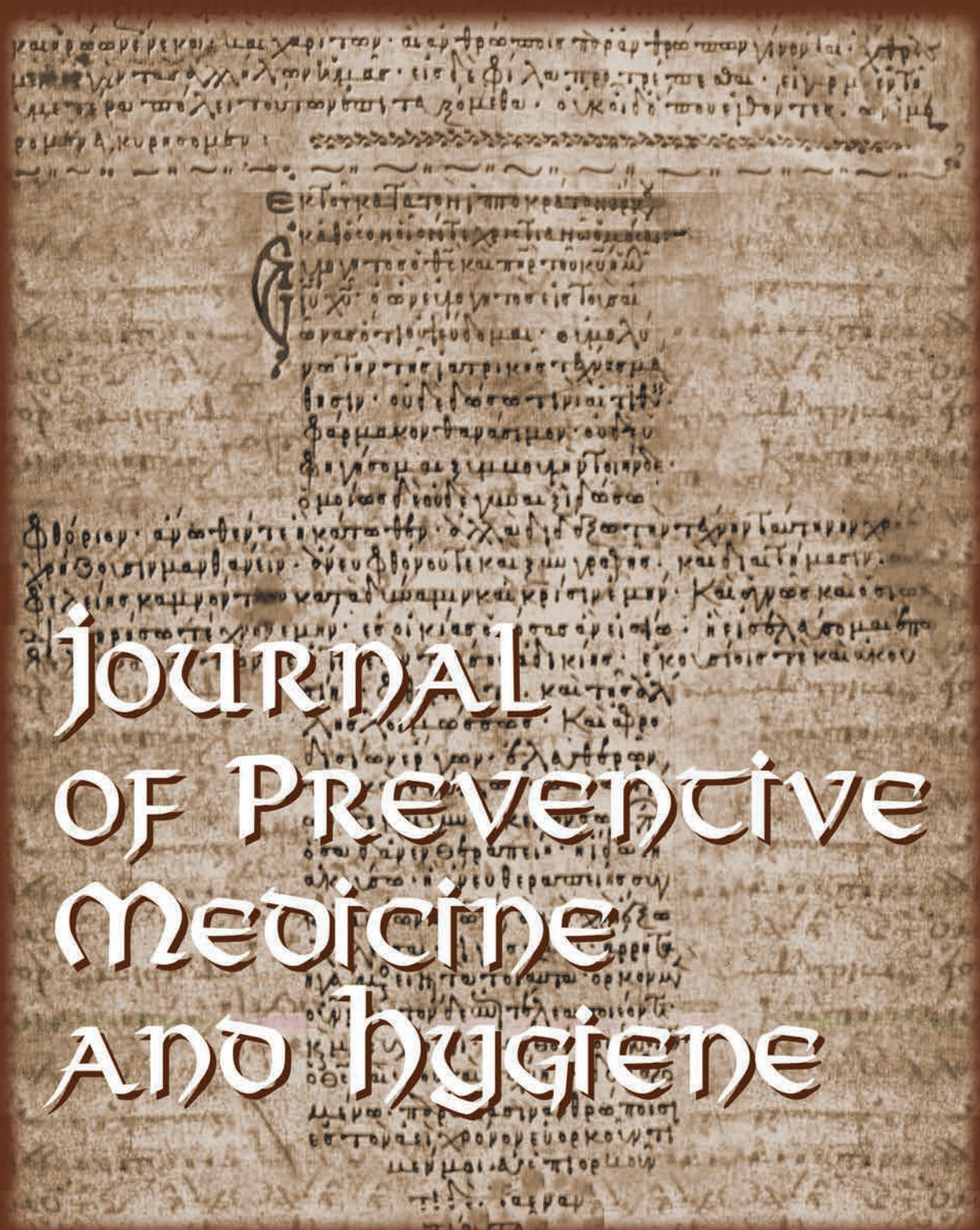
ISSN 2421-4248

vol. n.
57/4

Cited in Index Medicus / Medline
NLM ID 921440 (Pub-Med)

December
2016



# Journal of Preventive Medicine and Hygiene

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PACINI EDITORE MEDICINA

# JOURNAL OF PREVENTIVE MEDICINE AND HYGIENE

http://www.jpmh.org





Managing Editor: Patrizia Alma Pacini
Publisher: Pacini Editore Srl, Via Gherardesca 1, 56121 Pisa, Italy

Published online December 2016

Authorization Tribunal of Genoa, Italy n. 507 - 10/6/1960
Journal registered at "Registro pubblico degli Operatori della Comunicazione" (Pacini Editore srl registration n. 6269 - 29/8/2001).

CONTENTS

**Original articles**

Original article

# Estimation of mean number of daily hand hygiene procedures per patient can represent an effective and easy understandable method to evaluate adherence experience in a tertiary care pediatric hospital of Northern Italy

P. TATARELLI[1], I. LORENZI[2], I. CAVIGLIA[2], R.A. SACCO[2], D. LA MASA[2], E. CASTAGNOLA[2]
[1] Infectious Diseases Division, University of Genoa (DISSAL) and IRCCS San Martino-IST, Genoa, Italy; [2] Istituto Giannina Gaslini, Children's Hospital, Genoa, Italy

Keywords

Healthcare associated infections • Infection control • Hand hygiene • Hand washing

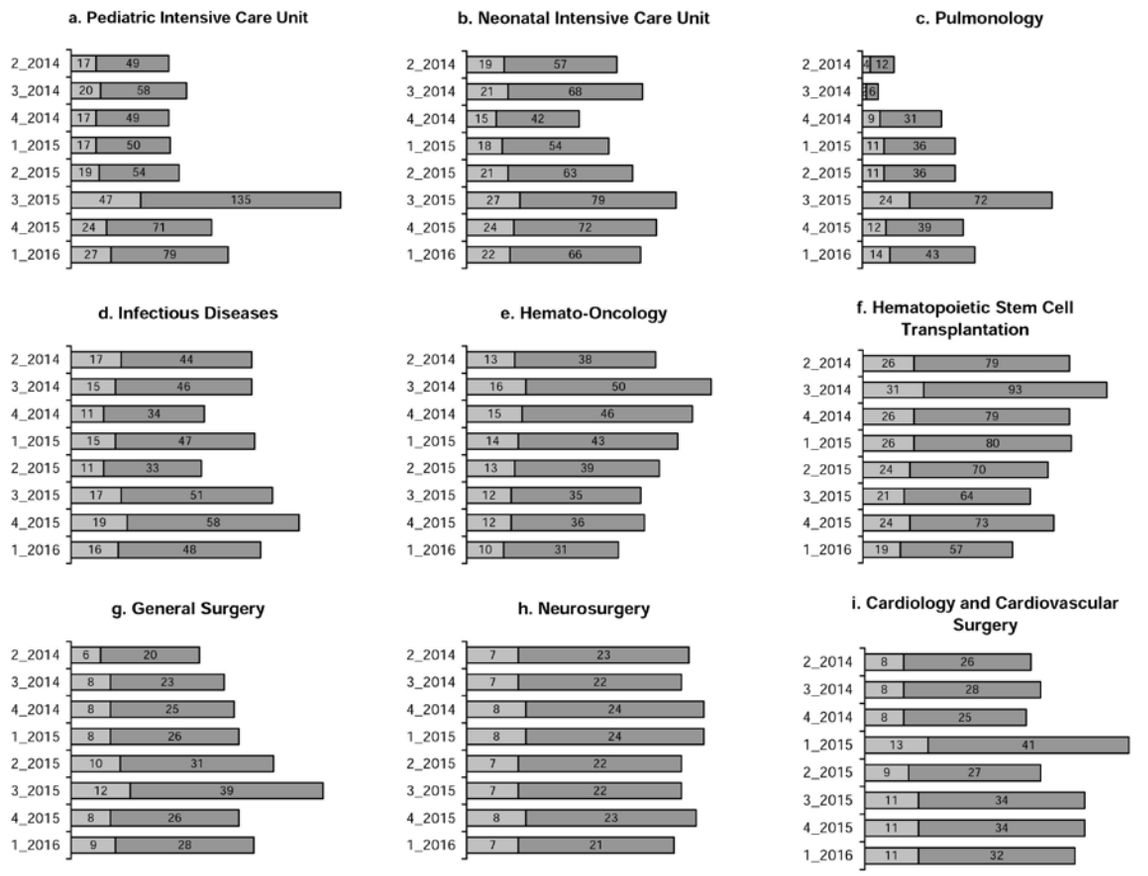
Summary

**Introduction**. *Hand decontamination with alcohol-based antiseptic agents is considered the best practise to reduce healthcare associated infections. We present a new method to monitor hand hygiene, introduced in a tertiary care pediatric hospital in Northern Italy, which estimates the mean number of daily hand decontamination procedures performed per patient.*
**Methods**. *The total amount of isopropyl alcohol and chlorhexidine solution supplied in a trimester to each hospital ward was put in relation with the number of hospitalization days, and expressed as litres/1000 hospitalization-days (World Health Organization standard method). Moreover, the ratio between the total volume of hand hygiene products supplied and the effective amount of hand disinfection product needed for a correct procedure was calculated. Then, this number was divided by 90 (days in a quarter) and then by the mean number of bed active in each day in a Unit, resulting in the mean estimated number of hand hygiene procedures per patient per day (new method).*
**Results**. *The two methods had similar performance for estimating the adherence to correct hand disinfection procedures. The new method identified wards and/or periods with high or low adherence to the procedure and indicated where to perform interventions and their effectiveness. The new method could result easy-to understand also for non-infection control experts.*
**Conclusions**. *This method can help non-infection control experts to understand adherence to correct hand-hygiene procedures and improve quality standards.*

## Introduction

Healthcare associated infections (HAI) represent an increasing problem in modern medicine. The impact of HAI includes prolonged hospital stay, long-term disability, increased resistance of microorganisms to antimicrobials, massive additional financial burden, high costs for patients and their families, and excess deaths [1-3]. In October 2005 the World Health Organization (WHO) started the "Clean Care is Safer Care" program to promote safe hand hygiene practices globally and at all levels of health care as a first step in ensuring high standards of infection control and patient safety [4]. The program provided technical recommendations and strategies to improve hand hygiene and included the development of WHO Guidelines on Hand Hygiene in Health Care along with a package of practical tools to facilitate implementation activities at a facility level [5]. WHO recommends hand rub with alcohol-based antiseptic agents as the gold standard procedure to protect patients from the multitude of harmful resistant and non-resistant organisms transmitted by health care workers' hands, and this easy and fast (20-30 seconds) procedure has been associated with reduction in nosocomial infections. Differently, standard hand washing with water and soap, is indicated only in case of visibly dirty hands or infection due to spore-forming pathogens [6, 7]. The monitoring of hand hygiene adherence is an integral part of infection control strategies. The Infection Control Team can use it to evaluate the effectiveness of specific interventions, as well as to introduce changes that minimize the risk of HAI. Several methods have been suggested to monitor hand hygiene compliance (direct observation, product use measurement, surveys, electronic systems), but the ideal one has not yet been identified [8, 9]. WHO considers direct observation the most effective method to monitor the health care workers adherence to the hand hygiene recommendations, meanwhile the alcohol-based antiseptic agents consumption is a proxy indicator of hand hygiene.
In 2006, Italy officially adopted the WHO program as a mainstay of its strategy for the promotion of hand hygiene in health care settings. "Istituto Giannina Gaslini" (IGG) Children Hospital, Genoa-Italy, a tertiary care

Fig. 1. Quarterly supply hand-hygiene products (litres/1000 hospitalization days, dark-gray bars) and estimates of hand-hygiene procedures (light-gray bars) and in different wards over 2 years of observation.




pediatric hospital in Northern Italy, complied with this program since 2007.

The aim of this study was to present a new method to estimate and report on adherence to correct hand hygiene procedures (CHHP)introduced in our hospital since 2007.

## Methods

Istituto Giannina Gaslini (IGG), Genoa-Italy is a tertiary care children's hospital in Northern Italy serving as local pediatric hospital for the Genoa area, but representing a tertiary care referring hospital for the whole Italy and many foreign countries.

In November 2007, IGG joined the "Clean Care is Safer Care" WHO program. This was firstly applied in the pediatric intensive care unit (PICU), and then gradually extended to all hospital wards. From January to March 2008, health care workers started a hand care hygiene educational program, conducted by the nurses of the IGG Infection Control Team. "How to" and "5 Moments" posters about hand washing were displayed close to the sinks. Beyond the standard antiseptic hand washing, hand decontamination with isopropyl alcohol gel was also recommended. Its use was implemented placing alcoholic gel dispenser at the ward entrance and next to each patient location. As the program began, random audits were performed to check the proper application of the procedures; re-training sessions and meetings with the Infection Control Team nurses were performed when needed. Retraining programs were performed in the following years.

Adherence to hand hygiene was initially estimated by means of the amount of antiseptic product supplied, expressed in terms of litres of isopropyl alcohol gel supplied to the Unit per 1000 hospitalization-days. However, since this approach could be hard to understand for non-infection control experts, in 2010 another hand hygiene reporting method was introduced, and associated to the first one. As the amount needed for an effective hand hygiene procedure is known for each product (manufacturer's instructions), we estimated the number of the performed procedures by dividing the total volume of isopropyl alcohol gel (hereinafter referred also as hand hygiene product) supplied in a period (a quarter) by the volume of the product indicated for an effective hand hygiene procedure. This number was then divided by 90 (days in a quarter) and then by the mean number of beds active in each day in the Unit in that period (average bed occupancy in a quarter). This calculation should estimate the number of daily CHHP procedures performed at each patient's bed. Data about alcoholic gel consump-

**Tab. I.** Changes in litres of hand hygiene products/1000 hospitalization days (l/1000 hd) and estimated hand hygiene (ehh) products in different quarters in different wards.

| | Quarter | 2_2014 | 3_2014 | 4_2014 | 1_2015 | 2_2015 | 3_2015 | 4_2016 | 1_2016 | Correlation coefficient |
|---|---|---|---|---|---|---|---|---|---|---|
| Pediatric intensive care unit | l/1000 hospitalization days | 48 | 58 | 49 | 50 | 54 | 135 | 71 | 79 | 0.9996 |
| | Estimated hand hygiene procedures | 17 | 20 | 17 | 17 | 19 | 47 | 24 | 27 | |
| Neonatal intensive care unit | l/1000 hospitalization days | 57 | 68 | 42 | 54 | 63 | 79 | 82 | 66 | 0.9528 |
| | Estimated hand hygiene procedures | 19 | 21 | 15 | 18 | 21 | 27 | 24 | 22 | |
| Pulmunology | l/1000 hospitalization days | 12 | 6 | 31 | 36 | 36 | 72 | 39 | 43 | 0.9966 |
| | Estimated hand hygiene procedures | 4 | 2 | 9 | 11 | 11 | 24 | 12 | 14 | |
| Infectious diseases | l/1000 hospitalization days | 44 | 46 | 34 | 47 | 33 | 51 | 58 | 48 | 0.9447 |
| | Estimated hand hygiene procedures | 17 | 15 | 11 | 15 | 11 | 17 | 19 | 16 | |
| Hemato-oncology | l/1000 hospitalization days | 38 | 50 | 46 | 43 | 39 | 35 | 36 | 31 | 0.9887 |
| | Estimated hand hygiene procedures | 13 | 16 | 15 | 14 | 13 | 12 | 12 | 10 | |
| Hemopoietic stem cell transplant | l/1000 hospitalization days | 79 | 93 | 79 | 80 | 70 | 64 | 73 | 57 | 0.9942 |
| | Estimated hand hygiene procedures | 26 | 31 | 26 | 26 | 31 | 39 | 26 | 28 | |
| General surgery | l/1000 hospitalization days | 20 | 23 | 25 | 26 | 31 | 39 | 26 | 28 | 0.9802 |
| | Estimated hand hygiene procedures | 6 | 8 | 8 | 8 | 10 | 12 | 8 | 9 | |
| Neurosurgery | l/1000 hospitalization days | 23 | 22 | 24 | 24 | 22 | 22 | 23 | 21 | 0.8133 |
| | Estimated hand hygiene procedures | 7 | 7 | 8 | 8 | 7 | 7 | 8 | 7 | |
| Cardiovascular surgery | l/1000 hospitalization days | 25 | 28 | 25 | 41 | 27 | 34 | 34 | 32 | 0.9663 |
| | Estimated hand hygiene procedures | 8 | 8 | 8 | 13 | 9 | 11 | 11 | 11 | |

tion were collected by each Unit head nurse, crossed with data from the Pharmacy service supply to any given Unit in a given period (a quarter), and communicated to the Infection Control Team. Since 2010 the program was gradually extended and in April 2014, after 4 years of progressive extension to different wards, the method was considered adequate to involve all IGG wards. From that moment the Infection Control Team drafted quarterly reports including both the amount of hand hygiene products used by each Unit and the estimated number of CHHP procedures performed. Moreover, the presence of epidemic clusters, defined as an aggregation of cases of infection, without regard to whether the number of cases was more than expected, was evaluated and reported in real time, and it was followed by specific retraining sessions [10].

Data were collected on an electronic spread sheet and graphically reported. In order to compare the 2 methods to report on CHHP we calculated the Pearson correlation coefficient that is a measure of the linear dependence between the 2 methods (litres/1000 hospitalization days and CHHP estimates). Graphics and correlation coefficient were obtained by means of Microsoft Office Excel 2007 (Microsoft Corporation Redmond WA).

## Results

Figure 1 presents the quarterly reports from April 2014 to March 2016 (eight trimesters) of nine selected Unites: Intensive Care Units [Pediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU)], Pulmonology, Infectious Diseases, Hemato-Oncology, Hematopoietic Stem Cell Transplantation, General Surgery, Neurosurgery, Cardiology and Cardiovascular Surgery. Table I reports data on adherence to the hand hygiene program estimated according to two different methods, together with the Pearson correlation coefficients between the 2 methods, and show the presence of a positive correlations between the 2 systems that described in

the same way changes in procedures for hand hygiene, while Figure 1 is the graphic representation of these results. For example, during the third trimester of 2015 in PICU, a cluster of *Acinetobacter baumannii* colonization/infection was followed by an intervention of the Infection Control Team and we observed an increase in hand hygiene procedures compared to the previous trimester, which is reported by both methods (47 CHHP vs. 19 and 135 litres of hand hygiene products *vs* 54, respectively) (Fig. 1a). During the third trimester of 2015 Pulmonology ward experienced a cluster of carbapenem resistant *Enterobacter* colonization, and after an intervention of the Infection Control Team we observed an increase of both parameters compared to the previous trimester (24 CHHP *vs* 11 and 72 litres of hand hygiene products *vs* 36, respectively). However, during the fourth trimester of 2015, when the Infection Control Team intervention became less pressing (and maybe less persuasive), a reduction in the number of hand hygiene procedures (from 24 to 12) was observed: this trend was considered worrisome and induced the Infection Control Team to retrain health workers. The result obtained in the following period (14 procedures per patient per day in the first trimester of 2016) was still considered not adequate, even if improving, so that a new educational intervention was programmed (Fig. 1c).
In general, in ICUs (Fig. 1a, Fig. 1b) the number of CHHP was generally larger compared to medical wards, like Pulmonology or Infectious Disease (Fig. 1c, Fig. 1d). On the other hand, in surgical wards (General Surgery, Neurosurgery, Cardiovascular Surgery) (Fig. 1g, Fig. 1h, Fig. 1i) the number of estimated procedures was quite similar, constant and relatively low, even if within the standard indicated by the WHO (20 liters/1000 hospitalization days), and in absence of any epidemic clusters or high levels of surgical site infections rate. In these wards, the decision was made to periodically retrain the health staff, though with quite frustrating results.

## Discussion

Hand hygiene is the most important precaution to reduce HAI and enhancing patient safety [11]. Thus, no effective infection control strategy can afford to neglect its monitoring. Unfortunately, all the methods currently used to monitor both standard hand washing and CHHP show some limits. For example, direct observation of staff members is currently considered the gold standard in hand hygiene compliance monitoring, but it is time-consuming and requires an adequate number of monitors to be performed frequently in a large number of hospital wards in a reasonably time. On the other hand, direct observation can influence and modify behaviour of health workers when realizing that they are being observed (so called Hawthorne effect), leading to falsely elevated compliance rates [12].
Starting from a conventional approach, based on measuring litres of isopropyl alcohol gel consumed per 1000 hospitalization-days, the IGG introduced a new method, still based on the amount of hand hygiene product, that estimates the mean number of daily CHHP performed per patient. Our estimate of CHHP can be performed using data that can be easily obtained by the hospital administration (litres of hand hygiene products, days of hospitalization, mean number of patients present in a trimester in a given ward). Furthermore, the method we adopted to estimate CHHP is unobtrusive and allows collecting and reporting real-life information without compartmental bias, even if it does not evaluate if the procedure of hand hygiene is performed in a correct way or not. As shown by this paper, these two methods provide overlapping results in terms of the description of hand hygiene performance over time. In fact there was a very strict positive correlation between these measure methods, with a coefficient $> 0.80$ in all cases (in 8/9 cases $> 0.94$), a value that can be considered very high in social science, where there may be a greater contribution from complicating factors [13]. However, the quality of information presented by the two methods is quite different. The new one provides the health staff with a (estimated) measure of its hand hygiene performance (estimated mean number of CHHP per patient per day), while the other one refers to the amount of hand hygiene products consumed in a Unit during a certain period (litres of product per 1000 hospitalization-days). This difference can prove relevant for non-infection control experts. Indeed, it is easier to understand one's own hygiene conduct by thinking in terms of number of hand hygiene procedures than in terms of gel consumption. This aspect can be crucial for health care workers training. Figure 1 is a realistic picture of the mean daily number of CHHP performed per patient per day in each Unit. Noteworthy these numbers can fluctuate, sometimes widely, from one trimester to another within the same ward. Therefore, the main helpfulness of this analysis is to compare each Unit against itself over time. However, some observations arise from the comparison among the hand hygiene trend of different Units. For example, the higher number of procedures found in intensive care Units compared to medical wards is probably due to their different case mix. Indeed, intensive care Units usually host critical patients, who require constant assistance and frequent invasive procedures and, therefore, a higher number of CHHP procedures is expected. On the other hand, the low number of procedures observed in surgical wards, even in absence of epidemic clusters or high levels of surgical site infections, can be at least partially explained by the fact that surgical patients undergoing more complex interventions are usually admitted to intensive care Units during the first postoperative phases, which are the most critical periods, while they are referred to surgical wards when a lower level of assistance, and therefore of hand hygiene procedures, is needed. If we consider that the risk of carbapenem resistant *Enterobacteriaceae* transmission can be significantly reduced by performing at least 50% of the required hand washings, it is at least partially understandable that surgical wards could need a relatively low number of procedures to prevent pathogens' transmission, provided that the volumes of products are adequate, at least according to WHO standards [14]. These considerations well applies to our pediatric reality and its type of patients and surgi-

cal procedures, but they cannot be simply transposed to adult or pediatric wards/hospitals with a different case mix. For this reason, we believe that our method of estimating CHHP can be used in each setting to compare its own performance in process of time, but can be roughly used to compare different realities in different hospitals or even in the same one. Further studies in different clinical settings are needed to validate our approach.
The proposed method has also limitations. First, it estimates the adherence to CHHP but does not provide the real number of performed procedures. Second, since it does not include direct observation, it does not allow fixing errors in real time, but only a "post-hoc" retraining. Third, it may underestimate adherence to hand hygiene because it does not consider standard handwashing, which is an effective procedure actually used by many health-care workers (since it is perceived as more secure), even if not the currently recommended standard.

## Conclusions

In conclusion, our experience provides a new, reliable method for measuring hand hygiene adherence that non-infection control experts may find easier to understand, compared to conventional ones. This is a key issue, since no satisfactory infection control can be obtained without the health-care workers collaboration and adherence to hygiene interventions.

## Acknowledgments

The authors declare no competing interest.

## Authors' contributions

P.T. performed data analysis and manuscript preparation.
I.L. carried out data collection and analysis.
I.C. carried out data collection and analysis.
R.A.S. performed data collection and review.
D.L.M. carried out data collection and review.
E.C. performed study design, data analysis and manuscript preparation.

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■ Received on July 13, 2016. Accepted on September 29, 2016.

■ Correspondence: Elio Castagnola, Infectious Diseases Unit, Istituto Giannina Gaslini, Largo G. Gaslini 5, 16147 Genoa, Italy - Tel. +39 010 56362428 - Fax +39 010 3763436 - E-mail: eliocastagnola@gaslini.org

ORIGINAL ARTICLE

# Caregivers' vector control methods and their impact on malaria outcome in under-five presenting in tertiary health institution in Nigeria

D.U. NWANERI[1], O.A. OLADIPO[2], A.E. SADOH[1], M.O. IBADIN[1]
[1] Department of Child Health, University of Benin Teaching Hospital, Benin City, Nigeria; [2] Department of Child Health, University of Benin, Benin City, Nigeria

Keywords

Bed-nets • Insecticide-treated • Malaria • Mortality • Strategy • Vector

Summary

**Background and aims**. *Integrated vector control especially use of insecticide-treated bed nets have been reported as effective malaria preventive strategies. This study aimed at documenting factors that influence regular use of insecticide-treated nets in under-fives and impact of vector control methods on malaria outcome (severe malaria prevalence and mortality) in under-fives presenting in a tertiary health institution in Nigeria.*
**Methods**. *Cross-sectional study carried out from June 2012 and July 2013. Data was obtained by researcher-administered questionnaire and malaria was confirmed in each child by microscopy.*
**Results**. *329 caregiver (31.2 ± 6.0 years) /child (20.7 ± 14.0 months) pair were recruited. Netting of doors/windows (80.0%) was the most practiced vector control method. 177 (53.8%) caregivers possessed insecticide-treated bed nets, and only a quarter of their under-5s regularly sleep in these nets. Children from lower social class statistically significantly sleep in the nets (p = 0.03), however, presence of 2 or more nets in a household independently predicted its regular use for the under-5s (β = 1.09, OR = 3, p = 0.02). Prevalence of severe malaria was 36.2% and mortality was 52 per 1000. Combination of regular use of insecticide treated nets, environmental sanitation, indoor insecticide spray and netting of household doors/windows significantly predicted low prevalence of severe malaria compared to each of the malaria vector control methods used singly by the caregivers (β = 1.66, OR =5.0, p = 0.04).*
**Conclusions**. *Integrated vector control remains the most effective method of malaria vector control at the community.*

## Introduction

In malaria endemic areas, factors such as poverty and poor environmental sanitation allow survival and proliferation of malaria vector [1, 2]. The emergence and rapid spread of resistance both of the malaria vector (female anopheles mosquito) to insecticides and of the pathogenic plasmodia to antimalarial drugs are the major causes of increased malaria disease morbidity and mortality. The malaria vectors predominantly found in the Northern and Southern regions of Nigeria include *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles funestus*, and *Anopheles melas* [3].
Malaria is responsible for nearly half a million deaths worldwide annually [1]. With a view to reducing the malaria burden, the World Health Organization (WHO)/ Global Malaria Programme (GMP) promotes personal and communal control measures against the malaria vector known as the Integrated Vector Control (IVC). IVC comprises the use of insecticide treated nets (ITN), and other control measures such as netting of house doors/ windows, regular environmental sanitation (clearing of bushes and drains/ gutters around the house) to eliminate and reduce the burden of malaria vector [1, 2, 4].

In Nigeria, the most common methods of malaria vector control include the use of window/door mosquito screens/netting, clearing of bushes/drains, mosquito repellants/insecticides and insecticide treated-bed nets (ITN) [5, 6]. In the last decade, the ITN has been observed to be a veritable tool of malaria vector control worldwide. However, its availability and regular usage in households have been major factors against the effectiveness of this malaria vector control strategy [7]. The Nigeria Malaria Indicator Survey (MIS) report of 2010 showed an overall ITN ownership of 42.0% (75.0% ownership in areas with recent mass ITN distribution campaign) and usage of 23.0% (41.0% utilization in areas with recent mass ITN distribution campaign) [7]. Since then, there has been increased mass campaigns to improve household ownership and utilization of ITN in most countries in Africa including Nigeria [7-10].
The use of ITN has been observed to reduce malaria morbidity and mortality, however, reports from some authors in Africa showed varying results. For example Afoakwah et al. [11] observed a reduction in under-five mortality from malaria in Northern Ghana, while Loha et al. [12] did not find any influence of free mass distribution of ITNs on malaria morbidity in South Ethiopia.

Although these studies assessed only ITN usage against malaria morbidity and mortality, the differences in the results have been criticized on the basis of the methods applied in establishing the association between ITN usage and the outcomes. For example, sleeping under the ITN the night before the survey was regarded as usage. This may not be an objective assessment as individual who possessed the ITN without sleeping in it until the night before a survey will be erroneously regarded as an ITN user. Again none of these studies assessed the impact of other vector control methods on malaria outcome in children.

To improve on the ITN utilization and IVC strategy in the communities, the National Malaria Elimination Programme (NMEP) in the last 5 years preceeding this study had instituted a scaled-up awareness programme on IVC strategy at all levels [13]. To provide insight into the progress made so far on malaria vector control programme especially with regards to ITN ownership and usage, this study aimed at documenting the malaria vector control practices of caregivers, ITN usage by under-fives and factors that influence the use of ITN by under-fives in households. Due to paucity of studies on the impact of vector control on malaria health indices of under-fives in the study locale, this study evaluated the impact of vector control methods by caregivers on malaria health indices (severe malaria prevalence and mortality) of their under-fives presenting in a tertiary health institution in Nigeria.

## Study hypothesis

### Null hypothesis

The IVC does not have favourable impact on malaria health indices (severe malaria morbidity and mortality) in under-fives.

### Alternate hypothesis

The IVC have favourable impact on malaria health indices (severe malaria morbidity and mortality) in under-fives.

## Study participants and methods

This study was carried out in Benin City, Edo State Nigeria. The State lies within the South-south region of Nigeria and the topography is that of tropical rain forest where malaria transmission is holoendemic and stable throughout the year [1-3]. The vegetation is mainly rain forest with some regions of creeks and swamp which support the breeding of the malaria vector (anopheles mosquito) especially *Anopheles gambiae* and *Anopheles arabiensis* [3]. It is a cosmopolitan City where most inhabitants are civil servants, traders, artisans and farmers. This was a cross-sectional descriptive study carried out from June 2012 to July 2013.

The study participants included caregivers and their apparently well-nourished children (6-59 months) who presented with malaria in the index tertiary health institution. Malaria was confirmed in each child by microscopy following standard protocols [14]. Each caregiver/child pair were recruited consecutively in the study.

Children excluded from the study were children who had clinical and/or laboratory evidence(s) of localized infection such as acute tonsillitis, otitis media, pneumonia, and urinary tract infection. These diseases are common cause of fever in children. Children who had malnutrition were also excluded from the study. The Z-scores for weight-for-age (WFA) were calculated for each child using the revised WHO growth charts from the Centre for Disease Control (CDC) as reference [15]. Children with WFA Z-score of minus 2 standard deviation of the reference median value were regarded as acute under-weight malnutrition. Malnutrition is associated with morbidities such as diarrhoea, pneumonia, urinary tract infection and sepsis; and has an increased mortality [16].

### Data collection and sampling technique

Data was collected by a researcher-administered semi-structured questionnaire. The tool was validated by extensive literature review and was pre-tested on 20 caregiver/child pairs who were excluded from the final analysis. The questionnaire sought information on the study participants' demographic features, their knowledge and attitudes on malaria vector control practices. Children with clinical features in keeping with WHO case definition of severe malaria were classified as severe malaria while children without such features were classified as uncomplicated malaria [1]. Children with severe malaria were admitted and treated according to the national guideline for management of severe malaria while those with uncomplicated malaria received full course of artemisinin-based combination therapy (ACT). The family social class was determined as described by Olusanya et al. using mother's level of education and the father's occupation. In this method of classification of social class, information on child's mother's level of education and father's occupation is required. This is obtained by history from the child's informant. Specific score is then allotted to the father's occupations as follows: 1, 2, or 3; and mother's educational qualifications – 0, 1 or 2 as shown below. The sum of these scores i.e from father's occupation and mother's educational qualification scores describes the family social class as High –1, High Intermediate – 2, Middle – 3, Low Intermediate – 4, and Low – 5. This is then further interpreted as follows: Upper social class for scores 1 and 2, Middle social class for score 3 and Lower social class for scores 4 and 5. Households were categorized as small if they contained ≤ 5 individuals and large if they contained ≥ 6 individuals [18].

### Research ethics

A written informed consent was obtained from the caregivers of all the children. Ethical certificate for this study was obtained from the Research and Ethics Com-

mittee of University of Benin Teaching Hospital, Benin City, Nigeria; protocol number ADM/E 22/A/ Vol.VII/ 741.

### Data handling and analysis

The data obtained in this study was analysed using the statistical package for social sciences (SPSS) version 16.0 (Chicago, Illinois, USA). Further analysis was by GraphPad InStat Software (GraphPad Software Inc, San Digeo 92130, USA) where applicable.

Malaria health indices sought for in this study were malaria morbidity at presentation (severe malaria prevalence) and mortality during the acute phase of the illness (within 72 hours of presentation). Such associations as the relationship between regular use of ITN and socio-demographic characteristics of the study participants as well as malaria vector control practices of caregivers and malaria health indices (severe malaria prevalence and mortality) of their under-fives were analyzed using Chi-square and Fisher's Exact Test where applicable. Regular use of ITN for the under-fives in this study was defined as sleeping in ITN every night for 6 weeks preceeding presentation in the health facility and not just necessarily sleeping in the ITN a night before the survey. Six weeks is the longest expected intrinsic incubation period of *Plasmodium falciparum* which is the commonest malaria parasite in the study locale [1, 2, 3] Sleeping in the ITN a night before the survey as it is conventionally used in some other studies [19] as demonstration of ITN usage was not employed in this study because such would not give objective assessment of impact of ITN usage on malaria health indices (severe malaria and mortality). All children were recruited in the study the same day of presentation at the health facility. Associations with p-values ≤ 0.05 were further analyzed with Binary Logistic Regression Model to identify factors that independently influenced the outcome variables – severe malaria and mortality. In order to ensure correct specification of the model and how well the model fit the data available (reliability of the model), goodness-of-fit test was performed which showed that N = 2313, correlation coefficient (R) = -0.08, 95% CI = -0.12, -0.04 and $R^2$ (the square of the explained sum of square of test) = 0.006; $p < 0.0001$(severe malaria) and p = 0.000 (mortality) respectively. This showed that the model has good fitness and reliable in predicting the desired outcomes i.e that the logit model has no omitted variables in failing to accept the null hypothesis. The level of significance of each test was set at $p < 0.05$.

## Results

Three hundred and twenty nine children/caregiver pairs were recruited for the study. Age (mean ± SD) of the children was 20.7 ± 14.0 months and that of their caregivers was 31.2 ± 6.0 years. Among the 329 children, 191/329 (58.1%) were males and 138/329 (41.9%) females. Majority of the caregivers had secondary 128/329 (38.9%) and tertiary 121/329 (36.8%) education; 111/329 (33.7%) belonged to the upper social class, 129/329 (39.2%) to the middle and 89/329 (27.1%) to the lower social class as shown in Table I.

Tab. I. Socio-demographic characteristics of the study participants.

| Socio-demographic characteristics | N = 329 (%) |
|---|---|
| **CHILDREN** | |
| *Gender* | |
| Male | 191 (58.1) |
| Female | 138 (41.9) |
| *Age group (months)* | |
| < 12 | 108 (32.8) |
| 12 – 23 | 108 (32.8) |
| 24 – 35 | 50 (15.2) |
| 36 – 47 | 31 (9.4) |
| 48 – 59 | 32 (9.7) |
| *Family social class* | |
| Upper | 111 (33.7) |
| Middle | 129 (39.2) |
| Lower | 89 (27.1) |
| *Household size* | |
| Small (≤ 5) | 314 (95.4) |
| Large (≥ 6) | 15 (4.6) |
| **CAREGIVER** | |
| *Type of caregiver* | |
| Mother | 322 (97.9) |
| Grand-mother | 5 (1.5) |
| Father | 2 (0.6) |
| *Age group of caregivers (years)* | |
| 16 – 25 | 44 (13.4) |
| 26 – 35 | 220 (66.9) |
| 36 – 45 | 60 (18.2) |
| > 45 | 5 (1.5) |
| *Level of education of caregivers/ mothers* | |
| Tertiary | 121 (36.8) |
| Secondary | 128 (38.9) |
| Primary | 65 (19.7) |
| No formal | 15 (4.6) |

Three hundred and eighteen (96.7%) caregivers mentioned that malaria is caused by mosquitoes and 312/329 (94.8%) caregivers stated that the disease is preventable. The most common malaria vector control method mentioned by the caregivers was the use of ITN by 92.0%, however, only about a quarter of these caregivers regularly used the ITN for their under-fives. Other methods of malaria vector control mentioned and practiced by the caregivers is shown in Figure 1.

Concerning ITN ownership, 177 (53.8%) of the 329 caregivers possessed at least one ITN in their homes while 152 (46.2%) did not have any ITN. Figure 2 shows the sources of ITN by the caregivers. Most of the ITNs (52.5%) were obtained during the ITN campaign programme in the State and 18.1% purchased their ITNs. The mean cost of one ITN was One Thousand, Five Hundred and thirty, 95% CI (1242.8, 1817.2) Naira (equivalent to USD 10.00).

Forty-three (24.3%) of the 177 ITN owners stated that their children regularly sleep in it, while 134 (75.7%)

Fig. 1. Malaria vector control methods mentioned and practiced by the caregivers.

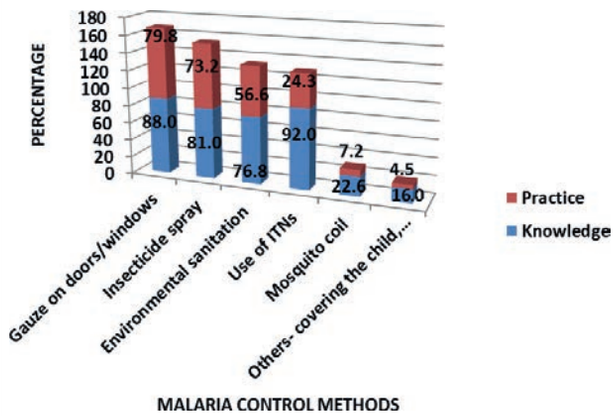



Fig. 2. Sources of insecticide treated nets.

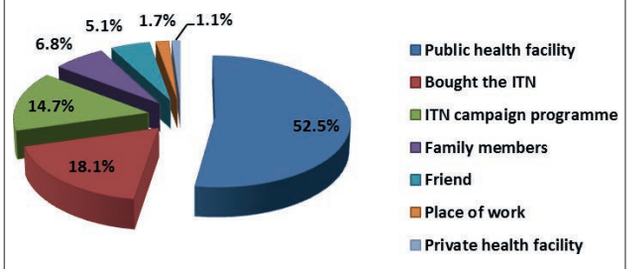
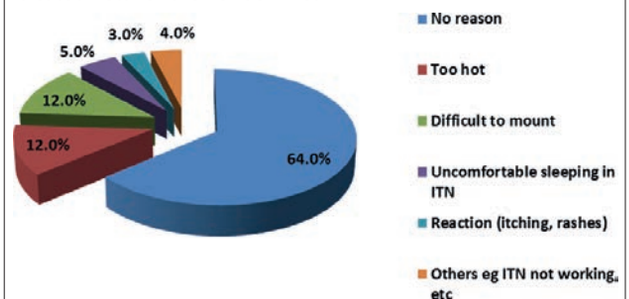



Fig. 3. Reasons given by caregivers for not using the insecticide treated nets for their under-fives.




did not regularly use the ITN. The mean duration of use of ITN by the children from time of acquisition of the ITN to the time of this study was 13.5, (95% CI 11.7-15.3) months. Of those who did not regularly use the ITN, 86/134 (64.0%) gave no reasons for not using ITNs while 16 (12.0%), said that it was too hot to sleep in. Reasons for not sleeping in ITN regularly are shown in Figure 3.

Tab. II. Factors associated with regular use of insecticide treated nets for the children by 177 ITN owners.

| Regular use of insecticide treated nets | | | | | |
|---|---|---|---|---|---|
| Socio-demographic factors | Yes n = 43 (%) | No n = 134 (%) | $\chi^2$ | df | p |
| *Age Group of children (Months)* | | | | | |
| < 12 | 15 (34.9) | 46 (34.3) | | | |
| 12 – 23 | 10 (23.3) | 44 (32.8) | | | |
| 24 – 35 | 9 (20.9) | 19 (14.2) | 4.68 | 4 | 0.32 |
| 36 – 47 | 6 (14.0) | 9 (6.7) | | | |
| 48 – 59 | 3 (6.9) | 16 (12.0) | | | |
| *Social class* | | | | | |
| Upper | 13 (30.2) | 58 (43.3) | | | |
| Middle | 14 (32.6) | 52 (38.8) | 7.09 | 2 | 0.03 |
| Lower | 16 (37.2) | 24 (17.9) | | | |
| *Household size* | | | | | |
| Small | 40 (93.0) | 130 (97.0) | | | |
| Large | 3 (7.0) | 4 (3.0) | * | ** | 0.36 |
| *Number of ITN in household* | | | | | |
| One | 13 (30.2) | 67 (50.0) | | | |
| Two or more | 30 (69.8) | 67 (50.0) | 4.37 | ** | 0.04 |
| *Age group of the caregivers (Years)* | | | | | |
| 16 – 25 | 8 (18.6) | 16 (12.0) | | | |
| 26 – 35 | 24 (55.8) | 94 (70.1) | | | |
| 36 – 45 | 11 (25.6) | 22 (16.4) | 4.18 | 3 | 0.24 |
| > 45 | 0 (0.0) | 2 (1.5) | | | |
| *Level of education* | | | | | |
| Tertiary | 3 (7.0) | 7 (5.2) | | | |
| Secondary | 12 (27.9) | 16 (11.9) | 7.72 | 3 | 0.05 |
| Primary | 15 (34.9) | 47 (35.1) | | | |
| No formal | 13 (30.2) | 64 (47.8) | | | |

Fisher's Exact Test*, ** odds ratio = 0.4, ITN = insecticide treated nets

**Tab. III.** Malaria prevention methods used by the 329 caregivers and their association with study outcomes.

| Prevention Methods | Severe malaria | | Mortality | |
|---|---|---|---|---|
| | Yes (%) | No (%) | Yes (%) | No (%) |
| Insecticide spray | | | | |
| Yes (n = 242) | 79 (32.6) | 163 (67.4) | 9 (3.7) | 233 (96.3) |
| No (n = 87) | 40 (46.0) | 47 (54.0) | 8 (9.2) | 79 (90.2) |
| | $\chi^2$ = 4.93, OR = 0.6, p = 0.03 | | $\chi^2$ = 3.92, OR = 0.4, p = 0.048 | |
| Nets on doors/windows | | | | |
| Yes (n = 263) | 88 (33.5) | 175 (66.5) | 10 (3.8) | 253 (96.2) |
| No (n = 66) | 31 (47.0) | 35 (53.0) | 7 (10.6) | 59 (89.4) |
| | $\chi^2$ = 4.17, OR = 0.6, p = 0.04 | | $\chi^2$ = 5.00, OR = 0.3, p = 0.03 | |
| Environmental sanitation | | | | |
| Yes (n = 188) | 57 (30.3) | 131 (69.7) | 5 (2.7) | 183 (97.3) |
| No (n = 141) | 62 (44.0) | 79 (56.0) | 12 (8.5) | 129 (91.5) |
| | $\chi^2$ = 6.51, OR = 0.6, p = 0.01 | | Fisher's Exact: OR = 0.3, p = 0.02 | |
| Mosquito coil | | | | |
| Yes (n = 24) | 12 (50.0) | 12 (50.0) | 1 (4.2) | 23 (95.8) |
| No (n = 305) | 107 (35.1) | 198 (64.9) | 16 (5.2) | 289 (94.8) |
| | $\chi^2$ = 2.15, OR = 1.8, p = 0.14 | | Fisher's Exact: OR = 0.8, p = 1.00 | |
| Regular use of ITNs | | | | |
| Yes (n = 43) | 20 (46.5) | 23 (53.5) | 0 (0.0) | 43 (100.0) |
| No (n = 134) | 41 (30.6) | 93 (69.4) | 17 (12.7) | 117 (87.3) |
| | $\chi^2$ = 3.65, OR = 2.0, p = 0.06 | | Fisher's Exact: OR = 0.1, p = 0.01 | |

Table II shows factors associated with ITN regular use for the children by 177 caregivers that owned the ITNs. Significantly more caregivers from the lower social class regularly used ITN when compared with caregivers from the middle and upper social classes ($\chi^2$ = 7.09, df = 2, p = 0.03). Also households that owned two or more ITNs were statistically significantly more likely to use ITN regularly when compared with households that owned only one ITN ($\chi^2$ = 9.61, df = 2, p = 0.01).

Prevalence of severe malaria in this study was 36.2%. Of the 329 children, 17 (5.2%) children died; a mortality rate of 52 per 1000.

Table III shows the relationship between malaria vector control methods utilized by the 329 caregivers and the study outcomes. Significantly more children whose caregivers did not use in-door insecticide spray (46.0%) ($\chi^2$ = 4.93, p = 0.03), nor had nets on the doors/windows of their houses (47.0%) ($\chi^2$ = 4.17, p = 0.04) and, who did not practice regular environmental sanitation (44.0%) ($\chi^2$ = 6.51, p = 0.01) presented with severe malaria when compared to children whose caregivers practiced these control methods. Mortality was significantly lower in children who used ITN regularly (p = 0.01) as well as in children whose caregivers used indoor insecticide spray ($\chi^2$ = 3.92, p = 0.048), used nets on the doors/windows of their houses ($\chi^2$ = 5.00, p = 0.03), and whose caregivers practiced regular environmental sanitation (p = 0.02)]. The duration of use of ITN was not significantly associated with severe malaria ($\chi^2$ = 5.64, p = 0.65, 95%CL = 0.58, 0.72), and, all the children that died had never slept in ITN.

The Logistic Regression Model using the combined malaria vector control practices of caregivers as dependent variables and the study outcomes (severe malaria and mortality) as independent is shown in Table IV. The model showed that sleeping in ITN regularly, use of

**Tab. IV.** The final Logistic regression model of malaria vector control methods utilized by the 329 caregivers and their predictor on the study outcomes (adjusting for demographic factors).

| Prevention methods | Severe malaria | Mortality |
|---|---|---|
| | β (OR) p-value | β (OR) p-value |
| ITN (n = 43) | 0.20 (1.2) 0.560 | 18.50 (1.1) 1.00 |
| IS (n = 242) | 0.51 (1.7) 0.30 | 1.51 (4.5) 0.18 |
| NDW (n = 263) | 0.43 (1.5) 0.34 | 1.06 (2.9) 0.23 |
| RES (n = 188) | -0.41 (0.7) 0.29 | 18.88 (1.6) 1.00 |
| ITN+IS+NDW+RES (n = 9) | 1.66 (5.2) 0.04 | *-0.65 (0.5) 1.00 |
| IS+NDW+RES (n = 141) | -0.19 (0.8) 0.69 | -18.58 (0.0) 1.00 |
| IS+NDW (n = 212) | -1.24 (0.3) 0.05 | -0.81 (0.4) 0.58 |

p < 0.05*, OR = odds ratio, β = measure of how strongly each predictor variable influences the outcome variables. ITN – Regular use of ITN, IS – Use of insecticide spray, NDW – Netting of doors/windows and RES – Regular environmental sanitation (clearing bushes and drainages around the house); Constant for the model was -0.28 (0.8) 0.41 for severe malaria and 1.15 (3.1) 0.01 for mortality.

insecticide spray, regular environmental sanitation and netting of doors/windows used in combination by caregivers significantly predicted low prevalence of severe malaria.

## Discussion

The majority of the caregivers in this study were aware that malaria is preventable. Most of the malaria vector control methods mentioned and practiced by the caregivers was similar to previous documentation by some authors in Nigeria [5, 6]. These included netting of house doors and windows, use of insecticide sprays and environmental sanitation. Knowledge of ITN as a malaria vector control method was high (92.0%). The high knowledge of malaria vector control methods especially of the use of ITN could be attributed to the intensified ITN campaign programme by the NMEP at the Local, State and Federal levels in Nigeria [7, 10]. In 5 years preceeding this study, over 10 million ITN including the Long Lasting Insecticide-treated Nets (LLIN) had been distributed to different households in Nigeria through the house-to-house distribution campaign and the various antenatal/ immunization clinics in the communities [7, 10].

Despite this positive finding on knowledge of malaria vector control methods, there is still a huge gap between knowledge and practice of these methods especially as regards to the use of ITN. In this present study, there was a gap of over 50.0% between ITN ownership (53.8%) and regular usage of 24.3% in under-5s. Hot weather condition was a major factor against regular use of ITNs. Nigeria is a tropical country and the environmental temperature in the study locale usually ranged between 28$^{o}$C and 38$^{o}$C [3, 18]. Such hot weather could deter many households from sleeping in the ITN. This is compounded by lack of basic amenities such as electric power and good housing [18]. Nigeria is currently characterized by erratic power supply and poor housing. Sleeping in ITN is usually uncomfortable in hot weather especially in absence of fans and air-conditioners as well as in overcrowded houses with its antecedent poor ventilation.

The number of ITN available in each household has been found to correlate positively with its use in under-5s [20, 21]. Although effort has been made by the NMEP and other Malaria Control Partners to improve on ITN coverage within the communities, but two ITNs given to each household during the distribution campaign is grossly inadequate[7, 10, 19]. The current Malaria Indicator Survey (MIS) Report still stated that the average number of LLIN per household in Nigeria was 1.6 [19]. This value grossly falls short of the ITN universal distribution goal of at least one net for every two persons [21]. Households that wished to purchase their own LLIN were unable to afford it due to financial constraints. This is because the mean cost of one ITN observed in this study was USD 10.00 which was far beyond the reach of many of the study participants. Most of the study participants were from the lower social class; and these groups also had the lowest ITN ownership rate when compared with those from middle and upper classes. These also were unlikely to access health faciies where they could obtain the LLIN during antenatal and immunization clinics. Therefore, for effective malaria control, universal coverage of ITN and intensified education on its usage for all individuals at risk of malaria in endemic regions remains the goal.

Extrinsic incubation period i.e parasite incubation period in the vector mosquito is temperature dependent.[3] The higher the environmental temperature the shorter the extrinsic incubation period. It has been described in literature that *P. falciparum* takes 8-11 days to complete the mosquito phase at an optimal ambient temperature of 28°C and 22 days at 20°C. It suffices to say that the extrinsic incubation period of *P. falciparum* may be shorter at the environmental temperature of the study locale which ranged between 28.0$^{o}$ C and 38.0$^{o}$C. This assertion coupled with poor environmental sanitation and poverty enhance mosquito breeding sites, allow survival and proliferation of malaria vector and malaria parasite [1, 3, 4]. The implication of these include increased malaria morbidity and antecedent mortality. Most children who presented with severe malaria as well as those who died from the disease in this study were from the lower social class. Despite the beneficial effect of ITN as a veritable tool for malaria vector control, a multi-prong approach to addressing other factors such as basic amenities, environmental sanitation and poverty should be employed in process of eliminating malaria in the country.

The IVC/M advocated by the WHO includes environmental management strategies in view of eliminating mosquito breeding sites (regular environmental sanitation), use of chemicals such as use of in-door insecticide spray, physical barrier methods such as netting of doors/ windows in houses and regular use of ITN [1, 3, 4]. These combined malaria vector control methods significantly predicted low incidence of severe malaria when compared with single vector control methods. Although there is paucity of studies on relationship between IVC/M and malaria outcomes (morbidity and mortality), the observation in this study that combined malaria vector control predicted low incidence of severe malaria is in keeping with the WHO assertion that IVC/M truly reduces malaria outcomes (morbidity and mortality).

### Limitations of study

Physical verification of ITN ownership and usage was not carried out. This could limit the strength of inferences drawn from the study.

## Conclusions and recommendation

Although, most caregivers had good knowledge of malaria vector control, their proportion that possessed and used ITN for malaria control was still low. IVC/M is a veritable tool for malaria vector control, there should be intensified advocacy for scaling-up of ITN distribution and education on utilization of ITN and these integrated malaria vector control methods.

## Acknowledgments

The authors thank Miss RoseMary Okonkwo who assisted in data collection, Mr Osas the microscopist, and Mrs Adaeze Nwaneri who edited the manuscript. The authors also thank the nursing staff and resident doctors in children emergency room University of Benin Teaching Hospital Benin City, Nigeria for providing the enabling environment for this study. The authors declare no competing interest.

## Authors' contributions

The concept and design of the study was by D.U.N. and M.O.I. The coordination and supervision of data collection and analysis were performed by D.U.N. and O.O.O. Drafting of the initial manuscript was by D U.N..
A.E.S. and M.O.I. designed the data collection instruments and critically reviewed the manuscript for intellectual content. All the authors approved the final manuscript as submitted.

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■ Received on July 11, 2016. Accepted on November 7, 2016.

■ Correspondence: D.U. Nwaneri, Department of Child Health, University of Benin, Teaching Hospital, PMB 1111, Benin City, Edo State, Nigeria - Tel. +2348056321577 +23480139172309 - E-mail: uchechukwu.nwaneri@uniben.edu

ORIGINAL ARTICLE

# Prevalence of high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* in an Iranian hospital

M. EMANEINI[1 2], B. KHORAMIAN[3], F. JABALAMELI[2], R. BEIGVERDI[2], K. ASADOLLAHI[4], M. TAHERIKALANI[5], A.R. LARI[1]
[1] Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran, Iran; [2] Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; [3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. Mashhad, Iran; [4] Department of Social Medicine, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran; [5] Razi Herbal Medicines Researches Center, Department of Microbiology, School of Medicine, Lorestan University of Medical Sciences, Khoramabad, Iran

Keywords

Enterococcus • HLGR • RAPD-PCR

Summary

*This study was designed to determine the molecular characteristics and antimicrobial resistance of enterococcal strains isolated from patients admitted to an Iranian Hospital. Enterococcal strains were isolated from the burn patients. All strains were screened for genes encoding resistance to aminoglycoside [aac(6')-Ie-aph(2")-Ia, aph (3'), ant (4')], resistance to vancomycin (vanA, vanB), resistance to tetracycline (tetK, tetL, tetM, tetO), and resistance to erythromycin (ermA, ermB, ermC) by PCR and multiplex PCR-based methods. Genetic diversity was evaluated via Random Amplified Polymorphic DNA (RAPD)-PCR. All enterococcal isolates showed complete sensitivity to vancomycin with MIC ≤ 0.5µg/ml. Resistance to gentamicin, tetracycline, erythromycin, ciprofloxacin or quinopristin-dalfopristin was detected, whilst more than 96.2% of isolates were high-level gentamicin-resistant (HLGR) and multiple drug resistant. The most prevalent aminoglycoside resistance gene was aac(6')-Ie-aph(2")-Ia, that was found in 96.2% (26/27) of the isolates. The most prevalent tetracycline resistance genes were tetM, found in 85.1% (23/27) followed by tetL and tetO found in 7.4% (2/27) of the isolates. The ermA and ermB genes were detected in 33.3% (9/27) and 44.4% (12/27) of the isolates respectively. RAPD-PCR analysis yielded 17 distinct profiles among 27 investigated isolates. One cluster of isolates shared the same RAPD pattern, while 16 isolates had unique RAPD pattern. Our study showed that during the examination time period one RAPD genotype was the common type and was disseminated among patients in the burn unit. Interestingly, most of these strains had an identical or very similar antibiotic and gene resistance pattern.*

## Introduction

Enterococci are Gram-positive, facultative anaerobic bacteria that are members of the normal flora in the human gastrointestinal tract but have been recognized as important pathogens worldwide [1, 2]. *Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent species isolated from the urinary tract and wound infections, which affect mainly patients admitted to the medical care centers [3-6]. The disruption of the normal skin barrier, the immunocompromised state, prolonged hospitalization and antibiotic therapy put burns patients at a high risk of acquiring nosocomial infections including enterococcal infections [7].
Enterococcal infections can be difficult to treat because they have a remarkable ability of adaptation when are exposed to antibiotics; they have intrinsic resistance to several antimicrobial agents and have a tremendous capacity to acquire high levels of resistance to antibiotics [8, 9]. Vancomycin-resistant enterococci (VRE) and high-level gentamicin-resistant (HLGR) isolates have emerged as important pathogen in Iran as well as in other countries, which create serious challenges for treating the infected patients [9-11].
There are several reports on the endemicity of VRE and HLRG in Iran but there is a lack of information on enterococcal stains isolated form burn centers [3, 12]. This study was designed to determine the molecular characteristics and antimicrobial resistance of *enterococcal* strains isolated from patients in a burn center in Iran.

## Materials and methods

### Bacterial isolates

Twenty-seven enterococcal isolates were collected from wound specimens of patients with burn injury, during April to September 2012, in a burn hospital in Tehran. Only one isolate per patient was included in the study. Identification of enterococci was performed based on a series of conventional microbiological tests including Gram reaction, catalase reaction, presence of pyrrolidonyl arylamidase (PYR), growth on bile-aesculin agar and 6.5% NaCl media, motility test, arginine decarboxylation in Moeller decarboxylase

media, pyruvate utilization, and fermentation of arabinose, raffinose, mannitol and ribose [3]. To confirm the identity of the isolates as *E. faecalis* or *E. faecium* the $ddl_E$ gene was amplified by a polymerase chain reaction (PCR) method as described by Dutka-Malen et al. [13].

### Antimicrobial susceptibility testing

Antibiotic susceptibility of the strains was performed by the disk diffusion method. The antibiotics (Mast Group Ltd., Merseyside, UK) tested were ciprofloxacin (5 µg), erythromycin (15 µg), tetracycline (30 µg), and quinupristin-dalfopristin (15 µg). High-level resistance to gentamicin was also determined by disk diffusion method on Mueller–Hinton agar (Conda S.A., Madrid, Spain) using 120 µg gentamicin disk. The minimal inhibitory concentration (MIC) of vancomycin was determined by the standard agar dilution test on *Brain Heart Infusion* agar (Conda S.A., Madrid, Spain). All Antibiotic susceptibilities were performed and interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) guidelines [14].

### DNA extraction and gene detection

DNA extraction was performed as described previously and this DNA was used as a template for PCR analysis [11]. All strains were screened for genes encoding resistance to aminoglycoside [*aac(6')-Ie-aph(2'')-Ia*, *aph (3')*, *ant (4')*], vancomycin (*vanA*, *vanB*), tetracycline (*tetK*, *tetL*, *tetM*, *tetO*), and resistance to erythromycin (*ermA*, *ermB*, *ermC*) by PCR and multiplex PCR-based methods, using specific primers [3, 11,12, 15, 16].

### Random Amplified Polymorphic DNA (RAPD)-PCR

The RAPD-PCR assay was carried out in 25 µl reaction volumes containing 0.5 µM of each primer (5´-AGCGGGCCAA-3´ and 5´- ACGGCCGACC-3´), 12.5 µl of PCR master (Sinaclone Inc, Iran) and 5 µl of DNA template. Cycling conditions were as follow: initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 38.5°C for 1 min, and 72°C for 2 min, and final extension at 72°C for 10 min. PCR products were then separated by electrophoresis in 1.4% agarose gel with 0.5X TBE buffer. DNA bands were observed by staining with KBC power load dye (Kawsar Biotech Co. Iran) and photographed under UV illumination. RAPD patterns were analyzed by visual inspection. Only the obvious, prominent and reproducible bands from repeated experiments (at least twice) were considered and any pattern differing by one or more bands was classified as a distinct RAPD type.

## Results

Distribution of species according to conventional microbiological tests and PCR method included 19 (70.3%) *E. faecalis* and 8 (29.7%) *E. faecium*. The prevalence of antimicrobial resistance pattern and RAPD types have been summarized in Table I. In the present study, no VRE was recovered and all enterococcal isolates showed complete sensitivity to vancomycin with MIC ≤ 0.5 $\mu$g/ml.

All isolates at least exhibited resistance to one antibiotic and 6 different profiles were observed on the basis of their antibiotic resistance patterns. High-level resistance to gentamicin and multiple drug resistance were observed in 96.2% of isolates. The most frequent profile was resistance to erythromycin, gentamicin, tetracycline, quinupristin-dalfopristin and ciprofloxacin that was observed in 74% of isolates.

The most prevalent aminoglycoside resistance gene was *aac(6')-Ie-aph(2'')-Ia,* found in 96.2% (26/27) of the isolates. These genes were detected in all HLGR isolates. The *ant (4')* gene was detected in 62.9% (17/27) of isolates. The most prevalent tetracycline resistance gene was *tetM*, found in 85.1% (23/27) of the isolates followed by *tetL* and *tetO* found in 7.4% (2/27) of the isolates. The *ermA* and *ermB* genes were detected in 33.3% (9/27) and 44.4% (12/27) of the isolates respectively. The *aph (3'), tetK*, *ermC*, *vanA*, and *vanB* genes were not detected in any of the enterococcal isolates in this study.

To determine the degree of clonality among enterococcal isolates, the RAPD typing was used. RAPD analysis yielded 17 distinct profiles among 27 investigated isolates (Fig. 1). One clusters of isolates shared the same RAPD patterns, while 16 isolates had unique RAPD patterns. A dominant RAPD type designated as A, that consisted 11 isolates of *E. faecalis* and were HLGR.

## Discussion

Among Enterococci isolates, *E. faecalis* and *E. faecium* are the most common species caused enterococcal nosocomial infections [1]. In our study, the most prevalent species was *E. faecalis* (70.3%) followed by *E. faecium* (29.7%) isolated from burn wound infections. Similar results were reported for clinical isolates by other studies [3, 11, 16, 17].

In the treatment of enterococcal infections, the combination of gentamicin with a beta lactam antibiotic or a glycopeptide is used to obtain a synergistic bactericidal effect. However, strains that are highly resistant to gentamicin are no longer susceptible to the combination therapy [18]. Several studies have demonstrated that VRE have caused both outbreaks and endemic infections in burn units [5, 6], However, in this study, there were no VRE isolates and more than 96% of isolates were HLGR and 74% of them were simultaneously resistant to erythromycin, tetracycline, quinupristin-dalfopristin and ciprofloxacin. Our study is the first investigation on enterococcal resistance among burn patients in Iran and the findings indicated that vancomycin keeps its therapeutic effects against enterococcal infections.

Resistance against aminoglycosides among enterococci is commonly due to enzymatic modification. Genes encoding aminoglycoside-modifying enzymes (AMEs) are often carried on transposable elements. One of the most prevalent AMEs genes among enterococci is *aac(6')-Ie-aph(2'')-Ia* encoding a bifunctional enzyme Aac(6')-Ie-Aph(2'')-Ia which confers resistance to virtually all of the clinically available aminoglycosides including gentamicin [11, 19].

**Tab. I.** The distribution of antimicrobial resistance genes, virulence genes and biofilm production among enterococcal species.

| Isolate | Date of Isolation | Species | Resistance pattern | Resistance genes | RAPD type |
|---|---|---|---|---|---|
| 1 | 20/4/2012 | *E. faecalis* | E*,GM, TET,SYN,CIP | *tetM,ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 2 | 22/4/2012 | *E. faecium* | GM, TET, SYN | *tetM, aac(6')-Ie-aph(2")-Ia, ant(4')* | B |
| 3 | 24/4/2012 | *E. faecium* | E,GM, TET,SYN,CIP | *tetM, aac(6')-Ie-aph(2")-Ia* | H |
| 4 | 26/4/2012 | *E. faecium* | E,GM, TET | *tetL, tetM, ermB, aac(6')-Ie-aph(2")-Ia, ant(4')* | G |
| 5 | 26/4/2012 | *E. faecalis* | E,GM, TET | *tetM,ermB, aac(6')-Ie-aph(2")-Ia* | P |
| 6 | 1/5/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM, ermB, aac(6')-Ie-aph(2")-Ia* | A |
| 7 | 4/5/2012 | *E. faecium* | E,GM, TET | *tetL, tetM, ermB, aac(6')-Ie-aph(2")- Ia, ant(4')* | J |
| 8 | 8/5/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *ermB, aac(6')-Ie-aph(2")-Ia* | A |
| 9 | 15/5/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM, aac(6')-Ie-aph(2")-Ia* | N |
| 10 | 2/6/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,ermB, aac(6')-Ie-aph(2")-Ia* | A |
| 11 | 12/7/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 12 | 14/7/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 13 | 14/7/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM, ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 14 | 21/7/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM, ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 15 | 23/7/2012 | *E. faecium* | TET | *tetM* | M |
| 16 | 25/7/2012 | *E. faecium* | E,GM, TET,SYN,CIP | *tetM,ermB, aac(6')-Ie-aph(2")-Ia* | F |
| 17 | 7/8/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM, ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 18 | 7/8/2012 | *E. faecalis* | GM,SYN,CIP | *tetM,ermB, aac(6')-Ie-aph(2")-Ia* | E |
| 19 | 14/8/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | D |
| 20 | 14/8/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,ermB, aac(6')-Ie-aph(2")-Ia, ant(4')* | I |
| 21 | 21/8/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,ermA, aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 22 | 25/8/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,ermA aac(6')-Ie-aph(2")-Ia, ant(4')* | A |
| 23 | 11/9/2012 | *E. faecalis* | E,GM, TET,SYN,CIP | *tetM,tetO, ermB, aac(6')-Ie-aph(2")-Ia, ant(4')* | K |
| 24 | 11/9/2012 | *E. faecium* | E, GM | *aac(6')-Ie-aph(2")-Ia, ant(4')* | Q |
| 25 | 16/9/2012 | *E. faecalis* | E, GM,TET,SYN,CIP | *tetM, ermB, aac(6')-Ie-aph(2")-Ia* | C |
| 26 | 16/9/2012 | *E. faecalis* | E, GM,TET,SYN,CIP | *tetM aac(6')-Ie-aph(2")-Ia, ant(4')* | L |
| 27 | 16/9/2012 | *E. faecium* | E, GM,TET,SYN,CIP | *tetM,tetO, ermB, aac(6')-Ie-aph(2")-Ia, ant(4')* | O |

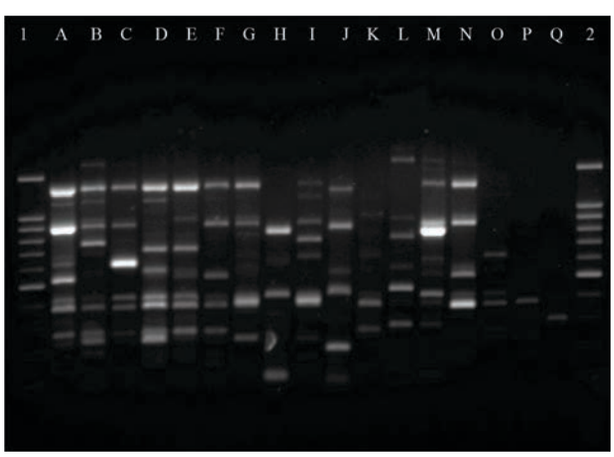
* E, Erythromycin; G, Gentamicin; SYN, Quinupristin-dalfopristin; TET, Tetracycline; CIP, Ciprofloxacin

In the current study, as in many other reports, the *aac(6')-Ie-aph(2")-Ia* gene was the most prevalent AME gene, encountered in 96.2% of isolates followed by the *ant (4')* gene and was detected in 62.9% of isolates [16, 20].

Acquired resistance to tetracyclines and macrolides in enterococci is often by mobile genetic elements [20]. In the present study, as in many previous reports, the most common tetracycline and erythromycin resistance mechanism was mediated by the *tetM* and *ermB* genes, respectively [21-25]. To take preventive measures and apply infection control, identification of the source of infection and nature of outbreaks are required. RAPD-PCR has become a reliable tool for the differentiation and identification of enterococci from clinical origin [26-28]. In this study, RAPD-PCR profiles showed that eleven *E. faecalis* isolates shared identical banding patterns. In addition, the results of RAPD typing corresponded with antibiogram findings and the resistance genes patterns for eight of these eleven isolates. This could be explained by the predominance or an outbreak of a particular clonal group of *E. faecalis* with high-level gentamicin resistance in the assessed burn unit.

In conclusion, we found that over the examination time period one RAPD genotype was the common type and was disseminated among patients in the burn unit. Interestingly, most of these strains had an identical or very similar antibiotic and gene resistance pattern.

**Fig. 1.** RAPD-PCR profiles of *Enterococcus faecalis* and *E. faecium* isolates. Lanes 1and 2; DNA size marker (100bp), Lanes A-Q; Enterococcal isolates with different RAPD patterns.




## Acknowledgments

This research has been supported by Iran University of Medical Sciences & Health Services grant number: 17989/91-03-13 and Tehran University of Medical Scienc-

es & Health Services grant 95-02-30-32393. Authors deny any existing or potential conflicts of interest of a financial, personal or any other nature that could affect or bias their research.

## Authors' contributions

ME: study design, project and protocol development, data analysis and manuscript writing. B.K., F.J. and R.B.: collected data and performed microbiological and molecular experiments. K.A.: was involved in the editing of the manuscript. M.T.: was performed the data quality control and manuscript writing. A.R.L.: conceived, designed and coordinated the research**.** All authors read and approved the final draft of the manuscript.

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■ Received on August 2, 2015. Accepted on September 19, 2016.

■ Correspondence: Mohammad Emaneini, Department of Microbiology, School of Medicine, Tehran University of Medical Sciences 100 Poursina St., Keshavarz Blvd., Tehran, Iran - Tel- Fax: 098 021 8895 5810 - E-mail: emaneini@tums.ac.ir

ORIGINAL ARTICLE

# High frequency of vancomycin resistant *Enterococcus faecalis* in children: an alarming concern

F. SABOUNI[1], Z. MOVAHEDI[2], S. MAHMOUDI[3], B. POURAKBARI[3], S. KESHAVARZ VALIAN[4], S. MAMISHI[1 3]
[1] Department of Infectious Diseases, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; [2] Department of Pediatric Infectious Diseases, School of Medicine, Qom University of Medical Sciences, Qom, Iran; [3] Pediatric Infectious Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran; [4] School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Keywords

*E. faecalis* • Vancomycin resistant • Children

Summary

**Introduction**. Enterococcus spp. *is considered as important etiological agents of nosocomial infections. However, a little is known about the epidemiology of vancomycin resistant Enterococcus faecalis (VREF). The aim of this study was to investigate the frequency of VREF and detecting of two prevalent resistance genes (vanA, vanB) at Children Medical Center Hospital, an Iranian referral pediatric Hospital.*
**Materials and methods**. *During January 2013 to December 2013, 180* E. faecalis *were isolated from clinical samples of hospitalized children. Antimicrobial testing was performed by Kirby-Bauer disk diffusion to gentamicin, amikacin, ceftriaxone, cefotaxime, ceftazidim, cefixime, piperacillin/tazobactam, cefepime, trimethoprim/sulfamethoxazole, erythromycin, clindamycin, linezolide and E-test method vancomycin and teicoplanin according to Clinical Laboratories Standards Institute (CLSI). Two prevalent resistance genes (vanA, vanB) were investigated in VREF isolates.*
**Results**. *Seventy-five (42%) of patients were male and 105 (58%) were female. Mean age of patients was 34.74 months. Cephalosporin resistance was found in majority of* E. faecalis *isolates (98.7 to ceftazidim, 95% to cefixime, 93.3% to ceftriaxone, and 89.4% to cefotaxime). Most of the isolated were susceptible to cefepime (91.7%). In addition, high level of erythromycin and clindamycin resistance was reported (93.4% and 91.2%). There were no linezolid-resistant* E. faecalis *among all isolates. Teicoplanin resistance was observed in 13.8% of* E. faecalis *(n = 25). Minimum Inhibitory concentration (MIC) ≥ 32 µg/ml for vancomycin was found in 29 isolates (16%) and vanA gene was detected in 21 (72%) VREF strains, while vanB gene was not detected in any of these isolates. The mortality rate of all cases was 3.4%.*
**Conclusions**. *This study revealed high rate of vancomycin resistance in E. faecalis strains. Therefore, periodic surveillance of antibacterial susceptibilities is highly recommended to detect emerging resistance.*

## Introduction

Enterococci have emerged as important nosocomial pathogens in the last few decades. Nowadays, few antimicrobials are active against enterococcal species and intrinsic resistance to several clinically used antimicrobials agents, making them important nosocomial pathogens [1]. *Enterococcus faecalis* can acquire resistance via various forms of conjugation and spread these genes through conjugative transposons, pheromone-responsive plasmids, or broad-host-range plasmids [1]. The increasing rate of vancomycin resistance Enterococcus (VRE) has emerged as the global concern [2].The prevalence of VRE varies widely according to outbreak situations [3]. In nosocomial settings, *Enterococcus faecium* accounts for majority of VRE infections and *E. faecalis* constitutes only 2-20% of VRE isolates, depending on geographical location and healthcare facility [4].

A little is known about the epidemiology of vancomycin resistant *E. faecalis* (VREF) [5, 6]. PCR-based screening can rapidly detects the presence of VRE and help early prevention of VRE spread [3]. The screening of critically ill patients at high risk of VRE colonization, is recommended to prevent and control of VRE transmission [3]. Currently, eight phenotypic variants of acquired glycopeptide resistance in enterococci have been reported (*VanA, VanB, VanD, VanE, VanG, VanL, VanM*, and *VanN*), with one type of intrinsic resistance (VanC) which belongs to *Enterococcus gallinarum* and *Enterococcus casseliflavus* [7]. The vanA and vanB phenotypes confer high-level vancomycin resistance (MIC > 64 µg/mL and is more prevalent among other phenotypes [8].
Data on the prevalence of VREF are scarce in Iran [5]. The aim of this study was to investigate the frequency of VREF and detection of two prevalent resistance genes (*vanA, vanB*) in pediatric population in an Iranian referral pediatric Hospital.

## Methods

### Study design

We performed a study of patients in whom *E. faecalis* were detected in clinical samples between January 2013, and December 2013, at Children Medical Center Hospital, tertiary care and teaching hospital in Tehran, Iran. A total of 180 *E. faecalis* isolates were analyzed. All isolates were identified using standard microbiology methods [9].

### Microbiological methods

Antimicrobial testing was performed by Kirby-Bauer disk diffusion method to detect resistance to gentamicin, amikacin, ceftriaxone, cefotaxime, ceftazidim, cefixime, piperacillin/tazobactam, cefepime, trimethoprim/sulfamethoxazole, erythromycin, clindamycin, linezolide according to Clinical Laboratories Standards Institute (CLSI) [10].

Antimicrobial resistance to vancomycin and teicoplanin was detected by measuring minimum inhibitory concentrations using E-test.

Vancomycin and teicoplanin sensitivity were evaluated by the E-test (AB BIODISK, Solna, Sweden) method. The results were read after 24h incubation at 37°C. MIC of ≤ 4 (μg/mL) was considered as susceptibility, MIC 8 to16 and ≥ 32 were considered as intermediate and resistant, respectively [10].

### DNA Extraction

DNA was extracted from VREF isolates using QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instruction

Polymerase chain reaction (PCR) amplification of vanA and vanB genes

The PCR assay was performed in a total volume of 25 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), and 0.5 U of Taq DNA polymerase with the following primer F:5′-CATGAATAGAATAAAAGTTGCAATA-3′ and 5′-CCCCTTTAACGCTAATACGATCAA-3′ for amplification of vanA and F: 5′-GTGACAAACCGGAGGCGAGGA-3′ and R: 5′-CCGCCATCCTCCTGCAAAAAA-3′ for amplification vanB gene [11]. DNA amplification was carried out with the following thermal cycling profile: initial denaturation at 94°C for 5 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 10 min. *E. faecium* BM4147 (*vanA*-positive) and *E. faecalis* V583 (*vanB*-positive) were used as positive controls. PCR products were analyzed on a 1% agarose gel with 0.5 × Tris-borate-EDTA buffer. A 100-bp DNA ladder (New England Biolabs, Beverly, Mass.) was used as the molecular size marker. The gels were stained with gel red and photographed under UV light.

## Results

In this study 180 samples of E. faecalis were obtained from children aged 1month to 12 years old. Seventy-five (42%) of patients were male and 105 (58%) were female. Mean age of patients was 34.74 months. Thirty eight of the patients were hospitalized in urology ward, whereas the others were distributed in gastroenterology ward (n = 19), nephrology ward (n = 20), infectious ward (n = 18), emergency ward (n = 40), hematology ward (n = 4), NICU (n = 17), PICU (n = 8), surgery ward (n = 7), cardiology ward (n = 6) and rheumatology ward (n = 3). The mortality rate of all cases was 3.4% and 10% of patients with VREF infection died.

Antimicrobial susceptibility was determined for a variety of antibiotics (Tab. I). Cephalosporin resistance was found in majority of *E. faecalis* isolates (98.7 to ceftazidim, 95% to cefixime, 93.3% to ceftriaxone, and 89.4% to cefotaxime). Most of the isolated were susceptible to cefepime (91.7%). In addition, high level of erythromycin and clindamycin resistance was reported (93.4% and 91.2%). More than 90% of isolated were resistant to ceftriaxone, cefotaxime, cefixime, ceftazidim and clindamycin. There was no linezolid-resistant *E. faecalis* among all isolates. Teicoplanin resistance was observed in 13.8% of *E. faecalis* (n = 25). MIC ≥ 32 μg/ml for vancomycin was found in 29 isolates (16%). Among resistant group, 12(41.4%) were male and 17(58.6%) cases were female with a mean age of 27.9 months. Ten patients with VREF were hospitalized in urology ward, the others were distributed in infectious ward (n = 3), CICU (n = 8), gastroenterology ward (n = 6) and emergency (n = 2). There were no significant differences between the age, sex and wards of the patients with VREF or vancomycin susceptible isolates (P value ≥ 0.05).

Antimicrobial susceptibility of VREF isolates was shown in Table 2.Among all patients with VREF isolates, 117 (65%) and 20 (69%) cases had underlining disease, respectively.

Tab. I. Antibiotic susceptibility in all samples by disk diffusion method.

| Antibiotics | Resistant (N, %) | Sensitive (N, %) |
|---|---|---|
| Gentamycin | 134 (74.4) | 46 (25.6) |
| Amikacin | 110 (61.1) | 70 (38.9) |
| Ceftrixone | 168 (93.3) | 12 (6.7) |
| Cefotaxime | 161 (89.4) | 19 (10.6) |
| Cefixime | 171 (95) | 9 (5) |
| Ceftazidim | 177 (98.7) | 13 (1.3) |
| Piperacillin/ tazobactam | 100 (56) | 80 (44) |
| Cefepime | 15 (8.2) | 165 (91.7) |
| Trimethoprim- sulphametoxazole | 139 (77.5) | 41 (22.5) |
| Erythromycin | 168 (93.4) | 12 (6.6) |
| Clindamycin | 164 (91.2) | 16 (8.8) |
| Linezolide | 0 (0) | 180 (100) |

Tab. II. Antibiotic susceptibility in VREF samples by disk diffusion method.

| Antibiotics | Resistant (N, %) | Sensitive (N, %) |
|---|---|---|
| Gentamycin | 28 (96.6) | 1 (3.4) |
| Amikacin | 25 (86.2) | 4 (13.8) |
| Ceftriaxone | 29 (100) | 0 (0) |
| Cefotaxime | 29 (100) | 0 (0) |
| Cefixime | 29 (100) | 0 (0) |
| Ceftazidim | 29 (100) | 0 (0) |
| Piperacillin/ tazobactam | 17 (58.6) | 12 (41.4) |
| Cefepime | 3 (10.3) | 26 (89.7) |
| Trimethoprim- sulfamethoxazole | 1 (3.4) | 28 (96.6) |
| Erythromycin | 29 (100) | 0 (0) |
| Clindamycin | 26 (90.5) | 3 (9.5) |
| Linezolide | 0 | 100 |

Amplification of *vanA*, *vanB* targets produced distinct bands corresponding to their respective molecular sizes (1,030 bp for *vanA* and 433 bp for *vanB*). Among VREF, *vanA* gene was detected in 21 (72%) isolates, while *vanB* gene was not detected in any of these isolates. *vanA* gene was found in 13 girls (62%) and 8 boys (38%) (p value ≥ 0.05).

## Discussion

The emergence of VRE as an important nosocomial pathogen is due to its propensity for colonization of the gastrointestinal (GI) tract, persistence in hospital environments, genome plasticity, mobile genetic elements, and increased mortality [12]. The epidemiology of VRE varies from one hospital to another, which depends on several factors including the hospital size, patient population, antibiotic usage patterns and geographic location. According to earlier reports, risk factors that increase the likelihood of VRE infection or colonization can be due to host factors, hospital-specific factors and antibiotic usage [4].

The antimicrobial susceptibility of *Enterococcus spp*. showed higher resistant pattern to a majority of antibiotics compare to our previous hospital report in 1996-2000 [13].

Analysis of our results similar to other studies indicate *vanA* gene as common determinant for glycopeptide resistance in *Enterococcus spp.* [14-17]. *VanA* is responsible for most of the human cases of VRE around the world [7]. In addition, the *vanA* operon can easily be transferred through acquired resistance [18]. Our previous study demonstrated that clonal dissemination was a major mechanism of the spread of these isolates [5]. The majority of E. faecalis colonization occurs in the gastrointestinal tract infection (GI) and to a lesser extent on the skin, in the genitourinary tract, and in the oral cavity [7, 8, 19]. When GI colonization with VRE occurs, it can persist for months to years. In addition, and efforts for decolonization are typically transitory and recurrence of VRE may occur days or weeks later [7, 19]. The common pathway of nosocomial VRE acquisition might be via person-to-person contact or exposure to contaminated objects. Health care workers' hands are the most consistent source of transmission and it has been reported that VRE can persist for up to 60 minutes on hands and as long as 4 months on surfaces [7, 20]. Therefore, healthcare facilities need a comprehensive infection control program in order to decrease the transmission of VRE among patients.

The emergence of VRE is also due to the inappropriate use of cephalosporin as well as poor hospital infection control measures [21]. Long duration of hospital stay and high rate of antibiotics treatment are the most frequently reported risk factor for multi-resistance Enterococci colonization and infection.

Another concern about VREF is the possible transfer of *vanA* from *E. faecalis* to *S. aureus* [22]. E. faecalis *vanA*-carrying plasmid was found to encode a response to sex pheromone and it raises concern about the potential uptake of *vanA* from Enterococci by a pheromone-related process in *S. aureus* [23].

Our study highlights further intervention for controlling the spread of VRE. Active periodic surveillance cultures (or molecular testing) of patients at highest risk for carriage, decontaminating the hands of healthcare workers using an antiseptic-containing preparation before and after all patient contact, adherence to barrier precautions (*i.e.*, gloves and gowns) and cohorting colonized and/or infected patients; and cleaning of occupied rooms by patient with VRE are highly recommended [24, 25].

In conclusion, in this study high frequency of vancomycin resistance in *E. faecalis* strains was fournd. Therefore, periodic surveillance of antibacterial susceptibilities is highly recommended to detect emerging resistance.

## Acknowledgments

This study was supported by a grant (grant number: 89-04-88-11642) from Tehran University of Medical Sciences to Dr. Farah Sabouni. The authors declare that they have no competing interests.

## Authors' contributions

FS and SM conceived designed and coordinated the research. ZM, SM, BP and SKV collected data. ZM and SM performed the statistical analyses. ZM, BP, SM and DA evaluated the results. ZM and SM wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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■ Received on May 21, 2016. Accepted on August 5, 2016.

■ Correspondence: Setareh Mamishi, Department of Pediatric Infectious Diseases, Children Medical Center Hospital, School of Medicine, Tehran University of Medical Sciences, No.62, Gharib St., Keshavarz Blvd., Tehran, Iran - E-mail: smamishi@sina.tums.ac.ir

Original article

# Relationship between lead exposure and mild cognitive impairment

C. FENGA[1], S. GANGEMI[1], A. ALIBRANDI[2], C. COSTA[3], E. MICALI[4]
[1] Department of Biomedical, Odontoiatric, Morphological and Functional Images, Occupational Medicine Section, University of Messina, Messina, Italy; [2] Department of Economics,University of Messina, Messina, Italy; [3] Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; [4] University of Messina, University Hospital of Messina, Italy

Keywords

Neurobehavioral effects • Lead exposed workers blood lead level • Low lead exposure • ALAD • ZPP

Summary

**Introduction.** *Since it is still controversial whether-low-to moderate long-term lead below current threshold values causes neurobehavioural deficits in adults.*
**Methods**. *Forty lead-exposed workers subjects with a mean blood lead (PbB) level of 56.4 µg/dL and 40 non-lead-exposed aged matched subjects (PbB: 15.4 µg/dL) with the same socio-economic background were investigated. Participants were administered a neuropsychological tests consisting of BAMT (Branches Alternate Movements Task), FT (Finger Tapping Speed), DS (Digit Span) POMS (Profile of Mood States).*
**Results.** *Authors noted a significant relationship between the exposed and the referent groups in tests mainly involving executive functions, short time memory and psycho-emotional variables. In addition, Poisson regression test performed on single psycho-emotional factors (POMS), has allowed to evidence a significant influence of Pb e ZPP levels on tension, anxiety and depression.*
**Conclusions**. *The present study showed that lead exposure among adults at levels previously considered safe, results in impairment of certain cognitive abilities.*

## Introduction

Lead (Pb) is a metal with many important industrial uses. Occupational exposure to lead can produce toxic effects on multiple organ systems including renal dysfunction, hematopoietic diseases, neurocognitive and reproductive disorders. Although occupational exposure to this neurotoxic agent has declined steadily over the past 20 years, the presence of lead in occupational settings continues to be a source of both acute and chronic exposure, resulting in blood levels ranging 40 to 120 µg/dL, as demonstrated by the Agency for Toxic Substances and Disease Registry [1].
Several studies showed an association between lead and cognitive abilities in children at blood levels even below 10 µg/dL without evidence of a safe lower threshold [2]. In 2015, United States National Institute for Occupational Safety and Health (NIOSH) indicated 5 µg/dL as the reference blood lead level (PbB) for adults. Nonetheless, the U.S. Occupational Safety and Health Administration (OSHA) recommends to remove workers from lead exposure when PbB is above 60 µg/dL and readmit them when it is below 40 µg/dL. Moreover, the American Conference of Governmental Industrial Hygienists (ACGIH) suggests a biological exposure index of 30 µg/dL for workers.
Workers exposed to lead often show impaired performance on neurobehavioral test involving attention, processing, speed, visuospatial abilities, working memory and motor function. It has also been suggested that lead can adversely affect general intellectual performance [3]. Exposure to inorganic lead in the environmental and occupational settings continues to be a serious public health problem. At high exposure levels, lead causes encephalopathy, kidney damage, anaemia and toxicity to the reproductive system. Even at lower doses, lead produces alterations in cognitive development in children and adults. A really safe level for lead exposure has not been defined, as health risks associated with this metal have been shown even at very low doses [4].
Recent meta–analyses reported worse neurobehavioral performances for exposed than unexposed workers with PbB levels lower than 50-60 µg/dL. The authors concluded that none of the individual studies were conclusive or adequate in providing information on the effects of lead on cognitive function [5-7]. Furthermore, mild cognitive impairment (MCI) is considered to be a high-risk state for developing dementia with about 50% of MCI patients progressing to dementia [8]. However, several lines of evidence have now suggested that environmental exposure to lead may play an important role. A recent study investigating cumulative lead exposure and cognitive function in adult men reported that the degree of performance impairment over time, particularly in visuospatial and visuomotor domains, increased with increasing bone lead concentration, a marker of cumu-

lative exposure [9]. Recent animal studies report that early developmental lead exposure in rodents resulted in an age-related elevation in amyloid precursor protein (APP) and its amyloidogenic Aβ product, markers of Alzheimer's Disease (AD) [10, 11] and over-expression of the β-amyloid protein precursor gene 20 months after exposure had ceased. Subsequent studies in non-human primates that were exposed to lead during development have shown similar effects [12]. Furthermore, lead may be indirectly linked to dementia through its demonstrated hypertensive effect [13-16], a risk factor considered to play an important role in the development of dementia [17]. In addition, lead could act on neurotransmission, such as the acetylcholine system which is known to be compromised in AD [18, 19]. One limitation of the current understanding of the potential risk posed by lead exposure for dementias or MCI is the lack of information on the specific behavioural profile with which lead may be associated. In particular, it is unknown whether lead exposure reproduces the specific behavioural deficits, many of which can also be directly evaluated in experimental animal models, that have proven to be predictive of dementias in human. The present study was conducted to evaluate the association between occupational lead exposure and MCI using biological markers and validated behavioural measures.

## Materials and methods

The present study was carried out at the Occupational Health Institute, Medical School, University of Messina, Italy.

Forty male workers, employed in a battery recycling plant placed in Messina, Italy, responders to an invitation to participate in the health surveillance program, fulfilled the inclusion criteria for the present study.

Inclusion criteria were: living in Messina metropolitan area, working in the battery storage plant for at least 5 h/day, willing and able to attend required study visits, lack of any systemic disease. Workers under medication with both cerebro-active drugs and any other substances able to interfere with neuro-behavioural performances were excluded from the study.

A total of forty workers, with mean age of 37.15 years (SD ± 8.09), matched the inclusion criteria. The control group included forty healthy male subjects with no present or past exposure to lead, age-matched, chosen from people working in several offices located in the urban area of Messina. Informed consent was obtained from workers.

All participants were interviewed by well-trained occupational physicians, and information about socio-demographic characteristics, disease history, alcohol consumption, cigarette smoking, dietary patterns (ethnic products intake), residential area (presence of nearby industries or factories), occupational history (of the last 3 years for possible lead-exposing occupation) were gathered.

Cognitive and behaviour measures were administered to workers by a specialist in clinical psychology after the working shift, in a standardized environment and using uniform procedures.

The evaluation of both biomarkers of exposure and effect (blood lead, PbB; aminolevulinate dehydratase, ALAD; Zn protoporphirin, ZPP; haemoglobin, Hb) and psychological tests in the exposed workers with respect to non exposed subjects was performed.

Environmental assessment of workplace lead levels was given by factory management and was over the threshold limit value of 0.05 mg/m$^3$ set by the ACGIH [20].

### Biological monitoring

Venous blood samples were taken for the determination of lead dose (PbB) and effect (Hb, ALAD and ZPP) biomarkers.

The whole blood specimens were collected using a lead-free heparinized evacuated tubes. Blood samples were stored at +4°C until the analysis, which was performed within 2 weeks.

### Psycho-diagnostic protocol

BAMT (Branches Alternate Movements Task) was performed on all subjects to assess motor coordination [21]. Subjects alternatively touch their knees crossing arms and the sequence is repeated alternatively for 30 seconds.

FT (Finger Tapping) speed measures the maximum number of repetitive movements made beating as quickly as possible a button with the index finger, holding the arm supported in a fixed position and comfortable and alternating hands (dominant/non-dominant) for a total of 6 tests in 10 seconds [22].

DS (Digit Span), a simple traditional evaluation of short term memory: a series of numbers, each time increasing in length, is repeated forwards and in reverse order. Subtest is on the basis of correct answers [23].

Profile of Mood States (POMS), administrable to adults with compulsory education in a maximum range of time of 10 minutes, in the Italian version is made up of 58 items that define the six factors of mood [24]:

- Tension-Anxiety = T
- Depression-Dejection = D
- Anger-Hostility = A
- Vigor-Activity = V
- Fatigue-Inertia = F
- Confusion-Bewilderment = C

To get the score of each of the six factors, the scores of the single answers to each single item that define the score itself are added to every item. 0,1,2,3 or 4 points are given except for the two terms "relaxed" in the scale Confusion-Bewilderment that must be inverted in the assignment result (4,3,2,1 or 0). The factor Vigor-Activity is evaluated with a negative sign and referred to male sex. The rough scores are converted into standard ones.

**Tab. I.** Socio-demographic characteristics and biomarkers of lead exposure and effect in exposed and non-exposed workers.

| | Exposed | Controls | p < |
|---|---|---|---|
| Population under study | 40 | 40 | NS |
| Age (years, mean ± SD) | 37.15 ± 8.09 | 37.3 ± 8.1 | NS |
| Education (years, mean ± SD) | 15.3 ± 2.73 | 12.0 ± 1.7 | NS |
| Seniority (years, mean ± SD) | 4.3 ± 2.05 | 4.6 ± 1.8 | NS |
| Alcohol consumption | | | |
| None | 0 (0) | 0 (0) | NS |
| At least 1 drink a day | 40 (100%) | 40 (100%) | |
| Smoking habits | | | |
| Never | 10 (25%) | 8 (20%) | NS |
| Current | 24 (60%) | 26 (70%) | NS |
| Former | 6 (15%) | 4 (10%) | NS |
| PbB (μg/dL, mean ± SD) | 56.4 ± 14.4 | 15.4 ± 1.5 | 0.005 |
| ZPP (μg/dL, mean ± SD) | 53.8 ± 22.9 | 23.4 ± 1.4 | 0.005 |
| ALAD (u/ml, mean ± SD) | 28.2 ± 9.4 | 58.0 ± 1.9 | 0.005 |

**Tab. II.** Comparison of neuro-behavioural and psycho-emotional variables (mean ± SD of score) between exposed and non exposed workers.

| | Exposed | Controls | p < |
|---|---|---|---|
| NEURO-BEHAVIORAL VARIABLES | | | |
| BAMT | 3.8 ± 1.1 | 4.4 ± 0.5 | 0.005 |
| Fingers | 3.8 ± 1.0 | 4.7 ± 0.5 | 0.005 |
| Direct Digitation | 5.5 ± 0.8 | 5.5 ± 0.8 | NS |
| Inverse Digitation | 3.5 ± 0.7 | 4.9 ± 0.4 | 0.005 |
| PSICO-EMOTIONAL VARIABLES | | | |
| Tension | 58.3 ± 9.1 | 50.6 ± 6.7 | 0.005 |
| Depression | 57.0 ± 10.6 | 48.3 ± 7.2 | 0.005 |
| Aggressiveness | 54.2 ± 9.9 | 47.2 ± 7.9 | 0.005 |
| Vigor | 57.0 ± 6.1 | 68.2 ± 3.7 | 0.005 |
| Tiredness | 61.2 ± 11.0 | 50.4 ± 7.3 | 0.005 |
| Confusion | 62.6 ± 11.4 | 51.5 ± 7.4 | 0.005 |

### Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science the Methodological S.R.L. NPC Test [25, 26]; the Minitab Release 13.31 [27] and R 2.1.1 [28] for the estimation of Poisson regression.

Descriptive variables were evaluated for differences between means for continuous variables and with non parametric analysis for not continuous variables.

The differences of both lead exposure indices and psychological tests between the group under study and the control group were analysed by Student's unpaired *t* test. Furthermore, correlation between lead exposure biomarkers and seniority and Hb level were tested using Pearson's linear correlation test.

In order to analyze the influence of lead exposure on neurobehavioral test (BAMT, FT, DS with Direct and Inverse Digitations) a multivariate ordinal logistic regression was performed because the scores of the neurobehavioral tests constitute ordinal categorical variables.

The influence of exposure to lead on the performance levels of each psycho-emotional test (POMS) was tested through the estimation of a generalized linear model of Poisson because the scores of the POMS are discreet and not negative. The fixed level of significance for the whole statistical analysis was $p < 0.005$.

## Results

The socio-demographic characteristics and biomarkers of exposed and non exposed workers are shown in Table I. PbB and ZPP mean levels were significantly higher in exposed than in non exposed workers. As expected, the mean value of ALAD was significantly lower in exposed than non exposed workers. A PbB level higher than the threshold limit value (60 μg/dL) was found in18 (45%) of the exposed workers. Current blood lead level (PbB) of the exposed workers ranged from 24 to 76 μg/dL. PbB of the controls ranged from 13 to 18 μg/dL.

Regarding to considered whole psycho-emotional variables, the authors evidenced significant differences between the two groups. The results of neuroemotional variables are showed in Table II. The values of tension, depression, aggressiveness, tiredness and confusion, resulted higher in the exposed workers than the controls. An inverse direction was found for the vigour, that resulted higher for the controls with respect to the exposed workers (Tab. II).

**Tab.III.** Pearson correlation test between biomarkers of lead exposure and effect vs seniority and Hb.

| | PbB | ALAD | ZPP |
|---|---|---|---|
| Hb | 0.153 | -0.459* | -0.264 |
| Seniority | -0.354** | 0.076 | -0.320** |

* $p < 0.01$ **$p < 0.05$

**Tab. IV.** Ordinal Logistic Regression between biomarkers of Pb exposure and effect vs neurobehavioral variables.

| | PbB | ALAD | ZPP |
|---|---|---|---|
| BAMT | -0.223 * | 0.025 | -0.018 |
| FINGER | -0.008 | -0.149 | -0.395 |
| D Digitation | 0.558* | 0.068 | 0.401* |
| I Digitation | 0.031 | 0.032 | 0.002 |

* $p < 0.005$

**Tab. V.** Poisson regression coefficients between biomarkers of Pb exposure and effect vs psycho-emotional variables (POMS test).

| POMS variables | PbB | ALAD | ZPP |
|---|---|---|---|
| T | *0.011 | 0.004 | *0.007 |
| D | *0.012 | *0.017 | *0.008 |
| A | 0.001 | 0.010 | 0.004 |
| V | 0.006 | 0.002 | 0.003 |
| S | -00.001 | 0.004 | 0.002 |
| C | 0.003 | 0.005 | 0.002 |

* $p < 0.005$

Table III shows correlation between biomarkers of lead exposure and effect and both seniority and Hb concentration in the groups under study. Seniority was inversely correlated to the PbB level while ZPP was negatively correlated to the working age.

The estimation of ordinal logistic regression model (gompit was the used link function) has allowed to evaluate the influence of some variables on the scores obtained by neurobehavioral tests. Table IV reports the estimation of two models of ordinal logistic regression: in the first model the answer variable is BAMT, that implies a score ranging from 1 to 5. In the exposed subjects it assumes the value 1 in 2 cases (5%), value 2 in 2 cases (5%), value 3 in 10 cases (25%), 4 in 14 cases (35%), 5 in 12 cases (30%), so that the presence of four constants was evidenced in the model.

In the second model the answer variable is FINGERS, that requires a score ranging from 1 to 6. In the exposed group it assumes the value 1 in any case (0%), value 2 in 4 cases (10%), value 3 in 14 cases (35%), value 4 in 10 cases (25%), value 5 in 12 cases (30%);no case with value 6 was found. Therefore the model includes three constants.

As showed in Table IV, the BAMT is significantly dependent from PbB level (the number of correct movements of exposed subjects decreases with the increase of lead levels); the variable FINGERS is dependent on working age: the number of correct movements performed with the fingers decreases as the number of years of exposure increases.

Finally, the Poisson regression performed on the levels of performance of the single psycho-emotional tests (POMS) has allowed to underline that the levels of PbB and ZPP significantly influence the tension and the depression (Tab. V).

## Discussion and conclusions

The findings of the present study showed that occupational lead exposure results in impairment of certain cognitive abilities at levels considered safe by certain scientific committees. At a mean PbB of 56.4 μg/dL, we observed a significant relationship between the exposed and the referent groups in tests mainly involving executive functions, short time memory (BAMT test, FT test and DS Inverse) and for all the psychiatric symptoms measured by the POMS test.

These results are consistent with previous studies. A cross-sectional analysis of 107 occupationally exposed individuals showed increased rates of depression, confusion, anger, fatigue, and tension as measured by the POMS test among those with blood levels > 40 μg/dL [29]; authors found that cumulative measures of blood lead levels in currently exposed lead workers were associated with tension, anxiety, hostility and depression measured by the POMS questionnaire. Lindgren et al. [30] examined the POMS factor structure in retired lead smelter workers and showed that the resulting "general distress" factor was significantly related to cumulative exposure but not to current PbB level. Psychiatric symptoms (as measured by POMS), were positively associated with both the risk of Alzheimer diseases and a steeper rate of cognitive decline [31]. Because late life symptoms of depression are closely associated with dementia, investigators have put forth a number of hypotheses that suggesting that depression a) may be a risk factor for cognitive decline, b) has risk factors in common with dementia c) is an early reaction to declining cognition and d) influences the threshold at which dementia emerges. The exact temporal and mechanistic relation remains unclear. Regardless of the exact relation between depressive symptoms and cognitive function, however, the assessment of the impact of lead exposure on these outcomes is not compromised.

The mechanism with which lead exposure affects cognition in older adults has yet to be revealed, but several pathways have been proposed such as lead's impact on oxidative stress neural apoptosis, neurotransmitter storage and release, mitochondrial damage, and hippocampal changes [31-33]. Of particular relevance to MCI on dementia is oxidative stress, with higher levels of oxidative stress markers (e.g. isoprostanes, nitrotyrosine, 8-hydroxyguanosine, 8-hydroxyguanine) among patients with MCI and AD [34].

Although it is known to induce oxidative stress [35] the relationship of lead exposure with these specific markers of effect is not known; lead may also affect cognitive function indirectly through its effect on hypertension,

which is increasingly being recognized as a target for the prevention of dementias.
According to the recent scientific literature on this topic our results support the hypothesis that increased blood lead levels can also be associated with measurable neurocognitive abnormalities. From a neurobiological point of view, it is of great interest that neuropsychological effects may occur at concentrations several orders of magnitude below the clinical threshold for acute lead poisoning [36-38]. It could therefore be argued that there is no "safe" level for the adverse effects of lead on neuronal functioning and that these can only be measured using neuropsychological tests.
There are some limitations of our study that should be pointed out. For example a variety of factors can influence a person's susceptibility, such socioeconomic status, genetic factors and it cannot be determined from our data to which extent these factors influenced test results. Despite these limitations, however, these findings were consistent with those of previous studies; anyway, the present report suggests the need to define an occupational exposure limit for PbB lower than 30 μg/dL [39].

## Acknowledgements

All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript. The Authors have no conflict of interest to declare.

## Authors' contributions

C.F. developed and planned the whole study by coordinating the various stages of research.
S.G. performed the medical examination of subjects.
A.A. made data processing and statistical analysis.
C.C. has performed the sampling and laboratory analysis of the exposed and control groups.
E.M. has chosen, administered and rated the psychodiagnostic protocol.

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■ Received on August 14, 2015. Accepted on November 2, 2016.

■ Correspondence: Elvira Micali, University of Messina, University Hospital of Messina, via S. Sebastiano 24, 98122 Messina, Italy - Tel. +39 090 2217197 - Fax +39 090 2930337 - E-mail: emicali@unime.it

Original article

# Observational study to evaluate the impact of internet reminders for GPs on colorectal cancer screening uptake in Northern Italy in 2013

E. GABRIELLI[1], A.J. BASTIAMPILLAI[1], M. PONTELLO[1], G. BEGHI[2], P. CERESA[2], M.E. PIROLA[2], D. CEREDA[3]
[1] Public Health, Department of Biomedical Health Sciences, University of Milano, Italy; [2] Milano 1 Local Health Unit, Italy; [3] General Directorate of Health, Lombardy Region, Milan, Italy

Keywords

Colorectal cancer • Screening uptake • Reminder interventions

Summary

**Introduction**. *Colorectal cancer (CRC) is the third most common cancer worldwide and CRC-related mortality can be effectively reduced by population-based screening. Screening uptake is a key indicator of performance, susceptible of several implementation methods. Participation in ASL Milano 1 area (northern Italy) is increasing thanks to reminder invitation sent to non-responders. Here we evaluate the implementation of another strategy among those proved to be effective.*
**Methods**. *In the years 2013-2014 we conducted an observational study in patients non-responder to first invitation and subsequent mailed reminder. A list of them was sent to their own GP, who had the task to evaluate possible exclusion criteria and make a reminder, either by personal interview, telephone call or via e-mail. Intervention could be conducted either by the GP himself or by an assistant. Primary outcomes were to assess the overall efficacy of the intervention and the efficacy of its single features (type of intervention and provider), measuring the consequent uptake of CRC screening.*
**Results**. *Participation in CRC screening was significantly higher (33,5%) in patients who received a reminder from GP, regardless of the type, vs those who did not (19,0%, $p < 0.01$). No statistically significant difference was detected either by method or by provider of the intervention.*
**Discussion**. *The results of our study demonstrate that even a modest intervention can have a significant effect in improving compliance to screening for CRC, one of the cancers with highest incidence in developed countries, for which an effective treatment is available in case of early diagnosis.*

## Background

Colorectal cancer (CRC) is the third most common cancer worldwide, causing approximately 1,360,000 new cases every year, and the fourth leading cause of cancer-related death. Almost 55% of the cases occur in more developed regions [1].
In Italy, CRC is by far the most common cancer in our population, ranking third in men and second in women, with nearly 59,000 estimated diagnoses in 2013. Moreover, it represents the second cause of cancer-related death [2]. In Lombardy, a northern region of Italy with nearly 11 millions inhabitants, CRC has a great impact in public health, accounting for 9,126 new cases and 3,215 deaths estimated in 2013 and 1,500 hospitalizations every year and representing the second leading cause of cancer-related death, as reported by the Local Health Unit of one large metropolitan area in Lombardy (ASL Milano 1) [3, 4].
Therefore, reducing mortality from CRC represents an important and challenging problem for public health and this target may be achieved by the introduction of population-based screening programs that allows to effectively reduce cancer deaths by detecting cancers at an early stage and by detecting and removing precancerous polyps before cancer develops [5-7].
In 2005 Lombardy started a screening program using fecal immunochemical blood test (FIT), implemented by ASL Milano 1 in 2006. The CRC screening campaign of ASL Milano 1 is addressed to a target population of nearly 240,000 subjects resident in this area, that covers 73 municipalities with a total population of 940,000 subjects [8].
In organised screening programs, broad participation in screening is critical to reduce CRC mortality at the population level. Screening uptake is a key indicator of performance because is susceptible of several implementation methods [9, 10]. Postal reminders, telephone calls, General Practitioners (GPs) signing the invitation have all proved to be effective in increasing participation [9-11].
Screening uptake in ASL Milano 1 area is steadily growing from 30% in 2006 to 47% in 2012, in line with reference data at the national level. One of the approaches locally used to increase participation is mailing a reminder invitation to non-responders three months after the first one. In 2014 a second reminder invitation was introduced for non-responder patients.
Our aim was to describe and evaluate the implementation of another strategy among those proved to be effective to increase CRC screening uptake by involving GPs in recruitment of their eligible non-responder patients.

## Methods

### Study design and setting

In the years 2013-2014 we conducted an observational study in patients non-responder to first invitation and subsequent mailed reminder after 3 months in a large metropolitan area north-west of Milan, covered by a Local Health Unit called ASL Milano 1. This area has population of nearly 1,000,000 inhabitants distributed in 73 municipalities and 604 GPs.

Target population is invited every two years by a personal letter to collect the FIT kit at the local pharmacies and to return the sample to the same pharmacies, that provide to send it to the central laboratory (Parabiago Public Health Laboratory). If FIT results positive, subjects are informed by a phone call from the Screening Center inviting them to an interview with a gastroenterologist, who will explain them the second level test, a colonoscopy.

The study was divided in two phases each year:

- In the first phase we analysed health administrative data to retrieve a list of non-responder patients, resident in ASL Milano 1 area and aged 50-69, who already received a reminder invitation from our Screening Center in the period January-May; each GP then received an email with a personal link with the list of their patients to be evaluated for a possible reminder.
- In the second phase (August - November), the GP had the task to check and report any reason for exclusion from reminder intervention, such as a colonoscopy performed in the past 5 years. Then the GP contacted eligible patients checking if they had received the invitation and asking them to participate and to contact the Screening Center for any further explanation.

### Study partecipants

Participants were GPs in charge in the ASL Milano 1 area that agreed to join the ASL project about CRC screening.

### Intervention

GPs received from ASL an e-mail reminder concerning a list of non-responder patients, as described above. After evaluating among possible exclusion criteria (performing a colonoscopy in the past 5 years, a concomitant severe disease, moving elsewhere, not being assisted by that GP), each GP could in turn choose among three different types of patient reminder: personal interview, telephone call or via e-mail. Intervention could be conducted either by the GP himself or by an assistant (i.e. a nurse or a secretary), if available.

The flow-chart of the project is shown in Figure 1.

### Outcome measures

The primary outcomes were to assess the overall efficacy of the intervention and the efficacy of its single features (type of intervention and provider), measuring the consequent uptake of CRC screening. Rates were adjusted for age and sex using as standard population the non-responder population of ASL Milano 1 area that received a reminder invitation in 2012.

An additional analysis performed on 2013 data evaluated the screening participation related to GP activity level by dividing GPs into four groups accordingly to the rate of patients contacted:

- Group 1 = ≤ 25% of patients contacted;
- Group 2 = 26%-50% of patients contacted;
- Group 3 = 51%-75% of patients contacted;
- Group 4 = 76%-100% of patients contacted.

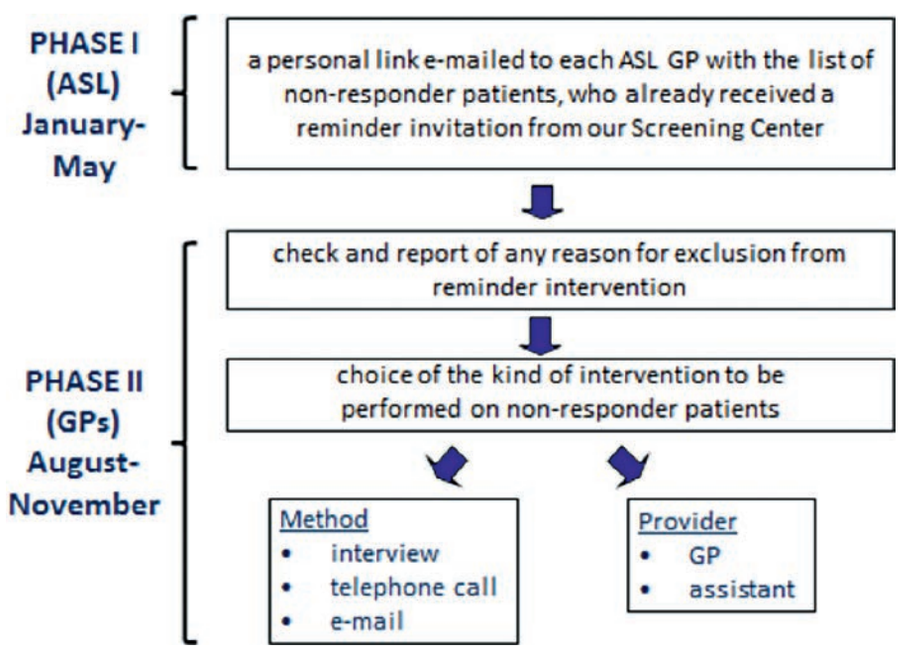


Fig. 1. Study flow-chart.

**Tab. I.** Patients non responder to the first invitation letter: participation rate by intervention type (year 2013; Rates were adjusted for age and sex using as standard population the non-responder population of ASL Milano 1 area that received a reminder invitation in 2012).

| 2013 participation rate by intervention type | | Interventions by | | Overall |
|---|---|---|---|---|
| | | GP | Assistant | |
| Patients of GPs joining ASL project | Interview | 33.4% | 32.7% | 33.3% |
| | Telephone call | 35.8% | 27.1% | 32.6% |
| | E-mail | | | 28.0% |
| | Intervention (all) | 33.8% | 28.8% | 33.1% |
| | No intervention | | | 16.7% |
| Patients of GPs not joining ASL project | No intervention | | | 13.7% |
| All | Overall uptake 2013 | | | 26.7% |

**Tab. II.** Patients non responder to the first invitation letter: participation rate by intervention type (year 2014; Rates were adjusted for age and sex using as standard population the non-responder population of ASL Milano 1 area that received a reminder invitation in 2012).

| 2014 participation rate by intervention type | | Interventions by | | Overall |
|---|---|---|---|---|
| | | GP | Assistant | |
| Patients of GPs joining ASL project | Interview | 33.9% | 32.4% | 33.8% |
| | Telephone call | 31.8% | 33.7% | 32.3% |
| | E-mail | | | 28.5% |
| | Intervention (all) | 33.5% | 32.9% | 33.1% |
| | No intervention | | | 20.4% |
| Patients of GPs not joining ASL project | No intervention | | | 16.8% |
| ALL | Overall uptake 2014 | | | 27.6% |

**Tab. III.** Patients non responder to the first invitation letter: participation rate by intervention type (pooled data years 2013+2014; Rates were adjusted for age and sex using as standard population the non-responder population of ASL Milano 1 area that received a reminder invitation in 2012).

| 2013+2014 participation rate by intervention type | | Interventions by | | Overall |
|---|---|---|---|---|
| | | GP | Assistant | |
| Patients of GPs joining ASL project | Interview | 33.6% | 32.1% | 33.5% |
| | Telephone call | 33.4% | 30.2% | 32.4% |
| | E-mail | | | 28.7% |
| | Intervention (all) | 33.5% | 30.9% | 32.8% |
| | No intervention | | | 19.0% |
| Patients of GPs not joining ASL project | No intervention | | | 15.4% |
| All GP | Overall uptake 2013+2014 | | | 27.3% |

**Tab. IV.** Non-responder patients participation rate according to the level of GP's activity volume (Year 2013).

| | Activity volume (rate of their patients contacted) | GPs distribution according to activity volume N°(%) | Non-responder patients participation rate |
|---|---|---|---|
| Group 1 | ≤ 25% | 103 (19%) | 16.3% |
| Group 2 | 26-50% | 25 (5%) | 24.0% |
| Group 3 | 51-75% | 135 (26%) | 26.2% |
| Group 4 | 76-100% | 267 (50%) | 31.6% |

### Statistical analysis

Results, after being collected and recorded in the personal link provided at the beginning of the study, were analysed using the statistical software package IBM SPSS (version 21.0).
We assessed the impact of the intervention and its features comparing screening uptake between patients who received the intervention and those who did not, using a Chi-square test. Finally, we compared the rate of screening uptake according to the level of participation of the GP in the study. For all analyses, a p-value of less than 0.05 was considered significant.
All data were been used according to the privacy laws and according to ethical standards.

## Results

A total of 59,579 (23,373 in 2013 and 36,206 in 2014) non-responder patients were identified using health administrative database. The mean age was 58 years old (SD 6.3) and 50% were female.
8,594 patients (14.4%) met exclusion criteria. The remaining 50,985 patients (85.5%) were included in the analysis. 23,281 (45.6%) did not receive any kind of intervention. 27,704 (54.3%) received one: 72% an interview, 27% a telephone call, 2% an e-mail. The interventions were been conducted in person by GP (87%), or their assistants (11%), or with (e-mail 2%).
Results separately for year are shown in Tables I and II. Overall results are shown in Table III. 33.5% of the 27,704 patients who received a reminder from GP, regardless of the type, subsequently participated to CRC screening. Conversely, only 19.0% of the 23,281 who did not receive any type of intervention participated. The difference resulted statistically significant ($p < 0.01$ Chi squared, yates correction, equals 1269.130 with 1 degrees of freedom).
Analysing each year separately, response rate without intervention increased from 15.0% in 2012, to 16.7% in 2013 and to 20,4% in 2014. Screening participation after intervention was 33.1% in 2013 and 32.6% in 2014 (Tab. IV).
Concerning the type of reminder from GP, no statistically significant difference was detected either by method (interview vs. telephone call) or by provider of the intervention (physician *vs* assistant).
Interview response rates were 33,3% in 2013, either performed by GP or assistant, and 33.8% in 2014 (33.9% if performed by GP and 32.4% if performed by an assistant), while telephone and e-mail remainders passed from response rates of respectively 32.6% and 28.0% in 2013 to 32.3% and 28.5% in 2014.
Screening uptake in non-responder patients raised from 15.0% in 2012 with only invitation reminder from ASL to 26.7% in 2013 and 27.6% in 2014 after GP or assistant intervention.
Evaluating screening uptake according to the level of participation of the GP in the study in 2013, the 530 GPs enrolled were stratified as follows according to the rate of patient contacted (Tab. IV).
Noteworthy, GPs most active in contacting non-responder patients had a significantly higher uptake rate compared to less active groups of physicians. Results were statistically significant ($p < 0.01$ Chi squared, yates correction, equals 377.489 with 3 degrees of freedom.).
The overall screening participation, including patients in the study and patients that had responded to the first call were: 45% in 2012, 49% in 2013, 53% in 2014.

## Discussion

The results of our study showed that receiving any kind of intervention in addition to invitation and mailed reminder from Screening Center can significantly affect uptake of CRC screening.
Public screening programs must achieve high compliance to be effective and efficient, yet participation is low in many countries despite standard invitations and recall systems. As high participation in screening is the primary goal of all organised programs, more and more attention has been paid recently to how to engage citizens in public health programs [11]. A systematic review showed that individuals who previously participated in screening were more likely to be screened subsequently, so efforts could be focused on identifying and encouraging attendance among those who have never previously participated in screening [12]. Several interventions have been proposed to increase participation. Scientific evidence confirmed that organised screening programs, based on invitation letter or on GP involvement, were consistently effective in increasing participation compared to spontaneous screening [9]. Although among the measures to increase participation in organised screening there is solid evidence of a modest positive effect of interventions such as postal reminders, telephone calls, GP signing the invitation, it is still controversial the efficacy of active GP involvement [9, 13-15].
In our case, the results show clearly that even a modest intervention, conducted either by the GP or by an assistant, can have a significant effect in improving compliance to screening for CRC, one of the cancers with highest incidence in developed countries, for which it is available an effective treatment if diagnosed early. Moreover, in 2013 screening uptake seemed to increase accordingly to GP's activity level and the result was significant also for groups with intermediate level of activity in comparison with the one with low or no activity.
It is important to underline how screening uptake increase without GP intervention from 16.7% in 2013 to 20.4% in 2014 may be due to the introduction of a second reminder invitation from ASL for non-responder patients. A possible explanation for the uptake improvement following the introduction of the second reminder can be that this kind of intervention is likely to involve people already disposed to participate in screening program. Conversely, GP intervention can be more effective in involving patients less prone to participation through a targeted counselling. The effect of second reminder alone can be showed in patients without intervention: uptake improves from 16.7% to 20.4% (+3.4%) in pa-

tients of GPs joining ASL project, and from 13.7% to 16.8% (+3.1%) in patients of GPs not joining ASL project. So we can suggest that the second reminder can help to improve the uptake of 3% in patients non responder. The effect of second reminder looks to be hide by the interventions of the GPs or their assistants. To confirm this hypothesis and compare these possible effects, a further study with a four-arm design is required.
Among limitations concerning our study, the observational design instead of a randomized controlled trial can limit the strength of the results. In fact the method of reminder (call, interview, mail) were simply due to a choice of the GPs. Furthermore the GPs were not randomized but they decided to join or not to ASL project. In this way probably GPs more interested in "CRC screening" attended ASL project. An other limit of this study is that we can't describe socio-economical status of responders/non responders.
But we have to consider that others studies [9, 10] already said us that the provider intervention is effective to improve uptake to screening. This study wants to show how literature suggestions can be applied to real setting. In fact our aim was to describe and evaluate the implementation of evidence-based strategies to increase CRC screening uptake. With this study we want to share our result to starts a factual benchmark with other "screening centers".

## Conclusions

In conclusion, our findings stress the pivotal role of health providers in counseling and could be relevant also in the light of the recent Italian reform concerning territorial health care, according to which similar interventions could be systematically operated by health care professionals other than GPs [16]. Moreover, Lombardy is going to review its Health System to improve the organisation of GPs and this could further facilitate the implementation of counseling concerning cancer screening.

## Acknowledgements

The project was realized with the collaboration of GPs who joined the study. The collection of data and the computational assistance were provided by the Screening Centre of ASL Milano 1 area. The Authors have no conflict of interest to declare.

## Authors contributions

Study conception and design: DC, GB, PC, MEP. Acquisition of data: DC, GBi, PC. Analysis and interpretation of data: EG, AJBi, DC. Drafting of manuscript: EG, AJB. Critical revision: EG, PC, AJBi, MP

■ Received on October 26, 2015. Accepted on June 8, 2016.

■ Correspondence: Eugenia Gabrielli, Public Health, Department of Biomedical, Health Sciences, University of Milano, via Pascal 36, Milan, Italy - E-mail: eugenia.gabrielli@unimi.it

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