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# Seroprotection after hepatitis B vaccination in children aged 1 to 15 years in central province of Iran, Semnan

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## Key words

Hepatits B antibody • Vaccination • Children

## Summary

**Introduction.** *There are controversies over the long-term persistence of post vaccination immunity to hepatitis B and the need for booster doses of the vaccine. The aim of this study was to verify antibody levels of antibody against hepatitis B virus surface antigen (anti-HBs) in children aged 1 to 15 years who received vaccination against hepatitis B in the central province of Iran, Semnan.*
**Materials and methods.** *We performed a seroepidemiological survey (n = 210) of anti-HBs in 2011 in the central province of Iran, Semnan using enzyme-linked immunosorbent assay (ELISA). The levels of anti-HBs < 10 mIU/mL were considered to be nega-*

*tive and samples showing an anti-HBs titer ≥ 10 mIU/mL was considered protective.*
**Results.** *Protective antibody levels were detected in 88% of the children less than 5 year after vaccination, decreased to 78% between 5 to 10 years after vaccination, and further declined to 74% in 10 years after vaccination, respectively.*
**Conclusion.** *The vaccination program has been proven effective in Semnan and immunological protection against hepatitis B infection was found in the majority of children even more than 10 years after being vaccinated.*

## Introduction

The vaccine against hepatitis B virus (HBV) is included in the routine immunization schedule for children in most countries with the ultimate goal of reducing the prevalence of chronic hepatitis B carriers, as well as preventing the occurrence of acute hepatitis B [1]. Although long-term reduction of chronic HBV after hepatitis B vaccination has been reported [2], decreasing the levels of antibody against hepatitis B surface antigen (anti-HBs) over the time can be alarming [3].

Hepatitis B virus (HBV) prevalence has decreased dramatically in Iranian population since 1993 when the mass vaccination program was started. The geographic distribution of HBV infection in Iran showed heterogeneous patterns of HBV prevalence from the highest prevalence rates of more than 3% in northeastern region of our country to less than 2% in central and western regions of Iran [4].

Several hundred million doses of plasma-derived HB vaccines are produced in the Republic of Korea, China, Vietnam, Myanmar, India, Indonesia, Iran and Mongolia [5, 6]. Engerix-B® (SmithKline Beecham, 1992) and Recombivax HB® (Merck & Co.) are considered as the two major yeast-derived hepatitis B vaccines that are licensed in most countries [5].

There are controversies over the long-term persistence of post vaccination immunity to HBV and the need for booster doses of the vaccine [7].

The aim of this study was to verify antibody levels of anti-HBs antibodies in children aged 1 to 15 years who received vaccination against HBV in accordance with the standard method in the central province of Iran, Semnan.

## Materials and methods

In this cross-sectional study, all children between ages of 1 and 15 years residing in Semnan, Iran in Amiralmoemenin hospital were tested for anti-HBs during 2009.

Informed consent was obtained from all children and/or their parents or guardians who agreed to participate in the study. The questionnaire was completed about the child's general data (e.g., family history of contact with HBV and knowledge about the possibility for the child to have any immunosuppressive disease, such as HIV, type 1 diabetes mellitus, or chronic renal failure).

We included immunocompetent participants without history of previous HBV infection. The participants were excluded from the study on the basis of the following criteria: (a) were not screened for serologic markers of HBV infection (HBsAg) before vaccination; (b) born to HBsAg carrier mothers; (c) had predisposing factors for any immunosuppressive disease such as HIV positive.

After blood sample collections, plasma samples were collected and tested for anti-HBs using enzyme-linked immunosorbent assay (ELISA)(Delaware Biotech Inc.

Dover, DE, USA) following the manufacturer's protocol.

The antigen and antibody formed a sandwich complex with the conjugated antibodies with the peroxidase (horseradish peroxidase) and the enzymatic activity was detected with the specific chromogen/substrate 3,3',5,5' -tetramethylbenzidine (TMB).

The TMB levels were quantified at 450 nm and the concentrations of the anti-HBs were determined on the standard curve. The levels of anti-HBs < 10 mIU/mL were considered to be negative and samples showing an anti-HBs titer ≥ 10 mIU/mL was considered protective [1].

The Chi-square test and Fisher's exact test were used with the SPSS 16 Package program (Chicago, IL, USA). Data were presented as mean ± SD or, when indicated, as an absolute number and percentage. Student's t-test was used for statistical analysis to compare the means between the two groups.

## Results

A total of 210 children were participated in this study. Totally, 67 cases (32%) were under 5 years, 67 (32%) were between 5 to 9 years old and 76 (36%) were more than 10 years. The male to female ratio was 1. Distribution of anti-HBs levels according to sex, age and duration after vaccination are shown in Table 1. Eighty four cases (80%) in the female group and 82 cases (78%) in the male group had protective levels of anti-HBs > 10 mIU/mL, with no statistically significant difference in anti-HBs positivity and genders (p = 0.735). Anti-HBs positivity was seen in 87% of cases less than 5 years, 81% of cases between 5 and 10 years and 71% of cases more than 10 years.

Protective antibody levels were detected in 88% of the children less than 5 year after vaccination, decreased to 78% between 5 to 10 years after vaccination, and further declined to 74% in 10 years after vaccination, respectively.

## Discussion

The immunity derived from the HBV vaccine was assessed by measuring the antibody in 210 children who were vaccinated in a routine vaccination program in central province of Iran, Semnan.

In our study, among 210 cases, 166 children (79%) had antibodies levels ≥ 10 mIU/mL. Protective antibody levels were detected in 88% of the children less than 5 year after vaccination, 78% in cases between 5 to 10 years after vaccination, and 74% in cases at 10 years after vaccination. Generally, 3-30% of vaccinated individuals lost their protective anti-HBs titres five years after the hepatitis B vaccination [8]. Long-term follow-up studies demonstrated that antibodies might become negative in 15-50% among the vaccine responders within 5 to 10 years [9, 10].

According to several studies among healthy children who had received a complete hepatitis B immunization program, the protective titer of anti-HBs antibody > 5 years after the last dose were seen in 50-100% of individuals [7, 11-13]. It has been reported that the variability in the anti-HBs antibody might be due to is the type of vaccine used, the amount of antigen delivered and the population immunized [14-17].

The HBV vaccination started in infants in two provinces (Zanjan and Semnan) in 1989, and since 1993 the vaccination was introduced in the expanded program on immunization in Iran. After implementation of HBV vaccination in our country, the coverage has reached an appropriate level 94% in 2005 compare with 62% in 1993 [18].

Jafarzadeh et al. found that 81.5% of children had protective levels of antibody [19] at five years after primary hepatitis B immunization while 47.9% of children had protective levels of antibody 10 years after primary vaccination [20].

In Aghakhani et al. study, protective antibody levels were detected in 65% of children one year after vaccination, which declined significantly over time to 24% in 15 years after vaccination [21].

Tab. I. Distribution of anti-HBs levels according to sex, age and duration after vaccination.

|  |  | Anti-HBs ≥ 10 mIU/mL | | Anti-HBs < 10 mIU/mL | | Total |
|---|---|---|---|---|---|---|
|  |  | N | % | N | % | N |
| Sex | Male | 82 | 78 | 23 | 22 | 105 |
|  | Female | 84 | 80 | 21 | 20 | 105 |
| Age | < 5 years | 58 | 87 | 9 | 13 | 67 |
|  | 5-10 years | 54 | 81 | 13 | 19 | 67 |
|  | ≥ 10 years | 54 | 71 | 22 | 29 | 76 |
| Duration after vaccination | < 5 years | 62 | 87 | 9 | 13 | 71 |
|  | 5-10 years | 54 | 78 | 15 | 22 | 69 |
|  | ≥ 10 years | 52 | 74 | 18 | 26 | 70 |

In Gilca et al. study, 88.2%, 86.4% and 76.7% of cases had a titer ≥ 10 IU/L after 5, 10 and 15 years post-vaccination [13].

In our study similar to other studies, no differences were observed between sex, age and anti-HBs titer following the vaccination [8, 21].

There are controversies over the long-term persistence of post vaccination immunity to hepatitis B. According to meta-analysis, protection which was provided by three or four doses of monovalent HB vaccine persists for at least two decades in the great majority of immunocompetent individuals and 3 doses of HB vaccine ensure a good protection against infection for up to 20 years [2], while some studies recommend a need for booster dose of vaccine in our country [20, 21]. Although a booster dose increases substantially anti-HBs titers, the clinical relevance of such an increase remains unknown.

In conclusion, the vaccination program has been proven effective in Semnan and immunological protection against HBV infection was found in the majority of children even more than 10 years after being vaccinated.

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# The colorectal cancer screening program in the Local Health Unit n. 6 of Livorno: evaluation of the screening activity in the period 2000-2011

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## Key words

Faecal occult blood test • Colorectal cancer screening program • Evaluation

## Summary

**Introduction**. *The colorectal cancer screening program in the Local Health Unit n. 6 of Livorno is running since July 2000 and is meant to residents, aged between 50 and 70, who are invited to perform the test for faecal occult blood every 2 years. The aim of this work is an evaluation of the screening activity in the period 2000-2011.*

**Methods**. *The evaluation is based on the analysis of the main quality indicators formulated by GISCoR (Italian Group for Colorectal screening).*

**Results**. *The screening activity extension reached 93% in 2006 and 100% in 2009. The compliance level was maintained above the acceptable GISCoR value (> 45%) with a maximum of 54.9%. Values around 80% were recorded for the compliance to colonos-*

*copy. The detection rate (DR) for cancer and advanced adenoma showed, as expected, the highest values in the early years and then move on values consistently lower than the regional average. In 2011, the raw DR for cancer was 0.9 x 1000 and the raw DR for advanced adenoma 5.3 x 1000.*

*The distribution by stage at diagnosis of screen-detected carcinomas shows that 58.1% of these were identified at stage I while the proportion of cases in stage III+ is 19.5%.*

**Conclusions**. *The overall analysis shows a good performance of the program. The proportion of colonoscopies performed on the total number of positive subjects remains a critical point of the system. The distribution by stage of screen-detected cancers shows an excellent diagnostic anticipation of the screening program.*

## Introduction

Colorectal cancer (CRC) is the most common newly-diagnosed cancer and the second most common cause of cancer deaths in Europe [1]. Screening for colorectal cancer using the faecal occult blood test has proven effective in reducing mortality from colorectal cancer [2]. Randomized trials in people of average risk invited to attend screening have shown a reduction in cause-specific mortality [3-6] and incidence [6, 7].

The Tuscany Region (TR) with the Deliberations n. 18, dated Feb. 3rd, 1998, and n. 24, dated Jan. 11th, 1999, has issued "Guidelines for Local Health Authorities in relation to cancer screening programs for cervical, breast, colorectal cancer and melanoma". According to those regional acts, the local health authorities all over the Region were required to activate cancer screening programs or to implement existing ones.

By the Decree of the President of the Council of Ministers, dated Nov. 29th, 2001, the screening of colorectal cancer has been defined an Essential Level of Care. In Local Health Unit (LHU) Livorno colorectal cancer screening has been running since July 2000.

The objective of this work is an evaluation of the screening activity in the period 2000-2011, through the analysis of the main indicators of quality set by the Italian Group for the Colo- Rectal Cancer Screening (GISCoR) [8] and

subject to monitoring by the Regional Reference Centre (RRC- ISPO).

## Methods

The program is aimed at people aged 50-70 years living (registered residence) in the territory of the Local Health Unit of Livorno who are invited to perform the faecal occult blood test (FOBT) every 2 years. The annual average target population of the period considered was 48 011 (min. 46 889, max. 48 980). The organizational model includes the recruitment by invitation letter signed by the Coordinator of Medical Screening Centre and the General Practitioner. The letter indicates the location (Social Health Centre) and the time of the delivery of the faecal occult blood test kit; at the same time are indicated the modes of redelivery. The test kits returned are sent to laboratory analysis, which employs the method of processing by latex agglutination for automatic reading and evaluation of the positivity with a cut-off of 100 ng/ml. Subjects with negative results are advised by mail accompanied by a letter of the Screening Centre which informed them that they will be invited to repeat the test in 2 years. Subject with positive results are contacted by telephone by health care workers of the Screening Centre, invited to a counselling by the Centre

and initiated to the subsequent diagnostic and therapeutic steps.

The program, like all cancer screening programs in the Tuscany Region, is the subject of continuous monitoring and epidemiological evaluation by the Regional Reference Centre through the annual production of indicators which become the basis for the drafting of an Annual Report that summarizes the activity and the quality of all the regional screening programs. In this paper the analysis considered the following indicators: *extension of invitations*: refers to the ratio between the number of subjects invited and the total number of eligible subjects in the period [8]; *compliance* to invitation: refers to the ratio between the number of subjects respondent and the total number of subjects invited [8]; *FOBT positivity rates*: represents the percentage of people with a positive test result on the total number of people tested [8]; *compliance to colonoscopy*: represents the percentage of people who have conducted the colonoscopy on the total number of people invited to conduct the examination [8]; *completeness rate of colonoscopies*: it is an indicator of the examination quality and represents the proportion of complete colonoscopies performed (caecal intubation) [8]; *detection rate for cancer and advanced adenoma*: this indicator expresses the ratio between the number of subjects diagnosed with cancer or advanced adenoma identified at screening and the number of subjects screened [8]; *positive predictive value* (PPV) *for cancer and advanced adenoma*: expresses the ratio between the number of subjects with histologically confirmed diagnosis of cancer or advanced adenoma or simple adenoma and the total number of subjects who performed colonoscopy after positive faecal occult blood testing [8]; *the stage at diagnosis* of screen-detected carcinomas.

## Results

In 12 years of activity of the programm there was a progressive increase of the extension with the attainment of the desirable standard as early as 2002 (GISCoR Standard: acceptable > 80%; desirable > 90%). Beyond the fluctuations due to changes in the allocation of resources, the level of extension is always maintained at high values and from 2009 has stabilized at more than 100%. The overall compliance, adjusted to subjects excluded after invitation and invitations without result, except for the first two years of program activation remains still above the rate considered acceptable in the GISCoR manual [8](Standard GISCoR: acceptable > 45%; desirable > 65%). The general trend shows an increase over the period and with values ranging from 38% in 2000 to 49.5% in 2011 an with a maximum of 54.9% in 2007. In the early activation of the screening program, the percentage of positive test is found to be higher than the regional average due to the use of a test with a lower specificity (Tab. I). The transition to a test of specificity in line with other tests used in the region, has led to positive values within the standards set forth by GISCoR [8]. Disaggregated data for the first and repeated screening was available from 2003 onwards.

From 2004 onwards, the positivity rate at repeated screening is, as expected, lower than those of the first screening (screening of prevalence), with some exception attributable to random fluctuations (Standard GISCoR: *first screening* acceptable < 6%; desirable < 5%, *repeated screening* acceptable < 4.5%; desirable < 3.5%).

About the compliance to the colonoscopy (GISCoR standards are > 85% acceptable and > 90% desirable) the graph (Fig. 1) shows that there was an initial gap in Livorno program compared to the regional average. From 2003 onwards, the rates align perfectly with the regional average. However, they remain below the acceptable standard pointing out a permanent feature related to the fact that about 20% of those with a positive test does not perform colonoscopy exam or, at least, the program does not become aware of it. Linking compliance to colonoscopy with sex and screening history (Fig. 2), it can be observed that men slightly adhere more than women to colonoscopy exam. Even the history of screening seems to have some influence on this indicator with a greater compliance of the subject at repeated screening, probably due to greater confidence in the program.

A complete scan of the colon is important because approximately 30% of carcinomas has a proximal location. For the Local Health Unit Livorno in the period under review the total of observed values are maintained at levels around the acceptable standard (GISCoR standards are: > 85% acceptable, > 90% desirable).

In Figure 3 and Figure 4 global Detection Rates (DR) (first screening + repeated screening) are presented for a need for

**Tab. I**. FOBT Positivity rates. Period 2000-2011: trends overtime and comparison with the average of the Tuscany Region.

| | LHU 6 | | T.R. | |
|---|---|---|---|---|
| Year | First screening + Repeated screening | | First screening + Repeated screening | |
| 2000 | 8,8 | | 5,7 | |
| 2001 | 6,8 | | 5,8 | |
| 2002 | 6,3 | | 5,0 | |
| | First screening | Repeated screening | First screening | Repeated screening |
| 2003 | 4,2 | 4,5 | 5,2 | 4,0 |
| 2004 | 4,2 | 3,7 | 4,7 | 3,8 |
| 2005 | 4,7 | 3,8 | 5,1 | 4,1 |
| 2006 | 4,0 | 3,1 | 5,2 | 3,9 |
| 2007 | 4,2 | 3,4 | 5,0 | 3,9 |
| 2008 | 4,8 | 4,4 | 5,2 | 4,3 |
| 2009 | 4,3 | 3,9 | 5,2 | 4,1 |
| 2010 | 4,0 | 4,1 | 5,4 | 4,0 |
| 2011 | 3,5 | 3,8 | 4,8 | 4,0 |

Fig. 1. Compliance to colonoscopy. Period 2000-2011 (first screening + repeated screening): temporal trends and comparison with the average of the Tuscany Region.
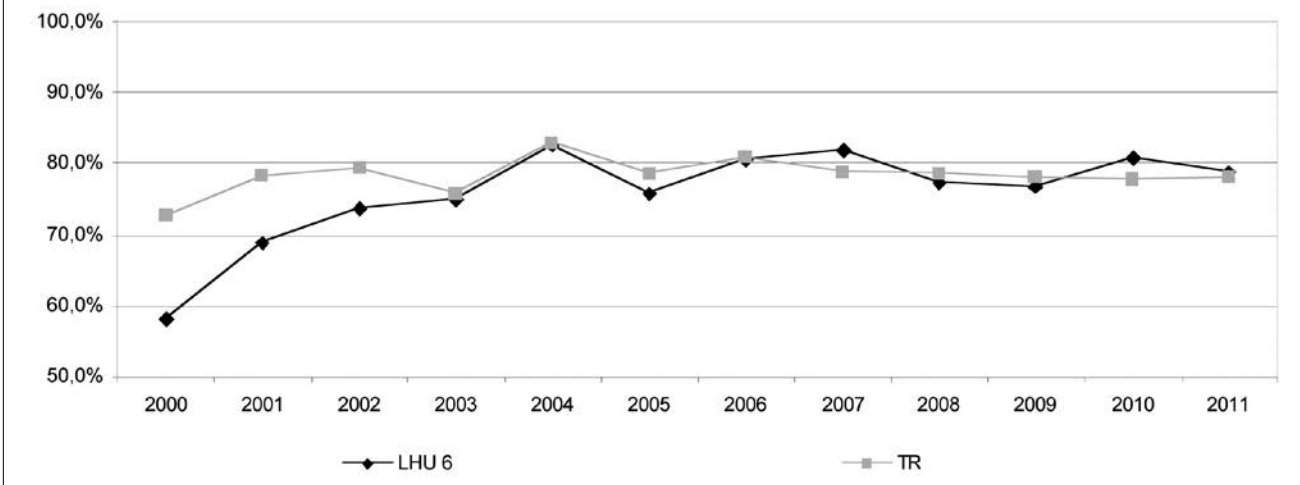


comparison with the regional average and a relatively low number of cases. Detection Rates adjusted for the compliance to colonoscopy are also calculated in order to allow a better comparison between years and to highlight the contribution of this to the overall effectiveness of the program. In the early activation of the screening program in Local Health Unit Livorno the highest values of DR for cancer and advanced adenoma had been observed, in line with the regional average and compatible with the initial phase of a screening program. It is evident from the graphs that the rates recorded in Livorno are consistently lower than the regional average, even after adjustment for compliance to colonoscopy and that there is, as expected, a trend to a decrease in the time of the DR for cancer (Fig. 3). A variability in the years of the DR for cancer and advanced adenoma is also noted; a different age distribution of the populations screened, as the data is not standardized for age, but also the low number of cases could contribute to this.

The PPV for cancer and advanced adenoma is consistently higher than that of simple adenomas during the period under consideration (Fig. 5). The analysis of the period under review showed, as expected, a very slight decline in the predictive power for cancer and a slight increase of the predictability for advanced adenoma and simple adenoma.

Overall, in 12 years of activity of colorectal cancer screening in the territory of Livorno were diagnosed 1051 simple adenomas, 1389 advanced adenomas and 285 invasive adenocarcinomas. For a significant proportion of cases (no. 75) it was not possible to retrieve information about the stage (Tab II). The distribution by stage of diagnosed cases, however, shows that just under half of these have been identified at the STAGE I, while the proportion of cases in STAGE III+ is 14.4%, definitely within the desirable GISCoR standard (GISCoR standard: acceptable < 30%; desirable < 20%). If the analysis is performed only on cases with known stage (Fig. 6) the

Fig. 2. Compliance to colonoscopy. Period 2000-2011 by sex and history of screening.
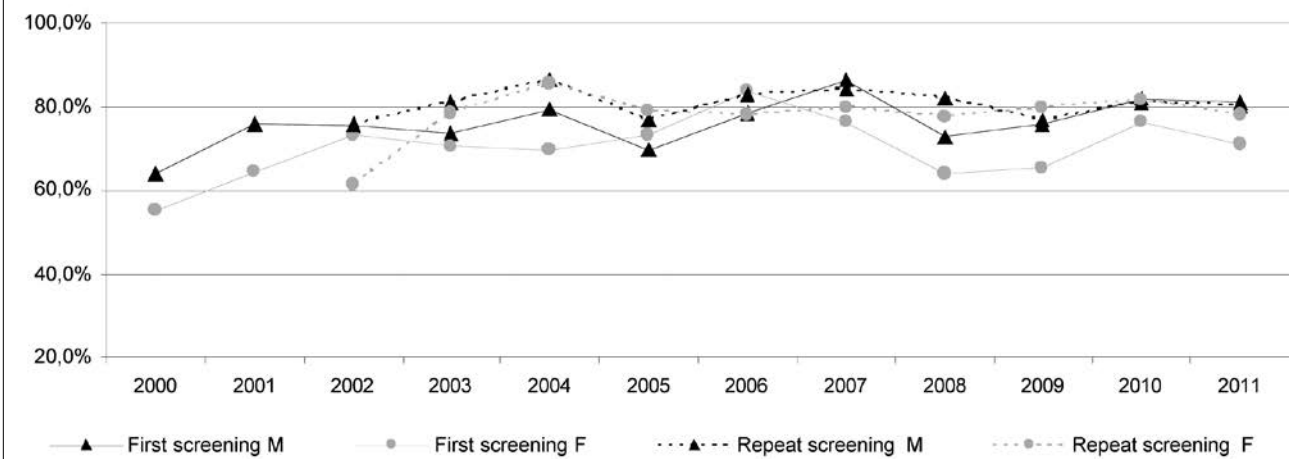
**Fig. 3.** Detection Rate (raw DR x 1.000 ) for cancer. Period 2000-2011 (first screening + repeated screening): temporal trends and comparison with the average of the Tuscany Region.



**Fig. 4.** Detection Rate (raw DR x 1,000) for advanced adenoma. Period 2000-2011 (first screening + repeated screening): temporal trends and comparison with the average of the Tuscany Region.
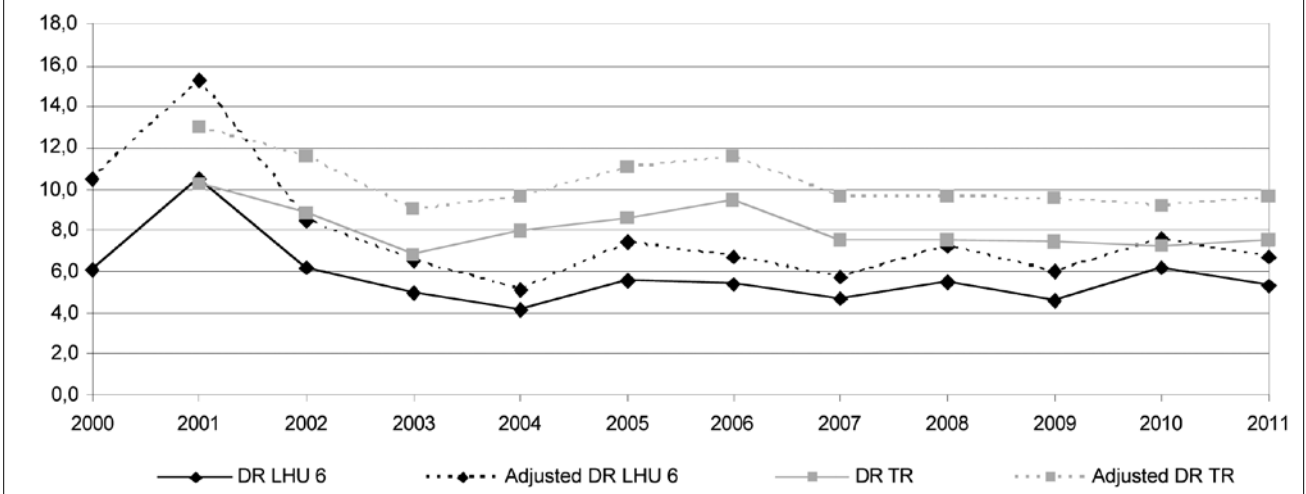
proportion of cases in STAGE I rose to 58.1% and that of cases STAGE III+ to 19.5% always remaining within the desirable GISCoR standard.

The criteria for the definition of colorectal cancer stage according to the TNM classification is summarized in Table III.

## Discussion

The analysis of the principal indicators includes a large period of time that allows to identify specific trends over time for all phases of the screening program. Extension and compliance are optimal and, especially for the extension, the values attest a consistent achievement of the target with the best performance among the regional and national scenario. "The compliance to the colonoscopy

is one of the key parameters to assess the impact of the program and its efficiency in reducing mortality" [8]. Its performances affect the overall diagnostic yield of screening intervention. The proportion of colonoscopies performed on the total number of FOBT positive subjects remains a critical point of the system at both local and regional level and should be the aim of all possible organizational efforts. The latest survey of the National Screening Observatory [9], indicates a national compliance rate to colonoscopy of 81,4%, not far from the recorded data in Local Health Unit Livorno and Tuscany Region [10]. A suboptimal compliance to the exam has a negative impact on the effectiveness of screening, because it leads to a loss of a portion of cancers and advanced adenomas that will not be diagnosed. A part of the non-compliance can be explained by the investigations carried out outside of the screening circuit, for which serve an organizational

effort for the recovery of information. A part, however, is attributable to a lack of education on the screening program and should be improved through training the general practitioners and implementing the attention to counselling subjects resulting FOBT positive. This is a weak point of the program that can invalidate a substantial part of the expected benefit, and therefore deserves special attention.

The difference between raw and adjusted detection rate confirmed as a high adhesion to colonoscopy optimize the program efficiency, minimizing the missed diagnosis of advanced lesions and adenocarcinomas of the colon and rectum. The detection rate for cancer and for advanced adenomas is lower than the regional average, and this result does not seem explained by the low compliance to colonoscopy. The DR consistently lower than the regional average are not easy to interpret: they could be due to loss of cases or errors/incomplete registration, but it is not excluded that since low rates of DR are also recorded in neighbouring areas of Livorno [11] they are related to a different incidence/prevalence of disease in this area. It would be useful to carry out a specific survey on the different ways of classifying lesions and registration of data from other areas of Tuscany

Tab. II. Stage at diagnosis of screen-detected cancer in the period 2000-2011 (first screening + repeated screening).

| Year | N° of cancers | Stage I | Stage II | Stage III-IV | Unknown |
|------|------|------|------|------|------|
| 2000 | 17 | 6 | 2 | 3 | 6 |
| 2001 | 24 | 10 | 3 | 3 | 8 |
| 2002 | 26 | 12 | 3 | 5 | 6 |
| 2003 | 24 | 10 | 4 | 2 | 8 |
| 2004 | 21 | 6 | 2 | 5 | 8 |
| 2005 | 23 | 10 | 5 | 3 | 5 |
| 2006 | 22 | 10 | 2 | 4 | 6 |
| 2007 | 21 | 10 | 4 | 1 | 6 |
| 2008 | 30 | 13 | 7 | 5 | 5 |
| 2009 | 24 | 13 | 3 | 5 | 3 |
| 2010 | 32 | 18 | 6 | 2 | 6 |
| 2011 | 21 | 4 | 6 | 3 | 8 |
| Total | 285 | 122 | 47 | 41 | 75 |
| % | 100% | 42,8% | 16,5% | 14,4% | 26,30% |



Fig. 6. Distribution by stage at diagnosis of screen-detected carcinomas in the 2000-2011 period. Cases with known stage.

Stage III - IV 19,5%
Stage II 22,4%
Stage I 58,1%



Fig. 5. Positive Predictive Value (PPV x 100) for cancer, advanced adenoma and simple adenoma. Period 2000-2011 (first screening + repeated screening): temporal trends and comparison with the average of the Tuscany Region.

□ Simple Adenoma
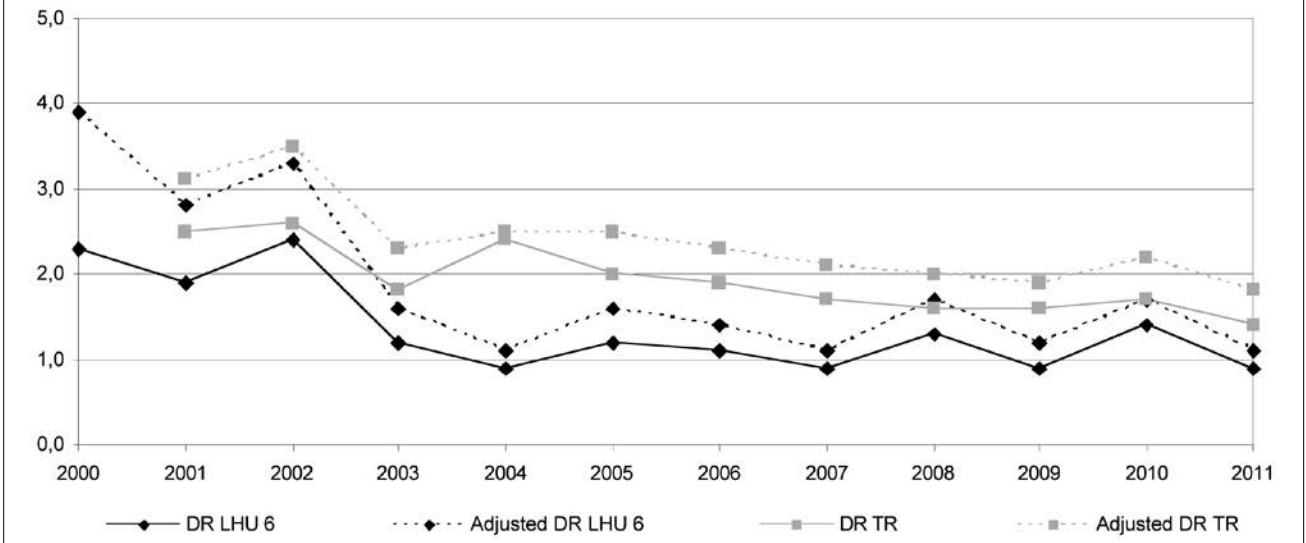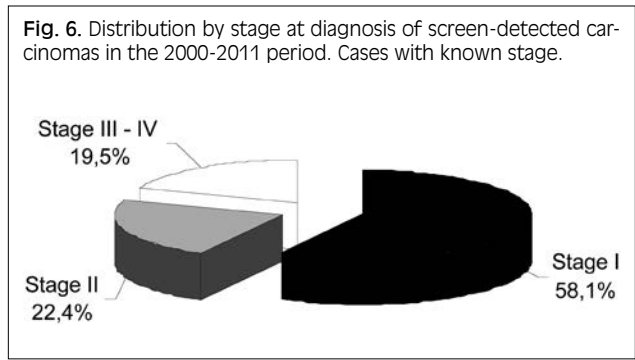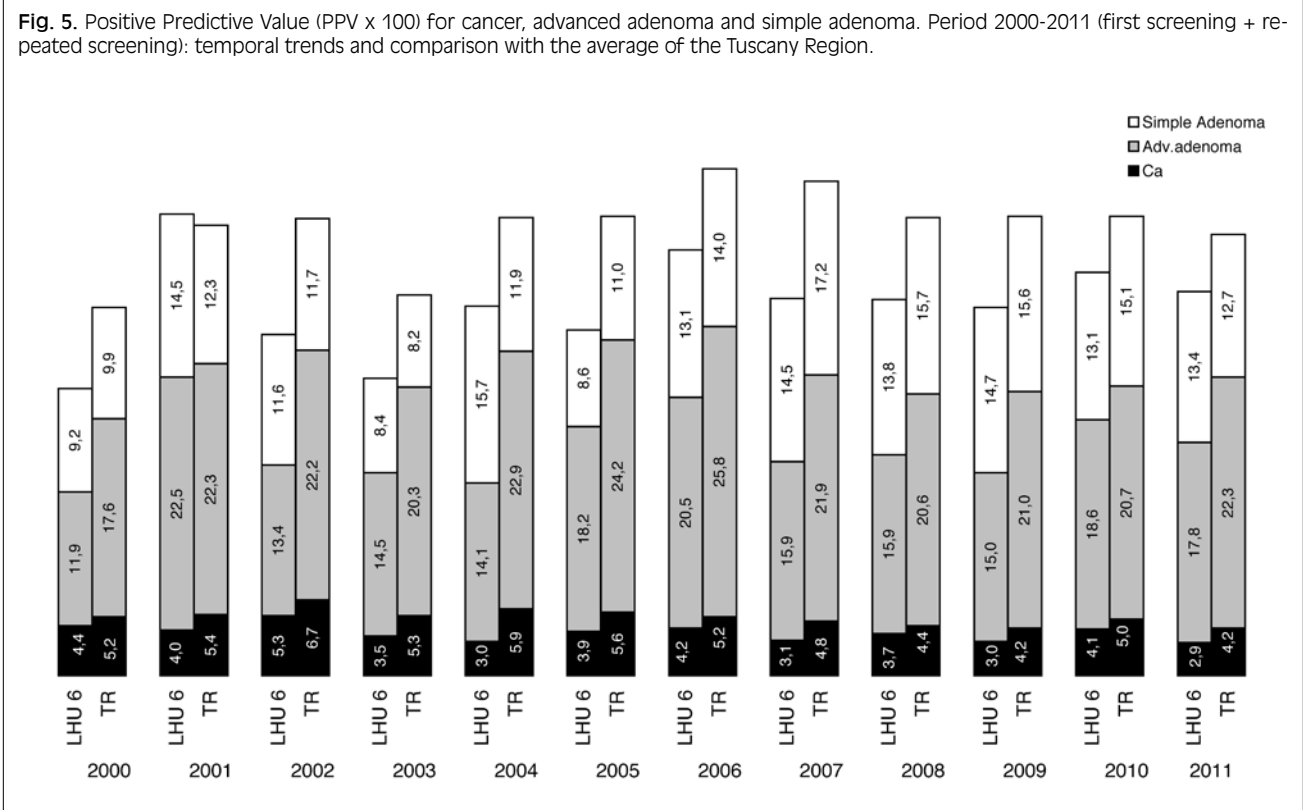◨ Adv.adenoma
■ Ca

Tab. III. Stage of colorectal cancer according to the TNM classification.

| Stage I | T1 or T2, N0, M0 |
|---------|------------------|
| Stage II | T3 or T4, N0, M0 |
| Stage III | Lymph nodes or distant metastasis |
| Stage IV | |

Region, in order to verify whether this anomaly can be traced, in whole or in part, to a selection bias of information. It is possible, once removed this suspicion, that this result is attributable to epidemiological differences that should be investigated.

Looking at the data of PPV confirms what has already been stated in the GISCoR Manual [8], i.e., the ability of the test to select subjects with significant lesions (cancer and advanced adenoma). Overall, in about a fifth of colonoscopies performed significant lesions are diagnosed. The distribution by stage of screen-detected cancers in the period proves an excellent diagnostic anticipation of the screening program in the Local Health Unit. n. 6 of Livorno. As a reference, in 2011 the stage distribution of screen-detected cancers in Tuscany Region is 46.6% of cases diagnosed in STAGE I and 17% of cases STAGE III+ [10]. The national data reported in the 10th Report of National Observatory Screening [9], calculated on cases with known stage, presents a proportion of cancers diagnosed in STAGE I ranging from 36.1% (at first screening) to 43.2% (at repeated screening) and a percentage of cases STAGE III - IV from 27.1% (at first screening) to 25.4% (at repeated screening).

## Conclusions

The comprehensive analysis highlights the good performance of the Livorno screening program over the years. The proportion of colonoscopies performed on the total number of positive subjects remains a critical point of the system. The distribution by stage of screen-detected cancers shows an excellent diagnostic anticipation of the screening program.

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# Safe meat-handling knowledge, attitudes and practices of private and government meat processing plants' workers: implications for future policy

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## Summary

**Introduction.** *Food-borne disease outbreaks remain a major global health challenge and cross-contamination from raw meat due to poor handling is a major cause in developing countries. Adequate knowledge of meat handlers is important in limiting these outbreaks. This study evaluated and compared the safe meat-handling knowledge, attitudes and practices (KAP) of private (PMPP) and government meat processing plants' (GMPP) workers in south-western Nigeria.*

**Methods.** *This cross sectional study comprised 190 meat handlers (PMPP = 55; GMPP = 135). Data concerning their safe meat-handling knowledge, attitudes and practices as well as their socio-demographic characteristics, such as age, gender and work experience were collected.*

**Results.** *A significant association was observed between the type of meat processing plants and their knowledge (p = 0.000), attitudes (p = 0.000) and practices (p = 0.000) of safe meat-handling. Meat handlers in the GMPP were respectively, about 17 times (OR = 0.060, 95%CI: 0.018-0.203), 57 times (OR = 0.019, 95% CI: 0.007-0.054) and 111 times (OR = 0.009, 95%CI: 0.001-0.067) less likely to obtain good knowledge, attitude and practice level of safe meat-handling than those from PMPP. Further, KAP levels were significantly associated with age group, education and work experience (p < 0.05).*

**Discussion.** *Study findings suggest the need for future policy in food industry in developing countries to accommodate increased involvement of private sector for improved food safety and quality delivery. Public health education on safe food handling and hygiene should be on the front burner among food handlers in general.*

## Introduction

Food-borne illness remains a significant source of human disease [1]. Recent food safety failures have attracted widespread attention resulting in public confusion and mistrust of the food industry and regulators [2]. Food-borne diseases have caused a significant morbidity and mortality around the world [3]. World Health Organization (WHO) reports that 18% of children aged below 5 years old in developing countries die due to diarrhea globally [4].

Meanwhile, food contamination from raw meat is an important cause of food-borne disease outbreaks or food poisoning [5] due to improper food handling. Such contaminations often occur when food that does not require cooking such as salad is prepared on the same chopping board that has been used to prepare raw meat without adequate washing [3]. Cross-contamination can also occur when raw meat is stored above ready-to-eat meals. Thus, separating raw and cooked food and using safe raw materials are some of the five main keys to safer food as developed by the World Health Organization [6].

On the other hand, however, the potential contaminating effects from meat can be limited with proper handling by the meat handlers. As reported, food handlers are a major cause of food contamination [7]. Food-borne disease outbreaks reported in the United States for instance were associated with mishandling; with 79% from commercial or institutional establishments and 20% from homes [8]. Another report indicated the presence of *Escherichia coli* and *Staphylococcus aureus* on the hands of food handlers [9] while multi-drug resistant *Staphylococcus aureus* has been isolated from meat being sold for human consumption [10].

Despite the fact that the international food management agencies have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices [11], the knowledge and perceptions of meat handlers on safe food handling in most developing countries particularly Nigeria remain largely unknown. Most studies [12, 13] conducted were based on food handlers in the restaurants, processed food establishments without any documented report on meat handlers; whereas, cases of food poisoning due to contaminated meat have been on the rise in recent years. The objective of this study was to evaluate and compare the safe meat-handling knowledge, attitudes and practices (KAP) of meat handlers between private and Government meat processing plants in south-western Nigeria. In addition, this paper was aimed at determining the relationship between the socio-demographic characteristics of the meat handlers and their KAP level.

## Methods

### STUDY SITE, DESIGN AND POPULATION

The study was carried out in Ibadan (7°21°N, 3°54°E), south-western Nigeria. This site is known with relatively high demand for meat [14], with meat being part of almost every diet. It has a high throughput municipal abattoir and other slaughter slabs owned by the Government in addition to privately owned meat processing plants (PMPP). This cross sectional study involved a total of 190 meat handlers. Of these, 135 from a population of 200 meat handlers in the Government meat processing plant (GMPP) and 55 from five of the 11 Local Government Areas where most PMPP were located volunteered to participate in the study after the study objectives had been communicated to them with technical assistance from the Veterinary Officer of the meat processing plant.

### QUESTIONNAIRE ADMINISTRATION

A self-administered semi-structured questionnaire was designed to obtain data on meat handlers' knowledge, attitudes and practices of safe meat handling. The questionnaire included four parts. In the first part, we attempted to determine their socio-demographic profiles, with the ages of the respondents categorized into three groups while educational levels were classified into "none" (no formal education), low (received only primary education) and "high" (received education up to secondary or tertiary level). In addition, working experiences were categorized into four different levels. The second part had ten questions to determine their knowledge on safe meat handling. The third and fourth parts contained seven and ten questions to respectively determine their work place practices and attitudes to safe meat handling. A pre-test was carried out after which some of the questions were modified in order to improve clarity. The potential participants were informed that they could choose either to participate in the study or not to. Consent was therefore obtained by their affirmative response to participation in the study.

### DATA ANALYSIS

Data were analyzed using Stata 12. Knowledge and attitudes were scored with reference to answers to ten questions each. Correct responses were scored 1 and incorrect 0 and scores ranged between 0 and 10. Scores ≥ 5 were taken as good knowledge or attitudes while scores < 5 were taken as poor knowledge or attitudes. Practices related to safe meat handling were similarly scored based on seven questions with scores ≥ 4 taken as good practices and scores < 4 as poor practices. Chi-square test was used to determine the relationships between the socio-demographic characteristics of the meat handlers and their KAP levels and between the KAP levels and the type of meat processing plants. Statistical significance was assessed using $p$-values and all results were considered significant if $p ≤ 0.05$. All variables significant at 10% significance level were included in the multivariate logistic regression model to determine the predictor variables for food handlers' KAP level. The odds ratios (OR) were reported with their 95% confidence intervals (CI).

## Results

### SOCIO-DEMOGRAPHICS OF THE PARTICIPANTS

The results show that more than 70% of the meat handlers were from GMPP and those within the age group 31-50 years comprised 47.4%. While 70% were males, 36.8% had low education and 35.3% had between 11-26 years of working experience.

Tab. I. Knowledge of safe meat-handling amongst meat handlers in private and Government meat processing plants.

| Statements | Private | | Government | |
|---|---|---|---|---|
| | Correct answers (%) | Incorrect answers (%) | Correct answers (%) | Incorrect answers (%) |
| Heard of the term HACCP. | 23.6 | 76.4 | 0.0 | 100.0 |
| Improper handling of meat could pose health hazards to consumers. | 81.8 | 18.2 | 91.1 | 8.9 |
| Insects and pests could be a source of contamination to raw meat. | 81.8 | 18.2 | 65.2 | 34.8 |
| Knew the symptoms associated with food poisoning. | 100.0 | 0.0 | 4.4 | 95.6 |
| Knew the causes of food borne illness. | 63.6 | 36.4 | 56.3 | 43.7 |
| Regular washing of hands during meat processing reduces risk of contamination. | 72.7 | 27.3 | 94.1 | 5.9 |
| High temperature or freezing is a safe method to destroy bacteria. | 80.0 | 20.0 | 70.4 | 29.6 |
| People with open skin injury, gastroenteritis, and ear or throat diseases should not be allowed to handle meat. | 85.5 | 14.5 | 31.1 | 68.9 |
| Washing and disinfection of working surfaces and tools are important to safety of meat. | 87.3 | 12.7 | 34.8 | 65.2 |
| Regular rotation of disinfectants for cleaning can reduce the risk of meat contamination from working surfaces and cutting tools. | 50.9 | 49.1 | 1.5 | 98.5 |

## KNOWLEDGE LEVEL OF MEAT HANDLERS ON SAFE MEAT-HANDLING

The majority from PMPP gave correct answers to most questions asked while those who gave correct answers from GMPP were only above average in five out the ten questions asked (Tab. I). Table II shows that 63.7% had good knowledge of safe meat-handling with significantly higher proportion of 94.6% from PMPP and 51.1% from GMPP ($X^2 = 31.877$; $p = 0.000$); the meat handlers in GMPP (OR = 0.060, 95%CI 0.018-0.203) being about 17 times less likely to demonstrate good knowledge than those from PMPP (Table III). In all, the age ($X^2 = 8.531$; $p = 0.014$), education ($p = 0.009$) and working experience ($p = 0.002$) were signifi-

cantly associated with knowledge level of safe meat handling (Tab. II). Meat handlers within age groups 31-50 years (OR = 0.387, 95%CI: 0.203-0.738) and ≥ 51 years (OR = 0.621, 95%CI: 0.204-1.886) were about 2.5 and 1.6 times less likely to obtain good knowledge level than those within 15-30 years. Similarly, those with high educational level (OR =1.187, 95%CI: 0.845-4.145) were about two times more likely than those without any formal education. In addition, meat handlers within 4-10 years working experience (OR = 0.269, 95%CI: 0.089-0.813) and 11-20 years (OR = 0.147, 95%CI: 0.051-0.422) were respectively 3.7 and 6.8 times less likely to obtain good knowledge level than those within ≤ 3years (Tab. III).

Tab. II. Relationship of meat handlers' knowledge, attitude and practice levels on safe meat-handling and their socio-demographic characteristics.

| Variables | Knowledge | | | | Attitudes | | | | Practices | | | | Total n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Good n (%) | Poor n (%) | $X^2$ | $p$-Value | Good n (%) | Poor n (%) | $X^2$ | $p$-Value | Good n (%) | Poor n (%) | $X^2$ | $p$-Value | |
| **Types of meat plants** | | | | | | | | | | | | | |
| Private | 52 (94.6) | 3 (5.4) | 31.877 | 0.000[a] | 50 (90.9) | 5 (9.1) | 92.440 | 0.000[a] | 54 (98.2) | 1 (1.8) | 67.313 | 0.000[a] | 55 (28.9) |
| Government | 69 (51.1) | 66 (48.9) | | | 22 (16.3) | 113 (83.7) | | | 44 (32.6) | 91 (67.4) | | | 135 (71.1) |
| **Age group** | | | | | | | | | | | | | |
| 15-30 | 62 (74.7) | 21 (25.3) | 8.531 | 0.014[a] | 51 (61.5) | 32 (38.5) | 35.265 | 0.000[a] | 59 (71.1) | 24 (28.9) | 22.633 | 0.000[a] | 83 (43.7) |
| 31-50 | 48 (53.3) | 42 (46.7) | | | 19 (21.1) | 71 (78.9) | | | 32 (35.6) | 58 (64.4) | | | 90 (47.4) |
| ≥51 | 11 (64.7) | 6 (35.3) | | | 2 (11.8) | 15 (88.2) | | | 7 (41.2) | 10 (58.8) | | | 17 (8.9) |
| **Gender** | | | | | | | | | | | | | |
| Male | 82 (61.7) | 51 (38.3) | 0.790 | 0.374 | 43 (32.3) | 90 (67.7) | 5.832 | 0.016[a] | 62 (46.6) | 71 (53.4) | 4.371 | 0.037 | 133 (70.0) |
| Female | 39 (68.4) | 18 (31.6) | | | 29 (50.9) | 28 (49.1) | | | 36 (63.2) | 21 (36.8) | | | 57 (30.0) |
| **Level of education** | | | | | | | | | | | | | |
| None | 33 (63.5) | 19 (36.5) | 9.354 | 0.009[a] | 14 (26.9) | 38 (73.1) | 36.877 | 0.000[a] | 26 (63.5) | 26 (36.5) | 28.039 | 0.000[a] | 52 (27.4) |
| Low | 36 (51.4) | 34 (48.6) | | | 13 (18.6) | 57 (81.4) | | | 21 (51.4) | 49 (48.6) | | | 70 (36.8) |
| High | 52 (76.5) | 16 (23.5) | | | 45 (66.2) | 23 (33.8) | | | 51 (64.4) | 17 (35.6) | | | 68 (35.8) |
| **Work experience (years)** | | | | | | | | | | | | | |
| ≤ 3 | 35 (87.5) | 5 (12.5) | 15.062 | 0.002[a] | 31 (77.5) | 9 (22.5) | 49.654 | 0.000[a] | 35 (87.5) | 5 (12.5) | 26.428 | 0.000[a] | 40 (21.0) |
| 4-10 | 32 (65.3) | 17 (34.7) | | | 24 (49.0) | 25 (51.0) | | | 22 (44.9) | 27 (55.1) | | | 49 (25.8) |
| 11-20 | 34 (50.8) | 33 (49.2) | | | 14 (20.9) | 53 (79.1) | | | 27 (40.3) | 40 (59.7) | | | 67 (35.3) |
| >20 | 20 (58.8) | 14 (41.2) | | | 3 (8.8) | 31 (91.2) | | | 14 (41.2) | 20 (58.8) | | | 34 (17.9) |
| **Total** | 121 (63.7) | 69 (36.3) | | | 72 (37.9) | 118 (62.1) | | | 98 (51.6) | 92 (48.4) | | | 190 (100.0) |

[a]Significant at p <0.05 REF: Reference

Tab. III. Logistic regression predicting food handlers' safe meat handling with knowledge, attitude and practice levels as independent variables.

| Variables | Knowledge | | Attitudes | | Practice | |
|---|---|---|---|---|---|---|
| | OR | CI | OR | CI | OR | CI |
| Types of meat plants | | | | | | |
| Private | 1.0 [a](REF) | | 1.0 (REF) | | 1.0 (REF) | |
| Government | 0.060 | 0.018–0.203 | 0.019 | 0.007-0.054 | 0.009 | 0.001–0.067 |
| Age group | | | | | | |
| 15-30 | 1.0 (REF) | | 1.0 (REF) | | 1.0 (REF) | |
| 31-50 | 0.387 | 0.203-0.738 | 0.168 | 0.086-0.329 | 0.224 | 0.118-0.426 |
| ≥ 51 | 0.621 | 0.204-1.886 | 0.084 | 0.018-0.0.390 | 0.285 | 0.097-0.835 |
| Gender | | | | | | |
| Male | [b]N/A | | 1.0 (REF) | | 1.0 (REF) | |
| Female | | | 2.168 | 1.150-4.086 | 1.963 | 1.038-3.711 |
| Level of education | | | | | | |
| None | 1.0 (REF) | | 1.0 (REF) | | 1.0 (REF) | |
| Low | 0.610 | 0.293-1.270 | 0.619 | 0.262-1.462 | 0.429 | 0.203-0.904 |
| High | 1.871 | 0.845-4.145 | 5.311 | 2.404-11.729 | 3.000 | 1.386-6.496 |
| Work experience (years) | | | | | | |
| ≤ 3 | 1.0 (REF) | | 1.0 (REF) | | 1.0 (REF) | |
| 4-10 | 0.269 | 0.089-0.813 | 0.279 | 0.110-0.706 | 0.116 | 0.039-0.347 |
| 11-20 | 0.147 | 0.051-0.422 | 0.077 | 0.030-0.198 | 0.096 | 0.033-0.277 |
| > 20 | 0.205 | 0.064-0.651 | 0.028 | 0.007-0.114 | 0.100 | 0.031-0.319 |

[a]REF: Reference

[b]N/A: Not applicable (Variable not significant at 10% significant level and was not entered into logistic model

## ATTITUDE LEVEL OF MEAT HANDLERS TO SAFE MEAT-HANDLING

Most of the meat handlers from PMPP had good attitudes towards the majority of the questions in contrast to the majority from GMPP (Tab. IV). Only 37.9% had good attitudes toward safe meat-handling with significantly higher proportion of 90.9% from PMPP and 16.3% from GMPP ($X^2$ =92.440; $p$ = 0.000) (Table II); with meat handlers in GMPP (OR = 0.019, 95%CI: 0.007-0.054) being about 57 times less likely to obtain good attitude level than those from PMPP (Tab. III). In all, the age ($p$ = 0.000), gender ($p$ = 0.016), education ($p$ = 0.000) and working experience ($p$ = 0.000) were significantly associated with attitude level toward safe meat-handling (Tab. II). Meat handlers within age groups 31-50 years (OR = 0.168, 95%CI: 0.086-0.329) and ≥ 51 years (OR = 0.084, 95%CI: 0.018-0.0.390) were respectively about 6 and 12 times less likely to obtain good attitude level than those within 15-30 years. The females (OR = 2.168, 95%CI: 1.150-4.086) were about two times more likely to obtain good attitude level than the males. Similarly, those with high educational level (OR =5.311, 95%CI: 2.404-11.729) were about five times more likely to obtain good attitude level than those without any formal education. In addition, meat handlers within 4-10 years working experience (OR = 0.279, 95%CI: 0.110-0.706) and 11-20 years (OR = 0.077, 95%CI: 0.030-0.198) were respectively about 3.6 and 13 times less likely to obtain good attitude level toward safe meat-handling than those within ≤ 3years (Tab. III).

## PRACTICE LEVEL OF MEAT HANDLERS ON SAFE MEAT-HANDLING

Table V shows that the majority of the meat handlers from PMPP had correct practices in most of the areas contrary to the poor practices by those from GMPP. Table II reveals that only 51.6% had good practice level of safe meat-handling with a higher proportion of 98.2% from PMPP and a much lower 32.6% from GMPP ($X^2$ = 67.313; $p$ = 0.000); the meat handlers in GMPP (OR= 0.009, CI 0.001 – 0.067) being about 111 times less likely to obtain good practice level than those from PMPP (Tab. III). In all, the age ($p$ = 0.000), gender ($p$ = 0.037), education ($p$ = 0.000) and working experience ($p$ = 0.000) were significantly associated with practice level of safe meat-handling (Tab. II). Meat handlers within age groups 31-50 years (OR = 0.224, 95%CI: 0.118-0.426) and ≥ 51 years (OR = 0.285, 95%CI: 0.097-0.835) were about 4.5 and 3.5 times less likely to obtain good practice level than those within 15-30 years; the females (OR = 1.963, 95%CI: 1.038-3.711) being about two times more likely than the males. Similarly, those with high educational level (OR = 3.000, 95%CI: 1.386-6.496) were three times more likely to obtain good practice level than those without any formal education. In addition, meat handlers within 4-10 years' working experience (OR = 0.116, 95%CI: 0.039-0.347) and 11-20 years (OR = 0.096, 95%CI: 0.033-0.277) were respectively 8.6 and 10.4 times less likely to obtain good practice level of safe meat-handling than those within ≤ 3years (Tab. III).

**Tab. IV.** Attitudes toward safe meat-handling amongst meat handlers in private and Government meat processing plants.

| Statements | Private | | | Government | | |
|---|---|---|---|---|---|---|
| | Yes (%) | No (%) | Uncertain (%) | Yes (%) | No (%) | Uncertain (%) |
| We should not handle meat with an open wound. | 83.6 | 5.5 | 10.9 | 23.7 | 58.5 | 17.8 |
| Sneezing or coughing without covering our noses or mouth could contaminate the meat. | 80.0 | 5.5 | 14.5 | 20.7 | 73.3 | 5.9 |
| Regular training could improve meat safety and hygiene practices. | 89.1 | 7.3 | 3.6 | 22.2 | 54.1 | 23.7 |
| Wearing protective clothing and shoes could help improve work safety and hygiene practices. | 80.0 | 14.6 | 5.4 | 19.3 | 52.6 | 28.1 |
| Putting on hair cover on the head is a good practice in food industry. | 87.3 | 3.6 | 9.1 | 17.8 | 48.9 | 33.3 |
| It is important to use potable water to wash working surfaces and cutting tools after disinfection. | 87.3 | 10.9 | 1.8 | 84.4 | 10.4 | 5.2 |
| We should not use non-potable water for meat processing. | 56.4 | 38.1 | 5.5 | 86.7 | 10.4 | 2.9 |
| Meat handlers can only contaminate meat when they are ill. | 47.3 | 47.3 | 5.4 | 10.4 | 39.2 | 50.4 |
| Meat handlers can get ill if they have contact only with the blood of animals during work activity. | 47.3 | 23.6 | 29.1 | 3.0 | 60.7 | 36.3 |
| Changing or sterilizing the knives in-between meat processing could limit cross contamination of meat. | 49.1 | 29.1 | 21.8 | 5.2 | 78.5 | 16.3 |

**Tab. V.** Practices of safe meat-handling amongst meat handlers in private and Government meat processing plants.

| Statements | Private | | Government | |
|---|---|---|---|---|
| | Correct answers (%) | Incorrect answers (%) | Correct answers (%) | Incorrect answers (%) |
| I wash my aprons after each day's work. | 47.3 | 52.7 | 3.7 | 96.3 |
| I replace my knives or sterilize them after each meat processing. | 58.2 | 41.8 | 0.0 | 100.0 |
| I wash my hands before and after handling meat. | 78.2 | 21.8 | 41.5 | 58.5 |
| I use potable water to process meat. | 81.8 | 18.2 | 97.8 | 2.2 |
| I protect any skin cut I sustain whenever I want to process meat. | 85.5 | 14.5 | 45.9 | 54.1 |
| I do not process meat when I am ill especially due to gastroenteritis, cough or skin diseases. | 76.4 | 23.6 | 33.3 | 66.7 |
| I freeze my left-over meat after each day's work. | 92.7 | 7.3 | 97.8 | 2.2 |

## Discussion

Adequate safe meat-handling knowledge and perception of meat handlers is very key to the overall safety and quality of food delivered on the table to man. The findings of this formative study provide a framework for future policy geared toward improving food safety and hence the health of man. As indicated, the type of meat plants was significantly associated with knowledge ($p = 0.000$), attitude ($p = 0.000$) and practice ($p = 0.000$) levels of safe meat-handling. The meat handlers in the PMPP had significantly higher good knowledge, attitude as well as practice level of safe meat-handling than those in the GMPP. This finding is a pointer to the observation that private establishments are generally better organized and tend to provide better quality services and products than the Government-owned facilities. The report of Irfan and Ijaz [15] which revealed that the private establishments in Pakistan were delivering better quality services than the public or Government establishment

further substantiated this finding. This might be consequent upon better supervision which often characterizes private establishments when compared to those owned by the Government. In addition, private facilities generally strive to gain competitive edge and remain on top and hence tend to provide superior services to their clients. The lower KAP levels among the meat handlers in the GMPP is a matter of public health concern since the bulk of meat supplied for human consumption particularly in the study area and in most developing countries is from the GMPP. As earlier stated, the GMPP in this study is a high throughput meat processing plant compared to the lower volumes of animals processed by all the PMPP combined. This therefore portends higher exposure risks considering the volume of consumers served by the plant.

From our study, only a few (23.6%) and none (0.0%) of the meat handlers from the PMPP and GMPP respectively had heard of HACCP. This is similar to the report of Gomes-Neves et al. [16] which showed that a

high proportion of meat handlers in Portugal were unacquainted with the concept. It seems to be very difficult therefore to implement an HACCP based system in meat processing industry in developing countries where a high proportion of employees are not familiar with the concept. Again, while Meyer [17] underscored the need for regular rotation of products for the purpose of disinfection, poor proportions gave correct responses regarding regular rotation of disinfectants. This observation coupled with the low dosages of disinfectants often being used generally in food establishments in a bid to reduce overhead costs therefore raises some concerns considering the possible resultant emergence of resistant pathogens.

Similar to the 83.6% of the meat handlers in PMPP who had correct attitudes toward avoiding handling meat with an open wound, Abdul-Mutalib et al. [3] reported that 95.3% of food handlers in Kuala Pilah, Malaysia agreed that food should not be touched with wounded hand. Although the hands of food handlers have been generally known as an important vehicle of food cross-contamination [9]; our results revealed that a much lower than half of meat handlers from GMPP washed their hands before and after handling meat. Improved personal hygiene and scrupulous hand washing should lead to the basic control of spread of potentially pathogenic transient microorganisms [9]. Besides, the practice of not replacing knives or sterilizing them after each meat processing by all the meat handlers from GMPP is a matter of public health concern. As reported, using the same utensils like cutting board or knife can cause cross contamination [3].

Furthermore, our findings show that the meat handlers in lower age groups generally demonstrated good knowledge, attitudes and practices of safe meat-handling. These findings are similar to the report [18] which indicated that age influenced food handlers' attitudes toward food hygiene. Therefore, incorporating younger age groups into the food industry by food hygiene policy makers should lead to improved food safety and quality delivery to the consumers.

In the same vein, the findings of this study show that gender influences meat handlers' attitudes and practices of safe meat-handling. This is consistent with a previous report [19] which indicated a significant difference in the mean percentage scores for KAP between male and females with the females scoring significantly better than the males on food safety knowledge and practices. Likewise, Altekruse et al. [18] reported that unsafe practices were reported more often by men and adults than by women.

Furthermore, this study reveals that the level of education is significantly associated with the knowledge, attitude and practice levels of safe meat-handling. This finding is in agreement with the report of Ansari-Lari et al. [20] who found that knowledge was significantly higher among food handlers with a higher educational level. Similarly, Abdul-Mutalib et al. [3] recommended that continuous education would strengthen knowledge of food hygiene. Again, the meat handlers with lower years of working experience obtained significantly higher knowledge, attitude and practice levels of safe meat-handling. This is contrary to the report [13] that the food handlers with longer years in food establishment had better practice of food hygiene and safety. However, our findings are in agreement with Lin and Sneed [21] who found a negative correlation between personal hygiene practices and length of employment in the food facility. Our finding suggests that there appears to be a general laxity among meat handlers as the length of time spent in food establishments increases. The tendency to therefore take learning new instructions or guidelines for improved food safety and quality delivery for granted might ensue.

Our findings notwithstanding, this study had some limitations. One, the issue of professional training among the meat handlers was not captured. This would have provided more insights into the impacts of training on the meat handlers' knowledge, attitude and practice levels of safe meat-handling. Two, the males constituted more of the respondents in this study. However, it is generally known that such activities as meat processing which require physical strength are generally dominated by the males.

## Conclusions

This study provides a framework for future policy geared toward improving food safety and hence the health of man. The authors propose that while the significance of intensifying general food safety awareness campaigns cannot be underestimated, increasing the involvement of private sector in the food industry especially in the developing countries holds the promise of improved safety and quality delivery. Our findings also show that higher education, lower years of working experience and younger age groups significantly enhance safe meat-handling knowledge, attitude and practice levels among meat handlers. It is therefore pertinent that Public Health education and regular refresher course on safe meat-handling and general hygiene should be intensified among meat handlers. In the same vein, the people in the lower age groups should be engaged more in the handling of food in the food industry.

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# Knowledge of sexually transmitted infections among younger subjects of the city of Messina (Sicily)

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### Key words

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Sexually transmitted infections • High school students

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### Summary

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**Introduction.** *Sexually transmitted infections (STIs) are considered a serious public health problem worldwide, affecting especially young people. The international monitoring data indicate that 70% of patients with STIs are aged between 15 and 24 years and the World Health Organization estimates that one out of 20 teenagers contracts an STI each year. We wanted to evaluate, through this work, what high school students of Messina know about the STIs.*
**Material and methods.** *From February to May 2013 questionnaires were distributed to students of the last three classes of seven high schools, three of which belonging to humanistic area and four to technical area. The completed questionnaires collected were 1588.*

**Results and discussion.** *The investigation carried out showed a better knowledge of the STIs by students of humanistic schools than students of technical-scientific schools, the percentage of correct answers was 74% and 60% respectively; this probably also depends on the family context, in fact, there is a statistically significant correlation between the percentage of correct answers and parents education level. Young people have a limited knowledge on the subject. We concluded our work by stressing the importance and the need to deepen and improve the training and information of the medical staff, teachers and families, as it is from their knowledge and their ability to provide complete and accurate information about the risks posed by STIs that can derive knowledge and choices of appropriate lifestyle between the youth population.*

## Introduction

According to 2008 World Health Organization (WHO) estimates, 499 million new cases of curable STIs (syphilis, gonorrhoea, chlamydia and trichomoniasis) occur annually throughout the world in adults aged 15-49 years. These figures do not include the additional health burden caused by HIV and other viral STIs such as HSV [1].

A real increase in incidence is due to the increased tendency to have promiscuous sex without using a condom [2, 3]. Another reason of the increased incidence is certainly related to the disinterest of most of population against these infections; this lack of interest is linked to lack of information devoted to this subject, especially by young people and consequently to poor knowledge of the problem represented by the STIs [4]. It's also important to point out that in most industrialized countries, where the widespread use of antibiotics in the past decades has led to a drastic reduction in the spread of STIs, there is now a marked increase in viral STIs such as genital herpes and warts, and the re-emergence of diseases almost completely disappeared, such as syphilis and lymphogranuloma venereum [5].

In fact, from the mid-1990s, the increase in diagnoses of sexually transmitted infections, including syphilis, gonorrhoea and chlamydia were reported in several European countries, especially among adolescents between 16-19 years [3]. In addition, the sexually transmitted infections are a major health problem that affects mostly young people, not only in developing but also in developed countries.

The problem with the most of STIs is that they can occur symptom-free and thus can be passed on unaware during unprotected sexual intercourse [3]. On an individual level, complications can include pelvic inflammatory diseases, ectopic pregnancies and infertility [4, 6, 7].

Female adolescents may have a higher risk of contracting a sexually transmitted disease than their male peers, being generally their sexual partners older and therefore with a greater probability of being infected [8, 9].

Certainly it is noteworthy, although we are in a period of great technological advances which fully involve information systems worldwide, that the STIs among adolescents continue to be an important public health problem for many industrialized and developing countries.

The international monitoring data indicate that 70% of patients with STI are aged between 15 and 24 years and the WHO estimates that one out of 20 teenagers contracts an STI each year [4].

In particular, some studies carried out in Italy [4] showed a significant prevalence of infections such as syphilis and hepatitis B. On the basis of this information it must be said that the rates of illness reported underestimate the true burden of infection because most STIs are asymptomatic.

The declining age of first sexual intercourse has been proffered as one possible explanation for the increase in numbers of STIs [10]. According to data from different European countries, the average age of first sexual intercourse has decreased over the last three decades, with increasing proportions of adolescents reporting sexual activity before the 16 years [11, 12]. The reluctance of adolescents to use condoms is another possible explanation for the increase in STIs. Some surveys of adolescents reported that condoms were seen as difficult to use by those who is sexually inexperienced, they diminish sexual pleasure and it is embarrassing to suggest its use [13, 14]. Condoms have also been reported to be primarily used as a contraceptive and not to prevent the occurrence of sexually transmitted diseases, and their use becomes irregular when other contraceptives are used [15, 16]. Furthermore, many adolescents do not perceive themselves to be at risk of contracting an STI. So the understanding and prevention of STIs among adolescents represent a critical aspect that would minimize the risk of sexual transmission and thus reduce the frequency of these infections [17]. Information on sexual practices of adolescents and, in particular, knowledge of risk behavior related to unprotected intercourse would lead to the dissemination of knowledge on preventive measures.

Therefore, since today sexually transmitted infections continue to pose a serious public health problem, especially among the younger subjects, the aim of this study was to evaluate both knowledge and information regarding STIs of high school students of Messina.

## Materials and methods

From February to May 2013, a survey was conducted on a sample of students aged 16-18 years in some public high schools in the city of Messina (Italy). In particular, the study involved students of the last three classes of seven high schools, three of which belonging to humanistic area and four to technical area.

All public high schools were approached by letter and visited by one of the researchers to request participation. A member of the research-team verbally explained the study to the students in their classroom. Participation is voluntary and to all students enrolled in the study were asked to complete an anonymous self-administered questionnaire (Fig. 1) after providing written informed consent for their participation. Belonging our research team at the center of reference for the epidemiological surveillance of HIV of Messina we have focused our attention primarily on the knowledge of the guys on this pathology. The completed questionnaires collected were 1588. Humanistic school's students were 735, 207 males and 528 females, however, the technical-scientific school's students were 853, including 378 women and 475 men (Tab. I).



**Fig. 1.** Self-administered questionnaire to students involved in the study.

The first part of the questionnaire collected information on nationality, place of birth, age, sex, sexual behavior and the level of education of their father and mother.

The second part of the questionnaire included questions with one correct answer and questions with subjective responses, with the aim of assessing the youths' knowledge about the main sexually transmitted infections, particularly HIV / AIDS, modes of transmission, methods of prevention, HIV seropositivity and progression of the disease, and also some questions related to the HIV test and the source of information for students on these topics.

Correlations were determined using either the standard Pearson correlation coefficient or the Spearman's rank correlation test. All analyses were performed using Prism 4.0 software.

## Results

Analysis of the questionnaire responses, given by students of seven schools under consideration, showed that the highest percentage of incorrect responses concerns the questions on the meaning of the term "HIV-positive", the concept HIV / AIDS and the future of a person with AIDS, this is a sign that unfortunately the students' knowledge is not complete. However the boys know the mode of infection of STDs, particularly HIV and biological fluids containing the virus (Fig. 2).

Comparing the correct answers between the humanistic group and the technical-scientific group we observed a higher percentage of correct answers in the first group (Fig. 3) with a significant difference (p = 0,0012).

We did not find significant difference assessing the correct answer according to gender and age regardless of the school address, however considering only the humanistic area there is a significant increase in correct answers directly proportional to the class belonging. In particular, as regards knowledge of sexually transmitted infections, the answers are similar between boys belonging to humanistic area and those of technical-scientific area. AIDS is the sexually transmitted infection better known followed by syphilis, HSV, Candida, EBV and HPV with 91%, 45%, 28%, 22%, 20% and 19% respec-

Tab. I. Study population.

| | Samples | School address |
|---|---|---|
| Students | 1588 | humanistic area 735 |
| | | technical-scientific area 853 |
| Female | 906 | humanistic area 528 |
| | | technical-scientific area 378 |
| Men | 682 | humanistic area 207 |
| | | technical-scientific area 475 |
| Average age | 16-18 | humanistic area 16-18 |
| | | technical-scientific area 16-18 |

tively (Fig. 4). Moreover humanistic school's students have a better knowledge of the mode of infection respect to technical-scientific school's students, in fact, the percentage of correct answers was 74% and 60% respectively.

In relation to the knowledge on the symptomatology related to infection, all students, independently of school membership, responded AIDS (63% humanistic school's students, 60% technical-scientific school's students), genital lesions (33% humanistic school's students, 28% technical-scientific school's students) and sterility (25% humanistic school's students, 23% technical-scientific school's students).

The question about the methods of sexually transmitted infections prevention showed a good understanding of the methods to be used, the majority of students reported to always use condom with both casual and regular partners.

Furthermore the questionnaire included questions related to knowledge of HIV infections. Our particular interest stems from the fact that there is very little information on this topic with a consequent lowering of the level of attention especially from the boys. With regard to the issues relating to the definition of HIV and AIDS, pupils



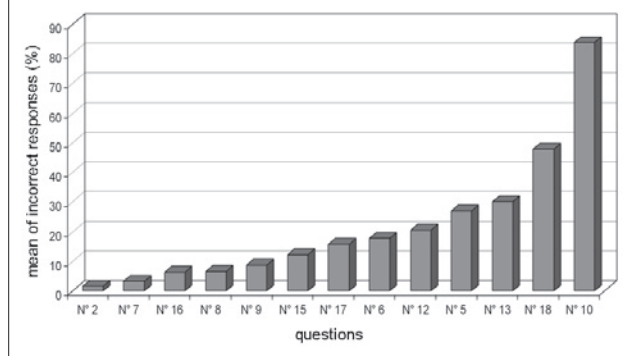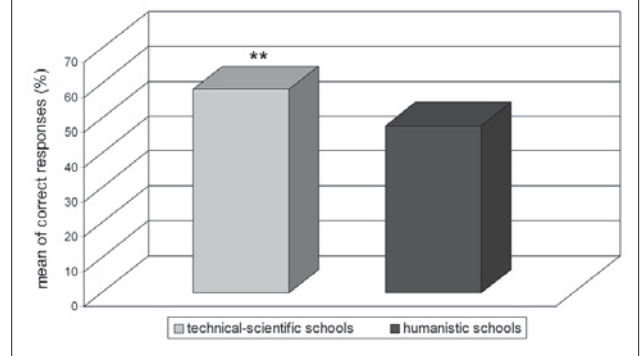Fig. 2. Questions with the highest percentage of incorrect answers.



Fig. 3. Comparison between the mean of correct responses among the humanistic schools and technical-scientific schools.

of humanistic secondary schools, have revealed a better understanding of the term HIV infection (64,82% vs 46,38%), and of the meaning of acronym AIDS (74,74% vs 63,77%). In fact, while only 5% of students in humanistic high schools do not know that HIV causes AIDS but that seropositivity does not mean that the person has the disease, among students of technical and scientific schools doubles this percentage. We didn't find a very significant difference between the two addresses school regarding questions about the mode of transmission of the virus (59,81 vs 60,74), instead, regarding the presence of the virus in body fluids, the percentage of correct answer (69,10 vs 51,38), indicating a greater knowledge of humanistic address students.

The answers or the lack of answers to the question regarding the actions to be taken against people with HIV, showed a common fear towards this disease, in fact, little more than half of the humanistic schools' students and just under half of scientific and technical schools' students do not would isolate HIV-positive subjects. The responses concerning the ability to diagnose the infection and the knowledge of the places where people can take the test, revealed here, as in the preceding questions, a greater knowledge of the students with humanistic address than those with technical-scientific address about this topic. Moreover, only the 50% of students are fully convinced of the need to maintain secrecy about the test and the results, and there is no significant difference in the responses between the two addresses.

To the question number eleven about "who would you say if you were HIV positive", the majority of students indicated in order: family, partner, doctor, best friend. Although a small percentage of the scientific-technical schools' boys has a greater tendency not to tell it to anyone (Fig. 5).

We also aimed to know more about the source of information of the boys. When we asked what was their best source of news, all students, regardless of school, replied that they had received more information from the school, followed by television. But on the basis of the replies to the question about the quantity and the quality of their knowledge, this information is limited, in fact, on average the 40% of respondents in all the schools makes a judgment of inadequacy of the information received, and therefore, would like to have more news especially from the school. Students from both schools' addresses have responded similarly to the questions. Undoubtedly there emerges a better understanding among the students attending the schools to humanistic address, probably also related to the family environment. In fact, the questionnaire included the collection of information about the cultural level of their parents, and comparing the two different addresses we found a higher degree of education of the parents of teens attending schools of humanistic address and comparing the percentage of cor-



**Fig. 4** Sexually transmitted infections better known among humanistic schools students and technical-scientific schools students.
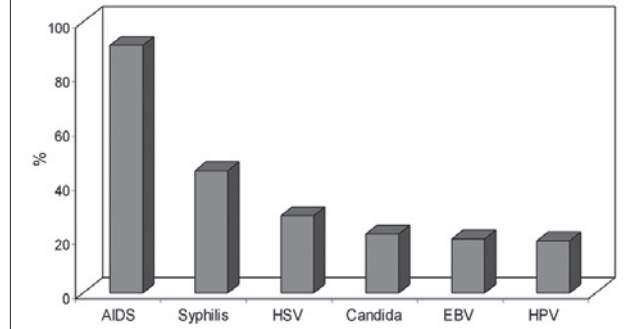


**Fig. 5.** Most frequent responses to the question number eleven among humanistic schools students and technical-scientific schools students.
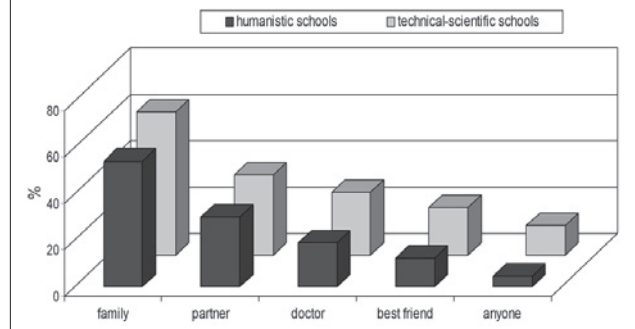


**Fig. 6.** Comparison between parents' cultural level and percentage of correct answers of the students from both schools addresses.
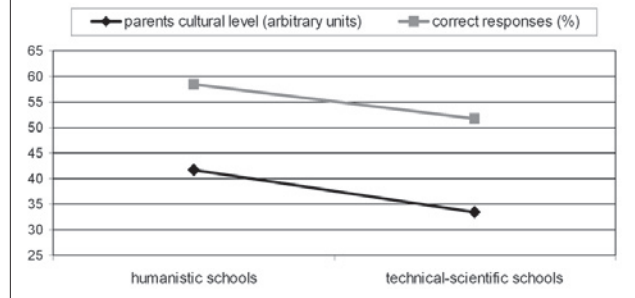
rect answers as a function of that, we noticed a directly proportional correlation (Fig. 6).

We must emphasize also the awareness of the teens in asserting to have a limited knowledge on the topic and while identified the school as the main source of information, is from this institution that they expect more information.

## Discussion

Through this study, we wanted to assess the degree of knowledge of secondary school students about STDs and what are their sources of information. The survey "Adolescents and reproductive health", carried out in 11 Italian regions in 1997 by the National Institute of Health, on knowledge, attitudes and behavior related to the reproductive health of students attending the first two years of high school, revealed that the majority of adolescents discover the sexuality with inadequate information often provided by untrained or unqualified sources. Over 95% of respondents would like to do in school sex education, 23% from the primary school and 58% from the middle school. Over 90% of respondents believe that sex education should stimulate a greater awareness. However, only 36% had the opportunity to participate in sex education programs in schools. Slightly less than 80% know that condoms can protect against sexually transmitted infections. Our results show a greater knowledge of AIDS compared to other sexually transmitted diseases much more common and the prevention of sexually transmitted diseases can be effectively implemented only with an integrated approach, starting from the physiology of reproduction and relational wealth of sexuality.

Even though in our study the children have shown to know the arrangements for the transmission of STI in general and HIV in particular, their knowledge is found to be incomplete. As reported in several works [18, 19], it is clear that the family is not for children as a source of information to the contrary of the school and mass media; this reflects a lack of communication between parents and children on topics related to sex. Despite the need to introduce reproductive health education in schools, there are still considerable impediments [20]. For examples, the principals of the schools involved in this study have given us permission to distribute the questionnaire only to students of the last three classes and this probably because it is still believed that talking to boys about sexuality will encourage them to have sex [21]. It should establish a school program on sexually transmitted diseases and decide who should teach the subject. It is clear from our results as the knowledge of the STI record levels well below expectations, especially after information campaigns on the subject done over the years. We think that these gaps are also due to the fact that high school teachers are not trained to carry out a comprehensive program of sexual health education and even the news spread by the mass media fail to be comprehensive and effective. Both teachers and parents should approach the topic in a different way in order to spread their knowledge correctly but also the government should intervene by requiring schools to introduce sex education and increasing news on the prevention of sexually transmitted diseases in television and radio.

## Conclusions

Health promotion among adolescents, understood as a real process of "empowerment" of individuals and communities is a top priority in public health. Precisely because of this feeling we decided to perform this study specifically investigating among the students of secondary schools since the process of health promotion becomes more feasible and meaningful if the target population studied may be the basis for more long-term strategies that may then involve the whole population. In addition, the intervention in high schools and in places where young people meet is crucial because it allows you to reach a large proportion of young people by offering a training program that will stimulate their direct involvement [22]. We want to mention here that adopt and develop appropriate health policies to promote global health but with particular attention to the vulnerable groups of the population, is one of the purposes of the "Declaration on Social Determinants of Health" sponsored by the World Health Organization in the conference in Rio de Janeiro on 19-21 October 2011. Due to the wide spread of sexually transmitted infections, the promotion of sexual and reproductive health is certainly an important step in the direction of health promotion and at the same time a topic of great importance with regard to the governments of different countries involved in the prevention of diseases such as AIDS, which continue to represent the wounds of our century [23]. We want to emphasize that the European Union is actively promoting sexual health to encourage the development of lifestyle that prevents the transmission of sexually transmitted diseases, including HIV, avoiding risky sexual behavior [24]. This goal is established in the European Programme for Health 2008-2013, with particular reference to young people as these, in general, are the most vulnerable to the transmission of sexually transmitted diseases, unwanted pregnancies, cultural, social and emotional issues related to their reproductive health. Sex education should ensure the complete formation of the person, through action involving families, schools, parishes, social and health centers. This study shows very clearly that the school should be a point of reference for sex education and intervention considered helpful by young people should be made to make them more aware, filling their doubts and uncertainties [25]. We conclude our work by stressing the importance and the need to deepen and improve the training and information of the medical staff, teachers and families, as it is from their knowledge and their ability to provide complete and accurate information about the risks posed by STI that can derive knowledge and choices of appropriate lifestyle between the youth population.

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# Antimicrobial susceptibility patterns of the gram-negative bacteria isolated from septicemia in Children's Medical Center, Tehran, Iran

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## Key words

Antimicrobial resistance • Antimicrobial resistant profile • Bloodstream infection

## Summary

**Introduction.** *The choice of antimicrobial treatment for septicemia is often empirical and based on the knowledge of local antimicrobial activity patterns of the most common bacteria causing such bloodstream infections. The current study aimed to study the prevalence of bacterial pathogens causing septicemia and their antimicrobial resistant profiles in hospital admitted patients.*

**Methods.** *This cross sectional study done at Children's Medical Center, Tehran, Iran. We examined 168 bacterial strains isolated from 186 clinically diagnosed septicemia cases refereed at Children's Medical Center, Tehran, Iran Over a period of twelve months from July 2010 to 2011 July. 11446 blood samples from patients of clinically suggestive septicemia were evaluated.*

**Results.** *Bacterial strains were isolated from 910 (7.95%) of blood cultures. Gram-negative bacteria identified were Pseudomonas species (20.5%), Pseudomonas aeruginosa (1.86%), Salmonella spp (1.09%), Acinetobacter naumannii (8.13%), Escherichia coli*
*(4.06%), Klebsiella spp (5.16%). Gram-negative pathogens were more than gram positive in bloodstream infections. Antimicrobial susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI, USA) guidelines against: amikacin ampicillin, amoxicillin, amoxiclav, cefuroxime, cefotaxime, ceftazidime, cefoperazone tetracycline, chloramphenicol, ciprofloxacin, gentamicin. Resistanc to different antibiotics in the most important isolated bacteria were: 32.1 %, 10.8%, 87.8%, 96%, 39.1%, 35.2, 49.4%, 69%, 80.02%, 22%, 59%, 30.1% respectively, for Pseudomonas spp, 32%, 3.7%, 84.2 %, 83.2%, 80.1%, 75.4%, 44.8%, 45.2%, 33.3%, 19%, 34.1, 11.5% respectively for Acinetobacter species.*

**Discussion.** *Resistant to majority of the antimicrobial agents for several pathogens implicated in bloodstream infections, particularly in Gram-negative bacteria, can make complication in treatment of infection cause by them.*

## Introduction

The increasing bloodstream infections mainly in developing countries is the one of most important health care systems concerne. Although the bloodstream infections mortality rate has reduced in latest two decades. Many studies worldwide have determined rising antibiotics resistance among causative bacteria for septicemia [1-3]. It can can lead to make a major challenge for the physicians for treatment of tham, because of they face to increase of antibiotics resistance in causative bacteria for septicemia [3-5].

Both Gram-negative and Gram-positive bacteria can cause bloodstream infections and that can be differs from different locality and/or different time [1, 2, 8]. Rapid and accurate diagnosis and select the appropriate antimicrobial treatment for patients with septicemia can help to decrease mortality and morbidity [7, 9]. Actually, for selection of empirical antibiotic therapy for bloodstream infections consider local antibiotic resistance paterns in frequent pathogens associated with septicemia is necessary and can be one of important point.

So, this study was performed to find the frequency of Gram-negative bacteria that causing septicemia in Children's Medical Center hospital admitted patients and determined antimicrobial resistance profile till clinicians can select the best choice antibiotic therapy.

## Methods

This study was performed at a Children's Medical Center, Tehran, Iran, over a period of twelve months from July 2010 to 2011 July. During the current study, 803 blood samples from patients of clinically suggestive bloodstream infection were surveyed, out of 4162 patients admitted. An informed consent was achieved from the patients. Blood samples from neonates and pediatrics were collected by pediatricians. Blood samples were gathered after careful cleaning of the venous with 70% alcohol and consequently followed by povidone iodine. Under the aseptic conditions 5 ml of blood was drawn by venipuncture and transmitted into two culture bottles each containing 50 ml of 0.5% bile-broth and 50 ml of 0.5% glucose-broth. Both the bottles were incubated

at 37°C for 10 days aerobically. Routine subculturing was performed on MacConkey agar and 5% sheep blood agar after 24 hours, 48 hours, 5th day and 10th day. In between these time points, subculturing was performed until there was observable turbidity. Microorganisms that isolated from blood culture were recognized by cultural characters, morphology and variety of standard biochemical tests such as oxidase,TSI, SIM, citrate, OF, Lysine decarboxilase, gelatinase, MR/VP, etc. [10-15]

Antimicrobial resistant patterns of the bacterial isolates to several antimicrobials was detected by using the Kirby Bauer disc diffusion method by Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The antibiotic concentration for each disc was as follows: gentamicin (10μg), amikacin (30μg), ampicillin (10μg), amoxicillin (30μg), amoxiclav (20:10μg), cefoxitin (30μg), cefotaxime (30μg), cefuroxime (30μg), ceftazidime (30μg), cefoperazone (75μg), ciprofloxacin (5μg), tetracycline (30μg) and chloramphenicol (30μg) for all organisms. All the antibiotic discs were purchased from MAST company (UK).

## Results

### Demographics

Over a period of twelve months from July 2010 to 2011 July. 11446 blood samples from patients of clinically suggestive septicemia were evaluated. The causative organism could be identified in 910 (7.95%) bacterial strains. Gram-negative bacteria identified were *Pseudomonas* species 187 (20.5%), *Acinetobacter baumannii* 74 (8.13%), *Klebsiella* spp 47 (5.16%), *Escherichia coli* 37 (4.06%), *Salmonella* spp 10 (1.09%), *Pseudomonas aeruginosa* 17 (1.86%) (Tab. I).

The rates of resistance in *Pseudomonas* spp to various antimicrobials are given below: gentamicin (30.1%), amikacin (10.8%), ampicillin (87.8%), amoxicillin (96%), amoxiclav (80.02%), cefuroxime (69%), cefotaxime (49.4%), cefazidime (35.2%), cefoperazone (39.1%), ciprofloxacin (22%), chloramphenicol (59%) and tetracycline (32.1 %) (Tab. II).

The rates of resistance in *Acinetobacter* species to various antimicrobials are given below: gentamicin (11.5%), amikacin (3.7%), ampicillin (84.2 %), amoxi-

**Tab. I.** Number and percentage of isolated Species of Bacterial.

| Species of isolated Bacterial | Number of isolated bacteria | Percentage of isolated bacteria |
|---|---|---|
| Pseudomonas species | 187 | 20.5% |
| Acinetobacter baumannii | 74 | 8.13 |
| Klebsiella spp | 47 | 5.16% |
| Escherichia coli | 37 | 4.06% |
| Pseudomonas aeruginosa | 17 | 1.86% |
| Salmonella spp | 10 | 1.09% |

cillin (83.2%), amoxiclav (80.1%), cefuroxime (75.4%), cefotaxime (44.8%), ceftazidime (45.2%), cefoperazone (33.3%), ciprofloxacin (19%),, chloramphenicol (34.1%) and tetracycline (32%) (table. 2).

The rates of resistance in *Salmonella* spp to various antimicrobials are given below: cefuroxime (21%), cefotaxime (39%), ampicillin (46.4%), amoxicillin (27.3%), amoxiclav (15.4%), cefoperazone (32.5%),, ciprofloxacin (6.3%), chloramphenicol (9.1%) and tetracycline (50%) (Tab. II).

The rates of resistance in *Escherichia coli* to various antimicrobials are given below: gentamicin (25%), amikacin (18%), ampicillin (92%), amoxicillin (90.9%), amoxiclav (73.9%), cefuroxime (54.5%), cefotaxime (52.9%), cefoperazone (36.8%), ciprofloxacin (31.6%), chloramphenicol (39%) and tetracycline (33.7%) (Tab. II).

The rates of resistance in *Pseudomonas aeruginosa* to various antimicrobials are given below: gentamicin (42.2%), amikacin (30.8%), ampicillin (96.1%), amoxicillin (92.9%), amoxiclav (95.8%), cefuroxime (81%), cefotaxime (48.4%), cefazidime (34.2%), cefoperazone (47.3%), ciprofloxacin (22.4%), chloramphenicol (32%) and tetracycline (42.6 %) (table. 2).

The rates of resistance in *Klebsiella* spp to various antimicrobials are given below: gentamicin (54%), chloramphenicol (49%) cefuroxime (77.4%), cefotaxime (52%), ampicillin (98.7%), amoxicillin (71.4%), amoxiclav (52%), cefoperazone (32%) and tetracycline (62%). Klebsiella pneumoniae did not show resistance to amikacin and ciprofloxacin (Tab. II).

**Tab. II.** Frequency of antibiotics resistance.

| | gentamicin | amikacin | ampicillin | amoxicillin | amoxiclav | cefuroxime | cefotaxime | cefazidime | cefoperazone | ciprofloxacin | chloramphenicol | tetracycline |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonas* spp. | 30.1% | 10.8% | 87.8% | 96% | 80.02% | 69% | 49.4% | 35.2% | 39.1% | 22% | 59% | 32.1% |
| *Acinetobacter* species | 11.5% | 3.7% | 84.2 % | 83.2% | 80.1% | 75.4% | 44.8% | 45.2% | 33.3% | 19% | 34.1% | 32% |
| *Salmonella* spp. | 21% | 39% | 46.4% | 27.3% | 15.4% | - | - | - | 32.5% | 6.3% | 9.1% | 50% |
| *Escherichia coli* | 25% | 18% | 92% | 90.9% | 73.9% | 54.5% | 52.9% | - | 36.8% | 31.6% | 39% | 39% |
| *Pseudomonas aeruginosa* | 42.2% | 30.8% | 96.1% | 92.9% | 95.8% | 81% | 48.4% | 34.2% | 47.3% | 22.4% | 32% | 42.6% |
| *Klebsiella* spp. | 54% | 0% | 98.7% | 52% | 52% | 77.4% | 52% | - | 32% | 0% | 49% | 62% |

## Discussion

Bacterial antibiotics resistant is remaining as an allarming problem in the therapy of bloodstream infections [16, 17]. Bacterial bloodstream infection mostly caused by strains that are resistant to a wide range of antimicrobial agents [18]. The current study investigated the antimicrobial resistance profile of 372 Gram-negative bacteria isolated from bloodstream infections. The data demonestrated the frequency of antimicrobial resistance among bacterial pathogens isolated from bloodstream infections.

Gram-negative bacteria is the most cause of bloodstream infections in many conties [1, 2, 7, 18]. Also, this type of bacteria have been the most common contributing pathogens of bloodstream infections in the present study. This is considerable that, different etiological agents of bloodstream infections can be related to the varying demography of bloodstream infections in developing countries because of different geographical area.

In some studies demonstrated that *Acinetobacter* species, Salmonella typhi and Escherichia coli were the most common Gram-nagative bacteria that involve in that bloodstream infections [19-21]. These results are approximately similar to our results in current study.

Gram-negative bacteria is more resistance to antibiotics than Gram-positive. *Acinetobacter* species demonstrated very high levels of resistance to beta-lactam antibiotics in some reports [22, 23], that it is similar to the results of current study. *Acinetobacter* species showed the lower levels of resistance to aminoglycoside and low level resistance rates to quinolones. Similarly, *Escherichia coli* demonstrated distinctly higher levels of resistance to beta-lactam antibiotics than other classes of antimicrobials. The organism demonstrated maximum resistance to ampicillin and minimum to amikacin [22, 23]. These results can support the finding of this study, because of our results of antibiotic susceptibility testing are similar to them.

## Conclusion

Extensive frequency of antimicrobial resistance levels were distinguished in our study, is similar to another studies [17, 18]. The high frequency of antibiotic resistance rates in Children's Medical Center might be because of indifferentiate and excess of use of treatment in our country because of their easy availability [19]. The other cause might be frequency of ESBL production among Gram-negative isolates from neonatal bloodstream infection [20]. Also, other cause could be the altering patterns of antibiotic utilize and varies in lifestyle. In the light of over findings there continues an increasing requirement for new agents, though a recent study reported polymyxin are being restored to as potential choices for the therapy [21]. Proper antimicrobial treatment for bloodstream infections is important in declining morbidity and mortality among patients with bloodstream infections caused by bacteria. Therefore, accurate microbiological diagnosis and their antimicrobial resistance profile can be very important for the rapid initiation of sufficient treatment for bloodstream infections [23].

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# Identification and molecular epidemiology of nosocomial outbreaks due to Burkholderia cepacia in cystic fibrosis patients of Masih Daneshvary Hospital, Iran

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## Summary

**Introduction.** *B. cepacia complex have emerged as an important opportunistic pathogen in hospitalized and immunocompromised patients. Small hospital outbreaks are frequent and are usually due to a single contaminated environmental source. In this study we were going to investigate the role of B.cepacia complex in those patients suspected to involve with cystic fibrosis and evaluate responsible types in Masih Daneshvary Hospital.*

**Methods.** *One hundred specimens were collected from all admitted patients who were suspected to cystic fibrosis to Masih Daneshvary hospital during one year April 2011 till end of March 2012. All were culture and identified standard procedure. All samples were checked by API system (API20NE) and by specific PCR method for genus Bulkhorderia and Bcc as well. Identified*

*strains were finally tested by PFGE system to identifying specific involving pulse-types.*

**Results.** *Isolation and identification methods revealed 5 specimens were B.cepasia, The frequency of the cystic fibrosis detected at this study was lower than other similar study previously reported. All these isolates showed similar pattern by PFGE standard protocol that may have spread from a single source and could not be attributed to cross infections from patient to patients.*

**Discussion.** *Application of PFGE and identification of pulse-type is a potential tool to enhance the investigation of apparent nosocomial outbreaks of B.cepacia. However it needs to be adjusted with environmental findings. Implementation of educational programs and adherence to infection control policies are obviously the main element for complete elimination of an outbreak.*

## Introduction

The Burkholderia cepacia complex (BCC) is a group of strictly aerobic, gram negative BCC. also known as ''B. cepacia like bacteria''. It currently divided into 17 species that mainly affect people with underlying disease such as cystic fibrosis or granulomatous disease, and show antimicrobial resistant to many of antibiotics [1-3]. The term ''complex'' emerged in the literature at the beginning of the genomic era associated to taxonomy and grouping organisms that belong to different species but exhibited similar patterns according to their morphological, physiological and/or other phenotypic features. DNA–DNA hybridization with 70% and high identity on 16S rRNA gene sequences [4].

Presently, the BCC includes 17 species: B. ambifaria, B. anthina, B. arboris, B. cepacia, B. cenocepacia, B. contaminans, B. diffusa, B. dolosa, B. lata, B. latens, B. metallica, B. multivorans, B. pyrrocinia, B. seminalis, B. stabilis, B. ubonensis, B. vietnamiensis. Among these organisms some authors have detected B. cepacia more frequently than the rest especially in non-CF patients [5].

Bcc is among the pathogens most frequently reported outbreaks and has been described in several healthcare centers around the world [6-13]. These organisms have also emerged as an important cause of morbidity and mortality in hospitalized patients because of high rate of antibiotic resistance [14]. Therefore, fast detection and elimination of the source of such these outbreaks is the great importance in guiding infection control measures and preventing additional cause of infections especially at Cystic Fibrosis centers.

The aim of this study was first isolation and identification of Bcc and then to perform molecular typing by pulsed-field gel electrophoresis (PFGE) in order to evaluate the epidemiology of this pathogen in CF patients at Masih Daneshvary Hospital.

## Methods

Sampling: One hundred specimens were collected from all admitted patients who were suspected to cystic fibrosis to Masih Daneshvary hospital during one year April

2011 till end of March 2012. Isolation and Identification: Bacterial identification was performed after culturing in BHI broth. One loop broth was then of transferred to blood agar after overnight incubation. Isolates were identified use standard identification tests and with API-I20NE system as well [15]. After species identification, isolates were stored at -20 oC as suspensions in 10% skim milk solution containing 10% glycerol.

PCR Methods: Microbial suspension was prepared for each isolated specimens and extracted use of QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The purity of all the extracted specimens was determined by measuring the optical density at the wavelengths of 260 and 280 nm. Primer pair sequences was order based on the Lynch's report (et al. 2008) [16]. He reported two set of Primer pairs one detecting Bulkhurderia species and the second specific for the BCC (Tab. I).

20 µl mixtures were prepared use of 200 µM of dNTP, 1.5 mM MgCl2,50 pg of each primers, 2U of taq polymerase, 1x PCR buffer and different concentration of each primer. The PCR were performed on Eppendorf thermal cyclers (Mastercycler Gradient) by following program: 96oC for 4 min, then 35 cycles at 96oC, 59oC, and 72oC each for 1 min, at the end 2 min at 59oC. PCR products were analyzed on 2% agarose gel with100 base pair ladder.

Genotyping: DNA polymorphisms of all isolates were evaluated by pulsedfield gel electrophoresis (PFGE) with SpeI (Boehringer Mannheim Biochemicals), as described elsewhere. 20 DNA banding patterns were compared by standard DNA marker interpreted by visual inspection.

PFGE was briefly performed as follows: bacterial suspension was prepared in a buffer (100 mmol l-1Tris, 100 mmol l-1 EDTA, pH 8.0) and adjusted to absorbance values of 0.8 - 1.0 at a wavelength of 610 nm after which plugs were prepared with SeaKem Gold agarose (Lonza, Rockland, ME, USA) and proteinase K. Bac-

terial plugs were lysed (50 mmol l-1Tris, 50 mmol l-1 EDTA, 1% sarcosine, and 0.5 mg of proteinase K,pH 8.0) and washed after which digested with forty units of Spe I restriction enzyme (Fermentase). DNA molecular weight size marker was prepared by XbaI digestion of Salmonella enterica serotype Braenderup H9812 plugs. CHEF Mapper XA System (Bio-Rad) was applied for electrophoresis.

## Results

Isolation and applied identification testes revealed 5 specimens were Burkholderia cepacia. PCR results also confirmed in these specimens (Tab. II and Fig. 1). The frequency of the cystic fibrosis detected at this study was 5%. It is obviously different at variously reports. Leite and coworkers studied a total of 244 CF patients from HCPA and observed a prevalence rate of BCC of 10.6%. BCC accounted for most BCC isolates in 244 examined specimens [17].

PFGE results: PFGE were performed by the standard procedures use of SpeI Restriction enzyme and provide one unique electrophoresis pattern (Fig. 2) meaning similar type involving in those patients suffering cystic fibrosis due to Burkholderia cepacia. Use of XbaI also revealed similar pattern among these 5 isolated organisms. The presence uniform pattern and similar pulsetype in all these identified B.cepacia was concluded just by visual inspection use of standard DNA marker since the system have set up recently and BioNumerics had not been delivered to the molecular lab on running date of experiment. The pattern of each sample was carefully analyzed and experiment repeated once more, although it was necessary to interpret these results by computer software such as gel compare or BioNumerics and drawing related dendrogram.

Tab. I. Primer sequence used in this study.

| Trarget | Primer | Sequence | Species | Product size |
|---------|--------|----------|---------|--------------|
| recA | BCC(F) | ATGACCAATCCGACCGATCTCAA | All | 429(bp) |
|  | BCC(R) | TCAGTGCTTGCGITNIGGGCAGTT |  |  |
| recA | BKF(F) | GGCNGAAGACGTCTACCGG | BCC (Group K) | 117(bp) |
|  | BKF(R) | TCGAAGTTGCTGCGCGAC |  |  |

Tab. II. API results for isolated specimens. All 5 isolated specimens had similar reaction to the standard strains by applied biochemical tests.

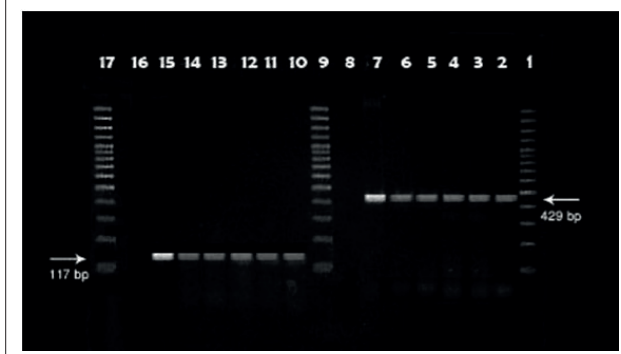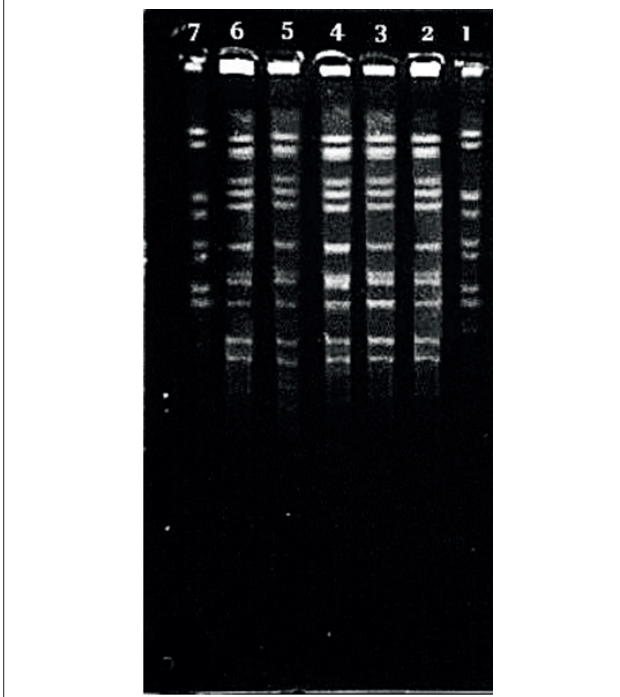| Polymixine | DNAase | Of Mannitol | Of Maltose | Of Lactose | Ornithine | Arginine | Lysine | Citrate | H2S in KIA | TSI | Indole | Motility | Oxidase | Pigment | Color Gram | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | - | + | + | + | + | - | + | w- | - | ALK/ALK | - | + | + | - | Rod g- | B. cepacia Standard strain (ATCC25416) |
| R | - | + | + | + | + | - | + | w- | - | ALK/ALK | - | + | + | - | Rod g- | Isolated organisms |

**Fig. 1.** PCR results.



**Fig. 2.** PFGE results.

## Discussion

In 2001, Agodi (et al. 2001) indicated a prevalence of Bcc infection of 9.5% in a study performed in four Italian CF Centers [18]. The frequency rate is obviously different in various reports. The possibility of patient-to-patient transfer of B. cepacia in the CF community has always been one of great concerns. Because patients colonized with Bcc may remain colonized for a long periods of time. In some patients, however, Bcc was not isolated for several years and then their first strain reappeared up to 10 years later. In some of these patients, it cannot be excluded that reacquisition from the environment occurred.

PFGE has the excessively high discriminatory and reproducible for the epidemiological study of BCC comparison with other typing method [19]. Based on the generated PFGE results by SpeI restriction enzyme all specimens had analogous PFGE pattern with 100% genetic similarity in this study.

It is possible that the observed infections may have spread from a single source since having identical PFGE pattern. This suggested hypothesis was supported by the isolation results because no other Burkholderia species was identified during the study over a period from April 2011 till end of March 2012. The unusual species distribution may be attributed to environmental sources besides to cross infections from patient to patients, although having similar pattern. Several epidemiological studies have shown most cases of BCC infection transmitted between CF patients [20]. However, occasionally detected B.cepacia distribution during one year of study may represent the acquisition from the environment as well as other sources cross infections from patient to patients.

It is frequently reported B.cepacia has high transmissibility rate and are the responsible microorganism for the mostly nosocomial infections among BCC. B.cepacia infection may result a significant illness, particularly in the cystic fibrosis patients, where patients are at greater risk of nosocomial infection due to their underlying illness. Therefore this finding suggests the importance of adequate patient follow-up in those hospitals that are the referral for CF centers.

## Conclusion

Application of PFGE and identification of pulse-type is a potential tool to enhance the investigation of apparent nosocomial outbreaks of B.cepacia. However it needs to be adjusted with environmental findings. Implementation of educational programs and adherence to infection control policies are obviously the main element for complete elimination of an outbreak.

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# Acute renal failure after influenza vaccination: a case report

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## Key words

## Summary

*A fifty-three years old surgeon had acute renal failure consisting with acute tubulo-interstizial nephropaty twelve days after influenza vaccination; he was on statin therapy since one month. He was given steroidal therapy and fully recovered two weeks apart. This is the fourth case report of acute renal failure after influenza vaccination in patients on statins therapy. The case we describe could account for a underestimated, even if very rare, phenomenon.*

A fifty-three years old surgeon was vaccinated against seasonal influenza on December 15, 2012, with 0,5 ml of split inactivated vaccine in a prefilled syringe (Vaxigrip). The surgeon had been previously vaccinated since year 2000, without any side effect, but this was the first time he took Vaxigrip. Standard hygiene procedures were followed during vaccination and no local or systemic side effect were reported soon thereafter. The surgeon was on statin therapy since one month and took low dose aspirin since a couple of years; the day after vaccination he took 10 mg ketorolac once by the oral route, for backpain; he rarely used ketorolac for the same reasons, always well tolerated. Twelve days after vaccination the surgeon had abrupt onset of extreme thirst with compensatory polyuria up to 3,5 liters daily, diffuse myalgias, weakness and one febrile peak at 38° C. On the fourth day of persisting symptoms he did laboratory exams consisting with a worsening acute renal failure: creatinine 2,34 mg/dl, urea 69 mg/dl. Urine analysis showed minimal leukocyturia and micro-haematuria, low level albuminuria with total protenuria not exceeding 1 gr daily and without myoglobinuria. Leukocytosis (12.300 WBC/ml) was also present, with increase of C-reactive-protein (1,6 mg/dl, range: 0-0,5); creatine phospho kinase was in the normal range and the autoimmune screening, including anti-neutrophil cytoplasmic antibodies (ANCA) was negative. The patient was admitted to Nephrology ward; he was otherwise well and had no symptoms suggestive of urinary tract infection: history was negative for exposure of any other nephrotoxic substance and he didn't report recent travels in tropical areas. Diuresis was unaffected. Given the urine pattern acute tubulo-interstizial nephropaty was suspected, the statin was stopped and the patient was given hydration and oral prednisolone 1 mg/kg for two weeks. The clinical picture rapidly improved, with regression of symptoms, improvement of the renal function, urine normalization without residual proteinuria; the renal biopsy was therefore not done and the patient was discharged on day three: renal function was normal on the follow-up visit two weeks after. The patient fully recovered and returned to work by the end of January.

At our knowledge and after extensive literature search this is one the very first cases of acute renal failure possibly due to influenza vaccination; our patient had a tubulo-interstizial pattern while in the other few cases a glomerular one was prevalent. In particular, we found three other previously published cases [1-3], who had acute renal failure as a consequence of rhabdomyolysis, possibly triggered by vaccination in patients taking statins, as our patient did. Also in another case [4] rhabdomyolysis caused renal failure, but it was unrelated to any other known cause. By contrast, we found just one case [5] of acute renal failure after influenza vaccination not due to rhabdomyolysis; in this patient the renal biopsy led to the diagnosis of minimal change disease [6]. Our patient took a small dose of ketorolac just after vaccination; this is a possible alternative explanation for acute renal failure, but has to be considered very unlikely [7]. Immunizations are a cornerstone of the nation's efforts to protect people from infectious diseases and vaccines are generally very safe [8, 9]; though generally very rare or minor, there are side effects, or "adverse effects," associated with some vaccines: importantly, some adverse events following a vaccine may be due to coincidence and are not caused by the vaccine. All this given, even if we cannot assume a causal relationship between acute renal failure and influenza vaccination in our patient, the association of the very few cases observed so far with statins therapy is noteworthy, and could account for a underestimated, albeit very rare, phenomenon.

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SNIPPET

# New measles vaccination schedules in the European countries?

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## Key words

Measles • Vaccine • Calendar • Europe • Outbreak

## Summary

Over the last 5 years, a number of outbreaks of measles have occurred in several European Union (EU) countries. Many of these outbreaks continue and/or continued for more than 1 year after the notification of the first case. Curiously in many of measles outbreaks about 10% of the patients were less than 12 months of age. All these patients according to the current EU countries vaccination calendars were not yet vaccinated against measles. Based on the results of measles outbreaks, especially age of affected patients, we should consider changing measles vaccination schedules in all EU countries. Most of mothers between 30 and 40 years of age are not vaccinated against measles, and many of them are not naturally immune against measles. These mothers do not pose antibodies against measles and in turn do not provide vertical protection for their infants. In conclusion, administrating the first dose of measles vaccine in the EU countries should be considered before 12 months of age, most probably at 9 months of age.

Measles is a highly infectious disease caused by the virus paramyxovirus, which is a linear single strand RNA. Measles was first described by Abu Becr Rhazes in the 10th century as Hasbah, which means eruption in Arabic. Since then measles epidemics continue to occur, with inter-epidemic periods of 3-5 years [1, 2]. This has been substantially changed since the inclusion of measles vaccine in the obligatory vaccine calendar in all European Union (EU) countries about 30 years ago. All EU countries included two doses of measles vaccine, with the first dose between 12 and 18 months of age [2, 3]. Previous studies showed that measles vaccine when given in two doses, at least 98% of vaccine recipients develop protective immunity against the disease [2]. In concordance, epidemiological investigation of a measles outbreak in a preschool in Incheon (Korea) showed that measles vaccine efficacy was 88.8% in the one dose group and 98% in the two doses group. The authors recommended improving the coverage with two doses vaccination [4].

Over the last 5 years, a number of outbreaks of measles have occurred in several EU countries [5]. Many of these outbreaks continue and/or continued for more than 1 year after the notification of the first case. One of the explanations for these outbreaks was the presence of non-vaccinated population over 30 years of age. Another explanation was vaccination rejection by several sectors of the population; anthroposophic, and ultra-orthodox Jewish communities, and immigrants [2, 6].

Curiously in many of measles outbreaks about 10% of the patients were less than 12 months of age. All these patients according to the current EU countries vaccination calendars were not yet vaccinated against measles [2, 3].

Old studies on measles infection showed that infants were protected against measles with maternal antibodies over the first year of their lives. Vaccination of infants after 12 months of age aimed at avoiding the neutralizing effect of maternal antibodies and in turn improving measles vaccine effectiveness [7]. Based on the results of measles outbreaks, especially age of affected patients, we should consider changing measles vaccination schedules in all EU countries. It is necessary to administrate the first dose of measles vaccine below 12 months of age, for example at 9 months of age, because of several reasons.

First, most of mothers between 30 and 40 years of age are not vaccinated against measles, and many of them are not naturally immune against measles. These mothers do not pose antibodies against measles and in turn do not provide vertical protection for their infants [3, 7]. In concordance with this observation, a recent study conducted in Sheffield (UK) evaluated the risk of healthcare workers (HCWs) of contracting and transmitting measles and mumps to patients and staff. To examine this risk at the point of entry to healthcare, the authors assessed the serological results of new HCWs presenting for pre-placement clearance without evidence of measles-mumps-rubella (MMR) immunity between 1 April 2010 and 31 March 2012. Overall rates of serological positivity to MMR across all age groups were 88.2%, 68.8% and 93.9%, respectively. With regard to measles and mumps, there were statistically significant decreases in the percentage of HCWs born after 1980 that had positive serology (P < 0.05) [5].

Second, over the last 30 years the prevalence of measles infections in the EU countries was progressively lowering. The contact of vaccinated women between 30 and 40 years of age was almost negligible. This contact acts like a booster dose and stimulates the immunity systems to generate measles antibodies. Again vaccinated mothers aged between 30 and 40 years old provide very low levels of antibodies to their infants, not sufficient to protect them over the first 12 months of their lives [2, 7].

Third, the prevalence of breast feeding is lowering or at least reduced in duration all over the EU countries, especially over the last 20 years because of several social and economic changes. Maternal milk provides antibodies which offers major protection for infants against many bacterial and viral infections including measles [1, 8].

The recent vaccine calendar of the Spanish Association of Paediatrics (2013) recommended administration of the first dose of measles vaccine at 12 months of age, not at 15 months as recommended before. The second dose is recommended between 2 and 3 years of age to achieve a protection > 98% [9].

In conclusion, administrating the first dose of measles vaccine in the EU countries should be considered before 12 months of age, most probably at 9 months of age.

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