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Antibiogram and Detection of ESBL Production in *Klebsiella* species Isolated from Various Clinical Samples

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A B S T R A C T

Klebsiella species belonging to the family *Enterobacteriaceae*, are known to cause a variety of human infections such as pneumonia, urinary tract infections, septicaemia, soft tissue infections and are an important cause of healthcare associated infections. The emergence of multidrug resistant *Klebsiella* is a growing problem worldwide. *Klebsiella* spp. are one of the most common organisms to carry plasmids encoding ESBL enzyme. They are associated with higher rates of treatment failure and death. In this study, we intend to detect the different species of *Klebsiella* causing infections and analyze their antibiogram including extended spectrum beta lactamase production. A retrospective study was conducted in the department of Microbiology in a tertiary care hospital. Patient samples in which *Klebsiella* species was isolated were included in the study. Sample processing and isolate identification was done using standard laboratory techniques. Antimicrobial susceptibility testing and ESBL screening was performed by Kirby Bauer's disc diffusion method as per CLSI guidelines. A total of 210 *Klebsiella* spp. were isolated. Sputum (35.7%) was the most frequently received specimen and 94.8% isolates were *K. pneumonia*. Highest sensitivity was seen towards imipenem. Among urinary isolates, norfloxacin was the least effective drug. 68.6% isolates were ESBL producers. There are limited therapeutic options for treatment with multidrug resistant organisms. Hence, it is becoming very important to prevent colonization and infection by implementing and periodically reinforcing appropriate infection control measures.

Introduction

Klebsiella species belonging to the family *Enterobacteriaceae*, which are usually opportunistic pathogens found in the environment, on mucosal surfaces and on the hands of hospital personnel.

The principal pathogenic reservoirs are the gastrointestinal tract of humans (Christensen and Korner, 1972). Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Klebsiella granulomatis*.

Organisms previously known as *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are considered non-fermenting subspecies of *K. pneumoniae* that have typical clinical manifestations (Mandell *et al.*, 2010).

K. pneumoniae is a primary pathogen capable of causing pneumonia, urinary tract infections and liver abscess in otherwise healthy people. However, most infections especially multidrug resistant infection caused by *K.pneumoniae* are healthcare associated infections such as wound infections, biliary tract infections, peritonitis, meningitis and device related infections. *K. oxytoca*, like *K.pneumoniae* can cause a variety of hospital acquired infections (Mandell *et al.,* 2010).

Over the last 15 years, numerous outbreaks of infection with organisms producing extended spectrum ß Lactamase (ESBL) enzyme have been observed worldwide (Slden *et al.,* 1971). *Klebsiella* spp. are one of the most common organisms to carry plasmids encoding ESBL enzyme and are associated with higher rates of treatment failure and death (Mandell *et al.,* 2010). Studies in Karnataka have showed detection rates of ESBL producing *K. pneumoniae* to vary from 9.6% to 81.1% (Rao *et al.,* 2014).

In this study, we intend to detect the different species of *Klebsiella* associated with infectious diseases in our hospital and analyze their antimicrobial resistance patterns including extended spectrum beta lactamase production. This will help in the selection of effective antibiotics for empirical treatment of Klebsiella infections as well as generating data on the prevalence of antibiotic resistance in our hospital.

Materials and Methods

A retrospective study was conducted in the department of Microbiology at a tertiary care centre in northern Karnataka from January 2016 to December 2017. Data of specimens such as sputum, urine, pus, blood, body fluids like peritoneal fluid, pleural fluid, synovial fluid, etc. received from patient's in the microbiology laboratory for culture during the study period were scrutinized. Patient samples in which *Klebsiella* species was isolated were included in the study. Sample received from the same patient twice and yielding the same growth was considered as a single isolate.

All samples were inoculated onto blood agar and MacConkey's agar and incubated overnight at 37^0C. Urine samples were inoculated by 'standard loop technique' using a calibrated loop. Preliminary identification of the organism was based on presence of large, mucoid, non – hemolytic colonies on blood agar and large, mucoid, pink lactose fermenting colonies on MacConkey's agar and Gram's stain showing short and stout Gram negative bacilli. Confirmation was done by biochemical reactions viz. Indole test, Simmon's Citrate Utilization test, Christensen's Urease hydrolysis test, triple sugar iron test and mannitol motility medium. Indole test was used to differentiate between *Klebsiella pneumoniae* and *Klebsiella oxytoca* (Koneman *et al.,* 2006; Overview of Bacterial Identification Methods and Strategies, 2007).

Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method on Mueller Hinton agar. Selection of antimicrobials to be tested and interpretation was done according to Clinical Laboratory Standards Institute (CLSI) guidelines. The antimicrobials tested were cefotaxime, ceftazidime, cefepime, piperacillin, piperacillin–tazobactam, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin andtrimethoprim–sulfamethoxazole. Only urinary isolates were also tested against nitrofurantoin and norfloxacin. Quality control was done using Escherichia coli ATCC 25922 (Clinical Laboratory Standards Institute, 2014).

Screening for Extended spectrum beta lactamase (ESBL) producing *Klebsiella* spp. was done as per CLSI guidelines. The isolate

was tested with ceftazidime (30µg), ceftriaxone (30 µg) and cefotaxime (30 µg) by disk diffusion. If the zone size for ceftazidime was < 22 mm and cefotaxime was < 27 mm, the isolate was an ESBL producer (Clinical Laboratory Standards Institute, 2014).

Descriptive statistics like percentage and proportions were used to describe data and difference was tested using chi square test.

Results and Discussion

During the study period, a total of 210 *Klebsiella* spp. were isolated in the microbiology laboratory. The age and sex distribution of the patients has been depicted in Table 1. Men were more frequently infected. Male to female ratio was 1.3: 1. The age group most commonly infected with *Klebsiella* spp. was from 61 to 70 years (19.5%), followed by 21 to 30 years (16.2%).

Out of the 210 Klebsiella spp., 199 (94.8%) isolates were *K. pneumoniae* and 11 (5.2%) were *K. oxytoca*. These isolates were obtained from various clinical samples received in the microbiology laboratory. Figure 1 shows the various samples that yielded both the species of *Klebsiella*. Overall, sputum (35.7%) was the most frequently received specimen, followed by urine (26.2%), pus (22.4%), blood (10%), endotracheal aspirate (3.8%) and body fluids (1.9%). It was seen that *K. oxytoca* was most frequently isolated from urine while *K. pneumoniae* was most commonly isolated from sputum. Among the isolates from blood, 16 out of 21 were from neonates in Neonatal Intensive Care Unit.

Antibiotic sensitivity testing was interpreted as per CLSI guidelines. The sensitivity pattern of the isolates is depicted in Figure 2. Highest sensitivity was seen towards imipenem, whereas least effective drug was ciprofloxacin and among urinary isolates, norfloxacin was the least effective drug. Phenotypic screening of extended spectrum beta lactamase (ESBL) producers as per CLSI guidelines is done routinely in the microbiology laboratory. It was seen that 68.6 % of isolates were ESBL producers by screening test. In the present study, a small proportion of resistance to imipenem (3.3%) was also noted.

The age and gender distribution of patients infected with *Klebsiella* species was studied (Table 1). In our study, maximum patients infected were in the age group of 61- 70 years. Similar results have been reported by several other investigators (Das and Debnath, 2015; Thomas and Ramyashree, 2016; Chakraborty *et al.,* 2016). There was no statistically significant difference between the number of male and females infected and no difference was observed in the age distribution.

In this study, majority of the infections were due to *K. pneumoniae* (94.8%), followed by *K. oxytoca* (5.2%). These findings are in concurrence with the fact that K *pneumoniae* is the most common pathogen amongst various species of *Klebisella* (Chakraborty *et al.,* 2016).

Various studies have reported either pneumonia (Das and Debnath, 2015; Anitha *et al.,* 2016) or urinary tract infections (UTI) (Akter *et al.,* 2013) as the most common infection caused by species of *Klebsiella*. In the present study, pneumonia was the most common infection followed by UTI. We noticed most of the isolates from blood were from neonates admitted in the Neonatal Intensive Care Unit [NICU] and all were due to *K. pneumoniae*. *K. pneumoniae* causing neonatal septicaemia has been well documented (Zaidi *et al.,* 2005). A previous study in the same hospital also found *Klebsiella pneumoniae* as the most common cause of neonatal septicaemia (Bhurle and Solabannavar, 2014)

Table.1 Age and sex distribution

Age gr (years)	Male (%)	Female (%)	Total (%)
<1	10 (4.8)	7 (3.3)	17 (8.1)
1 to 10	5 (2.4)	4 (1.9)	9 (4.3)
11 to 20	6 (2.9)	10 (4.8)	16 (7.6)
21 to 30	17 (8.1)	17 (8.1)	34(16.2)
31 to 40	10 (4.8)	10 (4.8)	20 (9.5)
41 to 50	11 (5.2)	7 (3.3)	18 (8.6)
51 to 60	17 (8.1)	16 (7.6)	33 (15.7)
61 to 70	28 (13.3)	13 (6.2)	41 (19.5)
>71	15 (7.1)	7 (3.3)	22 (10.5)
total	119 (56.7)	91 (43.3)	210 (100)

Fig.1 *Klebsiella* species from various clinical samples

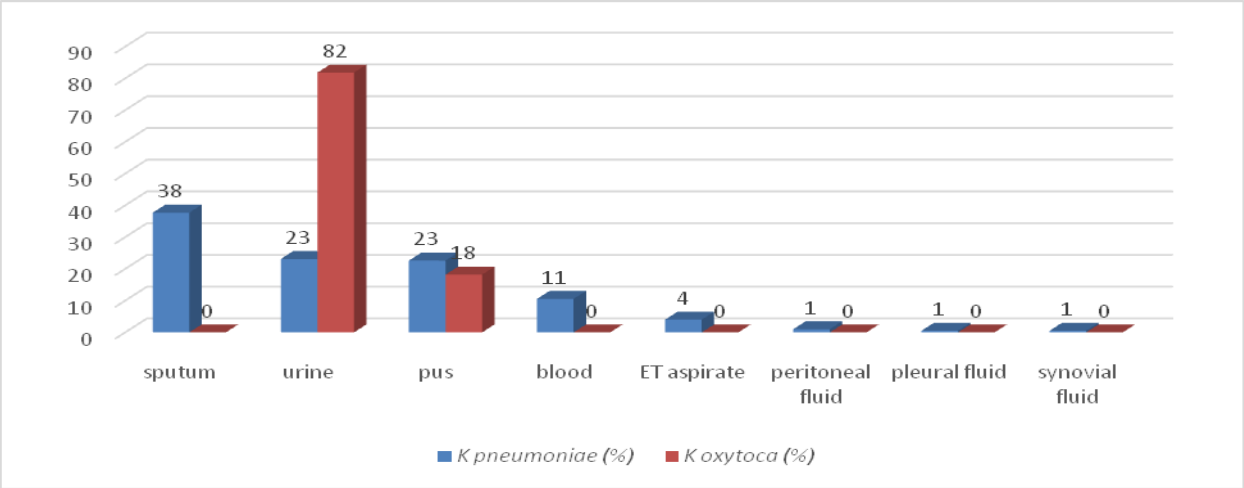
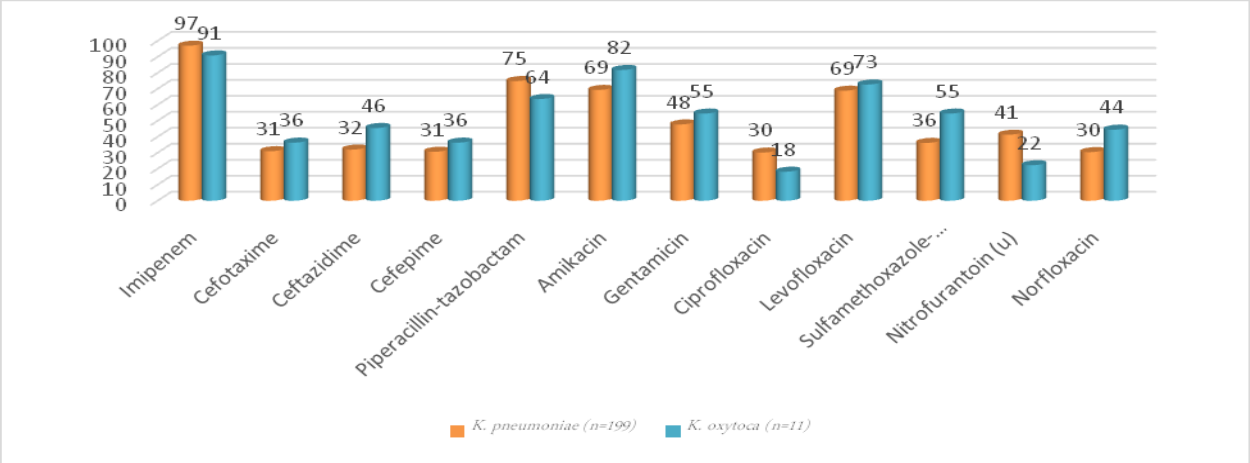


Fig.2 Antimicrobial Susceptibility Pattern of *Klebsiella* isolates



The various species of *Klebsiella* exhibit different levels of susceptibility to antibiotics employed in treatment. In the present study, the antibiogram of *Klebsiella* isolates showed higher resistance against cephalosporins and fluoroquinolones. Similar results were reported by other researchers (Panta *et al.,* 2013). Screening for extended-spectrum ß lactamase (ESBL) showed that 68.6% of the isolates were ESBL producers. Higher resistance to both cephalosporins as well as quinolones could be because the plasmids that contain the ESBL gene also contain resistance genes for quinolones (Paterson *et al.,* 2000).

Sridhar Rao *et al.,* (2014) reported a wide variation in detection rates of ESBL across regions. The authors stated that it could be due to a varied degree of exposure of these organisms to beta lactam antimicrobials or because of varied transferability of plasmids in the nature or due to the differences in the methodology employed (Rao *et al.,* 2014). In the present study, the high proportion of ESBL producers could be because only screening test was performed.

96.7% of the isolates were sensitive to imipenem. As it was a retrospective study, carbapenem resistance was not being confirmed. 100% sensitivity to imipenem was observed by the author in her previous study in the same setup (Bhurle and Solabannavar, 2014). 3.3% resistance is observed to imipenem in the present study, this gradual increase in resistance is an impending threat and cause for concern. This could be attributed to the increased use of carbapenems in the recent years.

Over the last 2 decades, the incidence of infections caused by multidrug resistant *Klebsiella* strains has increased significantly and this emergence of antimicrobial resistance has become a global health problem (Gupta *et al.,* 2003). Multidrug resistance strains causes challenges in healthcare and increases the morbidity and mortality. It increases the duration of hospital stay and cost incurred to the patient (Thomas and Ramyashree, 2016). The commonest reason for development of resistance is the high frequency use of antibiotics for empirical treatment thus strict adherence to antibiotic policy for empiric therapy is the need of the hour (Gupta *et al.,* 2003). Continuous monitoring of multidrug resistant organisms and their resistance pattern will help in managing the infections efficiently and also help in formulation of antibiotic policies.

Treatment of ESBL producing *K. pneumoniae* infections is challenging. There are limited therapeutic options for treatment with multidrug resistant organisms. Hence, it is becoming very important to prevent colonization and infection by implementing and periodically reinforcing control measures such as proper hand hygiene, barrier nursing, etc.

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