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ORIGINAL ARTICLE

Increase in prevalence of vancomycin resistant isolates of Enterococcous faecium at Labbafinejad hospital

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ABSTRACT

Background: Vancomycin resistant isolates of Enterococcous faecium (VRE) have previously been reported from Tehran Hospitals. However, little data were available on the genetic heterogeneity of VRE isolates among the Iranian population. Therefore the emergence of infections with the new clones of VRE needs to be investigated. The drug resistance surveillance program at Labbafinejad hospital has to be continued.

Patients and methods: Overall, 103 non-replicative isolates of enterococci grown from urine samples in the first quarter of 2005 were screened for their susceptibilities to different antibiotics. Ribotyping was then used to genetically characterize the isolates of VRE.

Results: Using disk diffusion method, all isolates were found susceptible to linezolid. Resistance to high level concentration of gentamicin was detected in 65.7% of isolates. All isolates of E. faecalis (n=86) were susceptible to vancomycin. Conversely, over 70% of E. faecium isolates (n=12) showed resistance to this glycopeptide. The VRE isolates recovered from patients in 2005 were heterogeneous comparing with those of 2000.

Conclusion: Conventional bacteriology confirmed the increase in the rate of VRE. It appears that a variety of new VRE clones have arisen recently at different wards of this hospital as determined by ribotyping.

Keywords: *Vancomycin resistant, Enterococcous faecium, Ribotyping, Drug resistance.*

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INTRODUCTION

The Strains of Enterococcous faecalis and E. faecium have emerged as important opportunistic pathogens in nosocomial infections (1,2). They cause a wide range of infections such as bacteremia, endocarditis, meningitis, post-surgical wounds, urinary tract infections and intra-abdominal sepsis (1,3,4). Although these organisms do not possess strong virulent factors, they are intrinsically resistant to several important antimicrobial agents including polymyxin, lincosamide, trimethroprim-sulfamethoxazole, low concentration of aminoglycosides, monobactams, and streptogramin (3,5). Importantly, enterococci can exchange resistant genes by conjugative plasmids or transposon.

Vancomycin-resistant isolates of E. faecium (VRE) with phenotype van A have already been isolated from the Iranian patients (6). However, none of the E. faecalis isolates from these patients were resistant to this glycopeptide. VRE isolates

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are usually resistant to other anti-cell wall antibiotics, making the treatment more difficult.

We had already determined the prevalence of enterococci and their sensitivities to antimicrobial agents at Tehran hospitals (6). However, with the current widespread use of broad spectrum antibiotics, regular surveillance of drug resistance patterns of enterococci in hospitals is imperative. This study was designed to investigate such changes in the population of enterococci cultured from patients with urinary tract infection at Labbafinejad hospital in the first quarter of 2005. Ribotyping was then used to trace any genetic relationships between the VRE isolates.

PATIENTS and METHODS

From December 2004 to April 2005, 103 isolates of enterococci were cultured from urine samples of patients hospitalized in transplant, paediatrics, urology wards of Labbafinejad Hospital, Tehran, Iran. All isolates had been cultured from cases with urinary tract infections. The biochemical tests were used for identification of bacterial isolates to species level as explained before (6).

Enterococcous faecalis ATCC 29212, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 1399 were used as control for identification and drug susceptibility testing.

The disk diffusion test was performed to assess the susceptibility of isolates to the following antibiotics: ampicillin (10µg), nitrofurantoin (300µg), penicillin G (10 unit), imipenem (10µg), tetracycline (30µg), chloramphenicol (30µg), gentamicin (120µg), ciprofloxacin (5µg), vancomycin (30µg), quinopristin/dalfopristin (15µg)), and linezolid (30µg) (7). All disks were purchased from Oxoid (Oxoid, Hampshire, England). The minimum inhibitory concentrations for gentamicin, ampicillin and vancomycin were determined in the selected isolates by macro broth dilution method (7). Selection of isolates was based on the inhibitory zones of 7-9mm for gentamicin, >18mm for ampicillin and 15-16 mm for vancomycin formed around the antibiotic disks (8).

Dilutions of antibiotics ranged from 32 to 2048 µg/ml for gentamicin, and from 0.25 to 256µg/ml for ampicillin and vancomycin. The results were read after incubation at 37°C for 18-20h (gentamicin and ampicillin) and 24 h (vancomycin) (8). Production of β-lactamase was examined by using a directed nitrocefin-based β-lactamase test (Oxoid, UK).

Vancomycin resistant strains were selected for ribotyping. To extract genomic DNA, the bacterial cells were treated in TE buffer (1mM EDTA, 10 mM Tris pH 8.0) containing lysozyme (10mg/ml) for three hours at 37°C followed by additional incubation at 65°C for 30min in the presence of proteinase K and SDS (20 mg/ml in SDS 10%). Proteins were removed by phenol-chlorophorm-isoamylalcohol (25:24:1) and DNA was precipitated by cold isopropanol followed by centrifugation at 12000 rpm for 15minute. The sedimented DNA was washed in 70% ethanol and dissolved in TE buffer containing RNAse (50mg/ml). DNA was stored at 4°C until used. Approximately 4 µg of genomic DNA from each strain was digested by Hin dIII endonuclease (Roche, Mannheim, Germany) and the fragments were separated on agarose gel (0.8%) electrophoresis in 1x TBE buffer (Tris 0.89M, EDTA 0.02M, boric acid 0.89M) for 16.5 h at °C. Lambda DNA (Roche, Mannheim, Germany) cleaved by Hin dIII was used as a molecular weight marker. DNA fragments were capillary transferred to the positively charged nylon membrane (Roche, Mannheim, Germany) and hybridized with digoxigenin-labelled oligo-nucleotide probes complementary to Escherichia coli 16S and 23S rRNA (9). The sequences of probes are as follows:

5´-AGAGTTTGATCATGGCTCAG-Dig, 5´-TTTGGCACCTCGATGTCGGCT-Dig, 5´-TGACGGGCGGTGTGTACAA-Dig, 5´-ACCGATAGIGAACCAGTACCGTG-Dig and 5´-GTACCICAAACCGACACAGGTIG-Dig. The oligo-nucleotide probes were labelled at 3´ end using labelling kit as instructed by the supplier (Roche).

RESULTS

A total of 103 isolates of enterococci were cultured from patients with UTI during the 4 months study at Labafinegad Hospital. These include isolates of E. faecalis (n=86, 83.5%) and E. faecium (n=17, 16.5%). All enterococcal isolates were susceptible to linezolid (table 1).

Table 1. Antibiotic resistance behavior of enterococci isolates with disk diffusion

	E.faecalis (n=86)			E.faecium (n=17)		
	S	I	R	S	I	R
Ampicillin	63.4	34.2	2.4	0.0	14.3	85.7
Tetracycline	3.24	0.0	96.76	28.6	28.6	42.8
Chloramphenicol	90.2	2.4	7.3	100	0.0	0.0
Quinopristin/ Dalfopristin	14.6	2.4	83	100	0.0	0.0
Vancomycin	100	0.0	0.0	28.6	0.0	71.4
Ciprofloxacin	17.2	2.4	80.4	14.3	14.3	71.4
Penicillin G	4.9	87.8	7.3	0.0	0.0	100
Linezolid	87.8	15.2	0.0	100	0.0	0.0
Imipenem	100	0.0	0.0	0.0	0.0	100
Nitrofurantoin	97.6	2.4	0.0	71.4	28.6	0.0
Gentamicin	34.2	0.0	65.8	28.6	0.0	71.4

S: Sensitive; I: Intermediate resistance; R: Resistant

Isolates of E. faecalis (100%) were sensitive to vancomycin and imipenem. The rates of resistance to other antibiotics were in the following order: tetracycline (96.76%), ciprofloxacin (80%), and gentamicin (65.8%).

All isolates of E. faecium were resistant to imipenem and penicillin G. However they showed good rate of sensitivity to quinopristin/dalfopristin (100%) and chloramphenicol (90.2%) (table 1).

The resistance rates for tetracycline, vancomycin, ciprofloxacin and gentamicin among isolates of E. faecium were 42.8%, 71.4%, 71.4% and 71.4% respectively. Intermediate resistance to ampicillin (inhibitory zone 18-25 mm) was found among 34.2% and 14.3% of E. faecalis and E. faecium isolates respectively.

The MICs of ampicillin against isolates of E. faecalis that showed intermediate level of resistance in disk diffusion test varied from 2 to 8μg. The MICs of ampicillin for isolates of E. faecium were higher than E. faecalis. Of 17 isolates of E. faecium, 3 had MIC128-256 μg/ml (high level resistant), and the remaining (n=14) had MICs between 16 to 64μg/ml.

Up to 87.8% of isolates of E. faecalis showed intermediate level of resistance to penicillin. The broth macro-dilution method showed that the MIC of penicillin was >2048 μg in 93.8% of these isolates. The MIC of vancomycin for all vancomycin resistant isolates of E. faecium were >256 μg/ml.

VRE isolates were grouped in four clusters by ribotyping. Between 2 to 6 DNA bands were detected when the bacterial genomic DNAs were digested with Hin dIII and probed with oligoprobes (figure 1). Vancomycin resistant isolates (n=4) had been recovered from patients at different hospital wards including transplant and pediatrics, during 2004.

Of 4 VRE isolates recovered from patients in 2005, 3 produced distinct banding patterns in ribotyping. They produced 2, 5 and 6 DNA bands with oligoprobes after hybridization. The fourth isolate was similar to the isolates recovered from patients in 2000. Three isolates within this group were originated from Labbafinejad Hospital, but one belonged to another hospital. These isolates were identical and produced 4 DNA bands after hybridization.

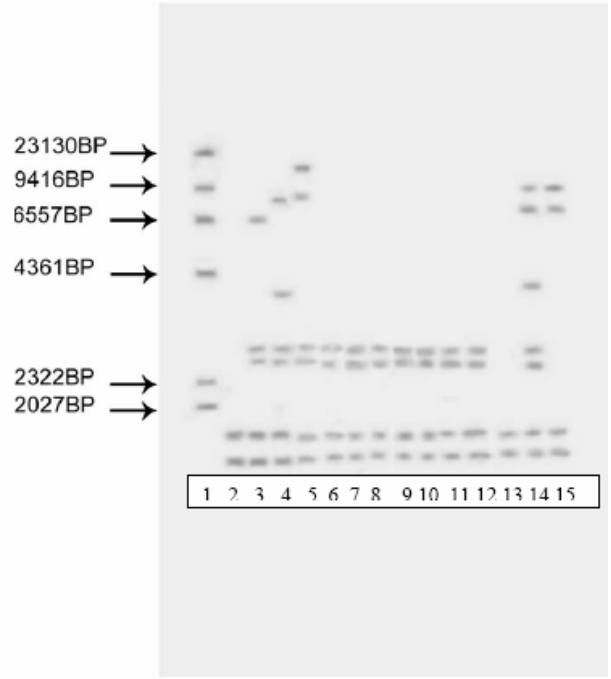

Figure 1. Ribotype patterns of vancomycin resistant strains of Enterococcous faecium obtained with HindIII restriction enzyme. (Lane 1: DNA ladder; Lane 2-5: VRE Enterococcus faecium 2005; Lane 6-9: VRE Enterococcus faecium 2000; Lane 9-15: Vancomycin sensitive Enterococcus faecium.)

DISCUSSION

This study showed E. faecalis (n=86, 83.5%) has remained as the dominant species at Labbafinejad hospital followed by E. faecium (n=17, 16.7%). Isolates belonging to other species have not been detected in the study hospital yet.

Our finding showed that linezolid and nitrofurantoin were the most active agents against enterococci during the study period. With the resistant rate of 96.7%, tetracycline has the least effect on the isolates of E. faecalis. It was more effective against E. faecium isolates. Similar findings have been reported from China and Kuwait (1,2,4).

Chloramphenicol was the third most active antibiotic against E. faecium after vancomycin and linezolid. The resistance rates to ciprofloxacin among the E. faecalis and E. faecium isolates were 80% and 71% respectively. These rates are much higher than Kuwait (33.5%, 55.5%) and Croatia (16.0%, 52.2%) (4, 10). Disk diffusion test showed that the rate of intermediate resistance to ampicillin among isolates of E. faecalis was high (34.2%). However, these isolates were found susceptible when they were screened by macro broth dilution assay (MIC= 2-8µg/ml). The MICs of ampicillin for three isolates of E. faecium were 16, 64 and 128µg/ml. Four isolates of E. faecium were found highly resistant to ampicillin since their MICs were >256µg/ml. Isolates showing MICs >64µg/ml has been defined as highly resistant to ampicillin (11). Resistance of Iranian isolates to ampicillin probably results from the production of a low affinity penicillin-binding protein since β-lactamase producing enterococci has not been detected in Iran yet (6).

In the current study there were an increase in prevalence of HLGR isolates of E. faecalis (from 42.4% to 65.8%) and E. faecium (from 60% to 71%) comparing with our previous study (6).The presence of HLGR is predictive of the loss of synergy between gentamicin and a cell-wall-active agent such as ampicillin or vancomycin (2).

Though the susceptibility of E. faecalis to vancomycin remained unchanged since previous study (100%), there was significant increase in rate of resistance (from 10.6% to 71.4%) to this glycopeptide among isolates of E. faecium (6).

Ribotyping has been applied to different population of pathogenic bacteria. Analysis of 8 VRE isolates by ribotyping showed that all isolates cultured from patients in 2000 produced identical ribotype. It demonstrates the circulation of a VRE clone at that time at different wards of hospital. The situation changed in 2005 since isolates recovered from patients in this period were genetically distinct from previous study by ribotyping. It suggests emerging of infection with new clones of VRE at Labbafinejad Hospital. Using pulsed field gel electrophoresis (PFGE), increase in prevalence of infections with different

clones of vancomycin resistant strains of E. faecium at German hospitals has recently been documented (12). Using different molecular techniques, dissemination of van A gene clusters among genetically diverse group of isolates has been reported (12).

A homogeneous patterns produced by ribotyping were observed among VRE isolates from different wards of Labbafinejad hospital in 2000, but this ribotype was observed only in one VRE strain in 2005. This ribotype has persisted in the hospital and infected patients. There were also two VRE strains from Labbafinejad and Shariati hospitals which produced similar pattern in ribotyping. It suggests the possibility of the inter hospital spread of vancomycin resistant E. faecium.

In conclusion, the prevalence of multi-drug resistant strains of enterococci in particular VRE isolates has increased in the study hospital. The VRE isolates recovered from patients in 2005 were heterogeneous comparing with those of 2000. It suggests that a variety of new VRE clones have arisen in different wards of the hospital.

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