Epidemiology of Multi-Drug Resistant Gram-Negative Uropathogens in South India

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ABSTRACT

Multi-drug-resistant bacteria surveillance (MDR) systems were used to identify the epidemiology of MDR bacteria in urinary tract infection (UTI) and non-UTI Patients for a period of 12 months collected from the regions of Tamil Nadu, South India. This study aimed to isolate colonies of MDR bacteria which were cultured on suitable selective media and identified by biochemical analysis as well as isolates of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* from the UTI and non-UTI patients. We screened a total of 1542 samples. A total of 532 strains of bacterial isolates belonging to 4 species were isolated. Among the clinical samples, *E. coli* was highly predominant in urine samples. Various antimicrobial activities were performed and higher resistance was observed at 86.2%, 95.2% and 46.0% to Ampicillin-sulbactum, and lowest resistance was observed to meropenem and colistin (7.8%). The MDR of four UTI and non-UTI causing bacteria was recorded in this study, which indicated that the bacteria were resistant to each antibiotic study in public health, particularly *E. coli, K. pneumoniae, A. baumannii* and *P. aeruginosa.* These findings confirm that antimicrobial resistance rates vary for different pathogens.

KEYWORDS: Multidrug resistance bacteria, Antimicrobial resistance, Urinary tract infection, Surveillance

INTRODUCTION

In general, antimicrobial resistance is a public health issue which is concerned with the size and characteristics of the problem may that may vary geographically according to healthcare settings (Harbarth *et al.*, 2001; Zinn *et al.*, 2004). About 150 million cases of urinary tract infections (UTIs) are estimated worldwide (Flores-Mireles *et al.*, 2015). Every day, the empirical treatment of bacteremia acquired in clinical practice in hospitals and infected by MDR microbes is the specific issue especially where PDR strains have been reported in hospital environments. One specific issue in everyday clinical practice is the empirical treatment of bacteremia acquired in hospitals and caused by MDR microorganisms, which is common (especially in hospitals where PDR strains have already been found). Recent researches indicated that MDR microbes infected people were hospitalized and the outcomes of health reports were worse with an increasing rate of mortality (Vardakas *et al.*, 2013).

In some cases, the causative agent based on the adequate antimicrobial therapy and the presumable infected pattern treatment is an essential method of bacteremia and reduces the mortality rate (Asgeirsson *et al.*, 2011; Kim *et al.*, 2014). Gram-negative bacilli such as *E. coli, K. pneumoniae*, *A. baumannii* and *P. aeruginosa*, are responsible for a variety of diseases including UTIs, nosocomial infections, septicemia, pneumonia and other opportunistic infections (Guentzel, 1996). Generally, extended-spectrum beta-lactamases (ESBL)-producing bacteria, methicillin-resistant *Staphylococcus aureus*, carbapenems-resistant *Enterobacteriaceae, A. baumannii, P. aeruginosa* and *Mycobacterium tuberculosis* are MDR/PDR bacteria. Common bacteria that show MDR/PDR are methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamases (ESBL)-producing bacteria, carbapenems-resistant *Enterobacteriaceae* (CRE), carbapenems-resistant *A. baumannii* (*A. baumannii*, CRAB), MDR/PDR *Pseudomonas aeruginosa* (*P.*

aeruginosa, MDR/PDRPA), and MDR *M. tuberculosis* (Chellat *et al.*, 2016; Seligman *et al.*, 2013; Wallinga *et al.*, 2015). The limited options to treat the MDR bacteria are limited to carbapenems, colistin and phosphomycin drugs. However, we need the potentially new MDR bacteria drug as an emergency. UTI is one of the most common infectious diseases influencing the social wellbeing of individual or society (Shahidul *et al.*, 2013).

According to researches, both males and females are susceptible to UTI with women being more susceptible than males. In the life span of women, 1 in every 3 women requires drugs at the age of 24, and 50% of women will suspected of UTI (Nerurkar *et al.*, 2012; Vasudevan, 2014). Patients with diabetes mellitus are most commonly affected by UTIs, because their urinogenital system condition has been altered. The major problem associated with UTI is the presence of biofilm associated pathogens that may lead to recurrence and persistence (Ejrnæs *et al.*, 2011). The extensive dissemination of MDR strains of Gram-negative bacilli is promoted by biofilm formation and beta-lactamases production. The catheterized patients are particularly susceptible to MDR pathogens because they form biofilm, which is really difficult to treat (Prigent-Combaret *et al.*, 2001). Our study was carried out to examine the cultural characteristics, biochemical analysis and antimicrobial susceptibility rates of Gram-negative MDR bacteria in clinical samples collected from different locations of south India.

MATERIAL AND METHODS

Study design

The study was conducted at Microtech Diagnostic Centre (MDC) and Department of Biotechnology at Nehru Arts and Science College, Coimbatore, Tamil Nadu, India from March

2021 to April 2022. MDC is a tertiary diagnostic and consultant center which serving a society from the zone of Coimbatore district, South India.

Specimens and procedure

A total of 1542 samples from different hospitals and diagnostic centers were collected. The specimens collected from different patients with UTI and patients with other infections or previous infections were included in this study. Patients' specimens like blood, urine, sputum, wound and fluid were collected in BHI broth medium and transported to MDC. The clinical specimens were preserved according to the clinical and laboratory standard guidelines.

Screening and identification of Gram-negative bacteria

The diagnosis of specimens contained bacterial population of at least to $\geq 10^5$ CFU/ ml were detected by (Jones, 1981). A culture of samples was done in Mac Conkey, Lactose electrolyte deficient (CLED) agar, EMB agar and Nutrient agar medium and was incubated at 37°C for 24hrs. Identification and isolation of bacterial cultures were performed by colony morphology and biochemical assays (Mshana *et al.*, 2009). In this experiment, the positive strains used for each bacterium acts as a reference control during the identification process.

Susceptibility test

Drug susceptibility assay was estimate on Muller Hinton agar by Kirby Bauer disc diffusion method followed by clinical and laboratory standard institute guidelines. The following antimicrobial drugs such as amikacin, gentamicin, tobramycin, piperacillin, cefepime, ceftazidime, piperacillin/ tazobactum, levofloxin, ofloxacin, imepenem, colistin, polymixin-b, carbenicillin, ciprofloxin and ampicillin were tested by disc diffusion method. Reference strains were used as control and the results were interpreted according to clinical laboratory standard institute (CLSI) guidelines (Sahu *et al.*, 2012).

Statistical analysis

All the experiments were conducted in triplicates to avoid errors and the values are represented as mean *P* value ≤0.05 is considered as significantly different.

RESULTS

Patients

In this study, we have examined a total of 1542 clinical specimens, of which 532 strains had pathogens. The most common gram-negative bacteria isolates were identified in urine (44%), wounds (22%), blood (20%), respiratory (12%) and fluids (2%), respectively (Fig. 1). The majority of clinical samples and bacterial isolates were detected from urology (685 samples, 44.4%) (Fig. 1). According to the samples, females (368 samples, 69.7%) were the most infected group of patients than male patients (164 samples, 30.82%) and the median age factor was 50 years (Table 1). In total bacterial populations, most isolated Gram-negative pathogens including *E. coli* (32.3%), *K. pneumoniae* (21.0%), *P. aeruginosa* (22.7%) and *A. baumannii* (23.8%) The higher frequency of Gram-negative bacteria isolated includes *E. coli* and *A. baumannii* (Fig. 2).

Fig. 1: Graphical representation of sources of clinical isolates used in this study

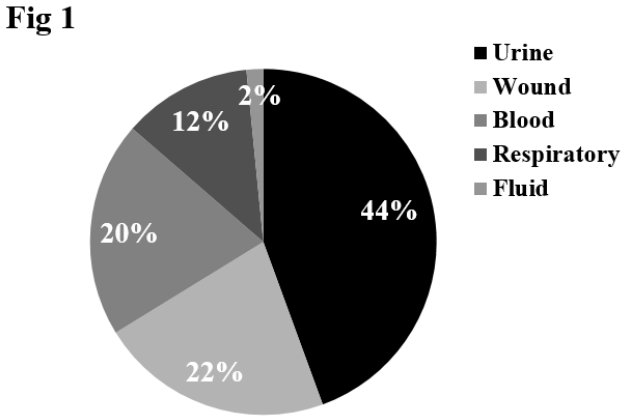
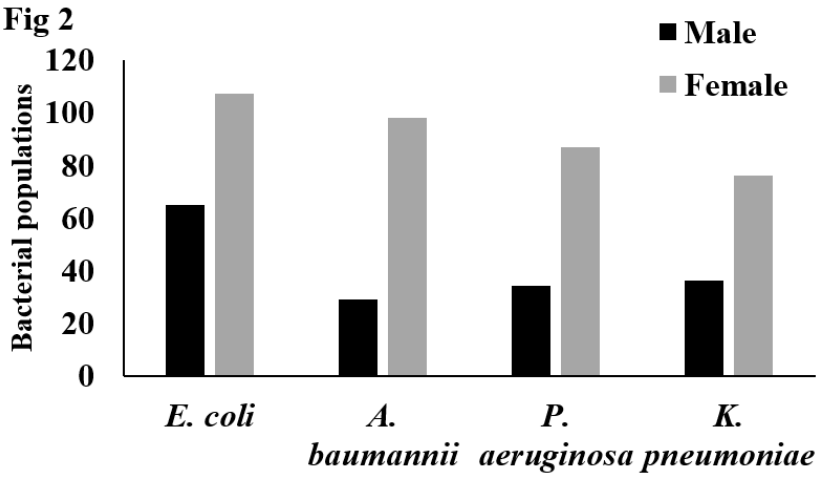


Fig. 2: Bacterial isolates from male and females



Cultural and biochemical characterization

Gram-negative bacterium, *K. pneumoniae* was identified by colony characteristics in Mac Conkey and CLED agar showing typical culture characteristics which grew as lactose fermenting, pink, mucoid and yellow mucoid colonies. *K. pneumonia* has been found positive for biochemical tests like catalase, Voges-Proskauer (VP), citrate, urease and nitrate, while indole, MR and oxidase tests were found to be negative. *K. pneumoniae* was verified using triple sugar iron (TSI) test which had shown the production of acid and gas (Table 2 & 3).

P. mirabilis and *P. vulgaris* had swarming and beta-hemolytic colonies on blood agar and appeared as translucent blue colonies on CLED agar. Positive results were observed in catalase, MR, urease citrate, and nitrate tests while other tests were negative. *P. aeruginosa* was identified using nutrient agar, which revealed that the irregular opaque with bluish green pigment was *P. aeruginosa*. *P. aeruginosa* positively responded to catalase, oxidase and urease tests, and negative responses were obtained for indole, MR and VP tests (Table 2 & 3).

E. coli colonies cultured on EMB agar appeared purple in color with flat dry, irregular shape and Mac Conkey agar appeared metallic green color with a pink LF mucosal colony. Biochemical tests were positive for catalase, indole, MR and nitrate, while it was negative for oxidase, VP, citrate and urease tests. In TSI test, acid is produced with gas (Table 2 & 3).

In addition, *A. baumannii* identified on Mac Conkey agar showed colorless smooth, opaque, raised, NLF and CLED agar showed blue colored opaque raised NLF. Biochemical tests were positive for catalase, VP and citrate test and were negative for oxidase, indole, MR and nitrate tests (Table 2 & 3).

Table 1: Age details of persons undergone treatment and percentage of infection

Gram-Negative Bacteria	Year (Number (n))	Frequency (%)
Age (in years)	>75	82 (15.6)
	61 - 75	93 (18>0)
	46 - 60	125 (23.4)
	31 – 45	151 (28.3)
	16 - 30	60 (11.3)
	≤ 15	21 (3.9)
Total		532

Table 2: Media used for isolation and maintenance of pathogenic bacteria from UTI and non-UTI samples and their colony characterizations.

Bacteria	Total bacterial stains	Cultural Medium used	Bacterial Colony Characterization
K. pneumoniae	35	CLED agar Mac Conkey agar	Yellow mucoid LF, pink, mucoid
A. baumannii	25	Mac Conkey agar, CLED agar	Colorless smooth, opaque, raised, NLF Blue coloured opaque raised NLF
P. aeruginosa	28	Nutrient agar	Large, irregular opaque with bluish green pigment
E. coli	53	EMB agar Mac Conkey agar	Purple coloured, flat dry, irregular colonies, with metallic green colour LF, pink, mucoid

CLED: Cysteine lactose electrolyte deficient

Table 3: Biochemical characterization of Gram-negative bacteria isolated from different patients. Where, MR: methyl red test; VP: Voges-Proskauer test; TSI: triple sugar iron test; A/AG: acid in slant and butt with gas production; Nd: Not done; +: Positive; -: Negative.

Bacteria	Cata-lase	Oxidase	Coa-gulase	Indole	MR	VP	Citrate	Urase	TSI	Nitrate	Bile Salt
K. pneumoniae	+	-	Nd	-	-	+	+	+	A/AG	+	Nd
A. baumannii	+	-	Nd	-	-	+	+	V	Nd	-	Nd
P. aeruginosa	+	+	Nd	-	-	-	+	+	Nd	+	Nd
E. coli	+	-	Nd	+	+	-	-	-	A/AG	+	Nd

Antibiotic susceptibility patterns

Gram-negative bacterial pathogens antibiotic resistant percentage patterns are shown in Table 3. The urinary pathogens such as *E. coli*, *K. pneumonia, P. aeruginosa* and *A. baumannii* showed high antimicrobial resistance against Ampicillin-sulbactum (86.2%), ciprofloxacin (181.9%), and amoxicillin/clavulanic acid (62.4%). The low resistance percentage were found against meropenem (0.1%) and colistin (7.8%) (Table 4). In *E. coli* isolates, the percentage of resistance to ampicillin-sulbactum (46.0%), co-resistant to amoxicillin-clavulanic (43.0%) and piperacillin (36.6%) was observed. Lower resistance was observed for meropenem (0.1%) and cephalexin/cephalotin (6.9%) and higher resistance was observed for Ampicillin-sulbactum (46.0%), respectively. *K. pneumoniae* isolates were resistant to beta-lactamine antibiotics such as ampicillin-sulbactum (95.2%), ceftazidime (64.5%) and piperacillin (62.9%). Colistin and ceftriaxone (7.8%), as well as piperacillin-tazobactum (40.1%) are low resistant.

Table 4: Percentage of antibiotic resistance shown by different bacteria. Where, Polymyxin B, Tobramycin and Carbenicillin were applied only for *Pseudomonas spp*.

S. no.	Antimicrobial agents	Resistant (%)			
		***K. pneumonia* n=112**	***A. baumannii* n=127**	***P. aeruginosa* n=120**	***E. coli* n=172**
1.	Amikacin	50 (45.0)	56 (44.9)	39 (32.5)	23 (19.0)
2.	Ampicillin		70 (55.1)		34 (19.7)
3.	Amoxicillin-clavulanic	74 (56.2)			18 (43.0)
4.	Ampicillin-sulbactum	84 (86.2)	90 (95.2)		17 (46.0)
5.	Carbenicillin			47 (39.1)	
6.	Cefepime	61 (54.9)	61 (48.0)	49 (40.3)	27 (22.3)
7.	Ceftazidime	70 (63.0)	82 (64.5)	48 (40)	39 (32.2)
8.	Cephalexin/cephalotin	35 (31.2)			12 (6.9)
9.	Ceftriaxone	17 (15.1)	10 (7.8)		21 (12.2)
10.	Ciprofloxacin	92 (81.9)	60 (47.2)	76 (63.3)	15 (8.7)
11.	Colistin		10 (7.8)		
12.	Gentamicin	55 (49.5)	57 (44.8)	45 (37.5)	36 (29.7)
13.	Imepenem	50 (45.0)	50 (39.7)	55 (45.8)	10 (8.2)
14.	Levofloxin	58 (52.2)	46 (36.2)	48 (40)	42 (34.7)
15.	Meropenem	76 (67.8)	63 (49.6)	14 (11.6)	2 (0.1)
16.	Nitrofuloxacin	58 (51.7)			18 (10.4)
17.	Ofloxacin	62 (55.8)	69 (54.3)	46 (46.6)	31 (25.6)
18.	Piperacillin	69 (62.1)	80 (62.9)	45 (37.5)	44 (36.3)
19.	Piperacillin-Tazobactum	43 (38.7)	51 (40.1)	37 (30.7)	26 (21.4)
20.	Polymixin-B			13 (10.8)	

P. aeruginosa was resistant to Ciprofloxacin (63.3%), Imepenem (45.8%) (Table 4). Most of the isolates were resistant to this imepenem antibiotic which is yet to be studied further. *A. baumannii* isolates were shown to be resistant to antibiotics like ampicillin-sulbactum (46.0%), ceftazidime (64.5%) and piperacillin (62.9%), and shown a lower range of resistance to d levofloxin (36.2%), respectively. The highest resistant of *A. baumannii* was seen against ampicillin-sulbactum (95.2%).

DISCUSSION

The current study reported Gram-negative MDR bacterial infections and antimicrobial activity in Coimbatore (South India). Out of 1542 bacterial colonies, 532 revealed that the populations of Gram-negative bacteria, which usually infect MDR pathogens which were 44.4% in urine and 21.7% in wound samples. In this data shows that female had 69.7% infected with UTI than male sex, which is agree with Zang *et al.,* 2009 (Zhang *et al.,* 2009). The most common identified microbes in UTI were Gram-negative *E. coli* pathogens, with a highest detection rate of 44.4% (n-172) observed and followed by *A. baumannii* 23.8% (n-127), *P. aeruginosa* 22.7% (n-121) and *K. pneumonia* 21.0% (n-112), respectively. Similar observations were reported by Ahmed S S *et al.,* such as *E. coli* (50.11%), *A. baumannii* (1.84%), *P. aeruginosa* (7.84%), *P. proteus species* (4.91%) *and K. pneumonia* (28.33%) were the most prevalent organisms in UTI patients, followed by MDR (Ahmed *et al.,* 2019). These results when compared with the prior research with a different percentage of MDR Gram-negative bacteria colonization (Ahmad and Ahmad, 1995). However, in our experimental result was found that the Gram negative resistance morphological test and MDR test were found in hospital infected samples (Bouchillon *et al.,* 2012). Therefore, the distribution of MDR bacteria, especially in hospitals environments is important for the control of infection as well as for the rational use of antimicrobial agents.

According to Rejendran *et al.,* study reported that, 90% of *E. coli* and *Klebsiella species* were potentially inhibited by fosfomycin (Rajenderan *et al.,* 2014). From 2010 to 2013, a Canadian national surveillance studies shows that the fosfomycin antibiotic susceptibility against *E. coli* in UTI (Al-Agamy *et al.,* 2014; Karlowsky *et al.,* 2014), as well as similar functions against *Enterrobacter species* (57%), *K. pneumoniae* (38%) and *Pseudomonas species* (30%). In India, Bradford PA stated that there has been an increase in the production of ESBLs, which is due to the

irrational use and ease of access to antibiotics over the counter, and such isolates are common in hospitals and the community (Bradford, 2001). The ESBL-producing *E. coli* exhibits the highest levels of resistance to ciprofloxacin, ceftazidime, ampicillin, trimethoprim-sulphamethoxazole and amoxicillin-clavulanic acid. This finding is consistent with many previous reports on *E. coli* resistance to nitrofurantoin (Al-Agamy *et al.*, 2014; Azap *et al.*, 2010; Moyo *et al.*, 2010), which while previously reported to be low in China (1.6%) (Ho *et al.*, 2015), North America (1.1%) (Zhanel *et al.*, 2005) and high in Latin American hospitals (13%) (Gales *et al.*, 2002). In our study, we observed that the meropenem, imepenem, cephalexin/cephalotin and ciprofloxacin were showed high resistance against to *E. coli*, respectively. Piperacillin-tazobactum, ceftriaxone and amikacin antibiotics is suggested as a viable treatment option against *E. coli.*

According to recent research, *K. pheumoniae* pathogens can increase their pathogenicity, accumulate and which lead to serious infections that are difficult to treat. When treating infections by bacteria that produced extended-spectrum beta-lactamase (ESBL) such as *K. pneumonia*e, the preferred b-lactam is carbapenem (Karuniawati *et al.*, 2013; Okoche *et al.*, 2015). However, the most recent and effective medications for the treatment of MDR-*K. pneumonia*e were carbapenems, amikacin and tigecycline, but according to a recent study showed that the overuse during treatment will increase persistence rate (Wu *et al.*, 2021a, 2021b). Trimethoprim sulfamethoxazole, gentamicin and ciprofloxacin resistance rate in India were 83.3%, 48.8% and 46%, respectively (Manikandan *et al.*, 2011). In our results we observed that the ampicillin-sulbactum, meropenem, piperacillin and ceftazidime antibiotics showed low significant resistance against *K. pneumoniae.* Ceftriaxone and cephalexin/ cephalotin showed that the inhibitory effect, which suggest that this could be the drug of choice against such resistant isolates.

Carbapenems, cephalosporin, fluoroquinolones or b-lactam inhibitors are recommended as primary treatment options for *P. aeruginosa*. For example, ceftolozane/ tazobactam's potent anti-pseudomonal agent to MDR *P. aeruginosa*. Interestingly, a recent report suggested that the quinolones and carbapenems antibiotics play a vital role in MDR *P. aeruginosa* acquisition (Seligman *et al.,* 2013). Rossi *et al.,* revealed that the resistance rate is significantly high (30 - 70%) to carbapenems antibiotics in Brazil, in the year 2010 and 2014 (Rossi *et al.,* 2017). In our result, *P. aeruginosa* isolates showed moderate rates of sensitivity to the studied antibiotics and which is lower than infections with *E. coli*, and *K. pneumoniae* and *Acinetobacter spp.* isolated from clinical samples (Davarzani *et al.,* 2021). Meropenem, polymixin-B and piperacillin-tazobactum antibiotics may be used as the drug choice for effective treatment and control against *P. aeruginosa*.

Recently, almost all antibiotics are being used to prevent *A. baumannii* which resulted in increased antibiotic resistance. For example, ampicillin, gentamicin, sulbactam, imipenem, leoflozacin and ciprofloxacin resistance were rapidly increasing. Meropenem, imepenem or doripenem and carbapenems have been shown to be the best therapeutic option for serious infections. However, Maragakis and Perl and Chen *et al.,* revealed that the limited choice of outbreak of carbapenems therapy options lead to *A. baumannii* infection resistance (Chan *et al.,* 2010; Maragakis and Perl, 2008). Moreover, colistin antibiotics agent are effectively used for the treatment against MDR *A. baumannii* (Amin *et al.,* 2019). In an Indian study by Behera *et al.,* 2009, and Bhose S *et al.,* 2013, A. baumannii had a 96.4% sensitivity to polymyxin B and 100% sensitivity of colistin and polymyxin B resistant (Behera *et al.,* 2009; Bose *et al.,* 2013). In our study, we suggested that the colistin and polymyxin B may be used as the drug of choice for treatment of infections of *A. baumannii*.

In conclusion, 18-month surveillance of UTI revealed that MDR isolates of 532 Gram-negative bacteria were prevalent in the environment around south India from diagnostic lab and hospitals. The prevalence of ampicillin, piperacillin or ciprofloxacin and ceftazidime resistant strains were higher in these regions. However, in individual patients, the use of extended spectrum penicillin, such as amoxicillin are highly resistant to common antibiotics. This could be due to the selective pressure of widespread antibiotic use in these regions. These results suggest a situation where monitoring of antibiotic resistance and infection prevention and control are required at regional level.

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