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PHENOTYPES OF ESBL-PRODUCING *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES RECOVERED FROM PEDIATRIC SUBJECTS IN CHENNAI, SOUTHERN INDIA

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ABSTRACT

Production of extended spectrum β -lactamase (ESBL) is a common mechanism by which most gram-negative bacteria develop resistance to third-generation cephalosporins. This prospective investigation examined the occurrence of ESBL-producing and multi-drug resistant phenotypes of *K. pneumoniae* and *E. coli* in pediatric subjects with certain underlying infectious conditions. Over a period of four months from April to June 2002, a total of 80 isolates recovered from clinical specimens of children between 0 and 5 years of age were investigated. Upon identification, the antibiogram profiles of the isolates were determined and those resistant to third-generation cephalosporins were tested for production of ESBL. Almost 96% of *K. pneumoniae* and 87.5% of *E. coli* isolates recovered showed resistance to at least one of the three third-generation cephalosporins [3GCs(ceftazidium, cefotaxime and ceftriaxone)] tested. Further, ~54% of the isolates showed resistance to all the 3GCs tested. All the isolates were susceptible to imipenem, and ESBL-production was detected in two *K. pneumoniae* and one isolate of *E. coli*. Our study documents the occurrence of ESBL-producing *K. pneumoniae* and *E. coli* among pediatric population in South India. This underscores the need for closely monitoring the incidence of ESBL-producing strains among clinical isolates of *K. pneumoniae* and *E. coli*.

Key Words: Antibiotic Resistance, ESBL, *Klebsiella Pneumoniae*, Multi-Drug Resistance, Double Disk Diffusion Synergy Test

INTRODUCTION

Ever since the first report of resistance of gram-negative bacteria to third- generation cephalosporins namely oxyimino β -lactams became available in the mid-1980s, there has been a dramatic increase in the occurrence of resistant strains worldwide (Sinha et al., 2008, Mehrgan et al., 2010). These bacteria reportedly produce enzymes called extended spectrum β -lactamases (ESBLs) that can hydrolyze oxyimino-cephalosporins, thereby conferring resistance to extended spectrum cephalosporins (Al-Agamy 2009). ESBLs are predominantly produced by *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, though there have been reports of ESBL production in salmonellae, *Pseudomonas aeruginosa* and certain members of enterobacteriaceae (Behroozi 2010). Extended spectrum cephalosporins being the drugs of choice against most nosocomial infections, ESBL-producing gram-negative organisms pose a serious threat to hospitalized patients, especially children (Andriatahina et al., 2010). This makes it necessary for the health care providers to be cognizant of their prevalence in community settings to devise empirical treatment policies in high-risk units in hospitals (Sinha et al., 2008, Taneja et al., 2008, Tsering et al., 2009). Importantly, indiscriminate use of antibiotics over the years has dramatically increased the frequency of occurrence of ESBL-producing bacteria in the general population. Furthermore, since conventional antibiotic susceptibility methods fail to detect these organisms, the development and implementation of easily accessible advanced screening methods is needed to effectively screen for resistant strains and devise infection control strategies (Leverstein-van et al., 2002, Sinha et al., 2008, Varaiya et al., 2008).

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We examined the occurrence of ESBL-producing and multi-drug resistant isolates of *K. pneumoniae* and *E. coli* in clinical specimens from children between 0 and 5 years of age. We chose to investigate children owing to their easy vulnerability to intestinal and extra-intestinal infections and due to paucity of reports on ESBL-producing bacteria in this age group in the Indian setting.

MATERIALS AND METHODS

A total of 80 non-repetitive gram-negative bacterial isolates were obtained from clinical specimens of children (0 - 5 years old) with septicemia, lower respiratory, urinary, wound infections and acute diarrhoea, attending the outpatient unit at the Institute of Child Health and Hospital for Children, Chennai, over a period of four months from April to June 2002. Only those predominant isolates obtained as pure culture from these specimens were investigated. The isolates were identified based on standard biochemical characterization methods (Collee 1996).

The sensitivity of isolates to third-generation cephalosporins (3GCs), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg) and to other antibiotics such as ampicillin (10µg), amikacin (30µg), cefuroxime (30µg), cefoxitin (30µg), gentamicin (10µg), imipenem (10µg) and ciprofloxacin (30µg) (HiMedia, Mumbai, India) was determined by disc diffusion method and the results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) guidelines (NCCLS 2000). *E. coli* ATCC 25922 was used as the quality control strain for each antimicrobial susceptibility assay. Isolates that showed intermediate or complete resistance to any of the 3GCs were selected for further ESBL detection by double disk diffusion synergy test (DDST) as described by others (Sridhar 2008, Subha 2002). A lawn culture of the test isolate on Mueller Hinton agar (HiMedia, Mumbai, India) was made with the 3GC discs and a disc of co-amoxycylav (20µg amoxicillin/10µg clavulanic acid) placed 30mm apart. The test organism was considered to produce ESBL if the zone size around the test disc increased towards the co-amoxycylav disc. This occurs because clavulanic acid present in the disc inactivates ESBL produced by the test organism.

RESULTS

Among the 80 isolates, 56 (70%) were *K. pneumoniae* (40 from urine, 10 from wound, 2 from blood, 2 from sputum and 2 from stool specimens) and 24 (30%) were *E. coli* (10 from urine, 10 from wound and 4 from faecal specimens). Table 1 shows the antimicrobial resistance pattern of isolates tested. *K. pneumoniae* (96.4%) and *E. coli* (87.5%) isolates showed resistance or decreased sensitivity to ceftazidime. All the isolates of *K. pneumoniae* and *E. coli* were resistant to ampicillin and other non-β-lactam antibiotics like amikacin and gentamicin. Whereas 35.7% of *K. pneumoniae* and 29% of *E. coli* isolates showed resistance to ciprofloxacin, all were susceptible to imipenem. Importantly, all the isolates were resistant at least to a minimum of three antibiotics and hence were multi-drug resistant. *K. pneumoniae* (26.8%) and *E. coli* (29%) isolates demonstrated complete resistance to all antibiotics tested excepting imipenem. The multi-drug resistance pattern pertinent to *K. pneumoniae* and *E. coli* is listed in table 2.

Fifty four isolates of *K. pneumoniae* (96.4%) and 21 *E. coli* (87.5%) isolates showed resistance to at least one of the three 3GCs tested, while 53.6% of *K. pneumoniae* and 54.2% of *E. coli* isolates showed resistance to all the 3GCs tested. This resistance pattern was found to co-exist with resistance to other antibiotics. All isolates were resistant to aminoglycoside derivatives, gentamicin and amikacin. ESBL production against ceftazidime, cefotaxime and ceftriaxone was detected in two *K. pneumoniae* isolates and one *E. coli* isolate. However, all the three ESBL-producing isolates demonstrated a similar four-drug resistance pattern against amikacin, ampicillin, ceftazidime and gentamicin.

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Table 1: Antimicrobial resistance phenotypes of *Klebsiella pneumoniae* and *Escherichia coli* isolates.

Antibiotic (μ g)	<i>Klebsiella pneumoniae</i> Resistance (%) n=56	<i>Escherichia coli</i> Resistance (%) n=24
Ceftazidime (30)	96.4	87.5
Cefotaxime (30)	53.6	54.1
Ceftriaxone (30)	53.6	54.1
Amikacin (30)	100	100
Ampicillin (10)	100	100
Gentamicin (10)	100	100
Cefoxitin (30)	89.3	5.4
Cefuroxime (30)	89.3	5.4
Imipenem (30)	0	0
Ciprofloxacin (30)	35.7	29.1

Table 2: Multi-drug resistance pattern of *Klebsiella pneumoniae* and *Escherichia coli* isolates.

Multi-drug Resistance Pattern	<i>Klebsiella pneumoniae</i> Resistance (%) n=56	<i>Escherichia coli</i> Resistance (%) n=24
A Ak Ca G	10.7	87.5
A Ak Cn Cu G	-	20.8
A Ak Cn Cu Cf Ca G	5.4	8.3
A Ak Ci Ce Ca Cn Cu G	17.9	45.8
A Ak Ci Ce Ca Cn Cu Cf G	26.8	29.1

Abbreviations: A-ampicillin 10 μ g, Ak-amikacin 30 μ g, Ca-ceftazidime 30 μ g, Ci ceftriaxone 30 μ g, Ce-cefotaxime 30 μ g, Cu-cefuroxime 30 μ g, Cn-cefoxitin 30 μ g, G-gentamicin 10 μ g, I-imipenem 10 μ g, Cf-ciprofloxacin 30 μ g.

DISCUSSION

Despite test organisms being reported to be susceptible to antibiotics by routine antimicrobial susceptibility testing, the risk of treatment failure persists while treating infections caused by ESBL-producing bacteria with extended spectrum cephalosporins. With over 17% of nosocomial urinary infections and other community-acquired infections reportedly being caused by *K. pneumoniae* and *E. coli*, it is imperative to monitor the incidence of ESBL-producing strains in clinical specimens (Subha and Anandan 2002, Oteo et al., 2006, Sridhar et al., 2008). Based on the physical properties and inhibition

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attributed by clavulanate, sulbactam and tazobactam, ESBLs are categorized into more than 30 types (Ensor et al., 2006, Al-Agamy et al., 2009). Several phenotypic tests have been developed to detect ESBL-producing organisms namely, identifying a reduction in susceptibility to extended-spectrum cephalosporins and inhibition by clavulanate in double-disk synergy test, Etest and the VITEK 1, VITEK 2, and Phoenix automated instruments based MIC determination for ceftazidime (Vercauteren et al., 1997, Tsering et al., 2009). Apart from routine antimicrobial susceptibility testing assays, detection of ESBL-production will provide clinicians with helpful information on making treatment decisions and ensures timely detection and prevention of nosocomial infection outbreaks.

The Clinical and Laboratory Standard Institute (CLSI) recommends screening of clinically relevant isolates of *E. coli*, *K. pneumoniae* and *K. oxytoca* for potential production of ESBL by an initial screening test followed by a phenotypic confirmatory test (CLSI 2007). Susceptibilities to cefpodoxime, ceftazidime, ceftriaxone, cefotaxime and aztreonam are evaluated using disc diffusion as the initial screening procedure. A decrease in susceptibility to one or more antibiotics tested may indicate production of ESBLs and warrant subsequent phenotypic confirmatory tests, where susceptibilities to cefotaxime and ceftazidime alone and those with clavulanate are compared using the disc diffusion method. If the susceptibility to either of the antibiotics tested increases significantly in the presence of clavulanate, the result is interpreted as confirmatory for ESBL production.

There have been reports of considerable geographical differences in ESBLs worldwide and within countries. The hospital-to-hospital variability that occurs possibly reflects the direct proportionality between use and misuse of antibiotics. In India, prevalence rate of ESBLs varies from 28 to 84% (Al-Agamy et al., 2009, Ensor et al., 2006). While earlier reports from North India have documented the prevalence of ESBLs ranging from 55 to 69%, a recent study from north India reported an alarming 64.8% of ESBL-producing isolates (Tankhiwale et al., 2004, Ensor et al., 2006). In Southern India, 21.6% ESBL-producing bacteria have been documented in a study from Karnataka and a Coimbatore report detected about 40% ESBL producers (Tsering 2009, Sridhar 2008). One similar report from Chennai found ESBL mediated resistance to 3GCs in 6.6% of the isolates recovered (Subha and Anandan 2002).

High prevalence of ESBL-producing strains has been reported predominantly in *Klebsiella* species (Tankhiwale 2004) and in the present study 3.75% of the isolates (two *K. pneumoniae* and one *E. coli*) tested positive for ESBL mediated resistance to 3GC. While it is encouraging that the rate of detection of ESBL-producing strains is much lower than other reports from India and abroad, this disparity could originate possibly owing to their lower occurrence in children, the target group in our study.

It is of interest to note that in a study comparable to ours conducted in pediatric patients with septicemic infections; the prevalence of ESBL-producers was much higher, 80% in *Klebsiella* spp. and 63.6% in *E. coli* (Anandan et al., 2009). However, this study was carried out for an extended duration of five years (2003 - 2007).

Treatment recommendations for infections caused by organisms producing ESBL's currently include carbapenems in the first-line therapy and ciprofloxacin and β -lactam/ β -lactamase inhibitor combinations as the second-line therapy. Since a substantial proportion of *K. pneumoniae* (35%) and *E. coli* (29%) isolates in our study showed resistance to fluoroquinolones like ciprofloxacin, these results urge the need for formulating an effective alternative strategy. Since all the isolates tested were sensitive to imipenem, it might serve as the drug of choice against ESBL-producing *K. pneumoniae* and *E. coli*. Thus, due care should be undertaken to minimize indiscriminate usage of imipenem and associated derivatives.

In conclusion, our study highlights the emergence of ESBL-producing strains endowed with extremely wide spectrum of antibiotic resistance and the resulting limitation on available therapeutic options. The results reveal the demand for newer control strategies of infections caused by gram-negative bacilli and emphasise the need to closely monitor the incidence of ESBL-producing strains among clinical isolates of *K. pneumoniae* and *E. coli*. Thus, the study warrants the detection of ESBL-producing organisms in clinical laboratories in addition to routine antimicrobial susceptibility testing assays.

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