



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prevalence of Multidrug Resistance in Uropathogenic *Klebsiella* Species With Reference to Extended Spectrum β -Lactamases Production

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ABSTRACT

Klebsiella species is the second most common uropathogen and important aspects of *Klebsiella* associated infection is the emergence of multi-drug resistance (MDR), mainly by means of extended spectrum β -lactamase (ESBL) production. To determine the prevalence of MDR and ESBL production among uropathogenic *Klebsiella* species. Isolation, identification and antimicrobial susceptibility of organism was done by standard Microbiological procedure. ESBLs production was detected by DDST as per CLSI guidelines. One thousands urine specimens were studied. Significant bacteriuria was present in 40% of specimen. Majority (90%) of urinary tract infections (UTI) were due to Gram-negative bacilli. *Klebsiella* species were isolated from 80 (20%) of the patients. *Klebsiella* species were highly resistant to Nalidixic Acid 80%, followed by 72.5% to Norfloxacin and Ciprofloxacin. Imipenem, Meropenem and Amikacin were the most suitable antibiotics having less resistance 6.25%, 6.25% and 32.5% respectively. MDR was seen in 68.75%, only 10% were sensitive to all the antibiotics tested. We found 60% *Klebsiella* species harbored the ESBLs. A high prevalence of MDR and ESBL production amongst *Klebsiella* species was observed, which is alarming in low-income settings. Our results suggest urgent need for regular screening and surveillance for these organisms.

Keywords: Urinary tract infections, *Klebsiella* species, Multi-drug resistance, Extended spectrum β -lactamase

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INTRODUCTION

A urinary tract infection (UTI) is a condition where one or more parts of the urinary system (the kidneys, ureters, bladder and urethra) become infected [1]. With over 150 million annual diagnoses of UTI worldwide, it remains one of the most common community-acquired as well as nosocomial infections [2]. The prevalence of UTI depends on age, sex, race and predisposing factors that impair the defence mechanism which maintain the sterility of normal urinary tract [3]. Predisposing factors in the development of UTI are anatomical, physiological, infective, social and environmental [4, 5]. *Escherichia coli* and *Klebsiella pneumoniae* have been reported as the most common organisms causing UTIs. Furthermore, *Klebsiella pneumoniae* is an important pathogen both in the community and the hospital setting to cause severe infections [6].

In present scenario, the essence of antimicrobial drug resistance among uropathogens has posed a global threat [7]. Multi drug resistant organisms (MDROs) are associated with certain risk factors that are institutionalization, previous use of any antibiotic, previous hospitalization, ICU stay, age, chronic underlying disease, urinary catheters and gut colonization [8]. MDROs represent one of the scourges of modern medicine. The more antibiotics that are used against them, the more resistant the pathogens seem to become, with few therapeutic options remaining for effective treatment regimens. Of the various resistance mechanisms that have been described in these organisms, production of β -lactamases is perhaps the well-studied [9].

During the last decade, extended spectrum β -lactamases (ESBLs) type have emerged in the community setting among *Klebsiella pneumoniae*, on plasmids that frequently bear additional resistance determinants [6]. The population of India of over one billion represents a potentially vast reservoir of antimicrobial resistance genes including those encoding ESBLs [10]. ESBLs are a group of enzymes capable to hydrolyze penicillin including the first, second, and third generation cephalosporins. The special characteristic of these enzymes is the ability to hydrolyze β -lactam structure in all classes of β -lactam antibiotics including penicillins, narrow spectrum and extended spectrum cephalosporins such as oxyimino cephalosporins (cefotaxime, ceftazidime), fourth generation cephalosporins (cefipime) and monobactams (aztreonam). These enzymes are sensitive to β -lactamase inhibitors (sulbactam, clavulanic acid and tazobactam) [11].

Detection of ESBLs is a challenge for microbiology laboratories because routine methods for monitoring a decrease in susceptibility to oxyimino-cephalosporins are not sensitive enough to detect all ESBL-producing strains, especially those that produce certain ESBLs like TEM-7, TEM-12 and SHV-2. The MICs of oxyimino-cephalosporins against these strains were raised only slightly. The simplest screen for ESBLs production is to test for synergy between ceftazidime and clavulanate. Ceftazidime is a good substrate for most ESBLs and is thus an appropriate indicator drug [12]. The knowledge of etiology and antimicrobial resistance pattern of the organism causing UTI is essential [13]. The purpose of this research is to find out the up to date

picture of MDR in uropathogenic *Klebsiella* species and to highlight the ESBLs production among them.

MATERIALS AND METHODS

This is prospective study conducted in Department of Microbiology, Yuvaraja's College (Autonomous), Mysore, India from December 2011 to November 2012. Urine specimens obtained from patients attending to K.R. hospital Mysore, clinically diagnosed as UTI and submitted to Microbiology, Department for bacteriological culture and sensitivity constitute the subject for study. Informed consent was taken from each subject included in the study. The study is ethically approved by Human Ethical Committee, University of Mysore.

Urine culture of the uncentrifuged urine was done by semi-quantitative method using standard wire loop. Sterilized inoculation loop was dipped in urine pot at 90°C, a loopful of urine holding 0.001 ml was taken. It was inoculated on cystein lactose electrolytes deficient agar (CLED) media, 5% sheep blood agar and Mac Conkey agar respectively. The inoculated plates were incubated aerobically in bacteriological incubator set at 37°C for 24-48 h [14, 15]. Identification was done on the basis of colony morphology, grams stain, catalase test, oxidase test and standard biochemical tests that include triple sugar iron agar (TSI) media, Simmon's citrate agar media, sulphide indole motility (SIM) media and Christensen's urease medium [16].

Antibiotic Sensitivity Test (AST)

It was done by Kirby-Bauer disk diffusion test method on Muller-Hinton agar (MHA) plate and interpreted according to Clinical Laboratory and Standards Institute (CLSI) guidelines, where the following antibiotics (from Hi media, Mumbai, India) were tested. amikacin (30µg), amoxicillin/clavulanic acid (20/10 30µg), cefixime (5µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cotrimoxazole (32.7µg), ciprofloxacin (5µg), nitrofurantoin (300µg), nalidixic acid (30µg), norfloxacin (10µg) and gentamicin (10µg), imipenem (10µg) and meropenem (10µg). *Escherichia coli* ATCC 25922 were used as control and tested along with the test strains daily as described for *Enterobacteriaceae* [17].

Detection of ESBLs Producing Strains by the Double Disc Synergy Test (DDST)

All uropathogens showing resistance to one or more third generation cephalosporins (3GCs) were tested for ESBL production by the double disc synergy test (DDST) using cefotaxime (30µg), cefotaxime/clavulanic acid (30/10µg), ceftazidime (30µg) and ceftazidime /clavulanic acid (30/10µg), a \geq 5mm increase in diameter of the inhibition zone of the cephalosporin/clavulanate disc when compared to the cephalosporin disc alone were interpreted as phenotypic evidence of ESBL production. *Klebsiella pneumoniae* ATCC 700603 was used as positive control and *E. coli* ATCC 25922 as negative control [18].

RESULTS

One thousand urine specimens were studied. Significant bacteriuria was present in 40% of specimen. Majority (90%) of UTI were due to Gram-negative bacilli and remaining (10%) Gram-positive cocci. *Klebsiella pneumoniae* were isolated from 60(15%) of the patients and *Klebsiella oxitoca* from 20(5%) of the patients (Fig.1). Based on distribution of age, sex and origin of patient having growth of *Klebsiella* species (Table 1), out of 80 *Klebsiella* species, 24 (30%) isolated from male patients and 54 (70%) from female patients were reported. *Klebsiella pneumoniae* were highly resistant to nalidixic acid 80%, followed by norfloxacin 75% and ciprofloxacin 75% (Fig. 2). Out of 60 *Klebsiella pneumoniae* isolates 42 (70%) were multiple drug resistant, only 6 (10%) were sensitive to all the antibiotics tested. *Klebsiella oxitoca* were highly resistant to nalidixic acid 80%, followed by 65% to norfloxacin, ciprofloxacin and cotrimoxazole (Fig. 3). Out of 20 *Klebsiella oxitoca* isolates 13 (65%) were multiple drug resistant, only 2 (10%) were sensitive to all the antibiotics tested

It was found that 66.66% *Klebsiella pneumoniae* and 40% of *Klebsiella oxitoca* harboured the ESBLs (Fig. 4). The sensitivity of *Klebsiella pneumoniae* was 93.33% to imipenem and meropenem; similarly the sensitivity of *Klebsiella oxitoca* was 95% to imipenem and meropenem.

Table: 1 Distribution of age, sex and origin of patients

Age in years		Sex				Origin of patient			
		Male		Female		OPD		IPD	
	No.	No.	%	No.	%	No.	%	No.	%
0-10	5	1	4.16	4	7.14	3	6.66	2	5.71
11-20	10	0	0	10	17.85	5	11.11	5	14.28
21-30	15	1	4.16	13	23.21	7	15.55	8	22.85
31-40	16	4	16.66	13	23.21	8	17.77	7	20.00
41-50	11	3	12.5	8	14.28	18	40.00	1	2.85
51-60	1	0	0	1	1.78	1	2.22	0	0
61-70	10	7	29.16	3	5.35	6	13.33	4	11.42
71-80	9	6	25.00	3	5.35	4	8.88	6	17.14
81-90	3	2	8.33	1	1.78	1	2.22	2	5.71
Total	80	24	30.00	56	70.00	45	56.25	35	43.75

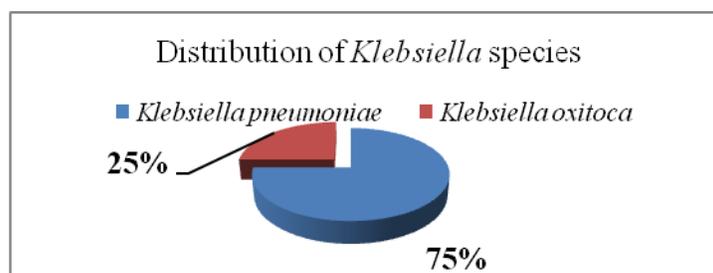


Fig. 1: The distribution of *Klebsiella* species

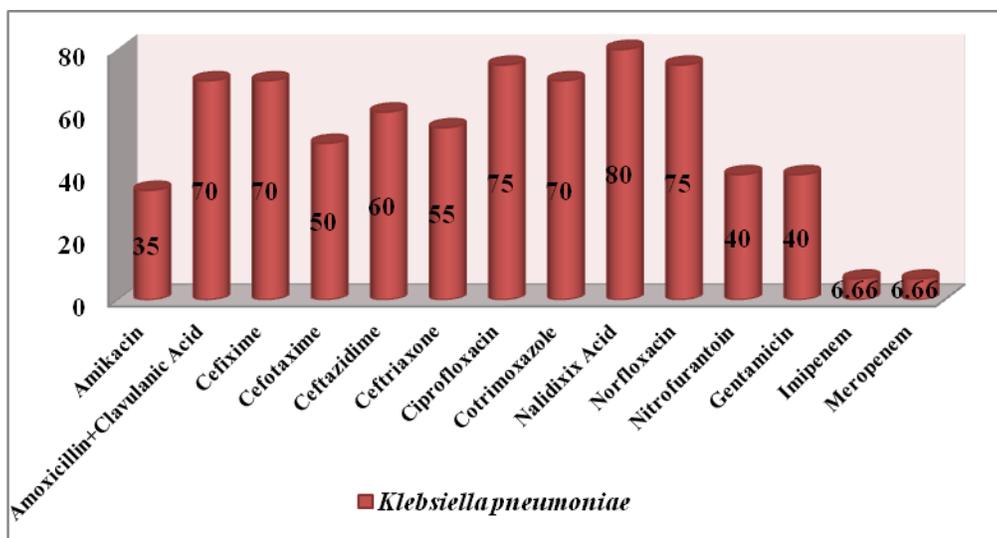


Fig. 2: Resistance pattern (%) of uropathogenic *Klebsiella pneumoniae*

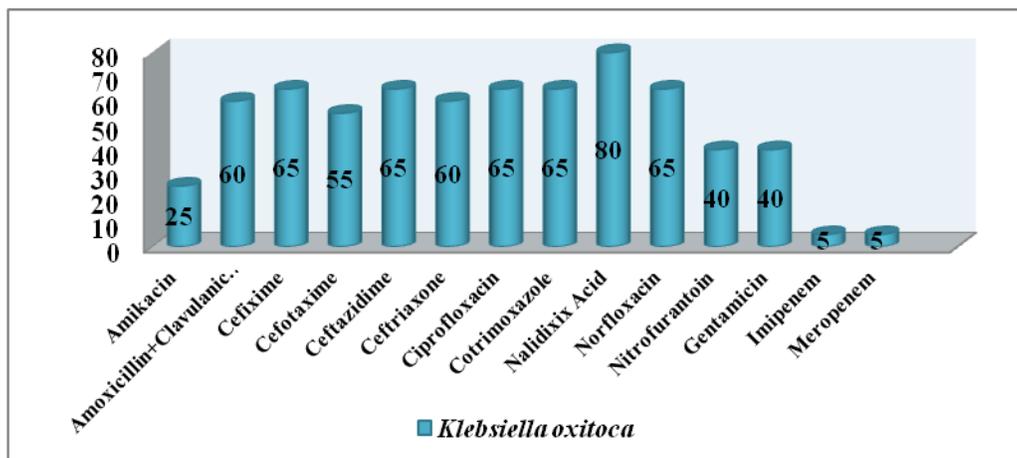


Fig. 3: The resistance pattern (%) of uropathogenic *Klebsiella oxitoca*

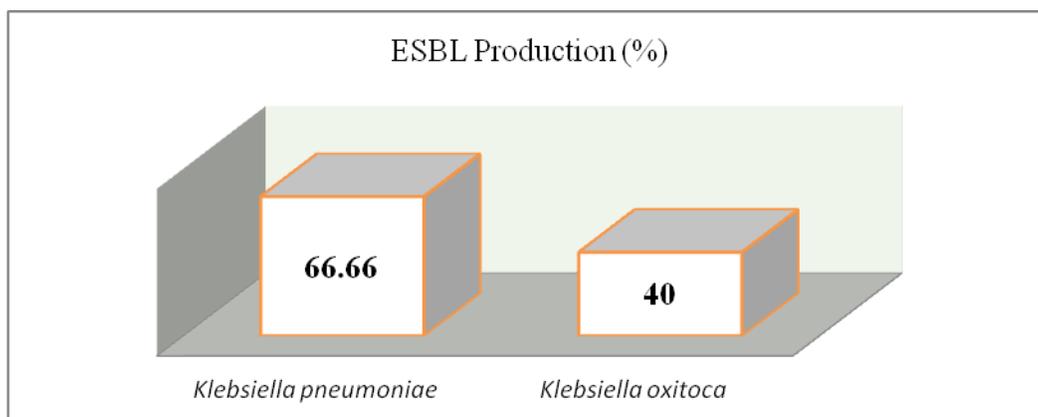


Fig. 4: Prevalence of ESBL production among *Klebsiella* species

DISCUSSION

Antimicrobial resistance is a global problem. It is now accepted as a major public health issue and has significant implication on health and patient care. Resistance to antimicrobial drugs is associated with high morbidity and mortality, high health-care cost and prolonged hospitalization. The problem of antimicrobial resistance is more troublesome to developing countries. World Health Organization (WHO) and the European Commission (EC) have recognized the importance of studying the emergence and determinants of resistance and the need for strategies for its control. Microorganisms and their resistance patterns vary from hospital to hospital and even from clinic to clinic in the same hospital [19].

In the present study, the significant bacteriuria was present in 40% of specimen, the similar finding (39.6%) were reported [20]. Majority (90%) of UTI were due to Gram-negative bacilli and remaining (10%) Gram-positive cocci. (GNB 95.4% and GPC 4.6%) [21]. *Klebsiella pneumoniae* were isolated from 60 (15%) of the patients and *Klebsiella oxitoca* from 20 (5%) of the patients, the similar finding were reported [22]. Out of 80 *Klebsiella* species, 24 (30%) isolated from male patients and 54 (70%) from female patients. In male patient UTI due *Klebsiella* species is seen mostly (54.16%) in age group 61-80 years. This might be due to prostate enlargement or some other reason, older male used to admit in hospital frequently. Likewise in female 46.42% *Klebsiella* species is seen in age group 21-40 years. This might be due to highly active in sexual activity during these ages, so faecal *Klebsiella* species get entry to the urinary tract.

Klebsiella pneumoniae were highly resistant to nalidixic acid 80%, even higher resistance (87.3%) [23]. The rate of resistance to norfloxacin 75% and ciprofloxacin 75%; from Bangalore a nearby result (73%) was reported [7]. The rate of resistance to ciprofloxacin is now concerning, as it is the most commonly prescribed antibiotics for UTIs. Furthermore, resistance to ciprofloxacin is particularly common among women with a history of prior UTI. Thus, resistance to fluoroquinolones may be significant issue only in high-risk groups, including patients with history of prior UTI and antibiotic use [24]. Resistance to amoxicillin/clavulanic acid was 70%, which is comparatively low [25].

In the current study, 70% of *Klebsiella pneumoniae* and 65% of *Klebsiella oxitoca* were resistance to cotrimoxazole, which is slightly high [26]. A lower resistance was seen to amikacin 25%, which is almost similar (24.4%). Lower resistance to amikacin is due to unavailability in the oral form like capsule or tablet in community, because it is injectable. The resistance to nitrofurantoin and gentamicin were 40% for both *Klebsiella pneumoniae* and *Klebsiella oxitoca*. Almost similar findings (41% to gentamicin and 39% to nitrofurantoin) were reported [27]. Out of 60 *Klebsiella pneumoniae* isolates 42 (70%) were Multiple drug resistant, only 6(10%) were sensitive to all the antibiotics tested. Out of 20 *Klebsiella oxitoca* isolates 13 (65%) were multiple drug resistant, only 2 (10%) were sensitive to all the antibiotics tested.

Overall prevalence of ESBLs in *Klebsiella* species was 60% (66.66% in *Klebsiella pneumoniae* and 40% in *Klebsiella oxitoca*), almost similar (60.27%) was reported [28] and

higher prevalence (70.73%) was reported from South India [29]. The prevalence of ESBL producers in this study was strikingly high, particularly given the fact that more than half (56.25%) of the isolates were obtained from outpatients. One of the reasons contributing to the high prevalence of ESBL may be the crowded hospital conditions precluding implementation of optimal hygiene practices. The epidemic in the community is then likely fuelled by unrestricted use of antimicrobials that may be purchased without prescription [30].

CONCLUSION

Significantly high prevalence of MDR to commonly used antibiotics among uropathogenic *Klebsiella* species was seen in present study. Furthermore, very high rate of MDR and ESBL production is of concern, which necessitate the re-evaluation of first and second line therapies for UTI and monitoring of the same is necessary to prevent treatment failure and increased morbidity and mortality with UTI.

ACKNOWLEDGMENT

Authors would like to acknowledge K.R. Hospital, Mysore for allowing us to collect the specimen for research work.

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