



SPECIATION OF ENTEROCOCCUS SPECIES IN RESOURCE LIMITED SETTINGS – BETTER WAY TO DEAL WITH CLINICAL INFECTIONS.

Dr. Kinjal P Patel

(M.B.B.S, MD) Resident, Government Medical College, Surat

Dr. Summaiya Mulla

(M.D Microbiology, PhD) Head And Professor, Government Medical College, Surat

ABSTRACT

TITLE- Speciation of *Enterococcus* species in resource limited settings – Better way to deal with clinical infections.

SUBTITLE- Significance of *Enterococcus* speciation

SUMMARY-

AIM- *Enterococcus* species are well known for its intrinsic resistance pattern to several antibiotics. Hence, appropriate management and prevention is essential in any healthcare facility. Present study was conducted to establish an accessible biochemical tests to differentiate *Enterococcus* species.

MATERIAL AND METHOD: *Enterococci* isolated from various clinical specimens were speciated using basic biochemical reactions and antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method. Results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS: Out of 107 enterococcal isolates, 63 (59%) were *E.faecium*, 40 (37%) were *E.fecalis*, 2 (2%) were *E.hirae*, 1 (0.9%) was *E.raffinosis* and 1 (0.9%) was *E.gallinarum*. *E.faecium* and *E.fecalis* showed 23% and 7% vancomycin resistance respectively, while *E.gallinarum* showed low level vancomycin resistance.

CONCLUSION: *Enterococcus* speciation can be done using simple biochemical reactions and its susceptibility pattern enables to distinguish Van phenotypes too. Hence, helpful for management of infections in resource limited settings to a greater extent.

KEYWORDS

Enterococcus, Biochemical Reactions, Antimicrobial Susceptibility, Phenotypes

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*Corresponding Author Dr. Kinjal P Patel

(M.B.B.S, MD) Resident, Government Medical College, Surat kinjal.1527@yahoo.co.in

INTRODUCTION:

Since from era of 'Streptococci of fecal origin' in late 19th century, *Enterococci* species was considered commensal organism and have been studied so far.⁽¹⁾ Many species of *Enterococci* known with clinical significance till date.⁽²⁾ Amongst them, *E.faecalis* and *E.faecium* accounts for upto 90% of clinical infections.⁽³⁾ However, other species were underestimated because of lack of speciation. But, recently an alarming increase in incidence of clinical infections related to non faecalis and non faecium *Enterococci* were noted.^(4,5) Amongst these, *E.gallinarum* and *E.casseliflavus* were found to be involved in causing serious infections,⁽⁶⁾ especially in hospitalized patients. Hence, there is a need of species differentiation because of varied antimicrobial susceptibility pattern of each species. Simple biochemical tests can be used in resource limited settings routinely because even only one phenotypic character can easily differentiates one species from another.⁽⁷⁾

Enterococci are intrinsically resistant to several antimicrobials. Especially, glycopeptide resistance which is mediated by six *van* genes. Amongst them, *vanA* and *vanB* are most clinically relevant. *vanC* phenotype shows intrinsic, low level resistance to vancomycin and but susceptible to teicoplanin. These genotype is seen in *E.gallinarum*, *E.casseliflavus* and *E.flavescens*.⁽⁸⁾ Species identification can be useful in reporting antimicrobial susceptibility pattern which help in differentiation of Van phenotypes also. So, present study aims to make a panel of biochemical reactions for enterococcal speciation which will help in appropriate interpretation at resource limited settings.

MATERIAL AND METHODS:

Retrospective study was conducted at Department of Microbiology after taking approval from ethical committee of institute. 107 *Enterococcal* isolates obtained from urine, swab, blood culture, CSF, body fluids and invasive medical devices were collected during the period of two years and species differentiation was done by panel of

conventional tests. Colony of *Enterococci* isolate was inoculated into 5ml todd-hewitt broth (HiMedia Lab., Mumbai) and incubated overnight at 37°C. This broth was added as an inoculum in all liquid media (Arginine dihydrolase, pyruvate broth, sugars) and was streaked on tryptic soy agar culture plate for pigment production. Carbohydrate fermentation tests were performed using 1 % solution of following sugars: mannitol, raffinose, sucrose and arabinose. All the above inoculated media were incubated at 37°C and results were interpreted after 24 hr.⁽⁹⁾

Antimicrobial susceptibility was determined by Kirby Bauer disc diffusion method. Various antibiotics tested were: Penicillin (10U/disc), Ampicillin (10 g), High level gentamicin (120 g), High level Streptomycin (300 g), Ciprofloxacin (5 g), Levofloxacin (5 g), Vancomycin (30 g), teicoplanin (30 g), Linezolid (30 g), Tetracycline (30 g) and Nitrofurantoin (300 g). Interpretation was done after 24 hours of incubation and zones were read using transmitted light for vancomycin as per CLSI guidelines.⁽¹⁰⁾ MIC of vancomycin greater than or equal to 32µg/ml was considered resistant, which was done by using HiComb™ MIC test (HiMedia Laboratories Pvt. Ltd., Mumbai) All required dehydrated media and antibiotic discs were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai) and *E.faecalis* ATCC 29212 used for quality control was procured from Microbiologics (USA).

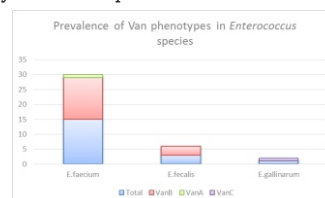


Figure No.1: Distribution of *Enterococcus* species isolated from various samples

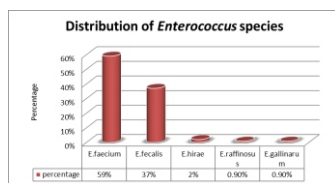


Figure 2: Presistance in *E.fecalis*. 1 isolate of *E.faecium* was resistant to both

vancomycin and teicoplanin indicating VanA phenotype. *E.hirae* showed resistance to penicillin (50%) and ampicillin (50%). However, *E.raffinosis* was resistant to beta lactams (100%) and High level resistance of Van phenotype among Enterococcus species.

RESULTS:

107 Enterococci isolated were speciated by using biochemical reactions. Amongst them, 63 (59%) were *E.faecium*, 40 (37%) were *E.fecalis*, 2 (2%) were *E.hirae*, 1 (0.9%) was *E.raffinosis* and 1 (0.9%) was *E.gallinarum*. [Figure 1].

E.faecium was found to be more resistant to beta-lactams, fluoroquinolones, tetracycline and high level aminoglycosides including 23% vancomycin resistance while 7 % vancomycin 1 streptomycin (100%). Besides, *E.gallinarum* which was identified by pigment production on tryptic soy agar was resistant to beta-lactams, fluoroquinolones, tetracycline and high level aminoglycosides with low level resistance to vancomycin having MIC of 8 – 16 µg/ml. [Figure

Table No.1: Panel of biochemical tests used for identification of Enterococcal speciation

Enterococci spp.	Arginine Deamination	Pyruvate utilisation	Mannitol fermentation	Arabinose fermentation	Raffinose fermentation	Sucrose fermentation	Pigment on trypticsoy	Motility
<i>E.faecalis</i>	+	+	+	-	-	+	-	-
<i>E.faecium</i>	+	-	+	+	V	+	-	-
<i>E.avium</i>	-	-	+	+	-	+	-	-
<i>E.hirae</i>	+	-	-	-	+	+	-	-
<i>E.raffinosis</i>	-	+	+	+	+	+	-	-
<i>E.dispar</i>	+	+	+	-	+	+	-	-
<i>E.durans</i>	+	-	-	-	-	-	-	-
<i>E.mundtii</i>	+	-	+	+	+	+	+	-
<i>E.casseliflavus</i>	+	V	+	+	+	+	+	+
<i>E.gallinarum</i>	+	-	+	+	+	+	-	+

Enterococci are intrinsically resistant to several antimicrobials. Especially, glycopeptide resistance which is mediated by six *van* genes. Amongst them, *vanA* and *vanB* are most clinically relevant because they are associated with transposons, hence easily transferred from one to other organisms. *VanA* gene mediates high level resistance to vancomycin and teicoplanin while *vanB* genotype have acquired inducible resistance to vancomycin but susceptible to teicoplanin. Besides these, *vanC* phenotype which is chromosomal in origin shows intrinsic, low level resistance to vancomycin and but susceptible to teicoplanin. These genotype is seen in *E.gallinarum*, *E.casseliflavus* and *E.flavescens*.⁽⁸⁾ Biochemical reactions like pigment production and motility test are helpful to distinguish species with acquired resistance to vancomycin (*VanA* and *VanB*) from those with intrinsic intermediate level resistance (*VanC*), such as in *E.gallinarum* and *E.casseliflavus*.⁽⁹⁾ In present study, 95% *E.faecium* were resistant to beta lactams and fluoroquinolones, 51% were resistant to high level aminoglycoside, 82% and 36% were resistant to tetracycline and nitrofurantoin respectively. While, *E.fecalis* showed less resistance as compared to *E.faecium*. Similarly, *E.raffinosis* was resistant to beta lactam, high level aminoglycosides and tetracycline but susceptible to fluoroquinolones. On contrary, *E.hirae* was resistant to beta lactams only. Above all, *E.gallinarum* showed intermediate level resistance to vancomycin and showed resistance to beta lactam, fluoroquinolones, tetracycline and high level aminoglycosides. [Table No.2] Resistance among Enterococcus species were higher in present study as compared to previous study in Jordan⁽¹⁷⁾ and Saudi Arabia⁽¹⁸⁾. Bekhit *et al* (2012)⁽¹⁵⁾ also reported VanC1 in 3 isolates of *E.gallinarum*.

Speciation will help to distinguish species with acquired resistance to vancomycin observed in *E.faecium* and *E.fecalis* from those with intrinsic intermediate level resistance such as in *E.gallinarum* and *E.casseliflavus*. It is also necessary to perform biochemical reactions

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On basis of biochemical reactions, *E.faecium* were identified by arginine dihydrolase test, mannitol and sucrose fermentation while *E.fecalis* by arginine dihydrolase test, pyruvate utilisation test and mannitol, arabinose, raffinose and sucrose fermentation. Amongst other species, *E.hirae* was identified by arginine dihydrolase test, raffinose and sucrose fermentation. *E.raffinosis* by pyruvate utilisation and mannitol, arabinose, raffinose and sucrose fermentation and *E.gallinarum* by pigment production on tryptic soy agar. [Table No.1]

DISCUSSION:

Species differentiation is helpful in clinical infections because of the naturally occurring differences in the susceptibility pattern of Enterococcus species. Also, it was helpful for epidemiologic surveillance within hospitals.

Most of the studies from India had reported *E.faecalis* and *E.faecium* as the only prevalent species.⁽¹¹⁻¹⁴⁾ In present study, *E.faecium* (59%) and *E.fecalis* (37%) were predominant isolates followed by *E.hirae* (2%), *E.raffinosis* (0.9%) and *E.gallinarum* (0.9%). Mohanty *et al* (2005)⁽¹⁵⁾ used the same panel of biochemical reactions for enterococcal speciation and isolated *E.mundtii*, *E.dispar*, *E.durans*, *E.avium*, *E.raffinosis* and *E.gallinarum*. While, Bekhit *et al* (2012)⁽¹⁶⁾ reported *E.fecalis*, *E.faecium*, *E.avium*, *E.hirae*, *E.casseliflavus* and *E.gallinarum* by using API strep. These Enterococci species were less frequently isolated but had a major clinical significance because of resistance to commonly used antibiotics.

when isolates shows vancomycin MICs 8-16µg/ml. These panel of tests will help resource limited settings to manage enterococcal infections more effectively.

Table No.2: Antimicrobial susceptibility pattern of Enterococcal spp. by Kirby Bauer disc diffusion method

Antibiotic tested	<i>E.faecium</i> (n=63)	<i>E.fecalis</i> (n=40)	<i>E.hirae</i> (n=2)	<i>E.raffinosis</i> (n=1)	<i>E.gallinarum</i> (n=1)
Penicillin (10units)	60(95)	40(100)	1(50)	100	100
Ampicillin (10µg)	60(95)	39(97)	1(50)	100	100
High level gentamycin (120µg)	54(85)	33(82)	0(0)	0(0)	100
High level streptomycin (300µg)	49(77)	24(60)	0(0)	100	100
Ciprofloxacin (5µg)	60(95)	31(77)	0(0)	0	100
Levofloxacin (5µg)	60(95)	31(77)	0(0)	0	100
Linezolid (30 µg)	0(0)	0(0)	0(0)	0	0
Vancomycin (30 µg)	15(23)	3(7)	0(0)	0	100
Teicoplanin (µg)	1(1)	0	0	0	0
Tetracycline (30 µg)	52(82)	31(77)	0(0)	100	100

Nitrofurantoin(300 µg)	23(36)	1(2)	0(0)	100	100
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(Values in parenthesis are percentages)

Present study had limitation that sample size was very less. More enterococcal isolates were needed to be studied for better correlation of biochemical reactions and antibacterial drug susceptibility pattern. Also, teicoplanin resistance was given by disk diffusion method which should be further confirmed by MIC method.

CONCLUSION:

Increasing resistance among *Enterococcus* species highlights the significance of rapid and accurate identification up to the species level. Therefore, developing simple and reliable tests for speciation and its antimicrobial susceptibility pattern is a need of present day therapeutics. Present study is a guide to resource limited settings so they can contribute in attenuation of coming pre-antibiotic era.

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