ORIGINAL ARTICLE

Prevalence of *Enterococcus faecalis* mediated UTI and its current antimicrobial susceptibility pattern in Lahore, Pakistan

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Abstract

Objective: To determine the prevalence of *Enterococcus faecalis* and recent trends in antimicrobial sensitivity profiling.

Methods: The study was conducted at Chughtais Lahore Lab, Lahore, Pakistan, from December 2013 to May 2014, and comprised urine specimens from suspected patients. Antimicrobial profiling of isolated strains of *Enterococcus faecalis* was determined by Kirby-Bauer disc-diffusion method.

Results: Of the230 specimens, 161(70%) were positive for *Enterococcus faecalis*. The prevalence of *Enterococcus faecalis*-mediated urinary tract infections was 120(74.53%) in females and 41(25.46%) in males.

Age-wise distribution of urinary tract infections among female patients was 41(34.16%) in >65 years age group. In males, the prevalence in the same age group was 19(46.34%). Besides, 145(90.09%) strains of *Enterococcus faecalis* exhibited resistance to gentamicin, 140(86.95%) to norfloxacin. Moreover, 138(85.71%) strains exhibited multi-drug resistance.

Conclusion: An overall pattern of drug resistance infections was observed in a majority of isolates.

Keywords: Urinary tract infection, *Enterococcus faecalis*, Antimicrobial susceptibility. (JPMA 66: 1232; 2016)

Introduction

Urinary tract infections (UTIs) are considered the most abundant bacterial infections commonly encountered in hospital environments. According to an estimate, UTI is responsible for approximately 40% of hospital-acquired infections.[1] UTIs occur in individuals of both sexes and of all ages but more abundant in females than males due to anatomical variations between the two genders.[2] "About 50% of women experience one episode of UTI at some point in their lifetime and about 20% to 40% of women have recurrent episodes, while approximately 20% UTIs occur in men. UTIs may lead to chronic renal failure, renal dialysis and eventually renal transplantations".[3]

Among the bacterial pathogens responsible for causing UTIs, include *Escherichia coli*, *Enterobacterspp., Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.[4] The patients exhibiting prolonged hospital stays and catheterisation are particularly susceptible to encounter multi-drug resistant (MDR) UTIs that will further increase the probability of morbidity, mortality and associated healthcare costs.[1] These infections may be symptomatic or asymptomatic and failure to accurate and timely diagnosis may lead to the emergence of MDR uropathogens.

Enterococcus faecalis (*E. faecalis*) has been recognised as the third-most important uropathogen responsible for intermittent and chronic UTIs among intensive care unit (ICU) patients.[5] These bacterial species are notorious for their widespread antibiotic resistance and are among the most frequently reported nosocomial pathogens having both intrinsic and acquired drug resistance.[6,7]

It has been extensively observed in Pakistan that most of the clinicians absurdly prescribe broad-spectrum drugs, even in the cases where not needed. In the recent years, unnecessary and short-term recommendation of latest generation antibiotics has resulted in the emergence of MDR bacterial strains.[8] Therefore, several strategies are proposed for preventing the spread of infection and they should be implemented on anurgent basis in order to prevent the situation from worsening further.[6]

The current study was conducted to determine the prevalence of *E.faecalis*-mediated UTIs along with its current antimicrobial susceptibility pattern among suspected male and female cases of different age groups in general population.

Materials and Methods

The present study was conducted at Chughtais Lahore Lab (CLL), Lahore, Pakistan, from December 2013 to May 2014. Midstream urine specimens (collected in sterile

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plastic containers following the standard clean-catch midstream procedure) from suspected cases of UTIs were processed for microbiological examination. Samples were collected from patients who were diagnosed with UTIs by the physicians on the basis of symptoms.

Specimens were cultured on cystine-, lactose- and electrolyte-deficient (CLED) agar plates followed by incubation under aerobic conditions at 37°C for 24 hours. After incubation, bacterial cultures were examined and those containing bacterial growth of $\geq 10^5$ colony forming unit (CFU)/ ml were considered significant culture results for bacteriuria.[9] Urine specimens displaying CFU count of less than 10^5 were considered non-significant for the analysis of bacteriuria. Isolated bacterial colonies were identified on the basis of their morphological characteristics. Furthermore, biochemical characterisation of bacterial isolates was carried out through gram's staining, catalase test and bile aesculin hydrolysis test.

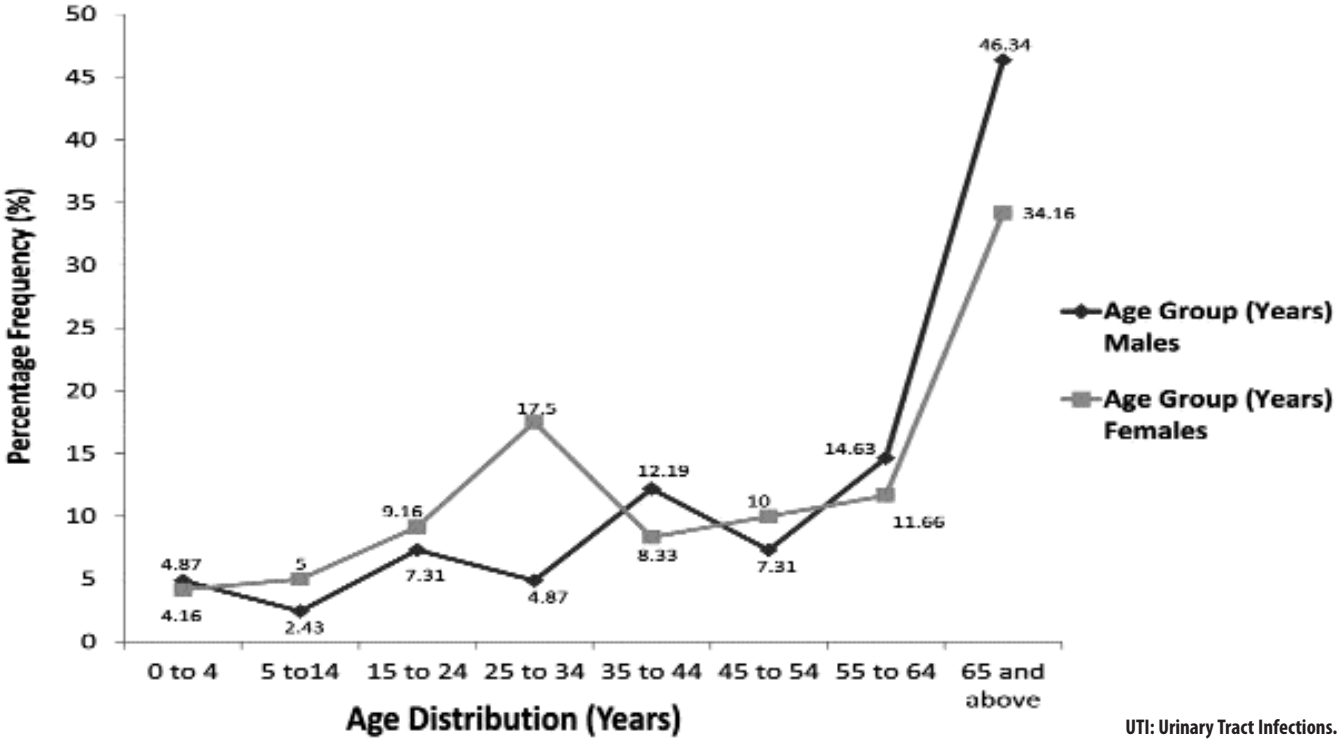
Identified bacterial isolates were then tested for antimicrobial susceptibility profiling by modified Kirby Bauer disc-diffusion method. Bacterial suspensions having turbidity comparable to that of 0.5 McFarland standard were applied on Mueller-Hinton agar plates with particular antibiotics to be tested.[9] Antibiotic discs of analytical grade (Oxoid, UK) used in the present study were as follows: ampicillin (10 µg), amoxicillin (10 µg), amoxicillin/clavulanic acid (20/10), sulbactam/ampicillin (10/10), vancomycin (30 µg), gentamicin (10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), fosfomycin (200 µg) and linezolid (30 µg). Sensitivity of bacterial strains to the above-described antibiotics was assessed by measuring the zones of inhibition in millimetres following the Clinical and Laboratory Standards Institute guidelines.[9,10] American type culture collection (ATCC® 25923) strain of *Staphylococcus aureus* was used as quality control standard.

Data was analysed statistically to determine the relation between the prevalence of disease and gender by t-test that was performed through Microsoft Office Excel 2007.

Results

Of the 230 midstream urine specimens, 161(70%) displayed positive results for *E.faecalis* bacterial culture.

The prevalence of *E.faecalis*-mediated UTI was 120(74.53%) in females and 41(25.46%) in males. The incidence of UTIs among female patients was 41(34.16%) in >65 years age group, followed by 21(17.5%) in 25-34 years, 14(11.66%) in 55-64 years, 6(5.00%) in 5-14 years and 5(4.16%) in 0-4 years age group. Among male



UTI: Urinary Tract Infections.

Figure-1: Age distributed incidence of Enterococcus faecalis mediated UTI in male and female patients.

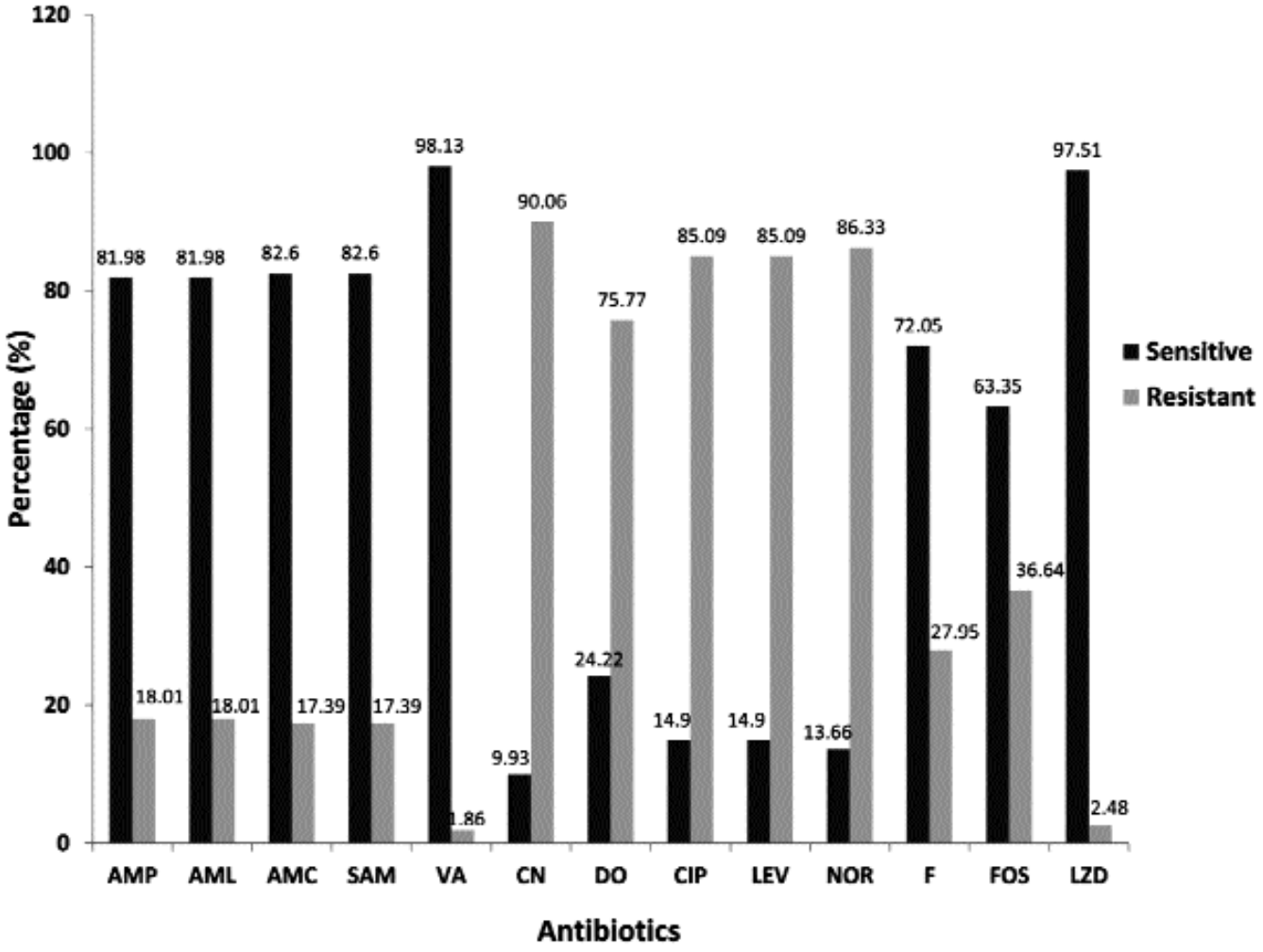


Figure-2: Current Antimicrobial Susceptibility Profile of Enterococcus faecalis; Antibiotics like Ampicillin (AMP, 10 µg), Amoxicillin (AML, 10 µg), Amoxicillin/Clavulanic acid (AMC, 20/10), Sulbactam/Ampicillin (SAM, 10/10), Vancomycin (VA, 30 µg), Gentamicin (CN, 10 µg), Doxycycline (DO, 30 µg), Ciprofloxacin (CIP, 5 µg), Levofloxacin (LEV, 5 µg), Norfloxacin (NOR, 10 µg), Nitrofurantoin (F, 300 µg), Fosfomycin (FOS, 200 µg) and Linezolid (LZD, 30 µg) were tested against Enterococcus faecalisstrains.

patients, the incidence was 19(46.34%) in the >65 years age group, followed by 6(14.63%) in 55-64 years, 2(4.87%) each in 0-4 and 25-34 years and 1(2.43%) in 5-14 years age group (Figure-1).

Besides, 145(90.09%) strains of E. faecalis exhibited resistance to gentamicin, 140(86.95%) to norfloxacin, 137(85.09%) each to ciprofloxacin and levofloxacin and 122(75.77%) to doxycycline (Figure-2). In contrast, 158(98.13%) strains were sensitive to vancomycin, 157(97.51%) to linezolid, 133(82.6%) each to sulbactam/ampicillin and amoxicillin/clavulanic acid, and 132(81.98%) to ampicillin and amoxicillin. Among these bacterial isolates, 138(85.71%) strains exhibited MDR as they showed resistance to at least four different members of aminoglycosides and quinolone.

Discussion

UTIs are most common human pathogenic infections that may be caused by different types of etiological agents such as bacteria, fungi or viruses. Bacterial infections dominate in majority of UTIs and the most commonly encountered etiological agents include *E. coli*, *E.faecalis*, *S. saprophyticus* and *S. aureus*.[11] Antimicrobial resistance among clinical isolates has been regarded as the most challenging problem worldwide that may pose a great threat to the society. The findings of the current study may help healthcare personnel to make some effective strategies for treatments.

In this study, urine specimens were processed which resulted in the isolation of 161 different clinical isolates of *E.faecalis*. In another relevant study,[12] *E.faecalis* was found to be the second-most important causative

agent of UTIs. Additionally, *Enterococciserves* is the major etiological agent and contributes towards the development of UTIs, especially under favourable conditions, i.e. the hospital environment where immunocompromised patients are particularly more susceptible to acquire bacterial infections.[13] *E.faecalis* exhibits a significant morphological advantageous feature, i.e. *E.faecalis* surface protein (Esp) that favours their adherence to the epithelial cell lines of the urinary tract.[5]

Key findings of the present study indicated higher incidence of *E.faecalis*-mediated UTI among females (74.53%) than in males (25.46%). Results were in accordance with another study[14] that reported 62.3% female cases of culture-positive urine specimens compared to 37.7% male cases. Similarly, in a comprehensive study regarding UTIs in women conducted by Salvatore et al.[15] it is described that 81% UTIs are found in females. Therefore, a higher frequency of UTI in females might be reflected due to anatomical variations between males and females; for instance females possess shorter urethra that can cause immediate bacterial penetration into the urinary tract.

This study showed maximum prevalence of UTI in old age females (34.16%) that might have been due to their weak immune systems. The second highest UTI cases were reported in females of 25-34 years age group (17.5%) because they represent sexually active proportion of tested population. Some other studies also described maximum prevalence of UTI among females of 16-45 years sexually active and child bearing age groups.[12,15] Therefore, married women in this age group frequently encounter hospital visits and are more likely to develop UTIs.[16] In contrast, young females of age groups 0-4 and 5-14 years displayed a significantly lower incidence of UTIs (4.16% and 5.00%, respectively) that is an illustration of least exposures to the hospital instrumentation and there is also difference in the composition of perineal flora in these age groups.[13]

Higher incidence of UTI among males of >65 years (46.34%) followed by those of 55-64 years (14.63%) is reported in this study. In the age groups of 0-4 years and 5-14 years, the least number of UTI cases (4.87 and 2.43%, respectively) are reported. These findings are supported by a Nepali study that reported the highest incidence (27.7%) of UTI in males aged >60 years.[14]

The incidence of UTI in males is found to be very low because of the natural defensive mechanisms of male urinary system. The increased incidence in old age people is indicative of high predisposition to infections due to their comparatively weak defensive mechanisms.

Antimicrobial susceptibility pattern of *E.faecalis* revealed that these strains exhibit maximum resistance to Gentamicin (90.06%) followed by antibiotics of the quinolones family such as Norfloxacin (86.33%), Levofloxacin and Ciprofloxacin (85.09% each). The work of Debnath et al.[12] on susceptibility of uropathogens reported comparatively less resistance profile of *E.faecalis* against Gentamicin (72.4%) and Ciprofloxacin (58.6%) compared to this study. Besides, 91.8% resistance of *E.faecalis* to Norfloxacin and 89.5% to Ciprofloxacin has been encountered by the study conducted in Nepal.[14] Therefore, such higher resistance of bacterial strains of *E.faecalis* to aminoglycosides (Gentamicin) and fluoroquinolones may be attributed to the intrinsic mechanisms of reduced uptake of drugs or acquisition of resistance by some foreign genetic materials.

Clinical isolates of the present study displayed 98.13% susceptibility against Vancomycin followed by Linezolid (97.51%), Sulbactam/Ampicillin and Amoxicillin/ Clavulanic acid (82.6% each), Ampicillin and Amoxicillin (81.98% each) and Nitrofurantoin (72.05%). The study conducted by Babar et al.[17] reported 100% susceptibility of Enterococci to Linezolid which closely resemble to the present study. Findings of a study[12] revealed 93.1% susceptibility of *Enterococci* isolates to Vancomycin, 79.3% to Nitrofurantoin and 81% sensitivity to Amoxicillin/Clavulanic acid that presents an analogy to the drug susceptibility patterns encountered in the present study. Furthermore, the study of Nepal reported 58.2% sensitivity of *E.faecalis* to Ampicillin, a β-lactam drug.[14]

The current study presented an overall 85.71% of MDR *E.faecalis* isolates that were resistant to more than four different types of antibiotics. However, in the study conducted by Wang et al.[18] a total of 83.9% MDR *E.faecalis* isolates were reported from urine specimens which widely exhibit resistance against aminoglycosides and glycopeptides. A maximum number of 55 isolate were non-susceptible to five different antibiotics, whereas among total isolates 26 showed resistance against more than eight different drugs. However, in the classification of drugs, maximum resistance was shown against Aminoglycosides (Gentamicin-145), Fluoroquinolones (Ciprofloxacin-137, Levofloxacin-137, Norfloxacin-140) and Tetracyclines (Doxycycline-122).

Conclusion

The prevalence of UTIs was found to be higher among female patients compared to males. Irrational use of antibiotics by practitioners as well as self-medication trends in Pakistan has further worsened the situation. An

overall pattern of MDR infections was observed in majority of the isolates. So, there is a need for an immediate strategy to efficiently diagnose and properly treat these infections prior to the development of further complications. Moreover, this study was an effort to appeal the attention of public health officials and researchers to consider this serious issue of public health.

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Conflict of Interest: None.

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