BACTERIAL AND FUNGAL ETIOLOGY OF CHOLESTEATOMA IN PATIENTS ATTENDING TERTIARY CARE HOSPITAL

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Abstract- The current study aims at bacterial and fungal etiology of Cholesteatoma in patients attending tertiary care hospital. Cholesteatoma is an abnormal, noncancerous skin growth that can develop in the middle section of ear, behind the eardrum. It is a complication of chronic suppurative otitis media. With the informed consent, 70 patients with Cholesteatoma were included; 42 (60%) were male and 28 (40%) were female, and the mean age was 11-50 years. **Materials and methods-** Cholesteatoma sac and 2 preoperative swabs were collected from ENT OPD patients complaining of earache and discharge. **Results-** Out of 70 patients, 28 were sterile samples, 40 (41.7%) showed aerobic bacteria and 2 (1.3%) had fungal etiologies. **Conclusion-** Gram negative pathogen forms most aural swabs and Cholesteatoma sacs. Pseudomonas aeruginosa was the predominant isolate 18(25.7%), followed by Staphylococcus aureus 11(15.7%) and their antimicrobial susceptibility testing was done. Though the treatment of Cholesteatoma is surgery but to avoid recurrence, the use of proper antibiotics by knowing the antibiogram will lead to a decrease in the prevalence of microbial growth and recurrence of Cholesteatoma.

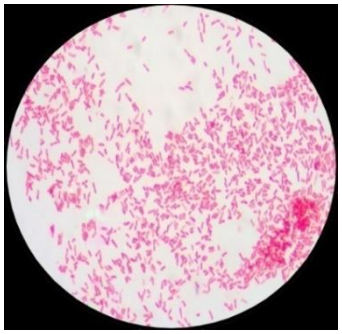
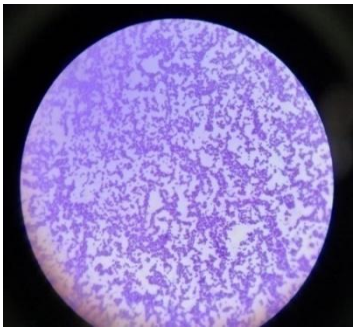
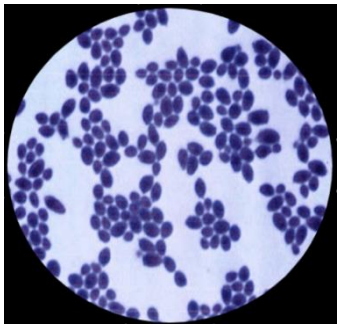
Key Words- Cholesteatoma, earache, discharge, otitis media.

INTRODUCTION - Hearing is one of the most important senses of man and helps him in one of the most important skills of man i.e., communication. Any imbalance in this disturbs the tranquility of life. Most common infections of ear are 1) Acute suppurative otitis media (ASOM) & 2) Chronic suppurative otitis media (CSOM). Chronic suppurative otitis media is classified

into two groups. i. Tubotympanic disease – safe type ii. Atticoantral disease - unsafe type. If untreated, CSOM leads to Cholesteatoma. Chronic suppurative otitis media with Cholesteatoma is typically a persistent disease. Insidious in onset, often capable of causing severe destruction and irreversible sequel and clinically it manifests with aural discharge and deafness. Thus, the present study with 70 samples were undertaken to detect the current bacterial and fungal etiology in Cholesteatoma patients attending tertiary care hospital in ENT Hospital, Hyderabad, Telangana.

MATERIALS & METHODS- After obtaining institutional ethical committee clearance, a total of 70 samples were collected from patients with Cholesteatoma in the Department of ENT, Koti, Hyderabad, Telangana, from October 2018 to September 2019 for bacterial and fungal etiology. Cholesteatoma sac and 1 aural swab from Amies transport medium was directly inoculated onto Blood agar (enriched media), MacConkey agar (differential media) and Nutrient agar (basal media) and incubated aerobically at 37^{oC} for 24 hours and a smear was made and stained by Gram stain to observe the morphology of the bacteria and presence of any inflammatory cells. KOH mount was done for 2^{nd} aural swab. After 24 hours, the plates inoculated were observed for colony morphology and Gram stain, hanging drop for motility, Catalase and Oxidase test for Gram negative bacilli and Coagulase test for Gram positive cocci were done. Antibiotic sensitivity testing was done by Kirby Bauer disk diffusion method. Bacterial species isolated are identified by morphology, culture characteristics and biochemical reactions according to standard microbiological techniques. Fungal cultures were identified, and anti-fungal susceptibility testing was done by disk diffusion and micro-broth dilution methods.

GNB on Gram stain **GPC on Gram stain** **Gram positive budding Yeast cells**

RESULTS- A total of 70 Cholesteatoma patients were included. Out of these, majority fall underage group of 11-20 years in both sexes. Among them, 42 showed positive culture which included 40 aerobic bacteria and 2 fungi and 28 showed negative cultures. Pseudomonas aeruginosa - 18(25.7%) was the predominant bacteria isolated followed by Staphylococcus aureus - 11(15.7%). The fungus isolated was Candida albicans (2.8%).

Chart 1– Showing the aerobic bacteria and fungi isolated in Cholesteatoma

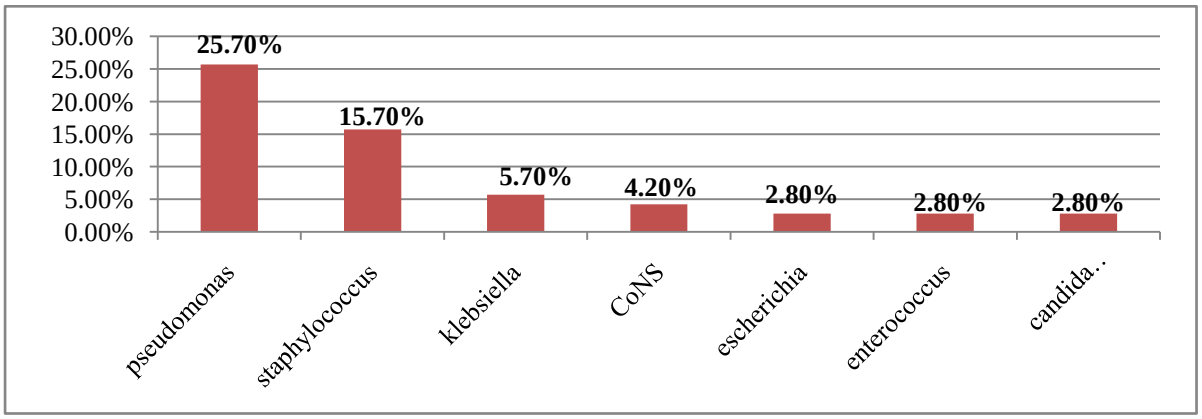


Table 1- Distribution of total no of organisms isolated in the study

Organism	**Total no. of samples**	**Total no. of organisms**	**Percentage**
Total Gram-negative bacilli	**70**	**24**	**(34.2%)**
Pseudomonas aeruginosa	70	18	(25.7%)
Klebsiella species	70	4	(5.7%)
Escherichia coli	70	2	(2.8%)
Total Gram-positive cocci	**70**	**16**	**(22.8%)**
Staphylococcus aureus	70	11	(15.7%)
CONS	70	3	(4.2%)
Enterococcus species	70	2	(2.8%)
Fungal isolates	**70**	**2**	**(2.8%)**
Candida albicans	70	2	(2.8%)

Table 2: Showing antibiotic sensitivity testing of Gram-negative bacilli

Antibiotics	**Pseudomonas aeruginosa**		**Klebsiella species**		**Escherichia coli**	
	S	**R**	**S**	**R**	**S**	**R**
Imipenem	100%	0%	100%	0%	100%	0%
Meropenem	100%	0%	100%	0%	100%	0%
Piperacillin-Tazobactum	100%	0%	100%	0%	100%	0%
Ciprofloxacin	75%	25%	75%	25%	50%	50%
Ampicillin	29.1%	70.9%	25%	75%	0%	100%
Amoxyclav	41.6%	58.6%	25%	75%	0%	100%
Cefperazone-Sulbactum	100%	0%	100%	0%	100%	0%
Ceftazidime	75%	25%	75%	25%	50%	50%
Cotrimaxazole	16.6%	83.4%	25%	75%	50%	50%

Majority of the isolated Gram-negative bacilli showed 100% sensitivity to Imipenem, Meropenem, Piperacillin-Tazobactam and Cefperazone-Sulbactum followed by 75% to Ceftazidime and Ciprofloxacin.

Chart 2: Antibiotic sensitivity pattern of Gram-negative bacilli

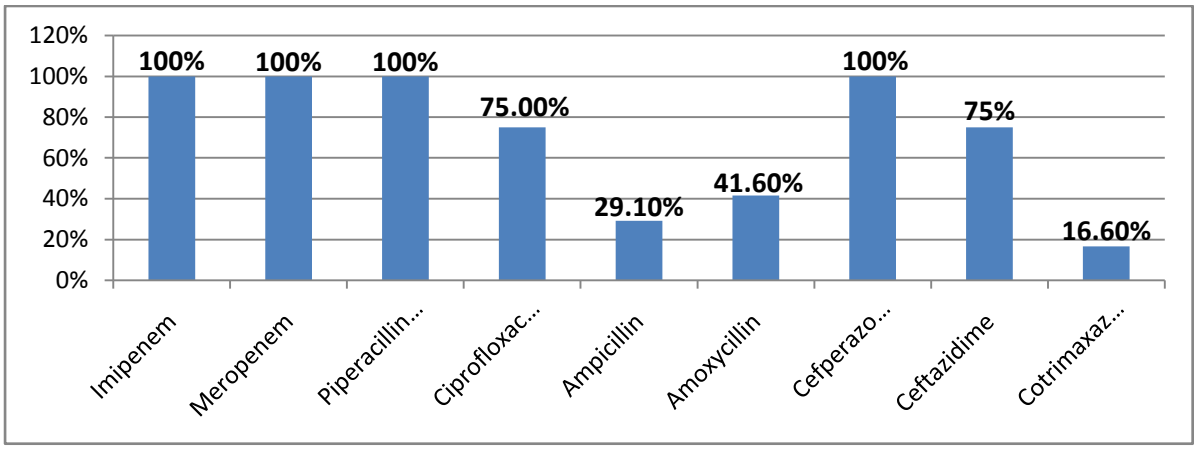


Table 3: Antibiotic sensitivity testing of Gram-positive Cocci

Antibiotics	Staphylococcus aureus		Cons		Enterococcus	
	S	R	S	R	S	R
Azithromycin	31.3%	68.7%	33%	66%	0%	100%
Ciprofloxacin	50%	50%	66%	33%	0%	100%
Cefoxitin	50%	50%	33%	66%	100%	0%
Tetracycline	68.6%	31.4%	66%	33%	50%	50%
Gentamicin	91.6%	8.4%	66%	33%	50%	50%
Clindamycin	100%	0%	100%	0%	100%	0%
Vancomycin	100%	0%	100%	0%	100%	0%
Doxycycline	100%	0%	100%	0%	100%	0%
Linezolid	100%	0%	100%	0%	100%	0%

Majority of the isolated Gram-positive cocci showed 100% sensitivity to Vancomycin, Linezolid, Doxycycline and Clindamycin followed by 81.2% to Gentamycin.

Chart 3: Gram Positive cocci Sensitivity Pattern

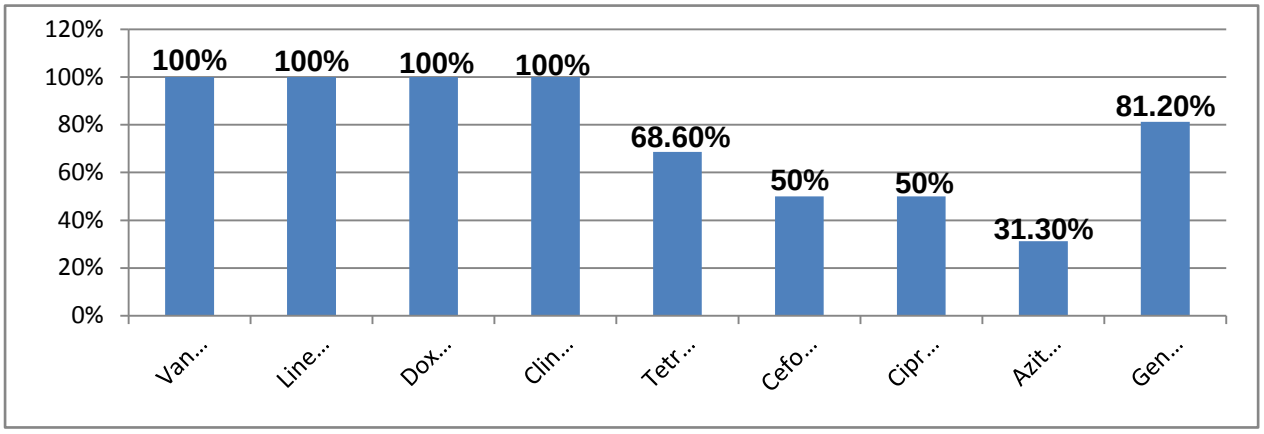


Table 4: Sensitivity pattern and MIC values of Fluconazole among the Candida albicans determined by microbroth dilution method of antifungal susceptibility

MIC value	Candida albicans
Susceptible (</=8)	2 (100%)
SDD (16-32)	0

Resistant (>/=64)	0

On observation, 2 (100%) isolates of Candida albicans with MIC value </= 8 were susceptible to Fluconazole.

Chart 4: Sensitivity pattern of Candida albicans isolated from Cholesteatoma cases to Fluconazole by Microbroth dilution method

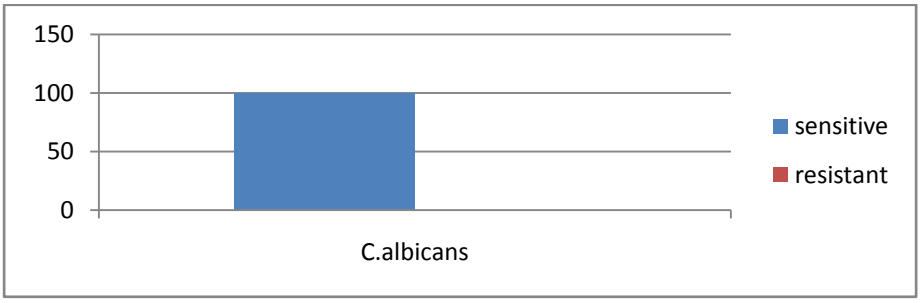


Chart shows that Candida albicans is 100% sensitive to Fluconazole by microbroth dilution method of antifungal susceptibility.

DISCUSSION

Cholesteatoma is a complication of the CSOM, characterized by persistent or recurrent discharge through a chronic perforation of the tympanic membrane. Due to perforation of the tympanic membrane, microorganisms may gain entry to the middle ear via the external ear. It is persistent disease with destructive sequel leading to fatal intra-cranial and extra-cranial complications which were very common in the pre-antibiotic era. Though such serious complications are low at present, few patients presented with nerve palsy, mastoiditis etc. Appropriate treatment at right time will avoid the chances of complications.

Knowledge of the local microorganism pattern and their antibiotic sensitivity is essential for empirical treatment prior to the results of microbiological culture. The present study was done to isolate the etiology of Cholesteatoma, with antimicrobial susceptibility testing of the isolates. Despite the advanced technology in medical care Cholesteatoma remains the most common cause of hearing loss in children and adults. Emergence of antimicrobial resistance makes it furthermore difficult for the otologists to reduce the risk of

complications. A total of 70 samples were collected from probable Cholesteatoma cases and were processed according to the standard operating procedures.

CONCLUSION

The bacteriological and fungal study of Cholesteatoma showed Pseudomonas aeruginosa as the commonest organism followed by Staphylococcus aureus, Klebsiella species, Coagulase negative Staphylococcus, Escherichia coli, Enterococcus and Candida albicans. Carbapenems, Piperacillin-Tazobactam were found to be the most suitable antibiotic for Gram negative bacilli and Vancomycin, Linezolid for Gram positive cocci. Fluconazole, Itraconazole, Voriconazole and Amphotericin B was found to be the most suitable drug for Candida albicans by disk-diffusion method.

The early detection of isolates and antibiogram helps in early institution of appropriate antimicrobial therapy and thus prevents the development and dissemination of these multidrug resistant strains in the hospital as well as in the community.

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