Uropathogens and Antimicrobial Resistance Pattern Among Patients of Surat, Gujarat

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Abstract
Antimicrobial resistance (AMR) has emerged as worldwide problem and constitute serious risk of community acquired infections with limited number of treatments. Nowadays, Urinary Tract Infection is becoming more susceptible amongst community due to certain MDR strain in developing and developed countries, leading to difficulty for establishing controlled preventive measurements. The study is focused to investigate antimicrobial resistance pattern on 500 infected urine samples. Isolation and identification of bacteria were carried out as well antibiotic susceptibility test were performed. Out of the 500 samples, uropathogens were identified in 211 samples (172 Gram negative and 39 Gram positive) by standard microbiological and biochemical tests. The predominant isolates were *Escherichia coli* (35.07%) followed by *Klebsiella spp.* (19.43%), *Enterococci spp.* (15.6%), *Pseudomonas spp.* (12.79%), *Citrobacter spp.* (6.16%), *Acenetobacter spp.* (4.2%), *Streptococcus spp.* (2.84%) and *Proteus spp.*(0.9%). The highest resistance rate was registered for gram negative bacteria towards Cefixime (85.46%) followed by Cefuroxime (79.62%), Co-Trimoxazole (68.72%), Ampicillin/Sulbactam (68.60%), Ciprofloxacin (61.53%), Levofloxacin (60.46%), Ofloxacin (58.72%), Meropenem (54.65%), Cefoperazone/ Sulbactum (51.16%), Amikacin (40.69%), Netillin (39.53%) and Nitrofurantoin (35.46%). The resistance rate for gram positive bacteria was highest for Cefuroxime (79.35%), Cefoxitin (79.35%), Penicillin-G (76.92%), Azithromycin (76.00%), Ciprofloxacin (71.79%), Clindamycin (76.00%), Amoxyclav (69.23%), Co-Trimoxazole (66.66%),Levofloxacin (30.76%),Vancomycin (23.07%),Linezolid (23.07%) and Teicoplanin (18.18%).

Keywords: UTIs, Multi-drug resistance, Uropathogens, Antibioticsusceptibility test.

Introduction

Today, misuse and overuse of antibiotics have directed global alarming situation throughout the world due to Antimicrobial Resistance (AMR). Healthy people, healthy animals and healthy environment are pivotal components of One Health Triad. Uropathogens are most common bacterial pathogens among human causing Urinary Tract Infections (UTIs). Urinary tract infections are the most common infectious disease (Abel *et al.*, 2019). It is estimated that more than 150 million UTIs in the world reported per year and it bears as economic and medical burden worldwide and about 35% of healthy individuals suffer from symptoms of UTI at some stages in their lives (Black *et al.*, 2004).

Increasing antibiotic resistance bacteria in urinary tract infections are serious health problem and greatest challenge in public health care and it referred as the evolution of microorganism such as bacteria, fungi,viruses and parasites that developed resistant nature to fight and neutralize an antimicrobialagent (Khawcharoenporn *et al.*, 2013; Tenney*et al.*, 2018; Mihankhah *et al.*, 2017). Each year, mortality rate is increasing due to antibiotic resistance in developing countries. The most influential factor of antibiotic resistance is the inappropriate use of antibio- tics (WHO, 2014). There are many potent antibiotics are available for the treatment of UTI, but due to increasing drug resistanceamong bacteria has made therapy of UTI difficult (Prakash *et al.*, 2013).

UTIs is commonly caused by gram negative pathogens such as *Escherichia coli, Klebsiella spp., Pseudomonas spp., Enterobacter spp., Acenetobacter spp., Proteus spp.* Among gram positive bacteria such as *Enterococci spp., Staphylococcus spp., Streptococcus spp.* are common bacteria which are responsible for causing UTIs. Gram negative bacteria found mostly in UTIs. Females are more affected than males and about 20% of women experience at least an episode of UTI during their life time and recurrence is very common (Foxman, 2010; Orrett, 2006). Therefore, proper diagnosis and use of antimicrobials for treatment and prevention of urinary tract infections are necessary to reduce the burden as well as long-term consequences (Kumar *et al.*, 2016).

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Materials and methods

Sampling

A total of 500 Urine samples were collected from the Surat Municipal Institute of Medical Education and Research (SMIMER), Surat,Gujarat, India, from both men and womenpatients in age between 12 to 75 years. Clean catch midstream urine specimens were collected in sterilized vials. The samples were clearly labelled and immediately stored at 4^{0}C for further analysis (Kumar *et al.*, 2016).

Isolation of Uropathogens

The urine samples from the UTI patients were streaked on MacConkey agar medium and blood agar medium in sterile condition (Osama *et al.*, 2021). Streaked plates wereincubated at 37°C for 24 h. Plates were observed for growth after overnight incubation. Plates which do not show any growth were considered for further 24h incubation. The morphological characteristicsof the microorganisms was observed for bacterial growth and recorded. Then, biochemical investigation was carried out for the obtained pure colonies.

Biochemical investigation

Characterization of bacterial isolates were further subjected to standard biochemical testing suchas gram staining, Idole productiontest, Citrate test, TSI (Triple sugar iron) test, Urease test, Methyl red, Voges-Proskauer reaction, catalase production, oxidase produc-tion and motility test (Cappuccino *et al.*, 1999; Holt *et al.*, 1994).

Antibiotic susceptibility test

After performing biochemical test, theidentification of pathogens was done, then the Antibiotic susceptibility test by the Kirby- Bauer's disc-diffusion method, using aMueller–Hinton (MH) agar medium were performed (Mishra *et al.*, 2017). Antibiotics used for Gram negative bacteria was Ampicilin/ Sulbactam (A/S, 10/10 mcg), Cefoperazone/ Sulbactum (CFS, 75/10mcg), Cefuroxime (CXM, 30 mcg), Cefixime (CFM, 5 mcg), Co-Trimoxazole (COT, 25 mcg), Ciprofloxacin (CIP, 5 mcg), Levofloxacin(LE, 5 mcg), Ofloxacin (OF, 5 mcg), Amikacin (AK, 30 mcg), Netillin(NET, 30 mcg), Nitrofurantoin (NIT, 300mcg), Meropenem (MRP, 10 mcg). Antibiotics used for Gram positive bacteriawas Cefuroxime (CXM, 30 mcg), Cefoxitin (CX, 30 mcg), Penicillin-G (P, 10 mcg),Amoxyclav (AMC, 30mcg), Ciprofloxacin (CIP, 5 mcg), Levofloxacin (LE, 5 mcg), Co-Trimoxazole (COT, 25 mcg), Vancomycin (VA, 30 mcg), Teicoplanin (TEI, 30 mcg),Linezolid (LZ, 30 mcg),Clindamycin (CD, 2 mcg), Azithromycin (AZM, 15 mcg).

Result

Prevalence of Uropathogens

The incidence of isolated uropathogens in urine sample was recorded sex-wise and age wise (Table 1). Among 500 samples, 211 samples showed growth, in which 42% male patients and 58% female patients was recorded. Out of 500 urine samples 211 (42.2%) were showing positive growth for UTI. Among them most predominant organism was Escherichia coli 74 (35.07%) followedbyKlebsiella41 (19.43%), Enterococci 33 (15.6%), Pseudomonas 27 (12.79%), Citrobacter 13 (6.16%), Aceneto-bacter 9 (4.2%), Enterobacter 6 (2.8%), Streptococcus 6 (2.84%) and Proteus 2 (0.9%) as shown in Figure 1.

Table 1. Demographic data of UTI patients.

Characteristics		Number of Cases	Percentage
Sex	Male	89	42%
	Female	122	58%
Age group	<50	162	77%
	50+	49	23%
Bacterial Growth	Growth	211	42.2%
	Non-Growth	289	57.8%
Gram staining	Gram negative	172	81.51%
	Gram positive	39	18.48%

Antimicrobial resistance rate of Gram negative uropathogens

Gram-negative isolates showed a high resistance rate towards Cefixime (85.46%) followed by Cefuroxime (79.62%), Co- Trimoxazole (68.72%), Ampicillin/ Sulbac- tam (68.60%), Ciprofloxacin (61.53%), Levofloxacin (60.46%), Ofloxacin (58.72%), Meropenem (54.65%), Cefoperazone/ Sulbac-tum (51.16%), Amikacin (40.69%), Netillin (39.53%) and Nitrofurantoin (35.46%). Escherichia coli, which accounted for 35.07% of gram-negative isolates showed highest resistance to cefixime and showed lowest resistance to Nitrofurantoin. The resistance rate of gram negative uropathogens had been shown in table 2 and 3.

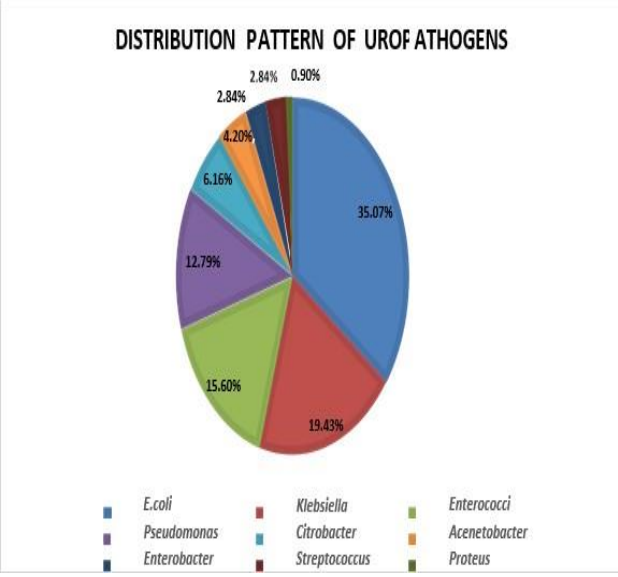


Figure 1. Distribution Pattern of Uropathogens

Table 2. Antimicrobial resistance rate of isolated Gram negative uropathogens

Antibiotics	*E.coli* (n=88)	*Klebsiella* (n=41)	*Pseudomonas* (n=27)	*Citrobacter* (n=13)
Ampicillin/ Sulbactam	56 (63.63%)	18 (43.90%)	12 (44.44%)	7 (53.84%)
Cefoperazone/ Sulbactam	33 (37.5%)	14 (34.14%)	17 (62.96%)	5 (38.46%)
Cefuroxime	65 (73.86%)	17 (41.46%)	21 (77.77%)	6 (46.15%)
Cefixime	70 (79.54%)	24 (58.83%)	18 (66.66%)	6 (46.15%)
Co-Trimoxazole	58 (65.90%)	18 (43.90%)	19 (70.37%)	5 (38.56%)
Ciprofloxacin	58 (65.90%)	17 (41.46%)	14 (51.85%)	5 (38.56%)
Levofloxacin	45 (51.13%)	12 (29.26%)	16 (59.25%)	3 (23.07%)
Ofloxacin	52 (59.09%)	15 (36.58%)	13 (48.14%)	4 (30.76%)
Amikacin	23 (26.13%)	14 (34.14%)	14 (51.85%)	4 (30.76%)
Netillin	19 (21.59%)	13 (31.70%)	16 (59.25%)	2 (15.38%)
Nitrofurantoin	18 (20.45%)	13 (31.70%)	14 (51.85%)	4 (30.76%)
Meropenem	46 (52.27%)	9 (21.95%)	15 (55.55%)	4 (30.76%)

(n=Number of total isolated pathogens)

Table 3. Antimicrobial resistance rate of isolated Gram negative uropathogens

Antibiotics	*Acenetobacter* (n=9)	*Enterobacter* (n=6)	*Proteus* (n=2)
Ampicillin/ Sulbactam	6 (66.66%)	2 (33.33%)	0
Cefoperazone/ Sulbactam	2 (22.22%)	1 (16.66%)	1 (50%)
Cefuroxime	4 (44.44%)	4 (66.66%)	0
Cefixime	4 (44.44%)	4 (66.66%)	2 (100%)
Co-Trimoxazole	6 (66.66%)	1 (16.66%)	0
Ciprofloxacin	5 (55.55%)	3 (50.00%)	2 (100%)
Levofloxacin	3 (33.33%)	2 (33.33%)	0
Ofloxacin	3 (33.33%)	2 (33.33%)	0
Amikacin	3 (33.33%)	1 (16.66%)	0
Netillin	4 (44.44%)	2 (33.33%)	2 (100%)
Nitrofurantoin	4 (44.44%)	2 (33.33%)	1 (50%)
Meropenem	5 (55.55%)	0	0

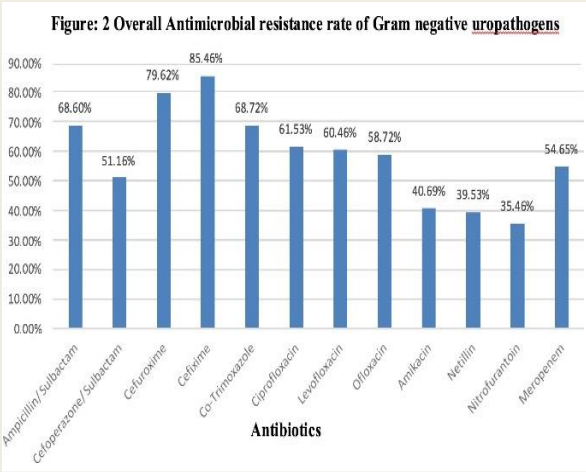
(n=Number of total isolated pathogens)

Antimicrobial resistance rate of Gram positive uropathogens

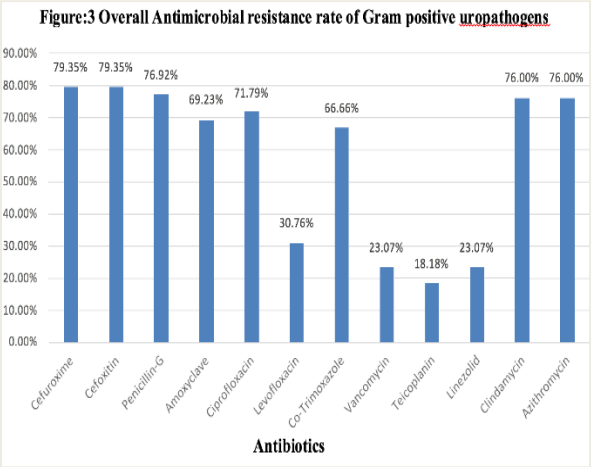
Gram-positive isolates showed a high resistance rate towards Cefuroxime (79.35%), Cefoxitin (79.35%), Penicillin-G (76.92%), Azithromycin (76.00%), Ciprofloxacin (71.79%), Clindamycin (76.00%), Amoxyclav (69.23%), Co-Trimoxazole (66.66%), Levofloxacin (30.76%), Vancomycin (23.07%), Linezolid (23.07%) and Teicoplanin (18.18%). The resistance rate of gram positive uropathogens had been shown in table 4. Figure 2 and Figure 3 shows overall resistance rate of gram negative and gram positive uropathogens against antibiotics.

Table 4. Antimicrobial resistance rate of isolated Gram positive uropathogens

Antibiotics	*Enterococci* (n=33)	*Streptococcus* (n=6)
Cefuroxime	30 (90.90%)	4 (66.66%)
Cefoxitin	29 (87.87%)	5 (83.33%)
Penicillin-G	28 (84.84%)	3 (50.00%)
Amoxyclav	28 (84.84%)	4 (66.66%)
Ciprofloxacin	25 (75.75%)	3 (50.00%)
Levofloxacin	12 (36.36%)	2 (33.33%)
Co-Trimoxazole	21 (63.63%)	3 (50.00%)
Vancomycin	09 (27.27%)	1 (16.66%)
Teicoplanin	07 (21.21%)	2 (33.33%)
Linezolid	08 (24.24%)	3 (50.00%)
Clindamycin	23 (69.69%)	4 (66.66%)
Azithromycin	26 (78.78%)	4 (66.66%)



(n=Number of total isolated pathogens)



Discussion

Urinary tract infection is generally detected inwomen of different age groups. The ratio of antimicrobial resistant rate is increasing day byday, so the selection of antibiotics should be based on the resistance pattern of pathogen in thelocality. Therefore, there is a need for constantobservation of the resistance and susceptibilitypattern of uropathogens.

In this study, commonly females are sufferingfrom UTIs more than males (Table 1), which similar with a study which is carried out by Haque et al., 2015. Women get more UTIs than males due to some reasons like shorter urethra, more sensitive skin, placement of urethra, sexual contact, specific type of contraception, menopause and pregnancy (Okonko et al., 2009) (Alyegoro, 2007)(Mishra et al., 2017). In India also researchersfound more cases of UTIs in females than in males which also correlate with this study (Orenstein et al., 1999).

In this study, a total of 211 (42.2%) uropathogens were isolated from 500 urinesamples. Among 211 bacterial isolates, 172isolates were gram negative and 39 isolates were gram positive. Escherichia coli was found out to be the predominant isolates 35.07% causing UTI, followed by Klebsiella 19.43%, Enterococci 15.6%, Pseudomonas 12.79%, Citrobacter 6.16%, Acenetobacter 4.2%, Enterobacter 2.84%, Streptococcus 2.84% and Proteus 0.9% (Table 2). The studies on uropathogens in different placesalso showed that Escherichia coli and Klebsiella spp. are the commonest uropathogens in UTI (Noormandi et al., 2015).

In cephalosporin group of antibiotics, cefuroxime showed highest resistance to Enterococci 90.90%, Pseudomonas 77.77%, Escherichia coli 73.86%; Klebsiella 41.46%; Acenetobacter 44.44%, Enterobacter 66.66%, Streptococcus 66.66%, Citrobacter 46.15%. In this study cefuroxime showed highest resistance to gram positive bacteria. Cefixime showed resistance to E.coli 79.54%, Pseudomonas 66.66%, Enterobacter 66.66%, Klebsiella 58.83%, Citrobacter 46.15%, Acenetobacter 44.44%. In this study we observed that gram negative bacteria showed highly resistant to cefixime. Cefoxitin showed resistance to Enterococci 87.87%, Streptococcus 83.33%. The high resistance rate against cephalosporin group ofantibiotics was also observed through the studydone in India (Orenstein, 1999).

Ciprofloxacin was considered as an antibiotic of choice for UTI but due to lack of use, this antibiotic lost its efficacy. So, actual use of fluoroquinolones should be restricted. About fluoroquinolone group, this study showed the resistance to Ciprofloxacin as in Proteus 100%, Enterococci 75.75%, E.coli 65.90%. Resistance to levofloxacin was 59.25% in Pseudomonas. Resistance to ofloxacin was 59.09% in E.coli. Nitrofurantoin showed resistance to Pseudomonas 51.85%, Acenetobacter 44.44%, Enterobacter 33.33%, Klebsiella 31.70%, Citrobacter 30.76%, E.coli 20.45%, Klebsiella 31.70%. In this study nitrofurantoin showed lowest resistance in gramnegative bacteria.

About aminoglycosides, Gram negative bacteria showed low resistance in this study which was similar with a study done in Bangladesh (Haque, et al., 2015). Co- Trimoxazole showed highest resistance to Pseudomonas 70.37%, Acenetobacter 66.66%, Enterococci 63.63%, E.coli 65.90%, Strepto-coccus 50.00%, Klebsiella 43.90%, Citrobacter 38.56%, Enterobacter 16.66%. Meropenem showed highest resistance to Pseudomonas 55.55%, Acenetobacter 55.55%, E.coli 52.27%, Citrobacter 30.76%, Klebsiella 21.95%. Netilllin showed highest resistance to Pseudomonas 59.25%, Acenetobacter 44.44%, Enterobacter 33.33%, Klebsiella 31.70%, E.coli 21.59%, Citrobacter 15.38%. The Resistance rates for Enterococci bacteria are increasing towards cefuroxime, for such resistantspecies, Vancomycin, Teicoplanin and Line- zolid is the effective choice of antibiotic. Enterococcus and streptococcus both were resistant to Penicillin-G, Amoxyclav, Co-Trimoxazole, Cephalosporins, Arythromycin in a different rate in this study (Table 4). UTI caused by Antimicrobial drug resistance is a burning issue in national and global perspective.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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