"To study the Molecular characterization of Metallo-beta lactamase gene with Special reference to blaIMP-1 gene in Imipenem Resistant *Pseudomonas aeruginosa* isolates from Patients of Chronic Suppurative Otitis Media at a Tertiary Care Centre in Uttar Pradesh, India" .

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Abstract

Introduction: Persistent suppurative otitis media (CSOM) is a persistent inflammation of the middle ear and mastoid cavity that persists for more than two weeks. *Pseudomonas aeruginosa* is a common pathogen that causes CSOM. One of the best medications for treating Pseudomonas infections is carbapenem. The development of bacteria resistant to carbapenems is frequently linked to metallo-ß lactamases. In an effort to stop and propagate resistance, identifying the strains that create MBLs can help guarantee that patients receive the best therapy possible.

Aim and Objective: To study the molecular characterization of metallo-beta lactamase gene with special reference to blaIMP-1 gene in imipenem resistant *Pseudomonas aeruginosa* isolates from patients of chronic suppurative otitis media.

Material and Methods: The present study was a cross sectional study carried out in the Department of Microbiology with collaboration with the ENT and the Pharmacology Department for a period of 1 year i.e between August 2022 to August 2023 at a tertiary care centre. A total of 352 patients clinically suspected cases for CSOM were studied. Swabs taken from discharging ears were sent for Gram's staining, culture and antibiotic sensitivity test was performed according to the CLSI guidelines 2022. 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, which was further proceeded for the blaIMP-1 gene detection for *Pseudomonas aeruginosa* by the conventional PCR.

Results: In the present study the clinically diagnosed suspected cases of CSOM were 352, out of which 118 (33.5%) was found to be positive for CSOM infection. The ratio of Male 74 (62.7%) was found to be more as compared to the Female 44 (37.2%), with the maximum cases in the age group of 0-10 and the least in the age group above 41 years. In our study it was observed that the maximum number of cases was observed in the Gram negative bacilli isolates (77.2%) as compared to the Gram positive isolates (19.4%) with sides of the ear almost equally affected.
P. aeruginosa 58 (49.1%) was the most common isolate followed by *Klebsiella* spp with 19 (16.1%) and among gram positive isolate *Staphylococcus aureus* was found to be 15.2 %, there was only 3 case found for *candida albicans* (2.5%).
The sensitivity observed for *P. aeruginosa* for Colistin was (96.5%), Piperacillin-tazobactam (74.1%), Amikacin (77.5%), and Cefipime (74.1%) were found to be the most effective Antibiotics. The resistance to ciprofloxacin was (58.6%), Levofloxacin (50%), Piperacillin(25.8%), Gentamicin (36.2%), Imipenem (36.2%), Tobramycin and Ceftazidime with 27.5% respectively. The molecular characterization of the blaIMP-1 gene was detected in 18 (31%) of the isolates of *Pseudomonas aeruginosa* which were screening test-positives for MBL by Imipenem – EDTA combined disc test, and MBL E test (Imipenem).

Conclusion: For a treatment to be effective and to prevent both medical problems and antibiotic resistance, it is essential to understand the etiological agents of CSOM and their antibiogram. It is imperative that wide spectrum antibiotics like imipenem be used for the need of the hour.

Keywords: Metallo-ßlactamases, CSOM, CLSI, Molecular characterization,Impenem, blaIMP-1 gene , DNA , PCR

INTRODUCTION

Chronic suppurative otitis media (CSOM) is defined as chronic inflammation of middle ear and mastoid cavity that may present with recurrent ear discharges or otorrhoea through a tympanic perforation [1]. Incidence of this disease is higher in developing countries especially among low socio-economic society because of malnutrition, overcrowding, poor hygiene, inadequate health care, and recurrent upper respiratory tract infection [2]. Infection can spread from middle-ear to vital structures such as mastoid, facial nerve, labyrinth, lateral sinus, meninges and brain leading to mastoid abscess, facial nerve, paralysis, deafness, lateral sinus thrombosis, meningitis and intracranial abscess [3,4]. Of all the complications, hearing loss associated with chronic ear discharge is nearly always significant, reported in 50% of cases and tending to be more severe than those reported in other types of otitis media [5].

Various studies have shown that both gram positive as well as gram negative organisms are responsible for CSOM [6,7]. Generally, microbiological culture of the ear discharge simplicates *Pseudomonas aeruginosa, Proteus* spp and *Staphylococcus* as the prevalent causative organism [8]. *Pseudomonas aeruginosa* is the most commonly identified organism in CSOM reported by various studies in India and abroad with incidence ranging from 21% -52.94% [9]. Among the organisms, pseudomonas infection is known to produce deep seated and progressive infection in middle ear and mastoid leading to various intracranial and extracranial complications. *Pseudomonas aeruginosa* is one of the most important hospital-acquired pathogens that causes miscellaneous opportunistic infections [10].

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 [11].

Complications associated with CSOM were frequent in pre-antibiotic era, however, the introduction of antibiotics gave clinicians a tool to be used even without the precise etiological diagnosis and the irrational use of antibiotics led to the emergence of multi-drug resistant bacterial strains and disease complication in return [12]. Changes in bacterial flora in CSOM in the last decade have been confirmed

The emergence of multidrug-resistant (MDR: was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories) and extremely drug resistant (XDR: was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories) *P. aeruginosa* isolates has been considered as a major concern for the treatment of infections caused by these isolates [13].

Carbapenemases are a wide spectrum group of beta-lactamase which hydrolyzes carbapenems to other b-lactams including monobactams, penicillins, and cephalosporins. Although carbapenems are a commonly last resort treatment used for MDR *P. aeruginosa* infection, the emergence of carbapenem-resistant *P. aeruginosa* is becoming a main public health concern and is associated with high rates of mortality and morbidity among hospitalized patients [14,15].

The MBLs encoding genes such as *bla*VIM and *bla*IMP are one of the most clinically important classes of beta-lactamases. Carbapenemases acquires resistance belongs to Ambler molecular classes A, B and D. Metallo–betalactamases (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.* The VIM, IMP and SPM types are the most clinically significant carbapenemases which is encoded by blaVIM, blaIMP, and blaSPM genes [15].

Resistance to carbapenems can be related to producing carbapenemase enzymes such as serine carbapenemases and the MBLs encoding genes such as IMP, VIM, and NDM [16].

Therefore, the present study was undertaken to study the molecular characterization of metallo-beta lactamase gene with special reference to blaIMP-1 gene in imipenem resistant *Pseudomonas aeruginosa* isolates from patients of chronic suppurative otitis media at a tertiary care centre in Uttar Pradesh, India.

MATERIAL AND METHODS

This was a cross sectional study carried out in the Department of Microbiology with collaboration with the ENT and the Pharmacology Department for a period of 1 year i.e between Auigust 2022 to August 2023 at a tertiary care centre. The ethical clearance was duly obtained from the Institutional Ethical committee. A total of 352 patients clinically suspected cases for CSOM were studied. Swabs taken from discharging ears were sent for Gram's staining, culture and antibiotic sensitivity

test was performed according to the CLSI guidelines 2022 [17]. The isolates were further tested for MBL by screening test, by Imipenem – EDTA combined disc test, and MBL E test (Imipenem). The participants in the study who gave their agreement were included and patients who were taking antibiotics at the time were excluded from the study.

Sample Collection and 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 processed to 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.

Screening, isolation and identification of organisms:

Identification of the bacteria 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 [18].

Antimicrobial susceptibility testing

Antibiotic susceptibility test of isolated bacteria was performed using modified Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines 2022 [17]. 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). The 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 (20/10µg), Ceftriaxone (30 µg), Gentamycin (10 µg), Ciprofloxacin (5 µg), Trimethoprim/sulfamethoxazole(1.25/23.75µg), 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, 100/10µg), 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 18–24h. Results were interpreted in accordance with CLSI guidelines [17]. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* (American Type Culture Collection; ATCC 25923 and *P. aeruginosa* ATCC 27853 were used as control strains.

The Phenotypic confirmatory test

Imipenem(IMP)- EDTA Combined disc test: The test organisms were inoculated by lawn culture technique on the plates of Muller-Hinton agar(MHA) as recommended by CLSI [17]. The 10 µg Imipenem Disk and 750 µg Imipenem EDTA Disk(Hi-media SD281) were placed on the plate. The inhibition zones of the imipenem and imipenem-EDTA disks were 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 ≥ 7mm than the imipenem disc alone, it is considered as MBL positive [19]

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 64 µg/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 [19]

Figure No. 1: Shows MBL positive by Imipenem (IMP)- EDTA Combined disc test MBL positive by E-Test

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

The DNA was extracted from *P. aeruginosa* isolates using the Qiagen DNA Extraction Kit as per manufactures guidelines. The DNA was eluted in 60 µl elution buffer and preserve at -20 °C till PCR analysis. For amplification of the target gene, PCR was carried out in a 50 µL reaction mixture with 30 no. of cycles. The primers were purchased and synthesized by Chromous Biotech. Pvt. Ltd. (Bangaluru) and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

Figure No.2:The DNA Extraction kit Figure No.3:The Reagents used for the DNA Extraction

Figure No. 4: The bla IMP-1 primers from the Chromous Biotech

Molecular Characterization of bla IMP-1 gene

Polymerase chain reaction (PCR) was carried out for detection of bla IMP-1, gene on a thermal cycler (Eppendorf, Germany). The primer pair sequences used in this study and the PCR conditions are 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*IMP1 *from Seratia marcesens* (sequenced by Bioneer company), and *bla*VIM . *P. aeruginosa* ATCC 27853 was used as a negative control [20].

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System

The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments. The resulting PCR product was subjected to 1 % agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA). A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample [21].

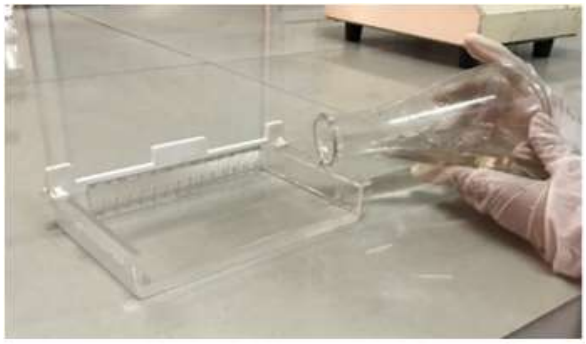


Figure No. 5: The Agarose gel preparation

Table No. 1: The Nucleotide sequences of primers used for detection of metalo-beta lactamase genes [20]

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

RESULTS

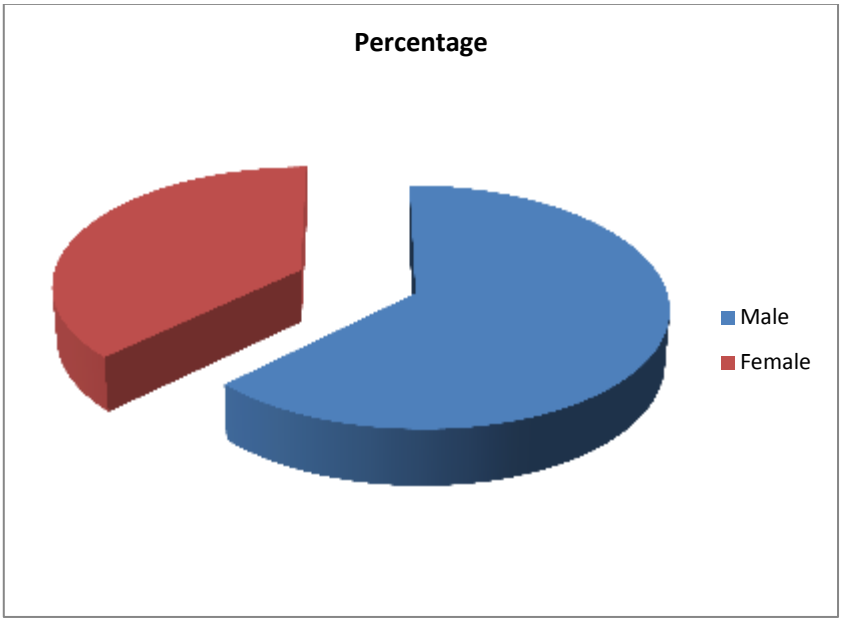
In the present study the clinically diagnosed suspected cases of having CSOM were 352, out of which 118 (33.5%) were found to be positive for CSOM infection [Table No. 2]. The ratio of Male 74 (62.7%) were found to be more as compared to that of Female with 44 (37.2%) which is illustrated in the Table No. 3. The maximum number of cases reported were observed in the age group of 0-10 years followed by 11-20 years of age and the least number of cases were seen in the age group above 41 years of age [Table No. 4] .

Table No. 2 : Total Number of Cases for chronic suppurative otitis media patients

S.N.	Type of Isolates	No. of Isolates	Percentage (%)
1.	Clinically diagnosed of having CSOM	234	66.4%
2.	Culture positive for CSOM infection	118	33.5%

Table No. 3 : Genderwise Distribution of chronic suppurative otitis media patients

S.N.	Gender	No. of Isolates N=118	Percentage (%)
1.	**Male**	74	62.7%
2.	**Female**	44	37.2%



Graph No. 1: Graphical Representation of Genderwise Distribution of chronic suppurative otitis media patients

Table No. 4: Age wise distribution of the chronic suppurative otitis media patients

S.N.	Age group (Years)	Male N= 74	Female N=44	Percentage (%)
1.	**0-10**	**28**	**13**	**34.7%**
2.	11-20	15	10	21.1%
3.	21-30	11	9	16.9%
4.	31-40	8	4	10.1%
5.	41-50	3	3	5%
6.	51-60	4	2	5%
7.	61-70	3	2	4.2%
8.	≤ 80	2	1	2.5%

It was observed that the side of the ear affected was almost in equal distribution, with the left ear being 68 (57.6%) and the right ear being 41 (34.7%). It was observed that 9 cases (7.6%) were bilateral Table No. 5. In our study it was observed that the maximum number of cases were found in Gram negative bacilli isolates (77.9%) as compared to the Gram positive isolates (23.7%). In the current study it was recorded that 110 isolates (93.2%) samples showed growth of single isolates while 8 (6.7%) were mixed isolates.

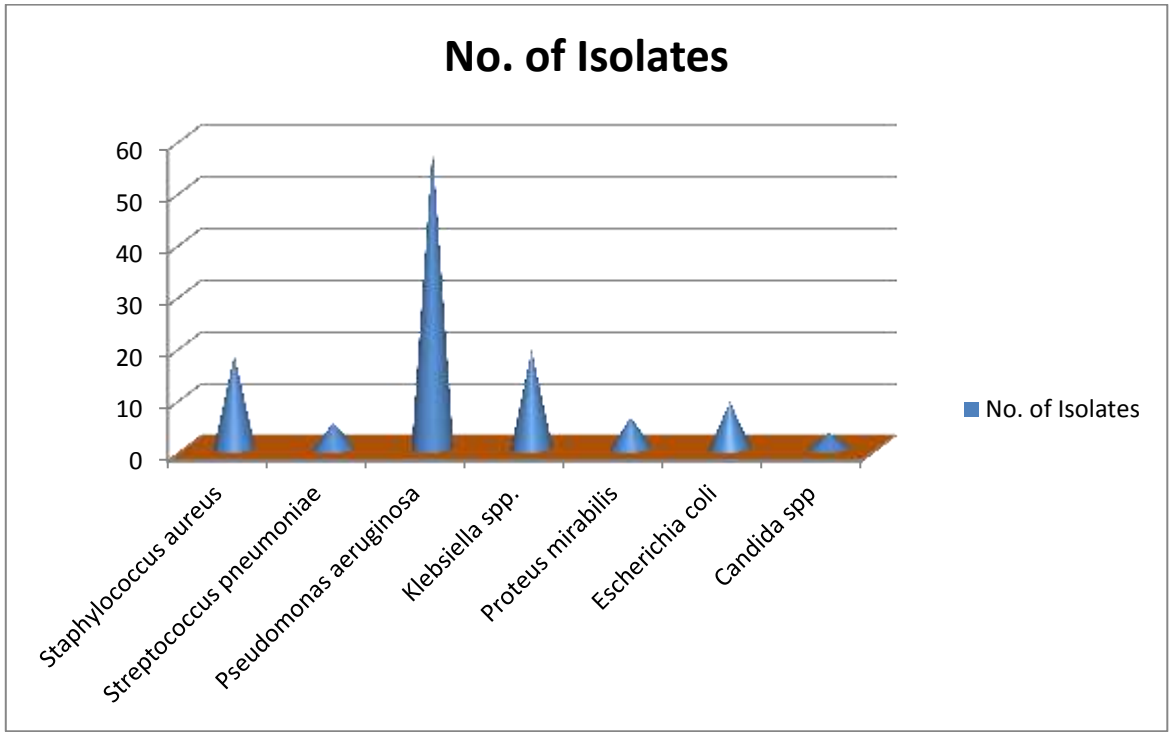
Table No. 5: Bilateral distribution of chronic suppurative otitis media culture positive patients

S.N.	Side of the Ear	No. of Isolates N= 118	Percentage (%)
1.	**Left**	68	57.6%
2.	**Right**	41	34.7%
3.	**Bilateral**	9	7.6%
4.	**Total**	118	100%

From the Table No. 6 it was clear that *P. aeruginosa* (49.1%) was the most common isolate followed by *Klebsiella* spp with 19 (16.1%) and among gram positive isolates *Staphylococcus aureus* was found to be 15.2 %. There was only 3 case found for *candida albicans* reported (2.5%).

Table No. 6: Distribution of bacterial species associated with chronic suppurative otitis media patients

Bacterial Isolates	**No. of Isolates N= 118**	**Percentage(%)**
Gram positive bacteria		
Staphylococcus aureus	18	15.2%
Streptococcus pneumoniae	5	4.2%
Gram negative bacteria		
Pseudomonas aeruginosa	58	49.1%
Klebsiella spp.	19	16.1%
Proteus mirabilis	6	5%
Escherichia coli	9	7%
Fungal		
Candida spp	3	2.5%
No growth	234	



Graph No. 2: Graphical Representation of the Phenotypic confirmatory test for MBL detection in Imipenem resistant Pseudomonas aeruginosa isolates from CSOM patients

Table No. 7: 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	18/58	18/58

Pseudomonas aeruginosa isolates 18 (31%) were screening test-positives for MBL by Imipenem – EDTA combined disc test and MBL E test (Imipenem).

Table No. 8: Shows isolation rate of *Pseudomonas aeruginosa* strains susceptible and resistant to each antibiotic class (n=58)

Antibiotic classs	Antibiotics	Percentage(%) Sensitivity	Percentage(%) Resistance
Polymyxins	Colistin	56 (96.5%)	2 (3.4%)
Aminoglycosides	Gentamycin	37 (63.7%)	21 (36.2%)
	Tobramycin	42 (72.4%)	16 (27.5%)
	Amikacin	45 (77.5%)	13 (22.4%)
Cephalosporins	Ceftazidime	42 (72.4%)	16 (27.5%)
	Cefipime	43 (74.1%)	15 (25.8%)
Antipseudomonal Penicillins	Piperacillin/ Tazobactam	43 (74.1%)	15 (25.8%)
Carbapenem	Imipenem	37 (63.7%)	21 (36.2%)
Fluoroquinolones	Ciprofloxacin	24 (41.3%)	34 (58.6%)
	Levofloxacin	29 (50%)	29 (50%)

In the current study the sensitivity observed in *P. aeruginosa* for Colistin was (96.5%), Piperacillin-tazobactam (74.1%),
Amikacin (77.5%), and Cefipime (74.1%) were found to be the most effective antibiotics. The resistance to ciprofloxacin was (58.6%), Levofloxacin (50%), Piperacillin(25.8%), Gentamicin (36.2%), Imipenem (36.2%), Tobramycin(27.5%) and Ceftazidime (27.5%). The blaIMP-1 gene was detected in 18 (31%) of the isolates of *Pseudomonas aeruginosa. S. aureus* showed a 100% sensitivity to Vancomycin, Linezolid . S.*Pneumoniae* showed 100% sensitivity to Gentamicin, Netilmicin, Levofloxacin, and Ofloxacin. *P. mirabilis* showed a sensitivity of 100% to levofloxacin and 100% to Ofloxacin, respectively, followed by Ceftazidime , Gentamicin 66.66% and Ceftriaxone 66.66%.

E. coli showed a sensitivity of 88.8% to Gentamicin and Levofloxacin, respectively, followed by Ofloxacin ,Ceftazidime , Ceftriaxone , Netilmicin with 77.7%, and Tetracycline 66.6%. *Klebsiella* species showed a sensitivity of 100% to Levofloxacin and Ofloxacin respectively, followed by Netilmicin, Ceftazidime, Ceftriaxone with 94.7%, Tetracycline and Gentamicin with 78.9 %.

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

Figure No. 5: The DNA Extraction

Figure No. 6: Thr Photograph of amplified bla_{IMP-1} gene in *P. aeruginosa* ; the amplified DNA band size was obtained 740bp, , Lane 1 and L 3 is the sample positive for $bla_{IMP;}$ L2 corresponding to 100bp DNA ladder used ; L4 corresponds to the Negative control and L5 corresponds to the Positive control

DISCUSSION

CSOM is a major public-health problem, and India is one of the countries with high-prevalence where urgent attention is needed [22].

It's a persistent disease with great risk of irreversible complications. CSOM is an important cause of preventable hearing loss particularly in the developing world [23] and a reason of serious concern, particularly in children, because it may have long-term effects on early communication, language development, auditory processing, educational process, and physiological and cognitive development [22]. Early, microbiological diagnosis ensures prompt and effective treatment to avoid such complications.

In people with impaired immune systems, *Pseudomonas aeruginosa* is an opportunistic infection that can cause immunocompromised. It has been identified as the most prevalent bacterium in many hospital wards around the world [24]. Nosocomial infections caused by strains of this organism that produce MBL have become more common in recent years.

The carbapenem-resistant *P. aeruginosa* causes serious infections, such as nosocomial pneumonia which based on the reports is increasing in the hospitalized patients [25]. Resistantce to carbapenems is often associated with production of metallo-ß-lactamases . Nosocomial infections caused by *Pseudomonas aeruginosa* remains the major cause of mortality, particularly because of emergence of multidrug-resistant strains [24, 25].

In the present study the clinically diagnosed suspected cases of having CSOM were 352, out of which 118 (33.5%) were found to be positive for CSOM infection . This study was similar to the study performed by the other research investigator where the prevalence of CSOM observed was similar ranging between 21 to 50% [9, 26] but in contrast with the study by Deepthi Maringanti et al.,where, the ear discharge swabs were sent for culture and sensitivity in which only 106 patients out of 180, showed culture positives with the prevalence rate of 58% [27]. There was another study by Shama A. Bellad *et a.,l* in 2019 ,where the prevalence of CSOM was 5.2% which is less than the prevalence reports of CSOM in other parts of Indian subcontinent. No significant difference was present in its prevalence with respect to age and gender of students [28] .

The ratio of Male 74 (62.7%) was found to be more as compared to that of Female with 44 (37.2%) in our study. This finding was similar to the study by Mohammed Jamiu Kazeem [29] where 198 (52.1%) patients were male while 182 (47.9%) were female. Other studies by Okesola

and Fasina and Akingbadeet al.,[30,31] was also in support with our study but in contrast with the study by Shrestha et al., [32] and Deepthi Maringanti et al. , [27] .

The maximum number of cases reported were observed in the age group of 0-10 years followed by 11-20 years of age and the least number of cases were seen in the age group above 41 years of age. This study was parallel to the study performed by the other research worker where the maximum number of cases recorded were in the age group of 11-20 years and least in the age group above 51 years [27]. Another study was also in support with the present study where maximum number of cases were 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 [29].

In the current study it was noted that the children and adolescents constitute the maximum patient population of CSOM. This may be because of the week immune system in the young age, lack of breastfeeding, overcrowding, poor hygiene, poor nutrition, passive smoking, high rates of nasopharyngeal colonization with potentially pathogenic bacteria and inadequate/unavailable health care and also because eustachian tubes are wider, shorter, and straighter compared to that of the adult.

CSOM has been described as disease more common among people of the poorer socio economic status, where there is overcrowding, more siblings under the age of five, poor sanitation and inadequate access to health care facilities; especially in children [33].

It was observed that the side of the ear affected was almost in equal distribution, with the left ear being 41(51.25%) and the right ear being 34 (42.5%) It was observed that 9 cases (7.6%) were bilateral. This study was in support with the study by Mohammed Jamiu Kazeem where the distribution pattern of the right and the left ear was equal while bilateral was 3.4% [29].

It was also observed that the maximum number of cases was found in Gram negative (77.2%) isolates as compared to the Gram positive (19.4%) isolates and only (2.5%) with Fungal isolates. Similar observation was made by the other author where gram negative isolates was observed to be the maximum. There was another study performed by Deepthi Maringanti et al. [27], which was in contrast to our study where there was no fungal growth recorded.

In our study it was observed that the maximum number of cases was found in Gram negative bacilli isolates (77.2%) as compared to the Gram positive isolates (19.4%). In the current study it was recorded that 110 isolates (93.2%) samples showed growth of single isolates while 8 (6.7%) were mixed isolates. This finding was in support with the other studies [29,34]. A study by M Chirwa *et al* in 2015 stated that there were 90 patients (86.5%) with unilateral disease and 14 (6.7%) with bilateral disease, which was in accordance to the current study [35].

In the current study the *P. aeruginosa* 58 (49.1%) was the most common isolate followed by *Klebsiella* spp with 19 (16.1%) and among gram positive isolates *Staphylococcus aureus* was found to be 15.2 %. There was only 3 case found for *candida albicans* (2.5%). This observation was compatible with the findings in other report where negative cultures were documented [36]. This study also 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% [26]. Another study by Loy et al. (33.3%) [37] and *Mansooret al.,* (40%) [28] also stated the rate of *P. aeruginosa* was more with no fungal isolate, but in Contrast with the study by Adoga *et al.,* [38] where Klebsiella species (40%) as the predominant organism. *Streptococcus pneumonia* , *Proteus mirabilis* were noted to be 3.75% and *Escherichia coli* with 8.75%. This correlate with the study by

the investigator Nwankwo and Salisu [39,40].There was another study which was in accordance to the present study where the most common bacterial isolates causing CSOM were *P. mirabilis*, *P. aeruginosa*, and *S. aureus* [35].

In case of Pseudomonas study, the sensitivity observed in *P. aeruginosa* for Colistin was (96.5%), Piperacillin-tazobactam (74.1%), Amikacin (77.5%) and Cefipime (74.1%) were found to be the most effective Antibiotics. This study was in support with the study performed by the other author where generally, ofloxacin (78.6%), gentamycin (76.9%), and ceftazidime (69.2%) were effective against *Pseudomonas* [41].

The resistance to ciprofloxacin was (55.26%), Levofloxacin (50%), Piperacillin(26.3%), Gentamicin (36.8%), Imipenem (36.8%), Tobramycin(28.9%) and Ceftazidime (28.9%). Similar finding was observed by the other coworkers [27,28] [41] . The study by Basavaraj Hiremath *et a*l in 2019 was also parallel to our study where *Staphylococcus aureus* showed maximum sensitivity to erythromycin (71.05%), followed by cotrimoxazole (63.15%) and ampicillin (55.26%). Maximum resistance was observed for ciprofloxacin (78.9%), followed by amoxiclave (55.26%). *Pseudomonas aeruginosa* showed maximum sensitivity to piperacillin (91.11%) followed by gentamicin (71.11%), amikacin (71.11%), moderate sensitivity to ceftazidime (51.11%); however resistance to carbpenicillin (60%) [42].

MBLs are a group of β-lactamase enzymes which need one or two zinc in their active site to cleave the amide bond of the β-lactam ring to inactive β-lactam antibiotics [43]. In the present study blaIMP-1 gene was detected in all the 18 isolates, screened test-positives for MBL by Imipenem – EDTA combined disc test, and MBL E test (Imipenem). In 2012, Fallah *et al*. checked 100 *P. aeruginosa* isolates from Shahid Motahari hospital in Teheran to detect bla_{IMP} and bla_{VIM} [44], where forty eight out of 83 (57.9%) imipenem-resistant *P. aeruginosa* showed MBL activity.

Since *Pseudomonas* was the predominant organism isolated in most CSOM cases and is mostly highly sensitive to ciprofloxacin which has none of the ototoxic risks of aminoglycosides and resistant to routinely used penicillin group of drugs and cephalosporins, it may be concluded that ciprofloxacin can be be adopted as a first line antimicrobial treatment for CSOM culture positive cases. Piperacillin –tazobactam, imipenem and meropenem though highly sensitive, are considered as reserve drugs in CSOM cases which are not responding to ciprofloxacin and gentamycin [27].

The genes responsible for the production of MBLs are typically part of an integron structure and are carried on transferrable plasmids but can also be part of the chromosome [45]. Due to integron-associated gene cassettes, MBL producing *P. aeruginosa* isolates are often resistant to different groups of antimicrobial agents, which can be transferred to another Gram-negative bacteria.

MBLs in Carbapenem-resistant *Pseudomonas aeruginosa* can be detected by different phenotypic methods and these methods are based on the ability of metal chelators to inhibit the activity of MBLs such as EDTA and thiol-based compounds.

The detection of MBL-producing *P. aeruginosa* help in appropriate antimicrobial therapy and avoid development and dissemination of these strains. Hence, routine detection of MBL production in *P. aeruginosa* should be undertaken.

A PCR detection assay is considered as a gold standard method for the detection of MBL producers. Because of the increasing rate of resistance to the carbapenems, the treatment of infections produced by MBLs producing *P. aeruginosa* is becoming critical.

Hence, regarding to horizontal transmission of integron-associated MBL genes, detecting MBL positive strains is necessary [46]. Moreover, by using new methods for rapid identification of MBL positive bacteria in the patients, we could prevent spreading of metallo-beta lactamase strains to other patients.

It is crucial to prevent the emergence and spread of resistant pathogens. Therefore, knowledge of the etiological agents of CSOM and their antibiogram data should be used when formulating antibiotic policy.

CONCLUSION

Like other chronic illnesses, CSOM can have an impact on a person's ability to work and quality of life. The most common strain in the study was *Pseudomonas aeruginosa*, and the best medicines to treat it were Colistin, Piperacillin-tazobactam, Amikacin, and cefepime were found to be the most efficient antibiotics against this strain.

Therefore, screening for MBL production in microbiology laboratories is crucial for optimal treatment of patients, particularly hospitalized patients and also to prevent the possible spread of resistance to other Gram-negative organisms because of their broad-spectrum drug resistance which creates a therapeutic challenge to clinicians. Finally, to understand the epidemiology, there is a need for genetic analysis and also typing of Metallo-ß-lactamase enzymes.

Declarations
Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: We have consent to participate.
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