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Comparative evaluation of ESBL detection in Bacterial isolates of ICU patient's samples by automated detection system and conventional methods

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Abstract
Introduction: Antibiotic resistance, a global concern, is a burning problem before whole medical fraternity and is further pressing in relation to developing nations, including India, where the burden of infectious disease is high and healthcare spending is low.

Aims and Objectives
1. To isolate the various micro-organisms from the various samples of ICU patients.
2. Detection of ESBL's by conventional methods and Vitek-2 (automated) methods.

Methods: This study was carried out on the samples of the patients who were admitted to the Intensive care unit of SRMS-IMS, Bareilly. Samples from 100 patients were evaluated by VITEK-2 method and conventional methods. All the samples were processed for culture on blood and MacConkey agar, the plates were incubated at 37 ^{0}c for 16-18hrs. Detection of ESBL producers is done by double disk synergy test, Modified Hodge test and E-strip test. Automated identification was done by VITEK 2 system.

Results: Out of the total micro-organisms isolated Acinetobacter baumannii were (26.63%), Klebsiella pneumonia (26.86%), Escherichia coli (20.39%), Pseudomonas aeruginosa (19.40%), Acineotbacter lwoffii (2.48%), Proteus mirabilis (1.99%), Enterobacter cloacae (1.49%), Alcaligenes faecalis (0.49%) and Serratia marcescens (0.49%). ESBL detection rate by Vitek-2 was highest among all the techniques i.e. 94.02%. The detection rate by Double disk synergy test was 91.54%, by E-strip 89.05% and Modified hodge test 36%.

Conclusion: Though the Vitek-2 excel automated system is better than conventional methods in the detection of ESBL producing organisms. But it has its own limitations in terms of costly infrastructure, high cost of sample processing and limited availability of the machine in developing countries like India.

Keywords: ESBL, DDST, MHT, VITEK-2, E-Strip Test

Introduction
Infections with resistant bacterial isolates are emerging as an important challenge in health care facilities. Mainly due to its extremely vulnerable population of critically ill patients, and the high use of (invasive) procedures, the intensive care unit (ICU) is the epicenter of infections. Among gram-positive organisms, the most important resistant microorganisms in the ICU are currently methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci. In gram-negative bacteria, the resistance is mainly due to the rapid increase of extended-spectrum Beta-lactamases (ESBLs) in Klebsiella pneumonia, Escherichia coli, and Proteus species and high level third-generation cephalosporin Beta-lactamase resistance among Enterobacter spp. and Citrobacter spp., and multidrug resistance in Pseudomonas aeruginosa and Acinetobacter species.

Two million people in India die each year due to infectious diseases. There is a need to integrate medicine and innovative technology in our public health system to provide rapid, efficient, accurate, and cost-effective results for identification and antimicrobial susceptibility testing [1].

ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid.

In the microbiology laboratory, detection of ESBLs can be done with phenotypic or genotypic tests. The phenotypic tests are routinely used in clinical diagnostic laboratories, whereas the genotypic tests are mainly used in reference or research laboratories.

Several methods including the double disc synergy test, modified hodge test, or specific ESBL E-test strips can be used in this regard [6, 2].

Vitek-2 excel system (bio Merieux), which uses a new fluorescence-based technology, was evaluated for the identification and susceptibility testing of gram negative clinical isolates [3]. The present study was designed to evaluate its performance in the identification of ESBL-producing isolates of *Enterobacteriaceae* and other organisms like Pseudomonas and Acinetobacter, etc.

Material and Methods

The present study was carried out on the samples of the patients who were admitted to the Intensive care unit of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly during 15th November 2016 to 31st May 2018.

Sample collection

All the samples were collected with aseptic precautions, in a sterile container and transported to the laboratory at the earliest possible time. The sample were collected by invasive and non-invasive techniques. The samples included in the study were blood, urine, CSF, bed sore swabs, body fluids, sputum, tracheal aspirate, BAL, etc.

Culture Media: Blood and MacConkey agar, a Gram's staining was performed with all the specimens. After subculture on blood and MacConkey agar, the plates were incubated at 37°c for 16-18hrs.

Detection of ESBL producers was done by double disk synergy test, Modified Hodge test and E-strip test.

Automated identification and detection of ESBL producers along with their type was done by VITEK 2 compact system.

VITEK 2 Compact System (bioMérieux, Marcy 1'Étoile, France)

- VITEK 2 Compact is an integrated system that automatically performs rapid identification using algorithms based on fluorescence and colorimetry, and antimicrobial susceptibility testing (AST) based on kinetic analysis of growth data.
- It features an advanced expert system (AES) that interprets the antibiotic resistance patterns, validates the results, and reports the resistance phenotype.

Double Disk Synergy Test

- Discs containing cephalosporin (cefotaxime or ceftriaxone, ceftazidime, cefepime) are applied next to a disc with clavulanic acid, amoxicillin + clavulanic acid or ticarcillin + clavulanic acid.
- Positive result is indicated when the inhibition zones around any of the cephalosporin discs are augmented in the direction of the disc containing clavulanic acid.
- The distance between the discs is critical and 20 mm center-to-centre has been found to be optimal for cephalosporin 30 µg discs.

Modified Hodge Test

- A 0.5 McFarland dilution of the Escherichia coli ATCC 25922 in 5 ml of broth or saline was prepared.
- A 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate. A 10 µg meropenem or ertapenem susceptibility disk was placed in the center of the test area.
- Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35±2°C in ambient air for 16–24 hours.
- After 24 hrs, MHT Positive test showed a clover leaf-like indentation of the *Escherichia coli* 25922 growing along the test organism growth streak within the disk diffusion zone. MHT Negative test showed no growth of the *Escherichia coli* 25922 along the test organism growth streak within the disk diffusion.

E-Strip test

- ESBL E-test (HIMEDIA) strips are thin, inert and non-porous plastic carriers measuring 5x60mm.
- MIC values are printed on both sides identically
- The upper half of the strip has Ceftazidime, Cefotaxime mixture + Clavulanic acid with highest concentration tapering downwards.
- Lower half is coated with Ceftazidime and Cefotaxime mixture in a concentration gradient in reverse direction.
- Production of ESBL is inferred by the appearance of a phantom zone or deformation in the ellipse. E-test confirmatory strips are convenient but are expensive.

Results

This present study was carried out in the Department of Microbiology on the sample of patients admitted in Intensive care unit of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly during 15th November 2016 to 31st May 2018. Samples from 100 patients were evaluated by VITEK-2(Excel) method and conventional methods.

A total of 261 samples were collected from 100 patients during the above defined time period. Out of 261 samples from ICU patients, 93 samples were sterile as their was no organism grown after 48 hrs. From the remaining the gram negative organisms were 201 and gram positive were 16. 21 fungal organisms were also grown. There was one sample in which commensal contaminant bacteria was grown.

Out of 261 samples, maximum number of samples were blood 108 (44.44%), followed by respiratory samples 95 (39.09%), urine samples 45 (18.51%), pus samples 5 (2.05%) and others were 8 (3.29%).

Out of the total micro-organisms isolated Acinetobacter baumannii were 53 (26.63%), Klebsiella pneumonia were 54 (26.86%), Escherichia coli were 41 (20.39%), Pseudomonas aeruginosa were 39 (19.40%), Acineotbacter lwoffii were 5 (2.48%), Proteus mirabilis were 4 (1.99%), Enterobacter cloacae were 3 (1.49%), Alcaligenes faecalis was 1 (0.49%) and Serratia marcescens was 1 (0.49%).

On comparative evaluation of ESBL results by conventional methods and Vitek-2, maximum percentage i.e 94.02% was detected by Vitek-2, followed by 91.54% by Double disk synergy test, 89.05% by E-Strip method and only 36% by Modified Hodge test method.

Discussion

Extended spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid-mediated beta-lactamases which confer resistance to broad spectrum beta-lactam antibiotics. They are commonly expressed by Entreobacteriaceae and the other gram-negative organisms like non-fermenters but the species of organisms producing these enzymes are increasing and this is a cause for great concern.

Previous studies from India have reported the presence of ESBL producers to be 6.6% to 68% [4].

The present study was conducted for ESBL detection from various bacterial isolates from the samples of ICU patients. Out of the 100 cases included in the study 69% were males and 31% were females. Few studies like Rakhee *et al*. [5] found out that out of the 183 patients, 66.78% were males and 33.21% were females. Another similar study by Hassan *et al*. [6] also showed that out of the 236 isolates, 54.7% were males while 45.3% were females.

In present study 261 samples were obtained from 100 patients. The samples included E.T. aspirate, pus, blood, urine, E.T. tip, etc. Out of these 261 samples, 93 samples were sterile. Out of 261 samples, maximum number of samples were blood 108 (44.44%), followed by respiratory samples 95 (39.09%), urine samples 45 (18.51%), pus samples 5 (2.05%) and others were 8 (3.29%).

The maximum number of isolates were from respiratory samples i.e. 154 (64.70%) followed by blood 46 (19.32%), urine 25 (10.50%), pus 5 (2.10%) and others were 8(3.36%). On culture 93(28.01%) samples showed no growth, 21(6.32%) were fungus, 16(4.81%) were Gram positive and 201(60.54%) were Gram negative bacteria. These results are consistent with the study done by Hecini-Hannachi *et al*. [7] who reported more Gram negative isolates than Gram positive isolates.

Out of these 201 gram negative bacilli, maximum number of gram negative bacilli were isolated from respiratory samples i.e. 139(69.15%), followed by blood 39 (19.40%), urine 13(6.46%), pus 4(1.99%) and others 6(2.98%).

In these 201 isolates, Acinetobacter species (28.85%) was the predominant bacteria followed by Klebsiella pneumonia (26.86%), Escherichia coli (20.39%), Pseudomonas aeruginosa (19.40%), Proteus mirabilis (1.99%), Enterobacter cloacae (1.49%), Alcaligenes faecalis (0.49%) and Serratia marcescens (0.49%).

Out of the 28.85% Acinetobacter species, the Acineotbacter baumannii were 53 (26.63%) and Acinetobacter lwoffii were 5 (2.48%).Our study well corroborated with the study done by Sofianou *et al*. [8] who found gram-negative bacteria (83.2%), were predominant organism in ICU with a high proportion of Acinetobacter spp. (35%) resistant to commonly used antimicrobial agents.

As far as ESBL detection is concerned, by Double disk synergy test, 100% Acineotbacter baumannii, Acinetobacter lwoffii, Enterobacter cloacae, Alcaligenes faecalis and Serratia marcescens were ESBL producers, while 94.4% Klebsiella pneumoniae, 87.8% Escherichia coli, 79.48% Pseudomonas aeruginosa, 75% Proteus mirabilis were ESBL producers.

On detection by E-strip method, 100% Acinetobacter lwoffii, Enterobacter cloacae, Alcaligenes faecalis and Serrtia marcescens were ESBL producers while the detection of ESBL in Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis was 98.11%, 92.5%, 82.9%, 76.9% and 75% respectively. Probably this 100% ESBL production in few bacteria by E-strip method is due to less number of isolates observed in my study.

By Modified Hodge test, only 58.49% Acinetobacter baumannii was found to be ESBL producers. The other organisms like Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumonia, Proteus mirabilis and Escherichia coli showed the detection rate of 46.15%, 33.33%, 27.78%, 25%, 14.63% respectively. None of the Alcaligenes faecalis, Serratia marcescens and Acinetobacter lwoffii was detected as ESBL producer.

The results of VITEK-2 excel showed that, 100% Acinetobacter baumannii, Acinetobacter lwoffii, Serratia marcescens, Alcaligenes faecalis, Enterobacter cloacae were found to be ESBL producer while it was 98.14% in Klebsiella pneumoniae followed by Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis which showed the ESBL production in the range of 92.68%, 82.05%, and 75% respectively.

On analysis of the other studies, lot of other people also used the same techniques for the production of the ESBLs in various clinical isolates.

In the study by Biswas SM *et al*. [194] they found by Double disk synergy method, 100% Acinetobacter baumannii were ESBL producer, followed by 85% of Pseudomonas aeruginosa, Escherichia coli (80.32%), Klebsiella pneumonia (71.42%), Enterobacter cloacae (50%).

So their results are also showing very high isolation rate of ESBLs and a total of 66.36% i.e. 73 out of 110 total isolates were ESBL producers.

In the study done by Shiju MP *et al*. [9], they found that 54.17% of Escherichia coli and 48.53% of Klebsiella pneumonia were ESBL producer by Double disk synergy method. In other studies by F Robin *et al*. [10], the ESBL production in Proteus was 100% followed by K. pneumoniae 91.30%, followed by E.coli (55.69%) by Vitek-2 method. Their Double disk synergy results were also same.

Another study done by Goyal Ankur *et al*. [11], they found 86% of A. baumannii were found to be ESBL producer when the analysis was done by Modified hodge test.

On statistical analysis, the results of Vitek-2, Double disk synergy test, E-strip and Modified hodge test were analysed. The kappa value and p-value for Vitek-2, DDST, E-strip and MHT were 0.81 and 0, 0.81 and 0, 0.68 and 0, 0.68 and 0.008 respectively. These all values are statistically significant.

Conclusion

Timely and appropriate diagnosis for ESBL producing organisms is crucial to the outcome of bacterial infection in ICUs. The present study concluded that gram negative organisms are more commonly encountered in intensive care units and most of them are ESBL producers. Vitek-2 excel automated system is better than conventional methods in the detection of ESBL producing organisms. But it has its own limitations in terms of costly infrastructure, high cost of sample processing and limited availability of the machine in developing countries like India.

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