Comparison of Antibiotic Resistance and Lipase Production in Extended Spectrum β-lactamases Producing and Non-producing Isolates of *Pseudomonas aeruginosa*

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**A B S T R A C T**

*Pseudomonas aeruginosa* is a major cause of nosocomial infections. Recently multidrug resistance and extended-spectrum β-lactamase (ESBL)-producing *P. aeruginosa* isolates are emerging worldwide. These isolates are reported to be more virulent than the non-multidrug resistance and non ESBL producing isolates. In order to find a correlation between ESBL production and virulence, we tested one virulence factor involved in pathogenicity- lipase production in ESBL and non ESBL producing isolates. A total of 100 samples were evaluated. ESBL was determined phenotypically by CLSI method while lipase production was determined using egg yolk agar. Forty nine isolates produced ESBL out of which 47 (95%) were positive for lipase while 51 isolates were non-ESBL producing out of which 34 (67%) were positive for lipase (p value < 0.05). Antibiotic resistance was also found more in ESBL producers compared to non-ESBL producers. Our data demonstrate that lipase production was higher in the ESBL producing isolates compared to the non-ESBL producing isolates. Lipase production therefore renders ESBL positive isolates more pathogenic.

**Keywords**

Antibiotic resistance, Lipase, *Pseudomonas*.

**Article Info**

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**Introduction**

*P. aeruginosa* is one of the most important pathogenic bacteria which cause clinical infection as a result of its high resistance to antimicrobial agents and is therefore a particularly dangerous and dreaded bug.[1] Infections cause by *P. aeruginosa* often are difficult to treat due to high level of resistance to multiple antibiotics as a result of both intrinsic genes and acquisition of resistance genes.[2] In addition to the constitutive low susceptibility of *P. aeruginosa* to antimicrobial agents, emergence of new resistance mechanisms such as extended-spectrum β-lactamase (ESBL) belonging to different classes have been identified in these organisms and therefore pose critical challenges to the health care giver.[2]

ESBLs are acquired plasmid-mediated β-lactamases and have the ability to inactivate β-lactams antibiotics containing an oxyimino-group such as oxyimino-cephalosporins (e.g. ceftazidime, ceftriaxone, cefotaxime) as well as oxyimino-monobactam (e.g. aztreonam). They are not active against cephemycins and carbapenems. Generally, ESBLs are inhibited by β-lactamase-inhibitors such as clavulanate and tazobactam.[3] Infection caused by ESBL-producers are associated with increased virulence of these strains and severe adverse
outcomes. This is often due to delay in effective therapy and the failure to use antibiotic active against ESBL-producing isolates.[2]

Pathogenesis of *P. aeruginosa* is multifactorial, involving several virulence factors that include structural components, toxins, and enzymes.[4] Extracellular enzymes may cause hemorrhage in internal organs in systemic disease, alter host cellular receptors, and alter microbial behaviour by promoting invasiveness, serum resistance, and evasion of host immune mechanisms.[5] Bacterial lipases are mostly extracellular and the major factor for the expression of lipase activity in Pseudomonas has always been carbon, since lipases are inducible enzymes and are thus generally produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids.[6,7] Most published experimental data have shown that lipid carbon sources (especially natural oils) stimulate lipase production, and peptone is one of the most suitable substrate for maximum lipase production by *P. aeruginosa*. [7] Lipase production is known for their role in disease production and establishment of infection.[5] Hence, this study was performed to evaluate and compare lipase production involved in pathogenicity in ESBL and non-ESBL producing clinical isolates of *P. aeruginosa*.

**Material and Methods**

A total of 100 isolates of *P. aeruginosa* from various clinical specimens like urine, pus, blood, body fluids and sputum, were collected from both indoor and outdoor patients, irrespective of age and sex, and identified by standard microbiological procedures.[8] The isolates were subjected to antimicrobial susceptibility testing performed by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) criteria. Mueller-Hinton Agar (MHA) was used for antibiotic sensitivity testing. Various antibiotics were put up and plates incubated at 37°C for 24 hours.

Isolates showing reduced susceptibility to third generation cephalosporins were tested for ESBL production as per CLSI method. The test organism was inoculated on MHA plate. One 30µg disc of ceftazidime and one 30 µg disc of cefotaxime and another 30/10µg disc of ceftazidime/clavulanic acid and 30/10µg disc of cefotaxime/clavulanic acid were placed on surface of agar plate. The plates were incubated at 35°C for 16-18 hours. A ≥5 mm increase in zone diameter for the antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was considered positive for ESBL production.[9]

For lipase production, the isolates were inoculated on egg yolk agar plates and the presence of pearly white iridescent sheen on the surface of the colonies after incubation at 37°C for 5 days, was taken as positive indicator for lipase production(Figure 1).[10]

**Results and Discussion**

Out of a total of 100 clinical isolates, maximum number of *P. aeruginosa* isolates were from urine (49%), followed by pus (20%), blood (19%), sputum (11%) and other body fluids (1%) samples. The antibiotic resistance pattern is depicted in Figure 2.

Among β-lactams group, the isolates of *P. aeruginosa* showed maximum resistance to ticarcillin /clavulanic acid (79%) followed by aztreonam (78%), ceftazidime (73%), cefoxitin (68%), ceftizoxime (50%), ceftriaxone (48%), cefepime (45%), meropenem (34%) and imipenem (33%), while resistance to piperacillin/ tazobactam was seen only in 12% isolates. Among
aminoglycosides group, the isolates of *P. aeruginosa* showed maximum resistance to netilmicin (64%) followed by amikacin (43%) and gentamicin (41%). Among fluoroquinolones group, isolates of *P. aeruginosa* showed maximum resistance to norfloxacin (67.3%) followed by ciprofloxacin (44%). Among others group, resistance to colistin and polymyxin B was seen in 3% and 2% strains respectively.

Out of these 100 isolates, 58 were multi drug resistant (MDR; isolates showing resistance to at least three of the four antimicrobial drugs i.e β-lactams, carbapenems, aminoglycosides, fluoroquinolones) and 20 were extreme drug resistant (XDR; isolates showing resistance to all the above mentioned four antimicrobial drugs). Forty nine (49%) isolates were ESBL producers, while 51 (51%) isolates were non-ESBL producers (Figure 3).

On comparing the antibiotic resistance among ESBL producing and non-ESBL producing *P. aeruginosa* isolates, a significant statistical difference (p value<0.05) was observed for all cephalosporins except cefoxitin, carbapenems, aminoglycosides and ciprofloxacin (Table 1).

Eighty one (81%) isolates were positive for lipase production out of which maximum was found in blood and other body fluids (100%), followed by pus (85%), sputum (82%) and urine (71%). Out of the total lipase positive isolates, 47 were ESBL producers and 34 were non-ESBL producers (Figure 4).

The rate of isolation of *P. aeruginosa* from various clinical samples taken in our study was similar with Pitout *et al*. who had also reported maximum rate of isolation of *P. aeruginosa* isolates in urine (43%), followed by pus (21%) sputum (20%) and blood (7%) samples. Khan *et al* reported maximum rate of isolation of *P. aeruginosa* from pus (57.64%) followed by urine (24.2%) samples. The difference in rates of isolation may be due to difference in type of samples received in different laboratories.

Studies done by various authors have reported the prevalence 84.5% and 68.75% multdrug resistant *P. aeruginosa* isolates. In contrast, Tam *et al*. reported 14% of MDR isolates. Delays in starting appropriate therapy may contribute to increased length of hospital stay and persistence of multdrug resistant *P. aeruginosa* isolates infection.

ESBL producing strains are usually found in those areas of hospitals where antibiotic use is frequent and the patient’s condition is critical. In India, prevalence rate of ESBLs ranging from 28% to 84% has been reported from various parts of the country: Bakshi *et al*. reported high prevalence (50%) of ESBL production among *P. aeruginosa* at Patiala (Punjab). Similar incidence of ESBL production among *P. aeruginosa* (42.31%) has been reported by researchers at AIIMS, New Delhi and from Coimbatore (40%). Our study corroborates these findings. This relatively high rate of ESBL production in our study may be due to selection pressure caused by extensive use of β-lactam antibiotics in our hospital. This ESBL production rate might be much higher but could not be detected due to presence of silent genes which do not express phenotypically because in the present study we have not confirmed the ESBL genes using genotypic methods. High antibiotic resistance in ESBL producing *P. aeruginosa* isolates have also been reported by authors. Tavajjohi *et al*. reported that resistant to antibiotics like piperacillin, cefotaxime, ceftriaxone, ceftazidime, imipenem, aztreonam was observed in 42.9%, 71.4%, 57.1%, 57.1%, 28.5%, 42.9% ESBL producing *P. aeruginosa* isolates, however no comparison in antibiotic resistance among
ESBL and non ESBL producers has been done by these authors.\cite{15} In the present study, a significant statistical difference (p value<0.05) was observed for resistance to aminoglycosides and fluoroquinolones in ESBL and non ESBL producing 
*P. aeruginosa* isolates. High antibiotic resistance to these antibiotics in ESBL producing *P. aeruginosa* isolates have been reported by other authors. Tavajohi et al reported that resistance to antibiotics like gentamicin and ciprofloxacin was observed in 42.9% and 17.4% ESBL producing *P. aeruginosa* isolates, respectively.\cite{15} Peshattiwar et al reported that, among 126 *P. aeruginosa* isolates, 28 (22%) were ESBL producers and total of sixty seven strains showed resistance to ceftazidime, of which 28 (41.79%) were found in ESBL producers.\cite{21}

**Table 1** Comparison of antimicrobial resistance pattern of ESBL producing and non-ESBL producing *P. aeruginosa* isolate to various antibiotics

| Antimicrobial drugs | ESBL producers (n=49) | Non-ESBL producers (n=51) | p value |
|---------------------|-----------------------|---------------------------|---------|
|                     | n (%)                 | n (%)                     |         |
| **β- lactams**      |                       |                           |         |
| Ceftazidime         | 49 (100)              | 24 (47.1)                 | <0.05   |
| Ceftizoxime         | 45 (91.8)             | 5 (9.8)                   | <0.05   |
| Ceftriaxone         | 47 (95.9)             | 1 (2)                     | <0.05   |
| Cefepime            | 32 (65.3)             | 13 (25.5)                 | <0.05   |
| Cefoxitin           | 36 (73.5)             | 32 (62.7)                 | >0.05   |
| Aztreonam           | 42 (85.72)            | 36 (70.6)                 | >0.05   |
| Imipenem            | 23 (47)               | 10 (19.6)                 | <0.05   |
| Meropenem           | 24 (49)               | 10 (19.6)                 | <0.05   |
| Piperacillin/tazobactam | 9 (18.4)         | 3 (5.8)                   | >0.05   |
| Ticarcillin/clavulanic acid | 40 (81.6) | 39 (76.5)             | >0.05   |
| **Aminoglycosides** |                       |                           |         |
| Gentamicin          | 27 (55)               | 14 (27.4)                 | <0.05   |
| Amikacin            | 29 (59.2)             | 14 (27.4)                 | <0.05   |
| Netilmicin          | 37 (75.5)             | 27 (53)                   | <0.05   |
| **Fluoroquinolones**|                       |                           |         |
| Ciprofloxacin       | 28 (57)               | 16 (31.4)                 | <0.05   |
| Norfloxacin         | 16 (32.7)             | 17 (33.3)                 | >0.05   |
| **Others**          |                       |                           |         |
| Polymyxin           | 0 (0)                 | 2 (3.9)                   | >0.05   |
| Colistin            | 1 (2)                 | 2 (3.9)                   | >0.05   |
**Fig. 1** Lipase production in egg yolk agar seen as a pearly white sheen on the surface of colony

**Fig. 2** Antimicrobial resistance pattern of the 100 isolates of *P. aeruginosa*
However, no comparison in antibiotic resistance pattern was made between ESBL producing and non-ESBL producing isolates. A decreased susceptibility of *P. aeruginosa* to the commonly used antibiotics has already been noted by previous researchers.\[^{22}\] In this study, the ESBL producing isolates were significantly more resistant to anti-bacterial agents compared with non-ESBL producing isolates (p < 0.05). The same results were reported for clinical isolates of enteric bacteria by Mansouri *et al* and the gram negative bacteria from urinary tract infections by Selvakumar *et al* and Mashouf *et al*.\[^{23}\],[^ {24}]\] This can be explained by the fact that ESBL producing bacteria usually
have mobile genetic elements coding gene for resistance to other antibacterial agents. Different efflux pumps in *P. aeruginosa* are able to eject multiple antimicrobials from cell, including beta-lactams and effect penicillin as well as non-beta-lactams drugs such as fluoroquinolones and aminoglycosides.\(^{[25]}\)

Lipase production in our study was high (81%), more in ESBL producers than non-producers which is similar to study done by Georgescu *et al* where lipase production was seen in 77% of *P. aeruginosa*.\(^{[26]}\) In another study by Mohammad on *P. aeruginosa* isolated from burn patients, 100% were positive for lipase production.\(^{[27]}\) In a study done by Maroui *et al* on virulence profiles of clinical and environmental *P. aeruginosa* isolates, lipase was produced in all the 68 (100%) clinical strains.\(^{[28]}\) In a similar study done by Khalil *et al*, the amount of lipase production was 81% and the incidence was more in ESBL producers than non-ESBL producers.\(^{[29]}\) In a study done by Finlayson and Brown comparing the antibiotic resistance and virulence factors in pigmented and non-pigmented *P. aeruginosa*, the incidence of lipase production in pigmented and non-pigment isolates was 82.5% and 17.5% respectively. However the total lipase production in 57 isolates taken in the study was 72%.\(^{[30]}\)

In conclusion, ESβL-producing *P. aeruginosa* clinical isolates are armed with a large arsenal of virulence factors. Lipase production in these pathogens enable them to breach the human innate immune system, to infect host cells, and to modulate human adaptive immune mechanisms, serving the goal of establishing systemic or more localized chronic colonization and hence associated with increase virulence. The obtained results clearly indicate the importance of the recommendation that the antibiotic resistance as well as virulence factors of *P. aeruginosa* must be periodically studied in order to understand the possible co-regulatory mechanisms that might be involved in their expression.

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