MOLECULAR IDENTIFICATION AND SPECIATION OF MALASSEZIA AND ITS SUSCEPTIBILITY PATTERN

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INTRODUCTION

Malassezia, a lipophilic yeast like fungus is a natural habitat of stratum corneum and has been implicated in pathogenesis of variety of opportunistic dermatological and systemic infections. But studies showed most patients did not respond to treatment and presented with recurrence of infection

Considering the importance of *Malassezia* in medical mycology, this study was performed to identify the various *Malassezia* species affecting the population under study.

OBJECTIVES

- Epidemiology - To know the age, sex and site of prevalence of *Malassezia* infection.
- Anti-fungal susceptibility -To identify the most effective anti-fungal drugs to treat infections caused by *Malassezia* and to obtain their MIC (minimum inhibitory concentration) values.
- Molecular study -To do DNA extraction, Polymerase chain reaction (PCR) and sequence the extracted genome, thereby identifying and speciation of *Malassezia*.

MATERIALS AND METHODS

Malassezia species were isolated from the skin scrapping's of the patients with hypo/hyper pigmented lesions in the Dermatology OPD of our tertiary care hospital for a period of two years from July 2016 to Feb 2018. Microscopic Examination with 10% KOH was done (fig1). KOH positive samples were cultured on Modified Dixons agar (MDA) and Sabouraud's Dextrose Agar (SDA) with olive oil overlay (fig 3,4) and incubated at 32°C. Gram stain (fig2) Catalase and Urease test was performed. Stock cultures were prepared and refrigerated.

fig 1

fig 2

fig 3

fig 4

Anti-fungal susceptibility- was done by broth Micro- dilution method in accordance with CLSI M27-A3 guidelines. Since *Malassezia* are lipid dependant yeast, To RPMI 40 ADD :-

GLUCOSE-1.8% PEPTONE-1% OX BILE-0.5% GLYCEROL-1%
TWEEN 80-0.05% TWEEN 40-0.5% CHLORAMPHENICOL-250mg/L

Stock inoculum was prepared. Anti-fungal stock solutions of drugs were prepared and stored at -70°C. Micro titre plates with 96 wells were incubated for 4 days at 32°C. Growth and sterility control wells were included in each test.

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MIC values to anti-fungal drugs - Fluconazole, Miconazole, Clotrimazole, Sertaconazole and Lulliconazole were read as MIC₅₀ and MIC₉₀ (where 50% and 90% inhibition of growth was seen).

Molecular study-

DNA extraction was done by phenol: chloroform method and stored at -20°C until used as a template for amplification.

DNA amplification

PAN FUNGAL PCR (TABLE 1)

Pan fungal PCR was done on 20 samples using ITS -1 and ITS-4 primers

ITS-1 5' TCCGTAGGTGAACCTGAGG3'

ITS-4 5' TCCTCCGCTTATTGATATGC3'

MALASSEZIA SPECIFIC NESTED PRIMER (TABLE 2)

Pan fungal PCR positive samples were subjected to species specific nested PCR using *Malassezia* specific primers

ITSIF-N 5' GGATCATTAGTGATTGCCTTTATA 3' and

ITS4-R 5' TCCTCCGCTTATTGATATG 3'

REACTION(35 cycles)	TEMPERATURE	TIME
INITIAL DENATURATION	94°C	10 MIN
DENATURATION	94°C	30 SEC
ANNEALING	51°C	45 SEC
EXTENTION	72°C	1 MIN
FINAL EXTENTION	72°C	10 MIN

Table 1

REACTION(35 cycles)	TEMPERATURE	TIME
INITIAL DENATURATION	94°C	3 MIN
DENATURATION	94°C	1 MIN
ANNEALING	57°C	1MIN
EXTENTION	72°C	50 SEC
FINAL EXTENTION	72°C	10 MIN

Table 2

Sequencing -Sanger's sequencing was done

Blasting-NCBI blast was done

RESULTS

Epidemiological result

Site of predilection

Sex predominance

Age distribution

Anti-fungal susceptibility and molecular study results

DRUG	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
FLUCONAZOLE	8-32	16->64
MICONAZOLE	2-4	4-16
CLOTRIMAZOLE	0.125-2	0.5-4
SERTACONAZOLE	4-16	8->16
LULLICONAZOLE	1-4	2-8

CONCLUSION

The commonest species causing infections was *MALASSEZIA sympodialis*, *M.furfur*, *M.globosa* and *M.obtusa*. Clotrimazole showed the lowest MIC values followed by Lulliconazole, Miconazole and Sertaconazole. Fluconazole showed comparatively higher MIC values.

Speciation and anti-fungal susceptibility is very important to prevent the chronicity in treatment and have a good patient compliance.

REFERENCE

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