TAJ December 2023; Volume 36 Number-2

ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

Fungal Agents Causing Chronic Otitis Externa with Their Antimicrobial Susceptibility Pattern at RMCH.

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Abstract

Background: Otomycosis is a well prevalent disease (9-30%) in our country which even might persist for more than 3 months (chronic cases). Now a days, development of antimicrobial drug resistant making difficult to treat such patients all over the world.

Objective: To identify fungal agents causing chronic otitis externa with their antimicrobial susceptibility in Rajshahi Medical College Hospital.

Material and Method: This cross-sectional descriptive study was conducted from January 2022 to December 2022 in the department of Microbiology at Rajshahi Medical College, Rajshahi. About 110 suspected chronic otitis externa patients were selected from outpatient department of ENT of RMCH & data were collected by a semi structured questionnaire. Fungal organisms isolated and identified by culture, LPCB stain and relevant tests including a specific Czapek Dox Agar media. Antimicrobial susceptibility was done by modified Kirby Bauer disc diffusion method. Data were analyzed using SPSS (version 24.0). Statistical significance was considered when p value < 0.05.

Result: In this study, near half (48.18%) aural swab from respondent of chronic otomycosis were culture positive for different fungal species. Among all, *A. niger* was most common species (50.9%) & 1/5th of them was *C. albicans* (20.8%) along with other species. Most sensitive drug for different fungal species were voriconazole &itraconazole and most resistant drug were fluconazole and ketoconazole.

Conclusion: As common antifungal drugs are resistant to different fungal species & their susceptibility pattern are changes day by day, among otomycosis patients the fungal culture & antimicrobial susceptibility should be done to reduce the morbidity of such patients as well as to reduce the generation of new resistance to antifungal drugs.

Key words: Antimicrobial susceptibility, Chronic otitis externa. Czapek Dox Agar.

TAJ 2023; 36: No-2: 185-192

Introduction

Otitis externa is an infection of the external auditory canal is similar to infection of skin and soft tissue elsewhere.[1] Higher temperature and humidity resulting higher incidence of chronic otitis externa in tropical zones. Risk factors included frequent swimming, insertion of foreign

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objects, cotton swabs, hearing aid, instillation of mustard oil.[2] Bacterial infections, topical use of antibiotics or steroids, immunodeficiency, ear surgery, diabetes mellitus, tympanic membrane perforation, and seborrheic dermatitis also involved.[3] All these resulting loss of the protective coating of cerumen of ear.[4] The global burden of Chronic superficial fungal infection of the external auditory canal also known as otomycosis. Prevalence of otomycosis is found to be between 9% to 30%.5 The higher numbers of patients fall within the lower- and middle-income family groups (up to 2,500 and 3,501–4,000 taka per month, respectively). Most patients were laborers, and most patients were illiterate. The majority lived semi-paka house and used tap water for bathing (53.5%). Most patients used cotton buds (49%) for cleaning of ears.6 Etiological agents vary among country to country & places to places. In the developing countries *Aspergillus spp.* are the predominant fungi among total fungal isolates; more frequently isolated fungi A. *niger* complex followed by *A. flavus*and A. *fumigatus*, *Candida albicans*, *Penicillium*, *Mucor* and *Trichophyton spp.*[7]A changing pattern of antimicrobial resistance is a growing global problem. Resistant for caspofungin, miconazole and econazole also for fluconazole, itraconazole and voriconazole has been reported for otomycosis.[8] This study result will help to develop antibiogram policy of chronic otitis externa of patients of Rajshahi medical college hospital which cover a big catchment area population of 10 district's around Rajshahi. So, this study was designed to isolate and identify fungal agents of chronic otitis externa with their antimicrobial susceptibility pattern to prevent antimicrobial resistance.

Materials and Methods

This cross-sectional descriptive study was conducted from January 2022 to December 2022 in the department of Microbiology in Rajshahi Medical College, Rajshahi. In this study, 110 clinically suspected chronic otitis externa patients were enrolled on the basis of inclusion criteria. Purposive sampling technique was adopted in this study. Data were collected after taking an informed consent form from the respondents by face-to-face interview on variables of interest with the help of semi-structured questionnaire. Aural swabs were collected by aseptic precaution. All the collected specimens were inoculated into Sabourauds Chloramphenicol Agar media with supplement of Gentamycin in screw capped test tube & Czapek Dox Agar media. The culture media were incubated aerobically at 35℃ for 24-48 hrs. About a period of 4 weeks of incubation of the culture media examined for presence of colonies every 3-4 days and declared negative. All the sample were collected on a sterile slide. Wet mount preparation, KOH (10-20%) staining, Gram staining and Lactophenol cotton blue staining were performed for fungal identification. Germ tube test was done for *candida albicans*. Identified fungus were sub cultured and preserved in distilled water for further use. Antimicrobial susceptibility was done by modified Kirby Bauer disc diffusion method by using Mueller-Hinton agar media with 2% glucose and 0.5 µg/ml methylene blue and commercially available antibiotic disc. After editing data were analyzed by using SPSS (version 24.0). Statistical significance was considered when p value < 0.05.

Results

Out of 110 respondents of aural swab showed near half (48.18%) were culture positive and remaining were culture negative. Among 53 culture positive cases, just above half (50.9%) was Aspergillus *niger* and about 1/5th was (*20.8*%) *Candida albicans*. Regarding *A. niger* highest sensitivity to itraconazole (92.60%) followed by voriconazole (88.80%), caspofungin (85.1%) and highest resistant to ketoconazole (88.89%) followed by fluconazole (66.67%). All *A. fumigatus* mostly sensitive to voriconazole (100%) followed by itraconazole & nystatin in equal proportion (85.71%) for each but proportionately more resistant to ketoconazole (71.43%) &>1/2 of them are resistant to amphotericin b & fluconazole in similar

proportion (57.17%) for each. *A. flavus* mostly sensitive to voriconazole, itraconazole, caspofungin (100%, 80%.80%) respectively but resistant to ketoconazole, fluconazole, amphotericin b (80%, 60%, 60%) respectively. Regarding *Candida albicans* voriconazole, caspofungin, nystatin, itraconazole were most sensitive drug (90.9%, 90.9%, 81.8%, 81.8%) respectively & most resistant fluconazole (63.63%) followed by amphotericin b & ketoconazole (54.5%). All *Trichophyton spp*. were sensitive to itraconazole & voriconazole (100%) for each & all were resistant to ketoconazole, fluconazole & caspofungin (100 %) for each. Regarding *Mucor spp.* caspofungin, voriconazole, itraconazole are 100% sensitive & amphotericin b, ketoconazole, fluconazole, nystatin 100% resistant.

Table 1: **Distribution of respondents according to their culture results (N=110)**.

Result of culture	Frequency	Percentage
Culture positive	53	48.18%
Culture negative	57	51.81%

Table 2: Culture positive fungus isolated by lactophenol cotton blue mount microscopy in chronic otitis externa cases (n=53).

Organism	Species	Number	Percentage (%)
Fungus (n=53)	*Aspergillus niger*	27	50.9%
	Candida albicans	11	20.8%
	Aspergillus fumigatus	07	13.2%
	Aspergillus flavus	05	9.4%
	Trichophyton spp.	02	3.8%
	Mucor spp.	01	1.9%

Figure I: Antimicrobial susceptibility pattern of *Aspergillus Niger* (n=27)

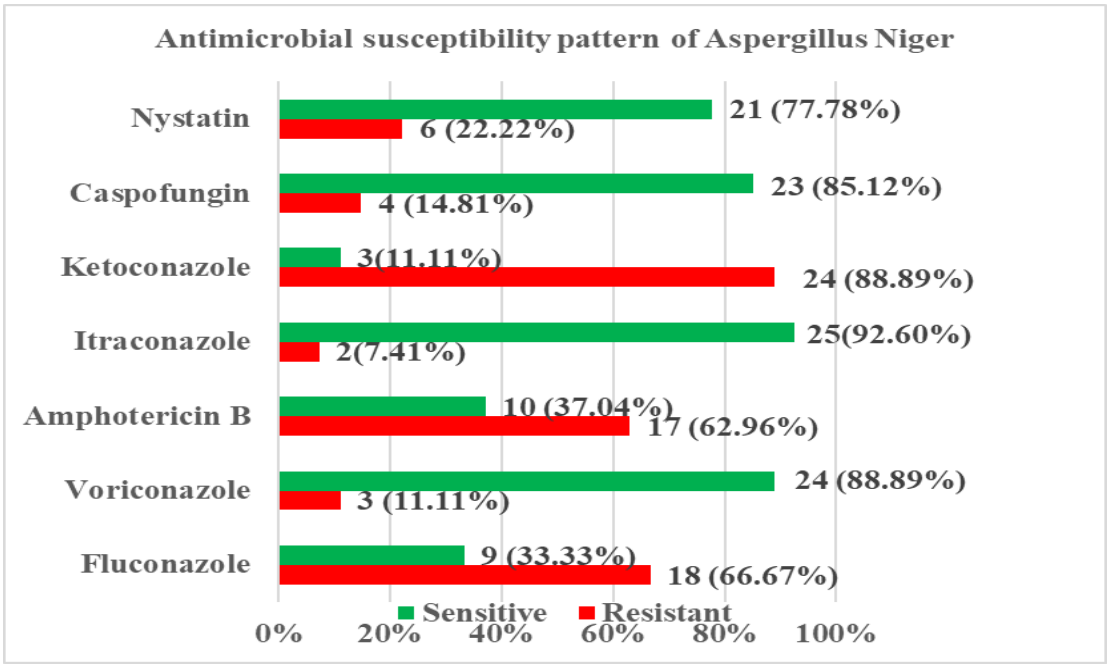


Figure II: Antimicrobial susceptibility pattern of *Aspergillus fumigatus* (n=7)

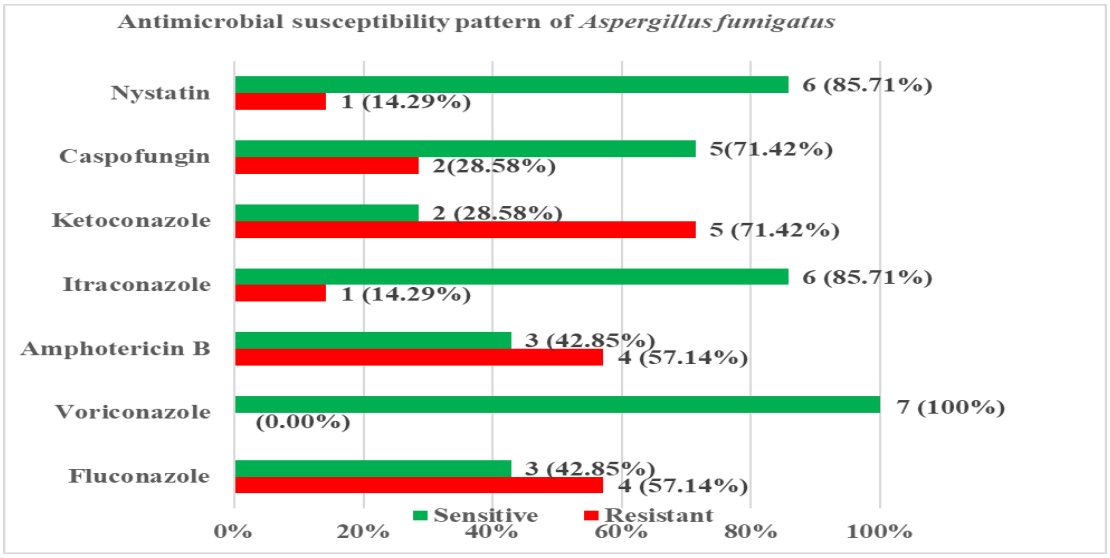


Figure III: Antimicrobial susceptibility pattern of *Aspergillus flavus* (n=5)

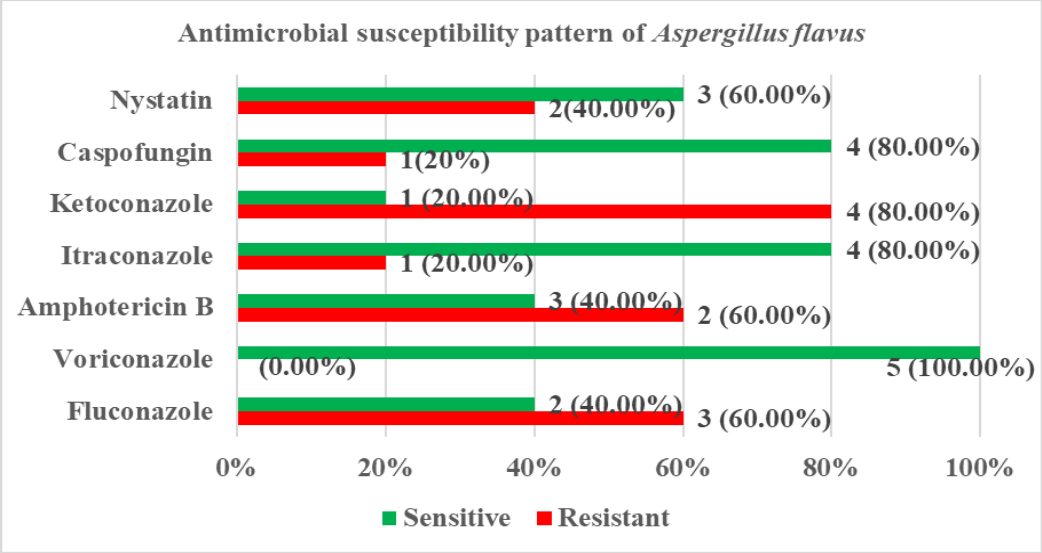


Figure IV: Antimicrobial susceptibility pattern of *C.albicans* (n=11)

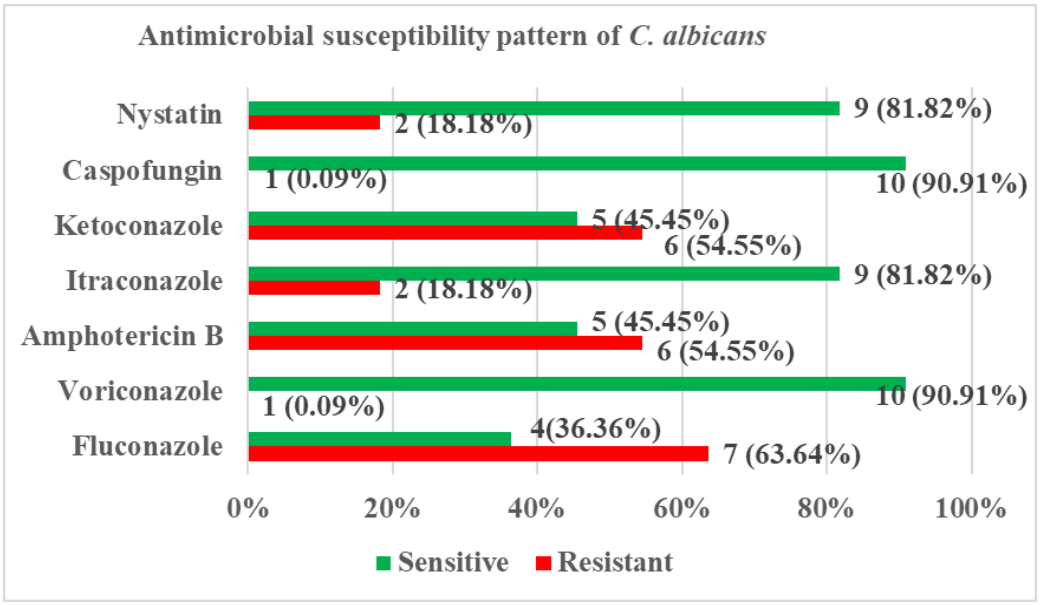


Figure V: Antimicrobial susceptibility pattern of *Trichophyton spp.*

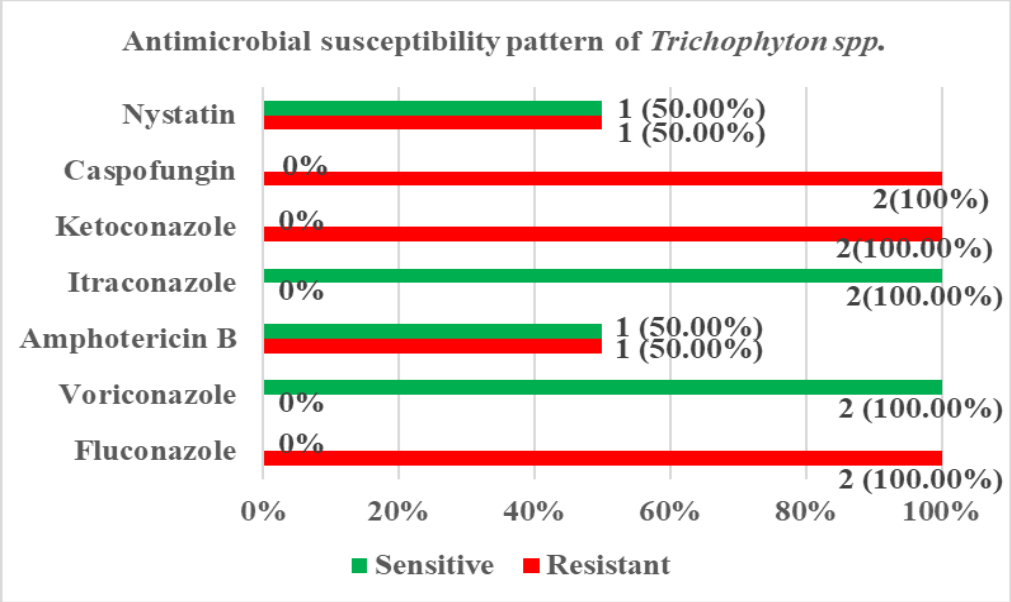
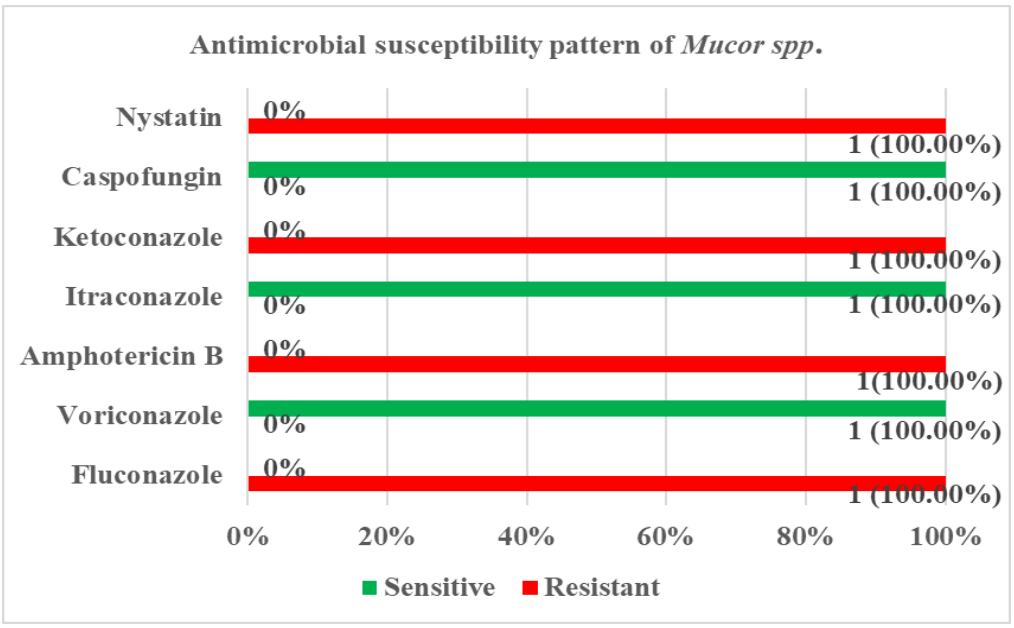


Figure VI: Antimicrobial susceptibility pattern of *mucor spp.* (n=1)



Discussion

Table 4 showed, among 53 culture and lactophenol cotton blue positive fungus, *Aspergillus niger* was the predominant fungus 27 (50.9%). Followed by *Candida albicans* 11 (20.8%), *A. fumigatus* 07(13.2%), *A. flavus* 05 (9.4%), *Trichophyton* 02 (3.8%), *Mucor* 01 (1.9 %). which is comparable with the study of Nipa *et al* in Bangladesh (2020),

A. niger (38%) was the most common followed by *A. fumigatus* (27%) and *A. flavus* (15%) &Agarwal *et al* in India (2017) *A. niger* 68.2% followed by *A. flavus* 19.9% and *A. fumigatus* 11.9%. Other fungiisolated were *Candida spp*. 10%, *Mucor* 0.58% and *Trichophyton mentagrophyte* 0.58% and Prasad *et al* in India (2014) *A. niger* was the commonest (38%) followed by *A. fumigatus* (27%) and *A. flavus* (15%). *Candida albicans* (4%), *Rhizopus* spp. (1%), and *Chrysosporium* spp. (1%) were the other fungi isolated.[7,9,10] But Pontes *et al*., (2009). in Brazil, found the most frequently isolated species were *C. albicans* (30%), *C. parapsilosis* (20%), *A. niger* (20%), *A. flavus* (10%), *A. fumigatus* (5%), C. tropicalis (5%), *Trichosporonasahii* (5%) and *Scedosporiumspp.* (5%).[11] This variation depending on geographical area, social, cultural, environmental and occupational factors.[12] Various studies reveal that distribution of fungi differs from temperate to tropical climate.[7]

In this study, seven antifungal drugs named fluconazole, ketoconazole, voriconazole, itraconazole, nystatin, amphotericin b, caspofungin were tested by disc diffusion method against 48 isolates of fungus. Antifungal test results revealed that voriconazole and itraconazole were the most effective antifungal drug followed by nystatin and caspofungin. ketoconazole and fluconazole and amphotericin b had the poorest activity. This study is comparable with Sathi*et al* (2022) in Bangladesh showed 95% sensitive to voriconazole and 67% resistant to fluconazole and Oparaodu *et al*. (2022) in Nigeria showed that highest

sensitivity to voriconazole (76.9%) and highest resistance was to fluconazole (84.6%) and nystatin

(53.9%) for yeast and highest sensitivity to voriconazole (66.7%) and 100% resistance to fluconazole for mold.[13,14] This study differ in resistant pattern with for mold showed nystatin (100%) resistant. ketoconazole, fluconazole, amphotericin b showed highest resistance due to its low cost and its widespread availability in all levels of health care centers which in turns turned into increase drug resistance.[15] In this study, isolation and identification of the causative fungus along with their antimicrobial susceptibility test was done. So, clinicians should follow the antimicrobial susceptibility test results to choose the appropriate drug for treatment of this type of chronic disease.

Conclusion

In this study *Aspergillus niger* followed by *C. albicans* were the predominant fungus. voriconazole, itraconazole were found effective against fungus. Knowledge of causative microorganism and their antimicrobial susceptibility pattern is essential, so that early and effective therapeutic measure can be initiated for better patient outcome. Antifungal susceptible is valuable also for the development of novel antifungal agents.

Conflict of interest: None declared

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