A study on prevalence of vancomycin-resistant enterococci carriers admitted in a children hospital in Iran

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ABSTRACT

Increased prevalence of Vancomycin-resistant Enterococci and their ability to transfer Vancomycin resistance genes to other bacteria have made them a subject of close scrutiny and intense investigation. This study was performed to determine the prevalence of Vancomycin-resistant Enterococci occurrence in children hospitalized in a Hospital in Tehran, Iran. During 12 months, we collected 780 stool specimens from 460 children hospitalized (age ranged from 1 to 180 months) in Mofid children’s hospital, Tehran, Iran. Resistance to Vancomycin and other antibiotics were evaluated by Epsilometer test for isolated Enterococci. We observed 16.9% of Enterococci were resistant to Vancomycin. Enterococci were mostly resistant to the other antibiotics. In conclusion regarding to the increasing prevalence of Vancomycin-resistant Enterococci we suggest more studies to evaluate the resistance patterns of Enterococci to new antibiotics are being introduced to treat Enterococci infections nowadays.

Keywords: Vancomycin Resistant Enterococci, Stool Carriage

INTRODUCTION

An increasing number of bacterial infections are now difficult or impossible to treat [1] because of the misuse of antimicrobial drugs and the epidemic spread of bacterial resistance to these drugs [2].

Enterococcus, long recognized as a pathogen in select populations, in the past two decades has become a common and particularly troublesome cause of hospital-acquired infection [3]. Enterococci are not aggressively invasive organisms and their dramatic emergence as a cause of nosocomial infection is predominantly due to their resistance to antibiotics commonly used in the hospital setting. Enterococci are highly resistant to Cephalosporins and semisynthetic Penicillin such as Nafcillin, Oxacillin and Methicillin. They are moderately resistant to extended spectrum Penicillins such as Ticarcillin and Carbenicillin. Ampicillin, Imipenem and Penicillin are the most active betalactamases against these organisms. Diseases caused by strains resistant to Ampicillin and Aminoglycosides can be treated with glycopeptides. Vancomycin has traditionally been effective against multi-resistant isolates, but resistance
to Vancomycin and other glycopeptides including Teicoplanin and Daptomycin is increasingly common [3].

Vancomycin-resistant Enterococci (VRE) recently have emerged as a nosocomial pathogen in worldwide [4]. They are the third most common cause of hospital-acquired bacteremia [5]. Outbreaks with VRE are also being reported with increasing frequency in many countries (6). VRE were initially described in Europe in 1987 [7].

Contaminated equipments, hands of health care workers and environmental surface can transmit the VRE [8]. As VRE survives for long periods of time on dry surfaces, it is a successful environmental contaminant causing some outbreaks [9]. The risk factors that have emerged are longer duration of hospitalization, longer lengths of stay in intensive care units (ICU), the need for nosocomial transfer to another ward, the need for surgical exploration following liver transplantation, and the use of enteral tube feedings or sucralfate [10]. Currently, there is no ideal therapy which yields the bactericidal activity for serious infections caused by VRE. Above all, assessing the efficacy of therapy remains difficult because VRE is often associated with severe underlying illnesses and can be a part of polymicrobial infection [9].

Colonization and infection with multi drug resistant (MDR) Enterococci occur worldwide. Early reports showed that in the United States, the percentage of nosocomial infections caused by VRE increased more than 20-folds between 1989 and 1993, indicating rapid dissemination [11]. The incidence of VRE has since increased rapidly, and VRE now comprise nearly 30% of Enterococci isolated from patients in ICU in the United States appeared [12].

In one study in the Czech Republic, 2691 rectal swabs from the hospitalized patients and 6529 rectal swabs from the subjects in community setting were examined. The prevalence of VRE in the gastrointestinal tract was 1.9% in the hospitalized patients and 0.4% of the community subjects [13]. In one study in Singapore, VRE was detected in the stool of 12.3% of patients [14].

This study was performed to determine the prevalence of VRE occurrence in patients hospitalized in Mofid Children Hospital in Tehran, Iran and to evaluate the Enterococci resistance to other antibiotics.

**MATERIALS AND METHODS**

This study was done from October 2007 to October 2008 at Mofid Children Hospital in Tehran, Iran. In this study, we collected 780 stool samples from 460 patients hospitalized in Mofid children's hospital with the average age of 19.14 months. The patients were included in our study without concerning the cause of their admissions. This study was performed after approval by the Ethics Committee of our university. At the same time we used questionnaire to ask about the patients' age, gender.

The first stool sample was taken on the first day of admission and the second sample was taken after 48 hours of hospitalization. One hundred and forty patients had one sample.

Samples were collected in sterile plastic containers which were used for stool collecting and then were transferred to the laboratory. Both stool samples were inoculated in the Enterococcovl agar. The plates were incubated for 24 hours at 37° C and cultures were evaluated for Enterococci growth by performing the catalase , and gram staining. Each colony that was gram positive cocci and catalase negative, subcultured in three medium: (1) Mueller Hinton agar to consider their growth at 15° C and 45° C(2) NaCl 6.5% (3) Bile esculine agar containing 6µg/ml Vancomycin and 64µg/ml Ceftazidim for screening. All media were kept at 37° C for 24 hours. Suspensions of presumptive Enterococci colonies from selective medium (bile Esclune azide agar containing 6µg/ml Vancomycin and 64µg/ml Ceftazidim) after 15 to 48 hours of incubation at 37°C were prepared to a density equivalent to a McFarland standard of 3 in 50 µl of 7.5% Chelex 100. Resistance to Vancomycin and other antibiotics were evaluated by Epsilometer test (E-test) method for isolated Enterococci.

We used SPSS Statistical software package version 12 for analysis. Statistical analysis was performed using T-test (post –Hoc multiple comparison test) and Chi -square test. Statistical significance was assumed at the P<0.05 level.
RESULTS

Seven hundred and eighty stool samples were obtained from 460 hospitalized patients during 12 months. One hundred and forty cases had one stool sample and 320 cases had two samples. As a result we have 780 cases stool samples. Forty seven percent of patients were males. The patients’ age ranged from 1 to 180 months (15 years old), with the average of 19.14 months.

Enterococci were isolated from 94.7% of samples. Enterococcus faecalis and Enterococcus faecium were detected from 85.3% cases and 10.8 cases% respectively and 3.9% cases had another types of Enterococci spp.

We observed 16.9% of Enterococci were resistant to Vancomycin. Among them, 73.2% were Enterococcus faecalis and 26.8% were Enterococcus faecium (Figure-1). Enterococci were mostly resistant to the other antibiotics. The antimicrobial susceptibility rate of VRE is shown in Table-1.

We found no relationship between VRE occurrence and patient’s gender and age.

![Figure-1. The prevalence of VRE strain in stool samples of patients who admitted in Mofid children Hospital](image)

| Antimicrobial resistance patterns for isolated Enterococci from stool samples of patients who admitted in Mofid children Hospital by E-test method |
|---------------------------------|----------|----------|
| Antibiotics                     | Sensitive % | Resistant % |
| Erythromycin                   | 1.8       | 98.2      |
| Clindamycin                    | 3         | 97        |
| Cephalexin                     | 10.5      | 89.5      |
| Oxacillin                      | 12.6      | 87.4      |
| Cefotaxim                      | 21        | 79        |
| Cefotetan                      | 24        | 76        |
| Nafcillin                      | 25.4      | 74.6      |
| Ceftriaxone                    | 58.5      | 41.5      |
| Imipenem                       | 62.6      | 37.4      |
| Chloramphenicol                | 64.7      | 35.3      |
| Trimetoprim sulfa metoxazol    | 74.4      | 25.6      |
| Rifampicin                     | 82.2      | 17.8      |
| Vancomycin                     | 83.1      | 16.9      |
| Ciprofloxacin                  | 88.6      | 11.4      |
| Gentamycin                     | 93        | 7         |
| Ampicillin                     | 93.3      | 6.7       |

DISCUSSION

Increasing antibiotic resistance in common bacterial pathogens, in both hospitals and communities, present a growing threat to human health worldwide [4]. VRE is a major concern in medical practice. Their increased prevalence and their ability to transfer Vancomycin resistance genes to other bacteria (including Methicillin-resistant Staphylococcus aureus) have made them a subject of close scrutiny and intense investigation [9]. In our study 16.9% of Enterococci isolated from patients were resistant to
Vancomycin and in comparison with other studies, it was moderate. Result of our study is similar to Lee k., et al study in Korea which showed that the rate of VRE was 16% and another study in Iran that was done in 2007 [15,16].

In 2004 one study in Brazil showed none of the Enterococcal isolates were resistant to Vancomycin or Teicoplanin [17]. The overall prevalence of VRE colonization was 0.06% in a study in Hong Kong [18] and 0.7% in a study that was performed in Spain [19]. The rate of VRE was less than 7% in a study in Argentina [20]. In 2006, N. Bruinsma et al reported that VRE remains below 10% in most European countries, but higher levels are reported from Portugal, Italy, Ireland, and Greece. Significant increases are observed in Germany, France, and Ireland [21]. In a multinational survey of nosocomial pathogens, none of the clinical Enterococcal isolates from South Africa, Egypt, Saudi Arabia or Lebanon were VRE. However, 7% of isolates from Germany and 16.7% from Switzerland and Greece were VRE [22].

The patients in our study were between one month and 180 months (15 years old). We observed no relationship between VRE and patient’s age. In some studies there was a direct relation between age and VRE colonization [23, 24,25]. We didn’t observe correlation between VRE occurrence and patient’s gender. Kolacheretis et al found that VRE-positive patients were more likely to be males [26]. On the contrary, one study in Germany and another in Singapore showed that VRE was significantly associated with female gender [27, 28].

In our study, the Enterococci were most resistant to many antibiotics. The resistance pattern for other antibiotics against VRE were respectively: Erythromycin 98.2%, Clindamycin 97%, Cephalosporins 89.5%, Oxacillin 87.4%, Cefotaxim 79%, Cefotetan 76%, Nafcillin 74.6 %, Ceftriaxone 41%, Imipenem 37.4%, Chloramphenicol 35.3%, Trimetoprim-sulfametoxyzol 25.6%, Rifampicin 17.8%, Ciprofloxacin 11.4%, Gentamycin7% and Ampicillin 6.7%. These findings are similar to many other studies that showed the wide resistance of Enterococci to many antimicrobials agents [17,29, 30,31,32]

CONCLUSION

VRE is an important nosocomial pathogen because of its rapid spread. With regard to the increasing prevalence of VRE we suggest more studies to evaluate the resistant patterns of Enterococci to new antibiotics are being introduced to treat Enterococci infections nowadays. Also we advise the adoption of infection control measures to prevent the spreading of VRE.

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