

Research Article

Chronic suppurative otitis media: a clinico-microbiological menace

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ABSTRACT

Background: Chronic Suppurative Otitis Media (CSOM) is an important cause of preventable hearing loss. Global emergence of resistant strains is of great concern. The aim of the present study was to determine the etiology and antibiotic sensitivity pattern of bacterial isolates from CSOM cases with special emphasis on ESBL (Extended Spectrum Beta- Lactamases) and AmpC beta lactamases.

Methods: Patients with sign and symptoms suggestive of CSOM, ESBL (Extended Spectrum Beta-Lactamases), AmpC beta lactamases and MBLs (Metallo beta lactamases) were included. Two ear swabs were taken from all the patients and cultured on blood agar and *MacConkey* agar. Bacterial identification of isolates was done using standard biochemicals. Antimicrobial susceptibility was performed by Kirby-Bauer's disc diffusion method as per the Clinical Laboratory Standards Institute (CLSI) guidelines using antibiotic discs (HI MEDIA).

Results: Out of 130 patients, 110(84.62%) had bacterial growth. The common pathogenic species were *Pseudomonas aeruginosa* 36(37.89%), *Staphylococcus aureus* 31(32.63%), *Citrobacter koseri* 9(9.47%) and *Proteus vulgaris* 6(6.32%). *P. aeruginosa* showed maximum sensitivity to colistin (94.4%), polymixin-B (91.3%) and imipenem (91.3%). Gram positive cocci showed maximum sensitivity to vancomycin (99%).

MRSA (Methicillin Resistant *Staphylococcus aureus*) and HLAR (High Level Aminoglycoside Resistance) were detected in 9(29%) *S. aureus* and 1(50%) *Enterococcus faecalis* respectively. ESBL and AmpC were detected in 11(18.3%) and 12(20%) Gram negative bacteria, respectively and MBL producer was not detected.

Conclusion: *P. aeruginosa* was found to be the most common isolate in CSOM cases and colistin, polymixin-B and imipenem was found to be most effective antibiotics.

Keywords: Chronic suppurative otitis media, *Pseudomonas aeruginosa*, Resistance

INTRODUCTION

Chronic Suppurative Otitis Media (CSOM) is one of the most common causes of preventable hearing loss especially in developing countries. It is defined as a condition of the middle ear that is characterized by persistent or recurrent discharge for three months or more through a perforation of the tympanic membrane.^{1,2} The incidence of CSOM is increasing in the developing countries due to poor nutrition, poor hygienic practices and lack of health education.³ According to World Health Organization (WHO) global burden of CSOM accounts for 28,000 deaths and a disease burden of over 2 million DALYs (Disability-Adjusted Life Year)⁴ and if

appropriate treatment is not given at the right time it leads to irreversible local destruction of middle ear structures resulting in complications such as labyrinthitis, facial nerve paralysis, lateral sinus thrombosis, mastoiditis, meningitis, and intracranial abscess. Both Gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and Gram negative bacterias (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus species*, *Klebsiella species*) are involved in the pathogenesis of CSOM. There are several studies on the CSOM regarding etiology and sensitivity pattern in India and other countries, but only few of them have reported the prevalence of ESBL, AmpC and MBLs. The aim of the present study was to determine the microbiological profile and antibiotic sensitivity pattern

in CSOM patients with emphasis on the prevalence of MRSA, HLAR in *Enterococcus species*, ESBL, AmpC and metallo-MBLs in Gram negative bacilli.

METHODS

Study design

The study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, AMU Aligarh (India), over a period of 18 months (January 2013- June 2014). Patients with unilateral or bilateral ear discharge and other sign and symptoms suggestive of CSOM (hearing loss, otalgia, perforation and tinnitus) attending Otolaryngology OPD (Out Patient Department) or admitted in the wards were included in the study.

Sample collection

Two swabs each of the infected ear cases were collected from each patient with sterile a swab taking sterile precautions and sent to the Microbiology Department for bacterial culture and sensitivity testing.

Direct microscopic examination

First swab was used to perform Gram staining to look for the presence of pus cells or microorganisms.

Bacterial Identification

Second swab was used to culture on 5% sheep blood agar and MacConky agar and incubated at 37°C for 24 hours. Bacterial species identification was done by using standard microbiological techniques⁵.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed by Kirby-Bauer's disk diffusion method on Mueller-Hinton agar (Hi Media, Mumbai, India) as per CLSI guidelines⁶ using commercially available antibiotic discs from HiMedia (Mumbai, India).

Antibiotics tested for Pseudomonas aeruginosa: Amikacin (30µg), gentamicin (10µg), ofloxacin (5µg), piperacillin (100µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), tobramycin (10µg), ticarcillin (75µg), imipenem (10µg), polymixin-B (300units) and colistin (10µg).

Antibiotics tested for other Gram negative bacilli: Amikacin (30µg), ofloxacin (5µg), tobramycin (10µg), gentamicin (10µg), cefepime (30µg), cefixime (5µg), ceftriaxone (30µg), cefoperazone (75µg) and cefoperazone-sulbactam (75/75µg).

Antibiotics tested for Gram positive cocci: Amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg),

erythromycin (15µg), ofloxacin (5µg), clindamycin (2µg), levofloxacin (5µg), erythromycin (15µg), cefaclor (30µg), cefazolin (30µg), oxacillin (1µg) and vancomycin (30µg) were used for the Staphylococcus species. Amikacin (30µg), amoxicillin (30µg), cefazolin (30µg), erythromycin (15µg), levofloxacin (5µg), and vancomycin (30µg) were used for Streptococcus species. Amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), cefazolin ((30µg), erythromycin (15µg), high content gentamicin (120µg), ofloxacin (5µg), high content streptomycin (300µg) and vancomycin (30µg) were used for the Enterococcus species.

Detection of MRSA: Test was performed on Mueller Hilton agar with 4% NaCl using oxacillin 1µg disc. An inhibition zone diameter of 10 mm was reported as methicillin resistant and 13 mm was taken as methicillin sensitive

Detection of HLAR: High content gentamicin (120µg) and high content streptomycin (300µg) disc were used for the detection of HLAR in Enterococcus species.

Detection of ESBL: Screening of possible ESBL production was done by using ceftriaxone (30 µg) and cefoperazone (75µg). Those isolates with zone diameters less than 25 mm for ceftriaxone and less than 22 mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone-sulbactam. An increase in a diameter of 5 mm was considered positive for ESBL detection.⁷

Detection of inducible and derepressed AmpC beta-lactamase: Isolates resistant to ceftriaxone, cefixime, cefoperazone and cefoperazone-sulbactam were tested for AmpC production. Organism resistant to cefoperazone and cefoperazone-sulbactam combination were considered AmpC producers.⁸

Detection of Metallo-beta-lactamases: If the zone of imipenem was reduced to 16-20 mm or less or heaping occurred, we tested the isolate for MBL production. Hodge test and Double Disc synergy test using EDTA were used for detection of MBL.⁹

Control strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* 25873 were used as control strains.

RESULTS

During the study period of total 130 patients were recruited of which, 74(56.92%) were males, 56(43.08%) were females. The male to female ratio was 1.3:1. The majority of patients 59(45.38%) were between 16-30 years of age group followed by 43(33.07%) patients between 0-15 years of age. Out of 130 ear swabs cultured, 110(84.62%) had bacterial growth, of which

95(95.96%) isolates were pathogenic and 4(4.04%) were identified as commensals. Amongst these 4 cases, 3(75%) were Coagulase negative staphylococcus (CONS) and 1(25%) was coryneform species thus excluded from the study. Mixed growth was observed in 11(10%) cases. Infection occurred predominantly in the months of July and August. Gram negative bacteria 60(60.60%) far exceeded Gram positive bacteria 39(39.39%). The common pathogenic species were *Pseudomonas aeruginosa* 36(37.89%), *Staphylococcus aureus* 31(32.63%), *Citrobacter koseri* 9(9.47%) and *Proteus vulgaris* 6(6.32%). Figure 1 sheds light on the etiology of CSOM.

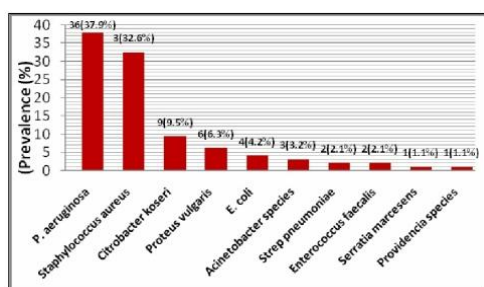


Figure 1: Distribution of pathogenic bacterial Isolates in CSOM cases.

Antimicrobial sensitivity testing of *Pseudomonas aeruginosa* revealed maximum sensitivity to colistin (94.4%) followed by polymyxin-B (91.3%), imipenem (91.3%), piperacillin-tazobactam (88.9%), ceftazidime (77.8%), amikacin (77.8%), piperacillin (75%), ticarcillin (69.4%) tobramycin (55.6%), ofloxacin (55.6%), and gentamicin (52.8%). Other Gram negative bacilli showed maximum sensitivity to amikacin (70.8%) followed by cefoperazone-sulbactam (66.7%), gentamicin (62.5%), cefepime (58.3%), ofloxacin (54.2%), tobramycin (50%), ceftriaxone (50%), cefixime (45.8%) and least sensitive to cefoperazone (37.5%) [Table 1]. Gram positive cocci showed maximum sensitivity to vancomycin (99%) [Table 2].

MRSA and HLAR were detected in 9(29%) *S. aureus* and 1(50%) *Enterococcus faecalis* respectively as shown in figure 2. ESBL and AmpC were detected in 11(18.3%) and 12(20%) Gram negative bacteria respectively. MBL producer was not detected in Gram negative bacteria.

Table 1: Antimicrobial sensitivity pattern of gram negative bacterial isolates.

Antibiotics	<i>P.aeruginosa</i>	<i>C. koseri</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>Acinetobacter Sp</i>	<i>Serratia marcessens</i>	<i>Providencia Sp</i>
Amikacin	28(77.8%)	7 (77.8%)	3 (50%)	3 (75%)	2 (66.7%)	1 (100%)	1 (100%)
Gentamicin	19 (52.8%)	7 (77.8%)	2 (33.3%)	3 (75%)	2 (66.7%)	0	1 (100%)
Tobramycin	20 (55.6%)	6 (66.7%)	2 (33.3%)	1 (25%)	2 (66.7%)	1 (100%)	0
Cefepime	-	7 (77.8%)	3 (50%)	2 (50%)	1 (33.3%)	1 (100%)	0
Cefixime	-	6 (66.7%)	2 (33.3%)	1 (25%)	1 (33.3%)	1 (100%)	0
Ceftriaxone	-	6 (66.7%)	2 (33.3%)	1 (25%)	1 (33.3%)	1 (100%)	1 (100%)
Cefoperazone	-	6 (66.7%)	0	1 (25%)	1 (33.3%)	1 (100%)	0
Cefoperazone-sulbactam	-	8 (88.9%)	1(16.7%)	3 (75%)	2 (66.7%)	1 (100%)	1 (100%)
Ofloxacin	20 (55.6%)	6 (66.7%)	2 (33.3%)	2 (50%)	1 (33.3%)	1 (100%)	1 (100%)
Ceftazidime	28 (77.8%)	-	-	-	-	-	-
Ticarcillin	25 (69.4%)	-	-	-	-	-	-
Piperacillin	27 (75%)	-	-	-	-	-	-
Piperacillin-tazobactam	32 (88.9%)	-	-	-	-	-	-
Imipenem	33 (91.7%)	-	-	-	-	-	-
Colistin	34 (94.4%)	-	-	-	-	-	-
Polymyxin-B	33 (91.7%)	-	-	-	-	-	-

Table 2: Antimicrobial sensitivity pattern of gram positive bacterial isolates.

Antibiotics	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Enterococcus faecalis</i>
Amikacin	27 (87.1%)	2 (100%)	2 (100%)
Amoxicillin	-	2 (100%)	-
Ciprofloxacin	8 (25.8%)	-	0
Cefaclor	20 (64.5%)	-	-
Cefazolin	29 (93.5%)	2 (100%)	0
Erythromycin	21 (67.7%)	0	0
Clindamycin	28 (90.3%)	-	-
Levofloxacin	5 (16.1%)	0	-
Gentamicin	23 (74.2%)	-	0
Ofloxacin	16 (51.6%)	-	1 (50%)
High content gentamicin	-	-	1 (50%)
High content Streptomycin	-	-	0
Oxacillin	22 (70.9%)	-	-
Vancomycin	31 (100%)	2 (100%)	2 (100%)

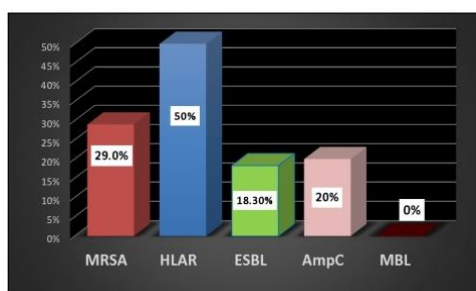


Figure 2: Resistance Pattern in CSOM Isolates.

DISCUSSION

CSOM is an important health problem in children and adults world-wide, but more so in developing countries. It can cause chronic hearing loss which has a negative impact on the development of speech, language and social interaction as well as school and workplace performance¹⁰ and is responsible for significant morbidity and mortality due to complications. According to a report by WHO, India belongs to the highest (>4%) CSOM prevalent countries.⁴ Topical antibiotics are the mainstay of therapy while systemic antibiotics are given in acute exacerbations and in complications due to CSOM.

In our study maximum patients (45.38%) were in the age group of 16-30 years, which is similar to results reported by Rakesh et al.,¹¹ Raghu et al.,¹² and Harvinder et al.¹³ The isolation rate was more in the months of August followed July, which is the monsoon season in the north India. Similar seasonal variation has been reported by a study from West Bengal.¹⁰ This season is marked by high humidity which allows bacteria to proliferate better and predispose to infection.

In our study, a large number of samples (84.62%) had a bacterial infection. Nazir et al.¹⁴ and Sanjana et al.¹⁵ have

reported similar results. Gram negative bacteria predominance (60.6%) matches other studies in India.¹³ *P. aeruginosa* was the predominant bacteria followed by *S. aureus* which are in concordance with other studies.^{12,16-18} In contrast, some studies reported *S. aureus* as predominant isolate followed by *P. aeruginosa*.^{3,19,20}

Citrobacter koseri was isolated as the third most common bacteria in our study, the prevalence of which was higher (9.47%) than previous studies.^{13,15} *Citrobacter koseri* may be considered an emerging pathogen for CSOM.

Amongst the various topical antibiotics tested for *P. aeruginosa*, colistin followed by polymixin-B was found to be most effective drugs. Among aminoglycosides sensitivity of tobramycin was found to be better than gentamicin and in the quinolone group ofloxacin was found to be equally effective as tobramycin. Amongst systemic antibiotics tested, imipenem and piperacillin-tazobactam were found to be best antibiotics for *P. aeruginosa*.

For other Gram negative bacteria, amongst the topically available antibiotics, gentamicin followed by ofloxacin and tobramycin (50%) were most active while amongst systemic antibiotics, amikacin followed by cefoperazone-sulbactam combination, cefepime, ceftriaxone and cefixime were most effective. Gram positive cocci showed maximum sensitivity to vancomycin (99%) as shown in Table 2.

In this study higher resistance demonstrated in Gram positive bacteria with 50% *E. faecalis* demonstrated HLAR and 29% *S. aureus* exhibiting methicillin resistance. On the other hand resistance among Gram negative bacteria was much lower with 18.3% ESBL and 20% AmpC producers and no MBL producer detected.

From the heart of our knowledge assessment of resistance marker like HLAR, ESBL and AmpC has not conducted in CSOM cases.

The low incidence of various resistance markers is heartening as they reflect the level of resistance in the community. In today's age, where there is increasing concern regarding antimicrobial resistance, the relatively low incidence of MRSA, HLAR, ESBL and AmpC is heartening.

CSOM is a common clinical health problem and topical antibiotic is the main treatment. However the emergence of antibiotic resistant strains is leading to increasing treatment failure. Continuous and periodic evaluation of microbiological profile and antimicrobial sensitivity pattern of bacterial is essential for optimum management of CSOM patients.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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