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 scraping’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 (fig 1). 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 (fig 2) Catalase and Urease test was performed. Stock cultures were prepared and refrigerated.

Anti-fungal susceptibility - was done by broth Micro-dilution method in accordance with CLSI M27-A3 guidelines. Since Malassezia are lipid dependent 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 tissue plates with 96 wells were incubated for 4 days at 32°C. Growth and sterility control wells were included in each test.

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‘ TCCGTAGGTGAACTCGAGG3’
ITS-4 5‘ TCCCGGTTATGATGTC3’

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‘ GGATCATTAGTATGAGC1CCCTATA 3’ and ITS4-R 5‘ TCCCGGTTATGATGTC 3’

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

<table>
<thead>
<tr>
<th>DRUG</th>
<th>MIC50 (µg)</th>
<th>MIC90 (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>8-32</td>
<td>16-64</td>
</tr>
<tr>
<td>Miconazole</td>
<td>2-4</td>
<td>4-16</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>0.125-2</td>
<td>0.5-4</td>
</tr>
<tr>
<td>Sertaconazole</td>
<td>4-16</td>
<td>8-36</td>
</tr>
<tr>
<td>Lulliconazole</td>
<td>1-4</td>
<td>2-8</td>
</tr>
</tbody>
</table>

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.