"Enterococci speciation and antibiogram: an assessment of data at a tertiary care hospital in Karnataka"

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Abstract

Background: Enterococci were known to be less virulent in healthy individuals, but recently have become important opportunistic pathogens, especially in hospitalized patients because of their ability to colonize the gastrointestinal tract for long periods which is a crucial factor in the development of drug resistance and have become a major obstacle for treatment.

Objective:

1. To determine the species of Enterococci isolated from various clinical samples.
2. To determine the antimicrobial susceptibility pattern of Enterococci.

Material and Methods: A cross sectional study was conducted at a private tertiary care hospital in Shivamogga district of Karnataka, using secondary data of Enterococcal species isolated from various clinical samples such as urine, blood, pus, sputum, sterile body fluids which were maintained in the Microbiology laboratory registers for a period of 1 year from January 2014 to December 2014. Standard protocols were followed for Enterococcus isolation, identification and to assess their antibiotic susceptibility. Analysis was done using MS Excel 2010.

Results: Out of 66 enterococcal isolates from various clinical samples majority i.e. 32 (48.48%) were isolated from urine followed by pus 22 (33.33%). Majority of the Enterococcus were isolated from females 34 (51.51%). *E. faecalis* was the predominant isolate i.e. 56 (84.84%). All the isolates were susceptible to Linezolid and Vancomycin. Maximum resistance was seen against Penicillin i.e. 26 (76.47%).

Conclusion: Considering the general scenario of increasing drug resistance and prevalence of wide variety of Enterococcus species there is a need to carry-out regular surveillance of antimicrobial resistance of enterococci to recommend appropriate therapy.

Key words: Antimicrobial susceptibility pattern, Clinical samples, Enterococcus species

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Introduction

Enterococci are widespread in nature. They are Gram-positive cocci, and are normal commensals of the gastrointestinal tract, genital tract, and anterior urethra.[1] They belong to group D streptococci as characterized by Lancefield in 1938, whose taxonomy has changed considerably in the last few years. Enterococci were known to be relatively avirulent in healthy individuals, but recently have become important opportunistic pathogens, especially in hospitalized patients because of the development of antimicrobial resistance.[2] Their ability to colonize the gastrointestinal tract of hospitalized patients for long periods is a crucial factor in the development of drug resistance.[3] In addition, an irrational and extensive use of broad spectrum antibiotics is responsible for their conversion into opportunistic nosocomial pathogens and an important cause of community-acquired infection.[4]

There are several important characteristics of the enterococci due to which they grow and survive in harsh conditions. They are the second most common cause of urinary tract infections (UTI) and third most common cause of bacteremia. Enterococci now exhibit intrinsic resistance to penicillinase-susceptible penicillin (low level), penicillinase-resistant penicillin, cephalosporin, nalidixic acid, aminoglycoside and clindamycin, which until recently, could be treated with ampicillin, or vancomycin with or without an aminoglycoside.(4) *E. faecalis* is the most predominant species implicated in human infections followed by *E. faecium*. Nevertheless, the increasing frequency of latter is a matter of concern as it is highly resistant to most of the currently available antibiotics. They acquire resistance either by mutation or by receiving the foreign resistant determinants through plasmids and transposons.[5]

Species identification of Enterococci and an assessment of their antibiotic susceptibility will be useful for epidemiological investigation of outbreaks as well as for clinical decisions, particularly with regard to therapy, as it would help in making optimal empirical choices.[1]

Moreover a policy for the judicious and rational use of antibiotics can be devised which would be helpful in delaying the emergence of even more resistant and virulent strains of Enterococci.[6] With this background, the following study was undertaken.

Materials and Methods

A cross sectional study was conducted in the Microbiology department of a private tertiary care hospital in Shivamogga district of Karnataka. Prior permission for the study was obtained from concerned authorities. Secondary data of enterococcal species isolated from various clinical samples such as urine, blood, pus, sputum, sterile body fluids which were maintained in the laboratory registers of Microbiology department for a period of 1 year from January 2014 to December 2014 were collected for the study. The following information was noted –name, age, sex, case history, organism isolated and their antibiotic susceptibility pattern.

Statistical analysis: Analysis was done using MS Excel 2010.

Bacterial isolation: Cases of clinically suspected infection are routinely sent for microbiological analysis. The samples sent will be processed in the laboratory for direct microscopy and aerobic culture and sensitivity as per the standard protocol. The samples will be inoculated on to Nutrient agar (NA), Mac Conkey Agar (MA) and Blood Agar (BA) plates and incubated at 37^0C for 24 hours aerobically. Identification of Enterococcus will be done by colony morphology on blood agar and Mac Conkey agar. All the Gram positive cocci which will be catalase negative will be confirmed as Enterococcus genus with growth on and blackening of bile-esculin agar, growth in the presence of 6.5% sodium chloride (salt tolerance test) and heat tolerance test i.e. growth at 60^0c for 30min.

Identification of enterococcal species: Subsequently, speciation will be performed by potassium tellurite reduction, pyruvate fermentation, arginine dihydrolase test, motility testing and sugar fermentation test including glucose, lactose, mannitol and arabinose.[7]

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing will be performed on Mueller Hinton agar as per CLSI guidelines.[8] The following antibiotics will be tested-

For urinary isolates- Ampicillin (10µg), Erythromycin (15µg), Chloramphenicol (30µg), Tetracycline (30µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Nalidixic acid (30µg), High level Gentamycin (120µg), Linezolid (30µg), Vancomycin (30µg).

Other clinical samples- Ampicillin (10µg), Tetracycline (30µg), Ciprofloxacin (5µg), High level Gentamycin (120µg), Linezolid (30µg), Vancomycin (30µg).

Result

Out of 66 enterococcal isolates maximum were isolated from urine i.e. 32 (48.48%) followed by pus 22 (33.33%), sterile body fluids 8 (12.12%), blood 2 (3.03%) and sputum 2 (3.03%) as shown in **Fig. 1**. Out of 66 enterococcal isolates *Enterococcus faecalis* were 56 (84.84%) and *Enterococcus faecium* were 10 (15.15%). (**Fig. 2**)

Majority of the Enterococcus were isolated from females 34 (51.51%) yielding a male: female ratio of 1:1.06. Majority i.e. 32 (48.48%) of the patients in whom Enterococcus were isolated belonged to the age group of 21-40 years followed by 14 (21.21%) in 41-60 years as shown in **Table 1**.

40 (60.6%) of the samples yielded pure enterococcal growth whereas 26 (39.9%) yielded mixture of growth and their pattern of isolation is depicted in **Table 2.**

Table 3 depicts the antibiotic susceptibility of Enterococcus isolated from urine. All the isolates were susceptible to Linezolid and Vancomycin. 28 (87.5%) were susceptible to nitrofurantoin, 22 (68.75%) to ampicillin and high level Gentamicin each followed by ciprofloxacin 20 (62.5%). Maximum resistance was seen against tetracycline 16 (50%), followed by norfloxacin and nalidixic acid in 14 (43.75%) each.

Table 4 depicts the antibiotic susceptibility of Enterococcus isolated from clinical samples excluding urine. All the isolates were susceptible to Linezolid and Vancomycin. 28 (82.35%) were sensitive to chloramphenicol followed by tetracycline 26 (81.25%), high level Gentamicin 26 (76.47%), ampicillin, erythromycin and ciprofloxacin 22 (64.7%) each. Maximum resistance was seen against penicillin i.e. 26 (76.47%).

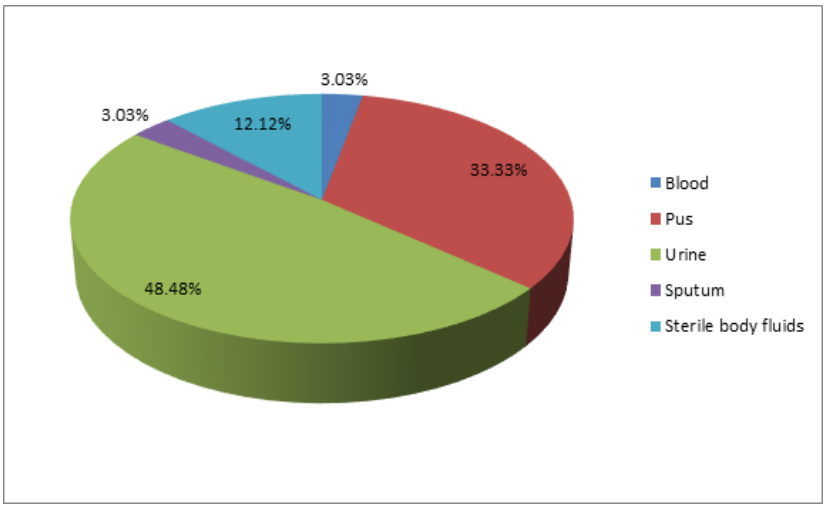

Fig. 1: Distribution of Enterococci isolates from various clinical samples (n = 66)

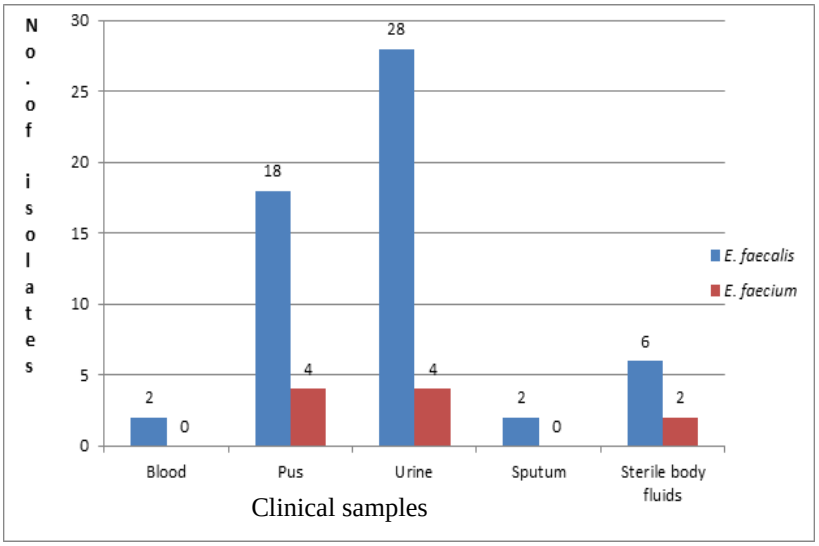

Fig. 2: Distribution of different species of Enterococci isolated from various clinical samples

Table 1: Characteristics of patients with Enterococcus infection (n = 66)

Variables		Enterococcal isolates	
		No.	%
Gender	Male	32	48.48
	Female	34	51.51
Age (years)	0- 20	10	15.15
	21- 40	32	48.48
	41- 60	14	21.21
	61-80	10	15.15
*OP/IP	OP	24	36.36
	IP	42	63.63
Enterococcus species	Enterococcus faecalis	56	84.84
	Enterococcus faecium	10	15.15

*OP- Out patient department
*IP- In patient department

Table 2: Pattern of Enterococcus isolation

Isolates	No.	%
Enterococcus	40	60.6
Enterococcus + Escherichia coli	12	18.18
Enterococcus +Klebsiella species	4	6.06
Enterococcus + *Pseudomonas aeruginosa*	6	9.09
Enterococcus + *Staphylococci aureus*	4	6.06
Total	66	100%

Table 3: Antimicrobial susceptibility pattern of urinary Enterococcus isolates (n = 32)

Antibiotics	Susceptibility pattern			
	Susceptible		Resistant	
	No.	%	No.	%
Ampicillin	22	68.75	10	31.25
Tetracycline	16	50	16	50
Ciprofloxacin	20	62.5	12	37.5
Norfloxacin	18	56.25	14	43.75
Nitrofurantoin	28	87.5	4	12.5
Nalidixic acid	18	56.25	14	43.75
High level gentamicin	22	68.75	10	31.25
Linezolid	32	100	0	0
Vancomycin	32	100	0	0

Table 4: Antimicrobial susceptibility pattern of Enterococcus from clinical samples excluding urine (n = 34)

Antibiotics	Susceptibility pattern			
	Susceptible		Resistant	
	No.	%	No.	%
Penicillin	8	23.52	26	76.47
Ampicillin	22	64.7	12	35.29
Erythromycin	22	64.7	12	35.29
Chloramphenicol	28	82.35	6	17.64
Tetracycline	26	81.25	8	23.52
Ciprofloxacin	22	64.7	12	35.29
High level gentamicin	26	76.47	8	23.52
Linezolid	34	100	0	0
Vancomycin	34	100	0	0

Discussion

The wide variety of infectious materials from which enterococci were isolated was found similar to those found in other studies.[1,2,9,10] Enterococci isolated suggest the frequency of their isolation from various clinical materials and do not reflect the true incidence of infection.

In our study maximum numbers of Enterococci were isolated from urine sample followed by pus which corroborates with other studies.[1,3,5,9,11] On the contrary few other studies found pus isolates to be higher compared to isolates from urine.[12,13]

Females presenting with Enterococcal infection were more compared to males in our study. This is comparable with the other studies.[10,11] On the contrary some studies showed more males being infected than females.[3,6] No gender difference with enterococcal infection was reported by Shinde RS et al.[1]

High prevalence of Enterococcal infection was seen in the age group of 21-40 years which is comparable with other studies.[3,10,11] However, Barros M et al. reported high prevalence of Enterococcal infection in the age group of 50-60 years.[14]

About 60.6% of Enterococcus were isolated in pure culture in our study which was almost similar to the study conducted by Palanisamy S. et al.[3] This was inconsistent with the finding of Desai PJ et al. where only 18% yielded pure growth.[23] 9.39% of Enterococci were found as one of the isolates in the clinical specimens with polymicrobial etiology which are better established as pathogens and are primary target of subsequent antibiotic therapy.

Only two species of Enterococcus, *E. faecalis* and *E. faecium* were isolated in our study which were comparable with other studies.[10,11] *Enterococcus faecalis* was found to be the predominant isolate from all clinical specimens followed by *E. faecium*. Similar

trend was reported by other studies,[1,2,3,12,15,16] even though there are recent studies which states that there is an increase in isolation of *Enterococcus faecium* and other enterococcal species.[4,17,18] Predominance of *Enterococcus faecalis* in the endogenous flora of the body could be the reason for this. Isolation of both these species is cause of serious concern as they are long known to be significantly associated with the clinical disease.

Penicillin along with gentamicin is the drug of choice for treatment of enterococcal infections. Therefore, resistance of Enterococci against these antibiotics has important clinical implications. Present study showed 76.47% resistance to penicillin, may be due to resistance mechanism involving low affinity penicillin binding proteins or production of β lactamases. Many studies have also demonstrated resistant to penicillin ranging from 16%-100%.[1,4,5,16,19]

Aminoglycoside resistance is of great concern because of its role in synergistic effect with cell wall synthesis inhibitors like penicillin or vancomycin. In the present study aminoglycoside resistance, especially high level gentamicin resistance (HLGR) was 31.25% in urine and 23.52% in other samples. Even higher resistance was observed in other studies.[1,3,4,12,20]

Emergence of vancomycin resistant enterococci is of great concern because of its epidemic potential and scanty therapeutic options. None of the isolates in the present study were resistant to Vancomycin and Linezolid. Similar findings were reported by other studies.[1,4,12] However few other studies have quoted resistance to vancomycin ranging from 1.7%-20%.[4,5,15,16] Some studies revealed 100% susceptibility to linezolid with low percentage of vancomycin resistance.[11,15] Study conducted by Jain S et al. revealed 100% susceptibility to vancomycin and 7% resistance to linezolid.[4]

Our study revealed results with quinolones as 37.5% of enterococcal urinary isolates being resistant to ciprofloxacin and 43.75% to norfloxacin. This fact is significant as quinolones are considered to be very potent urinary antimicrobials and being used extensively. A similar trend of enterococcal resistance to quinolones ranging from 58%-62% were noted in other studies.[6,14] Frequent use of penicillin and quinolones for the empirical treatment of endemic infectious diseases may be the cause of the high proportion of antibiotic resistant species seen in the isolates.

Encouraging results for nitrofurantoin were reported in our study with 87.5% of urinary isolates being susceptible. This correlates with other studies.[6,21] Since there is rise in drug resistance, it is mandatory that such antimicrobials should be given due importance because of their efficacy and low cost.

Conclusion

Considering the general scenario of increasing drug resistance and prevalence of wide variety of Enterococcus species there is a need to carry-out regular surveillance of antimicrobial resistance pattern of enterococci to recommend appropriate therapy thereby preventing the spread of resistant isolates. Prompt diagnosis and efficient infection control measures can restrict its spread.

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