Predominance of CTX-M genotype among extended spectrum beta lactamase isolates in a tertiary hospital in Saudi Arabia

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ABSTRACT

Objectives: To determine the molecular characterization of extended-spectrum beta-lactamases (ESBL) isolates from a tertiary center in Saudi Arabia using multiplex polymerase chain reaction (PCR) technique and assess their antibiotic susceptibility pattern.

Methods: Prospective study conducted at the Saudi Aramco Dhahran Health Center, Dhahran, Saudi Arabia between April-December 2006. Extended-spectrum beta-lactamases phenotype of isolates identified by automated methods was confirmed using E-test. Multiplex PCR for the detection of blaTEM, blaSHV and blaCTX-M was performed. Susceptibility to a panel of antibiotics was determined.

Results: One hundred isolates (Escherichia coli (E.coli) n=84; Klebsiella pneumoniae (K. pneumoniae) n=16) were studied and 71% harbored the blaCTX-M gene. For E.coli isolates 43 (51%) harbored CTX-M+TEM combination and 21 (25%) had CTX-M alone. In contrast, only one K. pneumoniae isolate (6.2%) harbored the CTX-M+TEM combination and 3 (18.8%) isolates had CTX-M only. One E.coli and 7 K. pneumoniae isolates were blaSHV positive. The blaCTX-M gene was found predominantly in urinary isolates (n=63/71; 88.7%). The presence of blaCTX-M was significantly higher in isolates from outpatients compared to inpatient (p<0.05). Sensitivity to imipenem was 100% and 78% to nitrofurantoin. Resistance to amoxicillin-sulbactam was significantly higher in blaCTX-M positive isolates (p<0.05).

Conclusion: The findings indicate a high-level of blaCTX-M positive ESBL isolates circulating in our setting with the dissemination of these in the community. The trend of multidrug resistance profile associated with carriage of blaCTX-M gene is cause for concern.
Pathogens producing extended-spectrum beta-lactamases (ESBL) now constitute a global problem as important causes of multidrug resistant nosocomial and community acquired infections.\(^1\) Extended-spectrum beta-lactamases are enzymes capable of hydrolyzing and thus mediating resistance to the penicillins and broad-spectrum cephalosporins as well as monobactams. However, they do not affect the cephemcins and carbapenems, and their activity is inhibited by clavulanic acid.\(^2\) Extended-spectrum beta-lactamases result from mutations in the TEM-1, TEM-2 or SHV-1 genes commonly found in the Enterobacteriaceae family.\(^3\) Currently, over 120 ESBL types including the CTX-M and OXA type enzymes have been described. In recent years, the emergence of the CTX-M ESBL isolates has been associated with increasing incidence of community-acquired infections and particularly in *Escherichia coli* (*E. coli*).\(^4\) Data from the Arabian Gulf region suggest ESBL isolates constitute a major problem in nosocomial and community acquired infections with rates as high as 31.7% in Kuwait and 41% in United Arab Emirates.\(^4,5\) However, there are limited works on the molecular characterization of ESBL isolates circulating in the Arabian Peninsula. This study was carried out to determine the molecular characterization of ESBL isolates from a tertiary center in Saudi Arabia using multiplex PCR technique. In view of the multidrug resistance phenotype commonly associated with ESBL pathogens, we also assessed the antibiotic susceptibility pattern of these isolates.

**Methods.** This study was carried out at the Saudi Aramco Dhahran Health Center, which is a 405-bed health care facility with primary and secondary care facilities catering to a catchment population of 360,000. Over an 8-month period from 1st April 2006 to 31st December 2006, 100 consecutive isolates comprising of *Escherichia coli* (*E. coli*) (n=84) and *Klebsiella pneumoniae* (*K. pneumoniae*) (n=16), which were phenotypically identified by the Vitek\textsuperscript{TM} 1 (bioMérieux, Vitek Inc, Hazelwood, USA) and BD Phoenix\textsuperscript{TM} automated microbiology system (Becton, Dickinson, USA) as ESBL positive were included in the study. Only one positive culture per patient was included.

**Identification of extended-spectrum beta-lactamases phenotype.** During the study period, bacterial identification and ESBL screening was carried out using the BD Phoenix\textsuperscript{TM} system (incorporating BD Xpert system) and VITEK\textsuperscript{TM} 1. *Escherichia coli* and *K. pneumoniae* isolates were reported as cephalosporin resistant or ESBL using these automated systems were manually screened for ESBL phenotype by disk diffusion method. In keeping with the clinical and Laboratory Standards Institute (CLSI) recommended guidelines, ceftriaxone (30µg) and aztreonam (30µg) were used and results expressed as susceptible or resistant according to CLSI criteria\(^6\) guidelines. Confirmation of ESBL phenotype was carried out using the ESBL E-test. Briefly, isolates were sub-cultured on Mueller-Hinton agar (MHA) plates supplemented with 5% sheep blood. Suspended colonies in Mueller-Hinton broth (SPML, Saudi Arabia) was adjusted to a turbidity of 0.5 McFarland then inoculated on MHA plates. The inoculum was allowed to dry, ESBL E-test strips (AB Biodisk, Solna, Sweden) were applied and the plates incubated in an inverted position at 37ºC for 24 hours. Reading and interpretation of the minimum inhibitory concentrations from the E-test was according to manufacturer's instructions and the CLSI interpretative standards. *Escherichia coli* ATCC 51446 and *K. pneumoniae* ATCC 700603 were used as positive controls. *Escherichia coli* ATCC 25922 was used as negative control.

**Determination of extended-spectrum beta-lactamases genotype.** The detection of blaTEM, blaSHV and blaCTX-M gene families was performed with multiplex PCR using 3 sets of specific primers (Thermo Fisher Scientific, Germany).\(^7,8\) All PCRs were conducted under standard conditions using plasmid DNA as templates. The PCR cycling parameters were initial denaturation at 95ºC for 2 minutes followed by 30 cycles of 95ºC for 1 minute, 55ºC for 1 minute and 72ºC for 1 minute, with a final extension at 72ºC for 10 minutes. Gel electrophoresis was carried out using 15µl of the PCR product mixed with 1µl of the loading dye and run on 1.2% agarose gel (Sigma chemical Co, USA). Deoxyribonucleic acid fragments were separated by horizontal electrophoresis cell (Bio-Rad, USA) at 80 volts/cm for 60 minutes, gels were stained with 0.5 µg/ml ethidium bromide (Sigma chemicals, USA) and photographed under UV light transilluminator (Bio-Rad, USA).

**Antibiotic susceptibility testing.** The antibiotic susceptibility pattern of the ESBL-producing isolates to a panel of antibiotics including Amikacin (AK), Ciprofloxacin (CIP), Imipenem (IMI), Nitrofurantoin (FD), Amoxicillin-sulbactam (AMS), Piperacillin-Tazobactam (TZP) and Trimethoprim-sulfamethoxazole (SXT) was recorded. The antimicrobial susceptibility testing were performed using Vitek 1 automated system in accordance with manufacturer guidelines.

Statistical analysis using Chi-squared test was performed on the SigmaStat version 3.5 software (Systat Software Inc, San Jose California, USA). *P* ≤0.05 was considered statistically significant.

**Results.** The findings indicate that most of the ESBL isolates (71%) harbored the CTX-M gene either alone or in combination with other ESBL genes. Of the
84 ESBL-producing *E. coli* isolates, 43 (51%) harbored CTX-M+TEM combination and 21 (25%) had CTX-M alone. In contrast, only one *K. pneumoniae* isolate (6.2%) harbored the CTX-M+TEM combination and 3 (18.8%) isolates had CTX-M only. Table 1 shows the distribution and combination of the beta-lactamase genotypes identified. Thirteen isolates were ESBL negative based on the primers used in this study. Most isolates (72/100) were obtained from outpatients. The distribution of ESBL genotypes in isolates from inpatients and outpatients sources is shown in Table 2. The presence of blaCTX-M alone or in combination with blaTEM and/or blaSHV was significantly higher in isolates obtained from outpatients (n=50) compared to those from inpatients (n=21); *p*<0.05. Overall, blaCTX-M gene (alone or in combination with other ESBL genotypes) was found predominantly in isolates obtained from urinary specimen (n=63/71; 88.7%). The distribution of the ESBL genotypes according to specimen type is shown in Table 3.

**Antibiotic sensitivity.** There was a high level of sensitivity to IMI (100%) and AK (>90%) in all isolates (Table 4). Only the isolates, which harbored CTX-M gene showed statistically significantly higher resistance to amoxicillin-sulbactam (*p*<0.05). Overall, 73% of isolates were sensitive to nitrofurantoin and this was higher among isolates positive for either blaCTX-M, blaTEM, blaSHV compared to the ESBL isolates negative for these genes.

**Discussion.** Extended-spectrum β-lactamases arise by point mutations in genes for common plasmid-
mediated β-lactamases TEM-1/2 and SHV-1. However, in recent years, non-TEM and non-SHV plasmid-mediated ESBLs have been reported in particular the CTX-M enzymes which have now emerged as the most frequent ESBL type worldwide.\(^3\) Available data is suggestive of independent evolution of these enzymes as well as clonal spread of CTX-M-type beta-lactamase producing bacteria.\(^9\) In recent years, data from the Arabian peninsula has shown high occurrence ESBL-producing isolates with rates as high as 31.7% in Kuwait, 41% in United Arab Emirates and 55% in Saudi Arabia.\(^4,5,10\) A recent report from Bahrain indicated that 22.6% of \textit{Enterobacteriaceae} at a major tertiary center were ESBL-producers.\(^11\) Although current data indicate ESBL-producing pathogens is an emerging public health concern in the region, there remains limited data on the molecular characterization of local isolates. The findings in this study indicate that most of ESBL isolates (71%) identified in our setting harbored the CTX-M gene. This much higher compared to other data from Saudi Arabia in which 34.1% of isolates were found to be blaCTX-M positive and blaSHV was found to be the dominant genotype.\(^10\) Characterization of \textit{Salmonella} isolates with ESBL phenotype from UAE and Kuwait also showed low CTX-M and carriage with 12.1% and 24.6% being blaCTX-M and blaTEM positive respectively.\(^12\) Although a high level of co-carriage of blaCTX-M and blaSHV genes was observed among our isolates (44%), previous report from Saudi Arabia showed a much lower proportion (2.27%) of isolates with this genotype.\(^10\) This suggests a changing trend or diversity of ESBL genotypes in the region and indicates the need for large-scale multicenter studies for a better understanding of the molecular epidemiology of ESBL. This is pertinent and as a recent report has indicated a single clone of \textit{K. pneumoniae} with CTX-M-15-like and SHV-112 enzymes causing blood stream infections in a neonatal ICU of a Kuwaiti hospital.\(^13\) A significantly higher number of these blaCTX-M ESBL isolates were identified among outpatients with majority being urinary isolates, which is in keeping with global data.\(^3,14\) This is of particular concern as it indicates an increasing dissemination of CTX-M ESBL isolates into the community in our setting. Indeed this is in keeping with a previous report from this center, which showed that 40% of ESBL isolates were community acquired with \textit{E. coli} being incriminated equally both in the hospital and community environments.\(^15\) With the exception of amoxicillin-sulbactam, the co-carriage of different ESBL genes did not significantly affect the pattern of susceptibility to the antibiotics tested. However, there was a trend of increasing multidrug resistance phenotype (resistance to 3 or more classes of antibiotics), with isolates harboring the CTX-M gene either singly or in combination. Carbapenems are widely regarded as the drugs of choice for treatment of infection caused by ESBL-producing organisms and remain useful in our setting, as all isolates were sensitive to imipenem. However, there is a need for good antibiotic stewardship policies as the spread of CTX-M type ESBLs, especially in \textit{E. coli} may provide a favorable background for selection of carbapenem resistance.\(^16\) In recent years, the increasing incidence of infections caused by ESBL-producers with associated multidrug resistance profiles has resulted in the re-evaluation of the old antimicrobials such as nitrofurantoin. The finding indicates that 73% of isolates were sensitive to nitrofurantoin, which is higher compared to recently reported data of 61.8% sensitivity for isolates from Bahrain.\(^11\) However, this remains lower in comparison >88% susceptibility reported in surveys from USA, 89% in India and 98% in Hong Kong.\(^17,19\) It has been suggested that nitrofurantoin should be considered as an alternative therapeutic agent for urinary tract infections caused by ESBL producers, but responses may be sub-optimal in our setting in view of the higher degree of resistance. The identification of CTX-M specific gene products by PCR is conclusive for determining the CTX-M genotype. However, determination of the CTX-M type is desirable because of the differences in the epidemiological distribution and antibiotic susceptibility profile of CTX-M types. The diversity of point mutations around the active sites of the TEM and SHV sequences makes the determination of TEM and SHV ESBL types more challenging. Nevertheless, the absence of further characterization of the CTX-M, TEM and SHV types is a limitation of our study and further work using nucleotide sequencing for the characterization of the specific CTX-M type and identification of the point mutation in the blaTEM, and blaSHV genes in our isolates is recommended.

In conclusion, these findings contribute to our understanding of the molecular characterization of ESBL isolates in our setting including the association between antibiotic resistance pattern and carriage of CTX-M gene. However, there is an urgent need for multi-center collaborative studies to fully understand the diversity, distribution and changing trend of the molecular epidemiology of ESBL isolates in the Arabian Gulf region.

References


Related topics


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