International Journal of Otorhinolaryngology and Head and Neck Surgery
Suneer R et al. Int J Otorhinolaryngol Head Neck Surg. 2017 Jul;3(3):666-670
http://www.ijorl.com
pISSN 2454-5929 | eISSN 2454-5937

Original Research Article

DOI: http://dx.doi.org/10.18203/issn.2454-5929.ijohns20173044

Micro biological evaluation of aural swab culture and antibiotic sensitivity in 100 cases of tubotympanic and attico antral disease

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Received: 04 April 2017
Revised: 15 May 2017
Accepted: 17 May 2017

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ABSTRACT

Background: Chronic suppurative otitis media and its complications is one of the commonest ENT problems in our country. The chronicity of the disease and poor response to routine treatment prompted us to isolate and identify the causative organisms and study the antimicrobial susceptibility pattern.
Methods: About 50 patients with tubo tympanic disease and about 50 patients with attico-antral disease were selected, complete clinical ENT examination carried out, aural swabs were collected, culture was done and antibiotic sensitivity was studied.
Results: Culture of aural swab demonstrate the polymicrobial bacteriology consisting of both gram positive and negative pathogens, while gram negative pathogens form the majority of both tubotympanic and atticoantral disease. Mixed growth is more common in attico- antral disease. In our study, Ciprofloxacin and Cefotoxime has found out to be the drug of choice for both tubotympanic and atticoantral disease with high sensitivity.
Conclusions: Periodical monitoring of microbiological profile is essential for making effective empirical protocol for the cases in a particular geographical location.

Keywords: Bacteriology, Antibiotic sensitivity, Chronic suppurative otitis media

INTRODUCTION

Chronic suppurative otitis media refers to the chronic suppurative inflammation of mucoperiosteal layer of the middle ear cleft. The disease is wide spread in India due to poor living conditions, overcrowding and poor hygiene and nutrition. Chronic suppurative otitis media is usually classified into two main groups. i) Tubotympanic disease ii) Attico antral disease. Tubo tympanic disease is characterized by a perforation of the pars tensa. Patients with this form of otitis media are generally not considered at risk of developing intracranial sepsis. Hence the name safe otitis media is applied to this condition. Attico antral disease most commonly involves pars flaccida and is characterized by the formation of a retraction pocket in which keratin accumulates to produce cholesteatoma. This cholesteatoma is divided into congenital and acquired types. Congenital cholesteatoma has no previous history of otitis media. However expansion of these lesion may lead to secondary infection.[1,2]

Acquired cholesteatoma is typically persistent disease, insidious in onset often capable of causing severe destruction and irreversible sequlae and clinically it manifest with aural discharge and deafness. It is also called unsafe disease due to its ability to erode bone and uncover neighbouring structures such as duramater, lateral sinus, facial nerve and labyrinth. Spread of bacterial infection into deeper tissues thus exposed by bone erosion leading to complications like meningitis, extradural abscess, subdural abscess, brain abscess, lateral sinus thrombosis, labyrinthitis or facial paralysis. This bacterial infection also plays a role in the destruction

of bone by causing changes in the surrounding perimatrix of cholesteatoma which include activation of osteoclasts and production of lysozymes. Thus infection plays a greater role in the complications of cholesteatoma.[3,4]

Various bacterial species have been found to be associated with chronic suppurative otitis media leading on to persistence of infection. They belong to both aerobic and anaerobic variety. The proportions of various organisms isolated vary from study to study. But *P*seudomonas aeruginosa, *P*roteus species, *Staphyloccus aureus* and *Escherichia coli* predominate.[1,5] The role of anaerobes in chronic suppurative otitis media has been the subject of speculation. Bacteroids melaninogenicus, Bacteroids fragilis and other species has been isolated.[2]

The present study was undertaken to study the microbial flora of tubotympanic and atticoantral disease and the sensitivity pattern of the study may be useful in prescribing the appropriate antibiotics there by controlling the infection which forms the first step in the management of chronic suppurative otitis media.

METHODS

The materials for the present study were collected from patients-both male and female suffering from chronic suppurative otitis media with the age group of 16-60 years, with clinical diagnosis of – Tubotympanic disease or cholesteatoma who were admitted and underwent mastoidectomy operation in a Tertiary care, Govt. Medical college Hospital for a period of 2 years from January 2015 to January 2017. Cases admitted as chronic suppurative otitis media for mastoid surgery were studied and a complete clinical examination of ear, nose and throat was carried out. Radiological investigation of mastoid was also done and pus obtained during aural toilet was also sent for bacteriological and antibiotic sensitivity studies. Thus a total of fifty cases, under each tubotympanic and atticoantral type were included in the study.

Containers and swabs for collection of specimens

Strong glass tubes of 4x 3/8 inches sterilized in autoclave were used for collecting aural swabs. Swabs were prepared from wooden sticks 5 – 6 inches in length with cotton – wool pledget wrapped around one end. The glass tube with the swab was plugged with cotton wool and autoclaved for use.

Collection of specimens

Aural swab

At the first examination, the external auditory canal was cleaned with a dry sterile swab and fresh pus was collected using another dry sterile swab under good illumination.

Culture study was done

1. To isolate the bacteria
2. To demonstrate their properties and
3. To determine sensitivity to antibiotics

Identification of bacteria was done by

1. Morphology
2. Staining reaction
3. Cultural characteristics and
4. Bio chemical reactions

Processing of the specimens

In the laboratory, the swabs collected were put in glucose broth for enrichment and was incubated at 37 degree centigrade for four hours. Then with a standard loop the inoculum (glucose broth) was spread over the following media.

Nutrient agar plate which forms the basal medium to carry out further bio chemical tests.

Blood agar plate (heated blood agar) which also serve as enriched media.

Mac Conkey's agar plate - differential media to differentiate lactose and non – lactose fermentors.

These plates were incubated aerobically at 37 degree centigrade for 18-24 hours. These plates were examined after 24 hours to observe the type, number and morphology of the colonies. Accordingly the biochemical tests were carried out for further identification and also their antibiotics sensitivity pattern were determined by using appropriate antibiotic discs. The anaerobic culture was not done due to limited facility.

RESULTS

In Tubotympanic disease, all the 50 patients complained of Aural discharge and hard of hearing followed by bleeding per ear in 2 patient and all patients presented with central perforation. In Attico antral disease, all the 50 patients complained of aural discharge and 46 patients complained of loss of hearing followed by bleeding form ear in 7 patients and vertigo in one patient. Of the fifty patients studied majority of the patient presented with postero superior retraction or posterior marginal perforation (52%). Four patients presented with aural polyp.

Culture results

In tubo tympanic disease mixed flora was isolated in 3 cases while 28 cases of unsafe variety presented with mixed flora.

Table 1: Culture results of aural swabs from tubotympanic and atticoantral disease.

Organisms	Total	Tubolympanic	Attico antral
Gram positive			
Staphylococcus aureus	50	11 (22%)	14 (28%)
Staphylococcus epidermidis	50	2 (4%)	2 (4%)
Alpha – hemolytic streptococci	50	1 (2%)	2 (4%)
Beta – hemolytic streptococci	50	2 (4%)	3 (6%)
Gram Negative			
Pseudomonas aeruginosa	50	14 (28%)	16 (32%)
Proteus mirabilis	50	8 (16%)	10 (20%)
Klebsiella species	50	8 (16%)	11 (22%)
Escherichia coli	50	7 (14%)	9 (18%)
Mixed	50	3 (6%)	14 (28%)

Table 2: Mixed flora.

Combination of Organism isolated	Tubotympanic disease	Attico antral disease
Staphyloccus and Klebsiella species	0	6
Pseudomonas and Klebsiella species	2	5
Staphylococcus and Escherichia coli	0	1
Escherichia coli & Klebsiella	1	2
Total	3	14
Total percentage	6	28

Table 3: Antibiogram of isolates of aural swab culture in tubotympanic disease.

Isolate	Total	Ampi (%)	Gara (%)	Cot (%)	Amoxy (%)	Cipro (%)	Cefot (%)	Doxy (%)
Staphylococcus aureus	11	4 (36.36)	10 (90.9)	4 (36.36)	2 (18.18)	11 (100)	3 (100)	3 (27.27)
Staphylococcus epidermidis	2	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)
Alpha – hemolytic streptococci	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Beta – hemolytic streptococci	2	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)
Pseudomonas aeruginosa	14	4 (28.57)	12 (85.7)	4 (28.5)	0 (0)	14 (100)	14 (100)	2 (14.2)
Proteus mirabilis	8	3 (37.07)	6 (75)	5 (62.5)	0 (0)	8 (100)	7 (87.5)	2 (25)
Klebsiella species	8	0 (0)	8 (100)	6 (75)	0 (0)	8 (100)	8 (100)	3 (37)
Escherichia coli	7	0 (0)	3 (42.8)	0 (0)	0 (0)	8 (100)	7 (100)	0 (0)

Amp – Ampicillin, Gara – Gentamycin, Cot - Co-trimoxazole, Amoxy –Amoxycillin, Cipro – Ciprofloxacin, Cefot – Cefotoxime, Doxy – Doxycycline.

Bacteriological study

Among the gram positive organisams isolated from the aural swab culture in tubotympanic disease, staphylococcus aureus formed the majority (22%) followed by beta hemolytic streptococci (4%), staphylococcus epidermidis (4%) and alpha hemolytic streptococci (2%). Among the gram negative organisms isolated from the aural swab, *Pseudomonas aeruginosa* formed the majority (28%) followed by *Escherichia coli* (14%) *Proteus* mirabilis (16%) and *Klebsiell*a species (16%). Mixed growth was isolated from 3 cases (6%). Among the gram positive organisms isolated from the aural swab of atticoantral disease again staphylococcus aureus formed the majority (28%) followed by alpha hemolytic streptococci (4%) beta hemolytic streptococci (6%) and staphylococcus epidermidis (3%). Among the

gram negative organisms isolated pseudomonas aeruginosa formed 32% followed by *e coli* (18%) *protens mirabilis* (20%) and *Klebsiella pneumonia* (22%). Mixed growth was isolated from 14 cases (28%).

Table 4: Antibiogram of isolates of aural swab culture in attico antral disease.

Isolate	Total	Ampi (%)	Gara (%)	Cot (%)	Amoxy (%)	Cipro (%)	Cefot (%)	Doxy (%)
Staphylococcus aureus	14	3 (21.4)	13 (92.8)	2 (14.2)	2 (14.2)	14 (100)	14 (100)	1 (7.1)
Staphylococcus epidermidis	2	0 (0)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	1 (50)
Alpha – hemolytic streptococci	2	2 (100)	2 (100)	2 (100%)	1 (50)	2 (100)	2 (100)	1 (50)
Beta – hemolytic streptococci	3	2 (100)	2 (100)	2 (100%)	2 (66.6)	3 (100)	3 (100)	2 (66.6)
Pseudomonas aeruginosa	16	4 (25)	12 (75)	3 (18.7)	4 (25)	16 (100)	15 (93.7)	5 (31.2)
Proteus mirabilis	10	3 (30)	4 (40)	5 (50)	0 (0)	9 (90)	8 (80)	3 (30)
Klebsiella species	11	0 (0)	10 (90.9)	8 (72.7)	0 ()	11 (100)	10 (90.9)	4 (36.3)
Escherichia coli	9	0 (0)	8 (88.8)	0 (0)	0 (0)	8 (88.8)	8 (88.9)	1 (11.11)

Amp - Ampicillin , Gara-Gentamycin Cot-Co-trimoxazole, Amoxy-Amoxycillin Cipro–Ciprofloxacin, Cefot–Cefotoxime, Doxy-Doxycycline

Antibiotic sensitivity pattern

Staphylococcus aureus from cultures of aural swab are more sensitive to gentamycin, ciprofloxacin and cefotoxime. They are more resistant to ampicillin, amoxicillin, cotrimoxazole and doxycycline. Beta hemolytic streptococci are more sensitive to ampicillin, ciprofloxcin, cefotoxine and alpha hemolytic streptococci to ampicillin, cotrimoxazole, amoxycillin and ciprofloxacin. Pseudomonas aeruginosa is more sensitive to ciprofloxacin and cefotoxime and resistant to amoxycillin, ampicillin and doxycycline. *Escherichia coli* and *Proteus* are more sensitive to gentamycin, ciprofloxacin and cefotaxime and resistant to ampicillin, amoxycillin and doxycycline. *Klebsiella* are more sensitive to garamycin, cotrimoxazole, ciprofloxacin and cefotoxime and resistant to ampicillin, amoxycillin and doxycycline.

DISCUSSION

In a study by Rao and Reddy on the bacteriology of aural swab culture of 120 cases in 1994 showed that staphylococcus aureus formed the predominant pathogen (42.5%) followed by *Pseudomonas aeruginosa* (21.67%), *Proteus* (18.35%) *Escherichia coli* (10.83%) and *Klebsiella* species (10%).[6] A single pathogen was isolated from 68.52% and mixed growth was isolated from the remainder. The antimicrobial sensitivity of the isolates showed that gentamycin formed the drug of choice for treating cases due to either gram positive or gram negative organisms which showed a high rate of activity against *Staphylococcus aureus*, *Psuedomonas aeruginosa* and *Proteus* species with sensitivity incidence of 92.15%, 84.61% and 90.9% respectively followed by cefotoxime and amikacin.

In the study by Itzhak Brook and Bethesda on the bacteriology of cholesteatoma, they have reported that *Staphyloccus aureus* was the predominant gram positive pathogen and *Pseudomonas aeruginosa* the most predominant gram negative organism followed by *Proteus mirabilis*, *Klebsiella* pneumonia and *Escherichea coli*. They have also reported mixed growth in 8 patients.[7]

In contrast to our present study, the study done by Loy et al, Ahmed et al, Shreshtha et al, Lakshmi et al and Kumar et al showed *Staphylococcus aureus* is the most commonest followed by *Pseudomonas aeruginosa*. [1,8-11] Studies done by Poorey et al showed *Pseudomonas* species as the most common isolate.[12] *Pseudomonas* has been shown to be the commonest organism responsible for CSOM by Bansal et al, Moorthy et al, Varshney et al and Shazia et al.[2,4,13] Its incidence has been reported to vary from 18% to 67% Predominance of aerobic bacteria is well known in studies by Bansal et al, Mishra et al and Poorey et al whereas anaerobic bacteria are responsible for a minority of cases only.[2,12,14]

The results obtained from the present study also concur with the data obtained from other studies. The most common gram positive organism obtained form aural swab culture in tubotympanic disease is staphylococcus aureus (22%) followed by beta hemolytic streptococci (4%) staphylococcus epidermidis (4%) and alpha hemolytic streptococci (2%), whereas those obtained from atticoantral disease is staphylococcus 28% followed by alpha and beta hemolytic streptococci with 4% and 6% respectively and staphylococcus epidermidis 3%.The most common gram negative organism obtained from aural swab culture of tubotympanic disease is *Pseudomonas aeruginosa* 28% followed by *Proteus mirabilis* 16%, *Escherichia coli* 14% and *Klebsiella* species 16% whereas those obtained from atticoantral disease is *Pseudomonas aeruginosa* 32% followed by

klebsiella Pneumonia 22% *Proteus mirabilis* 20% *escherichia coli* 18%. Mixed growth were isolated from 14 cases (28%).

In the present study, gentamycin shows a high rate of activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Euscherichia coli, Klebsiella* and *Proteus* with a sensitivity index of 90.9%, 85.7%, 88.8%, 90.9% and 75.0% respectively. Ciprofloxacin and Cefotoxime show a much better sensitivity nearing 100% against these organisms. Ciprofloxacin is the drug of choice with high degree of sensitivity against both gram positive and gram negative organisms.

CONCLUSION

Cultures of aural swab demonstrate the polymicrobial bacteriology consisting of both gram positive and negative pathogens, while gram negative pathogens form the majority of both tubotympanic and atticoantral disease. Mixed growth is five times more common in cultures of atticoantral disease compared to the tubotympanic disease. Ciprofloxacin and cefotoxime are the drug of choice for both gram positive and negative organisms nearing 100% sensitivity. A special note about the higher incidence of *klebsiella* pneumonia in our study requires mention. Around 20% of *klebsiella* is obtained from the present study compared to 10-12% in the previous studies. The microbial predominance and their antibiotic sensitivity pattern have been changing overtime. So periodical monitoring of microbiological profile is essential for making effective empirical protocol for the cases in a particular geographical location.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Suneer R, Sivasankari L. Micro biological evaluation of aural swab culture and antibiotic sensitivity in 100 cases of tubotympanic and attico antral disease. Int J Otorhinolaryngol Head Neck Surg 2017;3:666-70.