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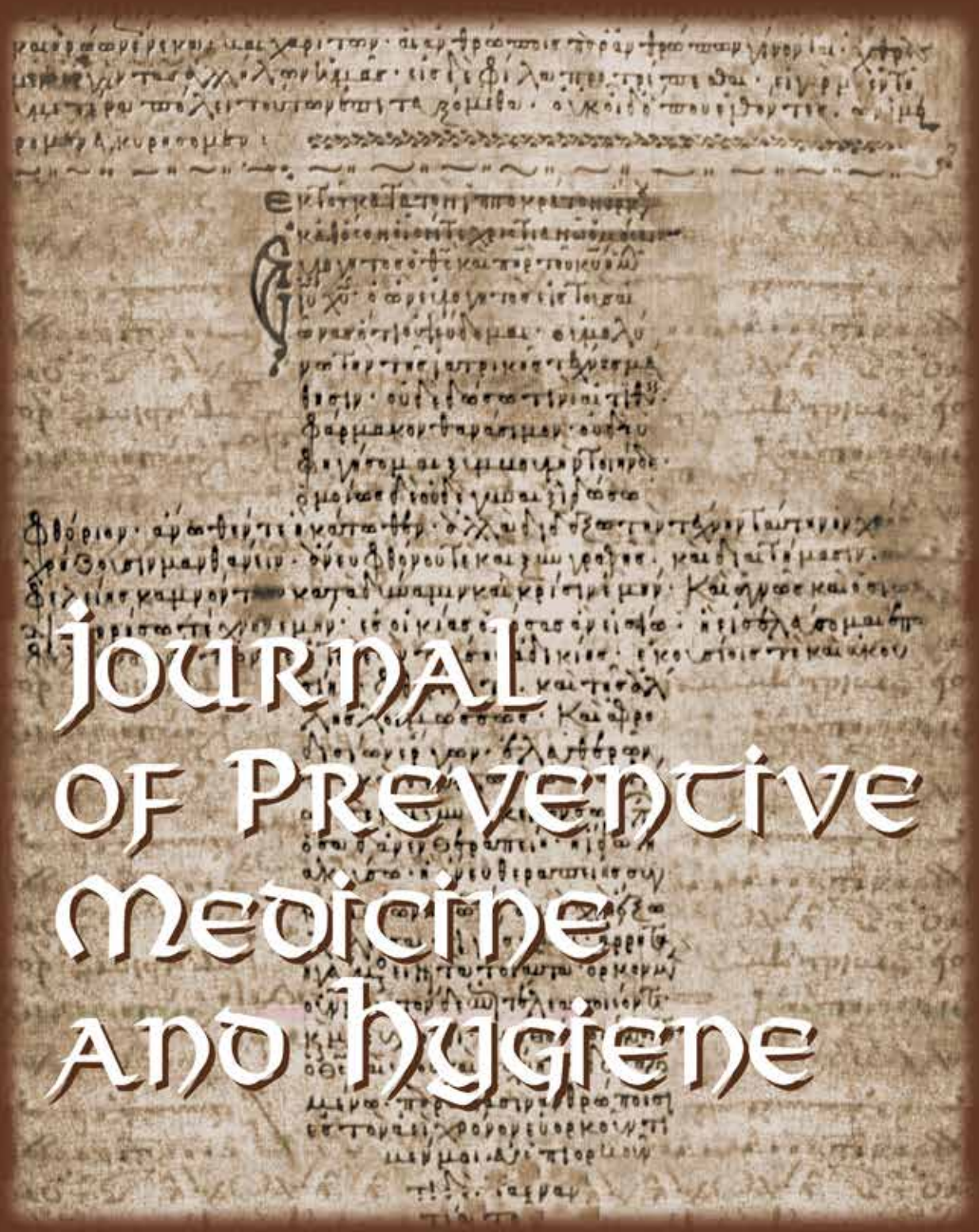

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CONTENTS

Original articles

ORIGINAL ARTICLE

Cervical Human Papillomavirus genotypes in HIV-infected women: a cross-sectional analysis of the VALHIDATE study

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Keywords

HIV-infected women • HPV types • Cervical lesions • Molecular Epidemiology

Summary

Introduction. *Primary-prevention by prophylactic vaccination against HPV-related cancers and HPV-based screening programs are based on HPV-type distribution in immunocompetent individuals. HIV-infected women are at high risk of invasive HPV-disease sustained by a broader range of HPV-types and have higher multi-type infection rates than immunocompetent hosts.*

Methods. *This is a cross-sectional analysis of High Risk HPV (HR HPV) type distribution in 805 HIV+ women (HIW) compared with a control group of 1402 immunocompetent HIV- women (SPW) enrolled in the VALHIDATE study in order to define HPV type-specific distribution according to cytology.*

Results. *HIW had a 3.8, 3.6, and 2.7 times higher risk of atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) than SPW respectively. HPV-DNA prevalence was 28.4% in HIW and 11.81% in SPW ($p<0.0001$). The prevalence of infection increased from normal cytology to HSIL both in HIW (from 21.45% to 90.91%) and SPW (from 9.54% to 75%). The OR for women with normal cytology of having a positive HPV-DNA test result of was 2.6 times higher in HIW than in SPW. The cumulative prevalence of HPV-16/18 in HSIL is much lower in HIW (36.4±28.4) than SPW (62.5±33.5).*

Conclusions. *A higher prevalence of infection and broader HPV type distribution were observed in HIV+ women compared to the general population. More than 60% of HSIL lesions of HIW patients are caused by single or multi-type infections from non-HPV16/18 HPVs. The potential 9v-HPV vaccine coverage could be even higher than that expected for the general population given the wide panel of HPV-types observed in the HSIL of HIV+ women.*

Introduction

Thirteen Human Papillomavirus (HPV) genotypes classified as carcinogenic and probably carcinogenic (group 1 and 2A) and six other HPV types with an invasive cervical cancer (ICC)/normal cytology ratio greater than 1.0, classified as possibly carcinogenic genotypes (HPV 26, 30, 67, 69, 73, and 82 – group 2B) are the cause of more than 90% of all ICCs worldwide [1, 2].

Their prevalence varies widely across world regions, but HPV-16 and -18 infections are the most prevalent and carcinogenic all over the world. Apart from the HPV type there are several co-factors that can contribute to invasive evolution [3].

Women affected by HIV/AIDS are at higher risk of invasive disease which is mainly due to the extent of immune-depression [4, 5]. However, the broader range of HPV types sustaining infections and the higher rate of multi-type infections in women living with HIV/AIDS could differently affect HPV type-specific carcinogenicity [6-8]. This is particularly relevant for cervical cancer prevention in immune-compromised hosts: in fact both vaccine primary prevention and HPV-based screening programs are based on the HPV-type distribution in immunocompetent individuals.

Three different HPV vaccines have been approved and licensed by the European Medicines Agency (EMA) and are available in Italy: the 2-valent (Cervarix®, GSK biologicals) vaccine, which prevents infections with High Risk HPV (HR HPV) types 16 and 18; the 4-valent vaccine (Gardasil®, Merck, Sanofi Pasteur MSD) which also targets Low Risk HPV (LR HPV) types 6 and 11 and the 9-valent vaccine (Gardasil9®, Merck, Sanofi Pasteur MSD) which, in addition to the four types of the 4-valent vaccine, also targets five additional HR HPV types (31, 33, 45, 52, and 58). Several studies [9, 10] have indicated that using a 9-valent vaccine could improve the prevention of invasive cervical cancers worldwide from 70% to 90%.

This paper reports a cross-sectional analysis of HPV-type distribution in HIV infected Women (HIW) com-

pared with a control group of immunocompetent HIV-negative women enrolled in the eVALuation and monitoring of HPV infections and relATEd cervical diseases (VALHIDATE) study [11]. The VALHIDATE study [11] was a 5-year (Dec. 2010-Dec.2015) multicenter open prospective cohort study aimed at gaining insight into the molecular epidemiology of HPV infection and cervical diseases in high-risk women in the Lombardy Region, Italy. HIW aged 26-64 years were one of the high-risk cohorts of the study. The control group was composed of women in the same age group attending spontaneous Pap screening-programs (SPW).

The aim of the study was to evaluate HPV type-specific distribution according to cytology among HIV infected women. Moreover, these data will enable us to establish the pre-vaccine type-specific prevalence of HPV–associated diseases in this population in order to evaluate the potential impact of the newly approved 9-valent HPV vaccine.

Methods

Study design

With the aim of evaluating the baseline HPV type-specific distribution stratified by the cervical cytological results, HIV-infected women (HIW) were compared with the control group (SPW). HIW and SPW cohorts were recruited consecutively for 12 months from 3 Infectious Diseases Units and 4 Gynecology Units of the four general hospitals located in Lombardy participating in the VALHIDATE study [11]. In particular, HIW were recruited from those followed up for HIV infection and SPW from those attending a spontaneous Pap screening program. Exclusion criteria were: history of histologically proven grade II or higher Cervical Intraepithelial Neoplasia (CIN) requiring treatment, pregnancy at the time of enrollment, inability to provide informed consent.

The protocol enrollment was approved by the Sacco Hospital Ethical Committees (Resolution n174/2010, 9 March 2010) and all participants provided written informed consent.

A total of 828 HIW and 1423 SPW were enrolled in the VALHIDATE study. The consenting women underwent basal co-testing with conventional Pap tests and HPV-DNA testing/genotyping. The cervical brush (Cytobrush Plus MedscandW Medical AB) sample collected at the baseline visit was used to perform the conventional Pap smear and then immersed and stored in a PreservCyt solution (ThinPrep® Pap Test, Hologic Italia Srl) to be analyzed for HPV-DNA and HPV genotyping.

The Pap tests were evaluated according to the 2001 Bethesda System terminology [12] by expert cytopathologists from the participating Centers. The cases were classified according to cytology at baseline evaluation as normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL) and high-grade SIL (HSIL).

DNA extraction, HPV detection and genotyping

DNA was extracted with a commercial kit (NucliSENS® EasyMAG®, bioMérieux, Lyon, France) and HPV-DNA was detected through PCR amplification of a 450 bp segment of ORF L1 using the degenerate primer pair ELSI-f and ELSI-r in the central reference laboratory of the University of Milan [11, 13]. HPV genotyping was performed on HPV-DNA positive cervical brushes using the commercially available Inno-LiPA® HPV Genotyping Extra (Innogenetics N.V., Belgium) method in the microbiology laboratories of the participating Centers. This test allows for the identification of 27 HPV types. All of the HPV-DNA positive cervical samples resulted as non-typeable by the Inno-Lipa test (HPV-X) were subjected to the Restriction Fragment Length Polymorphism (RFLP) type analysis which is capable of identifying all types of the High-Risk clade (HR-clade) and Low-Risk (LR) types of the alpha genus according to the 2011 IARC classification [1, 14].

Statistical analysis

HPV-DNA prevalence and type-specific HPV prevalence were expressed as crude proportions with corresponding 95% confidence intervals (95%CI) calculated assuming a normal distribution. The data are presented as the median (interquartile range, IQR) and percentages (with 95%CI) as appropriate. Comparisons between groups were made using the Chi-square test or Fisher's exact test. A P-value less than 0.05 was considered statistically significant (two-tailed test). All of the statistical analyses were performed using GraphPad Prism version 4.02 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

Results

Cytological results

Cytological results are provided for 805 HIW (97.2%) and 1402 SPW (98.5%). Women included in the HIW group had an overall 3.7-fold increased risk of ASCUS, a 3.6-fold increased risk of LSIL and a 2.7-fold increased risk of HSIL than those included in the control SPW group (Tab. I).

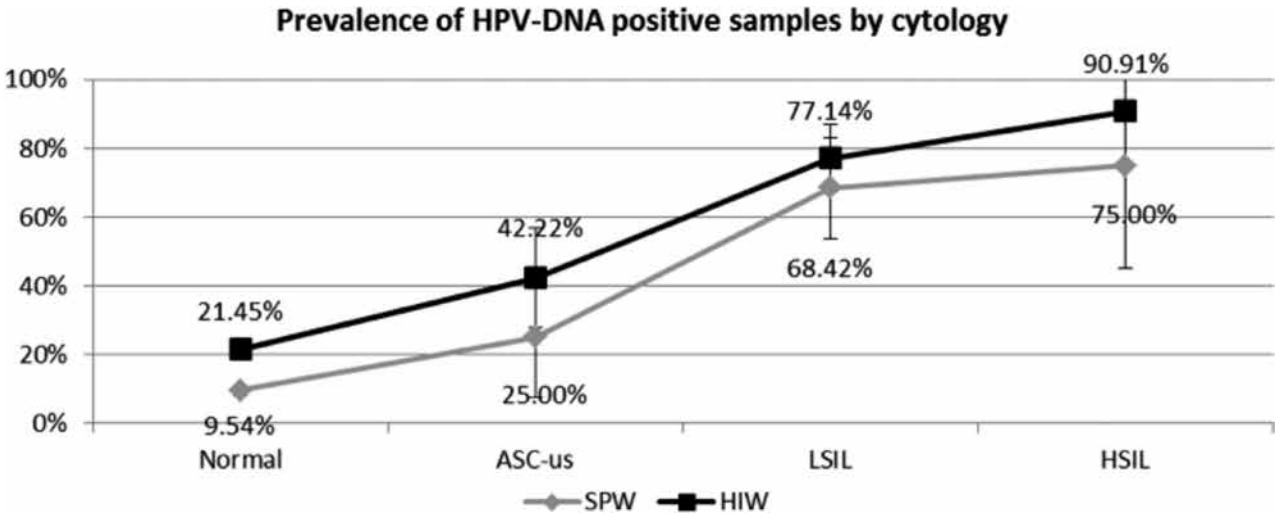
Human Papillomavirus infection by cytological status

HPV-DNA prevalence was 28.4% (95%CI 25.32-31.48) among HIW and 11.81% (95%CI 10.14-13.49) among SPW ($p < 0.0001$). HPV prevalence increased with the progression of the severity of cytological abnormalities from 21.45% to 90.91% in HIW and from 9.54% to

Tab. I. Normal and abnormal cytological results in 805 HIW and 1402 SPW at the baseline evaluation.

Cytology	HIW (n 805)	%	95%CI		SPW (n 1402)	%	95%CI		P value	OR 95%CI
Normal	678	84.22	81.71%	86.74%	1332	95.01	93.87%	96.15%	ref	1
ASCUS	46	5.71	4.11%	7.32%	24	1.71	1.03%	2.39%	< 0.0001	3.765 2.279 to 6.222
LSIL	70	8.70	6.75%	10.64%	38	2.71	1.86%	3.56%	< 0.0001	3.619 2.412 to 5.430
HSIL	11	1.37	0.56%	2.17%	8	0.57	0.18%	0.96%	0.0488	2.701 1.081 to 6.749

Fig. 1. Prevalence of HPV-DNA positive samples by cytology in HIW and SPW.



75% in SPW in normal cytology and HSIL respectively (Fig. 1).

The OR for women with normal cytology of having a positive HPV-DNA was 2.6 times higher in HIW (95%CI 2.0-3.3) than in SPW.

Different HPV-DNA prevalences were found for AS-CUS and LSIL, while no differences were observed for HSIL lesions between the two groups (Tab. II).

HPV typing by cytological status

Type-specific HPV prevalence is reported in Fig 2a: HPV-16 was the most prevalent type which was detected in 4.74% of HIW and in 4.61% of SPW. No significant differences were observed in the patterns of distribution of the HIW and SPW groups, except for the identification of infections sustained by HPV-67 and HPV-34 in HIW, absent in SPW and infections by HPV- 42 and HPV-72 types in SPW, absent in HIW.

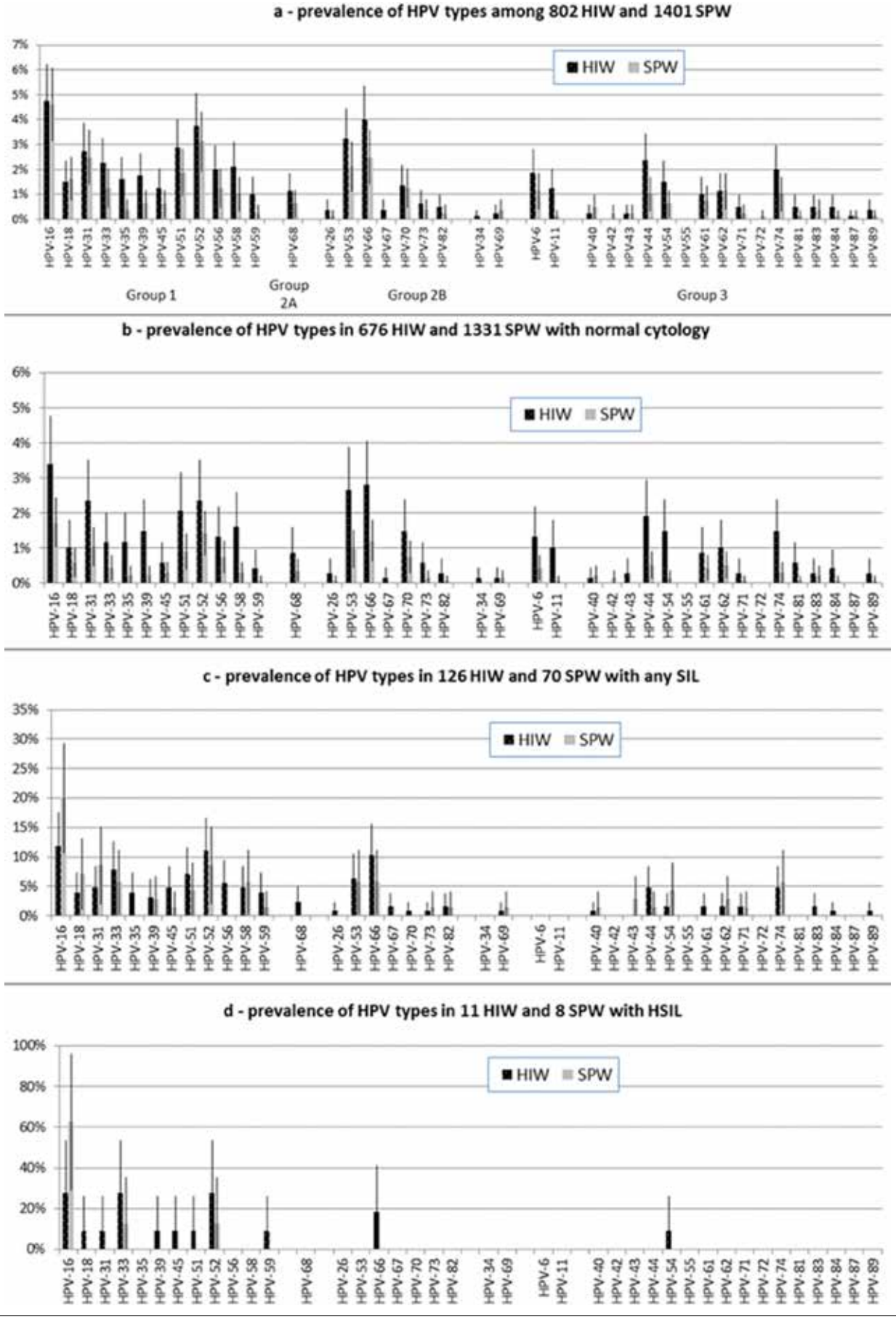
HPV typing stratified by cervical cytological results (Fig. 2b) showed very similar patterns in HIW and SPW with normal cytology. There is a lack of data concerning women with abnormal cytological results therefore we were unable to establish a different distribution pattern; however, HPV-16 is by far the most common type of HPV in women with abnormal cytology (any grade SIL, Fig. 2c) in SPW, while HPV-16 prevalence is similar to other high-risk HPV types (HPV-52, HPV-33 and HPV-66) in HIW. Several other HR-HPV types have been reported in HIW patients with cytological abnormalities (Fig 2c-2d). HSIL lesions sustained by non-HPV-16/18 types were 16.7% (1 out of 6) in SPW and 70% (7 out of 10) in HIW.

The cumulative prevalence of the two main oncogenic types (HPV-16/18), broken down by cytological outcome, is lower in LSIL and HSIL among the HIW than in the SPW and the general Italian population (Fig. 3).

Tab. II. Prevalence and OR with 95%CI of HPV-DNA by cytology in the HIW and SPW.

Cytology	HIW	%	SPW	%	P value	OR	95%CI
Normal	145	21.45	127	9.54	REF	1	
ASCUS vs normal	19	42.22	6	25.0	0.0351	2.774	1.074 to 7.161
LSIL vs normal	54	77.14	26	68.42	0.0290	1.819	1.076 to 3.076
HSIL vs normal	10	90.91	6	75.0	0.6082	1.460	0.5159 to 4.130

Fig. 2. Prevalence of HPV types in the whole population of HIW and SPW enrolled (a) broken down according to cytology (b, c, d).



Tab. III. Comparison of multi-type HPV infections according to cytology in HIW and SPW.

		Multi-type HPV/ HPV+ve	Prevalence	95%CI		P value	OR (95%CI)
Normal cytology	HIW SPW	65/119 40/107	54.62% 37.38%	45.68% 28.22%	63.57% 46.55%	0.0112	2.016 (1.184 to 3.435) ref
Any SIL	HIW SPW	40/75 15/36	53.33% 41.67%	42.04% 25.56%	64.62% 57.77%	0.3117	1.600 (0.7166 to 3.572) ref
HSIL	HIW SPW	4/10 1/6	40.00% 16.67%	9.64% 13.15%	70.36% 46.49%	0.5879	3.333 (0.2756 to 40.31) ref

Multi-type HPV infections (2 to 9 HPV types) occurred in 54.62% (95%CI 4.68-63.57) and 37.38% (95%CI 28.22-46.55) of HIW and SPW with normal cytology respectively (p 0.011). No differences were observed in the prevalence of multi-type HPV infections in women with cytological lesions (any grade or HSIL) (Table III).

Infection from vaccine HPV-types in women with normal cytology

No differences were observed in the proportion of infections sustained by at least one of the HPV types included in 2v-, 4v-, or 9v-HPV vaccines between HIW and SPW with normal cytology: 20.0% vs 23.62% for 2v-, 26.90% vs 27.56% for 4v-, and 51.7% vs 55.9% for 9v-HPV vaccine respectively. A highly significant difference in potential coverage was observed when we compared the 9v-HPV vaccine with the 2v- and 4v-HPV vaccines in both HIW and in SPW (Fig 4).

Discussion and conclusions

Worldwide, 69.4% of invasive cervical cancers are caused by HPV-16/18 infections [15]. In Italy, current estimates indicate that approximately 3.3% of females with normal cytology were infected with these two genotypes and the prevalence increased with disease progression [15]. The data reported in our study closely resemble the figures for SPW, even a slightly lower prevalence of HPV infection was observed in women with normal cytology; a comparable distribution of HPV types in women with normal cytology was found for HIW as well as a relatively lower presence of HPV-16/18 infections in the HSIL lesions compared to the Italian figures. HPV type distribution stratified by cytological results showed a substantial equivalence between women with HIV and SPW in normal cytology: HPV-16 is the most common viral type in both populations, followed by HPV-52, 66, 31, 53 for SPW and by HPV-66, 53, 52, 31 for HIW. Due to the

Fig. 3. Prevalence of HPV-16/18 infections by cytology in HIW, SPW and general Italian population [15].

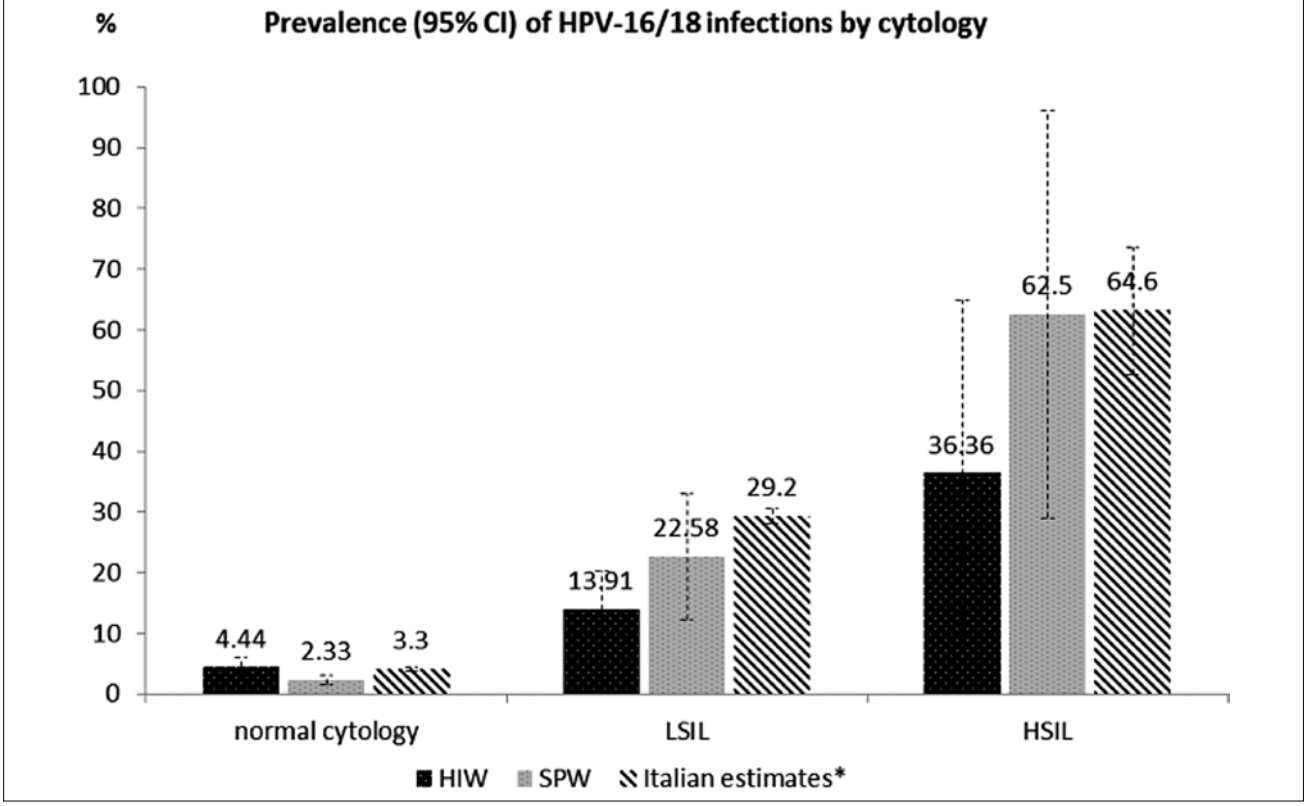
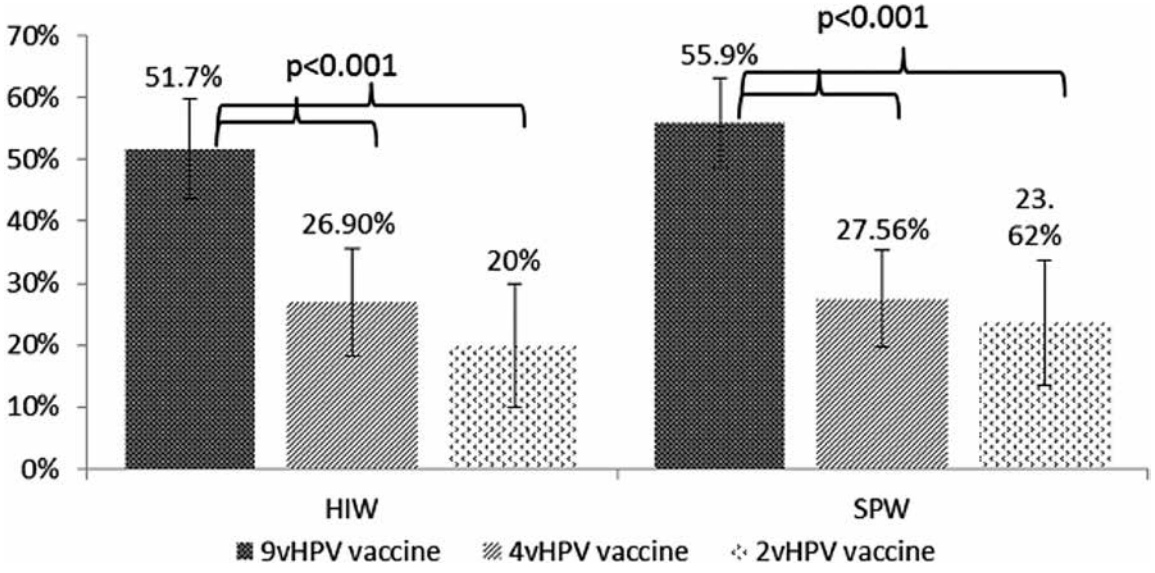


Fig. 4 . Proportion of women with normal cytology infected with at least 1 of the HPV types included in 2v-, 4v- or 9v-HPV vaccine.



limited number of cases with abnormal cytology observed in this study, it was impossible to identify statistically significant differences of distribution between HIW and SPW. However, although HPV-16 is by far the most common type expressed in HSIL of SPW, there is a greater heterogeneity of genotypes in women with HIV infection and HSIL. Keller et al. [16] highlighted that HIV-infected women with HPV-16 infection and normal Pap test results have a similar pre-cancer risk as those with LSIL and therefore referral for colposcopy is warranted. However the presence of non HPV-16/18 infections was observed in 70% and 16.7% of HSIL in HIW and SPW respectively. McKenzie et al. [17] reported that cervical dysplasia specimens from 23 HIV infected women were infected (55%) by non-16/18 high-risk HPV types. If confirmed by larger cohorts, this finding may have major implications in the screening and triage strategies for women infected with HIV.As described by several Authors [18-19], it was observed that HIV-infected women have a higher prevalence of HPV and multi-type infections than the control group. A high prevalence of rare HPV types was detected in HIW, both in normal cytology and in cervical abnormalities and to date little is known about their carcinogenicity. The IARC classification and the analysis of rare HPV types show a wide variability in the carcinogenic potential of the rare and common HPV types found in normal cytology [1, 2]. In HIV-infected women, variability towards oncogenesis can be further influenced by the simultaneous presence of other cofactors, in particular cell-mediated immunosuppression [5], while the additional risks associated with each HPV type in multi-type infections is difficult to establish. Although no statistically significant differences were found due to lack of data, the description of viral types in the cervical specimens of HIV-infected women and correlation with the severity of cytological lesions can contribute to the mapping of HPV-related pre-invasive or invasive disease and provide a better risk stratification of disease evolution in HIV-infected women. Even in the context of primary prevention through vaccination, the wide distribution of viral types found in high-grade lesions of HIV-infected women demonstrates the need for multivalent vaccines. This means that a primary prophylaxis with a 9v-HPV vaccine could have prevented infections in over 50% of the women included in this study, whether HIV infected or not. The assessment of the impact of HPV vaccines in the study population shows how immunization with a 9v-HPV-vaccine could prevent a significantly higher proportion of viral infections, both in HIV-infected people and in the control population, than 2v- and 4v-HPV vaccines. The ICC preventable fraction in the general population increased by 12-19% when the 9v-HPV vaccine was introduced due to the addition of 7 high risk HPVs to the 2v or 4vHPV vaccines [10, 20, 21] which could be even higher in HIV-infected people considering the wide range of HPV types observed in progressive diseases.

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The sponsor was involved in the study design, but not in collection, analysis, interpretation of data, or writing of the paper. All authors had full access to all the data in the study. All the authors declare that they have no conflict of interest with the sponsor.

Authors' contributions

GO: conceived and coordinate the study, evaluated the results and wrote the manuscript. ET: coordinated and contributed to the laboratory testing for HPV-DNA and HPV genotyping; contributed substantially to the manuscript writing. MMF, AM: contributed to the recruitment of the

participants, the acquisition of the clinical and epidemiological data, and critically revised the manuscript. SB, ERF, AA: contributed to the HPV detection and typing of the cervical samples and contributed to the manuscript writing. FM: contributed to the acquisition of the clinical and epidemiological data, to the samples handling and storage, and critically revised the manuscript. NZ: contributed to the HPV typing of the cervical samples and critically revised the manuscript. GR: contributed substantially to the conception, design and supervision of the study, and critically revised the manuscript.

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ORIGINAL ARTICLE

Knowledge, attitudes and practices (KAP) towards vaccinations in the school settings: an explorative survey

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Keywords

Immunization • Vaccination recommendation • Vaccine preventable disease • School environment • Vaccine hesitancy

Summary

Background. *Past researches have shown that schoolteachers' (STs) interventions maximize the consent for vaccination programs. European data regarding knowledge, attitudes and practices of STs towards vaccination are otherwise lacking.*

Objectives. *The aim of this study was therefore to evaluate knowledge and attitudes of STs regarding vaccinations in a sample from North Italy.*

Material and methods. *In this cross sectional study, 154 STs from Lombardy region (Northern Italy) responded to a specific questionnaire assessing their attitude towards vaccination and vaccine related knowledge.*

Results. *In general, 88.3% of subjects were somehow favourable to vaccinations. The main reason for declining vaccination was the risk of side effects whereas the main reason to be vaccinated was to avoid to be infected by VPDs (67.6%). Main information sources were health professionals (75.3%), and new media (13.1%), and the latter were STs more frequently associated with a negative attitude towards vaccinations ($p < 0.001$). Eventually, regression analysis identified risk perception as positively associated with propensity towards vaccinations, both for Students- and STs-recommended vaccinations (B = 0.372, 95% CI 0.247 to 0.496 and B = 0.005, 95%CI 0.004 to 0.006, respectively).*

Conclusions. *Our results are consistent with previous reports suggesting a significant knowledge gap in STs, with the risk perception of infectious diseases as the main predictor for vaccine propensity. Moreover, the better knowledge of official vaccination recommendations and policies among STs identifying Health Professionals as information source enlightens the role of the School Physicians and Occupational Physicians, whose intervention may ultimately increase the vaccination acceptance and vaccination rates.*

Introduction

Vaccinations have been repetitively acknowledged as a major tool for reducing the burden of infectious diseases and decreasing their related morbidity, mortality and healthcare costs [1-6]. In order to endure over time vaccination rates required by immunizations with the purpose to retain their efficiency [7], specific recommendations have to be progressively updated by competent Public Health Authorities [3, 4, 8-13]. In Italy, for example, official recommendations are issued by the Ministry of Health issues through the National Immunization Prevention Plan (in Italian, *Piano Nazionale di Prevenzione Vaccinale*, PNPV), a guidance document for immunization policies [11], listing vaccines actively offered free of charge to the general population, to high-risk subjects, as well as to certain occupational groups [11, 14, 15].

Sound evidence, built on previous studies from various occupational settings, suggests that the spreading of information across occupational groups may be affected by significant gaps and diffuse misconceptions. For instance, workers may be actually unaware that they are targeted by specific vaccination policies, or may receive incomplete and inappropriate information by employers and healthcare providers such as the Occupational Physicians (OPh) or the General Practitioner (GP) [1, 5, 9-16]. Individuals affected by knowledge gaps are usually affected by higher shares of doubts about safety and benefits of vaccines, frequently questioning the need for them, ultimately delaying (i.e. vaccine hesitancy, VH) or refusing vaccination (i.e. vaccine refusal, VR) [6, 17-19]. VH and VR have become a significant public health issue, as many European countries, including Italy, are experiencing increasing difficulties in reaching and maintaining targeted vaccination rates [17-18]. As under-vaccinated individuals tend to cluster together, certain population groups may exhibit even lower vaccination rates, similar to that usually reported by middle income countries [18], being potentially vulnerable to VPDs outbreaks, and possibly epidemics [6, 19].

Because of their large population, high level of close social interaction, frequent personal contact among students, faculty and staff and their interface with the community, schools are workplaces having the potential to

become outbreak centres for VPDs [7, 20-23]. Not coincidentally, school-based health programs have been appreciated among the most successful preventive approaches toward VPDs [24-27], significantly improving vaccination rates both in students and school employees (SEs) [28, 29]. In this regard, some analogies between SEs and healthcare workers (HCWs) may be identified. First at all, both HCWs and SEs work in settings at high risk of contracting VPDs and then transmitting the illness to others [21, 23, 30-32], involving also high-risk groups [33]. Second, they represent a significant occupational group in the adult population: recent estimates suggest that in Italy around 1 million people (i.e. 1.7% of total population and 2.6% of adults 18-67 years-old) are employed either as school teachers (STs) or school assistants (SAs). Third, both HCWs and STs are diffusely acknowledged as well trusted professionals. As the credibility of institution and professionals delivering information about vaccines is often more important than its content [34], they may be of significant relevance in the promotion of vaccine acceptance, also among subjects from difficult background (e.g. lower socioeconomic status).

In other words, appropriately informed STs can actively address individuals exhibiting VH because of inappropriate access to the facts or misinformation, and even vaccine objectors. On the contrary, STs sharing information deficits regarding vaccines and their official recommendations may ultimately hold and diffuse doubts or false beliefs about vaccines [34-37].

Knowledge, attitudes and personal beliefs (KAB) of STs towards immunizations have therefore the potential to significantly affect public health and occupational health [38-40]. Unfortunately, the abovementioned issues have been scarcely described, in particular in the European settings [21, 24-27]. In this questionnaire-based cross-sectional study, therefore, we aimed to assess knowledge of STs regarding vaccines and their official recommendations (i.e. knowledge of PNPV 2012-2014 recommendation) both in the paediatric age and for STs themselves, as well as their attitudes and personal beliefs, and whether knowledge and personal beliefs may be predictive of the personal attitude towards immunizations. In doing so, we sought to identify topics that may be target by specific and cost-effective formative and informative interventions.

Methods

Participants

The sample was collected between June and August 2016, and included STs from the provinces of Monza-Brianza and Brescia (2,130,181 out of 10,008,349 inhabitants of Lombardy Region, Northern Italy, at 2016 census) participating to a series of First Aid certification courses. Courses involved a total of 315 SEs from elementary, middle or high schools (i.e. secondary education, respectively of first and secondary grade) including 147 SAs and 168 STs. After the courses were completed and the First Aid certification achieved, STs from this initial sample having at least 6 months of seniority (n = 163, 97.0%) were invited to complete an anonymous questionnaire. As SAs usually don't share the trust that public opinion deserves to STs, it is implausible that their KAB have the potential to influence the acceptance of vaccinations, and were therefore excluded from the survey.

Instruments

Subjects giving their preliminary consent received by hand an anonymous and fully structured questionnaire inquiring attitudes and knowledge about vaccines and vaccinations. The questionnaire was a modified and adapted version of items previously developed for knowledge inquiry in the occupational settings [16, 41-43].

The questionnaire comprised 17 questions divided into 5 areas of inquiry:

1. *Demographic data*: age, sex, professional qualification, education level, household size and characteristics.
2. *Attitudes toward vaccinations*: participants' attitude was initially assessed asking their agreement towards vaccination practice through a 5-point Likert scale ("*strongly against vaccinations*", "*somehow against vaccinations*", "*neutral / no opinion*", "*somehow favourable to vaccinations*", "*strongly favourable to vaccinations*") , then explaining why they would get vaccinated (i.e. "*to avoid getting VPDs*", "*to avoid transmitting VPDs", "to avoid complications of VPDs*", "*to avoid VPDs in subjects who cannot be vaccinated*") or rather would refuse a vaccine or hesitate towards vaccinations (i.e. "*To avoid side effects of vaccinations*", "*No trust in vaccines*", "*Immunization by natural infections is more efficient*", "*Fear of shots*", "*Preference on other countermeasures*", "*Vaccines are superfluous (i.e. natural immunity ultimately overcomes any infectious disease)*", "*Vaccinations are against my personal beliefs (religious / ethical)*", "*Vaccines are useless (i.e. vaccines are eventually unable to enhance natural immunity)*"). Participants were then asked about their preferential information sources: TV/radio/newspapers, new media (i.e. wikis, blog, social media etc), friends, parents, school, health professionals.
3. *Knowledge of official recommendations*. Immunizations for sixteen VPDs were presented to the participants (i.e. diphtheria, tetanus, poliomyelitis, hepatitis B, pertussis, *H influenzae* type B, measles, rubella, parotitis, meningococcus C, pneumococcus, influenza, varicella, papillomavirus, hepatitis A, tuberculosis). For instance, PNPV 2012-2014 identified: (a) immunizations for paediatric age subjects, including 4 compulsory vaccinations for all newborns (i.e. diphtheria, tetanus, poliomyelitis and viral hepatitis B) and 9 recommended vaccinations (i.e. pertussis, *Haemophilus influenzae* type B, pneumococcus, meningococcus C, measles, parotitis, rubella, varicella, human papillomavirus); (b) immunizations for subjects working in the school environment, including 4 specifically recommended vaccination (i.e. measles,

parotitis, rubella, varicella). Participants eventually indicated which ones of presented vaccinations were recommended by PNPV 2012-2014 in paediatric-age subjects (i.e. Students-recommended immunization) and/or in STs (i.e. STs-recommended immunization) (possible answers: "*yes*", "*no*", "*don't know*"). As PNPV 2012-2014 recommendations for adults included that at least one of decennial boosters for tetanus and diphtheria incorporates acellular pertussis vaccine, and that subjects at occupational risk would receive vaccination against seasonal influenza (i.e. not only HCWs, but also subjects working in occupational settings at high risk for influenza, or whose sick leave during influenza season would severely impair public services), and both immunizations are recommended in SEs by the PNPV 2017-2019 [11, 15], they were also assessed among those recommended to STs. Knowledge score about official vaccine recommendations (KS-OR) was then calculated as the sum of correctly marked vaccines, separately for students and STs: when the participants correctly answered, +1 was added to a sum score [16].

4. *General knowledge*. The original knowledge test developed by Zingg [9] contains true-false statements such as "*vaccinations increase the occurrence of allergies*" (false) covering some typical misconceptions on vaccination. Both the original test and the revised version applied by Betsch and Wicker interpreted the sum of all incorrect answer as the degree of misconceptions held by the participant [9, 16]. In fact, this test successfully predicted influenza risk perceptions and vaccination intentions in previous studies [9, 16]. Briefly, a total of 13 statements were presented, including the 9 original items from Zingg questionnaire and 4 further items about vaccine misconceptions (i.e. "*The addictive used in the vaccines are not dangerous for humans*"; "*Multiple Sclerosis may be induced by HBV vaccine*"; "*Neurological disorders are possible side effects of measles vaccine*"; "*Autism is more frequent in subjects vaccinated against measles*"; "*Diabetes mellitus may be triggered by vaccination shoots*"; "*Vaccinations increase the occurrence of auto-immune diseases*"; "*Vaccinations increase the risk for allergic disorders*"; "*Vaccine are superfluous, as infectious diseases can be always treated with antibiotics*"; "*Without massive vaccination programs, smallpox would still exist*"; "*The efficacy of vaccines has been extensively proven*"; "*Children would be more resistant to infections if they were not always treated against all diseases*"; "*Many vaccinations are administered too early. As results, the immune system has no possibility to fully develop by itself*"; "*The immune system of children may be overwhelmed by a high number of vaccines*"; all statements presented as "*True*" / "*False*" / "*Don't know*"). General knowledge score (KS-G) was then calculated as the sum of correctly marked recommendations, by adding +1 to the sum score when the participants correctly marked presented statements.
5. *Risk perception*. We inquired the risk perception of STs about all the 16 VPDs we presented. In particular, we asked the STs about they perceived the severity of the natural infection through a 5-point Likert scale (i.e. 0, "*almost zero*"; 1, "*low*"; 2 "*moderate*"; 3 "*high*"; 4 "*very high*"). A risk perception score (RPS) was then calculated separately for all presented vaccination and as a cumulative score, in both cases dichotomized for pediatric-age and STs-recommended immunizations.
6. *Propensity towards vaccinations*. STs rated their specific propensity towards the sixteen vaccine-preventable diseases previously presented through a 5-point Likert scale (i.e. 1, "*strongly disagree*"; 2, "*disagree"*; 3, "*neutral"*; 4, "*agree"*; and 5, "*strongly agree*"). A cumulative vaccine propensity score (VPS) was calculated for the single attitudes, and separately for pediatric-age and STs-recommended immunizations by awarding a score of +1 for a specific propensity rated as "strongly disagree", +2 for "disagree", and so on.

Procedures

Subjects were informed that participation was on a strict voluntary basis, that completion and delivery of the questionnaire implied consent for study participation, and that all gathered data would be confidentially handled and collectively elaborated, having no other purpose than evaluation of vaccine attitudes and knowledge of participants. Because the individual participants cannot be identified through the questionnaire, it is implausible that this study caused them any harm. Moreover, as the consent for the participation was asked only after the course were actually completed and the First Aid certification achieved, it is also improbable that they have felt forced to participation. As the study design assured an adequate protection of study participants, and neither include clinical data about patients nor configure itself as a clinical trial, its preliminary assessment by Ethical Committee of the Provincial Agency of for Health Services (APSS) was not required.

Data Analysis

Two independent researchers, one of whom read the responses from each questionnaire while the other researcher reviewed the entered data, ensured the accuracy of data entry. The primary investigator examined unclear responses to determine the correct response. Described indices for general knowledge, knowledge about official recommendations, risk perception and propensity score, which assess the extent to which STs may pass on official recommendations, were eventually calculated. In order to more easily compare the scales, all results were normalized as per cent values.

Continuous variables were expressed as mean ± standard deviation and were analyzed through Student's t test for unpaired data or ANOVA when appropriate. The comparisons among risk perception scores and attitude scores was performed through the Dunnett's post-hoc test, arbitrarily assuming tetanus as the referent score.

Categorical variables were reported as per cent values. Analysis of discrete variables (i.e. age categories, specific attitudes …) was initially performed through bivariate analysis, and their associations with outcome variables were expressed as odds ratios (OR) with their 95% confidence intervals (95%CI). Adjusted OR (adjORs) were calculated through stepwise binary logistic regression analysis only for categorical variables that at bivariate analysis were associated with a general positive attitude towards vaccinations with p values < 0.05. In regression analyses we also assessed the relative influence of attitudes, general knowledge, and knowledge about recommendations on the propensity score. In the analyses, we controlled for age, sex and household characteristics. Significance level was < 0.05. All statistical analyses were performed using IBM SPSS Statistics 24.0 for Macintosh (IBM Corp. Armonk, NY).

Results

Demographic data

Demographic data of the study sample are summarized in Table I. A total of 154 questionnaires were returned (91.7% of the initial sample), including 38 STs from elementary school (24.7%), 40 from Middle school (26.0%), and eventually 76 from High School (49.4%). Mean age of the study population was 48.9 ± 7.8 years, with a mean seniority of 13.3 ± 8.7 years. Female sex was the most extensively represented (87.0%), and only 9 subjects (5.8%) had a migration background, being all other participants of Italian descent. Mean household size (including the participant) was 3.5 ± 1.2 people (min: 1, max: 7). For 41.6% of the participants, household included at least a subject younger than 18 years, whereas in 13.0% it included at least a subject older than 65 years. In total, 66 subjects (42.9%) referred a college degree or higher.

General attitude toward vaccinations

As shown in Table II, a total of 136 out of 154 respondents were "*strongly favourable*" or "*somehow favourable*" to vaccinations (88.3%). Negative attitudes peaked in age group 40 – 59 y.o. (n = 10/62 and 6.5% of the total sample), but positive and negative attitudes towards vaccines were actually unrelated with age (48.5 ± 7.7 y.o. vs. 51.8 ± 7.9 y.o., p = 0.088), seniority (12.7 ± 9.1 y vs. 15.1 ± 7.2 y, p = 0.310), household size (3.5 ± 1.2 vs. 3.6 ± 1.2, p = 0.668) and composition (p = 1.000 and p = 0.904 for household including subjects < 18 y.o. and > 65 y.o., respectively), and level of education (p = 1.000). Overall, female STs had more favourable attitude towards vaccinations than males (p = 0.018; OR 4.357, 95%CI 1.414-13.43), but this association was not confirmed by multivariate analysis (adjOR 2.976 95% CI 0.841-10.53).

Main information sources referred by study population were health professionals (75.3%), new media (17.5%), TV / radio (11.7%), and newspapers (11.7%), whereas 8.4% of participants recalled friends and/or relatives and 3.9% professional courses. STs identifying health professionals as their main referents exhibited a significantly more positive attitude towards vaccines (p = 0.003, OR 4.821 95%CI 1.741-13.35), and even though the majority of participants referring new media as information source were favourable to vaccinations, prevalence of negative attitude was significantly higher than in subjects not relying on such information media (p < 0.001, OR 0.153 95% 0.053-0.435). Eventually, both associations were confirmed at multivariate analysis (adjOR 4.599, 95%CI 1.540-13.74 and adjOR 0.143 95%CI 0.047-0.437, respectively).

Among the reasons for hesitate or even refuse vaccination (Tab. III), all subjects (100%) referred they aimed to avoid side effects, whereas 50% of the respondents

Tab. I. Demographics of the study population.

	Total	Males	Females
Participants (n, %)	154 (100%)	20 (13.0%)	134 (87.0%)
Mean Age (years ± SD)	48.9 ± 7.8	51.8 ± 8.7	48.5 ± 7.6
< 40 year-old	20 (13.0%)	1 (5.0%)	19 (95.0%)
40 – 49 year-old	60 (39.0%)	8 (13.3%)	52 (86.7%)
50 – 59 year-old	62 (40.3%)	6 (9.7%)	56 (90.3%)
≥ 60 year-old	12 (7.8%)	5 (41.7%)	7 (58.3%)
Seniority (years ± SD)	13.3 ± 8.7	14.1 ± 9.4	12.9 ± 7.7
Migration background (n, %)	9 (5.8%)	0 (-)	9 (100%)
Household size (mean ± SD)	3.5 ± 1.2	3.8 ± 1.1	3.4 ± 1.2
The participant (single)	14 (9.1%)	1 (7.1%)	13 (92.9%)
+1	18 (11.7%)	1 (5.6%)	17 (94.4%)
+2	29 (18.8%)	5 (17.2%)	24 (82.8%)
+3	71 (46.1%)	9 (12.7%)	62 (87.3%)
+4	16 (10.4%)	3 (18.7%)	13 (81.3%)
+5 or more	6 (3.9%)	1 (16.7%)	5 (83.3%)
Lives with subjects < 18 y.o.	64 (41.6%)	9 (14.1%)	55 (85.9%)
Lives with subjects > 65 y.o.	20 (13.0%)	5 (25.0%)	15 (75.0%)
Educational level			
High School	88 (57.1%)	8 (9.1%)	80 (90.9%)
University or greater	66 (42.9%)	12 (18.2%)	54 (81.8%)
Type of school			
Elementary	38 (24.7%)	3 (7.9%)	35 (92.1%)
Middle	40 (26.0%)	4 (10.0%)	36 (90.0%)
High School	76 (49.4%)	13 (17.1%)	63 (82.9%)

Tab. II. Attitudes towards vaccinations by demographic data and information sources. Continuous variables were compared through student's t test for unpaired data, where categorical ones were assessed through chi-squared test and their associations with outcome variables were expressed as odds ratios (OR) with their 95% confidence intervals (95%CI). Adjusted Odds Ratio (adjOR) were calculated through logistic regression analysis for variables that at bivariate analysis were associated with a general positive attitude towards vaccinations with p values < 0.05.

		Attitudes towards vaccinations				P value	OR	95%CI	adjOR	95%CI
		Somehow favorable (n = 136)		Somehow against (n = 18)						
Age (mean ± SD[1])	48.9 ± 7.8	48.5	7.7	51.8	7.9	0.088	-	-	-	-
< 40 y.o. (No., %)	20 (13.0%)	18	(90.0%)	2	(10.0%)	1.000	1.000	REF	-	-
40 – 49 y.o. (No., %)	60 (39.0%)	56	(93.3%)	4	(6.7%)	0.790	0.737	0.077 – 7.007	-	-
50 – 59 y.o. (No., %)	62 (40.2%)	52	(83.9%)	10	(16.1%)	0.231	0.274	0.033 – 2.284	-	-
≥ 60 y.o. (No., %)	12 (7.8%)	9	(75.0%)	3	(25.0%)	0.151	0.158	0.014 – 1.737	-	-
Seniority (mean ± SD)	13.3 ± 8.7	12.7	9.1	15.1	7.2	0.310	-	-	-	-
Household size (mean ± SD)	3.5 ± 1.2	3.5	1.2	3.6	1.2	0.668	-	-	-	-
Lives with subjects < 18 y.o. (No., %)	64 (41.6%)	57	(89.1%)	7	(10.9%)	1.000	1.134	0.414 – 3.104	-	-
Lives with subjects > 65 y.o. (No., %)	20 (13.0%)	17	(85.0%)	3	(15.0%)	0.904	0.714	0.187 – 2.727	-	-
Female Sex (No., %)	134 (87.0%)	122	(91.0%)	12	(9.0%)	0.018	4.357	1.414 – 13.43	2.976	0.841 – 10.53
University education level (No., %)	66 (42.9%)	58	(87.9%)	8	(12.1%)	1.000	1.076	0.400 – 2.985	-	-
Information source[2]										
TV / Radio (No., %)	18 (11.7%)	16	(88.9%)	2	(11.1%)	0.757	1.067	0.224 – 5.077	-	-
New Media[3] (No., %)	27 (17.5%)	18	(66.7%)	9	(33.3%)	< 0.001	0.153	0.053 – 0.435	0.143	0.047 – 0.437
Friends, relatives (No., %)	13 (8.4%)	11	(84.6%)	2	(15.4%)	1.000	0.704	0.143 – 3.466	-	-
Professional courses (No., %)	6 (3.9%)	5	(83.3%)	1	(16.7%)	1.000	0.649	0.071 – 5.889	-	-
Health professionals (No., %)	116 (75.3%)	108	(93.1%)	8	(6.9%)	0.003	4.821	1.741 – 13.35	4.599	1.540 – 13.74
Newspapers (No., %)	18 (11.7%)	15	(83.3%)	3	(16.7%)	0.757	0.620	0.161 – 2.393	-	-

[1] SD = Standard Deviation.

[2] As multiple choices were allowed, total sum may exceed 100%.

[3] New Media = wikis, blogs, social media, etc.

claimed lack of trust in vaccines, the belief that the immunization sustained by natural infections is more efficient, and the fear of vaccination shots. A third of the participants then referred preference for other countermeasures, and the belief that vaccines are superfluous as natural immunity ultimately is able to overcome any infectious disease (in both cases 33.3%). Eventually, 27.8% of the respondents claimed for "*religious/ethical reasons*", or shared the belief that vaccines are useless, as unable to enhance natural immunity.

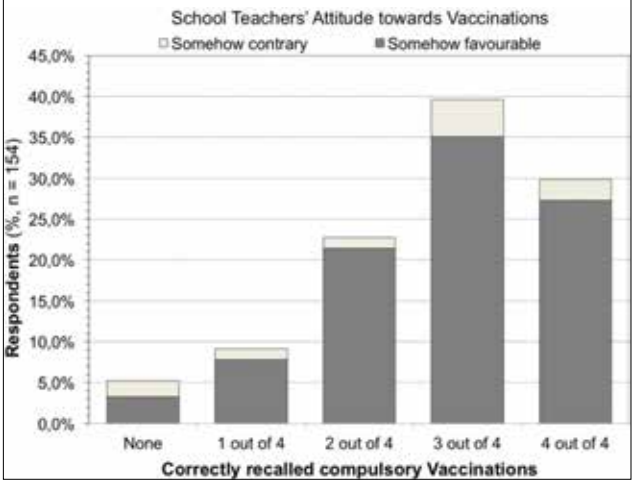
On the contrary, the most frequently referred reason to be vaccinated was to avoid be infected by VPDs (67.6%), followed by avoiding complications (50.7%) and transmission of VPDs (43.4%), whereas 19.9% of participants identified the avoiding of VPDs in subjects who cannot be vaccinated.

Tab. III. Reasons for hesitate or even refuse vaccination.

Why do you get vaccinations[1]?	**N# (/136)**	**%**
To avoid getting VPDs	92	67.6%
To avoid complications of VPDs	69	50.7%
To avoid transmitting VPDs	59	43.4%
To avoid VPDs in subjects who cannot be vaccinated	27	19.9%
Reasons to decline a vaccine or hesitate towards vaccinations[1]	**N# (/18)**	**%**
To avoid side effects of vaccinations	18	100.0%
No trust in vaccines	9	50.0%
Immunization by natural infections is more efficient	9	50.0%
Fear of shots	9	50.0%
Preference on other countermeasures	6	33.3%
Vaccines are superfluous (i.e. natural immunity ultimately overcomes any infectious disease)	6	33.3%
Vaccinations are against my personal beliefs (religious / ethical)	5	27.8%
Vaccines are useless (i.e. vaccines are eventually unable to enhance natural immunity)	5	27.8%

[1] As multiple choices were allowed, total sum may exceed 100%.

Fig. 1. Correctly recalled compulsory vaccinations in pediatric-age subjects (i.e. diphtheria, tetanus, Viral Hepatitis B, poliomyelitis), broken down by attitude towards vaccinations (i.e. somehow contrary vs. somehow favorable).



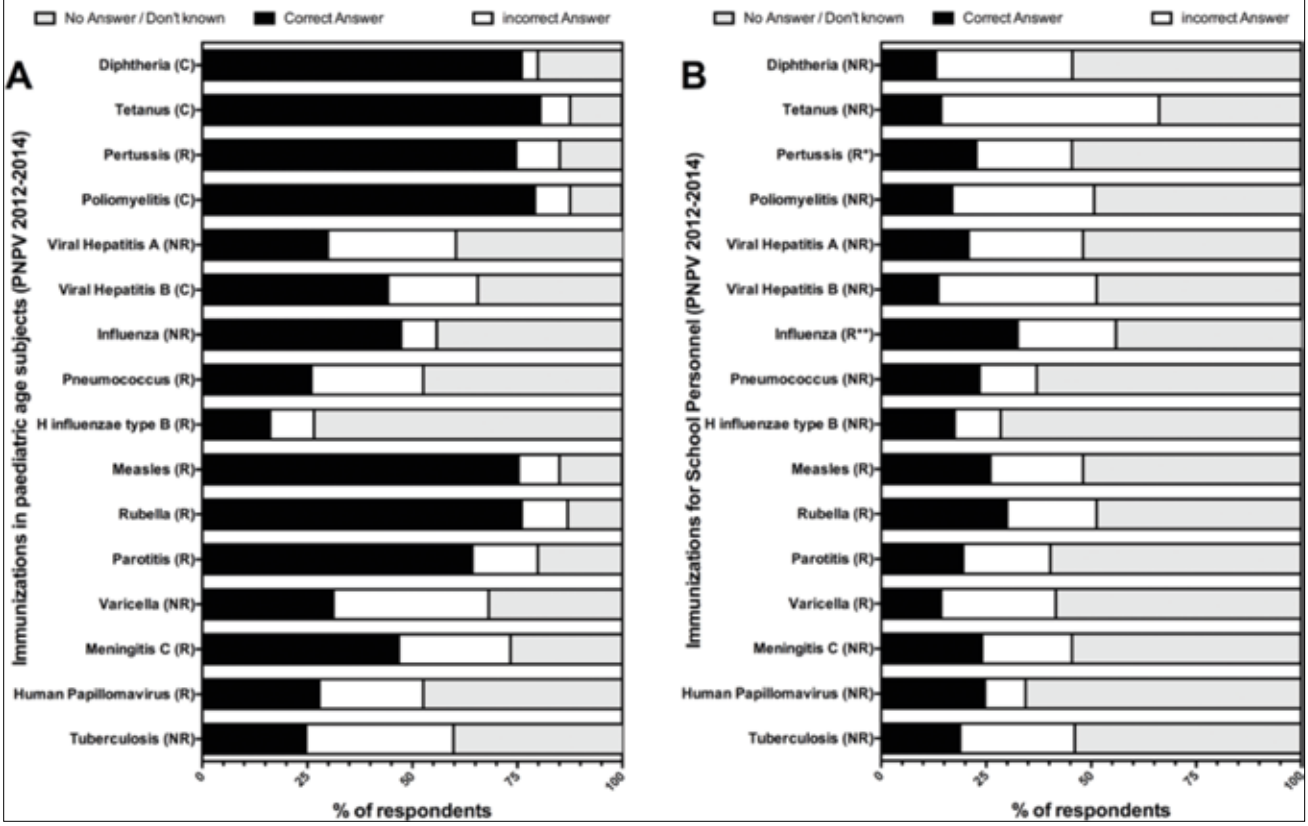
Knowledge of official recommendations

Mean KS-OR was 51.2 ± 17.8, with an actual range of 0.0 to 93.75 for Students-recommended vaccinations, and 20.7 ± 20.7 (range 0.0 to 75.0) for STs-recommended immunizations. Subjects exhibiting a positive attitude towards vaccinations had a significantly better knowledge of official recommendations for students (52.7 ± 16.9 vs. 39.9 ± 20.2, p = 0.004), whereas KS-OR for vaccinations in STs was not significantly different (21.14 ± 20.7 vs. 15.6 ± 20.9, p = 0.267). Overall, 46 out of 154 participants (29.9% of total sample) were able to identify all the 4 compulsory vaccines for paediatric subjects (Fig. 1).

Knowledge of participants on which vaccinations are recommended in paediatric age subjects by PNPV 2012-2014 is presented in Figure 2. In summary, most of participants (124 out of 154, 80.5%) correctly recalled tetanus, followed by poliomyelitis (n = 122, 79.2%), rubella and diphtheria (in both cases, n = 117, 76.0%), measles (n = 116, 75.3%) and pertussis (n = 115, 74.7%). Conversely, only 25 participants correctly recalled *H influenzae* type B (16.2%) as a recommended immunization, while 73.4% of the sample (n = 113) was unable to identify whether it was a recommended or not recommended vaccination.

Regarding the knowledge of recommended STs vaccinations, only 8 out of 154 participants correctly recalled all the six immunizations (5.2%), whereas around half of the sample (n = 69, 44.8%) recalled none of them. Focusing on single immunizations, around a third of the sample correctly identified influenza (n = 50, 32.5%) as a recommended immunization, followed by rubella (n = 46, 29.9%), measles (n = 40, 26.0%), pertussis (n = 35, 22.7%), parotitis (n = 30, 19.5%), and varicella (n = 22, 14.3%).

Fig. 2. Knowledge of participants on which vaccinations are recommended in paediatric age subjects and for School Personnel (PNPV 2012-2014). (C) = compulsory; (R) = recommended; (NR) = not recommended; * as a part of decennial tetanus-diphtheria booster in subjects > 18 years.** not explicitly recommended, but recalled for occupational settings associated with high risk and where sick leave during influenza season would severely impair public services.



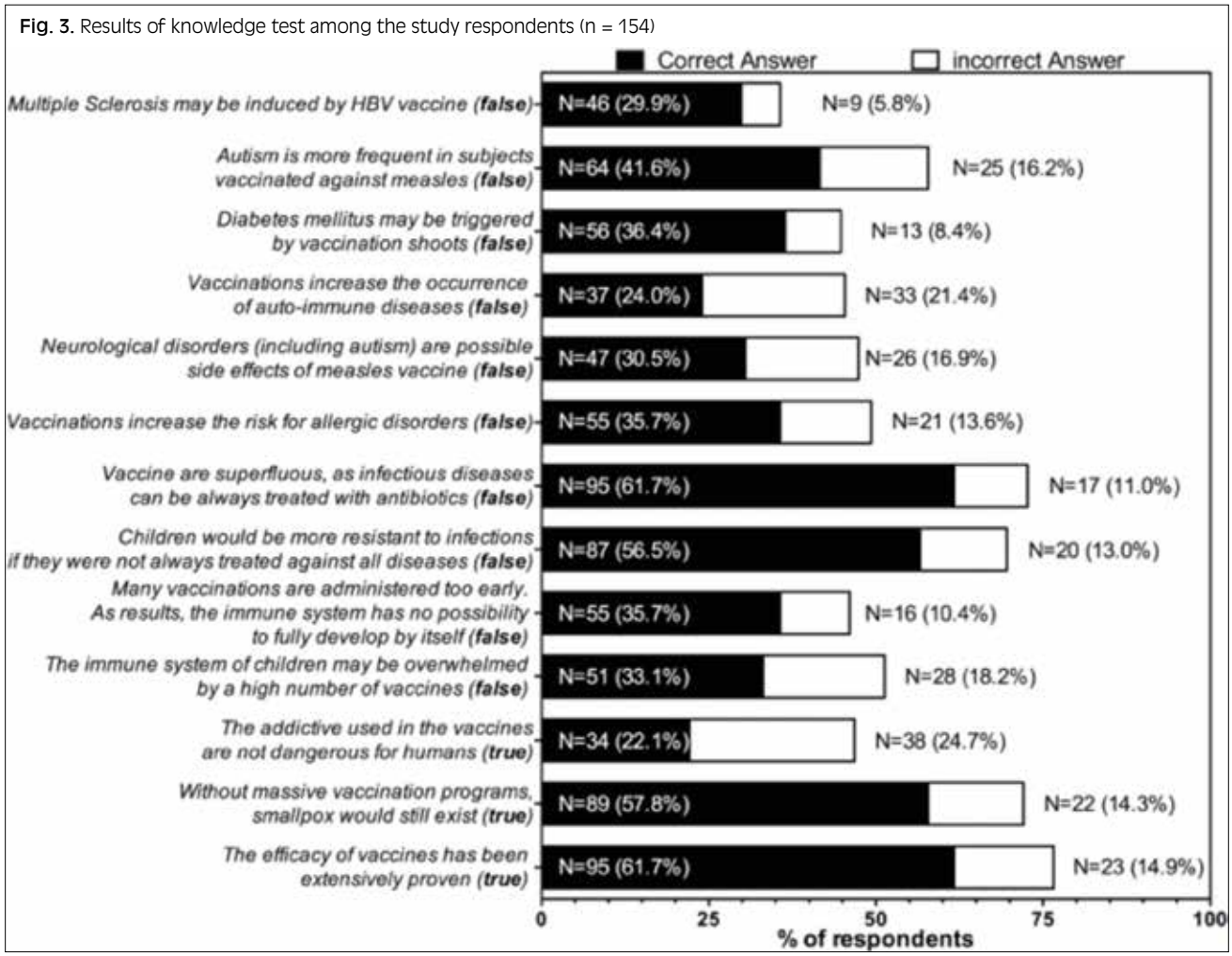


Fig. 3. Results of knowledge test among the study respondents (n = 154)

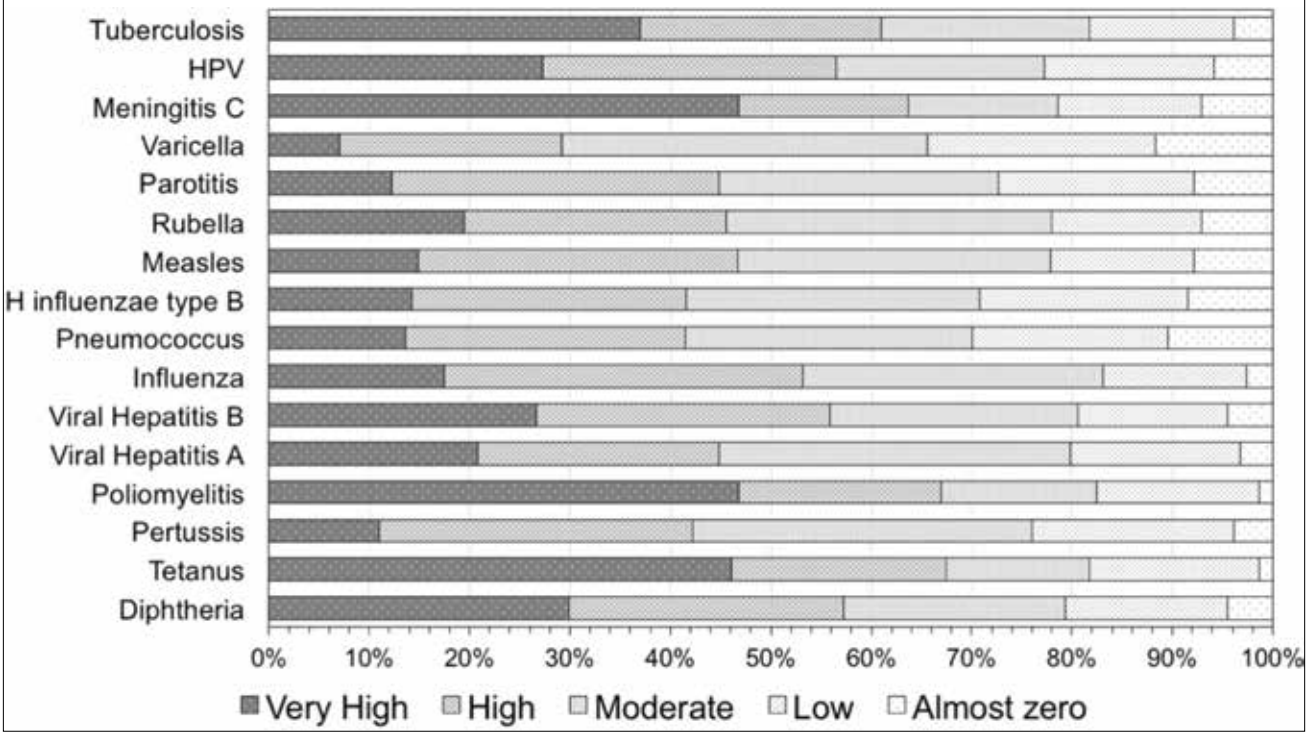
General knowledge

After normalization, the mean KS-G was 50.9 ± 26.8 (actual range 0.0 -100.0). Subjects exhibiting a favorable attitude towards vaccinations had a significantly higher score (66.7 ± 21.8 vs. 33.3 ± 19.4, $p < 0.001$) and exhibited a greater prevalence of false beliefs. For instance, the most reported false belief among the participants was that vaccine additives as dangerous for human health (38/154, 24.7%), with several subjects causatively associating vaccines and autoimmune diseases in general (33/154, 21.4%), the latter including diabetes (13/154 8.4%) and multiple sclerosis (9/154, 5.8%), whereas around one-sixth of the sample associated measles vaccine with neurological diseases (26/154, 16.9%) and autism (25/154, 16.2%) (Fig. 3). Moreover, misconceptions regarding vaccine practices and in particular vaccination schedules were exhibited by around a fifth of the sample, as 28/154 (18.2%) that the immune system may be overwhelmed by the high number of vaccines identified by the vaccine schedules, whereas 16/154 (10.4%) believed that many vaccinations are administered too early.

Assessment of the risk perception

Average RPS for natural infections were 61.2 ± 23.2 and 52.2 ± 21.9 for students- and STs-recommended vaccinations, respectively. Most of participants identified tetanus (67.5%), poliomyelitis (66.9%), and meningitis (63.7%) as infections whose severity was either high or very high, whereas only of 29.2% respondents acknowledged varicella as a severe or highly severe VPD (Fig. 4). Similarly, tetanus and poliomyelitis were associated with a greater risk perception score (73.5 ± 29.6 and 73.7 ± 29.5, respectively), followed by meningitis C (70.5 ± 33.6), tuberculosis (68.9 ± 30.1), diphtheria (65.4 ± 30.1), viral hepatitis B (64.6 ± 29.1). In ANOVA, assuming tetanus as the referent one, difference in risk perception score with all the aforementioned infections was not statistically significant (for all cases, $p > 0.05$). Contrarily, a significantly lower score was associated with HPV (63.8 ± 30.5, $p < 0.05$), viral hepatitis A (60.6 ± 27.4, $p < 0.01$), rubella (58.9 ± 29.1, $p < 0.001$), measles (57.9 ± 28.3, $p < 0.0001$), pertussis (56.3 ± 25.7, $p < 0.0001$), parotitis (55.5 ± 28.3, $p < 0.0001$), *H influenzae* type B (54.6 ± 29.2, $p < 0.0001$), pneumococcus

Fig. 4. Assessment of the perceived severity of presented natural infections by study participants.



(53.7 ± 29.8, p < 0.0001), and eventually influenza having the lowest score (37.2 ± 25.6, p < 0.0001).

Propensity score

Normalized mean VPS was 81.7 ± 19.4 for Students-recommended vaccines, and 78.9 ± 20.7 for STs-recommended vaccines: when single vaccinations were taken in account, higher VPS was associated with tetanus (84.1 ± 26.9), diphtheria (82.6 ± 26.3), poliomyelitis (82.2 ± 27.6), rubella (80.7 ± 28.5), followed by meningitis C (79.8 ± 27.7), tuberculosis (79.7 ± 26.7), pertussis (79.6 ± 26.8), parotitis (79.4 ± 27.9), measles (79.6 ± 28.6), viral hepatitis B (79.5 ± 28.2), HPV (77.0 ± 27.0), viral hepatitis A (76.0 ± 26.9). For all aforementioned vaccines, VPS was not significantly different from the referent one (i.e. tetanus) in post-hoc Dunnett's multiple comparison test. Contrarily, pneumococcus (72.4 ± 26.9, p < 0.01), varicella (72.2 ± 27.9, p < 0.01), *H influenzae* type B (70.7 ± 26.0, p < 0.001) and influenza vaccine had significantly lower acceptance (63.6 ± 28.9, p < 0.0001) (Table IV).

Regression analysis

Knowledge about vaccine recommendations was identified as a significant predictor for propensity towards Students-recommended vaccinations (B = 0.196, 95% CI 0.036 to 0.355, p = 0.016), similarly to RPS (B = 0.372, 95%CI 0.247 to 0.496, p < 0.001). In other words, STs having a lower knowledge of official recommendations and lower perception of the risk associated with natural infections had a lower acceptance of vaccines, and conversely a greater risk for vaccine hesitancy, whereas KS-G was unrelated with VPS (B = -0.043, 95%CI -0.147 to 0.060, p = 0.408). Contrarily, VPS towards STs-recommended immunizations was unaffected by the KS-OR (B = 0.001, 95%CI LL -0.001 to 0.002, p = 0.265), and KS-G (B = 0.000, 95%CI -0.001 to 0.001, p = 0.852), and only RPS was predictive for a better VPS (B = 0.005, 95%CI 0.004 to 0.006, p < 0.001).

Discussion

This cross-sectional study aimed to define KAB of STs towards vaccinations, as they represent a significant workforce at occupational risk for contracting and spreading VPDs through their workplaces [22, 31]. Moreover, as usually well trusted professionals, STs may be useful to address both the vaccine hesitant and more specifically individuals with uncertain attitude towards vaccinations (i.e. "*fence-sitter*") [34, 44, 45]. More specifically, previous reports actually suggest that STs may actively propagate better awareness and greater knowledge regarding vaccines and vaccination policies in populations groups otherwise affected diffuse lack of specific knowledge and training, whose informative gaps may be easily filled by uncontrolled information sources, with resulting false beliefs and misconceptions – a common feature of VH [1, 24, 34].

Overall, the attitude of the study participants towards vaccinations was largely positive: not only 88.3% of the STs self-declared either "*somehow favourable*" or "*strongly favourable*" to vaccinations, but the assessed VPS were similarly high, both for Students- and STs-recommended immunizations (81.7 ± 19.4 and

78.9 ± 20.7, respectively). However, our results were somehow disappointing.

First at all, among the 11.7% of participants self-declaring somehow against vaccination practice, the most frequently reported reason to hesitate and/or refuse vaccinations was identified in the fear of side effects, referred by all subjects self-assessing as against vaccinations, followed by the fear of shots (50.0%), and a series of statements such as the lack of trust in vaccines, or the belief that immunization by natural immunization would be more efficient than that promoted by vaccination.

As some reports from North America previously suggested that school environment may be associated with a significantly high degree of misbelieves about vaccines, such results were not unexpected [21-23], but they still remain somehow worrisome, as available evidence also indicates that knowledge gaps and high prevalence of false beliefs are strongly associated with low vaccination rates, not only in STs but also in the general population interacting with school professionals [7, 21-23].

Also the assessment of the general knowledge was largely ambiguous, and again supported the existence of a significant knowledge gap.

Firstly, although only the statement that "*the addictive used in the vaccines are not dangerous for humans*" was associated with higher prevalence of incorrect answers (24.7% vs. 22.1%), the share of false beliefs regarding the measles vaccine, including both the causative association with autism and neurological disorders, were noteworthy (16.2% and 16.9%, respectively), and also rates of "*don't know*" answers were indeed appreciably high, ranging from 23.4% to 64.3%. Again, this high rate of subjects either unable or unwilling to assess the presented statement may be interpreted as a consequence of incomplete or insufficient knowledge regarding the presented items, whose content was specifically designed in order to describe the prevalence of false beliefs and misunderstanding about vaccinations.

Second, knowledge of official recommendations as defined by PNPV 2012-2014 was unsatisfying, as the majority of participants were in facts either unable or unwilling to assess the status of STs-recommended vaccinations, whose specific knowledge was eventually strikingly low (i.e. 20.7 ± 20.7 with a potential range of 0 to 100). The knowledge gap probably reflects the somehow vague recommendations for occupational settings other than that of healthcare [11, 15], and the frequently inappropriate seeding of official recommendation to the targeted occupational groups [16, 41-44].

In facts, even though some uncertainties could have been expected for immunizations such as influenza and pertussis, whose occupational recommendations for SEs are actually ambiguous, most of participants were unable to characterize vaccinations having a far better defined status, as *H influenzae* type B, pneumococcus, and HPV. Not coincidentally, the upcoming PNPV 2017-2019, whose approbation is still underway, will identify more detailed occupational recommendations for the school settings [11, 15].

Also the knowledge of official recommendations targeting students as subjects of paediatric age was affected by several uncertainties. Even though around 70% of the sample was able to correctly recall at least three of the four compulsory immunizations, and diphtheria, tetanus and poliomyelitis were accurately reported as compulsory vaccines by more than 75% of the sample, the status of viral hepatitis B vaccine was acknowledged by only 44.2% of the participants. Similar uncertainties were associated with the recalling of significant immunizations such as parotitis, viral hepatitis A, varicella, meningitis C and pneumococcus. Moreover, *H influenzae* type B was apparently unknown to the large majority of the sample.

Tab. IV. Risk Perception Score (RPS) and Vaccine Propensity Score (VPS) for the single vaccinations presented to the study participants. Analysis was performed through ANOVA and Dunnett's test for multiple comparison by arbitrarily assuming tetanus scores as the referent ones.

Immunization	RPS (0.0 – 100)		VPS (Range 0.0 – 100)	
	Mean ± SD	P value	Mean ± SD	P value
Tetanus	73.5 ± 29.6	REFERENCE	84.1 ± 26.9	REFERENCE
Diphtheria	65.4 ± 30.1	> 0.05	82.6 ± 26.3	> 0.05
Pertussis	54.3 ± 25.7	< 0.0001	79.6 ± 26.8	> 0.05
Poliomyelitis	73.7 ± 29.5	> 0.05	82.2 ± 27.6	> 0.05
Viral Hepatitis A	60.6 ± 27.4	< 0.01	76.0 ± 26.9	> 0.05
Viral Hepatitis B	64.6 ± 29.1	> 0.05	79.5 ± 28.2	> 0.05
Influenza	37.2 ± 25.6	< 0.0001	63.6 ± 28.9	< 0.0001
Pneumococcus	53.7 ± 29.8	< 0.0001	72.4 ± 26.9	< 0.01
H influenza type B	54.6 ± 29.2	< 0.0001	70.7 ± 26.0	< 0.001
Measles	57.9 ± 28.3	< 0.001	78.6 ± 28.6	> 0.05
Rubella	58.9 ± 29.1	< 0.001	80.7 ± 28.5	> 0.05
Parotitis	55.5 ± 28.3	< 0.0001	79.4 ± 27.9	> 0.05
Varicella	47.6 ± 27.4	< 0.0001	72.2 ± 27.9	< 0.01
Meningitis C	70.5 ± 33.6	> 0.05	79.8 ± 27.7	> 0.05
Human Papillomavirus	63.8 ± 30.5	< 0.05	77.0 ± 27.0	> 0.05
Tuberculosis	69.0 ± 30.1	> 0.05	79.7 ± 26.7	> 0.05

Such results may again be explained as the consequence of a knowledge gap. Actually, infectious diseasese such tetanus, poliomyelitis, but also meningitis and tuberculosis are usually acknowledged as severe and potentially life-threatening infectious diseases, whereas the status of other VPDs may be more extensively disputed [41, 46-50]. For example, natural infections by measles, parotitis, varicella and pertussis are usually understood as indolent pediatric disorders, as general population usually ignores that such VPDs may be associated with severe complications such as acute respiratory failure, encephalitis or even death [41, 42, 48-52], and that sub-optimal immunization levels are revamping their incidence in EU countries [47, 51]. Not coincidentally, in our survey these vaccinations were associated with a RPS significantly lower than that identified not only for tetanus and diphtheria, but also for tuberculosis and meningitis.

In other words, the inconsistent and not up-to-date awareness of the risk associated with all the presented VPDs may have forced the participants to report "*common-sense"* rather than "*evidence based*" recommendations [41-42, 46, 49, 60, 52-55]. In this regard, the ambiguous status of rubella, diffusely acknowledged and publicized as both an indolent disease and a significant infection for childbearing-age women, may explain the relatively high rate of correct answers both for STs- (29.9%) and students-recommended vaccinations (76.0%).

Consistently with such remarks and previous reports, also in our survey the perceived severity of natural infections was identified as the most significant predictors for the acceptance of both Students- and STs-recommended vaccinations [31, 41-42, 49, 52-54]. Hence, the knowledge deficit of STs regarding the actual risk associated with natural infections may also have significantly affected the VPS. In other term, STs may be unaware of their specific risk towards the aforementioned natural infections, as they may consider themselves as either immune (because of previous natural infection or paediatric-age immunization) or unconcerned by somehow "not significant" VPDs. Even though the very low RPS assessed for seasonal influenza may therefore explain by itself the similarly unsatisfactory PS, it is possible that this result may have been also affected by the diffuse misunderstandings and alarming misconceptions about the efficacy of the vaccine [56], a critical issue well described in previous studies investigating knowledge, attitudes and practices of HCWs towards seasonal influenza vaccine [12, 57-63], and possibly shared by other immunizations such as measles vaccine [48-50].

As health professionals (including General Practitioners, OPh, etc.) were the main information source in STs about vaccines and immunizations, our results indirectly suggest that the knowledge gaps of STs may ultimately include not only an informative gap, but also a communicative one, with STs unable to obtain and/or retain up-to-date medical evidence regarding the morbidity and even the epidemiology of VPDs [46, 50, 55]. In this regard, it should be stressed that subjects referring to health professionals in order to be informed showed significantly higher propensity towards vaccinations (adjOR 4.599 95%CI 1.540-13.74), whereas STs referring to uncontrolled information sources such as new media, still retaining a generally positive attitude, were more frequently against vaccinations, and showed higher prevalence of false beliefs and misunderstanding, ultimately confirming the critical role for an appropriate information [1, 24, 34]. In this regard, it should be stressed that several Education systems, including the Italian one, lack of key vaccine stakeholders such as school nurses and school physicians [39, 62, 63]: in such settings, the OPh are therefore called to enhance vaccine acceptance among people who are vaccine hesitant or even refuse vaccinations [43, 64, 65].

Limits of the study

Our study is affected by several major limitations. Firstly, we assessed a sample of relatively small size, gathered through convenience sampling and a regional basis. As Italy is highly heterogeneous in term of vaccination rates and vaccine acceptance, our sample may therefore not represent neither the whole Italian SEs nor STs populations [11].

Second, our study included a very selected population (i.e. subjects participating to a First Aid course), presumptively encompassing subjects more sensitive to health themes: a significant selection bias cannot therefore be ruled out, ultimately suggesting that our survey overestimated actual vaccine acceptance of the parent occupational group.

Third, we lack data about the vaccination status of the participants. Despite this specific item has been repetitively described as a significant behavioural predictor [44], the self-referred vaccination rates are notoriously inaccurate, in particular for paediatric-age immunizations [7, 23, 32], and therefore we opted for a propensity assessment [41-42]. Unfortunately, this design ultimately hampers the ability of our study to accurately distinguish between vaccine hesitant and "*fence sitter*" peoples, but this assessment was not included in the endpoints of our study [41-43].

Eventually, our study assessed the risk perception of the participants towards VPDs without dichotomizing the perceived consequences in adults and children/adolescents. This a substantial limit, as health impacts of most VPDs are significantly influenced by age, sex and health status of the recipient, and also their perceptions may be significantly heterogeneous [1-4, 6]. For example, pertussis is frequently acknowledged as a severe disease in children, but general population is usually unaware of its possible consequences in adults and elderly, as well as of their potential role as spreaders of the pathogen among unprotected of partially protected groups. On the contrary, general population frequently ignore the potential severity of *H influenzae* type B infection in younger age groups, dismisses varicella as an annoying disease of children, undermining its consequences in adults, and similarly ignores that infections such as measles may have severe long term consequences in unvaccinated subjects [3, 14-15, 24-25, 34, 46-52].

Conclusions

In conclusion, our results support the usefulness for STs of educational campaigns and additional training about vaccines and misconceptions about vaccines and vaccine practices in order to fill their knowledge gaps. The primary objective of these interventions should be raising the awareness of STs regarding VPDs, emphasizing that the teachers themselves are at significant risk, and that they may potentially become the source of potentially life-threatening infections for their students and relatives. Moreover, these campaigns should also be aimed to convince STs about safety and efficacy of vaccines, in order that they could proactively share and disseminate up-to-date evidence across the school settings. Cornerstone of these campaigns should be identified in healthcare professionals strictly interconnected with the school environment and well aware of the specific characteristics of the school settings. In health system lacking significant stakeholders such as school nurses and school physicians, the contribution of OPh may be significant.

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All person who meet authorship criteria are listed as authors, and all authors certify that they have no affiliation with or involvement in any organization or entity with any financial interest, or non-financial interest (such as personal or professional relationship, affiliation, knowledge of beliefs) in the subject matter or material discussed in the manuscript.

Authors' contributions

MR, CS and GG conceived of the presented idea. MR developed the theory and performed the computations. GG and MR collected the data, with subsequent revision of LV. CS encouraged MR to investigate root causes of vaccine hesitancy, and supervised the findings of this work. MR, LV and GG contributed to the final version of the manuscript. All authors discussed the results and contributed to the final manuscript.

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ORIGINAL ARTICLE

Age- and risk-related appropriateness of the use of available influenza vaccines in the Italian elderly population is advantageous: results from a budget impact analysis

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Keywords

Budget impact analysis • Costs • Appropriateness • Influenza • Vaccination • Elderly • Italy

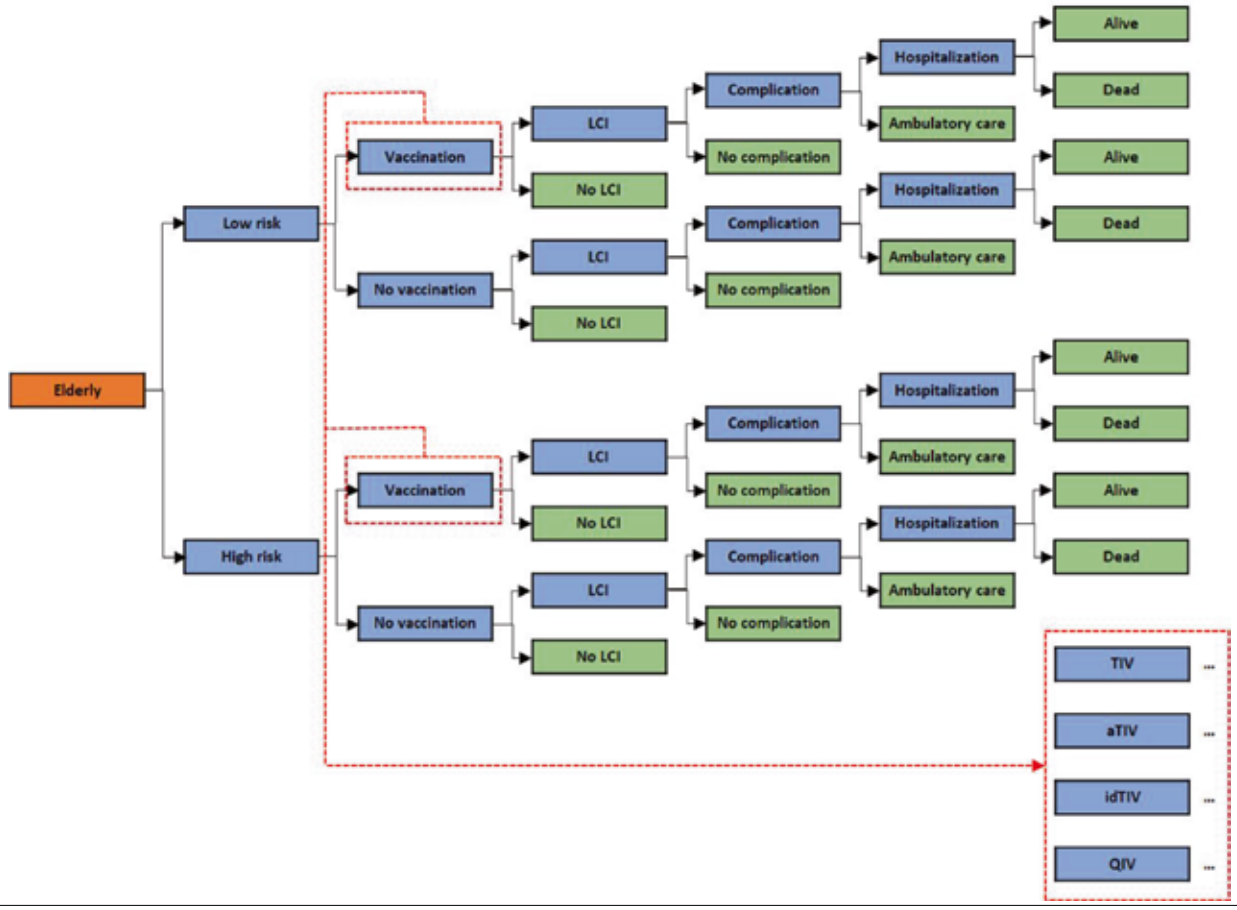
Summary

Introduction. *Nowadays, four different types of influenza vaccines are available in Italy: trivalent (TIV), quadrivalent (QIV), MF59-adjuvanted (aTIV) and intradermal TIV (idTIV) inactivated vaccines. Recently, a concept of the appropriateness (i.e. according to the age and risk factors) of the use of different vaccines has been established in Italy. We conducted a budget impact analysis of switching to a policy, in which the Italian elderly (who carry the major disease burden) received the available vaccines according to their age and risk profile.*
Methods. *A novel budget impact model was constructed with a time horizon of one influenza season. In the reference scenario the cohort of Italian elderly individuals could receive either available vaccine according to 2017/18 season market share. The alternative scenario envisaged the administration of TIV/QIV to people aged 65-74 years and at low risk of developing influenza-related complications, while aTIV/idTIV were allocated to high-risk 65-74-year-olds and all subjects aged ≥ 75 years.*
Results. *Switching to the alternative scenario would result in both significant health benefits and net budget savings. Particularly, it would be possible to prevent an additional 8201 cases of laboratory-confirmed influenza, 988 complications, 355 hospitalizations and 14 deaths. Despite the alternative strategy being associated with slightly higher vaccination costs, the total savings derived from fewer influenza events completely resets this increase with net budget savings of € 0.13 million.*
Conclusions. *An immunization policy in which influenza vaccines are administered according to the age and risk profile of Italian elderly individuals is advisable.*

Introduction

In Italy, influenza is still the third largest cause of infectious disease-related mortality [1]; most influenza-attributable deaths occur in the elderly [2, 3]. Vaccination remains the most important and effective public health measure able to dramatically reduce the large burden of seasonal influenza [4]. Indeed, given that the elderly are the most affected population, all member states of the European Union recommend seasonal influenza immunization for this particularly vulnerable group [5]. Both National [6] and supranational [5] authorities have recognized the value of influenza immunization among seniors and demand at least a 75% vaccination coverage (VC) or better still a 95% VC.
VC rates among single Italian regions are often described as "jeopardized" [7]. This figure may be exemplified by seasonal influenza VC rates among the elderly: in 2016/17 there was a 1.7-fold difference between regions, from 37.3% in South Tyrol to 63.1% in Umbria [8].
In the context of the Italian fiscal federalism, the Ministry of Health periodically issues, following approval of the State-Regions Conference, National Immunization Prevention Plans (the last edition for 2017–2019) aimed at guiding and harmonizing immunization strategies across the regions. Each Region then adopts its own immunization plan [9]. Moreover, prior to the commencement of an influenza season, the Ministry of Health issues "Prevention and Control of Influenza" recommendations for a given season [6, 10]. Each Region may then fully adopt the national recommendation or provide its own circular.
Unlike vaccines against several other diseases, the market of influenza vaccines is significantly diverse. In the last 2017/18 influenza season, four different vaccine types were available for immunization of the elderly, namely, trivalent inactivated vaccines (TIVs), MF59-adjuvanted TIV (aTIV), intradermal TIV (idTIV) and quadrivalent inactivated vaccines (QIV). These four vaccine formulations have different clinically important features, including age indication, route of administration, immunogenicity, vaccine effectiveness (VE), etc. [6, 11-13]. For instance, while TIV and QIV may be administered to all principal age-classes [6], aTIV and idTIV have been specifically developed for older adults in order to overcome the suppressive phenomenon of immunosenescence [6, 14-16].

Fig. 1. A simplified decision-tree model used in the budget impact analysis.



Procurement of influenza vaccines in Italy is based on regional tenders with four separate lots for the four aforementioned vaccine types. The vaccine mix (i.e. relative procurement distribution of single vaccine types) is changing continuously and partly reflects the evolving market situation (e.g. QIV has recently entered the market). Similarly to the "jeopardized" nature of influenza VC rates, the influenza vaccine mix is highly inhomogeneous among single regions. Several factors may have contributed to this inhomogeneity. For example, in some regions the vaccine acquisition price may be crucial, with TIV (as the "oldest" of the available alternatives) being the cheapest option.

It has been recently shown [13] that all available vaccines are cost-effective in the elderly Italian population when compared with non-vaccination. However, aTIV has the most favorable economic profile, being a highly cost-effective strategy compared with TIV and the dominant (both in terms of cost-saving and effectiveness) strategy compared with idTIV and QIV. However, while the cost-effectiveness analysis allows the value comparison of different strategies, it cannot directly address the issues of the affordability and sustainability of such interventions. This is why the budget impact analysis (BIA) is increasingly required by reimbursement authorities as a part of a comprehensive economic evaluation of a technology [17]. Here we quantified the impact of a "diversified" (i.e. according to the age and risk category) seasonal influenza offer to the Italian elderly population on the national budget with the ultimate goal of informing administrators of regional influenza vaccination programs about tender policies.

Methods

Model structure and basic assumptions

A novel budget impact model was built in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The model simulates the natural history of laboratory-confirmed influenza (LCI). Briefly, an elderly individual (defined here as subject aged ≥ 65 years) at lower or higher risk of developing an influenza-related complication (see below) may be vaccinated (according to the current VC rate) with one of the four available influenza vaccines (according to the assumed market share). The non-vaccinated sub-cohort will develop LCI according to its average natural attack rate; by contrast, the vaccinated sub-cohort will have some reduction – according to the vaccine efficacy of single vaccines – in the number of LCI and all consecutive events. People infected with virus may develop a complication that, in turn, may be treated in either outpatient or inpatient regimens. Finally, the hospitalized elderly may die or survive. A simplified version of the model is depicted in Figure 1.

Time horizon and perspective

Given that both consequences of seasonal influenza and associated healthcare expenditure are assessable in a short-term period and in order to facilitate the comprehension of major results by all relevant stakeholders, the time horizon was set to one year corresponding to one influenza season. As recommended [17], the analysis was conducted from the perspective of the National Healthcare Service (NHS), i.e. only direct costs were considered.

Target population

The latest Italian National Vaccine Prevention Plan [18] recommends a universal seasonal influenza vaccination for people aged ≥ 65 years. As of 2017, a total of 13,528,550 Italian people were aged 65 years or more [19]. The whole elderly cohort is not homogeneous with regard to different features; it, for example, is comprised of people with and without serious chronic conditions. The presence of at least one underlying serious disease significantly increases the likelihood of developing an influenza-related complication [20, 21]. According to the National Institute of Statistics [22], 44.1% of Italian ≥ 65-year-olds have at least one serious chronicity. The assumed parameter is very close to that used in a previous Italian economic evaluation [23]. Moreover, compared with elderly individuals aged 65-74 years, those aged ≥ 75 years (51.2% of the elderly [19]) have significantly more pronounced features of immunosenescence [24], which results in suboptimal immune responses to traditional influenza vaccines [14-16].

Immunization rate, current vaccine mix and hypothetical vaccine mix with rationale

The probability of receiving vaccination was set to 52.0%, which reflects the last available (season 2016/17) official VC data among the Italian elderly [8], and is independent of vaccine type.

As mentioned above, four different vaccine types (with four different lots in public tenders) are currently commercially available in Italy. The current (season 2017/18) market share was quantified through analysis of allotments requested by single Italian regions. However, these estimates do not exactly reflect the vaccine mix among the elderly since (i) no elderly-specific number of doses of single vaccines requested were known, (ii) all four types of vaccine are routinely administered to the elderly, (iii) and idTIV and aTIV may be administered only to older adults. To establish an elderly-specific market share we proceeded as follows. Given that, the Italian Ministry of Health provides data [8] on VC relative only to the elderly and general populations (15.1%), we first calculated a VC among people aged < 65 years (4.5%). We then assumed (following a consultation with external experts) that the off-label administration of idTIV and aTIV can be neglected; therefore, non-elderly people may receive TIV or QIV only. To figure out the elderly-specific market share, we adjusted the parameter of interest by subtracting the quota of TIV and QIV allocated to non-elderly individuals.

To establish a hypothetical market share, we both consulted experts and analyzed the available regional policies regarding seasonal influenza immunization among the elderly. We found annual recommendations issued by the Region of Emilia-Romagna [25, 26] very comprehensive and in line with a recently published paper by Bonanni et al. [27] on the appropriateness of the use of different influenza vaccines available in Italy. In particular, in the latest Emilia-Romagna circular [26] it is stated that subunit TIVs are the only available alternative for young children aged 6-35 months, and may be eventually administered to healthy people aged up to 65 years. QIVs should be given to people aged 3-65 years with chronic underlying conditions, healthcare professionals, and it is also possible to administer these vaccines to people aged 65-74 years without immunosuppressive pathologies. aTIV is recommended for people aged ≥ 65 years especially if affected by underlying conditions leading to immunosuppression and all elderly individuals aged > 75 years. idTIV, which is indicated for those ≥ 60 years old, may be also used – specifically due to the intradermal route of administration – in people with coagulopathies (that easily encounter hemorrhage or hematomas following an intramuscular injection) and those being immunized at home [26].

The imputation process of a hypothetical vaccine mix considered both the abovementioned statements from the latest Emilia-Romagna circular [26] and the fact that the elderly in low- and high-risk groups have different patterns of vaccination and clinical outcomes (see below). Thus, at the first step we established a vaccine mix for the elderly at high-risk of LCI-related complications. Considering the Emilia-Romagna circular [26], it was assumed that only aTIV and idTIV should be administered to this subpopulation in a ratio of 5.6, which reflects the latest tender allotment in Emilia-Romagna. To establish a vaccine mix for the low-risk elderly group (again, in order to reflect the Emilia-Romagna circular [26]), these were divided into two sub-cohorts, namely 65-74 year-olds and ≥ 75 year-olds. The first sub-cohort could receive either TIV or QIV according to their current countrywide elderly-specific market share ratio of 0.79, while the second sub-cohort may be immunized only with aTIV and idTIV in the above-described ratio of 5.6. The current and hypothetical vaccine mixes are summarized in Table I.

Tab. I. Current and hypothetical vaccine mixes, by vaccine type.

Vaccine	Current elderly-specific vaccine mix (low risk and high risk), %	Alternative elderly-specific vaccine mix (low risk/high risk), %
TIV	20	26/0
aTIV	40	37/85
idTIV	15	6/15
QIV	25	31/0

Tab. II. Input parameters used to populate the model (base case), by risk group.

Parameter		Low-risk elderly	High-risk elderly	Reference
Epidemiological and clinical, %	Vaccination	47.8	57.3	8, 28
	LCI	5.4	5.4	29, 30
	Outpatient visit | LCI	38.6	38.6	32
	P Bronchitis | LCI	2.69	3.46	21
	P Pneumonia | LCI	1.04	1.31	21
	P URTI | LCI	4.50	4.67	21
	P CV complication | LCI	0.09	0.80	21
	P CNS complication | LCI	0.21	0.31	21
	P Renal complication | LCI	0.05	0.16	21
	P Otitis media | LCI	0.21	0.15	21
	P GI bleeding | LCI	0.66	0.66	21
	P Hospitalization | complication	26.4	34.3	33–35
	P Death | complication	3.6	3.8	21, 37
VE, %	TIV	58.0		38
	rVE (vs TIV) of aTIV	25.0		39
	rVE (vs TIV) of idTIV	16.5		40
	rVE (vs TIV) of QIV	6.6		23, 41, 42
Costs, €	TIV price	2.11		Weighted average (regional allotments)
	aTIV price	5.28		Weighted average (regional allotments)
	idTIV price	5.37		Weighted average (regional allotments)
	QIV price	5.80		Weighted average (regional allotments)
	Vaccine administration	6.16		44
	GP visit	20.66		43
	Outpatient treatment of complications (except for otitis media)	80.0		45
	Outpatient treatment of otitis media	50.0		45
	Bronchitis (DRG 097)	1,832		23, 46
	Pneumonia (DRG 090)	2,291		23, 46
	URTI (DRG 080)	4,422		23, 46
	Cardiovascular complications (weighted mean DRGs)	3,544		29
	Renal complications (DRG 316)	3,734		23, 46
	CNS complications (weighted mean DRGs)	3,507		29
	Otitis media (DRG 069)	1,247		23, 46
	GI bleeding (DRG 175)	2,091		23, 46

Model input parameters and sources

Most input parameters relative to epidemiology, clinical outcomes of LCI, and costs were adapted from our recent CEA [13]; these are summarized in Table II. Some parameters were extracted as shown in Table II, some were averaged in order to reflect different seasons, while some were interpolated to reflect the Italian reality. Italy-based parameters were comprehensively searched and preferred providing that these were robust and nationally representative. For a detailed description of all model parameters, their strengths and shortcomings, readers are invited to assess a report by Di Pietro et al. [13]. Briefly, considering that the official VC data do not distinguish between low- and high-risk elderly, risk-specific VCs were imputed. It has been establishe [28] that compared to the Italian elderly at low risk of developing influenza-related complications those at high risk have a relative risk of being immunized of approximately 1.2. To establish a risk-category-specific VC we formulated the following equation: $(0.559 \times VC_{low\ risk}) + (0.441 \times VC_{high\ risk}) = 52.0\%$, i.e. $(0.559 \times VC_{low\ risk}) + (0.441 \times 1.2 \times VC_{low\ risk}) = 52.0\%$. This equation allowed us to assume VC rates of 47.8% and 57.3% for the elderly at low and high risk, respectively.

The natural attack rate of influenza was set to 5.4%. This came from an imputation approach: a baseline meta-analytically obtained influenza-like illness (ILI) attack rate among the Italian elderly (16.8%) [29] was weighted by an average Italy-specific influenza virus isolation rate (32%) [30]. The assumed parameter resulted congruent with an estimate (5.7%) from a pooled analysis of placebo arms in elderly-specific randomized controlled trials [31]. It was next assumed that approximately a third (38.6%) of the elderly with LCI will seek care from their general practitioners (GPs). This estimate is an elderly-specific average and came from a web-based Italian participatory surveillance system for influenza [32].

Tab. III. Comparison of the current and alternative scenarios in terms of laboratory-confirmed influenza and following events (base-case results).

Parameter	Current scenario	Alternative scenario	Difference
LCI	479,149	470,948	-8,201
Complicated LCI	49,419	48,431	-988
Hospitalizations	14,867	14,512	-355
Deaths	551	537	-14

Given the unavailability of large-scale elderly-specific Italian data on the consequences of LCI, these were established by experts from the international literature. A large-scale UK study by Meier et al. [21] was used to quantify influenza-related complications among the elderly belonging to low- and high-risk categories. The probability of hospitalization following a LCI-related complication was interpolated from the United States surveillance system FluSurv-NET (elderly-specific averaged data) [33] to the Italian elderly population. The estimated figure was then adjusted to the sensitivity of the polymerase chain reaction (PCR) influenza test that is relatively low in the elderly [34, 35]. By performing calculations, a total of 23,539 expected hospitalizations were established. It was assumed that, like in the case of LCI-related complications, the elderly at high risk had a greater probability of being hospitalized (relative risk vs elderly at low risk of 1.3 [21]). The resulting probabilities were 26.4% and 34.3% for the elderly at low and high risk, respectively.

Considering the mortality following LCI, it was decided not to use the statistically imputed excess mortality estimates since it is based on a statistical imputation. Indeed, a recent meta-regression analysis [36] highlighted that the statistical model selected has a significant impact on the estimate and that "no average estimate of excess mortality could reliably be made". Instead, we identified a study by Arriola et al. [37] that reported LCI-related mortality among non-vaccinated elderly being 3.8%. Given that almost all elderly in that subsample had at least one underlying health condition, we attributed this proportion to the at risk elderly. To figure out the mortality rate among the elderly at low risk, we deflated the estimate by Arriola et al. [37] by applying a correction factor derived from a ratio of the probability of death among low- and high-risk elderly reported by Meier et al. [21]. However, it should be considered that the number of deaths was considered only in the comparison between alternative scenarios from the point of view of Public Health impact and not budgetary impact (i.e. the cost of death was not quantified).

A meta-analytically obtained TIV VE of 58.0% [38] was used. The relative (i.e. *vs* TIV) VE (rVE) of aTIV, idTIV and QIV was assumed to be 25.0%, 16.5% and 6.6%, respectively. While the rVE of aTIV and idTIV were adopted from a large-scale multi-season observational study [39] and robust regression modeling [40], respectively, that of QIV was imputed, given the unavailability of VE data in the elderly. The additional benefit of the second B lineage strain was imputed, using a methodology similar to previous research on the topic [23], by weighting the relative frequency of B virus type in the elderly (17.9%), average level of B lineage mismatch (60.1%) [23] and rVE of QIV vs TIV against the mismatched B lineage (35%) [41, 42].

Vaccine acquisition price is a weighted average of awarded prices at regional tenders. Costs relative to vaccine administration and general GP visits derived from official documents [43, 44], while the ambulatory treatment of LCI-related complications were adapted from Marchetti et al. [45]. Diagnosis-related groups (DRGs) reimbursement tariffs were used to establish the costs of inpatient treatment of complications. Most DRGs (097, 090, 080, 316, 069 and 175) were taken directly from the current official reimbursement documents [46]. By contrast, given that cardiovascular and central nervous system complications are associated with several DRGs, we used a weighted average tariff estimated by Iannazzo [29].

Scenario analysis

In order to address the uncertainty, two scenario analyses were conducted by changing the vaccine mix and/or vaccine price. In particular, in the first analysis QIV took the whole quotum of TIV among low-risk people aged 65-74 years. The second scenario considered the same vaccine mix as the first, but with a decrease in QIV price by 20% (€ 4.64). Both scenarios were compared with the base case.

Results

Table III reports results of the base-case analysis relative to the most important clinical events. Over a single average influenza season, switching to the alternative scenario would, approximately, prevent an additional 8,201 cases of LCI, one thousand complications, 350 hospitalizations, and 14 LCI-related deaths.

From the point of view of budget implication, the alternative strategy would be associated with a slightly higher total investment (€ 1.1 million). On the other hand, the total savings derived from fewer influenza-related events completely reset (€ 1.3 million) the increase in vaccination campaign costs. Indeed, the alternative strategy would allow a saving of approximately 0.13 million (Tab. IV). In summary, the base-case results suggested that implementation of the alternative strategy would allow not only for significantly fewer number of LCI and LCI-related events, but also significant financial savings.

If the market share of TIVs is completely absorbed by QIV in the low-risk 65-74 year-olds (scenario analysis 1), some health-related savings would be observed

Tab. IV. Comparison of the current and alternative scenarios in terms of direct costs (base-case results).

Parameter	Current scenario	Alternative scenario	Difference
Total vaccination costs, €	77,012,493	78,131,409	+1,118,916
Total event costs, €	15,079,950	13,828,051	-1,251,899
Overall costs, €	92,092,443	91,959,460	-132,983

(Tab V). From the economic perspective (Tab. VI), a slight increase (3.6% or €0.24 per person) in overall costs would be seen. If, on the other hand, QIV price is reduced by 20% (scenario 2) the overall incremental cost goes up by only 1% corresponding to € 0.07 per person.

Discussion

The findings reported here suggest that a shift to the alternative scenario would be associated with both significant health gains and net financial saving. The alternative scenario tried to incorporate the issue on equity considering that the pool of elderly Italians is highly inhomogeneous in terms of both immune response following immunization and risk of developing LCI-related complications. Such a "diversified" influenza vaccination offer finds its grounds in epidemiological and immunological aspects of ageing. Thus, the prevalence of underlying chronicities increases sharply with age. If, for example, the prevalence of at least one serious chronic condition among elderly Italians aged 65-69 years is 31.7%, it growths by 21% and 68% among people aged 70-74 and ≥ 75 years, respectively [22]. In turn, several chronic conditions increase risk of immunosuppression [47-49]. Furthermore, it has been demonstrated that compared with subjects aged 60-75 years, in those aged > 75 years the number of lymphocytes falls below a critical threshold and the T cell receptor repertoire diminishes abruptly [24]. This is why the immune response to vaccination is less efficacious the most senior individuals and the elderly with underlying conditions [24, 50]; these subjects are therefore in need of enhanced influenza vaccines like aTIV, idTIV, or high-dose TIV (which is currently unavailable in Italy).

We also showed that a complete switch from TIV to QIV in the low risk elderly aged 65–74 years would be associated with substantial health benefits and a low budget impact of € 0.24 per person. If, however, the price of QIV drops, which is highly probable, the net impact would be of only € 0.07 per person. This strategy should therefore be considered in regions with a more flexible budget. This scenario would also be similar to the recently published paper by Bonanni et al. [27] on the appropriateness of the use of influenza vaccines in Italy; this paper states that subunit TIVs should be administered to children aged 0.5-3 years, split QIVs to subjects aged 3-70 years at high risk (except for people aged ≥ 60 and ≥ 65 years who due to health conditions need idTIV or aTIV, respectively) and aTIV/idTIV for all elderly individuals aged > 70 years [27].

More generally, our results confirm that the influenza immunization policy adopted by Emilia-Romagna [25, 26] is sound also from both public health and economic impact points of view. Emilia-Romagna may be considered as a benchmark Region in terms of both healthcare performance and immunization policies. The most recent *Meridiano Sanità* regional index [51] put Emilia Romagna at second place in the area "Effectiveness, efficiency and appropriateness of healthcare provision". In 2016, the Region was the first to introduce a mandatory character for some vaccines in order to access educational services for young children [52]. The seasonal influenza coverage rate in Emilia-Romagna is also higher than the national average [8].

Our discussion should include the comparison of our findings with previous research. However, only a few BIAs on this topic have been conducted so far. The early BIA by Iannazzo [29] compared the budget impact of a complete switch from TIV to aTIV; the budget impact of the use of either vaccine was also compared with non-

Tab. V. Scenario analysis: comparison of the base-case and scenarios 1 and 2 in terms of laboratory-confirmed influenza and following events.

Parameter	Base-case	Scenarios 1/2	Difference
LCI, N	470,948	469,019	-1,929
Complicated LCI, N	48,431	48,249	-182
Hospitalizations, N	14,512	14,464	-48
Deaths, N	537	536	-1

Tab. VI. Scenario analysis: comparison of the base-case and scenarios 1 and 2 in terms of direct costs

Parameter	Base-case	Scenario 1	Difference*	Scenario 2	Difference*
Total vaccination costs, €	78,131,409	81,599,501	+3,468,092	79,209,359	+1,077,950
Total event costs, €	13,828,051	13,644,505	-183,546	13,644,505	-183,546
Overall costs, €	91,959,460	95,244,006	+3,284,546	92,853,864	+894,404

*Difference between the base-case and a given scenario.

vaccination. In particular, the author established that the use of either vaccine would be associated with significant reduction in the number of cases of ILI and following events. However, compared with non-vaccination, the use of TIV would have produced a 4.6% increase (€ 50.2 million) in overall costs; while the universal use of aTIV among the elderly would have yielded a net saving of € 74 million. The results by Iannazzo [29] cannot be directly compared with our findings due to differences in model structures and several assumptions. First, given that, in the time frame only two alternatives were available, Iannazzo [29] compared the budgetary impact of only TIV, aTIV and no vaccination, assuming their universal use and therefore without establishing a vaccine mix. Second, the attack rate of ILI and not LCI were used, overestimating the economic output. Third, a significantly higher proportion of the elderly at high risk of developing complications was exploited. Fourth, the TIV acquisition price was mush higher (€ 3.81 *vs* € 2.25), while the relative (vs aTIV) increase in price was much lower (+46% *vs* +150%) [29].

The second more recent BIA [53] investigated the introduction of QIV; the model was not elderly-specific, i.e. the entire Italian population was included. The introduction of QIV with a market share of 9% was associated with both health benefits and some net savings (€ 674,089; € 0.01/person). However, the increase in QIV market share (from 0% to 9%) was mostly determined by reduction in the market share of aTIV (from 25% to 14%). Moreover, in that model the market share of TIV had a slight increase (from 49% to 52%) [54]. In real life, however, the increase in QIV was mostly driven by a progressive reduction in the quotum of TIV, while aTIV market share saw some increase.

Although all costs used in our model are specific to the Italian healthcare system, some input parameters relative to the disease natural history came from non-Italian sources. Indeed, it was sometimes not possible to identify robust, population-based representative and elderly-specific data from Italian literature. It is likely that some crucial model parameters were underestimated. For instance, the hospitalization rate was not adjusted by the likelihood of influenza testing that is relatively low among seniors. In this regard, Reed et al. [35] have estimated that the number of observed influenza-related hospitalizations in over-65-year-olds should be inflated by a factor of 5.2. Given that hospitalization is a major driver of LCI-related costs, our estimates are conservative. Moreover, the assumed rVE (25%) of aTIV vs TIV may be underestimated: it has been shown [54] that aTIV is more effective than TIV in preventing LCI among the Canadian elderly by 63%.

In conclusion, a seasonal influenza immunization policy targeting the elderly Italian population, in which enhanced vaccines (aTIV and idTIV) are administered to high-risk elderly individuals aged 65-74 years and all those ≥ 75 years old, while TIV/QIV are administered to the low-risk elderly individuals aged 65-4 years, would be associated with both substantial health benefits and net financial savings. Moreover, this strategy is more equitable and therefore decision-makers and other relevant stakeholders should consider the implementation of such age- and risk-tailored influenza vaccination policies in their regions.

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Authors' contributions

MB and SC conceived, designed and conducted the study, collected data on costs and wrote the manuscript; CdW, PB and DP collected epidemiological data. All authors approved the final manuscript.

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ORIGINAL ARTICLE

Practicing health promotion in primary care – a reflective enquiry

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Keywords

Health promotion • Health education • Primary care • Public health

Summary

Introduction. *Health promotion is an integral part of routine clinical practice. The physicians' role in improving the health status of the general population, through effective understanding and delivery of health promotion practice, is evident throughout the international literature. Data from India suggest that physicians have limited skills in delivering specific health promotion services. However, the data available on this is scarce. This study was planned to document the current health promotion knowledge, perception and practices of local primary care physicians in Odisha.*

Methods. *An exploratory study was planned between the months of January – February 2013 in Odisha among primary care physicians working in government set up. This exploratory study was conducted, using a two-step self-administered questionnaire, thirty physicians practicing under government health system were asked to map their ideal and current health promotion practice, and potential health promotion elements to be worked upon to enhance the practice.*

Results. *The study recorded a significant difference between the mean of current and ideal health promotion practices. The study reported that physicians want to increase their practice on health education.*

Conclusion. *We concluded that inclusion of health promotion practices in routine care is imperative for a strong healthcare system. It should be incorporated as a structured health promotion module in medical curriculum as well.*

Introduction

With time there has been a growing interest in the role of primary care, and general practice in particular, in public health activities. General practice and general physicians are often regarded as the basic building blocks of public health, and primary care is seen as a logical location for local public health activities [1].The Alma-Ata declaration in 1978 identified the role of general physicians in public health as important, and 30 years later in a report on primary care the World Health Organization (WHO) confirmed this special relationship (WHO 1978, 2008). Health promotion has been identified one of the most important public health activities of physicians working on primary care settings [2-4].

Health promotion has a holistic approach of promoting health intervention to stimulate health and wellbeing i.e. proper nutrition and physical activities, preventing diseases, identification and maintaining health of persons suffering from the chronic illnesses [5, 6]. The physicians' role in improving the health status of the general population, through effective understanding and delivery of health promotion practice, is evident throughout the international literature. The major role of the clinician in health promotion is at the individual level and involves screening for risk factors and disease, and providing early treatment, advice, counseling, and referral. Primary care physicians can further broaden their impact by assuming roles at organizational, community, and government levels (e.g., as an active member of an organization or a consultant to an outside organization, a community leader or an agent of change, an influential constituent or a lobbyist). These roles enable primary care physicians to have an impact both on individuals and on environments to reduce disease risk factors. For instance, randomized controlled trials addressing brief interventions in heavy alcohol consumers has clearly demonstrated the importance of behavioural-focused health promotion activities in addressing and lowering consumption trends [7]. Similarly in lifestyle modification behaviors, such as smoking cessation, increasing physical activity and tackling obesity [8-12]. However, much of this evidence occurs within Westernized countries with a more limited extent and importance attached to the health promotion role and function of physicians – particularly within South-East Asia [13]. Recent data from Global Adult Tobacco Survey (GATS), India show that less than half of smokers who visited health care providers were advised to stop smoking [14]. Data from India also suggests that physicians lack skills in delivering brief intervention and counseling in tobacco cessation [15, 16].

Potential reforms are needed in this geographical location to enhance the effectiveness of health promotion practice among general physicians. The intention of this study was to survey the current status of health promotion knowledge, perceptions and practices of local primary care physicians, with an intention to locate and improve such practice. It sought to identify both 'ideal' and 'actual' practice. To the best of our knowledge, no previous study of this kind has been conducted in Odisha state.

Methods

The present study was carried out in the month of January-February 2013 in the state of Odisha, India. This exploratory study was conducted, using a two-step self-administered questionnaire. Thirty physicians practicing under government health system were asked to map their ideal and current health promotion practice, and potential health promotion elements to be worked upon to enhance the practice. Physicians were purposively selected from the Community Health Centres (CHC). CHCs are the major primary health care providing institutions, under Indian healthcare system'. The physicians at CHCs are registered medical doctors (MBBS and MD) and are the first line of contact with the community. They are the focal person to engage in any kind of health promotion activity among the general population. At first step, different health promotion elements i.e. 1) Use of strategies, 2) Manifesting Features and 3) Expressing values were accessed. Sub-elements listed in each domains like health communication, health education, policy development, advocacy, determinants of health, empowerment and social justice and equity etc. were asked to be rated on a 10 point Likert scale, mapping both ideal as well as their current practice.

At second step, physicians were asked to choose and identify the health promotion elements which they think have big gap in their current and the ideal practice and to state the desired changes in terms of 'start' or 'stop' and 'increase' or 'decrease' terminologies. The quantitative data hence obtained were entered in the MS Excel Software and imported into SPSS Version 17.0. Mean score of each element is calculated and were compared between current and ideal practice using *t*-test statistics. Value of $p < 0.005$ were considered significant and $p < 0.001$ were considered highly significant. Results were represented in tabular formats. Health promotion elements listed as the area to be start or stop and increase or decrease are listed in the box according to their frequency as quoted by the participants.

Objective of the study was explained to the study participants before the execution of the questionnaire and informed consent has been taken before administration of the tool. Unique ID has been assigned to each participant and anonymity is maintained through the process.

Results

Table I illustrates mean score comparison of health promotion elements between the ideal and the current practices of the physicians. It is evident that for each of the 16 elements listed, the difference between the mean of current and ideal is highly significant. Amongst the three main domains i.e. Using Strategies, Manifesting Features and Expressing Values, maximum differences has been observed in health communication, participatory approaches and empowerment respectively under each category.

Table II represents the frequency of participants under each health promotion elements identified as the desired

Tab. I. Comparative table of ideal and current practice of Health Promotion Elements.

S. No.	Health Promotion Elements	Ideal Practice (+/-SD)	Current Practice (+/-SD)	Difference (Ideal – Current)
1	Health Communication	8.90 (0.93)	4.69 (1.36)	4.21*
2	Health Education	8.72 (1.16)	5.14 (1.86)	3.58*
3	Self Help Mutual Aid	8.10 (1.23)	4.41 (2.18)	3.69*
4	Organizational Change	8.03 (0.98)	4.21 (1.71)	3.82*
5	Community Development and Mobilization	8.55 (1.05)	4.45 (1.90)	4.10*
6	Policy Development	8.45 (1.42)	4.52 (1.95)	3.93*
7	Advocacy	7.97 (1.14)	4.28 (1.85)	3.69*
8	Holistic View of Health	8.59 (1.40)	4.66 (2.34)	3.93*
9	Participatory Approaches	8.52 (1.05)	4.45 (2.30)	4.07*
10	Determinants of Health	8.69 (0.96)	4.86 (2.03)	3.83*
11	Focus on strengths and assets	8.41 (1.05)	4.90 (1.98)	3.51*
12	Using multiple complementary strategies	8.52 (1.05)	4.72 (1.94)	3.80*
13	Empowerment	8.97 (0.98)	4.31 (1.98)	4.66*
14	Social Justice and equity	8.97 (0.86)	4.34 (2.05)	4.63*
15	Inclusion	8.41 (1.24)	4.69 (2.03)	3.72*
16	Respect	8.72 (1.36)	5.03 (2.51)	3.69*

* < 0.001 Significance

Tab. II. Health promotion elements listing.

S. No.	Health promotion elements	Frequency of participants reported a 'start' or 'increase'
Using strategies		
1	Health Communication	8
2	Health Education	14
3	Self Help Mutual Aid	2
4	Organizational Change	5
5	Community Development and Mobilization	5
6	Policy Development	8
7	Advocacy	3
	Sub-Total	**45**
Manifesting features		
8	Holistic View of Health	13
9	Participatory Approaches	7
10	Determinants of Health	7
11	Focus on strengths and assets	3
12	Using multiple complementary strategies	4
	Sub-Total	**34**
Expressing values		
13	Empowerment	9
14	Social Justice and equity	22
15	Inclusion	6
16	Respect	1
	Sub-Total	**38**

area of change, mostly, 'increasing' the already existing practice or to 'start' a new initiative under that element. In the current study, majority of the participants reported a desired change in use of strategy to practice health promotion. Under the domain using strategy, physician wants to increase their practice on health education, followed by holistic view of health, under manifesting features and social justice and equity under expressing values.

Table III represents the physicians' understanding of the American Journal of Health Promotion and the Ottawa Charter definition of health promotion.

Discussion

The aim of this study was to comprehend the present level of understanding on health promotion among in-service health professionals. The study would also enable an assessment of what is required to further enhance health promotion component in the context of primary care delivery. It is important that health professionals are able to understand and delineate exactly what constitutes health promotion practice. Effective health promotion practice is dependent on sound theory and clear conceptualization of the matter by the health professionals [17]. Even though health promotion is strongly built into the concept of all the national health programs with implementation envisaged through the primary health

Tab. III. Physicians' understanding of the American Journal of Health Promotion and the Ottawa Charter definition of health promotion.

S. No.	Core Themes	Domains	Major Contrasting difference between American Journal of Health Promotion & Ottawa Charter
1.	Emergence of definitions	Formulated by	'AJHP Definition has been formulated by a single person and Ottawa charter was formulated during a conference of many experts'. [3+]
		Dimensions	'Spiritual and intellectual dimensions were included in AJHP definition which is not there in Ottawa definition'. [3+]
		Process of promotion	'AJPH definition helps people to reach the optimal level of health whereas Ottawa enables people to attain optimal health'. [3+]
2.	Areas of development	Pre-Requisites	'AJHP definition has large number of pre-requisite like but Ottawa has very few'. [2+]
3.	Approach	Stakeholders	'AJPH has more of an individualistic Approach while Ottawa has programme, community and local need based approach'. [3+]
		Policy Perspective	'AJPH do not highlight the policy intervention in health promotion programme but importance of same has been highlighted in Ottawa charter'. [2+]
		Equity	'AJPH doesn't Emphasized on Equity in Health, while Ottawa highlighted the importance of the same'. [1+]
4.	Strategies of definition	Political Commitment	AJPH didn't highlight the importance of political commitment whereas Ottawa definition clearly quoted the importance of the same. [3+]
		Multi-Sectoral Collaboration	AJPH didn't discuss about multi-Sectoral collaboration whereas Ottawa definition clearly demonstrated the importance of the same. [2+]
		Accountability	Optimal Health through health promotion is much accounted at individual level by AJHP whereas Ottawa made community, government and society as a whole responsible for it. [3+]

care system based on the principles on equitable distribution, community participation, inter-sectoral coordination and appropriate technology, it has received lower priority compared to clinical care [18]. The present Indian medicine (MBBS) curriculum lacks health promotion component during formative training [19]. Evidence from earlier Indian studies on the student's beliefs and practices of health promotion reported that most students assessed preventive practices in their patients but did not feel well prepared and competent enough to counsel patients about health issues [20]. Furthermore, physicians have not been trained in-service. Though there are limited evidence published documenting the health promotion practices among physicians, some studies from other regions of the country demonstrated limited capacity of the physicians to practice health promotion related activities. Study from Chhattisgarh, an eastern state of India, reported that Fifty-four percent of practitioners were of the opinion that counselling is ineffective and 62% considered counselling as time-consuming process. Majority of physician expressed their willingness to undergo additional training in nutrition. Similarly, a study from Karnataka, southern state of India reported poor knowledge of primary care physicians about pharmacological as well as non-pharmacological methods of treatment of nicotine dependence. This could hampers the tobacco cessation practice among the physicians. In an another study conducted in Karnataka, physicians expressed requirement of continuing education about nutrition education, lactation management, and a greater awareness about the influence of inappropriate promotional practices by companies.

Health promotion and education plays a vital role in providing care for the Non-Communicable Diseases (NCDs). In response to current trends, the global health care community has begun to emphasize on health promotion as an essential tool to curtail the rise of individuals experiencing chronic diseases. Addressing the main determinants of these diseases such as tobacco use, improper diet, sedentary lifestyle and obesity, from a preventive approach could serve to be a cost-effective and sustainable strategy in heavily populous developing country like India. Tobacco, for instance, is a major risk factor for a number of morbidities and mortalities. Recent data from the Global Adult Tobacco Survey (GATS) in India showed that less than half of smokers who visited health care providers were advised to stop smoking [14]. Published data from India also suggest that physicians lack skills in delivering health promotion counseling services on tobacco cessation [24, 25]. One of the reasons identified for such lack of preparedness by health professionals is the fact that there is no well-established health promotion component during formative training in the country.

However, as a recently development, health promotion education has been launched by many elite government and private institutions of the country. Two year post graduate diploma on health education being run by Central Health Education Bureau (CHEB), which is an apex institute created in 1956 under the Directorate General of Health Services (DGHS), Ministry of Health and family welfare, India. Similarly, private and autonomous institutions like Public health foundation of India, The Gandhigram Institute of Rural Health and Family Welfare Trust, Ambathurai, All india Institute of hygiene and public health, Kolkata offers certificate and Post Graduate Diploma in Health Promotion (PGD-HP) [20]. The program aims to build public health capacity of the participants to enhance the understanding of health promotion and enhance their skills and proficiency in designing and implementation of health promotion programs. It can be inferred that health promotion is an intriguing field of public health gaining popularity steadily and significant efforts being made for capacity building of young public health workforce as well as in-service candidates (medical doctors and other staff). Though, an integrated health promotion in main stream curricula is still missing.

Against this backdrop, strengthening of health promotion and protection through development of an integrated education and health promotion programme, which has relevance to the local context, is important. There is a strong need of developing and incorporating a structured health promotion module in undergraduate and post-graduate medical curriculum to address the gap. Considering that health is essential for learning and development, health promotion should also be gradually built into all aspects of life in school as well as community. In-service physicians should be provided with compulsory hands-on training through specialized health promotion as part of their Continuing medical education.

A study conducted in Saudi Arabia to understand the health promotion practices of nurses reveals that while nurses had necessary skills, it was preferred that they focus on delivering acute care within the hospital setting and that the patients did not always appreciate nurses asking about health-related behavior switch were not directly linked to their present health problems [26]. Therefore, raising awareness among patients and educating them on the risks factors of NCDs through necessary health promotion initiatives is also a critical factor for prevention and control of NCDs.

It has also been observed that health promotion has never been incorporated in the duties or job responsibility of physician during primary care delivery services in India. This could have resulted in 'lay away' of health promotion practice compared to regular curative practices. Primary health care providers constitute the first point of contact between population and health system, and are suitably placed to assist individuals. Emphasizing health promotion at the primary care level is therefore important and can be addressed by introducing patient counseling or information dissemination on preventive aspects of prevalent diseases, as job responsibilities of primary care physicians.

The lack of awareness of the importance of health promotion has often prevented the proper recognition by managers and health workers. Physicians may have knowledge and skills but often their perception is that their role is as a sole point of care with curative services having

immediate outcome with immediate diagnosis and treatment [27, 28]. In the earlier studies, physicians have suggested that the main negative outcome associated with this role behavior was the de-prioritization of primary preventive care in favor of the immediate benefits of secondary care [29, 30]. . Second reason which could possibly lead to de-prioritization of health promotion practice could be the overburden of program implementation and increased patient load on physicians. Heavy inflow of patients for curative services might result in no choice but prioritization of curative services [29]. Training the allied health professionals like AYUSH practitioners, dieticians, physiotherapists etc. for counseling, nutrition education, hygiene, physical activities etc. could be a cost effective and efficient solution for the same.

The recent National Health Policy (NHP) 2017 recognizes and build upon the preventive and promotive care. The policy targets on school health- by incorporating health education as part of the curriculum, promoting hygiene and safe health practices by acting as a site of primary health care. Policy also promotes healthy living and prevention strategies from AYUSH (Indigenous system of medicine in India) and Yoga at the work-place, in the schools and in the community. However, there is very little documented evidence on health promotion practice in the country, which is limited to individual practices and motivation [31]. Due to recent policy push, we could expect an increase in health promotion practices among population and healthcare providers. An assessment in near future is desirable to ensure that the health promotion practice is incorporated and practiced effectively by primary healthcare providers.

Moreover, since it is difficult to measure the outcome of preventive services, the physician often tends to lacks motivation, given there is no official recognition, patient recognition, peer recognition, community recognition for the same. In addition to the above, there is also no incentive or financial benefits attached to it. Efforts should be made on designing framework for measurement of outcomes of preventive services and also generate sufficient awareness on the issue for patient and community recognition. The possibility of replicating successful global health promotion initiatives at the country level, customized according to country-level needs could also be explored.

The current study though provides a useful insight of physicians' health promotion practices but findings cannot be generalized to all physicians due to nonprobability sampling which includes purposive selection of physicians. However, findings from the study can be used as pilot exercise and more epidemiologically systematic studies can be undertaken to generate generalizable results.

Conclusions

Inclusion of health promotion practices in routine clinical care is imperative for building a strong healthcare system that ensures positive health outcomes, effectiveness and efficiency and health equity. This is all the more important in primary care settings as it is the first contact in a healthcare system for individuals and is characterized by longitudinally, comprehensiveness, and coordination. Health promotion should also be incorporated as a structured health promotion module in undergraduate and post-graduate medical curriculum. This will help the professional perceive health promotion as an integral part of health service delivery.

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Authors' contributions

SP1 and SP2 conceptualized the study. SP1 and SM did the data collection. ASC and RS has done the data analysis and interpretation. Manuscript was drafted by SP2, ASC, SM and RS. All the authors were involved in critical revision of the article and final approval of the version for submission.

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ORIGINAL ARTICLE

Changes in the incidence and antimicrobial susceptibility of healthcare-associated infections in a New York hospital system, 2006-2012

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Keywords

Antimicrobial resistance • Multidrug-resistant organisms • Bloodstream infections • Surgical site infections • Urinary tract infections

Summary

Introduction. *National efforts to curtail healthcare-associated infections (HAI) proliferated recently, though data detailing progress over time are limited. This retrospective cohort study aims to describe changes in incidence and antimicrobial susceptibility of HAI in four New York City hospitals over seven years.*
Methods. *Electronic data were collected retrospectively for all patients discharged from 2006 through 2012. Previously validated computerized algorithms based on National Healthcare Safety Network criteria detected bloodstream infections, pneumonia, surgical site infections, and urinary tract infections with Enterococcus spp., Staphylococcus aureus, Streptococcus pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Klebsiella pneumoniae. Antimicrobial susceptibilities were obtained from electronic laboratory records. Logistic regression was used to assess changes in odds of acquiring an HAI and odds of antimicrobial resistance over time, controlling for age, gender, severity of illness, previous hospitalizations, and admission source.*
Results. *In total, 19,052 HAI were identified among 761,426 discharges. HAI rates fell for all organisms, all infection types, and within all hospitals. Odds of acquiring an HAI decreased significantly over time for all organisms. Resistance levels were stable for Enterococcus spp., S. aureus, A. baumannii, and S. pneumoniae. Multidrug resistance increased for P. aeruginosa and decreased for K. pneumoniae, though imipenem resistance among K. pneumoniae climbed sharply in 2011.*
Conclusions. *This study suggests that HAI incidence rates are falling, possibly due to increased federal, state and local attention to healthcare quality and patient safety. Though we found no substantial reductions in resistance, recent national attention towards antimicrobial stewardship may precipitate a change in coming years.*

Introduction

Healthcare-associated infections (HAI) remain endemic in U.S. healthcare facilities despite growing emphasis on infection prevention and control programs designed to curtail their spread [1]. The latest national data released by the Centers for Disease Control and Prevention (CDC) National Healthcare Safety Network (NSHN) show notable decreases for some HAI including central line-associated bloodstream infections (BSI) and certain types of surgical site infections (SSI) [2]. However, national longitudinal data are available only for the selected types of HAI tracked by the NHSN, which are limited primarily to procedure- and device-associated infections. The NHSN data also reveal substantial differences across regions and states, emphasizing the importance of monitoring trends at the local level [2].

Antimicrobial resistance among common healthcare pathogens is similarly persistent in hospitals, even with advances in stewardship efforts and transmission-based precautions for patients with drug-resistant organisms [3-5]. Multidrug-resistant phenotypes are implicated in more than 20% of HAI nationally, though prevalence varies considerably by region and institution [6]. The CDC's most recent comprehensive report on antimicrobial susceptibilities shows only slight changes in resistance for most organisms over the past several years, but data are likewise limited to specific types of HAI and trends are evaluated for only a short time period [6].

In light of the need for longitudinal data at the local level following a period of sharp increases in regional Gram-negative resistance, this study aims to describe changes in the epidemiology of HAI in four New York City hospitals over a seven year period from 2006 through 2012. Specifically, this study assesses changes in incidence of HAI, prevalence of antimicrobial resistance, and patient-level factors at admission that are associated with these outcomes for six of the most common bacterial pathogens in healthcare settings.

Methods

Sample and setting

Data were collected from four hospitals in a single academically-affiliated network located in New York, NY. The facilities included a 221-bed community hospital, a 283-bed pediatric acute care hospital, a 647-bed adult tertiary/quaternary care hospital, and a 914-bed adult and pediatric tertiary/quaternary care hospital. All discharges occurring from January 1, 2006 through December 31, 2012 were included in the analyses. Although some patients were admitted multiple times throughout the seven-year study period, the unit of analysis for this study was each patient discharge.

Data collection

All data were collected retrospectively from the network's Clinical Data Warehouse, which stores information from a variety of electronic sources shared by the four hospitals [7]. Dates of hospital admission and discharge, source of admission, and previous in-network hospitalizations were obtained from the admission-discharge-transfer (ADT) record. Complete lists of International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) diagnosis and procedure codes associated with each admission were obtained from billing records. Time-stamped culture results and antimicrobial susceptibility patterns were obtained from clinical microbiology laboratory records. All data were linked using patients' unique medical record numbers and admission dates.

Definitions of infections, antimicrobial resistance, and patient characteristics

Four types of infections were included in this analysis: BSI, SSI, urinary tract infections (UTI), and pneumonia. Algorithms for identifying infections in the electronic data were designed in accordance with the NHSN guidelines for surveillance of HAI [8]. Figure 1 illustrates criteria used to adjudicate cases of each infection type: BSI were identified by blood cultures; SSI by post-operative wound cultures and documentation of an NHSN operating room procedure; pneumonia by respiratory culture and ICD-9-CM diagnosis code for pneumonia; and UTI by either urine culture 10^3-10^5 colony forming units per milliliter (CFU/mL) and less than two other species and pyuria within a 48 hour window before or after culture collection, or urine culture with at least 10^5 CFU/mL and less than two other species.

Dates of culture collection and hospital admission were used to determine whether infections were healthcare-associated, i.e., developed at least two days after hospital admission.

This study included HAI associated with *Staphylococcus aureus*, *Acinetobacter baumannii*, *Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis* and *Enterococcus faecium*. Binary classifications of antibiotic resistance were defined for *S. aureus* (oxacillin), *S. pneumoniae* (penicillin), *A. baumannii* (ampicillin-sulbactam), and *E. faecalis* and *E. faecium* (vancomycin). For *P. aeruginosa* we assessed resistance to cefepime, gentamicin, levofloxacin, meropenem, piperacillin/tazobactam, and tobramycin. For *K. pneumoniae* we assessed resistance to cefepime, ceftriaxone, gentamicin, imipenem, levofloxacin, meropenem, piperacillin/tazobactam, tobramycin, and trimethoprim/sulfamethoxazole. Multidrug resistance for *P. aeruginosa* and *K. pneumoniae* was defined as resistance to at least three antibiotic classes among those assessed [9]. Resistance to each antibiotic was determined by the hospitals' clinical microbiology laboratories.

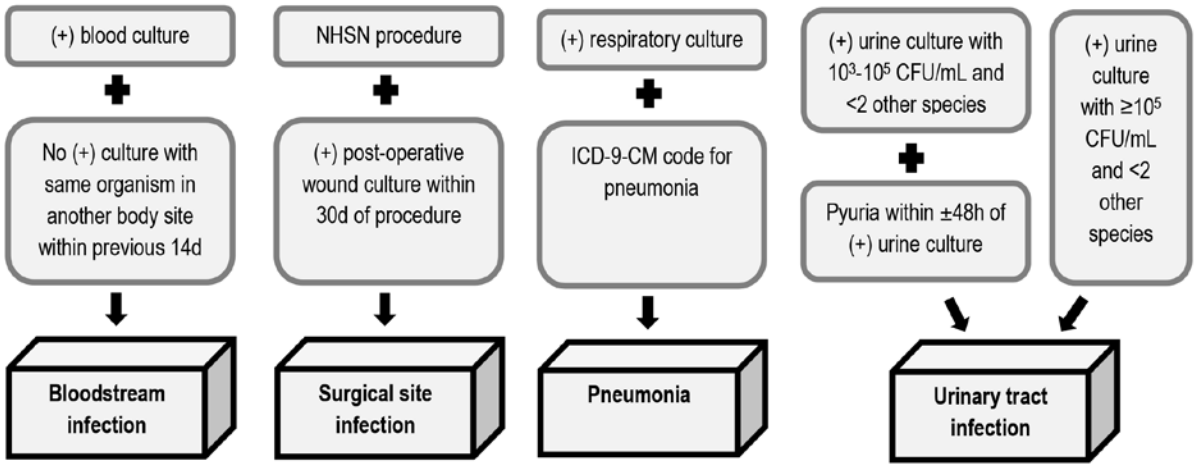
Patient characteristics at admission were assessed using several measures. A weighted Charlson Comorbidity Index was created using ICD-9-CM diagnosis codes for conditions indicated as being present upon hospital admission [10]. Patients who had at least one within-network inpatient hospitalization in the previous year were identified using ADT records. In addition, ADT records were used to determine patients' admission source, defined as either healthcare (i.e., transfer from another hospital, ambulatory surgery center, skilled nursing facility, hospice center) or non-healthcare (e.g., from home). Patient age, sex, admission hospital, and admission year were also collected from the Clinical Data Warehouse.

Statistical analysis

To assess changes in HAI over time we tabulated the number of HAI occurring each year and stratified by organism and body site of infection. Percent changes in HAI incidence per 10,000 discharges between 2006 and 2012 were calculated. Multiple logistic regression was used to evaluate changes in odds of infection over time, controlling for hospital, age (continuous), sex, within-network hospitalization in the previous year, admission source, and Charlson Comorbidity Index. A separate model was constructed for each of the six organisms. Patients who had an infection in more than one body site with the same organism during a single admission were represented only once in each multivariable model. In order to evaluate whether patient characteristics associated with HAI changed throughout the study period, we also assessed interaction between year and age, sex, prior hospitalization, admission source and Charlson Comorbidity Index for each body site of infection.

To assess changes in antibiotic sensitivities over time, we tabulated the annual proportion of infections resistant to each of the antibiotics identified *a priori* for each organism. Multiple logistic regression was used to evaluate changes in the odds of resistance over time, controlling for hospital, age, sex, within-network hospitalization in the previous year, admission source, and Charlson Comorbidity Index. Each organism was modeled separately. Patients who had an infection at more than one body site with the same organism during a single admission were represented only once in each multivariable model and were considered to have a resistant infection if at least one of the infections was caused by a resistant organism.

Fig. 1. Algorithms for identifying four types of infections using electronically available data from laboratory records and International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) diagnosis and procedure codes. Definitions are based on the Centers for Disease Control and Prevention National Healthcare Safety Network (NHSN) guidelines for surveillance of HAIs [7]. ICD-9-CM codes for pneumonia included 003.22, 020.3-020.5, 021.2, 022.1, 031.0, 039.1, 052.1, 055.1, 073.0, 083.0, 112.4, 114.0, 114.4, 114.5, 115.05, 115.15, 115.95, 130.4, 136.3, 480.0-480.3, 480.8, 480.9, 481, 482.0-482.3, 482.30-482.32, 482.39, 482.4, 482.40, 482.41, 482.42, 482.49, 482.8, 482.81-482.84, 482.89, 482.9, 483, 483.0, 483.1, 483.8, 484.1, 484.3, 484.5-484.8, 485, 486, 513.0, and 517.1. CFU/mL, colony forming units per milliliter.



Results

Characteristics of the 761,426 patient discharges that occurred during the study period are described by year in Table I. A total of 19,052 HAI with the six organisms of interest were identified. Forty-nine percent were UTI (N = 9,319), 23% were pneumonia (N = 4,414), 19% were BSI (N = 3,602), and 9% were SSI (N = 1,717). From 2006 to 2012, incidence per 10,000 discharges fell for each type of HAI within all four hospitals and for each of the six organisms included in this study (Fig. 2). Table II displays results of the multivariable regression analyses modeling the association between advancing year and odds of HAI. For each organism there was a statistically significant decrease in the odds of HAI over time, controlling for hospital and patient characteristics. Patients with a healthcare admission source were significantly more likely to develop an HAI with all organisms except *S. pneumoniae*, for which a positive but not statistically significant association was found. Within-network hospitalization in the previous year significantly increased the odds of developing an HAI with all organisms except *S. pneumoniae*, for which a statistically significant negative association was found. Advancing age and greater severity of illness were significantly associated with development of HAI for all organisms. Male patients were significantly more likely to develop an HAI with *S. aureus, P. aeruginosa, S. pneumoniae* and *A. baumannii,* while female patients were significantly

Tab. I. Characteristics of hospitalized patients by year.

Year	2006	2007	2008	2009	2010	2011	2012
N	104,645	106,783	105,177	109,631	112,656	112,122	110,412
% (no.) admitted to each hospital							
Community	13.1 (13,668)	12.6 (13,476)	12.7 (13,376)	11.7 (12,803)	11.6 (13,072)	11.2 (12,557)	11.1 (12,225)
Pediatric acute care	15.8 (16,507)	17.1 (18,281)	18.0 (18,959)	15.2 (16,694)	14.6 (16,487)	14.5 (16,260)	14.9 (16,405)
Adult tertiary/quaternary care	31.9 (33,355)	31.7 (33,839)	31.4 (33,054)	31.9 (35,005)	32.2 (36,283)	31.7 (35,579)	31.3 (34,608)
Adult/pediatric tertiary/quaternary care	39.3 (41,115)	38.6 (41,187)	37.8 (39,788)	41.2 (45,129)	41.6 (46,814)	42.6 (47,726)	42.7 (47,174)
% (no.) admitted from healthcare source*	9.6 (10,044)	10.5 (11,228)	10.7 (11,287)	17.3 (18,983)	16.7 (18,798)	17.5 (19,653)	16.6 (18,270)
% (no.) hospitalized within previous year**	22.6 (23,658)	32.4 (34,601)	37.2 (39,069)	40.2 (44,054)	43.3 (48,772)	44.4 (49,747)	45.1 (49,800)
Mean (standard deviation) Charlson Comorbidity Index	1.4 (2.75)	1.6 (2.95)	1.6 (2.93)	1.6 (2.99)	1.7 (3.08)	1.8 (3.30)	1.8 (3.36)
Mean (standard deviation) age in years	44.5 (27.82)	44.3 (27.89)	44.4 (27.99)	44.6 (28.11)	44.8 (28.11)	45.5 (28.3)	45.4 (28.46)
% (no.) male sex	44.9 (46,989)	44.5 (47,511)	44.2 (46,473)	44.4 (48,717)	44.3 (49,930)	44.5 (49,939)	44.4 (48,989)

*Admission from another hospital, ambulatory surgery center, skilled nursing facility, or hospice center.

**Within-network hospitalizations only.

Tab. II. Relationships between advancing year and odds of healthcare-associated infection, odds of antimicrobial resistance at four New York City hospitals, 2006-2012.

Organism	*Enterococcus faecalis and Enterococcus faecium*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Streptococcus pneumoniae*
Relationship between advancing year and odds of healthcare-associated infection[1]						
N	6,301	4,399	4,116	2,758	688	195
Year (continuous, 2006-2012)	0.86 [0.85,0.87]	0.89 [0.88,0.90]	0.89 [0.87,0.90]	0.90 [0.88,0.92]	0.88 [0.85,0.92]	0.85 [0.79,0.92]
Hospital*						
Community	0.44 [0.40,0.48]	0.59 [0.52,0.66]	0.55 [0.49,0.63]	0.49 [0.42,0.56]	0.76 [0.56,1.02]	0.82 [0.49,1.36]
Pediatric acute care	0.57 [0.51,0.65]	0.70 [0.61,0.79]	1.01 [0.86,1.17]	0.85 [0.71,1.02]	0.78 [0.54,1.13]	0.60 [0.32,1.13]
Adult/pediatric tertiary/ quaternary care	0.54 [0.51,0.57]	0.80 [0.75,0.86]	1.27 [1.20,1.36]	0.80 [0.74,0.87]	1.37 [1.15,1.61]	0.94 [0.69,1.30]
Healthcare admission source**	2.08 [1.96,2.21]	1.91 [1.78,2.05]	1.88 [1.75,2.02]	2.23 [2.05,2.43]	2.63 [2.23,3.10]	1.39 [0.96,2.00]
Hospitalized within previous year***	1.75 [1.66,1.85]	1.47 [1.39,1.57]	1.49 [1.40,1.59]	1.64 [1.52,1.78]	1.39 [1.19,1.63]	0.68 [0.50,0.93]
Charlson Comorbidity Index (continuous)	1.08 [1.075,1.085]	1.08 [1.07,1.09]	1.08 [1.07,1.09]	1.06 [1.05,1.07]	1.08 [1.07,1.09]	1.08 [1.05,1.11]
Age in years (continuous)	1.017 [1.016,1.018]	1.009 [1.007,1.010]	1.021 [1.019,1.022]	1.022 [1.020,1.024]	1.01 [1.007,1.014]	1.006 [1.00,1.01]
Male sex	0.87 [0.83,0.91]	1.57 [1.48,1.67]	0.81 [0.77,0.87]	1.11 [1.03,1.20]	1.42 [1.23,1.65]	1.84 [1.37,2.46]
Relationship between advancing year and odds of antimicrobial resistance for patients with healthcare-associated infections[2]						
N (%) resistant	2,716 (43.1)	1,964 (44.7)	404 (9.8)	165 (5.6)	276 (40.1)	75 (38.5)
Year (continuous, 2006-2012)	0.98 [0.95,1.01]	0.94 [0.91,0.97]	0.86 [0.82,0.91]	1.45 [1.32,1.59]	0.95 [0.88,1.03]	0.86 [0.73,1.01]
Hospital*						
Community	0.61 [0.49,0.76]	1.30 [1.03,1.65]	0.63 [0.42,0.95]	0.48 [0.20,1.13]	0.98 [0.52,1.85]	0.88 [0.28,2.76]
Pediatric acute care	0.40 [0.29,0.55]	1.34 [0.98,1.83]	0.17 [0.09,0.34]	0.34 [0.16,0.74]	0.11 [0.03,0.50]	0.67 [0.16,2.78]
Adult/pediatric tertiary/ quaternary care	2.77 [2.46,3.11]	1.00 [0.87,1.14]	0.37 [0.29,0.47]	0.78 [0.55,1.12]	2.01 [1.42,2.84]	0.74 [0.36,1.53]
Healthcare admission source**	1.36 [1.21,1.54]	1.28 [1.10,1.48]	1.98 [1.58,2.49]	1.47 [1.04,2.08]	1.05 [0.74,1.49]	2.26 [0.97,5.27]
Hospitalized within previous year***	1.46 [1.31,1.63]	1.62 [1.42,1.84]	1.59 [1.28,1.98]	1.18 [0.84,1.66]	0.82 [0.59,1.14]	1.40 [0.70,2.79]
Charlson Comorbidity Index (continuous)	1.04 [1.03,1.05]	1.01 [1.00,1.03]	1.00 [0.98,1.03]	0.98 [0.94,1.03]	1.00 [0.96,1.04]	1.12 [1.03,1.21]
Age in years (continuous)	1.002 [0.999,1.005]	1.017 [1.014,1.020]	0.99 [0.98,1.00]	0.98 [0.97,0.99]	1.00 [0.99,1.01]	0.99 [0.97,1.00]
Male sex	0.84 [0.75,0.93]	0.99 [0.87,1.13]	1.26 [1.02,1.56]	1.08 [0.78,1.49]	1.25 [0.91,1.73]	2.14 [1.11,4.14]

Results of logistic regression analyses controlling for hospital and patient characteristics. Data are odds ratios (95% confidence intervals).
[1] N for each organism is less than the total incidence for each organism because some patients had infections with the same organism in multiple body sites.
[2] Antimicrobial resistance was defined as resistance to: oxacillin for *Staphylococcus aureus;* penicillin for *Streptococcus pneumoniae;* ampicillin-sulbactam for *Acinetobacter baumannii;* vancomycin for *Enterococcus faecalis* and *Enterococcus faecium;* and ≥ 3 antibiotic classes for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*
* Reference: adult tertiary/quaternary care
**Admission from another hospital, ambulatory surgery center, skilled nursing facility, or hospice center.
***Within-network hospitalizations only.

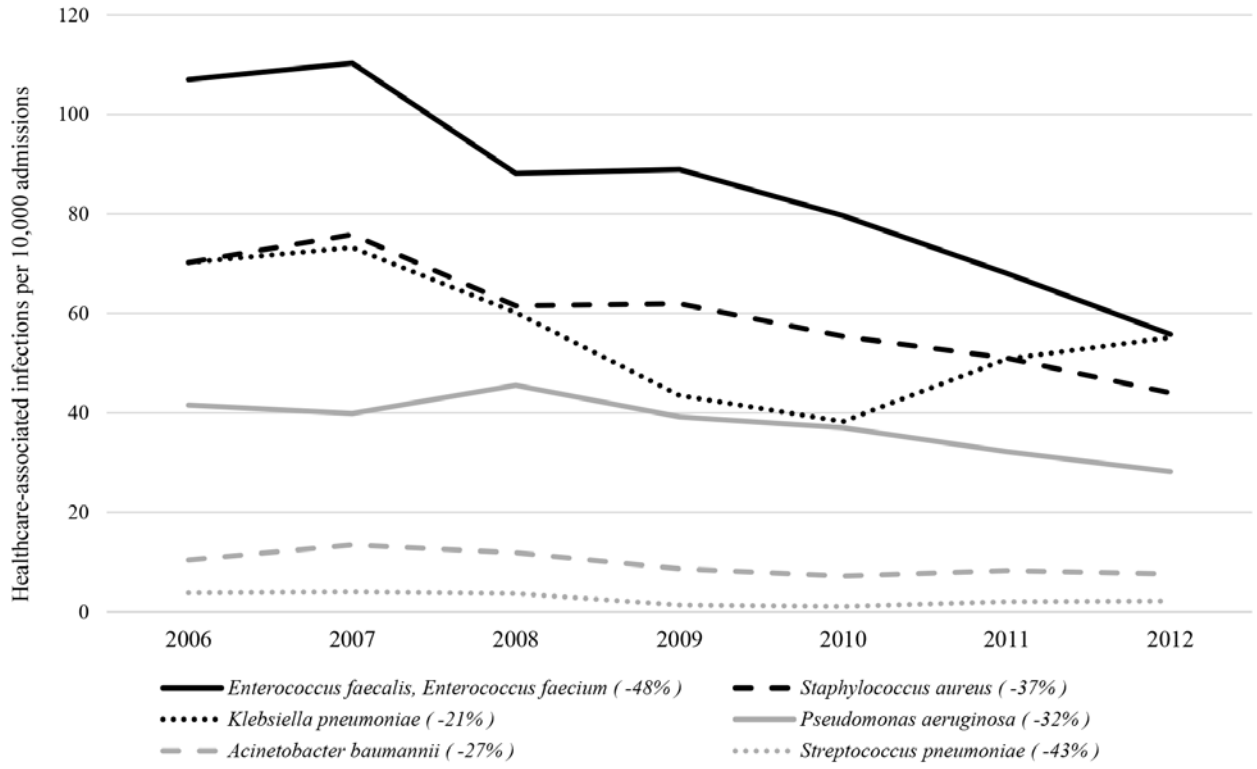
more likely to develop an HAI with *K. pneumoniae* and *E. faecalis/E. faecium*.

The impact of admission source and within-network hospitalization in the previous year decreased significantly over time, with the magnitude of association between HAI and admission source decreasing steadily throughout the study period and the magnitude of association between HAI and previous hospitalization decreasing steadily through 2010 and then rising slightly. Statistically significant interaction with year was not identified for any other factor.

The annual proportion of HAI caused by antibiotic-resistant organisms is presented in Table III. The multivariable logistic regression analyses show no appreciable change in levels of antibiotic resistance for any organism except *P. aeruginosa*, for which multidrug resistance increased significantly over the study period and *K. pneumoniae*, for which multidrug resistance decreased significantly (Table II). Resistance to all tested antibiotics increased for *P. aeruginosa*. For *K. pneumoniae,* resistance decreased slightly for all tested antibiotics except for tobramycin, for which resistance increased by 10%, and imipenem, for which there was a sharp increase from an average of 20% in 2006-2010 to over 70% in 2011.

Patients with a healthcare admission source were significantly more likely to develop a resistant infection for all organisms except *S. pneumoniae* and *A. baumannii*. Resistance was significantly associated with within-network

Fig. 2. Annual incidence of healthcare-associated infections per 10,000 admissions in four New York City hospitals, 2006-2012. The percent decrease in infection rate between 2006 and 2012 is displayed for each organism.



hospitalization in the previous year for *K. pneumoniae, S. aureus,* and *E. faecalis/E. faecium*. Odds of resistance were significantly higher for males among those infected with *K. pneumoniae* and *S. pneumoniae* and significantly higher for females among those infected with *E. faecalis/E. faecium*. There was a small but significant positive association between severity of illness and resistance among *S. pneumoniae* and *E. faecalis/E. faecium* infections. Advancing age was associated with resistance among *S. aureus* infections, while younger age was associated with resistance among *P. aeruginosa* infections.

Discussion

Using data from four hospitals in a major metropolitan center, we observed persistent and statistically significant declines in the incidence of healthcare-associated BSI, SSI, UTI, and pneumonia between 2006 and 2012. The reductions in BSI, SSI, and pneumonia paralleled trends tracked at the national level for selected conditions including central line-associated BSI, BSI with methicillin-resistant *S. aureus*, ventilator-associated infections, and SSI following common orthopedic, cardiac, gastrointestinal, and gynecological procedures [2, 11-13]. Though U.S. rates of catheter-associated UTI climbed in 2009-2012, our study sites continued to experience annual reductions of total UTI [2, 11].

The reduced incidence of HAI across the study institutions is noteworthy, particularly in light of changes to the patient population which occurred in tandem. Though severity of illness remained stable over the course of the study, the proportion of patients admitted from other healthcare facilities and who had been hospitalized in-network within the previous year increased considerably, rising from 23% to 45% and from 10% to 17%, respectively. Patients who have had prolonged contact with the healthcare system tend to be more vulnerable to infection and more likely to enter the hospital already having been colonized with common healthcare-associated pathogens [14]. Yet, the observed reductions in HAI were robust despite the demographic shift to include a higher burden of these patients. In fact, the results of the interaction models indicate that rates of HAI are falling even more among patients who had previous healthcare exposures compared with other patients, suggesting that the overall decline in HAI may be due, in part, to a reduced risk among this subset. Improved screening procedures for patients admitted from healthcare sources or with known history of hospitalization may have contributed to falling HAI rates, possibly because a higher proportion of infections that were present upon hospital admission would have been diagnosed within the first 48 hours and therefore not counted as HAI, or because interventions such as decolonization were effective at preventing HAI [15]. Similarly, the slight decrease in *S. pneumoniae* may be due to faster diagnosis

Tab. III. Changes over time in the proportion of healthcare-associated infections resistant to antibiotics, 2006-2012.

	2006	2007	2008	2009	2010	2011	2012	% change in proportion of resistant infections, 2006 to 2012
Enterococcus faecalis and *Enterococcus faecium* (N = 6,476)	483/1,120 (43)	529/1,178 (45)	361/927 (39)	406/975 (42)	425/897 (47)	322/763 (42)	279/616 (45)	+ 5%
Staphylococcus aureus (N = 4,553)	346/735 (47)	395/810 (49)	282/647 (44)	296/679 (44)	295/624 (47)	241/572 (42)	192/486 (40)	– 15%
Klebsiella pneumoniae (N = 4,237)	69/735 (9)	118/782 (15)	85/633 (13)	29/477 (6)	30/431 (7)	37/570 (7)	43/609 (7)	– 22%
Pseudomonas aeruginosa (N = 2,859)	3/435 (1)	7/426 (2)	11/479 (2)	35/430 (8)	42/417 (10)	34/361 (9)	33/311 (11)	+ 1,000%
Acinetobacter baumannii (N = 731)	34/109 (31)	69/144 (48)	69/125 (55)	38/95 (40)	32/81 (40)	40/93 (43)	24/84 (29)	– 6%
Streptococcus pneumoniae (N = 196)	15/40 (38)	21/43 (49)	19/39 (49)	2/15 (13)	0/12 (0)	10/23 (43)	9/24 (38)	0%
Total (N = 19,052)	950/3,174 (30)	1,139/3,383 (34)	827/2,850 (29)	779/2,671 (29)	824/2,462 (33)	684/2,382 (29)	580/2,130 (27)	– 10%

Data are no. resistant isolates/no. total isolates (% resistant). Antimicrobial resistance was defined as resistance to: oxacillin for *Staphylococcus aureus*; penicillin for *Streptococcus pneumoniae*; ampicillin-sulbactam for *Acinetobacter baumannii;* vancomycin for *Enterococcus faecalis* and *Enterococcus faecium*; and ≥ 3 antibiotic classes for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

and appropriate classification as non-HAI, since these infections are more likely to be acquired in the community. Changes in infection prevention practices at the study institutions such as hand hygiene improvement and implementation of a central line care bundle may have contributed to declining infection rates overall, though it is difficult to evaluate the impact of specific policies since they varied throughout the course of the study.

In addition to risk differences between patients with and without previous healthcare contact, we also identified risk differences based on gender. That male patients had higher odds of developing HAI caused by *S. aureus, P. aeruginosa, S. pneumoniae* and *A. baumannii* while female patients had higher odds of *K. pneumoniae* and *E. faecalis/E. faecium* may be related to the types of infections that these organisms are most likely to cause. For example, *S. aureus* is a common cause of bloodstream infections, which are more common in male patients, and *E. faecalis/E. faecium* have emerged as common causes of UTI, which are more common in female patients [16-18]. The fact that female patients were more likely to have a vancomycin-resistant strain of *E. faecalis/E. faecium* may be the result of previous antibiotic treatment for recurring UTI [19]. *S. pneumoniae* has been reported to occur more frequently among men, possibly due to higher rates of smoking and underlying conditions such as chronic heart and lung diseases [20]. Previous antibiotic treatment for *S. pneumoniae* and other causes of pneumonia may explain why resistance was found to be higher among men [21].

While the incidence of HAI was greatly reduced, little progress was made with regard to reducing antimicrobial resistance. The strongest trend occurred among *P. aeruginosa*, for which resistance to aminoglycoside, carbapenem, cephalosporin, and fluoroquinolone antibiotics increased and beta-lactamase inhibitor activity decreased. The proportion of multidrug-resistant *P. aeruginosa* isolates grew from less than 1% in 2006 to over 10% in 2012, and statewide data suggest that this upward trajectory has continued in recent years [22]. For *K. pneumoniae* we observed moderate decreases in resistance to aminoglycoside, cephalosporin, fluoroquinolone, and sulfonamide antibiotics as well as increased beta-lactamase activity, though resistance to imipenem more than tripled in 2011 following an outbreak of carbapenem-resistant *K. pneumoniae*. It's likely that this outbreak contributed to the overall rise in *K. pneumoniae* infections that occurred in 2011 after several years of steadily falling rates. The considerable uptick in carbapenem resistance is reflective of a national epidemic of *K. pneumoniae* carbapenemase, which first appeared in New York City in the early 2000s [23, 24]. Still, the percent of *K. pneumoniae* isolates that were multidrug-resistant was lower than statewide reports of 25% and decreased throughout the study period [22]. This discrepancy may be due to differences in the definition of multidrug resistance and the specific drugs for which antimicrobial activity was assessed. Similar to trends reported at the state and national levels, methicillin resistance among *S. aureus* remained relatively stable after 2007, following precipitous declines in

the previous decade [3, 6, 22, 25, 26]. Consistent with data available from the CDC, no meaningful changes in vancomycin resistance among *Enterococcus* spp. occurred in the study facilities during the observed time frame [6, 22].

This study was conducted during a period of heightened attention toward HAI prevention [1]. The application of evidence-based practices and bundles coupled with the adoption of new reimbursement policies that reframed many healthcare-associated conditions as fully preventable events likely played a role in reducing HAI [27, 28]. However, since many changes to infection prevention practice have been introduced during the last decade, it is not feasible to isolate the effects of any single initiative. Moreover, it is unlikely that any one factor was solely responsible for the reduction [29]. Analogous broad efforts towards reducing antimicrobial resistance among healthcare-associated pathogens were also introduced during this timeframe. Antimicrobial stewardship programs may have had some effect with regard to halting the upward trends in resistance for many organisms; nonetheless, data suggest that they have not yet had much impact with regard to lessening the burden of resistance at the state or national level [6, 16, 30]. The evidence of such impact is likely to require longer periods of time than other practices associated with prevention of HAI.

One of the major strengths of this analysis is its application of a consistent methodology for identifying HAI over time. Unlike other sources of longitudinal data, the electronic algorithms used to define infections in this study were not sensitive to changes in case definitions, infection prevention personnel training, or financial and regulatory incentives that may have altered reporting practices [31]. The algorithms were created and validated by an interdisciplinary team that included an infectious disease physician, and infection prevention nurse, an epidemiologist, a database manager, and an IT systems manager with expertise in hospital administrative data [7, 32, 33]. Still, the gold standard for diagnosis of an infection is clinician adjudication after full chart review, and disadvantages to using electronic data sources have been identified [34]. The SSI algorithm was designed to include only infections associated with NHSN operative procedures, so infections resulting from other procedures were not identified. Previous studies have reported low sensitivity for some of the ICD-9-CM codes used to create the Charlson Comorbidity Index, though specificity is generally high [35]. Data on previous out-of-network hospitalizations were not available, and it is possible that some within-network hospitalizations were not captured due to erroneous assignment of new medical record numbers to patients who were readmitted within one year. This type of misclassification, however, could only lessen the magnitude of the observed association between previous admission and odds of HAI. As the quality and availability of electronic patient data improves, the validity of some data elements may have changed over time, though we are not aware of any specific changes to the way data were collected or recorded that would have affected the study variables.

In addition to issues of data quality, there are also some limitations to our statistical analyses. We were unable to account for previous use of antibiotics, which is a known risk factor for resistance and may also confound the associations between infection and prior hospitalization or admission from a healthcare source, since patients with previous healthcare contact may be more likely to have taken antibiotics [36]. In addition, the multivariable model predicting antimicrobial resistance for *S. pneumoniae* was not adequately powered to detect differences over time.

Conclusion

Overall, this study provides strong support for the observation that the incidence of HAI is falling and that the reduction in HAI is not limited to device and procedure-associated infections. Although we were unable to measure the impact of any specific policy or practice changes due to the overlapping nature of their implementation, the reduction may be the result of increased federal and state attention to healthcare quality and patient safety. Accordingly, although we have yet to observe substantial reductions in antimicrobial resistance, the recent uptick in national attention towards antimicrobial monitoring and stewardship may precipitate a change in coming years.

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Authors' contributions

B.C. performed the analysis and wrote the manuscript. J.L. collected, prepared, and managed the data. E.L. oversaw the project and advised on the study design, analysis, and manuscript preparation.

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ORIGINAL ARTICLE

VIM-*Klebsiella oxytoca* outbreak in a Neonatal Intensive Care Unit. This time it wasn't the drain

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Keywords

VIM-*Klebsiella oxytoca* • NICU • Sink (drain)

Summary

Objective. *We describe an outbreak of VIM-carbapenemase Klebsiella oxytoca (VIM-Kox) in a NICU*
Materials and methods. *Prospective Epidemiological Surveillance:*
a) Systematically (weekly screening cultures) or on admission, if the patient had a history of previous colonization by VIM-Kox.
b) Clinical cultures, done if infection was suspected.
c) Other possible microorganism sources were investigated: their mothers (rectal microbiota), milk packages and preparation apparata in the lactodietary section, echocardiagram transductors, cribs, the sinks (faucets and drains), washing bowls, etc.
Molecular typing was performed using the DiversiLab (bioMérieux) system on all VIM-Kox isolated from environment or patients (one by neonate).
Results. *We identified 20 VIM-Kox cases, the most only presented colonization, but 4 showed infection. Three of the ten sinks (drains) in our NICU, were positive for VIM-Kox. Another four drains harbored P.aeruginosa, S. maltophilia and/or Enterobacter sp. Nevertheless the VIM-Kox bacteria in the sinks (drains) were not the same as those in the patients, who showed three different strains.*
Conclusions. *A VIM-Kox colonization or infection outbreak in a NICU is described. Rather than environment, not even drains, the source of the outbreak was other patients. The outbreak was relatively brief, as a result of the rapidness with which appropriate measures were taken and followed.*

Introduction

The frequency of carbapenemase-producing microorganism isolation in tertiary hospitals has been rising since 2007 [1], particularly *Enterobacteriaceae* [2-6]. Molecular biology techniques have detected antibiotic resistance genes like carbapenemase Ambler types A, B and D [7]. The Class B or metallo-betalactamases can be divided into three different types: the IMP, VIM and NDM.
When a new patient is colonized or infected by microorganisms with carbepenemases, it is necessary to determine whether this is due to microorganisms from other patients or from reservoirs that are difficult to clean during the inter-patient room cleaning/disinfection [8-12].
These reservoirs have sometimes been seen with hydrophilic bacteria like *P. aeruginosa*, *B. cepaciae*, or *Klebsiella oxytoca* with carbepenemase associated with sink contamination [13-18]. The drains of these sinks can harbor biofilms, which, in addition to hampering disinfection, can facilitate microorganism survival, gene interchange among microorganisms, and later, their dispersion as aerosols that can contaminate patients or healthcare workers when the sink is used for personal hygiene [18].
The microorganisms typing is useful to diagnose transmission and also allows us to detect if there is an outbreak or only a cluster of unrelated cases and establish monitoring systems to survey compliance with the prevention measures.
Carbepenemase-producing *K. oxytoca* were previously isolated in patients from some Spanish hospitals [1, 19-21]. Although this microorganisms can harbour plasmids with VIM-metallo-betalactamase (VIM-Kox), we had not found any reference to a previous outbreak among neonates. In this paper we report an outbreak by VIM-Kox in a NICU at a tertiary children's hospital, and describe the preventive measures taken to control further spread of this microorganism.

Materials and methods

La Paz Children's Hospital is a tertiary hospital with one NICU. Since 1985 monitoring and control of hospital infection is performed by one medical epidemiologist (part-time) and one nurse epidemiologist (dedicated full time).
Different multidrugs resistant microorganisms surveillance strategies are employed, including surveillance of clinical microbiology laboratory results, obtained during clinical care, and routine screenings to detect asymptomatic colonization.

Epidemiological surveillance for infection or colonization by these microorganisms is performed in two ways:
1. systematically, with an active surveillance methodology using weekly screening cultures taken from all children admitted to the NICU;
2. at admission, if the patient has a previous multidrugs resistant microorganisms colonization history.

A "VIM-Kox-case" is determined by the identification of *K. oxytoca* with VIM-carbepenemase in any biological sample taken from the patient (catheter tip, bronchoalveolar exudate, blood, conjunctiva, throat, rectal, etc.), regardless of the presence of symptoms. On some occasions, a patient was colonized by different *VIM-Enterobacteriaceae* genera.

Bacteria frequency was measured as a "cummulative incidence" of cases in a given time period (new cases divided by the number of children admitted during this period in our NICU, multiplied by 100).

A) Microbiological method

Surveillance studies in patients:

A.1) Clinical Samples

In neonates with any symptom of infection, urine, blood, broncho-alveolar lavages and other samples based on the most likely focus of infection were taken.

Antibiotic susceptibility was determined in clinical samples using the Wider (Fco. Soria Melguizo, Madrid, Spain) or Vitek (bioMérieux, Marcy l'Étoile, France) systems. Isolates were categorized as susceptible or resistant to any of the antibiotics tested following CLSI guidelines. Tigecycline Minimun Inhibitory Concentration (MIC) were evaluated according to the interpretative criteria of the FDA. Extended-spectrum β-Lactamase production was confirmed by E-test extended-spectrum β-Lactamase strips (bioMérieux) and carbapenem MIC were confirmed by E-test (BioMérieux). To rule out carbapenemase production, a modified Hodge test was performed on all *Enterobacteriaceae* isolates retrieved from clinical cultures having an MIC ≥ 1 mg/L to imipenem or meropenem and an MIC ≥ 0.5 mg/L to ertapenem. The inhibition tests with boronic acid and EDTA were used to screen for the production of class A and class B carbapenemases.

A.2) Surveillance Samples

Weekly samples were obtained from the neonates' pharynx, nose, feces, catheter entry points and connections, and the incubator water.

These samples were cultured in MacConkey agar supplemented with 4mg/L of cefotaxime (Tec-Laim, Madrid, Spain). Disc diffusion and a modified Hodge test were performed on all *Enterobacteriaceae* isolates to identify extended-spectrum β-Lactamase, plasmid-mediated AmpC and carbapenemase production.

We mapped the VIM-colonized (or infected) patients within the NICU. Carbapenemase genes were confirmed by PCR (Progenie Molecular, Valencia, Spain). Molecular typing was performed using the DiversiLab (bioMérieux) system on all KoVIM isolated from environment or patients (one by neonate).

B) Control measures

The bundles recommended for controlling VIM-*Enterobacteriaceae* were adapted from those described in CDC 2012 [21-24] (Tab. I).

Our Epidemiologist-Nurse evaluated implementation of these measures daily, reporting any compliance failures to the healthcare workers. The Medical-Epidemiologist reinforced these daily recommendations with the NICU supervisors for doctors and nurses.

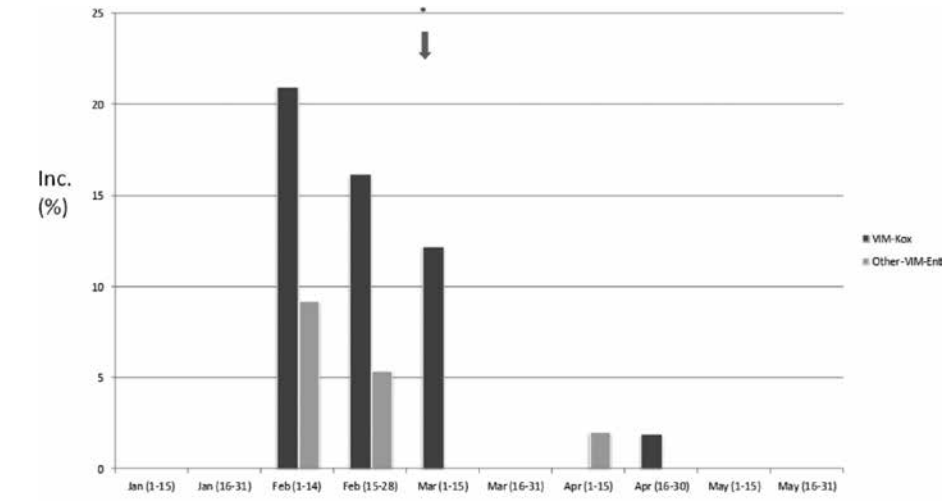
C) Statistical method

We studied the data for frequencies of children infected/colonized per week, (incident cases), plotting an epidemic curve.

Tab. I. Measures used in NICU by to control the outbreak of *VIM-K. pneumoniae*.

a) Early detection and implementation of contact precautions, emphasizing hand hygiene with alcohol solutions. The efficacy of the alcohol solution actually used in neonates was tested with VIM-microorganism recently isolated in our NICU.
b) Cohorting the VIM cases, grouping them in one specific area of the NICU.
c) Cohorting the healthcare workers, especially nurses. In the first month, physicians were also dedicated to the VIM-cases, but after that time, they also cared for other non-VIM patients.
d) Restriction of β -lactam-antibiotic use in neonates and limitation to sensitive antibiotics (according to the antibiogram) if the neonate carried VIM- microorganisms.
e) Flagging the patient's clinical history with a green-colored page stating the contact precautions, used when the child was taken out of the unit for clinical tests, etc. This same signalling page was used if the child was readmitted to our hospital.
f) Daily body washing used a 0.1%-0.5% aqueous chlorhexidine solution (0.1% in preterm < 4 weeks of life or a term < 2 weeks; in preterm > 4 weeks of life or a term > 2 weeks, chlorhexidine is used at 0.5%).
g) Restriction of number of healthcare workers from other specialties who came to visit neonates.
h) Information sessions for parents and refresher courses for NICU doctors, nurses, assistants, and specialists, held to explain the epidemiological evolution of VIM bacteria and the steps to be taken during each phase.
i) The possible environmental origins of this VIM bacteria outbreak were explored early on, and included sinks, NICU surfaces, disinfectants, eyewashes, echograph-transductors etc., that could be related with the outbreak. Milk from the Dietetary Service, instruments and milk recipients were sampled, and also the water faucets in the NICU were studied fortnightly (between February and March). The samples were taked with swabs immediately before bein immersed in Tood-Hewitt broth.
j) Other measures taken to interrupt the epidemiological evolution of the outbreak were to test the efficacy of disinfectant used on surfaces (double application of diluted quaternary ammonium and isopropyl-alcohol to the same surface) with VIM –microorganisms from our NICU, on a glass-germ-carrier, as previously described [21, 24].

Fig. 1. Cummulative incidence (%) for VIM-*K. Oxytoca* vs other VIM *Enterobacteriaceae* in NICU.



arrow = more extreme contact precautions

Results

The VIM-Kox NICU outbreak began in the first week of February 2014. The weekly microbiota control sample (performed weekly on Thursdays) identified 8 VIM-Kox cases (4 also had VIM-Serratia). There were another 3 patients in whom other VIM -*Enterobacteriaceae* were identified. The results for all the patients with multidrugs resistant microorganisms were received on the Monday of the second week of February, and all neonates with these microorganisms were placed under contact precautions, but it was not possible to establish an isolated cohort. Thursday that week, samples were again taken, and another six neonates had become VIM-Kox positive. Consequently, the first fortnight in February had a VIM-Kox cummulative incidence of 21% (Fig. 1), 40 times higher than during the two previous years.

The preventive measures mentioned in "Materials and methods" were taken.The effectivity of the hand antiseptic against the bacteria recently isolated from patients was studied and found to be very effective within 15 seconds. Moreover, the double application of diluted quaternary ammonium and isopropyl-alcohol to the same surface, destroyed, in vitro, all VIM-microorganisms studied. Consequently, it was not necessary to change these methods of antisepsis and disinfection.

Last, a cohort with VIM-neonates was established. However, it was not possible to include all the affected babies, since, due to their underlying disease not all could be moved into the cohort isolation area.

In the second fortnight of February, VIM-Kox cummulative incidence was slightly lower (16%). At this point other possible microorganism sources were investigated: their mothers (rectal microbiota), sink drains, milk packages and preparation apparata in the lactodietary section, echocardiagram transductors, cribs, the sinks (faucets and drains), washing bowls, etc. Everything, except for three of the ten sinks, were negative for VIM-Kox. Another four drains harbored *P.aeruginosa*, *S. maltophilia* and/or Enterobacter sp. (Fig. 2). Next, the susceptibility of these VIM-*Enterobacteriaceae* to different non-chlorinated desinfectants was determined. Chlorinated products were not used because they may emit gases that could irritate the neonate respiratory tract. All VIM-bacteria in this outbreak were susceptible to chlorhexidine, oxygen peroxide or alcohol, and moderately resistant to diluted quaternary ammonium. Once susceptibility was determined, the drains were disinfected by pouring 1 liter of 5% clorhexidine down the drain. This was effective in two of the three VIM-Kox-affected sinks. The third sink required, as we have done in other bacterial outbreaks, an application of steam (vapor) and after a chemical disinfectant (in this case a mixture of 3% hydrogen peroxide and lactic acid, which had given a good in vitro result). This treatment was applied in the ten affected sinks and the subsequent controls were negative in all cases for VIM-Kox as well as other multidrugs resistant microorganisms. Additionally, the number of HCW from other Services who were attending the neonates was restricted and in the third fortnight, the cumulative incidence had dropped to 12% and then 0% in the fourth fortnight after the outbreak began. There was only one new case in April with none in May or June, at which time the outbreak was considered over (Fig. 1).

As seen in Figure 2, the locations of incubators with cases were widely scattered throughout the unit, and were not particularly related with the sinks in which VIM-Kox had been isolated. Moreover, the Microbiology Laboratory confirmed via genetic analyses that the sink´s VIM-Kox were different to those of the cases and, among the 20 cases, there were three different strains. The first strain, isolated in the first fortnight of February (cases 1 to 8) remained until the first fortnight in March (case 15) affecting patients in the three "areas" (303, 304 and 308); The second strain spread during the second

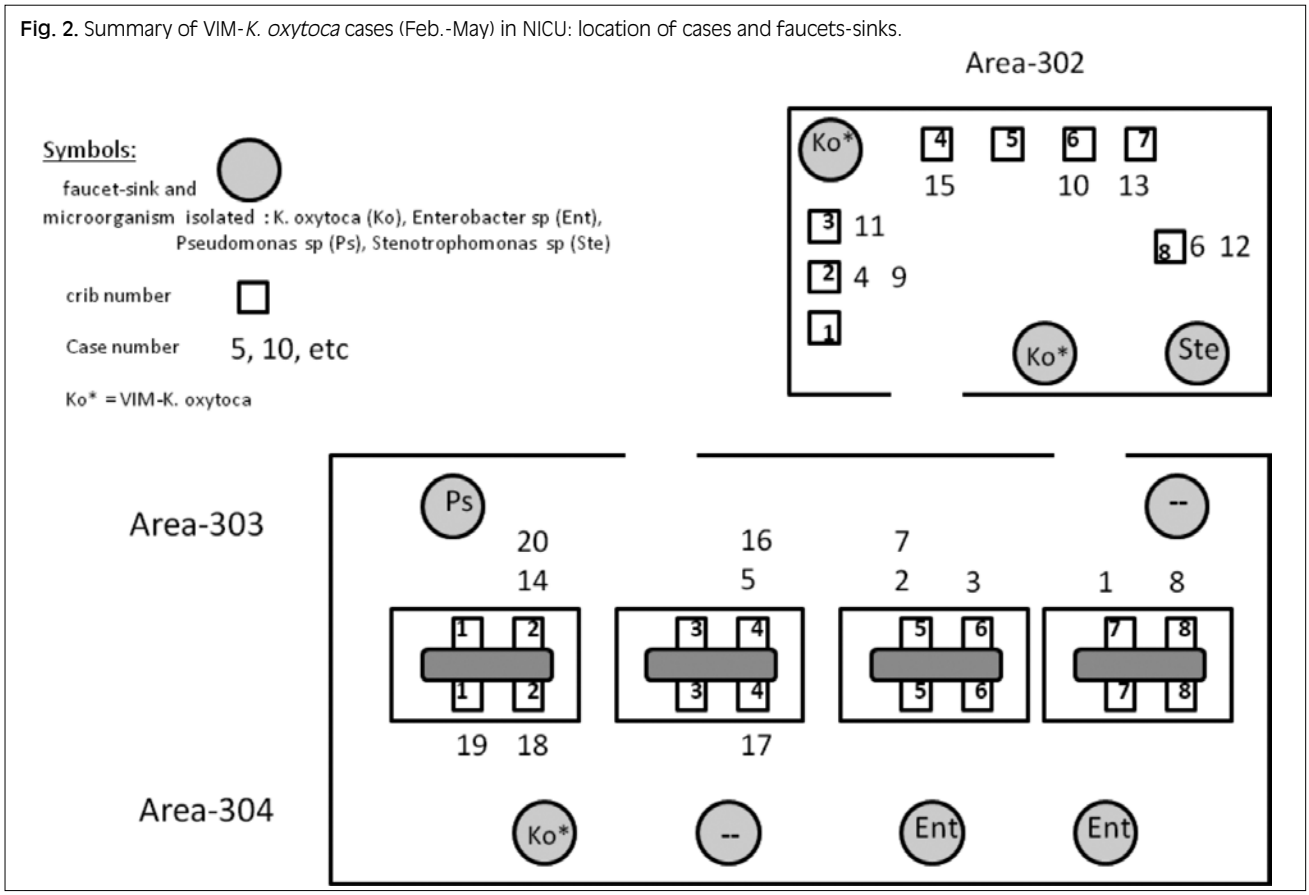


Fig. 2. Summary of VIM-*K. oxytoca* cases (Feb.-May) in NICU: location of cases and faucets-sinks.

fortnight in February (cases 9 to 14) and first fortnight in March (cases 17 to 19), also with cases in the three NICU areas, and, in this fortnight in March, a third strain was identified, but only in 303 area (case 16).

During the outbreak, three children were admitted to the hospital and found to be already VIM-Kox positive, colonized in another hospital, and their data are not considered in the analysis of the outbreak.

The third of these children was admitted in the first fortnight in April, and the last incident case to be detected in this outbreak was detected in the second fortnight of the month (case 20); he was probably, although we do not have the genetic study, infected by the outside case since:

1) it had been nearly a month since the last case;

2) they were near each other in the same room;

3) in the first two weekly controls in which they were both sampled, case 20 was still negative, only becoming positive at the end of the second fortnight in April.

In all, the NICU had 20 cases of VIM-Kox (4 of them had VIM-Kox and VIM-Seratia and 6 with other VIM-Enterobacteriaceae). Of the VIM-Kox cases most were only colonizations but 4 also had infection (3 pneumonias and one conjunctivitis, Table II). These bacteria were susceptible to various antibiotics: Ertapenem, Meropenem, Amikacin, Colistin, Tigeciclin and quinolones. In each patient, the median of surveillance studies from admission to event was 3.

VIM-Kox was also isolated in 6 other infants admitted outside the NICU, and there were 3 patients (coming from other hospitals) that entered the NICU already colonized with VIM-Kox.

Discussion

VIM-Kox has been few isolated in our neonates by epidemiological surveillance during the two previous years [21], with an incidence between 0.1% and 0.3%. Other hospitals, or even the community [25], may be the reservoirs of these microorganisms. Several studies [13-17] have related *K. oxytoca* outbreaks with very damp environmental reservoirs, like sink drains in patients' areas or the sinks in ICU's. On this occasion, these microorganisms were found in 30% of the sink (drains) in our NICU, but the genetic analysis showed that the sink VIM-Kox´s were different from those in the neonates. Nevertheless the sinks were disinfected with heat plus chemicals and the VIM-Kox microorganisms were eliminated from that reservoir (at least temporarily).

Separation into two cohorts (with and without VIM bacteria) as well as applying contact precautions have given good results in prior outbreaks (with an OR > 5 for infected and OR > 30 for colonized patients [24]), and were enacted as soon as possible, producing a large reduction in incidence (Fig. 1) as soon as the measures were in place.

However at times it was impossible to transfer the neonates in whom VIM- bacteria had been detected in the weekly sampling to the isolation area on the day colonization was microbiologically confirmed, and this may

Tab. II. Neonates with VIM-K. oxytoca, according to colonizatior or infection by these bacteria.

Case	Rectal col.	Ot. col. sites	Infection	Antibiotics adm.	Outcome
1	Yes	No	No	No	Favourable
2	Yes	Yes (pharinx)	BN	CTX;AM;Va;Cla	Favourable
3	Yes	Yes (pharynx)	No	No	Favourable
4	Yes	No (pharynx)	BN	CTX;G;Va;Me	Favourable
5	Yes	Yes	No	CTX; Va;Amp	Favourable
6	Yes	No	Conj.	CTX; Va	Favourable
7	Yes	Yes (pharynx)	No	No	Favourable
8	Yes	No	No	CTX; Va	Favourable
9	Yes	No	No	No	Favourable
10	Yes	No	No	Cla;G;Amp	Favourable
11	Yes	No	No	Amp;CTX; G	Favourable
12	Yes	No	No	No	Favourable
13	Yes	No	No	Amp;Va;CTX	Exitus*
14	Yes	No	No	No	Favourable
15	Yes	No	No	Va;AM	Favourable
16	Yes	Yes (pharynx)	BN	Amp;G;Cla;Me	Favourable
17	Yes	No	No	No	Favourable
18	Yes	No	No	No	Favourable
19	Yes	No	No	No	Favourable
20	Yes	Yes (pharynx)	No	CTX; Va;AM	Favourable

BN = bronchopneumonia; Conj = Conjunctivitis; col = colonization; Ot.Col. = other colonization; adm = administrated. CTX = Cefotaxime; AM = Amikacin; Va = Vancomycin; Cla = Clarithromycin; G = Gentamicin; Amp = Ampicillin; Me = Meropenem.
*exitus no related with any infection

have placed the neonates in their immediate surroundings at risk, although the neonate with possible multidrug resistant microorganisms had been placed under contact restrictions from the time of their first suspected diagnosis. From these facts, it follows that the main cause of VIM-Kox´s transmission was that the lack of adherence to contact precautions measures by some health care workers.
In any hospital outbreak it is necessary to evaluate the effectiveness of the existing antiseptic and disinfection protocols against the outbreak organisms. The already recommended alcohol solution for hands, and surface desinfectants, were effective against the patients' VIM-Kox strains.
In addition to all of the above, these measures, which suppose an organizational challenge within the NICU, must be accompanied by refresher hygiene training for all healthcare workers, as well as regular updates on the evolution of the outbreak so as to increase or maintain the existing measures and evaluate if there have been any failures so that these failures can be corrected as soon as possible.

Study limitations

1) Antibiotic effect has not been evaluated directly in neonate fecal microbiota, since microorganism diagnosis was qualitative, not quantative, and only detected the presence or absence of a given bacteria, but not its quantity in a given sample weight. Quantification would have made it possible to use this variable as an indicator of recent contamination or of susceptibility to VIM-bacteria multiplication (in competition with the other intestinal microbiota).
2) Microbiota studies more frequently than once a week have not been possible, even at the peak of the outbreak (differently from previous outbreaks), possibly delaying the establishment of precautionary measures like contact control and cohort grouping for children with recently acquired VIM-bacteria by a few days, and slightly increasing the possibility of multidrugs resistant microorganisms transmission to other neonates.
3) Compliance with control measures (hand samples, observation at established times, etc.) has not been objectively evaluated, and evaluation has only been qualitative by observation and speaking with healthcare workers who did not complete all the steps for controlling these multidrug resistant microorganisms. What is more, epidemiological surveillance was only done during the morning shift, occasionally in the afternoon, and never in the evening shift.

Conclusions

- A VIM-Kox colonization/infection outbreak in a NICU is described.
- The outbreak was relatively brief, as a result of the rapidness with which appropriate measures were taken and followed (at least during the time in which compliance was directly observed).
- The already recommended alcohol solution for hands and surface desinfectants were effective against the patients' VIM-Kox strains.
- The source of the VIM-Kox microorganisms was other patients, but not the mothers or environment (milk, milk preparation material, NICU apparata, etc.), or even the drains in the NICU (which, however, were reservoirs for other VIM-Kox strains).

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Authors' contributions

RH and FO conceived and designed the research. GR, AS and RH performed the microbiological analysis. SG and FO, collected the epidemiological data. RH and JD, performed the statistical analysis. RH and JD, wrote the manuscript. All authors revised and approved the final manuscript. Revision of the text by a native English speaker (C. Warren).

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ORIGINAL ARTICLE

Molecular analysis of immune evasion cluster (IEC) genes and intercellular adhesion gene cluster (ICA) among methicillin-resistant and methicillin-sensitive isolates of *Staphylococcus aureus*

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Keywords

Staphylicoccus aureus • MRSA • MSSA • Biofilm • IEC types • Personal hygiene

Summary

Introduction. *Resistance to antibiotics and presence of virulence factors play an important role in increased mortality associated with infection due to Staphylococcus aureus. In this study, we determine antibiotic resistance pattern, presence of the icaADBC locus as well as biofilm formation and distribution and diversity the immune evasion cluster (IEC) genes in clinical isolate of S. aureus from Kerman, Iran.*

Materials and methods. *During 15 months, 100 clinical isolates S. aureus recovered from different patients were admitted to Kerman University affiliated hospitals. Resistance to different antibiotic agents was determined by disk diffusion method. Phenotypic method was used to the determination of biofilm formation ability and methicillin-resistance S. aureus (MRSA). Polymerase chain reaction technique (PCR) was used to the detection of nuc, mecA, icaA, icaD, icaB, icaC, scn, sea, sak, sep and chp genes..*

Results. *Forty-four isolates were considered as MRSA and all of isolates were sensitive to vancomycin and linezolid. Our results showed, 77.2% (34/44) of MRSA and 8.9 % (5/56) of MSSA isolates were multidrug resistant. The predominant IEC variant was type B and our results displayed that 77.7% of the MRSA isolates harbor loci icaD and mecA. There was no significant difference in production biofilm between MSSA and MRSA isolates ($P \geq 0.05$). There was significant difference in presence IEC types between MSSA and MRSA isolates ($P = 0.000$).*

Conclusions. *The presence of icaADBC locus may not be a determining factor for biofilm formation in Staphtlocooci and other mechanisms might be involved in this process.The high prevalence IEC types in MSSA isolates can indicate that the presence of these genes can be an advantage for pathogenesis of these isolates in different infections.*

Introduction

Staphylococcus aureus is both a commensal and a versatile human pathogen causing a broad spectrum of disease, from mild skin and soft tissue infections to life-threatening sepsis, pneumonia, endocarditis and deep-seated abscesses [1, 2]. The emergence of methicillin-resistant strains as well as having multiple virulence factors are the main factors in increased mortality in hospital–acquired (HA) and community-acquired (CA) infections caused by *S. aureus* [3]. Recent studies have displayed an increase in the worldwide prevalence of MRSA. In a regional perspective, a higher prevalence of MRSA in Iran compared to neighboring countries in the Middle East, except Iraq has been reported [4]. One study which reported HA-MRSA rates for eight Asian countries showed higher percentage of MRSA in those countries compared to Iran. Mean prevalence of MRSA in Iran is moderately higher than Australia and lower than the United States [5]. However, recent reports have revealed that MRSA rates are decreasing in United States [6]. This organism produces a number of virulence factors that provide the ability to colonize for it, adhere to surfaces as biofilm, invade or evade the immune system, develop resistance to multiple antibiotics and cause toxicity to the host [7, 8]. The ability of *S .aureus* to produce an extracellular slime and constitutive a biofilm enables this organism to withstand the host immune response and to make clinical treatment extremely difficult because of biofilm creation protects bacteria from antimicrobial agent [9]. The intracellular adhesion (*ica*) cluster, *icaADB* and *C*, encodes enzymes mediating cell-cell the adhesion and synthesis of the polysaccharide intercellular adhesion (PIA) which is essential for biofilm establishment [10, 11]. Another attribute of *S. aureus* which enables the pathogen's escape from protective immune responses express a number of immune-modulating proteins [12]. One of the immune-modulating proteins is staphylococcal complement inhibitor (SCIN). SCIN is a complement inhibitor, blocking the

ability of human neutrophils to opsonophagocytose of *S. aureus* and neutrophil chemotaxis [13, 14]. The gene encoding SCIN (*scn*) was found to be portion of a so-called immune evasion cluster (IEC). All IEC variants harbor *scn* and a different combination of *sak*, *chp* and *sea* (or *sep*). So far seven different variants of IEC carried by several different βC-Φs[14, 15]. These encode the human-specific immune modulators including staphylococcal enterotoxin A (SEA), staphylokinase (SAK) and chemotaxis inhibitory protein of *S. aureus* (CHIPS). SEA is involved in the down-regulation of chemokine receptors of monocytes [16]. SAK is a bacterial plasminogen activator and blocker, the bactericidal effect of antimicrobial peptides, the α-defensins [17]. CHIPS can bind to the formylated peptide receptor and neutrophils so can block neutrophil chemotaxis [18, 19].

To our knowledge, there is no information about prevalence of IEC-carrying βC-Φs in human *S. aureus* isolates in Iran. Since different studies have shown the decisive role of the *ica* genes as virulence factors in staphylococcal infections [20, 21] and IECs are bacteriophage encoded, the aim of this study was to determine antibiotic resistance pattern, the biofilm formation ability, the presence of the *icaADBC* locus and investigate the distribution and diversity the immune evasion cluster (IEC) genes among clinical isolate of *S. aureus* from Kerman, Iran.

Materials and methods

Bacterial isolates

A total of 100 non repetitive clinical isolates of *S. aureus* recovered from different patients, were admitted to Kerman University affiliated hospitals during February 2015 to May 2016. Clinical samples such as urine, blood, discharged abscess, wound, cerebrospinal fluid (CSF), broncoalveolar (BAL), synovial fluid and pus were included in this study. Bacterial isolates were considered as *S. aureus* by conventional biochemical standard methods including Gram-staining, catalase, slide and tube coagulase, DNase and maninitol fermentation on *mannitol salt agar* medium. All the applied culture media were purchased from Merck, Germany. The final identification of *S. aureus* isolates was done by amplification of *nuc* gene in species level in PCR method [22]. We defined 'community acquired (CA)' and 'healthcare associated infections or hospitalized patients (HA)' according to the current CDC criteria [23].

Susceptibility of isolates to antibiotics

The disk diffusion method on Müller-Hinton agar medium (MHA) was used to determine susceptibility of isolates to ciprofloxacin (5μg), trimethoprim/sulfamethoxazole (1.25/23.75μg), gentamicin (10μg), amikacin (30μg), erythromycin (15μg), clindamycin (2μg), tetracycline (30μg) and linezolid (30μg) (Mast disks,UK) according to guidelines of Clinical Laboratory Standards Institute (CLSI) [24]. The Brain Heart Infusion (*BHI*) agar medium with 6 μg/ml vancomycin used for screening of vancomycin resistant *S. aureus* isolates (VRSA). *S. aureus* ATCC 25923 was used as standard strain in susceptibility tests to antibiotics. Multidrug resistance (MDR) was defined as resistance of isolate to three or more unique antimicrobial drug classes in addition to beta-lactams [25].

Screening of methicillin-resistant *S. aureus* isolates (MRSA)

The MRSA isolates were detected by susceptibility of isolates to cefoxitin (FOX: 30 μg) on MHA according to recommendations of the CLSI [24] and then confirmed for the presence of *mecA* gene by PCR technique as described previously [26]. Patients who have acquired CA-MRSA infections did not have typical MRSA risk factors such as recent history of hospitalization, kidney dialysis, residence in a long-term health care facility or intravenous drug use.

Biofilm formation

S. aureus isolates were cultured on Trypticase Soy Agar (TSA) at 37ºC for 24h. Few grown colonies suspended in sterile physiological saline with turbidity equal to 0.5McFarland. The 96 well polystyrene microtiter platessterile (Cell and Tissue Culture plates, flat well bottom, Guangzhou Jet Bio-Filtration Products Co., Ltd. Guangdong, China) were filled with 180μl Trypticase Soy Broth (TSB) supplemented with 1% glucose and 20μl of bacterial suspension added to each well. After incubation for 24h at 37ºC, broth was carefully drawn off and the plates were gently washed three times with sterile phosphate-buffered saline (PBS). The plates were inverted and allowed to dry for 1 hour at room temperature. For biofilm quantification, 200μl of 2% saffranin dye solution in water was added to each well and the plates were allowed to stand for 40 min at room temperature. The wells were subsequently washed thrice with sterile PBS to wash off the excess saffranin. Saffranin bound to the biofilm was extracted with 200ml of 95% ethanol**,** and the absorbance of the extracted saffranin was measured at 490 nm in an ELISA reader (BioTek, USA). Each assay was performed in triplicate. As a negative control, TSB+1%glucose medium was used to determine background optical density (OD). The cut-off ODc for biofilm formation was determined as average OD of negative control +3×standard deviation (SD) of negative control. OD value was calculated for each microtiter plate separately. OD > 4×ODc was considered as high biofilm formation ability; 2×ODc < OD ≤ 4×ODc categorized into moderate biofilm formation ability. ODc < OD ≤ 2×ODc and OD ≤ ODc were taken as weak or none biofilm formation ability respectively [27].

DNA extraction

Deoxyribonucleic acid (DNA) extraction was performed by using appropriate DNA extraction kit (Gene All, Korea) following manufacturer's instruction. The quality of isolated DNA was measured by determination of absorbency at the wave length A260 nm and 280nm that showed a high quality of the product.

Tab. I. PCR primers and cycling parameters for genes presented in this study.

Gene target	Primer/sequence(5'-3')	PCR condition	PCR product (bp)	Reference
chp	F-GAAAAAGAAATTAGCAACAACAG R-CATAAGATGATTTAGACTCTCC	30 sec 95°C,50 sec 48°C,1min 72°C	410	[18]
sak	F-AAGGCGATGACGCGAGTTAT R-GCGCTTGGATCTAATTCAAC	30 sec 94 °C,30 sec 50 °C,1min 72 °C	223	[14]
sea	F-AGATCATTCGTGGTATAACG R-TTAACCGAAGGTTCTGTAGA	30 sec 94 °C,30 sec 50 °C,1min 72 °C	408	[14]
sep	F-AATCATAACCAACCGAATCA R-TCATAATGGAAGTGCTATAA	30 sec 94 °C,30 sec 50 °C,1min 72 °C	500	[14]
scn	F-AGCACAAGCTTGCCAACATCG R-TTAATATTTACTTTTTAGTGC	30 sec 94 °C,30 sec 49 °C,1min 72 °C	258	[14])
icaA	F- TCTCTTGCAGGAGCAATCAA R-TCAGGCACTAACATCCAGCA	1 min 95 °C,45 sec 60 °C,1min 72 °C	188	[28]
icaB	F- ATGGCTTAAAGCACACGACGC R- TATCGGCATCTGGTGTGACAG	1 min 95 °C,45 sec 61 °C,1min 72 °C	526	[29]
icaC	R- CTCTCTTAACATCATTCCGACGCC F- ATCATCGTGACACACTTACTAACG	1 min 95 °C,45 sec 63 °C,1min 72 °C	1013	[29]
icaD	F -GAACCGCTTGCCATGTGTTG R- GCTTGACCATGTTGCGTAACC	1 min 95 °C,45 sec 61 °C,1min 72 °C	483	[53]
mecA	F-TCC AGA TTA CAA CTT CAC CAG G R-CCA CTT CAT ATC TTG TAA CG	1 min 95 °C,45 sec 56 °C,1min 72 °C	162	[26]
nuc	F-GCGATTGATGGTGATACGGTT R-AGCCAAGCCTTGACGAACTAAAGC	1 min 95 °C,45 sec 60 °C,1min 72 °C	279	[30]

Detection of ICA and IEC cluster genes by PCR

Amplification was conducted in temperature gradient thermal cycler (Biometra-T300, Gottingen, Germany) in a volume of 25µl. Each 25µl PCR mixture consisted of 1µl of bacterial DNA, 0.5 µl (10pM) of each oligodeoxynucleotide primers, 12.5 µl of 2× Master Mix Red (Ampliqon, Denmark) and 11µl DNase and RNase free water. PCR was used for detection *nuc, mecA, icaA, icaD, icaB ,icaC ,scn, sea, sak, sep, chb* genes. All primers and programs can be found in Table I [14, 18, 26, 28-30]. After amplification, the PCR products were analyzed by electrophoresis on 1.5% agarose gel in 0.5×TBE buffer (5.4 g Tris base, 2.75 g boric acid, 2 ml 0.5 M EDTA, in 1 L). DNA ladder was a ready to use plasmid double digest sized range 100- 3000bp obtained from SMOBIO Technology (Hsinchu, Taiwan). Specificity of the primers was checked by Primer Quest software tool (http://www.ncbi.nlm.nih.gov/GenBank).

Statistical analysis

Statistical analysis was performed with SPSS (v.22.0) statistics software. We used the Chi-Square test for the comparison of our data. A difference was considered statistically significant at a P-value of < 0.05.

Results

A total of 100 *S. aureus* isolates were obtained from 80 in patients (HA) and 20 outpatients (CA) with different clinical infections. The isolates corresponded to 61 males and 39 females. Most of the clinical isolates belonged to urine 30% (n = 30) followed by wound 28% (n = 28), blood 22% (n = 22), BAL 7% (n = 7), CSF 3% (n = 3), skin abscesses, synovial fluid, pus and other clinical samples 10 %(n = 10). Of the 100 *S. aureus* isolates, 44 (44%) were MRSA which were recovered from urine (n = 22, 50%), wound (n = 9, 20.4%), blood (n = 4, 9.1%), BAL (n = 4, 9.1%), CSF (n =1, 2.3%) and pus (n = 3, 7%) samples. Among the MRSA isolates, 68.2 %(n = 30) were classified as hospital-acquired MRSA (HA-MRSA) and 31.8% (n = 14) as community-acquired MRSA (CA-MRSA).

Antimicrobial susceptibility testing and PCR results

The resistance profiles of MRSA and MSSA isolates to antimicrobial agents tested were listed in Table II. All of isolates were sensitive to vancomycin and linezolid. It was found that 93.2% (n = 41) and 86.3 %(n = 38) of MRSA isolates were resistant to erythromycin and tetracycline respectively. In addition, the highest resistance MSSA isolates was to trimethoprim/sulfamethoxazole 30.4% (n = 17) and tetracycline 28.6 %(n = 16). The resistance rates of MRSA isolates to amikacin, ciprofloxacin, clindamycin, erythromycin, gentamicin and tetracycline were significantly higher than among MSSA isolates (Table II). Three CA-MRSA isolates had intermediate (borderline) resistance to clindamycin, while 19 MSSA isolates had intermediate (borderline) resistance as follows: 3(5.3%) to ciprofloxacin, 6(10.7%) to clindamycin and 10 (17.9%) to erythromycin. Overall, 77.2% (34/44) of MRSA isolates and 8.9%(5/56) of MSSA were multidrug resistant (MDR). HA–MRSA isolates were more resistant to multiple antibacterial classes than CA-MRSA isolates (73.5% vs. 26.5%). Fifty (50%) isolates contained an IEC-converting (βC-Φs),

Tab. II. Antimicrobial profiles of MRSA (HA-MRSA and CA-MRSA) and MSSA isolates from 100 patients of Kerman University affiliated hospitals.

Type of isolates	Rate of resistance to antimicrobial agents. n(%)							
	Isolates. n(%)	AK	GM	CD	E	CIP	T	SXT
CA-MRSA	14 (14%)	3 (6.8)	6 (13.6)	9 (13.6)	14 (31.8)	6 (13.6)	11 (25)	6 (13.6)
HA-MRSA	30 (30%)	21 (47.7)	25 (57)	20 (45.5)	27 (61.4)	25 (57)	27 (61.4)	15 (34.1)
MSSA	56 (56%)	1 (1.8)	2 (3.6)	2 (3.6)	11 (19.6)	8 (14.3)	16 (28.6)	17 (30.4)
Total	100 (100)	25 (25)	33 (33)	31 (31)	52 (52)	39 (39)	54 (54)	38 (38)

AK; Amikacin, GM; Gentamicin, CD; Clindamycin, E; Erythromycin, CIP; Ciprofloxacin, T; Tetracycline, SXT; trimethoprim/sulfamethoxazole,

as demonstrated by the presence of *scn*. The predominant IEC variant was type B (*sak*, *chp* and *scn*) present in 20 (40%) of 50 clinical isolates. Variant A (*sea*, *sak*, *chp* and *scn*), C (*chp* and scn), D (*sea*, *sak* and *scn*), E (*sak* and scn), F (*sep*, *sak*, *chp*, *scn*), and G (*sep*, *sak* and scn) were present in 2 (4%), 5 (10%), 3 (6%), 14 (28%), 3 (6%) and 0 (0%) of the fifty clinical isolates, respectively. Three isolates have both *scn* and *sea* which were non type able and negative *mecA* (Tab. III). Concerning the virulence factors in all isolates, *chp* was present in 36 (36%), *sak* was in 60 (60%), and the super antigens *sea* and *sep* were in 10 (10%) and 3 (3%) respectively. *scn* was present in 54% of these isolates.There was significant difference in presence of IEC types between MSSA and MRSA isolates ($P = 0.000$) (Tab. III).

Biofilm formation

The ability to produce biofilm in 9 (9%) isolates was strong, 26 (26%) isolates was moderate, 48 (48%) isolates was weak and 17 (17%) of them had no production biofilm. The prevalence of *icaA, icaB, icaC* and *icaD* in all of isolates was 2%, 1%, 2% and 84% respectively. Only in one MSSA isolate, all *ica* genes were positive and biofilm was strong. There was no significant difference in production biofilm between MSSA and MRSA isolates ($P \geq 0.05$). Although 69 of 84 (82.1%) producing biofilm isolates were positive for *icaD*, no significance difference between the presence of *icaD* gene and biofilm production was observed ($P \geq 0.05$).Thirty four MRSA was *icaD* gene positive.

Discussion

S. aureus is a powerful pathogen that is able to grow in nearly any part of the human body. This bacterium remains the most frequent cause of hospital and community-acquired infections with the high prevalence and rapid spread of drug-resistant *S. aureus* strains. *S. aureus* generates biofilm and an array of immune evasion factors that protect it from innate immune defense system [31]. According to the results of this study, 44% of isolates recognized as MRSA, were positive for *mecA* gene. A study conducted by Javan et al. [32] reported 42.6 % frequency of MRSA in Tehran. However, the frequency of MRSA isolates in present study is more than the results of some previous reports published from Iran and some other countries [33-36].The estimated prevalence in our study was lower than that found in the studies of Khosravi et al. [37], Heidari et al. [38], Sepehriseresht et al. [39], Saderi et al. [40] and Gudarsi et al. [25]. A systematic review displayed that prevalence of MRSA in Iran is high and varies between 20.4% and 90% in different parts of the country [41]. Discrepancy in MRSA prevalence may reflect differences in infection control policies, origin of the isolates and the characteristics of the participants and hospital wards [25]. Rapid and correct determination of the different *S. aureus* isolated from patients is a major help in understanding the epidemiology of this bacteria and its infection control.

The full susceptibility of MRSA and MSSA isolates recovered from clinical samples to vancomycin and linezolid observed in this study, is possibly as a consequence of limited usage of these antimicrobial agents and indicates that these antimicrobial agents are effective for the treatment of *S. aureus* infections in our population. Our data are in agreement with susceptibility rates in Iran and other countries [40-43].

Majority of the MRSA were resistant to tetracycline and erythromycin and these resistance patterns have been documented already by another study [43]. In view of the high resistance rates of MRSA to gentamicin, clindamycin, tetracycline, ciprofloxacin and erythromycin antibiotics which are probably due to misuse and overuse of these antibiotics, display that empirical treatment of MRSA infections at our hospitals with these may not be effective. Hence, these antibacterial agents should no longer be considered first-line drugs for the treatment of MRSA infections in our population.Our study revealed that 30.4% of MSSA and 47.7% of MRSA isolates were resistant to trimethoprim/sulfamethoxazole. In contrast of our data, several studies have been reported low rates of resistance to trimethoprim/sulfamethoxazole in *S. aureus* isolates [25, 45]. Our data is not in agreement with study by Wang*et al* [46] who reported trimethoprim-sulfamethoxazole susceptibility rates of 78.6% and 95.3% for MRSA and MSSA isolates respectively, recovered from patients in 12 cities across China.

Tab. III. Distribution of IEC types among MSSA and MRSA isolates.

Type of isolates	Isolates. n (%)	No. of IEC types						
		A	B	C	D	E	F	G
MSSA	39 (69.6)	2	19	5	2	11	-	-
MRSA	8 (18.2)	-	1	-	1	3	3	-
Total	47 (47%)	2	20	5	3	14	3	-

In this study, 77.2% of MRSA isolates were multidrug resistant, and this is of concern. Our results emphasize the need for persistent monitoring of antimicrobial resistance development in *S. aureus* isolates that are involved in hospital-acquired infections.
MSSA isolates exhibited intermediate resistance to ciprofloxacin, clindamycin and erythromycin, suggesting that more isolates can become resistant in the near future and the possible antimicrobial therapies for infections associated with such staphylococcal strains are confined.
Presence of staphylococci, especially those strains which generate an extracellular slime and constitute a biofilm, making clinical treatment extremely difficult [47]. Our results displayed that 77.7% of the MRSA isolates harbor locus *icaD* and *mecA* gene. Frebourg *et al* [48] have demonstrated that a large proportion of clinical isolates carrying the *ica* locus also harbor the *mecA* gene. Similar results have been reported by Martin-Lopez *et al* [47] and mirzaee *et al* [49]. We noted that 7 MRSA and 5 MSSA isolates were *ica* genes negative and biofilm producers and that 5 MRSA and 10 MSSA isolates were *icaA* positive and biofilm negative. In this regard our data may support some published data based on that biofilm formation may rely on environmentally regulated, *icaADBC*-independent mechanism(s) in MRSA [50, 51]. Eftekhar et al. [52, 53] showed that biofilm formation is independent of the *icaADBC* carriage in clinical and skin isolates of *S. epidermidis*. In contrast, namvar [54] reported that *S. aureus* isolates had no ability to form biofilm, unless they were positive for *icaD* gene. Relationship between the biofilm formation and the presence of these *ica* genes has been demonstrated in other studies [55, 56]. From clinical viewpoint, explanation of the main adhesive mechanisms in infections may help in developing preventive and therapeutic criterions, such as antiadhesive coatings or antiadhesin medicines [28].
In the present study, we demonstrated that 50% clinical *S. aureus* isolates contained an IEC-carrying bacteriophage. The predominant IEC variant was type B which has reported as the predominant variant in human infectious isolates [14]. Immune evasion cluster (IEC) is known to playan important role in human colonization [15]. To our knowledge, this study is the first report of prevalence Immune Evasion Cluster (IEC) genes in clinical isolates of *S. aureus* in Iran.
One study has shown that 90% of the human clinical *S. aureus* strains from a genetically diverse collection contain an IEC-carrying βC-Φs [14]. Some studies have demonstrated that the high incidence of IEC- carrying βC-Φs compared to other mobile elements carrying virulence factors such as *eta*, *lukS-PV*/*lukFPV* which are also carried by bacteriophages in human *S.aureus* strains, is a unique feature [57, 58]. One major reason for this observation is probably due to ability IEC to carrying by several different phages so they can cover a huge host range. IEC has spread successfully through the *S. aureus* population and will continue to do so. This enables *S. aureus* with a unique mechanism to adapt to, and counteract, the human host [14].On the one hand knowledge of the virulence strategies can help choose new ways to combat staphylococcal infections. On the other hand identification virulence genes provides potential targets in the treatment of *S. aureus* infection. For example the potent capacityof CHIPS to inhibit neutrophil chemotaxis, in vitro and invivo, makes this protein a promising candidate anti-inflammatory drug for those diseases in which C5a-induced damage by neutrophils plays an essential role [18].
In conclusion,this study reports that there was no correlation between antibiotic resistance and biofilm formation in under study clinical isolates of *S. aureus* and the biofilm formation ability of several MRSA and MSSA isolates in the absence of *icaABCD* genes suggests that further investigation is necessary to better understand *ica*-independent biofilm formation mechanisms. Different IEC types were detected among the isolates but these types were absent in many MRSA isolates.The high prevalenceIEC types in MSSA isolates can indicate that the presence of these genes can be an advantage for pathogenesis of these isolates in different infections.

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The authors declare that there are no conflicts of interest associated with this manuscript.

Authors' contributions

DKN conceived and designed the research; RA and DKN equally contributed to drafting the article and analyzed data. SFLK and YF contributed in collecting and processing samples. All authors read and approved the final article.

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ORIGINAL ARTICLE

Ultraviolet germicidal irradiation in tap water contaminated by *Aspergillus* spp

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Keywords

Tap water • *Aspergillus* spp • UV disinfection • Water disinfection • Fungi

Summary

We investigated the effect of ultraviolet germicidal irradiation (UVI) from a low-pressure mercury lamp on several pathogenic Aspergillus spp. including A. flavipes, A. flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. terreus, A. ustus and A. versicolor suspended in tap water under laboratory-scale conditions. It was shown that within 10 s of exposure, time species such as A. glaucus, A. niudulans and A. ustus were completely inactivated, while 40 s were needed for the elimination of all the species tested. A. flavus and A. niger were found to be less susceptible than other species. Based on these results we conclude that UV disinfection could effectively inactivate Aspergillus spp. in tap water. Such disinfection could be used to reduce potential exposure of high-risk patients to fungal aerosols, particularly in hospital settings, where point-of-use (POU) UV light devices could be installed to provide safe water at a very low cost.

Introduction

Fungi belonging to the genus Aspergillus are ubiquitous and are found in soil, decaying vegetation and various aquatic environments. Several studies have also demonstrated their presence in drinking water and their ability to resist chlorine or other chemical disinfectants [1-7]. Water distribution systems in hospitals could serve as potential indoor reservoirs for microorganisms such as *Aspergillus* species and other molds, mainly through aerosolization of fungal spores. The presence of *Aspergillus* spp. in hospital water distribution systems has been shown in many studies [8-12]. In addition, *Aspergillus* spp. could reside in biofilms in municipal water distribution systems, which might increase their overall concentration in tap water [13-15]. This could represent a significant public health problem since it has been established that several species are the cause of nosocomial aspergillosis, primarily affecting immunocompromised individuals, and are the second cause of nosocomial fungal infections, which are particularly difficult to prevent and treat [16]. Furthermore, although hospitals have adopted preventive measures such as high-efficiency particulate air (HEPA) filters and laminar air flow (LAF), the incidence of aspergillosis continues to rise [17, 18]. This suggests that, in addition to airborne infection, there may be other sources of aspergillus infection in hospitals, including water systems, the so-called *Wet route* of transmission for human systemic aspergillosis [8, 19, 20]. In this regard, it has been shown that the concentration of airborne *Aspergillus* spp. is significantly higher in the areas where the use of water is greater compared to other areas [19].

Ultraviolet germicidal irradiation (UVGI) is a physical technology used for the disinfection of drinking water thanks to its proven ability to act against many microorganisms, including (oo)cysts of *Cryptosporidium* and *Giardia*, two pathogens of major importance for the safety of drinking water [21, 22].

The UV irradiation technology has several advantages. Firstly, it does not produce undesirable secondary effects often associated with traditional chemical treatments, such as the generation of disinfection byproducts (DBPs), which appear to have mutagenic and/or carcinogenic effects [23]. Second, at the dosage used to treat drinking water (40 mJ cm^{-2}), UV irradiation does not significantly change the water's characteristics [24]. Finally, UVGI as a point-of-use sterilizing system can provide safe water at very low cost [25].

To our knowledge, few studies have been conducted on the inactivation of waterborne *Aspergillus* spp. by UV irradiation [26-28]. Furthermore, these investigations report the results of only a few species such as *A. fumigatus, A. flavus, A. ochraceus* and *A. niger*. Hence, the aim of the present study was to add to the current body of knowledge regarding UV-C irradiation efficiency in inactivating nine *Aspergillus* spp. in tap water.

Materials and methods

Fungi strains and test inoculum conidial suspensions

The following strains of *Aspergillus* spp. were selected for this study and obtained by environmental samples including hospital setting: *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor*. All strains were identified in

our laboratory using traditional techniques for the isolation and identification of filamentous fungi [29] and subcultured on Potato Dextrose Agar (PDA) at 35°C at least twice to ensure purity and viability. Conidial suspensions for each isolate were prepared according to the CLSI M38-A protocol with some modification [30]: the mould was grown on PDA agar slants for 7 days at 35°C, each slant was flooded with 2 ml of sterile 0.85% NaCl solution containing 0.05% Tween 80 and then gently probed with a pipette tip. The resulting mixture was collected in a sterile tube and the heavy particles were allowed to settle for 3 to 5 min. The upper homogeneous suspensions containing conidia were vortexed for 15 s and the transmittances (530 nm) of the mixture suspensions were adjusted by spectrophotometer to provide a final test inoculum of about 10^6 CFU ml^{-1}. The inoculum titres of each *Aspergillus* spp. were confirmed by plating serial dilutions of the suspensions onto PDA plates.

Tap water and UV source

Samples of water were taken from a municipal water supply and analyzed to exclude the presence of filamentous fungi using the spread plate technique, a standard method for the examination of water and wastewater [31]. UVT, namely the measure of the fraction of incident light transmitted through water sample, was made in a spectrophotometer at a wavelength of 254 nm. The main characteristics of this water are listed in Table I. UV-C irradiation was performed using a 18 W low pressure mercury lamp (Philips TUV PL-L 18 W 4 pin) with a monochromatic light output of 254 nm providing an average radiation intensity of 3.2 mW cm^{-2}.

Operation of laboratory-scale UV exposure conditions

Experiments were performed using a UV reactor (Fig. 1), which consists of a cylindrical plastic chamber (250 mm long, 115 mm inner diameter, effective volume 2.6 l) containing 2000 ml of water contaminated with 2 ml of each conidial suspension prepared as described above. The lamp was then immersed in the contaminated water. The final strength of the spores in the water was about 10^3 CFU/ml and during the experiment, the sample was maintained at 20 °C under continuous magnetic stirring to ensure the homogeneity of the suspension. The radiation fluence level ranged from 0 to 192 mJ cm^{-2} and was calculated as a product of average radiation intensity (mW cm^{-2}) and irradiation time (s). At time 0 s (not irradiated control) 10, 20, 40 and 60 s of UV irradiation sample aliquots were collected and tenfold serial dilutions were performed in 0.85% saline solution and 100 µl of each dilution suspension was spread-plated on PDA media to enumerate CFU/ml after incubation at 30 °C for 3-5 days. Experiments were repeated at least four times for each species.

Tab. I. Typical characteristics of the municipal water supply.

Parameter	Unit	Value
Conductivity	µs cm^{-1}	467.18
pH		7.92
Total hardness	mg l^{-1}	242
Turbidity	ntu	0.28
UVT 254 nm*	%	99
Aluminum	mg l^{-1}	33.83
Ammonium	mg l^{-1}	nd
Arsenic	mg l^{-1}	nd
Calcium	mg l^{-1}	80.7
Chlorides	mg l^{-1}	28.89
Chlorine residual	mg l^{-1}	0.16
Fluorides	mg l^{-1}	0.25
Iron	mg l^{-1}	14.81
Magnesium	mg l^{-1}	16.62
Manganese	mg l^{-1}	nd
Nitrate as N	mg l^{-1}	3.7
Nitrite as N	mg l^{-1}	nd
Phosphate	mg l^{-1}	nd
Potassium	mg l^{-1}	0.8
Sodium	mg l^{-1}	20.33
Sulfate	mg l^{-1}	57.41

Information from municipal authority; nd: below the limit of detection; * our determination.

Fig. 1. Ultraviolet germicidal irradiance testing unit for determining susceptibility of *Aspergillus* spp. to UV exposure.

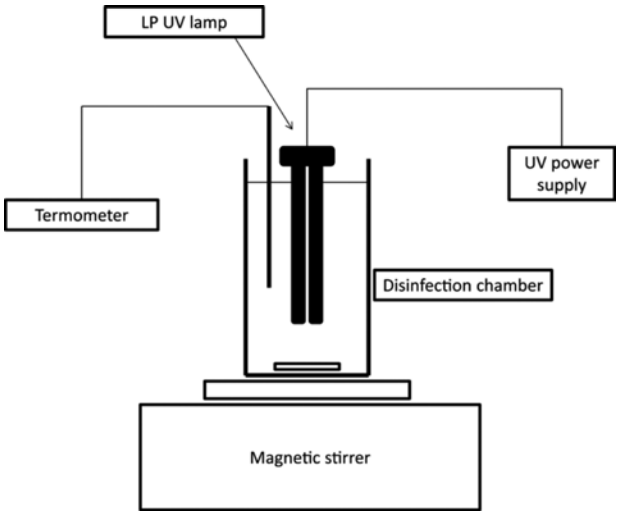

Photo-reactivation and dark repair assay

In order to assess the effective ability of complete inhibition of the UV radiation, tests of photo and dark repair were carried out at the exposure times that cause the complete inactivation of each species. Photo and dark repair experiments were assessed by CFU viability assay. Briefly, after the UV fluence, the sample of 10 ml of liquid conidial suspension, at the same concentration as described above, was introduced into 60- by 15-mm culture petri dishes. The sample was maintained with slow stirring at a distance of 50 cm from the fluorescent lamps (Osram 18 W/L20 4000K) and irradiated for 30 min at room temperature (photo-reactivation) in a laminar air flow cabinet. For the dark repair another 10 ml of liquid conidial suspension were taken and introduced into 60-by 15-mm culture Petri

dishes and left to stand for 4 h in darkness at room temperature. The sample was then serially diluted in 0.85% saline solution and 100 µl of each dilution suspension was spread-plated on PDA media to enumerate CFUml after incubation at 30 °C for 3-5 days.

Statistical analysis

Analysis of variance was performed by one-way ANOVA followed by Tukey's post hoc test. Statistical differences of $P < 0.05$ were considered to be significant.

Results

The susceptibility of the *Aspergillus* spp. to ultraviolet irradiation for various exposure times is shown in Figure 2. Ten seconds of exposure time, equivalent to 2 mJ cm^{-2} of irradiation fluence, is sufficient to cause the complete inactivation of the following species: *A. glaucus*, *A. nidulans* and *A. ustus,* while other species show reductions of conidial concentrations between 1.38 log and 2.67 log. After 20 s of exposition to UV irradiation (64 mJ cm^{-2}) *A. terreus* is also completely inactivated, whereas the other species such as *A. flavus, A. niger, A. flavipes*, *A. fumigatus* and *A. versicolor* undergo a reduction of 1.8 log, 1.91 log, 2.09 log, 2.11 log and 2.27 log, respectively. With an exposure time of 40 s (128 mJ cm^{-2}), only *A. flavus* and *A. niger* were still recovered, although viable biomass was significantly reduced (2.23 log and 2.37 log respectively) compared to initial concentrations. After 40 s of exposure time no viable species were found. Regarding DNA repair, we had no evidence of either light or dark repair of DNA damage caused by UV-C irradiation performed using a low pressure mercury lamp under the above mentioned conditions. Values of nephelometric turbidity units (NTU) and UV transmittance (UVT), on average 0.27 and 99% respectively, were always within the limits recommended for the treatment of tap water with UVGI.

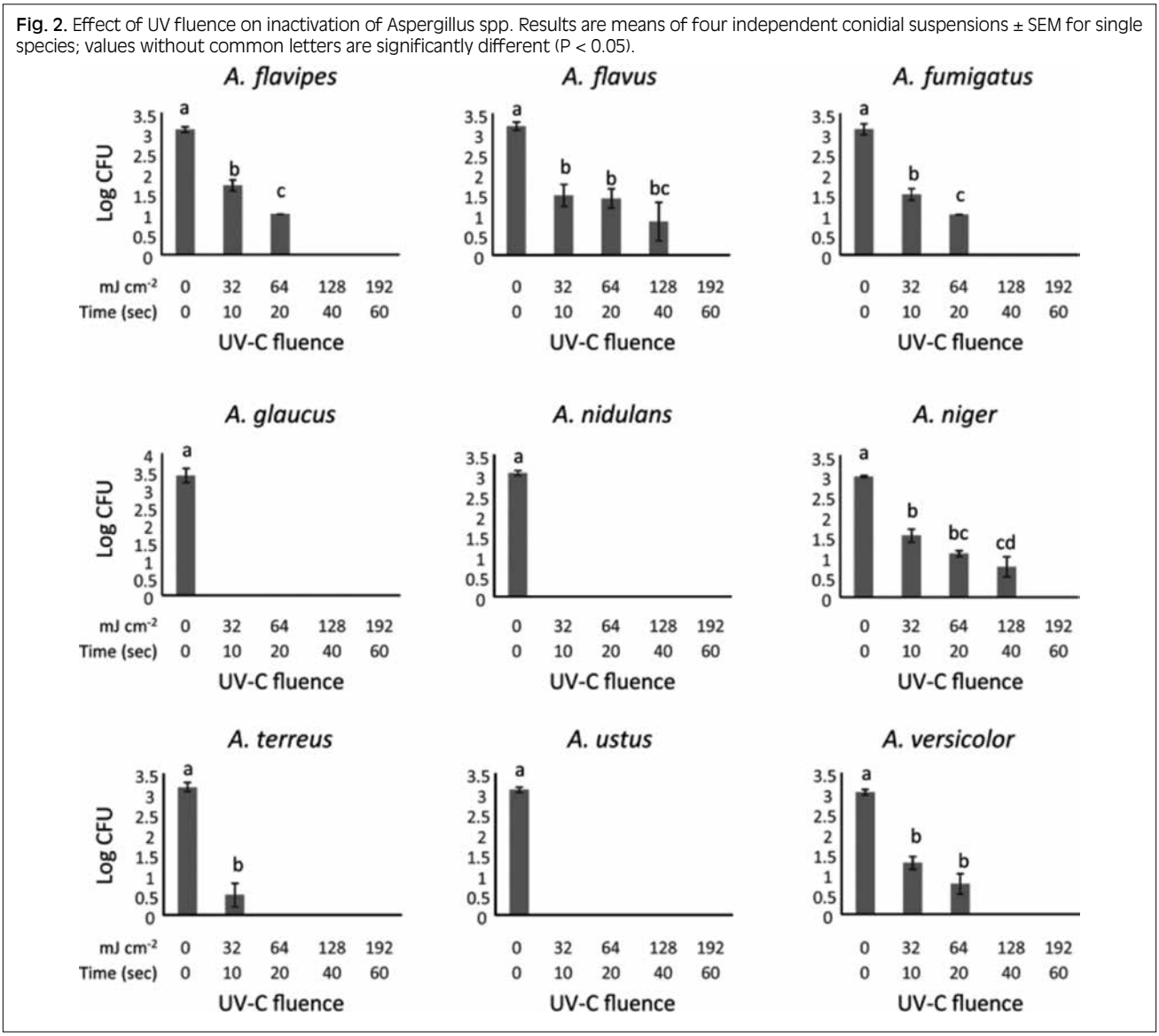

Fig. 2. Effect of UV fluence on inactivation of Aspergillus spp. Results are means of four independent conidial suspensions ± SEM for single species; values without common letters are significantly different ($P < 0.05$).

Discussion

Interest in using UV light treatment to disinfect drinking water is growing due to its ability to inactivate pathogenic micro-organisms without forming DBPs and its effectiveness against several pathogens that are resistant to commonly used disinfectants such as chlorine. Many investigations have been made to assess the dose of UV irradiation necessary for effective bacterial disinfection of drinking water [21, 32, 33], while less data are available concerning the effect of UV irradiation on fungi in potable water.

The efficacy of UV irradiation in inactivating fungi in potable water is very important with regard to water distribution systems in hospitals. Such systems are potential indoor reservoirs for these species and other molds, mainly through aerosolization of fungal spores, which could directly affect human health. Thus, we tested the antifungal efficacy of UV irradiation performed with a UV-C low-pressure mercury lamp in tap water to eliminate the spores of 9 species in the Aspergillus genera. Using this system, we showed that within 40 s of activity of the UV lamp (128 mJ cm^{-2}) it is possible to remove most of the species tested and achieve a significant reduction in the more resistant forms/species such as A. *flavus* and A. *niger.* The latter species are no longer detectable after 40 s of exposure time. In our previous study we found comparable effectiveness of UV radiation for the disinfection of swimming pool thermal water contaminated by dermatophyte fungi [34].

Standards for UV devices in some European countries establish a minimum UV fluence in drinking water of 40 mJ cm^{-2}, while others recommend doses that should never exceed 140 mJ cm^{-2} to avoid significant changes in the physical and chemical characteristics of the treated water [21]. The UV doses employed in this study were within these limits.

Our results confirm that 9 fungi belonging the Aspergillus genera*,* in particular *A. flavus* and *A. niger,* have a greater resistance to UV irradiation compared to other microorganisms, although the methodology used in our experiments is different from that proposed by other authors who employed continuous flow UV reactor testing or collimated beam testing [22, 26-28, 32, 33, 35, 36]. Furthermore, higher resistance of *A. flavus* and A. *niger* is also described by other authors in tests conducted in water [23], whereas *A. fumigatus* showed the highest resistance in tests conducted in air [37]. The higher UV tolerance of fungal spores belonging to the Aspergillus genera, such as *A. niger,* compared to other microorganisms is probably due to their particular cellular structure, which includes the presence of pigments as has been observed in melanized fungi [38].

Water turbidity can protect organisms against radiation and therefore significantly influence the process of UV radiation disinfection [21, 33]. However, in our experiments, both the values of turbidity and UVT were comfortably within the limits recommended for the use of this technology.

The data concerning photo and dark repair experiments confirm the complete inactivation of all fungal species tested within 40 s of UV light exposition.

Conclusions

Present study provides additional information on the effectiveness UV-C radiation in tap water to inactivate potential pathogenic species of fungi belonging to the Aspergillus genera. It has been widely shown that these species can survive conventional disinfection treatments, and therefore water distribution systems might be reservoirs for fungi, particularly the *Aspergillus* spp. Hence, in order to eliminate potential exposure to this risk, particularly in hospital settings such as in high-risk wards, we support the suggestion of several authors who call for the installation of point-of-use (POU) UV light devices that are easy to install and provide safe water at very low cost [11, 25]. In such systems working in full-scale UV reactors, the flow device must be adjusted taking into account the data we obtained regarding exposure time to UV radiation necessary to provide complete elimination of fungi.

Moreover future experiments will be conducted for the evaluation of UV-C radiation against the biofilm formation by *Aspergillus* spp. in combination with other filamentous fungi, especially those containing melanin pigments such as dermatophytes.

Acknowledgements

The authors declare no conflict of interest.

Authors' contributions

MS designed the study and performed data analysis and manuscript preparation. GFS and MDS carried out data collection and analysis and performed statistical analysis. GB carried out the technical revision of the manuscript. All authors have critically read and revised the manuscript and approved the final version.

Revision of the test by a english mother-tongue revisor.

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ORIGINAL ARTICLE

Food safety knowledge, attitude and practices of meat handler in abattoir and retail meat shops of Jigjiga Town, Ethiopia

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Keywords

Food borne disease • Food-handlers • Food safety • Hygienic practices • Meat hygiene • Personal hygiene

Summary

A cross-sectional survey was carried out among 91 meat handlers by using structured questionnaire to determine the food safety knowledge, attitude and practices in abattoir and retail meat shops of Jigjiga Town. The result shows that majority of the meat handlers were illiterate (30.8%) and primary school leaver (52.7%), and no one went through any food safety training except one meat inspector. The food-handlers' knowledge and safety practices were below acceptable level with the mean score of 13.12 ± 2.33 and 7.7 ± 2.1 respectively. Only few respondents knew about Staphylococcus aureus (3.3% correct answer), hepatitis A virus (19.8% correct answer), and E. coli (5.5% correct answer) as food borne pathogens. About 64% of meat handlers have good attitude about safety of food with mean of total score 14.4 ± 2. All respondents answer correctly questions about proper meat handling and hand washing but they did not translate into strict food hygiene practices. Chi2 analysis testing for the association between knowledge, attitude and practices did not show any significant association. It may be due to meat handlers' below acceptable level safety practices regardless of sociodemographic characteristics, knowledge and attitude. However, there was strong association between level of education and knowledge, and knowledge and hand washing ($p < 0.05$). There was also association between age and knowledge though it was not statistically significant. Thus, continuous education and hands on training for meat handlers that can enhance good safety practices through better understanding and positive attitude.

Introduction

Food safety that refers to the conditions and practices that prevent contamination of foods from toxic chemicals or microbes remains a major public health concern around the globe [1]. According to WHO global burden of foodborne diseases estimates 600 million people**,** almost 1 in 10 fall ill every year from eating contaminated food and 420, 000 die as a result [2]. Even in developed countries, every year one third of the total population are likely suffered from food borne diseases and from which 70% of the cases are linked with the consumption of contaminated food [3, 4].

The issue of food safety is much more complicated in developing country due to enormous reasons. Poverty is one of the leading causes of consumption of unsafe food attributing to lack of access to adequate food and clean water, poor arrangement in government structural, perpetuating infectious diseases in the community, inconvenient environmental conditions to assure food safety and poor food handling and sanitation practices [5, 6].

Food borne diseases are preventable, if food protection principles are followed from primary production to the level of consumer. However, it is practically unachievable to apply in developing countries. Ethiopia is not exceptional since the prevailing of poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest on food safety, and lack of education and training for food handlers [6].

A study conducted by Todd et al. revealed that most of the food borne outbreaks occurred worldwide are linked to food handlers [7]. According to Sharif & Al-Malki, food handlers' knowledge, attitude and practice are the three key factors that are playing vital role in food poisoning outbreaks [8]. Other studies also came across with a conclusion that knowledge of food handling is significantly related with food handling practices [9-11], whereas, studies done in Bangladesh, India and Nigeria indicated that food handling practices was related with educational status of food handlers [12-14].

Meat handlers have crucial role in controlling food borne pathogens either from contaminated utensils or from the animal itself such as *E. coli* and other pathogens. They may also carry some human specific food borne pathogens like *Hepatitis* A, Noroviruses, Typhoidal *Salmonella*, *Staphylococcus aureus* and *Shigella* in their hands, mouth, skin, hair and cuts or sores, and disseminate to

the consumer [4]. In Ethiopia very few studies have been conducted on food safety knowledge, attitude and practices of food handlers but none of them were focused to assess the knowledge, atitude and practices of meat handlers [15]. No study has been conducted on food safety knowledge, attitude and practices among meat handlers in abattoirs and meat retail shops in the country [6, 16]. It is also crucial to address the hygienic status of meat production and distribution as such information will be beneficial in designing any preventive strategies and control measures. It also serves as a baseline data for related researches. With the above motives, the objective of this study was to evaluate the level of knowledge, attitudes and practices among meat handlers from a municipality abattoir and retail shops in Jigjiga, Somali National Regional State of Ethiopia.

Materials and methods

Study design and sample collection

A cross-sectional survey was conducted among meat handlers from a municipal abattoir and ten retail meat shops in the Jigjiga town, Somali National Regional State of Ethiopia. Ninety-two meat handlers were interviewed by using structured questionnaire with 100% response rate; however, one questionnaire was filled incomplete during the interview and this questionnaire was excluded from analysis.

All (n = 92) workers involved in meat processing in the abattoir and retail meat shops of the town were included in the study and the respondents were interviewed face-to-face on a once-off basis during working hours without prior notice of the interview. Explanation on the purpose of the study was given before and the respondents were assured about the confidentiality of their status. The questionnaire was read and completed by an interviewer in individual interviews. The respondents were given sufficient time (30 min) to answer the questionnaire.

Questionnaire

A structured questionnaire was adopted from previous published research articles in order to meet the objective of this study [17-19]. The language of the questionnaire was translated to the local language (Amharic) in which all the participants can communicate. After pre-testing the questionnaire at a neighbour town (Harar) of the study area with 20 meat handlers the last version was prepared.

The questionnaire structured into four distinct parts including demographic information such as respondents' sex, age, years of experience, responsibility/duty, income, employment status, having health certificate and attending food safety training. The second section of the questionnaire is about food safety knowledge. Questions on knowledge referred to their personal hygiene, cross-contamination, causes and symptoms of food borne diseases, and time temperature control. It contains 22 close-ended questions and each question has three optional answers ("Yes", "No" and "I do not know"). The response was analyzed as categorical variables (right or wrong answer). A score of one was given to right answer and zero to the wrong and I do not know answer. A scale ranging between 0 and 22 which representing the total number of questions on food safety knowledge. Meat handlers that got overall score ≤ 14 points were considered to have "unsatisfactory" and those scored ≥ 15 points (≥ 68 % accuracy) "satisfactory" knowledge of food safety.

The third part of the questionnaire was about food safety attitude of meat handlers. It comprises 20 questions about hand washing, cross contamination, food handling, storage etc. In this section, the respondents' answers were "agree", "disagree", and "don't know". The response was analyzed as categorical variables (right or wrong answer). A score of one was given to right answer and zero to the wrong and I do not know answer. Each correct answer was given one point whereas incorrect answer including the answer I do not know was awarded zero point. For evaluation, food-handlers that answered 14 or more questions correctly were measured to have "good" attitude whereas respondents answer 13 or less questions correctly were measured to have "poor" attitude.

The last section dealt with food hygiene practices. The question comprises the issues of personal hygiene, hand washing practices, practices against food borne diseases and cross contamination. This section had 20 questions with two possible responses: "yes", and "no". Each correct practice reported scored one (1) point. For evaluation, a score ≥ 70% that means food-handlers practiced 14 or more out of 20 hygienic practices which are listed in the questions was considered as having "good" food hygienic practice [20].

Statistical Analysis

The statistical analyses of the data were performed by using SPSS (Statistical Package for the Social Sciences) software version 20. Descriptive statistics such as frequency (%) for categorical and mean and standard deviation (SD) for numerical data were used to sum up the data. Chi square ($\chi 2$) test was also used to find the relationship between the sociodemographic characteristics with knowledge and practice scores. p-value less than 0.05 was considered statistically significant.

Results

Sociodemographic characteristics of respondents

Sociodemographic profile of respondents is summarized in Table I. Of the 91 respondents participate in this study, 79.1% were males. Those respondents within the age of 31-40 years comprised 36.3% followed by age of 20-30 (31.9%). Average age of respondents was 29.7±5.78 with the minimum age of 16 and maximum 55. Education level of majority of the respondents (52.7%) were at the level of primary followed by considerable number of illiterate (30.8%). Only one person attained tertiary education and the remains (15.4%) were secondary school

Tab. I. Summary of sociodemographic characteristics of respondents (n = 91).

Characteristics	n	%	Mean ± SD	Range
Gender				
Male	72	79.1		
Female	19	20.9		
Age				
< 20	18	19.8		
20-30	29	31.9	29.7 ± 9.6	16-55
31-40	33	36.3		
40	11	12.1		
Level of education				
Illiterate	28	30.8		
Primary education	48	52.7		
Secondary education	14	15.4		
Tertiary	1	1.1		
Field of duty				
Butcher	33	36.3		
Helper	39	42.9		
Cook	17	18.7		
Other (meat inspector)	1	1.1		
Employment status				
Daily bases	52	57.1		
Contract	38	41.8		
Permanent	1	1.1		
Income				
< 1000 ETB	17	18.68		
1000-2000 ETB	46	50.55	1739.3 ± 681.8	500-3500
2001-3000 ETB	27	29.67		
> 3000ETB	1	1.09		
Year of service (experience)				
< 5	42	46.15	6.1 ± 4.2	1-19
5-10	34	37.36		
> 10	15	16.48		
Food safety training				
Yes	1	1.1		
No	90	98.9		
Health certificate				
Yes	1	1.1		
No	90	98.9		

leavers. Monthly income of majority of the meat handlers (50.5%) is in the range between 1000 and 2000 Ethiopian Birr (ETB), which is less than 100 USD per month. Only a single person get more than 3000 ETB and 18.68% monthly income were less than 1000 ETB. About 53.84% of respondents have been working in this sector for 5-10 years and 16.5% have more than 10 years' experience with an average length of 6.1 ± 4.2 years of experiance.

Food safety knowledge

The overall knowledge level of respondents about personal hygiene, cross-contamination, causes and transmission of food borne diseases, and time temperature control of food summarized in Table II. About 78% of respondents have unsatisfactory knowledge level with the mean score of 13.12 ± 2.33, which is below the cut of point 15 (≥ 68% accuracy). However, almost all meat handlers were aware of how to clean and sanitize food contact surface (95.6%), and hooks and knives (83.53%). Its also known by almost all (91.21 correct answer) meat handler about the role of insect and pets in food contamination. Many of the meat handler believe that diarrhea can be transmited by contaminated meat (93.41correct answer), and cooking with elevated temperature or freezing as safe method to destroy bacteria (91.21% correct answer). From 91 respondents 89% said people with open skin injury, gastroenteritis, and ear or throat diseases should not be allowed to handle meat.

On the other hand, the respondents had least knowledge about the importance of using gloves (41.8% correct answer) and rotation of disinfectants for cleaning (20.9% correct answer), the difference between cleaning and sanitization (42.9% correct answer), time and temperature control (44.8% correct answer) and correct storage temperature (24.2%). Almost no respondents knew about *Staphylococcus aureus* (3.3% correct answer), hepatitis A virus (19.8% correct answer), and *E. coli* (5.5% correct answer) as food borne pathogens. A study conducted by Soares et al. [19] mentioned that 56.6% of the food did not know that *S. aureus* is a pathogenic microorganism that is responsible for food-borne disease.

Tab. II. Summary of meat handlers' food safety knowledge in Jigjiga abattoir and retail meat shops.

	Statements	Response % (n)		
		Right Answer	Wrong answer	Do not know answer
1	Improper handling of meat could pose health hazards to consumers	100 (91)	0 (0)	0 (0)
2	Regular washing of hands before and during meat processing reduces risk of contamination	100 (91)	0 (0)	0 (0)
3	Using gloves while handling meat reduces the risk of contamination	41.8 (38)	26.4 (24)	31.9 (29)
4	Proper cleaning and sanitization of knives and hooks reduce the risk of meat contaminatio.	83.5 (76)	9.9 (9)	6.6 (6)
5	Eating and drinking in the work place increase the risk of meat contamination	15.4 (14)	72.5 (66)	12.1 (11)
6	Washing and disinfection of working surfaces and tools are important for safety of meat	95.6 (87)	4.4 (4)	0 (0)
7	Regular rotation of disinfectants for cleaning can reduce the risk of meat contamination from working surfaces and cutting tools	20.9 (19)	23.1 (21)	56.1 (51)
8	Insects and pests could be a source of contamination to raw meat	91.2 (83)	6.6 (6)	2.2 (2)
9	Diarrhea can be transmitted by food	93.41 (85)	3.3 (3)	3.3 (3)
10	E.coli is one of the food-borne pathogens	5.5 (5)	12.1 (11)	82.4 (75)
11	Hepatitis A virus is one of the food-borne pathogens	19.8 (18)	35.2 (32)	45.1 (41)
12	Staphylococcus is one of the food-borne pathogens	3.3 (3)	13.2 (12)	83.5 (76)
13	Microbes are on the skin, nose and mouth of healthy meat handlers	86.8 (79)	14.3 (13)	2.2 (2)
14	Clean is same as sanitized	42.9 (39)	36.3 (33)	20.9 (19)
15	Cross contamination is when microorganisms from a contaminated meat are transferred by the meat handler's hands or utensils to another	13.2 (12)	26.4 (24)	60.4 (55)
16	The ideal place to store raw meat is in the refrigerator	45.1 (41)	35.2 (32)	19.8 (18)
17	Freezing kills all the bacteria that may cause food-borne illness	51.7 (47)	46.2 (42)	2.2 (2)
18	High temperature or freezing is a safe method to destroy bacteria	91.2 (83)	7.7 (7)	1.1 (1)
19	The correct temperature for storing perishable foods is 5^{0}C	24.2 (22)	18.7 (17)	57.1 (52)
20	Contaminated meat always have some change in color, odor or taste	94.5 (86)	2.2 (2)	3.2 (3)
21	People with open skin injury, gastroenteritis, and ear or throat diseases should not be allowed to handle meat	89.1 (81)	11 (10)	0 (0)
22	The health status of workers should be evaluated before employment	95.6 (87)	4.4 (4)	0 (0)
	Total percentage mean of correct answer	59.3 ± 36.4		

Food safety attitudes

Table III summarized food safety attitude of meat handlers. Around 64% of respondents have good attitude about food safety with the mean of total score 14.4 ± 2. The overall attitudes of the food handlers were favorable with mean of total percentage scores of 71.4 ± 18.8. They also had good attitudes toward the cons of improper meat storage. Almost all the respondents said washing hands before and during food preparation is mandatory. Meat handlers' attitude towards taking regular training for better meat safety and hygiene practices were satisfactory (89.1%). High percentage (91.2%) of meat handlers were also aware of keeping working surfaces and utensils clean reduces the risk of illness. Beside this 80.2% were believe the fact that surfaces and equipment should be clean before reusing for meat processing. Approximately 87.9% handlers said knives and cutting boards should be properly sanitized to prevent cross contamination, for 59.3% the reason was hooks, knives and cutting boards can be a source of food contamination. About 78% respondents recommend that wearing protective clothing and shoes could help to improve work safety and hygiene practices whereas 68.1% said putting on hair cover on the head is a good practice in food industry. Higher percentage of the surveyed meat handlers (78%) stated that using potable water to wash meat contact surfaces and utensils. Approximately 71.4% respondents in this study also thought sneezing or coughing without covering noses or mouth could contaminate the meat.

Food handler's practices towards food hygiene and sanitation

In assessing food safety practices 20 questions enquired for 91 meat handlers. It was found that almost no respondents (98.9%) maintained food safety practices with the mean total score of 7.7 ± 2.1. The mean of total percentage scores of safety practices was 38.5 ± 27.3. Table IV shows food safety practices of meat handlers in the study area. Per the survey result, 69.2% of respondents eat and drink and 65.9% smoke at their work place. Almost no (98.9%) meat handlers use gloves during meat processing. Most of the respondents do not use aprons (55%), hairnet or cap (62.6%) and mask (98.9%) while doing their work. Concerning sanitizer use, 79.1% respondents do not use any sanitizer to wash utensils such

Tab. III. Summary of meat handlers' food safety attitude in Jigjiga abattoir and retail meat shops (n = 91).

	Statements	Responses % (n)		
		Right answer	Wrong answer	Not sure
1	Meat handlers with wounds, bruises or injuries on their hands must not touch or handle meat	98.9 (90)	1.1 (1)	0(0)
2	Using watches, earrings and rings will increase the risk of meat contamination	40.7 (37)	45.1 (41)	14.3 (13)
3	Improper meat storage is dangerous to health	87.9 (80)	12.1 (11)	0 (0)
4	Hand washing before handling meat reduces the risk of contamination	93.4 (85)	6.6 (6)	0 (0)
5	Regular training could improve meat safety and hygiene practices	89.1 (81)	7.7 (7)	3.3 (3)
6	Safe meat handling to avoid contamination and diseases is part of meat handler job responsibilities	75.8 (69)	9. 9 (9)	3.3 (3)
7	Keeping working surfaces and utensils clean reduces the risk of illness	91.2 (83)	7.7 (7)	1.1 (1)
8	Using different knives and cutting boards for meat and offal is worth	51.7 (47)	38.5 (35)	9.9 (9)
9	It is unsafe to leave meat out of the refrigerator for more than 2 hour.	68.1 (62)	20.9 (19)	11 (10)
10	Inspecting meat for freshness and wholesomeness is valuable	76.9 (70)	13.2 (12)	9.9 (9)
11	Surfaces and equipment should be clean before re-using for meat processing	80.2 (73)	15.4 (14)	4.4 (4)
12	After processing meat, any leftovers should be kept in a cool place within	44 (40)	14.3 (13)	41.8 (38)
13	Raw meat is healthier and nutritious than cooked	33 (30)	67 (61)	0 (0)
14	Knives, hooks and cutting boards can be a source of food contamination	59.3 (54)	37.4 (34)	3.3 (3)
15	Knives and cutting boards should be properly sanitized to prevent cross contamination	87.9 (80)	12.1 (11)	0 (0)
16	The same towel can be used to clean many places	53.8 (49)	44 (40)	2.2 (2)
17	Sneezing or coughing without covering our noses or mouth could contaminate the meat	71.4 (65)	23.1 (21)	5.5 (5)
18	Wearing protective clothing and shoes could help improve work safety and hygiene practices	78 (71)	19.8 (18)	2.2 (2)
19	Putting on hair cover on the head is a good practice in food industry	68.1 (62)	24.2 (22)	7.7 (7)
20	It is important to use potable water to wash working surfaces and cutting tools after disinfection	78 (71)	22 (20)	0 (0)
	Total percentage mean of correct answer	71.4 ± 18.8		

Tab. IV. Summary of meat handlers' food safety practices in Jigjiga abattoir and retail meat shops (n = 91).

	Food safety practices questions	Responses % (n)	
		Yes	No
1	Do you eat or drink at your work place?	69.2 (63)	30.8 (28)
2	Do smoke inside meat processing areas?	65.9 (60)	34.1 (31)
3	Do you use gloves while handling meat? If no, go to question no. 5?	1.1 (1)	98.9 (90)
4	Do you wash your hands properly before or after using gloves?	1.1 (1)	NA
5	Do you wash your hands before and after handling meat?	40.7 (37)	59.3 (54)
6	Do wash hands after handling waste/garbage?	35.2 (32)	64.8 (59)
7	Do wash hands after using toilet?	86.8 (79)	13.2 (12)
8	Do you wash your hand after smoking, sneezing or coughing?	13.2 (12)	86.8 (79)
9	Do you wear an apron while working?	45.1(41)	55 (50)
10	Do you wash your aprons after each day's work?	30.8 (28)	69.2 (63)
11	Do you wear a mask while working?	1.1 (1)	98.9 (90)
12	Do you wear a hairnet or a cap while working?	37.4 (34)	62.6 (57)
13	Do you wear nail polish when handling meat?	7.7 (7)	92.3 (84)
14	Do you properly clean the meat storage area before storing new products?	74.7 (68)	25.3 (23)
15	Do you use the sanitizer when washing service utensils (knives, hooks and cutting boards)?	20.9 (19)	79.1 (72)
16	Do you replace knives or sterilize them after each meat processing?	14.3 (13)	85.7 (78)
17	Do you remove your work equipment when using toilets?	51.6 (47)	48.4 (44)
18	Do you remove your personal stuffs such as rings, necklaces, watch etc. while processing meat?	79.1 (72)	20.9 (19)
19	Do you handle/process meat when you are ill?	44 (40)	56 (51)
20	Do you handle/process meat when you have cuts, wounds, bruises or injuries on your hands?	50.6 (46)	49.5 (45)
	Total percentage mean of practices	38.5 ± 27.3	

as knives, hooks cutting boards and the floor surface as well. Most of the handlers (86.6%) did not wash hands after smoking, coughing, and sneezing.

Discussion

Unlike other food processing, males most likely involved in meat processing [17, 21]. This is also true for our finding. The mean age of the respondents in this study is lower (29.7 ± 5.78) than the study conducted by Akabanda et al. [20] (41.5 ± 9.5), Soares et al. [19] (43.9 ± 8.4), and Sharif & Al-Malki [8] (43.9 ± 8.4) but higher than Farahat, El-Shafie, & Waly [22] (25.1 ± 9.6). Olumakaiye & Bakare [23] mentioned that food handlers at their older age have better hygienic practice score than their younger colleagues. In our study literacy rate of food handler were much lower than the finding of other studies [20, 21, 24]. However, a previous study indicated that regardless of educational level food safety knowledge was unacceptable that may trigger public health concern [20]. In our finding also there is not significant association between educational status and knowledge level. Lack of training among food handlers have negative consequence on performing behaviors [25], it was found that none of respondents attended training related to food safety except only one meat inspector working in the municipality. Several studies mentioned that food safety trainings should be provided to improve knowledge, attitude and safety practices of food handlers [20, 26].

All (100%) meat handlers have same thought and answer the question correctly about proper meat handling and hand washing which is similar finding to a study conducted by Haapala & Probart [28] reported that most participants gave correct answers for hand washing question. The overall knowledge level of meat handlers in our study area were lower (13.12 ± 2.33) comparing with a study conducted in Camaçari, Brazil schools' food handlers, which reported the mean score of food safety knowledge 16.3 ± 2.6 [19]. Our result also lower than the finding by Jianu [21], Siau [24] and Webb & Morancie [27]. The study conducted by Sani & Siow [29] mentioned that 98.2% of the respondents knew it is necessary to wash hands before processing or handling foods. Knowing the importance of proper handling of meat, proper hand washing and other important hygienic procedures by the meat handlers is very important since meat-handlers can serve as vehicles for cross contamination and spread of foodborne pathogens [30]. According to Xavier, Oporto, Silva, Silveira, & Abrantes [31], proper hand washing among meat handlers have significant impact on reducing threat of diarrheal disease transmission.

Improper temperature in meat processing and storage will also lead to the proliferation of microbes which ensuing to food borne infection and intoxication [32, 33]. Our result agrees with the findings by Baş et al. [9] that many of the respondents unaware of the correct refrigerator temperatures for food storage. Like our finding in a study by Akabanda et al. [20], 70.6% of food handler did not know/remember that hepatitis A is a foodborne pathogen. The motive of dealing with this question was to know whether the respondents are conscious about it and able to connect with disease outbreaks that occurred throughout the world [34]. Several studies reported isolation of methicillin-resistant *S. aureus* (MRSA) strains from food producing animals, processing plants and food handlers. The cross contamination of meat and its products mainly by infected food handlers at the time of further processing [35]. However, in our study most of the respondents were not aware in this regard. A study conducted in Malaysia reported that 73.4% of food handlers had acceptable knowledge of food borne pathogens [36]. This might be related to food safety training since majority of them (94.3%) attended one or more food safety training. Previous studies show that food safety training increased knowledge regarding food safety issues [37]. Training and education may be an effective tool to increase food safety knowledge among food handlers and thus improve food safety practices [38].

Attitude of meat handlers have key role that may influence food safety practice that helps to decrease the chance of food borne diseases outbreaks. Akabanda et al. [20] mentioned a strong linkage between positive attitudes and maintaining safe food handling practices. About 98.9% meat handlers agreed that a person with wounds, bruises or injuries on their hands must not touch or handle meat and 75.8% believe safe meat handling to avoid contamination and diseases is part of their responsibilities. Our finding was higher than Al-Shabib et al. [18] and Zanin, da Cunha, Stedefeldt, & Capriles [39], 82 and 85% of their respondents were aware of the risk of touching food with cut hands or fingers respectively. Around 53% of the workers thought that same towel can be used to clean many places. This observation divergent with the report by Sani & Siow [29] where 97.4% respondents recommended that use of different clean clothes to wipe different food utensils. Abdul-Mutalib et al. [36] and Al-Shabib et al. [18] did similar observation, where 49% and 40% of respondents use the same towel to clean different utensils respectively.

Personal hygiene practices play vital role to ensure safety of food and safeguard the consumer from food borne infection and intoxication. High percentage (86.8%) of respondents in this study said, they always wash their hands after using toilets. This result is lower than the finding by Soares et al. [19] and higher than Adesokan & Raji [17] who reported around 90 and 78.2% respondents wash their hands after using toilets respectively. About 79.1% of respondents removed personal stuffs such as watches, rings and jewelry during meat processing. Çakiroğlu & Uçar [40] demonstrated similar results that 84.2% indicated that they did not wear jewelry during food production. As per the CAC - Codex Alimentarius Comission [41], improper food handling and poor hand hygiene is the main risk factor in the occurrence of food contamination that leads to food borne diseases. The codex recommended that food handlers should al-

ways wash their hands at every stage of food production to safeguard the consumer from diarrheal and other food borne diseases. Particularly, before handling meat, after eating, smoking, coughing, sneezing, touching garbage and using toilet are critical time the meat handler should wash their hands. Meat handler with open skin injury, gastroenteritis, and ear or throat diseases should not deal with any meat production [41]. In our study, more than half of respondents (56%) handle meat while they are sick or having wounds and cuts. This is a substantial risk involved with the contamination of food by the sick and wounded.

As limitation, assessing hygienic practices would have been better through observational study rather than interview to avoid information bias. Due to this motive, the investigators had designed both observation and interview as sample collection tools. However, the venders at retail meat shops did not allow any observer while they process meat. On the other hand, we had chance to see how the slaughtering practice looks like in the municipality abattoir.

In our finding regardless of any demographic characterstics, level of knowledge and attitude the hygenic practices by all respondents were much lower than the acceptable level. In our study there was significant association between level of education and knowledge, and knowledge with handwashing at the value of $p<0.05$. There was association between age agroup and knowledge though it's not significant. Acording to Nigusse & Kumie [10] food safety knowledge of food handlers significantly related with food handling practices. Rabbi & Dey [13] indicated that food handling practices was related with educational status of food handlers. Nonetheless, more knowledge does not always lead to positive changes in food handling behaviors [30, 42].

Conclusions

In conclusion, meat handlers had unsatisfactory knowledge mainly on food borne pathogens, time temperature control, cross contamination, and difference between cleaning and sanitation. It may be due to high proportion of illiterate and primary school leaver meat handlers in the study area. Furthermore, no meat handler had taken any food safety training except one meat inspector. Though most of the meat handler have basic understanding and good attitude about personal hygiene, hand washing and proper cleaning, they did not translate into strict food hygiene practices. Therefore, continuous food safety education and hands on training for meat handlers should be given that can enhance good safety practices through better understanding and positive attitude. The last but not the least, the information gained from this study can be utilized to formulate essential safety measure to safeguard the consumer from food borne infection and intoxication.

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Authors' contributions

TH conceived, designed, coordinated and supervised the research project including data collection. PH performed the data quality control, performed the statistical analyses and evaluated the results, wrote the manuscript. Both authors revised the manuscript, gave their contribution to improve the paper and approved the final manuscript.

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ORIGINAL ARTICLE

Helminths and heavy metals in soils from a dumpsite in Ibadan city, Nigeria

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Keywords

Helminths • Heavy metals • Soil • Dumpsite • Ibadan city

Summary

Waste generation is inevitable because humans continue to generate waste due to increase in population, urbanization and advancement in technology. This generation of waste is of public health concern especially when the waste materials are deposited on dumpsites. This study assessed the helminths and heavy metal content of Awotan dumpsite in Ibadan city. Surface soils (0-15cm depth) of the dumpsite were randomly sampled at different dumpsite areas with the aid of a quadrat. Helminth content was determined using the zinc floatation method and the heavy metal concentrations of the soil were determined using Atomic Absorption Spectrophotometry (AAS). Some physicochemical properties of the soil were also determined. Results showed that the dumpsite soil was slightly alkaline with an average pH of 8.1 ± 0.2 while the overall mean electrical conductivity, temperature, moisture content and height above sea level were 545.9 ± 235.3µS/cm, 32.6 ± 2.2°C, 17.2 ± 4.8 % and 236±4.6 m respectively. The overall prevalence of soil helminths was 10.4% with Ascaris lumbricoides being more prevalent (8.8%). The heavy metal concentration of the soil followed the trend Pb (709.7± 1574.9mg/g) > Cu (316 ± 227.1mg/g) > Cr (48.8 ± 17.7mg/g) > Cd (9.7 ± 10.9mg/g). There was a low overall prevalence of soil helminths. However, the heavy metal concentrations exceeded USEPA permissible limits providing a possible source for underground water contamination in residential areas around the dumpsite. Human settlements close to the dumpsite should be discouraged by the government.

Introduction

Soil is a highly dynamic, ecologically complex and diverse living entity that is formed as a result of various biological and climatological interactions with the earth's bedrock [1]. Soil pollution is a phenomenon characterized by the loss of the structural and biological properties of the soil layers as a result of numerous human and natural factors [2]. Human activities that cause pollution include increased urbanization, disposal of untreated waste, indiscriminate use of agrochemicals, unscientific mining, dumping of industrial waste, accidental pollution and leakages, inadequate treatment and safety management of chemicals and toxic waste. As urbanization increases and human population grows, there is a need to manage the waste produced from human activities and this has led to the creation of dumpsites.

Dumpsites are waste depositing land (soil) areas where uncontrolled waste disposal activities occur in such a way that the environment is not protected from the detrimental effect that arises from these activities [3]. The ecological balance of any ecosystem gets affected due to the widespread contamination of the soil [2].

Accumulation of heavy metals can degrade soil quality, reduce crop yield and the quality of agricultural products, and thus negatively impact the health of humans, animals and the ecosystem [4]. Small life forms may consume harmful chemicals, accumulate and pass them up the food chain to larger animals leading to morbidity and an increased mortality rates of organisms. Human exposure to pollution is believed to be more intense now than any other time in human existence [5, 6].

Municipal and industrial solid wastes contain a variety of potentially significant chemical constituents and pathogenic organisms that could negatively affect public health, air, soil and groundwater qualities [7]. Some contaminants are carcinogens while others for example DDT are known to be toxic to humans and can also alter chromosomes. Others such as PCBs cause liver and nerve damage, skin eruptions, vomiting, fever, diarrhoea, and fetal abnormalities [8].

Over the last three decades there has been increasing global concern over the public health impacts attributed to environmental pollution [9]. The pathogenic organisms that can be found in solid waste include parasitic nematodes, protozoa and other microorganisms [10]. Parasitic nematodes represent a serious threat to humans, animals and plants. The parasitic nematodes whose infective stages can embryonate in the soil are soil transmitted helminths (STHs) [1]. Soil-transmitted helminths can cause a range of symptoms including intestinal manifestations (diarrhoea and abdominal pain), general malaise and weakness, impaired cognitive and physical development, and chronic intestinal blood loss which can lead to anaemia. Helminth infection is a ma-

jor cause of disease burden among children in developing countries especially in sub-Saharan Africa [11].
This study was carried out to assess the helminths and heavy metals in soils from a dumpsite in Ibadan city.

Materials and methods

Study area

This study was conducted in Awotan Solid Waste Dumpsite (ASWD) (Fig. 1) located within 07'27.719' – 07'27.811' North and 003'51.003' – 003'50.999' East, Ibadan, Oyo State. ASWD has been active since 1998, receiving 36000 tonnes of municipal wastes in an area of 14 hectares [3]. Residential buildings, markets, block industries, schools and churches are located within thirty meters radius of the dumpsite.

Soil sampling and preparation

The study area was divided into five sampling sites (A-E) and a total of twenty five samples were collected from each site. Soil sample (60g) was collected at each sampling point with the aid of a 0.25 m^2 quadrat thrown at random and with a soil auger at depth 0-15cm. The temperature of the soils was measured in-situ. Samples were collected in labelled airtight polythene bags and immediately transported to the laboratory for analysis. Sampling was done in the first three weeks of November, 2015.
Soil samples from the field were analysed for moisture content. Soils to be checked for helminths were kept moist in polythene bags pending time of checking and the remaining soils were transferred into a plastic tray for air drying; large clods of soil were broken to speed up drying. The soil was passed through 2mm sieve to remove stones and other unwanted materials, crushed and ground into fine powder using mortar and pestle and then analyzed for pH, conductivity and heavy metals.
Activities in each sampling site within the study area were observed. Site A had sorted and burnt waste materials while newly brought-in wastes were dumped in site B. Site C was the sorting region where the scavengers stored the recyclable materials such as plastic bottles, iron rods, aluminium cans and many more, site D was covered with wastes deposited for a long time while site E was a newly opened section for dumping activities (Plates A-E).

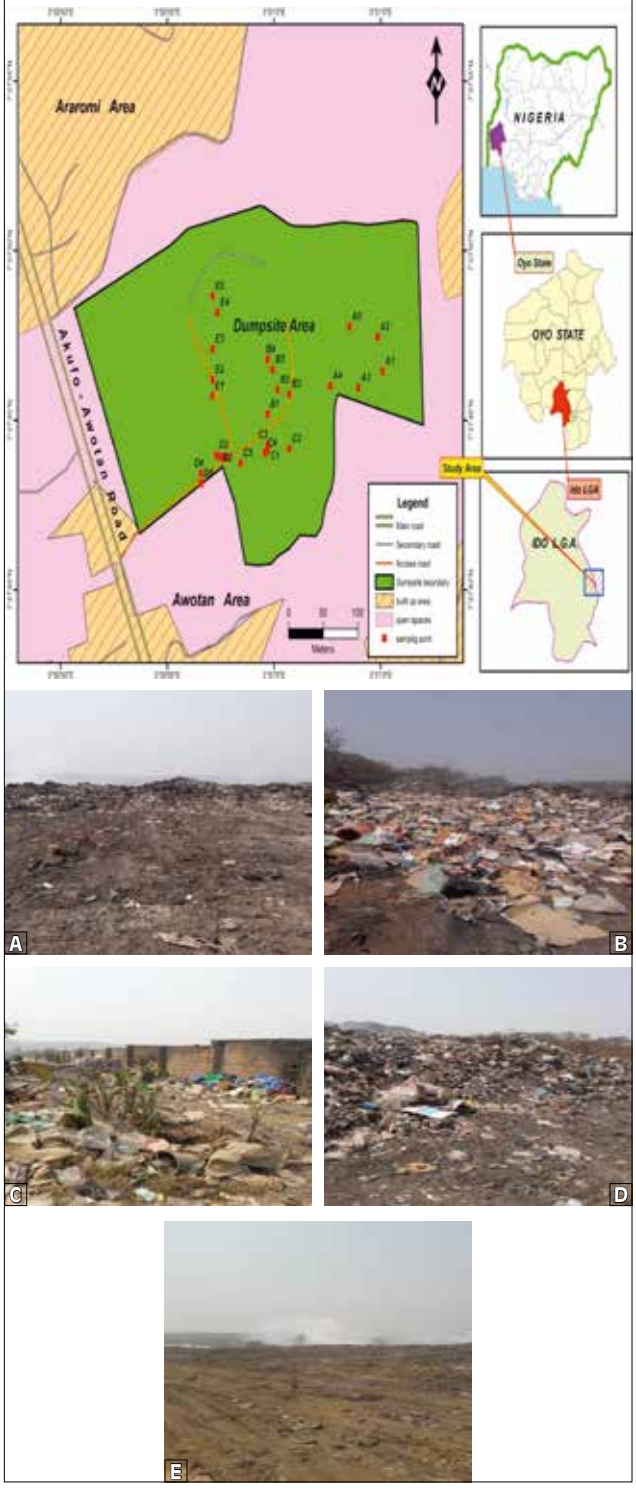
Laboratory Analysis

Physico-chemical parameters of soil

The pH of soil was measured in the supernant suspension of 1:2.5 soils: liquid mixture with the aid of a hand held pH meter [12] while soil electrical conductivity and in-situ temperature measurements were done according to established methods [13]. The height of the area above the sea level was measured using a GPS Garmin etrex 30 model. Moisture content was measured before air drying the soil samples and estimated in percentage. Ten grams of soil sample was transferred into a previously weighed tin plate, followed by the weighing of both the tin and

Fig. 1. Map of the study Area.

A. Repository of sorted and burnt materials
B. Repository for newly collected waste
C. Sorting areas for storing recyclable materials
D. Repository for long-term deposited waste materials
E. Newly opened area for dumping activities



Tab. I. Physicochemical parameters of soil samples from Awotan dumpsite.

Area/parameter	pH	EC (µS/cm)	Temp.(°C)	MC (%)	Height above sea level (m)
A	8.0 ± 1.8	724.2 ± 226.4	29.9 ± 1.3	24.6±4.5	232.8 ± 3.2
B	8.2 ± 0.3	680.4 ± 208.9	31.5 ± 1.0	18.3±2.8	240.2 ± 4.2
C	8.2 ± 0.2	600.7 ± 245.2	33.0 ± 1.1	15.6±2.1	238.2 ± 1.9
D	8.0 ± 0.2	396.9 ± 125.5	35.6 ± 1.7	13.3±1.8	231.8 ± 3.0
E	8.1 ± 0.1	327.1 ± 87.2	33.0 ± 1.1	14.0±1.0	236.8 ± 4.9
f- value	0.93	4.31	13.79	14.12	5.00
P-value	0.47	0.01	0.00	0.00	0.01
Overall mean	8.1 ± 0.2	545.9 ± 235.3	32.6 ± 2.2	17.2±4.8	236 ± 4.6

Keys: A-E are the subsampling areas; EC: Soil Electrical conductivity; Temp: Soil temperature; MC: Soil moisture content

soil sample (A gram). It was then dried in an oven until constant weight was obtained. This was then cooled in a desiccator and weighed (B gram) again [12]. The percentage soil moisture content was calculated using the formula,

$$\text{Moisturecontent \%} = \frac{A - B}{B - \text{tin}} \times 100$$

Total heavy metal contents were determined by acid digestion using aqua regia solution (Nitric acid and Hydrochloric acid in ratio 3:1). Three millilitres of distilled water was added to one gram of finely ground soil sample in a 250 mL conical flask. Freshly prepared aqua regia made from 15mL of HNO_3, 3 mL of HCl and 1mL of perchloric acid was added to the sample and the conical flask was then covered with a watch glass. The mixture was heated on a hot plate in a fume cupboard until brown fumes disappeared. The mixture was then removed from the hot plate, allowed to cool and was filtered through a Whatman No. 42 filter paper into a 50 mL standard volumetric flask. The volume was made up to mark by adding de-ionized water to get a meniscus. The concentrations of Pb, Cd, Cr, and Cu were determined on the Atomic Absorption Spectrophotometer (AAS) (Bulk Scientific Model GVP 210).
Dilution Factor (DF):

$$DF = \frac{C + B}{B}$$

C = Aliquot , B = Diluent
The measured concentrations of the metal were multiplied with the dilution factor to get the actual metal concentration.

Helminth analysis

The soil samples were checked for helminth using the Zinc Sulphate centrifugal floatation method. Five grams (5g) of the soil sample was measured and mixed thoroughly with distilled water. The suspension was filtered to remove coarse particles while the filtrate was centrifuged at 1500 rpm for 5 minutes and the supernant was decanted. Finally the sediment was mixed with 15mL saturated Zinc Sulphate solution in a centrifuge tube filled to the brim and allowed to stand for a few minutes with cover slip superimposed on the tube. The sample was centrifuged at 2500 rpm for 5mins then the cover slip was lifted onto a microscope glass slide and examined for the presence of parasite eggs and larvae under X 40 objective lens [14].

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. Quantitative data were summarized using mean, median and standard deviation. Inferential statistics were performed using ANOVA and chi-square at $P < 0.05$.

Results

Physicochemical properties of soils from Awotan dumpsite

Table I shows the physicochemical properties of soils from Awotan dumpsite. The values of pH of the areas were A (8.00 ± 1.80), B (8.20 ± 0.30), C (8.20 ± 0.20), D (8.01 ± 0.16) and E (8.10 ± 0.10). The soils' electrical conductivity (µS/cm) and temperature (°C) ranged from 327.14 ± 87.23 to 724.23 ± 226.37and 29.88 ± 1.34 to 35.56 ± 1.69 respectively. The soil moisture content (%) for the areas were A 24.56 ± 4.53, B 18.34 ± 2.76, C 15.62 ± 2.07, D 13.34 ± 1.81, E 14.02 ± 1.04 while the heights above sea level(m) were A (232.80 ± 3.19), B (240.20 ± 4.21), C (238.20 ±1.92), D (231.80 ± 2.95) and E (236.80 ± 4.87)

Prevalence of helminths in soils in Awotan dumpsite

The distribution and prevalence of helminths in soil samples from Awotan dumpsite is shown in Table II. The total number of soil samples examined within the dumpsite was 125 (100%), out of which 13 (10.4%) were positive for soil helminths. The prevalence of helminths in Areas A, B, C, D, E were 6 (24.0%), 0 (0%), 2 (8.0%), 3 (12.0%), 2 (8.0%), 3 (12.0%) respectively. The prevalence of *Ascaris lumbricoides* (Fig. 2) in areas A, D and E were 6 (24.0%), 3 (12.0%) and 2 (8.0%) respectively but was below detection limits in areas B and C while *Strongyloides stercoralis* (Fig. 3) was only found in area C 2(8.0%).

Tab. II. Distribution and prevalence of helminths in soil samples within Awotan dumpsite.

Area	Number examined	Helminth type (%)		Number infected (%)	Number uninfected (%)
		Ascaris lumbricoides	Strongyloides stercoralis		
A	25	6 (24.0)	0 (0.0)	6 (24.0)	19 (76.0)
B	25	0 (0.0)	0 (0.0)	0 (0)	25 (100)
C	25	0 (0.0)	2 (8.0)	2 (8.0)	23 (92.0)
D	25	3 (12.0)	0 (0.0)	3 (12.0)	22 (88.0)
E	25	2 (8.0)	0 (0.0)	2 (8.0)	23 (92.0)
Total	125	11 (8.8)	2 (1.6)	13 (10.4)	112 (89.6)

$\chi^2_{(8)} = 20.13;\ p = .01$

Heavy metal content of soils within Awotan dumpsite

The concentration of heavy metals in soils within Awotan dumpsite is shown in Table III. Concentrations of Cu, Cr, Cd and Pb respectively were A (342.2 ± 177.55, 49.69 ± 7.80, 14.69 ± 11.77, 421.38 ± 182.41), B (322.99 ± 90.51, 42.26 ± 21.69, 14.37 ± 18.26, 663.49 ± 375.64), C (18 441 ± 130.81,57.34 ±16.72,3.49 ± 3.48,1779.39 ± 3557 .45), D (459.09 ± 401.31, 55.63 ± 19.68, 9.36 ± 9.02, 488.04 ± 332.9) and E (271.34 ± 20.24, 39.12 ± 19.19, 6.38 ± 5.3, 796.17 ± 115.88).

Correlation matrix of soil parameters

Table IV shows the correlation matrix of soil parameters at the dumpsite. A significant ($p < 0.05$) negative correlation was observed between temperature and electrical conductivity ($r = -0.4$), height and copper ($r = -0.5$) and temperature and moisture content ($r = -0.7$), ($p = 0.01$) while a significant positive correlation was observed between cadmium and copper ($r = 0.4$)

Fig. 2. Egg of *Ascaris lumbricoides.*

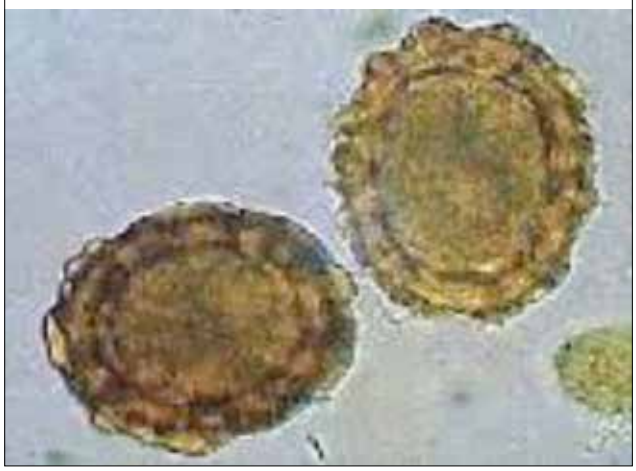


Fig. 3. Larva of *Strongyloides stercoralis.*

Discussion

The pH of the soil determines the viability of parasites in the soil because acidic pH inhibits the normal development of parasite infective stages while an alkaline pH level support the development of infective stages of parasites [15]. The range of pH from 8.0 to 8.2 observed showed that the study site was moderately alkaline. This finding is consistent with the study by Thomas et al. [16], where the mean dumpsite soil pH value was 8.0 but not in accordance with findings of Badmus et al. [17], who recorded acidic pH within the range of 5.45 – 6.45 at the Aba-Eku dumpsite area. The high average soil temperature (32.6°C) recorded in this study might be due to low

Tab. III. Variations of heavy metal concentrations within Awotan dumpsite.

Heavy metals (mg/g)	Sampling areas (mean± SD)							Overall mean	USEPA permissible limits
	A	B	C	D	E	F	P		
Cu	342.2 ± 177.6	323.0 ± 90.5	18441± 130.8	459.09 ± 401.3	271.3 ± 20.2	0.98	0.44	316.01 ± 227.11	4.3
Cr	49.69 ± 7.80	42.26 ± 21.69	57.34 ± 16.72	55.63 ± 19.68	39.12 ± 19.19	1.02	0.42	48.81 ± 17.74	0.05
Cd	14.69 ± 11.77	14.37 ± 18.26	3.49 ± 3.48	9.36 ± 9.02	6.38 ± 5.31	1.01	0.42	9.65 ± 10.91	.07
Pb	421.38 ± 182.41	663.49 ± 375.64	1779.39 ± 3557.45	488.04 ± 332.90	196.17 ± 1115.88	0.74	0.57	709.69 ± 1574.91	0.4

Tab. IV. Correlation matrix of soil parameters.

	Cu	Cr	Cd	Pb	pH	EC	Temperature	Heights	MC
Cu	1								
Cr	-.252	1							
Cd	.428*	.108	1						
Pb	-.153	.482*	-.037	1					
pH	-.315	.175	.239	-.064	1				
EC	.024	-.122	.081	.050	.137	1			
Temperature	.234	.010	-.181	-.124	.079	-.407*	1		
Heights	-.459*	.209	-.119	.189	.379	-.179	-.093	1	
MC	.175	-.140	.262	-.127	-.070	.708**	-.689**	-.207	1

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

moisture content ($r = -0.69$, $p < 0.05$) and the season of the year during which the study was carried out.

The mean electrical conductivity (EC) of the dumpsite soil (545.9µS/cm) was low when compared with the findings of Badmus et al. [17] who recorded 6280µS/cm from a study at Aba-Eku landfill Ibadan. The season of the year and moisture content of the soil determines the EC of the soil because salt moves in water therefore the presence of these ions in a moisture filled soil will increase the EC [17]. This can be supported by the result of the present study that showed a positive association ($r = 0.71$, $p < 0.05$) between EC and moisture content of dumpsite soil. The low level of soil moisture content (17.2%) observed may also be attributed to season of the year and high overall mean height (235.9 m) of the dumpsite above sea level.

The low overall prevalence of soil helminths (10.4%) recorded at the study area can be adduced to high temperature (32.6°C) and low moisture content (17.2%) as lower moisture content and higher temperature lead to faster inactivation process of soil helminth eggs [18-21]. High overall heavy metal content of the dumpsite soil might also contribute to the overall low prevalence of helminth parasite, although the association between soil heavy metal concentration and helminth parasite intensity was not investigated in the study.

The higher prevalence of *Ascaris lumbricoides* (8.8%) than *Strongyloides stercoralis* (1.6%) observed in this study can be corroborated by the findings of [22] where the percentage occurrence of *Ascaris lumbricoides* was (8.0%) and *Strongyloides stercoralis* was (5.7%). This might be due to the fact that eggs of *Ascaris lumbricoides* can withstand extreme environmental conditions because of the presence of the ascaroside layer [22].

Heavy metals are metallic elements that have their specific gravity above 5g/cm^3 [23] and are available in both natural and polluted environments. Heavy metals deposited do not disintegrate over a long time, thereby leading to health problems such as vomiting, abnormalities, nausea and gastrointestinal disorder. Some are known to be carcinogenic, mutagenic while others are known to cause brain disorders.

The findings of this study showed that the heavy metal content of the dumpsite followed the trend Pb > Cu > Cr > Cd which agrees with the work of Thomas et al. [16] who discovered the level of Pb (580.6 mg/kg) to be highest, followed by Cu (471 mg/kg), Cr (136 mg/kg) and Cd (16.8 mg/kg) from selected dumpsite soils in Ibadan metropolis.

The overall higher concentrations of Pb, Cu, Cr and Cd above USEPA limits might be due to the deposition of different forms of wastes like tyres, lubricant oil, old paint, pesticides, cans, tin, aluminum, plastics, glass bottles, old batteries, automobile parts, electronic waste and scraps of metals at the dumpsite over time.

Conclusions and recommendation

This study was carried out to investigate the helminths and heavy metals in soils from Awotan dumpsite, Ibadan. The overall prevalence of soil helminths was found to be low at the dumpsite. However, the heavy metal contents were dangerously higher than USEPA permissible limits, a possible source of underground water pollution which can cause harmful health effect to the population exposed to the environment.

Phytoextracting plants can be used to take up the heavy metals in soil. This will reduce the risk of the metals leaching into underground water. Scavengers and dumpsite workers should be encouraged to use personal protective gears at the site to reduce the risk of helminth infestation and exposure to heavy metals. Citing of residential buildings and social amenities close to dumpsites should be discouraged as dumpsites often have a high elevation due to accumulated refuse and this can support the movement of pollutants to residential areas with short and long term health effects.

Acknowledgements

The authors have no conflicts of interest.

Authors' contributions

OM designed and supervised the study. AA carried out field study. OM and AA analysed and interpreted data. Both authors read and approved the final manuscript.

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