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SPECTRUM AND ANTIBIOGRAM OF BACTERIA IN CHRONIC SUPPURATIVE OTITIS MEDIA AND BIOFILM FORMATION

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a common infection having a high prevalence rate of 7.8-16% in India and is one of the leading causes of conductive deafness particularly in the adolescent age group. CSOM is frequently associated with multidrug-resistant organisms with the ability to form biofilm, which is an important virulence factor and results in treatment failure. **Aim:** The main objectives of the study are to identify the spectrum of bacteria associated with CSOM, their antibiotic sensitivity pattern, and to detect the biofilm formation. **Methods:** This was a prospective cross-sectional study at a tertiary care hospital conducted from 2016-2019. Patients up to the age of 80, having otorrhea for more than 6-12 weeks and attending the ENT outpatient department were included. Samples of pus were collected from the deeper aspect of external auditory meatus and processed using standard techniques for culture and sensitivity. Biofilm detection was done. Results were compiled and statistically analysed. **Results**: 120 samples were processed and 86 yielded positive cultures. *Staphylococcus aureus* was the predominant organism, followed by *Pseudomonas aeruginosa*. Biofilm formation was seen in 38 (44.18 per cent) of the organisms. Biofilms were formed predominantly by *Staphylococcus aureus* (47.36 per cent). **Conclusion:** Multidrug resistance has become increasingly common amongst the causative organisms of CSOM, this trend being particularly evident among biofilm producers. Therefore, screening for biofilm formation, along with the usual antibiogram, needs to be performed as a routine procedure in CSOM to effectively manage the situation by choosing an appropriate modality of treatment.

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INTRODUCTION

Chronic suppurative otitis media (CSOM) is a common infection having a high prevalence rate of 7.8-16% in India and is one of the leading causes of conductive deafness in our country particularly in the adolescent age group. (1) CSOM is an otolaryngologic infection wherein the tympanic membrane is perforated, accompanied with persistent drainage lasting for >6-12 weeks from the middle ear. (2,3). It begins with inflammation ultimately leading to mucosal ulceration and rupture of the epithelial lining.

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This predisposes to infection by bacteria which are introduced into the middle ear either from the external auditory canal through the perforation or from the nasopharynx through the eustachian tube, thereby forming granulations. (4) CSOM may be life threateningas the infection can spread from middle ear to vital structures. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus species*, *Klebsiella pneumoniae*, *Streptococcus* and diphtheroids are the most common bacteria cultured from chronically draining ears of which most are multi-drug resistant. (5) Anaerobes and fungi may grow symbiotically with the aerobes. Recently, biofilms have been found to play a pivotal role in the pathogenesis of CSOM with an incidence of about 60-65%. (6) Biofilms are complex bacterial communities that adhere to the surface of implanted biomaterial or mucosa, embedded in a slime-like extracellular matrix composed of

proteins, polysaccharides, and nucleic acids known as extracellular polymeric substances (EPS) and are very difficult to treat and eradicate. (7,8) These add to bacterial resistance. The virulence provided by biofilm formation is a leading factor in the re-emergence of multi drug resistant bacteria. The formation of biofilms facilitates chronic bacterial infections and reduce the efficacy of the treatment by conventional antibiotics. (9) Therefore, identifying the biofilm producing bacteria will ensure better treatment of CSOM patients who do not respond well, having recurrence and hearing loss despite antibiotic treatment, as this is not done routinely.

METHODS

Study design: This prospective study was conducted at the Department of Microbiology, in a tertiary care research and referral hospital attached to a medical college and research institute from 2016-2019.120 patients attending the ENT outpatient department of the hospital were included in the study.Institutional ethical clearance was taken (Ref No. BMCRI/PS/105/2016-17), and informed consent was obtained from the subjects in their own language. All patients up to 80 years of age having Chronic Suppurative Otitis Media where they have otorrhea for more than 6-12 weeks (2,3), treatment naïve patients were included. Since biofilm formation leads to multi drug resistance, patients taking prior medication were also included. Samples were taken from patients coming to the ENT OPD and hence antibiotic free period was not assessed. Pregnant women and patients with other co-morbid conditions like HIV infection were excluded.

Sample collection and processing: The patients were assessed through detailed history and clinical examination by the ENT surgeon. The material for culture was collected with cotton tipped sterile swab from the deeper aspect of the external auditory canal by the surgeons. This was transported immediately to the microbiology department for culture and sensitivity and biofilm formation. Swabs received were cultured on Blood agar and McConkey agar and the plates were incubated overnight at 37^{o}C. Colonies obtained were identified by using standard techniques. (10)

Antibiotic sensitivity was done using Kirby Bauer's disc diffusion technique method as described in clinical laboratory standard institute guidelines 2015 (CLSI). (11)

Detection of biofilm: The biofilm formation was detected by tissue culture plate method in plastic microtitre plates as described by Stepanovic *et al*. (12) 230µl of Trypticase Soya Broth (TSB) was added on a sterile 96 well flat bottomed polystyrene microtitre plate. 20 µl of overnight bacterial culture was added into corresponding well (each strain in successive three wells). The negative control wells contained broth only. The plates were incubated aerobically for 24 hours at 35°C. The content of the wells was poured off and the wells were washed three times with 300 µl of sterile distilled water. The bacteria adhering to the wells was fixed with 250 µl of methanol for 15 minutes. Then the wells were stained with 250 µl of 1% solution of crystal violet for 5 minutes. Excess stain was removed by washing and will be air dried. The dye bound to the wells was re-solubilised with 250 µl of 33% (v/v) glacial acetic acid. The optical density (O.D.) of each well was measured at 490 nm using an ELISA auto-reader.

Statistical analysis: The tests were carried out in triplicate and the results were averaged. The cut-off O.D (O.D.c) were determined as three standard deviations above the mean O.D. of the negative control. Strains were classified as biofilm producer and no biofilm producer. Results were compiled and statistically analysed using Microsoft Excel 2010 Edition (Microsoft, Seattle, WA).

RESULTS

One hundred and twenty samples were collected from patients with chronic suppurative otitis media (CSOM). The study group comprised 58 male patients and 62 female patients, whose ages ranged from 3 months–80 years. The peak incidence was observed in the 0-20 years age group (43 per cent). From these samples, 86 isolates (71.67 per cent) were obtained. One polymicrobial infection was noted which had *Methicillin Sensitive Staphylococcus aureus* (MSSA) and *Candida sp*. Overall, 30 organisms (34.9 per cent) were gram-positive and 56 organisms (65.1 per cent) were gram-negative. Staphylococcus aureus was the most commonly isolated organism (34.9 per cent) followed by Pseudomonas aeruginosa (20.9 per cent), Gram negative non-fermenters (14 per cent), Proteus sp. (14 per cent), *Klebsiella sp.* (11.6 per cent), *Citrobacter freundii* and *Candida sp*. (2.3 per cent each). Amongst the Staphylococcus aureus, MRSA (9.3 per cent) and MSSA (25.6 per cent) were obtained. This is depicted in (Figure 1).

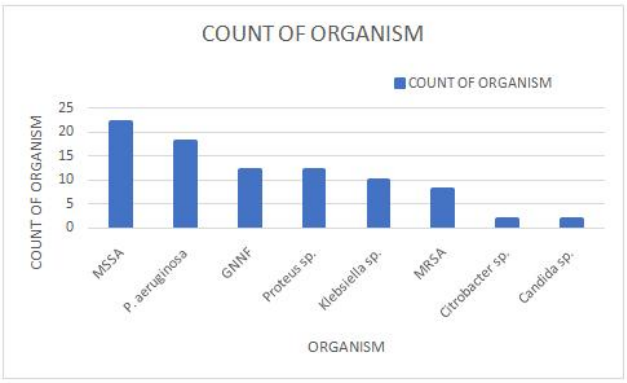

Figure 1. Organisms isolated in CSOM patients

Antimicrobial susceptibility: The MSSA isolates exhibited sensitivity to Cefuroxime (100 per cent), Gentamicin (82 per cent) and Ciprofloxacin (73 per cent) and were100 per cent sensitive to linezolid and vancomycin. This is shown in (Figure 2).

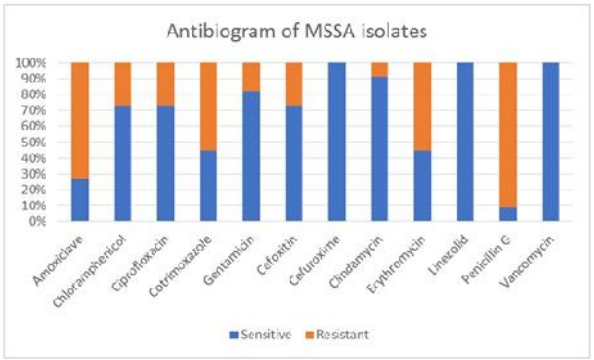

Figure 2. Antibiotic sensitivity of MSSA isolates

The *Pseudomonas aeruginosa* isolates showed good sensitivity to Imipenem (100 per cent) and Piperacillin-Tazobactam (100 per cent), Ceftazidime (90 per cent), Ceftazidime-clavulanic acid (90 per cent), Gentamicin (78 per cent) and Ciprofloxacin (78 per cent). This is shown in (Figure 3).

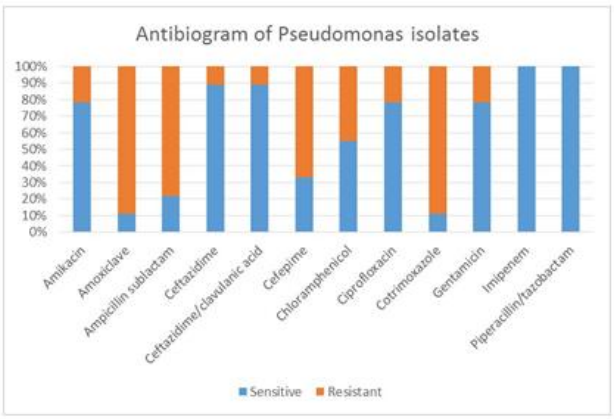

Figure 3. Antibiotic sensitivity of Pseudomonas aeruginosa isolates

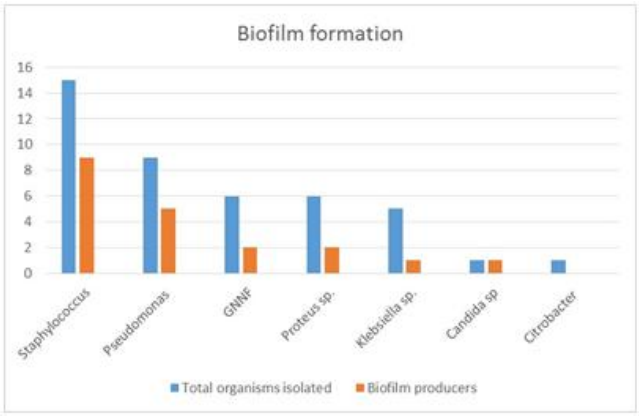

Figure 4. Comparison of biofilm-producing organisms

Other gram negative organisms showed sensitivity to Amikacin (100 per cent), Ceftazidime (100 per cent), Ceftazidime-clavulanic acid (100 per cent), Imipenem (100 per cent), Piperacillin-Tazobactam (100 per cent), Ciprofloxacin (88.8 per cent), Gentamicin (88.8 per cent) and Cotrimoxazole (66.6 per cent).

Biofilm formation: Thirty-eight (44.18 per cent) of the isolates showed biofilm formation. Staphylococcus aureus was the predominant biofilm former, with eighteen (47.36 per cent) of the isolates testing positive for biofilm formation. All eight (100 per cent) of the MRSA isolates were biofilm formers, while only ten (45 per cent) of the MSSA isolates formed biofilm. The second highest biofilm formation was by Pseudomonas aeruginosa (26.31 per cent) followed by GNNF (10.5 per cent), Proteus sp. (5.2 per cent), Klebsiella sp. (5.2 per cent) and *Candida sp.* (5.2 per cent). This is depicted in (Figure 4).

DISCUSSION

CSOM is a common infection having a high prevalence rate of 7.8-16% in India and hence urgent attention is needed. It is a chronic infection with a great propensity to lead to serious complications.

The main reason for concern is because CSOM is one of the leading causes of preventable conductive hearing loss in developing countries. Hearing loss, especially in children, can lead to various long-term effects on communication, language development, auditory processing, educational process, and cognitive development. (1) Therefore, early diagnosis of the causative organisms along with the antimicrobial susceptibility pattern ensures a more effective treatment to avoid such complications. In this study, CSOM was found to be most prevalent in the first two decades of life (43 per cent). This finding corroborates well with the observations made in various studies. (5, 13) High prevalence of CSOM in children may be attributed to the fact that they are more prone toupper respiratory tract infections (URTIs). In the present study, 34.9 per cent were found to be gram positive while 65.1 per cent were gram negative.

This is in agreement with the findings of various authorswhere the gram-negative organisms outnumbered the gram-positive organisms. (5, 13, 14) The predominant organism isolated in this study was Staphylococcus aureus (34.9 per cent) followed by Pseudomonas aeruginosa (20.9 per cent). This corresponds to the findings of *Nikakhlagh S et al*(15)which showed that *Staphylococcus aureus* is the most common isolate (32.4 per cent) followed by *Pseudomonas aeruginosa* (21.69 per cent),*Prakash, Rajat et al.* (16)and*Ettehad GH et al.*(17)However, studies by *VK Poorey et al, Maji PK et al* and*Abdelshafy et al* have shown *Pseudomonas aeruginosa* to be the predominant organism. (5, 18, 19) Such difference in results could have been due to the difference in the patient population studied and geographical variations of organisms.

Amongst the Staphylococcus aureus, MRSA (9.3 per cent) and MSSA (25.6 per cent) were obtained. The MSSA isolates exhibited sensitivity to Cefuroxime (100 per cent), Gentamicin (82 per cent) and Ciprofloxacin (73 per cent). All the organisms were sensitive to linezolid and vancomycin (100 per cent). This is like the study by *Prakash Rajat et al*., where the MSSA showed sensitivity to Cefuroxime (93.5 per cent), Gentamicin (84 per cent) and Ciprofloxacin (82.7 per cent). (16). The Pseudomonas aeruginosa isolates showed good sensitivity to Imipenem (100 per cent) and Piperacillin-Tazobactam (100 per cent), Ceftazidime (90 per cent), Ceftazidime-clavulanic acid (90 per cent) Gentamicin (78 per cent) and Ciprofloxacin (78 per cent).

These findings are in accordance with those of *Madana et al.*, (20) *PNS Moorthy et al*. (21) and *Prakash Rajat et al*. (16). In our study 44.18 per cent of the isolates showed biofilm formation. This was lower compared to prior studies in which it ranged from 60-70 per cent. (6, 22, 23) Staphylococcus aureus was the predominant biofilm former with 47.36 per cent of the isolates testing positive for biofilm formation. This is an expected result, with existing literature supporting the biofilm forming nature of Staphylococci**.** (24) Among the *Staphylococcus aureus*, all the MRSA isolates formed biofilms, thereby suggesting that biofilm formation can lead to greater resistance to antibiotics and add to the virulence. It is followed by Pseudomonas aeruginosa with 26.31 per cent testing positive. Some studies have reported Pseudomonas aeruginosa to be the most common organism to form biofilms in CSOM (19,23).

CONCLUSION

This study, in accordance with various other studies, shows that there can be variations in the organisms infecting and their susceptibility patterns, thereby emphasising the need for routine culture and sensitivity testing. Moreover, biofilm formation leads to difficulty in treatment of CSOM, mainly due to its frequent association with multi drug resistant organisms. But detection of biofilm is not being done and most of the times empirical antibiotic treatment is being given, which leads to multi drug resistance followed by complications and hearing loss. Detection of biofilm formation is an easy and cost-effective test that can be performed as a routine test in most of the laboratories.This will help doctors to effectively manage the situation by choosing an appropriate modality of treatment for CSOM and thereby prevent deafness.

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Conflicts of interest: None

Key points

- CSOM was found to be most prevalent in the first two decades of life (43 per cent).
- *Staphylococcus aureus* was the predominant organism, followed by *Pseudomonas aeruginosa*.
- **Staphylococcus aureus**, especially MRSA,was the predominant biofilm former, thereby suggesting that biofilm formation can lead to greater resistance to antibiotics and add to the virulence.
- Detection of biofilm formation is an easy and cost-effective test that can be performed as a routine test in most of the laboratories and can help doctors to effectively manage the situation by choosing an appropriate modality of treatment for CSOM and thereby prevent deafness.

Abbreviations: CSOM- Chronic Suppurative Otitis Media; ENT- Ear, Nose and Throat; OPD- Out patient Department; OD- Optical density; MRSA- Methicillin resistant Staphylococcus aureus; MSSA- Methicillin sensitive Staphylococcus aureus; GNNF- Gram negative non fermenters

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