Original Research Article

Antimicrobial Resistance Pattern of Gram Negative Bacteria Associated with Urinary Tract Infection at a Teaching Hospital in the Malwa Region of Punjab

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Abstract
Urinary tract infection (UTI) is one of the most common bacterial infections encountered by clinicians in developing countries. The aim of this study was to determine the type and antibiotic resistance pattern of the urinary pathogens isolated from patients attending a busy tertiary care teaching institute of Malwa region of Punjab. A total number of 10938 clean catch mid stream urine samples were collected andprocessed according to CLSI guidelines in year 2015. As drug resistance among bacterial pathogens is an ongoing evolving process so, knowledge of uropathogens and their antimicrobial susceptibility pattern is the need of the hour. Therefore, development of regional surveillance programs is necessary for implementation of national UTI guidelines.

Key Words:- Antimicrobial resistance, Multi Drug Resistant, Urinary Tract Infection

Introduction

Urinary tract infections (UTI) are among the most common bacterial infections affecting humans throughout their lifetime. They are one of the most common infectious diseases encountered by clinicians in developing countries. They are the frequent cause of morbidity in outpatients as well as most frequently involved in the cause of nosocomial infection in many hospitals accou-nting for as many as 35% of nosocomial infections.[1, 2,6]

In contrast to men, women are at three time's greater risk for UTI, this is mainly dueto several clinical factors including anatomic differences and hormonal affectsi.eshort, straight anatomy of the urethra, absence of prostatic secretion, pregnancy. Immuno-suppression, prolonged hospital stay, poor hygiene and poor infection control strategiesare few of the main predisposing factors causing UTI. [3,7]

Esch.coli is the major etiologic agent in causing UTI, which accounts for 75% to 95% of cases. *Pr.mirabilis, Klebsiella* species, *Ps. aeruginosa* and *Enterobacte*r species are less frequent offenders. Less commonly, *Enterococci, G. vaginalis* and *U.urealyticum* are also known agents in UTIs. Gram positiveorganisms are even less common in which Group *B Streptococcus,* S.*aureus, S. saprophyticus* and *S. haemolyticus* are recognized organisms. [4]

The introduction of antimicrobial therapy has led to profound improvements in the management of urinary tract infections; however antimicrobial resistance pattern of gram negative bacteria have been constantly changing due to the continuous development of new resistance mechanisms like the production of extended spectrum beta lactamases (ESBLs) or carbape-nemases by bacteria and the spread is because of

jumping genesi.e Transposons. Over the past several decades, resistance to many of the commonly prescribed antibioticsi.e ampicillin, co-trimoxazole, nitrofurantoin, and fluoro-quinolones - has emerged in the treatment of UTI.[4] Themain reason forthe increased antibiotic resistance in urinary tract infections that include inappropriate and empirical usage of wide spectrum of antibiotics.[7]

Objectives

Therefore, the aim of this study was to determine the type and antibiotic resistance pattern of the urinary pathogens isolated from patients attending a tertiary care teaching hospital of Malwa region of Punjab.

Materials and Methods

In present study a total of 10938 clean catch midstream urine samples were collected in a sterile container from both outpatient and inpatient fromJanuary 2015-December 2015. Urine samples were transported immediately for processing. Uncentrifuged urine samples were 1st examined under microscope for presence of pus cells, RBCs, epithelial cells and bacteria. Then the urine samples were inoculated on MacConkey's and Blood agar plates by using calibrated loop delivering 0.001 ml of sample and incubated at 37 ^{0}C aerobically for 24 hrs. For gram negative bacilli more than 10^{5} colonies per ml of single organism were considered significant. The organisms were identified by colony characters, gram's staining and biochemical reactions.

Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents (Hi – Media Mumbai India) by Kirby Bauer disk diffusion method on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute (CLSI) guidelines.[7] The antimicrobial tested for gram negative bacilli were ampicillin (10µg), amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg) ofloxacin (5µg), norfloxacin (10µg), ceftazidime (30µg),cefotaxime (30µg), ceftriaxone (30µg), cefepime (30µg), piperacillin-tazobactum (100/10µg), nitrofurantoin (300µg), cotrimoxazole (25µg), imipenam (10µg), meropenem (10µg), aztreonam (30µg). Resistance data were interpreted according to Clinical Laboratory Standards Institute.

ESBL production was tested by double disk approximation test and combined disk method:

Double disk approximation test: Double disk approximation test was performed by using amoxy-clav (20/10µg) + ceftazidime (30µg). The disks were placed 15 mm apart.

Combined disk method:Combined disk method was performed using cefatazidime (30µg) and ceftazidime + clavulanic acid (30/10µg). The disks were placed 20 mm apart.

All suspected isolates (from screening step) of *K. pneumoniae* were tested for the production of carbapenemase by Modified Hodge Test as described in CLSI guidelines and results were interpreted accordingly.

Modified Hodge Test (MHT):The MHT was performed as follows: First of all bacterial suspension of the carbapenem susceptible strain of *Esch.coli* ATCC 25922 was prepared in 5ml sterile saline and turbidity was adjusted to 0.5 McFarland. This suspension was then diluted to 1:10 using sterile saline.A lawn of the *Esch.coli* ATCC25922 was streaked on a Mueller Hinton agar plate and wasallowed to dry 3–5 minutes. A 10 µg meropenem or ertapenem susceptibility disk was placed in the centre of the test area. In a straight line, test organism was streaked from the

edge of the disk to the edge of the plate. The plate was Incubated overnight at 37 ^{o}C for 24 hours.

Interpretation: After 16–24 hours of incubation, the plates were examined for a clover leaf-type indentation at the intersection of the test organism and the *Esch. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk.

MHT Positive: Test had a clover leaf-like indentation of the *Esch.coli*25922 growing along the test organism growth streak within the disk diffusion zone.

***MHT Negative*:** Test had no growth of the *Esch.coli* 25922 along the test organism growth streak within the disc diffusion.

Quality control:The reference strainsused as control were *Esch. coli* ATCC 25922 and *Ps. aeruginosa* ATTC 27853.[17]

Results

Out of 10938 clinically suspected cases of UTI, culture for gram negative bacilli was positive in 1577 (14.3%) samples. Out of 1577 culture positive cases 1070 samples were from indoor patients while 507 samples were from outpatient department. Out of 1577 maximum patients were in the age group of 31-40 yrs 58.5 % (n≥922) followed by 21-30 yrs 25.5% (n≥402).Out of 10938 urine sample, culture for gram negative bacilli was positive in 14.3% (1577) samples. *Esch.coli* was the most common isolate 63.6% (n≥1003) followed by*K. pneumoniae* 19.2% (n≥302), *Pseudomonas aeruginosa* 8.7% (n≥138), *Proteus spp.* 7.0% (n≥110), *Citrobacter spp.*1.2 % (n≥19) and *Acinetobacter spp*. 0.4%(n≥5).

Gram negative isolates showed higher resistance towards, penicillins (92.3%), co-trimoxazole(81%), ciprofloxacin (48%), norfloxacin (47.2%) and cephalospoirins (41%). On the other hand very low levels of resistance were detected to antibiotics such as piperacillin –tazobactum (19.7%), amikacin (18.7%), nitrofurantoin(18%) and to carbapenems (0.3%).(Table 1)

412 (26%) isolates were resistant to penicillins, 1st generation and 2nd generation cephalosporins which were further tested by double disk and combined disk method for ESBL production. Out of 412 resistant gram negative bacilli, 302 isolates were ESBL-positive which included 216 isolates of *E. coli* (71.5%) and 86 isolates of *K. pneumoniae* (28.5%).(Figure 1)

Out of 302 ESBLs, 41% (n≥124) were from ICU, 31% (n≥93) were from Surgery Department, 14% (n≥43) patients were from Gynae Department 14% (n≥42) patients were from Medicine Department.

Carbapenem, piperacillin-tazobactum and amikacin were the antibiotics with the highest sensitivity against ESBL isolates. In *Eschcoli* the susceptibility to carbapenem, piperacillin-tazobactum and amikacin was 100%, 84%, and 68% respectively. The susceptibility among Klebsiella *spp.*was 94.2 % for carbapenem, 64% for piperacillin-tazobactum and 48% amikacin.ESBL producing isolates showed high level of resistance to gentamicin, ciprofloxacin and amoxicillin-clavulanicacid. (Table 2)

Out of 86 *K. pneumoniae,* 5 (4.5%) strains were carbapenem resistant. Out of these carbapenem resistant *K.pneumoniae* isolates, 2 (40%) were positive for Klebsiellapneumoniae-carbapenamase (KPC) by Modified Hodge Test. (Figure 2)Both the patients were females and

between the age group of 51-60 years and were admitted in ICU. In addition to meropenem, KPC producing isolates were found to be 100% resistant to penicillins ceftriaxone, cefotaxime, ceftazidime, piperacillin-tazobactem, ciprofloxacillin, and aztreonam. However, they were found to be sensitive to polymyxin -B andcolistin.

Figure 1: Phenotypic confirmation test of an ESBL producing strain showing zone size of more than 5 mm in the disk with ceftazidime and clavulanic acid (CAC) as compared to Ceftazidime (CA).

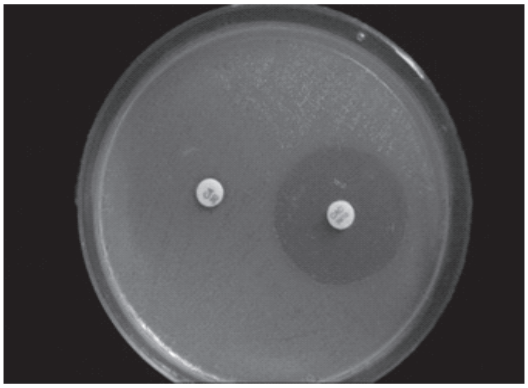

Figure: 2 : The Modified Hodge Test strain 1 and 3 had a clover leaf-like indentation positive result

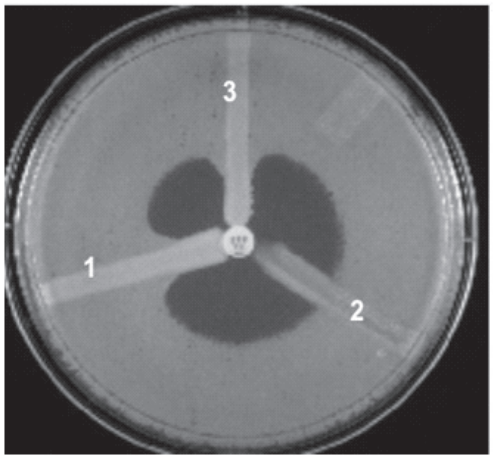

Table 1 : The Antimicrobial Resistance Pattern (%Age) Of 1577 Gram Negative Bacilli To Commonly Prescribed Antibiotics Against Urinary Tract Infection.

Name of antimicrobial agent	Esch coli N=1003	Klebsiellapneumoniae N=302	Proteus spp. N=100	Ps. aeruginosa N=138	Citrobacter spp N=19	Acinetobacter spp. N=5	Total Antimicrobial Sensitivity N=1577	Total Antimicrobial Resistance
Ampicillin	99 (9.8%)	15 (4.9%)	6 (6%)	NIL	3 (16%)	NIL	123 (7.7%)	92.3%
Co Trimoxazole	190 (19%)	46(16%)	20(20%)	40(29%)	3(16%)	Nil	299 (19%)	81%
Amoxy - clav	202 (20.1%)	50 (16.5%)	20 (20%)	40(29%)	5(26.3%)	Nil	317(20.1%)	79.9%
Ciprofloxacin	560 (56%)	145(48%)	55(55%)	50(31.2%)	11(58%)	Nil	821(52%)	48%
Ofloxacin	560 (56%)	145(48%)	55(55%)	50(31.2%)	11(58%)	Nil	821(52%)	48%
Levofloxacin	566 (56.4%)	145(48%)	55(55%)	50(31.2%)	11(58%)	Nil	827(52.4%)	47.6%
Norfloxacin	570 (56.8%)	148(49%)	55(55%)	50(31.2%)	11(58%)	Nil	834(52.8%)	47.2%
Cefatzidime	610(61%)	165(54.6%)	82(82%)	54(39%)	11	Nil	922(58.4%)	41.6%
Cefuroxime	614 (61.3%)	168(55.6%)	82(82%)	55(39%)	11	Nil	930(59%)	41%
Cefotaxime	620 (62%)	170(56.2%)	94(94%)	52(38 %)	10	Nil	946(60%)	40%
Ceftrioxane	817(81.4%)	243(80.4%)	91(91%)	119(86.2%)	14	Nil	1022(64.8%)	33.2%
Cefepime	ND	ND	ND	89 (100%)	ND	ND	%	
Gentamicin	781(79%)	236(78%)	81(81%)	100(72.5%)	12(63%)	Nil	1210(77%)	23%
Piperacillin+ Tazobactem	803(80%)	242 (80.4%)	90 (90%)	120 (86.2%)	12 (62%)	NIL	1267 (80.3%)	19.7%
Amikacin	812 (81%)	242 (80.1%)	90 (90%)	120 (87%)	19 (100%)	Nil	1283 (81.3%)	18.7%
Nitrofurantoin	813(81%)	252 (83.4%)	90 (90%)	120 (86.2%)	12 (62%)	NIL	1287 (82%)	18%
Imipenem	1003(100%)	297 (98.3%)	100 (100%)	138 (100%)	19 (100%)	5(100%)	1572 (99.7%)	0.3%
Meropenem	1003(100%)	297 (98.3%)	100 (100%)	138 (100%)	19 (100%)	5(100%)	1572 (99.7%)	0.3%
Aztreonam	1003(100%)	297 (98.3%)	100 (100%)	138 (100%)	19 (100%)	5(100%)	1572 (99.7%)	0.3%
Colistin	ND	5 (100%)	ND	ND	ND	ND	-	-
Polymyxin B	ND	5 (100%)	ND	138 (100%)	ND	ND	-	-

Table 2: The Antimicrobial Resistance Pattern (%Age) Of302 ESBLs Isolated From Urine Samples

Name of the Antibiotic	*Esch.coli* n=216		*Klebsiellapneumoniae*n=86	
	Sensitive	Resistant	Sensitive	Resistant
Amoxy –clav	15(7%)	201(93%)	16(9%)	70 (81%)
Gentamicin	25 (11.5%)	191(88.5%)	14 (16%)	72(84%)
Ciprofloxacin	27(12.5%)	189 (87.5%)	12 (14%)	74 (86%)
Amikacin	147 (68%)	69 (32%)	41 (48%)	45 (52%)
Piperacillin - Tazobactum	182 (84%)	34 (16%)	55(64%)	31(36%)
Imipenem	216 (100%)	0	81 (94.2%)	5 (5.8%)
Meropenem	216 (100%)	0	81 (94.2%)	5 (5.8%)
Aztreonam	216 (100%)	0	81(94.2%)	5(5.8%)

Discussion

Increasing drug resistant among uropathogens is a serious threat to public health and is a matter of great concern. The indiscriminate, inadequate usage of antibiotics has contributed to the emergence of resistance strains.Our study shows that females (65.8%) are more vulnerable to UTIs than males (34.2%), which is similar to previous studies done by Manjunath et al, V. Gupta et al and V. Niranjan et al[6,11,18]. Females are more prone to UTIs probably due to their short urethra and physiological changes.

Escherichia coli is the major aetiological agent in causing UTI, which accounts for up to 90% of cases [4, 6, 11]. In present study, *Escherichia coli* (63.6%) was the most predominant bacteria isolated from urine samples in both outpatients and inpatients of both sexes,followed by *Klebsiellapneumoniae* (19.2%), *Pseudomonas aeruginosa* (8.7%), *Proteus spp.* (7.0%), *Citrobacter spp*. (1.2%), and *Acinetobacter spp.* (0.3%). Results of this study are in concordance with the studies done by other authors. [6, 8, 11, 16, 18]

Resistance to antimicrobial agents has been noted since the first use of these agents and is an increasing world-wide problem. This study revealed that gram negative isolates showeda higher prevalence rate of resistance to the commonly prescribed antibiotic agents like penicillin (92.3%), co-trimoxazole (81%), ciprofloxacin (48%), norfloxacin (47.2%) and cephalospoirins (41%). Therefore these antibiotics cannot be used as empirical therapy for urinary tract infection. On the other hand very low levels of resistance were detected to antibiotics such as piperacillin -tazobactum (19.7%), amikacin (18.7%), nitrofurantoin (18%) and to carbapenem (0.3%). The comparable rate of antimicrobial resistance has been reported for these drugs in previous studies done in other parts of India and overseas[6, 11, 16,18]. Low resistance was observed for these drugs because they are relatively expensive in price compared to others. Thus, these drugs could be considered as alternative options in the empirical treatment of UTIs.

We observed that ESBL production among *Esch. coli* and*K. pneumoniae* isolates, and was more common among the hospitalized patients. Most of the ESBLs they were isolated from indoor patients admitted in different wards. Out of 302 ESBL isolates there were 216 (71.5%) *Esch.coli* and 86 (28.5%) *K. pneumoniae.*A similar study was conducted by GolamerzaIrajian et al (2010), in which *Esch coli* 75% was the most common isolate followed *K. pneumoniae* 25% , while Baby Padmini and Appalaraju (2004) reported *Esch coli* (41%) and *K. pneumoniae* (40%) as ESBL producer[13,14].

In present study, the most effective antibiotic against ESBL producers was found to be carbapenem, piperacillin-tazobactum and amikacin. ESBL producing *Esch. coli* showed good susceptibility to carbapenems 216 (100%), followed by piperacillin-tazobactum 182 (84%) and amikacin147 (68%). Similarly, the ESBL producing *K. pneumoniae* showed very good susceptibility to carbapenems (94.2%), followed by piperacillin-tazobactum (64%) and amikacin (48%). High level of resistance was seen among ESBL producer against antimicrobial agents like gentamicin, ciprofloxacin and amoxicillin-clavulanic acid etc. Our results are comparable with the study done byUma Devi et al (2011) and

Versha Gupta et al (2012)who showed good susceptibility of ESBL producers toamikacin 68%- 93.7% , piperacillin- tazobactam 63%-81% and 100% to carbapenems[12,21].

Recently, carbapenemase producing K. *pneumoniae* (CPKP) has rapidly emerged as one of the major nosocomial pathogens and we have limited therapeutic options for highly resistant, carbapenemase-producing organisms. In present study out of 86 *K.pneumoniae* isolates which were MDR, 5 were carbapenem resistant. Out of these 5 *K.pneumoniae* isolates 2 (40%) were positive for Klebsiellapneumoni-aecar-bapenamase (KPC) by Modified Hodge Test and they showed good sensitivity to these reserve drugs i.epolymyxin –B andcolistin. Current studyis comparable with the study done in Saudi Arabia (2016) who reported KPC production by Modified Hodge Test in(48.4%) isolates and all these carbapenem resistant isolates were sensitive to colistin and tigecycline[22].

Conclusion

An uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infectionsworldwide. The current study elaborates different antimicrobial resistance pattern among uropathogenes. High degree of resistance among gram negative bacilli was found commonly used drugs like co-trimoxazole, fluroquiolones and cephaolsporins. However these organisms showed good response to antibiotics like amikacin, nitrofurantoin, piperacillin -tazobactem and carbapenems. ESBL and carbapenem resistant *K. pneumoniae* (CRKP) producing organisms pose a major problem in treatment so misuse of carbapenems should be avoided. Thus it can be concluded from the present study that the drug resistance among pathogens is an ongoing evolving process, therefore routine surveillance, rationalize use of antibiotics and clinical trials should be done regularly with the assistance of treating physicians and to reach the most effective empirical treatment.

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Conflict of Interest None

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