Prevalence and antibiotic resistance profiles of Gram negative bacilli associated with urinary tract infections (UTIs) in Karachi, Pakistan

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Abstract: The aim of this investigation was to determine the prevalence and antibiotic resistance profiles of Gram negative bacilli (GNB) responsible for urinary tract infections (UTIs). Urine specimens were cultured on Cysteine Lactose Electrolyte Deficient Agar (CLED) medium and pathogenic GNB were identified by conventional biochemical methods and automated profile index (API) system and further subjected to antibiotic sensitivity testing by disk diffusion method. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii were encountered as most frequent GNB in sequence. Among them E. coli (71%) was the most prevalent GNB. About 77% E. coli isolates of indoor patients and 59% of outdoor patients were found resistant to Cefotaxime. Kleb. pneumoniae were 100% resistant to Ampicillin. Higher resistance in P. aeruginosa was noticed in isolates of indoor patients i.e. Ciprofloxacin (76%), Cefoperazone-sulbactam (60%), Ceftazidime (59%), Piperacillin-tazobactam (53%), Imipenem (49%) and Amikacin (39%) in contrast to that of outdoor patients. Slightly lower resistance in Acinetobacter baumannii against Ampicillin (86%), Nitrofurantoin (81%) and Fosfomycin (12%) was witnessed in indoor patients’ urine specimens compared to outdoor patients’ urine. Polymyxin B, Imipenem, Fosfomycin, Piperacillin-tazobactam, Cefoperazone-sulbactam, Amikacin and Nitrofurantoin were most effective in GNB induced UTIs. This study revealed elevated resistance profiles in GNB against Ampicillin, Amoxicillin-clavulanate, Cefotaxime, Aztreonam, Ciprofloxacin, Nalidixic acid and Trimethoprim/sulfamethoxazole. Emergence of antibiotic resistant GNB was due to the frequent use and misuse of antibiotics in our region.

Keywords: Prevalence, antibiotic resistance, urinary tract infections, resistance profiles, gram negative bacilli.

INTRODUCTION

Gram negative bacilli (GNB) are the significant cause of hospital and non-hospital associated infections (Jocelyn et al., 2004). They differ in the prevalence that they originate as the most prevalent types of nosocomial infections: pneumonia, urinary tract, bloodstream and many other infections of different anatomical sites and intensive care unit related infections like incision site (Chon, 2001; Barbier et al., 2013; Ruppe et al., 2015). Infections caused by G-ve infectious agents can involve every organ system of human, but appear commonly in the urinary tract (Rahal, 2009; Peleg and Hooper, 2010).

Urinary tract infections (UTIs) are the most common infections in infants, children and adults (Zare et al., 2018). Approximately, 1500, 000 cases of UTIs are being reported worldwide, out of which 35% are acquired through hospitals (Blair et al., 2015; Zowawi et al., 2015). Nearly all nosocomial UTIs are connected with catheterization in old and young age individuals (both male and female). Inaccurate treatment of UTIs may lead to severe conditions like abortion, high blood pressure, uremia and renal failure. Asperity of these infections relies upon the virulence factors and multi drug resistance of the etiological agent (Arjunan et al., 2010). A variety of pathogenic bacteria (E. coli, Citrobacter, Enterobacter, Enterococcus, Klebsiella pneumoniae, Morganella spp., Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Staph. saprophyticus and Staph. aureus) can lead to UTIs. But E. coli is the most frequent agent of urinary tract infection, both in men and women of all ages (Mahmoudi et al., 2016). Urinary tract associated E. coli invade the urinary system by a number of factors, consisting of adhesins, biofilm and antipathy of human immune reactions (Jacobsen et al., 2008; Asadi Karam et al., 2019).

Among GNB; E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa are found as most frequent cause of UTIs. The major difficulty in treating GNB associated UTIs is the emergence of multi drug resistant Gram negative bacilli (MDR-GNB). Aforesaid GNB strains are being recorded globally (Norouzi et al., 2011; Schwechheimer and Kuehn, 2015). Annually, around 600 deaths are caused by MDR-E. coli associated UTIs. In Pakistan, high ratio of nosocomial UTIs associated mortality and morbidity has been witnessed (Iqbal et al., 2002; Poolman and Wacker, 2016; Rafaque et al., 2019).

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Augmentin, Amikacin, Ceftriaxone, Ciprofloxacin, Fosfomycin, Imipenem and Tazocin are persistently used for the treatment of UTIs in Pakistan. But among these antibiotic resistance and susceptibility profile differ in E. coli, Kleb. pneumoniae and Ps. aeruginosa. Over 70% UTI associated E. coli have been found resistant to third generation cephalosporins (Farajnia et al., 2009). The resistance ratio in GNB against routinely recommended antibiotics may be different geographically. Assessment of regional antibiotic sensitivity and resistance profiles could assist in the management and empirical treatment of UTIs (Pitout et al., 2005).

Many investigations during the preceding ten years or before have provided satisfactory proof that adequate early empirical antibiotic treatment, prescribed by antibiotic sensitivity results, increases the viability rate. A profound knowledge and improved tests by laboratories, consisting of biochemical survey are needed to decrease therapeutic mess, to stop antibiotic resistant GNB access to healthcare settings and to avoid the transmission of evolving infectious agents within the community (Rahal, 2009). The increasing incidences of MDR-GNB associated with UTI in Pakistan, have become a threat in hospitals and in communities. This problem is responsible for day by day rise in illness rates and deaths. Antimicrobial resistance and susceptibility data suggests appropriate treatment options and strategies for the containment of MDR-GNB. Such notorious MDR-GNB can also pass on the resistance genes to the susceptible strains. Hence, resistance can disperse from hospitals to communities. The prevalence studies are helpful in this regard (Tenney et al., 2018). Therefore, this investigation was conducted to rule out the prevalence and in vitro antibiotic resistance sketch of GNB responsible for UTIs.

MATERIALS AND METHODS

Collection of specimen
Urine samples were obtained from hospitalized and outdoor patients in a tertiary care hospital, Karachi, Pakistan with overt symptoms of UTI. Midstream samples of urine were collected by clean catch method. One sample was collected per patient (Graham and Galloway, 2001).

Urine culturing and interpretation
The samples of urine were cultured by streak plate method on Cystine Lactose Electrolyte Deficient (CLED) agar medium with the help of calibrated (1 µl) disposable plastic loops. CLED agar plates were incubated for 18-24 hours at 37°C aerobically. Significant colony count, i.e. 10^5 CFU/ml or more were considered as the significant bacterial pathogen load and selected for further processing. Urine culture showing multiple (different) types of colonies with significant or insignificant count were refused (Scarparo et al., 2002).

Morphological and biochemical identification of E. coli
Isolated colonies were subjected to conventional biochemical identification. Single type isolated colonies (previously checked by Gram staining) from CLED agar plates were inoculated in different selective and differential media for identification such as Simmons citrate agar, urea agar, triple sugar iron agar (TSI) and sulfide indole motility (SIM) medium tubes. All tubes were incubated vertically in incubator at 37°C for 18-24 hours aerobically (Barrow and Feltham, 1993; Murray and Barron, 2007).

Automated profile index (API) 20 E (bioMerieux)
This system is a well regulated identification system for members of Enterobacteriaceae and other Gram negative bacilli, which utilizes 21 miniaturized biochemical tests and a database. The representatives of MDR-GNB were identified by this system. The processing of test strips was carried out according to manufacturer’s instructions (O’Hara, 2005).

Antimicrobial sensitivity testing (AST)
Finally, the identified GNB were checked for the antibiotic susceptibility as per CLSI guidelines. Disk diffusion method was used to check the antibiotic resistance profiles. Antibiotics were selected that are commonly used in hospitals and clinical settings for treatment of UTIs. These antibiotics are also mentioned in CLSI guidelines for the treatment of UTIs caused by Enterobacteriaceae (CLSI, 2009).

Disk diffusion method
Disk diffusion antibiotic sensitivity testing was achieved by Kirby-Bauer disc diffusion method on Muller Hinton agar on the basis of Clinical laboratory standard institute (CLSI) recommendations and guidelines of European committee on antimicrobial susceptibility testing (EUCAST). Quality control strains E. coli ATCC25922 and Pseudomonas aeruginosa ATCC27853 were utilized for the validation of antibiotic susceptibility testing. Specific sets of antibiotic disks were applied with different zone size criteria. Ampicillin (AMP), Amoxicillin-clavulanate (AMC), Cefotaxime (CTX), Amikacin (AK), Piperacillin-tazobactam (TZP), Cefoperazone-sulbactam (SCF), Nalidixic acid (NA), Trimethoprim/Sulfamethoxazole (SXT), Ciprofloxacin (CIP), Imipenem (IPM), Fosfomycin (FOS), Nitrofurantoin (F), Ceftazidime (CAZ), Aztreonam (ATM) and Polymyxin B (PB) were used (Andrews, 2009; CLSI, 2009).

STATISTICAL ANALYSIS
Frequency of bacterial pathogens encountered in urine and their resistance patterns were analysed in percentages by using MS Excel. The p-values (significant i.e. <0.05) have been calculated by Chi-square formula covering the antibiotic resistant profiles of GNB isolated from hospitalized and outdoor patients. Data has not shown in
order to maintain the credible length of the manuscript (Perla and Carifio, 2005).

RESULTS

Urine specimens (n=67262) were collected during Jan, 2011 to Dec, 2015 in a tertiary care hospital, Karachi. These specimens included 60% (n=40,100) from outdoor patients and 40% (n=27,162) from hospitalized patients. Out of these samples 22% (n=14680) were positive for bacterial pure culture, consisting of 56% (n=8233) outdoor patient’s specimens and 44% (n=6447) hospitalized patient’s specimens. E. coli, Klebsiella pneumoniae, Ps. aeruginosa and Acinetobacter baumannii were found to be the top four GNB encountered in clinical specimens. Among them E. coli (71%) was the most prevalent GNB among top 4 GNB as shown in table 1. Prevalence of E. coli was found as 58% and 42% in outdoor patients’ and hospitalized patients’ urine samples respectively. Kleb. pneumoniae was recorded same as of E. coli. Ps. aeruginosa was 44% in outdoor patients’ and 56% in indoor patients’ urine specimen. Acinetobacter baumannii were noted as 53% in outdoor and 47% in indoor patients’ urine (table 1).

E. coli

Approximately, equal rates of antibiotic resistance were observed in indoor and outdoor patients of UTIs except Cefotaxime (CTX). About 77% of E. coli isolates of indoor patients and 59% of outdoor patients were found resistant to Cefotaxime (CTX). About 90% of E. coli of both indoor and outdoor patients was resistant to Ampicillin. Comparison of antibiotic resistance profiles of E. coli isolated from indoor and outdoor patients’ samples was also done (fig. 1).

Antibiotic resistance profiles of E. coli were checked during 2011 to 2015. No significant change in resistance rates of urinary isolates was noticed against Ampicillin (AMP), Cefotaxime (CTX), Ciprofloxacin (CIP), Nalidixic acid (NA) and Trimethoprim/sulfamethoxazole (SXT) during five year study while minor changes in resistance to other antibiotics were observed (fig. 2).

Klebsiella pneumoniae

Slightly decreased rates of antibiotic resistance were observed in Klebsiella pneumoniae encountered in outdoor patients’ urine specimens in contrast to that of indoor patients’ urine samples except Ampicillin (AMP), Nalidixic acid (NA) Ciprofloxacin (CIP) and Nitrofurantoin (F) as shown in fig. 3. Klebsiella pneumoniae (both indoor and outdoor patients) were found totally (100%) resistant to Ampicillin.

Ps. aeruginosa

Fig. 4: Antibiotic resistance profiles of Klebsiella pneumoniae encountered in urine specimens during 2011-2015.

Fig. 5: Comparison of antibiotic resistance profiles of Ps. aeruginosa encountered in outdoor patients’ urine specimens and indoor patients’ urine specimens.
In five year (2011-2015) study of antibiotic resistance in urinary isolates minor fluctuations in antibiotic resistance in each year were found except Ampicillin (AMP) as given in fig. 4.

**Ps. aeruginosa**
Higher resistance rates were noticed in isolates of indoor patients’ urine, i.e. Ciprofloxacin (CIP) 76%, Cefoperazone-sulbactam (SCF) 60%, Ceftazidime (CAZ) 59%, Piperacillin-Tazobactam (TZP) 53%, Imipenem (IMP) 49% and Amikacin (AK) 39% in contrast to that of outdoor patients (fig. 5).

In five year (2011-2015) study of Ps. aeruginosa from urine, >50% of isolates were found resistant to Ciprofloxacin (CIP), Cefoperazone-sulbactam (SCF) and Ceftazidime (CAZ) (except 2011) while, fluctuations were seen in resistance profiles of other antibiotics except Fosfomycin (FOS) as depicted in fig. 6.

**Acinetobacter baumannii**
Slightly lower resistance rates against Ampicillin (AMP) 86%, Nitrofurantoin (F) 81% and Fosfomycin (FOS) 12% were witnessed in indoor patients’ urine specimens in contrast to outdoor patients’ urine. This difference is quite insignificant. Indoor urinary isolates were found more resistant to Amoxicillin-clavulanate (AMC) 65%, Cefotaxime (CTX) 61%, Trimethoprim/sulfamethoxazole (SXT) 54%, Piperacillin-tazobactam (TZP) 42%, Cefoperazone-sulbactam (SCF) 28% and Imipenem (IPM) 36%, while approximately equal rates of resistance were observed in Nalidixic acid (NA), Ciprofloxacin (CIP), Aztreonam (ATM) and Amikacin (AK) in both indoor and outdoor urinary isolates (fig. 7).

Five year (2011-2015) study of A. baumannii resistance in urinary isolates showed variations in resistance profiles each year as given in fig. 8. However, >50% of urinary isolates offered resistance to Ampicillin (AMP) and Nitrofurantoin (F) throughout the five years.

**DISCUSSION**

Higher rates of UTIs (22%) were recorded in the hospital which requires proper management of such infections in the hospital and in the community as well. Increased rates of UTIs may be due to the high prevalence of diabetes mellitus and kidney stones in the patients (Al-Badr and Al-Shaikh 2013; Schwechheimer and Kuehn, 2015).

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**Table 1: Frequency of Gram negative bacilli in hospitalized and outdoor patients of UTI**

<table>
<thead>
<tr>
<th>Gram negative bacilli</th>
<th>In hospitalized patients</th>
<th>In outdoor patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>4378 (42%)</td>
<td>6045 (58%)</td>
<td>10423 (71%)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>863 (42%)</td>
<td>1192 (58%)</td>
<td>2055 (14%)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1068 (56%)</td>
<td>840 (44%)</td>
<td>1908 (13%)</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>138 (47%)</td>
<td>156 (53%)</td>
<td>294 (2%)</td>
</tr>
<tr>
<td>Total</td>
<td>6447</td>
<td>8233</td>
<td>14680</td>
</tr>
</tbody>
</table>

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Among top four GNB encountered in UTIs, *E. coli* was found 71% and followed by *Klebsiella pneumoniae* 14%, *Ps. aeruginosa* 13% and *Acinetobacter baumannii* 2%. *E. coli* (58%) was isolated predominantly from outdoor patients’ urine, and 42% in hospitalized patients’ urine specimens, indicating a high prevalence of *E. coli* in community acquired UTIs. Similar prevalence of GNB in UTIs was observed in Kohat, Khyber Pakhtunkhwa, as *E. coli* (41%), *Klebsiella* spp. (15.5%) and *Ps. aeruginosa* (8.62%) (Ullah et al., 2018). UTIs caused by *E. coli* (76.6%) were also reported in a tertiary care hospital of Peshawar, Pakistan (Shabbir et al., 2017). *Klebsiella pneumoniae* being placed at 2nd position because it was encountered frequently (56%) in community associated UTIs and 42% in hospitalized patients’ urine. Interestingly, *Ps. aeruginosa* was more prevalent in hospitalized patients of UTI, among all four GNB while with low frequency in outdoor patients’ urine. The prevalence of GNB was found consistent with the previous studies conducted at Lahore and Karachi, Pakistan and Iran (Nizami et al., 1997; Noor et al., 2004; Farajnia et al., 2009; Sabir et al., 2014).

More than 50% resistance in *E. coli* to Ampicillin, Cefotaxime, Ciprofloxacin, Trimethoprim-sulfamethoxazole and Aztreonam was found approximately equal in both indoor and outdoor patients. Surprisingly, increased rate of Cefotaxime resistance was witnessed in indoor patients of *E. coli* associated UTI, because of the extensive and irrational use of third generation cephalosporins in hospital settings and emergence of ESBL-producing strains of *E. coli* (Park, 2014). No significant change in antibiotic resistance patterns of urinary isolates of *E. coli* was witnessed in five year (2011-2015) survey. The antibiotic resistance profiles of *E. coli* (present study) are in agreement with the past studies of Peshawar, Kohat and Karachi. Accordingly, *E. coli* was found resistant to Ampicillin (80-90%), Cefotaxime (50-66%) and Ciprofloxacin (70-80%) (Noor et al. 2004; Shabbir et al., 2017; Ullah et al., 2018). However, indoor urinary isolates of *Klebsiella pneumoniae* showed high resistance profiles against Cefotaxime, Trimethoprim-sulfamethoxazole, Aztreonam, Amoxicillin-clavulanate, Piperacillin-tazobactam and Imipenem. This may be the outcome of extensive use of selected antibiotic and injudicious use of antibiotics against *Klebsiella pneumoniae* in hospitals. During five year study (2011-2015) of urinary isolates of *Klebsiella pneumoniae*, minor fluctuations in resistance every year was observed while resistance to Ampicillin and patterns of other antibiotics resistance was same. These results were found consistent with the findings of Abdullah et al. (2013). Imipenem, Fosfomycin (only for urinary isolates), Amikacin, Cefoperazone-sulbactam and Piperacillin-tazobactam were found effective for the treatment of *Ps. aeruginosa* induced UTIs. Fosfomycin and Polymyxin B were recorded as best choice for the treatment of *Ps. aeruginosa* associated UTIs (Michalopoulos et al., 2011). Slightly higher resistance rates were found in hospitalized patients’ urine specimens except against Fosfomycin. During five year (2011-2015) study of urinary isolates of *Ps. aeruginosa*, more than 50% of the isolates were found resistant to Ciprofloxacin, Cefoperazone-sulbactam and Ceftazidime. However, fluctuations in resistance rates were observed in other antibiotics except Fosfomycin (Bibi et al., 2015). Comparatively, slightly lower resistance rates against Ampicillin (86%), Nitrofurantoin (81%) and Fosfomycin (12%) were found in *Acinetobacter baumannii* of indoor patients of UTIs in contrast to that of outdoor patients of UTIs. However, higher resistance proportions against Amoxicillin-clavulanate (65%), Cefotaxime (61%), Trimethoprim-sulfamethoxazole (54%), Piperacillin-tazobactam (42%), Imipenem (36%) and Cefoperazone-sulbactam (28%) were witnessed in indoor isolates of UTIs. Five year (2011-2015) investigation of resistance profiles in urinary isolates indicated >50 of *Acinetobacter baumannii* were resistant to Ampicillin and Nitrofurantoin every year persistently, while variations in resistance profiles were noted every year. Results suggested that antibiotics especially, Fosfomycin, Imipenem and Polymyxin B are suitable choice for UTIs caused by GNB (Rasool et al., 2015). Variation in antibiotic resistance profiles of GNB in five year is due to extensive use and misuse of antibiotics both in community and hospitals. Elevated resistance profiles in GNB against Beta-lactams, Quinolones and Trimethoprim/sulfamethoxazole antibiotics may be due to prolonged and selective use of these antibiotics in our area. This antibiotic resistance has resulted due to the evolution of Extended Spectrum Beta-lactamases (ESBLs), Metallo Beta-lactamases (MBL) producing and mutated GNB strains (Shaikh et al., 2015; Karam et al., 2016).

**CONCLUSIONS**

This five year study concludes; Polymyxin B (PB), Imipenem (IMP), Fosfomycin (FOS), Piperacillin-tazobactam (TZP), Cefoperazone-sulbactam (SCF), Amikacin (AK) and Nitrofurantoin (F) were found most active (in vitro) for the treatment of *E. coli*. Polymyxin B (PB), Imipenem (IMP) and Fosfomycin (FOS) were suitable for the treatment of *Kleb. pneumoniae* induced UTIs. Polymyxin B (PB) and Fosfomycin (FOS) showed tremendous activity against *Ps. aeruginosa*. Polymyxin B (PB), Cefoperazone-sulbactam (SCF) and Fosfomycin (FOS) were the best choice for the treatment of *Acinetobacter baumannii* associated UTI. The five year data of GNB showed elevated resistance profiles against Ampicillin (AMP), Amoxicillin-clavulanate (AMC), Cefotaxime (CTX), Aztreonam (ATM), Ciprofloxacin (CIP), Nalidixic acid (NA) and Trimethoprim/ sulfamethoxazole (SXT).
REFERENCES


