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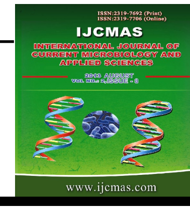

Original Research Article

Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from urine samples

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Keywords

Urine infection; Antimicrobial susceptibility; Resistant; Disk diffusion methods*;* antimicrobial resistance ;*Klebsiella* species.

A B S T R A C T

Urinary tract Infection (UTI) is among the most common infections described in outpatient setting and hospital patients. *Klebsiella* species isolated from urine samples from Gangasaras Diagnostic and Research Centre, Pattukkottai, Tamilnadu, were studied to determine the antimicrobial susceptibility pattern. Between January 2012 and December 2012, a total of 2400 specimens processed in the laboratory, of which 72 (11.2%) yielded *Klebsiella* species, from outpatients and inpatients. Organisms were identified by conventional methods. *Klebsilla* species were isolated mostly from female urine specimens. Antimicrobial susceptibility was done by the disk diffusion methods. The antimicrobial disk used include: amoxicillin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, cephalexin, ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin, amikacin, gentamicin, tobramycin, imipenem, nitrofurantoin, and cotrimoxazole. All were Himedia products. Antimicrobial susceptibility to various group drugs used was generally poor. The most sensitive antimicrobial was amikacin and imipenem with 62 (86.1%) isolates susceptible to it, followed by gentamicin and tobramycin with 58 (80.6%), ofloxacin with 57 (79.2%), ciprofloxacin with 55 (76.4%). 30 (41.7%) isolates were multiresistant to all the antimicrobial agents used. The result of this study will help in the empiric therapy of infection caused by *Klebsiella* species in Tamil Nadu State, but continuous surverillance of antimicrobial resistance of the organism is very necessary in the formulation of a sound antibiotic policy in the hospital.

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases (Kolawale *et al.*, 2009; Delanghe *et al.*, 2000; Hryniewicz *et al.*, 2000). They may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if not appropriately treated (Pezzlo, 1988). Although different causative agents can be responsible for UTIs, bacteria are the major cause being responsible for more than 95% of UTI cases (Bonadio *et al.*, 2001).

Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the Twentieth

Century. However, disease causing microbes that have become resistant to antibiotic drug therapy are increasing public health problem (Todar, 2011). There are three mechanisms that can cause antibiotic resistance: prevention of interaction of drug with target organisms, decreased uptake due to either an increased efflux or a decreased influx of the antimicrobial agent and enzymatic modification or destruction of the compound (Bonilla and Muniz, 2001). Antimicrobial resistance developed by microbes against antibiotics open serious debates in this issue and recognized as a serious problem by global medicinal and research community (Finch, 2004). Many factors play in the emergence of resistance (WHO, 2012) from poor utilization of antimicrobial agents, to the transmission of resistant bacteria from patient to patient and from healthcare workers to patients and vise versa, to a lack of guidelines for a appropriate and judicious use of antimicrobial agents, to lack of easy-to-use auditing tools for restriction (Mahmoud and Hanan, 2012). In addition, there is a clear misuse of antimicrobial in the animal industry, those are the same agents used in humans. All these factors together led to the inevitable rise and emergence of resistance.

Klebsiella pneumoniae is among the most common gram negative bacteria encountered by physicians worldwide (Lin *et al.,* 2010). *Klebsiella* spp. are often resistant to many antibiotics, including cephalosporins and aminoglycosides (Vinetz, 2007). These bacteria have become important pathogens in nosocomial infections (Nordamann *et al.,* 2009), which have been well documented in United States (Graybill *et al.,* 1973) and India (Mathur *et al.,* 1991). Epidemic and endemic nosocomial infections caused by *Klebsiella* species are leading causes of morbidity and mortality. *Klebsiella pneumoniae* can also cause a urinary tract infections in children and adults. In the United States, Klebsiella accounts for 3-7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals (Sarathbabu *et al.,* 2012). They are also important opportunistic pathogens clinical isolates of *K.pneumoniae* are generally resistant to a wider range of antibiotics, and virtually always naturally resistance to ampicillin and amoxycillin. β-lactam antimicrobial agents are most common treatment option for such infections (Savita *et al.,* 2012). The main scopes of the present studies are related to microbiological and biochemical methods are used to find *Klebsiella pneumoniae* in the clinical urine specimens and antimicrobial sensitivity profile.

Materials and Methods

Specimen collection

The surveillance was conducted for a period of 1 year from January 2012 to December 2012 among urinary tract infection patients in three hospitals in Pattukkottai. A total of 2400 urine samples were collected.

Bacteriology

In the laboratory, each sample was inoculated on McConkey agar, Nutrient agar, UTIChrom agar and Blood agar. The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop. The culture plates were incubated at 37°C for 24 hours and observed for growth through the formation of colonies. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Sharma (2008).

Antibiotic susceptibility test

Antibiotic susceptibility testing of *Klebsiella spp.*, was carried out by the disk diffusion technique using a commercially available disc (Himedia- CLSI, 2010). The antimicrobial sensitivity of the test strains to sixteen antibacterial drugs was done using the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). The antibiotics used were amoxicillin (AX, 30μg), ampicillin (AM, 10μg), cefotaxime (CTX, 30μg), ceftazidime (CAZ, 30μg), ceftriaxone (CT,30μg), cephalexin (CL,30μg), ciprofloxacin (CIP, 5μg), nalidixic acid (NA,30μg), norfloxacin (NX, 10μg), ofloxacin (OF, 5μg), amikacin (AK, 30μg), gentamicin (GEN, 10μg), tobramycin (TB, 10μg), imipenem (IPM, 10μg), nitrofurantoin (NIT, 300μg) and Cotrimoxazole (COT, 30μg). A lawn of test pathogen (1ml of an 18 hours peptone broth culture) was prepared by evenly spreading 100μl inoculums with the help of a sterilized spreader onto the entire surface of the agar plate. The plates were allowed to dry before applying antibiotic disc. Then, some commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

Result and Discussion

A total of 2400 urine samples yielded 650 (27.1%) strains of pathogens belonging to 12 species with four Gram-positive, seven Gram negative bacteria and *candida* spp. during the span of 12 months. The most common isolates in this study have been the Gram negative bacilli which accounts for 85.4% of the total positive isolates. *E. coli* (54.6%) was the maximally isolated UTI causing bacterium, followed by *K.pneumoniae* (11.2%), *P.aeruginosa* (10.5%), *Proteus* spp., (6.8%), *CONs* (4.1%), *Enterococcus* spp., (3.7%), *Staph saprophyticus* (2.3%), *Enterobacter* spp., (1.5%), *S. aureus* (1.1%), *Citrobacter* spp., (0.6%), and *Acinetobacter* spp., (0.3%). Figure 1 shows the detailed frequency of all the isolates identified. In the gram negative bacilli, the second most predominant isolate from UTI were 72 strains of *K.pneumoniae* (11.2%) included for this study.

Biochemical Tests

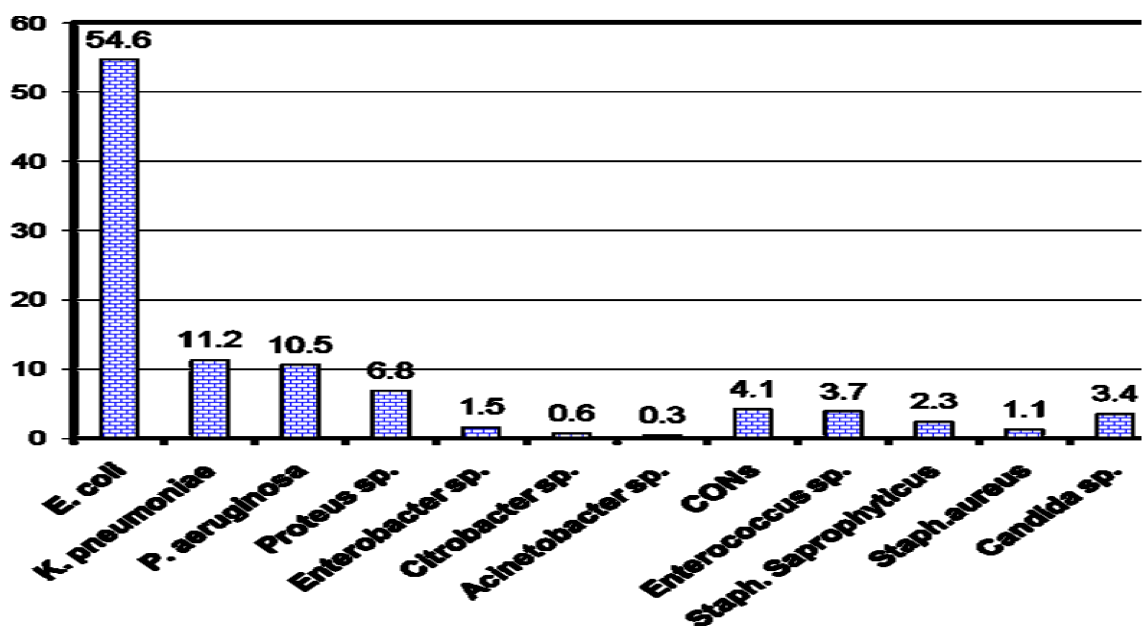
All the isolated *Klebsiella pneumoniae* were Gram negative straight rods, non motile, arranged singly or in pairs. Morphological examination revealed that their colonies were large, circular, convex, grayish white and mucoid on nutrient agar. While on MacConkey's agar, lactose fermenting (pink) colonies was detected. All strains were nonmotile and gave negative results for oxidase, indole, methyl red, gelatin liquefaction. On the TSI test, *K. pneumoniae* strains produced acids both in butt and slant along with gas production, while they showed positive

Table.1 Antibiotic sensitivity and resistant pattern of *Klebsiella* spp.

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	6 (8.3)	2 (2.8)	8 (11.1)	64 (88.9)
Ampicillin	10 (13.9)	2 (2.8)	12 (16.7)	60 (83.3)
Cefotaxime	43 (59.7)	5 (7.0)	48 (66.7)	24 (33.3)
Ceftazidime	35 (48.6)	4 (5.6)	39 (54.2)	33 (45.8)
Ceftriaxone	48 (66.7)	0	48 (66.7)	24 (33.3)
Cephalexin	19 (26.4)	3 (4.2)	22 (30.6)	50 (69.4)
Ciprofloxacin	55 (76.4)	0	55 (76.4)	17 (23.6)
Nalidixic acid	32 (49.4)	0	32 (49.4)	40 (50.6)
Norfloxacin	52 (72.2)	2 (2.8)	54 (75.0)	18 (25.0)
Ofloxacin	56 (77.8)	1 (1.4)	57 (79.2)	15 (20.8)
Amikacin	62 (86.1)	0	62 (86.1)	10 (13.9)
Gentamicin	58 (80.6)	0	58 (80.6)	14 (19.4)
Tobramycin	57 (79.2)	1 (1.4)	58 (80.6)	14 (19.4)
Imipenem	59 (81.9)	3 (4.2)	62 (86.1)	10 (13.9)
Nitrofurantoin	34 (47.2)	2 (2.8)	36 (50.0)	36 (50.0)
Cotrimoxazole	20 (27.8)	1 (1.4)	21 (29.2)	51 (70.8)

*Results are expressed as a percentage of 72 *Klebsiella pneumoniae* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial

Figure.1 Percentage of organisms involved in UTI



result for citrate utilization, Voges-Proskauer and urease tests (Figure. 2). Klebsiella grown on CHROMagar Candida, when *Klebsiella* form metallic blue colonies (Figure. 3).

This experiment was carried out to study the susceptibility of the bacterial isolates collected from urine specimens toward different 16 antibiotics. The percentages of susceptibility of *Klebsiella pneumoniae* isolates to the antibiotics which are commonly used to treat *Klebsiella* infections are shown in Table 1. The lowest percentage of susceptibility was manifested against amoxicillin (11.1%) followed by ampicillin (16.7%), Cotrimoxazole (29.2%), cephalexin (30.6%) and nalidixic acid (49.4%), whereas more susceptibility was observed with amikacin/imipenem (86.1%), followed by gentamicin/ tobramycin (80.6%), ofloxacin (79.2%), ciprofloxacin (76.4%), norfloxacin (75%), cefotaxime (66.7%), ceftriaxone (66.7%), ceftazidime (54.2%) and nitrofurantoin (50%) respectively.

Three aminoglycoside antibiotics, amikacin (13.9%), and gentamicin/ tobramycin (19.4%) were moderately resistant to species of pathogens used. Similarly, resistance patterns of the beta-lactam group, ampicillin, amoxicillin are detailed (Table 1). All these two antibiotics were almost equally resistant to the isolated UTI pathogens. Imipenem resistance patterns were 13.9% of K. *pneumoniae*. Further, resistance-percent values of UTI bacteria to cephalosporin antibiotics, Cefotaxime/ceftriaxone (33.3%), ceftazidime (45.8%) were moderately resistant and cephalexin (69.4%) was highly resistant to species of pathogens used. Similarly, resistance-percent values of UTI bacteria to antibiotics of the fluoroquinolone group, ofloxacin (20.8%), ciprofloxacin (23.6%), norfloxacin (25%) were low resistant and nalidixic acid (50.6%) were moderately resistant to species of pathogens used. These antibiotics were resistant to UTI pathogens in the order: nalidixic acid>norfloxacin>ofloxacin>ciprofloxacin. Among these four antibiotics, nalidixic acid was recorded to be more resistant to these pathogens. Lastly, detailed antibiograms of two stand-alone antibiotics, co-trimoxazole and nitrofurantoin were recorded. Nitrofurantoin was found resistance for 50% of 72 strains of *K. pneumoniae*. Surprisingly, resistance-percent values of UTI bacteria to antibiotic of the co-trimoxazole were 70.8%.

This study shows the distribution of microbial species and antibiotic susceptibility patterns of *Klebsiella pneumoniae* isolated from Pattukkottai area patients with UTIs. The majority of pathogens were isolated from women (69.8%). It has been extensively reported that adult women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors *(*Kumar *et al.*, 2006*).*

Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country (Kahan *et al.*, 2006; Sharma *et al.*, 2005). In Pattukkottai, there is an evidence for increase in antibiotic resistance. The highest percentage of resistance was noted against amoxicillin (88.9%) followed by ampicillin (83.3%), co-trimoxazole (70.8%), cephalexin (69.4%) and nalidixic acid (50.6%). This was previously reported in other hospital as well as in other institutions in the various country (Daoud *et al.,* 2006; Akujobi, 2005).

In most Indian studies *Klebsiella* spp. occupy second place among uropathogens. However in the present study also they were the second (11.2%) common uropathoges. Multiple drug resistance is common among members of *Klebsiella* spp. *Klebsiella* spp isolates of our study exhibited higher resistance to cephalosporins (ceftriaxone/cefotaxime, 33.3% and ceftazidime 45.8%) than other Indian studies (Khan and Zaman, 2006; Gupta *et al.,* 2007; Hasan *et al.,* 2007; Sonavane *et al.,* 2008; Bhargavi *et al,* 2010) as shown in Table 2. The other drugs for which *Klebsiella* spp. isolates showed higher resistance were amoxyclillin (88.9%), ampicillin (83.3%), cotrimoxazole (70.8%) nalidixic acid (50.6%), nitrofurantoin (50.0%) and norfloxacin (25.0%).

Among these nalidixic acid is not commonly prescribed and norfloxacin is active against gram negative rods. Amoxicillin resistance (88.9%) observed in the present study was lower than the reports of Sonavane *et al.,* (2008) (97.7%). The resistance pattern of *Klebsiella* spp. to norfloxacin, nitrofurantoin and nalidixic acid in other studies showed wide variation (Table 2). The resistance of *Klebsiella* spp. to cotrimaxazole (70.8%) was high, compared to most Indian studies (Khan and Zaman, 2006; Biswas *et al.,* 2006; Sonavane *et al.,* 2008; Hasan *et al.,* 2007). Only Arjunan *et al.,* (2010) from Tirunelvelli reported cotrimaxazole resistance, (14.3%) which was lower than what has been observed in our study. Cotrimaxazole is a broad spectrum antibiotic widely used in UTI treatment. Our study revealed high resistance to aminoglycosides, which is lower than most Indian studies (Table 2). Among antibiotics, ciprofloaxacin resistance was moderate (23.6%), amikacin (13.9%) and gentamycin (19.4%) were lower resistance to *Klebsiella* spp. isolates. As a whole *Klebsiella* spp. in our study area appeared to be still susceptible to ofloxacin (79.2%), ciprofloxacin (76.4%), norfloxacin (75%) and nitrofurantoin (50%), which are the common antibiotics prescribed for UTI.

The most of the isolates had a high level of resistance to examine antibiotics. Laboratory evidence of infection and antibiotic susceptibility testing should be carried out to help in the choice of systemic drugs. Continuous monitoring of antimicrobial susceptibility pattern in individual settings together with their judicious use is emphasized to minimize emergence of drug resistant bacteria. Thus, it is highly recommended that practicing physicians should become aware of the magnitude of existing problem of antimicrobial resistance and help in fighting this deadly threat by rational prescribing. We therefore recommended the following antibiotics for the treatment of Klebsiella infection in the order of the strength of their sensitivity; amikacin, imipenem, gentamicin, tobramycin, ofloxacin, ciprofloxacin, norfloxacin, cefotaxime, ceftriaxone, ceftazidime and nitrofurantoin.

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