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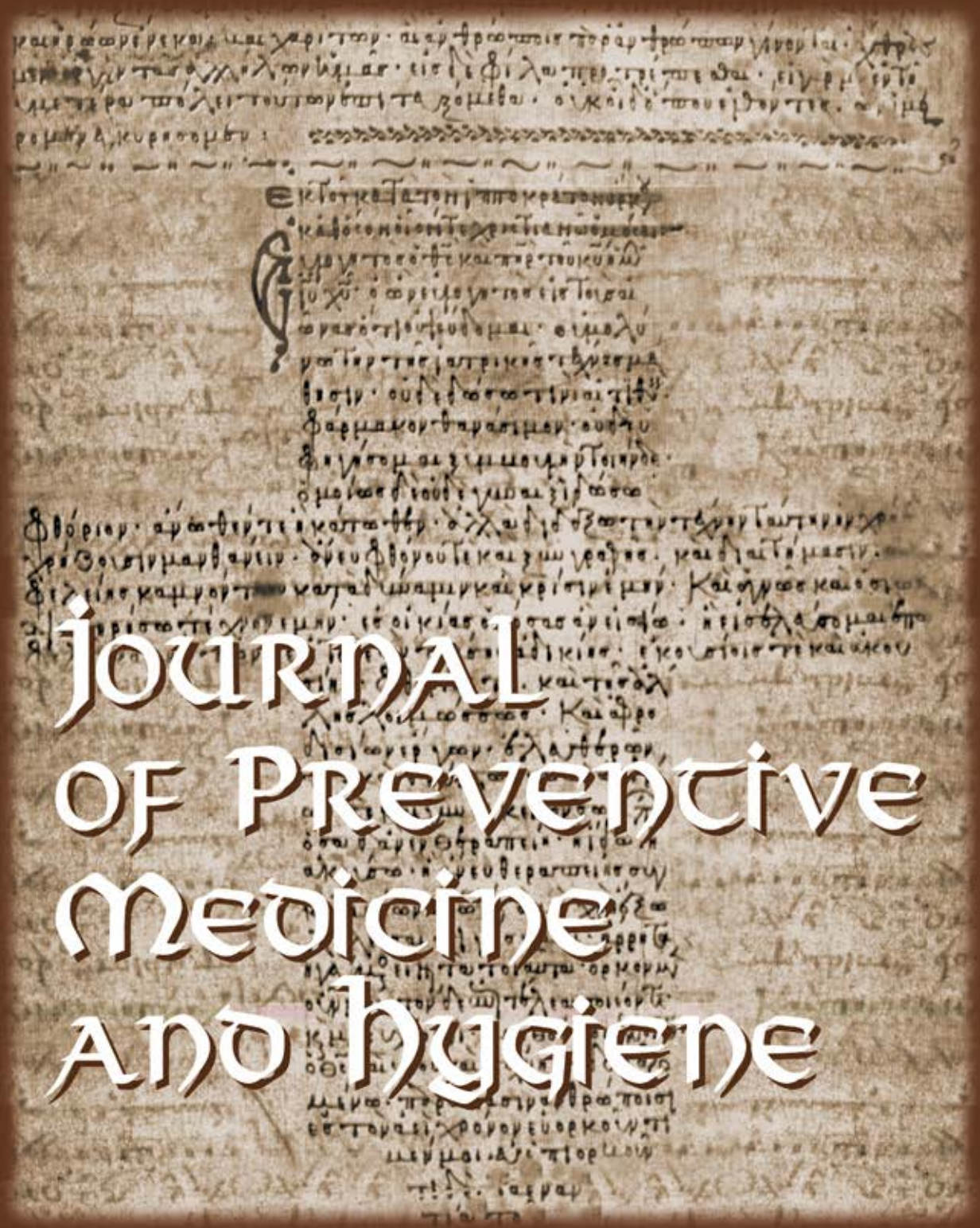

The original document of Hippocrates' oath

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CONTENTS

Original articles

ORIGINAL ARTICLE

The role of the general practitioner in the screening and clinical management of chronic viral hepatitis in six EU countries

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Keywords

General practice • Hepatitis B • Hepatitis C

Summary

Introduction. *Chronic viral hepatitis is still a major public health concern in the EU. In order to halt the progression of the disease and to prevent onward transmission, timely recognition and accurate clinical management are crucial. The aim of the present study was to investigate the role of the general practitioner (GP) in the screening of persons at risk and in the clinical management of chronic viral hepatitis patients in six EU countries.*
Methods. *An online survey among GPs and secondary-care specialists was conducted in the UK, Germany, the Netherlands, Hungary, Italy and Spain. In the GP survey, we used a four-point Likert scale to find out how commonly risk groups are screened. In both surveys, we measured GPs involvement in monitoring clinical indicators in patients undergoing antiviral treatment, and explored whether patients in four clinical scenarios are referred back to primary care.*
Results. *Between five and 10 experts per professional group were surveyed, except for Spain (GPs: n = 2; Specialists: n = 4) and, in the case of the GP survey, Hungary (GPs: n = 1) and Germany (GPs: n = 4). Migrants are variably or not routinely screened for hepatitis B/C in the majority of cases. The majority of GPs reported that hepatitis B/C screening was routinely offered to people who inject drugs. In Hungary, Italy and in the Netherlands, screening sex workers is not a regular practice. As to whether GPs offer screening to men who have sex with men, responses varied; in Germany, the Netherlands and Italy, screening was "variably" or "commonly" implemented, while in Hungary the practice seems to be sporadic. In the UK, screening for hepatitis B seems to be common practice among GPs, while hepatitis C testing is only occasionally offered to this risk group. Most GPs (> 44%) in all countries except Hungary reported that hepatitis B/C screening was very commonly offered to HIV patients.*
The role of GPs in monitoring hepatitis cases and the referral of cases back to GPs by specialists varied both within and between countries. GPs are unlikely to monitor clinical outcomes other than side effects in patients undergoing treatment. Patients who have had a sustained virological response are usually referred back to GPs, whereas patients undergoing antiviral treatment and those who do not respond to treatment are rarely referred back.
Conclusions. *The GP's decision to offer screening to risk groups often seems to be an individual choice of the healthcare professional. Raising GPs' awareness of the disease, for example through the adoption of effective strategies for the dissemination and implementation of the existing guidelines for general practice, is strongly needed. The role of GPs and specialists involved in the management of chronically infected patients should also be clarified, as opinions sometimes differ markedly even within each professional group.*

Introduction

Viral hepatitis B and C are of major public health concern in the European Union, although there are distinct geographical variations in the prevalence and incidence of viral hepatitis across countries. In the EU, the burden of disease is generally low in the north-western countries and higher in the south-eastern region: the prevalence in the general population varies from 0.4% to 5.2% for anti-HCV and from 0.1% to 5.6% for HBsAg [1, 2]. However, as there is a lack of representative data in higher-risk populations, such as migrants from countries where hepatitis is endemic [3], the true prevalence is probably higher. In order to halt the progression of the disease to advanced hepatic fibrosis, cirrhosis, and/or hepatocellular carcinoma, and to prevent onward transmission, timely recognition and accurate clinical management of the disease are of extreme importance. Both the general practitioner (GP) and the secondary-care specialist are involved in the diagnosis of chronic viral hepatitis and in the clinical management of infected patients. Several studies have explored the primary-care physician's role and experiences in treatment and shared-care with specialists in North America [4-6], in Australia [7-9] and in some parts of Asia [10, 11]. To the best of our knowledge, however, the remit of the GP in the clinical management of the disease in the EU member states has not been extensively evaluated. The aim of the present study, which is part of the EU funded Project "HEPscreen: Screening for hepatitis B and C among migrants in the European Union", was

Tab. I. Response rate by professional group and by country.

	UK n (%)	DE n (%)	NL n (%)	HU n (%)	IT n (%)	ES n (%)	Total n (%)
GPs	10 (25)	4 (10)	9 (22.5)	1 (2.5)	14 (35)	2 (5)	40 (100)
Specialists	10 (15.6)	9 (14.1)	22 (34.4)	10 (15.6)	9 (14.1)	4 (6.3)	64 (100)
Total n (%)	20 (19.2)	13 (12.5)	31 (29.8)	11 (10.6)	23 (22.1)	6 (5.8)	104 (100)

n: number of health professionals who participated in the survey.

to investigate, by means of a semi-quantitative online survey, the role of the GP in the screening of persons at risk and in the clinical management of chronic viral hepatitis patients in six EU countries: Germany, Hungary, Italy, the Netherlands, Spain and the United Kingdom.

Methods

Two semi-quantitative online surveys were developed and administered, respectively, to general practitioners (GPs) and to secondary-care specialists (SPs), i.e. gastroenterologists, hepatologists and infectious-disease specialists, working in the six EU countries. Both surveys were pilot tested, translated into the national languages of the study countries, uploaded into the open-source online software package LimeSurvey, and sent by email to healthcare professionals, who were board members of clinical associations and professional networks. Rather than reaching a large representative sample of practising clinicians, the aim was to involve 5-10 experts deemed able to reflect on practices within their specialty in both professional groups. Respondents were contacted via email in July 2012 and further reminded twice during data collection, which closed in September 2012. Data were exported from LimeSurvey to SPSS 19.2 (Inc. Chicago, IL) for descriptive analysis. In the GP survey, we aimed to find out how commonly population groups at higher risk, namely migrants from endemic countries, people who inject drugs (PWID), sex workers, men who have sex with men (MSM), HIV positive patients and patients with abnormal liver function test (LFT) results, are screened for hepatitis B/C by GPs in the six countries. To this end, we used a four-point Likert scale ("very common"; "variable or not routinely"; "rarely or never"; "unsure").

In both the GP and specialist survey, the same scale was used to determine whether GPs were involved in the clinical management of patients: specifically, whether they were involved in monitoring alanine aminotransferase (ALT), viral load and side effects in patients undergoing antiviral treatment. We also explored whether patients were referred back to primary care in four clinical/patient scenarios, i.e. i) patients not qualifying for treatment after the initial evaluation; ii) those undergoing antiviral treatment; iii) those who have a sustained virological response (SVR) due to treatment; and iv) those who are non-responders to treatment. The replies given by the two professional groups were compared.

Results

Respondent profile (Tab. I)

The respondent target of between five and 10 experts per professional group was achieved, except in the cases of Spain (GPs: n = 2; Specialists: n = 4), Hungary (GPs: n = 1) and Germany (GPs: n = 4) (Tab. I).

The majority of specialists (77%) were gastroenterologists/hepatologists; 21% were infectious-disease specialists and a small proportion were community/practice nurses. Overall, around half of the participating GPs see a few (1-10) chronic hepatitis patients per year, whereas more than 90% of the secondary-care specialists see chronic hepatitis patients on a weekly basis.

Screening by GPs for groups at higher risk (Tab. II)

Migrants from endemic countries. Results from the GP survey showed that 75% of respondents in Germany, 56% in the Netherlands, the one in Hungary and one of the two in Spain stated that it was very common to offer hepatitis B testing to migrants from endemic regions. On the other hand, approximately half of the respondents in the UK (60%) and Italy (50%) and the other respondent in Spain answered that this was not routine.

Except for Hungary, where the one respondent was unsure, most GPs in the study countries stated that they either routinely or variably offered screening for hepatitis C to migrants from endemic regions.

People who inject drugs. The majority of GPs from the UK, Germany and Italy, along with the one in Hungary and the two in Spain, reported that they routinely offered hepatitis B/C screening to PWID. In the Netherlands, although screening for hepatitis C appears to be commonly practised by GPs for PWID, screening for hepatitis B varied between very commonly (44%) or variably (44%) offering the test.

Sex workers. In Germany and the UK most GPs (75% and 70%, respectively) answered that it was very common to offer a hepatitis B test to sex workers, and the two respondents in Spain were also of this opinion. The single Hungarian GP stated that it was a variable practice. In the Netherlands, respondents were split between judgements of "very common" and "variable". In Italy, no apparent trend could be discerned.

The majority of GPs in the UK, Germany and the Netherlands, and both respondents in Spain, stated that it was very common to recommend hepatitis C testing to sex workers. In Italy, most replies were split between "very common" and "variable". The respondent in Hungary reported that it was not routinely practised.

Tab. II. Frequency of screening for hepatitis B or C for population groups at higher risk by primary-care physicians in the six countries.

Migrants						
HBV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	20%	75%	56%	100%	14%	50%
Variable or not routinely	60%	25%	22%	0%	50%	50%
Rarely or never	10%	0%	22%	0%	29%	0%
Unsure	10%	0%	0%	0%	7%	0%
HCV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	40%	75%	67%	0%	29%	50%
Variable or not routinely	30%	25%	11%	0%	57%	50%
Rarely or never	10%	0%	22%	0%	14%	0%
Unsure	20%	0%	0%	100%	0%	0%
People who inject drugs						
HBV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	90%	75%	44%	100%	64%	100%
Variable or not routinely	0%	25%	44%	0%	14%	0%
Rarely or never	0%	0%	0%	0%	14%	0%
Unsure	10%	0%	12%	0%	8%	0%
HCV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	50%	75%	67%	100%	72%	100%
Variable or not routinely	30%	25%	22%	0%	14%	0%
Rarely or never	0%	0%	11%	0%	14%	0%
Unsure	20%	0%	0%	0%	0%	0%
Sex workers						
HBV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	70%	75%	44%	0%	36%	100%
Variable or not routinely	0%	25%	45%	100%	29%	0%
Rarely or never	0%	0%	11%	0%	14%	0%
Unsure	30%	0%	0%	0%	21%	0%
HCV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	60%	75%	56%	0%	36%	100%
Variable or not routinely	20%	25%	33%	100%	43%	0%
Rarely or never	0%	0%	11%	0%	14%	0%
Unsure	20%	0%	0%	0%	7%	0%

Tab. II.

Men who have sex with men						
HBV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	60%	50%	44%	0%	36%	0%
Variable or not routinely	20%	50%	56%	100%	36%	50%
Rarely or never	0%	0%	0%	0%	21%	0%
Unsure	20%	0%	0%	0%	7%	50%
HCV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	20%	50%	44%	0%	36%	50%
Variable or not routinely	50%	50%	45%	100%	43%	50%
Rarely or never	0%	0%	11%	0%	21%	0%
Unsure	30%	0%	0%	0%	0%	0%
Patients with HIV						
HBV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	80%	75%	67%	0%	79%	50%
Variable or not routinely	0%	25%	22%	100%	0%	50%
Rarely or never	0%	0%	0%	0%	7%	0%
Unsure	20%	0%	11%	0%	14%	0%
HCV	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	50%	75%	44%	0%	71%	100%
Variable or not routinely	30%	25%	22%	100%	14%	0%
Rarely or never	0%	0%	11%	0%	7%	0%
Unsure	20%	0%	23%	0%	8%	0%
Screening for hepatitis B for patients with abnormal liver funcion test results						
1ST Abnormal Test Results	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	40%	50%	44%	100%	64%	50%
Variable or not routinely	50%	50%	45%	0%	22%	0%
Rarely or never	0%	0%	11%	0%	14%	50%
Unsure	10%	0%	0%	0%	0%	0%
2ND Abnormal Test Results	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	60%	100%	89%	100%	64%	50%
Variable or not routinely	30%	0%	11%	0%	36%	0%
Rarely or never	0%	0%	0%	0%	0%	50%
Unsure	10%	0%	0%	0%	0%	0%

Tab. II.

Screening for hepatitis C for patients with abnormal liver funcion test results						
1ST Abnormal Test Results	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	40%	50%	33%	100%	64%	50%
Variable or not routinely	40%	50%	34%	0%	29%	50%
Rarely or never	0%	0%	33%	0%	7%	0%
Unsure	20%	0%	0%	0%	0%	0%
2ND Abnormal Test Results	UK	DE	NL	HU	IT	ES
	(n = 10)	(n = 4)	(n = 9)	(n = 1)	(n = 14)	(n = 2)
Very common	60%	100%	56%	100%	79%	50%
Variable or not routinely	20%	0%	33%	0%	22%	50%
Rarely or never	0%	0%	11%	0%	0%	0%
Unsure	20%	0%	0%	0%	0%	0%

Men who have sex with men. As to whether GPs offer screening to MSM, replies indicated that it was "variably" and "commonly" practised in Germany, the Netherlands and Italy, while in Hungary it seems to be a sporadic practice. In the UK, while screening for hepatitis B seems to be common practice among GPs, the majority view is that hepatitis C testing is offered only occasionally to MSM.

Patients with HIV. Most GPs (> 44%) in all countries reported that it was very common practice to offer hepatitis B/C screening to HIV patients, except in Hungary, where the respondent stated that screening was not routinely offered.

Patients with abnormal liver function test results. A first abnormal LFT result would very commonly prompt approximately half of the GP respondents in each country and the one in Hungary to screen a patient for hepatitis B, while the others stated that this would not routinely be the case. On the other hand, a second abnormal LFT result would alert most GPs to recommend a hepatitis B test to their patients. While a first abnormal LFT result would only lead half of the GPs to request a hepatitis C test, apart from Italy and Hungary, where most would ask for a hepatitis C test, a second or repeat abnormal LFT would prompt the majority of GPs in all countries to screen for hepatitis C.

The involvement of GPs in the clinical management of the disease (Tab. III)

ALT. In Germany, the majority of respondents indicated that it was very common for GPs to monitor ALT in patients undergoing antiviral treatment. A similar, but less marked, trend could be seen in Italy, where over half the GPs selected "very common". GPs in Spain appeared to be involved in monitoring ALT variably according to the vast majority of respondents. The trends in these three countries contrast with that observed in the Netherlands, where nearly three quarters (71%) indicated that GPs were rarely or never involved in monitoring ALT. In the UK and in Hungary, over half (55%) indicated that GPs were rarely or never involved, the remaining replies being distributed across the other answer options.

Viral load. The results from both the GPs' and specialists' surveys show that, in the UK, the Netherlands, Hungary and Spain, most GPs are rarely or never involved in monitoring viral load among patients undergoing antiviral treatment. Also in Italy, despite the diverse spread of opinion, the largest proportion (39%) indicated that GPs were rarely or never involved. In Germany, a slight trend towards "very common" was observed.

Side effects. A diversity of opinion emerged from both surveys in most countries. The clearest picture emerged from Germany, where the majority view (62%) was that GPs were very commonly involved in monitoring side effects. The dominant view in Italy was more towards very common (35%) or variable (52%) monitoring of side effects by GPs, whereas the majority view inclined more towards variable to rarely or never in the UK, the

Tab. III. GPs' involvement in monitoring clinical indicators and side effects of antiviral treatment.

GPs involvement in monitoring ALT						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	20%	75%	0%	100%	64%	50%
Variable or not routinely	40%	0%	22%	0%	21%	50%
Rarely or never	20%	0%	56%	0%	14%	0%
Unsure	20%	25%	22%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	0%	56%	0%	10%	33%	0%
Variable or not routinely	10%	11%	23%	10%	67%	100%
Rarely or never	90%	11%	77%	60%	0%	0%
Unsure	0%	22%	0%	20%	0%	0%
COMBINED RESULTS	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	10%	62%	0%	18%	52%	17%
Variable or not routinely	25%	8%	23%	9%	39%	83%
Rarely or never	55%	8%	71%	55%	9%	0%
Unsure	10%	23%	6%	18%	0%	0%

Tab. III.

GPs involvement in monitoring viral load						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	0%	50%	0%	0%	36%	0%
Variable or not routinely	30%	25%	11%	0%	43%	50%
Rarely or never	50%	25%	67%	100%	21%	50%
Unsure	20%	0%	22%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	0%	33%	0%	0%	22%	0%
Variable or not routinely	0%	22%	0%	10%	11%	25%
Rarely or never	100%	22%	100%	70%	67%	75%
Unsure	0%	22%	0%	20%	0%	0%
COMBINED RESULTS	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	0%	38%	0%	0%	30%	0%
Variable or not routinely	15%	23%	3%	9%	30%	33%
Rarely or never	75%	23%	90%	73%	39%	67%
Unsure	10%	15%	6%	18%	0%	0%
GPs involvement in monitoring side effects						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	10%	75%	0%	0%	50%	100%
Variable or not routinely	50%	0%	22%	100%	36%	0%
Rarely or never	20%	25%	56%	0%	14%	0%
Unsure	20%	0%	22%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	10%	56%	0%	10%	11%	0%
Variable or not routinely	40%	11%	46%	20%	78%	50%
Rarely or never	50%	11%	55%	40%	11%	50%
Unsure	0%	22%	0%	30%	0%	0%
COMBINED RESPONSES	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	10%	62%	0%	9%	35%	33%
Variable or not routinely	45%	8%	39%	27%	52%	33%
Rarely or never	35%	15%	55%	36%	13%	33%
Unsure	10%	15%	7%	27%	0%	0%

Netherlands and Hungary. In Spain no majority opinion emerged.

Referral back to GPs/primary care from specialist secondary care (Tab. IV)

Patients who do not qualify for treatment after an initial evaluation. In the Netherlands and in Spain, the majority of respondents in both surveys agreed that patients who do not qualify for treatment after an initial evaluation are only variably or not routinely referred back to primary-care practitioners. Specialists' opinion was in contrast with that of GPs in the UK, where 90% of specialists (*vs* 10% of GPs) indicated that these patients were rarely or never referred back to Gps. In Italy, although the majority of specialists (56%) selected "variable", around one third indicated "rarely or never", while 57% of GPs selected "very common". In Germany, the majority opinion was divided between patients being very commonly (54%) and variably (39%) referred back to GPs. In Hungary, no dominant opinion could be observed.

Patients undergoing antiviral treatment. Overall, the majority of respondents in all countries (84% in the Netherlands, 83% in Spain, 65% in the UK, 64% in Hungary, 48% in Italy and 38% in Germany) stated that patients undergoing antiviral treatment were rarely or never referred back to GPs. Only in Germany and Italy did the majority of GPs (75% and 57%, respectively) indicate that these patients were very commonly referred back to GPs, although around one quarter selected "rarely or never". In Germany, although 44% of specialists reported "rarely or never" referring back patients undergoing antiviral treatment, the same percentage indicated that referral was variable. In Italy, 78% of secondary-care specialists indicated that these patients were rarely or never referred back to GPs (while 57% of GPs stated that it was very common).

Patients with a sustained virological response due to treatment. In the UK, despite divergent opinions from GPs, most GP and specialist respondents reported that referral back to GPs was very common for patients who have SVR on account of treatment. This was also the dominant opinion in Germany (61%) and in the Netherlands (49%), where, however, 42% stated that patients with these characteristics were variably or not routinely referred back to GPs. In Hungary, opinion was divided between "very commonly" and "rarely or never". In Ita-

Tab. IV. Frequency of referral back to GPs for: i) patients who do not qualify for treatment after the initial evaluation; ii) patients undergoing antiviral treatment; iii) patients with sustained virological response due to treatment; and iv) patients who are non-responders to treatment.

Patients who do not qualify for treatment after initial evaluation						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	50%	50%	33%	100%	57%	50%
Variable or not routinely	10%	50%	56%	0%	21%	0%
Rarely or never	10%	0%	11%	0%	21%	50%
Unsure	30%	0%	0%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	10%	56%	14%	20%	11%	0%
Variable or not routinely	0%	33%	59%	30%	56%	100%
Rarely or never	90%	0%	27%	30%	33%	0%
Unsure	0%	11%	0%	20%	0%	0%
COMBINED RESULTS	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	30%	54%	19%	27%	39%	17%
Variable or not routinely	5%	39%	58%	27%	35%	67%
Rarely or never	50%	0%	23%	27%	26%	17%
Unsure	15%	8%	0%	18%	0%	0%
Patients undergoing antiviral treatment						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	0%	75%	11%	0%	57%	0%
Variable or not routinely	40%	0%	22%	0%	14%	0%
Rarely or never	40%	25%	56%	100%	29%	100%
Unsure	20%	0%	11%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	10%	0%	0%	10%	22%	25%
Variable or not routinely	0%	44%	5%	0%	0%	0%
Rarely or never	90%	44%	95%	60%	78%	75%
Unsure	0%	11%	0%	30%	0%	0%
COMBINED RESULTS	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	5%	23%	3%	9%	43%	17%
Variable or not routinely	20%	31%	10%	0%	9%	0%
Rarely or never	65%	38%	84%	64%	48%	83%
Unsure	10%	8%	3%	27%	0%	0%

ly, although the majority judged referral to be very common, one third selected "rarely or never" and one quarter "variably or not routinely". Opinion was also divided in Spain, where half of the respondents selected "rarely or never", one third "very common", and 17% "variably or not routinely".

Non-responders to treatment. Non-responders to treatment are rarely or never referred back to GPs, according to the majority of respondents in all countries except Italy, where 44% stated that referral back to GPs was very common for these patients (the percentage was higher among GPs: 64%). In Germany, most reported that referral back to the GP occurred variably or not routinely.

Discussion

In patients with chronic viral hepatitis, shared management based on close collaboration between the GP and the specialist physician, through the identification of their respective tasks, is necessary for the appropriate diagnostic and therapeutic management of the patient along the care pathway. Since most people with chronic hepatitis are asymptomatic until cirrhosis or hepatocellular carcinoma are established, the initial diagnosis and management of chronic hepatitis relies on primary-care physicians to identify and screen high-risk individuals [12]. The GP can contribute significantly by promptly identifying and screening of those at risk, by providing counselling and information, by referring the patient to the specialist for disease staging and also by liaising/cooperating with the hospital services involved in the specialist management of patients.

Non-uniform practices are likely to create or exacerbate health inequalities, and might be an important cause of the "under-treatment" phenomenon, i.e. the disparity between the number of chronic hepatitis patients and the number of patients actually receiving treatment [13]. To our knowledge, this is the first study conducted contemporarily in six EU countries with the aim of investigating the role of the GP in the screening practices for risk groups and in the clinical management of chronic viral hepatitis patients. Given the careful selection of the survey participants and national representatives of the experts in their respective fields, it may justifiably

Tab. IV.

Patients with sustained virological response due to treatment						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	10%	50%	22%	0%	57%	0%
Variable or not routinely	30%	25%	56%	0%	14%	0%
Rarely or never	20%	25%	11%	100%	29%	100%
Unsure	40%	0%	11%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	70%	67%	59%	40%	22%	50%
Variable or not routinely	10%	22%	36%	0%	44%	25%
Rarely or never	10%	0%	5%	30%	33%	25%
Unsure	10%	11%	0%	30%	0%	0%
COMBINED RESULTS	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	40%	61%	49%	36%	44%	33%
Variable or not routinely	20%	23%	42%	0%	26%	17%
Rarely or never	15%	8%	6%	36%	30%	50%
Unsure	25%	8%	3%	27%	0%	0%
Patients non-responders to treatment						
ACCORDING TO GPs	UK (n = 10)	DE (n = 4)	NL (n = 9)	HU (n = 1)	IT (n = 14)	ES (n = 2)
Very common	20%	50%	11%	0%	64%	0%
Variable or not routinely	20%	25%	44%	100%	21%	0%
Rarely or never	20%	25%	22%	0%	14%	100%
Unsure	40%	0%	22%	0%	0%	0%
ACCORDING TO SPECIALISTS	UK (n = 10)	DE (n = 9)	NL (n = 22)	HU (n = 10)	IT (n = 9)	ES (n = 4)
Very common	10%	11%	0%	0%	11%	25%
Variable or not routinely	0%	44%	0%	10%	33%	0%
Rarely or never	90%	33%	100%	50%	55%	75%
Unsure	0%	11%	0%	40%	0%	0%
COMBINED RESULTS	UK (n = 20)	DE (n = 13)	NL (n = 31)	HU (n = 11)	IT (n = 23)	ES (n = 6)
Very common	15%	23%	3%	0%	44%	17%
Variable or not routinely	10%	38%	13%	18%	26%	0%
Rarely or never	55%	31%	77%	46%	30%	83%
Unsure	20%	8%	7%	36%	0%	0%

be assumed that the replies gathered provide a fair picture of the GP's remit in the countries considered. However, caution is needed in interpreting the results where the respondent target of five to ten experts could not be reached (in Spain and Hungary).

According to our results, the GP's role and referral back to GPs vary within and between countries. What seems certain is that GPs are unlikely to monitor any clinical outcomes (such as viral load) other than some side effects in patients undergoing treatment, indicating that this is considered the remit of specialists in secondary care.

Results from a Turkish study showed that GPs were not able to follow up chronic viral hepatitis B and C patients because of their limited awareness of diagnostic facilities and treatment options [14]. Indeed, while the majority of GPs had adequate knowledge of HBV and HCV transmission and of risk factors, a low percentage was well informed about the treatment of chronic patients with elevated ALT. In particular, the Turkish study identified gaps in GPs knowledge of the appropriate use of diagnostic tests and interventions to identify and manage patients with chronic viral hepatitis. The authors concluded that further coordination with secondary-care specialists was warranted in order to ensure that patients were followed up in the primary-care setting [14].

Strategic programmes of health education and awareness-raising, for both professionals and risk groups, should be established. In the EU, two different strategies are used to identify persons with HBV or HCV infection: population screening and healthcare provider-initiated testing (based on identified risk-factors). Population screening is not cost-effective, owing to the low prevalence of HBV and HCV infections in the general EU population, while the healthcare provider-initiated identification of HBV or HCV infection among defined risk groups is a valuable instrument in secondary prevention. Making GPs aware of risk factors, such as demographic, behavioural, occupational and medical risk factors, and clinical signs or symptoms of hepatitis, may efficiently improve case identification. Patients with chronic HBV or HCV infection should be referred for medical care and case-management, and those testing negative but with risk factors for acquiring HBV or HCV infection should receive counselling on prevention (those at risk of HBV infection should also be offered vaccination) [15].

In the USA, approaches to the screening, diagnosis and management of viral hepatitis patients vary considerably among primary-care physicians. Indeed, studies in the USA have shown deficiencies in the way some primary-care providers diagnose, treat or refer patients with HCV [16-23]. One such study investigated the association between the characteristics of the physician or practice and screening and treatment for HCV infection: more experienced physicians (longer in practice) and those based in affluent, suburban settings were more likely to order ALT tests [16]. In another study, a cross-sectional mail survey of 217 family physicians revealed insufficient levels of knowledge about screening and counselling for chronic hepatitis and hepatocellular carcinoma; in addition, around half of the physicians referred patients with chronic HBV or HCV to the specialist for further management [12]. Our results show that patients who have had a sustained virological response are generally referred back to the GP, while patients undergoing antiviral treatment and those who do not respond to treatment are rarely referred back to primary care.

As new treatment options, especially for hepatitis C, have become available in recent years, access and adherence to treatment are important determinants of the success of screening programmes [2].

Since 2012, population-based anti-HCV screening of all adults born between 1945 and 1965 has been recommended in the USA, where the prevalence of anti-HCV is highest in black non-Hispanics (6.42%) and in Mexican Americans (3.26%) [24]. In the EU, particular attention should be paid to providing screening and treatment for hepatitis B and C for migrant groups at high risk of chronic infection. The adoption of a targeted screening and treatment programme in primary care could be an effective strategy. Results from our study in the UK, suggest that standard screening practices are lacking, and allude to a shared role for GPs in the clinical monitoring of ALT, viral load and side effects. Referral back to the GP of patients undergoing antiviral treatment is not common, although GPs and specialists differed markedly in their estimates of the frequency of referral back to GPs of patients who do not qualify for treatment. In a recent UK study, GPs expressed concerns about screening and treating patients in primary care, considering their workload and also the sustainability of such a strategy [25]. Immigrants mentioned practical barriers, such as language and communication difficulties, limited time on account of long working hours, and, in some cases, limited trust and confidence in general practice-based care [25].

Indeed, chronic hepatitis B and C infections are often undiagnosed in primary care. According the 'Hepatitis B and C surveillance in Europe – 2012' report, in the minority of cases in which information on the testing facility was available, 27% of hepatitis B and 21% of hepatitis C cases were diagnosed in general practice [26]. One German study, involving 21,008 subjects, reported that the prevalence of HBsAg, anti-HCV and HCV-RNA was 0.52%, 0.95%, and 0.43%, respectively. Infections were previously unknown in 85% and 65% of HbsAg- and anti-HCV-positive individuals, respectively [27]. German hepatitis B and C treatment guidelines recommend HBsAg and anti-HCV screening in several pre-defined risk groups. According to the participants in our survey, most GPs in Germany report commonly screening population groups at higher risk. The management of patients undergoing treatment seems to be shared between GPs and specialists. Easy to apply guidelines with defined risk scenarios may help to diagnose previously unknown infections [27]. Previous results from the HEPscreen Project showed that the availability of training programmes to improve skills and knowledge of viral hepatitis differed across the six EU countries. Among the experts interviewed (268 health professionals), 80% and 73% were aware of hepatitis B and hepatitis C guidelines, respectively, in their country [28]. The findings of the present study could provide impetus to the formulation of precise and clear guidelines targeting primary-care physicians and secondary-care specialists. These should explicitly specify, in a shared-care model, the different responsibilities in the management of chronic hepatitis patients, so as to deliver more effective healthcare.

Conclusions

Although the GP's role in the screening and clinical management of chronic viral hepatitis is crucial to timely diagnosis and linkage to specialist care, the diversity of responses often observed suggests inadequate awareness of explicit recommendations, which results in a lack of uniform practices among experts. The GP's decision to offer screening to risk groups often seems to be an individually motivated choice of the healthcare professional. The inconsistencies observed in screening practices may mean that many chronic infections remain undetected. This underscores the need to raise GPs' awareness of this silent epidemic, for example through the adoption of effective strategies for the dissemination and implementation of the existing guidelines for general practice. The role of GPs and specialists involved in the management of chronically infected patients should also be clarified, as opinions sometimes differed markedly even within each professional group.

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Conflicts of interest: none declared

Disclaimer

Responsibility for the information and views set out in this study lies entirely with the authors. The European Commission is not responsible for any use that may be made of the information contained herein.

Declarations

Ethical approval: ethical approval was not required. All healthcare professionals identified as potential participants in the survey received written information about the project and its aims, and were subsequently invited to participate. We stressed that participation in this study was voluntary and withdrawal from the study was possible at any time. The anonymity of participants was maintained throughout.

Authors' contributions

AB and ML study concept and design, literature search, acquisition of data, analysis and interpretation of data, drafting of the manuscript; AF and AA interpretation of data, critical revision of the manuscript for important intellectual content; IV and ET critical revision of the manuscript for important intellectual content; PB critical revision of the manuscript for important intellectual content, supervised the study, obtained funding; all authors revised the manuscript and contributed to improving the paper; all authors read and approved the final manuscript.

References

[1] Hahné SJM, Veldhuijzen IK, Wiessing L, Lim T-A, Salminen M, Laar M van de. *Infection with hepatitis B and C virus in Europe: a systematic review of prevalence and cost-effectiveness of screening*. BMC Infect Dis 2013;13:181. doi: 10.1186/1471-2334-13-181.

[2] European Centre for Disease Prevention and Control. *Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies*. Stockholm: ECDC; 2010. Available at: http://www.ecdc.europa.eu/en/publications/Publications/TER_100914_Hep_B_C%20_EU_neighbourhood.pdf [Accessed 10/10/2015]

[3] European Centre for Disease Prevention and Control. *Assessing the burden of key infectious diseases affecting migrant populations in the EU/EEA*. Stockholm: ECDC; 2014. Available at: http://ecdc.europa.eu/en/publications/Publications/assessing-burden-disease-migrant populations.pdf [Accessed 10/10/2015]

[4] Sarkar M, Shvachko VA, Ready JB, Pauly MP, Terrault NA, Peters MG, Manos MM. *Characteristics and management of patients with chronic hepatitis B in an integrated care setting*. Dig Dis Sci 2014;59:2100-8. doi: 10.1007/s10620-014-3142-2.

[5] Burman BE, Mukhtar NA, Toy BC, Nguyen TT, Chen AH, Yu A, Berman P, Hammer H, Chan D, McCulloch CE, Khalili M. *Hepatitis B management in vulnerable populations: Gaps in disease monitoring and opportunities for improved care*. Dig Dis Sci 2014;59:46-56. doi: 10.1007/s10620-013-2870-z.

[6] Clark EC, Yawn BP, Galliher JM, Temte JL, Hickner J. *Hepatitis C identification and management by family physicians.* Fam Med 2005;37:644-9.

[7] Wallace J, McNally S, Richmond J, Hajarizadeh B, Pitts M. *Challenges to the effective delivery of health care to people with chronic hepatitis B in Australia.* Sex Health 2012;9:131-7.

[8] Dev A, Nguyen JNH, Munafo L, Hardie E, Iacono L. *Chronic hepatitis B: a clinical audit of GP management.* Aust Fam Physician 2011;40:533-8.

[9] Wallace J, Hajarizadeh B, Richmond J, McNally S. *Challenges in managing patients in Australia with chronic hepatitis B: the General Practitioners' perspective*. Aust N Z J Public Health 2013;37:405-10. http://doi.wiley.com/10.1111/1753-6405.12127.

[10] Peksen Y, Canbaz S, Leblebicioglu H, Sunbul M, Esen S, Sunter AT. *Primary care physicians' approach to diagnosis and treatment of hepatitis B and hepatitis C patients*. BMC Gastroenterol 2004;4:3.

[11.] Ren J-J, Liu Y, Ren W, Qiu Y, Wang B, Chen P, Xu KJ, Yang SG, Yao J, Li LJ. *Role of general practitioners in prevention and treatment of hepatitis B in China*. Hepatobiliary Pancreat Dis Int 2014;13:495-500.

[12] Ferrante JM, Winston DG, Chen PH, de la Torre AN. *Family physicians' knowledge and screening of chronic hepatitis and liver cancer.* Fam Med 2008;40:345-51.

[13] Cohen C, Holmberg SD, McMahon BJ, Block JM, Brosgart CL, Gish RG, London WT, Block TM. *Is chronic hepatitis B being undertreated in the United States?* J Viral Hepat 2011;18:377-83.

[14] Peksen Y, Canbaz S, Leblebicioglu H, Sunbul M, Esen S, Sunter AT. *Primary care physicians' approach to diagnosis and treatment of hepatitis B and hepatitis C patients*. BMC Gastroenterology 2004;4:3.

[15] Fretz R, Negro F, Bruggmann P, Lavanchy D, De Gottardi A, Pache I, Masserey Spicher V, Cerny A. *Hepatitis B and C in Switzerland – healthcare provider initiated testing for chronic hepatitis B and C infection*. Swiss Med Wkly 2013;143:w13793.

[16] Nicklin DE, Schultz C, Brensinger CM, Wilson JP. *Current care of hepatitis C-positive patients by primary care physicians in an integrated delivery system*. J Am Board Fam Pract 1999;12:427-35.

[17] Shehab TM, Sonnad SS, Lok AS. *Management of hepatitis C patients by primary care physicians in the USA: results of a national survey*. J Viral Hepat 2001;8:377-83. http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2893.2001.00310.x/abstract

[18] Shehab TM, Sonnad S, Gebremariam A, Schoenfeld P. *Knowledge of hepatitis C screening and management by internal medicine residents: trends over 2 years*. Am J Gastroenterol 2002;97:1216-22.

[19] Navarro VJ, St Louis TE, Bell BP. *Identification of patients with hepatitis C virus infection in New Haven County primary care practices.* J Clin Gastroenterol 2003;36:431-5.

[20] Shehab TM, Orrego M, Chunduri R, Lok AS. *Identification and management of hepatitis C patients in primary care clinics.* Am J Gastroenterol 2003;98:639-44.

[21] Coppola AG, Karakousis PC, Metz DC, Go MF, Mhokashi M, Howden CW, Raufman JP, Sharma VK. *Hepatitis C knowledge among primary care residents: is our teaching adequate for the times?* Am J Gastroenterol 2004;99:1720-5.

[22] Rocca LG, Yawn BP, Wollan P, Kim WR. *Management of patients with hepatitis C in a community population: diagnosis, discussions, and decisions to treat.* Ann Fam Med 2004;2:116-24.

[23] Clark EC, Yawn BP, Galliher JM, Temte JL, Hickner J. *Hepatitis C identification and management by family physicians.* Fam Med 2005;37:644-9.Gastroenterol 2004;4:3.

[24] Smith BD, Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Ward JW. *Hepatitis C virus testing of persons born during*

1945–1965: recommendations from the Centers for Disease Control and Prevention. Ann Intern Med 2012;157:817-22.

[25] Sweeney L, Owiti JA, Beharry A, Bhui K, Gomes J, Foster GR, Greenhalgh T. *Informing the design of a national screening and treatment programme for chronic viral hepatitis in primary care: qualitative study of at-risk immigrant communities and healthcare professionals*. BMC Health Serv Res 2015;15:97.

[26] European Centre for Disease Prevention and Control. *Hepatitis B and C surveillance in Europe.* 2012. Stockholm: ECDC; 2014. Available at: http://ecdc.europa.eu/en/publications/Publications/hepatitis-b-c-surveillance-europe-2012-july-2014.pdf [Accessed 10/10/2015].

[27] Wolffram I, Petroff D, Bätz O, Jedrysiak K, Kramer J, Tenckhoff H, Berg T, Wiegand J; German Check-Up 35+ Study Group. *Prevalence of elevated ALT values, HBsAg, and anti-HCV in the primary care setting and evaluation of guideline defined hepatitis risk scenarios*. J Hepatol 2015;62:1256-64.

[28] Bechini A, Falla A, Ahmad A, Veldhuijzen I, Boccalini S, Porchia B, Levi M. *Identification of hepatitis B and C screening and patient management guidelines and availability of for chronic viral hepatitis among health professionals in six European countries: results of a semi-quantitative survey*. BMC Infect Dis 2015;15:353.

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ORIGINAL ARTICLE

Impact of pneumococcal conjugate vaccination: a retrospective study of hospitalization for pneumonia in North-East Italy

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Keywords

Hospitalization • Epidemiology • PCV • Pneumonia • Streptococcus Pneumoniae • Vaccination

Summary

Introduction. *Pneumonia remains a common reason for hospitalizing infants and the elderly worldwide, and streptococcal infection is often responsible. The aim of this study was to assess the burden of pneumonia in a large general population.*
Methods. *All pneumonia-related hospitalizations from 2004 to 2013 in north-east Italy were identified from the hospital records with a first-listed diagnosis on discharge of bacterial pneumonia, or a first-listed diagnosis on discharge of meningitis, septicemia or empyema associated with a secondary diagnosis of bacterial pneumonia. We identified major comorbidities, calculated age-specific case-fatality rates (CFR), and estimated the related cost to the health care system.*
Results. *Of the 125,722 hospitalizations identified, 96.9% were cases of pneumonia, 2.4% of septicemia, 0.4% of meningitis, and 0.3% of empyema; 75.3% of hospitalizations involved ≥ 65-year-olds. The overall CFR was 12.4%, and it increased with age, peaking in people over 80 (19.6%).*
The mean annual pneumonia-associated hospitalization rate was 204.6 per 100,000 population, and it peaked in 0- to 4-year-old children (325.6 per 100,000 in males, 288.9 per 100,000 in females), and adults over 65 (844.9 per 100,000 in males, 605.7 per 100,000 in females).
Hospitalization rates dropped over the years for the 0-4 year-olds, and rose for people over 80. The estimated overall annual cost of these pneumonia-related hospitalizations was approximately € 41 million.
Conclusions. *This study shows that the burden on resources for pneumonia-related hospitalization is an important public health issue. Prevention remains the most valuable tool for containing pneumonia, and vaccination strategies can help in the primary prevention of infection, possibly reducing the number of cases in all age groups.*

Introduction

Pneumonia remains a common reason for the hospitalization of infants and elderly adults [1-3]. The disease can be caused by a variety of micro-organisms, but the pathogen most often responsible for pneumonia is the bacterium *Streptococcus pneumoniae*, especially in industrialized countries [4, 5].
Pneumonia pathogens may be reported in different ways, depending on the capabilities of in-hospital laboratories, how studies on them are designed, and the season or region in which studies are conducted [6]. They can cause a spectrum of diseases of variable severity and, when the organism invades normally sterile sites such as the bloodstream and meninges, the resulting forms are classified as invasive pneumococcal disease (IPD) [1].
Pneumococcal infections in adults can be a cause of community-acquired pneumonia (CAP), a respiratory disease with a high prevalence in the general population, a marked clinical heterogeneity, and a variable severity [2]. CAP remains a leading cause of morbidity and about 30% of CAP patients require hospitalization.
The introduction of a 7-valent pneumococcal conjugate vaccine (PCV7) that targets seven serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) as part of children's national immunization programs has led to a reduction in the hospital admission rates for all-cause pneumonia, particularly among children. A herd effect has also been reported in some countries [7-10].
The arrival on the market of more valent conjugate vaccines (10-valent and 13-valent) and the availability of 13-valent pneumococcal conjugate vaccine (PCV13) for adults, offers new opportunities to prevent the diseases caused by *S. pneumoniae* [11].
An immunization program based on a 7-valent pneumococcal conjugate vaccine (PCV7) was first introduced in the Veneto and Friuli Venezia Giulia regions of north-east Italy in the early 2000s as an optional vaccination only for infants at risk (at 2-3 and 14 months old). The program was subsequently extended to offer vaccination to all children in the Veneto and Friuli Venezia Giulia regions in 2005 and 2009, respectively. In 2010, PCV13 was adopted in both regions instead of the PCV7 vaccine, and a

catch-up program was implemented for infants up to 36 months old.

The present study investigated the pneumonia potentially preventable by vaccination, considering hospitalization for pneumonia and for meningitis, septicemia and empyema associated with pneumonia. We estimated: i) the pneumonia-related hospitalization (PRH) rate; ii) the age-specific case-fatality rate (CFR); iii) the trends of the PRH rates by PCV7 or PCV13 vaccination period for each region; and iv) the economic cost of PRH.

Methods

Setting and data source

We conducted this retrospective study in the Veneto and Friuli Venezia Giulia regions, which have populations of 4.9 and 1.2 million, respectively. We analyzed the hospital discharge records (HDRs) of public and accredited private hospitals in the two regions from 1 January 2004 to 31 December 2013. HDRs include the following data: details of the hospital; patient's name, date of birth, gender, place of residence, and date of admission; surgical and other procedures; and date and type of discharge. Each HDR contained one primary diagnosis (or first-listed diagnosis) and up to five secondary diagnoses based on the diagnostic codes of the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). Generally speaking, the first-listed diagnosis concerns the main condition identified during the patient's hospital stay, while other diagnoses indicate associated or contributing conditions (comorbidities and/or complications).

Inclusion and exclusion criteria

The PRH rate was calculated considering all hospitalizations identified from the HDRs as having a first-listed diagnosis on discharge of bacterial pneumonia potentially preventable by vaccination, or a first-listed diagnosis on discharge of meningitis, septicemia, or empyema associated with a secondary diagnosis of bacterial pneumonia potentially preventable by vaccination. Table I shows the diagnostic codes used as inclusion criteria. Only HDRs concerning residents of the Veneto or Friuli Venezia Giulia regions were considered. Day hospital admissions were disregarded for the purpose of this analysis.

Comorbidity

We obtained information on comorbid conditions from all hospital diagnoses mentioned in the HDRs. The ICD-9-CM diagnostic codes were selected on the basis of the current recommendations for pneumococcal vaccination in the Italian vaccination program [12] (see Table II). The presence of at least one of these comorbidity codes was used to classify patients by specific comorbidities.

Pneumonia-related hospitalization rate

Based on the total number of hospital admissions concerning Veneto and Friuli Venezia Giulia residents in each year considered, annual hospitalization rates were calculated by dividing the annual number of hospitalizations by the population in the year considered, according to the Veneto Regional Authority's statistical office, and expressing the rates as hospitalizations per 100,000 population. For patients readmitted within 30 days, only the first hospital stay was considered when calculating the hospitalization rate. The length of hospital stays was calculated as the days elapsing between the dates of admission and discharge, and the mean hospital stay was calculated. The case-fatality rate (CFR) was calculated by dividing the number of in-hospital deaths by the number of patients hospitalized with a diagnosis of pneumonia-related diseases, expressed as a percentage. Only first hospital stays were considered.

Temporal trends of the PRH rate by vaccination period

In the Veneto, the vaccination coverage for 24-month-old infants was low (10-40%) up until 2005, then rapidly increased from 2006 to 2010 (70-80%), and has exceeded 80% since 2011. In Friuli Venezia Giulia, the coverage rate was low up until 2009, then reached 70-80% in 2009, and has exceeded 80% since 2010.

Tab. I. Diagnostic codes used for hospital discharge record selection.

Pneumonia-related hospitalization Discharge Diagnoses Group	International Classification of Diseases 9 – Clinical Modification Codes	
	First-listed discharge diagnosis	Another discharge diagnosis
Pneumococcal pneumonia [*S. pneumoniae* pneumonia]	481	
Unspecified pneumonia [pneumonia without a causative organism identified]	485-487; 482.9	
Meningitis	321, 013.0, 003.21, 036.0, 036.1, 047, 047.0, 047.1, 047.8, 047.9, 049.1, 053.0, 054.72, 072.1, 091.81, 094.2, 098.82, 100.81, 112.83, 114.2, 115.01, 115.11, 115.91, 130.0, 320, 320.0, 320.1, 320.2, 320.3, 320.7, 320.81, 320.82, 320.89, 320.8, 320.9, 322, 322.0, 322.9	*plus* 481; 485-487; 482.9
Septicemia	038.1, 038.4, 003.1, 020.2, 022.3, 031.2, 036.2, 038, 038.0, 038.2, 038.3, 038.8, 038.9, 054.5, 790.7	*plus* 481; 485-487; 482.9
Empyema	510	*plus* 481; 485-487; 482.9

Tab. II. Diagnostic codes considered to identify comorbidities.

Code description	International Classification of Diseases 9 – Clinical Modification Codes
Asplenia or severe dysfunction of the spleen	282.5-282.6, 289.4-289.5; 759.0
Chronic renal disease or nephrotic syndrome	581, 582, 584, 585;
Immunodeficiency or immunosuppression	0420-0449, 279
Chronic heart disease	393-398, 414, 416
Chronic lung disease	490-496
Chronic liver disease	571-573
Diabetes mellitus	250
Malignant neoplasms	140-209

In classifying vaccination coverage at 24 months, we distinguished between: a "PCV7 period", when the coverage rate was < 40%; a "late PCV7 period" when it was 70-80%; and a "PCV13 period", when it exceeded 80% (Tab. III).
Throughout the study period, the 23-valent pneumococcal polysaccharide vaccine (PPV23) was optionally offered to 65-year-olds, achieving a low coverage (< 50%) in both regions.
For each region (considering only first hospital admissions), the trends of the PRH rates were calculated for the three vaccination periods and correlated with the pneumococcal vaccination coverage in children.

Estimated cost

The annual cost of the PCV13 vaccination campaign was calculated using the acquisition cost of the vaccine (€ 42.5 per dose; three doses for children and one dose for a one cohort strategy (in the elderly), the time per injection for immunization activities (physician, health visitor or nurse, records: 8.6 minutes [13]). To convert this time into a cost, we considered the hourly cost of each actor (€ 71/hour for a physician, € 18 for a health visitor). The vaccination coverage rate considered to estimate the overall cost was 90% of children and 50% of elderly people. The population considered was the resident population in 2014 (newborn and 65 year-olds).
The estimated cost to the health care system of PRHs was calculated using the diagnosis-related groups (DRGs) of hospitalized patients. The DRGs depend on the patients' ICD classification at the time of their discharge from hospital, their age and gender, and the consumption of resources during their hospital stay. According to the DRG-based reimbursement system, every hospitalized patient belongs to a group of diagnostically homogeneous cases. Patients within each category are therefore similar in clinical terms, and are expected to require the same level of hospital resources. As a result, patients in the same DRG are assigned the same reimbursement charges. All hospital stays were considered for patients readmitted within 30 days.

Tab. III. Study period and vaccination coverage in children by region.

Vaccine	Period		Vaccination coverage in children (24 months of age)	Type of offer
	Veneto	Friuli Venezia Giulia		
PCV7	2004-2005	2004-2008	< 40%	limited to groups at risk
	2006-2010	2009-2010	70-80%	extended to all children
PCV13	2011-2013	2011-2013	> 80%	

Statistical analysis

The data were analyzed using the chi-square test, Student's t-test for unpaired data, and Pearson's test, and 95% confidence intervals were calculated, as appropriate. A p value < 0.05 was considered significant. Analyses were performed using the Statistical Package for the Social Sciences (SPSS 16.0; SPSS Inc., Chicago, IL, USA). Significant trends over the years considered were assessed as average annual percent changes (AAPC), a summary measure of the trend over a given fixed interval that is computed as a weighted average of the annual percent change (APC) emerging from the joinpoint model, using weights equating to the length of the APC interval. If an AAPC lies entirely within a single joinpoint segment, the AAPC is the same as the APC for that segment [14].

Ethical principles

The study was conducted on data routinely collected by the health services and linked to anonymized records that make it impossible to identify the individuals concerned. The data analysis was performed on anonymized aggregated data in the Local Health Authority registries that are recorded with the patient's consent and can be used in aggregate form for scientific studies without further authorization [15]. This study complies with the Declaration of Helsinki and with Italian privacy law (Decree n. 196/2003 on the protection of personal data).

Results

Pneumonia-related hospitalizations

Overall, we identified 133,936 PRHs and 8,214 (6.1%) of them were disregarded because they concerned readmissions within 30 days. Of the 125,722 hospitaliza-

tions thus included in the analysis, 6.8% (8,510 hospitalizations) concerned children under five years of age, and 75.3% (94,715 hospitalizations) concerned adults over 65. The first diagnosis was pneumonia in 121,823 (96.9%) of cases, while it was pneumonia-related septicemia in 3,025 (2.4%), meningitis in 539 (0.4%) and empyema in 335 (0.3%).

The patients were male in 52.6% of cases (66,131 patients); the mean hospital stay was 11.9 ± 10.1 days, and did not vary between genders. The mean hospital stay increased significantly with age ($p < 0.001$), reaching about 13 days for patients aged 65 or more.

The characteristics of the sample are shown in Table I.

In the sample as a whole, 32.4% of patients had at least one chronic comorbidity and the number of comorbidities tended to increase with age ($p < 0.001$); their frequencies by principal type are shown in Figure 1. As for outcome, the overall CFR was 12.4% and remained stable during the study period. This rate increased with age, peaking in people over 80 years old (19.6%).

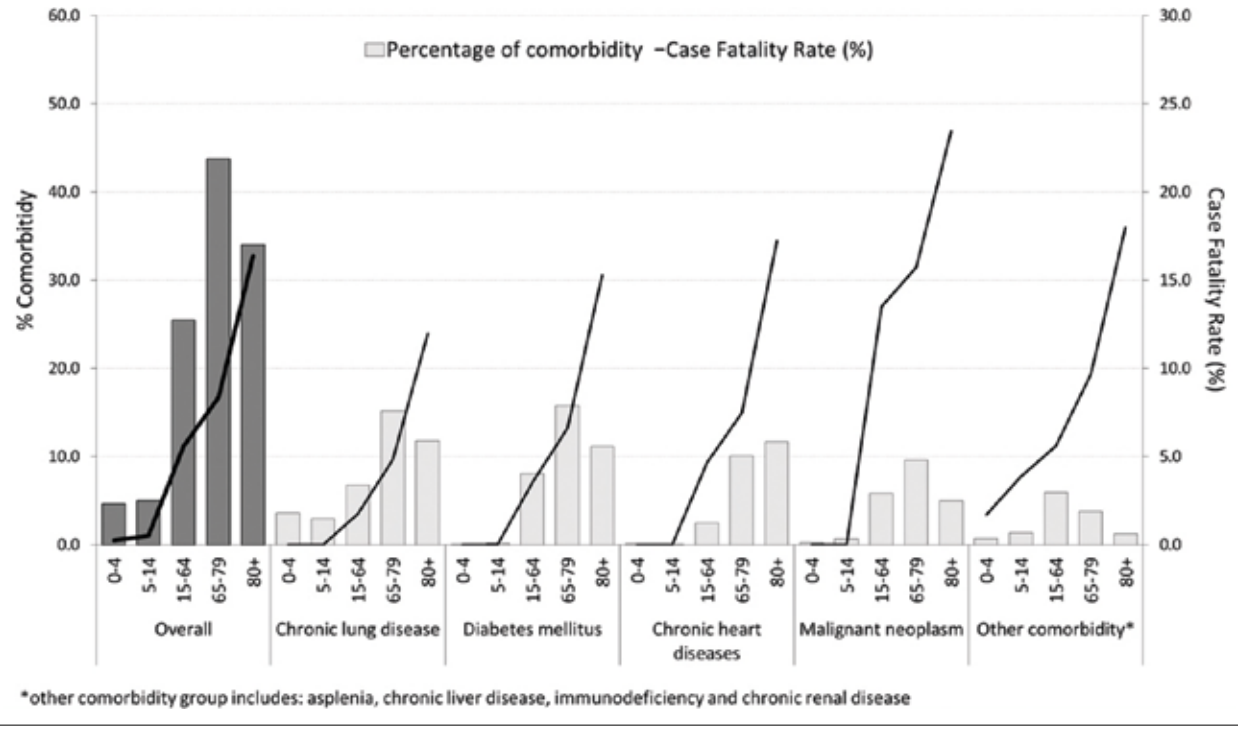
The overall annual PRH rate during the study period was 204.6 per 100,000 population (221.2 per 100,000 males and 188.8 per 100,000 females). Both genders showed two age-related peaks, one in children aged 0-4 years (325.6 per 100,000 in males, 288.9 per 100,000 in females), the other in adults over 65 years old (844.9 per 100,000 in males, 605.7 per 100,000 in females). The hospitalization rates for pneumonia were highest for the 0-4 and 80+ age groups, irrespective of the different types of vaccine used or their coverage. The rates for children up to 4 years of age were similar to those for 65- to 79-year-olds, at about 330 hospitalizations per 100,000 population a year. The PRH rate was stable during the study period, ranging from 200.9 per 100,000 in 2004 to 198.7 per 100,000 in 2013 [AAPC: 0.3%; (95% CI: -1.0; 1.6)]. The rate dropped significantly among the 0- to 4-year-olds, from 379.7 per 100,000 in 2004 to 211.9 per 100,000 in 2013 [AAPC: -4.6%; (95% CI: -6.5; -2.7)], while it rose significantly for adults aged 80 or more, from 1,370.3 per 100,000 in 2004 to 1,658.4 per 100,000 in 2013 [AAPC: 2.7%; (95% CI: 1.2; 4.3)] (Fig. 2).

Figure 3 shows that the PRH rate was consistent with the timing of the vaccination programs by region. The PRH rate was 204.9 per 100,000 in the Veneto, and 203.3 per 100,000 in Friuli Venetia Giulia. In the Veneto, the PRH rate dropped for the 0- to 4-year-olds from the early PCV7 period (2004-2005) to the late PCV7 period (2006-2010), and to the PCV13 period (2011-2013): -9.3% and -29.0% respectively; $p < 0.01$. A significant inverse trend was seen for people over 80 years old, whose PRH rates rose by 9.9% and 25.2%, respectively, over

Tab. IV. Hospital admissions for pneumonia by patients' characteristics and age group (2004-2013).

Variable	Total		Age groups									
			0-4		5-14		15-64		65-79		80 +	
Number of subjects	125,722		8,510		3,924		18,573		32,926		61,789	
Gender [n (%)]												
Males	66,131	(52.6)	4,625	(54.3)	2,167	(55.2)	11,450	(61.6)	20,646	(62.7)	27,243	(44.1)
Females	59,591	(47.4)	3,885	(45.7)	1,757	(44.8)	7,123	(38.4)	12,280	(37.3)	34,546	(55.9)
First-listed diagnosis [n (%)]												
Pneumonia	121,823	(96.9)	8,319	(97.8)	3,838	(97.8)	17,600	(94.8)	31,677	(96.2)	60,389	(97.7)
S. pneumoniae	4,002	(3.2)	659	(7.7)	371	(9.5)	967	(5.2)	930	(2.8)	1075	(1.7)
without a causative organism identified	117,821	(93.7)	7,660	(90.0)	3,467	(88.4)	16,633	(89.6)	30,747	(93.4)	59,314	(96.0)
Septicemia	3,025	(2.4)	88	(1.0)	28	(0.7)	582	(3.1)	1015	(3.1)	1,312	(2.1)
Meningitis	539	(0.4)	50	(0.6)	16	(0.4)	248	(1.3)	177	(0.5)	48	(0.1)
Empyema	335	(0.3)	53	(0.6)	42	(1.1)	143	(0.8)	57	(0.2)	40	(0.1)
Comorbidity [n (%)]												
Chronic lung disease	13,965	(11.1)	307	(3.6)	115	(2.9)	1,255	(6.8)	4,994	(15.2)	7,294	(11.8)
Diabetes mellitus	13,574	(10.8)	1	(0.0)	7	(0.2)	1,494	(8.0)	5,186	(15.8)	6,886	(11.1)
Chronic heart diseases	10,990	(8.7)	10	(0.1)	1	(0.0)	454	(2.4)	3,319	(10.1)	7,206	(11.7)
Malignant neoplasm	7,380	(5.9)	19	(0.2)	26	(0.7)	1,088	(5.9)	3,154	(9.6)	3,093	(5.0)
Chronic liver disease	2,882	(2.3)	9	(0.1)	6	(0.2)	1,000	(5.4)	1,161	(3.5)	706	(1.1)
Immunodeficiency	135	(0.1)	16	(0.2)	10	(0.3)	62	(0.3)	36	(0.1)	11	(0.0)
Asplenia	93	(0.1)	33	(0.4)	31	(0.8)	17	(0.1)	6	(0.0)	6	(0.0)
Chronic renal disease	110	(0.1)	1	(0.0)	5	(0.1)	27	(0.1)	39	(0.1)	38	(0.1)
Length of hospital stay [days (DS)]	11.9	(10.1)	4.7	(3.9)	4.9	(3.7)	10.9	(10.3)	13.,0	(10.2)	12.9	(10.2)

Fig. 1. Percentage of comorbidity and case fatality rate by type of comorbidity and age group (2004-2013).



the same periods. In Friuli Venezia Giulia, the figure was much the same for the 0- 4-year-olds, with reductions of 21% and 31.7% from the early to the late PCV7 period, to the PCV13 period ($p < 0.01$); while the PRH rate rose by 8.4% ($p < 0.01$) from the first to the last period among individuals aged 80 years or more (Fig. 3).

The overall estimated costs of the vaccination campaign for new-born and 65-year-olds was € 8.5 million (€ 6.4

Fig. 2. Pneumonia-related hospitalization rate (per 100,000 population) by age group (2004-2013).

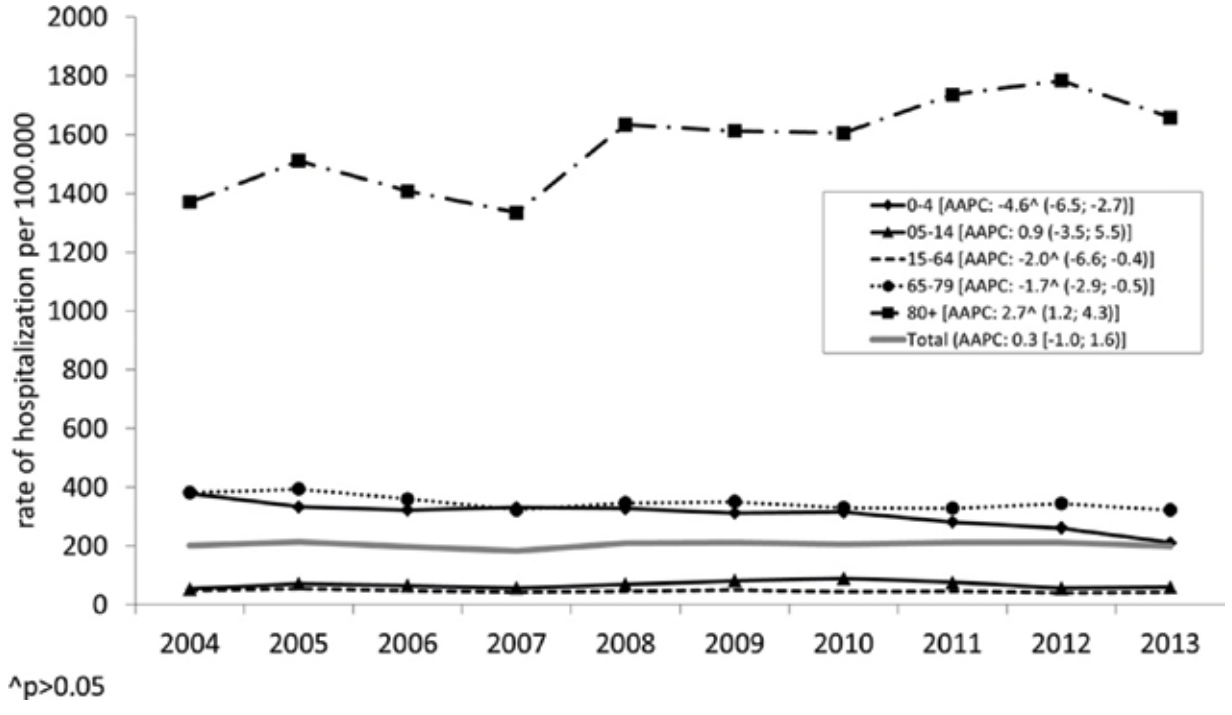
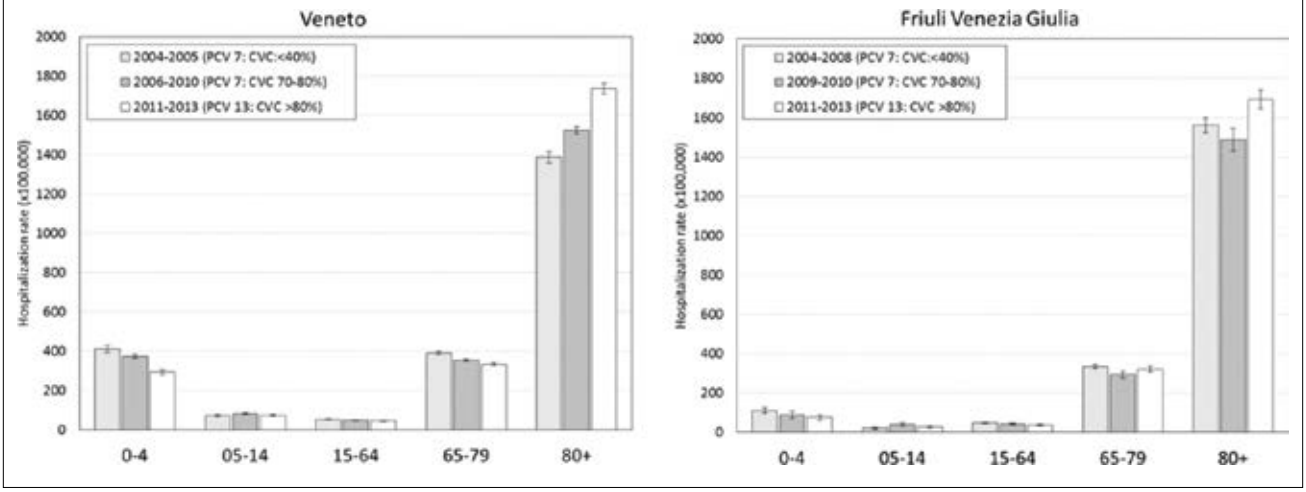


Fig. 3. Average annual pneumonia-related hospitalization rates for children by vaccination coverage (CVC), age group and region



million in children and € 2.1 million in the elderly). The purchase of PCV13 vaccine cost € 7.6 million (€ 5.9 for children and € 1.7 for the elderly).

The estimated overall annual cost of the PRHs was approximately € 41 million, with an estimated cost per patient of € 3,059. People over 65 accounted for 74.9% of the estimated annual overall pneumonia-related cost (€ 30.6 million in all, corresponding to € 22.9 per person over 65 in the general population).

Discussion

This retrospective observational study assessed the trends of pneumonia-related hospitalization in a population of 6.2 million, confirming that the burden on hospital resources is an important public health issue.

The PRH rate was stable on the whole, but dropped significantly for infants (0-4 years old), and this trend may be thanks to a greater vaccination coverage in this age group since 2008. Hospital admissions for pneumonia involving children aged 0-4 years dropped once the PCV7 coverage rate improved, and even more after the PCV13 vaccine was introduced. The same situation was confirmed in both regions, but Friuli Venezia Giulia had a lower hospitalization rate than the Veneto, probably due to differences in the two regions' admission modalities for this age group. Much the same picture has emerged in other areas [9, 10, 16].

The reduction in PRHs for infants seen in our sample was initially lower than the figures reported in previous observational studies [8, 17]. This may be due to a different diffusion of pneumococcal serotypes in our region, leading to a better coverage after the addition of more serotypes in the 13-valent conjugate vaccine (especially 19A, 3 and 1) [18].

The risk of pneumonia increases considerably with age and our analysis revealed a rising trend in the hospitalization rate for the very elderly (80 + years old). In contrast with our data, a similar retrospective study conducted in the US on hospital discharge records for the years 1997 to 2009 showed a substantial reduction in adult hospital admissions for pneumonia [7]. This difference could have several explanations. The magnitude of the indirect effects of vaccine-derived immunity depends on multiple influences relating to the transmissibility of the infection, the nature of the vaccine-induced immunity, the serotypes circulating, the patterns of mixing and transmitting infections in a given population, the type and schedule of vaccine used, the coverage achieved and, more importantly, the population's immunity. The heterogeneity of immunity and the complexity of populations make it difficult to draw comparisons [19, 20]. Active surveillance systems for monitoring invasive bacterial diseases show that serotypes not covered by the 7-valent pneumococcal conjugate vaccine (such as 19A) play a significant part in the burden of pneumonia-related disease [18], suggesting the likelihood of a reversal of the herd effect [21].

Our analysis confirms the mortality rate reported in other studies, with a CFR of 12%, and even higher in high-risk patients [22].

The introduction of PCV13 for infants in north-east Italy in 2010, and its subsequent use in people over 65 years old, can be expected to produce some benefits in years to come.

A limitation of this retrospective study lies in that it is impossible to rule out external factors that might confound the apparent effect of vaccination on the age-specific rates of hospitalization for pneumonia, such as changes in patient management or the diagnostic coding of pneumonia. Any systematic shift in the management of pneumonia is unlikely to have coincided with the introduction of the pneumococcal vaccine, however [8].

A better understanding of the effects of higher-valence vaccines could improve the sensitivity of surveillance systems and add to our understanding of the potential invasiveness of serotypes not included in PCVs (the prevalence of their carriage could be compared with their prevalence in invasive pneumococcal disease). This is an aspect of surveillance that has hitherto been largely overlooked.

A large trial to ascertain the efficacy of PCV-13 in adults > 65 years of age was begun in the Netherlands in 2008. The main objective of this trial was to establish the efficacy of PCV-13 in the prevention of a first episode of vaccine-serotype specific CAP (vaccine efficacy 45.6%). The study also tested the efficacy of PCV-13 against first episodes of non-bacteremic vaccine-serotype-specific CAP and vaccine-serotype-specific invasive pneumococcal disease (IPD). The results indicated that PCV-13 was effective in preventing vaccine-serotype pneumococcal, bacteremic, and non-bacteremic CAP, and vaccine-serotype IPD, but not all-cause CAP [23].

It is not easy to estimate the morbidity of *S. pneumoniae* diseases due to variable levels of accuracy in reporting the cases diagnosed, and to differences in surveillance methods and microbiological methods, but HDRs can be useful for assessing the cases severe enough to warrant hospitalization [14].

In our sample, 96.9% of the patients with pneumonia were first-listed diagnoses, so their disease was probably community-acquired (since hospital-acquired infections are not normally recorded as a first diagnosis). Vaccination can only have an impact on CAP, not on hospital-acquired pneumonia, because of the different types of agent involved. *S. pneumoniae* is considered the most common pathogen responsible for pneumonia, accounting for about 24% of all cases [6, 24].

One of the difficulties in this setting concerns the availability of microbiological data and the dubious accuracy of the total number of hospital admissions identified as being related to *S. pneumoniae* (SP) due to coding errors in the hospital records. In our dataset, the number of SP-specific diagnoses was very low and varied by age group. The use of different diagnostic testing procedures may have influenced the pattern emerging between the diagnostic subgroups. This is the impression that emerges on comparing the percentage of pneumonia cases of unspecified etiology in our study (93.7%) with the 55% found in a recent study conducted in Australia, where cases hospitalized for CAP underwent a standardized, detailed assessment for bacterial and viral pathogens [8]. A review on the burden of CAP among adults in Europe showed that Italy had the lowest rate of identification of pneumococcal disease and recommended improvements in the diagnostic assays used to detect pneumococcal pneumonia with a view to enhancing the detection rate and generating a more accurate epidemiological picture [25].

It is important to ensure a good vaccination coverage among older people for both influenza and pneumococcal disease. In the latter period of our study, the annual influenza vaccination coverage in our elderly population dropped from about 75% in 2004 to about 60% in 2013, meaning that other measures are needed to prevent pneumonia in seniors [26].

Continued monitoring and longitudinal analyses are warranted to assess the longer-term effects of various contributing factors such as serotype replacement, and vaccination coverage, as well as catch-up programs. This will be particularly important as new pneumococcal conjugate vaccines offering additional serotype protection are licensed and included in immunization programs.

From the economic point of view, the implementation of PCV13 vaccination in the elderly is justified by the cost-effectiveness of this preventive measure. Our estimated costs are similar to those indicated in a model used in another study conducted in the same geographical area [27], presumably indicating that the benefits of vaccination could be extended to our context.

Conclusions

Prevention remains the most valuable tool to help reduce the burden of pneumonia, and vaccination strategies can be used for the primary prevention of infection and possibly to contain this disease in all age groups. The availability of new-generation pneumococcal conjugate vaccines with a broader antigenic spectrum and suitable for people of all ages suggests interesting new opportunities for improving the control of pneumococcal disease in the population as a whole.

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The authors have no competing interests to disclose.

Authors' contributions

VB and SC: study conception and design, data analysis, supervision, drafting of the manuscript. TG and MS: study conception and design, data collection. PF: statistical analyses, drafting of the manuscript. EC and SDZ: performed the data quality control and collection. CB, AB and TB: intellectual content, drafting of the manuscript and data interpretation. All Authors revised the manuscript and gave their contribution to improve the paper. All authors have read and approved the final manuscript.

References

[1] World Health Organization. *Immunization surveillance, assessment and monitoring.* [Update 2014 Nov; cited 2015 Jul 28]. Available at: http://www.who.int/immunization/monitoring_surveillance/en/ [Accessed 30/09/2015].

[2] Wardlaw T, Salama P, Johansson EW, Mason E. *Pneumonia: the leading killer of children.* Lancet 2006;368:1048-50.

[3] McIntosh K. *Community-acquired pneumonia in children.* N Engl J Med 2002;346:429-37.

[4] Bohte R, van Furth R, van den Broek PJ. *Aetiology of community-acquired pneumonia: a prospective study among adults requiring admission to hospital.* Thorax 1995;50:543-7.

[5] Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Kleemola M, Koskela M, Leinonen M, Rönnberg PR, Saikku P, Stén M, Tarkiainen A, Tukiainen H, Pyörälä K, Mäkelä PH. *Microbial etiology*

of community-acquired pneumonia in the adult population of 4 municipalities in eastern Finland. Clin Infect Dis 2001;32:1141-54.

[6] Woo JH, Kang JM, Kim YS, Shin WS, Ryu JH, Choi JH, Kim YR, Cheong HJ, Uh ST, Park CS, Chung MH, Chung KS, Lee CJ, Ryu J. *A prospective multicenter study of community-acquired pneumonia in adults with emphasis on bacterial etiology.* Korean J Infect Dis 2001;33:1-7.

[7] Griffin MR, Zhu Y, Moore MR, Whitney CG, Grijalva CG. *U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination.* N Engl J Med 2013;369:155-63.

[8] Jardine A, Menzies RI, McIntyre PB. *Reduction in hospitalizations for pneumonia associated with the introduction of a pneumococcal conjugate vaccination schedule without a booster dose in Australia.* Pediatr Infect Dis J 2010;29:607-12.

[9] Durando P, Crovari P, Ansaldi F, Sticchi L, Sticchi C, Turello V, Marensi L, Giacchino R, Timitilli A, Carloni R, Azzari C, Icardi G; Collaborative Group for Pneumococcal Vaccination in Liguria. *Universal childhood immunisation against Streptococcus pneumoniae: the five-year experience of Liguria Region, Italy.* Vaccine 2009;27:3459-62.

[10] Baldo V, Cocchio S, Baldovin T, Buja A, Furlan P, Bertoncello C, Russo F, Saia M. *A population-based study on the impact of hospitalization for pneumonia in different age groups.* BMC Infectious Diseases 2014;14:485 doi:10.1186/1471-2334-14-485.

[11] Torres A, Bonanni P, Hryniewicz W, Moutschen M, Reinert RR, and Welte T. *Pneumococcal vaccination: what have we learnt so far and what can we expect in the future?* Eur J Clin Microbiol Infect Dis 2015;34:19-31.

[12] Conferenza permanente per i rapporti tra lo stato le regioni e le province autonome di Trento e Bolzano. *Piano Nazionale Prevenzione Vaccinale 2012-2014.* Gazzetta Ufficiale della Repubblica Italiana, numero 60 del 12 marzo 2012 (supplemento ordinario n. 47).

[13] Judith E. Glazner, Brenda Beaty, Stephen Berman. *Cost of vaccine administration among pediatric practices.* Pediatrics 2009;124 (Suppl 5):S492-8.

[14] Boehmer TK, Patnaik JL, Burnite SJ, Ghosh TS, Gershman K, Vogt RL. *Use of hospital discharge data to evaluate notifiable disease reporting to Colorado's electronic disease reporting system.* Public Health Rep 2011;126:100-6.

[15] Garante per la protezione dei dati personali: Autorizzazione generale al trattamento dei dati personali effettuato per scopi di ricerca scientifica - 1° marzo 2012. Gazzetta Ufficiale della Repubblica Italiana numero 72 del 26 marzo 2012.

[16] Martinelli D, Pedalino B, Cappelli MG, Caputi G, Sallustio A, Fortunato F, Tafuri S, Cozza V, Germinario C, Chironna M, Prato R; Apulian Group for the surveillance of pediatric IPD. *Towards the 13-valent pneumococcal conjugate universal vaccination: Effectiveness in the transition era between PCV7 and PCV13 in Italy, 2010-2013.* Hum Vaccin Immunother 2014;10:33-9.

[17] Grijalva CG, Nuorti JP, Arboqast PG, Martin SW, Edwards KM, Griffin MR. *Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis.* Lancet 2007;369:1179-86.

[18] Russo F, Pozza F, Napoletano G, Zanella F, Baldovin T, Lazzari R, Cocchio S, Baldo V. *Experience of vaccination against invasive bacterial diseases in Veneto Region (north-east Italy).* J Prev Med Hyg 2012;53:113-5.

[19] Longini IM, Halloran ME, Nizam A. *Model-based estimation of vaccine effects from community vaccine trials.* Stat Med 2002;21:481-95.

[20] Fine P, Eames K, Heymann DL. *"Herd immunity": a rough guide.* Clin Infect Dis 2011;52:911-6.

[21] Kim TH, Johnstone J, Loeb M. *Vaccine herd effect.* Scand J Infect Dis 2011;43:683-9.

[22] Stupka JE, Mortensen EM, Anzueto A, Restrepo MI. *Community-acquired pneumonia in elderly patients.* Aging Health 2009;5:763-74.

[23] Bonten MJ, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, van Werkhoven CH, van Deursen AM, Sanders EA, Verheij TJ, Patton M, McDonough A, Moradoghli-Haftvani A, Smith H, Mellelieu T, Pride MW, Crowther G, Schmoele-Thoma B, Scott DA, Jansen KU, Lobatto R, Oosterman B, Visser N, Caspers E, Smorenburg A, Emini EA, Gruber WC, Grobbee DE. *Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults.* N Engl J Med 2015;372:1114-25.

[24] Fine MJ, Smith MA, Carson CA, Mutha SS, Sankey SS, Weissfeld LA, Kapoor WN. *Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis.* JAMA 1996;275:134-41.

[25] Welte T, Torres A, Nathwani D. *Clinical and economic burden of community-acquired pneumonia among adults in Europe.* Thorax 2010;67:71-9.

[26] Ministry of Health: Italian Vaccine Coverage. *In Sintesi Anni 1999–2015.* Available at: http://www.salute.gov.it/imgs/C_17_pagineAree_679_listaFile_itemName_6_file.pdf. [Accessed 15/8/2015].

[27] Boccalini S, Bechini A, Levi M, Tiscione E, Gasparini R, Bonanni P. *Cost-effectiveness of new adult pneumococcal vaccination strategies in Italy.* Hum Vaccin Immunother 2013;9:699-706.

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ORIGINAL ARTICLE

Current preventive policies and practices against Vaccine-Preventable Diseases and tuberculosis targeted for workers from hospitals of the Sardinia Region, Italy

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Keywords

Vaccine-preventable disease • Healthcare workers • Infectious diseases

Summary

Introduction. *Health care Workers are exposed to infectious diseases more than the general population. Many of these infections are preventable by vaccination. The objective in this study is to investigate whether, how, and which vaccination underwent Sardinia Health Care Workers (HCWs) and the variability of policies in different Hospital Health Managements of the whole region.*
Methods. *In March 2013, we enrolled the Hospital Health Management of all the 32 Sardinia hospitals. We investigate on immunity against vaccine-preventable diseases and education campaigns about recommended vaccinations for HCWs. Flu, hepatitis B, measles-mumps-rubella, varicella and tuberculosis were the objects of our research.*
Results. *In most of the hospitals, influenza vaccination coverage among HCWs is less than 6%. Hepatitis B antibody assay was performed in all the respondent hospitals but only 14 had available data as collected electronically. Most of the hospitals did not perform serological tests for the evaluation of antibodies against Varicella, Measles, Mumps and Rubella in their HCWs. In 30 hospitals Mantoux test was replaced or integrated by "in vitro" test for health surveillance protocols.*
Conclusions. *This method produced a large amount of data in small time and at a low cost. Sending back data to respective Hospital Health Management (HHM) we took a step towards greater awareness of the issue of biological risks of HCWs and of vaccine coverage.*

Introduction

Health care workers (HCWs) are defined as persons working in health-care milieu having potential exposure to patients and to infectious materials; they are exposed to infectious diseases more than the general population although many of these infections are preventable by vaccination. When infected, HCWs may transmit the infection to patients or colleagues [1].
Outbreaks due to vaccine-preventable diseases in health care facilities are often associated with serious morbidity and mortality among patients, disruption of healthcare services, and high costs for the National Health Service [2].
Immunization of HCWs is a major infection prevention measure [3] to protect HCWs and, indirectly, the patients who may not be naturally immunized or vaccinated [4]. Vaccination is a very effective tool to promote safety within health care facilities for both HCWs and patients. Nevertheless, inadequate coverage of HCWs against vaccine-preventable diseases is a global problem [5].
In Italy, vaccination policies for HCWs are based – on one hand – on the "National Vaccination Plan" [6] which reinforces the recommendation to vaccinate HCWs against selected vaccine preventable diseases. On the other hand, the Italian Legislative Decree 81/08 reorganizes and revises all the provisions regarding health and safety at work. In particular, the Decree ratifies the obligation to the employers to provide vaccination to workers exposed to biological risk and emphasizes the key role of the Occupational physician for assessing biological risk, identifying and managing susceptible workers, and organizing and implementing vaccine campaigns. Therefore, employers and Occupational physicians have to manage vaccination activities within hospitals for their workers. In this sense, the following vaccinations are strongly recommended: anti-hepatitis B, anti-influenza, anti-measles-mumps-rubella, anti-varicella and anti-pertussis. The Italian law has drastically limited the use of the anti-TB vaccination to very few categories of HCWs: those who are at high risk of exposure to multi-drug-resistant tubercular bacterial strains, and those who work in high-risk environments and – in the case of positive Mantoux Test – they cannot undergo preventive therapy because of clinical contraindications to the use of specific drugs. The aim of our study was to investigate (1) whether, how, and which vaccination underwent the Sardinia HCW's; (2) the variability of policies and solutions in different Hospital Health Managements (HHM) of the whole region.
Consequently, an indirect aim was to raise awareness about HHMs' compliance rate to vaccination programs

and orient them in a positive approach towards vaccination in Sardinia.

Methods

In March 2013, we enrolled by both phone and a written questionnaire sent by e-mail the 32 HHM of all Sardinia hospitals which have totally 5,650 beds serving the whole Sardinia population (1.600.000 inhabitants).
A self-reported structured form was completed by HHMs. In case of lacking or inadequate response HHMs were successively contacted by phone to complete information. According to some critical aspects highlighted in 2010 by a team of occupational physicians of the Italian Society of Occupational Health and Industrial Hygiene (SIMLII), the questionnaire included 10 items based on multiple-choice questions. Each hospital was allowed to mark more than one answer per item.
The study investigated about the immunological pattern of HCWs towards Influenza, hepatitis B (HB), measles-mumps-rubella (MMR), varicella and tuberculosis (TBC). Furthermore, information was collected about the performed educational campaign for influenza vaccination. Specifically, five questions concerned flu vaccination and investigated (a) effective accomplishment of the educational campaign on vaccination and its starting, (b) the campaign planners, (c) the different strategies used to inform health professionals about the opportunity to undergo vaccination, (d) the topics used during the campaign, and (e) available data on vaccination coverage. One question regarded HB vaccination and the gathered data about compliance to vaccination. Two questions were addressed to serological surveillance: the first one asked whether serological tests for detection of MMR and Varicella viruses' antibodies were included in routine surveillance of HCWs by Occupational Health Physicians. The other one asked whether serological results were collected. The last two questions concerned TBC: one investigated whether and in which occasions HCWs underwent Mantoux skin test, the other one detected whether Mantoux was replaced or integrated by "in vitro" test.
The results were returned to each HHM in order to provide them a comparison with the other hospitals. In addition, we suggested recommendations about vaccination in HCWs according to National laws and National and International scientific communities.

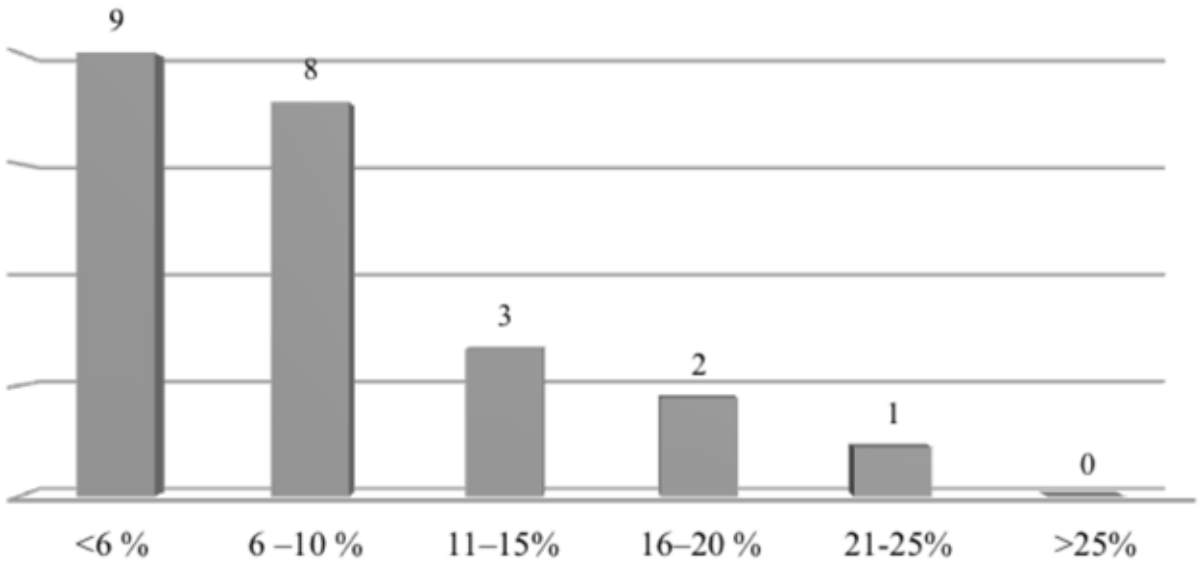
Results

Out of the 32 hospitals enrolled in the study, 30 joined our survey (94% compliance): three were University Hospitals and 27 were General Hospitals. The two non-responding hospitals were long-term care institutes. The total number of HCWs of the 30 hospitals was 12,977. Among these, 26% were medical staff, 47% nurses, and 27% represented other hospital staff.

Influenza

Twenty-five out of the 30 hospitals (77%) accomplished the vaccination campaign against flu carried out between October and December 2011, particularly November, in 18 hospitals.
All the hospitals answered to the questions concerning the communication methods during the flu campaign: 18 hospitals (72%) informed HCWs about the chance to be vaccinated by newsletter to unit managers, nursing and technicians coordinator, 8 (32%) sending newsletter to unit managers only, 7 (28%) through active call, 2 (8%) using posters and advertising.

Fig. 1. Influenza vaccination coverage rate in HCWs of 23 Sardinia Hospitals.



The coordination of the campaign was performed by HHM in 20 hospitals (80%), by Health visitors in 8 (32%) and by Occupational Health Physicians in 7 hospitals (28%). In 8 (32%) hospitals the Prevention Department managed the campaign. Therefore different professionals were involved in managing and coordinating the campaign in the different hospitals at the same time. This heterogeneity may depend on the hospital characteristics in terms of structure, complexity, size, and (urban or rural) area.
Concerning the topics used during the vaccination campaign to inform HCWs all 25 hospitals enlightened about professional and social responsibility in patients' protection; 20 reported also efficacy and safety of the vaccine and 17 warned about the frequency and severity of influenza.
Data of vaccination coverage among HCWs were reported only by 23 hospitals. The coverage rate is shown in Figure 1; 74% of the hospitals indicated a coverage rate lower than 10%.

Hepatitis B

Data on immunization coverage were available in 14 of 30 hospitals (Fig. 2). Regional average was 76%; 5 hospitals were below the average and 9 were above, only one hospital had 92% vaccination coverage. Antibody assay (anti-HBs) was carried out in all the 30 respondent hospitals but only 14 had electronically collected data.

Varicella, Measles, Mumps, Rubella

Most hospitals did not perform serological tests to detect antibodies against Varicella, Measles, Mumps and Rubella viruses in HCWs.
Three (10%) hospitals applied health surveillance for those viruses and other two hospitals performed serological test for Rubella in higher risk occupational categories.
No hospital had available data on immunity to those infections.

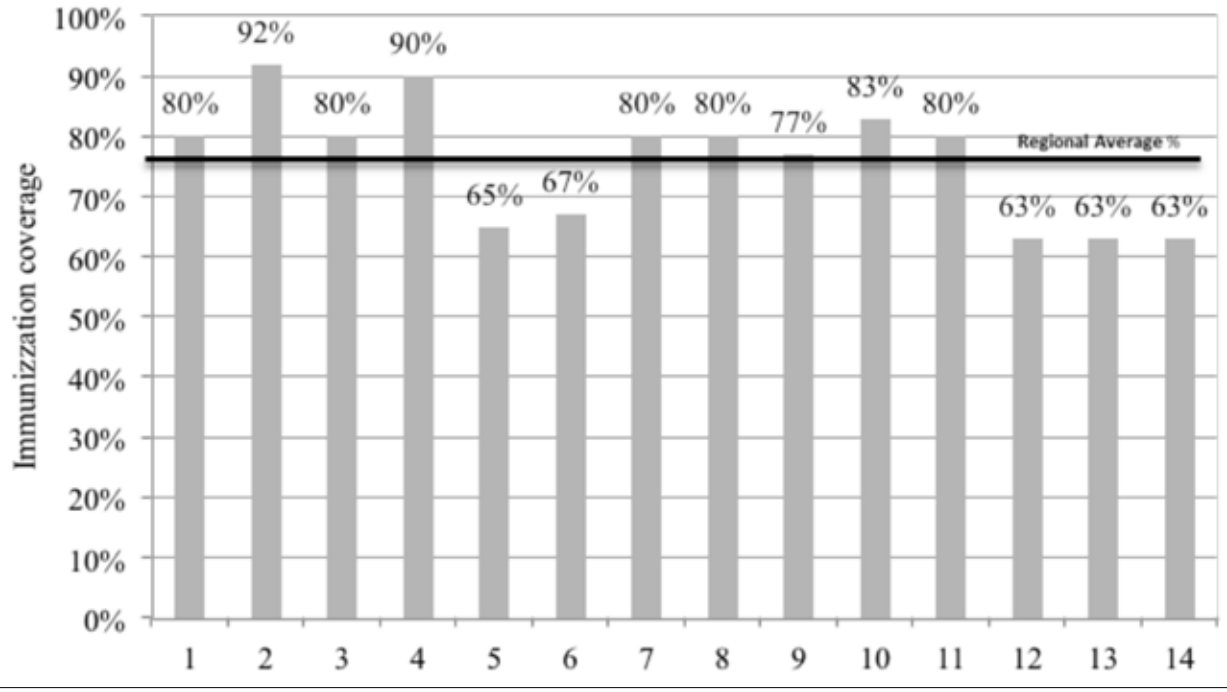
Tuberculosis

Intradermal Mantoux test was used in 29 of 30 hospitals: in 32% of hospitals HCWs were submitted to the Mantoux test at the hiring time and/or in post exposure situations.
In 1 hospital, Mantoux test was replaced by "in vitro" test (Quantiferon) and in 20 hospitals Quantiferon integrated Mantoux test.

Discussion

Vaccination is an important prophylactic action to reduce the number of susceptible HCWs and to indirectly protect susceptible patients and colleagues [7]. An additional benefit is a reduction in work time lost due to illness. Despite the persistence of outbreaks of vaccine-preventable diseases in health care facilities, HCWs vaccination rates remain suboptimal globally. Higher vaccination coverages among HCWs than the actual would be useful both to reinforce occupational safety in health care facilities and to prevent nosocomial outbreaks [1, 8, 9].
The Italian Health Ministry recommends HCWs to undergo vaccinations (i.e. anti-hepatitis B, anti-flu, anti-measles-mumps-rubella, varicella, anti-pertussis and, when indicated, anti-tuberculosis) not only to reduce

Fig. 2. Immunization coverage for Hepatitis B in 14 Sardinia Hospitals.



employees' absenteeism that may increase workload for staff, but also to prevent the nosocomial infection risk, especially in elderly or immunocompromised patients [6]. The same Institution have also included among the new objectives of the National Prevention Plan 2014-2018 an increasing vaccination coverage for both general and high-risk population including HCWs, by looking for a cooperation between regions in which regions more experienced can support regions with the lowest vaccination coverage [10].

Several outbreaks of health care facility-acquired influenza, including immunosuppressed or old patients, have been documented as a consequence of low vaccine effectiveness, thus requiring consequently an indirect protection. Influenza vaccination of all HCWs is recommended by the World Health Organization (WHO), [11] US Centers for Disease Control and Prevention (CDC) [1], and by the Italian Ministry of Health [6] in order to prevent transmission of influenza from HCWs to patients. Nevertheless, the same countries show a low uptake of influenza vaccine in HCWs [12]. A global literature review on vaccination programs in HCWs reported a percentage of vaccination coverage ranging from 2.1% to 82%, with highest uptake rates occurring in USA [13] (from 40% to 87.4%) [14, 15, 16, 17], followed by Germany (26.9%) [18], and Spain (from 14.7% to 38%) [19]. In Italy, data on vaccination coverage among HCWs are not regularly collected and the few ad-hoc studies have shown low coverage rates of 0-29% [20], and 20.8% [21]. In the 2012-2013 season, the coverage rate for the working-age population was 10% [22]. In our survey most of Sardinia hospitals had an influenza coverage rate lower than 6%.

Some studies highlighted how compulsory influenza vaccination with forfeit for non-adhesions was associated with larger compliance to vaccination programs [23]. Other authors asserted that voluntary vaccination of HCWs against influenza would represent the most effective strategy [24]. Hospitals that used personal contact approach had higher vaccination rates [24, 25]. Differently, newsletters sent by HHM to unit managers, and both nurse and technician coordinators were the most frequent tool used by Sardinia hospitals. Nevertheless, this tool obtained a vaccination coverage lower than 10% in 74% of the involved hospitals. A recent Italian study implemented different actions such as education, promotion, and easy access to staff vaccination in order to increase HCWs vaccination coverage [26]. Despite these efforts, the results showed that the vaccination coverage in the hospital staff was underperforming. On the light of these results, new techniques of information and empowerment on the importance of flu vaccination for HCWs should be considered and activated.

In 1997, CDC recommended HBV vaccination for all HCWs [27]; nevertheless, HBV vaccine coverage rate in USA was 63.4%, well below the 90% goal [28]. Similar percentages were reported in other countries (85% for Belgium, 55.4% for India, and 55% for Morocco) [29, 30, 31].

In 1991, Italy introduced HBV mandatory vaccination for infants and 12-year-old adolescents until 2003 when the two cohorts joined together [32]. In persons who received primary immunization in the first year of life, immune memory for HBsAg lasted for at least 17 years and additional booster doses were not needed to enhance long-term immunization [33, 34, 35, 36]. In Italian HCWs who did not receive HBV vaccination as adolescents and are older than 35-36 years, testing HCWs at hiring time and administering the vaccine to those who prove susceptible to HBV infection is strongly advised. In Italy, the coverage rate of HBV vaccination ranged from 43% to 87% [37]. Thus, we emphasize that efforts should be made to increase the number of vaccinated HCWs for HBV protection.

Measles, mumps, rubella, and varicella (MMRV) are highly contagious diseases, and pose a high risk for both HCWs and patients [2]. In Italy, the percentage of immunization in HCWs ranged from 85.7% to 95.1% for varicella, from 47% to 96.8% for rubella, from 71.4% to 97.8% for measles, and from 52.5% to 87.6% for mumps [38, 39]. These results show a significant proportion of unprotected workers. In fact, most of the Sardinia hospitals did not perform serological tests for MMVR on HCWs.

Furthermore, tuberculosis still remains a severe worldwide health problem, especially among immunocompromised patients. High percentage of latent Mycobacterium tuberculosis infection among HCWs and the increasing number of Mycobacterial strains resistant to the main drug therapies increases the need for prevention programs health care workers.

Tuberculin skin test (TST) is still globally recommended to screen HCWs [40] despite its limitations. In effect, the need for a return visit to assess the test effect, often, results in a low compliance to follow-up [41]. These limits could be overtaken by advancement in the in vitro test, which detects the interferon-g production in response to other Mycobacterium tuberculosis antigens. QuantiFERON-in tube test (QFT-in tube) does not need for a second visit to interpret results and it has a higher specificity than TST [42]. An Australian study compared QFT-in tube test to the TST in HCWs. The results demonstrated that positive QFT-in tube test strongly correlated with risk factors for TB exposure more than positive TST, especially in a low tuberculosis prevalence population. Yet, a positive TST strongly correlated with a prior history of BCG vaccination [43].

The main limitation of the study regards the data collection method. Specifically, we have used a non-validated questionnaire, which can result in a low level of reliability of the data. Furthermore, we did not examine the vaccination coverage according to the different occupational categories and healthcare settings. This did not allow us to identify specific professionals or healthcare areas that mainly need for specific intervention strategies to increase vaccination coverages.

Nevertheless, there are several advantages from this research. The data from the whole Sardinia district have

shown in HHM a low level of awareness about HCWs vaccination. Furthermore, we have highlighted some critical aspects that can be considered by the local policy-makers to organize tailored preventive interventions. We, thus, emphasize the strong need to implement information programs addressed to HCWs about vaccination-preventable diseases for potentially spreading in hospital settings complying with a patient-centered care system. Another key-point of our study is a pragmatic low-budget method to communicate with health managers; this method has allowed us to get a large amount of data in a small lapse of time, thus reducing costs. Finally, our study provides evidence for stimulating health managers of Sardinia about the need to identify HCWs susceptible to threats of some *infectious* diseases damaging the hospital setting. This step is essential to achieve preventive strategies aimed to guarantee the health of both patients and HCWs.

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Authors' Contributions

MC, FA and RCC conceived, designed and coordinated the research. BS, NMM and AL collected data. BS, NMM, AL and MG performed the statistical analyses. MC, FA and RCC evaluated the results. BS, NMM, AL, and MG wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

References

[1] Centers for Disease Control and Prevention. *Immunization of health care workers. Immunization of Health Care Personnel: recommendations of the Advisory Committee on Immunization Practices (ACIP) MMWR Recommendations and Reports*. 2011/60(RR07);1-45.

[2] Maltezou HC, Poland GA. *Vaccination policies for healthcare workers in Europe.* Vaccine 2014;32:4876-80.

[3] Maltezou HC, Wicker S, Borg M, Heininger U, Puro V, Theodoridou M, Poland GA. *Vaccination policies for health care workers in acute health care facilities in Europe.* Vaccine 2011;29:9557-62.

[4] Poland GA, Jacobson RM. *The age-old struggle against the antivaccinationists*. New Engl J Med 2011;364:97-9.

[5] Trevisan A, Frasson C, Morandin M, Beggio M, Bruno A, Davanzo E, Di Marco L, Simioni L, Amato G. *Immunity against infectious diseases: a predictive value of self-reported history of vaccination and disease*. Infect Control Hosp Epidemiol 2007;28:564-9.

[6] *Piano Nazionale Prevenzione Vaccinale (PNPV) 2012-2014.* Ministero della Salute, pp. 24-5.

[7] Dinelli MI, Moreira Td, Paulino ER, da Rocha MC, Graciani FB, de Moraes-Pinto MI. *Immune status and risk perception of acquisition of vaccine preventable diseases among health care workers*. Am J Infect Control 2009;37:858-60.

[8] Bolyard EA, Tablan OC, Williams WW, Pearson ML, Shapiro CN, Deitchman SD, *Hospital Infection Control Practices Advisory Committee (HICPAC). Guideline for infection control in healthcare personnel*, 1998. Infect Control Hosp Epidemiol 1998;19:407-63.

[9] Maltezou HC, Katerelos P, Poufta S, Pavli A, Maragos A, Theodoridou M. *Attitudes toward mandatory occupational vaccinations and vaccination coverage against vaccine-preventable diseases of health care workers in primary health care centers*. Am J Infect Control 2013;41:66-70.

[10] *Piano Nazionale della Prevenzione 2014-2018.* Ministero della Salute, pp. 61-5.

[11] Summary of WHO Position Paper – Immunization of Health Care Workers. Updated 30th May 2014.

[12] Hollmeyer HG, Hayden F, Poland G, Buchholz U. *Influenza vaccination of health care workers in hospitals A review of studies on attitudes and predictors*. Vaccine 2009;27:3935-44.

[13] Hofmann F, Ferracin C, Marsh G, Dumas R. *Influenza vaccination of healthcare workers: a literature review of attitudes and beliefs*. Infection 2006,34:142-7.

[14] Quan K, Tehrani DM, Dickey L, Spiritus E, Hizon D, Heck K, Samuelson P, Kornhauser E, Zeitany R, Mancia S, Thrupp L, Tiso SM, Huang SS. *Voluntary to mandatory: evolution of strategies and attitudes toward influenza vaccination of healthcare personnel*. Infect Control Hosp Epidemiol 2012;33:63-70.

[15] Helms C, Polgreen P, Polgreen L, Evans T, Roberts LL, Clabaugh G, Quinlisk P. *Voluntary reporting of employee influenza vaccination rates by acute care hospitals in Iowa: the impact of a four year provider-based statewide performance improvement project.* Vaccine 2011;29:3483-8.

[16] Sawyer MH, Peddecord KM, Wang W, Deguire M, Miskewitch-Dzulynsky M, Vuong DD. *A public health initiative to increase annual influenza immunization among hospital health care personnel: the San Diego Hospital Influenza Immunization Partnership.* Am J Infect Control 2012;40:595-600.

[17] Cadena J, Prigmore T, Bowling J, Ayala BA, Kirkman L, Parekh A, Scepanski T, Patterson JE. *Improving influenza vaccination of healthcare workers by means of quality improvement tools*; Infect Control Hosp Epidemiol 2011;32:616-8.

[18] Wicker S, Rabenau HF, Doerr HW, Allwinn R. *Influenza vaccination compliance among health care workers in a German University Hospital*. Infection 2009;37:197-202.

[19] Jiménez-García R, Hernández-Barrera V, Carrasco-Garrido P, López de Andrés A, Pérez N, de Miguel AG. *Influenza vaccination coverage among children, adults, health care workers and immigrants in Spain: related factors and trends, 2003-2006.* J Infect 2008;57:472-80.

[20] Placidi D, Bacis M, Belotti L, Biggi N, Carrer P, Cologni L, Gattini V, Lodi V, Magnavita N, Micheloni G, Negro C, Polato R, Puro V, Tonelli F, Tonozzi B, Porru S. *La tubercolosi. Focus sulla valutazione del rischio e la sorveglianza sanitaria dei lavoratori della sanità: risultati e prospettive di un gruppo di lavoro multicentrico*. G Ital Med Lav Erg 2010;32:273-81.

[21] Barbadoro P, Marigliano A, Di Tondo E, Chiatti C, Di Stanislao F, D'Errico MM, Prospero E. *Determinants of influenza vaccination uptake among Italian healthcare workers*. Hum Vaccin Immunother 2013;9:911-6.

[22] Istituto superiore di Sanità. *Vaccinazione antinfluenzale: stagione 2012-2013. Coperture vaccinali per 100 abitanti*. Ministero della Salute, 2013.

[23] Nowalk MP, Lin CJ, Raymund M, Bialor J, Zimmerman RK. *Impact of hospital policies on health care workers' influenza vaccination rates*. Am J Infect Control 2013;41:697-701.

[24] Gazmararian JA, Coleman M, Prill M, Hinman AR, Ribner BS, Washington ML, Janssen A, Orenstein WA. *Influenza vaccination of health care workers: policies and practices of hospitals in a community setting*. Am J Infect Control. 2007;35:441-7.

[25] Talbot TR, Dellit TH, Hebden J, Sama D, Cuny J. *Factors associated with increased healthcare worker influenza vaccination rates: results from a national survey of university hospitals and medical centers*. Infect Control Hosp Epidemiol 2010;31:456-62.

[26] Alicino C, Iudici R, Barberis I, Paganino C, Cacciani R, Zacconi M, Battistini A, Bellina D, Di Bella AM, Talamini A, Sticchi L, Morando A, Ansaldi F, Durando P. *Influenza vaccination among healthcare workers in Italy*. Hum Vaccin Immunother 2015;11:95-100.

[27] Immunization of Health-Care Workers: Recommendation of advisory committee on immunization practice (ACIP) and the Hospital Infection Control Practice Advisory committee (HICPAC). MMWR, Recommendation and report 26;12/26/97.

[28] Byrd KK1, Lu PJ, Murphy TV. *Hepatitis B vaccination coverage among health-care personnel in the United States*. Public Health Rep 2013;128:498-509.

[29] Vranckx R, Jacques P, De Schrijver A, Moens G. *Hepatitis B vaccination coverage in Belgian health care workers.* Infection 2004;32:278-81.

[30] Sukriti, Pati NT, Sethi A, Agrawal K, Agrawal K, Kumar GT, Kumar M, Kaanan AT, Sarin SK. *Low levels of awareness, vaccine coverage, and the need for boosters among health care workers in tertiary care hospitals in India*. J Gastroenterol Hepatol 2008;23:1710-5.

[31] Djeriri K, Laurichesse H, Merle JL, Charof R, Abouyoub A, Fontana L, Benchemsi N, Elharti E, El Aouad R, Chamoux A, Beytout J. *Hepatitis B in Moroccan health care workers*. Occup Med (Lond) 2008;58:419-24.

[32] Mele A, Stroffolini T, Zanetti AR. *Hepatitis B in Italy: where we are ten years after the introduction of mass vaccination.* J Med Virol 2002;67:440-3.

[33] Zanetti AR, Mariano A, Romanò L, D'Amelio R, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti C, Stroffolini T, Mele A; Study Group. *Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study*. Lancet 2005;366:1379-84.

[34] Campagna M, Siddu A, Meloni A, Murru C, Masia G, Coppola RC. *Epidemiological impact of mandatory vaccination against hepatitis B in Italian young adults*; Hepat Mon 2011;11:750-2.

[35] Coppola RC, Meloni A, Campagna M. *Impact of universal vaccination against hepatitis B: the Italian model*. Hepat Mon 2012;12:417-9.

[36] Spada E, Romanò L, Tosti ME, Zuccaro O, Paladini S, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti CM, Mele A, Zanetti AR; Study Group. *Hepatitis B immunity in teenagers vaccinated as infants: an Italian 17-year follow-up study.* Clin Microbiol Infect 2014;20:680-6.

[37] Carrer P, Micheloni G, Campagna M, Bacis M, Belotti L, Biggi N, Cologni L, Gattini V, Fostinelli J, Lodi V, Magnavita N, Negro C, Omeri E, Placidi D, Polato R, Puro V, Tonelli F, Porru S. *Focus sulla sorveglianza sanitaria dei lavoratori della sanità esposti ad agenti biologici trasmissibili per via ematogena: risultati e prospettive di un gruppo di lavoro multicentrico.* G Ital Med Lav Erg 2010;32:3,249-55).

[38] Campagna M, Bacis M, Belotti L, Biggi N, Carrer P, Cologni L, Gattinis V, Lodi V, Magnavita N, Micheloni G, Negro C, Oppini M, Placidi D, Polato R, Puro V, Tonelli E, Porru S. *Exanthemic diseases (measles, chickenpox, rubella and parotitis). Focus on screening and health surveillance of health workers: results and perspectives of a multicenter working group*. G Ital Med Lav Ergon 2010;32:298-303.

[39] Porru S, Campagna M, Arici C, Carta A, Placidi D, Crotti A, Parrinello G, Alessio L. *Susceptibility to varicella-zoster, measles, rosacea and mumps among health care workers in a Northern Italy hospital*. G Ital Med Lav Ergon 2007;29(Suppl 3):407-9.

[40] Centers for Disease Control and Prevention. *Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care facilities*, 1994. MMWR Recomm Rep 1994;43:1-132.

[41] Alvarez-León EE, Espinosa-Vega E, Santana-Rodríguez E, Molina-Cabrillana JM, Pérez-Arellano JL, Caminero JA, Serrano-Aguilar P. *Screening for Tuberculosis Infection in Spanish Healthcare Workers: Comparison of the QuantiFERON-TB Gold In-Tube Test with the Tuberculin Skin Test*. Infect Control Hosp Epidemiol 2009;30:876-83.

[42] Centre for Disease Control and Prevention, *Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting Mycobacterium tuberculosis Infection, United States.* MMWR Recommendation and Reports. 2005;54(RR15);49-55.

[43] Vinton P, Mihrshahi S, Johnson P, Jenkin GA, Jolley D, Biggs BA *Comparison of QuantiFERON-TB Gold In-Tube Test and tuberculin skin test for identification of latent Mycobacterium tuberculosis infection in healthcare staff and association between positive test results and known risk factors for infection*. Infect Control Hosp Epidemiol 2009;30:215-21.

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ORIGINAL ARTICLE

Measuring and benchmarking the quality of two different organizational ways in delivering infant vaccination

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Keywords

Health Services Administration • Quality of Healthcare • Vaccination

Summary

The aim of this study was the quality of service evaluation of two different organizational ways in delivering infant vaccination according to a Regional Vaccination Plan.

Eleven vaccination centres were selected in two Local Health Units (ASLs) belonging to the Regional Health Service of the Lazio Region, Italy. The services offering paediatric vaccinations for children under three years of age, delivered without an appointment (VACP) or with the need for an appointment (VACL), were investigated. The quality aspects under evaluation were communicational efficiency, organisational efficiency and comfort. Subjective data were collected from different stakeholders and involve the elicitation of best and worst feasible performance conditions for the ASLs when delivering VACP/VACL services. Objective data consists in the observation of current performances of the selected vaccination centres. Quality scorecards were obtained from the combination of all data. Benchmarking between VACP and VACL, i.e., two different organisational ways in delivering infant vaccination, can be performed as a result of the probabilistic meaning of the evaluated scores.

An expert of vaccination services, i.e., a virtual combination of patients, doctors and nurses, claims the quality of service delivery of the ASLs under investigation with probability 78.03% and 69.67% for VACP and VACL, respectively. In other words, for short, the quality scores of the ASLs were 78.03% for VACP and 69.67% for VACL. Furthermore our results show how to practically improve the current service delivery.

The QuaVaTAR approach can result in improvements of the quality of the ASLs for the two different ways of delivering paediatric vaccinations in a simple and intuitive way.

Introduction

The concept of quality, which was embraced during the second half of the last century, has spread exponentially in many areas. In the healthcare field, providers and policy makers have engaged in an extended search for useful approaches to measure and improve service performance in terms of quality, volume and user's perspective [1-5].

In Donabedian's classic framework, service quality is represented by considering structure, process and outcome using classes of key performance indicators (KPIs). The first class considers the organisational resources that ensure the functioning of health services and may be obtained from data generated "routinely" at healthcare facilities. The second class measures relief provided to the patient using data obtained from properly completed records. The third aims to assess the outcomes of healthcare interventions [6].

The Declaration of Alma-Ata in 1978 established the right and duty to participate individually and collectively in the planning and implementation of healthcare [7].

A main problem in measuring the quality of healthcare service could result from the fact that often, due to the lack of standards, different KPIs can be chosen to evaluate different providers; and this could lead to some benchmark issues. Furthermore, the evaluation process should include directly and actively both users and other stakeholders who participate in the organisation and delivery of services. To manage the KPIs heterogeneity and overcome the partiality of the decision maker's "absolutistic" point of view, an innovative and quantitative approach was defined and applied in different hospital settings [8, 9]. Later, the focus of the project has shifted from the hospital to local services, with the "Quality in Vaccination: Theory And Research" (QuaVaTAR) project, to assess the quality of vaccination services [10, 11].

The aim of this work is to illustrate a new application

of the QuaVaTAR approach for the quality evaluation of selected paediatric vaccination centres of the Lazio Region of Italy according to the Regional Vaccination Plan 2012-2014 [12].

Materials and methods

In April-July 2010, two of the twelve Local Health Units (ASLs) within the Regional Health Service of the Lazio Region of Italy were involved in this case study according to their willingness to participate: ASL RMH, located in the edge of Rome; and ASL RMF, located outside the city. Eleven vaccination centres were selected: two centres in one of the five districts of the ASL RMH and nine centres in the four districts of the ASL RMF. The two different ways of offering paediatric vaccinations to children under three years of age, with the need for an appointment (VACP) and without an appointment (VACL), were evaluated. The service quality aspects under evaluation were communicational efficiency, organisational efficiency and comfort. For each of these aspects, two quality KPIs were selected. Communicational efficiency was represented as the means and time of communicating information related to the vaccine with oral or written support before or during vaccination. Organisational efficiency was represented by the time a user spent in the waiting room and the time a user spent for vaccination. Comfort was represented by the opening times, and the presence of toys and/or a nursing room. KPIs and their possible values were the same for VACP and VACL, except for waiting time ranges.

Subjective data

An opinion survey was conducted at the two ASLs to collect "subjective data" related to the selected quality aspects of VACP and VACL. A well-defined questionnaire was administered to different groups of stakeholders: parents and escorts of immunised children (P/E), medical doctors (D) and nurses (N) working in the ASL. There were three sections in the questionnaire: an anonymous demographic section with questions on age, education, marital status and job; an informative section on the functioning of a generic vaccination service as presented by institutional guidelines; and a judgment section to assess specific performance conditions of delivery (defined in terms of the selected KPIs) containing 8 questions on communicational efficiency, 16 questions on organisational efficiency and 8 questions on comfort. Judgments expressed by stakeholders were used to assess probabilities (i.e., values ranging from 0-100%) of specific events related to communicational efficiency, organisational efficiency and comfort of the vaccination delivery. Different weights were chosen to combine judgments of each stakeholder: 0.3, 0.4, and 0.3 for P/E, D and N, respectively. This allowed to obtain the Expert, i.e. a "super" virtual stakeholder, point of view. The maximum and minimum probabilities which were assessed with this opinion survey define the best and worst achievable service quality scores for the ASLs.

Objective data

In the same period of the survey, April-July 2010, the selected KPIs were measured at the vaccination centres of the ASLs during the service delivery. These observations were recorded in an "objective data" set. They included waiting times (minutes), duration of vaccinations (minutes), indicators of means of communication and indicators of comfort features (true/false values).

Quality scorecard

Subjective and objective data were entered into a spread sheet that easily implements a quality scorecard according to a well-defined algorithm [9, 11]. Specific weights were chosen to combine the quality aspects under investigation and evaluate the overall quality score.

The overall quality for VACP/VACL is given by the weighted sum of the corresponding quality aspects (i.e., organisational efficiency, communicational efficiency and comfort).

The relevance of communicational efficiency, organisational efficiency and comfort was equal to 0.5, 0.3 and 0.2, respectively for both VACP and VACL.

Results

Questionnaires were administered to 416 stakeholders. The main socio-demographic characteristics of the interviewees are summarised in Table I. Statistical difference was present only for educational level as for VACP 88% of parents or escort had a high educational level in contrast with 77% in VACL ($p < 0.003$).

Table II shows the results of the survey related to the communicational efficiency.

From the Expert's point of view (i.e., the combination of all stakeholders' points of view), the minimum value of communicational efficiency (1.84% for VACP and 6.30% for VACL) is obtained if the information is not provided. In contrast, the maximum value for VACP (90.95%) and VACL (90.22%) were assessed if the information is provided through a brochure with the aid of a person. The difference was related to the moment considered more efficient for communication: during vaccination for VACP, in the waiting room for VACL.

The maximum values of organisational efficiency (94.91% for VACP and 81.22% for VACL) were assessed if the service is provided with waiting time less than 10-15 minutes (for VACP/VACL) and duration time less than 10 minutes. In both cases, however, a vaccination time of 10-20 minutes was considered efficient (84.21% for VACP and 74.21% for VACL).

The maximum values of comfort (95.61% for VACP and 90.17% for VACL) were assessed if the vaccination ambulatory is open alternatively in the morning and in the afternoon with the presence of children's toys and availability of a nursing room.

During the study 198 vaccination deliveries were observed.

Table IIIa shows different performance conditions related to the communicational efficiency which were ob-

Tab. I. Socio-demographic characteristics of the interviewed stakeholders.

Stakeholder demographic characteristic										
	VACP					VACL				
Stakeholder	n°	Median age (SD)	High School	Married	Job	n°	Median age (SD)	High School	Married	Job
P/E	226	34 (6.7)	200	213	173	162	34.9 (5.7)	125	147	98
N	8	39.2 (8.4)	8	5	8	8	39.7 (13.2)	8	5	8
D	9	46.1 (9.6)	9	7	9	3	39.0 (9.8)	3	2	3
Total	243	35.6 (7.2)	217	225	190	173	35.2 (6.2)	136	154	109

VACP: paediatric vaccinations to children under three years of age with the need for an appointment
VACL: paediatric vaccinations to children under three years of age without an appointment
P/E: Parents and escorts of immunised children
N: Nurses
D: Medical doctors

Tab. II. Communicational efficiency of the vaccination delivery assuming different performance conditions.

Subjective data related to the communicational efficiency								
Performance	VACP				VACL			
	P/E (%)	N (%)	D (%)	Expert (%)	P/E (%)	N (%)	D (%)	Expert (%)
Information is provided through a brochure with the aid of a person in the waiting room	86.65	60.00	63.33	69.33	84.73	96.00	90.00	90.22
Information is provided through a brochure with the aid of a person during the vaccination phase	79.83	90.00	100	90.95	75.93	93.00	87.78	85.79
Information is provided through a brochure without the aid of a person at the time of the vaccination service direct call	54.89	60.00	51.67	55.13	53.40	57.80	57.78	56.47
Information is provided through a brochure without the aid of a person in the waiting room	53.22	40.00	43.33	45.30	51.79	51.00	60.56	55.06
Information is provided through a brochure without the aid of a person during the vaccination phase	41.39.	25.00	26.67	30.58	37.74	39.50	48.89	42.73
Information is provided orally by a person in the waiting room	71.63	75.00	50.00	63.99	69.02	75.50	70.00	71.35
Information is provided orally by a person during the vaccination phase	72.38	95.00	76.67	80.88	65.20	77.50	62.22	67.70
information is not provided	6.14	0	0	1.84	10.01	11.00	0	6.30

VACP: paediatric vaccination to children under three years of age with the need for an appointment
VACL: paediatric vaccinations to children under three years of age without an appointment.
P/E: Parents and Escorts of immunised children
N: Nurses
D: Medical doctors

served at the ASLs during the vaccination delivery. The majority of performances were observed whit information provided orally by a person during the vaccination phase.

For organizational efficiency the majority of performances were observed with a waiting time less than 10-15 minutes (for VACP/VACL) and a vaccination time less than 10 minutes (Tab. IIIb).

Vaccination time greater than 30 minutes were never observed.

Considering comfort, for VACP, 58.00% of the services offered vaccination in the morning and in the afternoon; 48.00% have a nursing room and 75.00% toys (48.00% have both); while for VACL vaccination are administered only in the morning; in most cases the service provided toys (84.00%), and in 27.50% a nursing room (18.80% both).

Table IV shows the quality scorecard of the ASLs under evaluation for the two ways of offering vaccination and for different stakeholders. From the cumulative result the probability that an expert claims the quality of VACP was 78.03%, while the probability that an expert claims the quality of VACL was 69.67%.

Discussion

By adopting the QuaVaTAR approach it is possible to evaluate quantitatively and qualitatively the organizational characteristics of the vaccination services. In a first study it was applied to evaluate the different quality in the provision of HPV vaccination in three ASLs of the Lazio Region of Italy [11]. In the present work the same method was applied to evaluate different ways of delivering the same children vaccination,

Tab. IIIa. Performance conditions related to the communicational efficiency of the ASLs vaccination services.

Objective data related to the communicational efficiency		
Performance	VACP	VACL
	Performance frequency (%)	Performance frequency (%)
Information is provided through a brochure with the aid of a person in the waiting room	17.10	0
Information is provided through a brochure with the aid of a person during the vaccination phase	17.10	32.40
Information is provided through a brochure without the aid of a person at the time of the vaccination service direct call	0	0.60
Information is provided through a brochure without the aid of a person in the waiting room	0	0.60
Information is provided through a brochure without the aid of a person during the vaccination phase	0	0.60
Information is provided orally by a person in the waiting room	0	9.00
Information is provided orally by a person during the vaccination phase	65.80	56.80
Information is not provided	0	0

VACP: paediatric vaccinations to children under three years of age with the need for an appointment
VACL: paediatric vaccinations to children under three years of age without an appointment.

Tab. IIIb. Performance conditions related to the organisational efficiency of the ASLs vaccination services.

Objective data related to the organisational efficiency					
VACP			VACL		
Performance		Performance frequency (%)	Performance		Performance frequency (%)
Wt (minutes)	Dt (minutes)		Wt (minutes)	Dt (minutes)	
< 10	< 10	41.00	< 15	< 10	51.70
10 e 20	< 10	22.60	[15-30]	< 10	23.60
20 e 30	< 10	12.10	[30-45]	< 10	7.90
> 30	< 10	13.60	> 45	< 10	1.10
< 10	[10-20]	4.50	< 15	[10-20]	7.90
10 e 20	[10-20]	3.10	[15-30]	[10-20]	5.60
20 e 30	[10-20]	3.10	[30-45]	[10-20]	1.10
> 30	[10-20]	0	> 45	[10-20]	0
< 10	[20-30]	0	< 15	[20-30]	0
10 e 20	[20-30]	0	[15-30]	[20-30]	0
20 e 30	[20-30]	0	[30-45]	[20-30]	0
> 30	[20-30]	0	> 45	[20-30]	1.10

VACP: paediatric vaccinations to children under three years of age with the need for an appointment
VACL: paediatric vaccinations to children under three years of age without an appointment
Wt = Waiting time (minutes)
Dt = Vaccination Time (minutes)

i.e. VACP and VACL. Identical KPIs chosen for the first study were used here. This is not a limitation. In fact, due to the flexibility of the method, other KPIs could be chosen, depending on users' interest.

In this study VACP obtained a better result than VACL, and the only significant difference was seen in the instruction level of parents/escorts, where those with an higher instruction level preferred VACP, showing a greater interest in that type of service organization that allowed a better management of time.

Subjective data measure how stakeholders perceived the VACP and VACL service quality of providers. Focusing on communicational efficiency, the results suggest that the best way to perform communication is to provide information through a brochure with the aid of a person during the vaccination phase for VACP and in the waiting room for VACL. For organisational efficiency, as expected, the maximum value for VACP and VACL was for a waiting time of 10-15 minutes, respectively, and a vaccination time of less than 10 minutes. A short waiting time with a vaccination time of 10-20 minutes was however considered efficient for both VACP and VACL. For comfort, as expected, the maximum value corresponded to the opening time both in the morning and in the afternoon with the presence of toys and nursing room. These were the so called targets for VACP and VACL. These values do not correspond to the theoretical maximum

Tab. IV. Quality scorecard of the ASLs vaccination services.

Quality scorecards						
	VACP Overall quality = 78.03%			VACL Overall quality = 69.67%		
Stakeholder	Organizational efficiency (weight 0.3)	Communicational efficiency (weight 0.5)	Comfort (weight 0.2)	Organizational efficiency (weight 0.3)	Communicational efficiency (weight 0.5)	Comfort (weight 0.2)
P/E	71.07	76.11	65.95	74.09	68.66	62.12
N	81.82	88.14	69.23	76.63	81.78	38.59
D	83.48	78.38	72.91	68.38	71.04	63.57
Expert = 0.3P/E + 0.3N + 0.4D	79.26	80.63	69.72	72.57	73.55	55.64

VACP: paediatric vaccinations to children under three years of age with the need for an appointment
VACL: paediatric vaccinations to children under three years of age without an appointment P/E: Parents and escorts of immunised children
N: Nurses
D: Medical doctors

score values (i.e., 100%). This is not a "limitation" of the model; better performance measured by different KPIs could exist. In this study, however, only performances that could be effectively implemented by the ASLs were modelled.

It is worth noting that different stakeholders may have a different perception of the service quality also related to the modality of providing vaccination. For example, for communicational efficiency, while in VACL all category considered better to give information through a brochure in the waiting room, and this was the best achievable target, in VACP parents considered better this way while doctors and nurses considered more efficient to give information during the vaccination phase, and this was the best achievable value considered by the Expert. This seems logical; in fact when access is planned the vaccination phase could be the best moment for communication, as a long waiting time is not foreseen. When access is free, a longer waiting time can be expected by the P/E and this time could be used for communication.

Despite the best efficient way of communication considered also the use of a brochure, this was used during vaccination or in the waiting room only in 34% and 32% of VACP and VACL providing. The use of a brochure could therefore improve the quality of both VACP and VACL. For organisational performance does not seem that there is a difference between VACP and VACL, although the considered waiting times were different. A difference was seen for comfort related to the opening times of the service. In VACP in the majority of cases it is possible to have an appointment both in the morning and in the afternoon, while VACL is offered only in the morning. Although the double possibility proved to be the most efficient, the values reached 95.61% for VACP and 90.17% for VACL.

Conclusions

The top management of the ASLs needs to take in serious consideration that, in terms of business risks, 21.97% and 31.33% are the probabilities that an expert "do not" claim the quality of the ASLs for the two ways of delivering the pediatric vaccination, VACP and VACL, respectively. The good news is that there exist margins of improvements. And the general criterion to obtain this is simple and intuitive by using the QuaVaTAR approach. It is necessary to transform the performance conditions currently observed during the vaccination delivery in those which are better for the stakeholders, as suggested by the opinion survey which involved parents/escorts, nurses and doctors.

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Authors' contributions

MM, LP, EF, AC conceived, designed and coordinated the study. MM, LP, EF, AC, CC, SS, RC discussed and approved the design of the study. MM, LP, EF, AC contributed to the acquisition of data. MM, LP, AC optimized the informatics database.

References

[1] Parasuraman A, Zeithaml VA, Berry LL. *SERVQUAL: A multiple-Item Scale for measuring consumer perceptions of service quality*. J Rating 1988;1:12-40.

[2] Ravichandran K, Tamil Mani B, Arun Kuma S, Prabhakara S. *Influence of service quality on customer satisfaction: application of servqual model*. Int J Business Manag 2010;4:117-24.

[3] Lin DJ, Sheu IC, Pai JY, Bair A, Hung CY, Yeh YH, Chou MJ. *Measuring patient's expectation and the perception of quality in LASIK services*. Health Qual Life Outcomes 2009;7:63.

[4] Ryan M. *Discrete choice experiments in healthcare*. BMJ 2004;328:360-1.

[5] Ryan M, Skåtun D. *Modelling non-demanders in choice experiments*. Health Econ 2004;13:397-402.

[6] Donabedian A. *Evaluating the quality of medical care.* Milbank Memorial Fund Quart 1966;44:166-203.

[7] Declaration of Alma-Ata International Conference on Primary Health Care, Alma-Ata, USSR, 6-12 September 1978.

[8] Coletti G, Paulon L, Scozzafava R, Vantaggi B. *Measuring the quality of health-care services: a likelihood-based fuzzy modeling approach symbolic and quantitative approaches to reasoning with uncertainty lecture notes in computer.* Science 2007;4724:853-64.

[9] Paulon L. *Mathematics in Health Care with applications* (Doctoral dissertation, Sapienza University, Rome).

[10] Maurici M, Meleleo C, Campolongo A, D'Anna C, Mangia ML, Sgricia S, Serino L, Paulon L, Franco E, Ferrante M. *Application of the QuaVaTAR model to vaccination services in Latium, Italy*. Ig Sanita Pubbl 2010;66:793-801.

[11] Maurici M, Paulon L, Campolongo A, Meleleo C, Carlino C, Giordani A, Perrelli F, Sgricia S, Ferrante M, Franco E; QuaVaTAR Group. *Quality measurement and benchmarking of HPV vaccination services: a new approach.* Hum Vaccin Immunother 2014;10:208-15.

[12] Regione Lazio. Decreti del Commissario ad Acta. Decreto del Commissario ad Acta 5 novembre 2012, n. U00192. Presa d'atto dell'Intesa ai sensi dell'art. 8 comma 6 della Legge 5 giugno 2003 n. 131 tra il Governo, le Regioni. e le Province autonome di Trento e Bolzano sul documento recante "Piano Nazionale Prevenzione Vaccinale 2012 – 2014". Piano Regionale Prevenzione Vaccinale 2012 – 2014. Available at:http://www.epicentro.iss.it/temi/vaccinazioni/pdf/Normative/Lazio_%20Novembre%202012/PRPV%202012-14%20(CALENDARIO%20+%20PNPV12-14).pdf [Accessed on 21/12/2015]..

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ORIGINAL ARTICLE

PCR-based assay for the rapid and precise distinction of *Pseudomonas aeruginosa* from other Pseudomonas species recovered from burns patients

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Keywords

Pseudomonas aeruginosa • Burns patients • *OprL, OprI*

Summary

Background. *Pseudomonas aeruginosa is an important life-threatening nosocomial pathogen which plays a prominent role in wound infections in burns patients. We designed this study to identify the isolates of P. aeruginosa recovered from burns patients at the genus and species levels by means of primers targeting oprI and oprL genes.*
Methods. *During a 5-month period, wound samples were taken from burns patients and plated on MacConkey agar. All suspected colonies were screened for P. aeruginosa by means of a combination of phenotype tests. Specific primers for oprI and oprL genes were then used for the molecular identification of colonies.*
Results. *During the 5-month period, bacterial isolates recovered from burn wound infections were analyzed. Phenotype identification tests identified 171 (34.8%) P. aeruginosa isolates. However, molecular techniques that used species-specific primers to detect the amplicon of the oprL gene confirmed the exact identification of P. aeruginosa in only 133 cases; in the other isolates, the use of genus-specific primers detected the amplicon of the oprI gene, which confirmed the identification of fluorescent pseudomonads.*
Conclusions. *This study indicates that molecular detection by means of an assay targeting the oprL gene is a useful technique for the rapid and precise detection of P. aeruginosa in burns patients. In addition to phenotype testing, PCR detection should be carried out in order to promptly ascertain the best aggressive antibiotic therapy for P. aeruginosa infections, thereby significantly improving clinical outcomes.*

Introduction

Burn injury, one of the most common and devastating forms of trauma, is a major public health problem worldwide. Burn wounds can easily become infected because the skin no longer acts as an effective physical barrier against microbes. *P. aeruginosa* is the most common source of burn wound infections [1]. While these bacteria rarely cause disease in healthy individuals, they may do so in immuno compromised patients, such as those with AIDS or cystic fibrosis, and in burns patients [2]. The accurate identification of *P. aeruginosa* and detection of their susceptibility to antimicrobials are critical components of the management of burned patient. *P. aeruginosa* colonisation is normally detected by culturing wound swabs on artificial media. Typical isolation media used in wound infections include blood agar and chocolate agar and selective agars such as MacConkey agar and cetrimide-based media. Although large numbers of *P. aeruginosa* isolates are often present in clinical samples from burns patients, their detection and precise identification can often be challenging. For example, other species of Pseudomonas, as well as *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Ralstonia pickettii,* have been shown to grow on cetrimide-based media [3], and may be indistinguishable from non-pigmented strains of *P. aeruginosa*. Difficulties in recognizing *P. aeruginosa* are compounded by difficulties in biochemical identification. Biochemical test kits such as API 20 NE are commonly used for identification [4]; however, this technique has been seen to display a high rate of misidentification of oxidase-positive Gram-negative rods, including *P. aeruginosa* [5]. In addition, testing requires the use of a pure bacterial subculture and a minimum incubation time of 48 h. Hence, identification by means of this method requires at least 3 days. Another limitation of the conventional culture technique is that *P. aeruginosa* can easily be mistaken for closely related Gram-negative bacilli. The use of molecular techniques such as PCR could enable accurate and rapid identification of *P. aeruginosa* [6, 7]. L and I lipoproteins are two outer membrane proteins of *P. aeruginosa, and* are responsible for the inherent resistance of the bacterium to antibiotics and antiseptics. As these proteins are found only in this organism, they could be used as a reliable marker for the rapid identification of *P. aeruginosa* in clinical samples [8, 9]. In this study, we examined a technique for the rapid and precise identification of *P. aeruginosa* strains isolated from burns patients hospitalized in a main burns center in Iran. The performance of this technique, which utilizes PCR amplification of I lipoprotein (*OprI*) to detect the genus and

L lipoprotein (*OprL*) to detectthe species of *P. aeruginosa* strains, was compared with that of phenotypic and routine biochemical identification used in laboratories.

Materials and methods

Qualitative conventional detection

This study was carried out during a 5-month period, at a major center for the admission of burns patients in Tehran, Iran. Samples were obtained from burn wounds by swabbing. As in the routine phonotype tests usually performed in clinical laboratories, we inoculated burn wound swabs onto several selective media for the isolation of *P. aeruginosa*, including blood agar, MacConkey agar and Muller Hinton agar, and carried out incubation at 37ºC for 24-48 h. The isolates were presumptively identified by means of routine tests: colony morphology and pigment formation on selective medium, positive oxidase test, glucose fermentation, hydrolysis of gelatin and growth at 42ºC [10, 11].

Molecular (PCR) detection

DNA extraction. In order to minimize contamination and hence the possibility of false-positive results, all DNA isolation procedures were carried out in a room physically separated both from that used to set up nucleic acid amplification reaction mixtures and from the post-PCR room. Bacterial genomic DNA was extracted from all phenotypically and biochemically tested strains, as well as from the reference strain *P. aeruginosa* ATCC 27853, by means of a boiling method. For this purpose, depending on colony size, three to six colonies were picked from bacterial plates and mixed into 0.25 ml DNase/RNase-free water in sterile 1.5 ml eppendorf tubes in order to obtain a turbid suspension of bacteria (~ 1-2 × 109 cells/ml). The cell suspensions were kept in a boiling water bath for 10 minutes to lyse the cells, and were then centrifuged at 10000 g at 4°C for 10 minutes. Finally, the supernatant was transferred, in sterile conditions, into another tube and used as a DNA template. Extracted DNA was stored at -20°C prior to PCR amplification [12].

Primer selection. The primers used in this study are shown in Table I. PCR amplification of I lipoprotein (*OprI*) for the detection of *Pseudomonas* genus and L lipoprotein (*OprL*) for the detection of *P. aeruginosa* species was performed on all phenotypically tested strains of *P. aeruginosa*.

PCR amplification. In order to minimize contamination, all reaction mixtures were set up in a PCR room separate from that used for DNA extraction and amplification and from the post-PCR room. PCR was completed in adapted PCR micro centrifuge tubes according to the thermocycler used. Following optimization, reaction mixtures (25 µl) were set up as follows: 11 µl DNase/RNase-free water, 8 µl 2x PCR Master Mix (1.5 mM mgcl2, Denmark), 0.5 µl of each set of primers (*OprL* or *OprI*) and 5 µl of DNA template. The reaction mixtures were subjected to the following empirically optimized thermal cycling parameters in a thermocycler (Senso-Quest Labcycler, Germany): 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. Positive (*P. aeruginosa* ATCC 27853 DNA) and multiple negative (water) amplification controls were included in every set of PCR reactions.

Detection of amplicons

Following amplification, aliquots (10 µl) were removed from each reaction mixture and examined by means of electrophoresis (80 V, 45 min) in gels composed of 1% agarose in TBE buffer (40 mM Tris, 20 mM boric acid, 1 mM EDTA, pH 8.3). Gels were visualized under UV illumination by using a gel image analysis system (UVitec, Cambridge, United Kingdom) and all images were archived. Where a band was visualized at the correct expected size for *OprI*, the specimen was considered positive for *Pseudomonas* genus; if a band was visualized at the correct expected size for *OprL* loci, the specimen was considered positive for *P. aeruginosa* species.

Results

During the 5-month period, 491 bacterial samples recovered from burn wound infections were analyzed. The above-mentioned routine phenotype and biochemical tests enabled *P. aeruginosa* isolates to be recovered from 171 (34.8%) patients. By contrast, molecular techniques detected only 133 (27.08%) samples positive for *P. aeruginosa* species and 38 (7.73%) samples positive for pseudomonas genus. PCR assays employing each primer pair yielded DNA products of the predicted sizes (Fig. 1 and 2). The *OprI* and *OprL amplicon* genes were detected in all 133 *P. aeruginosa* isolates simultaneously. Table II shows the comparison of phenotype and biochemical testing with the molecular detection of *P. aeruginosa* in samples from burn wound infections.

Tab. I. Primers used in this study.

Primer	5′-sequence-3′	Product length (bp)	Reference
OprI-F	ATGAACAACGTTCTGAAATTCTCTGCT	249	7
OprI-R	CTTGCGGCTGGCTTTTTCCAG		
OprL-F	ATGGAAATGCTGAAATTCGGC	504	7
OprL-R	CTTCTTCAGCTCGACGCGACG		

Fig. 1. PCR amplification using Pseudomonas genus-specific primers (*Opr I* gene), M: marker, line 1: positive control, lines 2, 3, 4: clinical isolates of Pseudomonas genus, line 5: negative control.

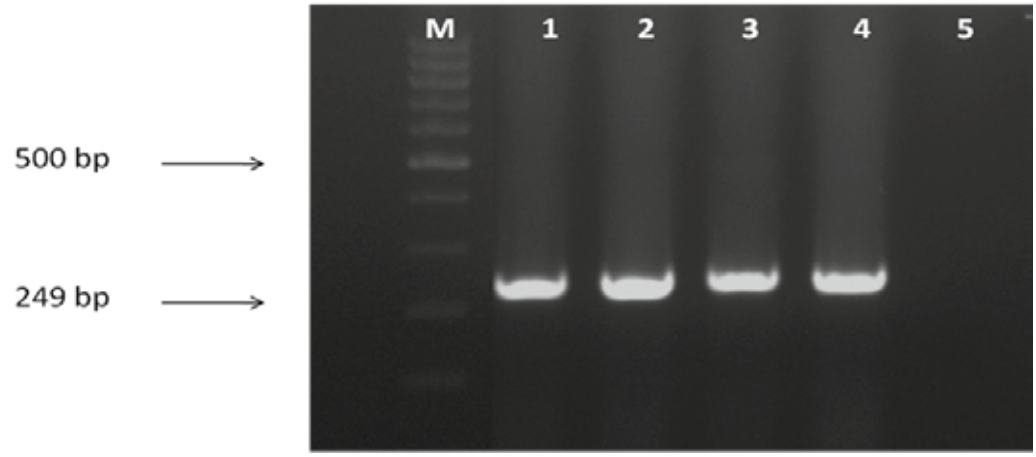
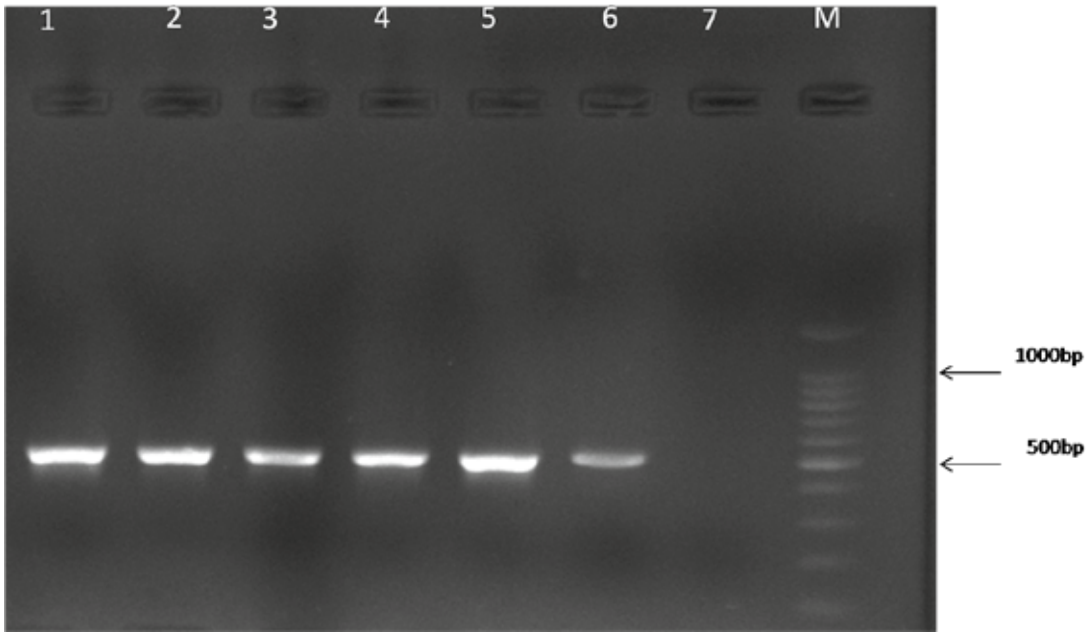


Fig. 2. PCR amplification using *P. aeruginosa*-specific primers (*Opr L* gene), M: marker, line 1: positive control, lines 2, 3, 4, 5, 6: clinical isolates of *P. aeruginosa*, line 7: negative control.



Tab. II. Comparison of phenotype and biochemical tests with molecular detection of *P. aeruginosa* in samples from burn wound infections.

Phenotypically & biochemically + (no. of isolates tested)	PCR(*OprI*)+ no. of isolates	PCR(*OprL*)+ no. of isolates
171	171	133

Biochemical method+: strains confirmed as *P. aeruginosa* on phenotype and biochemical testing.
PCR (OprI)+: strains confirmed as *Pseudomonas* genus by PCR amplification of *OprI*.
PCR (OprL)+: strains confirmed as *P. aeruginosa* genus by PCR amplification of *OprL*.

Discussion

Bacterial infections in burn wounds are common and are difficult to control. In recent decades, following the introduction of antibiotic therapy, *P. aeruginosa* has emerged as one of the most problematic Gram-negative bacteria in modern hospital settings. This organism is increasingly isolated as a nosocomial pathogen, and is responsible for high morbidity and mortality rates in burns patients, mechanically ventilated patients and those with cystic fibrosis [13, 14]. Infection by this bacterium is particularly problematic, since the organism is intrinsically

resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs. Some studies carried out in Iran have also indicated that infections caused by multi-drug resistant (MDR) *P. aeruginosa* are widespread in Iranian hospitals [15, 16]. It is therefore important to identify *P. aeruginosa* accurately and rapidly and to ascertain the susceptibility pattern of this organism; this may avoid prolonged and sometimes unnecessary antibiotic treatments, which could select other antibiotic-resistant pathogens [12]. The identification of *P. aeruginosa* has traditionally relied on phenotypic and biochemical methods. These tests take a long time to perform and require extensive hands-on work by technicians, both for setup and for ongoing evaluation. Various methods have been developed to identify *P. aeruginosa* species rapidly and accurately. According to our literature review, PCR has the potential to identify microbial species rapidly and precisely through the amplification of gene sequences unique to a particular organism [17]. Indeed, several PCR-based, DNA probe methods have been developed to detect various pathogens in clinical samples and water and food samples [18]. Also in the case of *P. aeruginosa*, molecular methods have been reported to be superior to phenotypic methods in identifying *P. aeruginosa* species [19]. The outer membrane proteins of *P. aeruginosa* play important roles in the interaction of the bacterium with the environment [20]. In the present study, two PCR assays were performed individually for the molecular detection of two outer membrane lipoprotein genes, *oprI* and *oprL,* in samples from burn wounds [21]. According to the phenotype and biochemical tests carried out in this study, 171 (34.82%) of 491 bacterial samples recovered were classified as *P. aeruginosa*. However, while the results of our molecular tests *on oprI* and *oprL* genes confirmed that many of these isolatesbelonged to the *pseudomonas* genus, only 133 (77.7%) of them were confirmed as *P. aeruginosa* species. Thus, there was nearly complete agreement between molecular and conventional phenotype and biochemical detection techniques with regard to the attribution of the *Pseudomonas* genus, but not the *P. aeruginosa* species. This may account for the potential phenotype misidentification of *P. aeruginosa* which has been recently described [22]. Alternatively, discrepant results (PCR- /biochemical+) may emerge in the case of true *P. aeruginosa* colonization, in that a false negative culture result may be due to sample overgrowth by other bacteria, or to the presence of non-cultivable organisms or auxotrophic mutations of the organism. Indeed, it has been shown that *oprI* is conserved among members of fluorescent pseudomonads [23, 24]. De Vos et al. designed a multiplex PCR assay based on *oprI* and *oprL* genes for the molecular detection of *P. aeruginosa*, and showed that its specificity and sensitivity were 74 and 100%, respectively [7]. Lavenir et al. also noted that all 268 of the *P. aeruginosa* strains that they detected contained the *oprI* and *oprL* genes (sensitivity = 100%, specificity = 80%) [25]. This is in line with our findings. Although our PCR and DNA sequence analyses revealed some isolates that had been misidentified by phenotype testing, it must be said that our study was not designed to ascertain the frequency of misidentification of isolates from burn wound infections or to compare the relative accuracy of different phenotype identification systems. The isolates analyzed in this study constituted a biased set of atypical isolates that were difficult to identify. Nevertheless, these isolates were well suited to providing a rigorous test of our PCR assays and represented strains for which molecular analysis would be expected to be most useful. Our study also confirms that various non-aeruginosa pseudomonal species can occasionally be recovered from burn wound infection cultures. In this regard, genotype-based identification methods circumvent the problem of phenotype variation and provide more accurate species identification.

Conclusions

It is important that primary diagnostic bacteriology methods have the ability to detect transient and early Pseudomonas colonization in burns patients as soon as possible, so that: (i) aggressive antibiotic regimes may be reconsidered; (ii) the patient can be managed optimally, with a view to avoiding early biofilm formation and chronic colonization with *P. aeruginosa*, and (iii) appropriate infection control precautions can be taken.

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Authors' contributions

Maryam Adabi and Abbas Gholami conceived; designed and coordinated the research.
Mina Boostanshenas collected data. Mahshid Talebi-Taher and Ali Majidpour evaluated the results.
Maryam Adabi and Abbas Gholami wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

References

[1] Church D, Elsayed S, Reid O, Winston B, Lindsay R. *Burn wound infections*. Clin Microbiol Rev 2006;19:403-34.

[2] Lyczak JB, Cannon CL, Pier GB. *Establishment of* Pseudomonas aeruginosa *infection: lessons from a versatile opportunist.* Microbes Infection 2000;2:1051-60.

[3] Kodaka H, Iwata M, Yumoto S, Kashitani F. *Evaluation of a new agar medium containing cetrimide, kanamycin and nalidixic acid for isolation and enhancement of pigment production of Pseudomonas aeruginosa inclinical samples.* J Basic Microbiol 2003;43:407-13.

[4] Van Pelt C, Verduin CM, GoessensWHF, Vos MC, Tümmler B, Segonds C, Reubsaet F, Verbrugh H, Belkum A. *Identification*

of Burkholderia spp. in the clinical microbiology laboratory: comparison of conventional and molecular methods. J Clin Microbiol 1999;37:2158-64.

[5] Wellinghausen N, Kothe JK, Wirths B, Sigge A, Poppert S. *Superiority of molecular techniques for identification of Gram negative, oxidase-positive rods, including morphologically non typical Pseudomonas aeruginosa, from patients with cystic fibrosis.* J Clin Microbiol 2005;43:4070-5.

[6] Anuj SN, Whiley DM, Kidd TJ, Bell SC, Wainwright CE, Nissen MD, Sloots TP. *Identification of* Pseudomonas aeruginosa *by a duplex real-time polymerase chain reaction assay targeting the ecfX and the gyrB genes.* Diagn Microbiol Infect Dis 2009;63:127-31.

[7] De Vos D, Lim A, Pirnay JP, Struelens M, Vandenveld C, Duinslaeger L, Vanderkrlrn A, Cornelis P. *Direct detection and identification of Pseudomonas aeruginosa in clinical samples such asskin biopsy specimens and expectorations by multiplex PCR based on two outer membrane genes*, *oprI* and *oprL*. J Clin Microbiol 1997;35:1295-9.

[8] Douraghi M, Ghasemi F, Dallal MM, Rahbar M, Rahimiforoushani A. *Molecular identification of Pseudomonas aeruginosa recovered from cystic fibrosis patients.* J Prev Med Hyg 2014;55:50-3.

[9] Osayande J. *Easy identification of difficult-to-type* P*seudomonas aeruginosa clinical and environmental isolates.* Internet J Microbiol 2008;7:2.

[10] Masuda N, Sakagawa E, Ohya S. *Outer membrane proteins responsible for multiple drug resistance in Pseudomonas aeruginosa.* Antimicrob Agents Chemother 1995;39:645-9.

[11] Hall GS. *Non fermenting Gram negative bacilli*. In: Mahon CR, Manuselis G (Eds.). *Text book of Diagnostic Microbiology.* 2nd ed. Philadelphia: WB Saunders;2000:542-62.

[12] Adabi M, Talebi-Taher M, Arbabi L, Afshar M, Fathizadeh S, Minaeian S, Moghadam-Maragheh N, Majidpour a. *Spread of Efflux Pump Overexpressing-Mediated Fluoroquinolone Resistance and Multidrug Resistance in Pseudomonas aeruginosa by using an Efflux Pump Inhibitor*. Infect Chemother 2015;47:98-104.

[13] Forbes BA, Sahm DF, Weissfeld A (Eds.). *Bailey and Scott's Diagnostic Microbiology*. 11th ed. St. St Louis:Mosby Inc 2002.

[14] Poh CL, Yeo CC. *Recent advances in typing of Pseudomonas aeruginosa*. J Hosp Infect 1993;24:175-81.

[15] Adabi M, Talebi Taher M, Arbabi L, Afshar M, Fathizadeh S, Minaeian S, Moghadam-Marageh N, Majidpour A. *Determination of antibiotic resistance pattern of Pseudomonas aeruginosa strains isolated from patients with burn wounds.* J Ardabil Uni Medl Scienc 2015;15:66-74.

[16] Shahcheraghi F, Feizabadi MM, Yamin V, Abiri R, Abedian Z. *Serovar determination, drug resistance patternsand plasmid profiles of Pseudomonas aeruginosa isolated from burn patients at two hospitals of Tehran (IRAN)*. Burns 2003;29:547-51.

[17] Nikbin VS, Abdi-Ali A, Feizabadi MM, Gharavi S. *Pulsed-field gel electrophoresis & plasmid profile of Pseudomonas aeruginosa at two hospitals in Tehran, Iran*. Indian J Med Res 2007;126:146-51.

[18] Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. *Primer directed enzymatic amplification of DNA with thermostable DNA polymerase.* Science 1988;239:487-91.

[19] Bej AK, Mahbubani MH, Dicesare JL, Atlas RM. *Polymerase chain reaction gene probe detection of microorganismsby using filter-concentrated samples*. Appl Environ Microbiol 199;1:57:3529-34.

[20] Qin X, Emerson J, Stapp J, Stapp L, Abe P, Burns L. *Use of real-time PCR with multiple targets to identify Pseudomonas aeruginosa and other non fermenting gram negative bacilli from patients with cystic fibrosis.* J Clin Microbiol 2003;4:4312-7.

[21] Hancock REW, Siehnel R, Martin N. *Outer membrane proteins of Pseudomonas.* Mol Microbiol 1990;4:1069-75.

[22] Cornelis P, Bouia A, Belarbi A, Guyonvarch A, Kammerer B, Hannaert V, Hubert JC. *Cloning and analysis of the gene for the majorouter membrane lipoprotein from Pseudomonas aeruginosa.* Mol Microbiol 1989;3:421-8.

[23] Kolmos HJ, Thuesen B, Nielsen SV, Lohmann M, Kristoffersen K, Rosdahl VT. *Outbreak of infection in a burns unit due to Pseudomonas aeruginosa originating from contaminated tubing used for irrigationof patients*. J Hos Infect 1993;24:11-21.

[24] De Vos D, Lim A Jr, De Vos P, Sarniguet A, Kersters K, Cornelis P. *Detection of the outer membrane lipoprotein I and its gene in fluorescentand non fluorescent pseudomonads: implications for taxonomy and diagnosis.* J Gen Microbiol 1992;139:2215-23.

[25] Lavenir R, Jocktane D, Laurent F, Nazaret S, Cournoyer B. *Improved reliability of Pseudomons aeruginosa PCR detection by the use of the specific ecfx gene target*. J Microbiol Methods 2007;70:20-9.

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ORIGINAL ARTICLE

Molecular detection of human papillomavirus from abnormal cervical cytology of women attending a tertiary health facility in Ido-ekiti, southwest Nigeria

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Keywords

Cervical cytology • HPV DNA • *women*

Summary

Background. *Human papillomavirus (HPV) has been implicated as one of the principal causes of cervical cancer, which is the second highest cause of cancer deaths among Nigerian women.*
Objective. *This study was aimed at determining the presence of HPV DNA in abnormal cervical cytology of a group of women who were screened using Papanicolaou staining technique.*
Methodology. *A total of 200 women attending the Obstetrics and Gynaecology clinic of the Federal Teaching Hospital, Ido-Ekiti, were screened by means of conventional Pap smear screening, while positive samples underwent molecular analyses by means of DNA isolation techniques and polymerase chain reaction (PCR).*
Result. *Results revealed that 14 (7%) of the subjects were positive for abnormal cytology. Abnormalities found among the subjects included: low-grade squamous intraepithelial lesions (LSIL), which constituted 50% of the total abnormal smears, high-grade squamous intraepithelial lesion (HSIL) and atypical squamous cells of undetermined significance (ASCUS), which were 28.6% and 21.4%, respectively. Molecular analyses showed that all the samples from abnormal cervical cytology subjected to HPV DNA extraction and gene amplification contained HPV DNA.*
Conclusions. *The high prevalence of HPV DNA in abnormal cytology gives credence to the fact that the presence of HPV is a critical indicator of the development of cervical cancer. Thus more effort should be put into vaccine production and distribution in order to reduce the incidence of cervical cancer in Nigeria.*

Introduction

Human papillomavirus (HPV) belongs to the family Papillomaviridae, which is capable of infecting humans. Like all papillomaviruses, HPV establishes productive infection only in keratinocytes of the skin or mucous membranes [1]. HPV is a small non-enveloped icosahedral DNA virus that replicates in the nucleus of squamous epithelial cells [2]. High-risk human papillomaviruses (HR-HPVs) are able to infect several types of epithelial cells, but more frequently cause cancer in the uterine cervix [3].
Various risk factors are associated with the occurrence of HPV infection; these include: smoking, multiparity, early onset of sexual intercourse, oral contraceptive use and Human Immunodeficiency Virus. They contribute greatly to an individual's chances of developing cervical cancer [4]. Cervical cancer ranks as the second most frequent cancer among women in Nigeria, and the most frequent cancer among women between 15 and 44 years of age. About 23.7% of women in the general population are estimated to harbor cervical HPV infection at any given time [1]. The prevalence of HPV genotypes in cervical cytological samples varies greatly in different geographical regions and shows a strong correlation with cervical cancer incidence. Epidemiological studies have consistently shown that the most important determinants of HPV infection in women are the number of sexual partners, the age of initiation of sexual activity, and the sexual behaviour of the male partner [5].
Clifford *et al.* randomly selected women from 11 countries for HPV DNA testing, using GP5+/6+ PCR analyses [6]. Women aged 15-74 years who did not have cytological abnormalities were included (n = 15613). They found that age-standardized prevalence varied nearly 20-fold among different populations: from 1.4% in Spain, to 25.6% in Nigeria. Overall, age-standardized HPV prevalence was five times higher in sub-Saharan Africa than in Europe, with intermediate prevalence in South America and Asia. In terms of types, HPV type 16 was the commonest type in all regions except sub-Saharan Africa, where HPV type 35 was equally common.

Materials and methods

Ido-Ekiti is located in Ido/Osi Local Government Area of Ekiti State, Nigeria. It is situated in the Northern part of the state, where the routes from Oyo, Osun and Kwara states converge. Ido-Ekiti is the headquarters of Ido/Osi local council. The hospital is located in Ido-Osi Local Government Area of Ekiti State, which lies south of

Kwara and Kogi State, East of Osun State and north of Ondo State, and has a total land area of 5887.890 sq km. The 2006 population census by the National Population Commission put the population of Ekiti State at 2,384.212 people.

Study population

The study was carried out among women attending the Gynaecology Clinic of the Federal Teaching Hospital, Ido-Ekiti who were between the ages of 15-64 years, willing to participate and met the inclusion criteria. A structured close-ended questionnaire was administered to these patients after informed consent had been obtained, followed by clinical examination.

Inclusion criteria

Female patients attending the Obstetrics and Gynaecological unit, between 15 to 64 years of age, who consented to take part in the study, and non-patients who met other inclusion criteria and agreed to participate in the study.

Exclusion criteria

Female patients over 65 years or under 15 years; male patients; patients who do not give consent to take part in the study and patients who have had total abdominal hysterectomy.

Ethical clearance

Approval for this study was obtained from the Ethics Review Committee of the Federal Teaching Hospital, Ido-Ekiti, after which informed consent was obtained from patients and/or parents and guardians. The study was done at no financial cost to the subjects, and information from the patients and/or parents and guardians were confidential.

Sample collection and analysis

A structured close-ended questionnaire was administered to the subjects after due consent had been obtained; cervical smears were collected by a gynaecologist after visual inspection. The smears collected were immediately fixed to slides before being transferred to the laboratory for processing. The fixed smears were stained by the cytotechnologist using the Papanicolaou staining procedure and read by a histopathologist using a light microscope. The smears were classified as normal, inflammatory, abnormal (epithelial lesion) or unsatisfactory.

Data Analysis

Descriptive statistics (mean, frequency, standard deviation and percentage) and a graph were used to present the results, in order to provide a clear representation of the data analyzed. The statistical package for social science version 17.0 for windows was used to test for the level of significance of the results obtained. Both continuous and discrete variables were generated. The relationship between categorical variables and outcome of interest was tested by means of Chi-square test at 95% ($p < 0.05$) confidence interval.

PCR/DNA extraction

Molecular analyses began with the extraction of DNA from the cervical smears that were positive for intraepithelial neoplasia. The coverslips present on the slides during Pap staining were removed with the aid of xylene, and the smears carefully removed by means of sterile scapel blades. A DNA extraction kit produced by Integrated DNA Technologies, USA, was utilized for DNA extraction. The extracted smears were transferred to 1.5ml tubes for DNA extraction. Buffy coats and proteinase K were used to lyse the cells of the virus, and DNA extraction was done by means of chloroform. PCR components were produced by Integrated DNA technologies (USA); the PCR reaction contained primers Gp5: 5'TTTGTTACTGTGGTAGATACTAC-3'and Gp6: 5'GAAAAATAAACTGTAAATCATATTC-3'. The reaction was programmed as follows. Initial denaturing step at 95°C for 15 min, 10 cycles of 30s at 94°C, 90s at 72°C, followed by 30 cycles of 30s at 94°C, 90s at 63°C and 90s at 72°C, with a final extension of 72°C for 10 min. A DNA band of 150 kb was considered a positive result.

Gel electrophoresis procedure

Agarose (3g) was heated in solution in a microwave until it had completely dissolved, and was then allowed to cool in a water bath set at 50-55°C. The required numbers of combs were placed in the gel tray; 5 ul of ethidium bromide was added to the cooled gel, which was then poured into the gel tray. The gel was allowed to cool for 15-30 min at room temperature. The combs were removed and placed in an electrophoresis chamber and covered with buffer (TAE). DNA and standard (ladder) was loaded onto the gel, which was electrophoresed for at least an hour. DNA bands were visualized by means of a gel imaging system.

Results

Table I shows the socio-demographic and reproductive characteristics of the respondents; about two-thirds 133 (66.5%) of the women had attained their sexual debut at ≤ 15 years of age. Married women accounted for 176 (88.0%), and 146 (73%) had a tertiary education.

Figure 1 shows the distribution of the abnormal cervical cytology by type of abnormality. Three different types of abnormality were found among the subjects. Low-grade squamous intraepithelial lesions (LSIL) constituted the main form of abnormal cytology, accounting for 50% of the total abnormal smears. High-grade squamous intraepithelial lesions (HSIL) were 28.6%, while atypical squamous cells of undetermined significance (ASCUS) were 21.4%.

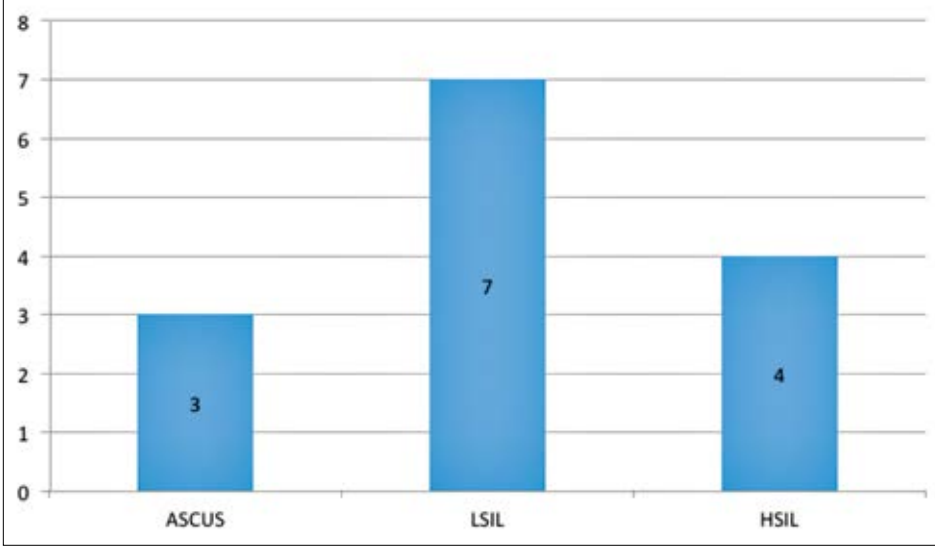
All the samples presenting abnormal cervical cytology underwent HPV DNA extraction and gene amplification; all contained HPV DNA (Fig. 2). Fourteen abnormal

Tab. I. Socio-demographic and reproductive characteristics of the respondents.

Variables	Frequency (%)	Variables	Frequency (%) N = 200
Age (years)		**Age at sexual debut (years)**	
15-24	5 (2.5)	≤ 15	133 (66.5)
25-34	50 (25.0)	> 15	67 (33.5)
35-44	80 (40.0)		
45-54	47 (23.5)	**Parity**	
55-64	18 (9.0)	0	45 (22.5)
		1-3	116 (58.0)
		> 3	39 (19.5)
Level of education			
Primary	4 (2.0)	**Previous genital infection**	
Secondary	23 (11.5)	Yes	126 (73.0)
Tertiary	146 (73.0)	No	74 (37.0)
None	27 (13.5)		
Marital status		**Number of sexual partners**	
Single	19 (9.5)	0-1	174 (87.0)
Married	176 (88.0)	> 1	26 (13.0)
Divorce	5 (2.5)		
Alcohol intake		**Male partner circumcision**	
Yes	31 (15.5)	Yes	192 (96.0)
No	169 (84.5)	No	8 (4.0)
Tobacco use			
Yes	9 (4.5)		
No	191 (95.5)		
Type of marriage**			
Monogamy	57 (31.5)		
Polygamy	124 (68.5)		
Use of oral contraceptives			
Yes	111 (55.5)		
No	89 (44.5)		

** N=181

Fig. 1. Distribution of abnormal cervical cytology types amongst subjects in relation to acquisition of HPV infection.

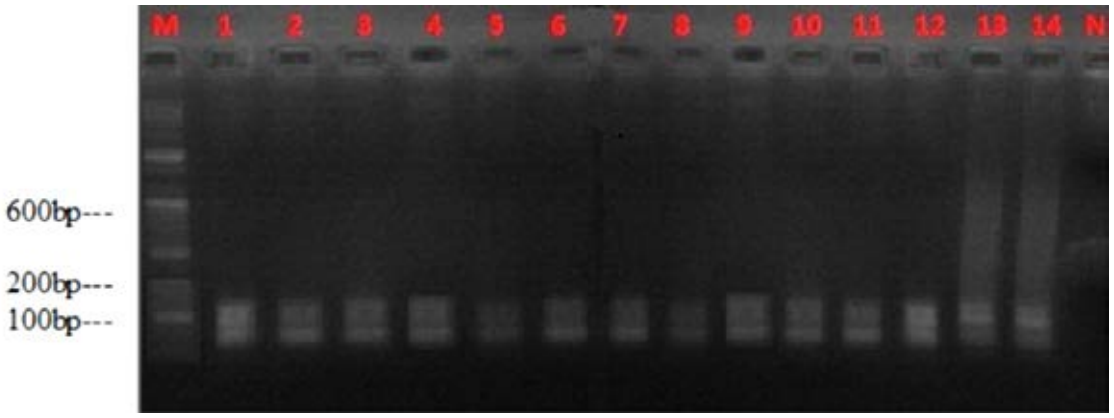


ASCUS: Atypical cells of uncertain significance

LSIL: Low-grade squamous intraepithelial lesion

HSIL: High-grade squamous intraepithelial lesion

Fig. 2. Positive PCR bands with corresponding HPV genotypes (1kb plus DNA ladder, 0.9% agarose gel stained with ethidium bromide).



Lane 1 represents (M) High ranger 1kb DNA Ladder: lanes 2-15 are amplified HPV genome: 1,2,3,4,5,6,7,8,9,10,11,12,13,14; lane 16 represents negative control (NC). Left hand side: molecular size of marker bands.

cervical cytology samples underwent molecular studies. Of these 14 samples, 7 had low-grade squamous intraepithelial lesions, 4 were high-grade squamous intraepithelial lesions and the remaining 3 atypical squamous cells of uncertain significance. The molecular results showed the analysis of HPV DNA in cervical samples to be a predictor of seropositivity. The 1-14 bands indicated in the gel electrophoresis image (1-14) are indicative of the presence of HPV DNA. Lane 1 represents (M) High ranger 1kb DNA Ladder; lanes 2-15 are amplified HPV genome: 1,2,3,4,5,6,7,8,9,10,11,12,13,14, and lane 16 shows a negative control (NC).

Discussion

Results showed that the largest number of abnormal smears were of low-grade squamous cell carcinoma (LSIL), which accounted for about 50% of abnormalities. This is similar to the results obtained by Obaseki and Nwafor (2013), who reported that LSIL accounted for the highest percentage (66.27%) of abnormalities found [7].

In this study, HPV DNA was extracted from all the samples with abnormal cytology; all of the subjects with abnormal cervical cytology were also positive for the presence of HPV DNA. This is in line with the results of the study by Sharifah et al. (2009), in which all 38 abnormal cervical cytology smears analysed were positive for the presence of HPV DNA [8]. HPV DNA was discovered in all the cases of low-grade quamous cell carcinoma, high-grade squamous cell carcinoma and atypical squamous cells of uncertain significance. This high prevalence of HPV DNA in abnormal cytology observed in this study also lends credence to several studies which have cast doubt on the existence of HPV DNA-negative abnormal cervical smears [8]. Indeed, various studies have shown a high prevalence of HPV DNA in abnormal cytology; this is in agreement with the presence of HPV DNA in all our abnormal cervical smears [4]. The frequency of HPV in abnormal cervical cytology also underlines the strong correlation between cervical cancer, HPV DNA positivity and abnormal cervical cytology.

Even though genotyping could not be done in this study, the presence of HPV DNA in all abnormal cervical smears reveals a dire need for a national program for vaccination against HPV infection in Nigeria. There is also a need to look at the various screening methods utilized for detecting HPV infection and, where possible, molecular techniques should be incorporated alongside with serology and the Papanicolaou test.

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Authors' contributions

OMK and KTO conceived and designed the research. KAD and KTO co-ordinated the data and Pap smear collection on the field. OMK, KTO and KAD performed the data quality control and optimized the informatics data base. KAD and KTO performed the statistical data analyses. KTO, OMK and KAD evaluated the results. KAD, KTO and OMK wrote the manuscript. All authors revised the manuscript and gave their contribution to improve the paper. All the authors read and approved the final manuscript.

References

[1] World Health Organization. (2008). *Country Strategy: Federal Republic on Nigeria 2002-2007, WHO, Regional office for Africa, Brazzaville.* Available at: http://www.who.int/countries/nga/about/ccs_strategy02_07.pdf [Accessed 10/06/2015].

[2] Parkin DM, Pisani P, Brary F. *The global health burden of infection associated cancer*. Int J Cancer 2006;118:3030-14.

[3] Timmons B, Akins M, Mahendroo M. *Cervical remodeling during pregnancy and parturition.* Trends Endocrinol Metab 2010;21:353-61.

[4] Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, Shah KV, Meijer CJ, Bosch FX; International Agency for Research on Cancer. Multicentric Cervical Cancer Study Group. *Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study*. Lancet 2002;359:1093-101.

[5] Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walbomers JM, Schiller JT, Bock JE, Sherman ME, Lowy DR, Meijer CL. *High-risk human papillomavirus is sexually transmitted: evidence from a follow-up study of virgins starting sexual activity (intercourse).* Cancer Epidemiol Biomarkers Prev 2001;10:101-6.

[6] Clifford GM, Gallus S, Herrero R, Muñoz N, Snijders PJ, Vaccarella S, Anh PT, Ferreccio C, Hieu NT, Matos E, Molano M, Rajkumar R, Ronco G, de Sanjosé S, Shin HR, Sukvirach S, Thomas JO, Tunsakul S, Meijer CJ, Franceschi S; IARC HPV Prevalence Surveys Study Group. *Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys*. Lancet 2005;366:991-8.

[7] Obaseki DE, Nwafor CC. *Cervical cancer screening in Benin City, South-South Nigeria*. J Dental Medical Sci 2013;5:16-9.

[8] Sharifah NA, Seeni A, Nurismah MI, Clarence-Ko CH, Hatta AZ, Ho NP, Rafaee T, Adeeb N, Jamal R. *Prevalence of human Papillomavirus in abnormal cervical smears in Malaysian patients.* Asian Pacific J Cancer Prev 2009;10:303-6.

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ORIGINAL ARTICLE

School-based anti-smoking intervention for physiotherapy students: a three-year non-randomized trial

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Keywords

Tobacco • Smoking habits • Education • Healthcare students • School-based intervention

Summary

Introduction. *The aim of the study was to assess the efficacy of a school-based intervention for reducing tobacco consumption among physiotherapy students.*
Materials and methods. *This controlled trial involved two groups of physiotherapy students: a treatment group (A) and a control group (B). Treatment consisted of a seminar on smoking-related diseases (3 hours) and training (at least 10 months) in a respiratory diseases or thoracic surgery unit. The control group (B) did not undergo any treatment. The main outcome was the prevalence of smokers. Follow-up lasted 3 years.*
Results. *Groups A and B comprised 78 and 72 students, respectively. The two groups did not differ in terms of gender distribution, mean age, prevalence of smokers and nicotine dependence. In group A, the prevalence of smoking declined from 36% to 33% between T0 and T1 (3 years), the relative risk (RR) at T1 being 0.93 (95% CI: 0.6-1.44). In group B, the prevalence increased from 28% to 35% between T0 and T1, with a RR at T1 of 1.26 (0.76-2.11). The prevalence reduction "attributable" to the intervention in group A 7.7%, while a 27.8% increase in prevalence "attributable" to the absence of intervention was found in group B. However, the differences were not statistically significant.*
Conclusions. *School-based interventions seem to be effective in reducing the prevalence of smoking among healthcare students. Further studies on larger samples and with standardized methodology are required in order to confirm these preliminary findings.*

Introduction

Reducing tobacco consumption is a public health priority at the international and national levels. According to the WHO, smoking kills nearly 6 million people each year [1].
Although the prevalence of smokers has declined by more than 4 percentage points in Italy in the last 20 years, it began to increase again between 2013 and 2014 [2]. Among Italian hospital personnel, including physician, healthcare workers, nurses and students, the prevalence of smokers is, paradoxically, higher than in the general population. According to recent surveys, about 44% of health professionals smoke, which is almost twice the prevalence in general population [3, 4]. Similar rates have been shown by a survey conducted among more than 800 nursing students, 42% of whom currently smoke [5]. The majority of hospital workers who smoke do so in the hospital, and more than 90% of healthcare workers have seen colleagues smoking cigarettes inside the hospital at least once (47.4% in staff toilets, 33.4% in kitchens and 4.7% in patients' rooms) [3].
Physicians and healthcare workers should play a key role in encouraging smokers to quit and to achieve long-term abstinence. Their behaviors can set an example and contribute to the spread of healthy lifestyles [6]. Thus, it is crucial that they receive good training on smoking-related diseases and smoking prevention. However, this is uncommon; for instance, UK Medical Colleges include factual knowledge of nicotine addiction and withdrawal symptoms in only 50% of curricula [7]. In Italy, although 90% of medical residents in Public Health report hearing about smoking-related issues during their undergraduate courses, only 17% claim to have received specific smoking cessation training during specialization [8]. Surveys conducted among Italian Health Professional School students reveal that 94.3% of the respondents should receive specific training to quit smoking, but that only 21.3% do so during their study courses [9].
School-based interventions to reduce and prevent smoking have been widely implemented and results have been summarized in a systematic review by Murphy-Hoefer that concludes that, while some promising results have been achieved, rigorous evaluation of a wider range of programs is needed [10].
The present study aims to contribute to the international debate on school-based anti-smoking interventions by evaluating the efficacy of a school-based intervention in reducing the prevalence of smokers among physiotherapy students.

Materials and methods

Study design

A non-randomized trial with two independent arms was carried out between 2008 and 2013.

Sample and setting

Students on two different physiotherapy courses at a teaching hospital in Rome were enrolled for the study: course A students participated in a seminar on smoking-related diseases and were enrolled for training in respiratory and thoracic surgery units. Course B students constituted a control group and did not receive any treatment. All students were in their first academic year, and were followed up for three consecutive years. To calculate the sample size, we assumed a 35% prevalence of smokers in the experimental group and a prevalence reduction of 15%. In order to obtain a power of 80% with α set at 0.05, we had to enroll at least 72 subjects in the experimental group.

Interventions

Group A underwent two different treatment steps:

1. Seminar on smoking-related diseases.

The seminar took place during the first semester of training and lasted three hours. Contents regarded individual and community risks and the costs of tobacco consumption in terms of health, life quality and economic aspects.

2. Training in respiratory and thoracic surgery units.

Training lasted at least 6 months in clinical units and four months in surgical units. Each student treated attended at least 10 months of training in services dealing with tobacco-related diseases.

Outcome and questionnaire

An anonymous questionnaire was administered to all participants of both courses in the first year (T0) and the third and last year of their university studies (T1). Personal data were collected: age and gender, smoking (yes/no), number of family members who smoked (asked only in the first year). Students who smoked were asked about their own level of nicotine addiction (*Fagerström score*) [11] at T0. On this test, subjects who scored 1 or 2 were considered to have low addiction to nicotine, while those who scored 3 or more were considered to be from moderately to highly addicted.

Statistical analysis

The demographic characteristics of both groups were recorded, and differences between the groups at T0 and at T1 were evaluated by means of Pearson's chi2 test for qualitative variables and the Student's t-test for quantitative variables.

The prevalence of smokers in the two groups at T0 and T1 was calculated. The relative risk (RR) of smoking at T0 and T1 and the 95% confidence interval (95% CI) were calculated in both groups. The attributable risk (AR) of smoking was calculated for both groups. In this study, AR was used to quantify the risk attributable to treatment (group A) or to the lack of the treatment (group B).

Ethics

The study was performed in accordance with the principles of the Declaration of Helsinki [12]. The research protocol was submitted to the research ethics committee concerned for consideration, comment, guidance and approval, and informed consent was obtained from all subjects enrolled.

Results

At T0 (before intervention), the treated group (A) was composed of 43 males and 35 females, with an average age of 22 years, 36% of whom smoked; group A subjects had an average of 1.2 smoking relatives. At T0, the control group (B) was made up of 75 members, 39 males and 33 females, with an average age of 22.5 years and an average of 0.9 smoking relatives; only 28% of group B subjects smoked at T0. The level of nicotine dependence estimated by means of the *Fagerström score* at T0 *was low among smokers in both groups.* No statistically significant differences in demographic factors or the level of nicotine dependence emerged between the two groups at T0.

After the intervention (T1), group A comprised 75 participants, 38 males and 37 females, with an average age of 25 years. The percentage of smokers was seen to have decreased over time, from 36 (T0) to 33 (T1). At T1, group B counted 57 participants, 28 males and 29 females, with an average age of 25 years. In this group, the percentage of smokers increased from 28% (T0) to 39% (T1).

In group A, only three participants where lost between T0 and T1 (4%), while in group B 15 participants were lost (21%).

The table above shows four comparisons and the relative risks that emerged. The first comparison is between the prevalence of smoking in groups A and B before the intervention. The prevalence was higher in group A than in group B at T0 (A: 36% *vs* B: 28%). The relative risk (RR) for group A was 1.29 (95% CI: 0.8-2.1). The second comparison shows an inversion in the prevalence of smokers between the two groups after the intervention: 35% in group B and 33% in group A; the RR of group A compared with group B was 0.95 (95% CI: 0.59-1.53). On comparing the prevalence of smokers in group A between T0 and T1, a decrease from 36% to 33% was seen, the RR at T1 being 0.93 (95% CI: 0.6-1.44). In group B, the prevalence increased from 28% to 35% between T0 and T1, with a RR of 1.26 (0.76-2.11) at T1.

Table III shows the Attributable Risks (AR%) of smoking in the two groups: the first AR indicates the percentage of students in the treated group who stopped smoking because of the intervention; the second AR indicates the percentage of group B students who started smoking that can be attributed to the lack of intervention. In group A, the reduction in smoking prevalence "attrib-

Tab. I. Demographic characteristics and smoking behaviors of participants.

Demographic characteristics and smoking behaviors		T0				T1			
		A	B	Tot	P	A	B	Tot	P
Gender	M (%)	43 (55)	39 (54)	68 (45)	0.91	38 (51)	28 (49)	66 (50)	0.86
	F (%)	35 (45)	33 (46)	82 (55)		37 (49)	29 (51)	66 (50)	
Age	Mean (SD)	21.8 (6.0)	22.5 (5.5)	22.1 (5.8)	0.46	25.3 (6.5)	25.3 (5.3)	25.3 (6.0)	0.99
Smoker	Yes (%)	28 (36)	20 (28)	48 (32)	0.29	25 (33)	22 (39)	47 (36)	0.53
	No (%)	50 (64)	52 (72)	102 (78)		50 (67)	35 (61)	85 (64)	
N° of smokers in family	Mean (SD)	1.2 (1.6)	0.9 (0.9)	1.1 (1.3)	0.16	-	-	-	-
Nicotine dependence: Fagerström's score	Mean (SD)	1.25 (1.8)	1.55 (1.9)	-	0.58	-	-	-	

T0 = baseline, first academic year
T1 = three years later, last academic year
Not asked

Tab. II. Relative risk of smoking, stratified by intervention and group (A *versus* B) and times (T0 versus T1).

Group and time	Non smokers (%)	Smokers (%)	RR	95% CI
Bt0	52 (72.2)	20 (27.8)	1	0.8-2.1
At0	50 (64.1)	28 (35.9)	1.29	
Bt1	37 (65.0)	20 (35.0)	1	0.59-1.53
At1	50 (66.7)	25 (33.3)	0.95	
At0	50 (64.1)	28 (35.9)	1	0.66-1.44
At1	50 (66.7)	25 (33.3)	0.93	
Bt0	52 (72.2)	20 (27.8)	1	0.76-2.11
Bt1	37 (65.0)	20 (35.0)	1.26	

Tab. III. Percent smoking prevalence attributable (AR%) to the presence or absence of intervention.

Groups	T0	T1	AR%	95% CI (AR%)
Smokers A ($N_{group\ A}$)	28 (78)	25 (75)	-7.69*	-52.93;37.55
Smokers B ($N_{group\ B}$)	20 (72)	20 (52)	27.78**	-15.57;71.13

* -7.69% is the decrease in smoking prevalence which can be attributed to the intervention
** 27.78 % is the amount of smoking prevalence which can be attributed to lack of intervention

utable" to the intervention amounted to 7.7%, while a 27.8% increase in smoking prevalence "attributable" to the lack of intervention was found in group B.
The difference between the AR of group A and the AR of group B provides a measure of the effectiveness of the intervention in reducing the risk of smoking. None of the risk measures calculated was statistically significant.

Discussion

The intervention seems to have been effective in reducing the risk of smoking in the experimental group, especially in comparison with the control group, in which the prevalence of smoking increased in the absence of intervention. At the international level, evidence of the effectiveness of school-based interventions against smoking is not homogeneous. While the latest systematic reviews on the issue are controversial, there is a consensus that results in terms of reducing or preventing smoking mainly depend on the approach of the intervention implemented. Thomas reports that school-based interventions that combined social competence and social influences showed a significant effect both at one year and over longer follow-up. By contrast, studies adopting a social-influences program alone showed no overall effect at any time point, and multimodal interventions and those with an information-only approach proved similarly ineffective. He argues that interventions involving social competence and those combining social competence and social influences have yielded positive results [13]. Santon agrees that complex approaches show promise, with some persistence of abstinence (30 day prevalence of abstinence or continuous abstinence at six months), especially those that incorporate elements sensitive to the stage of change and use motivational enhancement and CBT [14]. Carson concludes his systematic review by claiming that there is some evidence to support the effectiveness of community (including school-based) interventions in reducing the number of young people who take up smoking. However, the evidence is not strong and the studies reviewed contain a number of methodological flaws [15].
This study has several limitations.
First, the small sample size did not allow us to obtain statistical significances in the analysis performed. Sec-

ond, the intervention was not standardized and consisted of two different activities (seminar and practical training); it is therefore unclear which part of the intervention mostly contributed to the results obtained. Third, the percentage of group B subjects who were lost to follow-up was high; failure to investigate the reason for this phenomenon could have introduced a bias into the findings of the study. Fourth, as the T0 and T1 data were not paired, it is likely that the statistical analysis was not very accurate. Fifth, the data collected were insufficient to enable us to determine whether other important factors, in addition to the intervention, may have influenced the prevalence of smoking. Finally, the fact that the study was neither randomized nor blind could reduce the reliability of the results.
Nevertheless, the study suggests that the intervention was effective. Further studies with larger samples and better defined intervention should be conducted in order to assess the impact of campaigns to improve awareness and prevention among young adults enrolled in healthcare training.

Conclusions

School-based interventions could play a key role in the global fight against smoking. Moreover, education and training on tobacco-related diseases could improve awareness and promote healthy behaviors among healthcare practitioners, who have a leading role to play in fostering healthy lifestyles in the community. However, as evidence of the success of anti-smoking initiatives is not strong, further research on the efficacy and cost-effectiveness of school-based interventions should be undertaken.

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Authors' contributions

DM designed the study, performer the statistical analyses and wrote the manuscript, AM collected data, optimized the informatics database and performed the statistical analyses. CP conceived the study and realized the intervention, GLT conceived the study and coordinated the research group.

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DM, AM, CP and GLT evaluated the results. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

References

[1] WHO Tobacco. WHO. 2015. Available at: http://www.who.int/mediacentre/factsheets/fs339/en/ [Accessed 01/10/2015]

[2] OSSFAD- Doxa-ISS survey, 2014. [2015]. Available at: http://www.salute.gov.it/imgs/C_17_notizie_1602_listaFile_itemName_0_file.pdf [Accessed 01/10/2015]

[3] Ficarra MG, Gualano MR, Capizzi S, Siliquini R, Liguori G, Manzoli L, Briziarelli L, Parlato A, Cuccurullo P, Bucci R, Piat SC, Masanotti G, de Waure C, Ricciardi W, La Torre G. *Tobacco use prevalence, knowledge and attitudes among Italian hospital healthcare professionals*. Eur J Public Health 2011;21:29-34.

[4] Nardini S, Bartoletti R, Rastelli V, Ravelli L, Donner C. *Personal smoking habit and attitude toward smoking among the health staff of a general hospital.* Monaldi Arch Chest Dis 1998;53:74-8.

[5] Biraghi E, Tortorano AM. *Tobacco smoking habits among nursing students and the influence of family and peer smoking behaviour*. J Adv Nurs 2010;66:33-9.

[6] Stead LF, Buitrago D, Preciado N, Sanchez G, Hartmann-Boyce J, Lancaster T. *Physician advice for smoking cessation.* Cochrane Database Syst Rev 2013;5:CD000165.

[7] Raupach T, Al-Harbi G, McNeill A, Bobak A, McEwen A. Smoking Cessation Education and Training in U.K. *Medical Schools: a national survey.* Nicotine Tob Res 2015;17:372-5.

[8] La Torre G, Saulle R, Unim B, Angelillo IF, Baldo V, Bergomi M, Cacciari P, Castaldi S, Del Corno G, Di Stanislao F, Panà A, Gregorio P, Grillo OC, Grossi P, La Rosa F, Nante N, Pavia M, Pelissero G, Quarto M, Ricciardi W, Romano G, Schioppa FS, Fallico R, Siliquini R, Triassi M, Vitale F, Boccia A. *Knowledge, attitudes, and smoking behaviours among physicians specializing in public health: a multicentre study.* BioMed Res Int 2014;2014:e516734.

[9] Ferrante M, Saulle R, Ledda C, Pappalardo R, Fallico R, La Torre G, Fiore M. *Prevalence of smoking habits, attitudes, knowledge and beliefs among Health Professional School students: a cross-sectional study.* Ann Istituto Super Sanità 2013;49:143-9.

[10] Murphy-Hoefer R, Griffith R, Pederson LL, Crossett L, Iyer SR, Hiller MD. *A review of interventions to reduce tobacco use in colleges and universities.* Am J Prev Med 2005;28:188-200.

[11] Fagerström K, Russ C, Yu C-R, Yunis C, Foulds J. *The Fagerström Test for nicotine dependence as a predictor of smoking abstinence: a pooled analysis of varenicline clinical trial data.* Nicotine Tob Res Off J Soc Res Nicotine Tob 2012;14:1467-73.

[12] WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. 2013 Available at: http://www.wma.net/en/30publications/10policies/b3/ [Accessed 01/10/2015]

[13] Thomas RE, McLellan J, Perera R. *School-based programmes for preventing smoking*. Cochrane Database Syst Rev [Internet] 2013. From: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD001293.pub3/abstract

[14] Stanton A, Grimshaw G. *Tobacco cessation interventions for young people*. Cochrane Database Syst Rev 2013;(8):CD003289. doi: 10.1002/14651858.CD003289.pub5.

[15] Carson KV, Brinn MP, Labiszewski NA, Esterman AJ, Chang AB, Smith BJ. *Community interventions for preventing smoking in young people*. Cochrane Database Syst Rev 2011;(7):CD001291. doi: 10.1002/14651858.CD001291.pub2.

ORIGINAL ARTICLE

Smoking during pregnancy: a difficult problem to face. Results of a French multi-center study

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Keywords

Pregnancy • Tobacco consumption • Environmental tobacco smoke

Summary

Introduction. *Smoking tobacco during pregnancy is a preventable risk factor for adverse pregnancy outcomes. The aim of the study was to assess the impact of an information and training program implemented by the perinatal network of Auvergne, France, on smoking during pregnancy.*
Methods. *A multi-center before-and-after population-based study, based on two cross-sectional surveys, was carried out between July 2003 and June 2004, and between December 2008 and January 2010. Pregnant women aged over 18 years, with a fluent command of written and spoken French, were eligible. The main outcome was the prevalence of pregnant women who smoked daily. The preventive program consisted of informing women and healthcare providers and training healthcare providers. Multivariate analysis was performed by means of manual logistic regression and crude and adjusted Odds Ratios were calculated.*
Findings. *"Before" and "after" surveys involved 1027 and 720 women, respectively. In the "after" survey, a higher percentage of women smoked daily at the time of diagnosis (43.49% vs 51.94%, adjusted Odds Ratio 1.45 [1.10; 1.90]) and during the third term (40.53% vs 51.94%, adjusted Odds Ratio 1.62 [1.24; 2.12]). Environmental tobacco smoke exposure among non-smokers was higher in the "after" survey: 52.83% vs 69.57% adjusted Odds Ratio 1.95 [1.54; 2.47].*
Conclusions. *The program did not reduce smoking during pregnancy. Exposure to environmental tobacco smoke increased. French public health authorities should introduce a new policy aimed specifically at tackling tobacco use during pregnancy and exposure to second-hand smoke, and which takes into account the entire environment of pregnant women.*

Introduction

It is well documented that maternal smoking during pregnancy is a preventable risk factor for adverse pregnancy outcomes. Tobacco interferes with the development of brain structures, which results in a higher risk of low birth weight, stillbirth, pre-term delivery and placental detachment [1-3]. There is also evidence of an association between tobacco consumption during pregnancy and both infant mortality and the risk of nicotine dependence during adolescence [3, 4]. In addition, several studies have identified environmental tobacco smoke (ETS) as a risk factor for adverse effects on both fetuses and children [1-3, 5, 6].
The Prochaska cycle of the process of change has been applied to the various stages of tobacco dependence: pre-contemplation, contemplation, preparation, action, maintenance and relapse. Pregnant women commonly go through the Prochaska cycle from the pre-contemplation to the contemplation stages. McBride et al. identified pregnancy as an appropriate moment to stop smoking [7] and advocated developing preventive procedures during this period.
A national survey conducted in France in 2010 showed that tobacco consumption remained a major health problem and, in particular, that consumption had increased among women [8]. The Association of Users of Computerized Data in Paediatrics, Obstetrics and Gynaecology (AUDIPOG) reported in 2008 that, although consumption was lower during pregnancy, 14% of pregnant women still smoked.
Two special interest groups at the university hospital of Clermont-Ferrand, Auvergne, France – the DATAMATER group, a regional hospital research program, and the CAFE group, an inter-regional hospital research program - and the perinatal network of Auvergne (Réseau de Santé Périnatale Auvergne-RSPA) have been working on tobacco consumption during pregnancy [9-11]. They developed an information and training program targeting the general public and healthcare providers, to deal with tobacco use in pregnant women.
The main aim of the present study was to assess the im-

pact of the program on reducing tobacco consumption during pregnancy.

Methods

This study was approved by the ethics committee of Clermont-Ferrand (N°2003-AU509 for the "before" survey and 2007-AU 735 for the "after" survey).

The perinatal network of Auvergne

Auvergne is a rural region of south-central France with an estimated population of 1 343 964 , i.e. 2.1% of the French population (http://www.insee.fr/fr/bases-de-donnees). Auvergne currently has 10 maternity units (3 level-I with obstetric units only, 6 level-II with obstetric and neonatology units and 1 level-III with obstetric, neonatology and neonatal hospitalization units). Maternity units were reorganized between 2003 and 2009, and six units were closed. The number of births in 2003 and 2004, during the period of the "before" survey, was 13769 and 13779, respectively, and in 2008 and 2009, during the period of the "after" survey, 13852 and 13849, respectively. The rate therefore remained stable over time (http://www.insee.fr/fr/themes/detail.asp?reg_id=99&ref_id=etat-civil-naissances). Created in 1994, the RSPA includes all healthcare professionals working in the gynecology-obstetric and paediatric units of the maternity hospitals of Auvergne, 364 healthcare providers working in local mother-and-child protection centres and in the surgeries of general practitioners (Gps), and outpatient gynecologists (https://www.auvergne-perinat.org/). The RSPA aims to improve pregnant women's health through better coordinated management, the improvement of quality of care and the development of preventive and educational procedures. To this end, it manages computerized medical records throughout the regional area and organizes yearly scientific meetings to present its research and actions to its members.

Patients

Women aged 18 years or more, with a fluent command of spoken and written French, who lived in the administrative area of Auvergne and had given birth to a baby after at least 22 weeks of gestation (or 500 grams in weight) were deemed eligible for the study. They were recruited at the maternity hospitals at the time of delivery or in the immediate post-partum.

Methods

A multi-center cross-sectional before-and-after population-based study was conducted. The "before" survey was performed between July 2003 and June 2004 in 16 maternity hospitals, and the "after" survey between December 2008 and January 2010 in 11 such facilities. The month in which the survey should be carried out in each maternity hospital was randomly selected by the statisticians of the university hospital of Auvergne. Data were collected over four consecutive weeks.

Questionnaires were composed of items regarding the women's social characteristics, the course of pregnancy and delivery conditions. Women were questioned about daily smoking before pregnancy, on diagnosis of pregnancy, in the third term and during the immediate post-partum. Yes/no items investigated ETS at home, at work and in the company of family or friends.

The main outcome was the prevalence of pregnant women who smoked daily.

All participants gave their informed consent to be enrolled. The surveys were approved by a French ethics research committee (N°2003-AU 509 for the "before" survey and 2007-AU 735 for the "after" survey).

Information and training program

The information and training program was implemented by the RSPA, and was addressed both to women and their family circles and to healthcare professionals. It was divided into three waves: information of the women and family circles, information and training of the healthcare providers (Fig. 1). Healthcare providers were midwives, obstetricians, gynecologists, pediatricians, psychiatrists and general practitioners.

The first wave consisted of publicizing the results of the "before" survey in regional newspapers in September 2004 and June 2008. The RSPA website created the session "addictive behavior" in February 2007 and, in April 2007, published the national guidelines on addictive behavior during pregnancy. The RSPA then produced a poster in collaboration with communication advisers and smokers. The poster was displayed in the waiting rooms of regional maternity hospitals, in local mother-and-child protection centers, and in the surgeries of general practitioners (GPs) and outpatient gynaecologists. It was sent to healthcare professionals in January 2008.

The second wave began in June 2005 with one RSPA "scientific day" of training devoted to tobacco consumption during pregnancy. In May 2007, the actions implemented by the RSPA to deal with tobacco consumption during pregnancy were presented to participants in the "scientific day" of the Perinatal Prevention Research Information Association (*Association Périnatalité Prévention Recherche Information* – APPRI).

The third wave was composed of two sessions (Level 1 and Level 2) of continuous medical training and education (CME). The aims of the Level 1 session were to explain tobacco dependence, to train healthcare personnel in the management of pregnant women smokers at all stages of the Prochaska cycle, and to improve the screening of pregnant women. Level 2 sessions aimed to strengthen the knowledge of healthcare professionals who had already attended session 1, and was based on medical histories, role plays, advice on prescribing, and an introduction to cognitive behavioral therapies. An instrument to measure carbon monoxide levels was given to participants after the Level 2 session. All the sessions were held over two days, 6 hours a day, by a specialist in addictions. Five Level 1 sessions and four Level 2 sessions were held, beginning in December 2007 and Sep-

Fig. 1. Chronology of the surveys and the information and educational program.

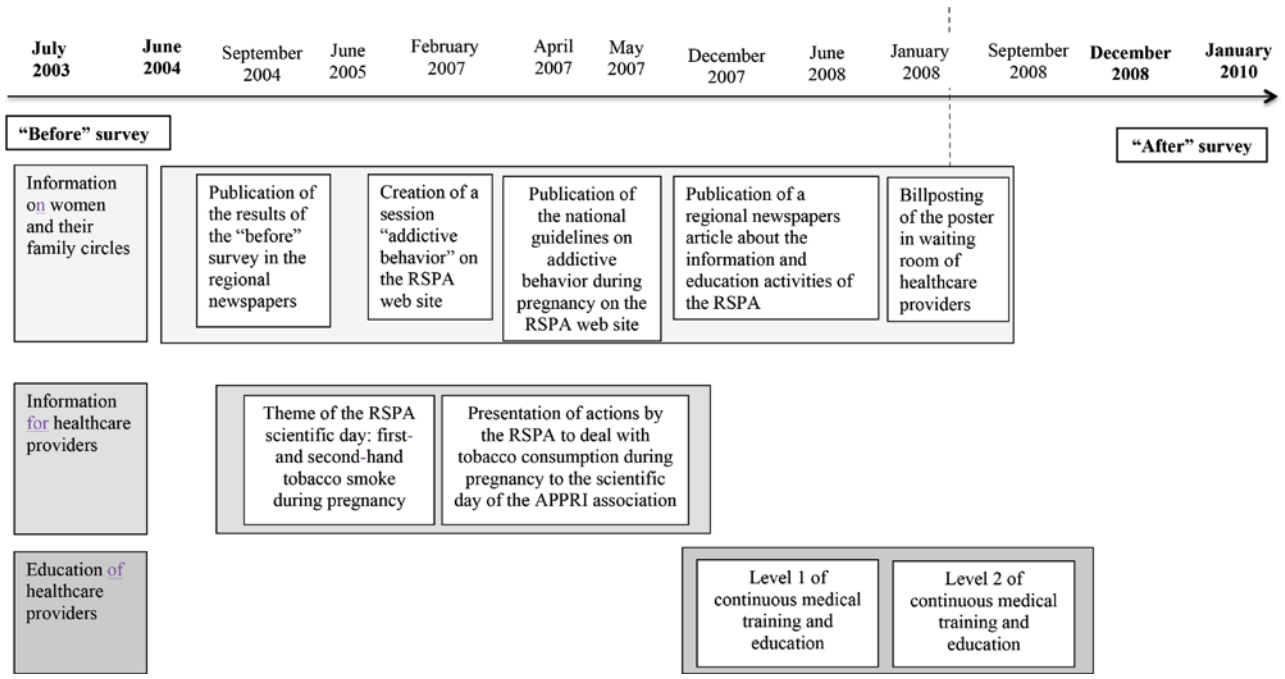
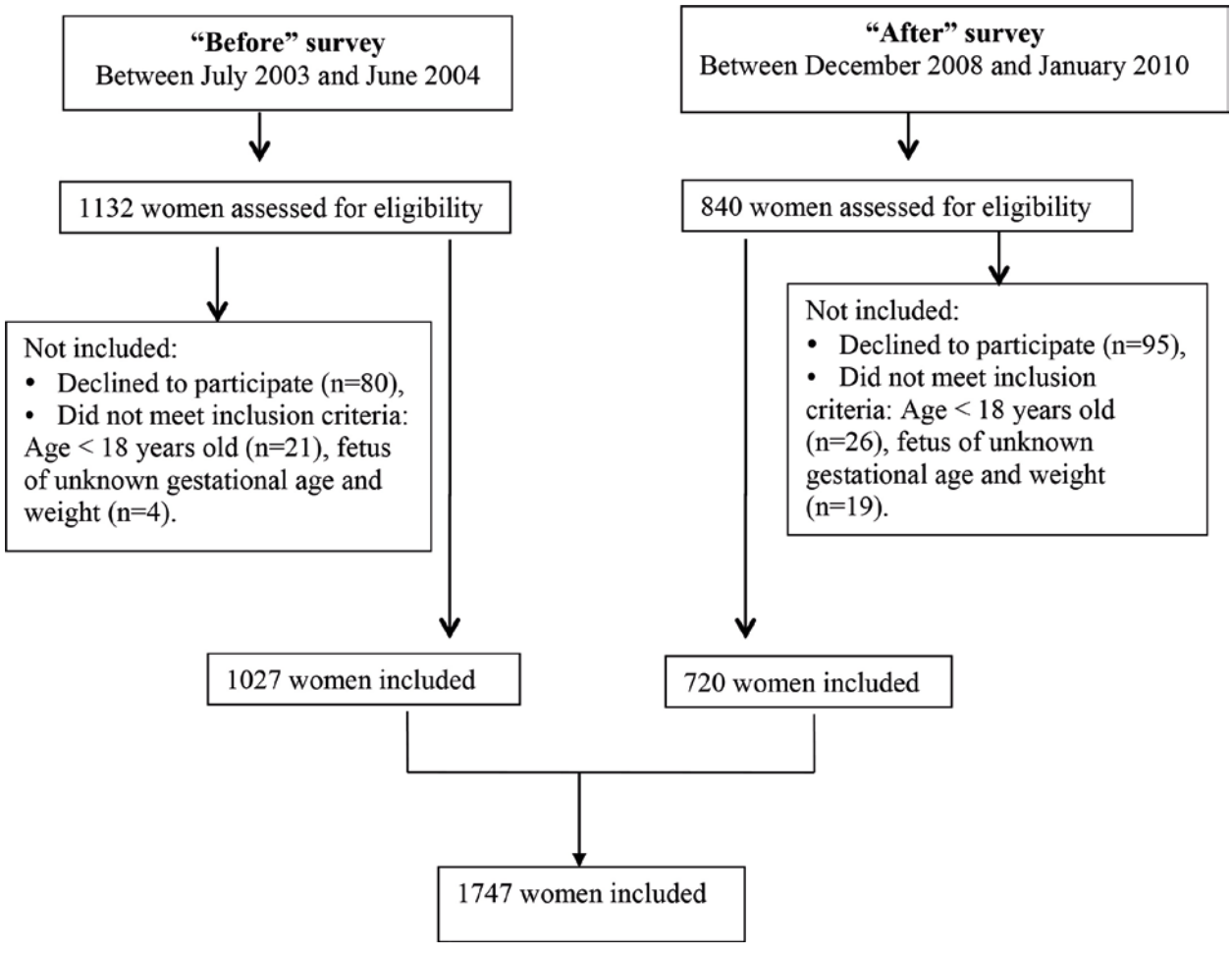


Fig. 2. Flow chart of women involved in the "before and after" study.



tember 2008, respectively. Fifty-eight healthcare professionals took part in Level 1 and 14 in Level 2.

Statistical methods

Descriptive analysis was performed to assess the women's characteristics and the prevalence of smoking. As the samples from the "before" and "after" surveys were non-comparative, a Mantel-Haenszel method was used to identify any socio-demographic and medical variables that might be confounding factors. A threshold of 10% was applied in the Mantel-Haenszel method. Bivariate analysis was performed by means of logistic regression and calculation of crude Odds ratio (OR) and adjusted Odds ratio (aOR) with their 95% Confident Intervals (95% CI) for qualitative variables, and by means of Student's t-test for quantitative variables. Multivariate analysis was then performed by means of manual logistic regression, taking into account all significant interactions. Tobacco smoking was compared before pregnancy, at the time when pregnancy was diagnosed, during pregnancy and in the post-partum period, taking into account the confounding factors previously identified. Crude Odds ratio (OR) and adjusted Odds ratio (aOR) with their 95% CI were calculated on confounding factors. A significance threshold of 5% was applied in all the statistical analyses. Statistical analysis was performed by means of SAS software (V9.3. SAS Institute Inc., Carry, NC, 2002-2003).

Results

Of the 1132 eligible women in the "before" survey and the 840 eligible women in the "after" survey, 1027 and 720, respectively, were finally included in the present study (Fig. 2).

The descriptive analysis showed no significant difference in socio-demographic characteristics between the "before" and "after" groups; the only exception concerned occupational status, in that a higher percentage of women were in employment in the "after" survey: 73.42% *vs* 80.06%, OR 1.45 [1.15; 1.83]. Regarding the women's medical data, a significant difference was observed only for parity, which was higher in the "before" survey: 64.97% *vs* 52.48%, OR 0.60 [0.49; 0.73]. Concerning the data on newborns, the only difference observed was in the 5-minute Apgar score, with lower Apgar scores in the "after" group: 1.37% *vs* 2.92% OR 2.17 [1.09; 4.29] (Tab. I).

Two confounding factors were identified by the Mantel-Haenszel method: occupational status and parity. In the multivariate analysis, occupational status was the sole explanatory variable that had an effect.

The trend in tobacco consumption indicated that a higher percentage of women in the "after" survey continued smoking: 43.49% *vs* 51.94%, aOR 1.45 [1.10; 1.90] on diagnosis; 40.53% *vs* 51.94%, aOR 1.62 [1.24; 2.12] during the third term, and 39.87% *vs* 46.46% aOR 1.31 [1.00; 1.72] in post-partum period. The majority of women quit smoking spontaneously before the diagnosis rather than after (Tab. II).

Pregnant women involved in the "after" survey were more exposed to at least one kind of ETS: 63.58% *vs* 75.24% aOR 1.74 [1.41; 2.15] overall and 52.83% vs. 69.57% aOR 1.95 [1.54; 2.47] among non-smokers. They were also more exposed to ETS at work and in the company of family or friends (Tab. III).

Discussion

Main results

The information and training program failed to reduce tobacco consumption during pregnancy. One unexpected finding was the higher exposure of pregnant women to ETS at work and with family or friends.

Comparisons with other studies

Previous studies have identified distinct trends in tobacco consumption during pregnancy, including a significant rate of "spontaneous quitters" before pregnancy or at the time of diagnosis [12]. Four studies have assessed the efficacy of training programs on healthcare professionals – midwives and the personnel of obstetric and pediatric units – and medical students [13-16]. Un-

Tab. I. Descriptive and bivariate analysis of socio-demographic, medical and newborn data among the 1027 and 720 women included in the "before" and the "after" surveys.

	"Before" survey % [m[a] ± sd[b]] (N[c])	"After" survey % [m[a] ± sd[b]] (N[c])	Odds Ratio CI (95%)
Age of the mothers[d]	[29.73 ± 4.87] ***(1027)***	[29.71 ± 5.19] ***(720)***	-
Family status (couple)	95.52 ***(1027)***	95.55 ***(719)***	1.01 [0.63; 1.60]
Occupational status (work)	73.42 ***(1027)***	80.06 ***(697)***	1.45 [1.15; 1.83]
Age on first cigarette[d]	[16.44 ± 2.85] ***(607)***	[16.18 ± 2.88] ***(367)***	-
Parity (≥ 1)	64.97 ***(1019)***	52.48 ***(707)***	0.60 [0.49; 0.73]
Type of pregnancy: single	98.44 ***(1027)***	98.16 ***(708)***	0.85 [0.40; 1.77]
Weeks of gestation at birth	[39.17 ± 1.55] ***(1027)***	[39.24 ± 1.56] ***(715)***	-
≥ 37 weeks	95.81 ***(984)***	94.83 ***(678)***	0.80 [0.51; 1.26]
Birth weight[e]	[3261.74 ± 498.91] ***(1024)***	[3243.5 ± 508.4] ***(717)***	-
5-Minute Apgar score < 7	1.37 ***(1023)***	2.92 ***(720)***	2.17 [1.09; 4.29]

[a]m: mean; [b]sd: Standard Deviation; [c]N: number of women with information on the variable; [d]age in years; [e] birth weight in grams.

Tab. II. History of smoking before, during and after pregnancy among the 1027 and 720 women included in the "before" and the "after" surveys, according to occupational status.

	"Before" survey 2003-2004 n[a] (%)[b] N[c]	"After" survey 2008-2010 n[a] (%)[b] N[c]	Crude Odds Ratio [CI[d] 95%]	Adjusted Odds Ratio[e] [CI[d] 95%]
Before pregnancy	407 (67.05%) ***(607)***	253 (70.28%) ***(360)***	0.81 [0.67; 0.99]	0.83 [0.67; 1.01]
Diagnosis of pregnancy	264 (43.49%) ***(607)***	187 (51.94%) ***(360)***	1.40 [1.08; 1.82]	1.45 [1.10; 1.90]
Third term	246 (40.53%) ***(607)***	187 (51.94%) ***(360)***	1.59 [1.22; 2.06]	1.62 [1.24; 2.12]
Post-partum period	242 (39.87%) ***(607)***	164 (46.46%) ***(360)***	1.31 [1.00; 1.71]	1.31 [1,00; 1.72]
	Stop smoking rate	Stop smoking rate		
Diagnosis of pregnancy	143 (35.14%) ***(407)***	89 (35.18%) ***(253)***	1.00 [0.72; 1.39]	1.00 [0.72; 1.40]
Third term	39 (14.77%) ***(264)***	19 (10.16%) ***(187)***	1.53 [0.85; 2.75]	1.51 [0.84; 2.71]
Post-partum period	5 (2.03%) ***(246)***	27 (14.59%) ***(185)***		
	Relapse rate	Relapse rate		
Third term	21 (6.12%) ***(343)***	19 (10.98%) ***(173)***	1.89 [0.99; 3.62]	1.85 [0.95; 3.58]
Post-partum period	1 (0.28%) ***(361)***	4 (2.41%) ***(166)***		-

[a]n: number of individuals in that response category; [b]%: percentage; [c]N: number of individuals with information on the variable; [d]CI: Confident Interval; [e]according to women's occupational status.

like the present study, these studies revealed a beneficial effect of the training program. The survey involving midwives revealed a positive impact on the number of cigarettes smoked a day [13]. The study performed in obstetric and pediatric units showed that healthcare providers changed their behaviour, though the difference was not statistically significant for all the outcomes measured [14]. Finally, the two studies involving medical students showed an impact on the students' confidence and attitude to dealing with tobacco consumption during pregnancy [15, 16].

Concerning exposure to at least one kind of ETS, the self-reported prevalence among non-smokers in the "after" survey (69.2%) was higher than that recorded by Aurrekoetxea in Spain (55.5%) [17]. In the present study, ETS exposure at home (23.35%) did not differ from the values measured by Aurrekoetxea in Spain (24.7%) and the WHO report for EU15 (25%) [17, 18]. By contrast, ETS exposure at work (32.15%) was higher than the values registered by Aurrekoetxea in Spain (9.8%) and the WHO report for EU15 (13%) [17, 18].

Implications

The rate of spontaneous quitters before pregnancy or on diagnosis remained moderate. The advice and support of physicians are reported to be effective in encouraging patients to give up smoking [19]. Training healthcare professionals is also recognized to have a positive effect on smoking cessation, although none of the 15 studies included in the recent Cochrane review targeted pregnant women [20]. The consensus is that anti-smoking interventions are effective on the both outcomes of both mothers and babies and should be implemented in all maternity hospitals [12]. It is important to take advantage of this 'teachable moment', to use the term of McBride et al. [7], because, if the opportunity is passed up, the rate of smoking cessation will be significantly lower for several years [21]. Consequently, prevention programs such as that organized by the RSPA are of great value. However, they could be considerably improved. First, such programs should target not only physicians but also medical students, through the use of both classic training methods and web-based training [22]. Sec-

Tab. III. Exposure to environmental tobacco smoke among the 1027 and the 720 women involved in the "before" and the "after" surveys and among non-smokers.

	"Before" survey n[a] (%)[b] N[c]	"After" survey n[a] (%)[b] N[c]	Crude Odds ratio [CI[d] 95%]	Adjusted Odds ratio[e] [CI[d] 95%]
Overall population				
At home (yes)	376 (36.61%) ***(1027)***	24 (31.77%) ***(705)***	0.81 [0.66; 0.99]	0.86 [0.70; 1.05]
At work (yes)	237 (31.43%) (***754***)	197 (37.38%) ***(527)***	1.30 [1.03; 1.65]	-
With family or friends (yes)	495 (48.20%) ***(1027)***	461 (66.91%) ***(689)***	2.17 [1.78; 2.65]	2.26 [1.84; 2.78]
At least one type of tobacco exposure	653 (63.58%) ***(1027)***	538 (75.24%) ***(715)***	1.74 [1.41; 2.15]	-
Among non-smokers				
At home (yes)	168 (22.64%) ***(742)***	117 (23.35%) ***(501)***	1.04 [0.8; 1.36]	1.07 [0.82; 1.41]
At work (yes)	144 (25.31%) ***(569)***	127 (32.15%) ***(395)***	1.40 [1.05; 1.86]	-
With family or friends (yes)	275 (37.06%) ***(742)***	282 (57.55%) ***(490)***	2.30 [1.82; 2.91]	2.36 [1.86; 2.99]
At least one type of tobacco exposure	392 (52.83%) ***(742)***	349 (69.57%) ***(509)***	1.95 [1.54; 2.47]	-

[a]n: number of individuals in that response category; [b]%: percentage; [c]N: number of individuals with information on the variable; [d]CI: Confident Interval; [e]according to women's occupational status

ond, they should make healthcare professionals aware of pregnant women's environment (familial and occupational status, deprivation), psychological health and ETS, in order to identify those women's needs [3, 23]. Particular attention should also be paid to pregnant women's partners [3, 24, 25]. Indeed, it is noteworthy that, during a woman's pregnancy, her partner also goes through the Prochaska circle. Interventions should therefore aim at helping couples to definitively quit smoking. Third, preventive programs could also provide specific support for women who continue to smoke by including face-to-face interventions and group therapy. Incentive measures and other strategies, such as physical activity, also seem to have a positive impact on tobacco consumption [1, 12, 26-29]. Fourth, the program should also target the couple at different time-points, such as before pregnancy (pre-wedding and family planning consultations) and in the post-partum period, which is a high-risk period for smoking relapse.

Study limitations

The study has certain limitations. First, "before" and "after" surveys should be performed in exactly the same conditions. Assessment of the impact of the program may have been distorted by the public health measures against tobacco consumption introduced by the French government. The price of a packet of cigarettes increased by about 40% between 2002 and 2004, during the period of the "before" study (http://www.inpes.sante.fr/10000/themes/tabac/consommation/marche-tabac.asp). In addition, a ban on smoking in public areas was imposed by decree in November 2006 and enforced in February 2007 and January 2008, before the "after" survey. The presence of strong legal regulations may explain the increase in ETS exposure between the "before" and "after" surveys. This increase might have impaired the effects of our prevention program. Second, the preventive program was assessed by means of a multi-center population-based survey without a control group. Third, no secondary outcomes were measured, such as those included in the Cochrane review (percentage of follow-up appointments made, percentage of self-help materials given, number of "quit dates" prescribed), an omission that prevented us from identifying the positive effect of the program [12]. Fourth, the inclusion criterion of a fluent command of spoken and written French might have excluded immigrant women, who are more exposed to tobacco consumption and ETS [30]. Finally, the information poster may have given rise to feelings of guilt that were counterproductive.

Conclusions

In conclusion, the information and training program seemed not to have reduced tobacco smoking during pregnancy. Moreover, unexpectedly high levels of ETS were revealed. Consequently, government authorities in France need to introduce new public health policies aimed specifically at tackling the problem of tobacco use and exposure to ETS during pregnancy. Programs that are too broad may leave out parts of the population, as shown by the increase in ETS exposure.

The solution is therefore to build pregnancy-based programs and to prevent smoking among men and women who are going to have children (such as pre-wedding intervention). We also need a better understanding of the image of tobacco and its dangers and how people consider its harmful effects on fetuses. Questions related to the environment of pregnant women also need to be addressed. Impact measurement and cost-effectiveness analyses of every program should be undertaken in order to assess how best to implement the strategies.

Public health stakeholders should be aware that some of the programs usually developed may not reach their objectives, and that any newly funded program should focus more closely on specific targets and provide strong evidence of efficacy.

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Authors' contributions

FV conceived and designed the original idea of the research. SL and MB performed the data quality control and optimized the informatics database. SL performed the statistical analyses. MB, SL, LG and FV evaluated the results. MB, SL, LG and FV wrote the article. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

References

[1] Murin S, Rafii R, Bilello K. *Smoking and smoking cessation in pregnancy*. Clin Chest Med 2011;32:75-91.

[2] Maritz GS, Mutemwa M. *Tobacco smoking: patterns, health consequences for adults, and the long-term health of the offspring*. *Glob J Health Sci.* 2012; 4: 62-75.

[3] World Health Organization. *Recommendations for the prevention and management of tobacco use and second-hand smoke exposure in pregnancy*, 2013, pp. 104.

[4] Rydell M, Cnattingius S, Granath F, Magnusson C, Galanti MR. *Prenatal exposure to tobacco and future nicotine dependence: population-based cohort study.* Br J Psychiatry 2012;200:202-9.

[5] DiFranza JR, Aligne CA, Weitzman M. *Prenatal and postnatal environmental tobacco smoke exposure and children's health*. *Pediatrics* 2004;113:S1007-15.

[6] Kharrazi M, De Lorenze GN, Kaufman FL, Kharrazi M, DeLorenze GN, Kaufman FL, Eskenazi B, Bernert JT Jr, Graham S, Pearl M, Pirkle J. *Environmental tobacco smoke and pregnancy outcome*. Epidemiology 2004;15:660-70.

[7] McBride CM, Emmons KM, Lipkus IM. *Understanding the potential of teachable moments: the case of smoking cessation*. Health Educ Res 2003;18:156-70.

[8] Guignard R, Beck F, Wilquin J-L, Andler R, Nguyen-Thanhl V, Richard J-B, Arwidson P. *Augmentation récente du tabagisme en France: principaux résultats du Baromètre santé*, France 2010. BEH 2011;20-21:229-46.

[9] Malet L, De Chazeron I, Schwan R, Llorca PM, Falissard B. *L'AUDIT en question.* L'Encéphale 2005;31:517-22.

[10] De Chazeron I, Llorca PM, Ughetto S, Coudore F, Boussiron D, Perriot J, Vendittelli F, Sapin V, Lemery D. *Occult maternal exposure to environmental tobacco smoke exposure.* Tob Control 2007;16:64-5.

[11] Chazeron Id, Daval S, Ughetto S, Richard D, Nicolay A, Lemery D, Llorca PM, Coudoré F. *GC-MS determined cotinine in an epidemiological study on smoking status delivery.* Pulm Pharmacol Ther 2008;21:485-8.

[12] Lumley J, Chamberlain C, Dowswell T, Oliver S, Oakley L, Watson L. *Interventions for promoting smoking cessation during pregnancy.* Cochrane Database Syst Rev 2009;8:CD001055.

[13] Dunkley J. *Training midwives to help pregnant women stop smoking*. Nurs Times 1997;93:64-6.

[14] Moss DR, Cluss PA, Watt-Morse M, Pike F. *Targeting pregnant and parental smokers: long-term outcomes of a practice-based intervention.* Nicotine Tob Res 2009;11:278-85.

[15] Bland E, Oppenheimer L, Brisson-Carroll G, Morel C, Holmes P, Gruslin A. *Influence of an educational program on medical students' attitudes to substance use disorders in pregnancy*. Am J Drug Alcohol Abuse 2001;27:483-90.

[16] Ramirez-Cacho WA, Strickland L, Beraun C, Meng C, Rayburn WF. *Medical students' attitudes toward pregnant women with substance use disorders.* Am J Obstet Gynecol 2007;196:86.e1-5.

[17] Aurrekoetxea JJ, Murcia M, Rebagliato M, Fernández-Somoano A, Castilla AM, Guxens M, López MJ, Lertxundi A, Espada M, Tardón A, Ballester F, Santa-Marina L. *Factors associated with second-hand smoke exposure in non-smoking pregnant women in Spain: self-reported exposure and urinary cotinine levels*. Sci Total Environ 2014;1:1189-96.

[18] World Health Organization. *Environmental health inequalities in Europe.* Assessment Report WHO 2012, pp. 212.

[19] Stead LF, Buitrago D, Preciado N, Sanchez G, Hartmann-Boyce J, Lancaster T. *Physician advice for smoking cessation*. Cochrane Database Syst Rev 2013;5:CD000165.

[20] Carson KV, Verbiest ME, Crone MR, Brinn MP, Esterman AJ, Assendelft WJ, Smith BJ. *Training health professionals in smoking cessation.* Cochrane Database Syst Rev 2012;16;CD000214.

[21] Rattan D, Mamun A, Najman JM, Williams GM, Doi SA. *Smoking behaviour in pregnancy and its impact on smoking cessation at various intervals during follow-up over 21 years: a prospective cohort study.* BJOG 2013;120:288-95.

[22] Tong VT, Dietz PM, England LJ. *Smoking cessation for pregnancy and beyond: a virtual clinic, an innovative web-based training for healthcare professionals.* J Womens Health 2012;21:1014-7.

[23] Flemming K, Graham H, Heirs M, Fox D, Sowden A. *Smoking in pregnancy: systematic review of qualitative research of women who commence pregnancy as smokers*. J Adv Nurs 2013;69:1023-36.

[24] Hemsing N, Greaves L, O'Leary R, Chan K, Okoli C. *Partner support for smoking cessation during pregnancy: a systematic review.* Nicotine Tob Res 2012;14:767-76.

[25] Xu H, Wen LM, Rissel C, Baur LA. *Smoking status and factors associated with smoking of first-time mothers during pregnancy and postpartum: findings from the healthy beginnings trial*. Matern Child Health J 2013;17:1151-7.

[26] Cluss PA, Levine MD, Landsittel D. *The Pittsburgh STOP program: disseminating an evidence-informed intervention for low-income pregnant smokers*. Am J Health Promot 2011;25:S75-81.

[27] Marteau TM, Thorne J, Aveyard P, Hirst J, Sokal R. *Financial incentives for smoking cessation in pregnancy: protocol for a single arm intervention study.* BMC Pregnancy Childbirth 2013;13:66.

[28] Ussher M, Aveyard P, Manyonda I, Lewis S, West R, Lewis B, Marcus B, Taylor AH, Barton P, Coleman T. *Physical activity as an aid to smoking cessation during pregnancy (LEAP) trial: study protocol for a randomized controlled trial*. Trials 2012;4,13:186.

[29] Windsor R, Clark J, Cleary S, Davis A, Thorn S, Abroms L, Wedeles J. *Effectiveness of the Smoking Cessation and Reduction in Pregnancy Treatment (SCRIPT) Dissemination Project: a science to prenatal care practice partnership*. Matern Child Health 2014;18,180-90.

[30] Jiménez-Muro A, Samper MP, Marqueta A, Rodríguez G, Nerín I. *Prevalence of smoking and second-hand smoke exposure: differences between Spanish and immigrant pregnant women.* Gac Sanit 2012;26:138-44.

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ORIGINAL ARTICLE

Mycotic and aflatoxin contamination in *Myristica fragrans* seeds (nutmeg) and *Capsicum annum* (chilli), packaged in Italy and commercialized worldwide

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Keywords

Aflatoxins • Moulds • Spices

Summary

Aflatoxins are secondary metabolites of moulds known to be carcinogenic for humans, and therefore should not be ingested in high doses. This study aimed to determine the level of mould and aflatoxin contamination in dehydrated chilli and nutmeg imported from India and Indonesia, respectively, packaged in Italy, and commercialized worldwide.

We tested 63 samples of chilli (22 sanitized through heat treatment and 41 not heat-treated) and 52 samples of nutmeg (22 heat-treated and 30 not heat-treated) for aflatoxin, moulds and moisture content.

Heat-treated samples were less contaminated than untreated samples. Spices in powder form (both chilli and nutmeg) were more contaminated than whole ones. In untreated spices, we observed a positive correlation between mould and moisture content. Of the powdered nutmeg and chilli samples, 72.5% and 50% tested positive for aflatoxin contamination, with a range of 0-17.2 μg kg^{-1} and 0-10.3 μg kg^{-1}, respectively.

The steam treatment of spices would be useful in reducing the initial amount of moulds. Although the risk from the consumption of spices contaminated with aflatoxins is minimal, owing to the small amount used in food, preventive screening of the whole food chain is very important, especially because the most frequently identified toxin was B_1, which is the most dangerous of the four toxins (B1, B2, G1, G2).

Introduction

Aspergillus, *Penicillium* and *Fusarium* are ubiquitous, saprophytic moulds. They may contaminate natural foods and animal feeds, producing mycotoxins that exert toxic effects on human and animal health [1]. Mycotoxins are produced by the secondary metabolism of moulds, the most common being the aflatoxins (AFs). These are mainly produced by two species of environmental filamentous fungi, *A. flavus* and *A. parasiticus*, and rarely by *A. nomius* [2], which can grow in many types of food, particularly in cereals.

Fungal growth in food is favoured by poor conditions in the producing countries, temperatures of 25-30°C, humidity between 88% and 95%, and water activity values greater than 0.78. Furthermore, in these environmental conditions, mycotoxins are very likely to be produced [3, 4].

Since 1993, AFB_1 and a natural mixture of aflatoxins have been classified as "carcinogenic to humans" (group 1) by the International Agency of Research on Cancer [5], while AFM_1, a metabolite of AFB_1, has been classified as "probably carcinogenic to humans" (group 2A). Today, AFs are well known to be toxic, mutagenic, carcinogenic, immunosuppressive, and teratogenic agents [5], capable of crossing the placental barrier [6]. Extensive experimental evidence shows that AFs can induce liver cancer in most species, including humans, notably among HBV carriers, as AFs and hepatitis B virus are co-carcinogens [7].

Mycotoxins can be found in a large variety of foods, such as cereals, fruit, infusion herbs, spices, coffee, cacao, fodder, etc. Fungal contamination can occur throughout the production chain, from the harvesting, drying and storage phases to product transportation [8]. Zinedine et al. [1] reported that about 50% of samples of some Moroccan cereals and spices were contaminated by mycotoxins. In a survey on cereals and cereal products conducted in the UK retail market, the Food Survey Information sheet [9] reported that the vast majority of the samples (71.8%) contained mycotoxins, although at levels below the regulatory limits for contamination in Europe [10]. However, the survey also showed that only 7 samples from the 220 analysed (3.2%) were found to contain levels of mycotoxins above the regulatory limits laid down in EU legislation [10], and in most cases these levels were only marginally above the limit.

In Europe, there are two specific regulations regarding mycotoxins: one concerns the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs [11], while the other establishes maximum levels of certain contaminants in food-

stuffs [10, 12, 13], such as AFB_1 in spices at 5 µg/kg and total aflatoxins at 10 µg/kg.
In Italian cuisine, chillies, the fruit of the *Capsicum annum* plant of the *Capsicum* genus (family: *Solanaceae*), are among the most frequently used spices. The chilli is appreciated for its pungency, caused by the presence of capsaicinoids, which are known to have chemo-preventive, anti-carcinogenic [14, 15], antioxidant [16], anti-inflammatory [17], antiviral, antibacterial, and antifungal properties [18]. Capsaicinoids are not stable in dehydrated chillies; they can lose their activity through oxidation [19]; consequently, chilli powder can lose up to 5% of its capsaicinoid content each month of storage. Capsaicinoids are present in different amounts in chilli varieties and cultivars [20]. Their concentration ranges from 0.001% to 0.01%, in fresh red pepper varieties, especially paprika, from 0.1% to < 1% in strong chilli varieties [21]. Pino et al. [22] found that the content of capsaicinoids varied between 0.42% and 0.66%. Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide) account for about 77-98% of capsaicinoids present in chillies [21], followed by minor capsaicinoids, such as nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nonivamide and other compounds [23, 24, 25].
Another spice often used in Italian cuisine is nutmeg, a dried ovoid seed of *Myristica fragrans* Houtt (family: *Myristicaceae*). Nutmeg is widely used both as a food spice and in alternative medicine, as it has been reported to have antidiarrheal [26], anti-inflammatory, anti-cancer [27], antioxidant, antibacterial and antifungal [28] properties. Nutmeg contains a mixture of hydrophobic and volatile compounds; among these, the most relevant are monoterpene hydrocarbons, followed by oxygenated monoterpenes, and others such as β-caryophyllene, which is reported to be anti-inflammatory and antifungal [29].
The aim of this study was to investigate the occurrence of aflatoxins and moulds, and to measure moisture content, in dehydrated chillies and nutmeg imported from India and Indonesia, packaged in Italy, and commercialized worldwide, in order to evaluate the health risk related to the consumption of these aflatoxin-laden foods, which are widely used in cooking worldwide.

Methods

A total of 115 samples of commercial spices, all imported and packaged by "Drogheria e Alimentari Spa" (Scarperia e San Piero, FI, Italy), were analysed for the presence of aflatoxins and moulds, and for the quantification of moisture. The samples analysed were divided as follows: 63 samples of chilli (13 samples in whole form and 50 samples in powder) and 52 samples of nutmeg (12 samples in whole form and 40 samples in powder). For convenience, crushed samples (28 chilli and 13 nutmeg) were included within those in powder form.
In order to reduce microbial contamination of the samples, and consequently to avoid microbial multiplication during the storage of packaged products, 22 chilli and 22 nutmeg samples had been subjected to steam treatment (100°C for a few minutes) by the supplier before shipment to the Italian factory (Drogheria & Alimentari Spa).

Detection of moulds

Analyses were performed in conformity with Standard ISO 7218:2007/Amd1 [30] and ISO 21527-2 [31]. A 25 g portion of each sample was aseptically taken, placed in 225 ml of Buffered Peptone Water (BPW) (Oxoid Spa, Rodano, MI, Italy), and homogenized by means of a Stomacher for 60 s at normal speed. Three subsequent decimal dilutions in BPW were prepared. A 0.5 ml portion of each dilution was streaked with Yeast Extract Dextrose Chloramphenicol Agar (YEDC) (Oxoid Spa, Rodano, MI, Italy) and incubated for 5 days at 25 ± 1°C. After incubation, colonies were counted and results were expressed as CFU *per* g of foodstuff (CFU/g).

Quantitative determination of aflatoxins B1, B2, G1, G2

Sampling procedures were performed according to Regulation (EC) n° 401/2006 [11]. All organic solvents, methanol and acetonitrile, were HPLC-grade. A 25 g portion of each sample was extracted with 100 ml of acetonitrile/water (84:16 v/v) by means of a sonicator (Falc instruments, Treviglio, BG, Italy) for 20 minutes. The solution was filtered through filter paper and 5 ml of filtrate was purified with "Mycosep 228 AflaPat" (Romer Labs, Tulln, Austria). The eluate was directly used to perform HPLC analysis (Varian – Chicago, IL, USA). The analytical procedure was performed in accordance with Bononi et al. [32].
The HPLC instrument was equipped with a fluorescence detector and a post-column derivatization system (coring cell). Aflatoxins were detected at the excitation and emission wavelengths of 365 nm and 435 nm, respectively, as per Golge et al. [33]. The column was an OmniSpher C18 (250 mm x 4.6 mm I.D., 5 µm particle size), and was maintained at 40°C during the analysis, which was performed at a flow rate of 0.8 mL/min. The mobile phase was a mixture of water/methanol/acetonitrile (61/23/16, v/v/v). HNO_3 (4M) and KBr (23.8g/l) were always added to the water, which was the derivatizing agent. The retention time of aflatoxins was approximately 12-23 min and the total run time was 30 min.
Calibration curves were constructed by using fortified samples at 4 incremental concentrations of total aflatoxins: standards for AFB_1, AFB_2, AFG_1, AFG_2 were purchased from Or Sell (Limidi di Soliera, MO, Italy). The uncertainty value was calculated according to NMKL [34]. The validation parameters assessed were: recovery (80-95%), limit of detection (LOD = 0.13 µg/kg for B1, G1 and G2 and LOD = 0.07 µg/kg for B2), limit of quantification (LOQ = 0.4 µg/kg for B1, G1 and G2 and LOQ = 0.2 µg/kg for B2), repeatability (intra-day precision RSDr = 0.068 for B1 and 0.142 for B2), and reproducibility (inter-day precision RSDR = 0.088 for B1 and 0.174 for B2).

Determination of moisture content

The moisture content of samples was detected by measuring the weight loss of 1-2 g of the samples in a thermobalance (Ohaus, Switzerland) at 80°C. At the end of the process, the instrument showed the percentage of moisture of the sample on a wet-weight basis.

Statistical analysis

The regression used for the correlation was linear, and the Pearson coefficient (p) was used to evaluate the behaviour of the variables. Microsoft Office Excel 2007.

Results

Detection of mould and aflatoxin content

Mycotic contamination was lower than the detection limit (< 10 CFU/g) in 39.7% of chilli samples and in 30.8% of nutmeg samples (Tab. I).

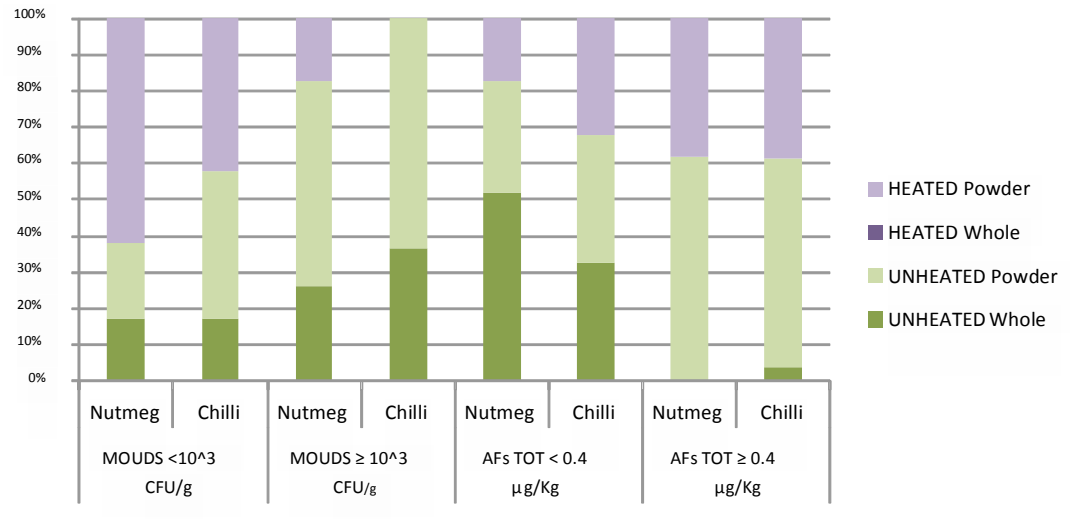
The percentages of samples with high contamination (≥ 10^3CFU/g) were higher in nutmeg (44.2%) than in chilli (17.5%) (Tab. I). Our results showed that in heat-treated samples there was a statistically significant reduction in the presence of mould: this reduction was more marked in nutmeg than in chilli samples ($p = 0.0001$ and $p = 0.067$, respectively). Few nutmeg (18.2%) and no chilli samples had mould concentrations higher than 10^3CFU/g. Undetectable AF content (< 0.4 µg/kg) was found in 58.7% and 44.2% of chilli and nutmeg samples, respectively (Tab. I). The percentage of samples with contamination equal to or higher than 5 µg/kg was 4.8% for chilli and 7.7% for nutmeg. Interestingly, higher levels of aflatoxin contamination (concentration > 1 µg/kg) were found in treated samples (data not shown): a possible explanation for this could be that treatment abated the competitive effect by reducing the number of moulds, thereby promoting toxinogenesis by surviving moulds.

In order to study the different occurrences of moulds and AFs, the trend in contamination was plotted as a function

Tab. I. Moulds and total aflatoxins in chilli and nutmeg samples (total, unheated, heated).

		chilli			nutmeg		
Contamination		N total samples (%)	N unheated samples (%)	N heated samples (%)	N total samples (%)	N unheated samples (%)	N heated samples (%)
Moulds (CFU/g)	< 10	25(39.7)	13(31.7)	12(54.5)	16(30.8)	2(6.7)	14(63.6)
	10 - < 10^2	13(20.6)	7(17.1)	6(27.3)	1(1.9)	0(0)	1(4.5)
	10^2 - < 10^3	14(22.2)	10(24.4)	4(18.2)	12(23.1)	9(30.0)	3(13.6)
	10^3 - < 10^4	8(12.7)	8(19.5)	0(0)	16(30.8)	12(40.0)	4(18.2)
	≥ 10^4	3(4.8)	3(7.3)	0(0)	7(13.4)	7(23.3)	0(0)
Total Aflatoxins (µg/Kg)	< 0.4	37(58.7)	25(61.0)	12(54.5)	23(44.2)	19(63.3)	4(18.2)
	0.4 - < 3	19(30.2)	12(29.2)	7(31.9)	21(40.4)	9(30)	12(54.5)
	3 - < 5	4(6.3)	2(4.9)	2(9.1)	4(7.7)	2(6.7)	2(9.1)
	≥ 5	3(4.8)	2(4.9)	1(4.5)	4(7.7)	0(0)	4(18.2)
	Total	63 (100)	41(100)	22(100)	52(100)	30(100)	22(100)

Fig. 1. Mould and aflatoxin content of heat-treated and non-heat-treated samples.



of the granulometry (whole and powdered) of the two spices: mycotic contamination higher than 10^3CFU/g was found in 11 chilli samples, 63.6% of which were ground (Fig. 1).
The mean level of mould contamination in powdered chilli samples was a little lower ($1.6 \times 10^3 \pm 3.9 \times 10^3$ CFU/g) than in whole chillies ($4.7 \times 10^3 \pm 1 \times 10^4$ CFU/g). This difference is greater if we consider the contamination level ≥ 10^4 CFU/g: 15.4% of whole chilli samples fell within this range, whereas only 2.0% of the ground samples did.
The two types of nutmeg samples displayed similar levels of mould contamination: whole ($5.8 \times 10^3 \pm 1 \times 10^4$ CFU/g) and powder ($8.4 \times 10^3 \pm 1 \times 10^4$ CFU/g). If we consider the contamination level higher than10^4 CFU/g, the results were 8.3% and 15.0% of whole and powder samples, respectively. In this case, it was interesting to subdivide ground nutmeg into crushed samples (7.7%) and fine powder samples (18.5%). Interestingly, and probably owing to the absence in nutmeg of antimycotic compounds, such as capsaicinoids, none of the whole nutmegs (Tab. II) and only 7.7% of chilli samples presented AFs (data not shown), whereas they were all contaminated by moulds. Ultimately, Table II shows that there was a statistical difference between powder and whole samples ($p < 0.0001$) regarding the aflatoxin content of both spices.

Influence of moisture on non-heat-treated nutmeg and chilli

A positive correlation (linear correlation r = 0.19 and line gradient m = 998.4) was found between moisture and mould content in non-heat-treated nutmeg samples (Fig. 2), prevalently due to whole samples (linear correlation r = 0.28 and line gradient m = 1349.8).
A negative correlation was also found between moisture and mould content in chilli samples (Fig. 3) (linear correlation r = -0.17 and line gradient m = -779.63), mostly due to powder samples (linear correlation r = -0.44 and line gradient m = -1215.8), which showed a higher moisture content (mean = 6.61; SD = 1.42) than whole ones (mean = 6.31; SD = 1.50). A positive correlation (Fig. 4) was found between aflatoxin production and moisture content in non-heat-treated nutmeg samples (linear correlation r = 0.10 and line gradient m = 0.0593), prevalently in ground samples (linear correlation r = 0.32 and line gradient m = 0.2274). No correlation was found in chilli samples. The moisture content of products was always low; only one sample of nutmeg had a moisture content of 12.68%; the mould concentration of this sample was high (3.9×10^4CFU/g).
Whole chilli samples presented lower moisture content than powdered ones; together with the lesser availability of nutrients, this caused a slowdown in the multiplication of mould. Indeed, the percentage of samples with a detectable level of mould contamination was lower for whole chilli samples than for powdered ones.

Mycotoxin occurrence

Only six samples exceeded the B_1 limit of 5 μg kg^{-1} set by European Commission Regulations [13]: three (5.77%) samples of heat-treated nutmeg powder, two (3.17%) of untreated chilli powder, and one (1.59%) of heat-treated chilli powder. We did not find any sample contaminated with aflatoxin G2, and aflatoxin G1 was only found in three powder samples (two nutmeg and one chilli).

Discussion

The quantification of moulds and aflatoxins in chilli and nutmeg is important because of their widespread culinary use and consequent ingestion by consumers, especially since storing spice products for long periods of time is one of the predisposing factors for aflatoxin production. Given that moulds produce mycotoxins, it is very important to evaluate the quality of spices that are shipped around the world [4].
Similar levels of contamination were found by Hammami et al. [35] and Mandeel [36].
We could conclude that the antimycotic effect of capsaicinoids in chilli was more marked in ground samples

Tab. II. Number and percentage, moisture and AF content of each mould contamination range.

Unheated samples	Mould contamination Range	Samples N	Samples %	Moisture mean %	Moisture range %	AF mean μg/Kg	AF range μg/Kg
Chilli powder	< 10	7	17.1	7.16	5.74-8.22	2.06	0-10.3
	10-10^3	14	34.2	6.47	4.94-7.95	1.03	0-3.9
	≥ 10^3	7	17.1	7.21	5.4-9.58	1.2	0-7.0
Whole chilli	< 10	6	14.6	6.01	3.14-6.94	0.23	0-1.4
	10-10^3	3	7.3	5.93	5.82-6.9	0	0
	≥ 10^3	4	9.7	7.07	5.09-9.61	0	0
Nutmeg powder	< 10	2	6.7	9.84	9.38-10.3	1.1	0-2.1
	10-10^3	4	13.3	7.91	4.02-9.9	2.5	0-4.8
	≥ 10^3	12	40.0	9.04	4.71-11.1	0.6	0-2.0
Whole nutmeg	< 10	0	0	-	-	-	-
	10-10^3	5	16.7	10.99	9.83-12.0	0	0
	≥ 10^3	7	23.3	9.00	5.32-12.7	0	0

Fig. 2. Correlation between moulds and moisture contents in total (blue/grey) and whole (black) non-heat-treated nutmeg samples.

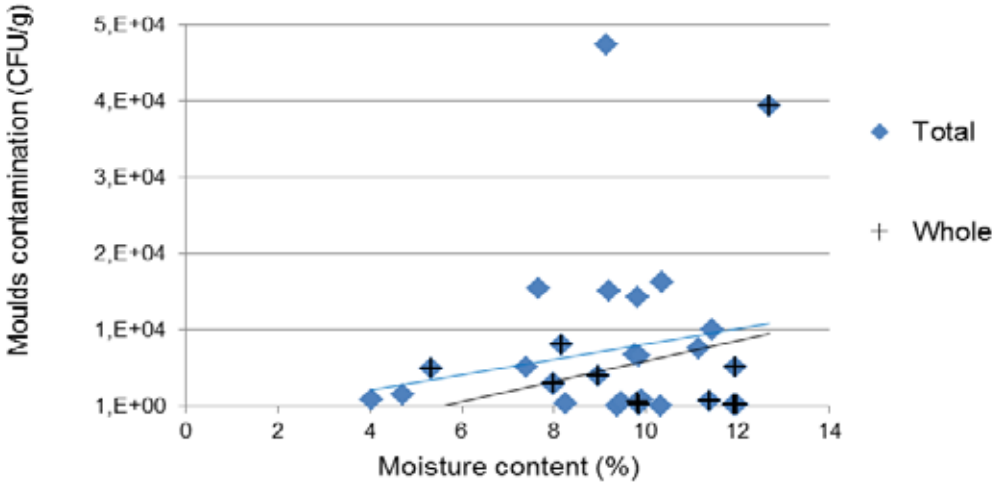


Fig. 3. Correlation between moulds and moisture contents in total (blue/grey) and powder (black) non-heat-treated chilli samples.

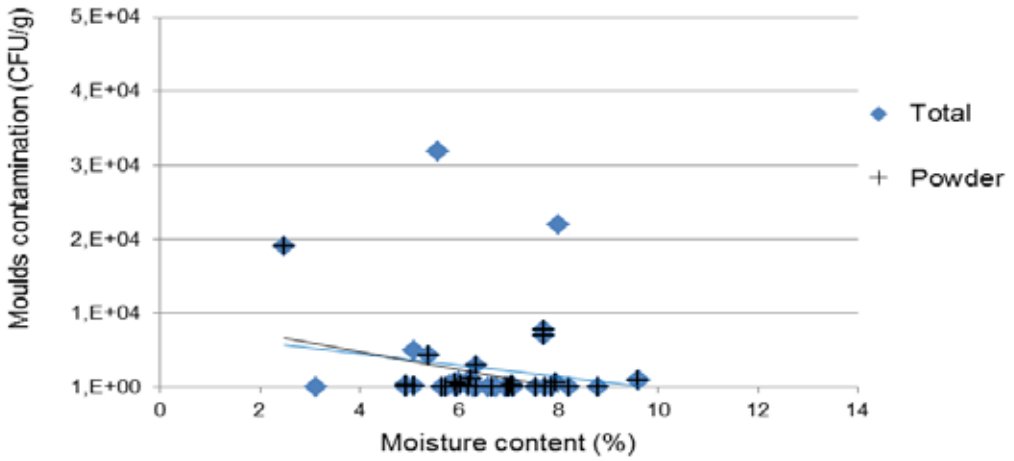
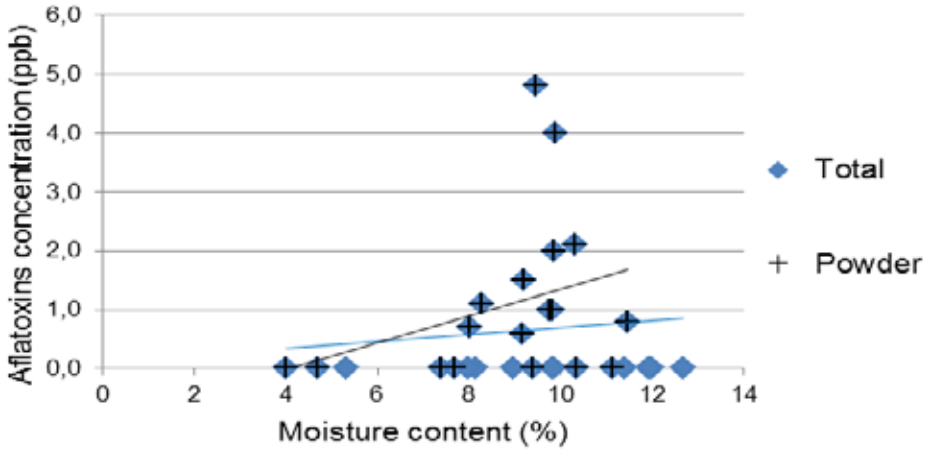


Fig. 4. Correlation between aflatoxins and moisture contents in total (blue/grey) and powder (black) non-heat-treated nutmeg samples.



Tab. III. Aflatoxins detected in nutmeg and chilli samples (heated and unheated).

Samples		Powdered nutmeg	Whole nutmeg	Powdered chilli	Whole chilli
AFs negative	N	11	12	25	12
	%	27.5	100	50.0	100
B_1 positive	N	21	0	22	1
	%	52.5	0	44.0	8.33
	Mean	2.02 ± 1.81	ND	1.95 ± 1.68	0.11 ± 0.39
$B_1 + B_2$ positive	N	6	0	2	0
	%	15.0	0	4.0	0
	Mean B1	3.18 ± 3.45	ND	6.85 ± 4.59	ND
	Mean B2	0.48 ± 0.41	ND	0.25 ± 0.07	ND
$B_1 + G_1$ positive	N	1	0	0	0
	%	0.25	0	0	0
	B1	3.1	ND	ND	ND
	G1	0.9	ND	ND	ND
$B_1 + B_2 + G_1$ positive	N	1	0	0	0
	%	0.25	0	0	0
	B1	14.8	ND	ND	ND
	B2	1	ND	ND	ND
	G1	1.4	ND	ND	ND
G_1 positive	N	0	0	1	0
	%	0	0	2.0	0
	G1	ND	ND	1.0	ND

ND: Not detected (below the detection limit)

than in whole ones because the moulds were more exposed to these molecules. As can be seen, ground chillies were also those with the highest percentage (75.0%) of samples with detectable contamination (≥ 10 CFU/g). This can be explained by the fact that ground samples had the highest nutrient availability. Another aspect to consider is that the antimycotic effect of capsaicinoids is not sufficient to prevent mycotic multiplication in highly contaminated samples.

Higher percentages of mould-contaminated samples in powdered than in crushed spices may be due to the greater surface area and availability of nutrients in ground spices (as opposed to whole ones), together with the absence in nutmeg of antimycotic compounds, such as capsaicinoids.

As Kaaya & Eboku [37] reported with regard to cassava products, we found a positive correlation (linear correlation r = 0.19 and line gradient m = 998.4) between moisture and mould content in non-heat-treated nutmeg samples (Fig. 2), prevalently due to whole samples (linear correlation r = 0.28 and line gradient m = 1349.8). It is plausible that the negative correlations observed in chilli samples (Fig. 3) were due to the presence of capsaicinoids, which could affect mould growth and consequently toxinogenesis. No correlation was found between aflatoxin production and mould contamination in non-heat-treated nutmeg samples, while a weak positive correlation was found in chillies. It is encouraging to note that the moisture content of the products analysed in this study was always lower than 12%; as a percentage greater than 12% allows microbial growth [38].

Given that aflatoxins are considered of great concern by health authorities, we detected the content of total aflatoxins, B_1, B_2, G_1 and G_2 in our samples (Tabs. II and III). Our results were almost always below the limit of 5 μg kg^{-1} for B_1 and 10 μg kg^{-1} for total AFs, according to European Commission Regulations [13] for spices, and lower than those found by other authors [35, 39, 40].

Conclusions

The current study showed a high incidence of mould, in both chilli and nutmeg. These moulds can multiply and produce AFs if spices are preserved in a critical condition for a long time, as may happen in the case of products that are commercialized worldwide. Interestingly, nutmeg samples were more contaminated by mould than chilli samples; this could be due to the antimycotic effect of capsaicinoids. Most studies have reported that a high mycotoxin content is due to the susceptibility of spices to fungal contamination and multiplication resulting from environmental and packaging conditions, such as high humidity and temperature [41]. We also saw that it could be useful to steam-treat spices before packaging, in order to reduce the initial amount of moulds. A possible way to reduce moisture inside packages could be to use appropriate materials, such as sorbents; the reduction in moisture would then increase the replication time of mould, and consequently inhibit AF production. However, we did not test this in this study. Positive correlations were found between moisture and mould and between moisture and aflatoxins only in untreated nutmeg samples; in untreated chilli samples, the presence of capsaicinoids probably negatively affects these correlations.

Concerning the occurrence of moulds and aflatoxins, we did not find any correlation (untreated samples).
Although the risk from the consumption of spices contaminated with aflatoxins is minimal, owing to the small amount used in food, preventive screening of the whole food chain remains very important, especially since the most frequently identified toxin was B_1, which is the most dangerous of the four toxins (B1, B2, G1, G2).

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Authors' contributions

ALN, GP conceived, designed and coordinated the research. MO and SR collected data. MO and GP optimized the informatics database and performed the statistical analyses. GP, CC, MO, SR and ALN evaluated the results. GP wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

References

[1] Zinedinea A, Brerab C, Elakhdarid S, Catanob C, Debegnachb F, Angelinib S, De Santisb B, Faidc M, Benlemlihd M, Minardib V, Miragliab M. *Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco.* Food Control 2006;17:868-74.

[2] Varga J, Frisvad JC, Samson RA. *Two new aflatoxin producing species, and an overview of Aspergillus section flavi.* Stud Mycol 2011;69:57-80.

[3] Lozano-Ojalvo D, Rodríguez A, Bernáldez V, Córdoba JJ, Rodríguez M. *Influence of temperature and substrate conditions on the omt-1 gene expression of Aspergillus parasiticus in relation to its aflatoxin production.* Int J Food Microbiol 2013;166:263-9.

[4] Schatzmayr G and Streit E. *Global occurrence of mycotoxins in the food and feed chain: facts and figures.* World Mycotoxin J 2013;6:213-22.

[5] IARC. *Some naturally occurring substances: food items and constituents, Heterocyclic Aromatic Amines and Mycotoxins. IARC monographs on the evaluation of carcinogenic risks to humans.* IARC Scientific Publication 1993; 56.

[6] Fung F, Clark RF. *Health effects of mycotoxins: a toxicological overview*. J Toxicol Clin Toxicol 2004;42:217-34.

[7] Iqbal SZ, Asi MR, Ariño A. *Aflatoxins*. In: *Brenner's Encyclopedia of Genetics (Second Edition).* Elsevier Inc. 2013, pp. 43-7. doi:10.1016/B978-0-12-374984-0.00022-X

[8] Czerwiecki L, Wilczyfiska G, Kwieciefi A. *Mycotoxins in several Polish food products in 2004-2005.* Mycotoxin Res 2006;22:159-62.

[9] Food Survey Information Sheet: 04/10 November 2010 Surveillance programme for mycotoxins in foods (year 1: cereals and cereal products) Food Standard Agency Available at: http://fera.co.uk/food/nationalReferenceLaboratory/chemicalFoodSafety/mycotoxins/documents/fsis0410.pdf [Accessed 24/04/2015]

[10] Commission Regulation (EU) No 1058/2012 of 12 November 2012 amending Regulation (EC) No 1881/2006 as regards maximum levels for aflatoxins in dried figs Text with EEA relevance. Official J Eur Commun 2012; L 313. Brussels.

[11] Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the Methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official J Eur Commun 2006*;* L 70. Brussels.

[12] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting Maximum levels for certain contaminants in foodstuffs. Official J Eur Commun 2006; L 364. Brussels.

[13] Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official J Eur Commun 2010; L 50. Brussels.

[14] Chanda S, Erexson G, Riach C, Innes D, Stevenson F, Murli H, Bley K. *Genotoxicity studies with pure trans-capsaicin.* Mutat Res Genet Toxicol Environ Mutagen 2004;557:85-97.

[15] Surh YJ, Lee SS. *Capsaicin, a double-edged-sword – toxicity, metabolism, and chemopreventive potential.* Life Sci 1995;56:1845-55.

[16] Alvarez-Parrilla E, de la Rosa LA, Amarowicz R, Shahidi F. *Antioxidant activity of fresh and processed jalapeno and serrano peppers.* J Agric Food Chem 2011;59:163-73.

[17] Spiller F, Alves MK, Vieira SM, Carvalho TA, Leite CE, Lunardelli A, Poloni JA, Cunha FQ, de Oliveira JR. *Anti-inflammatory effects of red pepper (Capsicum baccatum) on carrageenan- and antigen-induced inflammation.* J Pharm Pharmacol 2008;60:473-8.

[18] Careaga M, Fernández E, Dorantes L, Mota L, Jaramillo ME, Hernandez-Sanchez H. *Antibacterial activity of Capsicum extract against Salmonella typhimurium and Pseudomonas aeruginosa inoculated in raw beef meat.* Int J Food Microbiol 2003;83:331-5.

[19] Estrada B, Bernal MA, Díaz J, Pomar F, Merino F. *Capsaicinoids in vegetative organs of Capsicum annuum L. in relation to fruiting.* J Agric Food Chem 2002;50:1188-91.

[20] Govindarajan VS. *Capsicum production, technology, chemistry, and quality. Part II. Processed products, standards, world production and trade CRC.* Crit Rev Food Sci Nutr 1986;24:207-88.

[21] Govindarajan VS, Rajalakshmi D, Chand N. *Capsicum – production, technology, chemistry, and quality.4. Evaluation of quality CRC*. Crit Rev Food Sci Nutr 1987;25:185-282.

[22] Jorge Pinoa, Marilú Gonzálezb, Liena Ceballosc, Alma Rosa Centurión-Yahb, Jorge Trujillo-Aguirred, Luis Latourneríe-Morenoe, Enrique Sauri-Duch. *Characterization of total capsaicinoids, colour and volatile compounds of Habanero chilli pepper (*Capsicum chinense *Jack.) cultivars grown in Yucatan.* Food Chem 2007;104:1682-6.

[23] Constant HL, Cordell GA, West DP. *Nonivamide, a constituent of Capsicum oleoresin*. J Nat Prod 1996;59:425-6.

[24] Giuffrida D, Dugo P, Torre G, Bignardi C, Cavazza A, Corradini C, Dugo G. *Characterization of 12 Capsicum varieties by evaluation of their carotenoid profile and pungency determination.* Food Chem 2013;140:794-802.

[25] Huang XF, Xue JY, Jiang AQ, Zhu HL. *Capsaicin and its analogues: structure-activity relationship study.* Curr Med Chem 2013;20:2661-72.

[26] Grover JK, Khandkar S, Vats V, Dhunnoo Y, Das D. *Pharmacological studies on Myristica fragrans – antidiarrheal, hypnotic, analgesic and hemodynamic (blood pressure) parameters.* Methods Find Exp Clin Pharmacol 2002;24:675-80.

[27] Olajide OA, Ajayi FF, Ekhelar AI, Awe SO, Makinde JM, Alada AR. *Biological effects of Myristica fragrans (nutmeg) extract.* Phytother Res 1999;13:344-5.

[28] Ashish Deep Guptaa, Vipin Kumar Bansalb, Vikash Babuc, Nishi Maithila. *Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans *Houtt).* J Genet Eng Biotechnol 2013;11:25-31.

[29] Sabulal B, Dan M, J AJ, Kurup R, Pradeep NS, Valsamma RK, George V. *Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: chemical characterization and antimicrobial activity.* Phytochemistry 2006;67:2469-73.

[30] ISO 7218: 2007. *Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations*.

[31] UNI EN ISO 21527-2:2008. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeast and moulds. Part 2: Colony count technique in products with water activity less than or oqual to 0.95.* Geneva: International Organization for Standardization.

[32] Bononi M, Andreoli G, Tateo F. *Estrazione di Aflatossine con una nuova metodica di purificazione atta alla determinazione per HPLC/FD in spezie*. Industrie Alimentari 2008; 47:730-6è.

[33] Golge O, Hepsag F, Kabak B. *Incidence and level of aflatoxin contamination in chilli commercialized in Turkey.* Food Control 2013;33:514-20.

[34] NMKL – Nordisk Metodik Komité for Levnedsmiddel (Nordic Committee on Food Analysis). Procedure N°5; *Estimation and expression of measurement uncertainty in chemical analysis.* 1997.

[35] Hammam W, Fiori S, Al Thani R, Ali Kali N, Balmas V, Migheli Q, Jamouam S. *Fungal and aflatoxin contamination of marketed spices.* Food Control 2014;37:177-81.

[36] Mandeel QA. *Fungal contamination of some imported spices.* Mycopathologia 2005;159:291-8.

[37] Kaaya AN, Eboku D. *Mould and aflatoxin contamination of dried cassava chips in Eastern Uganda: association with traditional processing and storage practice.* J Biol Sci 2010;10:718-29.

[38] Aryeea FNA, Oduroa I, Ellisa WO, Afuakwab JJ. *The physicochemical properties of flour samples from the roots of 31 varieties of cassava.* Food Control 2006;17:916-22.

[39] Abdulkadar AHW, Al-Ali AA, Al-Kildi AM, Al-Jedah JH. *Mycotoxins in food products available in Qatar*. Food Control 2004;15:543-8.

[40] Yerneni SG, Hari SS, Jaganathan R. *Determination of the level of aflatoxin present in the marketed spices.* World J Sci Technol 2012;2:31-4.

[41] Patharajana S, Reddya KRN, Karthikeyana V, Spadarob D, Lorea A, Gullinoa ML, Garibald A. *Potential of yeasts antagonists on in vitro biodegradation of ochratoxin A.* Food Control 2011;22:290-6.

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ORIGINAL ARTICLE

Efficacy of safety catheter devices in the prevention of occupational needlestick injuries: applied research in the Liguria Region (Italy)

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Keywords

Healthcare workers • Safety catheter device • Needlestick injuries

Summary

Healthcare workers who use or may be exposed to needles are at risk of needlestick injuries, which can lead to serious infections by bloodborne pathogens. These injuries can be avoided by eliminating the unnecessary use of needles and using safety devices. The present study was aimed at evaluating the impact of a safety-engineered device, with passive fully automatic needlestick protection, on the rate of needlestick injuries among healthcare workers.
The setting of the study was a network of five public healthcare institutions situated in a Northern Italian Region. Data on the type of device, the number of employees and the number of catheter devices used per year were collected through regular meetings with healthcare workers over a period of five years.
The most notable result of this study was the huge risk reduction associated with safety devices. Indeed, the risk of needlestick injuries due to conventional devices was found to be 25-fold higher than that observed for safety devices. However, it is noteworthy that a considerable part of this excess can be explained by the different background number of devices used.
Moreover, descriptive analysis suggested that individuals with a poor/moderate training level had a lower risk than those with good/high training, though the difference was not statistically significant.
In conclusion, there is convincing evidence of a causal connection between the introduction of safety devices and the reduction in needlestick injuries. This consideration should prompt the introduction of safety devices into daily clinical practice.

Introduction

A "sharp injury" is a penetrating stab wound caused by a needle or other sharp object, and may result in contact with blood or other body fluids. Needlestick injuries (NSIs) are among the most prevalent occupational accidents, with hollow-bore needles and disposable syringes as the primary sources of injury [1-3]. In hospitals, healthcare workers (HCWs), particularly nurses and physicians [4, 5], are at higher risk, but cleaning staff and other workers may also be exposed to NSIs, owing to the inappropriate disposal of sharp objects [6]. HCWs are at risk of sharp injuries and subsequent infection by more than 40 bloodborne pathogens or species [7]. The risk of HBV, HCV and HIV infection attributable to contact with infected blood has been estimated to be about 30.0%, 0.5%, and 0.3%, respectively. In Italy, the estimated yearly number of HCWs at risk of bloodborne infections is about 900,000, with nearly 96,000 NSIs [6, 8]. The importance of monitoring and preventing NSIs has been recognized in U.S. and European laws. In recent years, healthcare authorities, initially in the U.S. (Public Law, September 19, 2000), have focused their attention on identifying and utilizing proper medical devices to prevent NSIs and other sharp injuries in the workplace [9].
In Europe, Directive 2010/32/EU, approved on May 10, 2010, requires EU member states to implement a global strategy to prevent occupational exposure to bloodborne pathogens in healthcare settings as a result of needlestick and sharp injuries, including the adoption of devices incorporating safety features, on the basis of risk assessment [10].
The use of needlestick safety devices is an essential mean of protecting HCWs from NSIs [7, 11]. Several new devices are rapidly entering the market. However, not all devices are alike or equally effective. To significantly decrease the risk of injury, the design of safety devices should take into account specific features. In particular, they should be needle-less and work passively; if user activation is necessary, the safety feature should be activated by means of a one-handed technique and allow the worker's hands to remain behind the exposed sharp point. Moreover, they should be easy and practical; they should be safely and effectively usable for patient care and should possess ad-

ditional features, according to the American Nurses Association [12].
Several studies have reported that passive safety devices offer better protection against accidental NSIs than active devices [13-16]. However, certain authors have concluded that there is very low quality evidence that NSIs are significantly reduced by the using of safety devices [17].
In the present paper, we evaluated the impact of a safety-engineered device on the prevention of NSIs in five public healthcare institutions in a Northern Italian Region (Liguria). This study, conducted at the Protection and Prevention Department of San Martino Hospital in Genoa, was aimed at assessing whether a reduction in the number of NSIs caused by catheters could be observed as a consequence of the introduction of the Introcan Safety® IV Catheter Straight (ISCS IV). The ISCS IV is a safety device equipped with a fully automatic passive safety shield, and was investigated because it won the supply tender of Regione Liguria. This safety catheter requires no user activation; with regard to design and handling, it is identical to the conventional catheter [18, 19].
This device was phased in over the study period, starting with a replacement rate of 24% in 2006 and reaching full replacement in 2010 in almost all institutions, except for one in which only a very low replacement rate (30%) was reached.

Methods

The present study, designed as a quasi-experimental study, was performed in order to evaluate the impact of the ISCS IV (B. Braun Medical Inc., Germany) on the number of NSIs among HCWs over a five-year period (2006-2010). The ISCS IV was used for peripheral venipuncture and possessed a passive fully automatic needlestick protection. The setting of the study was a network of five public healthcare institutions located in Liguria, a Northern Region of Italy. The following healthcare institutions participated in the investigation: San Martino Hospital (SMH), Galliera Hospital (GH), Local Health Agency 1 (ASL 1), Local Health Agency 4 (ASL 4) and Local Health Agency 5 (ASL 5). SMH and GH are hospitals located in Genoa, while ASL 1, ASL 4 and ASL 5 represent local levels of the National Health Service, consisting of small-sized hospitals and outpatient departments situated in Imperia, Savona and La Spezia, respectively. To participate in the study, detailed information on the yearly number of NSIs and the type of device involved was required. Specifically, through regular meetings with HCWs, data were collected on the type of device, its classification as a conventional or safety intravenous catheter, the number of users and the number of catheter devices used per year by each institution.
As both conventional and safety devices were used concurrently during the study period, it was impossible to establish, even approximately, the yearly number of HCWs who used each type of catheter. We therefore assumed that the same number of HCWs were exposed to both catheters. The average number of yearly training hours in occupational health and safety per HCW in each institution was used as a measure of HCW expertise and knowledge of the proper use of catheters and the prevention of sharp injuries. In this respect, HCWs were classified as having poor/moderate or good/high-level training, with 2 hours per year being set as a threshold value.
The relative frequency of NSIs was the main response variable of this investigation. For this reason, the overall number of employees per year in each healthcare institution was assumed to be the number of person-years at risk of NSIs. Accordingly, the relative frequency was calculated as the ratio of the number of NSIs per person-year at risk, and indicated as the NSI rate (NSIR). The distribution of the NSIR was then analyzed according to the categories of each study characteristic or covariate (i.e., type of catheter, healthcare institution, calendar year, staff training level). In addition, 95% confidence limits (95% CL) were computed for each rate, assuming the number of NSIs as a Poisson random variable [20].
Calendar year was taken as a continuous covariate (i.e., linear time trend) in order to estimate the mean yearly percent variation (MPV). The joint effect of all covariates on NSIR was assessed by means of the Poisson regression model, and rate ratio was used as a measure of relative risk (RR). For each RR, 95% CL were also computed. Overall and covariate-specific statistical significance was assessed by means of the likelihood ratio test. A two-tailed P-value < 0.05 was considered significant. All analyses were performed by means of STATA [21]. The Poisson regression analysis was applied in order to estimate the relative risk between NSIR and corresponding 95% confidence limits (95% CL). All analyses were repeated on using the yearly number of catheters as a denominator (offset) of NSIR in the Poisson model [22].

Results

The analysis of NSIs was performed on HCWs from five different Italian healthcare institutions. Table I shows the main features of each institution. SMH and GH are hospitals with yearly catchment area populations of 1,500,000 and 100,000, respectively; while ASL 1, ASL 4 and ASL 5 include 3 to 4 small-sized hospitals, with yearly catchment area populations between 150,000 and 217,000.
Table II describes the distribution of the number of medical devices, person-years at risk, NSIs, and the relative frequency of NSIR. During the study, the total number of person-years at risk was 122,464, and 286 NSIs occurred. These data show an overall average NSIR of 23.4 per 10^4 person-years (95% CL = 20.8-26.2). The total number of catheter devices employed was 4,785,345, which corresponded to a yearly average of 39.1 devices per HCW. Table II also shows that the risk of NSIs due to conventional and safety catheters was 44.9 and 1.8, respectively, while the ratio of the number of conventional and safety catheters used per person-year was 51.8 and 26.4, respectively. Descriptive analysis revealed three risk levels: a lower

Tab. I. Main features of each healthcare institution.

Healthcare institution	Population[a]	Small-sized hospitals[b]	Bed availability[c]	Bed occupancy rate[d]	Admissions[e]
SMH	1,500.000	-	1500	86.74	63.35
GH	100,000	-	500	90.75	27.09
ASL 1	217,000	3	700	83.14	33.32
ASL 4	150,000	4	530	91.85	22.46
ASL 5	213,000	3	400	83.08	32.40

[a] Yearly population of catchment area
[b] Number of hospitals included in each ASLs
[c] Number of available hospital beds
[d] Percent ratio of the number of occupied hospital beds to the number of available beds per year
[e] Number of hospital admissions per year
SMH: San Martino Hospital; GH: Galliera Hospital; ASL: Local Health Agency

Tab. II. Risk of needlestick injuries.

	Number[a]	Person-years[b]	Ratio[c]	NSI[d]	NSIR[e]	95%CL[f]
Catheter device						
Conventional	3,170.695	61,232	51.8	275	44.9	39.9-50.5
Safety	1,614.650	61,232	26.4	11	1.8	1.0-3.2
Healthcare facilities						
GH	513,595	17,630	29.1	10	5.7	3.1-10.5
SMH	842,000	44,872	18.8	10	2.2	1.2-4.1
ASL 1	499,250	27,786	18.0	38	13.7	10.0-18.8
ASL 5	610,000	12,090	50.5	24	19.9	13.3-29.6
ASL 4	2,320.500	20,086	115.5	204	101.6	88.5-116.5
Staff training level						
Poor/moderate	609,250	24,054	25.3	46	19.1	14.3-25.5
Good/high	4,176.095	98,410	42.4	240	24.4	21.5-27.7
Calendar year						
2006	886,750	24,768	35.8	67	27.1	21.3-34.4
2007	908,900	24,220	37.5	64	26.4	20.7-33.8
2008	951,900	24,234	39.3	55	22.7	17.4-29.6
2009	1,022.895	24,526	41.7	65	26.5	20.8-33.8
2010	1,014.900	24,716	41.1	35	14.2	10.2-19.7
Whole sample	4,785.345	122,464	39.1	286	23.4	20.8-26.2

[a] Total number of catheter devices; [b] Employees considered at risk of needlestick injuries per year; [c] Ratio of total number of catheter devices to person-years at risk; [d] Number of needlestick injuries; [e] Occurrence rate of NSIs per 10,000 person-years; [f] 95% confidence limits for NSIR; SMH: San Martino Hospital; GH: Galliera Hospital; ASL: Local Health Agency

level (from 2.2 to 5.7 NSIR) for two hospitals (GH and SMH), an intermediate level (from 13.7 to 19.9 NSIR) for ASL 1 and ASL 5, and a higher level (101.6 NSIR) for ASL 4. Moreover, individuals with a poor/moderate and good/high training level had a NSIR of 19.1 and 24.4, respectively, while the NSIR calculated by calendar year showed a trend from 27.1 to 14.2.

Table III reports the result of the Poisson regression. The number of medical devices used during the study and the person-years at risk were considered in the model: the former as a log-transformed continuous covariate, the latter as an offset. A significant difference was found between RR calculated for conventional devices and that calculated for safety devices (RR = 12.50 *vs* RR = 1; P-value < 0.001).

All ASLs were found to be at higher risk of NSIs: these institutions showed RRs which were greater than 1.80 when the NSIR of GH was used as a reference. By contrast, from the comparison between the two hospitals (SMH *vs* GH), quite a small difference in risk (RR = 1.16; 95% CL = 0.42-3.24) was observed. In addition, a statistically significant two-fold increase in risk emerged when the overall rate of all ASLs was compared with the overall rate of the two hospitals (RR = 2.00; 95% CL = 1.01-3.92; P-value < 0.001) (data not shown). Regression analysis showed no significant difference in NSIR between the two training categories (good/high *vs* poor/moderate: RR = 0.88, 95% CL = 0.48-1.62). Lastly, regression modeling confirmed the downward trend obtained in the descriptive context, even though in a weaker and not statistically significant manner (RR = 0.95, 95% CL = 0.87-1.05). In practice, a 5% reduction in NSI risk was expected to occur in the various institutions during the study period (MPV -5%, 95% CL = -13.1% / +4.5%).

Tab. III. Effect of catheter type and staff training on needlestick injury occurrence estimated through the Poisson regression model.

	RR[a]	95%CL[b]	P-value
Constant[c]	7.2	2.5-20.2	-
Catheter device			< 0.001
Safety	1.00	Ref.[d]	
Conventional	12.50	5.56-25.00	
Healthcare facilities			0.236
GH	1.00	Ref.	
SMH	1.16	0.42-3.24	
ASL 1	1.83	0.80-4.21	
ASL 5	2.00	0.80-4.96	
ASL 4	3.19	1.17-8.67	
Staff training level			0.680
Poor/moderate	1.00	(Ref.)	
Good/high	0.88	0.48-1.62	
Calendar year			0.307
Linear trend	0.95	0.87-1.05	

[a] Needlestick injury occurrence rate ratio (relative risk) adjusted for the total number of catheter devices used; [b] 95% confidence limits for RR; [c] Baseline needlestick injury occurrence rate per 10,000 person-years at risk in all reference categories (year 2006) evaluated at the yearly median value (16,500) of catheter devices used; [d] Reference category. MH: San Martino Hospital; GH: Galliera Hospital; ASL: Local Health Agency

Discussion

Assessment of the risk of HCW exposure to biohazards is one of the main issues for occupational health professionals. The present investigation provides convincing evidence that the implementation of safety catheters is related to the reduced occurrence of NSIs, confirming reported previously results [14, 16, 23].

Through this non-concurrent prospective investigation, we assessed the impact of safety-engineered devices in five Ligurian public healthcare institutions, following a specific regional competitive tender that offered the opportunity to start adopting safety needles. During the study, a marked downward trend in NSIR by calendar year was observed. Specifically, the NSIR declined by approximately 47% from 2006 to 2010, which corresponds to a mean yearly reduction of about 9%. Over the same period, the number of medical devices employed per HCW increased by about 15%. Notably, conventional catheters were gradually replaced by safety catheters, starting from a replacement rate of about 24% in 2006, and reaching full replacement in 2010 in almost all healthcare institutions considered in the study, except for ASL 4, which only reached 30% replacement.

The most striking result of this study was the huge and statistically significant risk reduction associated with the use of safety devices. Indeed, the risk of NSIs due to conventional catheters was found to be 25-fold higher than that observed for ISCS IV. However, it is noteworthy that a fairly large portion of this excess can be explained by the different background number of devices used, in that the number of conventional catheters used per person-year was almost double the number of safety devices used.

Our analysis suggested that individuals with a poor/moderate training level had a lower NSIR than those with better training, though the difference was not statistically significant. This paradoxical result could also be explained by the large difference in the number of medical devices per person-year used in the study.

The present study certainly suffers from some epidemiological limitations, the main one being due to the study design itself; a substantial bias stems from the fact that the exposure-disease relationship was only estimated on the available lumped data (institution level) and could not be extended to each individual (HCW level). Indeed, we did not know whether a worker who reported a NSI had previously received adequate training in occupational safety, since we only knew the yearly average of training hours per worker in each institution. Unfortunately, this drawback, which is typical of this type of study design, can only be avoided by conducting epidemiological investigations based on individual records (i.e., case-control study).

A second limitation is the lack of information on healthcare personnel truly at risk of exposure to NSIs, in that the concept of person-years at risk included the time contributions of all employees (healthcare providers, administrative and maintenance workers), regardless of their actual jobs. All healthcare facilities belong to the same Regional Health Authority and, accordingly, are subject to the same health policy guidelines and service standards, which set the priorities in clinical care, define the quality of assistance, and establish the number of medical and allied health professionals engaged in the public health sector. Considering the moderate extension of the regional catchment area, which definitely reflects a small variability in the overall disease burden, it is reasonable and realistic to assume that the proportion of medical and healthcare professionals truly at risk of exposure to bloodborne pathogens was constant across institutions. However, this does not guarantee that all healthcare providers within a public institution have a homogeneous risk level. In this respect, a moderate degree of extra-Poisson variation or over-dispersion, due to the lack of some important covariates, was found. This was properly addressed by using a specific extension of the Poisson model, namely the negative binomial regression, which did not yield important changes.

Conclusions

This investigation revealed that the NSIR ratio associated with the use of the ISCS IV safety device was significantly lower than that of the traditional device. It can therefore be concluded that, despite the limitations of the investigation, there was a causal relationship between the introduction of the ISCS IV and the reduction in NSIs. In conclusion, convincing evidence in favor of the ISCS IV should prompt the introduction of this new catheter device into daily clinical practice, especially when a fair trade-off between clinical performance and HCW safety can be achieved.

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Authors' contributions

DS conceived, designed and coordinated the research, and participated in all stages of the work. MDG, RF, AP and LR collected data and performed the data quality control. RP and VF performed the statistical analyses. MM and LO evaluated the results and drafted the final manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All Authors read and approved the final manuscript.

References

[1] Jagger J, Perry J, Parker G, Phillips EK. *Nursing2011 survey results: Blood exposure risk during peripheral I.V. catheter insertion and removal.* Nursing 2011;41:45-9.

[2] Cho E, Lee H, Choi M, Park SH, Yoo IY, Aiken LH. *Factors associated with needlestick and sharp injuries among hospital nurses: a cross-sectional questionnaire survey*. Int J Nurs Stud 2013;50:1025-32.

[3] Riddell A, Kennedy I, Tong CY. *Management of sharps injuries in the healthcare setting*. BMJ 2015;351:h3733. doi: 10.1136/bmj.h3733.

[4] Workbook for Designing, Implementing, and Evaluating a *Sharps Injury Prevention Program*, 2008. Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/sharpssafety/pdf/WorkbookComplete.pdf. Accessed October 3, 2008. [Accessed 15/02/2015]

[5] Pruss-Ustun A, Rapiti E, Hutin Y. *Sharps injuries: global burden of disease from sharps injuries to health-care workers*. World Health Organization. Available at: http://www.who.int/quantifying_ehimpacts/publications/9241562463/en/. [Accessed 15/10/2015]

[6] Di Bari V, De Carli G, Puro V, Gruppo Collaborativo dello Studio Italiano sul Rischio Occupazionale da HIV e Altri Patogeni a Trasmissione Ematica (SIROH). *Prevention of accidental needle sticks before the Directive 2010/32/EU in a sample of Italian hospitals*. Med Lav 2015;106:186-205.

[7] De Carli G, Abiteboul D, Puro V. *The importance of implementing safe sharps practices in the laboratory setting in Europe*. Biochem Med (Zagreb) 2014;24:45-56.

[8] Puro V, De Carli G, Cicalini S, Soldani F, Balslev U, Begovac J, Boaventura L, Campins Martí M, Hernández Navarrete MJ, Kammerlander R, Larsen C, Lot F, Lunding S, Marcus U, Payne L, Pereira AA, Thomas T, Ippolito G; European Occupational Post-Exposure Prophylaxis Study Group. *European recommendations for the management of healthcare workers occupationally exposed to hepatitis B virus and hepatitis C virus*. European Occupational Post-Exposure Prophylaxis Study Group. Euro Surveillance 2005;10:260-4.

[9] Occupational Exposure to Bloodborne Pathogens; Needle-stick and Other Sharps Injuries; Final Rule, 2001. US Department of Labor, Occupational Safety & Health Administration. Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=FEDERAL_REGISTER&p_id=16265. [Accessed 15/10/2015].

[10] Directive 2000/54/EC of the European Parliament and of the council of 18 september 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of article 16(1) of Directive 89/391/EEC). The Council of European Communities. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:262:0021:0045:EN:PDF. [Accessed 15/10/2015]

[11] Adams D, Elliott TSJ. *Impact of safety needle devices on occupationally acquired needlestick injuries: a four year prospective study*. J Hosp Infect 2006;64:50-5.

[12] American Nurses Association. *ANA's needlestick prevention guide*. 2002. Available at: www.nursingworld.org/MainMenuCategories/OccupationalandEnvironmental/occupational-health/SafeNeedles/NeedlestickPrevention.aspx. [Accessed 15/10/2015]

[13] Sossai D, Puro V, Chiappatoli L, Dagnino G, Odone B, Polimeri A, Ruzza L, Palombo P, Fuscoe MS, Scognamiglio P. *Using an intravenous catheter system to prevent needlestick injury*. Nur Stand 2010;24:42-6.

[14] Hoffmann C, Buchholz L, Schnitzler P. *Reduction of needlestick injuries in healthcare personnel at a university hospital using safety devices*. J Occup Med Toxicol 2013;8:20. doi: 10.1186/1745-6673-8-20.

[15] Whitby M, McLaws ML, Slater K. *Needlestick injuries in a major teaching hospital: the worthwhile effect of hospital-wide replacement of conventional hollow-bore needles*. Am J Infect Control. 2008;36:180-6.

[16] Fukuda H, Yamanaka N. *Reducing needlestick injuries through safety-engineered devices: results of a Japanese multi-centre study*. J Hosp Infect 2016;92:147-53.

[17] Lavoie MC, Verbeek JH, Pahwa M. *Devices for preventing percutaneous exposure injuries caused by needles in healthcare personne*l. Cochrane Database Syst Rev 2014; doi: 10.1002/14651858.CD009740.pub2.

[18] Trim JC. *A review of needleprotective devices to prevent sharps injuries*. Br J Nurs 2004;13:144-53.

[19] Wilburn SQ. *Needlestick and sharps injury prevention*. Online J Issues Nurs 2004;9:3-5.

[20] StataCorp. Stata Statistical Software. Release 11.2. Stata Corporation, College Station, TX, 2007.

[21] Rothman KJ, Greenland S. *Modern Epidemiology*, 2nd Edition. Philadelphia: Lippincott-Raven Publishers 1998.

[22] Cameron AC, Trivedi PK. *Regression analysis of count data*. Cambridge: Cambridge University Press 1998.

[23] Prunet B, Meaudre E, Montcriol A, Asencio Y, Bordes J, Lacroix G, Kaiser E. *A prospective randomized trial of two safety peripheral intravenous catheters*. Anesth Analg 2008;107:155-8. doi: 10.1213/ane.0b013e318174df5f.

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