

Abstract

Enterococci are a group of microorganisms that commonly inhabit the gastrointestinal tract of animals and humans. They are incriminated in causing severe life threatening infections.

Enterococci are recognized as a major cause of nosocomial infections worldwide. The majority of infections are caused by *E. faecalis* followed to a much lower extent by *E. faecium*.

Enterococci have both intrinsic and acquired resistance to several classes of antibiotics.

The goal of this study was to determine the scope of resistance of enterococci to antibiotics and to correlate that with resistance genes. In addition, we determined the relatedness of the isolates by pulsed field gel electrophoresis.

A total of 89 vancomycin resistant enterococcus isolates were collected from major hospitals in Jerusalem. Antimicrobial susceptibility and minimal inhibitory concentration was performed on all isolates following the CLSI guidelines. Subsequently, DNA was extracted by lysing the cell wall with lysozyme followed by applying the DNazol method to isolate the DNA. Molecular characterization for *vanA* and *vanB* genes was determined by PCR. Plugs for pulsed field gel electrophoresis were prepared from an overnight culture. After preparing a cell suspension in TE buffer with an optical density of 0.9-1.1 at a wave length of 610, Lysozyme and proteinase K were added to the suspension and mixed with 1.2% agarose. The plugs were then restricted for two hours with *smaI* enzyme and loaded in 1% pulsed field certified agarose prepared in TBE buffer and electrophoresis was performed using CHEF-DRIII instrument for 18 hours. The gels were stained with ethidium bromide, then viewed and photographed using a gel documentation system. Lambda ladder was applied in the first and last well of each gel to determine the size of the bands obtained.

The results reflected that 68.5% (61/89) of the isolates were *E. faecium* and 31.5% (28/89) were *E. faecalis*. All *E. faecalis* isolates were susceptible to ampicillin while all *E. faecium* isolates were resistant to it. Both *E. faecalis* and *E. faecium* isolates were resistant to vancomycin, ciprofloxacin and erythromycin. Resistance of *E. faecalis* isolates to teicoplanin, chloramphenicol and tetracycline was 85.7% (24/28), 21.4% (6/28) and 17.9% (16/28) respectively. Resistance of *E. faecium* to teicoplanin, chloramphenicol, and tetracycline was 77% (47/61), 8.2% (5/61) and 90.2% (55/61) respectively.

The MIC results for vancomycin in both *E. faecalis* and *E. faecium* were ≥ 256 ug/ml for all isolates. The MICs for *E. faecalis* isolates tested with teicoplanin were ≤ 4 ug/ml in 14.3% (4/28) and ≥ 64 in 85.7% (24/28) of the isolates. On the other hand, the MICs for *E. faecium* isolates for teicoplanin were ≥ 64 ug/ml in 77% (47/61) and ≤ 8 ug/ml in 22.9% (14/61).

Molecular characterization of the VRE isolates revealed that 24/28 of *E. faecalis* carried *vanA* gene while 4/28 carried *vanB* gene. There was 45/61 of *E. faecium* isolates carried *vanA* gene while 14/61 carried *vanB* gene. Interestingly 2/61 harboured genes, *vanA* and *vanB*. None of the enterococcus isolates tested carried *vanD* gene.

PFGE results showed a wide range of variation between the *E. faecium* isolates. Although the 52 *E. faecium* isolates were divided into 31 PFGE patterns, two patterns showed high relatedness: Pattern I had a cluster of 6 strains, most were isolated from the same hospital ward. Pattern II had a cluster of 5 strains isolated from 2 hospital wards. The rest of the isolates showed considerable variation that made it impossible to cluster them in groups.

The PFGE results for *E. faecalis* showed tremendous variation that the 26 vancomycin resistant *E. faecalis* isolates were divided into 17 different profiles.

In conclusion, the vancomycin resistant enterococci (*E. faecalis* and *E. faecium*) pose a great risk for hospitalized patients on one hand and stand defiant and resistant to most antibiotic classes. Therefore, the health officials in this country must take drastic steps to curb the spread of this hard to treat genus.