Sarwat et al., Yemeni J Med Sci. 2022;16:1-6.
https://doi.org/10.20428/YJMS.16.1.A1

ORIGINAL ARTICLE OPEN ACCESS

Prevalence of Extended Spectrum Beta-Lactamase in *Pseudomonas aeruginosa* isolated from patients at Sharda Hospital, Greater Noida, Western UP

Tarana Sarwat [1], Sneha Mohan [1], Satendra P. Singh [1], Dalip K. Kakru [1]

[1] *Department of Microbiology, Sharda University, Greater Noida.*

ABSTRACT

Introduction: *Pseudomonas aeruginosa* is an important pathogen causing healthcare-associated infections especially in immunocompromised patients. It poses a threat to public health due to its inherent resistance to various antimicrobial agents and its ability to acquire new resistance through multiple mechanisms. Infections due to extended spectrum β-lactamase (ESBL) producing isolates of *P. aeruginosa* continue to be a challenge for clinicians as these result in high mortality and morbidity due to antimicrobial resistance. The aim of this study was to determine the prevalence of Cefotaximase-Munich (CTX-M) producing strains of *P. aeruginosa* in Sharda hospital, Greater Noida.

Methods: Strains of *P. aeruginosa* isolated from various clinical samples were subjected to phenotypic detection for ESBL production by disc combination method. Positive strains were then subjected to polymerase chain reaction (PCR) for detection of $bla_{\text{CTX-M}}$ gene.

Results: Out of 166 isolates of *P. aeruginosa,* 54 (32.53%) were phenotypically confirmed to produce ESBL. Out of these 54 isolates, 39 (72.22%) were positive for $bla_{\text{CTX-M}}$ gene. Multidrug resistance was found in 70 (42.17%) isolates. Imipenem was the most effective drug with a sensitivity of 64.86% whereas aztreonam was found to be least effective with sensitivity of only 36.74%.

Conclusion: Current study highlights the phenotypic and molecular characterization of CTX-M gene in *P.aeruginosa* in our hospital set-up. With judicious use of antimicrobials and strict infection control practices, it might be possible to limit the effect of these drug destroying enzymes.

Keywords: CTX-M gene, Extended spectrum beta lactamases, Hospital acquired infections, multidrug resistance, Pseudomonas aeruginosa.

***Corresponding author:** T. Sarwat (taranasf@gmail.com)

1. Introduction

Pseudomonas aeruginosa is an epitome of hospital acquired pathogens, which causes a wide spectrum of infections and leads to high morbidity especially in immune-compromised patients.[1] It is a notorious pathogen known to cause nosocomial septicemia and burn wound infections, which are difficult to treat particularly in burn wards and intensive care units (ICUs).[2,3]

Unfortunately, even the selection of most appropriate antibiotic is negated by the ability of *P. aeruginosa* to acquire resistance to multiple classes of antibacterial agents, during the course of treatment itself. The organism can develop resistance to antibacterial agents either through acquisition of plasmids or through mutations that alter the expression and/or function of chromosomally encoded genes.[4] Both mechanisms of developing drug resistance can severely limit the therapeutic options for treatment of serious infections.[4] Extended spectrum beta-lactamases (ESBLs) producing *P. aeruginosa* have spread rapidly worldwide and pose a big challenge as healthcare-associated infections [5]. Cefotaximase-Munich (CTX-M) has evolved as the prevalent member of the ESBL group of enzymes, which is involved in imparting resistance against the third and even fourth generation of the cephalosporin.[6] The first explanation of the harboring of CTX-M type of ESBLs by *P. aeruginosa* was given by Al Naiemi et al. [7] The threat of infections caused by multidrug resistant and ESBL producing *P. aeruginosa* has become a major concern in healthcare settings and implementation of infection control policies are the mainstay to avoid the spread of this threat.[1] With prior knowledge of susceptibility pattern in a particular geographical region, it becomes easier to choose appropriate antimicrobials. The present study was therefore conducted to determine the phenotypic and genotypic surveillance of ESBL producing isolates of *P. aeruginosa* and hence, to guide control of nosocomial infections in our healthcare set-up.

2. Materials & Methods

The present study was conducted in the Department of Microbiology, Sharda Hospital, Greater Noida, Western UP. The study was approved by the Institutional Ethics Committee, Sharda University. It was a cross sectional study conducted over a period of 2 years from January 2018 to December 2019. The clinical samples from were collected from patients who had developed symptoms 48 hours after admission to hospital and hence, were suspected to be suffering from hospital acquired infections. The samples were transported immediately to the laboratory. These were processed according to standard bacteriological procedures available[8] Identification of the organism was done by conventional biochemical methods which included catalase and oxidase test, hanging drop for motility, Hugh-Leifson's oxidative fermentative test, triple sugar iron test, nitrate reduction, arginine dihydrolase and lysine decarboxylation test and pigment production. Ambiguous strains were identified by the VITEK 2-compact automated system (BioMerieux, France) following the manufacturer's instructions.

Antibiotic sensitivity was done by Kirby Bauer disc diffusion method as per the standard Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. Commercially available antibiotic disks (Himedia) were used for antimicrobial susceptibility testing. The zone diameters were interpreted as per the CLSI guidelines [9].

Multidrug resistance (MDR) was defined as resistance of the isolate to three or more classes of antibiotics [10].

2.1 Screening of ESBL production:

The strains showing resistance to third generation cephalosporins were screened out as ESBL producers and were subjected to phenotypic confirmation.

2.2 Phenotypic confirmation of ESBL production:

The CLSI recommended combined disk method involving ceftazidime and cefotaxime alone and with the inhibitor clavulanic acid (30 µg) was used to confirm phenotypically the presence of ESBL [9].

Escherichia coli American Type Culture Collection (ATCC) 25922 was used as a negative control for ESBL and *P. aeruginosa* ATCC 27853 was used as a control strain for a positive ESBL.

The strains were stocked in l% semisolid agar in SV-5 vials and were stored at 4°C till further processing for gene detection.

2.3 Genotypic confirmation of ESBL production:

The phenotypically confirmed strains were further subjected to conventional PCR for detection of CTX-M gene.

i. DNA extraction:

The bacterial DNA of test and control strains was extracted as per the kit manufacturer's instructions. The kit used was QIAamp DNA mini kit from QIAGEN, Germany with lot no. 157036210.

ii. Amplification:

The primers for amplification Table (1) were procured from Imperial Life Sciences Ltd. (Gurgaon, India) [11] and PCR was conducted in BIO-RAD CFX Connect thermocycler.

Table 1. Primers used for PCR to detect CTX-M gene

Primer	Sequence (5' →3')	Gene	Size of amplicon
CTX-M-F*	ATGTGCAGYACCAGTAARGTC	CTX-M	593 bp
CTX-M-R**	TGGGTRAARTARGTSACCAGA		

*Forward primer **Reverse primer

The cycling conditions for gene amplification were as shown in table (2):

Table 2. PCR cycles

Stages		No. of cycles – 30
Stage 1	95°C	5 min
Stage 2	95°C	30 sec
	55°C	30 sec
	72°C	30 sec
	72°C	5 min
Stage 3	4°C	∞

Analysis of PCR results was done by electrophoresis of the amplicons on 1.5% agarose gel which were then visualized by BIO-RAD GEL DOC set with UV/VISIBLE trans-illuminator (BIO-RAD).

3. Results

A total of 166 isolates of *P. aeruginosa* were isolated from various samples received in bacteriology lab during the study period. Maximum isolates were obtained from pus samples followed by endotracheal secretions, sputum, pleural fluid, urine, blood and ear swab as shown in table (3).

Table 3. Distribution of various sources of *P. aeruginosa* isolates

Sample	No. of isolates
Pus	67
Endotracheal secretions	36
Sputum	27
Pleural fluid	12
Urine	11
Blood	09
Ear swab	4

Maximum isolates were obtained from samples received from ICU followed by Surgery and Medicine wards as shown in table (4).

Table 4. Ward wise distribution of *P. aeruginosa* isolates

Sample	No. of isolates
ICU	56
General Surgery	45
Medicine	27
Obstetrics & Gynecology	16
Orthopedics	13
Pediatrics	5
ENT	4

The antibiotic susceptibility pattern showed maximum sensitivity towards imipenem (64.46%) followed by piperacillin/tazobactam (57.23%), ticarcillin/clavulanic acid (56.63%), and levofloxacin (54.22%) (Fig.1). Aminoglycosides were not found to be very effective with a sensitivity of only 40.36% with gentamicin and 42.17% with tobramycin. Aztreonam was found to be least effective with a sensitivity of only 36.74% as shown in table (5).

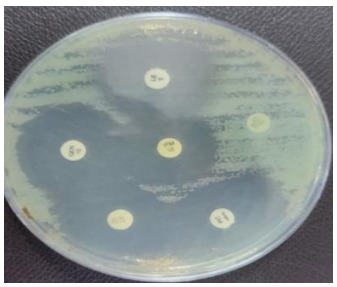

Fig. 1. Antibiotic susceptibility test for *P. aeruginosa*

Table 5. Antibiotic susceptibility pattern of *P. aeruginosa* isolates

Antibiotic	Sensitive n (%)	Resistant n (%)
Aztreonam (30µg)	61 (36.74)	105 (63.25)
Ceftazidime (30µg)	72 (43.37)	94 (56.62)
Ciprofloxacin (5µg)	82 (49.40)	84 (50.60)
Gentamicin (10µg)	67 (40.36)	99 (59.64)
Imipenem (10µg)	107 (64.46)	59 (35.54)
Levofloxacin (5µg)	90 (54.22)	76 (45.78)
Piperacillin (100µg)	67 (40.36)	99 (59.64)
Piperacillin/Tazobactam (100/10µg)	95 (57.23)	71 (42.77)
Ticarcillin (75µg)	72 (43.37)	94 (56.63)
Ticarcillin/Clavulanic acid (75/10 µg)	94 (56.63)	72 (43.37)
Tobramycin (10 µg)	70 (42.17)	96 (57.83)

Out of 166 isolates of *P. aeruginosa*, 70 (42.17%) were found to be multidrug resistant showing resistance towards cephalosporins, aminoglycosides and fluoroquinolones. On phenotypic confirmation, 54 (32.53%) isolates were found to be ESBL producers Table (6) and Fig. (2).

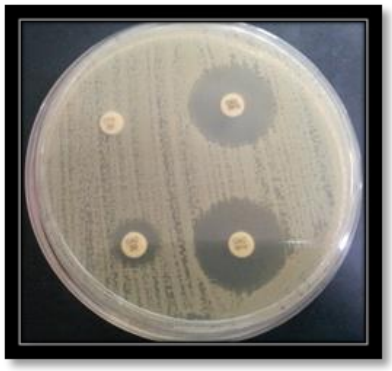

Fig. 2. Combined disk test for ESBL detection

Out of these 54 isolates, 39 (72.22%) were confirmed genotypically as ESBL producers harboring the $bla_{\text{CTX-M}}$ gene Table(6) and Fig. (3)

Table 6. Percentage of ESBL producing strains of *P. aeruginosa*

Total no. of isolates (n)	Phenotypically confirmed ESBL isolates, n (%)	Genotypically confirmed ESBL isolates, n (%)
166	54 (32.53%)	39 (72.22%)

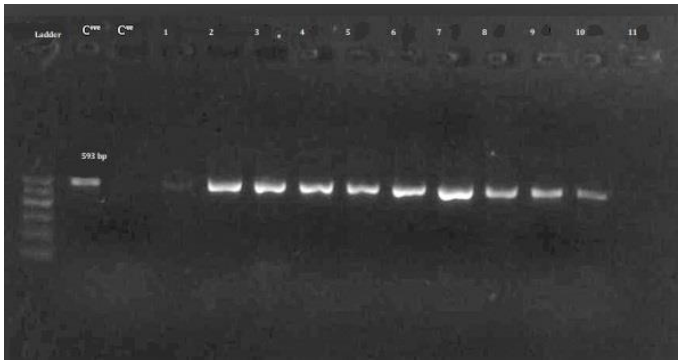

Fig. 3. Gel electrophoresis for $bla_{\text{CTX-M}}$ gene (Lane 2-10 positive for $bla_{\text{CTX-M}}$, Lane 1&11 negative for $bla_{\text{CTX-M}}$ gene)

4. Discussion

P. aeruginosa has rapidly emerged as a leading hospital acquired pathogen because of its ubiquitous nature, ability to survive in moist environments and acquired resistance to the great majority of antimicrobial agents.[12] Hospitalized patients are particularly susceptible to infections because their skin and mucosal barriers are already compromised due to the use of invasive devices. There has been an increased incidence of ESBL producing strains of *P. aeruginosa* over the past few years. Their detection becomes significant because these strains are most likely to be even more prevalent than it is currently recognized.[13] We therefore aimed to conduct phenotypic and genotypic characterization of ESBL producing isolates of *P. aeruginosa* in our hospital set up.

In the current study, 166 strains of *P. aeruginosa* were isolated from samples received from various wards of hospital. Maximum strains were isolated from pus samples. This was in agreement with other studies done in Bhubaneswar, Maharashtra and Devangere where too maximum strains were isolated from pus samples. [14-16] This high isolation rate of *P. aeruginosa* from pus samples is due to the fact that *P. aeruginosa* is notorious for causing wound infections in hospital set-ups because of its ubiquitous nature.

According to various studies, the strains of *P. aeruginosa* isolated from nosocomial infections tend to be quite resistant to various antimicrobial agents [17]. In the present study, we observed that *P. aeruginosa*

showed highest resistance against aztreonam (63.25%) followed by piperacillin and gentamicin (59.64%), tobramycin (57.83%) and ceftazidime (56.62%) while least resistance against imipenem (35.54%). This was in agreement with a study conducted in Amritsar which showed similar resistance pattern with aztreonam showing 64.4% resistance and imipenem 34.7%.[18] Another study conducted in Maharashtra also reported similar results with 57.15% resistance against ceftazidime and 57.80% resistance against gentamicin.[19]

A study conducted in Iran showed 57.3% resistance against tobramycin which was in concordance with our study but resistance against imipenem was quite high (77.1%) as per their study.[20] Gill et al. reported a lower resistance against ceftazidime (47.5%) as compared to our study.[18] This variation in antibiogram of *P. aeruginosa* is due to the difference in antibiotic policies being followed in different healthcare settings and also because of the difference in population that each setting caters to.

P. aeruginosa is notorious for being a multidrug resistant organism, part of its resistance being because of its innate resistance to some antimicrobials and rest, because of injudicious use of antimicrobials. The percentage of multidrug resistance in *P. aeruginosa* has been reported to be as low as 11.36% by Idris S *et al*. to as high as 91.6% as reported by Paranjothi S *et al*.[21, 22] In the current study, 56.02% [23] strains were found to be multidrug resistant which falls in the above range. Our results are also concordant with the study conducted by Sivanmaliappan *et al.* who reported 55.5% MDR in *P. aeruginosa*.[23] The emergence of multidrug resistant strains of *P. aeruginosa* warns of unlimited use of antimicrobials.

The prevalence of ESBL producing strains of *P. aeruginosa* varies across different regions of the globe and also within the hospitals. In our study, we detected ESBL production in 32.53% strains of *P. aeruginosa* which is in accordance with a study done by Tsering *et al.* who reported 32.61% ESBL strains of *P. aeruginosa* [24]. However, a lower percentage of 27.9% was reported by Taneja et al. in their study.[25] On the contrary a higher percentage of 42.30% was reported by Goel *et al.* [26]. Hence, it is important to detect ESBL production so that appropriate antibiotics can be chosen for proper treatment.

On genotypic detection, we found 72.22% strains of *P. aeruginosa* to produce CTM-M gene which was quite high as compared to a study done in Iran which reported 23.9% strains of *P. aeruginosa* to produce CTX-M gene.[18] Komijani *et al.* also reported a lower incidence of 24.22% for CTX-M production in the same organism.[27] However, a higher prevalence of 59.46% was reported by Jamali *et al.* [13] Hence, it can

be concluded that the prevalence of ESBL varies from region to region depending upon the population and antibiotic policies followed. Therefore, it should be a routine practice in labs to detect ESBL strains so that appropriate and judicious use of antibiotics can be done.

5. Conclusion

The study concludes that there is a high prevalence of ESBL producing strains of *Pseudomonas aeruginosa* in the area catered by Sharda Hospital, Greater Noida. It also highlights the existence of $bla_{\text{CTX-M}}$ group of ESBL producers among the hospital strains in the organism. The ability of these strains to transfer plasmid-borne resistance genes to the different bacteria is of great concern in health-care settings. Although environmental factors and mutations in bacterial genes cannot be overlooked but the key factor in the emergence of antimicrobial resistance is the inappropriate use of antibiotics. Unless judicious use of antibiotics is done, multi-drug resistant organisms, untreatable by every known antibiotic, may emerge, reversing the progress made in medical field and dumping us back to the pre-antibiotic era. Spread of multidrug resistant strains of *P. aeruginosa* is one such example.

Limitations:

One limitation of this study is that the AmpC producing strains of *P. aeruginosa* could have been missed out on phenotypic ESBL detection as AmpC interferes with ESBL detection. Another limitation is that we detected only CTX-M gene and hence presence of other ESBL

producing genes in phenotypically confirmed isolates could not be determined.

Financial support: Sharda University, Greater Noida

Conflicts of interest: The authors declare that they have no competing interest associated with this article.

References

1. Sarwat T, Rashid M, Rastogi V, Chander Y. A Comparative Study of Antibiogram of Pseudomonas aeruginosa in Hospital and Community Acquired Infections. Int.J.Curr.Microbiol.App.Sci 2015; 1: 286-91.
2. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis 2002; 34: 634-40.
3. Cao B, Whang H, Sun H, Zhu Y, Chen M. Risk factors and clinical outcomes of nosocomial multi-drug-resistant Pseudomonas aeruginosa infections. J Hosp Infect 2004; 57: 112-8.
4. Lister PD, Wolter DJ, Hanson ND. Antibacterial-Resistant Pseudomonas aeruginosa: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin Microbiol Rev. 2009; 22(4): 582–610.
5. Goosssens H. Susceptibility of multi-drug-resistant Pseudomonas aeruginosa in intensive care units:results from the European MYSTIC study group. Clin Microbiol Infect 2003; 9: 980-3.
6. Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. Korean J Intern Med 2012;27:128–42.
7. Al Naiemi N, Duim B, Bart A. A CTX-M extended-spectrum beta-lactamase in Pseudomonas aeruginosa and Stenotrophomonas maltophilia. J Med Microbiol 2006;55(Pt 11):1607-8.
8. Mackie and McCartney Practical Medical Microbiology, Tests for the identification of Bacteria, 14th Edition, Delhi: Elsevier Publication 2006:131- 50.
9. CLSI. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement CLSI: Document M100-S28. Wayne, PA, 2018.
10. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al.. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x. Epub 2011 Jul 27. PMID: 21793988.
11. Bhasin A, Chander Y, Manocha H. Molecular Characterization of blaCTX-M, blaTEM and blaSHV Beta Lactamases Produced by Uropathogens. Journal of Advances in Medicine and Medical Research. 2020; 32(23):97-106. https://doi.org/10.9734/jammr/2020/v32i2330721
12. Naas T, Poirel L, Nordmann P. Minor extended spectrum b-lactamases. Clin Microbiol Infect 2008; 14 Suppl1: 42-52.
13. Jamali S, Shahid M, Sobia F, Singh A, Khan HM. Phenotypic and molecular characterization of cefotaximases, temoniera, and sulfhydryl variable β-lactamases in Pseudomonas and Acinetobacter isolates in an Indian tertiary health-care center. Indian J Pathol Microbiol 2017;60:196-201.
14. Pathi B, Mishra SN, Panigrahi K, et al. Prevalence and antibiogram pattern of Pseudomonas aeruginosa in a tertiary care hospital from Odisha, India Transworld Med. J 2013; 1(3): 77- 80.
15. Satyajeet K, Pawar P, Mane M, et al. Pseudomonas aeruginosa and its antibiogram from clinical isolates in a tertiary teaching hospital from Western Maharashtra, India. J. Evidence Based Med. Healthcare 2014: 1(7): 574-81.
16. Mohan BS, Lava R, Prashanth HV, et al. Prevalence and antibiotic sensitivity pattern of Pseudomonas aeruginosa; an emerging nosocomial pathogen. Int. J. Biol. Med. Res 2013: 4(1): 2729-31.
17. Syed A, Thakur M, Shafiq S, Sheikh AU. In-vitro sensitivity patterns of Pseudomonas aeruginosa strains isolated from patients at skims - role of antimocribials in the emergence of multiple resistant strains. JK-Pract 2007; 14(1): 31-4.
18. Gill MK, Khanna A. Antibiogram of Pseudomonas aeruginosa Isolated from Infected wounds. Int J Med Microbiol Trop Dis 2019;5(3):163-5.
19. Raytekar NA, Choudhari MR, Das S. Antibiotic profiling of Pseudomonas aeruginosa isolates from pus sample of rural tertiary care hospital of Western Maharashtra, Loni, India. Int J Res Med Sci 2017;5:3076-81.
20. Bahrami M , Sichani MM, Karbasizadeh V. Prevalence of SHV, TEM, CTX-M and OXA-48 β-Lactamase Genes in Clinical Isolates of Pseudomonas aeruginosa in Bandar-Abbas, Iran. Avicenna J Clin Microbiol Infect 2018 ;5(4):86-90.
21. Idris S, Desa M, Aziz MN, Mohd Taib N. Antimicrobial susceptibility pattern and distribution of EXO U and EXO S in clinical isolates of Pseudomonas aeruginosa at a Malaysian hospital. Southeast Asian J Trop Med Public Health. 2012;43(1):116-23.
22. Paranjothi S, Dheepa R. Screening for multidrug resistance bacteria Pseudomonas aeruginosa in hospitalized patients in Hosur, Krishnagiri(dt). Int J Pharma Biosci.2010;1(3):281
23. Sivanmaliappan TS, Sevanan M. Antimicrobial Susceptibility Patterns of Pseudomonas aeruginosa from Diabetes Patients with Foot Ulcers. Int J Microbiol. 2011:1-4.
24. Tsering DC, Das S, Adhiakari L, Pal R, Singh TS. Extended spectrum beta-lactamase detection in Gram-negative bacilli of nosocomial origin. J Glob Infect Dis 2009;1:87-92.
25. Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL & Amp-C beta-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res 2008;127:85-8.
26. Goel V, Hogade SA, Karadesai SG. Prevalence of extended-spectrum betalactamases, AmpC beta-lactamase, and metallo-beta-lactamase producing Pseudomonas aeruginosa and Acinetobacter baumannii in an Intensive Care Unit in a tertiary care hospital. J Sci Soc 2013;40:28-31.
27. Komijani M, Shahin K, Barazandeh M, Sajadi M. Prevalence of Extended-Spectrum β-Lactamases Genes in Clinical Isolates of Pseudomonas aeruginosa. Med Lab J. 2018;12(5):34-41