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Detection of ESBL in *Pseudomonas aeruginosa* from Various Clinical Specimen-A Hospital Based Study

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Abstract
Extended Spectrum Beta-Lactamases (ESBLs) are beta-lactamases resist against bacteria to Pencillins, Ceprhalosporins and Aztrenam (not to Cephamycins or Carbapenems) and inhibited by Beta-lactamase inhibitors such as Clavulanic Acid. ESBLs- producing strains can resist to Cephamycins due to loss of porin protein on the outer membrane. ESBLs enzyme coded by gene TEM1, TEM2 and SHV1 and due to presence of same genes in Pseudomonas aeruginosa, consider as wide spread reservoir of ESBL enzymes. Identication of P.aeruginosa based on colony morphology and it's biochemical reactions from various positive clinical samples. Also, Double Disc Synergy Test has been performed for the detection of ESBL detection. Polymycin, Colistin have maximum sensitivity against P.aeruginosa. Among all positive samples, only 7% ESBL produced. ET secretion have high degree of isolation followed by urine and pus samples. Due to presence of Clavulanic Acid which act as Beta-lactamase inhibitor help to screen the production of ESBL, that's why, only 7 % production of ESBLs production by Phenotypic Confirmatory Test combined Disk Diffusion Test. The present study help to prevent the ESBLs production by applying Clavulanic Acid as main drug of choice for physicians.
Keywords: *ESBL, Clavulanic Acid, Double Disc Synergy Test.*

Introduction

Extended Spectrum Beta-Lactamase (ESBL) first discovered in Western Europe in mid 1980s and subsequently in the US in the late 1980s. According to Bush Jacoby Medeiros classification, ESBLs belong to group 2be or group 2d and belong to Ambler's molecular class A. The majority of ESBLs contain a serine at the active site. ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins; first-, second- and third-generation cephalosporins; and aztreonam (but not the cephamycins or carbapenems) and which are inhibited by β-lactamase inhibitors such as clavulanic acid. ESBLs contain a number of mutations that allow them to hydrolyze expanded- spectrum β-lactam antibiotics. The ESBL enzyme coded by gene TEM1, TEM2 and SHV1 confer high level resistant to early penicillins, cephalosporins and aztreonam is believed to major cause of mutation

in these enzymes that has led to the emergence of the ESBLs. These enzymes mediate resistance to Cefotaxime, Ceftazidine and other broad spectrum cephalosporins and monobactams but ESBLs are not active against cephamycins, and most strains expressing ESBLs are susceptible to Cefoxitin and Cefotelan. However, it has been reported that ESBL-producing strains can become resistant to cephamycins due to the loss of an outer membrane porin protein. These gene found in *Pseudomonas aeruginosa* which suggested that these organism are wide spread reservoir of the ESBL enzyme.[1]

Materials and Methods

In that study total 50 positive *Pseudomonas aeruginosa* isolated from various clinical samples. The identification of *Pseudomonas aeruginosa* was based on colony morphology on nutrient blood and macconkey agar and their biochmecial reaction like indole, methyl red, voges prausker, urease, citrate and triple sugar iron agar.

The antibiotic susceptibiity was performed by Kirby Bauer method against various antibiotic like Ampicillin(30µg), Ceftazidine(30µg), Cefotaxime (30µg), Ceftriaxone(30µg), Aztreonam(30 µg), Amikacin(30µg), Tobramycin(10µg), Piperacillin/ Tazobactum(75µg/10µg), Ticarcillin/Tazobactum (75µg), Cefoperazone/Salbactum(75 µg /30 µg), Ciprofloxacin (5 µg), Imipenam(10 µg), Meropenem (10µg), Polymyxin(300 unit)and Colistin(10µg). on Muller Hinton agar.

Phenotypic confirmatory test for ESBL detection (Double disc synergy test):- As per the CLSI guidelines use of Ceftazidime disk with or without Clavulanate for phenotypic confirmation of the presence of ESBLs production on the confluent growth on Muller Hinton agar. A difference of ≥5 mm between the zone diameters of Ceftazidime and its Ceftazidime/Clavulanate disks was taken to be phenotypic confirmation of ESBL production. When there was an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid(CAC) versus the inhibition zone diameter around Ceftazidime (CAZ) disk alone, it confirms ESBL production.

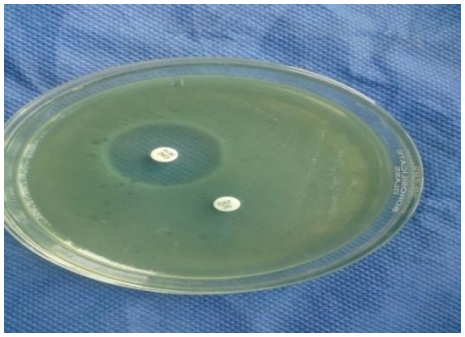

Figure 1: Phenotypic Confirmatory Test with combination disc using Ceftazidime disc 30 µg and Ceftazidime/Clavulanate disc 30/10 µg. (ESBL Positive).

Result & Discussion

Out of 300 isolates of *Pseudomonas aeruginosa*, maximum sensitivity against Polymyxin 98%, Colistin 98, Imipenem 89%, Cefeparazone/ Salbactum 86%, Meropenem 84%, Pipercillin / Tazobactum 82%, ciprofloxacin 72%, Cefepime 80%, amikacin, colistin 62% each, tobramycin 60%, ceftazidime 42%, Ceftriaxone and aztreonam 40% each, least cefotaxime 38% and Ampicillin show no sensitivity and maximum number of antibiotics show resistance against *P.aeruginosa*.

Table 1: ESBL production from all out of specimen

Total specimen	ESBL(%)
300	24(8%)

Table 1 shows, 24 strain of *Pseudomonas aeruginosa* ESBL producer has been detected out of 300 various samples.

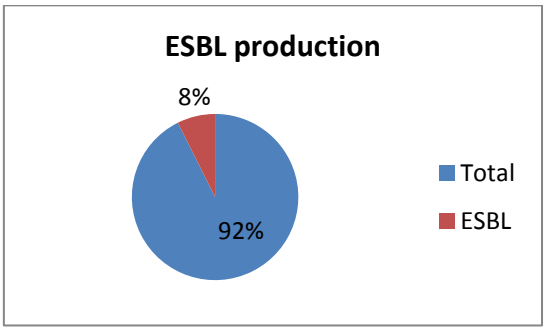

Figure 2: Isolation of ESBL Production

The figure shows that isolation of ESBL production is only 8%.

Table 2: Sample wise distribution of ESBL production

Clinical specimen	Total no. of isolates	ESBL isolates
Foleys tip	6	0(0%)
Catheter tip	6	0(0%
Blood	12	0(0%)
ET secretion	12	6(50%)
Sputum	42	0(0%)
Urine	54	12(2.22%)
Ear swab	78	0(0%)
Pus	90	6(6.66%)

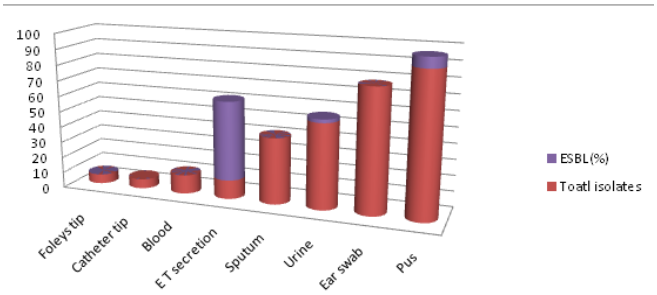

Figure 3: Graph shows sample wise distribution of ESBL Production

The above figure shows that out of all specimen ESBL production shows E T secretion 50%, pus 6.66% and urine 2.22% followed by Foleys tip, catheter tip, blood, sputum and ear swab.

Extended spectrum β-lactamases (ESBLs) are rapidly developing β-lactamases which are talented of conferring bacterial resistance to penicillins, first, second, third generation cephal-osporins and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics[1]. Clavulanic acid can be used as β-lactamase inhibitor to screen for ESBL production. Because of possibility of inoculum effect, their use for treatment of ESBL producing bacteria is not encouraged. In the present study show 8% ESBLs production by the phenotypic confirmatory test combined disk diffusion test in this test use Ceftazidime and Ceftazidime Clavulanic acid because Clavulanic acid inhibit the ESBL production. The present study similar with Jacoboson K L et al (1995)[2] was also produced 7.7% ESBLs Upadhyays et.al2010[3] reported very low incidence of ESBL among *P. aeruginosa* (3.3%), which contrasts in present study which showed 7% of ESBL production.

Conclusion

The present study is beneficial for the Physicians to prevent against *Pseudomonas aeruginosal* infection.

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