

Prevalence and Antimicrobial Susceptibility of Aerobic Bacterial Pathogens in Chronic Suppurative Otitis Media

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ABSTRACT

Objective: To determine the prevalence of aerobic bacterial organisms in chronic suppurative otitis media (tubotympanic variety), difference in isolation of aerobic bacterial organisms and to establish pattern of antimicrobial sensitivity of these isolates.

Study Design: Prospective comparative study

Place and Duration of Study: This study was conducted at the Department of Microbiology, Institute of Molecular Biology & Biotechnology, The university of Lahore, Pakistan from January 2015 to December 2015.

Materials and Methods: Two hundred patients with chronic suppurative otitis media were included. An ear swab was taken from the middle ear by insertion of nichrome wire loop and conventional sterile swab separately.

Results: Thirty-six (18%) were <10 years of age, 104 were females. Only a few cases were seen in higher age groups. Hundred samples were collected using nichrome wire loop and conventional sterile swabs each. Yield ratio of organisms was 94.5% of samples. Nichrome wire loop showed no advantage over the conventional sterile swab. Staphylococcus aureus was the most common organism isolated, followed by Pseudomonas aeruginosa and Proteus species. 92.9 % of the organisms were susceptible to ciprofloxacin and only 20.1% were susceptible to trimethoprim-sulfamethoxazole.

Conclusion: Bacterial isolates from ear samples with conventional sterile swab were found to be identical to those with nichrome wire loop. Conventional sterile swab was as good as nichrome wire loop for collection of ear samples.

Key Words: Chronic suppurative otitis media (CSOM), Ear swab culture & sensitivity pattern

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INTRODUCTION

The term CSOM, chronic suppurative otitis media, is employed when discharge from middle ear continues beyond duration of more than 8 weeks.¹ It is still prevalent around the world² and is a source of concern to the treating physician and patients/families.³ Chronic suppurative otitis media may occur at any age. Western literature quotes the incidence rate of this condition as 15-20% in the paediatric population.⁴

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In young children it is a worldwide problem, with socioeconomic consequences at a later age.⁵ Its predisposing factors include, age, sex, race, socioeconomic status, bottle feeding, cold climate, respiratory disease, nasopharyngeal masses, cleft palate and chronic illness.⁶

Specimen of discharge from middle ear is collected inserting a sterile cotton tip on an applicator into the external ear canal. The material is then transported in sterile conditions for culturing. This technique is inadequate as the tip of the applicator may not reach to middle ear to get representative sample. It becomes contaminated with the discharge collected in the external ear canal and may not represent the organisms of the middle ear. This is especially important in children whose external auditory canal is narrow.⁷

A 28 gauge pre-reduced nichrome wire swab can be used for collection of middle ear specimens. The swab can be prepared, 10 cm length of 28 gauge nichrome wire can bent completely back on itself at a length of 1 mm from the tip end and small wisp of cotton wool applied at the tip to make swab and this swab immersed in a screw capped tube of 12.5 cm length filled with Stuarts medium (transport medium) sterilized by autoclaving. The nichrome wire loop can pass into the middle ear through the perforation in the tympanic

membrane and the middle ear sample can be collected.^{8,9}

In this method specimen is aspirated through the perforation in the tympanic membrane, using 2 ml disposable syringe fitted with 24 gauges or 18 gauge needle covered by plastic cannula. The tip of the needle is bent to avoid accidental trauma.¹⁰

The presence of multiple strains of both Gram negative and Gram positive aerobes were seen to be the rule rather than exception.¹¹ Infective complications of CSOM range from mastoiditis, ossicular damage, meningitis to cerebral abscess with resulting disability and handicap and developing cholesteatoma.⁶ In CSOM, both aerobic Gram positive, Gram negative and anaerobes are found but *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacteriaceae* are the most frequently isolated micro-organisms.¹²

MATERIALS AND METHODS

This prospective comparative study was conducted at Department of Microbiology, Institute of Molecular Biology & Biotechnology, The University of Lahore, Pakistan from 1st January 2015 to 31st December 2015. Two hundred patients with CSOM of tubotympanic type, seen in ENT and Paediatric Clinics were eligible. Diagnosis of tubotympanic type of CSOM was established on the basis of history, ear examination and otoscopic examination of tympanic membrane, showing tubotympanic type of (central) perforation. Patients having marginal tympanic membrane perforation, showing cholesteatoma, granulation tissue or polyp were excluded from the study. Similarly, patients on topical or systemic antibiotic therapy were also excluded. Demographic information was collected including, age, sex, weight, place of residence, number of family members, number of rooms in the house and socioeconomic conditions. A detailed history of present illness, its duration and the symptomatology was recorded. External auditory canal was mopped dry using sterile ear swab. The samples for bacteriological studies were obtained from the middle ear by insertion of nichrome wire loop and the conventional sterile swab separately. The swabs were inoculated on chocolate agar, blood agar and MacConkey agar, incubated aerobically at 37°C, and read after 24 hours. Laboratory standard operating procedure for the identification of these organisms include colonial morphology, Gram staining, catalase, DNase, oxidase, motility, citrate and where needed API identification system of Biomerieux France was used.⁷ A Gram stain was done on all specimens gathered. Microorganisms were characterized and identified by using conventional scheme.¹³ A rapid method of identification for *Candida albicans* and *Candida* species in general was based on its ability to form germ tubes within two hours of incubation in human serum at 37°C.¹⁴ Antibiotic susceptibilities were determined using a comparative

disc method. Control organisms included *Staphylococcus aureus* NCTC6571 and *Escherichia coli* NCTC10418 susceptibility were confirmed by determining MIC's of these drugs. Data were checked for completeness, entered and analyzed by using SPSS-20.

RESULTS

There were 104 females and 96 males patients. 20% patients fell in the upper income group, 62% in the middle, and 18% in lower income group. Thirty patients (24 males and 12 females) were 1-10 years old, 50 were 11-20 years old (22 males and 28 females) and 52 were 21-30 years old (24 males and 28 females), 20 were 31-40 years old (with 8 males and 10 females) and 24 were 41-50 years old (10 males and 14 females) respectively (Table 1). Swabs were classified according to their nature of discharge; ear discharge was mainly thick and non-odorous in 90 patients (45%) cases. Mucopurulent ear discharge in 68 patients (34%) and mucopurulent with smell in 18 patients (9%), Discharge was watery in 16 patients (8%) and blood tinged discharge was seen in only 8 patients (4%). No discrepancy was observed in both types of samples (Table 2). *Staphylococcus* (coagulase negative) species were isolated from 30 patients with conventional swabs accounting for 6.7% of total isolates and in 12 patients with nichrome wire loop, accounting 2.8% of total isolates. *Streptococcus pyogenes* was observed in samples from 30 patients taken with nichrome wire loop and sterile swab, representing 7.2% and 6.7% of total isolates respectively (Table 3).

Single organism was isolated in 38 patients with nichrome wire loop and in 24 patients with conventional sterile swab. Two organisms (double growth) were isolated in 120 patients with nichrome wire loop and in 130 patients with conventional sterile swabs samples. With nichrome wire loop growth of three organisms was obtained in 30 cases and four organisms were in 12 cases. While with conventional sterile swab, three organism growths were observed in 34 patients and four organism growth in 12 patients (Table 4). *Staphylococcus aureus*, as a single isolate was observed in 24 out of 38 patients (63.15%), of those presenting with single organism growth with nichrome wire loop while in 18 out of 24 patients (75%) with sterile swab ear samples. *Pseudomonas aeruginosa* as a single pathogen isolates were seen in 10 of total patients (26.33%), nichrome wire loop culture and in 6 patients (25%) with conventional sterile swab isolates. *Proteus* species, *Streptococcus pyogenes* both were observed as a single pathogenic organism in one patient (5.2%) each with nichrome wire loop isolates (Table 5).

Methicillin resistance in *Staphylococcus aureus* was observed in 12 patients (7.89%) out of 152 patients' isolates whereas remaining 140 isolates showed

sensitivity to methicillin, 92.11%. Methicillin resistance in *Staphylococcus* (coagulase negative) species was observed in only 2 isolates (16.6%) while sensitivity was observed in 10 cases [83.3%] (Table 6).

Table No.1: Distribution of Patients according to age in male and female groups (n=200)

Age (years)	Males		Females	
	No.	%	No.	%
< 1	-		2	1.0
1 – 10	24	12.0	12	6.0
11 – 20	22	11.0	28	14.0
21 – 30	24	12.0	28	14.0
31 – 40	8	4.0	12	6.0
41 – 50	10	5.0	14	7.0
51 – 60	2	1.0	6	3.0
61 – 70	4	2.0	-	-
71 – 80	2	1.0	2	1.0

Table No.2: Classification of ear swabs according to nature of discharge

Nature of Discharge	No.	%
Non-odourous and thick	90	45.0
Mucopurulent	68	34.0
Mucopurulent with smell	18	9.0
Watery	16	8.0
Blood stains	8	4.0

Table No.3: Comparison of isolates between sterile swab and nichrome wire loop

Organism	Sterile swab (n=442)		Nichrome wire loop (n=416)	
	Isolates	%	Isolates	%
<i>Staph aureus</i>	152	34.3	152	36.5
<i>Pseudomonas spp.</i>	108	24.4	108	25.9
<i>Proteus spp.</i>	52	11.7	52	15.5
<i>Staph. Spp.</i>	30	6.7	12	2.8
<i>Strep. pyogenes</i>	30	6.7	30	7.2
<i>E. coli</i>	14	3.1	14	3.3
<i>Klebsiella spp.</i>	14	3.1	14	3.3
<i>Strep. pneumonia</i>	12	2.7	12	2.8
<i>Bacillus spp.</i>	16	3.6	12	2.8
<i>Candida spp.</i>	10	2.2	6	1.4
<i>Enterobacter spp.</i>	4	0.9	4	0.9

Table No.4: Correlation of growth pattern to sample collection technique

Type of growth	Nichrome Wire Loop	Sterile Swab
Single organisms growth	38	24
Two organisms growth	120	130
Three organisms growth	30	34
Four organisms growth	12	12

Table No.5: Type and frequency of single isolates

Isolate	Nichrome Wire Loop (n=416)	Sterile Swab (n=442)
<i>Staph. aureus</i>	24	18
<i>Pseudomonas spp.</i>	10	6
<i>Proteus spp.</i>	2	00
<i>Strep. pyogenes</i>	2	00
Total	38	24

Table No.6: Methicillin sensitivity in *Staph. aureus* and *Staph. Spp*

Isolates	No.	%
<i>Staph. Aureus</i> (n=152)		
MRSA	12	7.89
MSSA	140	92.11
<i>Staph. Spp.</i> (n=12)		
MRSS	2	16.6
MSSS	10	83.6

DISCUSSION

Prevalence of microorganism along with their sensitivity pattern was studied in 200 patients of tubotympanic type of CSOM. There were 104 females and 96 males. Few studies showed similar finding in gender distribution as observed in our study.^{14,15} Whereas western literature also quotes a significant male preponderance.¹⁶ This difference may be attributed to more common swimming habits in males in west as compared to our population.

Patients were arranged in different age groups, 36 patients were seen in 1-10 years age group. In the 11-20 and 21-30 age groups, there were 50 and 52 patients respectively. Number of patients decreased with increasing age and only 20 and 24 patients fell in 31-40 and 41-50 years age group. While only few cases were seen in higher age group. CSOM has been reported to be more common in third decade of life (30%–35% cases) that is comparable in our study and other studies in the world.^{4,17}

In the present study, ear discharge was thick and non-odourous in 90 patients (45%) cases mucopurulent in 68 (34%) and mucopurulent with smell in 18 patients (9%). Discharge was watery in 16 patients (8%) and blood stained in 8 patients (4%). Two other different studies in which non-odourous and thick discharge was reported in 42.5%, mucopurulent 30%, mucopurulent with smell 11.5%, 10% watery and 6% blood stained samples.^{18,19}

Nichrome wire loop samples yielded 208 isolates as compared to conventional sterile swab samples which yielded 221 isolates. Isolates both with Nichrome wire loop and conventional sterile swab were almost identical in 94.5% and samples showing same type of growth by both methods. The results were comparable with the observation of Raju et al⁹ who reported 88%

isolates of same type by both methods in a study of 25 isolates.

The commonest organism was *Staphylococcus aureus* (40%) cases followed by *Pseudomonas* (28%), *Proteus* species (11%), *Streptococcus pyogenes* (5%) and *Staphylococcus* species (1%) with decreasing frequency. Many workers have reported similar incidences in which *Staphylococcus aureus* was isolated 30.3-40.3% of cases, *Pseudomonas* 24-30%, *Proteus* species 8-11%.^{8,20}

In the present study, 60% of cultures yielded 2 organisms and 19% single organism whereas 15% and 6% yielded 3 and 4 organisms respectively. Earlier workers have also reported 40-85% of cases yielding 2 organisms.²¹ Whereas Aslam et al¹⁹ (1998) from Aligarh (India) have reported monobacterial isolates in 87% and polybacterial only in 13%. Maximum samples yielded 2 organism growth. This was followed by one, three and four organism growth in decreasing order of frequency.

In cases with two organisms' growth *Staphylococcus aureus* and *Pseudomonas* were the commonest associated infecting organisms and were isolated in 27% cultures which were followed by *Staphylococcus aureus* and *Proteus* association (7%). Many workers had reported *Staphylococcus aureus* and *Pseudomonas* association in 20-30% cases, *Staphylococcus aureus* and *Proteus* species association 5-8% cases.²²

Based on in vitro sensitivity the most effective antimicrobial agent, if all isolates are taken into account was observed to be ciprofloxacin (susceptibility 92.9%) followed by sparfloxacin (92.4%), cefpirome (91.9%), ofloxacin (91.4%) and imipenem, cefepime (90.4%). Septran was the least effective antimicrobial showing 20.1%. The results reported by Deosthale²³ is comparable with the results of our study, who reported ciprofloxacin >85 %, and gentamicin >81 % effective. When vancomycin and teicoplanin were tested against *Staphylococcus aureus* and *Staphylococcus* species, both were found to be 100% effective. As in the present study no vancomycin resistance was observed among 1829 *Staphylococcus* isolates from United States and Canada collected from January to June 1997. Similarly, all these strains were also sensitive to teicoplanin.²⁴ Methicillin resistance was seen in 7.9% of *Staphylococcus aureus* isolates in the present study. Methicillin resistant strains of *Staphylococci* emerged by the late 1970s have become increasingly more prevalent as nosocomial pathogens.²⁵ In Italy, the incidence of methicillin resistant *Staphylococcus aureus* among *Staphylococcus aureus* strains isolated at the Institute of Microbiology of the University Hospital of Genova exceeds 35%.²⁶ In France (Saint-Joseph Hospital), the incidence of methicillin resistant *Staphylococcus aureus* in 1992, was reported to be 44%.²⁷ Other reports show that the proportion of methicillin resistant *Staphylococcus aureus* in hospitals

in Spain, France and Italy ranges to the rate of 30-40%.^{26,28} In Lahore, the overall prevalence of methicillin resistance in *Staphylococcus aureus* isolates from indoor cases in the year 2000 was 46.5%.²⁹

In present study, methicillin resistant *Staphylococcus aureus* is 7.89% and *Staphylococcus* species is 16.6% only (Table 13) which is very low as compared to the other studies. Low level of methicillin resistant *Staphylococcus aureus* in current study is due to the fact that samples are collected from the community patients attending outpatients and were not exposed to the ward/ ICU environment.

CONCLUSION

Conventional sterile swab was as good as nichrome wire loop for collection of ear samples. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus* species continue to be the major infecting pathogens in chronic suppurative otitis media. Sensitivity pattern of isolates shows that all organisms are sensitive to commonly used drugs.

Author's Contribution:

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