

Aerobic Bacterial Profile and Antibiotic Susceptibility Pattern of Chronic Suppurative Otitis Media in a Tertiary Care Centre in Kerala

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ABSTRACT

BACKGROUND

Chronic suppurative otitis media is a notorious middle ear infection in the developing countries which if left untreated can lead to serious intra and extracranial complications. We wanted to identify the bacterial pathogens causing CSOM and to determine their antibiotic susceptibility pattern.

METHODS

The in-patients & out-patients from the Department of E.N.T. over a period of one year were recruited and evaluated in the study. Ear swabs collected from the 135 clinically diagnosed cases of CSOM were processed in the microbiology laboratory. The bacterial isolates were identified, and their antibiotic susceptibility testing was done.

RESULTS

Bacterial growth was obtained from 100 out of the 135 samples of which 37.5% of the isolates were *Pseudomonas aeruginosa*, 21.5% *Staphylococcus aureus*, 5.9% *Klebsiella pneumoniae*, 3.7% *Escherichia coli* etc. Maximum antibiotic resistance was observed towards Ciprofloxacin, Gentamicin and Ceftazidime in *Pseudomonas aeruginosa*. Among the multi-drug resistant Gram-negative isolates, 20 were Extended Spectrum Beta Lactamase producers, 10 were AmpC beta lactamase producers and 10 were Metallo Beta Lactamase producers.

CONCLUSIONS

The bacterial isolates were found to be highly resistant to fluoroquinolones and aminoglycosides, the most commonly used topical antibiotics for CSOM, in this study. Culture and antibiotic susceptibility testing of the bacterial isolates are therefore essential to help clinicians in better management of the cases and reduce the emergence of antibiotic resistant strains.

KEY WORDS

CSOM, Antibiotic Susceptibility, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

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BACKGROUND

Chronic suppurative otitis media is characterized by intermittent or persistent purulent discharge through the perforated tympanic membrane.¹ It is a disease of multiple etiologies and is known for its persistence and recurrence despite treatment. Infection can spread from middle ear to adjacent vital structures like the mastoid, facial nerve, labyrinth, lateral sinus, meninges and brain resulting in mastoid abscess, facial nerve paralysis, deafness, lateral sinus thrombosis, meningitis and intracranial abscess. Of all the complications, preventable hearing loss is nearly always significant particularly in the developing countries. The incidence of CSOM is at a rise in the developing countries as a result of poor hygienic practices and lack of health education. The study of bacterial profile and drug susceptibility is essential to help the treating physician plan the general management of CSOM and it is necessary for the ENT surgeon to make the discharging ear dry by myringoplasty and ossiculoplasty. However, the antibiogram of these organisms causing CSOM has been found to vary with time and geographical area as well as from continent to continent mostly because of the indiscriminate use of the antibiotics. The most commonly obtained bacterial isolate from the ear discharge of a patient with CSOM is *Pseudomonas aeruginosa*. The other bacterial isolates include Staphylococci, *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus* species. Detection of ESBL, MBL & Amp C enzymes are difficult, as their presence does not always produce a resistant phenotype on conventional disc diffusion or automated susceptibility testing methods. These enzymes are often associated with potentially fatal laboratory reports of false susceptibility to β -lactams phenotypically.² Thus, their accurate detection and characterization is important from epidemiological, clinical, laboratory and infection control point of view.

METHODS

This cross-sectional study was done in a tertiary care setting. In patients & Outpatients from the Department of E.N.T. were recruited and the evaluation was done in the Department of Microbiology, Government Medical College, Thrissur. The study was conducted over a period of one year. Sample size was calculated to be 135 (Calculated from former similar study with formula $4pq/d^2$ where p is the prevalence of Gram-Positive organism (42.62%),³ q is 100-p and d is 20% of p). Patients who were suffering from CSOM diagnosed after thorough clinical evaluation by ENT Surgeon and who were willing to participate in the study were included in the study. Patients below 5 years were excluded, in view of difficulty in obtaining proper sample. Patient's with history of ear discharge of less than 3-month duration were also excluded. Before collecting the ear discharge, the external auditory canal was cleared of cerumen, using normal saline and

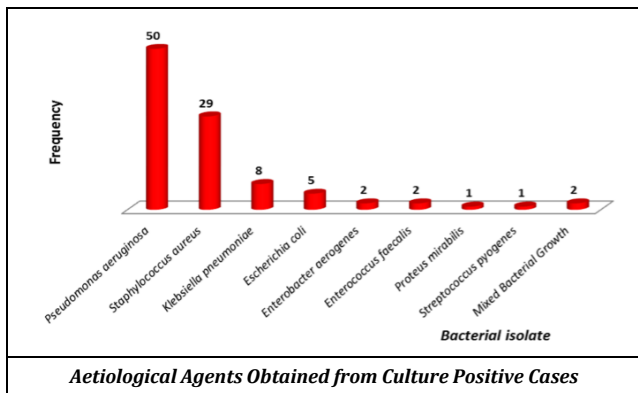
allowed to dry. The middle ear discharge was then collected aseptically by the otorhinolaryngologist from the tympanic cavity with two thin, sterile cotton swabs. These swabs were labeled and then transported immediately to the microbiology laboratory for further processing.

A thin smear was made on a clean glass slide which was fixed by heating. Gram staining was done for the smears and was examined under oil immersion objective of the light microscope to note the presence or absence of inflammatory cells, Gram reaction, morphology and arrangement of the bacteria. The swabs were then inoculated on Blood agar (BA), MacConkey agar (MA), and Tellurite blood Agar (TA) and incubated at 37°C overnight. If there was no growth, the plates were further incubated for 48 hours and then reported as no growth. If growth was seen then the colony morphology was studied on BA, MA and TA plates after which the bacterial species were identified based on the standard biochemical reactions. Antibiotic susceptibility testing for each organism was done according to the Kirby-Bauer disc diffusion method on Muller Hinton Agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines.⁴ Extended Spectrum Beta Lactamases, Metallo Beta Lactamases & Amp C Beta lactamases were tested for in the resistant strains of *Enterobacteriaceae*. The data were analysed using descriptive statistical tools like percentages and frequency distributions. SPSS software version 20 was used for this purpose.

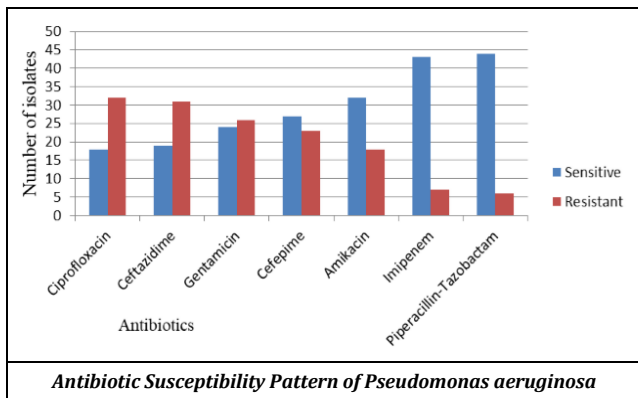
RESULTS

135 samples collected over a period of one year were studied. 37% of the isolates obtained were *Pseudomonas aeruginosa*, 21.5% were *Staphylococcus aureus*. *Klebsiella pneumoniae* contributed to 5.9%, *Escherichia coli* 3.7%, *Enterococcus faecalis* 1.5%, *Enterobacter aerogenes* 1.5%, *Streptococcus pyogenes* and *Proteus mirabilis* 0.7%. *Pseudomonas aeruginosa* was highly susceptible to Piperacillin-tazobactam (88%) and Imipenem (86%). But only few isolates were susceptible to Ciprofloxacin (36%) and Gentamicin (48%). Similarly, among *Enterobacteriaceae*, 50% of *Klebsiella pneumoniae*, 50% *Enterobacter aerogenes*, 20% *Escherichia coli* were susceptible to Ciprofloxacin. Out of the 29 isolates of *Staphylococcus aureus*, all were susceptible to Cefoxitin (Surrogate marker for mecA mediated oxacillin resistance). 20 isolates were found to be positive for ESBL which consisted of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*. 10 isolates each were positive for AmpC and MBL production.

Age Group of Patients	Frequency	Percent
15 & Below	21	15.6
16-25	23	17
26-35	19	41.1
36-45	28	20.7
45-55	25	18.5
56-65	12	8.9
Above 65	7	5.2
Age Group of Patients		

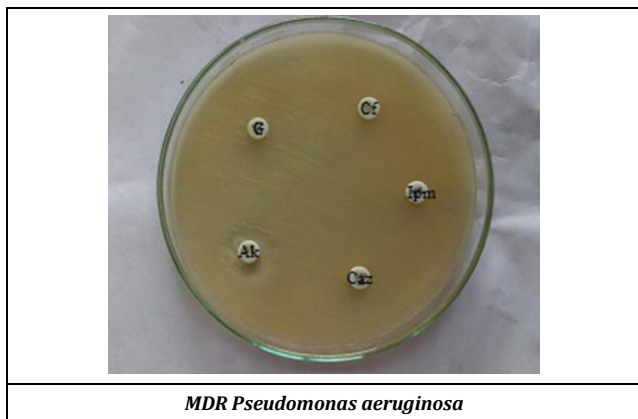


Aetiological Agents Obtained from Culture Positive Cases



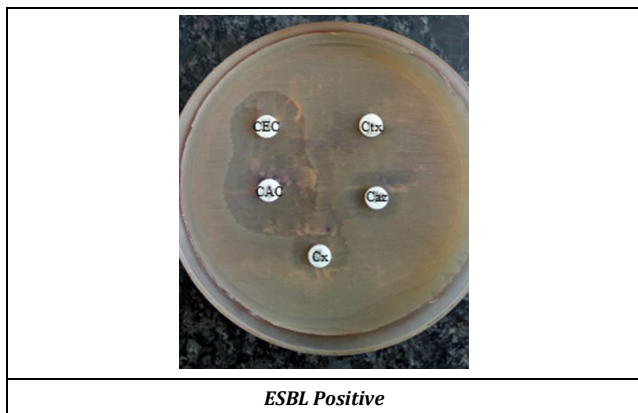
Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa*

Antibiotic Susceptibility Testing of *Pseudomonas aeruginosa*



MDR *Pseudomonas aeruginosa*

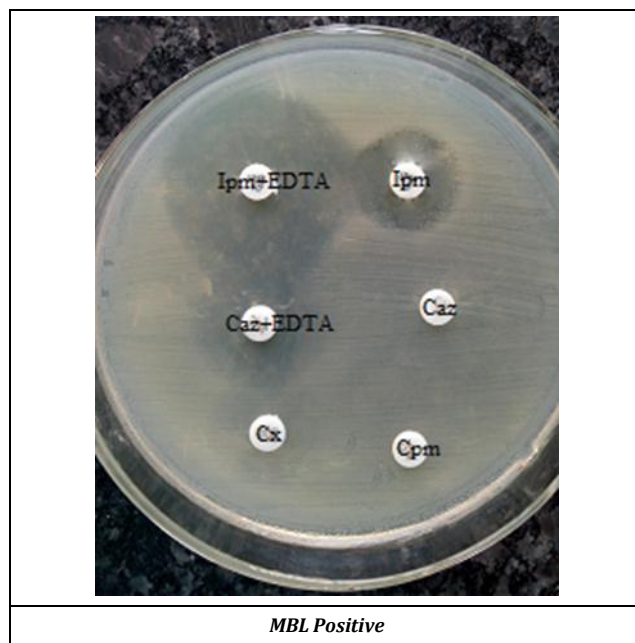
Ak- Amikacin, Cf- Ciprofloxacin, Caz- Ceftazidime, Cpm- Cefepime, G- Gentamicin, Ipm- Imipenem



ESBL Positive



AmpC Positive



MBL Positive

CAC- Ceftazidime + Clavulanic acid, Caz- Ceftazidime, CEC- Cefotaxime + Clavulanic acid, Ctx- Cefotaxime, Cx- Cefoxitin, Cx + PBA- Cefoxitin + Phenylboronic acid, Imp- Imipenem, Imp + EDTA- Imipenem + EDTA.

DISCUSSION

The microbial flora of CSOM varies regionally, depending on the type of otitis media, use of antibiotics in that area etc. Nowadays, it is also common for an otolaryngologist to encounter bacterial flora of a chronic discharging ear that has already been modified by previous topical antibiotic therapy. Knowledge of the pathogen is therefore mandatory before initiating therapy in CSOM. The most commonly affected age group in the present study was between 36-55 years. 61% of the affected patients were females and only 39% were males. This was consistent with the study by Loy AH et al.⁵ But most of the other studies have shown opposite trend with male preponderance. This is because males are usually considered

to be more vulnerable to infection than females. The reason for this could be that the androgens in males and estrogens in females modulate host immunity, and these sex steroids affect disease resistance genes and behaviour.⁶ 2% had history of allergy. Because of the close anatomical relationship between eustachian tube and nasopharynx, allergic disorders like allergic rhinitis may cause eustachian tube dysfunction by inflammation and swelling in the area.⁷

Unilateral ear involvement (That too more on left side) in CSOM has been found more commonly than bilateral ear involvement. 49% of the cases had discharge from the left ear, 38% from the right ear and 13% from both ears. This was in concordance with study of Kumar N. et al. where 58.3% of the patients had pathology in the left ear. Pure culture was obtained from 98 samples, two samples yielded mixed bacterial growth and no bacterial growth was obtained in 35 samples. Negative culture can be attributed to: (1) Anaerobic growth (2) Prior treatment (3) Presence of antimicrobial enzyme. Out of the pure cultures, 66 isolates were Gram negative bacilli, 32 were Gram positive cocci and two contained both Gram positive cocci and Gram-negative bacilli. The predominant isolate obtained was *Pseudomonas aeruginosa* constituting 37% (50 isolates) of the isolates followed by *Staphylococcus aureus* which contributed to 21.5% (29 isolates) of the total isolates. Many studies from different countries including India, Nepal, Nigeria and Singapore have reported that *P. aeruginosa* is the most common pathogen that caused CSOM, followed by *S. aureus* (Yeo et al.⁸ Madana et al.(32%),⁹ Afolabi et al.(31.3%).^{10,11} Whereas, studies from Pakistan, Iran and Saudi Arabia have shown that *S. aureus* as the predominant pathogen, followed by *P. aeruginosa* (Mariam et al. (65.2%).¹² Possible explanation to this difference in isolation rate might be related to the effect of climate.

Pseudomonas survives competition with other pathogens due to its minimal nutritional requirements and production of antibacterial products like pyocyanin and bacteriocin. *Klebsiella pneumoniae* constituted 5.9% of the cases whereas among other *Enterobacteriaceae*, *Escherichia coli* contributed to 3.7% of the cases, *Enterobacter aerogenes* 1.5% and *Proteus mirabilis* 0.7%. When the immunological resistance is low, organisms like *Escherichia coli* and *Klebsiella* species can become opportunistic pathogens in the middle ear. More frequent isolation of fecal bacteria like *E. coli*, *Klebsiella* and water bacteria like *Pseudomonas* indicates that the individuals are at a high-risk of infection due to poor hygienic conditions.

Ciprofloxacin (Quinolones) and gentamicin are usually given as topical antibiotics to patients diagnosed with CSOM. Quinolones are the preferred and most commonly used antibiotics due to their established effectiveness (Aslan et al.) and also because of them having lesser side effects (Cochleo toxicity and vestibulo toxicity). So, they are superior to aminoglycosides.¹³ In the present study, *Pseudomonas aeruginosa* showed only 36% susceptibility to Ciprofloxacin, 48% susceptibility to Gentamicin and 38% to the cephalosporin with anti-*Pseudomonas* activity, Ceftazidime. The other antipseudomonal cephalosporin, Cefepime though showed 54% susceptibility. The decreased susceptibility of *Pseudomonas aeruginosa* to the quinolone family is indicative of rapid appearance of antibiotic resistant strains. Quinolones are a critical group of antibiotics widely used in diverse

infections like respiratory, urinary, bone and joint infections which could be the one of the reasons for the drug to develop resistance. The empirical treatment of patients with CSOM by quinolones without antibiotic susceptibility testing or culturing may reinforce resistance to quinolones. Parenteral antibiotics are given when the pathogen is found resistant to both the topical and oral antibiotics. The increasing resistance to parenteral antibiotics like the cephalosporin group is a warning sign, as these are potent anti-*Pseudomonas* antibiotics. The most sensitive antibiotics in this study were Piperacillin-tazobactam, Imipenem and Amikacin. This finding was in agreement with the recent study which recommended the use of Piperacillin plus tazobactam like combinations against *P. aeruginosa* infections.¹⁴ Jang C.H et al. had similar findings in their study with Imipenem being the most susceptible antibiotic with an overall susceptibility rate of 96.5%, followed by Amikacin (55.6%), Piperacillin-tazobactam (37.5%) and Ceftazidime (31.8%).

Staphylococcus aureus was found to be susceptible to most of the antibiotics except Penicillin, to which only two out of the 29 isolates were susceptible. Among the 29 isolates of *Staphylococcus aureus*, all were Methicillin Sensitive *Staphylococcus aureus* (MSSA). This finding was contrary to the expectation in the current scenario that infections caused by MRSA would rapidly increase due to the misuse of antibiotics. Among the MDR Gram Negative isolates, organisms resistant to the Penicillins, first, second and third generation cephalosporins but not the cephamycins or carbapenems were tested for the presence of ESBL and AmpC. 20 isolates (50%) among the total of 40 multidrug resistant isolates were found to be ESBL producers in this study, which contained 15 isolates of *Pseudomonas aeruginosa*, two isolates each of *Klebsiella pneumoniae* and *Escherichia coli* and one isolate of *Proteus mirabilis*. Study by Baguma A et al. had 89% of the bacteria being ESBL producers with the predominant isolate being *Klebsiella pneumoniae* (52%), followed by *Escherichia coli* (44%) and *Proteus mirabilis* (4.5%).¹⁵ Most of the ESBL producing isolates were found to be resistant to Ciprofloxacin, Gentamicin along with Cefazolin, Ceftriaxone/ Ceftazidime and Cefepime. This was in concordance with study conducted by S.Shaikh et al. where the ESBL positive *Pseudomonas aeruginosa* showed 95% resistance to Gentamicin, 91% resistance to Ceftazidime, 85% resistance to Amikacin and 74% resistance to Ciprofloxacin and 65.96% resistance to Cefepime. It was 100% sensitive to Imipenem and Meropenem.¹⁶ High percentage of ESBL producing isolates in the study could be due to selective pressure imposed by the frequent use of antibiotics.

In this study, 10 multidrug resistant isolates were AmpC producers. AmpC producers were also 100% susceptible to Imipenem and Piperacillin-tazobactam. About 50% of the AmpC producers were susceptible to Ciprofloxacin and Gentamicin. 9 isolates of which were *Pseudomonas aeruginosa* and one isolate was *Enterobacter aerogenes*. This was in concordance with Wassef M et al, where out of the 50 isolates, 32 of AmpC producers were *Pseudomonas aeruginosa*, 10 were *Klebsiella* species, three *Escherichia coli*, three *Enterobacter* species and two *Acinetobacter* species.¹⁷ In a similar study conducted by Jameel NA et al. AmpC β -lactamases producing *E. coli* was found to be significantly

resistant to Co-trimoxazole (91.8%), Cefpodoxime (87.1%), Aztreonam (69.4%), Gentamicin (62.4%), Amikacin (61.2%) and Ciprofloxacin (34.1%). They were found to be less resistant to Cefepime (7.1%) and Piperacillin-tazobactam (5.9%).¹⁸ High prevalence of AmpC beta lactamase producing *Pseudomonas aeruginosa* (22%) has also been reported in a study done by Bhattacharjee A et al¹⁹ and 17% in a study conducted by Arora S et al. Therefore, compared to ESBL producers, isolates producing AmpC β -lactamase were resistant to additional β -lactams and to the currently available β -lactamase inhibitors. They also have the potential for developing resistance to carbapenems. Once the isolate was found to be resistant to carbapenems also, presence of carbapenemase was tested. 10 isolates of MBL producers were found in this study, majority being *Pseudomonas aeruginosa* (Seven isolates) followed by *Klebsiella pneumoniae* (two isolates) and *Enterobacter aerogenes* (One isolate). In a study on prevalence of MBL conducted by Jena J et al, out of the 112 Multi drug resistant strains, 20 isolates were found to be MBL producers.²⁰ Maximum production was for *Klebsiella oxytoca* (75%) and *Citrobacter* species (75%). Resistance to newer drugs like Meropenem and Imipenem is a warning sign for the judicious use of carbapenems because Imipenem is regarded as the final medication for MDR *Pseudomonas* infection. Strains resistant to Ceftazidime, Cefepime and Imipenem are difficult to treat due to lack of antibiotics. Such cases require treatment with Polymyxin which is associated with severe nephrotoxicity and neurotoxicity. The high level of resistance seen in the isolates of *P. aeruginosa* could attribute to the fact that incomplete or partially treated cases are the ones mostly reaching tertiary care referral center.²¹

CONCLUSIONS

In the present study, high level of resistance to the commonly used antibiotics for CSOM like Ciprofloxacin and Gentamicin was observed. The second line drugs like Amikacin, Linezolid, Imipenem and Piperacillin-tazobactam were more effective. But as most of these drugs are intravenous antibiotics which are costlier and require hospital admission, it is difficult to start them for all the patients with uncomplicated CSOM. This situation can only be controlled by the judicious use of antibiotics. Since empirical therapy is often started for the patients attending the clinic with features of CSOM, it is prudent to do the antibiotic susceptibility testing regularly and be familiar with the susceptibility patterns in the hospital. Regular monitoring of the antibiogram will aid in the development of an antibiotic policy in the hospital which in turn will help in promoting rational use of antibiotics and prevent emergence of resistance. Also, testing for the β -lactamases are not routinely done in the laboratory. This may cause dissemination of ESBL producing strains within and between hospitals which may remain undetected for long periods.

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