4.1. INTRODUCTION

Antimicrobial resistance is a matter of serious concern, because of the drug resistance developed microbes. The effect of ‘miracle drug’- antibiotics is fading away. Many pathogenic bacteria and fungi have developed multi drug resistance. It is reported that India has become hot spot of (Multi Drug Resistant) MDR TB transmission and 30 percent of world’s TB patients are in India. (The Hindu, Nov 6, 2016). In 2050- ten million death expected due to antimicrobial drug resistant bacteria and fungi (WHO, 2016 Bulletin). A US report says in the year 2013, 2,049,442 cases of illness and 23,000 deaths are reported in USA due to antimicrobial drug resistance (www.cdc.gov/drug resistance/threat - report 2013).

Like pathogenic bacteria fungi have also developed drug resistance. Drug resistant Candida sp has been reported and it was found to have mutant gene for ergosterol synthesis (Rajapandian, Ph D thesis, MS University, 2014) Candida albicans with multi drug resistance was reported to co exist in TB patients studied in Chennai. Hence in the present study, an attempt was made to find out whether Candida albicans isolated from patients staying in the hospital for a long time for urinary tract infection and also from the fungal isolates collected from other public utility devices or things, also the gene sequence of drug resistant fungus was made and it was compared with non drug resistant, similarly fungal sequence catalogued in NCBI.

Resistance to antibiotics in pathogenic fungi is a problem of special importance in the control of infections caused by these organisms. The same extraordinary
conservation of the basic eukaryotic cellular biology exhibited by fungal and animal cells that has allowed these smaller eukaryotes to serve as outstanding model organism limits the range of fungus-specific antibiotics that have been described. In addition, mutant fungi are readily isolated, both in the laboratory and in the clinic, that demonstrate resistance to a wide range of antibiotics beyond that initially used for treatment. This broad-spectrum drug tolerance is referred to as multidrug resistance and occurs in organisms ranging from bacteria to humans. The limited number of antifungal drugs makes this phenotype an acute problem in the chemotherapeutic eradication of fungal infections. (Kailash et al., 2011). The common fungal infections are mostly cutaneous. Several species of fungi are significant pathogens of human and other animals. In nature fungi generally grow by secreting enzymes that digest tissues but some are actually predacious. Hence it is decided to find out the antifungal resistance nature I the fungal strains isolated in the present study. The outcome of the study will help to find out the drug resistant pattern so as to develop new effective drugs and to initiate the steps to be taken to control the rising drug resistance in fungal strain (Kailash et al., 2011).

### 4.2. SAMPLE COLLECTION AREA

For the isolation of fungal strains for human environment, samples were collected in the month of July - August 2013. Samples were taken from 3 different sources, 1. Public utility devices and things in Railway station which includes train seat, window, and toilet. Also from waiting hall of bus stand, bus handle, ATM machine, public telephone, computer key buttons, hotel juice cup, 5, 50, and 1000 rupee notes, currency coin. 2. The samples were collected from hospital area like hospital floor, bed, window, toilet, waiting hall, and wall. 3. The samples were also taken from disease infected patients. To isolate the drug resistant fungi, saliva, skin scrapings, sputum,
urine samples were collected from patients from hospitals located in Tenkasi, Kadayanalloor, Alwarkurichi, and Tirunelveli.

4.3 ISOLATION OF FUNGAL STRAINS

4.3.a) Material and methods

For collection of samples (Hi-media) tubes attached with sterile swabs were used. The swabs were taken from the public places mentioned earlier and introduced into yeast peptone water. Clinical samples were collected from patients with urinary tract infections, skin infections, tooth decay, respiratory tract infections, and eye infections.

For wound infection – swabs was taken from the infected portion of diabetic wound. Swabs were also taken from skin, for tooth decay, swabs were taken from infected tooth. For respiratory tract infection, first sputum of the morning was collected. Also urine samples, and a vaginal swabs were collected and diluted up to $10^{-5}$ and antibiotic resistance was tested using Kirby-Bauer Test. Those clinical samples were collected from diagnosed patients with their permission.

The samples collected from different locations were inoculated directly on to the surface of solid media such as saboured dextrose agar (SDA) and potato dextrose agar (PDA). All inoculated media were incubated aerobically at $27^\circ$ C and examined daily. From the colony grown on culture plates, samples were taken for identification of the strain. Agar slants were prepared to store the culture for identification.

4.4 IDENTIFICATION OF RESISTANT FUNGAL STRAINS

4.4.a Morphology

Among the 16 colonies obtained, 10 perfect fungal pathogens were choosen for further analysis. The 10 fungal pathogens separated were tested for basic morphological
testing called Bromophenol cotton blue staining technique. (Bergey’s manual) and examined through high power microscope.

Identification of fungal species were carried out by observing the development of the colonies, and their groups and microscopic characteristic features. The surface, colour, and growth rate of fungal colonies along with the pigmentation of the reverse side of the colonies were also considered for identification. Fungus grown on primary isolation media were subcultured in SDA and PDA agar and identification was carried out based on their microscopic features.

4.5 ISOLATION OF RESISTANT FUNGAL STRAINS

4.5.a Material and Methods

The strains were tested for antifungal resistance. The isolated fungi were tested for their susceptibility against the following drugs such as Fluconazole (FLC), Metrinozole (MT), Doxycycline (DX), Amphoterin (AMP), and Cycloheximide (C). The susceptibility to antifungals were evaluated with the Kirby-Bauer disc diffusion method, and interpreted according to the guideline established by the (NCCLS, 2000) National Committe on Clinical Laboratory Standards.

The swabs were immediately transported to the laboratory after collection. During the transportation the swabs were maintained in peptone water. After transportation the swabs were inoculated into SDA Saboured Dextrose Agar plates and were kept for incubation in room temperature for a week. After 5-7 days the colonies were isolated. The separated colonies were noted and stored in PDA slants (Bergey’s manual – Holt 1994, Konemann, 1998). For antibiotic sensitivity analysis antimycotic sensitivity agar was used. 5 antibiotic disc were used are Fluconozole, Metrinozole, Doxycycline, Amphoterin, and Cycloheximide.
4.6 RESULT AND DISCUSSION

4.6.1 Presence of different types of fungal strains in public utility places

Prevalence of fungi

Table 4.6.2 Percentage of prevalence of fungal species in different places

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Percentage of prevalence of fungal species in different places</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Public places</td>
</tr>
<tr>
<td>Candida sp</td>
<td>46.11*</td>
</tr>
<tr>
<td>Aspergillus sp</td>
<td>74.17*</td>
</tr>
<tr>
<td>Pencillium sp</td>
<td>32.64*</td>
</tr>
<tr>
<td>Cryptococcus neoformis</td>
<td>18.14*</td>
</tr>
<tr>
<td>Trichophyton sp</td>
<td>-</td>
</tr>
</tbody>
</table>

*statistically significant at < 0.05 level
**Candida sp.**

The sensitivity of *Candida sp* collected from the urinary tract infected cases was tested against five different types of antifungal agents. The *Candida* isolates were found to be highly sensitive to Doxycycline and less sensitive to Fluconazole. But for other drugs like Metrinozole, Amphotericin, and Cyclohexidine the *Candida* isolates showed resistance pattern. This indicates how the *Candida sp* are slowly became drug resistant.

*Candida* species were also isolated from the skin infected cases and from the surface of beds in hospitals. The *Candida sp* isolates from skin showed resistance to all drugs except Amphoterin. However the *Candida sp* isolated from the beds of hospital showed a higher degree of resistance to all the drugs tested.

The study clearly indicates that the most dreadful *Candida sp* is in the process of over winning the power of all drugs available today.

**Aspergillus sp**

The fungi *Aspergillus sp* were isolated from the toilets of hospitals, handles in public transport system. Buses, the surface of seats in train and from the surface of keybutons in ATM vendors. *Aspergillus sp* isolated from all the devices showed high degree of resistance to all the drugs tested. Doxycycline is alone able to inhibit the growth of *Aspergillus sp* to a small extent. The drug resistance in *Aspergillus sp* will create medical a lot of medical problems and intiate the necessity to develop new generation drugs.

**Cryptococcus neoformis**

*Cryptococcus neoformis* collected from the frames of windows in hospital also showed resistance to all the drugs tested. Only an insignificant level of sensitivity was noticed for Metrinozole exposure.
Penicillium sp

Penicillium sp was isolated from the rim of cups used to drunk juices in juice centre with less hygienic condition. Penicillium sp was found to show resistace to all the drugs tested. However a little sensitivity was observed for Cycloheximide.

Trichophyton rubrum

Trichophyton rubrum a dangerous fungal pathogen were isolated from patients with urinary infection, skin infection, and also from hospital toilets, hospital beds, and other public places. Antifungals like Fluconozole, Metrinozole, Doxycycline, Amphoterin, and Cycloheximide were used. The pathogen showed resistance to Metrinozole, Doxycycline and Cycloheximide. However the little inhibition was seen for Fluconozole, and Amphoterin.

Conclusion

Five types of fungal strain such as Candida sp, Aspergillus sp, Cryptococcus sp, Penicillium sp, Trichophyton rubrum were found repeatedly in the isolation. Candida sp, Aspergillus sp was isolated dominantly from the samples taken from all the environment. But Trichophyton rubrum was isolated from only the skin infection. Similarly Cryptococcus sp and Penicillium sp were isolated from hospitals and in tumblers used for drinking juices respectively. Rajapandian, (2012) had isolated drug resistant fungi, Trichophyton rubrum from clinical samples. Starvo et al., (2010) also reported Trichophyton rubrum to develop drug resistance. Nweze and Emeka et al., (2010) also reported an increase in resistance to drug in many fungal strain.