COMPARISON OF DOUBLE DISC AND THREE DIMENSIONAL METHODS TO SCREEN FOR ESBL PRODUCERS IN A TERTIARY CARE HOSPITAL

*T Menon, D Bindu, CPG Kumar, S Nalini, MA Thirunarayan

Abstract

Extended spectrum β-lactamases (ESBLs) continue to be a major problem in clinical setups world over, conferring resistance to the expanded spectrum cephalosporins. An attempt was made to study ESBL production among Enterobacteriaceae members from a tertiary care center in Chennai. A total of seventy randomly collected isolates of the family Enterobacteriaceae from a tertiary care center was studied for their susceptibility patterns to various antibiotics and detection of ESBL producers by double disc synergy (DDS) test and three dimensional test (TDT). Eighty percent of the isolates were multidrug resistant (MDR) and 20% were ESBL producers. TDT detected 85.7% whereas only 14.2% were detected by DDS. In the present study, a large number of isolates were found to be MDR and ESBL producers. TDTs were found to be better than DDS in the detection of ESBLs. Continued monitoring of drug resistance is necessary in clinical settings for proper disease management.

Key words: Extended spectrum β-lactamases, multidrug resistance, enterobacteriaceae

Materials and Methods

Setting and samples

Seventy clinical isolates of Enterobacteriaceae from cancer patients admitted in Apollo Specialty Hospital, a tertiary care center in Chennai, South India from March 2002 to December 2003 were studied. Thirty three isolates were Klebsiella spp, 26 E. coli, seven Enterobacter spp. and four Citrobacter spp. Twenty nine isolates were from urine, 13 from endotracheal aspirates, 12 from pus, six from sputa, four from blood, three from bronchial wash, two from aspiration fluid and one from vaginal swab. Identification of isolates was done based on cultural characteristics and reactions in standard biochemical tests.

Antibiotic susceptibility testing

The susceptibility of the isolates was determined against 21 antibacterial agents by disc diffusion method. They included cefazolin (Cz-30 µg), cefuroxime (Cu-30 µg), ceftriaxone (Ci-30 µg), cefotaxime (Ce-30 µg), ceftazidime (Ca-30 µg), ceftizoxime (Ck-30 µg), cefpirome (Cpo-30 µg), gentamicin (G-10 µg), tobramycin (Tb-10 µg), isepamicin (Ise-30 µg), amikacin (Ak-30 µg), netilmicin (Nt-30 µg), ofloxacin (Of-5 µg), piperacillin (Pc-100 µg), imipenem (I-10 µg), aztreonam (Ao-30 µg), cefepirone-sulbactum (Cs-75/35 µg), cotrimoxazole (Co-25 µg), piperacillin-tazobactum (Pt-100 µg), tetracycline (T-30 µg) and chloramphenicol (Cl-30 µg). Susceptibility and resistance was determined based on the interpretative criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS).5 E. coli ATCC 25922 was used as the quality control strain.
Tests for determination of ESBL activity

Double disk synergy method (DDS)

Test strains were preincubated in brain heart infusion broth (BHIB) at 37°C to an optical density matching that of 0.5 McFarland turbidity standard. This suspension was then used to inoculate Mueller Hinton agar (MHA) plates by swabbing them with a sterile cotton swab. 30 µg discs of aztreonam, ceftazidime, ceftriaxone and cefotaxime were placed 15 mm (edge to edge) from an augmentin (amoxycillin-clavulanate; 20/10 µg) disc. Inoculated plates were incubated overnight at 37°C. Enhancement of the zone of inhibition between the clavulanate disc and any one of the β-lactam discs indicated the presence of an ESBL.4,6,7

Three dimensional tests (TDTs)

(i) Direct TDT

MHA plates were inoculated with test strains matching 0.5 McFarland turbidity standard as described for DDS and a disc of ceftazidime, ceftriaxone, cefotaxime or aztreonam was placed in the center of the plate. A well of 4 mm (diameter) was punched at a distance of 2 mm from the antibiotic disc. The inoculum (30 µL) of the test strain in BHIB preadjusted to 5.0 McFarland standard was seeded into the well. Plates were then incubated at 37°C for 24 hours. Heart shaped distortion of zone of inhibition with growth of test organism appearing behind the well and reaching the well was indicative of a positive TDT.4

(ii) Indirect TDT

MHA plates were seeded with the inoculum of a standard sensitive strain (E. coli ATCC 25922) adjusted to McFarland 0.5 standard. A disc of ceftazidime, ceftriaxone, cefotaxime or aztreonam was placed in the center of the plate. A well of 4 mm (diameter) was punched at a distance of 2 mm from the antibiotic disc. The test strain (30 µL) in BHIB preadjusted to 5.0 McFarland standard, was seeded into the well. Plates were then incubated at 37°C for 24 hours. Heart shaped distortion of zone of inhibition around the β-lactam disc as in the direct TDT was indicative of ESBL activity.4

Results

Among the 70 isolates of family Enterobacteriaceae analysed, eight isolates (4 E. coli, 3 Klebsiella spp. and 1 Enterobacter spp.) were susceptible to all antibiotics tested. Fifty-six isolates (80%) were resistant to three or more antibiotics tested. The sensitivity was 100% with imipenem, 85.7% with ertapenem and 72.8% with cefepime-sulbactum. The resistance was 70% against cefazolin followed by 67.1% resistance against ceftriaxone, ofloxaciln and gentamicin. The isolates that were resistant or showed decreased susceptibility to all four 3GC were tested 14.28% and 30% of isolates showed resistance to three 3GC.

Of the 70 strains tested, 14 (20%) were found to be ESBL producers. Seven (21.2%) were Klebsiella spp., five (19.2%) E. coli and two (28.5%) were Enterobacter spp. None of the Citrobacter spp. demonstrated ESBL activity. Antibiogram profile of ESBL producers revealed susceptibility to imipenem and piperacillin-tazobactum to be 100% and against ertapenem, amikacin and cefepime-sulbactum to be 85.7, 78.6 and 78.6% respectively (Figure).

Two of the ESBL producers were positive by DDS (14.2%) with ceftriaxone and aztreonam indicator disks detecting one ESBL each. Theses two ESBL producers were also detected by the indirect TDT. Twelve (85.7%) were positive by the TDTs (7 - direct, 5- indirect TDT) with aztreonam detecting nine, ceftriaxone and ceftazidime detecting six each and cefotaxime detecting five ESBL producers.

Discussion

Antibiotic resistance surveillance has a central role among all strategies to manage the problem of antibiotic resistance. Since their first description in the mid 1970s, ESBLs have been isolated worldwide and form a major contributor of drug resistance in many genera of Enterobacteriaceae.

Previous studies from India have reported prevalence of ESBL producers to be 6.6 to 68%9-17 ESBL production (68%) was reported among gram negative bacteria from a teriary care hospital by Mathur et al.12 Tankhiwale et al reported that 48.3% of urinary isolates tested were ESBL producers.13 Jain et al reported 86.6% of Klebsiella spp, 73.4% of Enterobacter spp and 63.6% of Escherichia coli strains from cases of neonatal septicaemia to be ESBL producers.14 In South India, Subha et al15 have reported 6.6% ESBL prevalence among Klebsiella pneumoniae from children, whereas Babypadmini et al16 have shown 40% and 41% ESBL positivity among K. pneumoniae and E. coli respectively in their study cohort. Another study reported an incidence of 58.06% for ESBL producing E. coli and 57.14% for ESBL producing Enterobacter spp.17 The occurrence of ESBL producers among the Enterobacteriaceae isolates in the present study was 20% while 28.5% Enterobacter, 21.2% Klebsiella and 19.2% E. coli were found to elaborate ESBLs.

As indicated in many previous studies, the 100% imipenem sensitivity in the present study, advocates usage of carbapenem antibiotics as the therapeutic alternative in the wake of the increasing resistance rates observed with conventional β-lactam and non β-lactam antibiotics.

In the present study, TDTs were found to be better than DDS in the detection of ESBLs. The combined positivity of both the TDTs was 85.7% against the 14.2% by the DDS. Vercauteren et al7 observed 93% positivity by DDS whereas, 79% positivity was reported by Thomson et al.4 Datta et al, have reported TDT to be more sensitive than DDS.11 As observed in the present study, use of only one disk
Figure: Susceptibility pattern of ESBL and Non-ESBL producing isolates of Enterobactericeae

combination might fail to detect ESBL production resulting in under reporting of prevalence. Simultaneous use of 3GC and aztreonam disks with an augmentin disk is recommended in screening for ESBL-producing organisms.

The susceptibility data collected in the present study demonstrates the high degree of resistance among the major members of Enterobacteriaceae. Continued monitoring of their susceptibility pattern is necessary in clinical settings to detect the true burden of antibiotic resistance for proper disease management.

References


12. Mathur P, Tatman A, Das B, Dhawan B. Prevalence of extended beta lactamase producing gram negative bacteria in a tertiary care


