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Original Article

Antibiogram of Extended-spectrum beta-lactamase (ESBL) producing Pseudomonas aeruginosa isolates from pus samples

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Abstract
Pseudomonas aeruginosa is one of the most common opportunistic nosocomial pathogens, which causes a wide spectrum of infections and leads to increased mortality and morbidity. Due to indiscriminate use of antibiotics, significant changes in microbial genetic ecology is seen and this has led to the spread of multidrug resistance globally. The present study was undertaken to detect the extended spectrum β lactamases (ESBL) in Pseudomonas aeruginosa isolated from pus samples and to evaluate their susceptibility patterns. A total of 90 isolates of P.aeruginosa were analyzed to study their sensitivity patterns. The presence of the ESBL enzyme was detected by the phenotypic test-Double Disc synergy test. Out of 90 isolates of P.aeruginosa, 20(73.3%) were ESBL producers (of the 30 P.aeruginosa resistant to cetazidime). All the ESBL producing isolates were sensitive to Imipenem which is a carbapenem and is the drug of choice for treating infections caused by ESBL producing P.aeruginosa. Thus, we recommend a routine surveillance on antibiotic resistance in all hospitals so that control measures can be taken to prevent the spread of these strains in the hospitals at a very early stage itself.
Keywords: *Pseudomonas aeruginosa, ESBL, Imipenem, DDST.*

Introduction

P. aeruginosa is a physiologically versatile microorganism possessing the capability to flourish as a saprophyte in multiple environments like sinks, drains, respirators, humidifiers and disinfectant solutions. Pseudomonas aeruginosa is the most common opportunistic pathogen with innate resistance to many antibiotics and disinfectants.

Apart from its innate resistance, acquired resistance is seen in P.aeruginosa which is due to plasmids. Plasmid-mediated resistance is known to be due to indiscriminate antibiotic use, which has led to modifications in the preexisting enzymes in the microorganism.[1] A large number of enzymes are known to be responsible for resistance in organisms and Extended-spectrum beta-lactamases (ESBLs) being one of the most important of them. ESBL are the enzymes that mediate resistance to third generation cephalosporin's as well as monobactams.[2] Generally, ESBLs are a group of β-lactamases that

hydrolyzepenicillin's and cephalosporin's, including oxyimino-β-lactams (third- and fourth-generation of cephalosporins) and aztreonam. These ESBL enzymes are known to be inhibited by β-lactamase inhibitors, such as clavulanic acid, sulbactam and tazobactam.[3]Genes SHV-2a and TEM-42 are found to be responsible genes for ESBL production in P. aeruginosa.[4,5]Clinical Laboratory Standards Institute (CLSI) guidelines doesn't describe any method for the detection of ESBL in P.aeruginosa.[6]Hence the present study was taken up for the detection of ESBL producers in P. aeruginosa isolates from pus samples and to know the antibiotic sensitivity of these positive isolates.

Material and Methods

A total of 1000 pus samples were screened in one year which were received at the Department of Microbiology, J. N. Medical College, KLE university, from hospitalized patients of K.L.E.'S DR. Prabhakar Kore's Charitable Hospital and MRC, Belagavi were processed. Only those isolates of P. aeruginosa which were obtained from pus samples as pure cultures and predominant growth were included in the study. Using Kirby Bauer disc diffusion method, sensitivity of the isolates to any one of the third-generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, 30 µg each) was determined using P. aeruginosa ATCC 27853 as control strain. Results were interpreted according to the CLSI guidelines, which suggest a diameter of inhibition zone ≥22 mm for ceftazidime, ≥27 mm for cefotaxime and ≥25 mm for ceftriaxone as susceptible.[7] Only those isolates showing resistance to third generation cephalosporins were tested for ESBL production - Double Disc synergy test (DDST).[8]

Mueller Hinton agar (MHA, Hi-Media) was prepared by inoculating with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture. 30mcg disc of each third generation cephalosporin antibiotics: Cefotaxime, Ceftriaz-one and Ceftazidime, are placed on MHA at distance of 15mm center to center from Augmentin disc (Amoxicillin/Clavulanic acid-20mcg/10mcg) followed by incubation for overnight at 37°C.

Increase in the inhibition zone of any one of the three third generation antibiotic disc towards augment disc was considered as an ESBL producer. Increase in zone size occurs because the clavulanic acid present in the amoxyclav disc inactivates the ESBL produced by the test organism.

Results

Out of 1000 pus sample screened, 90 P. aeruginosa isolates were isolated. Of the 90 P.aeruginosa isolates, 50(55.5%) were sensitive to ceftazidime and 30(33.33%) were resistant. Of the 30 P.aeruginosa resistant to ceftazidime, DDST detected 20(73.3%) of ESBL producers. All the 20(100%) ESBL producing P. aeruginosa were sensitivity to Imipenem.

The antibiotic sensitivity pattern of all the ESBL and Non-ESBL producing P.aeruginosa are depicted in table no.1

Table no.1: Antibiotic sensitivity pattern of all the ESBL and Non-ESBL producing P.aeruginosa

Antibiotics	ESBL (n=20) % resistant	Non ESBL (n=90) % resistant
Ceftazidime	100	32.6
Ampicillin	40.41	78.2
Ofloxacin	30.12	10
Piperacillin	58.24	20.3
Piperacillin + Tazobactam	40.52	22.5
Cotrimoxazole	66.7	40.41
Tetracycline	45.34	42.3
Ciprofloxacin	60.16	20.2
Gentamicin	55.4	35.15
Amikacin	20.2	79.4
Imipenem	0	20

Discussion

In the recent years ESBL producing Pseudomonas aeruginosa had created a significant problem in treatment, mainly after being detected to be responsible for various nosocomial infections. Their control and prevention of spread is also a major challenge in the present scenario as there are a very limited treatment options for these organisms available.[3]The prevalence of ESBL producing organisms varies from different continents, countries and also between the different wards of the same hospitals.[3,9,10,11,12,13,14,15,16]

Study done by Das.A et al in New Delhi; showed that, the prevalence rates of ESBL producing organisms varies between different institutions from 28 to 84%.[17]

In our study 73.3% is the prevalence rate of ESBL producing P.aeruginosa detected by DDST method, when compared with the prevalence of 34.03% in a study done by Hansotia.JB et al in Nagpur and Jarlier V et al.[18,19]

The sensitivity of DDST in the detection of ESBL producer varies in different studies from 79%, 87% and 3%.[20,21,22]

This detection rates of ESBL producer by DDST was found to be affected by a number of factors like precise placement of the disc, use of appropriate storage temperature forclavulanate containing disc and use of a known strain of ESBL producer as control while performing the test each time.[18,21,23]

33.3% of the P.aeruginosastrains though were resistant to cetazidime did not show positive results in DDST for ESBL production. The possible reason for this could be the inability of the DDST to detect the strains of P.aeruginosa producing chromosomal cephalosporinases, as explained by Moland.ES et al.[23]

In our study the use of cetazidime for segregation of ESBL producing P.aeruginosa so as to carry out DDST on those resistant strains, was more effective than the other third generation cephalosporins. This finding is in ordinance with the observation done by Cormican MG et al in their study.[21] In contrast to this Coudron PE et al; and Datta P et al; in their study found that ceftriaxone followed by cefotaxime and lastly ceftazidime detected the maximum ESBL producing organisms.[24,25]

In our study it was noted that all the ESBL producers were 100% sensitivity to imipenem, which is in accordance with findings of other studies.[26,27,28] Majority of the ESBL producers showed a comparatively good sensitivity to amikacin followed by pipracillin+tazobactam, and this pattern of sensitivity is same as seen in a study done in Bangladesh by Begum S et al; and Farzana R in Bangladesh.[27,28]The probable reason for this type of low resistance at amikacin and piperacillin+ tazobactam could be due to lesser use of these antibiotics in our hospital for empirical treatment. So these two drugs may be considered as an alternative drug in the treatment of ESBL producing P.aeruginosa causing infections. As observed in the table no.1 that ESBL producer are resistant to most of the drugs, we are left with very limited antibiotic choice for their treatment. Hence, early detection and appropriate antibiotic therapy remains the main priority in controlling the development and spread of ESBL producing organisms.

The main limitation of our study was that we could not carryout advanced molecular methods for the confirmation of ESBL producers, due to lack of infrastructure.

Conclusion

To sum up, the prevalence of ESBL producing P.aeruginosa was found to be 73.3% in the pus samples received from our hospital which cannot be ignored. Since ESBL producers can be easily detected by tests like DDST, we recommend their use routinely as a screening tests in all microbiology units, to prevent the dissemination of ESBL producing organisms.

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