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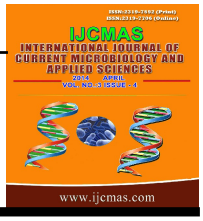

Original Research Article

ESBL & AmpC detection in *Klebsiella* species by Non Molecular methods

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A B S T R A C T

The β-lactamase are the most significant group of enzymes involved in conferring resistance to β-lactam antibiotics in gram-negative bacteria. They work by hydrolysing the β-lactam bond of any number of substrates thus rendering the antibiotic ineffective. In this study, a total of 92 Klebsiella species isolates obtained from 693 sputum samples and processed for antibiotic sensitivity pattern. The Isolates were also checked for ESBL & AmpC detection by simple, non molecular methods. Total 10.20/% isolates were ESBL producers and 7.14% were AmpC producers. Most effective antibiotics for Klebsiella species were Amikacin and Gentamicin.

Keywords

ESBL; AmpC; β-lactamase; Klebsiella species.

Introduction

Klebsiella species are opportunistic pathogens that cause hospital and community acquired infections such as pneumonia, urinary tract infection, septicaemia, soft tissue infections, liver abscess, and meningitis (Abdelgayed Metwaly younes, 2010). Extended spectrum β- lactamases (ESBLs) are plasmid mediated, TEM and SHV derived enzymes, first isolated in Western Europe in mid 1980s, most commonly in Klebsiella species, followed by Escherichia coli. These enzymes are capable of hydrolyzing broad spectrum Cephalosporins, Penicillins and Monobactams such as Aztreonam, but inactive against Cephamycins and Imipenem & are usually inhibited by β-Lactamase inhibitors such as Clavulanic acid. In addition, Plasmids responsible for ESBL production tend to be large (80 Kb or more in size) and carry resistance to several agents, an important limitation in the design of treatment alternatives. The most frequent co-resistances found in ESBL producing organisms are Aminoglycosides, Fluoroquinolones, Tetracyclines, Chloramphenicol and Sulfamethoxazole + Trimethoprim resulting in limitation of therapeutic option

(Chaudhary, et al., 2004). AmpC β-lactamase have gained importance since the late 1970s as one of the mediators of antimicrobial resistance in Gram negative bacilli. These enzymes are Cephalosporinases capable of hydrolyzing all β–lactams to some extent. AmpC β-lactamases are of two types plasmid-mediated and chromosomal or inducible AmpC (Hemalatha et al., 2007).

Plasmid-mediated AmpC β-lactamases produced by isolates of Klebsiella pneumoniae associated with decreased outer membrane permeability can even confer resistance to the Carbapenems (Jennifer A. Black et al., 2005). Plasmid-encoded AmpC genes have been found around the world since 1989 , in nosocomial and non nosocomial isolates, having been most easily detected in those enterobacteria not expected to produce an AmpC β-lactamase (George A.Jacoby 2009). Plasmid-mediated AmpC-resistance has arisen through the transfer of chromosomal genes for the inducible AmpC betalactamases onto plasmids. Plasmids with these genes can spread among other members of the family Enterobacteriaceae (Nasim, et al., 2004).

Materials and Methods

The present investigation comprised of a total of 92 Klebsiella species isolates obtained from 693 sputum samples collected in Mahatma Gandhi Mission Hospital Kamothe Navi Mumbai from February 2012 to February 2013. All the samples were processed and identified as per the standard microbiological protocols and procedures (Ho, et al., 2000). Isolates confirmed as Klebsiella species were studied for their antimicrobial susceptibility pattern, extended spectrum β-lactamase, combined ESBL+AmpC and pure AmpC production.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method with the following set of antibiotics- Netilline (30mcg), Amikacin (30mcg), Cefaperazone (30mcg), Lomefloxacin (30mcg), Cefotaxime (30mcg), Ceftazidime (30mcg), Cefuroxime (30mcg), Ciprofloxacin (5mcg), Gentamicin (10mcg), Pefloxacin (5mcg), Ofloxacin (5mcg)

Method for ESBL detection

National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test. A disc of Ceftazidime (30μg) alone and Ceftazidime + Clavulanic acid (30μg/10) were placed at a distance of 25 mm center to center , on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37˚C. An increase in inhibition zone diameter of ≥5 mm for a combination disc versus Ceftazidime disc alone confirmed ESBL production (Cormican et al., 1996).

AmpC detection: (Inhibitor based test)

All isolates were tested for AmpC β-lactamase production on disks containing Boronic acid. A disk containing 30 μg of Cefoxitin and another containing 30 μg of Cefoxitin with 400 μg of Boronic acid was placed on the agar. Boronic acid were prepared by dissolving 120 mg of phenylboronic acid (Benzeneboronic acid;Hi media.) in 3 ml of Dimethyl Sulfoxide. 3ml of sterile distilled water was added to this solution. 20 μl of the stock solution was dispensed onto disks containing 30 μg of Cefoxitin. Disks were allowed to dry for 30 min and used.

Inoculated plates incubated overnight at 35°C. An organism demonstrating a zone diameter around the disk containing Cefoxitin and Boronic acid ≥5 mm than the zone diameter around the disk containing Cefoxitin alone will be considered an AmpC producer (Kim et al., 2002; Steward et al., 2001).

Results and Discussion

Out of total 693 Sputum samples, 98 (14.14%) Klebsiella species were isolated (Chart 1). Out of 98 Klebsiella species, 77 (77.10%) were isolated from Male patients and 21 (22.90%) were isolated from Female patients (Chart 2). The patients of all age groups were included in this study but highest 41.30% patients were of 56 -75 years of age group (Chart 3). Out of 98 Klebsiella species, 10 (10.20%) were ESBL producers, 7 (7.14%) were AmpC producers and 16 (16.32%) were both ESBL as well as AmpC producers (Chart 4). Klebsiella species showed the variable sensitivity pattern against different antibiotics. The most effective antibiotics were Amikacin and Gentamicin (94.89%) whereas the least effective antibiotic was Cefuroxime (73.4%).

The knowledge on the extent of the ESBL mediated resistance appears to be limited due to the inability of the standardized methods of susceptibility testing or the commercially available systems to detect this resistance (Bonnet, 2004). The emergence and the spread of the ESBL producing strains have led to questions regarding the optimal therapy for infections which are caused by the ESBL producing strains (Philippon, et al., 2002). The confirmation of the ESBL production by clavulanic acid inhibition can be difficult in some strains, not only because the activity of the β-lactamase varies with different substrates, but also because the organism may contain additional resistance mechanisms that can mask the presence of the ESBL activity (Hanson, 2003).

The prevalence of Klebsiella in sputum sample was 14.14%. Study by Shamweel Ahmad et al. showed the prevalence of Klebsiella in sputum 7.69%. (Shamweel Ahmad. 2009) In another study by D.O Acheamporg et al. showed the prevalence from sputum 14.0% (Acheampong et al. 2011).

Out of total 98 klebsiella isolates, 77 (77.10%) were isolated from males and 21(22.90%) were from females. The male: female ratio was 2.4:1. In the study by A.O.Okesola et al. (2012) Who reported that 30 (34.1%) were males and 58 (65.9%) were females. The male: female ratio was 0.6:1. Renuka Rampure et al. (2013) have reported that 199 (51.8%) were males and 185 (48.2%) were females in their study.

Prevalence of total ESBL in sputum samples was 10.20%. Prevalence of both ESBL and AmpC was 16.32 %. The total AmpC producers were 7.14%. A study by Shamweel Ahmad et al. showed 20% prevalence of ESBL producing Klebsiella in sputum samples. (Shamweel Ahmad, 2009) Laghawe Avinash showed 11.7% prevalence of AmpC in Klebsiella species. (Laghve Avinah et al., 2005)

The prevalence of Klebsiella species was 14.14% in sputum samples. 10.20% of Kebsiella isolates were ESBL producers. 7.14% of Klebsiella showed AmpC production. 16.32% of Klebsiella isolates were both ESBL as well as AmpC producer.

Chart.1

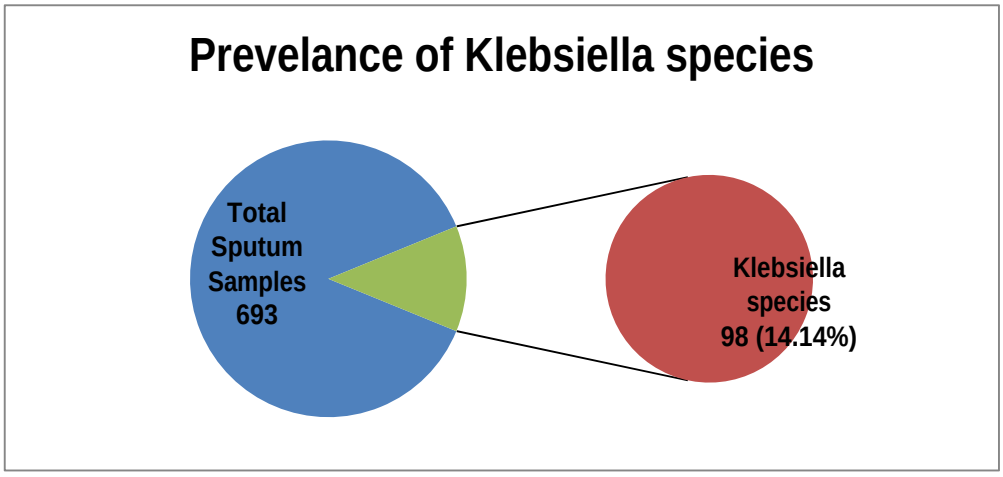


Chart.2

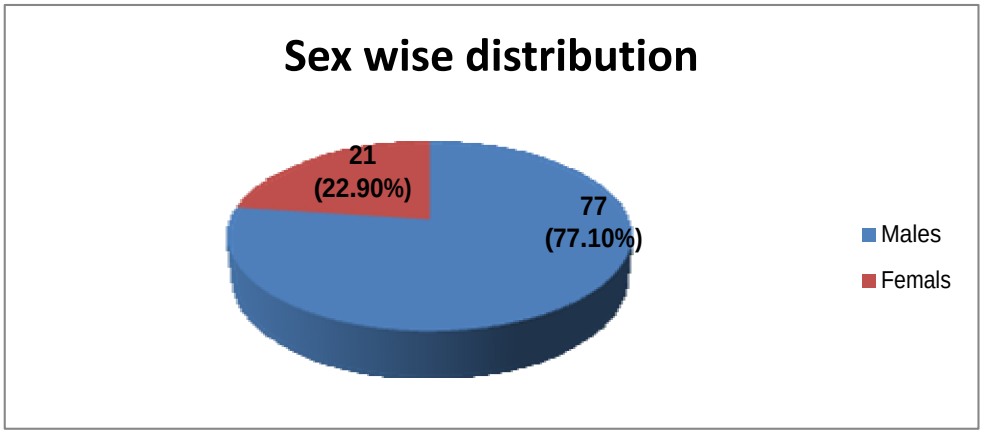


Chart.3

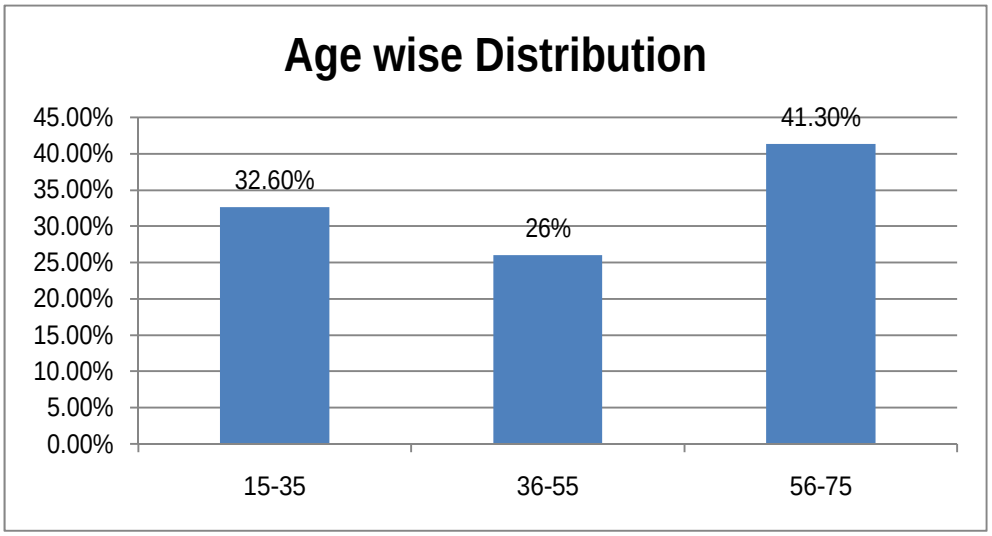


Chart.4 Prevalence of ESBL & AmpC Producers among Klebsiella species

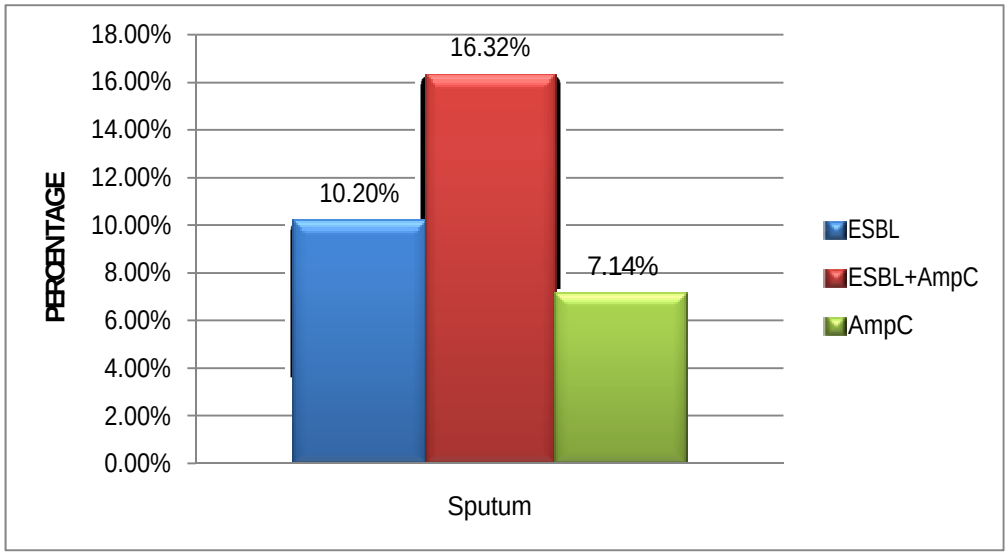


Chart.5 Antibiotic sensitivity pattern of Klebsiella species

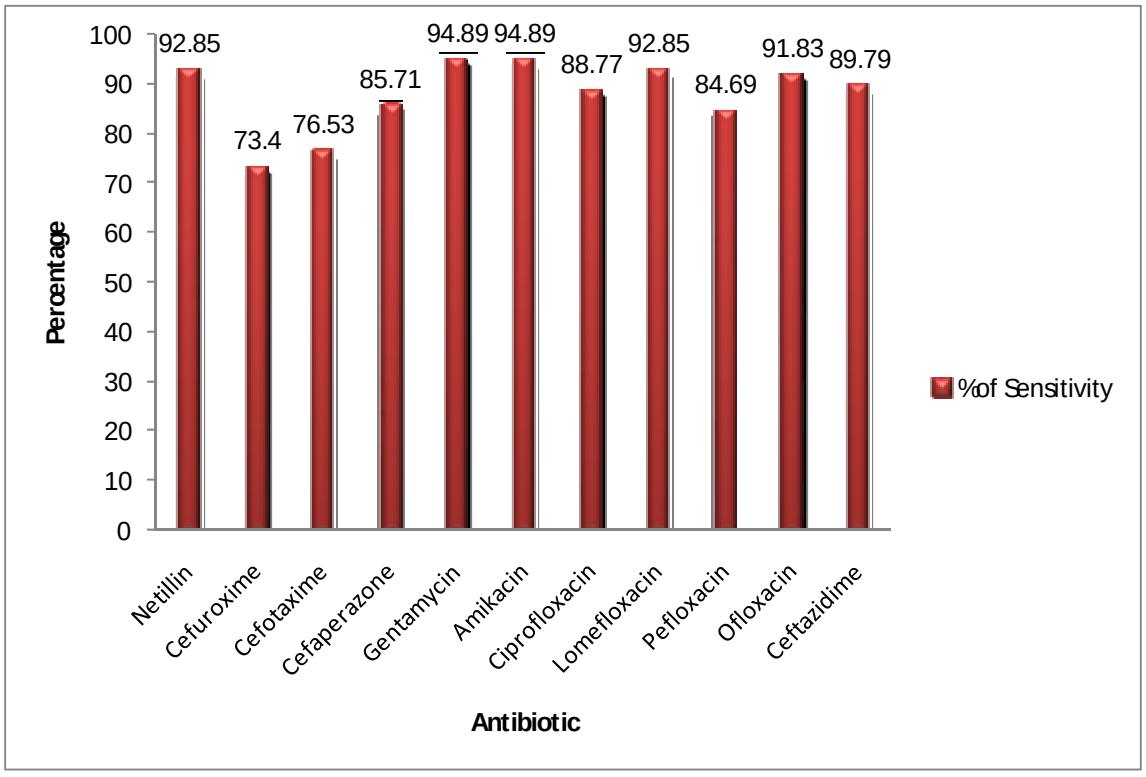
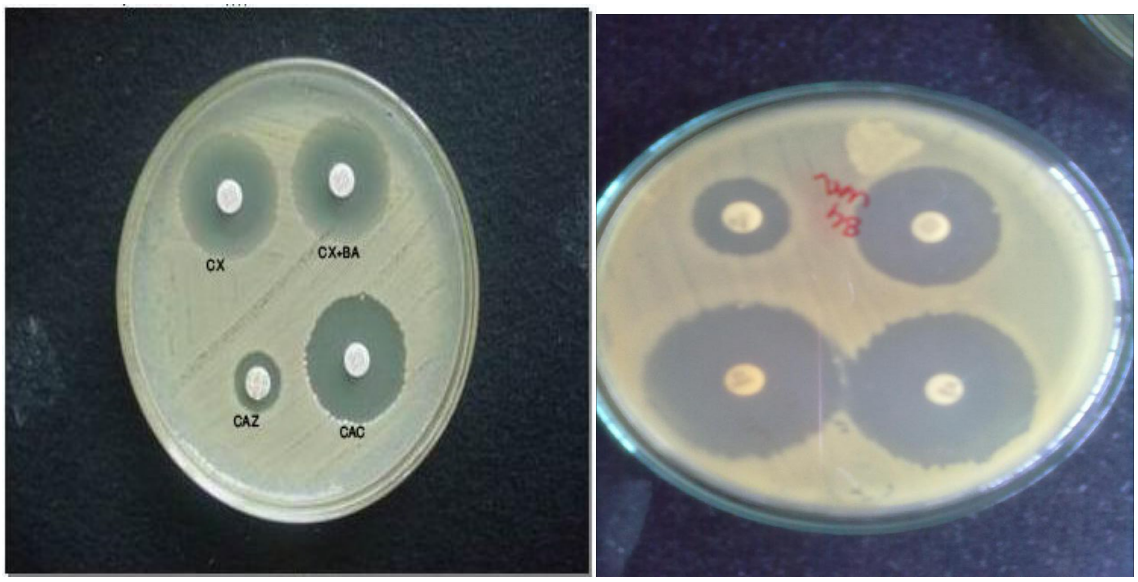


Fig.1 ESBL positive & AmpC negative AmpC positive & ESBL negative



Maximum sensitivity was observed against Gentamicin (94.89%) and Amikacin (94.89%). The least effective antibiotic was cefuroxime (73.4%).

The detection of ESBLs and AmpC β-lactamases by this method is simple and any microbiology laboratory can do it along with the routine antibiotic susceptibility testing

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