ISSN- O: 2458 - 868X, ISSN –P: 2458 - 8687

International Journal of Medical Science and Innovative Research (IJMSIR)

IJMSIR : A Medical Publication Hub

Available Online at: www.ijmsir.com

Volume – 2, Issue –5, September- October - 2017, Page No. : 442 - 450

Fungi Causing Chronic Suppurative Otitis Media and Antifungal Susceptibility Testing of Candida Isolates in A Tertiary Care Centre in Kerala.

Dr.Jyothi.R, Associate Professor, Department of Microbiology, Govt Medical College, Trivandrum

Dr. Samitha, Consultant in DDRC, Trivandrum

Dr.Harilal, Consultant ENT Surgeon

Dr. Ramani Bai .J.T, Professor and Head, S.R. Medical College, Varkala

Dr. Lancy.J, Additional Professor, Govt. Medical College, Trivandrum

Correspondence Author: Dr. Lancy.J, Additional Professor, Govt. Medical College, Trivandrum, Kerala, India.

Conflicts of Interest: Nil.

Abstract

Fungi play a significant role in causing chronic suppurative otitis media both in children and in adults. A study was conducted in the Department of Microbiology, Govt. Medical College, Thiruvananthapuram in co-ordination with the Dept. of ENT for one year period from March 2013 to February 2014 with the objective of compiling and analyzing the aetiological spectrum of CSOM with special attention to fungi. This study aims in helping to devise an optimal antimicrobial therapeutic regimen that can be adopted as an institute protocol. A total no. of 322 cases of CSOM including all ages and both sexes attending the out patient department of ENT were studied in detail. Culture positivity was 95% of the total isolates of 306, bacterial pathogens constituted 73% and fungal pathogens (22%) culture was sterile in 5% of cases. Among the 72 fungal solates obtained in culture, 29 (40.3%) were Aspergillus niger, 17 (23.6%) were Aspergillus flavus 12 (16.7%) were Asperigillus fumigatus, only one isolate (1.4%) was Aspergillus terreus, 3 (4.2%) were pencillium species and 10 isolates (13.8%) were candida species. Among the candida species, 80% isolates were nonalbicans and only 20% were candida albicans. Identification of the candida species was made by germ tube test, chalamydospore formation in command agar, characteristic distinct colour on CHROM agar, Carbohydrate assimilation and fermentation tests. Antifungal susceptibility testing of the isolates were done by Disc diffusion method according to CLSI guidelines. Three Antifungal drugs – Amphotericin B Fluconazole and Itraconazole were tested. All cardia isolates were 100% sensitive to Amphotericin B. Candldaalbicans isolates were 100% sensitive to Fluconazole Isolates of C. tropicates, 50% sensitive to Fluconazole, and 75% sensitive to Intraconazoles. C. parapsilosis showed 50% sensitivity to fluconazole and 100% sensitivity to Intraconazole. C. glabrata was 100% resistant to Fluconazole and 50% resistance to Itraconazole.

Keywords: Candida albicans, C.tropicalis, Amphotericin B, Fluconazole Itraconazole.

Introduction

Chronic suppurative otitis media (CSOM) is characterized by persistent discharge from the middle ear through a tympanic perforation. It is an important cause of preventable hearing loss, particularly in the developing world. Prevalence surveys show that the global burden of illness from CSOM involves 65 – 330 million individual with draining ears. CSOM is uncommon in developed countries. The WHO requires only 2 weeks of otorrhoea.

Once CSOM sets in the change in Microbiology is relevant to the patient and the clinician in order to salvage hearing ability and to prevent the more devastating complications associated with it.

Materials and Methods

Study design	: Descriptive study
Study population	: Patients with CSOM attending outpatient department of ENT, Govt. Medical College, Thiruvananthapuram
Study setting	: Dept. of Microbiology & ENT
Study period	: One year (from March 2013 to February 2014)
Study group	: Age group between 12 years to 60 years

Collection of Specimen

Ear discharge was collected under aseptic precautions. Excess discharge was mopped and the external auditory canal cleaned using sterile normal saline. The specimen was then collected using four sterile cotton swabs. One swab was subjected to microscopic examination by KOH wet mount preparation and Gram staining. Second swab was used for bacterial culture. Third swab for fungal culture and the fourth swab was inoculated into Robertson's cooked meat medium for anaerobic culture. All swabs were processed immediately in the 24 hours clinical microbiology laboratory at Govt. Medical College Hospital, Thiruvananthapuram, Kerala.

Culture media used for bacterial culture are blood agar, Mac conkey agar, Mannitol salt agar, Anaerobic blood agar and Rokertson's cooked meat medium. For fungal culture, the specimen was inoculated into 2 tubes of sabouraud's Dextrose Agar slopes. All the inoculated media for bacterial culture and one tube of SDA were incubated at 37^0 C and the other SDA tube was incubated at room temperature. The incubated plates were then examined at 24 and 48 hours. The specific identification of bacterial pathogen was done based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory procedures. Antibiotic Sensitivity testing of bacterial isolates was done using Kiroy-Baner's disc diffusion technique. Antibiotics were selected depending on the type of organism. Mueller – Hinton agar was used for sensitivity testing. A report of bacteriological examination was given only after 48 hrs of incubation.

Identification of fungal isolates

1. **Aspergillus fumigatus**

Macroscopic colony morphology of the fungus observe – velvety or powdery at first, turning to smoky green. Reverse is white to tan. On LPCB tease mount, septate hypha with flask shaped vesicle, multiseriatephilalides, conidia covering the upper half of the vesicle.

2. **Aspergillus Niger**

Obverse is first white to yellow, then turning dark brown to black. Reverse is colourless to ivory or pale yellow. On LPCB tease mount, septate hypha, globose vesicle with biseriatephialides, jet black conidia covering entire vesicle.

3. **Aspergillus Terreus**

Observe is velvety, cinnamon to buff brown. Reverse is white to brown. On LPCB tease mount, dome shaped vesicle with biseriatephialides and conidia covering only the upper half of vesicle.

4. **Aspergillus Flavus**

Colonies – yellow green, velvety, reverse white. Microscopic examination shows globular vesicle. Phialides in one or two rows. Conidia arise from entire vesicle. Conidia are hyaline.

5. **Pencillium species**

Observe is velvety bluish green in colour. Reserve is yellowish cream. On LPCB tease mount, brush like

appearance. Branching of conidiophore into primary metulae and secondary phialides, from which chains of conidia arise.

6. **Candida albicans and Nonalbicans**

Candida isolates were identified by standard protocols that included germ tube formation chlamydospore production on corn meal agar with Tween 80 and sugar assimilation and sugar fermentation tests. Antifungal susceptibility testing was done by disc diffusion method and MIC using E-test.

Germ tube test

The germ tube test is rapid screening test used for the presumptive identification of candida albicans. Production of germ tubes (GT) within 2 hours of contact with the human / rabbit. Serum is indicative of candida albicans. This test must be confirmed with corn meal agar test.

Method

Candida colonies are inoculated into 1 ml of sterile serum taken in a sterile test tube and incubated at 37^0C for 2 – 3 hrs. After that, a drop of this serum was taken on a clean glass slides and covering was placed on it and examined under high power objective lens for germ tube formation. Germ tube appear as a cylindrical filament originating from the year cell without any constriction at the point of origin and without obvious swelling along the length of the filament.

Chlamydospore Formation

Polysorbate (Tween 80) added to corn meal agar to reduce the surface tension to allow for the development of psudohyphae, hyphae and blastoconidia. Different species of candida develop characteristic morphological features on this medium.

Method

Small portion of yeast colony was taken with a straight wire and the medium was inoculated onto corn meal agar such that the agar was stabbed all the way to the bottom of the plate at an angle of 45^0 (Dalmau inoculation technique). The end of the wire was pushed under the agar and only a small amount of inoculum was used. A sterile covering was placed on the inoculated surface of the agar. (This provided partial anaerobic environment at the margins of the covership). The plate was incubated at 25^0C for upto 3 – 5 days. The petri dish itself was placed on the microscope stage and examined at low power and high power objective. Observations were made for hyphae, pseudokyphae, blastospores and chalmydospores near the margin of the coverslip.

Interpretation

Candida albicans : Elongated pseudohyphal cells with large grape like clusters of blastoconidia along the length of the hyphae. Large, usually single, terminal chlamydospore is characteristic formed most likely near the edge of the covershipchlamydospores are seen as spherical, thick double walled, hyaline structures usually at the terminal end of a hypha.

Candida tropicalis : Abundant branching pseudohyphae radiating with clusters of blastoconidia at the centre. Blastoconidia singly or in very small groups all along graceful, long pseudohyphae are seen.

Candida parapsilosis : Blastoconidia, single or in small clusters are along the pseudohyphae which are crooked or curved in appearance and relatively short. Occasional giant cells, which are large hyphalelements are seen.

Candida glabrata : No pseudohyphae are seen. Small oval yeast cells with terminal budding / short chains of ovoid cells are seen.

Carbohydrate Assimilation Test

Yeasts and yeast like fungi utilize specific carbohydrate substrates alone. Organisms were inoculated onto a carbohydrate free medium and carbohydrate containing filter paper discs were placed. Utilization was determined by the presence of growth around the discs.

Characteristics carbohydrate utilization profiles were used to identify species of yeast.

Method : The test was done on yeast Nitrogen base agar. A suspension of colonies in 0.85%. Saline or distilled water was prepared and turbidity was adjusted to a density equivalent to Mc.Farland No.4. The surface of yeast nitrogen base was covered with the suspension. The various carbohydrate impregnated discs (Hi Media) were placed onto the surface of the agar plate in a well spaced manner (30mm apart) and the plates were incubated at 37^0C for 24 – 48 hrs Growth around individual discs indicated assimilation of that carbohydrate.

Interpretation

Species	Glucose	Maltose	Sucrose	Lacobse	Galactose	Trehalose
Candida albicans	+	+	+	-	+	+
C. tropicalis	+	+	+	-	+	+
C.Parapsilosis	+	-	-	-	-	+
C. glabrata	+	+	+	-	+	+

Carbohydrate Fermentation Test

This is done to test the ability of candida species to ferment a number of carbohydrates by producing acid and gas and hence pink colour in the presence of Andrade's indicator.

Sugar Fermentation test medium

Liquid medium was prepared by dissolving 10 g peptore and 5 gm sodium chloride in 1 litre of distilled water and Andrades indicator of 0.05 ml (PH 7.5) is added. Sterilized at 121^0C / 15 mts.Appripriate sugars at the concentration of 2% (20gms) added to the medium – aseptically poured into small test tubes along with a single sterile Durham's tube inverted into each tube and plugged with cotton.

Method The organism was grown on SDA at 37^0C for 24 hours. Then it was inoculated in test tubes containing 2% carbohydrate solution of dextrose, maltose, sucrose, lacrosse, galacrose and trehalose. These tubes were incubated at 25^0C for 7 days and examined every 48 – 72 hrs interval for the production of acid (pink colour) and gas (Durhams' tube). Fermentation was indicated by production of gas in the tube while only acid production might simply indicate that carbohydrate was assimilated.

Species	Glucose	Maltose	Sucrose	Lacobse	Galactose	Trehalose
Candida albicans	F	F	F	-	F	F
C. tropicalis	F	F	F	-	F	F
C.Parapsilosis	F	-	-	-	-	F
C. glabrata	F	-	-	-	-	-

CHROM Agar

Readymade CHROM agar media in petri plates were obtained from Hi Media. Colonies of the isolates from a fresh 24 – 48 hrs subculture were directly streaked on the plates and incubated at room temperature for 24 – 48 hrs. Colour of the growth of each isolate was noted and compared with the manufacturer's standard.

Species	On chrom agar
Candida albicans	Light green
C. tropicalis	Blue with purple halo
C.Parapsilosis	Cream
C. glabrata	Pink

Identification characteristics of Candida

Isolate	Germ tube formation	Colour on chrom agar	Chlamydospore on CMT
C. albicans	+	Green	+
C. tropicalis	-	Blue	-
C. glabrata	-	Pink	-
C. parapsilosis	-	Cream	-

Antifungal susceptibility testing method for Disk diffusion susceptibility testing of yeasts (M 44-A, CLSI)

Medial used: Mueller – Hinton agar supplemented dye (GMB) was used.

Antifungal discs tested : Amphotericin B (10mg) Fluronazole (25mg) and Itraconazole (8mg)

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, Ba2SO4 suspension with turbidity, equivalent to a 0.5 McFarland standard or its optical equivalent was used. This turbidity standard was prepared by adding 0.5ml of

0.048 mol/L Bacl2 (1.175% W/v Bacl2 H20) to 99.5 ml of 0.18 mol/L H2SO4 (1% v/v) with constant stirring to maintain a suspension. 4 – 6 ml was distributed into screw cap tubes of the same size as those used in growing or diluting the broth culture inoculum.

Procedure for performing the disk diffusion test

Inoculum was prepared by picking four to five distinct colonies of approximately 1mm in diameter from a 24 hour old culture of candida species. Colonies were suspended in 5ml of sterile 0.8% saline. The resulting suspension was vortexed for 15 seconds and its turbidity was matched with 0.5 McFarland standards by adding sufficient saline or more colonies. This procedure produced a semi-confluent growth. Antifungal disks were dispensed onto agar surface of the inoculated agar plate. Each disc was pressed down to ensure its complete contact with the agar surface. They were distributed evenly so that they were no closer than 24mm from centre to centre. Each plate was examined after 20 to 24 hours of incubation. If the plate is satisfactorily streaked and the inoculum is correct, the resulting zone of inhibition will be uniformly circular and there is a semi-confluent lawn of growth. The zone diameter was measured to the nearest whole mm at the point at which there was a prominent reduction in growth. It was read at 48 hours, only when sufficient growth was observed after 24 hrs of incubation.

Reference strams for quality control

To control the precision and accuracy of the results obtained with disc diffusion test procedure two quality control strains were used.

1. Candida parapsilosis (ATCC 22019)
2. Candida albicans (ATCC 9002)

Antifungal drug	Susceptible	Susceptible dose dependent	Resistant
Fluconazole (25mg)	>/- 19mm	15 – 18mm	</- 14mm
Itraconazole (8mg)	>/- 15mm	14 – 10mm	< 10mm
Amphotericin B (10mg)	>/- 15mm	14 – 10mm	< 10mm

Interpretation

For Fluconazole, CLSI recommended zone diameter is used.

For Itraconazole and Amphotericin B zone diameter was interpreted according to manufacturer's standard.

Further, antifungal susceptibility testing was confirmed using MIC detection by E-test Vitek 2 system.

Results

A total no. of 322 patients diagnosed with chronic suppurative otitis media who attended the ENT outpatient department were examined and the samples obtained were processed in the 24 hrs clinical microbiology laboratory at Govt. Medical College, Thiruvananthapuram.

Table 1 : Gender distribution of CSOM

Sex	Culture positive	Culture negative	Total
Female	163 (94.2%)	10 (5.8%)	173 (54%)
Male	143 (95.9%)	6 (4.1%)	149 (46%)
Total	306 (95%)	16 (5%)	322

Table 2 : Age and sex distribution of CSOM

Age group	Number of cases		Total
Female	Male	Female	
0 – 10	2	3	5 (1.55 %)
11 – 20	24	13	37 (11.5%)
21 – 30	49	77	126 (39.1%)
31 – 40	55	61	116 (36%)
40	19	19	38 (11.8%)
	149	173	322

Table 3: Profile of isolates

Pathogen	No. of Isolates
Bacteria	234 (73%)
Fungi	72 (22%)
Total	306

Table 4 :Profile of bacterial isolates

Sl. No	Bacteria isolated	No. of isolates
1	Pseudomonas aeruginosa	131 (56%)
2	Staphylococcus aureus	81 (34.7%)
3	Klebsiella pneumoniaE	9 (3.8%)
4	Escherichia coli	7 (3%)
5	Proteus mirabilis	4 (1.7%)
6	Proteus vulgaris	1 (0.4%)
7	Acinetobacterbaumanii	1 (0.4%)
	Total	234

Table 5 : Profile of fungal isolates

Sl. No.	Fungus isolated	No. of cases
1	Aspergillus Niger	29 (40.3%)
2	Aspergillus Flavus	17 (23.6%)
3	Aspergillus Fumigatus	12 (16.7%)
4	Aspergillus Terreus	1 (1.4%)
5	Pencillium species	3 (4.2%)
6	Candida tropicalis	4 (5.55%)
7	Candida albicans	2 (2.7%)
8	Candida parapsilosis	2 (2.7%)
9	Candida glabrata	2 (2.7%)
	Total	72

Table 6 : Antifungal susceptibility pattern of Candida species

Sl. No.	Isolate	Amphotericin B	Flurocanzole	Itraconazole
1	C.albicans (2)	2 (100%)	2 (100%)	2 (100%)
2	C.tropicalis (4)	4 (100%)	2 (50%)	3 (75%)
3	C. parapsilosis (2)	2 (100%)	1 (50%)	2 (100%)
4	C. glabrata (2)	2 (100%)	0	1 (50%)

Table 7: Complications of CSOM

Sl. No.	Complications	No. of cases
1	Mastoiditis	12
2	Facial nerve palsy	5
3	Temporal lobe abscess	1
4	Post aural fistula	1
	Total	19 (5.9%)

Table 8 : Methods of management (n = 322)

Sl. No.	Management	No. of cases
1	Symptomatic / conservative	285 (88.5%)
2	Tympanoplasty / myringoplasty	15 (4.6%)
3	Cortical mastoidectomy with tympanoplasty	10 (3.1%)
4	Modified Radical Mastoidectomy for atticoantral disease	12 (3.8%)
	Total	322 (100%)

Discussion

The present study was done to know the spectrum of bacterial and fungal aetiologicalagents o chronic suppurative otitis media and the Antibiotic sensitivity pattern of the bacterial isolates and the antifungal sensitivity pattern of the candida species. Bacteria were the more common causative agents of CSOM (73%) and fungi constituted 22% culture was sterile in 5% of cases. A total no. 322 patients with CSOM were studied in details of which 306 (95%) were culture positive. Among the bacterial isolates, pseudomonas aeruginosa was the mnost common organism isolated (56%) followed by staphylococcus aurens (34.7%), Klebsiella pneumonia (3.8%), E-coli (3%), Ratens species (2.1%) and Acinetoblactabaumenii (0.4%).

Among the fungal isolates Aspergillus species were the predominant fungi accounted for 82%. Rest of the 18% werepencillium and candida species. There were 10 isolates of candida species. Non albians are more than the candida albicans. Candida tropicadis was the most common candida nonallocious group causing CSOM (40%). Other species of candida isolated within study wee candida globrata (20%), candida parapsitosis (20%) and candida albicans (20%).

As per the results of the present study 22% of cases of CSOM had fungal aetiology. This is significantly higher than the results of most other reported studies. Fungal aetiology accounted for 7% of cases in a study done in Nigeria (1983). Fungi were isolated in only 89% of cases

of CSOM in a study conducted by Rio de Janeiro. However in a study done in Saudi Arabia, fungi were isolated from 18.5% of cases of CSOM, which is similar to our study.

Out of the 306 culture positive cases of CSOM Aspergillus species were the major pathogens 59 (19.28%) followed by Candida species 10 (32%) and pencillium species 3 (1% only).

UrmilMohan et al in their study on CSOM in punjab observed that Aspergillus species and candida species were the most common fungal isolates causing CSOM.

Talwar et al conducted a study in 1988 observed that Aspergillusniger was the most common fungal pathogen followed by Candida species.

Loy etal in their study on CSOM reported Aspergellisniger and candida species were the most common fungal pathogens.

Studies by Ashok et al showed equal distribution of Aspergillusniger and candida albicans. The major difference seen in the current study was that Non-albicans candida constituted the majority of fungal isolates (80%). Of these candida tropicalis was the most common (40%). Two isolates of C. parapsilosis (20%) and C. glabrata (20%) were also detected. 20% of the isolates were candida albicans.

Candida parapsilosis is an important emerging fungal pattern. It is now the second most common isolated organism after candida albicans in invasive candidiasis. It shows a striking predilection to grow in hyperalimentation solution (TPN) and prosthetic devices as well as indwelling catheters by biofilm formation. C. parapsilor's has shown the largest increase in incidence recently the risk factors for infection include neuropaenia, immunosuppressive therapy, prolonged antibiotic therapy and malignancy.

Clinical spectrum consists of blood stream infections, endocarditis, meningitis, vulvovaginitis and UTI. Treatment is by Amphotericin B or Fluconazole.

In the present study, monomicrobialaetiology was seen in 287 (93.8%) cases and polymicrobialaetiology was seen in 10 (3.3%). It was also observed that combined bacterial and fungal infection was 9 (2.9%). Similar results were observed in many other studies – Taneja Mk etal (1995). AsiriSaad et al (1999), Hiremath SL et al (2001), A.A. Gul et al (2003), Koppad M et al (2005) etc.

In India, multicentric studies investigating antifungal susceptibility pattern are few with many other studies reporting conflicting results. In a recent publication by ARTEMIS, one of the largest and longest running programmes on antifungal drug susceptibility testing based on more than 190,000 isolate collected from 2001 to 2007, it was shown that Fluconazole resistance has to be expected in c. glabrata and few other rare species.

A number of mechanisms of azole resistance have been demonstrated for candida species. Alteration of the target enzyme (cytochrome P 450) catalyzing the 14 – x – demethylase step of ergosterol synthesis) either in structure or quantity has been described. This common made of action by inhibition of the cytochrome P 450 dependent 14 x – sterol demethylase may be an important factor for development of cross resistance. Resistance associated with energy dependent multidrug efflux pumps might also lead to cross – resistance.

Antifungal drug resistance in Candida species continuies to increase in response to the wide spread application of triazole therapeutics among immunosuppressed patients. Many authors have noted a strong correlation in the rise of non albicans candida species with the use of Fluconazole and this was observed in our study also.

Fluconazole resistance was high for c. glabrata and Itracona resistance was higher in a c. glabrata isolates.

Other researchers have also documented high antifungal resistance among non albicans candida species as compared to candida albicans.

Treatment and Outcome.

All patients with CSOM were treated with aural toileting with acetic acid and topical amikacin or ciprofloxacin with 88.5% cure rate. In longstanding cases, oral antibiotics like ciprofloxacin amoxicillin + clavulanic acid were given based on the antibiotic sensitivity patterns. Patients who were culture positive with MRSA isolates were treated with oral Linezolid. A total of 37 (11.5%) cases of CSOM were treated with definitive surgery. 15 patients (4.6^) underwent tympanoplasty / myringoplasy. 19 patients (5.9%) developed complications like mastoiditis, temporal lobe abscess and facial nerve palsy, which were managed accordingly. No case fatalities were reported. Radical mastoidectomy for atticoantral disease in 12 patiets (3.8%), cortical mastoidectomy with tympanopalasty was done in 10 patients (3.1%).

Conclusion

Being a disease that is associated with significant morbidity if untreated, CSOM requires diligent laboratory evaluation with comprehensive antibiotic sensitivity testing. The incidence of pure fungal and continued bacterial and fungal growth in our study, only highlights the need for a fungal culture in all cases referred for isolation of organisms in CSOM. When treated adequately the complication rates of CSOM are very low and almost nil in cases of tubotympanic disease.

Conflicts of interest

There are no conflicts of interest

The authors are greatful to Dr.Mohan J, Professor and Head of Dept. of ENT, Govt. Medical College, Thiruvananthapuram for granting permission to collect necessary samples from cases of CSOM attending the outpatient department and for providing necessary help to carry out the study.

Financial help and sponsorship: Nil

References

[1]. Pfaller MA, Diekema J, Gibbs L et al and the global antifungal surveillance group. Results from the ARTEMIS disc global antifungal surveillance study, 1997 to 2007, analysis of susceptibilities of candida species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion method. Journal of clinical Microbiology 2010 : 48 (4) : 1366 – 1367

[2]. Sanglard D, Kuchlea K, IscherFet et al Mechanisms of resistance to azole antifungal agents in candida albicans. Antimicrobial agents and chemotherapy 1995 : 39 (11): 2378 – 2386.

[3]. P. Talwar, Fungal infections of the ear with special reference to chronic suppurative otitis media. Mycopathologia1988 : 104 (1) : 47 – 50.

[4]. Poorcy VK, AratiIyer, study of bacterial flora and its clinical significance. Indian journal of otolaryngology and Head and neck surgery 2002, 54 (2): 91-95

[5]. Odds FC and Bernaerts R. CHROM agar, a new differential isolation medium for presumptive identification of candida species Journal of clinical Microbiology. 1994 : 32 (8) : 1923 – 1928.

[6]. JagadishChander, Text book of Medical Mycology, 3rd Edition 2012 : 266-290

[7]. National committee for clinical and laboratory standard performance standads for Antinicrobial Disc susceptibility testing. NCCLS document 7th Edition 2000; 20(1) : M2-A7.

[8]. National committee for clinical and laboratory standard performance.l Standards for sutimicrobial disc susceptibility testing, 15th Informational supplement (M 100 – 515) 2005, NCCLS, Wayne PA.

[9]. Rippon JW. Medical Mycology : Pathogenic fungi, WB Saunders, 1988 : 3rd edition.

[10]. Capoor MR, Nair D, Deb M et al. Emergence of Non-albicans candida and antifungal resistance in a tertiary care hospital. Japanese journal of infectious Diseases 2005, 58 (6) : 344 – 348.

[11]. VandenBossche H, Marichal P, OddsFcetal, Characterisation of an azole resistant candida glabrata isolate. Antimicrobial agents and chemotherapy 1992; 36 (12); 2602 – 2610.

[12]. Ballal M, Shivaranda PG et al. A bacteriological and mycological study of chronic suppurative otitis media. Indian journal of otolaryngology and Head and Neck surgery. 1992; 1 (i); 10 – 13.