Antibiotic Resistance Pattern in Nosocomial Urinary Isolates of *Enterococcus*

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Abstract

Background
Recent years have witnessed advancement in medical facilities and as a consequence increase in healthcare related infections. The leading problem in majority of hospitals is nosocomial infections. Among nosocomial infections UTI has utmost importance. *Enterococcus* is among one of nosocomial organisms. *Enterococcus* emerges as important nosocomial pathogen during past few decades. The present research project was designed to study drug susceptibility of clinical isolates of *Enterococcus* species to have better knowledge in understanding the emerging organism and making treatment more accurate.

Results
52.72% (29) were isolated from urine samples collected from female patients whereas 47.27% (26) from male patients. *Enterococcus faecalis* was present in 87.27% (48) clinical cases and *Enterococcus faecium* in 12.7% (07). *Enterococcus* was frequently isolated from medicine 42% (23) and ICU 29% (16) wards. In this study, Quinolones group showed high resistance where as fosfomycin, vancomycin and linezolid were found useful against *Enterococcus* infections.

Methods
55 positive culture of *Enterococcus* were analyzed after collection of urine sample from different hospitals. Urine samples were streaked on CLED medium for isolation of organisms. Organisms were identified by their colonial characteristics and gram staining. For further identification bile esculin test and mannitol salt agar test was performed.

Conclusion
Enterococcus is emerging as important nosocomial uropathogen. Antibiotic resistance among *Enterococci* spp. are on rise and become great concern for hospital setup. Quinolones prescription should be eluded as empirical treatment for *Enterococcus* urinary tract infection whereas fosfomycin, Vancomycin and linezolid are potent drugs but should be used with caution.

Key words
Urinary tract infection, *Enterococcus*, Nosocomial infection or hospital acquired infection, antimicrobial susceptibility.

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Introduction

Past decades have witnessed great progress in medical field. Currently, increase in invasive and complicated procedures lead to increase in opportunistic infections.[1] Nosocomial or hospital acquired infections (HAI) are those infections which are acquired by patient during hospital stay. HAIs are considered continuous threat and extra burden to patient and health authorities.[2] Among hospital acquired infections urinary tract infection are a frequent cause. About 40% of all nosocomial infections are urinary tract infection.[3] The financial implication and morbidity and mortality related to these infections are major problem for hospital resources.

Approximately 80% of nosocomial UTIs are related to urinary catheterization.[3] However, old age, diabetes mellitus, renal diseases, any debilitating disease and other several factors are associated with UTIs. Even hospital-acquired urinary tract infections can lead to urosepsis and septic shock. The ultimate treatment of these infections is antibiotic but the irrational use of antibiotics especially in Pakistan has contributed significantly in development of drug resistance and evolution of multidrug resistance strains. Nosocomial uropathogens show more antibiotic resistance than community acquired UTIs.[3] This trend can be seen in isolated strains of *Enterococcus* from clinical samples.

Enterococci are gram positive organisms that are part of normal gut micro-biota. Over the last few decades *Enterococcus* emerge as important health care associated pathogen due to their potential to withstand extreme and harsh circumstances. In addition, intrinsic and increasing acquired resistance among *Enterococcus* isolates to many antibiotics also creating challenges for health-providing authorities.[4]

E. faecalis and *E. faecium*, are among the main pathogenic strains. *Enterococcus* is involved in serious nosocomial infections such as urinary tract infection. But the organism can cause other diseases like endocarditis, bacteremia, sepsis and rarely meningitis.[5,6] The contributing factors for *Enterococcus* infection include catheterization, impaired host immunity, use of antibiotics, old age etc.

The ability to resist multiple antibiotics makes *Enterococcus*

a real threat. *Enterococcus* exhibits intrinsic and acquired resistance. The intrinsic resistance is present in all *Enterococcus* species. *Enterococci*are intrinsically resistant toco-trimoxazole, aminoglycosides and cephalosporins. These are empirically prescribed antibiotics to cure Urinary tract infections and *Enterococcus* infection. By acquiring antimicrobial resistance, *Enterococci* can survive for more period of time. The indiscriminate use of antibiotics allows Enterococci to develop acquired resistance against different classes of antibiotics which include glycopeptides, tetracycline, chloramphenicol, nitrofurantion and quinolones.

The substantial rise in antibiotic resistance of *Enterococcus* species across the world emphasizes on the better understanding of this organism. Infection control programs and policies are imperative in minimizing the chance of hospital-acquired UTIs. Knowledge of *Enterococcus* resistance pattern is essential for treatment purpose. This would be helpful in determining the importance of *Enterococcus* infection.

Materials and Methods

In present study urine samples were collected from Civil hospital Karachi and Zubaida Medical Centre, Karachi during a period from December 2014 to March 2016 and analyzed. Midstream urine samples were collected from patients suffering from acute or chronic urinary tract infection, catheterized or uncatheterized, school going children, young and grownup adults of either sex while menstruating women, toddlers, infants and those having renal, bladder and genital tumors were not included.

From uncatheterized patients samples were collected in a properly labeled sterile plastic container whereas in catheterized patients urine sample were taken from part of catheter after taking consent from patient/attendant after sample collection, samples were analyzed within 6 hours. Urine samples were streaked on Cysteine Lactose Electrolyte Deficient (CLED) (Oxoid) agar. Urine sample was considered positive by presence of bacterial count 1000-10,000cfu/ml.[7] Colonies were examined and identified on the presence of peculiar colony characteristics and further identified by gram staining and catalase test. Bile esculin test and mannitol salt agar test were performed for further identification of *Enterococcus* spp.

The susceptibility of *Enterococcus* spp. to empirically used antibiotics against urinary tract infection was tested by using Kirby-Bauer disc diffusion method[8] and interpreted in reference to CLSI.[9] Bacterial suspension was prepared to match the standard 0.5 McFarland index. Sterile cotton swab was used to prepare bacterial lawn on Mueller-Hinton agar plates (Oxoid) in three different directions to obtain a uniform inoculum over the entire plate. Antibiotics disc were placed on medium with the help of forceps. Antibiotic discs of different concentration were used. These include: imipenem(10µg), ampicillin (10µg), piperacillin (100µg), levofloxacin (5µg), norfloxacin (10µg), nalidixic acid (30µg), pipemidic acid (20µg), fosfomycin (50µg), Vancomycin (30µg) and linezolid (30µg)and co-amoxiclav (20/10)µg (Oxoid). Plates were examined after 24 hours of incubation at 37°C and cleared areas around discs were measured and noted in mm. *E. faecalis* ATCC 29212 was used as reference strain for antibiotic susceptibility testing.

Results

In total 55 urine samples showing positive growth of *Enterococcus* spp. were analyzed. Out of 55 *Enterococcus* isolates, 42% (n=23) strains were isolated from medicine ward followed by ICU 29% (n=16) and gynecological ward 14% (n=8) (Fig 1), among which 53% (n=29) were isolated from urine samples of female patients whereas 47% (n=26) were from male patients (Fig 2). *Enterococcus faecalis* was present in 87% (n=48)samples and *Enterococcus faecium* in 13% (n=7) (Fig 2). The age of patient ranged between 5-90 years. The median age was 40 years.

Antibiotic Resistance Patterns in *E. faecalis* and *E. faecium*:
The drug susceptibility of *Enterococcus* against different antimicrobials was showed in table (Table 1). High resistance against quinolones was observed in *E. faecalis* isolated from oncology, ICU and surgical ward (88%, 84% and 80% respectively) (Fig 3). *E. faecium* (64%) were observed resistant to beta lactam drugs isolated from various wards of hospital. Vancomycin resistant was noticed particularly in *E. faecalis* isolated from ICU ward. 29% VRE were isolated from ICU and gynecological ward preceded by 15% in medicine.

Discussion

Recently *Enterococcus*has emerged as multifaceted and important nosocomial uropathogen.[1,10] As antimicrobial resistance is high among *Enterococcus* species, it is wise to find out the sources to control *Enterococcus* infections. Kahlmeter (2003)[11] found *Enterococci* amongst the leading cause of UTI in hospitals.

In recent study prevalence of *Enterococcus* spp. were mainly

Number of Organisms Isolated from Different Wards of Hospital

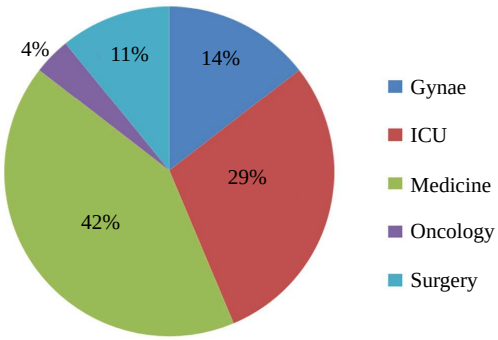

Fig 1. Frequency of *Enterococcus* spp collected from different wards of hospitals

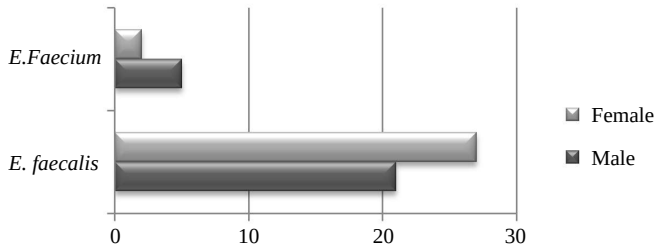

Fig 2. Gender and Age wise distreibution of *E. faecalis* and *E. feacium*

Table 1: Antimicrobial susceptibility of *Enterococcus* spp.

Antimicrobial disc (Oxoid)	Susceptible	Inter-mediate	Resistant
Linezolid(30µg)	49 (89%)	-	06 (11%)
Vancomycin(30µg)	43 (78%)	2(4%)	10 (18%)
Fosfomycin(50µg)	38(69%)	-	17(31%)
Co-amoxiclav(20\10µg)	35(64%)	-	20 (36%)
Imipenem(10µg)	34(62%)	-	21(38%)
Ampicillin(10µg)	33(60%)	-	22(40%)
Piperacillin(100µg)	24 (43.6%)	-	31(56.36%)
Levofloxacin(5µg)	17 (31%)	1(2%)	37(67%)
Norflaxacin(10µg)	11(20%)	-	44(80%)
Nalidixic acid(30µg)	11(20%)	-	44(80%)
Pipedemic acid(20µg)	11(20%)	-	44(80%)

observed in urine samples collected from medicine ward followed by ICU. Majority of patients admitted to these wards via emergency department suffered from chronic or acute illness. These patients may require catheterization, complex invasive procedures and antibiotics administration more frequently as compared to patients admitted via OPD. In US approximately 5% catheterized patients develop urinary tract infections.[12] In addition, excessive antibiotics administration disturb normal flora hence more chances of infection. In China, a study conducted in a hospital showed that *Enterococcus* was mainly isolated from burns center.[1] In another study by Richards and his colleagues (2000)[13] revealed 20-30% UTIs in ICU wards.

In present study *E. faecalis* was one of the important uro-pathogen isolated from catheterized patients. Pascale *et al*., (2013)[14] demonstrated *E.faecalis* as main pathogen in catheterized patients. In another study in Mexico *E. faecalis* and *E. faecium* was found to be 81.2%. However Salem-Bekhit (2012)[15] and partners reported 69% *E. faecalis* and 11% *E. faecium* isolates from clinical samples in hospital in Saudi Arabia.

High antibiotic resistance among *Enterococcus* species is probably due to easy survival of these organisms in extreme conditions. Quinolones are frequently prescribed medicine for treatment of urinary, respiratory, and gastrointestinal tract infections.[16] Current study revealed high resistance against quinolones (nalidixic acid, pipemidic acid and norfloxacin). About 80% *Enterococcus* isolates showed resistance against quinolones. Altaf and Tuba (2015)[17] came across with similar observations. Variation in quinolones resistance can be observed across the world depending on various factors. An Indian study demonstrated 75% quinolones resistance,[18] UK, Australia and France, showed low resistance rate.[17] This happens due to

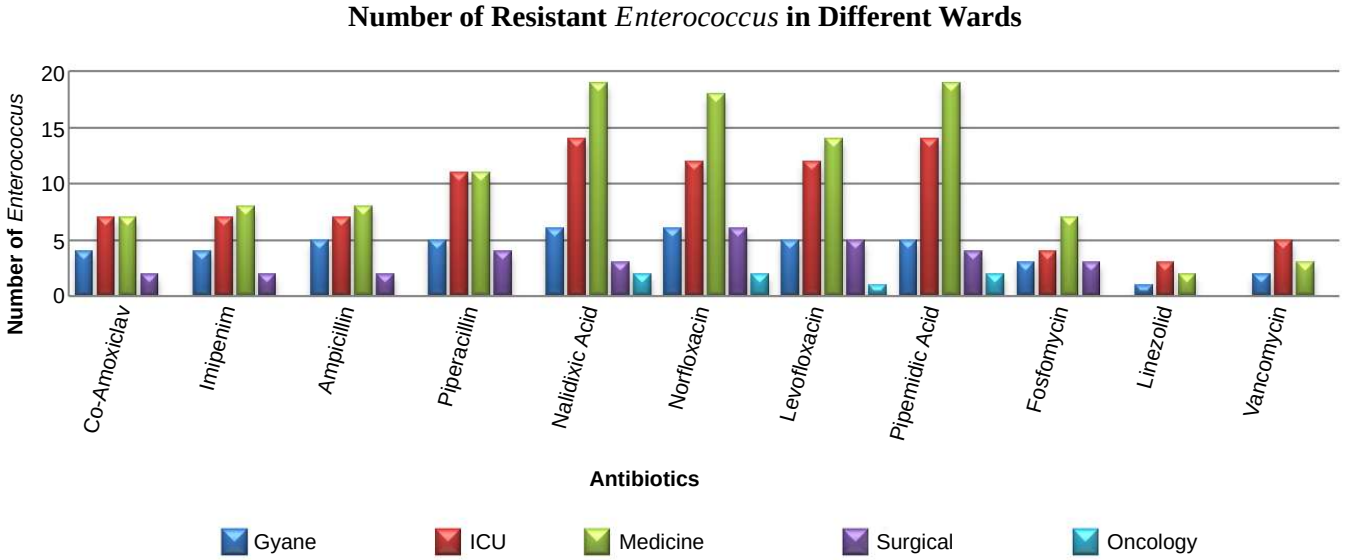

Fig 3. Numbers of resistant *Enterococcus* spp isolated from different wards

discipline in prescribing and controlled sale of antimicrobials. Rattanaumpawan *et al,* (2011)[19]described that quinolones resistance in *Enterococcus* was particularly noticed who were subsequently exposed to clindamycin, cephalosporin and flouroquinolones.

The resistance pattern of beta lactam drugs; imipenim, ampicillin, piperacillin and amoxiclav against Enterococcus was observed. Because of low affinity of penicillin binding proteins, the effectiveness of β-lactam drugs has been reduced among gram positive *Enterococci*.[20] In this study 64.28% isolated *E. faecium*were found resistant to β-lactams drugs. These results are in close association with Wei *et al* (2014).[1] However in another study Shamshad *et al* (2014)[21] demonstrated 25%, 12% and 8% resistant isolates against ampicillin, co-amoxiclav and imipenem, respectively. Farhan and hiscolleagues(2006)[22] observed 45% ampicillin resistant isolates.

Vancomycin aglycopeptides was introduced due to resistance in other drugs. Vancomycin resistance was first observed in *Enterococcus* and afterwards in *S. aureus*.[23] In present study 18% VRE strains were isolated from urine samples. These results are very much similar to Asif *et al* (2014)[24] in Lahore (Pakistan) revealed 16.2% VRE. However, 3% Vancomycin resistance was observed by Shamshad *et al*., (2014).[21] Farhan and Essa, (2006)[22] and The Laboratory, Karachi, demonstrated only one% VRE. However Vancomycin resistance vary considerably.[25] In accordance with CDC, in US 30% Vancomycin resistant strains were observed while in a Brazilian, Iran and Indian tertiary hospital VRE observed 15.5%, 20.5% and 12% respectively.[26] Literature survey also pointed towards transferring of resistant gene to other organisms via VRE.[27] Beyond all, Vancomycin is still considered as better treatment option for *Enterococcus* infection.

In present study antibiotic resistance in different hospital's ward varied. Quinolones resistance was mainly observed in strains, isolated from urine samples collected from medicine unit. This may be attributed to condition of patient, depressed immunity and prolonged use of antibiotic. Prevalence of beta lactam resistance was observed mainly in isolates collected from gynaecological ward. In another study by Shah (2004)[28], use of ampicillin and co-amoxiclav was studied. He reported that maximum numbers of antibiotics were given for LSCS. In our hospitals drastic increase in LSCS without proper indications is important contributing factor. In recent study VRE strains were mainly isolated from ICU ward. VRE ranks second among resistant organisms in ICU patients.[29]

Conclusion

Due to presence of drug resistance, *Enterococcus* treatment become challenging as fewer options are available. For betterment antibiotic should be selected after culture and susceptibility and narrow spectrum antibiotic should be preferred.With limited health resources, a policy should be constructed for resistant strains.

Authors Contribution

Aqeel Ahmad (Help in study design, planning, analysis, interpretation, discussion, facilitated for reagents, critical review); Ghulam Fatima (facilitated for reagents, critical review); Sabiha Mirza (facilitated for reagents, critical review) and Mehveen Iqbal (Concept, study design, study conductor, analysis, interpretation, discussion, manuscript writing, finalize research paper).

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