

NON-FAECALIS AND NON-FAECIUM ENTEROCOCCI AS UROPATHOGENS: AN UPDATE FROM A TERTIARY CARE CENTRE

Medical Microbiology

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ABSTRACT

Background: The identification of non-faecalis and non-faecium *Enterococcus* spp. necessitate elaborate diagnostic tests for speciation and are under-reported. Their role in urinary tract infection (UTI) is deficiently documented. **Materials And Method:** Among 18,712 urine specimens, 250 *Enterococcus* isolates obtained from UTI were speciated, antimicrobial susceptibility testing was done by modified Kirby Bauer disc diffusion method and Minimum inhibitory concentration (MIC) of Vancomycin was determined by E-strip. **Results:** Prevalence of non-faecalis and non-faecium *Enterococcus* spp. among all culture positive urine samples was 0.74%. *Enterococcus dispar* constituted 3.6% (9/250), *Enterococcus hirae* 3.2% (8/250) and *Enterococcus raffinosus* 0.8% (2/250). Their MICs of Vancomycin ranged between (0.38-1.5) µg/ml. **Conclusions:** very little is known about the burden and implication of non-faecalis and non-faecium *Enterococcus* spp. in UTI. *Enterococcus hirae*, is rarely reported as a human pathogen, with only a handful of cases being reported worldwide. The non-faecalis non-faecium *Enterococcus* isolates merit further evaluation to elucidate their virulence.

KEYWORDS

UTI, non-faecalis, non-faecium, *Enterococcus*

INTRODUCTION:

Enterococcus is a Gram-positive facultative anaerobic coccus belonging to the lactic acid bacteria of the phylum Firmicutes. They constitute a major part of the normal flora of human and animal intestines. Traditionally considered as low grade pathogens, *Enterococcus* spp. have been implicated in a range of infections comprising blood stream infection, urinary tract infection (UTI), endocarditis, pyogenic, intra-abdominal and pelvic infections. Since its first implication in UTI in 1906, over the recent years, *Enterococcus* related UTIs have risen significantly with the increasing use of urethral catheters, broad-spectrum antibiotic therapy, as well as the increasing use of cephalosporins.^{1,2}

Currently, 23 different species of *Enterococcus* have been described, with *E. faecalis* and *E. faecium* being the most clinically relevant species, isolated in the US at a ratio of 1.6 : 1, respectively.³ The other strains of *Enterococcus* known to cause human infections include- *Enterococcus avium*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *Enterococcus raffinosus*, *Enterococcus mundtii* and *Enterococcus durans*. With the evolution and rapid dissemination of multidrug resistance, the lesser studied, non-faecalis and non-faecium *Enterococcus* spp. are emerging as a diagnostic and therapeutic concern worldwide.

Most of the studies till date have focused on the clinico-epidemiological profiles and properties of *E. faecalis* and *E. faecium*. Literature regarding the prevalence, clinical spectrum and properties of the non-faecalis and non-faecium *Enterococcus* isolates is handful. Most of the work pertaining to the non-faecalis and non-faecium enterococci have been from bacteremia. Whereas with an accruing burden on health care settings, the incidence of infections caused by non-faecalis and non-faecium enterococci has been rising steadily.⁴ Additionally, the conventional tests employed in the laboratory are difficult to incorporate routinely for speciation. Thus, these non-faecalis and non-faecium *Enterococcus* isolates, may be easily missed or misidentified and are consequently under reported. Thus, the present study was aimed at determining the prevalence of UTI associated with the non-faecalis and non-faecium *Enterococcus*, delineating their clinico-epidemiological profile, phenotypic characters and antimicrobial susceptibility profile including minimum inhibitory concentrations (MIC) of vancomycin.

MATERIALS AND METHOD:

A prospective study was conducted over a period of one year at the Department of Microbiology of a tertiary care center. The study was approved by the institutional ethics committee. All the urine samples received in the laboratory for routine bacterial culture were assessed for the presence of significant pyuria (0-1 pus cells per 7 high power

field)⁵ and cultured semi-quantitatively on Cysteine Lactose Electrolyte Deficient (CLED) agar.

Colonies resembling *Enterococcus* spp., in pure and significant counts ($\geq 10^5$ colony forming units /ml) were analyzed further. Growth on Bile-esculin semi-solid agar, 6.5% NaCl at 9.6 pH, PYR test and a battery of biochemical tests including mannitol, sucrose, raffinose, pyruvate, sorbose, arabinose, lactose and arginine di hydrolase were used for identification and speciation of *Enterococcus* spp. as per standard microbiological techniques.⁶ The nature of hemolysis was observed on 5% sheep blood agar.

Antimicrobial susceptibility of all isolates to standard antimicrobials were determined by the modified Kirby Bauer disc diffusion method and interpreted as per CLSI 2021.⁷ While the minimum inhibitory concentration (MIC) of vancomycin for all isolates were performed by Epsilometer strip (Himedia laboratories, Mumbai, India) test. (Quality Control: *S. aureus* ATCC 25923 & *E. faecalis* ATCC 29212)

RESULTS:

Over a period of one year, 250 isolates of *Enterococcus* spp. were obtained from 18,712 samples of urine processed in the laboratory. The prevalence of *Enterococcus* spp. in 2557 culture positive samples was 9.78% (250/2557). *Enterococcus faecalis* was the predominant species accounting for 62% (155/250) followed by 30.4% (76/250) *Enterococcus faecium*. The prevalence of non-faecalis and non-faecium *Enterococcus* spp. (non-faecalis and non-faecium) among all culture positive *Enterococcus* spp. isolates, in urine samples was 7.6% (19/250). The non-faecalis and non-faecium group of *Enterococcus* spp. were isolated in following percentages in pure and significant counts ($\geq 10^5$ cfu/ml) from the urine samples of study patients: 3.6% (9/250) *Enterococcus dispar*, 3.2% (8/250) *Enterococcus hirae* and 0.8% (2/250) *Enterococcus raffinosus*.

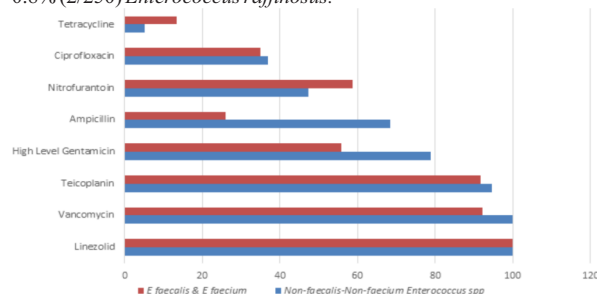


Figure 1: Comparison Of The Antimicrobial Susceptibility Between The Non-faecalis Non-faecium Group And *E. Faecalis-e. Faecium* Group Of Isolates

The majority of the patients with UTI due to the above non-*faecalis* and non-*faecium* *Enterococcus spp.* were females (63.2%) while (36.8%) were male patients.

Figure-1 depicts a comparative illustration of the antimicrobial susceptibility between the non-*faecalis* non-*faecium* group of *Enterococcus spp.* and the other common *Enterococcus spp.* isolates. While the antimicrobial susceptibility of all the non-*faecalis* and non-*faecium* *Enterococcus* isolates are depicted in Table 1.

Table 1: Antimicrobial Sensitivity Profile Of Unusual *Enterococcus Spp.*

Antibiotics	E.raffinosis n=2(%)	E.hirae n=8(%)	E.dispar n=9(%)
Ciprofloxacin	1(50)	2 (25)	4(44.44)
Nitrofurantoin	2(100)	4(50)	2(22.22)
Gentamicin (120µg/ml)	1(50)	8(100)	8(88.89)
Ampicillin	1(50)	7(87.5)	5(55.56)
Fosfomycin	2(100)	8(100)	8(88.89)
Teicoplanin	2(100)	7(87.5)	9(100)
Linezolid	2(100)	8(100)	9(100)
Vancomycin	2(100)	8(100)	9(100)
Tetracycline	0(0)	1(12.5)	0 (0)

Table 2: Demography, Comorbidities And Microbiological Profile Of The Non-*faecalis* And Non-*faecium* Group And *E. Faecalis* & *E. Faecium* Group Of *Enterococcus* Isolates.

	Non- <i>faecalis</i> And Non- <i>faecium</i> Group	<i>E. faecalis</i> & <i>E.</i> <i>faecium</i> group
Age distribution Range	6 – 70 years	6 months – 90 years
Mean	(38.9 ± 20.5) years	(33.5 ± 19.6) years
Proportion of hospitalized subjects	47.3% (n=9)	61% (n=141)
Mean duration of hospitalization	(1.33 ± 1.75) days	(3.2 ± 1.9) days
Rate of catheterization	37% (n=7)	48.5% (n=112)
Mean duration of catheterization	(2.3 ± 1.1) days	(3.4 ± 1.4) days
Proportion of CAUTI	26.3% (n=5)	40.7% (n=94)
MIC range of Vancomycin	0.38 – 1.5 µg/ml	0.38 – 256 µg/ml
Mean MIC of Vancomycin	(0.97 ± 0.38) µg/ml	(9.0 ± 40.9) µg/ml
MIC 50 of Vancomycin	0.5 µg/ml	1.5 µg/ml
MIC 90 of Vancomycin	1.5 µg/ml	3 µg/ml
Vancomycin resistance	0% (n=0)	8.2% (n=19)
Associated Factors:		
Pre-existing renal failure	5.3% (n=1)	3% (n=7)
Diabetes mellitus	26.3% (n=5)	12.1% (n=28)
Genito-urinary instrumentation/ surgery	31.6% (n=6)	10.8% (n=25)
Antibiotic therapy before sample collection	47.4% (n=9)	44.2% (n=102)
Use of 3 rd Generation cephalosporin ≥ 1 day prior to sample collection	31.6% (n=6)	34.2% (n=79)

After overnight incubation, 62.5% (5/8) isolates of *E.hirae* showed alpha hemolysis while 37.5% (3/8) isolates produced β-hemolytic colonies. One isolate each of *E.dispar* isolates exhibited alpha and β hemolysis, while majority (77.8%, n=7) of them showed no hemolysis on 5% sheep blood agar. *E.raffinosis* isolates exhibited both alpha and gamma hemolysis. Table 2 compares the demographic settings, associated conditions as well as microbiological properties of the non-*faecalis* and non-*faecium* group and *E. faecalis* & *E. faecium* group of *Enterococcus* isolates.

E.hirae were isolated from wards (62.5%, n=5) and ICUs (37.5%, n=3). 50% (n=4) of patients *E.hirae* isolates were from patients who have been catheterized for ≥2 days before collection of urine sample, thus amounting to catheter associated UTI (CAUTI). 62.5% (n=5) of the above CAUTI patients had pre-existing renal comorbidities like glomerulonephritis, renal failure and diabetic nephropathy. Whereas

37.5% (n=3) patients gave history of urinary tract surgery or genito-urinary instrumentation within the past 6 months.

E.dispar was predominantly isolated from out-patient settings (88.9%, n=8) followed by hospital ward (11.1%, n=1). Among the patients whose urine samples yielded *E.dispar*; 33.3% (3/9) of patients were catheterized for ≥2 days before collection of urine sample resulting in CAUTI. *E.raffinosis* was isolated in equal numbers from ward (1/2) and OPD (1/2); while only one patient was catheterized for ≥2 days prior to specimen collection. None of the above patients from whom *E.dispar* and *E.raffinosis* were isolated, had history of renal surgeries, genitourinary instrumentation, previous UTI or comorbidities like diabetes mellitus and immunosuppression.

DISCUSSION:

The prevalence of UTI due to *Enterococcus*, in our study (9.78%) was coherent with other studies from various geographical regions of India.⁸ However, higher prevalence of *Enterococcus* has been reported from women in reproductive age group.⁹ *E. faecalis* followed by *E. faecium* were the commonest *Enterococcus spp.* isolated from our study subjects, which was in agreement with several Indian studies.^{10,11} The predominance of *E. faecalis* infections, could be attributed to the plentitude of *E. faecalis* in the gut microbiome. Nevertheless, the no typed enterococci including the non-*faecalis* non-*faecium* enterococci contribute a staggering 24.6% of all enterococcal infections. Since these non-*faecalis* non-*faecium* enterococci are seldom reported separately, infections caused by these species are often overlooked.⁴

Unlike several studies where *E.faecalis* and *E.faecium* were the only two *Enterococcus spp.* isolated from urine samples, *E.dispar* (3.6%), *E. hirae* (3.2%) and *E. raffinosus* (0.8%) were isolated from urine specimens in the present study. Other studies have isolated non-*faecalis* non-*faecium* enterococci like: *E.dispar*, *E.pseudoavium*¹² and *E.raffinosis* from urine specimens.¹³ Several authors have raised concerns about the under reporting of the real incidence of these non-*faecalis* non-*faecium* enterococci.¹⁴ The elaborate range of tests for phenotypic identification at species level, confer major limitations in speciation. The possibility of species misidentification and unavailability of the full range of biochemical in most laboratory settings often impose restrictions in reporting. Since there is variation in antimicrobial resistance among *Enterococcus spp.*, the need of a larger range of tests for species recognition within the genus, and a holistic interpretation of key tests becomes imperative. Nevertheless, to the best of our knowledge, studies regarding the prevalence and significance of these isolates in urine samples are rare.

E. mundtii, *E.solitarius*, *E.malodoratus*, *E.durans*, *E.gallinarum*, *E. psedoavium*, *E. avium* and *E. casseliflavus* have been isolated from clinical samples in different parts of India.^{15,16} The above non-*faecalis* non-*faecium* enterococci have been isolated from heterogeneous clinical samples like blood, muscle tissue, ear swabs, burn wounds, ascitic fluid, pus and renal aspirate.¹⁷⁻¹⁹ *E.hirae*, a frequent pathogen in birds, cats and rats, is rarely reported as a human pathogen. It accounts for <1% of *Enterococcus* isolates in human clinical samples.²⁰ Only a few cases of *E.hirae* associated acute pyelonephritis in Korea,²¹ bacteremia associated with pancreatitis in USA²² and septicemia associated with end stage renal disease from Israel have been reported.²³ *E.hirae* has also been reported to cause native and prosthetic valve endocarditis and spondylodiscitis in diabetic patients.²⁴⁻²⁶ Following in accord, 62.5% of our study subjects from whom *E.hirae* was isolated, had pre-existing renal comorbidities like glomerulonephritis, renal failure, diabetic nephropathy; while 37.5% of the subjects gave history of urinary tract surgery/ genito-urinary instrumentation. The non-*faecalis* non-*faecium* enterococci like *E. hirae*, *E. casseliflavus*, *E. gallinarum*, *E. durans*, *E. mundtii*, *E. avium*, and *E. raffinosus* have been implicated in infections in people with hematological malignancies, neutropenia and previous corticosteroid therapy.⁴ Several of these non-*faecalis* non-*faecium* enterococci are intrinsically resistant to high end glycopeptide as well as commonly used cephalosporins. Considering the higher predisposition to infection and subsequently higher antimicrobial use in these group of patients, the need for species level identification of enterococci cannot be emphasized enough.

E.dispar have been isolated in pure and significant numbers from study subjects diagnosed with CAUTI. However, majority of *E.dispar* were isolated from outpatient settings. Though occasionally isolated from urine samples of UTI patients, little is known about the pathogenicity

and biology of *E. dispar*.¹⁵ Other studies have also reported *E. dispar* from urine samples.²⁷

The natural habitat of *E. raffinosus* is unknown, but it has been isolated from oropharynx of cats. Apart from urinary tract infections, *E. raffinosus* has also been associated with wounds, ulcers, intra-abdominal and inguinal abscesses, Bartolin gland abscesses, biliary and peritoneal infections, vertebral osteomyelitis, endocarditis and bacteraemia.²⁸ *E. raffinosus* is usually isolated from patients on empirical antibiotic therapy, prolonged hospitalization, urinary tract surgeries and catheterization.²⁹ Standard antimicrobial susceptibility pattern for *E. raffinosus*, is difficult to establish and it is gradually emerging as a multidrug resistant *Enterococcus spp.*, with resistance to penicillin, aminoglycosides, fluoroquinolones, carbapenems and glycopeptides already being reported.²⁹ To the best of our knowledge no *E. raffinosus* have been erstwhile isolated in pure and significant number from patients with significant pyuria.

Antimicrobial susceptibility testing revealed Tetracycline, followed by Ciprofloxacin and Ampicillin to be the least effective antibiotics against the non-*faecalis* non-*faecium* enterococci. The prevalence of high level aminoglycoside resistance in the present study was lower than a study conducted in north India, however the same study had concurrently reported vancomycin resistance among these non-*faecalis* non-*faecium* enterococci.²⁷ All the non-*faecalis* non-*faecium* *Enterococcus spp* isolates in our study were however, uniformly susceptible to vancomycin. The discordance may be at least partially explained by the differences in geographical settings as well as the possible antibiotic pressure owing to differences in antimicrobial prescribing practices in the two study settings. Interestingly, the widely used fluoroquinolone in UTI patients, ciprofloxacin as well as the urinary antiseptic nitrofurantoin, were found to be a poor therapeutic choice for these non-*faecalis* and non-*faecium* *Enterococcus spp.* The non-*faecalis* and non-*faecium* enterococci had higher predilection to patients with renal failure, diabetes mellitus, previous genito-urinary instrumentation/surgery and antimicrobial therapy prior to specimen collection. The altered immune response in the these group of patients, as well as selection of intrinsically resistant pathogens by administration of antibiotics, enhance colonization and subsequent infection with these bacteria. The epidemiology, associated risk factors, clinical spectrum and phenotypic characters of these species of *Enterococcus* have been studied deficiently and merit further deliberation.

CONCLUSION:

E. faecalis and *E. faecium* are the predominant *Enterococcus spp.* typically reported from urine samples. The elaborate range tests, conventionally used for speciating *Enterococcus*, may be partially responsible for the relative lower rates of reporting of non-*faecalis* and non-*faecium* group of *Enterococcus*. Despite the rising rates of isolation of these *Enterococcus spp.* from various clinical specimens, importance of routine deployment of the entire spectrum of tests for complete speciation is still debated. Selective antibiotic pressure along with indiscriminate usage of antibiotics in animal feed have been implicated for the emergence of these species as human pathogens. Hence, more studies are required to elucidate the clinico-epidemiological profile of these non-*faecalis* and non-*faecium* enterococci, in order to curb their spread and acquisition of multidrug resistance.

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