DESCRIPTIVE STUDY OF ENTEROCOCCAL SPECIES WITH THEIR ANTIBIOTIC STEWARDSHIP IN TERTIARY CARE HOSPITAL

Ram Murugan Navaneetha Krishnan¹, Senthil Kumar Jayakumar Jayaraj²

¹Associate Professor, Department of Microbiology, Government Sivagangai Medical College, Sivagangai, Tamilnadu, India.

²Senior Assistant Professor, Department of Microbiology, Government Sivagangai Medical College, Sivagangai, Tamilnadu, India.

ABSTRACT

BACKGROUND

Objectives of this study were

1. Isolation and speciation of enterococci from urine, pus sample, blood and other body fluids.
2. To identify the species of enterococci mostly isolated in ICU and non-ICU samples.
3. To know antibiotic susceptibility pattern of the various isolates.

MATERIALS AND METHODS

1. Samples such as blood, urine, pus, respiratory samples were collected from patients attending as outpatients and also from different clinical department wards and intensive care units by observing standard specimen collection procedure and aseptic conditions.
2. Identification of Enterococcus species is based on routine bacteriological culture methods and biochemical tests.
3. Antibiotic susceptibility testing was performed with Ampicillin, Penicillin, Levofloxacin, Nitrofurantoin, HL Gentamycin, HL Streptomycin and Teicoplanin with the disc diffusion method (modified Kirby-Bauer method) using Mueller-Hinton agar.
4. Vancomycin Sensitivity test was done with Enterococcus species by E Test Strip using Mueller-Hinton agar. MIC determination paper strip which is coated with Vancomycin in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the Enterococcus organism.

RESULTS

1. Out of 97 samples isolated, 42% were from Enterococcus faecalis, 41% were from Enterococcus faecium, 3% were from Enterococcus avium, 1% from Enterococcus gallinarum and 11% were from other Enterococcus Species.
2. Enterococcus faecalis showed high sensitivity to Ampicillin, Penicillin, Levofloxacin, Nitrofurantoin, Vancomycin and Teicoplanin. Enterococcus faecium and all other species showed high sensitivity to Vancomycin and Teicoplanin only.
3. Enterococcus faecalis were most commonly isolated in ICU and Surgery post-operative ward (IP). Enterococcus faecium were most commonly isolated in OPD, Medical-, Paediatrics- and OG-Wards (IP).
4. Vancomycin and Teicoplanin were most commonly sensitive in OP, Medicine, Paediatrics, & O&G Wards. Ampicillin, Penicillin and Levofloxacin, were most commonly sensitive in Surgery& ICU Wards.

CONCLUSION

Infection control measures like hand washing, ideal disinfectant usage, and strict antibiotic policy reduces Vancomycin resistance to zero Level in our tertiary care hospital. Enterococcal faecium slowly increasing with most of the antibiotics should be continuously monitored. ICU and surgical ward need more attention to bring down the bugs with antibiotic resistance.

KEY WORDS

Enterococcus Faecalis, Enterococcus Faecium, Vancomycin.

HOW TO CITE THIS ARTICLE: Krishnan RMN, Jayaraj SKJ. Descriptive study of enterococcal species with their antibiotic stewardship in tertiary care hospital. J. Evolution Med. Dent. Sci. 2019;8(01):37-41, DOI: 10.14260/jemds/2019/9

BACKGROUND

Enterococci are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci based on physical characteristics. Two species are common commensal organisms in the intestines of humans, *E. faecalis* (90–95%) and *E. faecium* (5–10%). Rare cases of infections occur with other species, including *E. casseliflavus*, *E. gallinarum*, and *E. raffinosus*.¹

Financial or Other Competing Interest: None.

Submission 29-11-2018, *Peer Review* 25-12-2018,

Acceptance 31-12-2018, *Published* 07-01-2019.

Corresponding Author:

Senthil Kumar Jayakumar Jayaraj,

ED Vairam Kamalam Apartments,

Sambakulam, Madurai-7,

Tamilnadu, India.

E-mail: dr_jsenthil@yahoo.com

DOI: 10.14260/jemds/2019/9

These organisms become a prominent cause of Nosocomial infection. *E. faecalis* is most commonly isolated, but incidence of *E. faecium* is on the rise in many hospitals.¹ Enterococci are tolerant to extreme temperature (10–45 °C), pH (4.5–10.0), and high sodium chloride concentrations.² Enterococci exhibit alpha-haemolysis on sheep's blood agar.³ Members of the genus Enterococcus were classified as group D Streptococcus with genomic DNA analysis.⁴ Clinical infections caused by Enterococcus include urinary tract infections, bacteraemia, bacterial endocarditis, diverticulitis, Wounds and meningitis.^{2,3} Sensitive strains of these bacteria can be treated with ampicillin, penicillin, Levofloxacin, Teicoplanin, High level Gentamycin, High level Streptomycin, Nitrofurantoin and vancomycin.⁵ Some enterococci are intrinsically resistant to β -lactam-based antibiotics (Penicillins, cephalosporins, carbapenems), as well as many aminoglycosides. This genus acquires and disseminates resistance to Vancomycin. This is emerging as a nosocomial

infection of hospitalized patients. It produces a serious public health concern.⁶ VRE may be treated with quinupristin/dalfopristin with response rates around 70%. Tigecycline has also been shown to have anti-enterococcal activity.⁷ Enterococcus faecium is a leading cause of multi-drug resistant enterococcal infections over Enterococcus faecalis. 40% of medical intensive care units reportedly with E. faecium. Central lines, urinary drainage catheters, and ventilators were related to vancomycin- and ampicillin-resistant E. faecium.⁸ The rapid increase of VRE has made it difficult for physicians to fight infections caused by E. faecium. A genome-wide sRNA study suggested that some sRNAs are linked to the antibiotic resistance and stress response in E. faecium.⁹ E. faecalis causes cytotoxicity in combination with high-level gentamicin resistance is associated with a five-fold increase in risk of death in human bacteraemia patients.¹⁰

MATERIALS AND METHODS

This study was conducted after getting the ethical committee clearance.

Sample Size

97

Study Period

Six months (March 2018 to September 2018)

Inclusion Criteria

Newly admitted patients without any antibiotic treatment.

Exclusion Criteria

Patients already with antibiotic treatments, HIV Patients.

This descriptive study was conducted by Department of Microbiology, Government Sivagangai Medical College. Samples were collected from Sivagangai Medical College Hospital. Clinical data were descriptively collected by us using a standardized case-report form. The data that were routinely collected included the patient's age, sex, locations namely ICU, non-ICU ward and outpatient patients with clinical History. The identification of Enterococcal species and antimicrobial susceptibility patterns from 2900 Patients samples. Total number of Enterococci isolates processed was 97. Samples such as Blood, Urine, Pus, Respiratory samples were collected from patients attending as outpatients and also from different clinical departments, wards and intensive care units by observing standard specimen collection procedure and aseptic conditions. The samples were transported to the lab and streaked on MacConkey Agar and Blood Agar medium. After the incubation period at 37°C, the culture plates were examined. On MacConkey Agar medium, they produce tiny deep pink colonies. They are Heat resistant, surviving at 60°C for 30 minutes. On Blood Agar medium, they are non-hemolytic, some strains may show alpha or beta colonies. On selective media, Bile esculin agar slant produces black color colonies. On Gram staining Gram positive cocci appeared as pairs of oval cocci. Catalase test was performed, it was Negative. Motility test was performed with hanging drop method, except E. gallinarum, E. casseliflavus, other species showed non-motile. Identification of Enterococcus species is based on biochemical tests.¹¹ E. faecalis was identified with ability to ferment mannitol, Arginine, and

Pyruvate. E. faecium was identified with ability to ferment mannitol, arabinose, arginine. E. avium was identified with ability to ferment mannitol, sorbose, arginine. E. gallinarum was identified with ability to ferment mannitol, raffinose.

Antibiotic Susceptibility testing was performed with Ampicillin, Penicillin, Levofloxacin, Nitrofurantoin, HL Gentamycin, HL Streptomycin and Teicoplanin with the disc diffusion method (Modified Kirby-Bauer method) using Mueller-Hinton agar.¹² Nitrofurantoin was used for urine samples only. High Level Gentamycin and Streptomycin was used for Blood samples only.

Vancomycin Sensitivity test was done with Enterococcus species by E Test Strip using Mueller-Hinton agar. MIC determination paper strip which is coated with Vancomycin in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the Enterococcus organism. The ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection. Vancomycin showing reading of 0.75 mcg/ml should be rounded up to next concentration i.e. 1.0 mcg/ml. When growth occurs along the entire strip, report the MIC as > the highest values on the MIC strip. When the inhibition ellipse is below the strip (Does not intersect the strip), report the MIC < the lowest value on the MIC scale. MIC of 4 and below reported as sensitive, 8-16 as Intermediate, 32 and above as Resistant. The results were interpreted as per CLSI 2018 (Clinical and Laboratory Standards Institute) guidelines corresponding to that period. All antibiotic discs were obtained from HiMedia (India).¹³

RESULTS

Age	Numbers (n=97)
Neonates	6
up to 1 yr.	3
1-12 yrs.	14
12-20 yrs.	8
20-40 yrs.	18
40-60 yrs.	21
more than 60 yrs.	27
Total	97

Table 1

A total of 97 isolates from patients attending the tertiary care teaching hospital. Most common age group of isolation of Enterococci were 20-40 yrs., 40-60 yrs. and above 60 yrs. [Table. 1]

Sex	Numbers (n=97)
MCH	12
FCH	11
Male	46
Female	28
Total	97

Table 2

Out of 97 isolates isolated, 46 isolates were from male patients and 28 isolates were from female patients. 12 isolates were from male children and 11 isolates were from female children. [Table. 2]

	Numbers (n=97)
OP	6
IP	67
ICU	24
Total	97

Table 3

Out of 97 isolates isolated, most common area of isolation of Enterococci were from medicine, Surgery, O& G Wards and ICU (NICU, CCU, PICU, IMCU, and IRCU). [Table. 3]

Samples	Numbers (n=97)
Urine	57
Blood	15
PUS	9
Wound Swab	12
Other Respiratory Samples	4
Total	97

Table 4

Out of 97 isolates isolated, most common Samples isolated from Urine, Blood and Wound Swab. [Table. 4]

Organism	Numbers (n=97)
E. faecalis	42
E. faecium	41
E. gallinarum	1
E. avium	3
E. species	10
Total	97

Table 5

Table 5, Fig. 1, Out of 97 samples isolated, 42% were from Enterococcus faecalis, 41% were from Enterococcus faecium, 3% were from Enterococcus avium, 1% from Enterococcus gallinarum and 11% were from Other Enterococcus Species. [Table. 5, Fig. 1]

Organisms	Amp				Pen				Levo				NIT				HL GM/HLSM				Van/Tei			
	s	%	R	%	s	%	R	%	s	%	R	%	s	%	R	%	s	%	R	%	s	%	R	%
E. faecalis	34	81	8	19	34	81	8	19	31	74	11	26	20	90	2	10	2	50	2	50	42	100	0	0
E. faecium	4	10	37	90	3	10	38	90	4	10	37	90	7	32	15	68	2	10	9	80	41	100	0	0
E. species	5	50	5	50	5	50	5	50	4	40	6	60	6	60	4	40	0	0	0	0	10	100	0	0
E. avium	1	33	2	67	1	33	2	67	2	67	1	33	0	0	0	0	0	0	0	0	3	100	0	0
E. gallinarum	1		0		1		0		1		0		0	0	0	0	0	0	0	0	1	100	0	0

Table 6. Antibiotic Sensitivity Analysis of Enterococci

Figure 2

Table 6, Fig. 2, Chart. 1 showed, Enterococcus faecalis showed high sensitivity to Ampicillin, Penicillin, Levofloxacin, Nitrofurantoin, Vancomycin and Teicoplanin. Enterococcus faecium and all other species showed high sensitivity to Vancomycin and Teicoplanin only. [Table. 6]

Sl. No.	Organisms	Vancomycin Mic's with E Strip Method			
		1 and below 1 in Nos.	%	Between 1- 2 in Nos.	%
1	E. faecalis	15	36	27	64
2	E. faecium	36	87	5	13
3	E. species	8	80	2	20
4	E. avium	3	100	0	0
5	E. gallinarum	1	100	0	0
	Total	63		34	

Table 7

Chart 1

Figure 3

Table 7, Fig. 3 showed, All Species are Sensitive to Vancomycin. *Enterococcus faecalis* showed 64 % sensitive to MIC between 1 and 2. *Enterococcus faecium* showed 87% sensitive to MIC between 1 and below 1. Other *Enterococcus* species showed 80% sensitive to MIC between 1 and below 1.

Most Commonly Isolated Bacteria in Hospital Areas						
Sl. No.	Out Patient	Medical	O.G	Paediatrics	Surgery	ICU
1	<i>E. faecium</i>	<i>E. faecium</i>	<i>E. faecium</i> & <i>E. faecalis</i>	<i>E. faecium</i>	<i>E. faecalis</i>	<i>E. faecalis</i>

Table 8

Table 8 showed, *Enterococcus faecalis* were most commonly isolated in ICU and Surgery Post Operative ward (IP). *Enterococcus faecium* were most commonly isolated in Op, Medical, Paediatrics and OG Wards (IP). [Table. 8]

OP, Medicine, Paediatrics, & O&G Wards	Surgery & ICU
VAN AND TEICO	AMPI, PENICILLIN, LEVO, TEICO, VANCO

Table 9. Drugs Most Commonly Sensitive in Hospital Area

Table 9 showed Vancomycin and Teicoplanin were most commonly sensitive in OP, Medicine, Paediatrics, & O&G wards. Ampicillin, Penicillin and Levofloxacin, were most commonly sensitive in Surgery& ICU Wards.

DISCUSSION

In this study isolates were highest from urine followed by blood. Most of the studies done on Enterococci support the same findings as Enterococci is identified as one of the most frequent uropathogen. In present study, most common species isolated was 42% *E. faecium* and 41% *E. faecalis*. In a study by Shweta Jaiswal et al¹⁴ from north India found also correlates with this study. This may be due to increasing incidence of *E. faecium* infection in hospitalized patients in present circumstances. Iwen et al.¹⁵ have also reported an increase in *E. faecium* isolates from 12.9 to 36.3% over a period of 8 years during 1987 to 1995.3. *Enterococcus* has become resistance to many antibiotics due to adhere to indwelling medical devices, and ability to survive in adverse environmental conditions. Antimicrobial resistance in *Enterococcus* has been increasing mainly in hospitalized patients. 3. In Current study, *E. faecalis* showed 81% sensitive to Ampicillin and Penicillin, 74% sensitive to Levofloxacin, 90% sensitive to Nitrofurantoin, 100% sensitive to Vancomycin and Teicoplanin. *E. faecium* showed higher rates of resistance to Ampicillin, Penicillin, Levofloxacin, Nitrofurantoin. Many studies have also demonstrated that *E. faecium* is comparatively more resistant than *E. faecalis*.¹⁶ 4. Zero prevalence of resistance to vancomycin and teicoplanin was detected in both *E. faecium* and *E. faecalis*.¹⁷ In this study, *Enterococcus faecalis* were most commonly isolated in ICU correlates with Tripathi et al.¹⁸ Intrinsic risk factors related to the need for intensive care, such as severe underlying disease, multiple illnesses and extremes of age; (2) invasive

medical devices, such as endotracheal tubes for mechanical ventilation, intravascular catheters and urinary tract catheters; (3) animate reservoirs (e.g., colonised or infected patients), which increase the risk of cross-infection in the ICU. All Species are Sensitive to Vancomycin. *Enterococcus faecalis* showed 64 % sensitive to MIC between 1 and 2. *Enterococcus faecium* showed 87% sensitive to MIC between 1 and below 1. Other *Enterococcus* species showed 80% sensitive to MIC between 1 and below 1

CONCLUSION

Infection control measures like hand washing, ideal disinfectant usage, and strict antibiotic policy reduces Vancomycin resistance to Zero Level in our tertiary care Hospital. *Enterococcus faecium* slowly increasing with most of the antibiotics should be continuously monitored. ICU and surgical wards need more attention to bring down the bugs with antibiotic resistance.

ACKNOWLEDGEMENT

The author thanks the Dean, Government Sivagangai Medical College, Sivagangai. The author gratefully acknowledges the technicians and other co staff who have helped to conduct this study successfully.

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