JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2017. Vol. **11**(1), p. 15-21

The Effect of Some Antiseptics on Molds and Yeasts Isolated from Wards in Al-Diwaniya Teaching Hospital, Iraq

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http://dx.doi.org/10.22207/JPAM.11.1.03

(Received: 09 December 2016; accepted: 11 January 2017)

The study involved 120 samples collected from wards of the Al-Diwaniya Teaching Hospital, Iraq during the period from 5 April to 5 June 2016. The results showed that the percentage of the fungal contamination in the wards was 77/120 (64.1%), highest of the fungal contamination was recorded in the floors and walls and in the air 18(90%), followed by patients (Nose, Ear and Hands) 14(70%) and lowest contamination was in the instruments 3(15%) when compared. A total of 125 fungal isolates were identified and the isolates belong to 9 genera comprising of *Aspergillus* spp with 52 isolates (mainly *A. niger*= 24, *A. flavus*= 19, *A. fumigates*= 5 and *A. ochraceus*= 4). *Penicillium* spp with 17 isolates, *Alternaria alternata* with 14 isolates, *Candida albicans* with 11 isolates, *Fusarium* spp 9 isolates, *Mucor* spp 7 isolates, *Rhizopus stolonifer* 7 isolates, *Cryptococcus* spp 5 isolates and *Rhodotorula* spp 3 isolates. The highest occurrence and frequency percentages of fungi recorded for *A. niger* reaching (18.947%) and (19.2%) respectively, followed by *A. flavus* reaching (16.842%) and (15.2%) respectively with significant differences when compared with other fungi isolated in this study, while the lowest occurrence and frequency percentages recorded for *Rhodotorula* spp reaching (2.105%) and (2.4%) respectively. The antiseptics (Dettol, Hibitane and Formalin) have high inhibitory activity against the growth of all fungi studied when used at original concentration which was 10% compared with other concentrations 7.5, 5 and 2.5%. Formalin showed highly significant effect in inhibitory activity for the growth of fungi from Dettol and Hibitane, the percentage of inhibition when using Formalin at concentration 10% ranged between (85.2-95.4%) while for Hibitane between (68.1-79.7%) and for Dettol between (40.6-65.8%) at the same concentration.

Keywords: Antiseptics; Molds; Yeasts.

Various microorganisms found in hospitals, molds and yeasts are considered the most important of it because it play a major role in making various infections to the inpatients in hospitals and these infections may be dangerous systemic infections especially for some people who suffer from Immunocompromised or who are taking Immunosuppressive drugs[1]. Some opportunistic fungal infections that can occurs in people who do not have any factors help infection and this is what happens when inhaling very large number of spores of various fungi, which are small in size, making it easier to enter and adhesion within the various tissues of the body[2,3]. In hospitals the infection occurs with various molds and yeasts either directly from one person to another or indirectly by its transition from the air and other instruments[4]. Air play a major role in the spread of various pathogens in hospitals is considered major factor in making contamination of the wards and halls of various surgery as it contains various germs

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on the dust[5]. The most common fungi in indoor environments for hospitals are saprophytic molds and yeasts, such as some species of *Aspergillus* spp, *Penicillium* spp, *Cladosporium* spp and *Candida* spp[6,7]. Antiseptics are considered one of the most important materials used to reduce the number of pathogens and remove them from various materials and instruments to prevent the transmission and spread of diseases, the disinfection process is affected by various factors such as germs concentration, antiseptics concentration, disinfection period, temperature and pH[8,9]. The aim of this study is investigate the types of molds and yeasts contaminated the wards of the Al-Diwaniya Teaching Hospital and the effect of some antiseptics on the growth of these fungi.

MATERIALS AND METHODS

Culture media

1. Sabouraud Dextrose Agar medium: Prepared according to the manufactured company's instructions (Himedia India) by dissolving 65 g of it in 1 liter of distilled water in a flask was then sterilized by autoclave at 121°C for 15 minutes and then remove the flask and left to cool and then added to 25 mg/L of antibacterial Chloramphenicol and adjust the pH at 5.6. This medium is used to isolate the fungi under study.
2. Urea Agar medium: Prepared by dissolving 2.4 g of urea base agar in 95 ml of distilled water in a flask and adjust the pH at 6.8, then sterilized by autoclave at 121°C for 15 minutes and then remove the flask and left to cool and added to it 5 ml of sterile urea solution by filtration with concentration 40% then distributed into test tubes with size 5 ml as a slant[10]. This medium is used to detect the production of urease enzyme.

Sample collection

120 samples were collected from wards of the Al-Diwaniya Teaching Hospital for the period from 5 April to 5 June 2016. In the collection of the samples were used sterile cotton swabs by opening it and move it in the required places which include (Floors and walls, Instruments, Patients, Patients beds and Patients clothes) then these swabs were transported to the laboratory and cultured on Petri dishes containing (SDA) Sabouraud Dextrose Agar medium, the air samples were collected by open Petri dishes containing SDA medium and exposed to air of wards for 10 minutes with continuous movement to increase its exposure to the air, and then put all the dishes in the incubator at 25°C for 3 to 4 days for the purpose of fungal growth and identification this fungi[11].

Isolation and identification of molds and yeasts

Identified fungi under study after purification depending on morphological features such as shape, color, diameter colony and height and also depending on the microscopic features such as the shape, size and color of fungal hyphae, spores and other reproductive structures according to the taxonomic placement[12,13,14]. also used some additional tests to identification yeasts according to[15] as follows:

1. Growth at 37 ° C: This test involved the growth of yeasts on SDA medium at 37° C for 7 days, the test is positive when the yeasts growth.
2. Germ tube formation test: 0.3 ml of human blood serum put in test tube and then transfer part of the colony of yeast under study in age (2-3) days to the test tube containing serum was then shaken well and incubated in incubator at 37° C for (2-3) hours after it put one drop of suspended yeast on a slide and then put the cover slide, It was microscopic examination to see germ tube as in the case of its formation, it is an indicator that a positive test and this special test of *Candida albicans*.
3. Urease test: This test used to identify the *Rhodotorula* spp and *Cryptococcus* spp and it involved inoculated test tubes containing the sterile urea agar medium with yeast to be identified using the sterilized needle and incubated in incubator at 25° C for (2-5) days and recorded the result, if the medium color changed from yellow to pink the result is positive.

All pure fungal isolates obtained were saved in test tubes with a capacity of 20 ml containing slant SDA medium and maintained in refrigerator at 4°C until used, and then calculated the percentages of fungal contamination and occurrence and frequency of fungi according to the following equations[16].

$$\text{Fungal contamination \%} = \frac{\text{The number of swabs that gave growth}}{\text{The total number of swabs}} \times 100$$

$$\text{Occurrence \%} = \frac{\text{The number of samples that appeared the same genus or species}}{\text{The total number of samples}} \times 100$$

$$\text{Frequency \%} = \frac{\text{The number of isolates of the same genus or species}}{\text{The total number of isolates}} \times 100$$

Sensitivity test of isolated fungi to some antiseptics

Testing involved use of Dettol (Chloroxylenol) and Hibitane (Chlorhexidine) and Formalin (Formaldehyde) at concentration 10% and then was prepared concentrations 7.5%, 5% and 2.5% by dilution with distilled water and sterilized by filtration using Millipore filter paper 0.45 um and then was transferred 3 ml of each concentrations by sterile pipette into flasks containing 27 ml of the SDA medium which placed in a water bath at a temperature 50 ° C and then distribute into plastic petri dishes with 90 mm in diameter with three replications for each concentration and left to become a solid and made a standard cylinder in the gel of dishes that containing antiseptics by sterile cork borer with diameter of 6 mm and then filled with cylinders in the same size that cut from each pure colonies of fungi under study and incubated at (28-30)° C for 7 days, the results recorded by measuring the diameter of inhibition zone in units of a millimeter[17], then calculated the percentage of inhibition according to the following equation:

$$\text{Inhibition \%} = \frac{\text{Diameter of colony control} - \text{Diameter of colony treatment}}{\text{Diameter of colony control}} \times 100$$

Statistical analysis

One Way Analysis of Variance (ANOVA) was used to test significant differences between

Table 1. Percentages of fungal contamination in the hospital wards

Location	Number of samples	Number of swabs that gave fungal growth	Fungal contamination (%)
Air	20	18	90
Floors and walls	20	18	90
Instruments	20	3	15
Patients (Nose, Ear and Hands)	20	14	70
Patients beds	20	11	55
Patients clothes	20	13	65
Total	120	77	64.1

Table 2. Percentages of occurrence and frequency of fungi isolated from hospital wards

Fungi	Number of samples that appeared the fungus	Occurrence (%)	Number of isolates	Frequency (%)
Alternaria alternata	11	11.578d	14	11.2d
Aspergillus flavus	16	16.842b	19	15.2b
Aspergillus fumigatus	4	4.210gh	5	4.0h
Aspergillus niger	18	18.947a	24	19.2a
Aspergillus ochraceus	3	3.157hi	4	3.2hi
Candida albicans	9	9.473e	11	8.8e
Cryptococcus spp	3	3.157hi	5	4.0h
Fusarium spp	6	6.315f	9	7.2f
Mucor spp	5	5.263fg	7	5.6g
Penicillium spp	14	14.736c	17	13.6c
Rhizopus stolonifer	4	4.210gh	7	5.6g
Rhodotorula spp	2	2.105i	3	2.4i
Total	95		125	

Key- Percentages of occurrences and frequencies with the same letters did not differ significantly ($p>0.05$) between species of isolates.

the rates by Duncan multiple range test at 0.05 probability level [18].

RESULTS

Fungal contamination in the hospital wards

The results showed that the percentage of the fungal contamination in the wards was 77/120 (64.1%), highest of the fungal contamination was recorded in the floors and walls and in the air 18(90%), followed by patients (Nose, Ear and Hands) 14(70%) and lowest contamination was in the instruments 3(15%) (Table 1).

Isolation and identification

In this study, 125 fungal isolates were obtained and identified as belonging to 9 genera

Table 3. Percentages of inhibition the growth of fungal isolates using different concentrations of Dettol

Fungi	Percentages (%) growth inhibition for Dettol concentrations			
	2.5 %	5 %	7.5%	10 %
Alternaria alternata	38.0 d	58.4 c	61.5 b	65.8 a
Aspergillus flavus	22.6 d	31.7 c	35.5 b	48.4 a
Aspergillus fumigatus	36.4 d	43.2 c	47.0 b	53.2 a
Aspergillus niger	34.7 d	58.1 c	60.3 b	64.5 a
Aspergillus ochraceus	23.9 d	35.7 c	42.2 b	48.6 a
Candida albicans	21.7 d	28.5 c	40.2 b	61.2 a
Cryptococcus spp	32.2 c	49.5 b	49.9 b	52.2 a
Fusarium spp	43.5 d	51.2 c	56.7 b	65.0 a
Mucor spp	30.2 d	47.5 c	49.1 b	53.8 a
Penicillium spp	20.6 c	21.4 c	37.6 b	54.6 a
Rhizopus stolonifer	15.7 c	16.4 c	38.7 b	40.6 a
Rhodotorula spp	30.7 d	46.3 c	49.5 b	52.3 a

Key-Percentage inhibitions by various species of fungi with the same letter at a particular concentration and between concentrations are not significantly different ($p>0.05$) at 95% confidence level.

Table 4. Percentages of inhibition the growth of fungal isolates using different concentrations of Hibitane

Fungi	Percentages (%) growth inhibition for Hibitane concentrations			
	2.5 %	5 %	7.5%	10 %
Alternaria alternata	38.8 d	62.6c	67.3b	78.5a
Aspergillus flavus	32.6 d	36.3c	50.2b	68.1a
Aspergillus fumigatus	35.2 d	44.0c	66.8b	73.3a
Aspergillus niger	37.8 c	58.3b	60.7b	72.6a
Aspergillus ochraceus	28.6 c	38.9b	40.0b	72.7a
Candida albicans	22.4 d	33.5c	40.6b	70.7a
Cryptococcus spp	39.7 d	46.6c	69.4b	79.7a
Fusarium spp	46.8 d	58.3c	62.5b	71.4a
Mucor spp	34.0 d	52.4c	57.7b	78.2a
Penicillium spp	31.0 d	34.9c	44.8b	69.5a
Rhizopus stolonifer	29.6d	35.5c	52.6b	68.5a
Rhodotorula spp	20.8d	46.0c	55.8b	75.6a

Key-Percentage inhibitions by various species of fungi with the same letter at a particular concentration and between concentrations are not significantly different ($p>0.05$) at 95% confidence level.

Table 5. Percentages of inhibition the growth of fungal isolates using different concentrations of Formalin

Fungi	Percentages (%) growth inhibition for Formalin concentrations			
	2.5 %	5 %	7.5%	10 %
Alternaria alternata	59.0 c	61.5 c	78.3 b	90.0 a
Aspergillus flavus	60.9 d	67.9 c	76.0 b	91.2 a
Aspergillus fumigatus	64.5 d	75.8 c	80.2 b	95.4 a
Aspergillus niger	59.8 d	65.7 c	75.4 b	88.7 a
Aspergillus ochraceus	68.4 d	74.6 c	82.5 b	90.4 a
Candida albicans	62.9 d	70.3 c	76.6 b	92.6 a
Cryptococcus spp	65.4 d	73.7 c	83.0 b	91.5 a
Fusarium spp	65.3 d	77.2 c	85.6 b	90.2 a
Mucor spp	66.7 c	75.9 b	78.5 b	85.2 a
Penicillium spp	58.7 d	65.5 c	77.4 b	87.3 a
Rhizopus stolonifer	60.2 d	69.3 c	77.7 b	86.7 a
Rhodotorula spp	62.6 c	64.6 c	74.7 b	93.0 a

Key-Percentage inhibitions by various species of fungi with the same letter at a particular concentration and between concentrations are not significantly different ($p>0.05$) at 95% confidence level.

comprising of *Aspergillus* spp with 52 isolates (mainly *A. niger*= 24, *A. flavus*= 19, *A. fumigates*= 5 and *A. ochraceus*= 4). *Penicillium* spp with 17 isolates, *Alternaria alternata* with 14 isolates, *Candida albicans* with 11 isolates, *Fusarium* spp 9 isolates, *Mucor* spp 7 isolates, *Rhizopus stolonifer* 7 isolates, *Cryptococcus* spp 5 isolates and *Rhodotorula* spp 3 isolates. The highest occurrence and frequency percentages of fungi recorded for *A. niger* reaching (18.947%) and (19.2%) respectively, followed by *A. flavus* reaching (16.842%) and (15.2%) respectively with significant differences when compared with other fungi isolated in this study, while the lowest occurrence and frequency percentages recorded for *Rhodotorula* spp reaching (2.105%) and (2.4%) respectively. (Table 2).

Sensitivity of isolated fungi to antiseptics

The results showed that the antiseptics (Dettol, Hibitane and Formalin) have high inhibitory activity against the growth of all fungi studied when used at original concentration which was 10% and differing significantly ($p<0.05$) from other concentrations 7.5, 5 and 2.5% that used. Formalin showed highly significant effect in inhibitory activity for the growth of fungi from Dettol and Hibitane, the percentage of inhibition when using Formalin at concentration 10% ranged between (85.2-95.4%) while for Hibitane between (68.1-79.7%) and for Dettol between (40.6-65.8%) at the same concentration (Tables 3, 4 and 5).

DISCUSSION

Hospitals environment containing various microorganisms such as viruses, bacteria and fungi, so it is considered one of the most complex environments, the level of contamination in the hospital environment depends on many various factors such as: hospital size, hospital design, patients number, visitors number, ventilation system and methods used in the disinfection and sterilization processes[19]. In this study may be due reason for the high fungal contamination percentages in the floors and walls and in the air of the wards to the use of incorrect methods of ventilation process such as opening windows continuously and the entry of large amounts of dust which contains various germs and do not use antiseptics correctly in the disinfection process[20,21]. The decrease of fungal contamination percentages in the instruments may be due to these instruments often be immersed in antiseptics during use and these processes reduce the chances of occurrence of microorganisms contamination[22]. Generally species of *Aspergillus* spp have a high ability to produce various primary and secondary metabolites, which enables it to

use various nutrients under various environmental conditions and this explains the recording of *A. niger* highest occurrence and frequency in the wards compared with other fungi under study[23]. As for the effect of antiseptics in the growth of various fungi has been reported[24] that the effect of antiseptics least whenever they are used in diluted concentrations and that the reason for the presence of fungi as endemic in the wards despite disinfection and sterilization processes that occur may be due to random dilution of antiseptics or the use of only non-sterile water in the cleaning process which provides adequate moisture for fungal growth and spread in the environment, in addition to that these water may contain various germs. The high effect of Formalin on the growth of fungi under study compared with the effect of other antiseptics that used can be due to a little use of Formalin in hospitals because it irritate the tissue and has a strong odor and an expensive compared with Dettol and Hibitane which are used extensively in hospitals and in random concentrations which led to the development of resistance to them by various fungi as the frequent use of antiseptic without the other leads to a resistance factor due to a genetic mutation[25]. The presence of fungal contamination in the wards is require attention by the authorities responsible for it may cause secondary infections more dangerous than the primary infections that the patients came to the hospital for treatment and complications may occur lead to death[26].

CONCLUSION

Depending on the results of the current study, the use of antiseptics in the original concentrations without dilution is the best way to reduce the contamination inside the hospital as well as the use of F0rmalin mainly in sterilization processes for their high effectiveness in eliminating contamination with microorganisms.

ACKNOWLEDGMENTS

I thank all workers in the wards and laboratory of Al-Diwaniya teaching hospital for provide some materials and collect the samples that required in this study.

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