Multidrug Resistant Escherichia coli and Klebsiella pneumoniae Causing Urinary tract Infection in Pregnant Women

Naveen Qadeer*, Muhammad Asif Durrani*, Syed Manzoor Iqbal**

*Basic Medical Sciences Institute, Jinnah Postgraduate Medical College Karachi,
**Department of Pathology, Poonch Medical College, Rawlakot, Azad Kashmir

Abstract

Objective: Identify the multidrug resistant *Escherichia coli* and *Klebsiella pneumoniae* occurring in pregnant women with urinary tract infection.

Materials and Methods: A total of 200 clean catch midstream urine specimens (MSU) were collected from pregnant women in a sterile container. Specimens were then inoculated on Blood agar, MacConkey agar and cysteine lactose electrolyte deficient (CLED) agar medium. Uropathogens were identified by using battery of tests. Antimicrobial susceptibility was determined by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guideline.

Results: *E. coli* constituted 39.70% of bacterial population followed by *K. pneumonia* 20.58%. Antimicrobial tests, of isolated strains showed 92.68% sensitivity to imipenem, 87.8% to piperacillin/ tazobactam while 100% resistance to ampicillin.

Conclusion: *E. coli* remained the most common cause of UTI followed by *K. pneumonia*. Imepenem showed high sensitivity to UTI as compared to piperacillin/ tazobactam in our set up.

Keywords: MDR, *E. coli*, *K. pneumoniae*, UTI, pregnancy.

Introduction

Urinary tract infection is a common problem in pregnancy and the enteric bacteria particularly E. coli remains the most frequent cause of UTI. The widespread inappropriate use of antibiotics is a well recognized causal factor for the spread of bacterial infection which is resistant to antimicrobial agents.

A common clinical problem, which may involve urethra, bladder and kidney is Urinary tract infection (UTI). UTI affects all age groups and both genders, but women are more prone than men due to short urethra, absence of prostatic secretions, pregnancy and easy contamination of urinary tract with faecal flora. [8] UTI is a major health problem that has been reported amongst 20% of pregnant women and is the most common cause of admission in obstetrical wards.[9] In general, gravid females are considered immunocompromised UTI hosts because of the physiologic changes associated with pregnancy. These changes place the pregnant women at increased risk of serious infectious complications from symptomatic and asymptomatic urinary infection

Correspondence: Dr. Naveen Qadeer, Basic Medical Sciences Institute, Jinnah Postgraduate Medical College Karachi.
khanzadajunaid@hotmail.com

in a healthy pregnant woman.[1] Major laborious work load in clinical microbiology laboratories pertains to UTI, and enteric bacteria particularly *E. coli* which has remained the most frequent cause of UTI, although the distribution of pathogens that cause UTI changes from time to time and place. [5,16]

Antimicrobial drug resistance is on the increase day by day, rising at an alarming rate with very few latest treatment options for Gram negative bacteria in non-pregnant and pregnant patients. [12] Monitoring studies regarding time and places are aimed to gain knowledge about the type of urinary pathogens which are responsible for UTIs and their resistance patterns that may help the clinicians to choose the right empirical treatment. [7] It is well known that the mechanism of antimicrobial resistance occurs as a result of enzymatic inactivation, modifications in receptors or by alteration in antibiotic transport mechanism. [13] The most common cause of bacterial resistance to β-lactam antibiotics is the production of beta lactamases. Many of the second and third generation cephalosporins were specifically designed to resist the hydrolytic action of major β-lactamases. [11] Drug-resistant bacterial infections are associated with higher rates of mortalities and morbidities, which have a preposterous effect on costs of health care. [15]

Materials and Methods

This study was carried out in the Department of Microbiology, Basic Medical Sciences Institute with the collaboration of Gynecology & Obstetrics Departments of Jinnah Postgraduate Medical Centre, Karachi, during the period of February 2011 to November 2011.

After at least four hours stay of urine in bladder, a sample of 30 to 50 ml mid-stream urine (MSU) was collected in a sterile wide mouth covered glass container and processed within one hour of collection for different parameters of screening. [3]

A total of 200 clean catch midstream urine specimens (MSU) voided were collected by patients as instructed. A Performa was also filled which was specifically designed for this study.

Urine microscopy and culture: A loop full of well mixed centrifuged urine was examined by wet preparation procedure to detect pyuria while any red cells, casts, parasite, and fungi were also noted when present. Urine culture was done with 0.001ml of well mixed urine delivered by a sterile calibrated wire loop and plated on CLED, MacConkey and blood agar plates, which were incubated aerobically at 35-37°C for 24 hours. Reculturing was done for contaminated specimens. Each significant isolate was identified by colonial morphology, Gram staining and biochemical reactions according to the standard procedure. Antibiotic sensitivity testing was done by emulsifying single colonies of isolates in normal saline at a turbidity compared to 0.5 Mac Farland's standard. Using sterile swab, suspensions were inoculated on Muller-Hinton agar in accordance with modified Kirby-Bauer method and incubated at 35-37°C for 18-24 hours. [4]

Antibiotic discs used were:
Ampicillin (10μg), Gentamicin (10 μg), Amoxiclav (20/10μg), Ceftazidime (30 μg), Ceftriaxone (30 μg), Cefotaxime (30 μg), Aztreonam (30 μg), Imipenem (10 μg), Piperacillin/ Tazobactam (100/10μg), Amikacin (30 μg)
Nitrofurantoin (300 μg), Nalidixic acid (3 μg) and Norfloxacin (10 μg)

Inclusion Criteria: All pregnant women with urinary complaints
Exclusion Criteria: Non pregnant subjects, pregnant women on antibiotics and contaminated samples.

Results

Table 1 shows the percentage of isolated Gram

negative and Gram positive organisms and yeast cells amongst culture positive UTI patients. (Table 2). E.coli found to be the commonest uropathogen amongst other culture +ve organisms (Table 2). The susceptibility pattern of E. coli and Klebsiella showed high sensitivity to imipenem as compared to others (Table 3).

Table 1: Percentage of Isolated Pathogens among Culture Positive UTI Patients (n=68)

Pathogens	Percentage (number)
Gram negative bacteria	72.06% (49)
Gram positive bacteria	22.06% (15)
Yeast cells	5.88% (4)

Table 2: Distribution of Gram-VE, Isolated From Culture Positive Cases of UTI

Organism	Number N=(49)	Percentage (out of 68)
E.coli	27	39.70%
Klebsiella pneumoniae	14	20.58%
Proteus	4	5.88%
Enterobacter	3	4.41%
Pseudomonas species	1	1.47%

Discussion

Over the last few years the infection caused by Multi drug resistant (MDR) uropathogens especially *E. coli* and *K. pneumoniae* have been associated with increased maternal and perinatal morbidity and mortality.

Antibiotic resistance in uropathogens is increasing worldwide. It varies according to geographical locations and is directly proportional to the use and misuse of antibiotics. Understanding the impact of drug resistance is of critical importance as the changing rate of antibiotic resistance has a large impact on empirical therapy of UTIs. Some bacteria, especially *E. coli* and *Klebsiella spp.* that are more frequent agents of UTI, show increasing resistance to cephalosporins. [10]

Distribution of uropathogen in our study was G-ve bacilli 72.05%, Gram positive (G+ve) cocci 22.05% and Candida spp 5.88%. The results were very close to the findings of Al-Haddad (2005) who also found G-ve bacilli 68.4%, G+ve cocci 24.4% and Candida albicans 7.3% respectively. In the present study the antimicrobial resistance pattern of *E. coli* (27) isolates revealed 100% resistance to ampicillin, 48.15% to ceftazidime,

Table 3 : Susceptibility pattern of *E. Coli* and *klebsiella*

Antibiotics	*E. coli* (27)		*Klebsiella* (14)	
	S	R	S	R
Ampicillin (10 µg)	0%	100%	0%	100%
Amoxiclav. (20/10 µg)	51.85%	48.15%	57.14%	42.86%
Ceftazidime (30 µg)	48.14%	51.86%	50%	50%
Ceftriaxone (30 µg)	37.04%	62.96%	35.72%	64.28%
Cefotaxime (30 µg)	22.23%	77.77%	35.72%	64.28%
Aztreonam (30 µg)	70.38%	29.62%	42.86%	57.14%
Imepenem (10 µg)	92.59%	7.41%	92.85%	7.15%
Piperacillin/ tazobactam (100/10µg)	88.88%	11.12%	85.71%	14.29%
Gentamicin (10 µg)	62.96%	37.04%	50%	50%
Amikacin (30 µg)	59.25%	40.75%	42.85%	57.15%
Nitrofurantoin (300 µg)	29.62%	70.38%	57.14%	42.86%
Nalidixic acid (3µg)	22.22%	77.78%	35.71%	64.29%
Norfloxacin (10 µg)	25.92%	74.08%	21.42%	78.58%

77.77% to cefotaxime and 48.15% to amoxiclav 37.04% to gentamicin and 40.75% resistance to Amikacin while it showed reasonable sensitivity to imipenem and piperacillin/tazobactam amikacin i.e. 92.59% and 88.88% respectively.

Klebsiella (14) showed 92.85%, 85.71% and 42.85% sensitivity to imipenem, piperacillin/tazobactam and amikacin respectively while resistance to ampicillin 100%, gentamicin 50%, ceftazidime 50%, cefotaxime 64.28% and amoxiclav 42.86% in this study.

Data of study indicates that E. coli was susceptible to 51.85% and 62.96% to amoxiclav and gentamycin and Klebsiella was susceptible to 57.14% to amoxiclav and 50% to gentamicin, which was in close approximation with the study by Oli et al (2010) which showed that E. coli was susceptible 57.14% to amoxiclav and 66.67% to gentamicin and Klebsiella were susceptible to 63.64% amoxiclav and 54.5 4% to that of gentamicin.

In the present study the overall sensitivity of norfloxacin, nitrofurantoin and nalidixic acid was 24.39%, 31.70% and 26.82% respectively which is relatively compareable with the study conducted by Aggarwal et al (2009) who found the sensitivity of norfloxacin, nitrofurantoin and nalidixic acid 25%, 30.8% and 25% respectively.

Overall pattern of resistance of ceftriaxone, cefotaxime, ceftazidime and amikacin was 78.7%, 65.9%, 51.1% and 44.7% respectively by Dalela et al (2012) while in the present study the overall resistance pattern of ceftriaxone, cefotaxime, ceftazidime and amikacin was calculated to be 58%, 64.58%, 62.5% and 33.33% respectively which shows the relevance of studying antimicrobial sensitivity pattern from time to time and place to place.

In our data of study overall percentage of imipenem and piperacillin/tazobactam resistance was calculated to be 7.32% and 12.2% respectively, which showed that imepenem is highly sensitive and piperacillin/tazobactam is relatively less sensitive in our studied population. This is in accordance to the study conducted by Teneja et al (2008) (add reference in the list)which shows the most effective antibiotics against ESBL producers were imipenem having 8.2% resistance and piperacillin/ tazobactam 9.5% resistance.

Increasing resistance to broad spectrum cephalosporins amongst E. coli and Klebsiella species predominantly due to the production of ESBLs that were reported from different countries. [13]

Conclusion

It is concluded that E. coli remains the most common cause of UTI followed by K. pneumonia. Imepenem is highly sensitive in UTIs followed by piperacillin/ tazobactam as compared to rest of the antimicrobials available.

It is emphasized that infections with resistant bacteria are emerging as an important challenge in health care facilities and antimicrobial resistance is associated with adverse outcomes, including increased mortality, hospital stay and costs. Third generation cephalosporin resistant organisms should be subjected to appropriate tests for detection of mechanism of resistance to avoid indiscriminate use of third generation cephalosporins. Administration of new antibiotics should be with caution and, this will reduce the rate of injudicial use of modern antibiotics which results in decreasing the rate of bacterial resistance.

References

1. Adabara NU, Momoh JA, Bala JD, Abdulrahaman AA and Abubakar MB. The prevalence of bacterial urinary tract infections (UTI) among women attending antenatal clinic in the general hospital, minna in Niger State. Intern J Biomed Res, 2012; 3(3):171-173.
2. Aggarwal R, Chaudhary U and Sikka R. Detectio n of extended spectrum β-lactamase production

among uropathogens. J Lab Physicians, 2009; 1(1):7-10.

3. Ahmed E, Durrani MA, Qamar S and Kharal SA. Detection of extended spectrum β-lactamase (ESBL) producing organisms in *Enterobacteriaceae* family like *Escherichia coli* (*E.coli*) and *Klebsiella pneumoniae* by double disc diffusion method (DDDM). Pak J Pharmacol, 2009; 26(1):25-30.

4. Ajayi AB, Nwabuisi C, Aboyeji AP, Ajayi NS, Fowotade A and Fakeye OO. Asymptomatic bacteriuria in antenatal patients in Ilorin, Nigeria. Oman Med J, 2012; 27(1):31-35.

5. Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B and Agegn A. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. BMC Research Notes, 2012; 5:197-211.

6. Al-Haddad AM. Urinary tract infection among pregnant women in Al-Mukalla district, Yemen. Eastern Mediterranean Health J, 2005; 11(3):505-510.

7. Dalela G, Gupta S, Jain DK and Mehta P. Antibiotic resistance pattern in uropathogens at a tertiary care hospital at Jhalawar with special reference to ESBL, AmpC β-Lactamase and MRSA production. J Clin Diag Res, 2012; pp.1-7.

8. Haider G, Zehra N, Munir AA and Haider A. Risk factors of urinary tract infection in pregnancy. JPMA, 2010; 60(3):213-216.

9. Hamdan HZ, Ziad AHM, Ali SK and Adam I. Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Ann Clin Microbiol Antimicrob, 2011; 10:1-5.

10. Irajian G and Moghadas AJ. Frequency of extended spectrum *β*-lactamase positive and multidrug resistance pattern in Gram-negative urinary iso-lates, Semnan, Iran. Jundishapur J Microbiol, 2010; 3(3):107-113.

11. Jalalpour S. Antibiogram pattern in extended spectrum *β*-lactamase nano enzyme producing gram negative bacilli in Iranian urinary tract infection. African J Pharmacy Pharmacolo, 2012; 6(12):899-903.

12. McGready R, Wuthiekanun V, Ashley EA, Tan SO, Pimanpanarak M, Viladpai-nguen SJ, Jesadapanpong W, Blacksell SD, Proux S, Day NP, Singhasivanon P, White NJ, Nosten F, and Peacock SJ. Diagnostic and Treatment Difficulties of Pyelonephritis in Pregnancy in Resource-Limited Settings. Am J Trop Med Hyg, 2010; 83(6):1322–1329.

13. Mekki AH, Hassan AN and Elsayed DEM. Extended spectrum β-lactamases among multi drug resistant *Escherichia coli* and *Klebsiella* species causing urinary tract infections in Khartoum. J Bacteriol Res, 2010; 2(3):18-21.

14. Oli AN, Okafor CI, Ibezim EC, Akujiobi CN and Onwunzo MC. The prevalence and bacteriology of asymptomatic bacteriuria among antenatal patients in Nnamadi Azikiwe University teaching hospital Nnewi; South Eastern Nigeria. Nigerian J Clin Prac, 2010; 13(4):409-412.

15. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X and Kluytmans J. Extended Spectrum β-Lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emer Infect Dis, 2011; 17(7):1216-1222.

16. Zaria LT, Raufu IA and Mohammed HS. Isolation and antibiotic sensitivity of Escherichia coli from pregnant and non-pregnant women attending the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria. Intern J Biomed Health Sci, 2010; 6(3):159-164.