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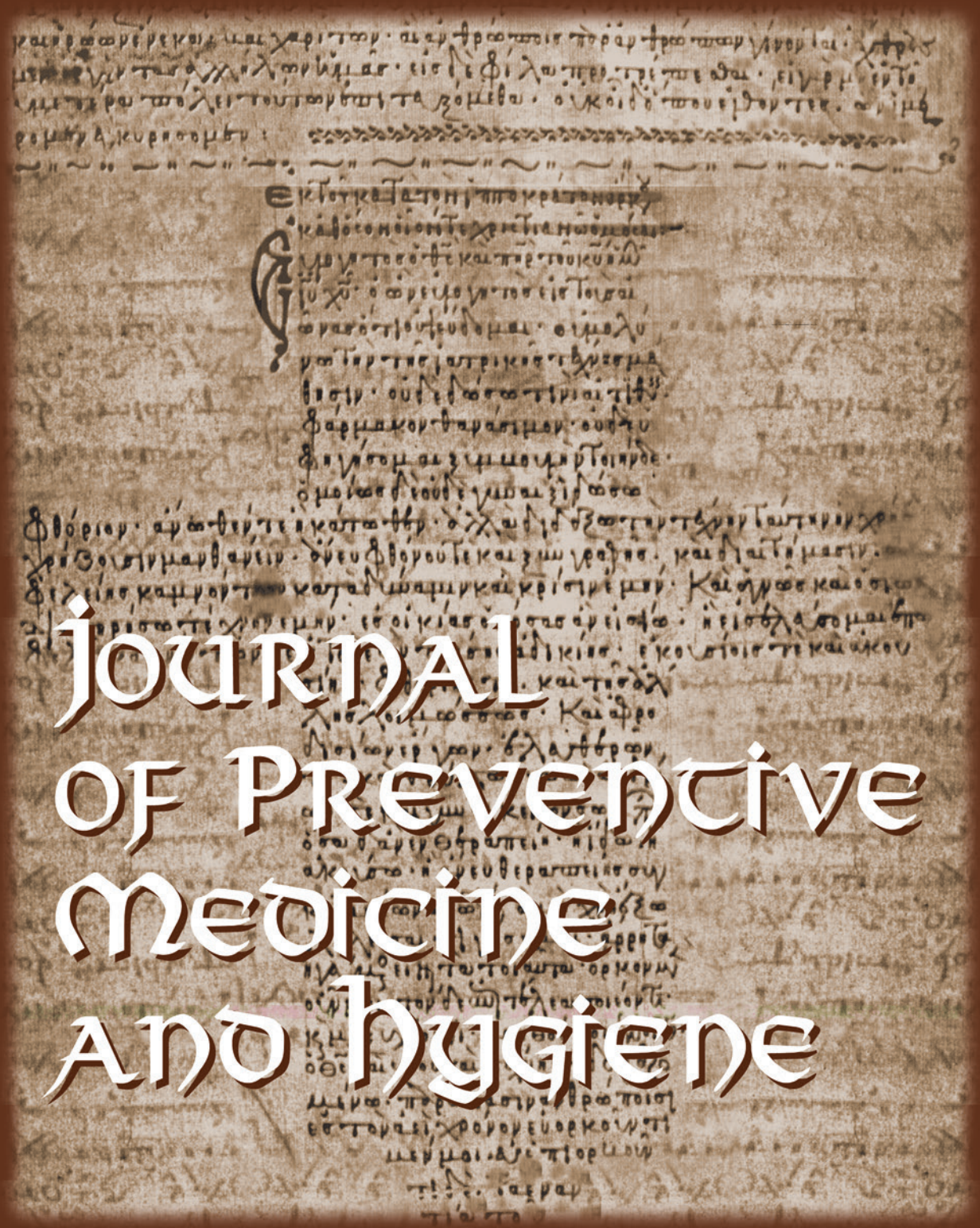

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Letter to the editor

Political and medical role in the last Ebola outbreak

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Dear Editor,
M.D. Drasher and G.W. Schlough in 2016 [1], presented an interesting work about the Ebola outbreak that occurred in 2014 and which represented an important challenge for public health. Previous Ebola outbreaks occurred in remote regions and were generally contained in a single country. This outbreak, in contrast, was regional in scale, spreading into densely populated urban areas and across multiple international borders [2].The countries mostly involved by the epidemics were Sierra Leone, Guinea, and Liberia, which registered almost 28,000 cases [3]; of those, 7,905 died. The fatality rate of Ebola can be as high as 90 percent when access to effective supportive therapies – replacement of electrolytes and fluids, blood transfusions, and other forms of intensive palliative care – is limited. This outbreak showed that there is a too little expertness for managing Ebola cases, because of the deficit of enough hospital beds, supplies, and well trained personnel to provide intensive care for the increasing number of seriously ill patients [2].
As Walker et al. affirmed [4], the failure of early control in the Ebola outbreak was multifactorial, and the policy and the behavioural factors played a key role. Outbreak response strategies must be guided by the epidemiology and route of transmission and the doctors on the front line should adopt a unequivocal strategy to screen patients and to identify suspected cases, because the typical presentation is not universal. Moreover, healthcare workers are extremely vulnerable and their welfare should be prioritised. Simple supportive management strategies, such as rehydration and antibiotic therapy, are likely to reduce mortality from Ebola and should be a priority in patient management.
In a so big outbreak, healthcare workers resulted essential and very vulnerable. They were indispensable to isolate and treat suspected and confirmed cases, to detect and report early cases and for continuous surveillance. Because of the healthcare worker safety was not considered a paramount, there was a high incidence of infection among the healthcare worker, with the following spread of Ebola in healthcare settings, increasing stigma associated with healthcare workers and healthcare facilities, and the increasing fear among the staff [4]. Healthcare worker infections and deaths could be easily prevented through an adequate knowledge and training of the PPE (personal protective equipment) that represent useful preventive measures also for the Lassa Fever, which is endemic in Sierra Leone.
So, it is quite wrong to identify a single factor as responsible for the epidemic and the initial delay was not a failure of surveillance because suspected cases in Guinea were soon reported after the first Ebola death. Ebolavirus was identified as the cause of the epidemic in March 2014 and subsequently this was widely publicised by Médecins Sans Frontières among others [4].
The Ebola outbreak showed the absolute need for policymakers and experts in public health to stenghten all the highlighted weaknesses in the whole management of the phenomenon, including bilateral agreements for providing health care, the adequacy of the health personnel, infrastructural, financial, and institutional barriers for an efficient public health system; and a better management of the needs of the populations living in the most affected countries [2].
Dradher and Schlough affirmed that the effects of Ebola on social, biological, and economic livelihood necessitates the provision of comprehensive care with universal coverage in West Africa and that a restructuring of international aid limitations must occur for an increase in comprehensive approaches to care for survivors [1]. As public health doctor we hope that the local and the international policy could act in order to improve the strategies of surveillance and control of this kind of infection to avoid another so big epidemics.

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Authors' contributions

GT conceptualized the letter, NN provided support and suggestions.

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LETTER TO THE EDITOR

New approach for promoting HPV vaccination in college men based on multi-theory model (MTM) of health behavior change

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Dear Editor,

Human papilloma virus (HPV) is among the most common infections in college-aged men [1]. In men, it causes genital warts and several cancers that include penile, anal, and oropharyngeal carcinomas [2]. To prevent genital warts and HPV-related cancers, HPV vaccination has been recommended by the Advisory Committee on Immunization Practices for young adult males since 2011 [3]. College students are a salient target population for HPV vaccination surveillance and promotional activities because they are at an increased risk for HPV infection and also represent a priority group for HPV catch-up vaccinations [4]. Furthermore, about 48% young adults ages 18 to 24 have been enrolled in or have completed college between 2013 and 2015 [5]. However the rates of HPV vaccination in college males has been quite low and was recorded as 42.9% in 2013 with the odds of HPV vaccination being 2.97 (95% CI 2.48-3.55) [4]. While the rates for HPV vaccination in the ages 18-21 years were 49.5% the rates in ages 22 -26 years were only 28.6% [4]. These rates were far lower than in women. Hence, there is a need to design and evaluate HPV vaccine promotion interventions for college men.

Very few interventional studies have been undertaken to assess the efficacy of educational interventions to promote HPV vaccination in college men. One such study was undertaken by the researcher in 2013 that utilized health belief model (HBM) [6, 7]. Using a randomized controlled trial, HBM-based intervention was compared with a traditional knowledge-based intervention in college-aged men ages 18-25 years. Repeated measures ANOVA demonstrated significant positive changes in the HBM-based group for knowledge and HBM constructs. Pretest/posttest regression analysis found that self-efficacy for taking the vaccine ($p < 0.001$), perceived barriers ($p = 0.007$), and perceived severity ($p = 0.004$) were significant positive predictors of vaccine acceptability. The model had an adjusted R^2 of 0.351. However, the actual rates of HPV vaccination were not measured in this study. Also this study did not apply this approach for effectiveness testing.

HBM is an old theory and a newer theory has been proposed that is the multi-theory model (MTM) of health behavior change that is parsimonious, utilizes proven constructs from multiple theories, is culturally robust and can be used to design interventions at individual, group and community levels [8, 9]. This theory breaks the behavior change into initiation and sustenance. The theory proposes that participatory dialogue in which advantages outweigh disadvantages, behavioral confidence and changes in physical environment are crucial for health behavior change. For sustenance of behavior change the constructs of practice for change, emotional transformation and changes in social environment are important. The theory is new and in its initial applications to physical activity behavior in adults [10], portion size behavior in adults [11], and sleep behavior [12] has shown very good predictability. Hence, we propose that this theory be utilized for designing and evaluating the efficacy and effectiveness of brief MTM-based interventions to promote HPV vaccination in college men. The studies can utilize randomized controlled trials (RCTs) in which the independent variables will be the experimental MTM-based interventions which can be compared against knowledge-based interventions. The dependent variables can be the constructs of MTM, intent for vaccinations and actual vaccinations received. An instrument for quantitative study is attached in the Appendix 1.

The experimental MTM-based intervention can consist of two-sessions of 60 minutes each. The first session can entail administration of baseline instrument and activities to influence initiation of behavior of acquiring HPV vaccination. These can include a large group participatory dialogue on advantages and disadvantages of HPV vaccination in which the advantages would outweigh disadvantages. In order to build behavioral confidence, the steps for getting HPV vaccination and overcoming barriers in acquiring this vaccination can be underscored through a lecture and role play. In order to influence physical environment, the sites for getting this vaccination and support in getting HPV vaccination can be underscored through a lecture, group discussion, and provision of incentives. The second session after one week of the first session can aim at influencing sustenance of behavior of completing three doses of HPV vaccination. In order to influence the construct of practice for change the participants can be provided a check sheet and explained how to maintain it. Ways to overcome barriers in completing the schedule and adjusting one's routine can

also be discussed during the session. In order to influence the construct of emotional transformation ways to direct one's emotions/feelings to the goal of getting all three doses of the HPV vaccination, ways for self-motivation and ways for overcoming self-doubt can be discussed. Finally, in order to influence changes in social environment, ways to enlist social support from friends, family and researcher can be discussed. Data can be collected post intervention for intent of getting vaccinated and after six months for actual vaccination rates.
The control knowledge-based intervention can also consist of two equivalent sessions of 60 minutes each. The first session can entail administration of baseline instrument and lectures on overview of sexually transmitted diseases, signs and symptoms of sexually transmitted diseases, HPV, consequences of HPV, significance of HPV, transmission of HPV, and prevention of HPV. The second session after one week of the first session can include lectures on history of vaccines, common vaccines, and HPV vaccine and will collect data through the 41-item instrument for the second time (at the conclusion of the intervention). We implore practitioners and researchers in HPV prevention to design and evaluate such efficacy and effectiveness trials

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Authors' contributions

MS conceptualized the letter and developed the instrument. VKN provided suggestions and is the corresponding author.

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Appendix 1

Measuring Change in HPV Vaccination Behavior Code # _____

Directions: This survey is voluntary, which means you may choose not to complete it or not to answer individual questions. There is no direct benefit of this survey to you; however your responses will help in developing effective human papillomavirus (HPV) vaccination programs. All data from this survey will be confidential. Please put an X mark by the response or fill the response that correctly describes your position. Thank you for your help!

- Are you a male? [Screening question]
  - ☐ Yes
  - ☐ No

If respondent selects no, she will not be enrolled.

- Are you between the age of 18 and 26 years? [Screening question]
  - ☐ Yes
  - ☐ No

If respondent selects no, he will not be enrolled.

- Do you suffer from any medical condition that prevents you from getting vaccinations? [Screening question]
  - ☐ Yes
  - ☐ No

If respondent selects yes, he will not be enrolled.

- How many doses (shots) of the human papillomavirus (HPV) vaccine have you received? [Screening question]
  - ☐ 0
  - ☐ 1
  - ☐ 2
  - ☐ 3

If respondent selects, "1, 2, or 3", they will receive the following message, "You have initiated taking HPV vaccine; therefore, you do not qualify for this study. Thank you for your time!"

1. How old are you today? _____ years

2. What is your race/ethnicity?
   - ☐ White or Caucasian
   - ☐ Black or African American
   - ☐ Asian or Pacific Islander
   - ☐ American Indian, Alaskan Native, or Native Hawaiian
   - ☐ Biracial or Multiracial
   - ☐ Hispanic or Latino
   - ☐ Other _____

3. What is your current overall GPA?
   - ☐ Less than 1.99
   - ☐ 2.00 – 2.49
   - ☐ 2.50 – 2.99
   - ☐ 3.00 – 3.49
   - ☐ 3.50 – 4.00

4. What is your year in school?
   - ☐ 1st year undergraduate
   - ☐ 2nd year undergraduate
   - ☐ 3rd year undergraduate
   - ☐ 4th year undergraduate
   - ☐ 5th year or more undergraduate
   - ☐ Graduate or professional
   - ☐ Not seeking a degree
   - ☐ Other

5. Where do you live?
   - ☐ On campus
   - ☐ Off-campus

6. Do you work?
   - ☐ No
   - ☐ Yes, _____ hours/week (put a single number not a range)

7. What is your primary source of health insurance?
   - ☐ My college/university sponsored plan
   - ☐ My parents' plan
   - ☐ Another plan
   - ☐ I don't have health insurance

8. Prior to being asked to participate in this study, had you ever heard of human papillomavirus (HPV)?
   - ☐ Yes
   - ☐ No

9. Prior to being asked to participate in this study, had you ever heard of the vaccine for human papillomavirus (HPV)?
   - ☐ Yes
   - ☐ No

10. What is your relationship status?
    - ☐ Not in a relationship
    - ☐ In a relationship but not living together
    - ☐ In a relationship and living together

11. What is your marital status?
    - ☐ Single, never married
    - ☐ Married
    - ☐ Separated
    - ☐ Divorced
    - ☐ Widower

Please rate the following items by placing a [X]

	Never	Hardly ever	Sometimes	Almost always	Always
Participatory dialogue: Advantages					
If you get the HPV vaccination you will…					
12. … be protected against genital warts.	☐	☐	☐	☐	☐
13. … be protected against cervical cancer (females) or anal cancer (males).	☐	☐	☐	☐	☐
14. …improve your sexual health.	☐	☐	☐	☐	☐
15. …feel better about yourself.	☐	☐	☐	☐	☐
16. …feel less stress.	☐	☐	☐	☐	☐

	Never	Hardly ever	Sometimes	Almost always	Always

Participatory Dialogue: Disadvantages

If you get HPV vaccination you will…					
17. … have side effects.	☐	☐	☐	☐	☐
18. … not have enough money to pay for it. (cost is around $150 for each dose)	☐	☐	☐	☐	☐
19. … be inconvenienced.	☐	☐	☐	☐	☐

	Never	Hardly ever	Sometimes	Almost always	Always

Participatory Dialogue: Disadvantages

If you get HPV vaccination you will…					
20. … suffer from adverse effects.	☐	☐	☐	☐	☐
21. … have a false sense of protection against all sexually transmitted diseases (STDs).	☐	☐	☐	☐	☐

	Not at all sure	Slightly sure	Moderately sure	Very sure	Completely sure

Behavioral confidence

How sure are you that you will get the first dose of the HPV vaccine …					
22. … in the near future?	☐	☐	☐	☐	☐
23. … in the near future without insurance (cost is around $150 for each dose)?	☐	☐	☐	☐	☐
24. … in the near future with your present schedule?	☐	☐	☐	☐	☐
25. … in the near future even if it is offered at a large distance from your home?	☐	☐	☐	☐	☐
26. … in the near future even if it causes side effects?	☐	☐	☐	☐	☐

	Not at all sure	Slightly sure	Moderately sure	Very sure	Completely sure

Changes in physical environment

How sure are you that you will…

27. … be able to arrange payment for the first dose of the HPV vaccination?	☐	☐	☐	☐	☐
28. … be able to arrange a place to get the first dose of the HPV vaccination?	☐	☐	☐	☐	☐
29. … be able to arrange transportation to get the first dose of the HPV vaccination?	☐	☐	☐	☐	☐

	Not at all sure	Slightly sure	Moderately sure	Very sure	Completely sure

Emotional transformation

How sure are you that you can…

30. … direct your emotions/feelings to the goal of getting all three doses of the HPV vaccination?	☐	☐	☐	☐	☐
31. … motivate yourself to get all three doses of HPV vaccination?	☐	☐	☐	☐	☐
32. … overcome self-doubt in accomplishing the goal of getting all three doses of HPV vaccination?	☐	☐	☐	☐	☐

	Not at all sure	Slightly sure	Moderately sure	Very sure	Completely sure

Practice for change

How sure are you that you can…

33. … keep a record to monitor getting three doses of the HPV vaccination?	☐	☐	☐	☐	☐
34. … get three doses of HPV vaccination even if you encounter barriers?	☐	☐	☐	☐	☐

	Not at all sure	Slightly sure	Moderately sure	Very sure	Completely sure

35. … change your schedule to get three doses of HPV vaccination if you face difficulties?	☐	☐	☐	☐	☐

	Not at all sure	Slightly sure	Moderately sure	Very sure	Completely sure

Changes in social environment

How sure are you that you can get the help of a…

36. … family member to support you with getting three doses of the HPV vaccination in the next 12 months?	☐	☐	☐	☐	☐
37. …friend to support you with getting three doses of HPV vaccination in the next 12 months?	☐	☐	☐	☐	☐
38. … health professional to support you with getting three doses of HPV vaccination in the next 12 months?	☐	☐	☐	☐	☐

	Not at all likely	Somewhat likeky	Moderately likely	Very likely	Completely likely

Behavior change: Initiation

How likely is it that you will…

39. … get the first dose of the HPV vaccination in the next month.	☐	☐	☐	☐	☐

	Somewhat all likely	Moderately likeky	Very likely	Completely likely	likely

Behavior change: Sustenance

How likely is it that you will…

40. … complete all three doses of HPV vaccination within the next 12 months.	☐	☐	☐	☐	☐

Thank you for your time!

SCORING

Construct of advantages: Scale: Never (0), Hardly ever (1), Sometimes (2), Almost always (3), Always (4). Summative score of Items 13-17. Possible range: 0- 20. High score associated with likelihood of initiation of behavior change.

Construct of disadvantages: Scale: Never (0), Hardly ever (1), Sometimes (2), Almost always (3), Always (4). Summative score of Items 18-22. Possible range: 0- 20. Low score associated with likelihood of initiation of behavior change.

Subtract disadvantages score from advantages score to calculate **participatory dialogue** construct score. Positive score will be indicative of initiation of behavior change.

Construct of behavioral confidence: Scale: Not at all sure (0), slightly sure (1), moderately sure (2), very sure (3), completely sure (4). Summative score of Items 23-27. Possible range 0-20. High score associated with likelihood of initiation of behavior change.

Construct of changes in physical environment: Scale: Not at all sure (0), slightly sure (1), moderately sure (2), very sure (3), completely sure (4). Summative score of Items 28-30. Possible range 0-12. High score associated with likelihood of initiation of behavior change.

Construct of emotional transformation: Scale: Not at all sure (0), slightly sure (1), moderately sure (2), very sure (3), completely sure (4). Summative score of Items 31-33. Possible range 0-12. High score associated with likelihood of sustenance of behavior change.

Construct of practice for change: Scale: Not at all sure (0), slightly sure (1), moderately sure (2), very sure (3), completely sure (4). Summative score of Items 34-36. Possible range 0-12. High score associated with likelihood of sustenance of behavior change.

Construct of changes in social environment: Scale: Not at all sure (0), slightly sure (1), moderately sure (2), very sure (3), completely sure (4). Summative score of Items 37-39. Possible range 0-12. High score associated with likelihood of sustenance of behavior change.

For **modeling initiation** dependent variable can be Item 40: not at all likely (0), somewhat likely (1), moderately likely (2), very likely (3), and completely likely (4) and multiple regression can be used. For **modeling sustenance** dependent variable can be Item 41: not at all likely (0), somewhat likely (1), moderately likely (2), very likely (3), and completely likely (4) and multiple regression can be used.

Flesch Reading Ease 68
Flesch-Kincaid Grade Level 5.5

ORIGINAL ARTICLE

Epidemiological HIV infection surveillance among subjects with risk behaviours in the city of Messina (Sicily) from 1992 to 2015

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Keywords

HIV infection epidemiology • Unprotected sex • HIV testing • Risk behaviours • Prevention strategies

Summary

Introduction. *Epidemiological studies are a key element in determining the evolution and spread of HIV infection among the world population. Knowledge of the epidemiological dynamics improves strategies for prevention and monitoring.*
Methods. *We examined 2,272 subjects who voluntarily underwent HIV testing from January 1992 to December 2015. For each subject, an anonymous form was completed to obtain information on personal data, sexual habits and exposure to risk factors.*
Results. *The number of subjects undergoing the screening test has increased over the years and the average age of the tested subjects has decreased over time. The main motivation for undergoing HIV testing is unprotected sex. Although heterosexual subjects taking the test were more numerous than homosexuals in this study, an increase in the latter over time should be highlighted.*
Conclusions. *Although the number of tests performed has increased over the years, the persistence of unprotected sex shows an inadequate perception of risk. Therefore, it is necessary to implement programmes to increase the general awareness of HIV infection. It is also essential to undertake constant monitoring of behaviour, risk perception and the application of the screening test via surveillance systems in order to implement effective and efficient prevention.*

Introduction

Epidemiological HIV data are the most important element showing the evolution and diffusion of HIV infection among the world population. Moreover, epidemiological studies allow the planning of specific prevention strategies monitoring the spread of this infection [1-4]. The AIDS surveillance systems provide useful information on HIV epidemic dynamics [5-7]. For this reason, since 1988 several Italian provinces and regions (Lazio, Veneto, Friuli Venezia-Giulia, Trento, Modena, Sicilia) have established local surveillance systems for new HIV diagnoses [8, 9]. AIDS surveillance has three complementary objectives in HIV-infected individuals: (1) reducing high-risk sexual behaviours [10-13]; (2) reducing the use of injected and non-injected drugs [10, 14]; (3) improving clinical care [15]. The last objective aims to attain adherence to antiretroviral therapy, the suppression of HIV viral load and the prevention of the development of HIV drug resistance [15, 16]. At the Epidemiological Observatory, the Regional Registry (activated in Sicily in 1985) collects all cases of AIDS occurring among Sicilian residents or diagnosed in Sicily. The register is part of the National Register, which is maintained by the National Institute of Health and it collects all cases of AIDS occurring or diagnosed in all Italian regions. However, the monitoring of new cases of HIV infection has always been difficult to implement due to the significant problems related to privacy. Subsequently, following the issue of the Ministerial decree of 2008.03.31, a national surveillance system of HIV infection was established which included the notification of new cases; this system is similar to that used for cases of AIDS. For this purpose, the regional representatives of the Infectious Diseases Units have been specially appointed and a regional surveillance system has been implemented since 2010. In addition, since 2009 our laboratory has adhered to the system of regional surveillance, although it was already in place in the province of Messina from 1984. This system offers the screening test to all people who voluntarily request it after engaging in risk behaviours. Therefore, our study is a surveillance survey of the number of subjects who underwent a voluntary HIV test in the city and province of Messina from January 1992 to December 2015, monitoring in the territory risk behaviour, risk perception, HIV infection and the application of the screening test via surveillance systems in order to implement effective and efficient prevention.

Methods

Overall, 2,272 people, 1,554 males and 718 females, voluntarily underwent HIV testing from January 1992 to

December 2015 at the HIV laboratory of the Policlinic G. Martino in Messina, which is one of the reference centres for monitoring HIV infection in Sicily. Messina lies on the north-eastern tip of Sicily and it has around 240,000 inhabitants. Before blood sample collection, the volunteers underwent counselling to ascertain the need for the test considering the risk factor and if the time between exposure and the test was sufficient to detect the presence of antibodies. During counselling, we collected information about gender, age, place of birth, occupation and sexual habits (heterosexual, homosexual or bisexual). We also asked about the motivation for coming and the kinds of risk to which the subjects may have been exposed recently, such as blood transfusion, dental visits, accidental punctures, drugs and/or alcohol use, piercing and/or tattoos and unprotected sex.

Comparisons and correlations were determined using the standard Pearson test and linear regression. Significance was assessed at the $p < 0.05$ level. All analyses were performed using the Prism 4.0 software.

Results

Table I shows information about gender, age, sexual habits, occupation and exposure to risk factors.

The analysis of the data collected from 1992 to 2015 clearly showed a statistically significant increase ($p < 0.0001$) in the number of HIV tests performed. The number of subjects undergoing the screening test has increased from 8 to 245 persons/year (Fig. 1).

The data analysed by gender shows a predominance of male subjects who carried out the test (1,554 males vs. 718 females). As confirmed by the regression analysis, from 1992 to 2015, the mean age of the tested subjects steadily decreased from 48 to 32 ($p < 0.0001$) (Fig. 2).

Analysing the sample according to profession, most of the subjects in the study were students (38.13%), followed by employees (16.54%) and unemployed (15.52%). These categories were prevalent in all the years examined in this study. All categories were uniformly represented over the years under investigation, with the exception of the category "unemployed" and "retired" significantly more

Tab. I. Overview on subjects who voluntarily underwent HIV testing from January 1992 to December 2015.

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
N° test	8	14	37	29	10	5	25	122	100	82	53	105
Male	6	6	28	17	4	4	18	70	63	49	30	63
Female	2	8	9	12	6	1	7	52	37	33	23	42
Mean age	48	50	46	47	44	52	46	43	42	42	42	43
Occupation												
Student	3	7	15	11	7	1	11	51	51	35	25	49
Employee	0	2	7	3	1	0	4	23	17	18	5	18
Freelancer	0	1	0	4	0	0	3	2	2	3	2	6
Workman	0	0	0	4	0	1	3	7	3	5	2	5
Housewife	0	1	1	2	0	0	0	4	6	2	2	3
Physician*	0	0	1	0	0	0	0	12	5	5	5	13
Dealer	2	1	4	2	0	1	2	6	7	2	0	2
Police,army	2	0	4	0	0	0	0	2	1	3	3	2
Retired	0	0	0	0	0	2	0	1	1	1	0	0
Prostitute	0	0	0	0	0	0	1	0	0	0	0	0
Unemployed	1	1	4	1	2	0	0	14	6	7	8	6
Sexual habits												
Heterosexual	7	13	33	29	10	4	21	104	85	69	44	96
Homosexual	1	0	2	0	0	1	3	5	7	3	3	3
Bisexual	0	0	1	0	0	0	1	4	1	3	1	6
Transsexual	0	0	0	0	0	0	0	0	0	0	0	0
Unresponsive	0	1	1	0	0	0	0	9	7	7	5	0
Risk factor												
No risk reported	0	0	1	1	0	0	0	0	1	0	0	1
Unprotected sex	6	9	22	18	5	4	20	85	64	46	28	71
Prevention/control	0	1	10	6	1	1	1	16	8	8	2	13
Accidental injury	1	0	0	1	1	0	1	13	14	21	12	15
Invasive practices$	0	0	0	0	1	0	0	1	4	0	1	0
Drug addicts/ partner drug addicts	1	2	4	2	2	0	1	2	5	4	7	0
Partners of HIV+ subjects	0	1	0	1	0	0	1	4	4	2	3	5
Transfused or dialysis patients	0	0	0	0	0	0	0	0	0	0	0	0
Suspected diagnosis	0	0	0	0	0	0	0	0	0	0	0	0

* physician, paramedic, dentist
$ surgery, dental care, tattoo, piercing

Tab. I. Overview on subjects who voluntarily underwent HIV testing from January 1992 to December 2015.

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
N° test	99	90	218	143	89	83	84	193	83	147	208	245
Male	69	62	144	96	65	63	60	124	55	97	164	197
Female	30	28	74	47	24	20	24	69	28	50	44	48
Mean age	41	41	38	36	36	40	34	31	35	32	33	32
Occupation												
Student	28	25	79	63	38	20	36	96	25	60	55	73
Employee	15	20	49	24	13	18	11	23	11	21	26	47
Freelancer	3	9	22	13	10	6	8	14	8	15	23	18
Workman	5	5	6	8	8	5	6	13	3	8	18	17
Housewife	2	2	1	0	2	3	5	4	2	2	5	2
Physician*	13	8	13	7	3	10	6	9	2	8	9	17
Dealer	3	0	10	8	2	2	4	1	2	4	6	2
Police,army	5	2	0	1	0	5	1	5	1	1	4	7
Retired	1	3	7	1	2	2	1	0	4	4	5	4
Prostitute	0	0	0	0	0	0	0	0	0	0	0	0
Unemployed	23	16	30	17	10	11	4	28	24	23	56	58
Sexual habits												
Heterosexual	71	67	191	111	54	72	57	155	56	117	134	128
Homosexual	15	15	13	23	24	10	20	17	23	20	57	75
Bisexual	10	6	9	7	4	1	4	7	2	10	5	25
Transsexual	0	0	0	0	0	0	0	0	0	0	0	0
Unresponsive	3	2	5	2	7	0	3	14	2	0	12	17
Risk factor												
No risk reported	0	0	0	1	0	0	0	6	1	0	2	5
Unprotected sex	68	64	144	115	76	68	69	127	68	106	158	186
Prevention/control	7	5	58	11	5	4	6	31	4	19	25	14
Accidental injury	17	12	13	14	8	9	6	4	10	11	9	10
Invasive practices$	0	0	0	1	0	2	2	25	0	2	1	3
Drug addicts/ partner drug addicts	0	0	3	1	0	0	0	0	0	0	9	1
Partners of HIV+ subjects	7	8	0	0	0	0	0	0	0	4	0	14
Transfused or dialysis patients	0	0	0	0	0	0	0	0	0	1	0	0
Suspected diagnosis	0	0	0	0	0	0	0	0	0	3	4	11

* physician, paramedic, dentist
$ surgery, dental care, tattoo, piercing

present in recent years ($p = 0.0078$ and $p=0.0046$ respectively), in contrast to the "dealer" category, most represented in the 1990s ($p = 0.0076$).

The information collected on sexual habits showed that, on average, 76.06% of the sample comprised heterosexual subjects, 14.96% homosexual subjects and 4.71% bisexual subjects. The statistical analysis showed a significant inverse correlation ($p < 0.0001$) between the number of heterosexual and homosexual subjects; in particular, the former decreased while the latter increased over the years (Fig. 3a).

The most common reason prompting the subjects to carry out the test was unprotected sex (71.17%). In addition, 9.95% of people were persuaded to take the test for prevention and 10.03% due to contact with potentially contaminated objects. The category of drug users, on average, accounted for 4.36% of the subjects examined. The comparison between the two risk factors most implicated – unprotected sex and the exchange of syringes among drug users – showed that the former increased significantly from 1992 to 2015, while the latter declined significantly over the years (Fig. 3b).

In all, 35 of 1,554 tested males (2.25%) were HIV positive and their average age was 35 ± 11.73. Only 4 female subjects out of 718 were HIV positive (0.5 %); they were on average 45 ± 11.21 years old. As shown in Figure 4a, the number of positive HIV tests increased over the years, especially from 2013 onwards, reaching 5.7 new cases of HIV infection per 100,000 residents in 2015. Furthermore, the number of positive HIV tests increases significantly with an increase in the number of tests performed (Fig.4b). Of the subjects who were positive on the test, 48% were heterosexual, 41% were homosexual and 11% were bisexual. Analysing the reasons that led the subjects to carry out the test, it was found that 56% of them did so because of occasional unprotected sex, 19% because of sexual contact with an HIV-positive partner, 18% because of apparent symptoms related to HIV and only 7% due to being drug users (Fig. 5a-b).

Fig. 1. Number of 2 screened for HIV from 1992 to 2015.

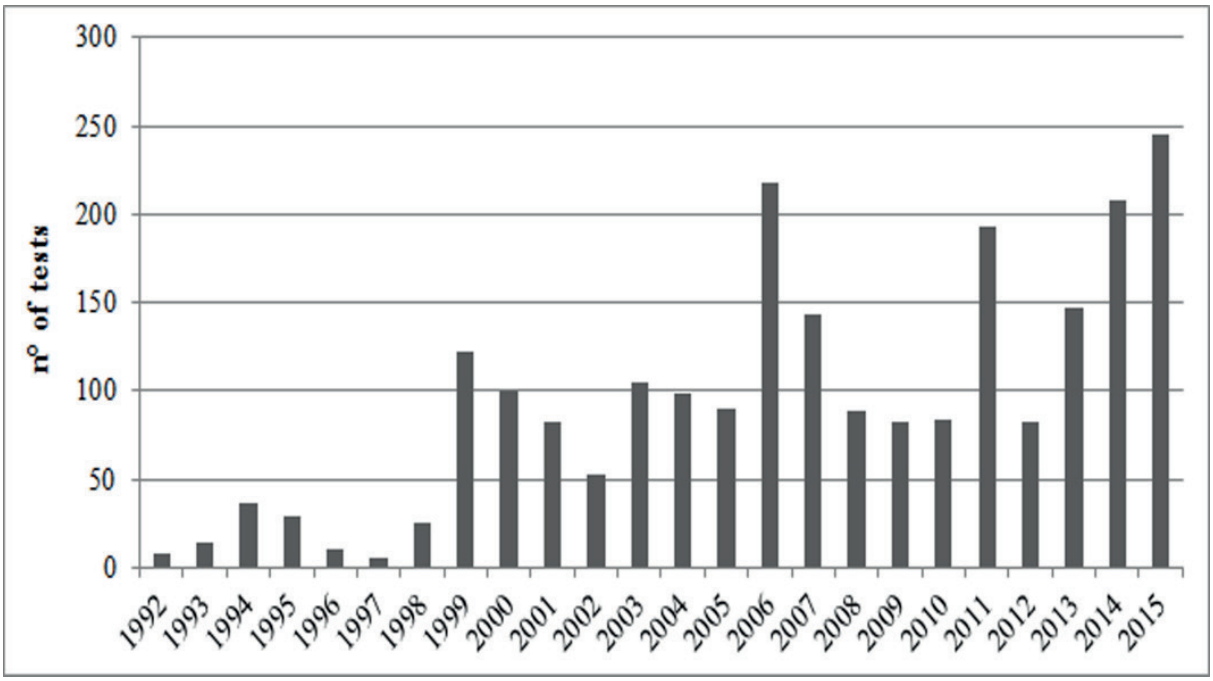
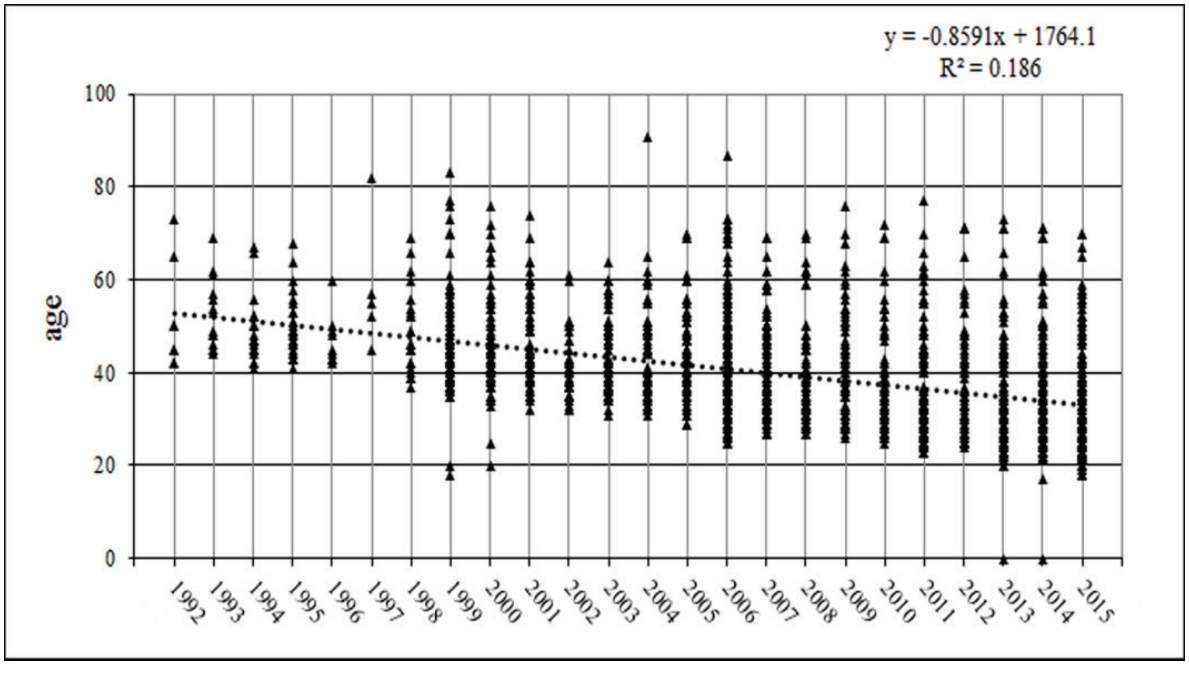


Fig. 2. Regression analysis shows the distribution of subjects undergoing screening for HIV by age in the period 1992-2015.



Discussion

Our study is an observational survey that drew on epidemiological surveillance carried out in our laboratory from 1992 to 2015 among subjects who underwent a voluntary test. The Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) has defined second generation surveillance (SGS) [17, 18] as the "regular, systematic collection, analysis and interpretation of information for use in tracking and describing changes in the HIV/AIDS epidemic over time". This kind of surveillance provides an

Fig. 3. Comparison between heterosexuals and homosexuals categories, (a) and the most involved risk factors (b).

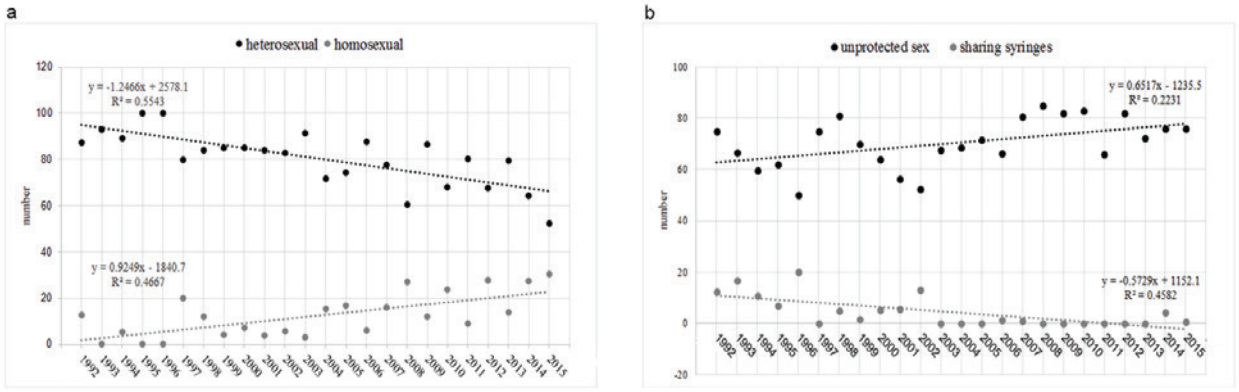


Fig. 4. The scatterplots show the results of regression analysis to assess the increase in the number of tests during the years examined (a) and the relationship with the number of HIV tests performed in the same period (b) (1992–2015).

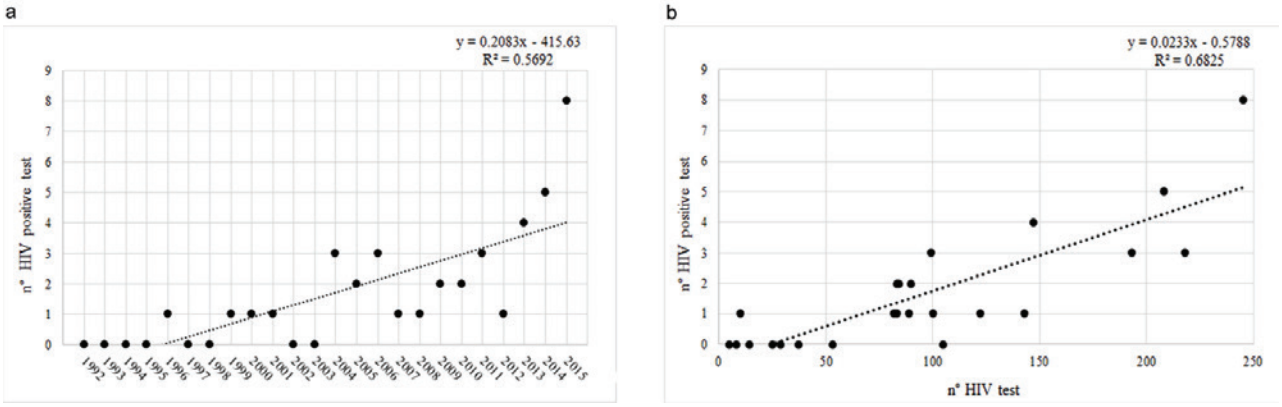
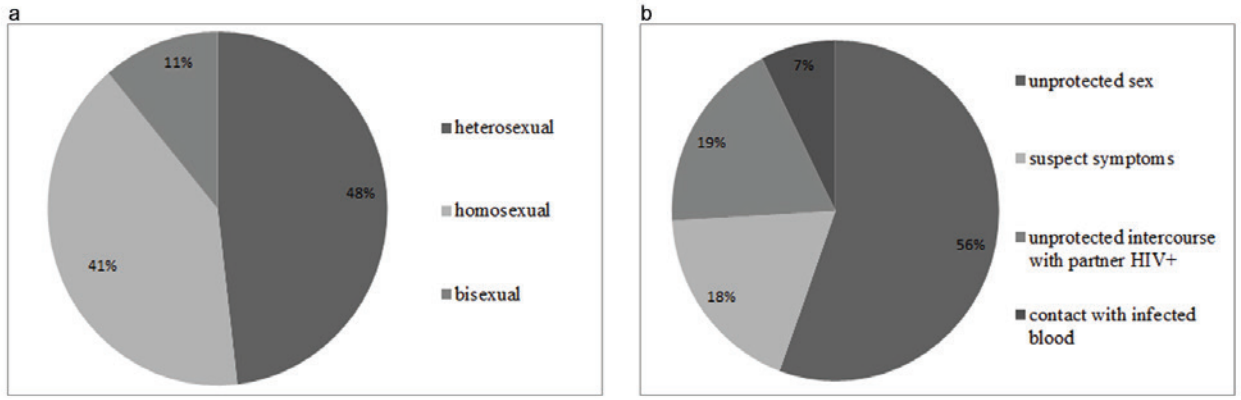


Fig. 5 Sexual habits (a) and main cause of transmission (b) of the subjects who tested HIV positive.



understanding of local epidemics, including sources of new infections over time, as well as the behavioural and biological factors driving epidemic spread. Most new transmission is still attributed to those with higher risk behaviours and their immediate sexual partners. The monitoring of risks related to the transmission of HIV provides a key source of information, not only to understand the drivers of epidemics, but also for advocacy and the planning and evaluation of preventive interventions [19, 20].

From the data described above, the greater presence of males than females could be attributed to the fact that men pay less attention to prevention than women and therefore their behaviours are less cautious. Alternatively, it could reflect a heightened perception of risk among men. The results showed that during the entire period of observation, the average age of the tested subjects consistently decreased, whereas the number of individuals who voluntarily took the test increased over time. This is an unequivocal sign that information has become more effective over the years. During the period observed, we highlight the increase in the number of those undergoing voluntary testing in the years 2006 and 2011. In these years, our laboratory, in collaboration with the Red Cross of Messina, organized prevention campaigns, with information points distributed in the main squares of the city, offering the chance to do an on-site test.

The results obtained show that from 1992 to 2015 individuals who requested testing and those who tested positive were predominantly subjects with at-risk sexual behaviours. Despite the fact that heterosexuals remain the category most represented, our results show that in recent years the number of heterosexuals voluntarily taking the test has decreased, while the number of homosexuals has increased. This can be attributed to greater awareness among the latter of the greater risk of transmission of HIV through anal intercourse [21], or perhaps finding it easier to declare their sexual orientation because the homosexuality taboo is greatly reduced in society. Some associations, particularly active in our city, help us in the field of sexual health and the prevention of HIV and sexually transmitted infections (STIs) to increase the promotion of testing in this group given the increased vulnerability of gay and bisexual men.

Only a few subjects belonged to the category of drug addicts, in contrast to the 1980s in Italy, when they were the predominant category in the spread of the virus [22, 23]. In addition, the prevalence of people who reported that they practised unprotected sex denotes a lack of knowledge of the risk, in particular evidence of inadequate risk perception, condom use and HIV testing, suggesting the need to implement programmes aimed at increasing the general awareness of HIV infection according to the guidelines issued at the European level [24].

Regarding the subjects who tested positive for HIV, our data show a lower incidence of HIV infection in women, in line with the latest data from the AIDS Operating Center (COA) of the National Institute of Health. Unlike women, only the age group of affected men is in line with the national figure, at 39 years for males and 36 for females.

The increase in the number of positive HIV tests could suggest an increase in the incidence of new diagnoses, but this would not be in line with current Italian data. According to the data provided by the COA in 2015, the incidence of new HIV infection diagnoses has decreased slightly from the previous three years, with 3,444 new HIV infection diagnoses equal to 5.7 new cases of HIV infection per 100,000 residents reported. As new diagnoses increase with the number of tests performed, we believe it plausible that the incidence of infection has not increased, but rather the increase in the number of tests has simply led to the discovery of infection that would otherwise be hidden.

Since the mid-1980s, the distribution of new HIV transmission diagnoses has changed dramatically: the proportion of IDUs declined from 76.2% to 3.2% in 2015, while the cases attributable to sexual transmission, in particular those attributable to heterosexual transmission, increased from 1.7% in 1985 to 44.9% in 2015 and cases attributable to homosexual transmission over the same period increased from 6.3% to 40.7%. Our data correlate with this trend, 48% of HIV-positive people were heterosexual and 41% were homosexual. Yet, our data show that the majority of new diagnoses of infection by HIV can be attributed to unprotected sexual intercourse at a rate of 83%, in line with COA data that attributes 85.5% of all cases to unprotected sexual intercourse [25, 26].

Conclusions

Data from the National Institute of Health in Italy (the AIDS Operating Center) show that the distribution of HIV methods of transmission has undergone remarkable changes in recent years. As the major cause of the risk of HIV exposure is represented by unprotected sex, we believe it is essential to promote knowledge about diseases and their complications. Since students are more vulnerable to unprotected sex, it is clear that there is an urgent need to introduce sex education as a proper subject in Italian schools in order to promote the practice of sexual relationships in safe and responsible manner and to enhance awareness of the risk of contracting and transmitting STIs [27].

It is essential to undertake constant monitoring of behaviour and risk perception, as well as the application of the screening test in Italy and other countries via a surveillance system and other methods in order to implement effective and efficient prevention [28, 29].

In agreement with other studies [7, 30], we affirm that the changed pattern of the HIV epidemic in Italy highlights the need for a nation-wide surveillance system for HIV infection, aimed also at the allocation of adequate economic resources and the planning of specific prevention.

Over the last decade, the proportion of late presenters or those with indications of advanced infection has increased, from 20.5% in 2006 to 74.5% in 2015. This means that these individuals are diagnosed only when their immune system is already failing. There is no cure for HIV, but early diagnosis allows access to lifesaving treatment that prolongs healthy life.

The above provides an understanding of the importance of promoting HIV testing. This is important because it allows the emergence of previously hidden non-diagnosed cases of HIV infection; later diagnosis of HIV is worse

for the immune system, while timely diagnosis helps manage the infection effectively. Providing antiretroviral therapy in the early stages of HIV infection allows people with HIV to live longer and healthier lives. It also reduces costs for the healthcare system and above all reduces the risk of transmitting HIV to others.

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Authors' contributions

IP and GV conceived, designed and coordinated the research; EA collected data; GV, MPB,AD, PSand PL contributed to the acquisition, interpretation of data,identified the endpoints analysed and prepared the figures and tables; IP, AD and GVwrote the paper.
All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Atherosclerosis is associated with a higher risk of hepatic steatosis in HIV-infected patients

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Keywords

Atherosclerosis • Hepatic steatosis • HIV • Cardiovascular diseases • Intima Media Thickness

Summary

Introduction. *Cardiovascular Diseases (CD) have emerged as a leading cause of morbidity and mortality in HIV population. Some studies have reported higher carotid Intima Media Thickness (c-IMT), a measure of subclinical atherosclerosis (AT), in this cohort of patients.*
Methods. *Here, we evaluate the role of Hepatic Steatosis (HS) as likely marker for AT in 128 HIV-infected patients without hepatitis C infection. c-IMT has been detected non-invasively by carotid ultrasonography to assess the progression of AT. HS has been evaluated using a process based on vibration-controlled transient elastography (Fibroscan) by a novel ultrasonic controlled attenuation parameter (CAP). The cut-off value for defining the presence of significant HS was CAP > 259 dBm^{-1}.*
Results. *AT has been detected in 26 patients (20.3%), whereas steatosis of grade 2 (S2) in 31 (24.2%). The variables statistically related to AT were age, obesity, diabetes, hypertension and S2. In the multivariate analysis, AT was only associated ($p < 0.001$) with age and S2. The optimal cut-off value indicated by ROC curve for predicting AT was CAP > 250 dB/m^{-1}.*
Discussion. *Our results highlight the presence of AT in HIV-infected persons and its association with fatty liver disease; therefore, HS assessment in HIV population results crucial to predict AT and CD.*

Introduction

Since the advent of highly active anti-retroviral therapy (HAART), the prognosis of HIV infection has been dramatically altered, transforming it from an inexorably fatal disease into a manageable chronic condition [1, 2].
In this context, cardiovascular diseases (CD) result an important cause of morbidity and mortality [3, 4]. Atherosclerosis (AT), a complex, active and progressive disease with inflammation involved at every stage, is closely correlated to an increased risk of heart diseases [5].
Epidemiological data show a relationship between HIV and AT, in fact in the last few years AT prevalence has increasingly been found in this cohort of patients [6].
Several factors may contribute to its development among HIV-infected individuals. First and foremost the role of inflammation has been recognized as the key pathologic process [7, 8]. Furthermore, other determinants including dyslipidemia, lipodystrophy, insulin resistance, tobacco abuse, and metabolic and mitochondrial dysfunctions may be involved in AT disease [9-13]. Previous researches have demonstrated an association between various components of HAART and the development of AT, as well [14, 15]. Finally, HIV infection itself may lead AT via monocyte or T-cell activation and likewise concomitant infections in the HIV-infected subjects could also promote this process [16, 17].

Epidemiological studies have showed strong association between AT and hepatic steatosis (HS) [18, 19]. The latter is a clinical condition associated with inflammation and hepatocyte changes [20]. The different grade of steatosis (S) is defined as S0 = 0-10%; S1 = 11-33%; S2 = 34-66% and S3 = 67-100% of hepatocytes that have a fatty accumulation [21]; it can also be a co-factor in many chronic liver diseases that can lead to fibrosis, cirrhosis and hepatocellular carcinoma, as well as the possibility of developing metabolic alterations that can lead to AT [22, 23].
Mounting evidence suggests that HS is common among HIV-infected individuals with or without HCV co-infection [24, 25], though data on factors associated with steatosis in HIV-mono-infected patients are scarce [26].
To date, liver biopsy is considered the gold standard for the assessment of HS [27]. But recently, ultrasound-based vibration-controlled transient elastography device has been developed to detect it [28] using controlled attenuation parameter (CAP). This tool is a non-invasive, quantitative, non-ionizing and inexpensive method that provides immediate results and it can be performed by an operator without specific radiological competence and individual interpretation. Furthermore, CAP is able to explore a liver volume ~ 100 times larger than liver biopsy [21].

Measurement of AT activity results fundamental for early detection of CD [29]. It can be detected non-invasively by carotid ultrasonography to assess carotid Intima Media Thickness (c-IMT) [30, 31]. IMT is a characteristic of arterial aging related to AT; the cellular and molecular alterations that underlie IMT are implicated in the development, progression or both of AT [32]. c-IMT is widely used as a validated marker for subclinical atherosclerosis in HIV-negative populations [33, 34]; moreover, its value greater than or equal to the 75th percentile for age, sex and ethnicity has been associated with an increased risk of CD [32]. For its low cost and the absence of exposure to radiation, this technology has also been used to identify the predictors of subclinical AT in HIV-infected individuals [10, 35, 36].

Few data are available on the relationship between AT and HS in HIV subjects and since fatty liver diseases are common among this population [37], determining the link with AT may be useful for predicting CD risk [38, 39].

Therefore, objective of our study is to evaluate this relationship in a cohort of HIV patients without concomitant hepatitis C virus (HCV) infection.

Methods

Patients

We performed a cross-sectional study by consecutively enrolling HIV-infected outpatients from January to June 2012. All patients approached for the study gave consent to participate.

Exclusion criteria were: age less than 18 years, HCV infection, liver cirrhosis, active psychiatric disorders, alcoholism and drug abuse.

At enrolment time the following demographic, clinical and laboratory variables were collected for each subject through a patient interview (using a predefined form) and a chart review: gender, age, ethnicity, duration of HIV infection, CD4 cell count, nadir CD4, HIV viral load, therapy, CD risk factors (current smoking habit; obesity, defined as a body mass index above or equal to 30 kg/m^2; diabetes, defined as fasting plasma glucose (FPG) levels above 126 mg/dl; hypertension, defined as blood pressure (BP) above 140/90 mmHg and/or treatment with antihypertensive medications; dyslipidemia, characterized by increased plasma concentration of triglycerides, reduced high-density lipoprotein cholesterol and increased numbers of small, dense low-density lipoprotein particles).

Ultrasonography examination

A Logiq 5 ultrasound scanner (General Electric Medical Systems, Wallingford, Connecticut, USA) was used to determine AT defined as c-IMT > 0.9 mm. The c-IMT was defined as the distance between media-adventitia and lumen-intima interfaces and it was measured at about 1 cm proximal to the bifurcation of the common carotid artery using a 7.5 MHz linear probe. The probe was placed so that the near and far walls were parallel to it and lumen diameter was maximized in the longitudinal plane. Mean common c-IMT was defined as mean IMT of the right and left common carotid arteries, calculated after 3 measurements on each side.

The patients were placed in the supine position with their head in the midline position, tilted slightly upwards and the heart in systole. Sonographic evaluations were performed by a single trained sonographer blinded to the patients' data [40].

Fibroscan examination

In all patients the liver steatosis was evaluated by CAP measuring ultrasonic attenuation in the liver at 3.5 MHz using signals acquired by the FibroScan M probe (Fibroscan 502, Echosens, Paris, France) based on vibra-

Tab. I. Baseline characteristics: Demographics, HIV factors, clinical characteristics of HIV patients (N = 128).

Variable	n (%)	Range
Male sex	92 (71.9)	
Age, years*	44	37-49
Ethnicity, Caucasian	123 (96.0)	
Smoke use	52 (40.6)	
Duration of HIV infection, years *	11	5-16
Current CD4, cells/mm^3*	543	360-700
Nadir CD4, cells/mm^3*	233	112-313
HIV RNA <20 cp/ml	107 (83.5)	
Current cART	128 (93.7)	
Duration of cART, years*	8	4-13
Obesity (BMI≥30 kg/m^2)	8 (6.2)	
Diabetes (FPG>126 mg/dl)	7 (5.4)	
Hypertension (BP>140/90 mmHg)	23 (17.9)	
Dyslipidemia	101 (78.9)	
Atherosclerosis (IMT > 0.9 mm)	26 (20.3)	
S2	31 (24.2)	

*median (interquartile range); BMI, body mass index

tion-controlled transient elastography. The principles have been described elsewhere [28]. An elastographic evaluation has been performed by a single trained sonographer blinded to patients' data. S2 was defined as CAP > 259 dBm^{-1} according to previously published data demonstrating that this cut-off was the most discriminating value [21].

Statistical analyses

The factors associated with AT were identified by logistic univariate regression analysis. The variables showing a p-value < 0.100 in the univariate analysis were evaluated in a multivariable analysis afterwards. The ROC (Receiver Operating Characteristic) curve and corresponding AUC (Area Under Curve) were calculated in order to evaluate the CAP cut-off value able to predict AT.
A two-tailed p-value < 0.05 was considered statistically significant. Statistical calculations were performed with MedCalc software, version 11.6.0.0.

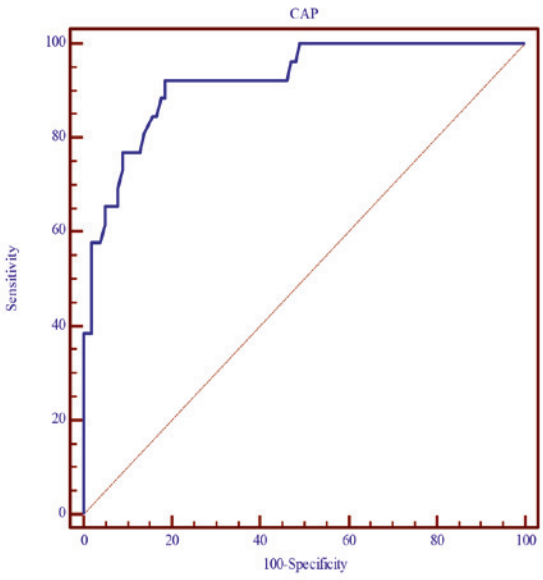
Results

In total, 128 patients [71.9% males, median age 44 years (IQR 37-49), median CD4 543 cells/µl, (IQR 360-700), 83.5% with HIVRNA < 20 cp/ml] were enrolled.
At enrollment time, 93.7% of patients were receiving ART and were using it for an average of 8 years (IQR 4-13). Obesity was diagnosed in 8 patients (6.2%), diabetes mellitus in 7 (5.4%) and hypertension in 23 (17.9%). Instead, dyslipidemia was detected in a large proportion of patients (78.9%) (Tab. I).
26 (20.3%) patients had AT while 31 (24.2%) subjects had liver S2 (Table I). HS was observed in 12 patients (11.8%) considering the subjects (n = 102) without AT, whereas among those (n = 26) with AT, 19 patients had also S2 (73.1%). Subjects with AT showed a higher significant difference (p < 0.001) of S2 than those without AT.
In univariate analysis, age (5.80, CI 1.8-19.5), obesity (15.0, CI 2.8-79.7), diabetes (6.0, CI 1.3-28.7), hypertension (3.90, CI 1.5-5.3), and liver S2 (20.3, CI 7.0-58.4) were significantly associated with subclinical AT (Tab. II).
In the multivariate model, subclinical AT was associated with age [odds ratio (OR) 5.8; p < 0.001] and liver S2 (OR 20.3; p < 0.001). ROC curve indicated that the most discriminant CAP value for predicting AT was > 250 dB/m^{-1} (AUC = 0.92, sensitivity 92.31%, specificity 81.37%, p < 0.0001) (Fig. 1).

Discussion

Several reports have shown that CD and in particular AT are common among HIV infected patients [29, 41, 42].
In this cross-sectional study, conducted in a cohort of HIV-infected subjects receiving care at the Infectious Disease Department in a tertiary hospital in southern Italy, 20.3% of the study population was affected by AT. These results, similar to those of other studies, underline the importance of CD in this group, also with regard to

Fig. 1. ROC curve.



Tab. II. Factors associated with atherosclerosis.

Variable	Univariate analysis OR (95% CI)	p	Multivariate analysis OR (95% CI)	p
Male sex	1.78 (0.68-4.70)	0.23		
Age	5.80 (1.8-19.5)	0.004	5.8 (2.2-24.5)	< 0.001
CD4 at nadir (per 100 cells increase)	1.08 (0.90-1.42)	0.29		
CD4 cells count (per 100 cells increase)	0.99 (0.78-1.13)	0.66		
HIVRNA < 20 cp/ml	0.44 (0.05-1.67)	0.26		
Current smoking	1.25 (0.5-13)	0.62		
Obesity (BMI≥30 kg/m^2)	15.0 (2.8-79.7)	0.005	5.82 (0.6-39.5)	0.14
Diabetes (FPG > 126 mg/dl)	6.0 (1.3-28.7)	0.02	2.5 (2.3-20.7)	0.30
Hypertension (BP > 140/90 mmHg)	3.90 (1.5-5.3)	0.005	2.14 (0.53-8.6)	0.28
Dyslipidemia	3.80 (0.9-17.6)	0.07	4.39 (0.45-42.7)	0.20
S2	20.3 (7.0-58.4)	<0.001	20.3 (4.5-60.1)	< 0.001

age; therefore, to address this issue is important for normalizing the life expectancy of HIV population [43, 44]. In our study, we have found that 24.2% of HIV-infected subjects had S2, 78.9% dyslipidemia and 17.9% were affected by hypertension. Our results regarding the univariate analysis have shown that age, diabetes, hypertension, obesity and S2 were associated with AT. This correlation did not remain significant in multivariate analysis unless age and S2.

The elevated prevalence of CD and its correlation with age suggest that HIV patients may present accelerated vascular aging [44]. Nevertheless further studies are needed to clarify the mechanisms unrelated to natural aging process. Furthermore, the study has shown an association between carotid AT disease and S2, and the CAP value > 250 dB/m^{-1} discriminant in predicting AT has been detected. Thus, in patients with a value higher than the discriminant CAP value, an early evaluation of c-IMT may be strongly recommended.

Previous studies on the correlation between fatty liver disease and CD have displayed a prevalence of fatty liver of 37% and 13% in population with coronary artery disease [44, 45], *versus* 73.1% founded in our study.

The cause-effect relationship between these two factors still remains confusing, but the inflammatory state may be the common risk factor for AT [46].

The ultrasonographic system used to measure c-IMT seems a well-established method for assessing subclinical AT in the HIV-negative population and it is the only non-invasive imaging recommended by the American Heart Association for risk assessment for CD [47]. Moreover, c-IMT may be a useful methodology to evaluate the intima-media thickening implicated in the development and progression of atherosclerotic disease in HIV patients, as well [40, 48].

Our study have several strengths, in fact to the best of our knowledge, it is one of the few studies that examines the association between fatty liver disease and AT in HIV-infected persons.

Furthermore, the results of this paper may have important clinical implications. The elastography, used to detect HS, is a useful tool not dependent on operator skill and does not expose the patient to ionizing radiation. Other advantages of CAP include its simplicity, accurate quantification, inexpensive and sensitivity to lesser degrees of HS [49]. Therefore, its use in the normal clinical routine could be strongly recommended.

Weaknesses of the research include the cross-sectional design, therefore prospective studies might be appropriate to evaluate the progression of fatty liver disease in CD. Moreover, the sample size was limited to arrive at definite conclusions, thus the study needs to be confirmed in a larger population. The assessment of inflammatory markers to explain the association between HIV serostatus and c-IMT should be included in future studies also consequently to conflicting evidences founded [50].

In summary, HIV-infected populations have a high prevalence of subclinical AT. Moreover, AT is associated with fatty liver disease and HS assessment results crucial both in clinical practice for management of patients with chronic liver disease and for reducing risk factors, and in clinical research for epidemiological and therapeutic studies.

Early identification of CD risk in HIV patients could permit to modify lifestyle and to take therapeutic measures in order to prevent or delay the onset of chronic diseases difficult to manage.

Acknowledgments

The authors declare that they have no conflict of interest.

Authors' contributions

AZ, MG, PG conceived, designed and coordinated the research. GP performed ultrasonography and fibroscan examination. AZ, MRT and DDA performed the data quality control. MG and FB optimized the informatics database. MG performed the statistical analyses. AZ, MG, MRT, DDA and PG evaluated the results. AZ, MG, and MRT wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Universal proposal strategies of anti-HPV vaccination for adolescents: comparative analysis between school-based and clinic immunization programs

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Keywords

HPV • School-based • Adolescents • Prevention

Summary

Introduction. *A promising approach to increase teenager's adherence to immunization against HPV is the administration of vaccinations within the school facilities. The Local Health Unit of Taranto experienced two different vaccine strategy proposals in the twelve-year-olds: the first one was the usual active call strategy in the outpatient clinic, while the second one provided the involvement of the schools in the area. The aim of the study is to evaluate the results of the proposed vaccination strategies in both sexes and in towns of different sizes in order to identify an effective path for achieving vaccine coverage improvement.*
Methods. *To estimate the number of anti-HPV vaccine doses administered in adolescents of the 2003 cohort, we used the computerized vaccination system data of the Apulia Region. Then, once analyzed, the data for anti-HPV vaccine were broken down by gender, vaccine strategy and size of the town of residence. Analyses performed by using STATA SE 14.*
Results. *The multiple logistic regression points out that, females (OR = 3.2; p < 0.01), living in small towns (OR = 1.3; p < 0.01) and school vaccination strategy (OR = 2; p < 0.01) increase the likelihood of completing the anti-HPV vaccine cycle in adolescents. The comparative assessment of anti-HPV coverage strategies, suggests that school vaccination has resulted in significantly better outcomes than outpatient clinic one, for all the groups considered (overall 72.3% vs 55.6%).*
Conclusions. *The involvement of school institutes can define a winning organizational model to get a wider adolescent's adherence to immunization programs, especially in bigger towns. The school vaccination strategy could improve anti-HPV vaccine adherence also in males, who perceives a lower HPV-related diseases risk than females.*

Introduction

The human papillomavirus (HPV) infection is the more frequent sexually transmitted disease (STD) in the world [1]. A meta-analysis conducted on over one million women estimated that HPV infection worldwide was 11.7%, with a very high peak in the population under the age of 25 [2].
It has been amply described that the role of HPV in determining uterine cervix cancer [3-7] is the fourth cause of cancer among women and is responsible for approximately 275,000 deaths per year [8]. Recently it has developed a great interest in the relationship between HPV infection and some diseases in males, as invasive penile cancer. It has been estimated that 30% of the HPV-related cancers affects males, corresponding to 17,000 cancer cases per year in the European population [9].
The availability of effective vaccines against HPV offers the possibility to decrease morbidity and mortality rates associated with this pathology: an estimation based on 179 countries shows that the vaccination of 12 years old girls only (for a total of 58 million) would be able to prevent 690,000 cancer cases, including 420,000 deaths [10]. Indeed, since the very first years of the introduction of HPV vaccination, various international organizations and scientific associations such as World Health Organization (WHO), the Atlanta Centers for Disease Control and Prevention (CDC), the American Academy of Pediatrics (AAP), the European Centre for Disease Prevention and Control (ECDC), recommended the administration to 11-12 years old girls with a catch-up between the age of 13 and 18 [11].
Subsequently, the quadrivalent vaccine demonstrated its effectiveness in preventing more than 90% of HPV-related male genital lesions [12] and different organizations have extended this indication to teenager males [13-15]. However this group is not considered by the WHO as a priority, especially in contexts with limited resources [16]. Currently, with about 120 national anti-HPV immunization programs for women [17], only USA, Canada, Australia, Austria, Croatia, Liechtenstein, Saxony and Czech Republic (the latter only for the catch-up) extended to the males the vaccination program [13, 18-21]. This is due to the fact that the cost-

benefit ratio of the anti-HPV vaccination in males varies considerably in relation to the duration of the protective effect induced by the vaccination, to the coverings reached in the female population and, of course, the cost of the vaccination [22, 23].
A promising approach in order to increase teenagers' adherence to the anti-HPV immunization is the administration of the vaccines within school institutes. In Sweden and some areas of the United Kingdom, Spain and Australia, the coverage of the complete anti-HPV vaccine cycle among teenagers reached optimal levels, mainly thanks to the school programs [24-28].
In the USA, the school programs in cooperation between local school system and public health department, greatly increased the adherence to different kinds of vaccines [29-31].
More than 50% of the European countries providing an organized anti-HPV vaccination program, mainly lean on school distribution-based strategy [32], although in the literature there are few comparative evaluations between schools and outpatient clinic interventions, in order to increase the coverage of this vaccination [33]. Particularly, the impact of the HPV vaccination program offered by schools wasn't adequately analyzed in relationship to the context the teenagers live in: the assessment of the vaccination strategy effectiveness should consider that, living in a rural area or in a big city can change the access to vaccines [34-36].
Since 2007 the Italian Health Authorities recommended the active and free anti-HPV vaccination offer for thne 12 years old girls starting, entrusting the Regions with the task of deciding whether to extend the program to other categories [37]. The anti-HPV vaccine price reduction and the opportunity to reduce the number of doses needed to confer protection have allowed to expand target groups for immunization [23]. On 2014, Apulia and some other Italian Regions (Liguria, Sicily, Friuli Venezia Giulia, Molise and Veneto) introduced free vaccination for 12 year old males starting with those born in 2003, by using the quadrivalent vaccine and overlapping their vaccination schedule to the one of the female sex [38]. Currently, the goal to reach by the new National Immunization Program is the universal anti-HPV vaccination all over the Italian Regions [39].
The Local Health Unit of Taranto, experienced two different vaccine strategies: the first one is the usual active offer, performed by sending an invitation letter to target subjects' address, and the vaccine administered in the vaccine outpatient clinics; the second one involved secondary schools so that the vaccine was promoted and administrated within the school institutes.
In this context, at the end of the first year since the introduction of the universal vaccine, including teenager males, the goal of this work is to evaluate the vaccination strategies outcomes for both sexes and towns of different sizes in order to identify the most efficient path for a rapid achievement of optimal coverages.

Materials and methods

The anti-HPV vaccine has been offered to subjects born in 2003 by means of two different proposals:
10 vaccination centers, randomly selected, implemented the traditional active offer. The family of the target subjects received an invitation letter to go to the vaccination center for the immunization. The letter also contained information on benefits and risks of anti-HPV vaccination. This vaccination strategy don't need a date, but the teenagers and their parents can freely access to the outpatient clinic.
Other 14 vaccine centers involved the secondary schools in their jurisdiction. After acquiring the list of the enrolled students born in 2003, were organized counselling and anti-HPV vaccines promotional meetings with the participation of the Local Health Unit's healthcare professionals, the teachers, the students and their parents. During the meetings, planned outside of school time, the parents provided written consent for the vaccine administration within the schools. Both doses administration was scheduled within the schools during the class time.
In order to estimate the number of anti-HPV doses administrated to teenagers born in the period between January 1st and December 31st 2003, have been used the routine data of annual vaccines updated to December 31st 2016. The data were collected by infectious disease representatives of the Public Health Services and entered in the computerized vaccine registry of the Puglia region (GIAVA). To calculate the coverage has been used as reference population of those born in 2003, the one existing in the computerized vaccination registry system GIAVA, (data updated on December 31st 2016).
The anti-HPV vaccine coverage has been analyzed based on gender, vaccine strategy and size of the town. For this last feature, was considered the data of the resident population as of January 1st 2016 from the National Institute of Statistics, which allowed to classify the districts in small and big centers assuming a population of 30,000 as a cut-off (maximum limit of patients for the general medicine functional aggregation in Italy [40]).
Quantitative variables were identified as medians of the samples, with the related interquartile range, while qualitative variables were expressed as proportions with a 95% confidence interval. Mann-Whitney rank-sum test has been used for the median values comparison, while the chi-squared test has been used for the proportions comparison.
We assessed the possible correlations among the explored variables by defining double-entry contingency tables and calculating Chi-Square (Chi2) and Odds Ratio (OR) with 95% CIs. The variables considered in univariate analyses were evaluated in a logistic regression model to study the relationship between the vaccination coverage and the explanatory variables, while adjusting for confounding factors and effect modification if needed. OR with 95% CI was used to evaluate the strength of an association. The significance level was considered when $p < 0.05$. Analyses were performed by using STATA SE 14 for Mac OS.

Results

In the Local Health Unit of Taranto, there are 5720 subjects belonging to the 2003 cohort. The sample distribution by gender, town size and vaccination strategy it's quite uniform as shown in Table I.

The complete cycle administration coverage of the anti-HPV vaccine for the 2003 cohort it's 63% (n = 3603; 95% CI = 61.7-64.2%). The interval median between the 2 doses administration for those teenagers who completed the cycle is 194 days (p25-p75 = 184-225).

In the univariate analysis, the female sex, living in a small town and the school vaccine strategy increase the likelihood of anti-HPV vaccine cycle completion for teenagers (Tab. II).

The multiple logistic regression confirms the existence of these connections, that are more evident for the female sex (OR = 3.2; 95% CI = 2.8–3.5; p << 0,01) and for the school strategy (OR = 2; 95% CI = 1.8–2.3; p << 0,01), rather than small towns (OR = 1.3; 95% CI = 1.1–1.4; p << 0,01) (Tab. III).

The coverage for the teenagers immunized at school is 84.5% (n = 1089/1289; 95% CI = 82.5-86.5%) for the females and 59.7% (n = 741/1241; 95% CI = 57-62.4%) for the males (Fig. 1).

Tab. I. 2003 cohort teenagers characteristics. Taranto Local Health Unit (n = 5720).

Gender	N	%
Male	2861	50.02
Female	2859	49.98
District size		
Small	2404	42.03
Big	3316	57.97
Vaccine strategy		
School	2530	44.23
Clinic	3190	55.77

By considering the town size as a discriminating parameter, the coverage reached by school vaccination is 71.7% (n = 1066/1487; 95% CI = 69.4-74%) in the small towns and 73.2% (n = 764/1043; 95% CI = 70.6-75.9%) in the big ones.

The comparative evaluation of the coverage, by considering the different vaccination strategies shows that the school vaccination leads to significantly better results than the outpatient clinic one, for all the considered groups (Fig. 1).

The proportion of subjects who did not completed the vaccine cycle, after receiving the first dose, is 15% of those vaccinated in the outpatient clinics (n = 314/2087; 95% CI = 13.5-16.7%) and 8.5% of those immunized at school (n=169/1999; 95% CI = 7.3-9.8%); that is a significant difference according to the Chi square test ($\chi^2 = 42.6$; p << 0.01).

The median of the interval time between the two doses, for those who completed the cycle, is 184 days (p25-p75 = 183-209) for the vaccines administrated in school, and 217 (p25-p75 = 196-259) for the vaccines administrated in outpatient clinic; that is a significant difference according to the Mann-Whitney rank sum test (p < 0.01).

Discussion and conclusions

After one year since the introduction of the global immunization, the comparative analysis between the school and the outpatient anti-HPV immunization programs gives interesting and useful information to keep in mind for setting the most effective vaccination strategy.

The anti-HPV school vaccination for the 12 year olds in the Taranto Local Health Unit has been far more effective than the outpatient one, leading to the best overall results. The gap between the coverage, got by the two strategies is 16% for both genders. This result it's aligned with the data existing in the literature, confirming that school vaccination is associated to better coverage [41], and clearly showing that school institutes could

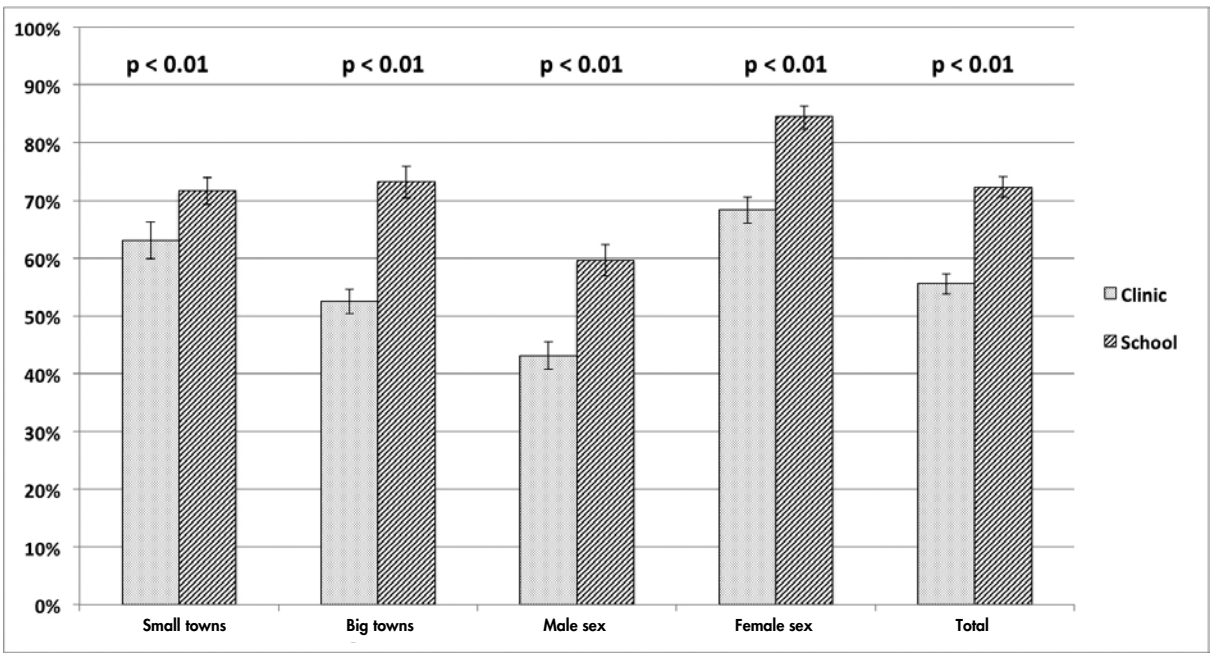
Tab. II. Univariate analysis about determinants of HPV vaccination, Taranto Local Health Unit, 2003 cohort (n = 5720).

Gender	N	%	OR (95% CI)	χ^2	P value
Female	2164	60.1%	3.1 (2.7-3.4)	392.8	0.00
Town size					
Small	1645	45.7%	1.5 (1.3-1.7)	52.6	0.00
Vaccine strategy					
School	1830	50.8%	2.1 (1.9-2.3)	169.9	0.00

Tab. III. Multivariate analysis about determinants of HPV vaccination, Taranto Local Health Unit, 2003 cohort (n = 5720).

Gender	z	OR (95% CI)	P value
Female	19.6	3.2 (2.8-3.5)	0.00
District size			
Small	3.7	1.3 (1.1-1.4)	0.00
Vaccine strategy			
School	11.5	2 (1.8-2.3)	0.00

Fig. 1. Coverage for anti-HPV complete cycle. Taranto Local Health Unit, 2003 cohort, by gender and town size.



be the key to getting greater participation to teenage vaccination programs.

That's especially important for males, since the coverage it's significantly lower than the one recorded for females. The males, according to other previous studies, are in fact associated to a lower chance of completion of the anti-HPV cycle [42]. The main limiting reason which adversely affects the prevention campaign against the HPV-infection in men, it could be the lack of risk perception connected to this STD [43]. That's why the school vaccination, by means of a more direct and effective counselling activity, could be an important promotional way to the immunization and adhesion to the anti-HPV vaccine among the teenagers [44]. Meetings with healthcare professionals about vaccine counselling represents a better approach compared to the traditional invitation letter to the outpatient clinic and allows to show in a thorough way the benefits of vaccination, especially for the males.

The impact of the anti-HPV school administration strategy it's particularly evident in towns with more than 30,000 people, where there is an increase in coverage of 20.5% compared to the outpatient vaccination. Right in those contexts in which the study found the greatest difficulties in reaching the target population of the vaccine offer, the school proposal has been undoubtedly effective. A possible interpretation may be the greater accessibility of school vaccination, this hypothesis is also supported by other data such as the reduced number of teenagers who didn't complete the vaccine cycle after receiving the first dosage and the median interval shortening between the two doses.

It should be underlined that the reported coverage estimate for school vaccination, also include the catch-up doses administrated in outpatient clinics to those school children who were absentees when the school vaccination was scheduled. In addition, those who were already privately vaccinated before 2014, were excluded from the calculation.

To set up a school vaccination campaign needs a considerable organizational effort. First, the vaccinations must be performed in compliance with the best safety standards: healthcare professionals must have Pediatric Basic Life Support and Defibrillation (P-BLSD) certifications, as well as a portable pharmacological and instrumental kit for emergencies. In the future, it will be important the cooperation with the schools to set up permanent outpatient clinics for all the students' health needs, including vaccinations. Moreover it's proper to allow real time access to the computerized vaccine registry, data entry from a laptop and a dedicated network connection.

Anyway, the school is the best place to perform health promotion and collective health education programs against vaccine-preventable diseases. Some countries included the school vaccination strategy into a wider school-based health approach, thus improving the impact of those interventions [45].

In this regard, integrating the health promotion and school vaccination programs seems to be the most sustainable solution also with regard to human resources management, especially if it's associated with teacher's involvement. Furthermore the healthcare professionals in schools could administrate much more vaccinations than

in the outpatient clinics, since they could save time by doing counselling vaccination in a single group session.
The Taranto Local Health Unit experience suggests that the school is the ideal context to get the wider adherence to the anti-HPV immunization programs. The most important factors for the success of the school-based vaccination strategy are the "good" relationships between the Local Health Unit and the Local School management, the working skills improvement and the communication with the students and their parents.
If the application of the aforementioned organizational model to different contexts and wider samples will confirm the outcomes, it could be adopted as a regional or national strategic plan able to oppose the hesitancy and stimulate resilience phenomena to vaccination.

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Authors' contributions

AG and MC conceived and designed the study. CR, TB and GC performed a search of the literature on epidemiology of HPV related-diseases and anti-HPV vaccination proposal strategies. FD carried out statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

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ORIGINAL ARTICLE

Assessing hand hygiene compliance among healthcare workers in six Intensive Care Units

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Keywords

Intensive care unit • Hand hygiene compliance • Hand washing technique • Healthcare workers

Summary

Introduction. *Healthcare associated infections (HAIs) are a cause of high morbidity, disability and reduced quality of life, as well as mortality and rising costs for health systems. Preventing the HAI risk by planning and implementing effective preventive strategies is important to safeguard patient health.*

Methods. *The study aimed to evaluate the presence of procedures and protocols for infection control, to assess the adhesion to the different aspects of hand hygiene (HH) and hand washing technique by healthcare workers in six ICUs. A perspective observational study was conducted in six ICUs. In each ICU, the adherence by health care workers to both hand hygiene practices and standard precautions was assessed, as well as the presence of procedures and written protocols.*

Results. *The findings showed that in all the involved ICUs, 73 of 142 required protocols and procedures were available. Specifically, 59 of 79 were available for general measure of risk control, 12 of 15 for hand hygiene, and 24 of 48 for standard precautions and isolation measures. Also, the results showed highly variable levels of adherence to the best hygiene practices in all the ICUs involved in the study, with compliance rates ranging from 3% to 100%, and 73 of 142 required protocols were available at the study time.*

Conclusions. *Overall, the involved ICUs showed low levels of adherence to best hygiene practices. This suggests the need to implement immediate strategies for infection control in the ICUs. A multidisciplinary intervention could be effective in preventing and control the HAI risk.score was reached only by the third year students with regard to the proper HH. The level of knowledge about HAI was inadequate.*

A periodically check of nursing students' knowledge would be advisable in order to fill any gaps, improve training, reduce HAI and increase prevention measures compliance.

Introduction

Healthcare associated infections (HAIs) are caused by environmental pathogens or patient's endogenous flora [1]. HAIs are related to both pathogens and host characteristics. Virulence, infectious load, and multiple resistances against antibiotics are the aspects related to the pathogen. The factors associated with the host characteristics include age, chronic diseases, iatrogenic or pathological immunosuppression. Furthermore, the use of invasive diagnostic and therapeutic procedures is an additional risk factors contributing to HAIs. In intensive care unit (ICU), these risk factors are very common, making ICUs the hospital wards with the highest incidence of HAI [2]. Health care workers (HCWs) hands are the more frequent carriers for the responsible microorganisms. For these reasons, infections are often associated to inadequate hand hygiene practices among health care workers and poor clinical conditions during hospitalization.

Scientific literature shows significant differences among Countries and among departments of the same Country with regard to the rates of infection, microorganisms, sites of infection, and antibiotic resistance profiles [3]. Such discrepancies denote the need for aligning with and disseminating knowledge and skills for healthcare workers regarding best practices for preventing infections at the European and Italian levels [4, 5], as well as the importance of an adequate surveillance system.

HAIs are not all preventable. Nevertheless, research has shown that the majority of infections are preventable through interventions based on effectiveness evidences [6]. The vascular catheter-related and urinary catheter-related avoidable infections percentage has been estimated to be 65% to 70%. The determinants of HAIs on which it is possible to intervene for improving quality of care are: a) deficiencies in technology (e.g. appropriate safety devices) and structural work environment; b) poor human resources management and work organization related to poor quality of interpersonal relationship, emotional disorder, and inadequate communication among staff members; c) health care practices that do not meet gold standards in reducing the infection risk (e.g. inappropriate application of standard precautions for spe-

cific diseases, and use of antibiotics) d) lack of information for workers about infection control systems, and poor participation in interventions for preventing or reducing the infection risk. Promoting these aspects would mean improve capacity in measuring and managing the HAI risks. Fostering a correct adhesion to hand hygiene (HH) and to the use of personal protective equipment, could be a simple, efficacious and cost-effective strategy. Despite it is well documented that compliance with HH can reduce HAI rates and the antibiotic-resistant pathogens cross-transmission [7, 8], non-compliance is still a main problem in hospital care. In both developed and developing Countries, health care workers have difficulty in adhering to hand hygiene practices [5, 9]. An association was found between workload, infections and poor adherence to HH practices. In fact, a Swiss study showed that adherence to HH practices before device contact was 25% during understaffing and workload period, but increased to 70% at the end of this period [10]. Therefore, a way to reduce the HAI risk is to achieve enduring improvements in HH by implementing effective programs able to increase compliance with best practices in healthcare workers.

This study aimed to measure the adhesion to the best practice in HH in six ICUs from four Italian hospitals. Specifically, we considered the presence of procedures and protocols for infection control and assessed the adhesion rate to different aspects of HH by HCWs, as well as the hand washing technique.

Methods

A perspective observational study was conducted in six ICUs from six hospitals in the Centre and North of Italy, which voluntarily participated in the research. Each ICU had mixed (medical and surgical) patients with a separate ICU team (physicians, nurses, and nurse aide). Table I shows the descriptive characteristics of the units involved in the study.

A multidisciplinary work group was created to perform the study. The study was carried out from October 2012 to June 2013.

In a first step, we developed a compendium of best practice in preventing infection named "Standard", according to the National and International scientific literature. The Standard was adopted by the ICUs and used to create a check list in order to assess the presence of procedures and protocols in each unit, as well as compliance with both HH practice and technique by HCWs. Specifically, the check list consisted in two parts. The first part (check list A) aimed to verify the existence of required protocols for infection control, guidelines, and standardized procedures reported by head physician or head nurse of the ICU. The second part (check list B) aimed to verify by direct observations the adherence by HCWs to the best evidence-based practices of HH during the clinical practice [10]. The observations took into account physicians, nurses and nurse aides.

The check list A data were collected by the research team in two times: (1) at the beginning of the study and (2) after six months in order to identify a baseline and consequently analyze potential improvements in terms of procedures and protocols for infection control.

The check list B data were collected from February to May 2013 for two weeks by two external expert observers who were known by all other healthcare workers. Specifically, data were collected in the morning shift under suggestion of both head physician and head nurse, due to high workload in the shift. From January to February 2013, a pilot phase was performed to validate the check list.

HCWs were observed during the recommended HH practices including standard hand washing with soap and water, or with an alcohol-based solution. As recommended by WHO, the study analyzed five HH steps: (1) HH before touching a patient, (2) HH before clean/aseptic procedures, (3) HH after body fluid exposure risk, (4) HH after touching a patient, and (5) HH after touching patient surroundings. Furthermore, we evaluated hand washing technique by considering different steps: (I) wetting the hands with water, (II) applying the required amount of product (e.g. soap or alcohol solution) (III) covering all surfaces of the hands, (IV) rubbing the hands with a rotary movement, (V) rinsing the hands with water and drying them with a disposable towel, (VI) using the towel to close the faucet.

To assess potential statistical differences in compliance by HCWs, Chi-square test (χ^2) was used.

Results

Results from Check list A

General measures

At the beginning of the study, the required protocols for infection control were 79. At the time of the assessment, 75% (59 of 79) of them were available in the units. Among the available protocols we found: protocols for environment cleaning, written procedures for verifying the cleaning techniques, and written directions for refraining from direct contact with equipment or patient for HCWs with exudative dermatitis.

Hand hygiene

The protocols required at the beginning of the study were 15. At the time of the assessment, 80% (12 of 15) of them were available. Among the available protocols we found: written procedures for HCWs and visitors for hand hygiene with hydro alcoholic gel solution, written directions for adequate techniques of hand washing with alcohol solution, and indications for the use of gloves.

Standard precautions and isolation measures

The protocols required were 48. At the time of the assessment, 50% (24 of 48) of them were available. Among the

available protocols we found: written directions for do not routinely using gloves when entering the room or the box in which the patient is located, do not putting on gloves before contact with the intact skin of the patient or do not using gloves before contact with surfaces and objects (e.g. equipment, bed protections, etc.) placed in close contact with the patient, and using gloves whenever it is expected to come into contact with blood or other potentially infectious materials (i.e. mucous membranes or non-intact skin).

Results from Check list B

A total of 347 workers were involved in the study. Among these, 34% (118) were physicians, 56% (195) were nurses, and 10% (34) were nurse aide. A total of 17 items were analyzed and 332 hours and 67 days of observation were performed.

Considering the overall results, we found that compliance rates with HH procedures and standard precautions was significantly different among HCWs ($\chi^2 = 17.56$, $p < 0.001$). Nurse aides had the higher compliance rates compared to nurses and physicians (Tab. II).

Hand washing (with soap and water or antiseptic soap or with an alcohol-based product) before handling medication and before preparing or serving food ranged from 30% to 100%). Hand washing after contact with inanimate objects including medical equipment in the immediate proximity of the patient ranged from 28% to 45%. Hand washing before direct contact with the patient and after direct contact with the patient ranged from 37% to 42% and from 55% to 97%, respectively (see Tab. III for the results).

Regarding the hand washing technique, ICUs workers showed high compliance in all the phases of the hand washing process, even if we found variability among the ICUs (ranging from 47.6% to 100%). Finally, we found poor adhesion (ranging from 3.1% to 5%) regarding using a towel to close faucet after hands washing with soap and water (Tab. IV).

Discussion

The main aim of this study was to assess the adherence to the best preventive practices by HCWs in different ICUs.

The focus on ICUs is due to the specific epidemiology and the risk profile of patients in these units, in which use of invasive devices, aging, unstable immunological conditions are common. This study emphasizes the need to reduce the infection risk in ICU by addressing control systems on the best evidence-based practices. In the ICUs involved in our study, 73 of 142 protocols and procedures were available. Hence, standard precautions and isolation measures were weak points of the infection control system of the studied ICUs. This result denotes the need to constantly verify and update indications, procedures and protocols as a way to improve the infection control system. In this sense, the check list A was a useful and practical instrument for identifying problems, stimulating solutions, and encouraging cooperation between hospital management and both head physician and nurse in the units. Finally, available and updated protocols and procedures are also important in case of litigation to safeguard the organization under the jurisdiction of a medical examiner.

Furthermore, the results suggest orienting the efforts of the ICUs towards a system of continuous monitoring in order to increase compliance with best practice by workers, thus safeguarding patient health. In the ICUs involved in the study, the level of adherence to the hygiene practices was highly variable, with a range from 3% to 100%. Among the analyzed procedures, "Hand hygiene after contact with inanimate objects including medical equipment"which is the most studied moment according to WHO showed a compliance range from 28% (i.e. physicians) to 86% (i.e. nurse aide). This result is in line with the findings from a recent study [11] in which the rate of compliance by HCWs was 38.7% in the absence of specific interventions. The excessive variability in the results of our study can be explained by the fact that the ICUs were different in terms of number

Tab. I. Characteristics of the ICUs included in the study.

ICU	Room type	Number of beds for each ICU	Number of beds for each Hospital
ICU 1	Single room	5	649
ICU 2	Single room	7	380
ICU 3	Single room	9	630
ICU 4	Bay room and single room	5	396
ICU 5	Single room	14	1357
ICU 6	Bay room and single room	13	1700

Tab. II. Contingency table for Chi-square test.

	Physicians	Nurses	Nurse aide	Total
Observations	1014 (951.55) [4.10]	1843 (1888.49) [1.10]	154 (170.96) [1.68]	3011
Adhesions	550 (612.45) [6.37]	1261 (1215.51) [1.70]	127 (110.04) [2.61]	1938
Total	1564	3104	281	4949

Note. Observed values, expected values in parenthesis, and chi-square statistic for each cell in square bracket.

Tab. III. Compliance with hand hygiene procedures and standard precautions for the different HCWs.

Hand hygiene procedure	HCW	%
(CI 95%)		
Hand hygiene with soap and water or antiseptic soap when hands are visibly dirty or contaminated with biological fluids	Physicians	63.9
(58.4- 69.4)		
	Nurses	95.5
(93.5-97.5)		
	Nurse aide	100.0
(100.0-100.0)		
Hand hygiene before direct contact with the patient	Physicians	38.4
(31.3-45.6)		
	Nurses	42.4
(36.4-48.5)		
	Nurse aide	37.0
(18.8-55.3)		
Hand hygiene after direct contact with the patient	Physicians	55.1
(47.3-62.8)		
	Nurses	81.6
(77.2-86.1)		
	Nurse aide	96.9
(90.8-100.0)		
Hand hygiene after removing gloves	Physicians	60.9
(53.1-68.7)		
	Nurses	71.0
(66.3-75.7)		
	Nurse aide	82.9
(71.4-94.4)		
Hand hygiene before using invasive devices for patient care	Physicians	63.0
(50.1-75.8)		
	Nurses	63.8
(51.4-76.2)		
	Nurse aide	100.0
(100.0-100.0)		
Hand hygiene after contact with fluid or bodily excretions, mucous or non-intact skin, or wound medications	Physicians	67.3
(54.2-80.5)		
	Nurses	63.2
(53.1-73.4)		
	Nurse aide	100.0
(100.0-100.0)		
Hand hygiene when moving from a contaminated body site to a clean	Physicians	56.8
(40.8-72.7)		
	Nurses	57.7
(46.7-68.7)		
	Nurse aide	100.0
(100.0-100.0)		
Hand hygiene after contact with inanimate objects including medical equipment	Physicians	28.0
(18.3-37.8)		
	Nurses	40.1
(33.0-47.2)		
	Nurse aide	85.7
(67.4-100.0)		
Hand hygiene before manhandle drugs	Physicians	40.0
(15.2-64.8)		
	Nurses	30.3
(20.8-39.9)		
	Nurse aide	Not recorded
Hand hygiene before manhandle food	Physicians	Not recorded
	Nurses	94.7
(84.7-100.0)		
	Nurse aide	100.0
(100.0-100.0)		

Tab. IV. Compliance with hand hygiene techniques and standard precautions for the different HCWs.

Hand washing technique	HCW	%
(CI 95%)		
Applying a quantity of sufficient product to cover the whole surface of the hands	Physicians	86.0
(79.2-92.8)		
	Nurses	95.6
(92.9-98.2)		
	Nurse aide	97.6
(92.8-102.3)		
Rubbing hands and fingers with alcohol solution, until hands are dry	Physicians	70.2
(61.0-79.5)		
	Nurses	76.9
(71.4-82.5)		
	Nurse aide	53.5
(38.6-68.4)		
Washing hands with soap and water: wet the hands with water	Physicians	47.6
(36.9-58.3)		
	Nurses	100.0
(100.0-100.0)		
	Nurse aide	100.0
(100.0-100.0)		
Washing hands with soap and water: apply a quantity of sufficient product to cover the whole surface of the hands	Physicians	97.4
(93.8-100.0)		
	Nurses	92.1
(88.1-96.1)		
	Nurse aide	92.3
(77.8-100.0)		
Washing hands with soap and water: rub the palm and the back of the hands with a rotatory movement, including the fingers between them to cover all the surfaces of the hands and the fingers	Physicians	59.3
(48.6-70.0)		
	Nurses	68.9
(62.1-75.7)		
	Nurse aide	84.6
(65.0-100.0)		
Washing hands with soap and water: rinse the hands with water and mop up with a disposable towel	Physicians	95.3
(90.9-99.8)		
	Nurses	99.5
(98.6-100.0)		
	Nurse aide	100.0
(100.0-100.0)		
Washing hands with soap and water: use the towel to close the faucet	Physicians	3.1
(2.9-9.2)		
	Nurses	4.4
(0.6-8.2)		
	Nurse aide	5.0
(4.6-14.6)		

of beds, quality of fan and air conditioning systems, as well as to have a water-alcohol solution dispenser hanging at the wall. These differences could have been a risk source and have determined the variability. Furthermore, we found differences in the levels of adherence to the HH practices among HCWs. Specifically, physicians had lower compliance rates than other HCWs especially "After direct contact with the patient", "After contact with inanimate objects including medical equipment", and "Hand hygiene with soap and water or antiseptic soap when hands are visibly dirty or contaminated with biological fluids". These results are in line with data from literature[16] and may be partially explained by a lack in basic training in HH and preventive measures by physicians. Differently from nurses, the university programs for physicians do not include a specific training in infection prevention. In addition, some studies [12] refer some inappropriate physicians' attitudes regarding

HH, because they consider HH as a practice to protect themselves and not to protect patients. In our study we analyze the compliance results for "Hand hygiene before direct contact with the patient" (38.4%) and "Hand hygiene after direct contact with the patient" (55.1%), confirming that finding.
Regarding the hand washing technique, ICUs workers show a considerable compliance in all the stages of the hand washing process, although some variability among the ICUs was found. Regarding the rubbing hands and fingers with alcohol solution and with soap and water technique, the compliance rate is quite poor (from 53.5% to 70.2% for hand washing with alcoholic solution and from 59.3% to 84.6% for hand washing with water and soap), but in line with findings in recent studies [13]. These data suggest that even if HCWs are familiar with all the steps of hand washing technique, solicitations to improve the overall process is always required like a continuous training system. In fact, a recent study [14] showed that 72% of staff implicated in a HH education and assessment program achieved a satisfactory coverage. This denotes a real difficulty to comply with all the required procedures. Thus, ICU Managers should encourage their workers towards a behavioral change by clarifying the importance to complete correctly all the steps of the hand washing technique to guarantee high HH quality.

Limitations

In line with several studies [5, 9, 15], we used an observational method to assess compliance with HH, which is considered by WHO the "gold standard". Nevertheless, this method can represent a limitation because of the "observer effect" (Hawthorne effect). It concerns an individual's psychological response that generates an improvement in performance due to the awareness of being observed. A way to reduce the Hawthorne effect is to increase the observation time so that HCWs may become accustomed to the presence of the observers. In this study, the HCWs of each ICU were observed for five consecutive days and five hours per day. In this way, we tried to undergo the effect, thus helping workers in perceiving the observers as be part of the staff and displaying their natural behavior.

Conclusions

Although "zero risk" cannot be achieved in ICUs, the infection risk can be easily assessed with simple instruments and efficaciously managed by implementing adequate protocols and procedures to increase quality of care. The results of our research suggest that even if HH is still the main action line in reducing infection risk, its importance is not yet well known among the studied ICUs staff. In this sense, multidimensional hand hygiene intervention programs [16] based on specific needs of the ICUs, should sensitize the staff on the importance to adhere to the best clinical practices. Finally, the results of this study suggest that a good way to increase compliance by HCWs is to provide continuous improvements in quality of protocols and procedures, and to support them in terms of communication, education and training.

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Authors' contributions

MM and AL equally contributed to the study design and drafting the article. NMM drafted the article and analyzed data. MFP and LS drafted the article. MG analyzed data regarding chi-square test, edited the manuscript and performed critical revision. MC, MT, MVM, SC, PM, and RCC made considerable contributions to critical revision for important intellectual content. All authors read and approved the final article.

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ORIGINAL ARTICLE

Monitoring biodiversity in libraries: a pilot study and perspectives for indoor air quality

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Keywords

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Summary

Indoor Air Quality (IAQ) in libraries is influenced by the presence of specific factors which can impact on both paper storage as well as people health. Microclimatic conditions induce and support a biodiversity pattern involving environmental and anthropic microorganisms. We used a multidisciplinary monitoring model to characterize microflora biodiversity by Next Generation Sequencing (NGS). Biodiversity indexes were adapted to evaluate anthropic vs environmental pollution by combining Shannon mean index (H), species representativeness (E_H), human/environmental pollution ratio (S_A) to better characterize the NGS output and acquire synthetic information on Indoor Air Microbial Biodiversity (IAMB). Results indicate a frequently low microbial load (IGCM/m^3 < 1000) characterized by different species (n = 10^2), including several cellulose metabolizing bacteria. Workers and visitors appeared a relevant source of microbial contamination. Air biodiversity assayed by NGS seems a promising marker for studying IAQ.

Introduction

Indoor air quality (IAQ) is recognized as a key factor for human health, due to the fact that people spend 80%-90% of their time in indoor environments [1-4]. Biological, physical and chemical agents can affect the characteristics of the indoor atmosphere [5]. Biological agents relevant to health are widely heterogeneous, including pollen, allergens, plants spores, bacteria, fungi, algae and some protozoa. Moreover, activities like talking, sneezing, coughing, can generate airborne biological particulate, and this is considered a main factor contributing to the buildup and spread of airborne microbial contamination [6, 7]. The exposure to microbial pollution can be associated with respiratory symptoms, allergies, asthma and immunological reactions [8]. Microclimatic conditions, as humidity, temperature and ventilation rates, together with geometry of building, influence individual comfort and environmental health [9-11]. Volatile compounds (VOCs) can be released from building materials, furnishings, office machinery, or cleaning products [12]. Libraries represent an exemplificative model of sensitive indoor environments such as hospitals, schools, museums, laboratories, drug or food factories, where IAQ impacts on both people health and materials, products or procedures. During the last decades, studies on specific indoor environments have identified risk sources and threshold levels for different pollutants, proposing targeted control measures. Even the role of the structure itself has been investigated and analysis of the Sick Building Syndrome has been considered for the implications on the health of workers, dwellers, as well as for the preservation of materials and procedures [13-15]. However, the combined effects of biological agents and chemical mixtures still require further investigation, and innovative tools are needed to improve quality of indoor air [16, 17]. When it happens to specific facilities, as libraries, archives and schools, it needs further consideration to identify most appropriate markers to assess indoor air quality for people, paper materials and activities. Libraries are indoor facilities where air quality is influenced by both environmental and anthropic microorganisms, interacting with chemical and physical pollutants.

Microclimatic conditions influence the growth of several microorganisms (e.g. *Aspergillus, Penicillium, Trichoderma, Alternaria, Rhizopus*) that can attack library collections, eventually favoring the biodeterioration processes [3]. Traditional cultural methods or classical molecular approaches cannot address a comprehensive microflora analysis [18, 19]. The limit is mainly due to technical restrictions in growing, detecting or classifying the environmental species as well as in providing an overall view of the whole microflora pattern in that setting. The rapid diffusion of Next Generation Sequencing (NGS) and new bioinformatic tools are overcoming this problem, offering a wider approach to microflora biodiversity, at increasingly affordable costs. Starting from the microflora DNA (mfDNA) it became possible to detect simultaneously different species even from

very complex matrices [20, 21]. NGS satisfies the need for qualitative and quantitative information on microbial complexity, disclosing innovative solutions for prevention and hygiene. Since the strategy is based on analysis of genomic properties, the method is independent from the knowledge of the microorganisms or their culture offering new potentials as already shown for other DNA based techniques [22-24]. Moreover, even unknown or uncultivable species can be identified, quantified and characterized following an established phylogenetic approach, by amplification of rDNA, massive sequencing and bioinformatics analysis.

Following a traditional approach, previous studies have already investigated the effects of biological and chemical conditions on paper conservation, outlining also issues related to the health of library users, such as students, visitors and employed personnel [12, 26, 27]. However, most of these studies focused on independent categories of risk factors, such as chemical agents [12, 26, 28, 29], physical [30-32], without adopting an integrated approach. The characterization of microbial air contamination using single culture-based sampling not only provides a limited understanding of the phenomenon, but also restrains a comprehensive evaluation of IAQ and the implementation of corrective actions [33]. Analysis of microbial biodiversity in indoor air (IAMB) can play an important role in characterizing specific environments, identifying possible biological risks and acquiring information for the anthropic component.

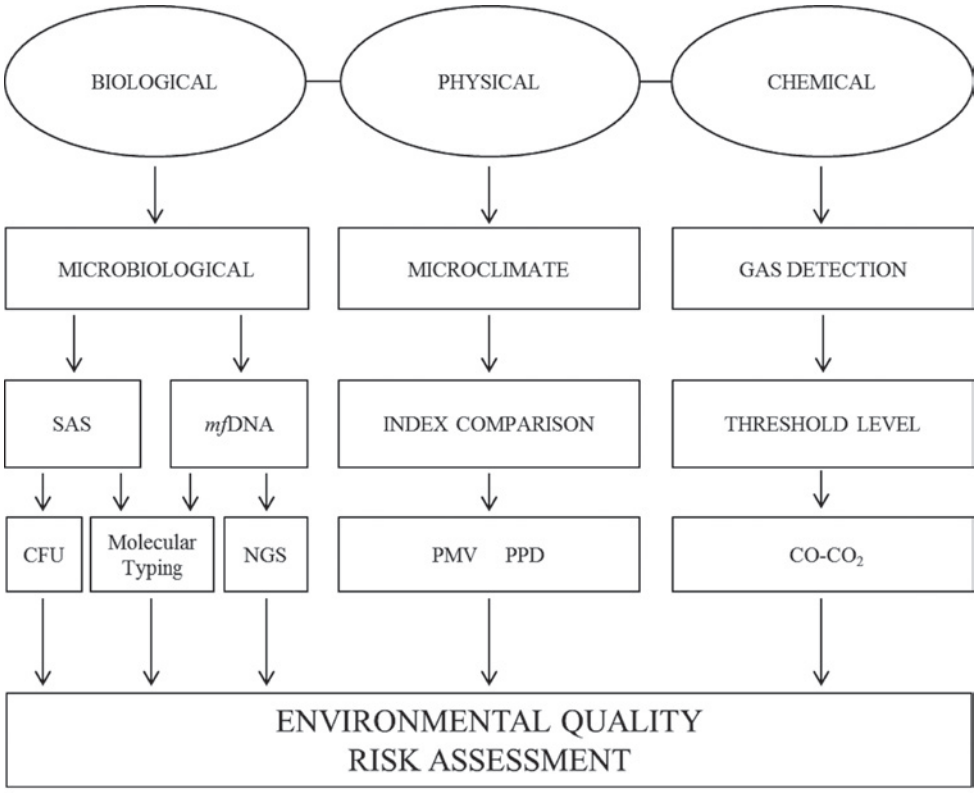
In the present study, environmental samplings have been simultaneously performed in order to obtain a wider overview focused on biological issues and integrated with microclimate and environmental parameters. Moreover, for the first time, an advanced approach for microorganism identification was adopted through NGS, with the final aim of typing the indoor microflora and acquiring information on the impact of anthropic and environmental microflora on IAQ.

Air microflora is influenced by environmental conditions and may play a role on human health as well as on safety of library resources, especially when storing valuable ancient books. NGS seems to offer an extraordinary and promising opportunity for making microflora biodiversity as a new marker for monitoring specific indoor environments.

Materials and methods

In order to evaluate IAQ, three different kinds of libraries were chosen: Site I, a university library; Site II, an old scientific library; Site III, a digital library. Several environmental samplings were performed following an integrated approach (Figure 1), to the aim of acquiring an overview on microclimate, chemical and biological

Fig. 1. Flowchart with panel of sampling techniques and integrated monitoring approach.



parameters. Biological monitoring was performed by a novel passive method adapted for DNA extraction and by classical active sampling techniques (SAS). Microorganisms were characterized by both culture-based methods and by molecular techniques based on mfDNA analysis.

Study design

Environmental sampling was performed to provid an overview of microclimate and microbiological parameters in these facilities and to show the feasibility of an integrated approach for IAQ surveillance. Biological monitoring was performed with different sampling techniques (active and passive) and methods (classical microbiology and DNA techniques). Statistical analysis was performed to determine the distribution of indoor mesophilic, psichrophilic bacteria and fungi concentrations in association with independent variables, such as the buildings age, light exposure, air exchange rate and ventilation systems. Similarly, the indoor mesophilic, psichrophilic and fungi concentration have been correlated with the relative humidity, the external and internal temperature.

Study area

Three scientific libraries in the city of Rome (Italy), were selected as exemplificative of different typologies. The first site (Site I) is a "university library" founded in 1994, consisting of a single reading room with desks for students, computers and a loft with shelves for the books storage. The documentary print heritage consists in over 5000 monographs and 2000 periodicals, providing access to about 2000 electronic journals and scientific databases. The library also has books storage room, located in the same building, in the basement. The second site (Site II) was founded in 1930s and represents a centralized reference, specialized in scientific documentation. The print documents legacy consists in more than 9000 periodicals and about 200000 monographs including publications of national and international institutes, pharmacopoeias, official documents, texts of health legislation and grey literature. This library also collects thousands of electronic journals in full text, and accesses to biomedical databases. It also stores historic 16th-18th century dated books, and precious documents. The library is organized on three levels, where the books are stored in shelves both fixed and mobile. The library has also a book storage room, located in an independent building, and divided into two rooms. The third one (Site III) is a "digital library": it was founded in 1930 and in 2005 was moved in an independent building and organized as an electronically accessible library, associated to a hospital and research centre. The collection currently amounts to about 10000 online magazines and different local databases. It offers a reading room with 15 PC workstations intended to health consulting and health education activities open to visitors, including both patients and professionals; in the same building the upper floor houses the management offices. Libraries have an usual access of about 10-50 visitors per day, employing 3-6 workers dedicated to reading rooms, office and management activities.

In Site I, sampling points (n = 2) were: the reading room and the book storehouse. In Site II (n = 7): Flat A Room 1, Flat A Room 2, Flat B room 3, Flat B room 4, Reading room, Book storehouse 1, Book storage 2. Site III samplings (n = 2): the reading room and the manager office. The samplings were performed in the period ranging from September 2014 to July 2015.

Daily averages of outdoor temperature data were obtained from a monitoring station at an average distance of about 10 km from the library sites. For each library, site elevation, proximity to agricultural/industrial processes or major arterial roadways was verified also by the support of satellite geographic maps (Google Map) to exclude major air pollution sources. Building characteristics were considered, including construction materials, restorations, heating and ventilation systems. Signs of moisture, water damage or fungal growth on building materials were assessed visually and annotated in the sampling form.

Physical and chemical parameters

The instrument HD32.3 DataLogger® (*Delta Ohm s.r.l.*), conforms to ISO 7730, 7726, 27243, 7933, 11079, 8996 (Supplementary Table, S1), was adopted for microclimate analysis. The unit has three probes, thermohygrometric, anemometer and globe thermometer to determine the discomfort indices, PMV (Predicted Mean Vote) and PPD (Percentage of Person dissatisfied). The PMV is an individual wellness index. It is a mathematical function which gives as result a numerical value in the range between -3 (feeling too cold) and +3 (feeling too hot), where 0 represents the thermal comfort. The PPD expresses the percentage of dissatisfied people in a particular environment. Through DeltaLog10® software (*Delta Ohm s.r.l.*) the data were downloaded. During sampling, the instrument was positioned in the sites center, for 15 minutes, with 15 sec intervals in measurement at the worker's chest height.

Carbon dioxide (CO_2) and carbon monoxide (CO) quantification was also performed by HD21AB17 Datalogger® (*Delta Ohm s.r.l*). It has a single probe which can simultaneously measure the level of CO_2, CO and atmospheric pressure. The instrument can also measure the temperature, the relative humidity and calculate the dew point, the wet bulb temperature, absolute humidity, mixing ratio and enthalpy. Through DeltaLog10® software (*Delta Ohm s.r.l.*) data were downloaded and analyzed. The tool has been positioned in the room centre at the worker's chest height and the measures were performed for 15 minutes, with 15 seconds of intervals between each measure. We referred to the European standard EN 13779-2008 on the Ventilation for non-residential buildings to evaluate the CO_2 levels and to the European directive 2008/50/ EC to evaluate the CO levels.

Microbiological air sampling

Active sampling

The microbiological air sampling was performed by SAS® (Surface Air System, *VWR International, LLC,*

Radnor, USA), a plate impact active sampler, slot type, using Petri dishes Ø 55mm. The air aspiration volume was 180 L for each sampling. We used two culture media types: TSA (Tryptic Soy Agar - *Oxoid, Germany*) for bacteria, SDA (Sabouraud Dextrose Agar - *Oxoid, Germany*) supplemented with Chloramphenicol® (*Oxoid, Germany*) for molds and yeasts. The TSA and SDA plates were incubated both at 37°C (for 48 hours) and at 22°C (for 72 hours) respectively. For each plate, the calculation of the CFU/m^3 was obtained as follows: CFU/m^3 = (MPN/plate x 1000) / air volume (L). MPN (Most Probable Number)/plate was obtained by comparing the CFU of each plate with the conversion table, in attachment to the device manual.
In addition, from each TSA plate, colonies were collected by sterile loop, in a 1.5 ml tube containing 200 µl of sterile water. From each SDA plate, representative colonies of each fungal species were collected by sterile loop in a 1.5 ml tube, containing 200 µl of sterile water. All tubes were centrifuged at maximum speed (16000 g for 5 minutes). The supernatant was removed and the pellet was frozen at - 4°C. The DNA extraction by pellets was performed with GenElute® Bacterial Genomic DNA Kit, following the manufacturer's instruction.

Passive sampling

In Site II, a tissue-based passive sampling approach was developed and tested to collect bacteria and extract DNA. Sterilized cotton tissues (size 5 cm^2) were placed for one month on the shelves of Flat A Room 1, Flat A Room 2, Flat B Room 3, Flat B Room 4, and named respectively T1, T2, T3, T4. The collection was followed by direct DNA extraction. Briefly, a piece of 1 cm^2 was cut from each tissue. After exposure, each sample was transferred in a 1.5 ml tube at room temperature and then frozen at -20°C for 20 minutes for cryofreezing fracturing. The samples were pretreated with glass beads® (*Sigma Aldrich, USA*) and 200 µl of Lysozyme Solution® (*Sigma Aldrich*, *USA*), adapting the protocol as previous described (Giampaoli 2012) In a second phase we followed the standard protocol procedure of GenElute® Bacterial Genomic DNA Kit (*Sigma Aldrich, USA*). The DNA extraction by pellets was performed with GenElute® Bacterial Genomic DNA Kit, following the manufacturer's instruction.

DNA analysis

Fungal ITS PCR amplification and Sanger sequencing

DNA was amplified with primer designed for fungal ITS (Internal Transcribed Spacer) region [34]. The PCR amplification was performed in 25 µl reaction mixture consisting of 1× Taqmastermix® (*Promega, USA*), 1 µM of forward and reverse universal primers and template DNA on TechneTC-PLUS® thermalcycler (*VWR International, LLC, Radnor, USA*). Thermocycling conditions were the following: for 35 cycles (each cycle is 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 60 s), with an initial hot start (94 °C for 15 min) and a final extension (72 °C for 10 min). The purified PCR products were sequenced using AB Big-Dye 3.1® dye chemistry (*Applied Biosystems*). A total of 20 µL sequencing reactions contained 2 µL of cleaned PCR product, 1 µL of BigDye Terminator v3.1 Ready Reaction Mix, 2 µL of 5× Sequencing Buffer, 1.6 pmol of Forward or Reverse sequencing primer and PCR grade water. Sequencing reactions were performed for 25 cycles (each cycle is 96 °C for 30 s, 50 °C for 15 s, and 60 °C for 4 min in a GeneAmp PCR 9700® thermocycler (*Applied Biosystems*). After the cleanup step with the Performa® DTR Gel Filtration Cartridges (*Edge Bio, Gaithersburg, MD, USA*) the reaction was run on AB 3500 XL® automated DNA sequencers (*Applied Biosystems*).

PCR amplification and NGS sequencing

Samples were prepared according to the "16S Metagenomic Sequencing Library Preparation" guide (Part# 15044223 rev. A; *Illumina, San Diego, CA, USA*). The amplicon PCR has been performed using the following primers (containing overhang adapters): Ba27F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCCTGGCTCAG-3', Ba338R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCTGCCTCCCGTAGGAGT-3'[35]. Libraries have been quantified through PicoGreen dsDNA quantitation assay (*Thermo Fisher Scientific, Waltham, MA, USA*) and validated on Bioanalyzer DNA 1000 chip (*Agilent, Santa Clara, CA. USA*). The sequencing was performed on MiSeq desktop sequencer (*Illumina*).

Analysis of sequence reads and IAQ evaluation by Microbial Biodiversity parameters

The sequence reads were analyzed in the cloud environment BaseSpace through the 16S Metagenomics app (version 1.0.1; *Illumina®*): the taxonomic database used is an Illumina-curated version of the May 2013, a release of the Greengenes Consortium Database (greengenes.secondgenome.com); the classification algorithm is an implementation of the Ribosomal Database Project (RDP) Classifier [36]. In order to acquire information on IAQ by Microbial Biodiversity (MB) a novel putative parameter (Indoor Air Microbial Biodiversity, IAMB) was assumed by composing the following indexes: Shannon mean index, Shannon's Equitability index and calculating and an Anthropogenic pollution index based on NGS reads. In particular, community richness and indoor air microbial biodiversity were computed through Shannon mean index (H) and Shannon's Equitability index (E_H) using EstimateS software [37, 38]. Briefly, a cut-off corresponding to taxonomic species was applied to the NGS data, and the indices were calculated on the filtered matrix. Moreover, to verify the origin of air pollution we calculated the proportion of anthropogenic species S_A compared to the total identified taxonomic units (Number of species of human source/total selected species). The combination of microbial load (H), species representativeness (E_H), human/environmental pollution ratio (S_A) was used to acquire information for IAMB.

Statistical analyses

Statistical analysis was performed by IBM SPSS statistical software, version 23.0 for Windows (*SPSS, Chicago, IL*). Continuous variables were reported as arithmetic mean, whereas categorical variables were reported as absolute values. A multiple linear regression equation was created for the outcome interesting of indoor mesophilic, psichrophilic bacteria and fungi concentration. All continuous variables were tested for a linear correlation with each other (Pearson r). Similarly, all categorical variables were tested for statistically significant relationships with other independent variables and choices made as for continuous variables.

Results

The comfort parameters were acceptable in all sampled sites, as shown in Table I: in Site I the value of PMV was 0.94 for the Reading room and 1.02 for the Books store. The PPD was similar for both environments with a percentage of the 25%. The CO_2 concentration was 572.08 and 721.13 ppm respectively for Reading room and Books store. In Site II the PMV value was less than 1 for all environments. The maximum PPD value was detected in Flat B room 3 and in the Reading room (respectively of 18.47% and 13.58%); in the other environments was around the 5%. The CO_2 concentration was around 550 ppm, with a lower value detected in the Reading room (445.97 ppm). In Site III, the PMV values were 0.35 and 0.88 respectively for the Reading room and the Manager office. The maximum PPD value was detected in the Manager office (21.37%), where the CO_2 concentration was 614.21, whether 752.89 in Reading room.

SAS air sampling data were analyzed according to "American Conference of Governmental Industrial Hygienists" and reported in Table II [39]. The IGCM index (Global Index of Microbiological Contamination) was very low in Reading room and low in the Books storage of Site I. In Flat A room 2, Flat B room 3, Reading room and books storage of Site II was very low as in other environments. The values of Reading room of Site III and manager office were respectively intermediate and low. IAQ was estimated according to the "European Collaborative Action" [40], showing in the Reading room of Site I an intermediate level of bacterial pollution at 22°C (101-500 CFU/m^3) and a low one at 37°C (80 CFU m^3); in the Books storage, it was intermediate at both temperatures. The fungal pollution was lower for both environments. In Flat A room 1, Flat A room 2, Flat B room 3, Flat B room 4 of Site II bacterial pollution was intermediate at both temperatures, while in the Reading room it was low (50-100 CFU/m^3) at 37°C and very low (< 50 CFU/m^3) at 22°C; in the Books storage 1 and 2 it was intermediate at both temperatures. The fungal pollution was very low in Flat A room 1, Flat A room 2, Flat B room 3, Flat B room 4 sites; while it was low (< 100 CFU/m^3) in the Books storage 1 and 2. In Site III a low bacterial pollution at 37°C was observed for both environments; at 22°C, the Reading room showed high bacterial pollution (500-2000 CFU/m^3), while the Manager office an intermediate one (101-500 CFU/m^3). The fungal pollution in the Reading room and in the Manager office was respectively 500-2000 CFU/m^3 and 100-500 CFU/m^3 (Supplementary Tables, S2-S3).

Table III shows the categorical variables for indoor mesophilic, psichrophilic and fungi microbial load used for statistical analysis: the buildings age, light exposure, air exchange rate and ventilation systems. Table IV shows the continuous variables with statistically significant relationship with indoor mesophilic, psichrophilic and fungi concentration. These variables have been correlated with the relative humidity, the external and internal temperature. Significant correlations were observed for mesophilic concentrations *versus* air exchange rate (+1; $p < 0.05$), psychrophilic concentrations and fungi concentration *versus* light exposure (+1; $p < 0.05$) and mean indoor relative humidity (respectively 0.831-$p < 0.01$; 0.848-$p<0.05$). Negative correlations were observed for fungi concentration *versus* air exchange rate (-1; $p < 0.05$) and ventilation system (-1; $p < 0.01$), air exchange rate *versus* psychrophilic concentrations (-1; $p < 0.05$), mesophilic concentrations *versus* light exposure (-1; $p < 0.05$) and mean indoor temperatures (-0.73; $p < 0.01$). Moreover, the table shows the results obtained for continuous variables with statistically significant relationship to indoor mesophilic, psichrophilic and fungi concentration in books storage. These variables have been correlated with the relative humidity, the external and internal temperature. Significant correlation was observed only for psichrophilic concentration compared to the three variables in all storage. In particular, mean indoor relative humidity showed a correlation coefficient of 0.999 ($p < 0.01$), while both mean indoor temperature and mean outdoor temperature showed a correlation coefficient of +1 ($p < 0.05$).

Tab. I. Evaluation of PMV, PPD, CO-CO_2 at Sites I, Site II and Site III.

	Sampling site	PMV	PPD	CO_2	CO
Site I	Reading room	0.94	23.83	572.08	0
	Books storage	1.02	27.35	721.13	0
Site II	Flat A room 1	0.03	5.06	560.87	0
	Flat A room 2	0.16	5.53	470.43	0
	Flat B room 3	0.8	18.47	542.79	0
	Flat B room 4	0.14	6.1	583.23	0
	Flat C Reading room	0.64	13.58	445.97	0
	Books storage 1	-0.52	11.01	442.03	0
	Books storage 2	-0.61	12.91	444.75	0
Site III	Reading room	0.35	7.51	752.89	0
	Manager office	0.88	21.37	614.21	0

Tab. II. Evaluation of IGCM, according to ACGIH [39], in Site I, Site II and Site III.

Group of microbes	Range of value (CFU/m³)	Pollution degree	Site I		Site II							Site III	
			Reading room	Books storage	Flat A room 1	Flat A room 2	Flat B room 3	Flat B room 4	Flat C Reading room	Books storage 1	Books storage 2	Reading room	Manager office
IGCM/m³	< 500	Very low	√			√	√		√		√		
	< 1000	Low		√	√					√			√
	> 1000	Intermediate										√	
	> 5000	High											
	> 10,000	Very high											

Tab. III. Categorical variables for indoor mesophilic, psichrophilic and fungi concentration.

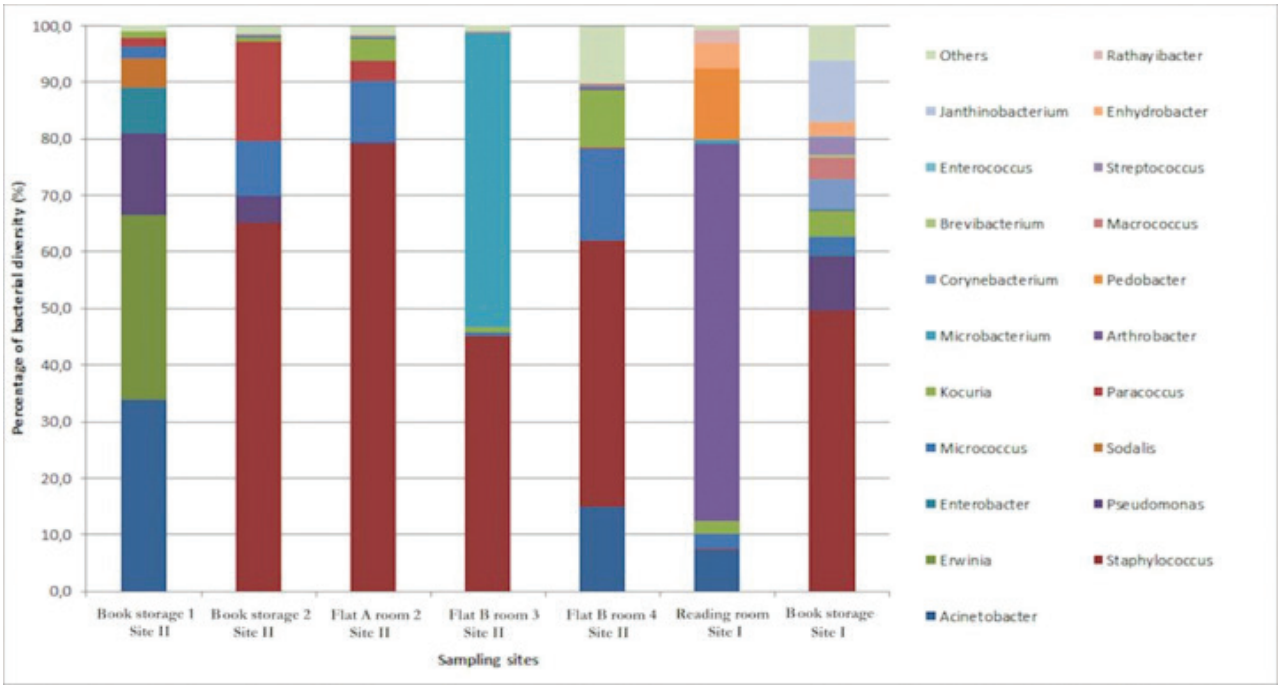
Variable	N	Mesophilic bacteria (Mean CFU/m³)	Psichrophilic bacteria (Mean CFU/m³)	Fungi (mean CFU/m³)
Air exchange rate (a.c.h.)				
Yes	4	160.8	397.5	219.5
No	7	198.6	255.7	88.3
Building age (years)				
11	1	71.5	748.0	559.0
22	1	208.5	250.0	44.5
82	1	210.4	197.7	41.3
Ventilation system				
Natural	2	235.5	695.0	417.0
Artificial	5	143.4	170.0	18.6
None	4	211.3	285.0	142.3
Light exposure				
Yes	6	138.7	360.3	196.5
No	5	240.2	243.6	63.4

Tab. IV. Continuous variables with statistically significant relationship to indoor mesophilic, psichrophilic and fungi concentration.

		Mesophilic Bacteria (Mean CFU/m³)	Psichrophilic Bacteria (Mean CFU/m³)	Fungi (Mean CFU/m³)
	Variable	Correlation Coefficent (Pearson *r*)		
Others ambient	Building age	0.629	-0.685	-0.624
	Air exchange rate	+1*	-1*	-1*
	Light exposure	-1*	+1*	+1*
	Ventilation system	-0.897	-0.992	-1**
	Mean indoor relative humidity	-0.424	0.831**	0.848*
	Mean indoor temperature	-0.73**	-0.005	0.187
	Mean outdoor temperature	-0.523	0.458	0.525
Books storage	Mean indoor relative humidity	0.657	0.999**	-0.967
	Mean indoor temperature	0.689	+1*	-0.977
	Mean outdoor temperature	0.669	+1**	-0.971

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

Fig. 2. Percentage of bacterial diversity at genus level of isolates sampled in Site I and II by NGS approach.



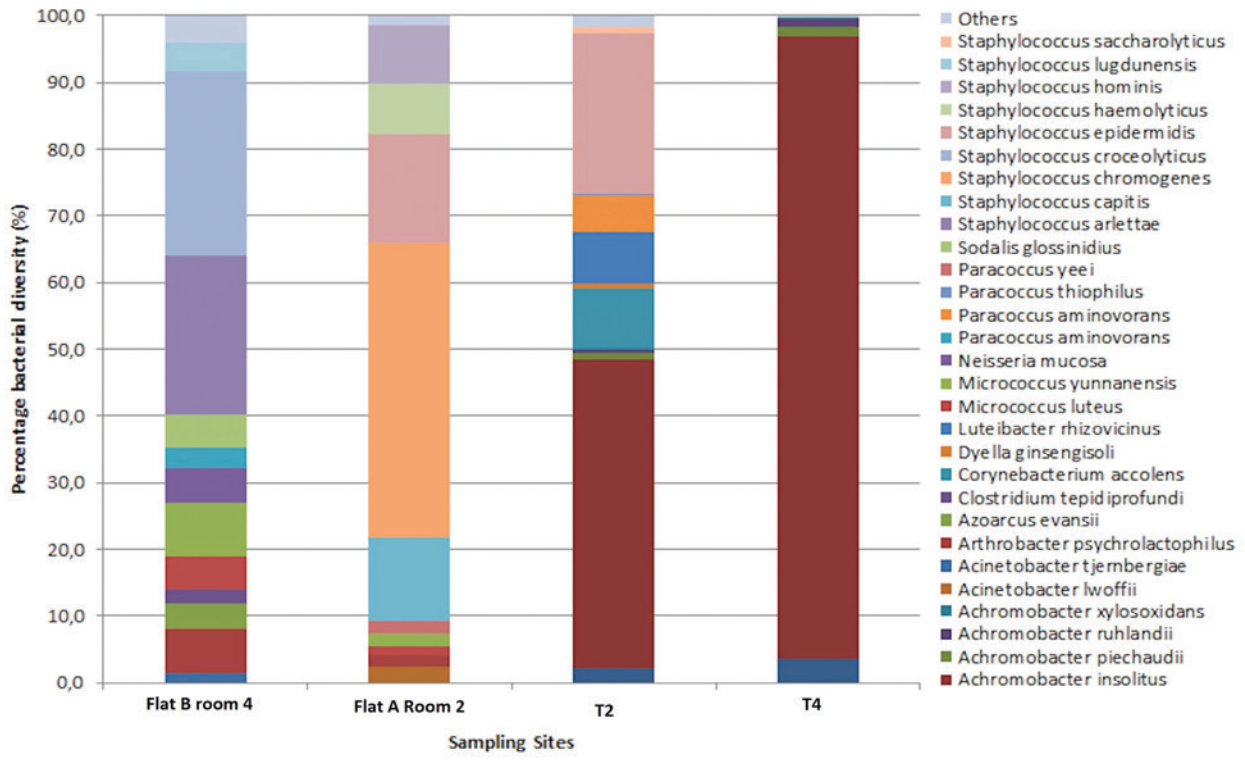
Microbiological identification of environmental mycotic species was performed through DNA sequencing. The most of fungi isolates belonged to the *Cladosporidium spp*. and were found in all Sites. Two isolates belonged to *Penicillium* genus, in particular *P. rivolii* (Site I) and *P. viticola* (Site II). In Site I, one sample was identified as *Cladosporidium uredinicola* and one as *Fusarium sp.* Finally, in Site II only a sample belonged to *Ascomycota* genus (Supplementary Table, S4).

The relative abundance of bacterial diversity at the genus level was detected by NGS (Figure 2). A high abundance of *Staphylococcus spp*. was observed in most of the samples (65% in Books storage 2; 79% in Flat A Room 2; 45% in Flat B room 3; 47% Flat A Room 1 of Site II and 49% in Books storage of Site I). The most prevalent bacterial species identified among all the observed samples were *Staphylococci*, including *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. Some other bacterial species were detected such as *Acinetobacter* (33% in Books storage 1 of Site II); *Erwinia* (32% in Books storage1 of Site II); *Arthrobacter* (66% in the Reading room of Site I), *Microbacterium* (52% in Flat B room 3 of Site II); *Pseudomonas* (14.4% and 4.7% respectively in Books storage 1 and 2 of Site II; 9.7% in Books storage of Site I); *Janthinobacterium*, *Corynebacterium* and *Macrococcus* (10.9%, 5.1% and 3.8% respectively in Books storage of Site I); *Kocuria* (3.8% and 10.3% in Flat A Room 2 and Room 1 of Site II respectively; 2.4% and 4.6% in Reading room and Books storage of Site I respectively); *Sodalis* (5.2% in Books storage1 of Site II); *Pedobacter* (12.5% in Reading room of Site I); *Paracoccus* (17.7%, 1.7% and 3.6% respectively in Books storage 1, 2 and Flat A Room 2 of Site II); *Enterobacter* (8.2% in Books storage1 of Site II). A total of 30 bacterial species were detected in all tissues sampled in this study.

The predominant bacterial species in the analyzed surface samples were reported, considering their relative (cutoff > 1.0%) abundance (Figure 3). These species include *Achromobacter insolitus* (46.4% in T2 and 93.4% in T4) and *Achromobacter arsenitoxydans* (2.1% in T2 and 3.6% in T4). Only in T2 tissue the species detected were *Corynebacterium accolens* (9.1%), *Luteibacter rhizovicinus* (7.6%), *Paracoccus aminovorans* (5.6%) and *Staphylococcus epidermidis* (23.9%). Figure 3 also shows the comparison between the microflora which was found in tissues (T2 and T4), and bacterial isolates of the respective sampling areas (Flat A Room 2, Flat B room 4): T2 - Flat A Room 2 showed a matching, detecting *Staphylococcus epidermidis* in similar percentage (16.4% in Flat A Room 2; 23.9% in T2).

The bacterial community diversity was calculated on a total of 83 taxonomic units which fulfilled the cut off limit. The values of Shannon mean index (H) and Shannon's Equitability index (E_H) are showed for each sample and compared with the control values for outdoor air and soil (Table V). In passive sampling (T1, T2, T3, T4), the H index was between 2.02 and 2.86 while the E_H index was between 0.46 and 0.65. In active sampling (Site II: Flat B Room 3, Flat B Room 4, Flat A Room 2, Books storage1, Books storage2; Site I: Reading Room, Books store) the highest value of H index was 3.36 (Reading Room of Site I) and the lowest was 2.97 (Books storage 1 of Site II), same trend holds for E_H index (0.67 and 0.76 respectively in Reading Room of Site I and Books

Fig. 3. Representative distribution of bacterial diversity at species level of tissue samples and corresponding sampling points in Site II.



Tab. V. Bacterial species distribution: results of Shannon diversity index richness (H), Evenness (E_H) and Proportion of Anthropogenic species (S_A) values.

Samples	Shannon mean index (H)	Shannon's Equitability (E_H)	Proportion of Anthropogenic species (S_A)
T1 (Flat A Room 1 Site II)	2.02	0.46	0.01
T2 (Flat A Room 2 Site II)	2.43	0.55	0.23
T3 (Flat B Room 3 Site II)	2.69	0.61	0.18
T4 (Flat B Room 4 Site II)	2.86	0.65	0.02
Book storage 1 Site II	2.97	0.67	0.62
Book storage 2 Site II	3.4	0.77	0.68
Flat A Room 2 Site II	3.28	0.74	0.82
Flat B Room 3 Site II	3.17	0.72	0.85
Flat B Room 4 Site II	3.2	0.72	0.89
Book storage Site I	3.34	0.76	0.73
Reading Room Site I	3.36	0.76	0.82
Outdoor Air [44]	4.38	0.98	0.05
Soil [48]	4.33	0.90	0.03

storage1 of Site II). The distribution of anthropogenic niche (Table V) shows that passive sampling values were between 0 and 0.23, while the bacterial population in air samples as measured by active sampling provided a variety of human origin species between 0.62 and 0.89. For each indoor environment, the parallel consideration of the three indices provides information on the microflora biodiversity in the air (IAMB).

Discussion

Libraries represent one of those public places where monitoring IAQ is relevant not only for people's health, but also for the preservation of materials, in this case the paper of the books and their appropriate storage or restoration [12, 31]. Therefore, even if libraries do not represent settings at high occupational risk, they are exem-

plificative indoor environments to test monitoring strategies and improve air quality over the minimal requirements for occupational safety. Several studies regarding IAQ in libraries have been reported, focusing on the different categories of risk factors [12, 26, 29, 30, 42]. A monitoring program should include biological, physical and chemical parameters, following a "holistic method" to provide an extended vision of the phenomenon and an overall evaluation of IAQ.

In this study, we considered an integrated approach (Figure 1). Interestingly, both chemical and physical parameters are known to influence microflora composition, and, otherwise, each environmental microflora is typical of that ecological niche [24, 25, 42, 43]. Biodiversity analysis showed the presence of paper-related bacteria and allowed to define extent and diffusion of airborne microorganisms.

The collected data defined the safety of libraries air, the degree of comfort and wellness for users and workers. IAQ data comply with standards for non-industrial premises. The PPM and PMV indicators were acceptable for most sites, with some slight discrepancies related with external climatic conditions [30]. No main differences in PPM and PMV were observed for the different libraries, although these buildings have very different structures, use, age, light exposure suggesting a major role for the air conditioning/heating system.

Collected microclimatic and microbiological data suggested the presence of disadvantageous conditions for psychrophilic bacteria and fungi proliferation. Higher air exchange/ventilation rates correspond to a decrease in microbial load. Slight variations involved the psychrophilic count in Reading room of Site III that can be related to interference of ventilation and intense turnout of users, in accordance with previous reports [11, 41]. In storage room of Site II, the psychrophilic bacterial counts and fungal pollution are significantly higher than in the reading room, although the microclimate was acceptable even if visible molds were observable on walls, suggesting external factors or discontinuous indoor management. Overall mesophilic and psychrophilic growth was in line with data surveyed in other rooms within the same library, suggesting that similar disposition and typology of books, such as age, materials and storage conditions, may play a role. This observation agrees with statistical analysis showing a significant correlation with the relative humidity, external and internal temperature (Tab. IV).

Sequencing analysis by NGS allowed a comprehensive evaluation of microflora biodiversity, and the indoor distribution of microorganisms. The relative abundance of different bacteria in the indoor atmosphere is of high interest to evaluate IAQ. Airborne bacterial fluxes, indeed, represent an important way for microorganisms to colonize remote environments. In this context, the identification of levels and type of microbial pollution and analysis of its variations in space and time can contribute to implement IAQ assessment and dynamics[44]. A total of 110 ± 10 species were detected by NGS for each sampled point. To analyze this microflora complexity bioinformatic tools and several indices can be used [45-47]. The Shannon diversity (H) and Shannon equitability (E_H) indices provide characterization of biodiversity, overcoming microbial richness. To evaluate microflora variations in indoor environments, we proposed the combined assessment of microbial load (H) and species representativeness (E_H) parameters, adding a human/environmental pollution ratio (S_A) to better characterize the NGS output and acquire synthetic information on Indoor Air Microbial Biodiversity (IAMB). As expected, the indices values were less rich in biodiversity than those reported for soil [48] or heavily polluted outdoor air [44] and the proportion of anthropogenic species S_A compared to the total identified taxonomic units was higher (Tab. V). Passive sampling allowed acquisition of data for longer periods (1 month), showing a comparable trend for H and E_H values but lower S_A values (0.001-0.23) while the bacterial population collected by active techniques (60 seconds) showed a larger variety of human origin species, with S_A values between 0.62 and 0.89. This can be explained considering the different sampling times, the drying effect of the tissue, the overall day/night exposure periods without visitors and the selective advantage of environmental resistant species respect to the survival capabilities of human anthropogenic mesophilic. *Staphylococci* were identified in the environments by 1 minute active sampling during working hours, but not in the corresponding 30 days passive sampling, except for *Staphylococcus epidermidis* that was identified in one site (T2 - Flat Room 2). In conclusion, there was a mismatch between the species detected by passive *versus* active sampling, therefore these microorganisms might be deposited, but were not cultivable on plate. However, in studying environmental microflora, plate culture may represent a kind of bias, because species are selected on the growth property we have available. The adoption of sampling and analysis systems strictly based on molecular methods without culture steps would bypass this limit, as shown by DNA analysis and NGS data.

In air samples analyzed by active techniques, the numerous sequences corresponding to anthropic bacteria (e.g. *Micrococci*, *Paracocci*, *Staphylococci*) suggested contaminations by people [49]. Besides, all the sampled libraries shared a similar microflora, including *Micrococci* ranging from 0.7% (Flat B room 3) to 10.9% (Flat A room 2) and *Kocuria* with a maximum of 10% in Flat A room 2. Amongst identified genera some as *Corynebacterium*, *Macrococcus* and *Janthinobacterium* were already reported as predominant flora in indoor air [50]. Furthermore, the presence of symbiotic (as *Kocuria* spp.) or phytopathogenic bacteria (as *Erwinia* and *Sodalis*) in the community illustrated the potential role of air in the dissemination of organisms interacting in different biological processes. The book storehouse in Site II showed a different scenario: although similar in terms of microclimate, fungal and psychrophilic count, a different mesophilic growth was observed, impacting on microflora biodiversity. *Staphylococcus* and *Pseudomonas* genera prevailed in the most accessible area

(books storage 2), while *Acinetobacter, Erwinia*, *Enterobacter* and *Sodalis* were present only in the farthest part dedicated to long term storage (books storage 1). These environments selected a different microflora. Bacteria present in books storage 1, less adjacent to the external environment, are typical of paper and include species described papermaking industries [50]. Similarly, the presence of *Pseudomonas* spp. in the storehouse of Site I, can be related to the capability to grow on different carbon sources including cellulose [51-54]. Fungi presence in libraries and archives has been largely investigated, mainly in correlation with paper deterioration and possible adverse effects on workers due to the release of secondary metabolites [3, 32, 41]. Opportunistic pathogens were sporadically detected, as *Fusarium* genus that produces mycotoxins and secondary metabolites which can trigger allergic reactions. Most of the isolates collected in our study belong to the genera *Penicillium* and *Cladosporidium*, in line with previous studies [55, 56].
The proposed model and the microflora data by IAMB can provide a comprehensive evaluation of IAQ, suggesting whether a particular environment may fall within one of the different risk categories, including: i) the site is clean or poses acceptable risk and no further action is mandatory; ii) the site poses borderline risk levels and a further monitoring or follow up may be required; iii) the site is highly contaminated and remedial action is needed. Evaluation of the potential impact of the microflora biodiversity on IAQ depends on the specific environment, activities and procedures carried out in that context. Several guidelines and strategies are already available for air risk assessment and can be improved by the contribute of new molecular biology technologies.

Conclusions

A comprehensive evaluation of Indoor Air Quality requires a multidisciplinary approach and the identification of critical points. The analysis of microflora by Indoor Air Microbial Biodiversity is revealed as a valuable tool to assess IAQ and plan further corrective actions, based on reconstruction of contamination sources and routes. We can suggest that microflora biodiversity itself may represent an informative and valuable marker for IAQ, also when assayed by NGS. This NGS integrated approach may represent a promising tool for surveillance of IAQ in libraries and other occupational environments.

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Authors' contributions

FV was involved in the design of the research, performing experiments and writing the paper. CC performed experiments and statistical analysis and collaborated in writing the paper. GG contributed in collecting and processing samples. SS was involved in acquisition of microclimatic data. VRS designed the study and supervised laboratory steps. NM designed the study, coordinated the research and edited the manuscript. All authors read and approved the final version of this manuscript.

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Supplementary tables

S1. Reference values of thermal comfort according to ISO 7730, 7726, 27243, 7933, 11079, 8996.

PMV	PPD%	Evaluation of thermal comfort
3	100	Very hot
2	75.7	Hot
1	26.4	Slightly hot
0.85	20	Thermal environment within acceptable
-0,5 < PMV < 0,5	< 10	Thermal comfort
-0.85	20	Thermal environment within acceptable
-1	26.8	Slightly cold
-2	76.4	Cold
-3	100	Very cold

S2. Evaluation of air quality in Site I, Site II according to the sanitary standards for non-industrial premises [40].

Group of microbes	Range of value (CFU/m3)	Pollution degree	Site I					Site II													
			Reading room		Books storage			Flat A room 1		Flat A room 2		Flat B room 3		Flat B room 4		Flat C Reading room		Books storage 1		Books storage 2	
			37°C	22°C	37°C	22°C		37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C
Bacteria	< 50	Very low															√				
	50-100	Low	√													√					
	101-500	Intermediate		√	√	√		√	√	√	√	√	√	√	√			√	√	√	√
	500 -2000	High																			
	> 2000	Very high																			
Fungi	< 25	Very low		√		√			√		√		√		√		√				
	< 100	Low																	√		√
	< 500	Intermediate																			
	< 2000	High																			
	> 2000	Very high																			

S3. Evaluation of air quality in Site III according to the sanitary standards for non-industrial premises.

Group of microbes	Range of value (CFU/m3)	Pollution degree	Site III			
			Reading room		Manager office	
			37°C	22°C	37°C	22°C
Bacteria	<50	Very low				
	50-100	Low	√		√	
	101-500	Intermediate				√
	500-2000	High		√		
	>2000	Very high				
Fungi	< 25	Very low				
	<100	Low				
	< 500	Intermediate				√
	< 2000	High		√		
	> 2000	Very high				

S4. Type of fungi isolated from each site.

Fungi isolate	Site I	Site II	Site III
Penicillium rivolii	+		
Penicillium viticola		+	
Cladosporidium sp.		+	+ +
Cladosporidium uredinicola	+		
Fusarium sp.	+		
Ascomycota genus		+	

ORIGINAL ARTICLE

The effect of musculoskeletal problems on fatigue and productivity of office personnel: a cross-sectional study

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Keywords

Discomfort • Fatigue • Musculoskeletal system • Office • Pain • Productivity

Summary

Introduction. *Work-related Musculoskeletal Disorders (WMSDs) can impact on fatigue and productivity of office workers. This study aimed to investigate the effect of musculoskeletal problems on fatigue and productivity among office personnel.*
Methods. *This study was performed on 101 Iranian office workers. Data were gathered through a demographic questionnaire, Nordic Musculoskeletal Questionnaire, Numeric Rating Scale, Persian version of Multidimensional Assessment of Fatigue Scale, and Persian version of Health and Work Questionnaire.*
Results. *The results revealed that the highest prevalence rates of musculoskeletal symptoms in the past week were related to neck (41.6%), lower back (41.6%), and shoulders (40.6%). The mean score of discomfort/pain was 1.67, 1.55, and 1.31 in the neck, lower back, and shoulders, respectively. Additionally, the severity of discomfort/pain in neck, shoulders, lower back, and thighs was correlated to total fatigue. The severity of discomfort/pain in neck, lower back, buttock, and thighs was also correlated to the concentration/focus subscale of productivity.*
Conclusions. *Improvement of working conditions is suggested to reduce musculoskeletal problems and fatigue and enhance productivity.*

Introduction

Musculoskeletal Disorders (MSDs) are widespread throughout the world [1] and are the second most common cause of disability in work setting [2]. These disorders are responsible for 40-50% of the costs of all work-related diseases. In addition, 50% of all more-than-3-day absences from work and 49% of all more-than-two-week absences are caused by MSDs [3].
Workplace risk factors for development of Work-related Musculoskeletal Disorders (WMSDs) include heavy physical work, forceful overexertion, awkward and sustained postures, repetitive movement, and vibration [4]. Previous surveys indicated a significant positive association between MSDs and fatigue [5, 6], stress [6], psychosocial distress, and sleep disruption [5]. MSDs are also a significant workplace issue resulting in loss of productivity at work and sickness absence [7]. Outcomes of MSDs can range from symptoms to major impairment losses [8], such as reduction of quality of life [9], reduction of productivity (e.g., lost time) [10], and increase in medical expenses due to disability [11]. The results of some studies have also shown a significant association between the reported prevalence of MSDs and productivity loss in terms of "presenteeism" [12].
The economic and social pressure of MSDs in the working-age population are so huge that employers spend about $103,000 for every 100 employees annually [13]. In the USA (2012), 29% of injuries and diseases leading to days off work were attributed to WMSDs. Besides, Haufler et al. reported that the total cost of lost productivity linked to MSDs in the European Union's population [10] might be about 2% of the Gross Domestic Product (GDP) [14]. On the other hand, direct costs (medical costs) arising from MSDs were $576 billion (4.5% of GDP) between 2004 and 2006. In the same period, indirect costs (calculated as lost wages) related to MSDs were $373 billion (2.9% of GDP).
Office workers are exposed to risk factors of WMSDs [15, 16]. The findings of the study by Maakip et al. revealed that the 6-month prevalence of MSDs symptoms was 92.8% and 71.2% among Malaysian and Australian office workers, respectively [15]. Alavi et al. also conducted a study in Iran and found that the prevalence rates of MSDs symptoms in shoulders, hands/wrists, and elbows of office workers were 18.1%, 13.9%, 5.3%, respectively. Additionally, MSDs in shoulders, elbows, and hands/wrists were associated with poor mental health ($p < 0.001$) [17]. In the same line, Choobineh et al. demonstrated that the prevalence rate of MSDs symptoms was 56.6% in the neck, 46.7% in wrists/hands, 44.6% in

lower back, 39.2% in upper back, 38.2% in shoulders, 34.4% in knees, 24.1% in legs/feet, 15.0% in elbows, and 14.6% in thighs among Iranian office workers [18]. Generally, office workers comprise a big workforce group employed in any organization and workplace. These workers usually work in sedentary posture for a long time. This condition can be conducive for developing MSDs, fatigue, and loss of productivity. In the present study, it was hypothesized that MSDs could be effective in developing fatigue and loss of productivity. Therefore, this study was performed to assess musculoskeletal symptoms and discomfort/pain in office personnel and to examine the relationship between MSDs and fatigue and productivity.

Methods

Data gathering tools

a) Demographic questionnaire: This questionnaire included questions about age, weight, height, job tenure, daily sitting working time, sex, marital status, and education level.

b) Nordic Musculoskeletal Questionnaire (NMQ): NMQ examines the reported prevalence of MSDs in different body regions among the study population [19]. In the present study, reported musculoskeletal symptoms were limited to the past week. Each participant received the questionnaire in his/her workplace. The validity and reliability of the Persian version of NMQ had been surveyed by Choobineh et al. [20].

c) Numeric Rating Scale (NRS): NRS is a unidimensional measure of discomfort and pain intensity [21].

d) Persian version of the Multidimensional Assessment of Fatigue (P-MAF) Scale: Multidimensional Assessment of Fatigue (MAF) scale was developed by Belza et al. (1993) among older adults with rheumatoid arthritis [22]. This scale is in fact a revision of the Piper Fatigue Scale developed and tested among oncology patients [23]. MAF scale contains 16 items that assess various aspects of fatigue. This scale is a self-administered questionnaire to assess four dimensions of fatigue, including degree and severity, amount of distress it causes, its timing, and the degree to which fatigue interferes with daily living activities. Respondents are asked to reflect their experiences of fatigue in the past week [22]. In our study, the psychometric properties of P-MAF scale were examined among Iranian office workers. Accordingly, the internal consistency of the scale was acceptable ($\alpha \geq 0.854$) for all subscales. Indeed, the convergent validity ranged from 0.466 to 0.948 for all subscales. Moreover, factor analysis of P-MAF scale revealed that its items were related to severity, distress, timing of fatigue, interference with activity at home, and interference with activity outside the house.

e) Persian version of Health and Work Questionnaire (P-HWQ): Health and Work Questionnaire (HWQ) was developed by Shikiar et al. (2004) among a sample of reservation agents at a US-based international airline. HWQ assesses various aspects of workplace productivity. It consists of 30 questions responded through a 10-point Likert scale. These questions are categorized into six subscales, namely productivity, concentration/focus, supervisor relations, work and non-work satisfaction, and impatience/irritability [24]. In our study, the validity and reliability of P-HWQ were examined among Iranian office workers. Accordingly, the internal consistency of the Persian version of the questionnaire was acceptable for all subscales ($\alpha \geq 0.65$). Besides, factor analysis was acceptable (> 0.4) for each item related to the subscales.

Implementation of the study

This study was conducted on 101 Iranian office workers with at least one year of working experience. Employees with underlying diseases or accidents affecting the musculoskeletal system were excluded from the study. The participants were selected from Shiraz University of Medical Sciences through simple random sampling using the table of random numbers. All subjects voluntarily participated in the study after receiving information about the study objectives. They also signed written informed consents before commencement of the study. The study was conducted in accordance with the Helsinki Declaration of 1964 as revised in 2008. The required data were gathered in two weeks consecutively:

Week 1) In the first week, demographic questionnaire was completed by the participants. In order to assess the intensity of musculoskeletal discomfort/pain, the subjects were required to rate NRS on Saturday, Monday, and Wednesday at the beginning, middle, and end of the shift. Then, difference between the NRS scores at the beginning and end of the shift during the three working days was calculated and the mean of differences was considered as musculoskeletal discomfort/pain in the working week.

Week 2) NMQ was used to determine the prevalence rate of MSDs symptoms in the past week. Besides, P-MAF scale and P-HWQ were used to assess the participants' fatigue and productivity in the past week (week 1), respectively.

Statistical analysis

In this study, the Statistical Package for Social Sciences 16 (SPSS Inc, Chicago, IL, USA) was used to analyze the data. Because the data did not appear to follow a normal distribution, Spearman's correlation coefficient was used to evaluate the relationship between musculoskeletal discomfort/pain, and fatigue and productivity. It is worth mentioning that Kolmogorov-Smirnov test was used to test the normality of the data.

Results

Some personal characteristics of the studied office workers have been presented in Table I.

Tab. I. Some personal characteristics of the studied office workers (n = 101).

Quantitative variables		Mean ± Standard deviation
Age (years)		37.91 ± 13.52
Weight (kg)		70.81 ± 15.80
Stature (cm)		166.32 ± 13.66
Job tenure (years)		12.30 ± 8.37
Working hours per day		8.13 ± 1.88
Working hours per week		44.40 ± 14.24
Hours of exercise per week		2.94 ± 1.96
Qualitative variables		No. (%)
Sex		
Male		56 (55.4%)
Female		45 (44.6%)
Marital status		
Single		30 (29.7%)
Married		71 (70.3%)
Education level		
Associate degree and lower		34 (33.7%)
Bachelor of science and higher		67 (66.3%)

Tab. II. The frequency of reported musculoskeletal symptoms in different body regions among workers during the past week (n = 101).

Body region	MSDs symptoms	
	No.	%
Neck	42	41.6
Shoulders	41	40.6
Elbows	15	14.9
Wrists/hands	27	26.7
Upper back	31	30.7
Lower back	42	41.6
Thighs	13	12.9
Knees	36	35.6
Legs/feet	31	30.7

Tab. III. Mean ± standard deviation of the severity of discomfort in different body regions of the participants (n = 101).

Body region	Severity of pain
	Mean ± standard deviation
Neck	1.67±2.24
Shoulders	1.31±2.28
Elbows	1.01±2.02
Wrists/hands	1.05±1.97
Lower back	1.55±2.29
Buttock	1.12±2.28
Thighs	0.97±1.99
Ankles	1.28±2.19

Tab. IV. The correlations between the severity of discomfort/pain in different body regions and the scores of P-MAF and its subscales.

			P-MAF subscales				
			Degree and severity	Distress that it causes	Degree of interference with activities of daily living	Timing of fatigue	Total fatigue/ Global Fatigue Index
Body regions	Neck	r*	0.363	0.418	0.274	-0.351	0.344
		p**	0.000	0.000	0.010	0.001	0.002
	Shoulders	r	0.304	0.262	0.245	-0.355	0.229
		p	0.003	0.012	0.024	0.001	0.048
	Elbows	r	0.225	0.238	0.154	-0.351	0.104
		p	0.029	0.022	0.154	0.001	0.369
	Wrists/hands	r	0.271	0.279	0.123	-0.370	0.158
		p	0.008	0.007	0.263	0.000	0.174
	Lower back	r	0.283	0.316	0.159	-0.230	0.285
		p	0.006	0.002	0.144	0.027	0.013
	Buttock	r	0.166	0.171	0.121	-0.343	0.104
		p	0.111	0.104	0.265	0.001	0.366
	Thighs	r	0.210	0.274	0.251	-0.215	0.305
		p	0.041	0.008	0.019	0.039	0.007
	Ankles	r	0.160	0.184	0.118	-0.219	0.125
		p	0.123	0.078	0.275	0.036	0.278

*r: Spearman's correlation coefficient; **p: p-value, significance level $\alpha = 0.05$

Tab. V. The correlations between the severity of discomfort/pain in different body regions and the score of productivity subscales.

			P-HWQ subscales							
			Productivity	Productivity own assessment	Productivity others assessment	Concentration/ focus	Supervisor relations	Non-work satisfaction	Work satisfaction	Impatience/ irritability
Body regions	Neck	r*	-0.044	-0.091	-0.035	0.216	-0.009	-0.078	-0.059	0.107
		p**	0.682	0.386	0.735	0.032	0.928	0.450	0.565	0.297
	Shoulders	r	-0.069	-0.007	-0.125	0.179	-0.046	-0.094	-0.047	0.170
		p	0.518	0.947	0.230	0.079	0.661	0.367	0.649	0.101
	Elbows	r	-0.008	-0.049	-0.005	0.188	-0.006	-0.076	-0.073	0.173
		p	0.940	0.639	0.960	0.063	0.955	0.457	0.475	0.091
	Wrists/ hands	r	-0.002	-0.037	-0.044	0.167	-0.013	-0.006	-0.017	0.083
		p	0.983	0.729	0.671	0.103	0.899	0.954	0.866	0.427
	Lower back	r	-0.018	-0.072	-0.003	0.216	-0.006	-0.026	-0.047	0.008
		p	0.867	0.496	0.980	0.033	0.955	0.799	0.645	0.937
	Buttock	r	-0.103	-0.156	-0.088	0.223	-0.028	-0.097	-0.032	0.167
		p	0.330	0.137	0.392	0.027	0.787	0.346	0.753	0.105
	Thighs	r	-0.102	-0.104	-0.021	0.206	-0.056	-0.039	-0.049	0.102
		p	0.334	0.320	0.840	0.041	0.587	0.707	0.630	0.324
	Ankles	r	-0.017	-0.094	-0.004	0.145	-0.059	-0.035	-0.007	0.038
		p	0.873	0.375	0.966	0.155	0.568	0.732	0.946	0.716

*r: Spearman's correlation coefficient; **p: p-value, significance level $\alpha = 0.05$

The prevalence rates of the reported musculoskeletal symptoms in different body regions among office workers during the past week have been presented in Table II. Mean ± standard deviation of severity of discomfort/pain in different body regions among the studied subjects has been displayed in Table III. As the table depicts, the means of severity of discomfort/pain were higher in the neck, lower back, and shoulders compared to other regions.

The correlations between the severity of discomfort/pain in different body regions and the scores of P-MAF and its subscales have been depicted in Table IV. Accordingly, the scores of discomfort/pain in neck, shoulders, lower back, and thighs were correlated to total fatigue. Based on the rule of thumb in interpreting the size of the correlation coefficient, these correlation coefficients were in the negligible or low correlation category (0-0.5) [25].

The correlations between the severity of discomfort/pain in different body regions and the score of productivity subscale derived from P-HWQ have been presented in Table V. Based on the results, the severity of discomfort/pain in the neck, lower back, buttock, and thighs was only correlated only to the concentration/focus subscale of productivity. Based on the rule of thumb in interpreting the size of the correlation coefficient, these correlation coefficients were in the negligible correlation category (0-0.3) [25].

Discussion

The present study was carried out to assess the effect of musculoskeletal symptoms on fatigue and productivity among office workers. The mean ± standard deviation of age and working hours per week were 37.91 ± 13.52 years and 44.40 ± 14.24 hours, respectively. Additionally, 55.4% of the subjects were male and the rest (44.6%) were female.

The results of the study revealed that the highest prevalence rates of musculoskeletal symptoms in the past week were related to the neck (41.6%), lower back (41.6%), and shoulders (40.6%). A previous study noted that the prevalence rate of musculoskeletal symptoms among office workers ranged from 40% to 80% [26]. Besides, Rempel et al. stated that most MSDs symptoms in office workers were reported in upper limbs, neck, and shoulders and that these disorders constituted nearly 30% of all workplace injuries [27]. Moreover, the findings of the study by Choobineh et al. showed that the prevalence rate of musculoskeletal symptoms was 56.6% in the neck, 46.7% in wrists/hands, and 44.6% in lower back among Iranian office workers in the past 12 months [18]. The results of our previous study indicated that the highest prevalence rates of MSDs were related to the lower back (45.1%), neck (41.7%), and knees (33.8%) among the studied office workers [28]. Persistence of these problems can be attributed to static and awkward postures as well as repetitive movements [29].

The findings of the present study revealed that the mean scores of musculoskeletal discomfort/pain in the neck, lower back, and shoulders were 1.67, 1.55, and 1.31, respectively. This shows that the reported symptoms of musculoskeletal problems were in accordance with the participants' perceived discomfort/pain.

The results also showed that the severity of musculoskeletal discomfort/pain in different body regions was correlated to different aspects of fatigue, including degree and severity, distress that it causes, degree of interference with activities of daily living, and timing of fatigue. Indeed, the severity of musculoskeletal discomfort/pain in

neck, shoulders, lower back, and thighs was associated with total fatigue derived from P-MAF scale. In some studies, researchers found that musculoskeletal discomfort/pain was associated with fatigue [5, 6, 30], psychosocial distress, sleep disruption [5], and stress [6]. Furthermore, the findings of previous studies have shown that holding a static and awkward posture for long periods during the work could lead to discomfort/pain and chronic fatigue [31, 32]. The findings of the research by Chavalitsakulchai and Shahnavaz indicated a close relationship between musculoskeletal discomfort/pain and fatigue among workers [33]. Another study also demonstrated that the prevalence of discomfort/pain in the lower back and neck was higher in supermarket cashiers and that subjects reported perception of high fatigue levels after work days [34].

The results of the current study showed that the severity of discomfort/pain in the neck, lower back, buttock, and thighs was correlated to the concentration/focus subscale of productivity derived from P-HWQ. In other words, as discomfort/pain severity increased, concentration/focus decreased. This reduction could eventually lead to loss of productivity.

The findings of previous studies have revealed that some aspects of productivity were related to musculoskeletal problems [35]. Moreover, it has been pointed out that discomfort/pain might have an adverse impact on several aspects of an individual's performance, such as concentration, cognitive capacity, rationality/mood, mobility, stamina, and agility, as well as physical aspect [36]. Also, the findings of studies have shown that individuals with musculoskeletal pain might suffer from psychophysiological symptoms, such as lack of concentration, insomnia, stress-related pain, ability, and other disabling conditions [37, 38].

In addition to what was mentioned above, the consequences of WMSDs are considerable for employees as well as for employers. MSDs can be related to lost working days, early retirement and unemployment (significant for employees), decline of productivity, rise in sickness payments, and staff absenteeism (significant for employers) [39]. A prior study reported that WMSDs were the biggest single factor of medical bed days and lost working days (loss of productivity) in the United States [40]. On the other hand, WMSDs negatively affect productivity because workers are not only injured when they are fatigued, but they are also inclined to decelerate working [41]. Based on Ng et al., there was a significant association between the reported prevalence rate of WMSDs and productivity loss in terms of "presenteeism" [12]. Also, Van den Heuvel et al. stated that 26% of subjects with MSDs symptoms in neck/shoulders or hands/arms reported loss of productivity [42, 43]. Manzoli et al. mentioned in their study that promotion of health in the society is the main factor for smart, sustainable, inclusive growth, which is one of the objectives of Europe 2020 Europe's growth strategy. Based on this strategy, healthy and active people have a positive impact on productivity and competitiveness. Indeed, workplace factors (physical, psychosocial, and organizational factors) have a significant impact on improving individuals' health, especially the musculoskeletal system [44].

The results of the present study indicated a direct relationship between the presence of MSDs and fatigue in individuals and that presence of these disorders could affect individuals' concentration/focus as well as productivity. Therefore, pre-employment or periodic medical examinations are recommended to be carried out in order to control WMSDs, which are the key factor contributing to increase of fatigue and loss of productivity. In this context, workplace analysis, control of risk factors, medical management, and training individuals for prevention and elimination of WMSDs are necessary.

Limitations of the study

Regarding the cross-sectional nature of the study and self-report data gathering method, the findings are to be interpreted cautiously. Moreover, this study was carried out among office workers in Shiraz. Therefore, the results may not be generalized to other office personnel and working groups.

Conclusions

Work-related musculoskeletal discomfort/pain and symptoms that mainly occur due to physical (static and poor postures, repetitive movements, non-ergonomic workstation design, etc.), psychological (stress, mental workload, etc.), and organizational (improper work-rest cycle, lack of job enrichment, etc.) factors in the workplace may result in fatigue and affect productivity among office workers. Thus, improvement of working conditions, proper organization of work, and implementation of ergonomic interventions in the workplace are recommended as necessary measures to decrease musculoskeletal discomfort/pain among office workers.

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Authors' contributions

HD: idea, data interpretation, article drafting, final approval of the article. AC: idea, data interpretation, article drafting, final approval of the article. HG: data analysis and interpretation, article drafting, final approval of the

article. MA: data gathering, article drafting, final approval of the article. AF: data gathering, article drafting, final approval of the article

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