Original Article

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Microbiology Section

Antibiotic Susceptibility Pattern of ESβL Producing *Klebsiella pneumoniae* Isolated from Urine Samples of Pregnant Women in Karnataka

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ABSTRACT

Background: *Klebsiella pneumoniae* possess a new problem to health care professionals worldwide, which complicates and limits therapeutic options. It is one of the leading nosocomial bacterial pathogens, and the present study aims to determine the prevalence of ESβL producing *K. pneumoniae* isolates with their antibiotic susceptibility pattern in urine samples of the pregnant women with UTI.

Materials and Methods: Using standard isolation and identification procedures a total of 41 isolates were obtained from 417 midstream urine samples of pregnant women with suspected UTI in Karnataka. The antibiotic resistance profile of each isolate was performed by Kirby-Bauer disc diffusion method and ESβL production by standard phenotypic method.

Results: Isolation rate of *K. pneumoniae* in pregnant women was 19.9% and overall incidence rate was 9.8%. Among the 41 *K. pneumoniae* isolates, 26 (63.4%) were ESβL producers and all were found to be Multi Drug Resistance (MDR). The antibiotic susceptibility test (AST) for the isolates revealed that the highest number of *K. pneumoniae* were resistant to ampicillin (75.6%) followed by, nitrofurontoin and cefuroxime (73.1%) and least to chloramphenicol (12.1%). ESβL producers were highly resistance to nitrofurontoin (69.2%) and cotrimonazole (65.2%) and lower resistance was (7.6%) to amaikacin, observed. A higher resistance pattern to these two antibiotics was observed against ESβL non producing *K. pneumonia* but lowest to polymyxin B (13.3%) instead of amikacin (26.6%). All the isolates were found to be susceptible to imipenem.

Conclusion: Present investigation revealed high prevalence of MDR- ESβL producing *Klebsiella pneumoniae*, which indicates dire need for effective ESβL surveillance in the community by using cost effective antimicrobials agents.

Keywords: ESβL, *Enterobacteriaceae K. pneumoniae* , Uropathogens, UTI. Pregnant women.

INTRODUCTION

Despite the widespread availability of antibiotics, UTI remains the most common bacterial infections in the human population. Incidences of UTIs are more in women than men and it was reported that upto 15% of women will have one episode of UTI at some time during their life [1]. The incidence of UTI reported among pregnant women is 8-10% [1]. If not treated asymptomatic bacteriuria increases the frequency of premature delivery and neonates with low birth weight [2] and also is likely to cause acute pyelonephritis at a rate of 20 to 30% [3].

During pregnancy UTI are more frequently caused by the indigenous microfllora especially from gastrointestinal tracts. Members belonging to the family *Enterobacteriaceae* are the most frequent pathogens (84.3%) detected in UTI cases and *E. coli* alone accounts for 80-90% [4]. *Klebsiella pneumoniae* is a successful opportunistic pathogen that has been associated with various ailments such as urinary tract infection, pneumonia, septicaemia, respiratory tract infection, wound infections and diarrhoea [5]. *K. pneumoniae* have become important pathogens in nosocomial infections [6], leading to more morbidity and mortality, which has been well documented in US and India [7]. In the United States, it accounts for 3-7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals [8]. Prevalence of *K. pneumoniae* has been reported to be 20.96% in Southern India [9].

Even though, *K. pneumoniae* accounts for only 10-15% of the total UTIs throughout the world, it occupies second place after *E. coli* among uropathogens. Infections caused by *Klebsiella* are treated with broad spectrum of antibiotics such as, cephalosporins, fluoroquinolones, aminoglycosides and carbapenems [10] and it has been found that resistance has developed to these antibiotics [11]. Resistance to beta-lactams has been reported to be associated with ESβL [12], which hydrolyze oxyimino beta-lactams like cefotaxime, ceftriaxone, ceftazidime and monobactams, but have no effect on cephamycins, carbapenems and related compounds [13]. ESβL producing *Klebsiella sps* in this part of the world has been observed by several workers; its prevalence reported to be more than 55% [11]. Studies have reported high prevalence of ESβL-producing members of family *Enterobacteriaceae* in India, where use of antimicrobials is relatively unrestricted. Indian studies have reported 26 to 48% of uropathogens belonging to *Enterobacteriaceae* were ESβL producers [14]. A recent report, from a hospital in rural Southern India, described a high prevalence of ESβL producers [15], while other report showed 96.1%[9].

Antimicrobial therapy is initiated in UTI even before the reports of urine culture are available. It is necessary to treat UTI in pregnancy as it is essential to maintain sterile urine without causing toxicity to the foetus, and there is no consensus on the choice of antimicrobials, duration of therapy or on prophylactic use of antimicrobials in pregnancy [16]. The aim of the study is to determine the incidence of *K. pneumoniae* in pregnant women with suspected UTI antibiotic profile of ESβL producers.

MATERIALS AND METHODS

Study was conducted on pregnant women suspected with signs and symptoms of UTI or symptomatic urinary tract infection

Antibiotics	Concentration (mcg/disc)	No: of isolates resistant to antibiotics	Percent resistance
Amikacin	30	6	14.6%
Ampicillin	10	31	75.6%
Aztreonam	30	27	65.8%
Azithromycin	15	15	36.5%
Cefepime	30	22	53.6%
Cefotaxime	30	26	63.4%
Cefoxitin	30	17	41.4%
Cefuroxime	30	30	73.1%
Chloramphenicol	30	5	12.1%
Ciprofloxacin	30	15	36.5%
Cotrimoxazole	25	28	68.2%
Gentamicin	10	15	36.5%
Imipenem	10	0	0%
Nitrofurontoin	30	30	73.1%
Piperacillin/ tazobactam	100/10	16	39.0%
Polymyxin B	300 units	9	21.9%
Tetracycline	30	19	46.3%

[Table/Fig-1]: Percent resistance of *K. pneumoniae* isolates against each antibiotic

Month of pregnancy	No: of *K. pneumoniae* isolates	Percent of *K. pneumoniae* isolated
3	0	0
4	2	4.8
5	3	7.31
6	9	22
7	8	19.5
8	13	31.7
9	6	14.6
Total	**41**	**99.9**

[Table/Fig-2]: Frequency of *K. pneumoniae* isolated in relation to different month of pregnancy

characterized by frequency, urgency, dysuria, or supra pubic pain in a woman with a normal genitourinary tract as in uncomplicated UTI and complicated UTI with functional or structural abnormalities of the genitourinary tract which involve either the bladder or kidneys [17,18].

Urine samples were collected from the pregnant women attending Government and Private hospitals/clinics and pathological laboratories in Gulbarga, Belgaum and Bangalore regions of Karnataka, Southern India during the period from December 2009 to August 2011.

Isolation and Identification of Uropathogens

Diagnosis of UTI was based on the microscopic findings of more than 10 pus cells/ high power field (40X) in centrifuged urine. Further, uropathogens were isolated from freshly voided midstream urine samples by inoculating with calibrated loop on nutrient agar plate, which were incubated aerobically at 37°C for 24h and extended to 48h in culture (growth) negative cases. Colonies were further identified and characterized based on morphological and biochemical tests [19]. As recommended by Kass [20] in distinguishing genuine infection from contamination, culture of a single bacterial species from urine sample at a concentration of $>10^5$ CFU/ml was included in the study. Isolated and characterized uropathogens were preserved on agar slants at 4°C and nutrient broth containing 25% glycerol at -20°C.

No: of antibiotics	No: of *K. pneumoniae* isolates (%)
4	0 (0)
5	3 (7.3)
6	0 (0)
7	2 (4.8)
8	3 (7.3)
9	3 (7.3)
10	8 (19.5)
11	8 (19.5)
12	5 (12.1)
13	3 (7.3)
14	1 (2.4)
15	1 (2.4)
Total MDR isolates	**37 (90.2%)**

[Table/Fig-3]: Multidrug resistance pattern of *K. pneumoniae* isolates

Antimicrobial Susceptibility

The 17 antibiotics of different classes (groups) with concentration in mcg/disc used in this study were taken as shown in [Table/Fig-1]. Antimicrobial susceptibility of each isolate was tested by following the CLSI guidelines on Mueller Hinton agar [21]. Antibiotic strength in discs used as recommended by CLSI. All chemicals required for culture media, reagents and antibiotic discs were procured from HiMedia laboratories Pvt Ltd., Mumbai.

Detection of ESβL producing *K pneumoniae*

ESβL producing *K. pneumoniae* isolates were determined using double disc synergy test recommended by CLSI guidelines [21]. The test organisms were grown in Luria Bertani broth and log phase was adjusted to 0.5 McFarland's standard, and inoculated on the Mueller Hinton agar plate with sterile cotton swab. Then discs of cefotaxime (30 mg) and ceftazidime (30 mg) separately and each of these in combination with clavulanic acid (10 mg) were placed 20mm apart on the surface of the agar plates preincoculated with test cultures and incubated at 37°C for 18h. Increase of inhibition zone diameter by ≥ 5mm around the antibiotic disc in combination with clavulanic acid compared to antibiotic alone was considered as ESβL producers. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as standards.

RESULTS

Overall 206 uropathogens were isolated from 417 samples collected from the UTI suspected pregnant women. Majority of isolates belonged to *Enterobacteriaceae* family, among them *E. coli* isolation rate was highest (56.79%), followed by *Klebsiella pneumoniae* (19.9%), Further the frequency of *K. pneumoniae* isolation rate increased with age of pregnancy. It was observed that the prevalence was highest at 8th month of pregnancy (31.7%), followed by 22% in the 6th month. Least prevalence was observed at 4.8% at 4th month of pregnancy [Table/Fig-2]. No clinical correlation has been done.

The susceptibility pattern of all the *Klebsiella pneumoniae* isolates in the study revealed that highest number of 75.6% isolates showed resistance to ampicillin, followed by cefuroxime and nitrofurontoin (73.1%) and lowest to chloramphenicol (12.1%). Low to moderate level of resistance was exhibited to the other antibiotics and all the isolates were (100%) susceptible to imipenem [Table/Fig-1].

Out of 41 *K. pneumoniae* isolates 90.2% were found to be multidrugresistant (MDR), defined as resistant to at least one agent in three or more antimicrobial classes. Majority of them were

ANTIBIOTICS	ESβL producers (n =26) (63.4 %)		Non ESβL producers (n= 15) (36.5%)	
	Sensitive	Resistant	Sensitive	Resistant
Gentamicin	14 (53.8%)	12 (46.1%)	12 (80%)	3 (20%)
Amaikacin	24 (92.3%)	2 (7.6%)	11 (73.3%)	4 (26.6%)
Ciprofloxacin	14 (53.8%)	12 (46.1%)	12 (80%)	3 (20%)
Polymyxin B	19 (73%)	7 (26.9%)	13 (86.6%)	2 (13.3%)
Tetracycline	12 (46.1%)	14 (53.8%)	10 (66.6%)	5 (33.3%)
Nitrofurontoin	8 (30.7%)	18 (69.2%)	3 (20%)	12 (80%)
Cotrimonazole	9 (34.6%)	17 (65.3%)	4 (26.6%)	11 (73%)
Azithromycin	16 (61.5%)	10 (38.4%)	10 (66.6%)	5 (33.3%)
Chloramphenicol	22 (84.6%)	4 (15.3%)	14 (93.3%)	1 (6.6%)

[Table/Fig-4]: Comparison of susceptibility to antibiotics between ESβL producers and ESβL non producer *K. pneumoniae* isolates

resistant to 8 or more antibiotics (78%). The maximum of 8 (19.5%) *K. pneumoniae* isolates were found to be resistant to 10 and 11 antibiotics [Table/Fig-3].

ESβL phenotypic detection test showed 9.8% of *K. pneumoniae* isolates were ESβL producers and maximum of them showed high resistance to nitrofurantoin (69.2%) followed by, cotrimoxazole (65.3%), tetracycline (53.8%), gentamicin and chloramphenicol (46.1%) as compared to amikacin (7.6 %) [Table/Fig-4].

DISCUSSION

In the present study, of the 417 urine samples from pregnant women suspected with UTI, 49.4% samples were culture positive while, incidence of *K. pneumoniae* accounted for 19.9% and the overall prevalence rate of *K. pneumoniae in pregnant women with UTI was found to be 9.8%.*

In our study *K. pneumoniae* has shown high antibiotic resistance which is in similarity with the study reported by Tonkic et al*.,* [22]. Carbapenems are the drugs of choice for many infections caused by Gram positive and Gram negative bacteria and were found to be the most effective antibiotics, and our study revealed 100% susceptible whereas, consistent rise was observed with other studies [23]. Maximum isolates of *K.pneumoniae* (75.6%) were resistance to ampicillin as comparable with other studies [24]. Cephalosporins, particularly second and third generation have been used to treat *Klebsiella pneumoniae* infections *[25].* Low resistance to second and third generation cephalosporins (63.4%) is seen in our studies compared to (84%) other reports [26]. Aminoglycosides have shown good activity against clinically important Gram negative bacilli [27]. In our study 14.6% and 36.5% of *K pneumoniae* isolates wrer resistant to amikacin and gentamicin resistance in contrast to other studies were 39.10% to amikacin and 16.70% to gentamicin in Klebsiella [28].

The resistance rate in *K. pneumoniae* isolates was 36.5% to ciprofloxacin which is lower than other studies conducted in India [27] and higher than reported in USA [28].

Multi-drug resistance (MDR) is a major problem in the management of uropathogens [29]. This MDR may be due to plasmids harboring several resistance genes which are transferred from one bacterium to another [29] and have linked such resistance pattern to the presence of integrons [30]. Multidrug Resistance (MDR) in *K. pneumoniae* is increasing throughout the world [31]. In our studies 90.2% isolates were MDR as reported by others with 100% multidrug resistance [31].

Prevalence of ESβL has been reported 40%, 58% and 58.7% from Iran Pakistan and India respectively [11,15,32]. In our study, 26(63%) *K. pneumoniae* isolates were ESβL producers and have higher antibiotic resistance to ampicillin and cephalosporins, which are important drugs for the treatment of UTIs. Our results showed ESβL producing *K. pneumoniae* susceptibile to nitrofurontoin (30.7%), amaikacin (92.3%) and imipenem (100%), whereas, other studies in India showed 100%, 89% and 86% respectively [11]. Another study revealed 48.1% susceptibility to amakacin, 81.4% to imipenem and 25.9% to ciprofloxacin [32], whereas, our studies revealed 53.8% susceptibility to ciprofloxacin. Such isolates are also resistant to flouroquinolones, tetracyclines and cotrimoxazole. Ampicillin, gentamycin, cephalosporin group and nitrofurontion are the choice of drugs during pregnancy.

Untreated UTI may lead to pre-term premature rupture of membrane, maternal chorioamnionitis, intrauterine growth retardation, and low birth weight baby. Early treatment with antibiotics reduces the above complications. Strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing this resistance.

REFERENCES

[1] Delzell JE, Lefevre ML. Urinary tract infections during pregnancy. *Am Fam Physician.* 2000; 61(3):713-21.

[2] Kahlmeter G, THE ECO-SENS project. A prospective multinational multicenter epidemiological survey of the prevalence and antimicrobial susceptibility of urinary tract pathogens. *J Antimicrob Chemother.* 2000;46:15-22.

[3] Raz R, Chazan B and Dan M. Cranberry juice and urinary tract infection. *Clin Infect Dis*. 2004;38:1413-19.

[4] Gales CA, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalised patients in Latin America; reports from the second year of the SENTRY Antimicrobial Surveillance Program (1998). *J Antimicrob. Chemothrep*. 2000;45(3):295-303.

[5] Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev*. 1998;11(4):589-603.

[6] Nordamann P, Cuzon G and Naas T. The real threat of *Klebsiella* pneumonia carbapenemase -producing bacteria. *Lancet Infec Dis*. 2009;9(4):228-36.

[7] Mathur NB, Khalib A, Sarkar R and Puri RK. Mortality in neonatal septicaaemia with involvement of mother in management. *Ind J Pediatri.* 1991; 28: 1259-64.

[8] Sarathbabu R., Ramani TV, Bhaskara rao K and Supriya Panda. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *IOSR J Pharma Biol Sci.* 2012;1(2):4-9.

[9] Gaddad SM, Muzaheed and Shivannavar CT. Incidence of ESBL producing *Klebsiella pneumoniae* among inpatients and outpatients from North eastern part of Karnataka. *Res Rev Bioscience.* 2008;2(2-6):160-63.

[10] Roussel-Delvallez M, Wallet F. Bactericidal activity of three betalactams alone or in combination with a beta-lactamase inhibitor and two aminoglycosides against *Klebsiella pneumoniae* harboring extended-spectrum beta-lactamases. *Clin Microbiol Infect.* 1998; 4(10): 570-76.

[11] Jain A, Mondal R. Prevalence & antimicrobial resistance pattern of extended spectrum beta-lactamase producing *Klebsiella* spp isolated from cases of neonatal septicaemia. *Indian J Med Res.* 2007;125(1):89-94.

[12] Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* sps collected from intensive care units in Southern and Western Europe in 1997-1998. *J Antimicrob Chemother.* 2000;45(2):183-87.

[13] Philippon A, Labia R. Extended-spectrum beta-lactamases. *Antimicrob Agents Chemother*. 1989;33(8):1131-36.

[14] Babypadmini S, Appalaraju B. Extended spectrum β lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* - Prevalence and susceptibility pattern in a tertiary care hospital. *Indian J Med Microbiol.* 2004;22(3):172-74.

[15] Navaneeth BV. Extended-spectrum and AmpC β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from a rural South Indian tertiary care hospital. *Int J Antimicrob Agents.* 2007; 29:602-03.

[16] Beckford- Ball J. Related Articles, Management of suspected Bacterial urinary tract infection. *Nurs Times.* 2006;102(32):25-26.

[17] Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. *Infect Dis Clin North Am.* 1997;11:551–82.

[18] Nicolle LE. A practical approach to the management of complicated urinary tract infection. *Drugs Aging.* 2001;18:243.

[19] Cheesbrough M, Medical Laboratory Manual for Tropical Countries. Vol II. Microbiology. *Cambridge, Great Britain;* 1989: 248-63.

[20] Giron E, Rioux C, Brun-Buisson C, Lobel B. Infection committee of the French Association of Urology. The Postoperative bacteriuria score; a new way to predict nosocomial infection after prostate surgery. *Infect Control Hosp Epidemol.* 2006; 27(8): 847-54.

[21] CLSI 2006. Clinical and Laboratory Standards Institute (CLSI): Performance Standard for Antimicrobial Susceptibility Testing. 16th Informational supplement. *CLSI document.* M100-S16.

[22] Tonkic M, Goic-Barisic I, Prevalence and antimicrobial resistance of extended-spectrum beta-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in a university hospital in Split, *Croatia. Int Microbiol*. 2005;8(2):119-24.

[23] Nicolau DP, Carbapenems: a potent class of antibiotics. *Expert Opin Pharmacother*. 2008; 9(1):23-37.
[24] Aktas E, Yigit N, Detection of antimicrobial resistance and extended-spectrum beta-lactamase production in *Klebsiella* pneumonia strains from infected neonates *J Int Med Res*. 2002; 30(4):445-48.
[25] Jett BD, Ritchie DJ. In vitro activities of various beta-lactam antimicrobial agents against clinical isolates of *Escherichia coli* and *Klebsiella* spp. resistant to oxyimino cephalosporins. *Antimicrob Agents Chemother*. 1995;39(5):1187-90.
[26] Singh NP, Goyal R. Changing trends in bacteriology of burns in the burns unit, Delhi, India. *Burns.* 2003;29(2):129-32.
[27] Revathi G, Puri J. Bacteriology of burns. *Burns*. 1998;24(4):347-49.
[28] Fedler KA, Biedenbach DJ. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. *Diagn Microbiol Infect Dis*. 2006;56(4):427-36.
[29] Akram M, Shahid M. Etiology and antibiotic resistance pattern of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Ann Clin Microbiol Antimicrob.* 2007; 6:4.
[30] Mathai E, Grape M. Integrons and multidrug resistance among *Escherichia coli* causing community-acquired urinary tract infection in southern India. *APMIS.* 2004;112(3):159-64.
[31] Tokatlidou D, Tsivitanidou M. Outbreak caused by a multidrugresistant *Klebsiella pneumoniae* clone carrying blaVIM-12 in a university hospital. *J Clin Microbiol.* 2008; 46(3):1005-08.
[32] Farhat Ullah, Salman Akbar Mallik, Jawas Ahmed. Antimicrobial susceptibility pattern in *Klebsiella pneumoniae* from urinary tract infections in North-West of Pakistan. *African Journal of Microbiology.* 2009;3(11):676-80.

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