Prevalence of extended-spectrum beta-lactamase and carbapenemase producing klebsiella isolates in a tertiary care hospital

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Abstract:

Background: Klebsiella is one of the commonly encountered gram-negative pathogens in hospital-acquired infection. Multidrug resistance associated with the production of enzymes such as Extended-Spectrum Beta-Lactamases (ESBL) and carbapenemases are being encountered with increasing frequency nowadays, leading to very limited therapeutic options. Aim: To study the antibiotic susceptibility profile of Klebsiella isolates from clinical samples. To detect the presence and prevalence of ESBL and carbapenemase producing strains by phenotypic methods. Materials and Methods: 170 Klebsiella isolates from various clinical samples were taken up for the study. The antibiotic susceptibility testing was done by Kirby-Bauer disk-diffusion method. ESBL production was confirmed by Double-Disk Synergy Test. Carbapenemase producers were confirmed by Modified Hodge Test. Results: Out of the 170 isolates, maximum resistance was observed towards cefazolin (51.7%) and least resistance to imipenem (9%). 79 (46%) isolates were ESBL producers. 15 isolates out of 170 (8.8%) were resistant to carbapenems. 9 out of 15 isolates (60%) produced carbapenemase enzyme. Conclusion: As the available treatment options are limited, antibiotic policies together with implementation of infection control measures are of utmost importance in the health-care institutions, to curb the rapidly increasing resistance rates of microorganisms.

Key words: Antibiotic susceptibility profile; Carbapenemase; Double Disk Synergy test; ESBL; Klebsiella; Modified Hodge test

Introduction

Klebsiella is one of the commonly encountered gram-negative pathogens in hospital acquired infections. Klebsiella is a well known as a cause of community acquired bacterial pneumonia occurring particularly in chronic alcoholics, causing a severe pyogenic infections with a high fatality rate if left untreated [1]. As an opportunistic pathogen, Klebsiella usually infects patients with underlying disorders such as diabetes mellitus or chronic
obstructive pulmonary disease [2]. Multidrug resistance Klebsiella isolates are a major problem faced in treatment of nosocomial infections. Similar resistance patterns are being increasingly observed in community-acquired Klebsiella infections. The injudicious use of empirical antibiotics has been a major cause in the development of multidrug resistance among bacteria. Resistance due to production of Extended-Spectrum Beta-Lactamases (ESBL) has also been reported with increasing prevalence nowadays. Extended spectrum beta lactamases are enzymes that mediate resistance to extended spectrum (third generation) cephalosporins (e.g., ceftriaxone, cefotaxime and ceftizoxime), and monobactams but do not affect cephemycins or carbapenems [3]. ESBL falls under group 2be in class 2 of Bush, Jacoby and Medeiros functional classification scheme, and under class A of Ambler’s molecular classification [4].

Carbapenemases are beta-lactamases with the ability to hydrolyse penicillins, cephalosporins, monobactams and carbapenems. Carbapenemases are members of molecular class A, B and D beta-lactamases. Bacteria producing these beta-lactamases may cause serious infections in which the carbapenemase activity renders many beta-lactams ineffective.

ESBLs are usually plasmid-mediated and since these plasmids are easily transmitted among the Enterobacteriaceae, the accumulation of resistance genes results in strains that contain multiresistant plasmids [1]. ESBL-producing organisms often also possess resistance determinants to other important antibiotic groups, such as aminoglycosides and fluoroquinolones, leaving an extremely limited range of effective agents. The aims of this study are to detect the prevalence and production of ESBL and carbapenemase enzymes in Klebsiella isolates by phenotypic confirmatory methods, in a tertiary care hospital.

Materials and Methods

In this study, 170 isolates of Klebsiella sp. were collected from various clinical specimens like urine, exudates, respiratory samples and blood over a period of 1 year and 6 months (from January, 2012 to July, 2013). Informed consent was obtained from the patients to use the isolates for study purposes. Out of the 170 isolates, 98 were from urine, 46 from pus and other exudates, 21 from respiratory specimens and 5 were from blood. Biochemical reactions [2,5] were used to speciate the Klebsiella isolates.

Antibiotic susceptibility testing was done by the Kirby-Bauer disk diffusion method, according to CLSI guidelines [6]. The isolates that were resistant to either cefotaxime or ceftizoxime were further subjected to phenotypic confirmatory test (Double Disk Synergy test) for ESBL production. Double-Disk Synergy test [7-9] was performed using ceftazidime (30μg) and piperacillin-tazobactam (100μg/10μg), and ceftazidime (30μg) and ceftazidime-clavulanic acid (30μg/10μg). The plates are then incubated at 37°C in ambient air for 16-20 hours. This test does not require critical disc spacing. The plates are then observed and the diameter of zones of inhibition around the disks is measured.

A >=5mm diameter increase in a zone around a disk for either antimicrobial agent tested in combination with clavulanic acid vs the zone diameter of the agent when tested alone confirms ESBL production by the strain (Figure 2).

Detection of carbapenemase production:

Phenotypic confirmatory testing for the carbapenemase producing Enterobacteriaceae has to be done for all the isolates that show decreased susceptibility to carbapenems in the disk diffusion method of testing. Modified Hodge Test: [10-12] A lawn culture of a susceptible strain of Escherichia coli (ATCC 25922) is made on a Mueller-Hinton agar plate. A carbapenem disk (meropenem or ertapenem 10μg) was placed in the centre. Isolates of suspected carbapenemase are streaked from the disk to the outer margin of the plate and incubated at 37°C for 16-24 hours. Growth of E.coli near the disk or along the isolate streak, giving the appearance of a clover-leaf pattern indicates carbapenemase production by the strain (Figure 4).

Institutional Ethical Committee approval was obtained for the study.

Results

Out of the total 170 isolates of Klebsiella species, 159 were Klebsiella pneumoniae subsp. pneumoniae and 11 were Klebsiella oxytoca.

Antibiotic susceptibility pattern: 86 isolates were found to be sensitive to all the antibiotics, except ampicillin due to inherent resistance of Klebsiella to ampicillin. The percentage of isolates resistant to the following antibiotics were: Cefazolin–51.7%, Cefuroxime–49.4%, Cefotaxime–45.8%, Cefepine–38.2%, Gentamycin–28.2%, Amikacin–20%, Ciprofloxacin–37%, Norfloxacin–28.2%, Nitrofurantoin–25.8%, Cotrimoxazole–40%, Piperacillin-Tazobactam–22.3%, Meropenem–30% and Imipenem–8.8%. (Figure 1)
Figure 1: Percentage of *Klebsiella* isolates resistant to various antibiotics.

**ESBL producers:** The isolates that were resistant to third generation cephalosporins were also tested for the production of Extended Spectrum Beta Lactamases by the double-disk approximation test. The enhancement of zone of inhibition on the side of the combination disk containing Beta lactam with Beta-lactamase inhibitor (Piperacillin- tazobactam-100/10μg) as compared to the zone on the side of third generation cephalosporin (cefotaxime-30μg), was noted (Figure 2).

Figure 2: ESBL producer in Double disk approximation test

79 isolates out of 170 (46.4%) were found to be ESBL producers. 51 (64%) strains were isolated from urine, 19 (24%) strains from exudates, 3 isolates from blood (4%) and 6 isolates from respiratory specimens (8%) (Figure 3).

**Figure 3: ESBL producers**

![ESBL producers graph](image)

**Carbapenemase producers:** Among the 170 Klebsiella isolates, 15 strains (8.8%) were found to be resistant to the carbapenems (imipenem and meropenem). All 15 strains were tested for carbapenemase production using Modified Hodge test. 9 isolates (60%) were positive for carbapenemase production, out of which, 3 isolates were from urine, 4 from exudate samples and 2 from respiratory specimens (Figure 5).

**Figure 4: Modified Hodge test for carbapenemase detection**

![Modified Hodge test](image)

(The clover-leaf type of indentation noted along the streak line of test strain indicates carbapenemase production by the isolate.)
Discussion

*Klebsiella* is the second most common uropathogen after *E.coli*. *Klebsiella* spp. are among the most common pathogens isolated in intensive care units and *K.pneumoniae* is the most frequently encountered carbapenemase producing Enterobacteriaceae [13]. The most important aspect of *Klebsiella* associated infection is the emergence of multidrug resistance, mainly mediated by the production of Extended spectrum beta-lactamases. It often leads to treatment failure, which has serious consequences in critically ill patients if resistance is not detected well in time. Inadequate empirical antibacterial therapy, defined as initial use of antibacterial agents to which pathogen was not susceptible, is associated with increased mortality rates. Increased prevalence of *Klebsiella* infection was observed in the age group of 20-60 years. This correlates to the observation by Kelmani Chandrakanth R [14] et al where the maximum prevalence was seen in 26-50 year age group.

86 isolates out of the total (50%) were sensitive to all the antibiotics tested. Among the resistant isolates, maximum resistance was found to be to the first generation cephalosporin, cefazolin (51%), followed by cefuroxime (49%) and cefotaxime (46%) resistance. The lowest resistance rates were observed against imipenem (9%), amikacin (20%) and piperacillin- tazobactam (23%).

According to a study by Kaur M and Aggarwal A [15] the percentage of *K.pneumoniae* resistant to Piperacillin-tazobactam combination was 12.24%, which was lower than the current study finding. According to a study conducted by Vemula Sarojamma [16] and Vadde Ramakrishna, 39% of *Klebsiella* isolates were resistant to third generation cephalosporins which was slightly lower than this current study where 49% isolates were found resistant. Highest percentage of susceptibility was found to imipenem (96%) in that study, which relates to this study finding of 91% susceptibility to imipenem. 46% of the total isolates were found to be ESBL producers. This is almost similar to the report by Kingsley J [17] (45.9% ESBL producers) in a hospital in Chennai in 2008. A prevalence of 79% of ESBL producing *Klebsiella* was reported in a study by Kelmani Chandrakanth et al from Gulbarga [14].

Baby Padmini et al, [18] from Coimbatore in 2004 have reported a prevalence of 40% ESBL producers in *K.pneumoniae* isolates which is also similar to this study. In the current study, the highest rate of ESBL producers were from blood (60%), followed by urine (52%), exudate samples (41%) and respiratory specimens (28%).

In the present study, 15 patients (8.8%) were infected with multi drug resistant *Klebsiella* strains which were susceptible only to polymyxin B and colistin. All these isolates were identified as
**Klebsiella pneumoniae subsp. pneumoniae** except one strain which was *Klebsiella oxytoca*, isolated from a diabetic patient with catheter associated urinary tract infection. They were treated with intravenous colistin along with other antibiotics and supportive therapy. 3 out of the 15 patients expired. Those three patients were more than 75 years of age and had multidrug resistant polymicrobial infections with septicemia when they succumbed to infection. The common organisms that were isolated along with *Klebsiella pneumoniae* from these patients were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The cause of these deaths could be multifactorial. None of the patients who died had *Klebsiella pneumonia* bacteremia.

**Conclusion**

This study highlights the prevalence of ESBL-producing *Klebsiella* species having a significant percentage (46%). Routine detection of ESBL-producing microorganisms is required to be done by each laboratory by standard detection methods so as to control the spread of these infections and also institute proper therapeutic strategies. For the detection, the phenotypic confirmatory disk diffusion tests are simple, sensitive and cost-effective. As the available treatment options are limited, antibiotic policies together with implementation of infection control measures are of utmost importance in the health care institutions. Owing to the indiscriminate use of cephalosporins, most strains are becoming resistant to fourth generation cephalosporins also. Therefore, cycling of antibiotics for treating patients with *Klebsiella* infection may help to curb the rapid rates of emerging drug resistance.

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