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Extended spectrum beta lactamase production & multidrug resistance in *Klebsiella* species isolated from children under five with intestinal & extraintestinal infections

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Background & objectives: **Extended spectrum β-lactamases (ESβL) are enzymes produced in some Gram-negative bacilli that mediate resistance to third generation cephalosporins (3GC) and aztreonam. These are common in *Klebsiella* spp. and *Escherichia coli* and in other members of the family enterobacteriaceae. ESβL production is accompanied by resistance to other antibiotics as these are encoded by multi drug resistance conjugative plasmids. The present study was undertaken to study the incidence of multi drug resistant and ESβL producing *Klebsiella* spp. in children under five years of age suffering from intestinal and extraintestinal infections.**

Methods: **A total of 90 strains of *Klebsiella* spp. (76 isolates of *K. pneumoniae* and 14 of *K. oxytoca*) were tested for resistance to 3GC antibiotics (ceftazidime, cefotaxime, ceftriaxone), amikacin, ampicillin, erythromycin, gentamycin and streptomycin by disc diffusion method. Isolates found resistant to 3GC antibiotics were tested for the production of ESβL by double disc diffusion synergy test. Transconjugation experiments were done to study the transfer of drug resistance and ESβL production from *Klebsiella* isolates to an *Esch. coli* strain (K12 J62-2).**

Results: **All the 90 isolates showed multi drug resistance; 87 (96.6%) isolates showed resistance or decreased susceptibility to at least one of the three 3GC. ESβL production was detected in four strains of *K. pneumoniae* and two *K. oxytoca*. ESβL activity could be experimentally transferred to recipient *Esch. coli* in all the 6 isolates. Resistance to β-lactam antibiotics was co-transferred along with resistance to gentamycin.**

Interpretation & conclusion: **This study has shown the incidence of ESβL producing *Klebsiella* strains in children in Chennai, and possibly poses a threat in the treatment and management of *Klebsiella* associated infections. The incidence of ESβL producing strains of *Klebsiella* and other members of enterobacteriaceae should be carefully monitored in children to prevent unnecessary use of antibiotics especially 3GC and aminoglycoside antibiotics. Hence, tests for the detection of ESβL producing *Klebsiella* strains should be carried out routinely for better therapeutic management.**

Key words Cefotaxime - ceftriaxone - ceftazidime - ESβL-*Klebsiella* spp. - multidrug resistance - third generation cephalosporins (3GC)

Klebsiella spp. are an important group of bacteria, which are involved in various ailments such as urinary tract infections, septicaemia, respiratory tract infections and diarrhoea[1]. Klebsiellae and several

members of enterobacteriaceae produce extended spectrum beta lactamases (ESβL), which confer resistance to oxyimino-β-lactams such as cefotaxime, ceftazidime and aztreonam antibiotics, that were designed to be effective against strains producing β-lactamases[2,3]. After the recognition of ESβL producing *Klebsiella pneumoniae* in Germany in 1983[4], these have been increasingly reported from around the world[5]. ESβL producing strains are resistant to a variety of antibiotics due to accumulation of resistance genes transmitted through plasmids among different members of enterobacteriaceae[1]. Due to extensive spread of plasmid mediated multi-resistant strains, particularly ESβL producing strains, there has been renewed interest in *Klebsiella* infections[1].

ESβLs are distinguished into more than 30 types based on their physical properties and all are inhibited by clavulanate, sulbactam and tazobactam, a property which has been used to detect these in the laboratory[6]. Occurrence of ESβL producing *Klebsiella* spp. has been reported from south India[7] and central India[8]. ESβL producing Klebsiellae and other enterobacteria pose a serious threat to β-lactam therapy and demand new measures for the management of infections caused by these bacteria[1].

In view of the increasing reports of ESβL producing *Klebsiella* strains from clinical samples, the present study was conducted with an objective to examine the incidence of multi drug resistant and ESβL producing strains among the *Klebsiella* spp. recovered from children under 5 yr of age, suffering from intestinal and extraintestinal infections. Children of this particular age group (0-5 yr) were included in this study as they are considered more susceptible to such infections. Moreover, there is not much information available on incidence of ESβL producing *Klebsiella* isolates in this age group. Since Klebsiellae are also considered as an important source of transferable drug resistance[9], transmissibility of drug resistance was also examined among these isolates.

Material & Methods

Clinical isolates: A total of 90 clinical isolates of *Klebsiella* spp. inclusive of 23 isolates from children with septicaemia, 48 from urinary tract infections, 16 from acute diarrhoeal cases and 3 from respiratory tract infections were obtained from patients 0-5 yr of age attending Institute of Child Health and Hospital for Children, Chennai during October 1998 to March 1999. *Klebsiella* isolates that were obtained as a pure and predominant growth from the clinical specimens were only considered for the present study. The organisms were identified and speciated based on colony morphology and biochemical reactions[10]. Sensitivity to third generation cephalosporins (3GC) *viz.*, ceftazidime, cefotaxime, ceftriaxone each 30 μg/disc (Hi-Media, India) was tested by disc diffusion method[11] and interpreted as per the criteria of National Committee for Clinical Laboratory Standards (NCCLS)[12]. Isolates with resistance or with decreased susceptibility (intermediate by NCCLS criteria) to any of the 3GC were selected for further study. All the 90 isolates were found to be resistant to at least one of the three 3GC and were selected for further study. *Escherichia coli* ATCC 25922 strain (culture collection of Department of Microbiology, Dr. ALM Postgraduate Institute of Basic Medical Sciences, Chennai) was used for quality control.

ESβL detection by double disc diffusion synergy test (DDST): In the DDST, synergy was determined between a disc of augmentin (20 μg amoxycillin and 10 μg clavulanic acid) and a 30 μg disc of each of the 3GC tested placed which were at a distance of 30mm apart on a lawn culture of the resistant isolate under test on Mueller-Hinton Agar (MHA) (Hi-Media, Mumbai)[13]. The test organism was considered to produce ESβL, if the zone size around the test antibiotic disc increased towards the augmentin disc. This increase occurs because the clavulanic acid present in the augmentin disc inactivates the ESβL produced by the test organism.

Susceptibility to other antibiotics: The sensitivity of the *Klebsiella* isolates was determined by the disc diffusion method[11] to amikacin (30 μg), ampicillin (10 μg), erythromycin (15 μg), gentamycin (10 μg), and streptomycin (10 μg), (Hi-Media, India). The results were interpreted as per NCCLS recommendations[12].

Transfer of 3GC resistance and ESβL production: Conjugal transfer of 3GC resistant and ESβL producing strains was done at 37°C using *Esch. coli* K12 J62-2 (provided by Dr M.V. Jesudason, Professor and Head, Department of Clinical Microbiology, Christian Medical College and Hospital, Vellore) as recipient. Aliquots of exponentially growing Brain Heart Infusion (BHI) broth cultures of donor and recipient strains were mixed (10:1 ratio) and allowed to mate in BHI broth at 37°C for 18 h. Transconjugants were selected on MHA plates containing ceftazidime (2μg/ml) and rifampicin (2.5 mg/ml)[14].

Serotyping of the *Klebsiella* isolates obtained from stool specimens was done at Statens Serum Institute, Copenhagen.

Results & Discussion

Of the 90 isolates of *Klebsiella* spp., 76 isolates (17 from blood, 43 from urine, 14 from stool and 2 from throat swab) were speciated as *K. pneumoniae* sub sp. *aerogenes* and 14 isolates (6 from blood, 5 from urine, 2 from stool and 1 from throat swab) were speciated as *K. oxytoca*. All the 90 isolates showed multi drug resistance and 87 (96.6%) isolates showed resistance or decreased susceptibility to at least one of the three 3GC tested. Fifty nine (65.5%) isolates showed resistance to all the three 3GC antibiotics (ceftazidime, cefotaxime, ceftriaxone) and this was found to coexist with resistance to other antibiotics.

ESβL production against ceftazidime, cefotaxime, ceftriaxone was detected in four strains of *K. pneumoniae* (2 from urine and 2 from stool specimens) and in two strains of *K. oxytoca* (one each from urine and stool specimen). The ESβL positive *Klebsiella* isolates, *K. pneumoniae* and *K. oxytoca*, recovered from stool specimens were found to belong to the K-types K20 and K54 respectively.

The incidence of ESβL producing *Klebsiella* strains was found to be 6.6 per cent in the present study which was relatively lower compared to a previous study from central India, where 76.5 per cent of *Klebsiella* isolates resistant to 3GC antibiotics were found to produce ESβLs by DDST[8].

During the past decade, ESβL producing *K. pneumoniae* have emerged as one of the major multi drug resistant organisms[15]. The incidence of ESβL-producing *Klebsiella* isolates in the United States has been reported to be 5 per cent[16]. In France and England 14 to 16 per cent ESβL producing *Klebsiella* isolates have been reported[17]. In particular regions or hospitals, the incidence of ESβL producing *Klebsiella* isolates has been reported to be 25 to 40 per cent[18]. In a report from France, 24.8 per cent ESβL positive *K. pneumoniae* were found in patients of more than 16 yr of age[15]. In another report from France, 3.5 per cent of ESβL producing *K. pneumoniae* was obtained in patients with a mean age of 66 yr[19].

The incidence of ESβL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past years and accounts for 6 to 17 per cent of all nosocomial urinary tract infections, 5 to 38 per cent of isolates from stool and 1 to 6 per cent from respiratory tract infections[1]. In our study 18.7 per cent of the isolates obtained from stool and 6.2 per cent of the isolates obtained from urine were found to be ESβL producers.

In addition to resistance to 3GC, (94.4% to ceftazidime, 63.3% to cefotaxime, 74.4% to ceftriaxone) 47 per cent isolates showed resistance to amikacin, 96.2 per cent to ampicillin, 93.5 per cent to erythromycin, 64.67 per cent to gentamycin and 61 per cent to streptomycin (Fig.); 26.2 per cent of the isolates showed 8 drug resistance pattern, 24 per cent showed 7 drug resistance pattern. The resistance to 3GC was found to coexist with resistance to other antibiotics. A recent report has highlighted the emergence of ESβL producing strains endowed with extremely wide spectrum of antibiotic resistance, including resistance to sulfonamides, trimethoprim, streptomycin, kanamycin, gentamycin and amikacin[20]. The resulting limitations on the therapeutic options demand new measures for the management of *Klebsiella* infections.

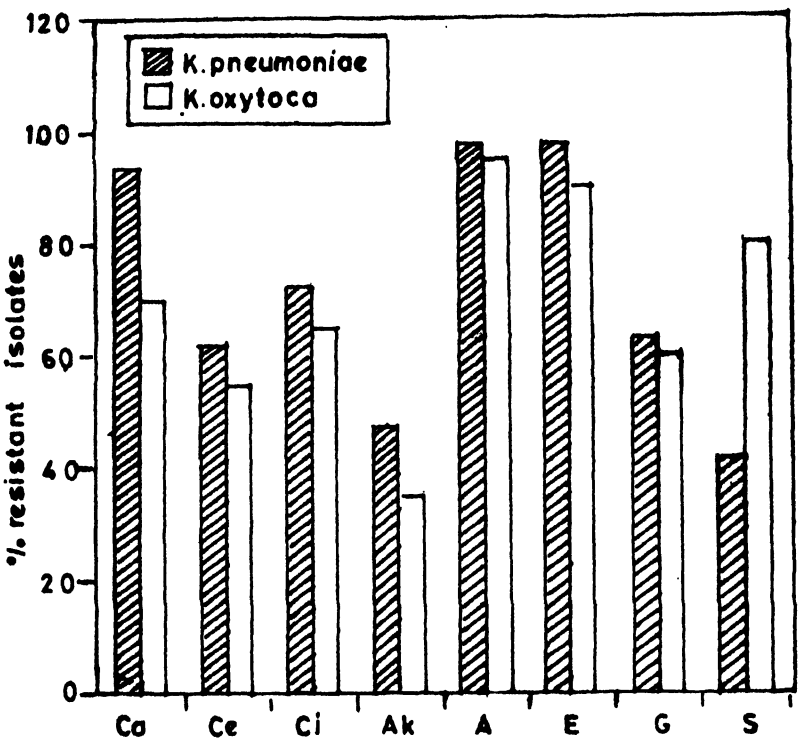

Fig. Antibiotic resistance pattern of the *Klebsiella* isolates. Ca-ceftazidime (30 μg), Ce-cefotaxime (20 μg), Ci-ceftriaxone (30 μg), Ak-amikacin (30 μg), A-ampicillin (10 μg), E-erythromycin (15 μg), G-gentamycin (10 μg), S-streptomcyin (10 μg).

Klebsiella spp. are an important source of transferable antibiotic resistance. In our study, the resistance to 3GC was transferred to the recipient *Esch. coli* strain along with resistance to gentamycin. In a recent study from Spain, all the *K. pneumoniae* isolates obtained in an outbreak in a paediatric ward were multi drug resistant, with resistance to 3GC, gentamycin and amikacin and resistance to β-lactams and gentamycin was cotransferred[21]. ESβL production is coded by genes that are located on large conjugative plasmids of 80-160 Kb in size[20]. In the present study ESβL production was transferred to the recipient *Esch. coli* strain from all the six ESβL positive isolates indicating plasmid mediated ESβL production. Since these plasmids are easily transmitted among different members of the enterobacteriaceae accumulation of resistance genes results in strains that contain multi drug resistant plasmids. For this reason, ESβL-producing isolates are resistant to a variety of antibiotics. Moreover, the emergence of these multiply resistant *Klebsiella* strains is accompanied by a relatively high stability of the plasmids encoding ESβLs[1].

There seems to be a wide disparity in the incidence of ESβL positive *Klebsiella* infections in different age groups. The incidence of ESβL producing strains of *Klebsiella* spp. and other members of enterobacteriaceae needs to be carefully monitored in children to prevent unnecessary use of 3GC and aminoglycoside antibiotics. Hence, it is suggested that routine diagnosis of ESβL producing strains should be carried out in children in order to avoid undesired effects of multi drug resistant and ESβL producing *Klebsiella* by strict control of antibiotic usage.

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