Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Molecular characterization of metallo-beta lactamase genebla$_{imp-1}$ in imipenem resistant *Pseudomonas aeruginosa* isolates from patients of chronic suppurative otitis media at a tertiary care hospital

R. Sujatha [1,*], Bipin Kishore Prasad [1], Nashra Afaq[1], Arunagiri[2], Deepak Sameerbind[1]

[1]*Dept. of Microbiology and ENT, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India*
[2]*Endodontics, MDC, Kanpur, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 25-09-2022
Accepted 08-11-2022
Available online 28-12-2022

Keywords:
CSOM
blaIMP-1
Pseudomonas
Amikacin

ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear and mastoid cavity, persisting for more than 2 weeks. *Pseudomonas aeruginosa* **,** is one of the most common organisms to cause CSOM. Carbapenems are among the most effective antibiotics used against *Pseudomonas* infections resistant to carbapenems is often associated with production of metallo-ß-lactamases strains. Detection of MBLs producing strains can be effective for optimal treatment of patients for the control and spread of resistance. The main purpose of the study is to determine the imipenem resistant *Pseudomonas aeruginosa* carrying metallo-ß-lactamase (MBL) genebla$_{IMP}$ in Patients of Chronic Suppurative Otitis Media at a Tertiary care hospital."

Materials and Methods: This was a cross sectional study carried out in the Department of Microbiology and ENT Department for a period of 1 year between July 2021 to July 2022. A total of 185 patients clinically suspected cases for CSOM were studied. Swabs taken from discharging ears were sent for Gram's staining, culture and antibiotic sensitivity test as per the latest CLSI guidelines. The isolates were further tested for MBL by screening test, by Imipenem – EDTA combined disc test, and MBL E test (Imipenem). The DNA was extracted by using Qiagen DNA Extraction kit and *bla*$_{IMP-1}$ gene for *Pseudomonas aeruginosa* isolates was detected by conventional PCR.

Results: In our study the number of cases clinically diagnosed of having CSOM was 185, out of which 63 (34%) was found to be culture positive for CSOM infection. Males were 38 (60.3%) as compared to that of female 25 (39.6%), Both the ears affected were almost in equal distribution, with the left ear being 32 (50.7%) and the right ear being 28 (44.4%) while in 3 cases (4.7%) it was bilateral. Gram negative bacilli were (74.6%), *Pseudomonas aeruginosa* being the most common isolate with 47.6% followed by *Klebsiella sp.* 19% and among the Gram positive isolates (25.3%). *Staphylococcus aureus*(12.6%) was the most common isolate. Colistin (96.6%), Piperacillin-tazobactam (73.3%), Amikacin (76.6.3%), and Cefepime (73.3%) were found to be the most effective Antibiotics. The resistance to ciprofloxacin was (56.6%), Levofloxacin (50%), Piperacillin (46.6%), Gentamicin(36.6%), Imipenem (36.6%), Tobramycin (30%), Ceftazidime (30%). Out of 30 *Pseudomonas aeruginosa* isolated 11(36.6%) were screening test-positives for MBL by Imipenem – EDTA combined disc test, and MBL E test (Imipenem). The bla$_{IMP-1}$ gene was detected in all 11 (36.6%) of the isolates of *Pseudomonas aeruginosa.*

Conclusion: In the present study *Pseudomonas aeruginosa* being the most common isolate with Colistin, Piperacillin-tazobactam, Amikacin, and cefepime to be the most effective antibiotics. Resistance of *P. aeruginosa* isolates to Imipenem due to MBL enzymes is increasing in the Kanpur region (36.6%). Judicial use of broad spectrum antibiotics, like Imipenem is the need of the hour, hence knowledge of the etiological agents of CSOM and their antibiogram is of paramount importance for an efficient treatment and prevention of both disease complications and antimicrobial resistance.

1. Introduction

Chronic suppurative otitis media (CSOM) is one of the most common diseases of the middle ear particularly in childhood.[1] It is a common health problem in a developing country like India especially in the lower socio economic status.Generally, microbiological culture of the ear discharge simplicates *Pseudomonas aeruginosa, Proteus* spp and *Staphylococcus* as the prevalent causative organism.[2] *Pseudomonas aeruginosa,* is one of the most common organisms to cause CSOM. Among the beta lactams, carbapenems are considered as the potent drug of choice for serious treatment of gram-negative bacteria infections. The most effective antibiotics that can be used against *Pseudomonas aeruginosa* are ß- lactam antibiotics in which imipenem as a carbapenem is considered as the most appropriate antibiotic to be used against the mentioned organisms.

Worldwide, the prevalence of gram negative bacteria with multi-drug resistance profiles is now recognized. Carbapenem resistance occurs because of the production of carbepenemases as there is decrease in antibiotics absorption. Carbapenemases acquires resistance belongs to Ambler molecular classes A, B and D. Metallo–beta-lactamases (MBL) enzymes are the most significant carbapenemases. Nowadays the emergence of antibiotic resistance strains is one of the challenges in treating patients, such as MBLs producing *Pseudomonas aeruginosa.*[3]

The VIM, IMP and SPM types are the most clinically significant carbapenemases which is encoded by blaVIM, blaIMP, and blaSPM genes.[4] Therefore, this study is undertaken to study the Molecular Characterization of Metallo-Beta Lactamase Genebla$_{IMP-1}$ in Imipenem Resistant *Pseudomonas aeruginosa* isolates from Patients of Chronic Suppurative Otitis Media at a Tertiary care hospital.

2. Materials and Methods

This was a hospital based cross sectional study carried out in the Department of Microbiology and ENT Department at RMCH & RC, Kanpur over a period of one year from July 2021 to July 2022. Ethical clearance was duly obtained from the Institute Ethical Committee for conducting the study. A total of 185 patients was included in our study. All adult patients who consented to participate in the study were included and Patients already on antibiotics were excluded.

2.1. Specimen collection ans sample processing

The sample was collected using Pus swab from the external auditory canal and introduced into Amies transport medium bottle and sent for laboratory analysis. The sample was processedto primary gram stain for pus cells and inoculated into Blood agar (Oxoid, UK), and MacConkey agar (Oxoid, UK) and incubated aerobically at 37 °C for 24–48 h.

2.2. Screening, isolation and identification of organisms

Identification of pathogens was based on Microscopy and colony characteristics (colony morphology, hemolysis on blood agar, changes in the physical appearance of the differential media). Gram positive isolates were tested for catalase and Coagulase tests while biochemical tests for gram negative isolated bacteria were tested for oxidase, Triple sugar Iron (TSI), Sulphur indole and motility (SIM), urease production and citrate utilization.[5]

2.3. Antimicrobial susceptibility testing

Antibiotic susceptibility test of isolated bacterial pathogens were performed using modified Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.[6]

A colony suspension with concentration equivalent to 0.5 McFarland solution was prepared for each identified isolate and inoculated into Mueller–Hinton-Agar (Oxoid, UK). Appropriate Selected Antibiotic discs were placed onto the media and incubated at 37 °C for 24 h. Gram positive isolates were tested against Ampicillin (10 μg), Amoxicillin/clavulanate (20/10 μg), Ceftriaxone (30 μg), Gentamycin (10 μg), Ciprofloxacin (5 μg), Trimethoprim/sulfamethoxazole (1.25/23.75 μg), Chloramphenicol (30 μg), Amikacin (17 μg) and Cephalexin (18 μg), Cefoxitin (30μg). Gram negative organisms were tested sensitivity to amikacin (AMK, 30 μg), gentamicin (GM, 10 μg), tobramycin (TOB, 10 μg), ceftazidime (CAZ, 30 μg), cefepime (CFP, 50 μg), piperacillin (PIP, 100 μg), PIP/tazobactam (PTZ, 100/10 μg), imipenem (IMP, 10 μg), ciprofloxacin (CIP, 5 μg), and levofloxacin (LFX, 5 μg) by modified Kirby Bauer disc diffusion method using Mueller Hinton agar (MHA) medium. A suspension of the isolated colonies of each test strain equivalent to a 0.5 McFarland's standard was prepared in sterile normal saline. Briefly, a suspension of each strain was made so that the turbidity was equal to 0.5 McFarland standards and then plated as a lawn culture on to MHA. Antibiotic discs were placed and plates were incubated at 37°C for 18–24 h. Results were interpreted in accordance with CLSI guidelines[6]*Escherichia coli* ATCC 25922, *Staphylococcus aureus* (American Type Culture Collection; ATCC 25923 and *P. aeruginosa* ATCC 27853 were used as control strains.

2.4. Phenotypic confirmatory test

Imipenem(IMP)- EDTA Combined disc test: The test organisms are inoculated by lawn culture technique on the plates of Muller-Hinton agar(MHA) as recommended by CLSI.[7] 10 μg Imipenem Disk and 750 μg Imipenem-

* *Corresponding author*.
E-mail address: drsujatha152@gmail.com (R. Sujatha).

EDTA Disk(Hi-media SD281) are placed on the plate. The inhibition zones of the imipenem and imipenem-EDTA disks are compared after 16 to 18 hours of incubation at 37°C. In the combined disc test, if the increase in inhibition zone with the imipenem and EDTA disc will be ≥ 7 mm than the imipenem disc alone, it is considered as MBL positive.[8]

MBL E test: The E-test MBL Strip contains a double sided seven-dilution range of IP(Imipenem) (4 to 256 μg/ml) and Imipenem (1 to 64μg/ml) in combination with a fixed concentration of EDTA is considered as the most sensitive method for MBL detection]. The E-test was done according to manufacturer's instructions. MIC ratio of IP/IPI (Imipenem+EDTA) of >8 or >3 log dilutions indicates MBL production.[8]

2.5. *Genotypic detection of blaIMP-1 gene in P. aeruginosa*

The DNA was extracted from P. aeruginosa using the Qiagen DNA Extraction Kit as per manufactures guidelines.

2.6. *Molecular analysis*

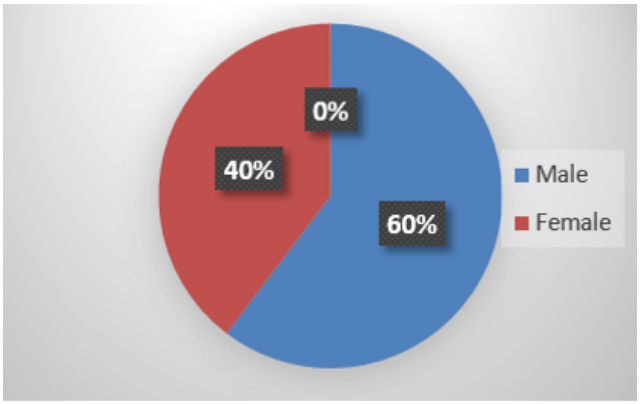
Polymerase chain reaction (PCR) was carried out for detection of bla$_{IMP}$, gene on a thermal cycler (Eppendorf, Germany). The primer pair sequences used in this study and the PCR conditions is described in the below Table 1. The DNA extraction was performed and the electrophoresis unit was run where 2% agarose gel was prepared with ethidium bromide. The bromophenonol blue dye was used for loading our DNA product which was then visualized in the gel documentation system. Positive controls used in this test were SPM-1 producing *P. aeruginosa* 16 strain (provided by Prof. Patrick Nordmann), *bla$_{IMP-1}$ from Seratia marcesens* (sequenced by Bioneer company), and *bla$_{VIM}$*. *P. aeruginosa* ATCC 27853 was used as a negative control.[9]

3. Results

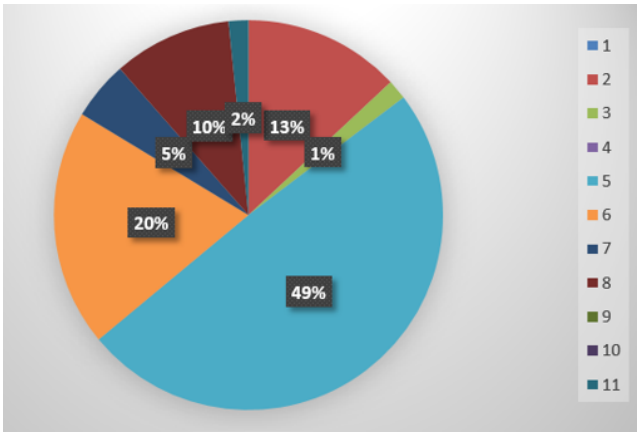
In our study the clinically diagnosed suspected cases having CSOM were 185, out of which 63 (34%) was found to be positive for CSOM infection. The ratio of Male 38 (60.3%) were found to be more as compared to that of Female 25 (39.6%), which is illustrated in the Table 2.

The maximum number of cases reported was observed in the age group of 0-10 years followed by 11-20 years of age and the least number of cases was seen in the age group above 51 years of age Table 3.

The side of the ear affected was almost in equal distribution, with the left ear being 32(50.7%) and the right ear being 28 (44.4%) while 3 (4.7%) were bilateral Table 4. In our study it was observed that the maximum number of cases was found in Gram negative bacilli isolates (74.6%) as compared to the Gram positive isolates (25.3%). It was also observed that 55 isolates (87.3%) samples showed growth of single isolates while 8 (12.6%) were mixed isolates.



Graph 1: Genderwise distribution of chronic suppurative otitis media patients



Graph 2: Distribution of bacterial isolates from CSOM patients

P. aeruginosa (47.6%) was the most common isolate followed by *Klebsiella* spp 19% and among gram positive isolates *Staphylococcus aureus* was 12.6%.

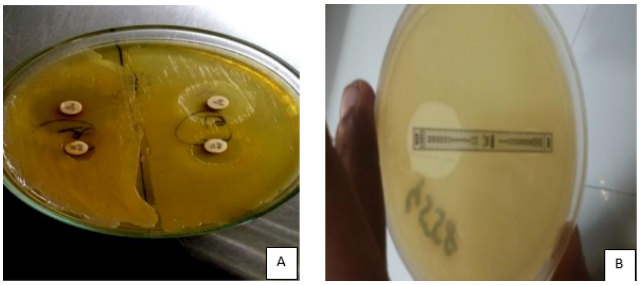


Fig. 1: A: and B: shows MBL positive by Imipenem (IMP)- EDTA Combined disc test MBL positive by E-Test

Pseudomonas aeruginosa isolated 11(36.6%)were screening test-positives for MBL by Imipenem – EDTA combined disc test, and MBL E test (Imipenem).

Table 1: The Nucleotide sequences of primers used for detection of metalo-beta lactamase genes.[9]

Primer name	Sequence	Denaturing	Anneal	Extension	Cycles	Size(bp)
bla_{IMP-1}	5′TGAGCAAGTTATCTG ATTC3′ 5′ TTAGTTGCTTGGTTTTGATG 3′	94°C, 60 s	57°C, 60 s	72°C, 2 min	35	740

Table 2: Genderwise distribution of chronic suppurative otitis media patients

S.N.	Gender	Isolates N=63	Percentage (%)
1.	Male	38	60.3%
2.	Female	25	39.6%

Table 3: Age wise distribution of the CSOM culture positive isolates

S.N.	Age group (Years)	Male N=38	Female N=25	Percentage (%)
1.	0-10	15	13	44.4%
2.	11-20	11	4	23.8%
3.	21-30	6	1	11.11%
4.	31-40	1	2	4.7%
5.	41-50	2	2	6.3%
6.	51-60	1	1	3.1%
7.	61-70	2	-	3.1%
8.	≤ 80	-	2	3.1%

Table 4: Bilateral distribution of chronic suppurative otitis media culture positive patients

S.N.	Side of the ear	Isolates N=63	Percentage (%)
1.	Left	32	50.7%
2.	Right	28	44.4%
3.	Bilateral	3	4.7
4.	Total	63	100%

Table 5: Distribution of bacterial isolates from CSOM patients

Bacterial Isolates	No. of Isolates N= 63	Percentage(%)
Gram positive isolates		
Staphylococcus aureus	8	12.6%
Streptococcus pneumoniae	1	1.58%
Gram negative bacilli		
Pseudomonas aeruginosa	30	47.6%
Klebsiella spp.	12	19%
Proteus mirabilis	3	4.7%
Escherichia coli	6	9.5%
Fungal		
Candida spp	1	1.58%
No growth	122	

Table 6: Phenotypic confirmatory test for MBL detection in Imipenem resistant Pseudomonas aeruginosa isolates from CSOM patients

Organisms	Imipenem(IMP)- EDTA Combined disc test:	E-test
Pseudomonas aeruginosa	11/30	11/30

Table 7: Shows isolation rate of *Pseudomonas aeruginosa* strains susceptible and resistant to each antibiotic class (n=30)

Antibiotic classs	Antibiotics	Percentage(%) Sensitivity	Percentage(%) Resistance
Polymyxins	Colistin	96.6%	
Aminoglycosides	Gentamycin		36.6%
	Tobramycin		30%
	Amikacin	(76.6.3%),	
Cephalosporins	Ceftazidime		30%
	Cefipime	73.3%	
Antipseudomonal Penicillins	Piperacillin/ Tazobactam	73.3%	
Carbapenem	Imipenem		36.6%
Fluoroquinolones	Ciprofloxacin		56.6%
	Levofloxacin		50%

The sensitivity observed in *P. aeruginosa* for Colistin was (96.6%), Piperacillin-tazobactam (73.3%), Amikacin (76.6.3%), and Cefepime (73.3%) were found to be the most effective Antibiotics. The resistance to ciprofloxacin was (56.6%), Levofloxacin (50%), Piperacillin(46.6%), Gentamicin(36.6%), Imipenem (36.6%), Tobramycin(30%), Ceftazidime (30%) and Gentamycin (36.6%). The bla$_{IMP-1}$ gene was detected in 11 (36.6%) of the isolates of *Pseudomonas aeruginosa. S. aureus* showed a 100% sensitivity to Vancomycin, Linezolid *Pneumoniae* showed 100% sensitivity to gentamicin, netilmicin, levofloxacin, and ofloxacin *P. mirabilis* showed a sensitivity of 80% sensitivity to levofloxacin and 80.9 % to ofloxacin, respectively, followed by ceftazidime and gentamicin 65%, ceftriaxone 57%. *E. coli* showed a sensitivity of 92.2% and 94.1% to gentamicin and levofloxacin, respectively, followed by ofloxacin 90.2%,ceftazidime 70.6%, ceftriaxone 78.4%, netilmicin 78.4%, and tetracycline 64.7%. *Klebsiella* species showed a sensitivity of 100% to levofloxacin and ofloxacin, respectively, followed by netilmicin, ceftazidime, ceftriaxone with 95.8%, tetracycline and gentamicin with 75 %.

The DNA Extraction was performed by the Qiagen DNA kit and the DNA was isolated from the samples.

Fig. 2: Photograph of DNA from *P. aeruginosa* isolates

Fig. 3: Photograph of amplified bla$_{IMP}$ gene in *P. aeruginosa;* the amplified DNA band size was obtained 256bp, Lane 1 and L 3 is the sample positive for bla$_{IMP}$; L2 corresponding to 100bp ladder used; L4 corresponds to the negative control and L5 corresponds to the positive control

4. Discussion

The CSOM presents as painless discharge and is the most common cause of deafness in India. Various studies have shown that both gram positive as well as gram negative organisms are responsible for CSOM.[10] In our study the number of ears clinically diagnosed of having CSOMs was 185, out of which 63 (34%) was found to be positive for CSOM infection. Our study was in support with the study performed by Deepthi Maringanti et al.,[11] where, the ear discharge swabs were sent for Culture and Sensitivity in which only 106 patients out of 180, showed culture positives.

The ratio of Male 38 (60.3%) were found to be more as compared to that of Female 25 (39.6%) in our study. This finding was similar to the study by Mohammed Jamiu Kazeem[12] where 198 (52.1%) patients were male while 182 (47.9%) were female. Other studies by Okesola and Fasina[13] and Akingbadeet al.,[7] was also in support with our study but in contrast with the study by Shrestha et al.,[14] The maximum number of cases reported was observed in the age group of 0-10 years followed by 11-20 years of age and the least number of cases was seen in the age group above 51 years of age. This study was in support with the study performed by the other author[12] where maximum

number of cases was reported in the age group of 10 years age with the fact that CSOM is predominantly a childhood disease, particularly the under 10 and least was observed above the age group of 50 years. This may be because of the week immune system in the young age and also because eustachian tubes are wider, shorter, and straighter compared to that of the adult.

The side of the ear affected was almost in equal distribution, with the left ear being 32 (50.7%) and the right ear being 28 (44.4%) while 3 (4.7%) were bilateral . This study was in support with the study by Mohammed Jamiu Kazeem [12] where the distribution pattern of the right and the left ear was in and the equal while bilateral was 3.4%.

In our study it was observed that the maximum number of cases was found in Gram negative isolates as compared to the Gram positive isolates and only 1.5% with Fungal isolates. It was also observed that 87.3% samples showed growth of single isolates while 12.6% were mixed isolates. This was in support with the study. [12]

This current study showed that *P. aeruginosa* (47.6%) has the highest prevalence of the isolated organism. This correlates with the studies performed by other authors where the incidence of *P. aeruginosa* as the most commonly isolated organism in CSOM ranging from 21%-52.94%. [15] Another study by Loy et al. (33.3%) [16] and *Mansooret al.* (40%) [17] also stated the rate of *P. aeruginosa* was more with no fungal isolate, but in Contrast with the study by Adoga et al. where Klebsiella species (40%) as the predominant organism. [18]

In our study *Klebsiella* spp. was the second most common isolate followed by *Staphylococcus aureus* with 12.6%, *Escherichia coli* (9.5%), *Proteus mirabilis* (4.7%) and *Streptococcus pneumonia* (1.58%) being the least observed. This correlate with the study by the Nwankwo and Salisu. [19]

Furthermore, this study showed that the commonly available antibiotics such as ciprofloxacin, and levofloxacin were generally ineffective against *P. aeruginosa,* species, which is the most isolate in our study. This is in support with the reports of Nwabuisi and Ologe. [20] Moreover, the ineffectiveness may be due to indiscriminate use of antibiotics, resulting in the emergence of resistant strains.

Piperacillin –tazobactam, Imipenem, Cefepime and Amikacin though highly sensitive, are considered as effective drugs in CSOM cases which are not responding to ciprofloxacin and Levofloxacin. Although still the sensitivity is declining so there is a need to quickly check the menace of inappropriate treatment of CSOM by quacks and intensify campaign against self-medication.

5. Conclusion

CSOM as similar to other chronic disease can limit an individual's employability and quality of life. In the present study *Pseudomonas aeruginosa* being the most common isolate with Colistin, Piperacillin-tazobactam, Amikacin, and cefepime to be the most effective antibiotics. Resistance of *P. aeruginosa* isolates to Imipenem due to MBL enzymes is increasing in the Kanpur region (36.6%). Judicial use of broad spectrum antibiotics, like Imipenem is the need of the hour, hence knowledge of the etiological agents of CSOM and their antibiogram data should be utilized for formulating antibiotic policy as it is paramount importance for an efficient treatment and prevention of both disease complications that it helps in preventing the emergence and spread of resistant pathogens.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Kong K, Coates HL. Natural history, definitions, risk factors and burden of otitis media. *Med J Aust*. 2009;191(9):39–43.
2. Thakur SK, Singh SK, Acharya R, Anwar A, Ghimire N. Sociodemographic profile and the associated factors of chronic otitis media in rural areas of eastern Nepal. *Int J Otorhinolaryngol Head Neck Surg*. 2017;3(2):222–7.
3. Sujatha R, Goyal R, Mishra V. Detection of Metallo Beta Lactamase Producing Pseudomonas aeruginosa among Clinical Isolates. *Int J Curr Microbiol App Sci*. 2017;6(2):1567–73.
4. Liakopoulos A, Mavroidi A, Katsifas E, Theodosiou A, Karagouni AD, Miriagou V. Carbapenemase-producing Pseudomonas aeruginosa from central Greece: molecular epidemiology and genetic analysis of class I integrons. *BMC Infect Dis*. 2013;13(505):1–7.
5. Cheesebrough M. District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge: ELBS University Press; 2010. p. 726.
6. Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of yeasts: approved standard, M44-A. Wayne (PA): CLSI; 2020.
7. Akingbade OA, Awoderu OB, Okerentugba PO, Nwanze JC, Onoh CC, Okonko IO. Bacterial spectrum and their antibiotic sensitivity pattern in children with otitis media in Abeokuta, Ogun state. *World Rural Obs*. 2013;5(3):11–7.
8. Sujatha R, Goyal R, Mishra V. Detection of Metallo Beta Lactamase Producing Pseudomonas aeruginosa among Clinical Isolates. *Int J Curr Microbiol App Sci*. 2017;6(2):1567–73.
9. Sarhangi M, Motamedifar M, Sarvari J. Dissemination of Pseudomonas aeruginosa Producing blaIMP1, blaVIM2, blaSIM1, blaSPM1 in Shiraz, Iran. *Iran J Microbiol*. 2013;7(6):302–3.
10. Mansoor T, Musani MA, Khalid G, Kamal M. Pseudomonas aeruginosa in chronic suppurative otitis media: Sensitivity spectrum against various antibiotics in Karachi. *J Ayub Med Coll Abbottabad*. 2009;21(2):120–3.
11. Maringanti D, Ganapuram J, Maringanti DA. Prevalence of Pseudomonas aeruginosa in CSOM cases at a tertiary care centre. *Int J Otorhinolaryngol Head Neck Surg*. 2018;4(1):71–5.
12. Kazeem RMJ. Current bacteriological profile of chronic suppurative otitis media in a tertiary facility of Northern Nigeria. *Indian J Otol*. 2016;22(3):157–61.
13. Okesola AO, Fasina OA. Trends in the resistance pattern of bacterial pathogens of otitis media in Ibadan, Nigeria. *Afr J Clin Exp Microbiol*. 2012;13(1):46–50.
14. Shrestha BL, Amatya RC, Shrestha I, Ghosh I. Microbiological profile of chronic suppurative otitis media. *Nepal J ENT Head Neck Surg*.

2011;2(2):6–7.
15. Shyamala R, Reddy PS. Incidence and Sensitivity Pattern of Pseudomonas aeruginosa in Chronic Suppurative Otitis Media in South Indian Rural Population. *J Microbiol Biotech Res*. 2012;2(2):346–50.
16. Loy AH, Tan AL, Lu PK. Microbiology of chronic suppurative otitis media in Singapore. *Singapore Med J*. 2002;43(6):296–305.
17. Mansoor T, Musani MA, Khalid G, Kamal M. Pseudomonasaeruginosa in chronic suppurative otitis media: Sensitivityspectrum against various antibiotics in Karachi. *J Ayub Med Coll Abbottabad*. 2009;21(2):120–3.
18. Adoga AA, Bakari A, Afolabi OA, Kodiya AM, Ahmad BM. Bacterial isolates in chronic suppurative otitis media: A changingpattern? *Niger J Med*. 2011;20(1):96–104.
19. Nwankwo EO, Salisu AD. Bacteriology of chronic discharging ears of patients in Kano, Nigeria. *J Med Lab*. 2005;14(1):57–62.
20. Nwabuisi C, Ologe FE. Pathogenic agents of chronic suppurative otitis media in Ilorin. *Nigeria East Afr Med J*. 2002;79:202–7.

Author biography

R. Sujatha, Professor and HOD https://orcid.org/0000-0002-1475-8598

Bipin Kishore Prasad, Professor https://orcid.org/0000-0002-6287-4139

Nashra Afaq, Research Associate

Arunagiri, Professor

Deepak Sameerbind, PhD Scholar

Cite this article: Sujatha R, Prasad BK, Afaq N, Arunagiri, Sameerbind D. Molecular characterization of metallo-beta lactamase genebla$_{imp-1}$ in imipenem resistant *Pseudomonas aeruginosa* isolates from patients of chronic suppurative otitis media at a tertiary care hospital. *IP Int J Med Microbiol Trop Dis* 2022;8(4):324-330.