ABSTRACT

Extended-spectrum β-lactamases (ESBLs) are enzymes that can hydrolyze extended-spectrum cephalosporins and monobactams. ESBL-producing *Klebsiella pneumoniae* are responsible for serious morbidity and mortality among paediatric patients. This study aimed to determine the frequency of ESBL-producing *K. pneumoniae*, phenotypic characterization techniques and antimicrobial resistance pattern. The study was also established to determine the molecular characterization of bla_{SHV}, bla_{TEM}, bla_{CTX-M} genes which are responsible for ESBL-mediated antibiotic resistance.

The study was conducted at The Children's Hospital & Institute of Child Health, Lahore, Pakistan during May 2010 to February 2012. The molecular studies of bla_{SHV}, bla_{TEM}, bla_{CTX-M} and integron genes were performed during October 2012 to April 2013 at the Microbiology and Immunology Department, The University of Melbourne, Australia. Various clinical samples were collected and studied from paediatric patients, including blood, central venous pressure line, cerebrospinal fluid, ear swab, endotracheal tube, peritoneal dialysis catheter, pleural fluid, pus, tracheal secretion, urine and wound swab. The organisms were identified using various biochemical tests and the API 20E system. ESBL production was determined using double disk synergy test (DDST) and Clinical and Laboratory Standards Institute (CLSI) confirmatory test. The antimicrobial resistance pattern of ESBL-producing *K. pneumoniae* was determined using Kirby-Bauer disc diffusion method with various antibiotic groups. The target genes were amplified and DNA sequencing was performed for bla_{SHV} and bla_{TEM} genes to find out the mutations responsible for ESBL genotype.

Screening of 710 *K. pneumoniae* isolates showed 214 (30.1%) were ESBL screen positive *K. pneumoniae*. The CLSI confirmatory test showed significantly greater sensitivity ($p<0.0001$) compared to DDST. There were 82 (38.3%) neonates infected with ESBL *K. pneumoniae* and 152 (71.0%) of the total cases were males. The most common sources of ESBL *K. pneumoniae* were blood (117; 54.7%) and urine (46; 21.5%). Of the 214 cases, 92 (43.0%) cases were isolated from Neonatal Nursery Unit and (47; 22.0%) Nephrology. Patients presented with various symptoms such as fever (125 cases; 58.4%) and respiratory distress (104 cases; 48.6%). Important interventions given to the patients

included intravenous line (209 cases; 97.7%), urinary catheters (46; 21.5%) and endotracheal tube (18; 8.4%). The outcome of the patients showed the successful discharge of 127 (59.0%) patients after treatment while there were 56 (26.0%) cases of mortality and 31 (15.0%) left against medical advice (LAMA). There was no significant correlation (p=0.1396) found between length of stay and mortality of the patient. Neonates infected with *K. pneumoniae* had a significantly higher chance of mortality than the older age groups (p=0.0140), while there was no association of outcome (p=1.0000) between the two genders. A higher mortality rate (p=0.0005) was seen among the septicemic patients. The mortality rate was significantly higher (p=0.0013) in patients who presented with respiratory distress symptoms.

An antibiotic resistance profile of ESBL-producing *K. pneumoniae* was performed against 18 antibiotics. All ESBL *K. pneumoniae* isolates were resistant to ceftazidime, ceftriaxone, cefotaxime and cefuroxime. The antibiotics that *K. pneumoniae* were most resistant to, include co-amoxiclav (212; 99.1%), cefpodoxime (210; 98.1%), co-trimoxazole (207; 96.7%), gentamicin (201; 93.9%), tobramycin (199; 93.0%), aztreonam (192; 89.7%), cefepime (171; 79.9%) and amikacin (164; 76.6%). Only 41 (19.2%) isolates were resistant to cefoxitin and 96 (44.9%) showed medium level resistance to ciprofloxacin. Only one (0.5%) isolate showed resistance to imipenem and meropenem. The number of isolates displaying resistance to sulbactam-cefoperazone and piperacillin-tazobactam were 13 (6.1%) and seven (3.3%) respectively. The number of antibiotics to which *K. pneumoniae* were resistant in each patient were compared in patients with (n=67) or without (n=147) history of antibiotic use in the last three months. No significant difference (p=0.5298) found between the two groups.

Amplification and analysis of *bla* genes showed the majority of *K. pneumoniae* isolates carry the bla_{SHV} (99.5%), bla_{TEM} (93.0%) and $bla_{CTX\text{-}M}$ (99.0%) genes. All of the TEM genes isolated in this study were wild type TEM-1 β-lactamases. The ESBL type SHV detected in the present study were SHV-28 (19.2%), SHV-12 (5.2%) and SHV-110 (0.5%), while non-ESBL type SHV were SHV-1 (20.2%), SHV-11 (31.5%), SHV-42 (1.9%) and SHV-27 (1.4%). The CTX-M-1 group β-lactamases was identified in 99% of the strains. *K. pneumoniae* isolates in the present study were also studied for the presence of an integrase gene and it was found that 94.9% of isolates had a class 1 integrase, while

the class 2 and 3 integrase genes were identified in 1.4% and 0.9% of isolates, respectively.

This is the first study conducted on clinical isolates of ESBL-producing *K. pneumoniae* among paediatric patients from a tertiary care paediatric hospital of Pakistan. The high prevalence of ESBL-producing *K. pneumoniae* among paediatric patients is responsible for prolonged hospital stay and an increased financial burden on parents and the government. Cephalosporins, monobactams, aminoglycosides and sulfonamide drugs do not prove to be a good choice for the treatment of ESBL-producing *K. pneumoniae* infections to high rates of resistance to these antibiotics. This study recommends the use of carbapenems, sulbactam-cefoperazone and piperacillin-tazobactam for the treatment of ESBL *K. pneumoniae* infections but they should be used as a last resort following culture and susceptibility testing. It is being recommended that a stricter infection control policy should be implemented to control the horizontal transfer of bla_{SHV}, bla_{TEM}, bla_{CTX-M} genes and integrons in clinical isolates of *K. pneumoniae* and other bacteria.