Original Research Article

Detection of ESBLs in *Pseudomonas aeruginosa* isolated from different body fluids.

ABSTRACT

Background: The present study was carried out in order to detect the production of ESBLS in *Pseudomonas aeruginosa* and their susceptibility pattern against different antibiotics.

Material and methods: A total of 100 isolates of *P. aeruginosa* was analyzed for the production of ESBLs by using phenotypic confirmatory test and the results were determined. The antibiotic susceptibility against various antibiotics was performed by the Kirby-Bauer Disc diffusion method on Mueller-Hinton Agar.

Results: Out of the 100 isolates tested 20 isolates were positive for the production of ESBLs, with a high prevalence of 36.3% found in the pus samples followed by the sputum (26%), blood (22.2%) and urine (5.4%), respectively. Imipenem was found to be the most effective antibiotic with 100 per cent susceptibility followed by Ofloxacin. A drug resistance of 57% for ceftazidime was noted against *P. aeruginosa* followed by cefotaxime. A high prevalence rate of *P. aeruginosa* was detected in males.

Conclusion: This study showed the rising tendency of multidrug-resistant strains of the Enterobacteriaceae family, especially *P. aeruginosa* in the hospital settings, which is a great concern for clinicians and patients to treat these resistant strains.

Keywords: *P. aeruginosa*, Drug resistance, ESBLs, β-lactams.

1. INTRODUCTION

One of the main mechanisms of resistance in Gram-negative bacteria is the manufacturing of β-lactamases, which hinder protein transpeptidases from participating in the creation of bacterial cell walls [1, 2]. Presently there are several such enzymes known but ESBLs and AmpC β-lactamases are of particular clinical and epidemiological significance that is capable of inactivating the broad-spectrum cephalosporins and penicillins. *Pseudomonas aeruginosa* is a primary cause of nosocomial infections, including pneumonia, urinary tract infections, and bacteremia. The infections can be predominantly brutal in patients with impaired immune systems, such as in neutropenic or cancer patients [3]. Extended-spectrum β-lactamases are enzymes that arbitrate resistance to Extended-spectrum Cephalosporins (ESCs), such as cefotaxime, ceftriaxone, and ceftazidime, and the monobactam aztreonam [4]. Although modern advances in sanitation amenities and the introduction of a wide range of antimicrobial agents with antipseudomonal activities, life-threatening infections caused by these agents continue to cause devastation in hospitals. The resistance in *P. aeruginosa* is primarily mediated by β-lactamases [5]. Although the most important ones are metallo beta-lactamases, several studies indicated the presence of (ESBLs) in *Pseudomonas* as well [6, 7].

2. MATERIALS AND METHODS

A total of 225 different specimens were processed for the isolation of *P. aeruginosa* out of which 100 isolates of *P. aeruginosa* were recovered by using various conventional methods. The identification was based on the various biochemical tests and colony morphology of the organism [8]. The antibiotic susceptibility was performed against different antibiotics including, cefotaxime (30 µg), ceftazidime (30µg), ofloxacin (5µg), gentamicin (10µg), amikacin (30µg), tobramycin (10µg), piperacillin (100 µg), piperacillin/tazobactum (100µg/10µg), meropenem (10µg) and imipenem (10µg) using Kirby disc diffusion method [9]. Results were interpreted according to CLSI guidelines [10]. All the isolates of *P. aeruginosa* were tested for the production of ESBLs by using a phenotypic confirmatory test [11]. A 0.5 McFarland's suspension of every isolate was spread on a Mueller-Hinton agar (MHA) plate (Hi-Media) and a 30µg disc of ceftazidime and ceftazidime/clavulanic acid 30 µg/10µg discs were positioned aseptically on the agar plate. The two discs were kept apart at a distance of about 15mm and the plates were incubated at 37℃ overnight. The results were interpreted and a distance of ≥ 5mm increase in the zone diameter of the antimicrobial agent that was tested in combination with

clavulanic acid, against its zone diameter when tested alone, confirmed the organism as an ESBL producer [Figure 1].

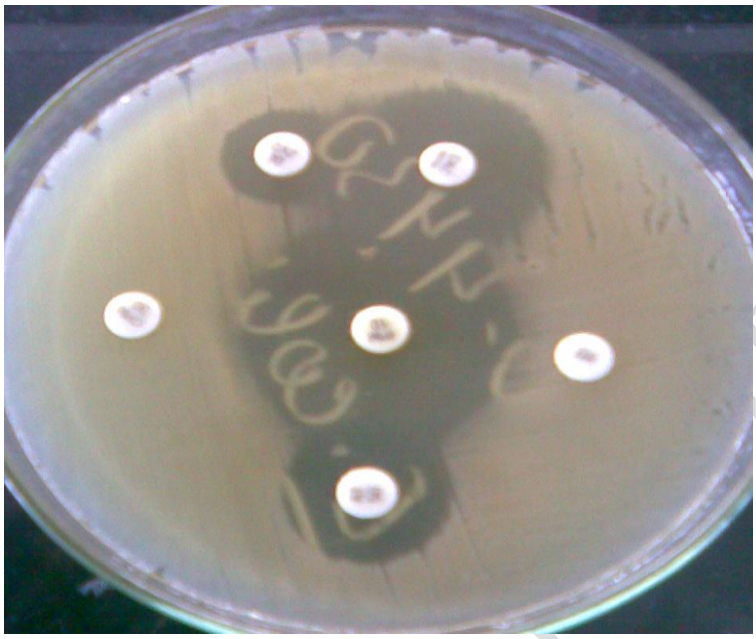

Figure 1: Detection of ESBL production in *P. aeruginosa*

3. RESULTS

Out of 100 *P. aeruginosa* isolates, 20 were found to produce ESBLs. The maximum prevalence of ESBLs was set up in the isolates detected from the pus specimens (36.3%) while a low 5.4% was detected in urine [Table 1]. All the ESBL-producing strains showed drug resistance to the maximum number of antibiotics. Imipenem was found to be the drug of choice with 100% sensitivity followed by ofloxacin, while as highest resistance of 57% was noted against ceftazidime followed by cefotaxime at 51% [Table 2]. The majority of the isolates (76%) was obtained from male patients.

Table 1: Distribution of *P. aeruginosa* (ESBL) producing strains in different specimens

Sample	**No. of isolates**	**No of ESBL strains**	**ESBL %age**
Sputum	23	6	26%
Pus	22	8	36.3%
Blood	18	4	22.2%
Urine	37	2	5.4%
Total	**100**	**20**	

Table 2: Antibiotic resistance of *P. aeruginosa* strains

Antibiotic	Resistance
cefotaxime (30µg)	51%
ceftazidime (30µg),	57%
ofloxacin (5µg),	8%
gentamicin (10µg),	37.5%
amikacin (30µg),)	32.3%
tobramycin (10µg),	29%
piperacillin (100µg),	40%
piperacillin/tazobactum (100µg/10µg)	21.2%
meropenem (10µg)	13.7%
imipenem (10µg)	0

4. DISCUSSION

Extended-spectrum β-lactamases (ESBLs) are rapidly developing β-lactamases, which are talented ot conferring bacterial resistance to penicillins, first, second, third generation cephalosporins and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics [12]. Clavulanic acid can be used as a β-lactamase inhibitor to screen for ESBL production. Because of the possibility of the inoculum effect, their use for the treatment of ESBL-producing bacteria is not encouraged [13]. In the present study, the prevalence of ESBLs-producing *P. aeruginosa* was found to be 20% which is quite similar to the studies conducted previously [14, 15], which reported 20.27% and 22.22%, respectively, while another study had reported a 64% incidence of ESBL producing *P. aeruginosa* [16]. However, another study noted a low ESBL production of 7.7% [17]. During the ongoing study, Imipenem was found to be the drug of choice with 100% sensitivity, which is similar to the studies reported [18, 19]. The current investigation has recorded a prevalence rate of 76% of *P. aeruginosa* in male patients, which is comparable to the findings of Garg *et al.* [20], who reported 78% isolates from the male

patients in her study. However, [21] reported a predominance of males as (68%). In our study ceftazidime showed a resistance rate of 57% which is related to the conclusion of [22] who showed a resistance rate of 55.4% while another finding recorded 53.17% [15], whereas one more study has noted 63% ceftazidime resistance [23]. The emergence of ESBLs and their wide-ranging spectrums and unrivalled drug resistance is creating a therapeutic challenge for clinicians and microbiologists throughout the world. Therefore, it is highly recommended to include the identification of ESBL in P. aeruginosa as a routine practice. In light of the growing threat of antibiotic resistance, it is advisable for hospitals to undertake periodic assessments in order to mitigate the risk of infection and safeguard patient health.

5. CONCLUSION

The current study highlights that *P. aeruginosa* relics a significant cause of nosocomial wound infections worldwide. The incidence of β-lactamase-producing *P. aeruginosa* is on the rise. Although this study underlines the exceptional problem of ESBL-mediated resistance, which has produced a therapeutic confrontation for clinicians and microbiologists. In order to address the issue of the emergence and the spread of multidrug-resistant *P. aeruginosa*, it is necessary for microbiologists, clinicians, and the infection control team to communicate and collaborate effectively. It is recommended to regularly monitor antibiotic resistance both inside and outside of hospitals.

6. ETHICAL APPROVAL

The authors have collected and preserved written ethical approval in accordance with international standards.

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