Original article:

Analysis of bacteriological pattern and antibiotic sensitivity of bacteria in ear discharge

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Abstract:

Introduction: Infections of middle ear space and their sequel have plagued mankind from the beginning of time. The objective of this cross sectional prospective study was to analysis of bacteriological pattern and antibiotic sensitivity of bacteria in ear discharge .

Materials and methods: This is an observational cross sectional study carried out in the OPD department of otorhinolaryngology, at a tertiary care hospital. Period of two months from July 2014 to August 2014 for this study. A total number of 50 patients of paediatric age group (upto 12 years) of both genders presenting with otorrhoea in the OPD of otorhinolaryngology.

Results: The present study shows that active CSOM in children is mainly due to staphylococcus aureus (42%), followed by Pseudomonas spp. 11 (22%), Proteus spp. 4(8%).

Conclusion: Staphylococcus aureus and Pseudomonas spp. were found to be the common cause of CSOM in our study.

Keywords: CSOM, Staphylococcus aureus

Introduction:

Infections of middle ear space and their sequel have plagued mankind from the beginning of time [1].Chronic suppurative otitis media (CSOM) is defined as a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent ear discharges or otorrhoea through a tympanic perforation. The disease usually begins in childhood as a spontaneous tympanic perforation due to an acute infection of the middle ear known as Acute Otitis Media (AOM) [2,3] .

In chronic otitis media, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia and Escherichia coli found in the skin of the external ear enter into the middle ear through a chronic perforation [4]. The frequency of such bacterial isolates could vary in different geographical areas.

The emergence of antibiotic resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant-*Enterococcus* (VRE), vancomycin-resistant *Staphylococcus aureus* (VRSA), and quinolone resistant Pseudomonas, has caused a significant change in the distribution of the dominant bacteria in patients with CSOM[5,6].

The objective of this cross sectional prospective study was to analysis of bacteriological pattern and antibiotic sensitivity of bacteria in ear discharge .

Materials and methods:

This is an observational cross sectional study carried out in the OPD department of otorhinolaryngology, at a tertiary care hospital. Period of two months from July 2014 to August 2014 for this study. A total number of 50 patients of paediatric age group (upto 12 years) of both

genders presenting with otorrhoea in the OPD of otorhinolaryngology.

Inclusion criteria:

- Paediatric patients presenting with ear discharge in OPD of department of otorhinolaryngology at a tertiary care hospital.
- Patients presenting with mucoid/ mucopurulent/ purulent ear discharge either unilateral or bilateral
- Children up to the age of 12 years.
- Willingness of parents. (*format of consent form attached)*

Exclusion criteria:

- Conditions causing otorrhoea due to:

1. Trauma- Cerebrospinal fluid otorrhoea
2. Otitis externa
3. Acute otitis media
4. Foreign body in ear

- Patients who have taken antibiotic therapy, local or systemic within 2 weeks before presenting to the department

Methodology :

Collection of sample:

According to standard procedures, the ear discharge samples were collected aseptically by using sterile cotton swab containing test tube obtained from microbiology department just before collection. The outer contaminated discharge is cleaned with sterile cotton. Discharge from deep area near tympanic membrane is taken on the sterile swab through a sterile ear speculum to avoid sample contamination, under Bull's eye lamp illumination in ENT OPD.

Transport of sample:

The swab is kept immediately in sterile bottle & sealed with cap & it is properly labelled with name, date and IP/ID number of the patient and the time of collection with a duly filled request form and sent to the microbiology laboratory. (*format of laboratory investigation form attached)*

Processing of sample:

Direct smear examination: Gram's stain was performed by Jensen's modification and then screened under oil immersion to note the various morphological types of bacteria, their number, the presence or absence of inflammatory cells and the number of squamous epithelial cells in the sample.

Aerobic culture: - The swab on reaching the laboratory was inoculated on the following culture media.

- MacConkey agar plate
- Blood agar plate
- Chocolate agar plate and
- Nutrient agar plate to isolate the organisms

The inoculated Blood agar and MacConkey agar plates were incubated aerobically at 37°C for 24 hours. After overnight incubation at 37° C the blood agar and MacConkey agar plates were examined for evidence of growth. The colony characters were studied; smears were stained by Gram's stain and examined under the 100x objective. After 48 hours incubation the chocolate agar plate was similarly examined and the colonies further processed. The bacterial species then isolated were identified by morphology, cultural characteristics and bio-chemical reactions according to the standard techniques[7].

Antibiotic sensitivity testing:

For antibiotic sensitivity, the bacterial growth suspension is flooded on the surface of plate containing culture media Mueller Hinton agar (MHA) & the excess is pipetted off. After the plate is dried, antibiotics (about 6 – 7 in a 9 cms plate) which are commercially available in 6 mm discs are kept at minimum distance of 24 mm from each other. After overnight incubation the inhibition zone is measured and compared against standard

guidelines. It is reported as sensitive or resistant.

Results:

Table 1: Distribution of organisms in the participants based on culture report

Organism	**Frequency**	**Percentage**
Methicillin sensitive staphlylococcus aureus (MSSA)	21	42%
Pseudomonas aeruginosa	11	22%
Proteus species	4	8%
Citobacter spp		
Freundii	2	4%
Koseri	1	2%
Klebsiella pnemoniae	2	4%
Methicilin resistant staphlylococcus aureus (MRSA)	2	4%
Group 'D' streptococcus	1	2%
E.coli	1	2%
Fungus		
Aspergillus fumigatus	2	4%
Aspergillus niger	1	2%
No growth	3	6%
Total	50	100%

Table 2: Antibiotic sensitivity and Resistance of *S.aureus*(21)

Antimicrobials	Sensitive	Resistant
Oxacillin	21 (100%)	-
Clindamycin	21 (100%)	-
Erythromycin	16 (76%)	5 (23.8%)
Gentamicin	20 (95%)	1 (4.7%)
Cotrimoxazole	13 (61.9%)	8 (38%)
Vancomycin	21 (100%)	-
Linezolid	21 (100%)	-
Ofloxacin	16 (76%)	5 (23.8%)
Ciprofloxacin	10 (47.7%)	11 (52.3%)

Amikacin	18 (85.7%)	3 (14.2%)

Table 3: Antibiotic sensitivity and Resistance of *Psedomonas aeruginosa*(11)

Antimicrobial	Sensitive	Resistant
Amikacin	11 (100%)	-
Gentamicin	10 (90%)	1 (10%)
Ciprofloxacin	10 (90%)	1 (10%)
Ofloxacin	11 (100%)	-
Carbenicillin	11 (100%)	-
Piperacillin	11 (100%)	-
Ceftazidime	10 (90%)	1 (10%)
Imepenem	11 (100%)	-
Ceftazidime + Clavulinic acid	11 (100%)	-
Ceftazidime+ Tazobactam	11 (100%)	-

Figure 1: Antibiotic sensitivity and Resistance of *Pseudomonas aeruginosa*

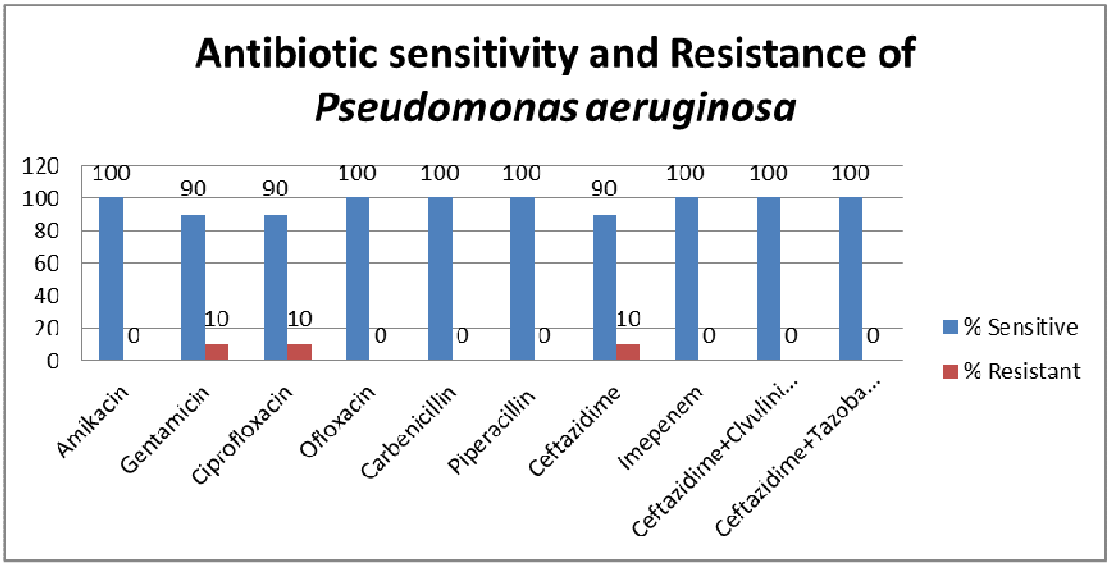

Table 4: Antibiotic sensitivity and Resistance of remaining organisms

Antimicrobials	*Proteus spp.*		*Klebsiella spp.*		*Citrobacter spp.*		*MRSA*		*Streptococcus spp.*		*E.coli*	
	%S	% R	% S	% R	%S	% R	% S	% R	% S	% R	%S	% R
Amikacin	100	-	100	-	100	-	100	-	-	-	100	-
Ampicillin	75	25	50	50	100	-	-	-	-	-	-	100
Norfloxacin	100	-	100	-	100	-	-	-	-	-	-	100
Gentamicin	100	-	100	-	100	-	100	-	100	-	100	-
Cefotaxime	100	-	100	-	100	-	-	-	-	-	-	100
Cotrimoxazole	75	25	100	-	100	-	100	-	-	-	-	100
Chloramphenicol	100	-	100	-	100	-	-	-	-	-	-	100
Ceftazidime	100	-	100	-	100	-	-	-	-	-	-	100
Imipenem	100	-	100	-	100	-	-	-	-	-	100	-
Oxacillin	-	-	-	-	-	-	-	100	100	-	-	-
Clindamycin	-	-	-	-	-	-	50	50	100	-	-	-
Erythromycin	-	-	-	-	-	-	50	50	100	-	-	-
Ceftazidime+Tazobactam	100	-	100	-	100	-	-	-	-	-	-	100
Ceftazidime+Clavulinic acid	100	-	100	-	100	-	-	-	-	-	-	100

%S -%Sensitive , %R- % Resistant

Discussion:

In the present study an attempt was made to know the bacteriology of CSOM, with antimicrobial susceptibility testing of the isolates. The results are compared with the other studies and discussed as follows.

The present study shows that active CSOM in children is mainly due to staphylococcus aureus (42%), followed by Pseudomonas spp. 11 (22%), Proteus spp. 4(8%). Gh. Ettehad, Rejahi S, Nemmati A, Pirzadeh A, Daryani A (2006) undertook a study on microbial and antibiotic susceptibility patterns from patients with chronic suppurative otitis media in Ardebil revealed that most frequently isolated organism was Staphylococcus aureus (31.95%), followed by Pseudomonas aeroginosa (26.35%), and Proteus species (19.67%) . In India, Saini reported that S. aureus was the commonest isolate in paediatric patients. My study correlates to the above study.

Many of the previous studies showed Pseudomonas to be the most common bacteria isolated from CSOM cases. But Pseudomonas spp. was the second most common organism in our study, isolated from 22% cases. This is similar to a study by Sharma et al[8] who reported Pseudomonas in 36% cases. In our study, Staphylococcus aureus and Pseudomonas sp. together account for about 64% of cases, which is in accordance with the study by Aslam et al[9].

The frequency of Staphylococcus aureus in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract. The organisms like Pseudomonas spp. and Proteus spp. are considered mostly as secondary invaders from external auditory canal gaining access to the middle ear via a defect in tympanic membrane. Possible explanation to this difference in isolation rate might be related to the effect of climate. Bacterial colonization of otitis media increases as temperatures rise which in turn increases the isolation rate of bacteria.

Among the gram negative pathogens, next to Pseudomonas, Klebsiella pneumoniae (4%) was the other common pathogen followed by Escherichia coli (2%).This is similar to study by Loy et al[10].

The presence of contaminants like E.coli from ear discharge indicates water contamination by faecal matter and poor hygienic conditions.

Staphylococcus species were Sensitivite with ciprofloxacin in 47.7% cases in our study. This is in accordance to study conducted by Sanjana R.K, Singh Y.I and Reddy N.S (2011) where sensitivity was only 26.1% to ciprofloxacin[6]. However, In some of the study, the Gh. Ettehad, Rejahi S, Nemmati A, Pirzadeh A, Daryani A, Staphylococcus species sensitivity with ciprofloxacin was higher (83.0%-95.0%). Vancomycin, linezolid, clindamycin and oxacillin were 100% sensitive and also against MRSA positive, thus making these agents as the drug of choice for same.

The sensitivity of pseudomonas spp. to aminoglycosides, flouroquinoles and cephalosporins were 90%. However, some results have shown much higher sensitivity pattern for flouroquinolone. A Srivastava et al reported 100% sensitivity to flouroquinolne and 97% sensitivity to cephalosporin[26]. The decreased sensitivity of pseudomonas spp. to quinolone family and cephalosporin family, to which it was highly sensitive until recently, is indicative of the rapid appearance of antibiotic resistant strains of Pseudomonas spp which is a matter of great concern.

Antibiotic susceptibility pattern was tested for all the isolated organisms. Most of the isolates were found to be susceptible to amikacin. But, almost 85% of the organisms showed resistance to amoxicillin which is in accordance with Chakraborty et al[11] (95.4%) and Malkappa et al[12] (90%). Staphylococcus aureus was found to be highly susceptible to aminoglycosides and oxacillin followed by cephalosporins and quinolones. The gram negative isolates were fairly susceptible to ciprofloxacin, third generation cephalosporins and gentamicin. One important fact to be kept in mind is that the antibiotic susceptibility pattern of the CSOM causing organisms keeps changing. Hence, routine antibiotic susceptibility testing before treatment is recommended.

Conclusion:

Staphylococcus aureus and Pseudomonas spp. were found to be the common cause of CSOM in our study.

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