with blood.

Evaluations of adenylate ratios in gloved-hand samples

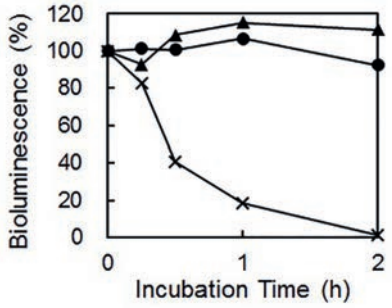
The RLUs of ATP, ATP + AMP, and A3 in gloved-hand samples (n = 10) were measured and the ratios of the results are shown in Table I. Gloved-hand samples generally contain sweat, skin cells, and bacteria. The means of (ATP + AMP)/ATP and A3/ATP were 8.2 and 20.1. The relative values of ADP and AMP were 11.8 and 7.2 fold higher than that of ATP. Since ATP was the minor

Tab. I. The means of ratios of ATP + AMP, ATP + ADP + AMP (A3), ADP, and AMP to ATP in hemolysate, sweat, and specimens from the hospital.

Sample	ATP + AMP	A3	ADP[a]	AMP[b]
Hemolysate (before incubation)	1.4	1.7	0.3	0.4
Hemolysate (after 1 h incubation)	8.9	10.1	1.2	7.9
Hemolysate (after 2 h incubation)	148.7	150.4	1.7	147.7
Gloved-hand samples	8.2	20.1	11.8	7.2
High touch surfaces	4.3	7.5	3.2	3.3
Debris from gastroscopes	2.4	3.5	1.2	1.4
Debris from colonoscopes	4.9	7.6	2.7	3.9

The ratios of each adenylate to ATP are expressed relative to ATP, which was set to 1. [a]: A3-(ATP + AMP); [b]: (ATP + AMP)-ATP.

Fig. 1. Stability of ATP (cross), ATP + AMP (triangle), and ATP + ADP + AMP (A3, circle) in hemolysate (10-fold dilution with water) at 35°C. The abundance ratios are expressed relative to RLU values before incubation, which were set to 100%.



and ADP was the major adenylate in the gloved-hand samples, 20 and 2.4 times higher sensitivity in detection of debris from hands can be achieved by the A3 method, compared with the conventional ATP test and the ATP + AMP test, respectively. Since the A3 method seemed to be useful for the evaluation of environmental cleaning and hand washing, a field study for high touch surfaces in the hospital was carried out to evaluate the practical performance of the A3 test.

The inhibitory effects of disinfectant and cleaning agent to the A3 assay

Hydrogen peroxide (3%) and commercially available enzyme-based immersion cleaning agent had little inhibitory effect on the A3 assays when they were added at 10% final volume (Tab. II).

Field study for the measurements of adenylates and VBC on high touch surfaces in the hospital

The cleanliness of high touch surfaces (n = 35) in the nurse station, the hospital room, the ward, the newborn nursery, the treatment room, and the communication room before and after cleaning with moistened microfiber cleaning cloths were evaluated by adenylate assays and VBC. After the cleaning, the amounts of A3 decreased at all measurement points, and the mean rate of decline was 87%. For instance, A3 values and VBC reduced from 1280 RLU and 1670 cfu before cleaning to 100 RLU and 0 cfu after cleaning of the door handle of the patient's room. For the bed rail, 1100 RLU and 50 cfu before cleaning was improved to 160 RLU and 0 cfu after cleaning. The means of (ATP + AMP)/ATP and A3/ATP for all measurement points were 4.3 and 7.5, respectively. The relative values of ADP and AMP were 3.2 and 3.3 fold that of ATP. The correlation between the adenylate assays and VBC is shown in Figures 2A, B. The samples containing large amounts of microorganisms tended to show high A3 values. If the benchmark is set at a typical value for high touch surfaces (e.g. 200 RLU), the ATP method showed many false-negatives, although these analytes were sampled before cleaning. These data indicate that the sensitivity of the ATP test may be insufficient for the hygiene monitoring of high touch surfaces in the hospital and the A3 test can better detect insufficient cleaning.

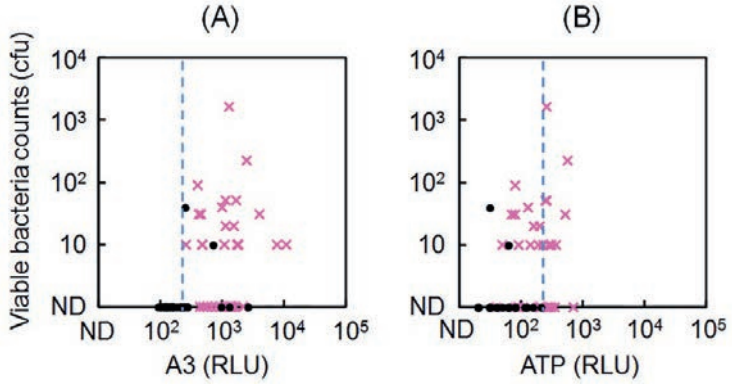
Tab. II. The inhibitory effects of disinfectant and cleaning agent to the A3 test.

	Hydrogen peroxide (3%)	Enzyme-based immersion cleaning agent (1%)
ATP	105	101
ADP	102	102
AMP	95	100

Field study for the measurements of adenylates, protein and VBC on gastrointestinal reprocessed endoscopes

The RLUs of ATP, ATP + AMP, and A3 in debris from working channels of the gastroscopes (n = 19) and the colonoscopes (n = 6) immediately after endoscopic examination were measured and the ratios of the adenylate content of these samples are shown in Table I. Debris derived from endoscopes immediately after endoscopic examination generally contains digestive juices, mucous membranes, and sometimes blood. For the debris from the gastroscopes, the means of (ATP + AMP)/ATP and A3/ATP were 2.4 and 3.5, respectively. The relative values of ADP and AMP were 1.2 and 1.4 fold that of ATP. For the debris from the colonoscopes, the mean of (ATP + AMP)/ATP was 4.9 and A3/ATP was 7.6; the ratio of ATP:ADP:AMP was 1:2.7:3.9. These results indicated that the A3 test can detect inadequate cleaning with 3.5 and 7.6 times higher sensitivity in comparison with the conventional ATP test for gastroscopes and colonoscopes, respectively. The ratio of A3/ATP for colonoscopes was twice as large as that for gastroscopes, probably due to the difference in the expression levels of adenylate metabolizing enzymes, such as alkaline phosphatase in the intestine [16]. The correlations be-

Fig. 2. Correlations of viable bacterial count and the amount of adenylates on high touch surfaces (n = 35) before cleaning (pink cross) and after cleaning (black circle) with moistened microfiber cleaning cloth. RLU, relative light units. **A)** ATP + ADP + AMP (A3), **B)** ATP. The typical benchmark value showing a clean surface, 200 RLU, is shown with the light blue dotted lines.

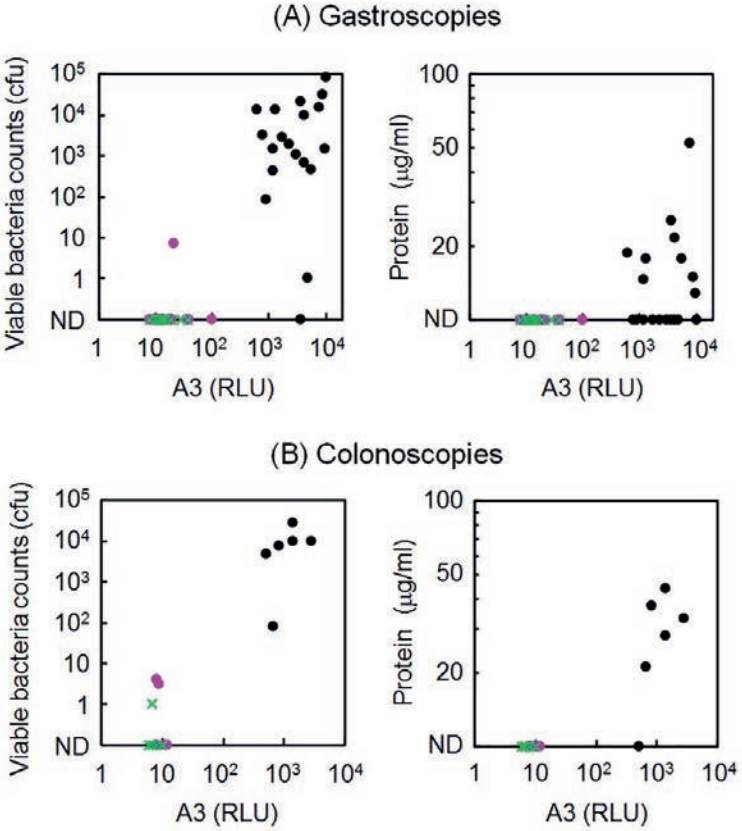


tween the protein assay, VBC, and the A3 test are shown in Figures 3A (gastroscopes), B (colonoscopes). As the cleaning steps progressed, the amount of A3 clearly decreased. Moreover, the samples that contained large amounts of protein and microorganisms tended to show high A3 values. The protein assay partly showed false-negative results although the analytes were sampled before cleaning. This data also suggests that the sensitivity of A3 method is higher than that of the Bradford protein assay.

Discussion

ATP hygiene monitoring tests are widely used in many clinical and hospital applications for the prevention of HAI because they are easy to use and provide immediate results and verification of cleaning processes. However, this study demonstrated that testing ATP levels alone can be ineffective in verifying cleaning procedures for instruments with blood contamination and high touch surfaces because contamination may be overlooked. In

Fig. 3. Correlations of viable bacteria count, protein, and the amount of ATP + ADP + AMP (A3) in the working channels of the endoscopes after removal (black circle), manual cleaning (pink circle), and automated reprocessing (before alcohol flushing, green cross). RLU, relative light units. **A)** gastroscopes, **B)** colonoscopes. The protein concentration was measured by Bradford protein assay.



hemolysates, ATP can be promptly degraded to AMP through ADP, and AMP is accumulated (Fig. 1, Tab. I). In gloved-hand samples and on high touch surfaces, ATP only accounts for approximately 5% and 13% of A3, respectively. As a result, if the benchmark value is not stringent, the ATP method may produce false-negative results for cleaning of high touch surfaces, as shown in Figure 2B. On the other hand, the concentration of A3 remains relatively stable in hemolysates (Fig. 1), and the A3 test would provide higher sensitivity due to a positive signal even in the event of ATP degradation and is less likely to produce false-negative results for high touch surfaces (Fig. 2A). The detection sensitivities of the A3 method for residues derived from gastroscopes and colonoscopes were between 3 and 8 times higher than those of the ATP method (Tab. I). Moreover, the comparison with VBC and protein assays demonstrated that the A3 test can assess the reliability of cleaning procedures of endoscopes (Fig. 3). Thus, the A3 test provides a rapid, sensitive, and reliable method for monitoring environmental contamination and verifying cleaning procedures in hospital rooms, operating rooms, and in instrument reprocessing operations. Since adenylate concentrations are modulated in a complicated process by breakdown and synthesis in human body [17], the simultaneous detection of A3 as an indicator of contamination of body fluid is reasonable for cleaning verification in healthcare settings.

Disinfectants are used to kill microorganisms, and cleaning agents are used for the degradation of soil and for rust prevention in instruments. The A3 tests, performed after thorough washing and rinsing, and before disinfection/sterilization, can ensure proper washing to achieve effective disinfection/sterilization [4, 5]. However, since insufficient rinsing may leave chemical residuals on surfaces, data regarding inhibition (or lack thereof) of the A3 test by these chemicals is important. A previous study demonstrated that inhibition by major sanitizer compounds for the food industry, such as sodium hypochlorite (500 ppm of effective chlorine concentration), ethanol (ca. 80%), and benzalkonium chloride (0.1%) were not inhibitory when ca. 10% volumes of disinfectants were added [17]. Additional study also demonstrated that the presence of hydrogen peroxide (3%) and cleaning agents containing detergent, enzymes, e.g. protease, and rust preventive compounds had little effect on the A3 tests under the same conditions (Tab. II).

Field studies for high touch surfaces and endoscopes show that A3 values become smaller with progressive cleaning and microbial populations decrease (Figs. 2A, 3). Though the A3 swabbing test is not specific for the presence of bacteria (similar to conventional ATP tests), these data demonstrate that using A3 as an indicator for sufficient cleaning is a promising method for the prevention of HAI. This is simply because inadequate cleaning can give rise to the possibility that microorganisms remain on a surface or instrument. Furthermore, data show that A3 can be detected, despite VBC not being detected in both field studies (Figs. 2A, 3A). Three reasons can explain this result. One, the bacteria present on the surface could not survive the osmotic pressure of tap water in the swab or sampling solution (5% glucose) or were unculturable on TSA medium under the general conditions (aerobic, 35°C, 1 day). For example, blood agar plates may show growth of bacteria from clinical specimens that are unculturable on TSA [20]. Two, since small amounts of bacteria exist within (and are protected by) organic debris, swabbing the surface will not capture them. This is particularly important because organic debris can interfere with the activity of germicides for disinfection/sterilization and/or can protect microorganisms by acting as a physical barrier [4, 5]. Third, there were indeed no bacteria present but only organic matter remaining on the surface. Because it is known that hospital environments contain a diverse range of bioaerosols, which include bacteria and fungi, it would be expected that these bioaerosols contribute viable cells, including opportunistic pathogens, that can adhere to, be protected by, proliferate, and form biofilms within any residual organic debris remaining [21]. Taking into consideration the cases mentioned above, the high A3 values, even in the absence of small amounts of bacteria, should be seen not as false-positive, but indicative of inadequate cleaning and as a risk for HAI.

Conclusions

Because the A3 test can detect total adenylate simultaneously, it provides a more sensitive and reliable indicator of cleanliness in hospital rooms, operating rooms, and in instrument reprocessing operations than the conventional ATP test. Since the A3 test is easy to use and provides immediate results and verification of cleaning processes, it guides and assures better sanitation outcomes and supports a more effective hygiene program in healthcare settings.

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Conflict of interest statement

Mikio Bakke is an employee of Kikkoman Biochemifa Company. Shigeya Suzuki is an employee of Kikkoman Corporation. E. Kirihara and S. Mikami declare that there is no conflict of interest regarding the publication of this paper.

Authors' contributions

MB conceived, designed the research, collected data, wrote the manuscript, SS planned the method, EK and SM coordinated the field studies in the hospital. All au-

thors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Do HCWs adequately know about meningitis and 4CMenB vaccine and recommend its use to parents? A cross sectional analysis in Campania Region, Italy

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Keywords

Healthcare workers • Vaccination strategy • Invasive meningococcal disease • Multicomponent meningococcal serogroup B vaccine • 4CMenB

Summary

Invasive meningococcal disease (IMD) is a severe disease caused by various Neisseria meningitidis serogroups that represents a serious public health problem worldwide. In Italy, serogroups B and C are the major causes of IMD. On 14 January 2013, the European Medicines Agency authorized the use of the first vaccine available to protect against meningococcal serogroup B (4CMenB).

The aim of this study was to assess the IMD epidemiology knowledge and 4CMenB vaccine attitudes of healthcare workers (HCWs) with regard to recommending this vaccine for use, vaccine practices and infectious disease control in the Campania region in Italy. A cross-sectional study was conducted among 293 HCWs (49.5% physicians and 46.4% nurses)interviewed using a self-administered questionnaire.

The majority of the HCWs had sufficient knowledge about the disease incidence and lethality, but they were less informed about the higher risk age categories and the serogroups most frequently involved. Additionally, their knowledge about the vaccine was poor with regard to the targeted categories and side effects. Approximately30.0% of the HCWs reported incidences of fever and pain and swelling at the injection site. Moreover,32.8% of the HCWs knew that the risk of developing adverse reactions increases when the 4CMenB vaccine is co-administered with other vaccines.

Overall, all of the HCWs were convinced that vaccinations are an important instrument for preventing infectious diseases, and they were aware of their central role in promoting the 4CmenB vaccination and their need to be better informed.

Introduction

An invasive meningococcal disease (IMD)is a severe and a life-threatening disease caused by various *Neisseria meningitidis* serogroups, and it represents a serious public health problem worldwide, with an annual number of cases estimated to be at least 1.2 million [1-3]. In Europe, the USA and other industrialized regions, serogroups B and C are the major causes of IMDs [4, 5]. Approximately one in ten cases are fatal, and among those who survive, long-term sequelae, such as hearing loss, neurological defects or amputation, can occur [6, 7]. Vaccination practices (MenC and ACWY135 vaccines) have significantly changed the epidemiology of this disease, so that serogroup B is currently the predominant cause of IMDs in Europe, Latin America and North America [5, 8, 9]. In Italy, the IMD incidence is higher in children 0 to 4 years old than in the other age categories, particularly in infants less than 1 year old (IMD incidence = 4.01/100,000 in 2013) [10, 11]. The serogroup mainly involved in infants less than 1 year old is type B (IMD incidence = 3.44/100,000 in 2013), with the highest incidence among children younger than 24 months, peaking at 4-8 months [10, 12]. Between 2014 and 2016, the IMD incidence among individuals 15-24 years old increased, from 0.30/100,000 in 2014 to 0.90/100,000 in 2016, and decreased in adults older than 25 years during the same time period [10].

In Italy, the IMD surveillance system based on laboratory-confirmed cases (almost 70%) revealed that between 2011 and 2017, serogroup B was the most common IMD-causing type [10], except between 2015 and 2016 when a hyper virulent meningococcal C strain was responsible for an unexpected increased IMD incidence in the Tuscany region. Following this episode, the Italian Health Authorities implemented immunization campaigns and enhanced IMD surveillance. On 14 January 2013, the European Medicines Agency authorized the use of the first vaccine available to protect against meningococcal serogroup B (Bexsero; GSK, Philadelphia, PA, USA) [13-16]. It is a multicomponent vaccine (4CMenB) composed of three purified recombinant antigenic proteins from *Neisseria meningitides* serogroup B and the outer membrane vesicles of the bacterium. The 4CMenB vaccination schedule for infants consists of three doses: the first dose is given at three months old, the second dose is given between 1 and 2 months after the first dose, and the third "booster" dose is given at 13 months old. As with other vaccinations, the most common adverse reactions from the4CMenB vaccina-

tion consist of fever, pain and swelling at the injection site, abnormal crying and irritability, eating disorders and gastrointestinal symptoms, sleepiness and a cutaneous rash. The uncommon adverse reactions consist of febrile or non-febrile convulsions and pallor, while the rarest adverse reactions are urticaria and Kawasaki syndrome [17].

The 4CMenB vaccination strategy differs across the European countries; for example, in Italy, the vaccination strategies are set out by different regions through the Regional Health Plan. This plan must respect the guidelines defined by the National Health Plan and the National Vaccine Prevention Plan (Piano Nazionale Prevenzione Vaccinale, PNPV) [18]. The latter defines the best vaccination policy to be carried out in each region in accordance with the best scientific evidence available. Each region can issue their own vaccination strategy in terms of the target population and costs that might being incurred by the citizens. This can lead to differences in the vaccine administration across Italian regions, resulting in heterogeneous vaccine coverage. The decreasing immunization adherence trend in Italy over recent years further compromises homogeneous and efficacious vaccine coverage, and this includes an unjustified fear of adverse reactions, scarce awareness regarding severe outcomes when not vaccinated, and the media's role in spreading incorrect information about vaccines. Many people's attitudes toward vaccinations may have been affected after an unverified association between the measles, mumps and rubella vaccine and autism was reported by the media, as well as after the Fluad case. The latter followed the withdrawal of the Novartis vaccine against influenza by the Italian Medicines Agency known as "Agenzia Italiana del Farmaco" after the occurrence of 3 deaths in 48 hours. Although there was prompt readmission of the Novartis vaccine, the media event had already affected people's attitudes towards vaccinations. These events have increased both citizens and healthcare workers' (HCWs) loss of faith in Italian institutions.

Previous studies have documented the strong influence that HCWs have on a patient's decision making process regarding whether or not to undergo vaccination [19-22]. However, some HCWs feel poorly informed and poorly trained on how to answer patient questions, and they often struggle when dealing with those who distrust the efficacy and safety of vaccines [23, 24]. Therefore, the aim of our study was to assess the knowledge and attitudes of HCWs involved in vaccination programs and infectious disease control with regard to recommending the 4CMenB vaccine in the Campania region of Italy.

Methods

Participants and setting

Within each Local Health Service (Aziende Sanitarie Locali, ASL) that manages public healthcare, the communicable disease prevention is run by two departments, the Maternal Childhood Health Protection Department, which delivers vaccines to children through its Maternal Childhood Operative Unit (Unità Operativa Materno Infantile, UOMI), and the Public Health Department, which looks at infectious disease surveillance and control through the Epidemiology Service (Servizio di Epidemiologia e Prevenzione) and Public Hygiene Service (Servizio di Igiene e Sanità Pubblica) and administers vaccines to adults and travellers through the Collective Prevention Operative Unit (Unità Operativa Prevenzione Collettiva).

A cross-sectional study was conducted from 1 January 2017 through 30 June 2017 at the ASLs in the metropolitan areas of Naples, Caserta and Salerno in the Campania region among the HCWs involved in the surveillance and control of infectious diseases or vaccine administration (the total number of HCWs at these ASLs was around 750). In each unit, a healthcare operator was identified as a reference contact, and they collaborated to explain the study objectives and raise awareness among the HCWs. In addition, this individual distributed the questionnaires and collected them immediately after they were completed anonymously by the participants. The questionnaires focused on the HCWs' knowledge about IMD epidemiology and preventability and their attitudes towards 4CMenB vaccine use. It consisted of 45 items gathered into 3 main topics described as follows:

1. Socio-demographic information (sex, age, marital status, how many children, any children < 5 years old, education, degree type and medical specialty) and professional characteristics (ASL, workplace, occupational category, type of activity and seniority).
2. Knowledge about serogroup B meningococcal disease and the 4CMenB vaccine (epidemiology of meningococcal meningitis in Italy and its lethality and mortality rate, knowledge about the 4CMenB vaccination and its side effects).
3. Attitudes toward vaccination practices, specifically toward the 4CMenB vaccination (opinions about the 4CMenB vaccine, its efficacy and safety, the opportunity to recommend it and make it mandatory; opinions about the reasons why parents do or do not vaccinate their children), and updating resources (self-evaluation of their own level of knowledge about the 4CMenB vaccine and updating resources used).

Sample size

The number of HCWs needed was determined on the assumption that 75% of the HCWs had appropriate knowledge regarding IMDs and the 4CmenB vaccine, a confidence interval of 95% and a ratio unexp/exp 1:2. The results showed that a total number of 365 HCWs needed to be enrolled in the study.

Data analysis

The data was analysed using Stata: Data Analysis and Statistical Software version 10.1 (Stata Corp LLC, College Station, TX, USA). Following the descriptive analysis, a univariate analysis was performed using a chi-squared test to identify the associations between each independent variable and the outcomes of interest. Then,

only those variables with a p value < 0.25 in the univariate analysis were included in the final multivariate logistic regression models. The p values were assessed using two-sided tests, with the statistical significance for p defined at a value of ≤ 0.05.

The independent variables were the sex, age, number of children, education, occupational category, department type and activity type. The dependent variables were the knowledge about meningococcal meningitis (incidence, most common serogroup in Italy, lethality and mortality rate) and knowledge about the 4CMenB vaccine (vaccine recommended age groups, vaccinations schedule, inclusion or exclusion among recommended vaccinations by the PNPV in Italy and by the Regional Vaccine Prevention Plan in Campania, and risk for adverse reactions when the 4CMenB vaccine is co-administered with another vaccine).

Ethical considerations

All of the participants were informed that the data was collected anonymously and stored in a confidential manner. None of the participants could be identified based on the material submitted, and no incentives were offered to the HCWs for their participation in this study.

Results

A total of 293 HCWs completed the survey, with a response rate of 80.3%. As shown in Table I, 63.8% were women, 65.9% were between 41 and 59 years old, 79.2% were married, 83.6% had at least one child, 6.1% had a child less than 5 years old, 55.6% had graduated, and 7.4% and 88.9% had graduated from nursing and medicine, respectively. Among those who were physicians, the majority were specialists in hygiene and preventive medicine (35.2%), 21.4% were in paediatrics and just a few of them were specialists in infectious diseases (4.8%). Moreover, 50.8% of the HCWs worked in ASLs in Naples, 51.9% worked in UOMIs, the majority were physicians (49.5%), 46.4% were nurses and only 4.1% were other types of HCWs, like medical assistants, biologists and professional educators. Additionally, 51.9% of the participants were directly involved in vaccination programs, 17.1% were in infectious disease surveillance and control, and 23.2% were involved in both activities.

Table II shows the results of the IMD knowledge among the physicians and nurses with relative confidence intervals; 24.1% of the physicians and 17.7% of the nurses reported that the meningococcal meningitis incidence in Italy was not high, while 50.3% of the physicians and only 38.2% of the nurses identified serogroup B as the most common. Regarding the age groups at a higher risk of contracting meningococcal meningitis, 27.3% of the HCWs indicated < 1 year old, 30.6% indicated from 1-4 years old and 27.3% indicated from 15-24 years old; however, only 7.6% of the physicians and 4.4% of the nurses indicated all three age groups that were at a higher risk (data not shown). Moreover, 80.4% of the HCWs indicated that the meningococcal meningitis lethality rate was high, while 48.3% and 36.0%,respectively, indicated that the mortality rate was very low.

Tab. I. Sociodemographic and professional characteristics of the healthcare workers (HCWs) (n = 293).

		n	%
Sex	98	33.5	98
	187	63.8	187
	8	2.7	8
	293	100.0	293
Age	≤ 40	8	2.7
	41–59	193	65.9
	≥ 60	84	28.7
	No response	8	2.7
	Total	293	100.0
Marital status	Married	232	79.2
	Unmarried	23	7.9
	Widow/Widower	4	1.4
	Separate/Divorced	11	3.7
	No response	23	7.8
	Total	293	100.0
Have children	Yes	245	83.6
	No	32	10.9
	No response	16	5.5
	Total	293	100.0
Children less than 5 years old [a]	Yes	15	6.1
	No	230	93.9
	Total	245	100.0
Type of degree [b]	Medicine	145	88.9
	Nursing	12	7.4
	Other	6	3.7
	Total	163	100.0
Medical specialty [c]	Hygiene and preventive medicine	51	35.2
	Paediatrics	31	21.4
	Infectious disease	7	4.8
	Other	17	11.7
	Not specialized	39	26.9
	Total	145	100.0

UOPC: Collective Prevention Operative Unit; UOMI: Maternal Childhood Operative Unit; SEP: Epidemiology Service ; SISP :Public Hygiene Service ; a Calculated only for the HCWs who reported at least one child (n = 245); b Calculated only for the HCWs who graduated (n = 163); c Calculated only for the physicians (n = 145).

The results from the HCWs' knowledge about the 4CmenB vaccine are described in Table III. Most of the participants (69.0%) had at least sufficient knowledge about the 4CMenB vaccine, and 79.4% had sufficient knowledge about its vaccination schedule. However, only 34.1% of the HCWs indicated < 1 year old as the targeted group for the 4CMenB vaccination, while 18.2% and 20.5% indicated 1-4 years old and immunesuppressed individuals, respectively. Only 2.7% of the physicians and 2.9% of the nurses correctly identified all three targeted groups (results not shown). In order to assess their knowledge about the vaccination schedule, the HCWs were asked about the number of doses, timing and whether a booster shot was needed. Only 31.0% of the physicians and 21.3% of the nurses knew the 4CMenB vaccination schedule for all of the age groups.

Tab. II. Healthcare workers' (HCWs) knowledge about the meningococcal meningitis epidemiology (n = 281).

		Physicians		Nurses		Total	
		%	(95% CI)	%	(95% CI)	n	%
In Italy the incidence of meningococcal meningitis is high, do you agree?	Strongly agree	3.4	(1.3-5.4)	4.4	(2.0-6.7)	11	3.9
	Somewhat agree	16.6	(12.3-20.8)	21.3	(16.6-25.9)	53	18.9
	Somewhat disagree*	53.8	(48.0-59.5)	45.6	(39.9-51.3)	140	49.8
	Disagree*	24.1	(19.2-29.0)	17.7	(13.3-22.0)	59	21.0
	Don't know/ No response	2.1	(0.4-3.7)	11.0	(7.4-14.5)	18	6.4
	Total	100.0		100.0		281	100
Which are the most common meningococcus serogroups in Italy?	Serogroup A	0.0		0.0		0	0.0
	Serogroup B*	26.2	(21.1-31.2)	19.1	(14.6-23.6)	64	22.8
	Serogroup B and C*	24.1	(19.2-29.0)	19.1	(14.6-23.6)	61	21.7
	Serogroup C	47.6	(41.8-53.3)	59.6	(53.9-65.2)	150	53.4
	Serogroup Y or serogroup W135	0.0		0.7	(-0.2-1.6)	1	0.3
	Don't know/ No response	2.1	(0.46-3.74)	1.5	(0.1-2.8)	5	1.8
	Total	100.0		100.0		281	100.0
Which are the age categories at higher risk in Italy? (more than one answer allowed)a	< 1 year*	29.2	(23.9-34.4)	25.4	(20.4-30.3)	114	27.3
	1-4 years*	33.0	(27.6-38.3)	28.2	(23.0-33.3)	128	30.6
	5-14 years	10.5	(6.9-14.0)	8.1	(4.9-11.2)	39	9.3
	15-24 years*	22.0	(17.2-26.7)	32.6	(27.2-37.9)	114	27.3
	> 25 years	4.3	(1.9-6.6)	4.3	(1.9-6.6)	18	4.3
	Don't know/ No response	1.0	(-0.1-2.1)	1.4	(0.0-2.7)	5	1.2
	Total	100.0		100.0		418	100.0
Is meningococcal type B meningitis a lethal disease?	Yes*	82.1	(77.7-86.4)	78.7	(74.0-83.3)	226	80.4
	No	10.3	(6.8-13.7)	12.5	(8.7-16.2)	32	11.4
	Don't know/ No response	7.6	(4.5-10.6)	8.8	(5.5-12.0)	23	8.2
	Total	100.0		100.0		281	100.0
How high is the meningococcal type B meningitis mortality rate in Italy?	Very high	2.1	(0.4-3.7)	5.1	(2.5-7.6)	10	3.6
	High	30.3	(25.0-35.5)	28.0	(22.8-33.1)	82	29.2
	Low*	11.0	(7.4-14.5)	14.7	(10.6-18.7)	36	12.8
	Very low*	48.3	(42.5-54.0)	36.0	(30.5-41.5)	119	42.3
	Don't know/ No response	8.3	(5.1-11.4)	16.2	(11.9-20.4)	34	12.1
	Total	100.0		100.0		281	100.0

CI: confidence interval; *Correct answer; a Calculated based on the total number of answers given by the HCWs (n = 418).

Of the HCWs, 62.6% knew that the PNPV recommends the 4CMenB vaccine, while only 30.6% knew that the Regional Campania Vaccine Prevention Plan has not implemented the 4CMenB vaccine.

When assessed with regard to the 4CMenB-related adverse reactions (Tab. IV), 32.0% of the HCWs reported fever and 31.0% reported pain and swelling at the injection site. A few of the HCWs identified gastrointestinal disorders, headaches, somnolence, asthenia, convulsions, pallor, Kawasaki syndrome, malaise and arthralgia among the possible adverse reactions to the vaccine (data not shown). Only 32.8% of the HCWs knew that the risk of developing adverse reactions increases when the 4CMenB vaccine is co-administered with other vaccines. In addition, 40.0% of the physicians and 52.2% of the nurses knew that the 4CMenB vaccine must be administered several days after the other recommended vaccinations, while 36.2% of the physicians and only 15.5% of the nurses knew that the main reason for this was the increased risk for adverse reactions. Most of the physicians (81.4%) and nurses (72.8%) disagreed with the idea that the inclusion of the 4CMenB vaccine in the vaccination schedule would reduce adherence to the other vaccinations.

As shown in Table V, 56.6% of the HCWs stated that the 4CMenB vaccine was extremely efficacious, and 65.5% stated that the vaccine was safe. Additionally, 90.4% of the HCWs stated that encouraging people to allow their children to receive the 4CMenB vaccine was their professional duty, and 53.4% would make it mandatory. The fear of the disease and its complications was the reason most often given by the HCWs (61.9%) to address why the parents chose to vaccinate their children, while the fear of severe adverse reactions (31.6%), disinformation (24.0%) and a poor perception of the disease severity (20.5%) were the most common reasons why the parents chose not to vaccinate their children. Almost all of the HCWs(91.5%) believed that the

Tab. III. Knowledge about the meningococcal serogroup B multicomponent (4CMenB) vaccine (n = 281).

		Physicians		Nurses		Total	
		%	(95% CI)	%	(95% CI)	n	%
What do you consider your overall knowledge about the 4CMenB vaccine to be?	Scarce	8.3	(5.1–11.4)	14.7	(10.6–18.7)	32	11.4
	Not sufficient	19.3	(14.7–23.8)	19.9	(15.3–24.4)	55	19.6
	Sufficient	36.6	(31.0–42.1)	30.1	(24.8–35.3)	94	33.5
	Good	30.3	(25.0–35.5)	35.3	(29.8–40.7)	92	32.7
	Excellent	5.5	(2.8–8.1)	0.0		8	2.8
	Total	100.0		100.0		281	100.0
Are you familiar with the4CMenB vaccination schedule?	Yes	82.0	(77.6–86.4)	76.5	(71.6–81.3)	223	79.4
	No	18.0	(13.6–22.4)	23.5	(18.6–28.3)	58	20.6
	Total	100.0		100.0		281	100.0
Which age groups are recommended to receive the 4CMenB vaccination? (more than one answer allowed)a	< 1 year*	34.9	(29.4–40.3)	33.2	(27.8–38.5)	199	34.1
	1-4 years*	20.5	(15.8–25.1)	15.5	(11.3–19.6)	106	18.2
	5-14 years	8.1	(4.9–11.2)	6.1	(3.3–8.8)	42	7.2
	15-24 years	14.7	(10.6–18.7)	16.3	(12.0–20.5)	90	15.4
	> 25 years	1.3	(0.0–2.6)	3.6	(1.4–5.7)	14	2.4
	Immunosuppressed*	19.5	(14.9–24.0)	21.7	(16.9–26.4)	120	20.5
	Don't know/No response	1.0	(-0.1–2.1)	3.6	(1.4–5.7)	13	2.2
	Total	100.0		100.0		584	100.0
	Infants						
Assessment of the HCWs' knowledge about the 4CMenB vaccination schedule for each of the following age groups:	Correct	47.6	(41.8–53.3)	36.8	(31.2–42.3)	119	42.3
	Incorrect	52.4	(46.6–58.1)	63.2	(57.6–68.7)	162	57.7
	Total	100.0		100.0		281	100.0
	Children						
	Correct	42.8	(37.1–48.4)	39.7	(34.1–45.3)	116	41.3
	Incorrect	57.2	(51.5–62.8)	60.3	(54.7–65.9)	165	58.7
	Total	100.0		100.0		281	100.0
	Teenagers						
	Correct	45.5	(39.8–51.2)	34.6	(29.1–40.0)	113	40.2
	Incorrect	54.5	(48.8–60.2)	65.4	(59.9–70.8)	168	59.8
	Total	100.0		100.0		281	100.0
	All ages						
	Correct	31.0	(25.7–36.3)	21.3	(16.6–25.9)	74	26.3
	Incorrect	69.0	(63.7–74.3)	78.7	(74.0–83.3)	207	73.7
	Total	100.0		100.0		281	100.0
Has the 4CMenB vaccination been included among those recommended by the National Vaccine Prevention Plan in Italy?	Yes*	64.2	(58.7–69.6)	61.0	(55.4–66.5)	176	62.6
	No	18.6	(14.1–23.0)	22.8	(18.0–27.6)	58	20.7
	Don't know	17.2	(12.8–21.5)	16.2	(11.9–20.4)	47	16.7
	Total	100.0		100.0		281	100.0
Has the 4CMenB vaccination been included in the Regional Campania Vaccine Prevention Plan in Campania?	Yes	40.7	(35.0–46.3)	33.8	(28.3–39.2)	105	37.4
	No*	27.6	(22.4–32.7)	33.8	(28.3–39.2)	86	30.6
	Don't know	31.7	(26.3–37.0)	32.4	(27.0–37.7)	90	32.0
	Total	100.0		100.0		281	100.0

CI: confidence interval, HCWs: healthcare workers; * Correct answer; [a] Calculated based on the total number of answers given by the HCWs (n = 584).

distrust that the parents had with regard to vaccinating their children had no scientific basis.

Of the participants, 98.9% stated that HCWs must constantly be updated on vaccination-related scientific evidence, and 85.8% stated that they needed better information. In addition, the HCWs reported courses and conferences (30.5%) and the internet (26.2%) as their major sources of information about the 4CMenB vaccine.

Table VI shows the results of the multivariate models for which significant associations were identified between the variables and the outcomes of interest. The knowledge about the most common serogroup was lower among those HCWs who were < 50 years old [odds ratio (OR) = 2.02, 95% confidence interval (CI) = 1.1-3.6, $p = 0.022$)]. Moreover, the HCWs who worked in the Public Health Department and those who were

Tab. IV. Knowledge about the risks related to the meningococcal serogroup B multicomponent (4CMenB) vaccine (n = 281).

			Physicians		Nurses		Total	
			%	(95% CI)	%	(95% CI)	n	%
How frequently occurring are the following 4CMenB vaccine side effects?	Fever	Correct	34.5	(29.0-39.9)	29.4	(24.1-34.6)	90	32.0
		Incorrect	65.5	(60.0-70.9)	70.6	(65.3-75.8)	191	68.0
		Total	100.0		100.0		281	100.0
	Pain and swelling at the injection site	Correct	32.4	(27.0-37.7)	29.4	(24.1-34.6)	87	31.0
		Incorrect	67.6	(62.2-72.9)	70.6	(65.3-75.8)	194	69.0
		Total	100.0		100.0		281	100.0
	Irritability	Correct	26.2	(21.1-31.2)	21.3	(16.6-25.9)	67	23.8
		Incorrect	73.8	(68.7-78.8)	78.7	(74.0-83.3)	214	76.2
		Total	100.0		100.0		281	100.0
	Cutaneous rash	Correct	6.9	(4.0-9.8)	8.1	(4.9-11.2)	21	7.5
		Incorrect	93.1	(90.2-96.0)	91.9	(88.7-95.0)	260	92.5
		Total	100.0		100.0		281	100.0
Does the risk of developing an adverse reaction increase when the 4CMenB vaccine is co-administered with another vaccine?		Yes*	34.5	(29.0-39.9)	30.9	(25.6-36.1)	92	32.8
		No	46.2	(40.4-51.9)	48.5	(42.7-54.2)	133	47.3
		Don't know	19.3	(14.7-23.8)	20.6	(15.9-25.2)	56	19.9
		Total	100.0		100.0		281	100.0
Must the 4CMenB vaccine be administered several days after any other vaccination?		Yes*	40.0	(34.3-45.6)	52.2	(46.4-57.9)	129	45.9
		No	28.3	(23.1-33.4)	22.8	(18.0-27.6)	72	25.6
		It makes no difference	20.7	(16.0-25.3)	10.3	(6.8-13.7)	44	15.7
		Don't know	11.0	(7.4-14.5)	14.7	(10.6-18.7)	36	12.8
		Total	100.0		100.0		281	100.0
Why must the 4CMenB vaccine be administered several days after any other vaccination? (more than one answer allowed) [a]		To avoid an increased risk for adverse reactions*	36.2	(30.7-41.7)	15.5	(11.3-19.6)	32	24.8
		Vaccination schedule is too busy	15.5	(11.3-19.6)	22.5	(17.7-27.2)	25	19.4
		To better asses adverse reactions	10.4	(6.9-13.9)	4.3	(1.9-6.6)	9	7.0
		To improve patient compliance	6.9	(4.0-9.8)	5.6	(2.9-8.2)	8	6.9
		To avoid hyperstimulation of immune system	6.9	(4.0-9.8)	1.4	(0.0-2.7)	5	3.9
		To reduce parents' anxiety	0.0		2.8	(0.9-4.6)	2	1.4
		No response	24.1	(19.2-29.0)	47.9	(42.1-53.6)	46	35.6
		Total	100.0		100.0		129	100.0
In your opinion, would any other vaccination be refused if the 4CMenB vaccination was added to the vaccination schedule?		Yes	2.1	(0.4-3.7)	7.4	(4.4-10.4)	13	4.6
		No	81.4	(76.9-85.8)	72.8	(67.7-77.9)	217	77.2
		Don't know	16.5	(12.2-20.7)	19.8	(15.2-24.3)	51	18.2
		Total	100.0		100.0		281	100.0
If yes, which of the following vaccinations would be refused? (more than one answer allowed) [b]		Trivalent vaccine	16.7	(12.4-20.9)	43.8	(38.1-49.4)	8	36.4
		Pneumococcus	33.3	(27.9-38.7)	25.0	(20.0-29.9)	6	27.3
		Hexavalent vaccine	16.7	(12.4-20.9)	25.0	(20.0-29.9)	5	22.7
		Meningococcal C	33.3	(27.9-38.7)	6.2	(3.4-8.9)	3	13.6
		Total	100.0		100.0		22	100.0

CI: confidence interval; * Correct answer; [a] Calculated based on the total number of answers given by the healthcare workers (n = 129); [b] Calculated based on the total number of answers given by the healthcare workers who answered yes to the previous question (n = 22).

Tab. V. Healthcare workers' (HCWs) attitudes toward the meningococcal serogroup B multicomponent (4CMenB) vaccine use and updating sources (n = 281).

		Physicians		Nurses		Total	
		%	(95% CI)	%	(95% CI)	n	%
Is the 4CMenB vaccine efficacious in preventing bacterial meningitis?	Extremely efficacious	55.9	(50.2-61.5)	57.4	(51.7-63.0)	159	56.6
	Efficacious	40.0	(34.3-45.6)	39.7	(34.1-45.3)	112	39.9
	Scarcely efficacious	4.1	(1.8-6.3)	2.9	(0.9-4.8)	10	3.5
	Inefficacious	0.0		0.0		0	0.0
	Total	100.0		100.0		281	100.0
Is the 4CMenB vaccine safe?	Extremely safe	28.3	(23.1-33.4)	30.9	(25.6-36.1)	83	29.6
	Safe	66.2	(60.7-71.6)	64.7	(59.2-70.1)	184	65.5
	Scarcely safe	5.5	(2.8-8.1)	1.5	(0.1-2.8)	10	3.5
	Not safe	0.0		2.9	(0.9-4.8)	4	1.4
	Total	100.0		100.0		281	100.0
Do you consider encouraging people to get the 4CMenB vaccination to be an HCWs' professional duty?	Yes	91.7	(88.5-94.8)	89.0	(85.4-92.5)	254	90.4
	No	2.1	(0.4-3.7)	2.9	(0.9-4.8)	7	2.5
	Don't know	6.2	(3.4-8.9)	8.1	(4.9-11.2)	20	7.1
	Total	100.0		100.0		281	100.0
Would you agree to make the 4CMenB vaccination mandatory?	Yes	56.5	(50.8-62.1)	50.0	(44.2-55.7)	150	53.4
	No	26.9	(21.8-31.9)	27.9	(22.7-33.0)	77	27.4
	Don't know	16.6	(12.3-20.8)	22.1	(17.3-26.8)	54	19.2
	Total	100.0		100.0		281	100.0
Why do people decide to vaccinate their children? (more than one answer allowed)[a]	Fear of the disease and its complications	60.7	(55.1-66.2)	63.3	(57.7-68.8)	245	61.9
	Vaccination is mandatory	25.6	(20.6-30.6)	24.9	(19.9-29.8)	100	25.3
	Vaccine is safe	10.4	(6.9-13.9)	9.7	(6.3-13.0)	40	10.1
	Correct information provided by HCWs	1.4	(0.0-2.7)	0.5	(-0.3-1.3)	4	1.0
	Trust the source that recommends the vaccination	0.5	(-0.3-1.3)	0.5	(-0.3-1.3)	2	0.4
	Increased number of new cases within the community	0.5	(-0.3-1.3)	0.0		1	0.3
	Don't know	0.9	(-0.1-1.9)	1.1	(0.0-2.2)	4	1.0
	Total	100.0		100.0		396	100.0
Why do people decide not to vaccinate their children? (more than one answer allowed)[b]	Fear of adverse reactions	31.8	(26.4-37.1)	31.3	(25.9-36.6)	208	31.6
	Disinformation	21.3	(16.6-25.9)	26.9	(21.8-31.9)	158	24.0
	Poor perception of disease severity	22.4	(17.6-27.1)	18.4	(13.9-22.8)	135	20.5
	Vaccine is not mandatory	11.1	(7.5-14.7)	10.4	(6.9-13.9)	71	10.8
	Fear of unknown long-term effects	8.2	(5.0-11.3)	8.9	(5.6-12.1)	56	8.5
	Vaccination schedule is too busy	4.0	(1.7-6.2)	3.2	(1.1-5.2)	24	3.6
	Costs	0.3	(-0.3-0.9)	0.6	(-0.2-1.4)	3	0.4
	Advertisement campaigns on websites	0.6	(-0.2-1.4)	0.0		2	0.3
	Don't know	0.3	(-0.3-0.9)	0.3	(-0.3-0.9)	2	0.3
	Total	100.0		100.0		659	100.0
Do you consider the parents' choice not to vaccinate their children to be scientifically based?	Yes	1.4	(0.0-2.7)	1.5	(0.1-2.8)	4	1.4
	No	93.8	(91.0-96.5)	89.0	(85.4-92.5)	257	91.5
	Don't know	4.8	(2.3-7.2)	9.5	(6.1-12.8)	20	7.1
	Total	100.0		100.0		281	100.0
Must HCWs be constantly updated on vaccination-related scientific evidence?	Yes	99.3	(98.3-100.2)	98.5	(97.1-99.8)	278	98.9
	No	0.0		0.0		0	0.0
	Don't know	0.7	(-0.2-1.6)	1.5	(0.1-2.8)	3	1.1
	Total	100.0		100.0		281	100.0
Do you need to be better informed about the 4CMenB vaccine?	Yes	82.1	(77.7-86.4)	89.7	(86.2-93.1)	241	85.8
	No	13.1	(9.2-16.9)	7.3	(4.3-10.2)	29	10.3
	Don't know	4.8	(2.3-7.2)	3.0	(1.0-4.9)	11	3.9
	Total	100.0		100.0		281	100.0

continues

Tab. V. *follows.*

How did you get informed about the 4CMenB vaccine? (more than one answer allowed) c	Updating courses/congresses	7.6	(4.5–10.6)	11.4	(7.7–15.0)	46	9.3
	Internet	9.7	(6.3–13.0)	5.0	(2.5–7.5)	38	7.7
	National journals	1.5	(0.1–2.8)	7.8	(4.7–10.8)	21	4.2
	Pharmaceutical promoters	0.7	(-0.2–1.6)	3.2	(1.1–5.2)	9	1.8
	International journals	0.0		3.6	(1.4–5.7)	8	1.6
	Television	0.4	(-0.3–1.1)	0.5	(-0.3–1.3)	2	0.4
	Vaccine information leaflets	2.2	(0.5–3.8)	3.6	(1.4–5.7)	14	2.8
	Colleagues	1.1	(0.0–2.2)	1.4	(0.0–2.7)	6	1.2
	National health plan	100.0		100.0		496	100.0
	No source of updating	7.6	(4.5–10.6)	11.4	(7.7–15.0)	46	9.3
	No response	9.7	(6.3–13.0)	5.0	(2.5–7.5)	38	7.7
	Total	1.5	(0.1–2.8)	7.8	(4.7–10.8)	21	4.2

CI: confidence interval; [a] Calculated based on the total number of answers given by the HCWs (n = 396); [b] Calculated based on the total number of answers given by the HCWs (n = 659); [c] Calculated based on the total number of answers given by the HCWs (n = 496).

involved in the surveillance and control of the disease (OR = 0.29, 95% CI = 0.1–0.5, $p = 0.000$ and OR = 0.35, 95% CI = 0.1-0.8, $p = 0.015$, respectively) were more likely to have this knowledge. Being male and being involved in the surveillance and control of the disease (OR = 0.51, 95% CI = 0.2-0.9, $p = 0.034$ and OR = 0.34, 95% CI = 0.1-0.8, $p = 0.014$, respectively) were associated with greater knowledge about the meningococcal B meningitides mortality rate.

Working in the Public Health Department (OR = 3.31, 95% CI = 1.6-6.7, $p = 0.001$) was the only variable associated with knowledge about the 4CMenB vaccination schedule. Additionally, knowledge about the National Health Plan was lower among those who worked in the Public Health Department (OR = 3.22, 95% CI = 1.8-5.5, $p = 0.000$). Being involved in the surveillance and control of the disease(OR = 2.83, 95% CI = 1.2-6.3, $p = 0.012$) was associated with lesser knowledge about the Regional Health Plan, while this knowledge was higher among the HCWs who worked in the Public Health Department (OR = 0.36, 95% CI = 0.2-0.6, $p = 0.001$). Not having children and working in the Public Health Department (OR = 0.41, 95% CI = 0.1-0.8, $p = 0.020$ and OR = 1.76, 95% CI = 1.0-2.9, $p = 0.037$, respectively) were associated with knowledge about the increased risk for adverse reactions when the 4CMenB vaccine was co-administered with another vaccine.

Discussion

This study was conducted after the 4CMenB vaccine was placed on the market. This vaccine specifically prevents serogroup B meningitis, which is the serogroup most frequently involved in this disease, and against which no traditionally made vaccines were previously available. Since its approval for use, many concerns about the most appropriate vaccination strategy have been raised within the international and national scientific communities [17]. In fact, in the years following the 4CMenB vaccine being placed on the market, the scientists and public health advisors in charge of health policies have had different opinions on how to provide the 4CMenB vaccine to the general population [14]. Therefore, the aim of the present study was to assess HCWs' knowledge about the 4CMenB vaccine and its vaccination strategy, while considering the role HCWs play in implementing vaccination coverage (whatever their position) within operative or decision-making units.

The present analysis determined that the majority of HCWs have sufficient knowledge about the lethality of the disease, but they are less informed about the incidence, higher risk age categories and most frequent serogroups involved. Many of them are confused about what is meant by mortality and lethality, and they mistakenly consider this disease to have a high mortality rate in the general population. The majority of the HCWs considered their knowledge about the vaccine and its vaccination schedule to be good, but only a few identified all of the targeted categories. This is particularly evident when considering immunosuppressed individuals, who are considered by the scientific community to be the group at highest risk and the most appropriate to receive the vaccine; however, they were identified as a target category by few of the HCWs. Moreover, some of the HCWs' answers were not consistent. For instance, one-third of the nurses believed that administering the 4CMenB vaccine with another vaccine enhanced the risk for adverse reactions, but only 15.0% indicated that the 4CMenB vaccine must be administered several days after another vaccine in order to reduce the risk for adverse reactions. Interestingly, those HCWs involved in surveillance and control activities had more knowledge about the epidemiological characteristics of the disease when compared with those working in the UOMI, where the HCWs are mainly involved in administering the vaccine to children. However, the HCWs working in the UOMI had better knowledge about the 4CmenB vaccination strategy and adverse reactions.

It was unexpected that only a few of the HCWs knew about the PNPV (60.0% of the HCWs) and Campania Vaccine Prevention Plan (30.0% of the HCWs) indications about 4CmenB vaccine use, considering that both of these documents represent reference tools for HCWs.

One limitation of this study was that the questionnaire was self-administered; therefore, we cannot be sure that

Tab. VI. Knowledge about the meningococcal meningitis epidemiology and meningococcal serogroup B multicomponent (4CMenB) vaccine.

	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
HCWs' knowledge about the most common meningococcal serogroup in Italy				
Sex (Male vs. Female*)	0.76 (0.4-1.2)	0.284	n.v.	
Age (< 50 vs. ≥ 50*)	1.74 (1.0-2.9)	0.037	2.02 (1.1-3.6)	0.022
Children (No vs. Yes*)	1.28 (0.6-2.7)	0.513	n.v.	
Degree (No vs. Yes*)	1.90 (1.1-3.1)	0.010	1.68 (0.4-6.3)	0.440
Profession (Doctor vs. Nurse*)	0.58 (0.3-0.9)	0.023	1.62 (0.4-6.0)	0.474
Department (Public health vs. Maternal childhood*)	0.17 (0.1-0.2)	0.000	0.29 (0.1-0.5)	0.000
Activity (Surveillance and control vs. Vaccination program*)	0.18 (0.0-0.3)	0.000	0.35 (0.1-0.8)	0.015
HCWs' knowledge about the meningococcal meningitis mortality rate				
Sex (Male vs. Female*)	0.47 (0.2-0.8)	0.005	0.51 (0.2-0.9)	0.034
Age (< 50 vs.≥ 50*)	1.54 (0.9-2.6)	0.108	1.15 (0.6-2.0)	0.645
Children (No vs. Yes*)	1.09 (0.5-2.3)	0.811	n.v.	
Degree (No vs. Yes*)	1.43 (0.8-2.3)	0.145	1.28 (0.3-4.5)	0.704
Profession (Doctor vs. Nurse*)	0.71 (0.4-1.1)	0.149	1.59 (0.4-5.8)	0.481
Department (Public health vs. Maternal childhood*)	0.54 (0.3-0.8)	0.013	0.80 (0.4-1.4)	0.464
Activity (Surveillance and control vs. Vaccination program*)	0.36 (0.1-0.7)	0.005	0.34 (0.1-0.8)	0.014
HCWs' knowledge about the 4CMenB vaccination schedule				
Sex (Male vs. Female*)	0.98 (0.5-1.7)	0.932	n.v.	
Age (< 50 vs.≥ 50*)	0.77 (0.4-1.4)	0.389	n.v.	
Children (No vs. Yes*)	0.69 (0.3-1.5)	0.358	n.v.	
Degree (No vs. Yes*)	1.53 (0.8-2.6)	0.133	0.90 (0.1-4.6)	0.901
Profession (Doctor vs. Nurse*)	0.60 (0.3-1.0)	0.066	0.32 (0.0-1.6)	0.174
Department (Public health vs. Maternal childhood*)	2.95 (1.6-5.2)	0.000	3.31 (1.6-6.7)	0.001
Activity (Surveillance and control vs. Vaccination program*)	2.04 (0.9-4.6)	0.087	1.40 (0.5-3.6)	0.487
HCWs' knowledge about the 4CMenB vaccination being included among those recommended by the National Vaccine Prevention Plan				
Sex (Male vs. Female*)	1.78 (1.0-2.9)	0.026	1.52 (0.8-2.6)	0.138
Age (< 50 vs.≥ 50*)	0.99 (0.5-1.6)	0.983	n.v.	
Children (No vs. Yes*)	0.51 (0.2-1.1)	0.118	0.46 (0.1-1.1)	0.086
Degree (No vs. Yes*)	1.18 (0.7-1.9)	0.515	n.v.	
Profession (Doctor vs. Nurse*)	0.88 (0.5-1.4)	0.590	n.v.	
Department (Public health vs. Maternal childhood*)	3.04 (1.8-5.0)	0.000	3.22 (1.8-5.5)	0.000
Activity (Surveillance and control vs. Vaccination program*)	1.07 (0.5-2.0)	0.842	n.v.	
HCWs' knowledge about the 4CMenB vaccination not being included among those implemented by the Regional Vaccine Prevention Plan in Campania				
Sex (Male vs. Female*)	0.81 (0.4-1.3)	0.446	n.v.	
Age (< 50 vs.≥ 50*)	0.93 (0.5-1.6)	0.811	n.v.	
Children (No vs. Yes*)	1.42 (0.6-3.3)	0.413	n.v.	
Degree (No vs. Yes*)	0.85 (0.5-1.4)	0.536	n.v.	
Profession (Doctor vs. Nurse*)	1.34 (0.8-2.2)	0.257	n.v.	
Department (Public health vs. Maternal childhood*)	0.50 (0.3-0.8)	0.010	0.36 (0.2-0.6)	0.001
Activity (Surveillance and control vs. Vaccination program*)	1.56 (0.7-3.2)	0.238	2.83 (1.2-6.3)	0.012
HCWs' knowledge about the increased risk for adverse reactions when the 4CMenB vaccine is co-administered with another vaccine				
Sex (Male vs. Female*)	1.02 (0.6-1.7)	0.931	n.v.	
Age (< 50 vs.≥ 50*)	0.97 (0.5-1.6)	0.919	n.v.	
Children (No vs. Yes*)	0.44 (0.2-0.9)	0.030	0.41 (0.1-0.8)	0.020
Degree (No vs. Yes*)	1.03 (0.6-1.7)	0.923	n.v.	
Profession (Doctor vs. Nurse*)	0.85 (0.5-1.4)	0.521	n.v.	
Department (Public health vs. Maternal childhood*)	1.78 (1.0-2.9)	0.025	1.76 (1.0-2.9)	0.037
Activity (Surveillance and control vs. Vaccination program*)	1.38 (0.6-2.7)	0.368	n.v.	

OR: odds ratio; CI: confidence interval; HCWs: healthcare workers; n.v.: not valuated (p > 0.250 in the univariate analysis); * Reference category

the participants responded without having first been informed about the topics of interest. However, the results from the present analysis did show that the HCWs' knowledge was often partial and incorrect.

Overall, it must be noted that all of the HCWs were still convinced that vaccinations are important instruments for infectious disease prevention, and they were aware of the key role that they play in promoting 4CmenB vac-

cinations, as well as other vaccines, and their need to be better informed.

Conclusions

This study highlights the importance of and need to implement professional training courses for HCWs with interactive teaching methods. These should be suitable for an audience of experienced HCWs, as focus group, specific to the epidemiological aspects of meningococcal disease and the 4CmenB vaccine. These interventions would be useful for ensuring that HCWs are able to correctly answer patients' questions about the vaccine risks and benefits, because they represent the interface between public institutions and citizens [23].

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Conflict of interest statement

None declared.

Authors' contributions

EA conceived the study and revised it critically for important intellectual content; DP participated in its design and coordination, in interpretation data and wrote the manuscript; MGC revised the manuscript and contributed to data interpretation; AD has been involved in acquisition data and performed data entry and statistical analysis and contributed to data interpretation.

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ORIGINAL ARTICLE

Practice of safety measures among inter-city commercial vehicle drivers in Kwara State, Nigeria

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Keywords

Practice • Safety • Measures • Commercial • Drivers

Summary

Introduction. *The incidence of Road Traffic Crashes (RTC) is rising world-wide, with 1.24 million people killed on the world's roads in 2010 due to non-compliance with safety measures. The objectives of the study was to determine the practice of safety measures and prevalence of road crashes among inter-city commercial vehicle drivers in Kwara State, Nigeria.*

Methods. *A descriptive cross-sectional study done by interviewer-administered questionnaire and blood alcohol concentration of respondents was determined using Breathalyzers. A total of 410 respondents were involved by multi-stage sampling technique; data analysis was done using EPI INFO version 3.5.1 software package. Level of significance was < 0.05 at 95% confidence level.*

Results. *More than eighty per cent of the respondents practiced safety measures and checked their vehicles before embarking on a journey. More respondents who practiced safety measures carried out driving test before issuance of license compared with those who did not (p = 0.001). Some respondents tested positive for alcohol with mean blood alcohol concentration of 23.28 ± 23.32 µg/dl. About a third of the respondents had road traffic crashes in the past.*

Conclusions. *The drivers demonstrated good safety measures and practices before embarking on a journey. Safety practices were influenced by driving test before issuance of license. Sensitization and orientation of drivers on relevance of driving test before issuance of driving license should be promoted by all stakeholders in road safety. The enforcement of laws by government is critical to addressing challenges of road safety and security by ensuring appropriate driving test before licensing.*

Introduction

Motorized road transport among others has changed the face of employment, trade, family life and health care, bringing benefits that were unimaginable 100 years ago. However, the price being paid as a result of road traffic injury for such benefits is too high. Worldwide, Road Traffic Crashes (RTC) lead to death and disability as well as high financial cost to the individual involved, family, community and society at large. The incidence of RTC is rising worldwide, in 2004, about 1.2 million people were known to die in road accidents worldwide [1] and 1.24 million people were killed on the world's roads in 2010 [2].

More than half the people killed in traffic crashes are young adults aged between 15 and 44 years, often the breadwinners in a family. In Nigeria, a study conducted in Ilorin, Kwara State revealed that over three-quarters of the victims of road traffic crashes are young people [3]. Furthermore, road traffic injuries cost low-income and middle-income countries between 1% and 2% of their gross national product, more than the total development aid received by these countries [1]. Enormous human potential was being destroyed, with grave social and economic consequences [1]. Losses are not limited to reduced worker productivity and trauma affecting a victim's private life. Equally significant are the rising costs in health services and the added burden on public finances.

The negligence of safety practices among motorists is one of the major factors affecting road traffic crashes. Vehicles are poorly maintained due to poverty, ignorance and corruption among enforcement agents and other stakeholders [4]. In 2013, WHO reported that deaths from road traffic crash in 2010 were 33.7/100,000 (Nigeria), 22.2/100,000 (Ghana) and 31.2/100,000 (South Africa). Among behavioural factors, alcohol plays an important role in car crashes, and accidents involving alcohol are more likely to result in injuries and deaths than crashes where alcohol is not a factor. Traffic laws such as National drink-driving law, drink-driving law defined by Blood Alcohol Concentration (BAC) and random breath testing at police check point used for enforcement are available in the countries however, the effectiveness of overall enforcement (respondent consensus: scale 0-10) vary between the three countries. While Ghana had a score of 3, Nigeria and South Africa had a score of 2 [2]. Despite global progress in strengthening drink-driving legislation, only 39 countries have their enforcement as "good" (8 or above on a scale of 0 to 10), indicating that better implementation of these laws needs urgent attention [5]. The objective of this study was therefore, to determine the safety practices of inter-city commercial drivers in Kwara State, Nigeria.

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Methods

The study was conducted in Kwara State, Nigeria. The State is one of the 36 states in the country located in the North central geo-political zone of the country. It was a descriptive cross-sectional study of road safety practices among inter-city commercial drivers in Kwara State, Nigeria. About 95% of the drivers in the State were licensed to drive. Quantitative data was collected using an interviewer administered questionnaire and blood alcohol concentration of respondents was determined using Breathalyzers. The questionnaire was pre-tested in a neighboring Oyo State which is 45 km to the study sites. Corrections and validation were done based on the outcome of the pre-test. The validation was done by senior researchers in the University. Only commercial drivers registered with Kwara State Chapters of the respective associations and those whose primary occupation or main source of livelihood is driving, and convey passengers on inter-city with motor vehicles which could be buses or cars were involved in the study. A total of 410 respondents were used for the study.

The study was carried out in the three senatorial districts of Kwara State. Proportional allocation of respondents was done based on population from each senatorial district. Multi-stage sampling technique was used. In stage one, simple random sampling technique using the balloting method was adopted to select four major parks in each of the Kwara Senatorial districts. In each of the parks selected, systematic sampling method was used to select desired number of respondents among commercial drivers based on proportional allocation. The list of the drivers in the selected parks served as the sampling frame. Replacement with the next person on the sampling frame was done for those not available or unwilling to participate in the study. Breathalyzer was used to determine the Blood Alcohol Concentration (BAC) of the commercial vehicle drivers. This assessment was done in a room within the garage office of the drivers to ensure confidentiality. The measurements were done by the Researcher and supported by two research assistants who are medical doctors in postgraduate training. The test subject must be able to provide the required minimum respiratory volume of 1.2 L. for this purpose; the breathing flow must be constant for a certain minimum blowing period [6].

The sampling is automatically triggered after reaching the minimum respiratory volume and the minimum blowing period. The test subject must breathe evenly and continuously in to the mouthpiece [6]. A continuous tone sounds and the green lamp flashes when a sufficient breathing flow volume has been reached. "Blow" appears on the display while the breath sample is taken.

After delivering a sufficient breath sample, the green lamp goes out and the continuous tone falls silent. "Wait Analyzing" appears on the display. After 5 to 25 seconds (depending on the device temperature and the measured concentration), the measuring result appears on the display [6]. The accuracy of the Drager AG and Co breathalyzer used was > 98%.

Data was collected and edited manually to detect omission and to ensure uniform coding, after which data was entered into the computer. Data analysis was done using EPI INFO version 3.5.1 and SPSS software package. Important variables from data collected were presented as frequency tables, and cross tabulation. An appropriate test of significance (Chi-square, t test) were used to test statistics and the level of significance was predetermined at less than 0.05 at 95% confidence level. Ethical approval for study was obtained from the Ethical Review Committee, University of Ilorin. Permission was sought from the different motor parks chairmen. Informed consent was obtained before interview from the respondents while explaining that any information provided would be confidential. The purpose and benefits of the study were explained accordingly.

Results

In Table I, the mean age of the respondents was 46.7 ± 11.27 years. Men constituted 99.8% of the respondents. Majority 370 (90.3%) of respondents were Muslims and Yoruba ethnicity accounted for 387 (94.4%) respondents. On literacy level, 18 (4.4%) had tertiary, 103 (25.1%) secondary and 123 (30.0%) had no formal education. Majority 94.6% were married, 2.2% were widowers and 3.2% were single. Participation was 100%.

From Table II, majority of the respondents practiced safety measures which include obeying speed limit 339 (82.7%), obeying traffic light 363 (88.5%), observing road signs 367 (89.5%), using belts 351 (85.6%), resting when fatigued 355 (86.6%), maintaining the vehicle in good condition 368 (89.8%), and not ingesting alcohol while driving 361 (88.0%).

Concerning safety checks of the vehicles, majority of the respondents checked water level in radiator 371 (90.5%), lighting system 371 (90.5%), brakes and clutches 372 (90.7%), horns and spare tyre 370 (90.2%) as seen in Table III.

In Table IV, about half 210 (51.2%) of the respondent that had good safety practices were on the job for between 11-30 years, while 103 (25.1%) who had good safety practices had spent more than 30 years as drivers. Only 59 (14.4%) of those who had within 10 years' experience had good safety practices. The difference in safety practices and years of experience on the job was not statistically significant $p = 0.680$. Possession and validity of driver's license have no statistical significant relationship with practice of safety measures among respondents $p = 0.949$, 0.679 respectively. Of all respondents with driver's license, 297 (75.6%) had good safety practices and did test before licensed. There was statistical significant relationship between practice of safety measures and subjected to driving test before issuance of license $p = 0.001$.

As shown in Table V, many respondents 262 (63.9%) who had good safety practices had no history of RTC compared with 110 (26.8%) who had RTC. The difference in the level of safety practices and ever had RTC was not statistically significant $p = 0.938$. The number of RTC, severity of injury sustained and the last time respondents had RTC

Tab. I. Socio-demographic variables of respondents (n = 410).

Socio-demographic variables	Frequency	Percent (%)
Age group (years)		
≤ 25	16	3.9
26-35	62	15.1
36-45	131	32.0
46-55	109	26.6
56 -65	74	18.0
> 65	18	4.4
Mean ± SD	46.78 ± 11.27	
Median (IQR)	45.00 (40.00-55.00)	
Range	22-73	
Sex		
Male	409	99.8
Female	1	0.2
Religion		
Islam	370	90.3
Christianity	39	9.5
Others	1	0.2
Ethnicity		
Yoruba	387	94.4
Hausa	8	2.0
Igbo	6	1.5
Others	9	2.1
Educational level		
None	123	30.0
Primary	166	40.5
Secondary	103	25.1
Tertiary	18	4.4
Marital status		
Single	13	3.2
Married	388	94.6
Widowed	9	2.2

SD: standard deviation; IQR: inter-quartile range.

had no significant relationship with safety practices while driving p = 0.506, 0.881, 0.902 respectively.

The respondents that tested positive for alcohol were 29 (7.1%), while 381 (92.9%) were negative. The mean level of blood alcohol concentration was 23.28 ± 23.32 µg/dl (Tab. VI).

Tab. II. Safety measures practiced by the respondents while driving (n = 410).

	Always	Sometimes	No
Safety measures	**n (%)**	**n (%)**	**n (%)**
Obey speed limit	339 (82.7)	23 (5.6)	48 (11.7)
Obey traffic lights	363 (88.5)	10 (2.4)	37 (9.0)
Observe road signs	367 (89.5)	5 (1.2)	38 (9.3)
Use seat belt	351 (85.6)	12 (2.9)	47 (11.5)
Rest when fatigued/ stressed during driving	355 (86.6)	13 (3.2)	42 (10.2)
Maintain vehicle in good condition	368 (89.8)	2 (0.5)	40 (9.8)
Avoiding alcohol and drugs (kolanut, tobacco, caffeine etc.) while driving	361 (88.0)	4 (1.0)	45 (11.0)

Discussion

The mean age of the respondents in this study was 46.78 ± 11.27 years. This reflected that active population group are involved in commercial driving. Although the age range from 22 to 74 years, this showed the wide spectrum of individuals involved in transportation at early stage of life and continue till older age. On literacy level, a quarter 25.1% of the respondents attended secondary school. Many of the respondents either had primary (40.5%) or no formal education (30.0%). Driving work does not require advance education, therefore, it was not a surprise that three-quarters either had primary or no formal education. In fact, it is because of challenges of job opportunities in Nigeria that probably made the 4.4% of those with tertiary education to be engaged in driving work.

About one-third of the respondents (30.0%) were in driving business for 10 to 20 years and 26.1% had spent between 21 and 30 years on the job. These findings were consistent with age distribution of the respondents because most drivers start learning the art of driving between 15 and 20 years of age. This implied that major motor parks in the State were saturated with experienced and mature drivers where it is expected that compliance to safety rules and regulations will be given high priority. Although, 95.9% had drivers' license, only 75.1% of respondents had valid license. This is far from expectation as these were professional drivers whose only occupation is driving yet possession of license is sub-optimal. It clearly showed that there were gaps in monitoring and enforcement of laws if one in every four commercial drivers has no valid license.

About a third (29.5%) of the respondents had experienced road traffic crashes in the past. Of those who had experienced RTC, 78.5% had one RTC, while 21.5% had two or more RTC. This reflected the magnitude of road crashes in Nigeria. This implied that concerted efforts are required by all stakeholders to address the challenges in road safety and reduce road traffic crashes. In addition, data generation and management should be supported as there may be under reporting of road traffic crashes. Almost two-thirds, 63.6% of respondents that were involved in road crashes reported that the crash occurred in the day time and 36.4%

Tab. III. Safety check conducted on the vehicles by respondents before embarking on journey (n = 410).

Safety check of vehicle	Always	Sometimes	No
	n (%)	**n (%)**	**n (%)**
Water level in the radiator	371 (90.5)	1 (0.2)	38 (9.3)
Oil level	371 (90.5)	1 (0.2)	38 (9.3)
Tyre pressure	370 (90.2)	3 (0.7)	37 (9.0)
Lighting system such as headlamps	371 (90.5)	1 (0.2)	38 (9.8)
Brake and clutches	372 (90.7)	1 (0.2)	37 (9.0)
Wipers	369 (90.0)	3 (0.7)	38 (9.3)
Horns	370 (90.2)	2 (0.5)	38 (9.3)
Gauge tyre before travelling	371 (90.5)	2 (0.5)	37 (9.0)
Battery	372 (90.7)	2 (0.5)	36 (8.8)

Tab. IV. Relationship between practice of safety measures and driving experience of the respondents.

	Practice of safety measures				
	Good	Poor	Total	χ^2	P value
Variable	n (%)	n (%)	n (%)		
Duration driving (years)					
≤ 10	59 (14.4)	5 (1.2)	64 (15.6)	0.769	0.680
11-30	210 (51.2)	20 (4.9)	230 (56.1)		
> 30	103 (25.1)	13 (3.2)	116 (28.3)		
Possession of driver's license					
Yes	356 (86.8)	37 (9.0)	393 (95.9)	0.004^{Y}	0.949
No	16 (3.9)	1 (0.2)	17 (4.1)		
Valid driver's license (n = 385)					
Yes	263 (68.3)	26 (6.8)	289 (75.1)	0.171^{Y}	0.679
No	86 (22.3)	10 (2.6)	96 (24.9)		
Did driving test before given the license (n = 393)					
Yes	297 (75.6)	23 (5.9)	320 (81.4)	10.021	0.001
No	59 (15.1)	14 (3.6)	73 (18.6)		

χ^2: Chi square; *: p value < 0.05; ^{Y}: Yates corrected.

Tab. V. Relationship between practice of safety measures and involvement in road traffic crash by the respondents.

	Practice of safety measures				
	Good	Poor	Total	χ^2	P value
Variable	n (%)	n (%)	n (%)		
Ever had RTC as a driver					
Yes	110 (26.8)	11 (2.7)	121 (29.5)	0.006	0.938
No	262 (63.9)	27 (6.6)	289 (70.5)		
Number of RTC had (n = 121)					
1	85 (70.3)	10 (8.3)	95 (78.5)	0.442^{Y}	0.506
≥ 2	25 (20.7)	1 (0.8)	26 (21.5)		
Severity of RTC (n = 121)					
No injury	78 (64.5)	7 (5.8)	85 (70.3)	0.252^{Y}	0.881
Mild to serious injury	26 (21.5)	4 (3.3)	30 (24.8)		
Fatal	6 (4.9)	0 (0.0)	6 (4.9)		
Last time RTC occurred (years) (n = 121)					
< 1	16 (13.2)	2 (1.7)	18 (14.9)	0.015^{Y}	0.902
≥ 1	94 (77.7)	9 (7.4)	103 (85.1)		

χ^2: Chi square; *: p value <0.05; ^{Y}: Yates corrected.

persons reported that it occurred at night. The occurrence of crash in the night reported in this study is similar to a study conducted in India where 44.16% of crashes occur in the evening hours [7]. However, less than 10% of those who travelled by road were involved in night travel. This implied that travel activity of less than 10% accounted for more than a third of the crashes. Therefore, if night travel is averted, a third of road crashed will be prevented. Additionally, rescue operations and provision of assistance and support services to victims of road crashes are often limited during the night hours.

Tab. VI. Blood alcohol concentration of intercity commercial vehicle drivers just before embarking on a journey (n = 410).

Variable	Frequency	Percent
Alcohol test		
Positive	29	7.1
Negative	381	92.9
Blood alcohol concentration (n = 29)		
Range (min-max)	3-105	
Median (inter-quartile range)	14.00 (7.00-34.00)	
Mean ± SD	23.28 ± 23.32	

On the severity of the RTC, 24.8% reported mild to severe injuries were sustained in this study. It has been reported that in many low-income and middle-income countries, the burden of traffic-related injuries is such that they represent between 30% and 86% of all trauma admissions, millions of others sustain injuries, with some suffering permanent disabilities. Enormous human potential were being destroyed, with serious social and economic consequences [1, 5]. Among those involved in crashes, 14.9% had the last RTC in less than a year while 8.3% had the last RTC in 1-2 years ago. This showed that road crashes is a frequent problem and concerted efforts are required by all relevant stakeholders to combat the menace.

Majority of the respondents practiced safety measures which include obeying speed limit 82.7%, obeying traffic light 88.5%, observing road signs 89.5%, using belts 85.6%, resting when fatigued 86.6%, maintaining the vehicle in good condition 89.8% and not ingesting alcohol while

driving 361 (88.0%). The respondents demonstrated good safety practices in many aspect of driving. This showed that they are experienced drivers and put knowledge garnered over the years to use. Regular and continuous maintenance of vehicle is fundamental to avert road crashes. Concerning safety checks of the vehicles, majority of the respondents checked water level in radiator 90.5%, check lighting system 90.5%, check brakes and clutches 90.7%, check horns and spare tyre 90.2%, have fire extinguishers 87.3% and did wheel alignment and balancing 89.8%. This demonstrated that respondents gave safety practice the necessary priority. One in every ten respondents 9.3% did not have driver's license, and of those who had it, 83.8% possess valid licenses. Monitoring of drivers by regulatory agencies is required because lack of valid license has serious implications on legality of driving. More respondents, 68.3% in the age group 26-55 years had good safety practices compared with those ≤ 25 years 3.2% and > 55 years 19.3%. However, the observed difference was not statistically significant p = 0.057. Literacy level of the respondents had no statistical significant relationship with practice of safety measures p = 0.373.

Of all respondents with driver's license, three-quarters (75.6%) had good safety practices and carried out driving test before issuance of license. There was statistical significant relationship between those that practiced safety measures and those who carried out driving test before issuance of license p = 0.001. It is obvious that those who carried out driving test were more likely to conform to standard practice. Many respondents 63.9% who had good safety practices had no history of RTC compared with 26.8% who had RTC. The difference in the level of safety practices and ever had RTC was however, not statistically significant p = 0.938. Though not statistically significant, it showed good safety practice may reduce road crashes. The respondents that tested positive for alcohol were 7.1%, and the mean level of blood alcohol concentration was 23.28 ± 23.32 μg/dl. Alcohol has strong association with RTC and this may be one of the major factors responsible for increasing road crashes in Nigeria. This implied that monitoring of commercial drivers is required to enhance road safety. This will provide the basis of strategic interventions and will reduce alcohol consumption while driving as many commuters are in danger with drunk driver.

Conclusions

Majority of inter-city commercial drivers demonstrated good safety practices in many domains. More respondents who practiced safety measures carried out driving test before issuance of license compared with those who did not (p = 0.001). Some respondents tested positive for alcohol and the mean blood alcohol concentration was 23.28 ± 23.32 μg/dl. About a third of the respondents had experienced road traffic crashes in the past. Night travel was responsible for more than a third of road traffic crashes but less than 10% travelled during this period. It is recommended that restriction of night travel, continuous orientation of the drivers and enforcement of relevant laws are critical to addressing the challenges of road safety in Nigeria while supporting research and development for informed decisions.

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Conflict of interest statement

None declared.

Authors' contributions

AGS-Conceptualization, data collection and analysis; manuscript preparation. KAD - Data collection, analysis, manuscript preparation and editing. ASY - Data collection and manuscript preparation. MFA - Data collection.

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