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Short Communication

Multi drug resistance and β-lactamase production by *Klebsiella pneumoniae*

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The extended spectrum β-lactamase (ESBL) production and multidrug resistant of *Klebsiella pneumoniae* in children below 5 years of ages are investigated. The *K. pneumoniae* strains isolated from patients suffering from intestinal and extra intestinal infection between the 0 - 5 year's ages of children showed resistant to the three antibiotics (ceftazidime, cefotaxime, ceftriaxone), and coexist with non β-lactam resistance and ESBL production. All the strains were susceptibility to the antibiotic, imipenem. Out off 110 strains only 9 strains produced ESBL. The plasmid responsible for the antibiotic resistance and ESBL production can be transferred to recipient *Escherichia coli* strain.

Key words: *Klebsiella pneumoniae,* drug resistance, ESBL production.

INTRODUCTION

Klebsiella pneumoniae is a successful opportunistic pathogen and has been associated with various ailments such as urinary tract infection, septicemia, respiratory tract infection and diarrhea (Podschu and Ullmann, 1998). In 1983, Extended Spectrum β-Lactamase (ESBL) was found to confer resistance to broad spectrum cephalosporin (Knoth, 1983). Since then the promising and usable spectrum of third generation cephalosporin (3GC) like cefotaxime, ceftriaxone and ceftazidime, in the treatment of multidrug resistant *K. pneumoniae* infection has been limited as resistant strain have been reported (Brun-Buisson et al., 1987; Jarlier et al, 1988; Legakis et al., 1995; Abigail et al., 1995, Hobson et al., 1996, Subha et al., 2003, Abdul Rahaman and Kumar, 2005). The ESBL are mediated by plasmids which encode enzymes that hydrolyze the oxyimino-β-lactams and monobactams (Aztreonam) but have no effect on cephamycins (cefoxitin and cefotetan), carbapenems (imipenem) and related compounds (Philippon et al., 1989). Most ESBL are mutant forms of sulphyl variable (SHV) enzymes coded by genes located on plasmid that can be easily spread from one organism to another (Sirot, 1995). These enzymes are capable of inactivating a variety of β-lactam drugs (Rice, 1999). plasmid that can be easily spread from one organisms to another (Sirot, 1995) these enzymes are capable of inactivating a variety of β-lactam drugs (Rice, 1999). The ESBL producing organisms often show multidrug resistant as the plasmid carry resistance to antibiotics (Paterson, 1997; Steward, 2001). The increasing antimicrobial resistance among ESBL producing bacteria makes therapy very difficult and leads to the use of expensive broad spectrum drugs, such as carbanpenems, which are known to be most effective antibiotics against these organisms (Livermore, 1998).

Children of less than five years of age group are very susceptible to intestinal and extra intestinal infections. In this study the ESBL prevalence and antimicrobial susceptibility of *K. pneumoniae* among the children under the 0 - 5 year's ages was investigated.

MATERIALS AND METHODS

Microorganisms

The clinical samples including blood, urine, stool and sputum are collected from the patient aged 0 - 5 years, at Government Hospital and Medical College, Anantapur, Andhra Pradesh, India, from the month of April 2005 to March 2006. The pathogenic microorganism *K. pneumoniae* was isolated and identified based on the colony morphology and biochemical reactions (Koneman et al., 1992) and microbial populations were counted. The *E. coli* ATCC (25922) and

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Table 1. ESBL producing *Klebsiella Pneumoniae* isolates (110) and in their susceptibility categories by disc diffusion method.

Susceptibility category	No. (%) of ESBL producing isolates with in each category with 30 mg disc of antibiotic			
	Aztreonam	Cefotaxime	Ceftazidime	Cefoxitin
Susceptible	18 (16.3)	17 (15.4)	19 (17.3)	79 (71.8)
Intermediately resistant	15 (13.6)	54 (49)	25 (22.7)	8 (7.3)
Resistant	77 (70)	39 (35.4)	66 (60)	23 (20.9)

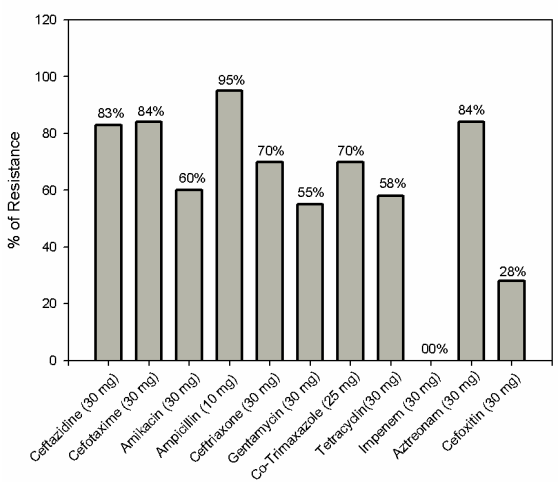

Figure 1. Antibiotic resistance pattern of *Klebsiella Pneumoniae* isolates.

K. pneumoniae (48188) were obtained from IMTECH, Chandigarh, India.

Double disc approximation test

ESBL production was carried out by double disc approximation test (Jarlier et al., 1988). The strains were pre-incubated in brain heart infusion broth (BHIB) at 37°C and the optimal density of 0.5. This bacterial suspension was swabbed with sterile cotton on to a Mueller- Hinton agar medium. The antagonistic tests were conducted with antibiotic discs of Amoxicillin/Clavulanic acid (20/10 mg) and cefotoxime (30 mg) were placed at a distance of 15 cm apart from each other and incubated. After incubation the antibiotic activity of Cefotaxime was determined by the formation of inhibition zone.

NCCLS confirmatory test

The Antibiotic resistance bacterial strain was confirmed by NCCLS (2000) confirmatory test. While performing antibiotic testing ceftazidime (30 mg) and ceftazidime and clavulanic acid (30/10 mg) were placed on Mueller Hinton agar media and incubated. After incubation the zone of inhibition was measured by standards method (Bal, 2000).

Transformation test

The antibiotic resistant and ESBL mediating gene was transferred to recipient *E. coli* K12 strains. The transconjugates were selected on Macon key agar containing antibiotics nalidixic acid (125 mg/ml) and cefotaxime (0.25 µg/ml) and identified biochemically by the method of Koneman et al. *(*1995).

RESULTS AND DISCUSSION

The antibiotic resistance pattern and activity of *K. pneumoniae* was detected and shown in the Table 1 and Figure 1. Out off 110 isolates of *K. pneumoniae*, all are found to be resistant to 3rd generation antibiotics (3GC) and these isolates showed multidrug resistance. 88 (80%) of the isolates showed resistance or decreased susceptibility to various 3GC antibiotics (ceftazidime, cefotaxime, ceftriaxone) and coexisted with other antibiotics (Figure 1.) According to Padmini (2004), 90% of *K. pneumoniae* strains showed resistance to the 3GC antibiotics. Jacoby (1999) also reported that a particular plasmid is responsible for drug resistance and ESBL production. All the isolates were found susceptibility to the antibiotic imipenem. The ESBL production against the antibiotics, ceftazidime, cefotaxime and ceftriaxone was detected in 9 strains of *K. pneumoniae* (6 from urine, 2 from stool and 1 from sputum). The gene responsible for resistance to the 3GC antibiotics and ESBL production was transferred to the recipient *E. coli* strain from 9 ESBL positive isolates. The Transconjugates were selected on Macon key agar containing the antibiotics nalidixic acid (125 mg/ml) and cefotaxime (0.25 µg/ml) and identified with the biochemical method of Koneman et al. *(*1995). Similar studies were made Jarlier et al. (1998).

This study has revealed the occurrence of β-lactamase producing strains of *K. pneumoniae* recovered from children with urinary tract infection, septicemia and respiratory tract infections. ESBL mediating resistance to 3GC was found in 9% of isolates. The prevalence rate is higher than the reported figure of *K. pneumoniae* in Canada (6.2%) and United States of America (Jones et al., 1999) and lower in India (Padmini and Applaraju, 2004). The incidence of ESBL producing strains among clinical *Klebsiella* isolates has steadily increased over the years and account for 6 to 17% of all nosocomial urinary tract infections. The detection rate of ESBL producing *Klebsiella* isolate in stool sample ranges from 5 to 38%. While rates in the nasophrynx range from 1 to 6%. In our study, isolates were obtained from stool (8.3%), urine (8.5%) and respiratory tract infection (16.6%). In addition to 3GC antibiotics, the isolates showed resistance to Amikacin (55%) Tetracycline, (58%) Co-trimoxozole (70%) and Gentamicin (55%).

The ESBL mediated and resistance gene was transferred to the recipient *E. coli* K 12 strain reported earlier (Jarlier et al., 1998). These plasmids are easily transmitted among bacteria and this accounts for ESBL producing isolates that are resistance to a variety of antibiotics. According to Podschu and Ullmann (1998), the multidrug resistant *Klebsiella* strain is unfortunately accompanied by a relatively high stability of the plasmids. The present study has shown the incidence of multidrug resistant and ESBL producing *Klebsiella* isolates among children in Anantapur. Hence routine diagnosis should be carried out to detect ESBL mediated 3GC resistant isolates.

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