A Study of Scanning Electron Microscope of Vancomycin Resistant Enterococcus faecalis from Clinical Isolates

Ajay Kumar Oli¹, Raju Sungar², Nagaveni Shivshetty¹, Rajeshwari Hosamani¹, Kelman Chandrakanth Revansiddappa*¹
¹Department of Biotechnology, Gulbarga University, Gulbarga, India
²ELLA Foundation, Hyderabad, India
Email: *ckelman@gmail.com

Received February 4, 2012; revised March 2, 2012; accepted April 5, 2012

ABSTRACT

Vancomycin-resistant Enterococcus faecalis pose an emerging health risk, but little is known about the precise epidemiology for vancomycin resistance. The glycopeptide resistant was studied using different techniques such as broth macrodilution, agar dilution combined with agar diffusion, morphology cell changes by scanning electron microscopy. Eight VREF isolated from different clinical samples were used. Results showed low level and high level resistant to vancomycin antibiotic at concentration of 64 to 128 µg/ml, but antibacterial activity was reduced to 256 µg/ml, the SEM revealed increased in the cell size with the antibiotic compared to control and standard culture. The technique constitutes simple method for the detection of organism.

Keywords: VREF; SEM; Vancomycin

1. Introduction

Enterococcus spp. are natural inhabitants of the gastrointestinal tract of humans and animals [1,2] but can be also found in soil, water, and vegetables [3]. The two most important species, Enterococcus faecium and E. faecalis, are most frequently implicated in human and animal infections [4]. E. faecalis is an opportunistic pathogen known to cause serious infections, such as bacteraemia, sepsicaemia, urinary tract infections, wound infections, meningitis, and endocarditis [2,5,6].

Prior to 1990s, enterococci also have been recognized as an important cause of bacterial endocarditis for almost a century [7]. However, during the past decade, there has been a worldwide trend in increasing occurrence of enterococci (in the hospitals), a shift in the spectrum of enterococcal infections, and emergence of antimicrobial resistance among such isolates. Enterococci were reported as the second most common cause of nosocomial infections in the US. The most frequent infections caused by enterococci are urinary tract infections (UTIs) [8].

The acquisition of high level aminoglycoside resistance (HLAR) and vancomycin resistance has limited the therapeutic options available for clinicians. The transfer potential of vancomycin resistant genes from Enterococci to S. aureus have been reported in clinical settings, increases the importance of findings ways to limit the spread of vancomycin resistant Enterococci (VRE) [9]. The problem of nosocomial enterococcal infection is compounded by emerging antibiotic resistance. The resistance alone does not explain the increase of Enterococci in nosocomial infections, microorganisms can adapt to different organic substances and other forms of environmental stress by several adaptive mechanisms. The exposure of bacteria to sub-MICs (Minimum Inhibition Concentration) of Vancomycin results in a significant alteration of cellular morphology and disturbance of metabolic activity in resistant E. faecalis [10]. The major adaptive responses of microorganisms to externally occurring changes in the environment are modifications of the cell envelope [11] and also coupled with changes in the overall morphology of the cells.

Scanning electron microscopy offers the unique ability to examine surface structures at relatively high resolution and proves particularly useful in the examination of the effect of antibiotics that act on the bacterial cell wall [12-14]. The present study describes the effect of antibiotic stress on the morphology of vancomycin resistant E. faecalis strains examined by scanning electron microscopy.

2. Materials and Method

2.1. Bacterial Strains

The E. faecalis strains used in the present investigation were isolated from clinical samples over six months pe-
period from September 2008 and January 2009 from Dist-

rit Govt. hospital and diagnostic centres from Gulbarga

region. The strains were isolated from blood, urine, pus and Cerebrospinal fluid sample.

Bacteria were isolated as previously described [15] and routinely grown in trypticase soy broth or agar at 37°C. They were purified by standard methods and iden-
tified to the species level by the conventional biochemi-

cal identification scheme of De Marques and Suzart [16].

Confirmed isolates were stored in trypticase soy broth containing 20% glycerol at −80°C until further charac-
terisation could be performed.

2.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (Hi-media, India) by the standard disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards [17]. The antibiotics used for the tests were vancomycin, ampicillin, oxacillin, rifamycin, ciprofloxacin, tobramycin, genta-

mycin, teicoplanin and streptomycin.

2.3. Minimal Inhibitory Concentration (MIC)

All strains were screened for vancomycin (Sigma Aldrich Ltd., Bangalore) MIC’s by the agar dilution method while the disc diffusion method was performed for screening susceptibility to other antimicrobials, by CLSI guidelines [18] E. faecalis NCIM 5025 used as control.

2.4. Scanning Electron Microscope Study

SEM is used to examine the minor changes in cell mor-

phology of the populations that have adapted to antibiotic stress [19]. The selected VREF strains were grown in BHI media with increasing vancomycin concentrations as described earlier. The bacterial cells from each culture were recovered by centrifugation at 6000 rev/min and the cells were washed twice with potassium phosphate buffer (50 mM, pH 7.0). Bacterial cells were then fixed by im-

mersing in 2.5% glutaraldehyde in potassium phosphate buffer (50 mM, pH 7) for overnight at 4°C. Then the specimens were washed twice with buffer and dehy-

drated by ethanol series (v/v) ranging from 30%, 40%, 50%, 60%, 70%, 80%, 90% to 100% and stored in 100% ethanol. For SEM, the specimens were dried to critical point, coated with gold and examined with an S-200C scanning electron microscope. [20] compared with stand-
dard NCIM 5025 and control EF122 strain.

3. Results

3.1. Bacterial Isolates

A total of 122 Enterococcus strains were isolated from different clinical samples on bile esculin agar. The spe-
cies identities of the clinical Enterococcal isolates, in-
cludes 76 (62.29%) strains were E. faecalis. The E. fae-
calis was the predominant isolates from urine, pus, CSF and blood samples.

3.2. Antimicrobial Susceptibility

E. faecalis strains showed resistance to the different anti-
biotics like vancomycin (77.63%), gentamycin (64.47%) and oxacillin (55.26%) antibiotics, and were found to be multi drug resistant. The isolates were found susceptible to rifamycin (61.84%), teicoplanin (55.26%) streptomycin (52.63%) and tobramycin (51.13%).

3.3. MIC’s in E. faecalis Isolates

All the vancomycin resistant E. faecalis were subjected for vancomycin MIC’s test. Twelve strains showing drug resistance to all the antibiotics tested were selected for the MIC studies. Among them, 8 strains showed MIC in the range of ≥64 μg/ml while other 4 strains exhibited MIC of ≥128 μg/ml. The bactericidal activity was observed at concentration of 256/256 μg/ml and low bacte-
icidal growth at 128/256 μg/ml. The concentration of antibiotic showed bacterial growth to about ten-fold at 24 hrs, with a concentration of 128/256 μg/ml. An increase in 100 fold at 24 hr was observed with a vancomycin concentration of 6/32 μg/ml.

3.4. Scanning Electron Microscope Study

The results of cell morphology of VREF strains exam-
ined by scanning electron microscopy (SEM) revealed that in the presence of vancomycin, the cells altered their morphology with respect to different concentrations of the antibiotic. In the absence of vancomycin the cell morphology of control were apparently normal (Figure 1(a)). However standard culture showed no alteration in their cell morphology (Figure 1(b)) but enlarged, mal-
formed and rough surfaced were observed in the antibi-

otic treated VREF culture with a concentration of 12 μg/ml (Figure 1(e)).

4. Discussion

Enterococci infections have become increasingly com-
mon because of their intrinsic resistance to several anti-
microbial agents and their propensity to acquire resist-
ance from the environment [21]. Approximately 80% to 90% of all enterococcal infections are attributed to E. faecalis, whereas E. faecium is responsible for about 5% - 10% of these infections [22]. E. faecalis has recently evolved from a generally a virulent commensal into an MDT healthcare-associated pathogen causing difficult-to
treat infections. Therefore, studies of E. faecalis resis
A. K. OLI ET AL. 95

(a) Control-strain EF 122 without antibiotic; (b) Standard culture NCIM 5025; (c) Strain EF 122 treated with antibiotic concentration of 12 µg/ml.

Figure 1. Scanning electron micrographs of VREF cells Magnification at 10,000 × 5 µm. (a) Control-strain EF 122 without antibiotic; (b) Standard culture NCIM 5025; (c) Strain EF 122 treated with antibiotic concentration of 12 µg/ml.

tance have increased. Determination of glycopeptides activity has a significant role in guiding antibiotic usage. The results of this study confirms that *E. faecalis* were more resistant to the vancomycin (77.63%), gentamycin (64.47%) and oxacillin (55.26%) and were sensitive to}

copyright © 2012 SciRes.

REFERENCES

[1] R. Creti, M. Imperi, L. Bertuccini, F. Fabretti, G. Orefici, D. R. Rosa and L. Baldassarri, “Survey for Virulence Determinants among *Enterococcus faecalis* Isolated from Different Sources,” *Journal of Medical Microbiology*, Vol. 53, No. 1, 2004, pp. 13-20. doi:10.1099/jmm.0.05353-0

[2] E. B. De Marques and S. Suzart, “Occurrence of Virulence-Associated Genes in Clinical *Enterococcus faecalis* Strains Isolated in Londrina, Brazil,” *Journal of Medical Microbiology*, Vol. 53, No. 11, 2004, pp. 1069-1073. doi:10.1099/jmm.0.45654-0

[3] M. J. G. Burgos, R. L. Lopez, H. Abriouel, N. B. Omar and A. Galvez, “Multilocus Sequence Typing of *Enterococcus faecalis* from Vegetable Foods Reveals Two New
“Epidemiology and Microbiology of Surgical Wound Infections,” Journal of Medical Microbiology, Vol. 38, No. 2, 2000, pp. 918-922.

A. Hällgren, C. Claesson, B. Saeedi, H.-J. Isaksson, H. Hanberger and L. E. Nilsson, “Molecular Detection of Aggregation Substance, Enterococcal Surface Protein, and Cytolysin Genes and in Vitro Adhesion to Urinary Catheters of Enterococcus faecalis and E. faecium of Clinical Origin,” International Journal of Medical Microbiology, Vol. 299, No. 5, 2009, pp. 323-332. doi:10.1016/j.ijmm.2009.09.006

[D. Greenwood and F. O'Grady, “Antibiotic-Induced Surface Changes in Microorganisms Demonstrated by Scanning Electron Microscopy,” Science, Vol. 163, No. 3871, 1969, pp. 1076-1078. doi:10.1126/science.163.3871.1076]

[D. Greenwood and F. O'Grady, “Scanning Electron Microscopy of Staphylococcus aureus Exposed to Some Common Anti-Staphylococcal Agents,” Journal of General Microbiology, Vol. 70, No. 2, 1972, pp. 263-270.

[T. S. J. Elliott and D. Greenwood, “The Response of Pseudomonas aeruginosa to Azlocillin, Ticarcillin and Ceftu- lodin,” Journal of General Microbiology, Vol. 16, No. 3, 1983, pp. 351-362. doi:10.1099/00222615-16-3-351]

[C. R. Jackson, P. J. Fedorka-Cray, J. B. Barrett and S. R. Ladely, “Effects of Tylosin Use on Erythromycin Resistance in Enterococci Isolated from Swine,” Applied and Environmental Microbiology, Vol. 70, No. 7, 2004, pp. 4205-4210. doi:10.1128/AEM.70.7.4205-4210.2004]

[E. B. De Marques, S. Suzart, “Occurrence of Virulence-Associated Genes in Clinical Enterococcus faecalis Strains Isolated in Londrina, Brazil,” Journal of Medical Microbiology, Vol. 53, No. 11, 2004, pp. 1069-1073. doi:10.1099/jmm.0.45654-0]

[Copyright © 2012 SciRes.]

[AI]
[27] G. Neumann, Y. Veeranagouda, T. B. Karegoudar, O. Sahin, I. Mausezahl, N. Kabelitz, U. Kappelmeyer and H. J. Heipieper, “Cells of Pseudomonas putida and Enterobacter sp. Adapt to Toxic Organic Compounds by Increasing Their Size,” *Extremophiles*, Vol. 9, No. 2, 2005, pp. 163-168. doi:10.1007/s00792-005-0431-x