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Speciation of *Enterococci* as a Urinary Pathogen with Its Resistance Pattern: A Study from Pondicherry.

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ABSTRACT

Enterococcus spp. is a common urinary pathogen, which is less commonly speciated. Ithas emergedas nosocomial pathogen with resistance to multiple antimicrobial agents including glycopeptides. This study was done to speciate *Enterococci*isolated from urine cultures and to study their resistance pattern.A descriptive study was performed at a tertiary care hospital in Pondicherry from June to December2011. The urine specimens received at the microbiology lab were processed by standard procedures. All *Enterococci*identified, were further speciated and the antibiogram was determined by disk diffusion method. Out of 55 *Enterococci* isolates identified from urine samples, 70.90% of isolates were from females and 29.09% were from males. Most of the patients were inpatients (58.18%). *Enterococcus faecalis*(78.18%) was the most commonly isolated followed by *Enterococcus faecium* (21.82%). 44.1% were resistant to ciprofloxacin and 39.5% were resistant to tetracycline, 20.93% of *E. faecalis* were resistant to high level gentamicin (HLG). All the *E. faecium* isolates were sensitive to HLG but 2.5% were resistant to tetracycline and 16.6% were resistant to ciprofloxacin. No vancomycin resistance was identified. *Enterococcus faecalis*is the commonest *Enterococcus*isolate causing UTI. No vancomycin resistance was identified among the urinary isolates from our centre.

Key words:*Enterococci*; UTI; resistance pattern; uropathogen

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INTRODUCTION

Urinary tract infection (UTI) is a commoninfection prevalent among various age groups. Although UTI is common in women, it can affect both gender and all age groups.[1]Untreated UTI in a long run can lead to many complications such as pyelonephritis and renal damage. It is the most common nosocomial infection accounting for 34- 46% of all infections acquired in the hospital.[2]UTI cause morbidity among the patients and add on to the financial burden worldwide.[3]Among the etiological agents causing UTI *E. coli* accounts for 50-80%. It is followed by other Enterobacteriaceae, *Streptococci, Enterococci*and *staphylococcus* which account for the remaining positivity.[1]*Enterococcus* is a common constituent of gastrointestinal tract which can colonize other areas.[4]The most common *Enterococcus* associated nosocomial infection is UTI followed by surgical wound infection and bacteremia.[5]The *Enterococcus* isolated from human infection may include *E. faecalis, E. faecium, E. casseliflavus, E. durans, E. gallinarum, E. durans, E. hirae*. Among the *Enterococcus i*solated,*E. faecalis* account for 90% and *E. faecium* account for 10%.[2] Although *Enterococcus* is commonly isolated from UTI it is less often speciated. Speciation and antibiogram of *Enterococci* is gaining relevance because of emerging antimicrobial resistance.[6]The resistance in *Enterococci* is high especially with glycopeptides .This has increased the treatment failure by 20%.[6]The vancomycin resistant *Enterococci* are common among hospitalized patients making the treatment cumbersome. The intrinsic resistance of *Enterococci* with dissemination of mobile genetic elements poses a treatment problem.[5]It is of great concern for the clinicians as the treatment options are less for these resistant organisms.The resistance pattern of the organisms isolated from UTI varies from region and hospital setting.[7]This study may aid as an epidemiological tool to curb antimicrobial resistance in the hospital setting in our region. The main aim of our study was to identify the prevalence of *Enterococcus* among the patients with UTI and to speciate them. Since the resistance is common among these isolates the next aim of our study was to identify their resistance pattern.

MATERIALS AND METHODS

Study design

A descriptive study was performed at a tertiary care hospital in Pondicherry from June 2011 to December 2011. The patient's samples were obtained from various disciplines in our hospital. Patients with complaints of fever, burning micturition and pain lower abdomen were included in the study. Both ambulatory and catheterized patients were included in our study. An informed consent was obtained from the patient before sample collection by the clinician concerned. The patients admitted to various medical and surgical conditions in this hospital were included in the study. Urine samples were collected and processed in the laboratory by standard methods.[8]Thename, age, sex, provisional diagnosis, date of onset of symptoms was noted. On isolation of *Enterococcus* the organism was speciated and antibiogram was done.

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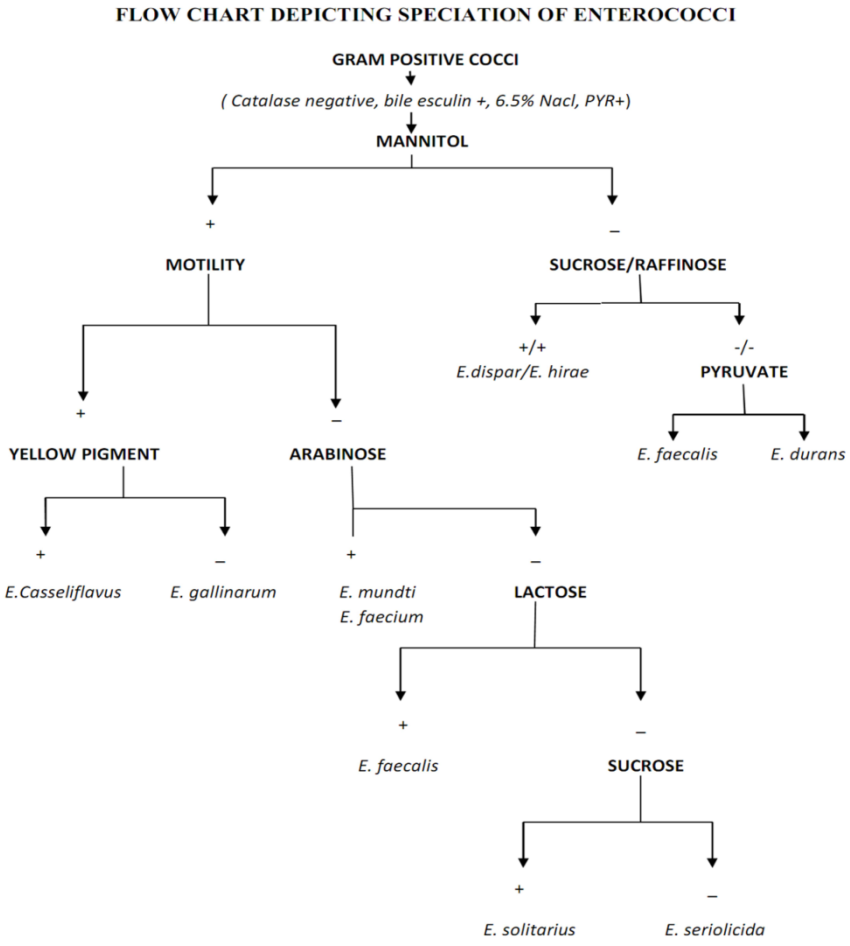
Diagnostic criteria

The urine samples were inoculated into blood agar and cysteine lactose electrolyte deficient (CLED) agar.A standard calibrated loop was used for sample inoculation after dipping in the sample perpendicularly. A perpendicular streak line was made followed by horizontal streaks. The samples were observed under microscope for the presence of pus cells, epithelial cells, and RBCs. The plates were incubated in at 37°C and after 24 hours the plates were observed. After 24 hours incubation, the colony count was read manually and recorded. For clean catch mid-stream urine, significance was decided if there were one lakh colonies forming units per ml (CFU/ml) of a single pathogen. However, significance for catheterized and aspirated samples was decided at lower colony counts.[8]

Small α or non-hemolytic grey coloured colonies, which were Gram positive cocci, arranged in pairs, catalase negative were subjected to further biochemical tests like salt tolerance,heat tolerance, bile esculin hydrolysis to identify the genus *Enterococcus*. Speciation of these was done by sugar fermentation tests using mannitol, sucrose, arabinose, pyruvate, and raffinose as per the scheme mentioned in Fig.1 (modified from Koneman).[9]

Figure 1.*Enterococcus* speciation scheme



The speciation scheme used for *Enterococcus* species identification was based on gram stain, biochemical reactions and sugar fermentation tests using mannitol, sucrose, arabinose, pyruvate, and raffinose.

The antibiogram of the isolates was determined by Kirby Bauer disk diffusion method as per CLSI guidelines.[10]The organisms were tested with Ampicillin (30µg), ciprofloxacin (5µg), tetracycline (30µg), high level gentamicin (120µg)and vancomycin (30µg). All the antibiotic discs were obtained fromHi-media (Mumbai, India).

Quality Control for Antibiotic sensitivity testing- The above mentioned antibiotics were tested for potency by using ATCC *Staphylococcus aureus* 25923.

Statistical analysis

All the data were recorded using MS Excel software. The statistical analysis included descriptive statistics like percentages. Chi-square test was used to determine statistical significance. All p values below 0.05 were statistically significant.

RESULTS

During the study period, 55 *Enterococcus* isolates were obtained from 2524 urinary samples during the study period accounting for 2.1% of the isolates. Of these*Enterococci* isolates,70.90% (n=39) of isolates were from females and 29.09% (n=16) were from males. Age group distribution of isolates shows that most of the isolates were from 21-30 age group (38.18%) (Table 1). Most of the patients were inpatients (58.18%).

The maximum number of isolations was from obstetrics and gynecology department (67.27%) followed by medicine (20%) and the least from surgical departments (1.81%)(Table 2).

Enterococcus faecalis(78.18%, n=43) was the most commonly isolated followed by *Enterococcus faecium* (21.82%, n=12). Among the *E. faecalis* isolated nearly 44.1% were resistant to ciprofloxacin and 39.5% were resistant to tetracycline (Table 3). Around 20.93% of *E. faecalis* were resistant to high level gentamicin (HLG) indicating that there was no synergistic activity if used with penicillins/glycopeptides,[10] though this finding was not statistically significant. All the *E. faecium* isolates were sensitive to HLG but 2.5% were resistant to tetracycline and 16.6% were resistant to ciprofloxacin.

Table 1. Age-wise distribution of *Enterococcus* isolates

Age group (years)	No. of isolates	Percentage
10-20	6	10.9%
21-30	21	38.18%
31-40	11	20%
41-50	5	9%
51-60	8	14.5%
More than 60	7	12.7%

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Table 2. Distribution of *Enterococcus* isolates in various departments

Department	No. of isolates	Percentage
Medicine	11	20%
Obstetrics	37	67.27%
Orthopaedics	2	3.63%
Surgery	1	1.81%
Urology	4	7.27%

Table 3. Resistance patterns of *Enterococcus* isolates

	Antibiotics resistant to *E. faecium*	Antibiotics resistant to *E. faecalis*	P value
High level gentamicin	0	9 (20.93%)	0.1964
Tetracycline	3 (2.5%)	17 (39.5%)	0.5577
Ciprofloxcin	2 (16.6%)	19 (44.1%)	0.1618

DISCUSSION

Enterococcus is an emerging nosocomial pathogen that is gaining attention due to the increasing antimicrobial resistance.*Enterococcus* is the third most common pathogen isolated from UTI following *Escherichia coli* and *Pseudomonas aeruginosa.*[5]*Enterococcus* is reported to be more common among patients above 60 years of age with obstructive uropathy.[11]In our study it was commonly isolated in 21-30 year age group followed by the elderly age group indicating that reproductive age group is also prone for *Enterococcus* infection.

Females are more prone for UTI due to the close proximity of the anus to the female urinary tract. In our study, predominant isolates(70.9%) were from females. Similarly in a study from Rajasthan, India, UTI was more common in females.[12]*Enterococci* can be either acquired from hospital or community. In our study,*Enterococci*were mostly isolated fromhospitalized patients which is consistent to similar other studies from India and worldwide.[13]Most of the hospital acquired strains pose a problem with muti-drug resistance.

Our speciation of the enterococcal isolates showed that two isolates were more common. *E. faecalis* and *E. faecium* were obtained from our samples. Around 78.18% were *E. faecalis*and 21.82 %were *E. faecium*which was consistent with similar other studies.[9, 13] As UTI is a common nosocomial infection management and cost effectiveness of treatment is of great concern. The knowledge of the resistance pattern of the organism aids in formulating an empirical therapy. The resistance pattern varies from each geographical area and hospital setting. Resistant organisms are common among hospital setup due to indiscriminate use of broad spectrum antibiotics. *Enterococcus*sppshows intrinsic resistance to penicillinase susceptible penicillin, penicillinase resistance penicillin, cephalosporins, nalidixic acid and clindamycin, thus limiting the treatment options available.

Among the *Enterococcus*spp, *Enterococcus faecalis*showed more resistance than *Enterococcus faecium to* antibiotics like gentamicin, tetracycline and ciprofloxacin. But high resistance to these antibiotics was reported in a similar study in India(72 % isolates for*E. faecalis* and 81 % for *E. faecium*).[14]

Vancomycin resistant *Enterococcispp* is increasingly reported in many parts of the world and India.Vancomycin resistant organisms are difficult to treat as the antibiotics of choice are linezolid or quinupristin/ dalfopristin. In a North American study - *Enterococci* obtained from urinary isolates were primarily vancomycin-resistant *E. faecium*(88.4%).[2] In our study, no vancomycin resistant *Enterococci*were isolated. The reason could be the small sample size, the lower consumption rates of glycopeptides like Vancomycin on account of their high cost and also possibly restricted use of glycopeptides due to good antibiotic prescription practices in our hospital. However, further studies with larger sample sizes are required to prove this.

CONCLUSIONS

*Enterococcus faecalis*is the commonest *Enterococcus* isolate causing UTI. No vancomycin resistance was identified among the urinary isolates from our centre.

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