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Pattern of Bacterial Isolates and their Antimicrobial Susceptibility Profiles of Chronic Suppurative Otitis Media Patients attended at a tertiary Care Hospital in Bangladesh

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Abstract

Background: Different bacteria can cause the chronic suppurative otitis media. **Objective:** The purpose of the present study was to assess the pattern of bacterial isolates and their antimicrobial susceptibility profiles of chronic suppurative otitis media patients. **Methodology:** This cross-sectional study was carried in the Department of Microbiology in collaboration with the Department of ENT at Sylhet MAG Osmani Medical College, Sylhet, Bangladesh from July 2017 to June 2018 for a period of one year. In this study, aural swab was taken from patients presented with CSOM and isolation and identification of bacterial species were done by standard microbiological methods. The Kirby Bauer's modified disc diffusion test was used to determine the antimicrobial susceptibility pattern of the recovered bacterial isolates. **Results:** Out of 80 ear swabs processed, 42(52.5%) cases were positive by culture and microscopy. No growth was reported in 38 (47.5%) samples. The most commonly isolated bacteria were Pseudomonas aeruginosa followed by *Escherichia coli, Staphylococcus aureus, Klebsiella species* and *Proteus species*. Most of the Gram-negative bacilli were sensitive to imipenem (100.0%), meropenem (90.9%), amikacin (85.0%), gentamicin (72.2%), levofloxacin (60.0%), azithromycin (52.4%), and ciprofloxacin (52.4%). Gram positive cocci were sensitive to imipenem (100.0%), meropenem (100.0%), amikacin (100.0%), gentamicin (83.3%), ceftriaxone (75.0%), azithromycin (60.0%), erythromycin (50.0%), and ciprofloxacin (28.6%). **Conclusion:** In conclusion the most commonly isolated bacteria are Pseudomonas aeruginosa followed by Escherichia coli, *Staphylococcus aureus, Klebsiella species* and *Proteus species*.

Keywords: Bacterial isolates; antimicrobial susceptibility profiles; chronic suppurative otitis media

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Introduction

Ear infection can be caused by viruses and fungi but the major cause is bacterial[1]. Various studies have shown that both Gram positive and Gram negative bacteria are responsible for infection of middle ear[2]. The aerobic microorganisms most frequently found in CSOM are *Pseudomonas aeruginosa, Staphylococcus aureus* and Gram negative organisms such as *Proteus species, Klebsiella species, Escherichia coli, Haemophilus influenzae and Moraxella catarrhalis*[3-4]. while anaerobes include Bacteroides fragilis and *anaerobic Streptococci*. In different studies *Pseudomonas aeruginosa* a Gram negative bacilli is implicated as the most common pathogen for CSOM in our country[5].

Pseudomonas aeruginosa is uniquely problematic because of its wide distribution in nature, hardiness, colonization in human GI tract as normal flora, a combination of inherent resistance to many drug classes and its ability to acquire resistance to all

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relevant treatments[6]. It has been found to be very resistant to chemical disinfectants and found to grow in hospital environment in presence of certain ammonium compounds, hexachlorophene soap and iodine solutions, phenolic and β-glutaraldehyde[7].

In a study conducted in Mumbai, out of total 80 specimens, 69 specimens showed growth of one or two organisms. *Pseudomonas species* (34.1%), *Proteus species* (8.8%), *Acinetobacter species* (5.5%), *Klebsiella species* (5.5%) and *Enterobacter species* (6.6%) were the most common Gram-negative bacteria isolated followed by *Staphylococcus aureus* and coagulase negative Gram-positive bacteria[8]. In a study conducted in Shaheed Monsur Ali Medical College, a total of 115 ear discharge samples were tested for bacterial isolation and 86(74.8%) cases were found positive[9]. Among the isolates, *Staphylococcus aureus* 21(24.4%)were predominant followed by *Pseudomonas aeruginosa* 20 (23.3%). In the same study they also tested the antibiotic sensitivity and found *Staphylococcus aureus* showed high sensitivity rate to chloramphenicol (80.7%), gentamicin and doxycycline (85.7%). *Pseudomonas species* showed high sensitivity to levofloxacin (88.9%) and relatively sensitive to ciprofloxacin and cefixime (77.8%). About 81.25% of coagulase negative *Staphylococci* were resistant to cefixime, 75% of *Klebsiella species* and 77.8% of Proteus were resistant to amoxicillin[9]. The purpose of the present study was to assess the pattern of bacterial isolates and their antimicrobial susceptibility profiles of chronic suppurative otitis media patients.

Methodology

Study Settings and Population: This was designed as cross-sectional study. This study was conducted in the Department of Microbiology at Sylhet MAG Osmani Medical College, Sylhet, Bangladesh. The specimens were collected from the Department of Otorhinolaryngology and Head-Neck Surgery of Sylhet MAG Osmani Medical College Hospital, Sylhet, Bangladesh. This study was carried out from July 2017 to June 2018 for a duration of one year. All patients with Chronic Suppurative Otitis Media (CSOM) attending the OPD of the department of Otorhinolaryngology and Head-neck Surgery of Sylhet MAG Osmani Medical College Hospital, Sylhet who were fulfilling the inclusion and exclusion criteria were selected as study population. Patient giving history of chronic aural discharge for three months or more, having perforation of the ear drum were considered as a case of CSOM. All diagnosed cases of chronic suppurative otitis media presented at ENT outpatient department, who were not on antibiotic treatment for CSOM for the last 5 days were included. Patients with CSOM, received antibiotics during last 5 days, patients who underwent ear surgery for CSOM complication or patients with purulent discharge from ear, after trauma were excluded from this study.

Study Procedure: The patient/attendant was informed of the details of the sample collection and written consent was taken. The type of specimen was aural swab. The collection and transport of the specimen were performed with full precaution. All aseptic measures were taken and cleansing external auditory meatus with sterile normal saline soaked cotton were performed. A cotton tipped sterile swab stick was introduced into the ear and rotated at 3600 to collect the aural discharge adequately. The specimen containing swab stick was placed into the sterile test tube with cotton plug. The test tube was labeled properly along with the patients' ID number and transported to the Microbiology laboratory as early as possible for further processing. Non-probability convenient sampling was done during collection of samples. The variables that were recorded were age of the patients, gender, residence, socio-economic status, education and nature of discharge.

Data collection tools and procedure: Data were recorded by preformed data collection sheets. A total 80 patients of CSOM were studied. Informed written consent were obtained from all the study population. The demographic data were taken as per questionnaire. All the necessary information such as duration of ear discharge, nature of discharge and treatment history were collected in the pre-designed data sheet. Samples were collected from the department of Otorhinolaryngology and Head-Neck surgery, Sylhet MAG Osmani Medical College Hospital, Sylhet, Bangladesh.

Laboratory Procedure: Culture media used for isolation of bacteria were blood agar (BA) media. This media was used for isolation of all possible bacterial species, present in the aural swabs and to observe the hemolytic properties of the pathogens. MacConkey (MAC) agar media was a selective medium and was commonly used for isolation of any Gram-negative bacteria present in specimen and for the differentiation between lactose-fermenters and non-fermenters. Isolation of *Pseudomonas species* from specimens containing a mixed flora was facilitated by the use of selective media. MacConkey agar is a useful selective

medium for the most *Pseudomonas species*. Pseudomonas aeruginosa does not ferment lactose and can easily differentiated from the lactose-fermenting bacteria. Bacterial colonies were identified presumptively on the Blood agar and MacConkey agar by colony characteristics against each of the organism. The isolates were further identified using standard identification protocol such as Gram's staining, motility test, pigment production and biochemical tests. Catalase test, coagulase test tests were done for the identification of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Gram negative bacteria such as *Escherichia coIi, Klebsiella species* and *Pseudomonas aeruginosa* were identified by their characteristics of sugar fermentation, indole production.

Antimicrobial Susceptibility test (AST): The performance of antimicrobial susceptibility test by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. AST was done by Kirby-Bauer disk diffusion method, in which inhibitory zone diameters (IZDs) against each of the antibiotic, for every isolate, was recorded. All the isolates were tested for antimicrobial susceptibility for the following antibiotics like ceftazidime (30μg), cefuroxime (30 μg), imipenem (10 μg), meropenem (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), amoxicillin-clavulanate (10 μg), levofloxacin (5 μg), amikacin (30 μg), cefotaxime (30 μg), cefepime (5 μg). Antimicrobial agents were selected following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2017). Mueller-Hinton agar (Himedia, India) was used for the determination of antibiotic sensitivity patterns by the Kirby-Bauer techniques.

Inoculation of sensitivity plates: A suspension of well-isolated colonies of the test organism on primary culture or subculture plates was made in sterile physiological saline, in an equivalent to McFarland standard 0.5 for inoculation over sensitivity plates. A sterile cotton swab was immersed into the suspension, the excess from which was removed by rotating the swab firmly against the side of the tube above the fluid level. The swab was then streaked evenly over the dried surface of the Mueller- Hinton agar plate in three different planes at 600 to ensure equal distribution of the inoculums throughout the medium surface. A final circular motion was made around the rim of the sensitivity plate by the bacterial suspension-soaked swab stick. The inoculated plate was then allowed to dry for approximately 5 minutes at room temperature with the lid closed.

Disc placement: Before starting the preparation of sensitivity test, the antimicrobial disks were kept out of refrigerator for half an hour in a sterile Petri dish to return to room temperature. The disks were then placed over the inoculated surface about 15 mm away from the edge of the Petri dish and 20-25 mm away from each disk.

Interpretation: The interpretive criteria to evaluate the susceptibility pattern of the isolates, whether it is sensitive, intermediate or resistant, were done according to CLSI. 2017. After placing the antimicrobial disks, the inoculated plates were placed into an incubator for incubation at 37° C for 24 to 48 hours. After proper incubation, the sensitivity plates were observed for zones of inhibition, which are the clear zone around each antibiotic disk, showing no growth of bacteria. The diameters of the zones of inhibition, measured in millimeters, were made in two directions at right angles to each other through the centre of the disks, and the average of the two was recorded.

Statistical analysis: All data were processed and analyzed with the help of SPSS (Statistical Package for Social Science) version 21.0 Quantitative data were presented as mean and standard deviation. Qualitative data were analyzed by frequency and percentage and comparisons were performed by Pearson's Chi-squire (x2) test. A probability value ($P<0.50$) was considered statistically significant.

Ethical consideration: After explaining the purpose of the study informed written consent was taken from each patient or legal guardian. All information was kept confidential with complete respect to the participants wish and without any force or pressure. Prior to the beginning of this study, approval of the research protocol was obtained from the ethical review committee of Sylhet MAG Osmani Medical College, Sylhet, Bangladesh.

Results

Five species of microorganisms were isolated from 80 patients with CSOM. The bacterial species most often isolated was *Pseudomonas aeruginosa*, which was present in 20(25.0%) patients followed by *Escherichia coli* 9(11.25.0%), *Staphylococcus aureus* 7(8.75%), and *Klebsiella species* 3(3.75%) and *Proteus species* 3(3.75%) cases (Table 1).

Table 1: Microbiology of Middle Ear Discharge from Patient with CSOM (n=80)

Species isolated	Frequency	Percent
Pseudomonas aeruginosa	20	25.0
Escherichia coli	9	11.25
Staphylococcus aureus	7	8.75
Klebsiella species	3	3.75
Proteus species	3	3.75
No growth	38	47.5

Distribution of the participants according to isolation of bacteria from ear discharge from patients with CSOM: The following pie chart revealed that bacteria was isolated from ear discharge from patient with CSOM in 42(52.5%) and remaining 38(47.5%) cases revealed no bacterial growth (Figure I).

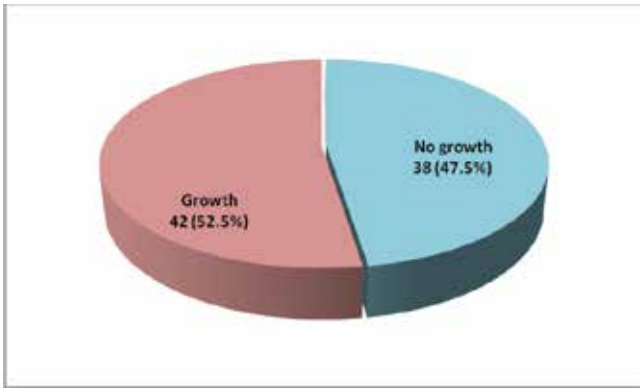

Figure I: Pie chart showing distribution of the participants according to isolation of bacteria from ear discharge from patients with CSOM (n=80)

Antimicrobial Susceptibility Pattern of isolated *Pseudomonas aeruginosa, Escherichia coli, Proteus species* and *Klebsiella species*: *Pseudomonas aeruginosa* were sensitive to imipenem (100.0%), meropenem (100.0%), amikacin (80.0%), ciprofloxacin (77.8%), gentamicin (60.0%), levofloxacin (60.0%), cefuroxime (40%) whereas all the isolates of *Pseudomonas aeruginosa* were resistant to cefotaxime and cefepime; 71.4% to erythromycin, 62.5% to amoxicillin-clavulanate, and 50.0% to ceftazidime. All the isolates of *Escherichia coli* were sensitive to imipenem, levofloxacin and amikacin; 88.9% organisms were sensitive to gentamicin, 85.7% to meropenem, 77.8% to ciprofloxacin. Whereas all the isolates of *Escherichia coli* were resistant to cefepime; 71.4% to cefotaxime, 66.7% to ceftazidime, 55.6% cefuroxime and 44.4% to erythromycin. All the isolates of *Proteus species* were sensitive to imipenem, meropenem, levofloxacin and cefuroxime, 66.7% were sensitive to ciprofloxacin, 50.0% to amikacin, and ceftazidime. whereas all the isolates of *Proteus species* were resistant to erythromycin and cefepime; 66.7% to amoxicillin-clavulanate, and ceftazidime. All the isolates of *Klebsiella species* were sensitive to imipenem, gentamicin and cefuroxime; 66.7% were sensitive to meropenem, amikacin. Whereas all the isolates of *Klebsiella species* were resistant to ceftazidime, cefotaxime, erythromycin and cefepime (Table 2).

Antimicrobial susceptibility pattern of isolated *Staphylococcus aureus*: All the isolates of *Staphylococcus aureus* were sensitive to imipenem, meropenem, amikacin and cefuroxime. 83.3% organisms were sensitive to Gentamicin, and 55.6% to Erythromycin. Whereas all the isolates of *Staphylococcus aureus* were resistant to cefotaxime, ceftazidime, and cefepime; 80.0% to levofloxacin, 44.4% to erythromycin, 57.1% to amoxicillin-clavulanate and 57.1% to ciprofloxacin (Table 3).

Table 2: Antimicrobial Sensitivity pattern of Isolated P. aeruginosa (n=20)

Antimicrobials	*P. aeruginosa*	*E. coli*	*Proteus spp.*	*Klebsiella spp.*
Gentamicin	60.0%	88.9%	66.7%	100.0%
Imipenem	100.0%	100.0%	100.0%	100.0%
Meropenem	100.0%	85.7%	100.0%	66.7%
Amikacin	80.0%	100.0%	66.7%	66.7%
Ciprofloxacin	77.8%	77.8%	66.7%	33.3%
Levofloxacin	60.0%	100.0%	100.0%	0.0%
Amoxicillin-Clavulanate	25.0%	25.0%	33.3%	33.3%
Ceftazidime	14.3%	22.1%	66.7%	0.0%
Cefuroxime	44.4%	44.4%	100.0%	100.0%
Cefotaxime	0.0%	0.0%	0.0%	0.0%
Erythromycin	0.0%	0.0%	0.0%	0.0%
Cefepime	0.0%	0.0%	0.0%	0.0%

Table 3: Antimicrobial Susceptibility Pattern of isolated *Staphylococcus aureus* (n=7)

Antimicrobials	Sensitive	Intermediate	Resistant
Gentamicin	83.3%	0.0%	16.7%
Imipenem	100.0%	0.0%	0.0%
Meropenem	100.0%	0.0%	0.0%
Amikacin	100.0%	0.0%	0.0%
Ciprofloxacin	28.6%	14.3%	57.1%
Levofloxacin	20.0%	0.0%	80.0%
Amoxicillin-Clavulanate	42.9%	0.0%	57.1%
Ceftazidime	0.0%	0.0%	100.0%
Cefuroxime	100.0%	0.0%	0.0%
Cefotaxime	0.0%	0.0%	100.0%
Erythromycin	55.6%	0.0%	44.4%
Cefepime	0.0%	0.0%	100.0%

Discussion

Chronic suppurative otitis media is a condition of the middle ear that is characterized by persistent or recurrent discharge through a chronic perforation of the tympanic membrane[7]. Untreated cases of chronic suppurative otitis media can result in a wide range of complications. Ear discharge due to chronic suppurative otitis media has a diverse bacteriological pattern and early diagnosis and proper treatment is mandatory to avoid extra and intracranial complications and hearing impairment. Moreover, antibiotic resistant bacteria are a significant issue for providing a cost-effective treatment for the patients of chronic suppurative otitis media[9]. So this study was an attempt to evaluate the bacterial pathogen commonly associated with chronic suppurative otitis media in our hospital setting to help clinicians for prescribing the specific medication. Development and spread of resistant bacteria due to over and indiscriminate use of antibiotics is a global public health threat. This study was designed to identify the common bacteria and their antimicrobial character and thereby an up-to-date information on microbial susceptibility pattern at national and local levels can be developed to guide the rational use of the existing antimicrobial drugs.

In this study revealed that bacteria were isolated from ear discharge from patient with chronic suppurative otitis media in 52.5% and remaining 47.5% cases revealed no bacterial growth. This study reveals that Gram negative bacteria (44.6%) are more responsible than Gram positive bacteria (8.8%) as causative agents of chronic suppurative otitis media. The predominance of Gram-negative aerobes indicate that the nasopharynx is not the source of infection, as it does not contain these organisms. The most predominant bacterial species isolated was *Pseudomonas aeruginosa*, which was present in 25.0% followed by Escherichia coli (11.25%), *Staphylococcus aureus* (8.75%), Chronic suppurative otitis media is a condition of the middle ear that is characterized by persistent or recurrent discharge through a chronic perforation of the tympanic membrane[7]. Untreated cases of chronic suppurative otitis media can result in a wide range of complications. Ear discharge due to chronic suppurative otitis media has a diverse bacteriological pattern and early diagnosis and proper treatment is mandatory to avoid extra and intracranial complications and hearing impairment. Moreover, antibiotic resistant bacteria are a significant issue for providing a cost-effective treatment for the patients of chronic suppurative otitis media[9]. So this study was an attempt to evaluate the bacterial pathogen commonly associated with chronic suppurative otitis media in our hospital setting to help clinicians for prescribing the specific medication. Development and spread of resistant bacteria due to over and indiscriminate use of antibiotics is a global public health threat. This study was designed to identify the common bacteria and their antimicrobial character and thereby an up-to-date information on microbial susceptibility pattern at national and local levels can be developed to guide the rational use of the existing antimicrobial drugs.

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Prevalence of coliforms bacteria such as *Escherichia coli* and *Klebsiella* in this study was 12.0% and 3.8% respectively which has similarity reported by Shyamala and Reddy[11] from India, where *Escherichia coli* was reported in 12% and *Klebsiella species* in 5.0% of cases. This result differs from reports by Prakash et al[12] where *Klebsiella species* were 9.42%

isolates and *Escherichia coli* were 7.33% isolates. Isolation of water bacteria like *Pseudomonas* and fecal bacteria like *Escherichia coli* and *Klebsiella species* may indicate that individuals are at risk of infection due to poor hygiene conditions.

The youngest patient was 1-year-old, while the oldest patient was 60 years old. Maximum number of patients (27.5%) belonged to the age group of 11 to 20 years, similar to a study by Kumar et al[13] who reported an incidence of 35.0% cases in the age group of 11 to 20 years. In contrast Loy et al[14] showed the increased prevalence in 30 to 40 years' age in his study.

There was no statistically significant association between bacteria and gender in this current study. This observation agrees well with reports from other researchers[10]. In this present study 76.2% cases were female and 23.8% cases were male and male to female ratio was 1:3.21. Thus females were affected more in this study which is in accordance with Loy et al[14], but differ from data of Ahmed[15]. According to the report of Hassan and Adeyemi[16], females were more affected by ear infections. A study conducted at Shaheed Monsur Ali Medical College, 64.35% cases were female and 35.65% cases were males[17].

Most of the patients (68.8%) in this study belonged to the rural group. Similar results were seen in studies by Agrawal et al[18] who reported 56% patient and Prakash et al[12] who reported 66.6% patients belonging to rural area. Factors like unhygienic conditions, overcrowding, ignorance regarding ear disease and lack of medical facilities might have been responsible for the high prevalence in this group of patients.

In this study, all the isolates of *Pseudomonas aeruginosa* were highly sensitive to imipenem and meropenem followed by amikacin, ciprofloxacin, gentamicin and levofloxacin. Our findings correlate with the study done by Kumar and Seth[19]. All the isolates of *Staphylococcus aureus* were sensitive to amikacin which correlate study done by Kumar et al[13] where *Staphylococcus aureus* was sensitive to amikacin.

Pseudomonas aeruginosa has long been known as an opportunistic pathogen, especially dreaded in the hospital environment. Opportunistic pathogen meaning that it exploits some break in the host defenses to initiate an infection. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner[15].

In this study *Escherichia coli* were 77.8% cases sensitive to ciprofloxacin which correlate reported by Altuntas et al[20] where *Escherichia coli* was highly sensitive to ciprofloxacin. On the other hand, study of Iqbal et al[21] showed that *Escherichia coli* were resistant to Ciprofloxacin. In our study all the isolates of *Escherichia coli* were resistant to Cefepime.

Conclusion

CSOM remains the common and important diseases responsible for chronic ear discharge. There may occurs a variation in the organisms infecting and their susceptibility pattern. This study revealed that Pseudomonas aeruginosa was the most common pathogen followed by Escherichia coli, *Staphylococcus aureus, Klebsiella species* and *Proteus species*. These organisms were found to be sensitive to Imipenem, Meropenem, Amikacin followed by Gentamicin, Levofloxacin and Ciprofloxacin. Therefore, continuous and periodic evaluation of microbial pattern and antibiotic sensitivity of CSOM is necessary. Rational use of antimicrobials should be practiced throughout the country under proper guidelines. Antimicrobials should be started after susceptibility testing to prescribe the most appropriate agents.

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Conflict of Interest
The authors have no conflicts of interest to disclose.

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Authors' contributions
Akter F, Khaled MS, Akhter F conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Akter F, Khaled MS contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Akhter F, Khan MJ, Yusuf MA involved in the manuscript review and editing. Akter F as collector of Data and Data Analyst. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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