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Research Article

Characterization and Antibiogram of *Klebsiella* spp. Isolated from Clinical Specimen in a Rural Teaching Hospital

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Abstract: *Klebsiella* spp. is ubiquitous in nature. They inhabit the environment and intestinal tract of humans and animals. *Klebsiella* spp causes variety of healthcare associated infections including pneumonia, urinary tract infections, blood stream infections, surgical wound infections, peritonitis, septicaemia and meningitis. *Klebsiella* are highly drug resistant bacilli causing significant morbidity and mortality. Foregoing study aims at isolation of *Klebsiella* spp. from different clinical specimens and their antibiogram, which would enable formulation of appropriate antimicrobial policy for patients suffering from *Klebsiella* infection. A total of 1957 various clinical samples at Microbiology diagnostic laboratory of KVG Medical College & Hospital, Sullia were processed. A total of 100 strains of *Klebsiella* spp. isolated were identified by standard procedures. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and interpreted as per CLSI guidelines. A total of 100 *Klebsiella* spp were isolated from 1957 clinical samples including sputum, urine, blood, wound swabs and various aspirates. 79% were *Klebsiella pneumoniae* and 21% were *Klebsiella oxytoca*. The highest percentage of *Klebsiella spp* (40%) were isolated from pus sample. The isolates were 100% sensitive to imipenem and showed sensitivity to fourth generation cephalosporin cefepime (77%), amikacin (74%) and gentamicin (70%). Isolates showed high resistance to ampicillin (89%), followed by aztreonam (83%) and piperacillin/ tazobactam (67%). Among the third generation cephalosporins tested *Klebsiella* isolates showed high resistance to cefotaxime (65%). Though majority of *Klebsiella* infections are caused by *K. pneumoniae*, a sizeable infection are caused by *K. oxytoca*. However, both species are totally sensitive to imipenem, variable degree of resistance was exhibited to other antibiotics. Males and above 60 years of age are more affected and infections are more commonly associated with diabetes mellitus, hypertension, alcoholism, steroid therapy and chronic smoking.
Keywords: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, Antibiotic resistance

INTRODUCTION

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in community and hospital settings [1]. Gram negative bacilli are arising as the major cause of nosocomial infections. *Klebsiella species* is a Gram negative opportunistic nosocomial pathogen and well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics, urinary tract infection, wound infections, blood infections and infections in the intensive care unit [2,3].

The vast majority of *Klebsiella* infections are associated with hospitalization. As an opportunistic pathogen, *Klebsiella* spp. primarily attack immunocompromised individuals, who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction, chronic cardiac, renal and neoplastic disease [2, 4, 5]. Nosocomial *Klebsiella* infection are mainly caused by the *Klebsiella pneumoniae* followed by *Klebsiella oxytoca*. *K. pneumoniae* has been identified as an important common pathogen for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15% of all cases), wound infections (2 to 4% of all cases) and neonatal septicaemia (3 to 30% of all cases) [6].

Klebsiella spp. are ubiquitous in nature. In addition to being the normal flora of the intestine are also found in respiratory tract of humans and animals [2]. These bacteria have been recovered from aquatic environments receiving industrial waste waters [7], plant products and fresh vegetables [8], Cockroaches were suggested to play the role of vectors in the hospital environment [9].

Klebsiella spp have several virulence factors such as capsular polysaccharides, lipopolysaccharide (LPS) and iron-scavenging systems (siderophores). Major risk factor for colonisation or infection with ESBL producing organisms are long term antibiotic exposure, prolonged intensive care unit stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporins use and instrumentation or catheterisation [10].

Nosocomial isolates are frequently resistant to numerous antibiotics as a result of the acquisition of multidrug resistance (MDR) plasmids. *K. pneumoniae* is one of the most common organisms to carry plasmid encoding extended-spectrum β-lactamases (ESBLs), and such strains are isolated with increasing frequency [11]. *K. oxytoca* is also resistant to multiple antibiotics due to the chromosomal beta-lactamase overexpression that leads to a characteristic antibiogram with high resistance to all the penicillins except temocillin, resistance to cefuroxime, cefotaxime, ceftriaxone and aztreonam, but susceptibility to ceftazidime except in ESBL producers [11, 12].

It is essential to understand the antimicrobial susceptibility pattern of *Klebsiella* spp because of the variation in antibiotic susceptibility in different geographical settings and to implement the measures to control the rapid spread of drug resistance. Hence, the present study was undertaken to characterize and find the antibiogram of *Klebsiella* spp. isolated from clinical specimens in a rural teaching hospital.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, KVG. Medical College and Hospital, Sullia, between December 2011 to October 2013. Approval from Institutional Ethical committee was obtained for the study. A total of 1957 various clinical samples such as urine, endotracheal tubes, catheter tips, exudates, throat swab, sputum, and pus obtained from inpatients and outpatients were included in the study. The samples were processed according to standard conventional methods and the isolates were identified by standard biochemical tests [13,14].

Antimicrobial Susceptibility testing

A total of 100 isolates were screened for antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Hi-Media, Mumbai) and interpreted as per CLSI guidelines [15]. A log phase broth culture inoculum of the isolate with a turbidity equivalent to McFarland 0.5 standard ($1.5x10^8$ CFU/ml) was prepared and lawn cultured on the Mueller-Hinton agar and allowed to dry. Antibiotic discs were applied to the Mueller Hinton agar surface with the help of sterile forceps. The antibiotics tested were ampicillin (10µg), gentamicin (10µg), amikacin (30µg), amoxicillin/clavulanic acid (20/10µg), piperacillin/tazobactam (100/10µg), cefepime (30µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), ciprofloxacin (5µg), imipenem (10µg), meropenem (10µg), cotrimoxazole (25µg), aztreonam (30µg), chloramphenicol (30µg), tobramycin (10µg), norfloxacin (10 µg), nitrofurantoin (300µg), sparfloxacin (5µg), nalidixic acid (30µg) (Hi-Media, Mumbai).

A parallel antibiotic susceptibility test put up using *Escherichia coli* ATCC 25922, served as control.

RESULTS

During the study period, a total of 1957 various clinical samples were processed and 100 *Klebsiella* spp were isolated. Among the 100 *Klebsiella* spp, 40% of the isolates were from pus, 26% each from urine and sputum, 6% from catheter tips and 1% each from vaginal swab and endotracheal tube. Out of the 100 *Klebsiella* spp, 79% of the isolates were identified as *K. pneumoniae* and 21% were *K. oxytoca*. Percentage of isolation of *Klebsiella* species from different clinical specimens is summarised in Table 1.

Table 1: Isolation rate of *Klebsiella* species from various sample

Sample	*K. pneumoniae* No. (%)	*K. oxytoca* No. (%)	Total No. (%)
Urine	21 (80.76%)	5 (19.24%)	26 (100%)
Pus	30 (75%)	10 (25%)	40 (100%)
Sputum	24 (92.30%)	2 (7.70%)	26 (100%)
Catheter tip	2 (33.33%)	4 (66.67%)	6 (100%)
Vaginal swab	1 (100%)	0 (0%)	1 (100%)
Endotracheal tube	1 (100%)	0 (0%)	1 (100%)
Total	79 (79%)	21 (21%)	100

Of the 100 *Klebsiella* isolates, 63 were from males and 37 were from females with a male: female ratio of 1.7: 1. Among the 63 isolates from males, 50 (79.36%) were *K. pneumoniae* and 13 (20.63%) were *K. oxytoca*. Out of 37 isolates obtained from females, 29 (78.37%) were *K. pneumoniae* and 8 (21.62%) were *K. oxytoca*. Isolation rate was highest in patient aged above 60 years (male 28.57% & female 32.43%) followed by 45-60 years of males (23.80%) and females (27.02%). Lowest rate of isolation was observed in male (7.93%) and female (5.40%) children aged 0-15 years. Table 2 shows the distribution of *Klebsiella* infection in various age groups.

Table 2: Frequency of age and sex wise distribution of *Klebsiella* species infection

Age in years	Male (n=63) No. (%)	Female (n=37) No. (%)
0-15	5 (7.93%)	2 (5.40%)
16-30	8 (12.69%)	8 (21.62%)
31-45	17 (26.98%)	5 (13.51%)
45-60	15 (23.80%)	10 (27.02%)
>60	18 (28.57%)	12 (32.43%)

Various risk factors associated with *Klebsiella* infection observed were diabetes mellitus, hypertension, steroid therapy, alcoholism and smoking. Among the 63 males, 21 (33.33%) were suffering from diabetes mellitus, 15 (23.80%) were alcoholic, 10 (15.8%) were hypertensive, 7 (11.11%) were chronic smokers and 5 (7.93%) were on steroid therapy. In females, out of 37, 9 (24.32%) were suffering from diabetes mellitus, 5 (13.51%) were hypertensive and 3 (8.10%) were on steroid therapy.

Antimicrobial susceptibility testing revealed all isolates were sensitive to imipenem (100%) followed by cefepime (77%), amikacin (74%), gentamicin (70%), ciprofloxacin (59%), sparfloxacin (56%), cefoperazone/sulbactam (53%) and nitrofurantoin (53%) (Fig 1).

Isolates were highly resistant to ampicillin (89%), aztreonam (83%) and piperacillin/tazobactam (67%). Among the third generation cephalosporins tested, *Klebsiella* isolates showed high resistance to cefotaxime (65%), followed by ceftazidime (57%) and ceftriaxone (52%). Isolates were also resistant to meropenem (48%). Antimicrobial susceptibility pattern of *K. pneumoniae* and *K. oxytoca* is shown in Table 3.

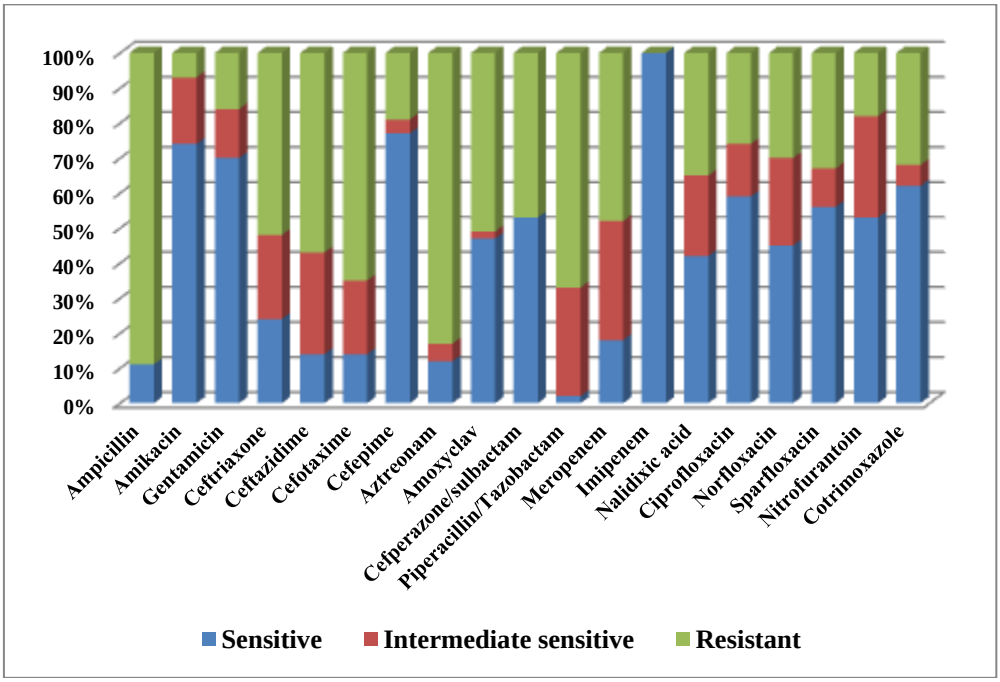

Fig. 1: Antimicrobial susceptibility pattern of *Klebsiella* spp

Table 3: Antimicrobial susceptibility pattern of *K. pneumoniae* and *K. oxytoca*

Antibiotics	*K. pneumoniae* (*n*= 79) No. (%)			*K. oxytoca* (*n*= 21) No. (%)		
	S	IS	R	S	IS	R
Ampicillin	9(11.59)	-	70(88.60)	2(9.52)	-	19(90.47)
Amikacin	56(70.88)	16(20.25)	7(8.86)	18(85.71)	-	3(14.28)
Gentamicin	54(68.35)	11(13.92)	14(17.72)	16(76.19)	3(14.28)	2 (9.52)
Ceftriaxone	18(22.78)	21(26.58)	40(50.63)	6(28.57)	3(14.28)	12 (57.1)
Ceftazidime	8(10.12)	26(32.91)	45(56.96)	6(28.57)	3(14.28)	12 (57.14)
Cefotaxime	10(12.65)	19(24.05)	50(63.29)	4(19.04)	2(9.52)	15(71.42)
Cefepime	62(78.48)	2(2.53)	15(18.98)	15(71.42)	2(9.52)	4(19.04)
Aztreonam	7(8.86)	5(6.32)	67(84.81)	5(23.80)	-	16(76.19)
Amoxyclav	39(49.36)	2(2.53)	38(48.10)	8(38.09)	-	13(61.90)
Cefoperazone/ sulbactam	40(50.63)	-	39(49.36)	13(61.90)	-	8(38.09)
Piperacillin/ tazobactam	2(2.53)	21(26.58)	56(70.88)	11(52.38)	-	10(47.61)
Meropenem	14(17.72)	28(35.44)	37(46.83)	4(19.04)	6(28.57)	11(52.38)
Imipenem	79(100)	-	-	21(100)	-	-
Nalidixic acid	34(43.03)	19(24.05)	26(32.91)	8(38.09)	4(19.04)	9(42.85)
Ciprofloxacin	44(55.69)	13(16.45)	22(27.84)	15(71.42)	2(9.52)	4(19.04)
Norfloxacin	36(45.56)	20(25.31)	23(29.11)	9(42.85)	5(23.80)	7(33.33)
Sparfloxacin	43(54.43)	7(8.86)	29(36.70)	13(61.90)	4(19.04)	4(19.04)
Nitrofurantoin	43(54.43)	22(27.84)	14(17.72)	10(47.61)	7(33.33)	4(19.04)
Cotrimoxazole	53(67.08)	2(2.53)	24(30.37)	9(42.85)	4(19.04)	8(38.09)

DISCUSSION

Klebsiella is an important nosocomial human pathogen that has the potential to cause severe morbidity and mortality. *Klebsiella* especially *K. pneumoniae* is gaining renewed interest because of emergence of multidrug resistance among *Klebsiella* associated infections. These are now being recognised as one of the major threats to effective management of patients in hospital, especially in developing country like India.

In the present study the isolation rate of *Klebsiella* spp is more from pus, followed by urine, sputum and catheter tip. This finding is similar to the previous report [16]. However, environmental including hospital environment, *Klebsiella* may be more responsible for wound infections (pus) and respiratory infections, unless otherwise there is direct hematogenous spread. In our study majority were community acquired infections, hence it may be probable that the infections other than urinary tract were acquired partly from non-hospital environment.

In the current study, isolation rate of *K. pneumoniae* was more compared to *K. oxytoca*. This finding is comparable to the report of Asmaa *et al*. [17] who isolated 65.5% of *K. pneumoniae* and 34.5% of *K. oxytoca* from clinical samples. A lower isolation rate of *K. pneumoniae* and *K. oxytoca* were reported by Kaur *et al*. [18] (14.8% & 4.2%), Rao *et al*. [19] (13.52% & 0.9%) respectively. *K. pneumoniae* was the predominant species isolated in all previous reports including the present study.

In general, the tendency is to identify *Klebsiella* only to the genus level especially in laboratories of private sectors. Our findings emphasise the need to identify the organism to the species level because a substantial percentage of the isolates were *K. oxytoca* (21%). This would enable us to understand if there is a gradual increase in infection with other species say *K. oxytoca* and shift from *K. pneumoniae* to the pathogenic species. High rate of colonisation of catheter tip by *K. oxytoca* further stresses the need to identify *Klebsiella* to the species level.

In our study, *Klebsiella* infection was predominantly observed in males than females, with a male to female ratio 1.7:1, which was almost similar with the study by Shah *et al* (1.6:1) [20]. *Klebsiella* infection was more commonly seen in persons aged above 60 years and 45-60 years of age which corroborates with previous reports [20, 21].

A gender difference of infection was observed in our study. A female dominance of infection was observed in age group 16-30 years (21.62%) and 46-60 (27.02%) than males (12.69% and 23.80% respectively), while it is not clear why female dominance of infection was observed in 16-35 years. The higher infection in females of 45-60 years may be attributed hormonal changes of the phase of menopause. Contrary to the above findings, in 30-45 years age group more males (26.98%) were infected than females (13.51%).

The common predisposing factors associated with *Klebsiella* infection were diabetes mellitus, hypertension, alcoholism and steroid therapy. The prevalence of Gram-negative bacilli varies widely depending upon the clinical settings and the geographic region. The reported risk factors for the infection with Gram negative organisms include increased length of stay in the ward or intensive care unit, severity of illness, respiratory or urinary tract infections, malignancy, in-dwelling urinary catheter, dialysis, age more than 65 years, functional dependence, emergency abdominal surgery, and prior administration of antimicrobials [22]. In our study, the most common predisposing factors associated with *Klebsiella* infection in age groups between 45-60 years and above 60 years was diabetes mellitus followed by hypertension, alcoholism, steroid therapy and chronic smoking.

Isolates of our study were susceptible to imipenem, which was similar to the findings by Gupta *et al*. [23], Shiju *et al*. [24] and Bhaumik *et al*. [25]. But 48% of them were found to be highly resistant to meropenem. This might be due to the loss of porin and the presence of plasmid mediated β- lactamases. Fourth generation cephalosporin, cefepime and aminoglycosides such as amikacin and gentamicin were the most effective antibiotic agents against *Klebsiella* spp, which was consistent with the previous study [16].

A significant high resistance (52%-65%) to third generation of cephalosporins (cefotaxime, ceftazidime and ceftriaxone) observed in the study corroborates with the report Shah *et al*. [20]. Studies from Chandigarh (87%-89%) and Nigeria (84.8%-96%) revealed markedly high cephalosporin resistance [23, 26]. The decreased susceptibility to third generation of cephalosporin could be due to production of extended spectrum beta- lactamase and Amp C beta- lactamase.

Contrary to previous reports [23, 27] our study showed that β- lactam and β- lactamase inhibitor combinations were ineffective. 67% of the isolates were resistant to piperacillin/tazobactam followed by amoxyclav (51%) and cefoperazone /sulbactam (47%). The high resistant to β- lactamase inhibitors could be due to the production of inhibitor resistance TEM β-lactamase.

In our study, 89% of the isolates showed resistance to ampicillin, which was close to the observation by Kothari *et al* [28]. The chromosomally encoded β- lactamases could be responsible for this intrinsic resistance. Among the fluoroquinolones tested, ciprofloxacin (59%) was the most effective antibiotic against *Klebsiella* spp followed by sparfloxacin (56%) and norfloxacin (45%). The previous studies by Gupta *et al* (63%) and Ali *et al* (76.9%) observed high resistance to ciprofloxacin [23, 16].

CONCLUSION

Though majority of *Klebsiella* infections are caused by *K. pneumoniae*, a sizeable infection are caused by *K. oxytoca*. Infection was predominant in males, although more females are affected in 16-30 years and above 60 years of age. The common predisposing factors associated with *Klebsiella* infection were diabetes mellitus, hypertension chronic, smoking, alcoholism and steroid therapy. Both *K. pneumoniae* and *K. oxytoca* were totally susceptible to imipenem. Both species of *Klebsiella* exhibited high level resistant to 3rd generation cephalosporin, aztreonam, amoxyclav, piperacillin/tazobactam and meropenem. Nosocomial *Klebsiella* infection continues to be heavy burden on the economy and life expectancy of patients. Therefore, study of *Klebsiella* associated infections and the antibiogram pattern of *Klebsiella* isolates are the requirements in the implementation of preventive and control measures against infections. Regular surveillance of antibiotic susceptibility pattern may help to overcome the indiscriminate use of antibiotics a major cause of emergence of drug resistance among pathogens and to develop antibiotic policies.

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