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Detection of contamination by opportunistic fungi in solid and liquid soaps in special medical labs and investigation of ability to producing toxins

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Abstract :
The purpose this study was determine the contamination of fungi in solid soap comparative between with liquid soap in use hand washing soaps in 10 labs in Al-Najaf city at four season .Swabs were taken from surfaces of solid soaps and from liquid soaps ; at toilets of special labs in the city. Were taken in four season in four months (January , march , July , October) 2017. the samples were cultured in lab by use conventional microbiologic methods of the swabs and identification of the isolates, and this lead to the conclusion that solid soap could be contamination with fungi excessively while was Liquid soap empty of contamination. 39 samples of 50 samples were contaminated with opportunistic fungi which shown in solid soap, *Aspergillus niger* was 30% , *A.flavus* 20% , *A.terreus* 12% , *A. fumigatus* 6%, *Alternaria alternata* 4% *Penicillium* spp. 4% ,and *Fusarium* spp. 2%. The study showed the wide spread of fungi in the spring where was 16 isolates, followed by the summer season was 12 isolates, while was the autumn 7 isolates, But winter was the least in isolation , where was 3 isolates. The liquid soap was empty of any contamination. ELISA examination showed that some isolates was it ability to produce of toxin.
Keywords : solid soap , Liquid soap , ELISA examination .

INTRODUCTION :

About 80, 000 to 120, 000 species of fungi have been described to date ; although the total number of species was estimated at around 1.5 million[7]. Fungi organisms Eukaryotic unicellular or multicellular cells , non hetero trophic [3].Many filamentous fungi have the ability to secrete Secondary metabolites known as mycotoxins , The most important species that produce such compounds are *Aspergillus* spp., *Penicillium* spp., *Fusarium, Alternaria*. As well as other species with the ability to secrete toxins[11] .Opportunistic fungal infections have emerged of important causes which morbidity and mortality in patients with some underlying illnesses and compromised host defenses [1] Human fungal infections have increased in incidence and severity in recent years[16]. This was mainly attributed to advancements made in cancer treatment ,surgery, HIV epidemics and use of immunosuppressive drugs, add to the wide application of antibiotics[20]. Diseases caused by species of more genera that fungi are gaining more and more prominence where more than 85% of the patients die , especially patients with immunodeficiency[22]. This led to the wide spread of fungal infections not only by the fungal pathogens such as Candida and Cryptoccoccus species but also by the opportunistic *Aspergillus* species[14]. Fungal toxins have significant public health effects causing kidney toxicity, immune inhibition, fetal malformation and congenital malformations , these toxins can cause severe and chronic effects in humans and animals ranges from death or disorder in the central nervous system, heart, blood vessels and pulmonary systems[16] .The purpose of this work is to detect the opportunistic fungi , contaminated solid and liquid soap[8] .

MATERIALS AND METHODS:

Samples collection and identification:

fifty samples of solid and liquid soap were collected randomly from lab in Al-Najaf city during period in four seasons (Winter , Spring , Summer , Autumn) at 2017. In four seasons, 25 swabs were collected from surfaces of solid soaps from toilets ; and 25 swabs another from samples were collected from liquid soaps approximately at the same time in 10 special labs of Al-Najaf city .Despite of the soaps in this settings were called anti-organism by the manufacturer; that was considered as preservative rather than anti-organism effect. All samples were transported to the laboratory in ice-cooled box. Microbiological analysis was performed on arrival of samples to determine the average fungi with special emphasis on the isolation and identification of isolates through their biochemical characteristics and other mycological analysis .

Preparation of media for growth of fungi :

Potato dextrose agar PDA and SDA were two of the solid culture media selected for the growth and characterization of fungi , after the media was prepared, fungi were activated in media and the contents were thoroughly mixed and pour in sterile petri dishes[7].

Culture :

Collected swabs were dipped into tubes containing 1 ml sterile normal saline (0.9%). Samples were brought to the microbiology laboratory without delay. PDA and SDA media are enforced with chloramphenicol (16 µg/mL) to inhibit the growth of contaminating bacteria; incubated at 28 C°, for 7days[4].

Statistical Analysis

Data were analyzed by using statistical analysis system. The significant differences were determined at $p< 0.05$ .The statistical analysis of the data was performed by the least significant differences (LSD) were conducted to find the significant differences among the different mean values.

RESULT AND DISCUSSION:

The result showed that found contaminants in solid soap samples which were collected in this study where it appeared *Aspergillus niger* by 30%, *A.flavus 20%* , *A.terreus* 12% , *A.fumigatus* 6%, *Alternaria alternate* 4%, *Penicillium* spp. 4% , and *Fusarium* spp. 2% **(**table-1**).** While was no contamination in liquid soap samples (table-2). The results revealed that fungal contaminants were appeared in four seasons of year (winter , spring , summer , autumn) from 10 labs in Al-Najaf city for 50 samples were collected to solid and liquid soaps .The solid soap samples could be contaminated due to the infections of patients This contamination was caused by the adhesion of the fungus parts to the surface of solid soap while not appearing in the liquid soap .

Table (1) fungi isolated from solid soap in special medical labs.

No.	Isolated fungus	Number of isolates in four seasons				Sum.	%
		The seasons					
		Winter	Spring	Summer	Autumn		
1	*Aspergillus niger*	2	5	5	3	15	30
2	*A.flavus*	1	4	4	1	10	20
3	*A.terreus*	0	3	2	1	6	12
4	*A.fumigatus*	0	1	1	1	3	6
5	*Alternaria alternata*	0	1	0	1	2	4
6	*Penicillium* spp.	0	1	1	0	2	4
7	*Fusarium* spp.	0	1	0	0	1	2
	Sum.	3	16	13	7	39	78
	L.S.D.	0.3	0.9	0.8	0.7	2.4	

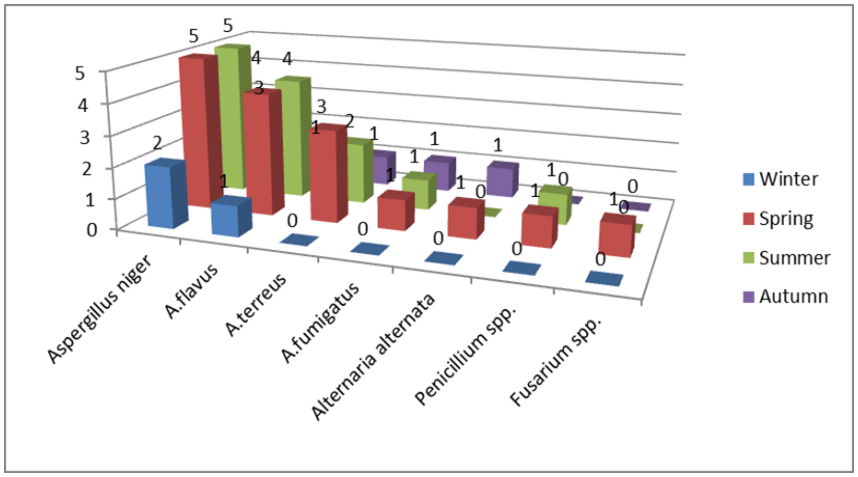


Figure (1) fungi isolated from solid soap in special medical labs.

Table (2) fungi isolated from liquid soap in special medical lab.

No.	Isolated fungus	Number of isolates in four seasons				Sum.	%
		The seasons					
		Winter	Spring	Summer	Autumn		
1	*Aspergillus niger*	0	0	0	0	0	0
2	*A.flavus*	0	0	0	0	0	0
3	*A.terreus*	0	0	0	0	0	0
4	*A.fumigatus*	0	0	0	0	0	0
5	*Alternaria alternata*	0	0	0	0	0	0
6	*Penicillium* spp.	0	0	0	0	0	0
7	*Fusarium* spp.	0	0	0	0	0	0
	Sum.	0	0	0	0	0	0
	L.S.D.	0	0	0	0	0	0

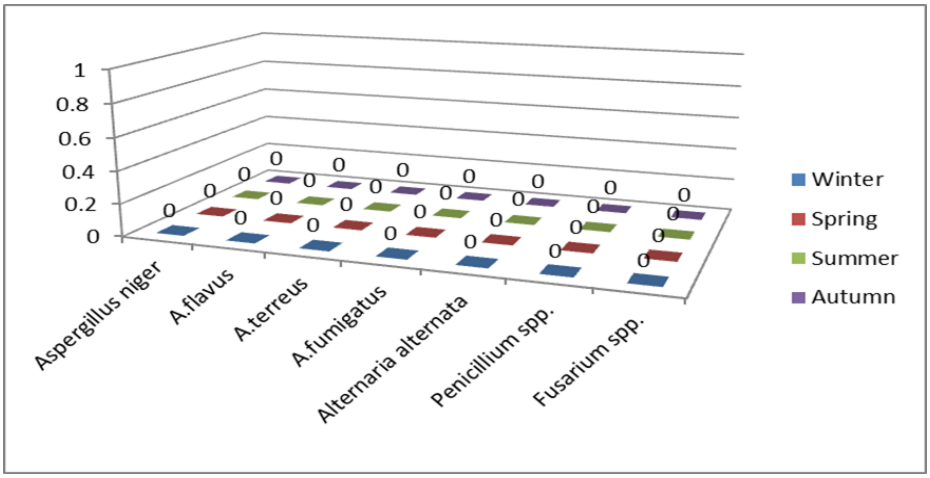


Figure (2) fungi isolated from liquid soap in special medical lab.

The result showed that found contaminants in solid soap samples which were collected in Winter Season where it appeared *Aspergillus niger* by 4%, *A.flavus 2%* , *A.terreus* 0% , *A.fumigatus* 0%, *Alternaria alternate* 0%, *Penicillium* spp. 0% , and *Fusarium* spp. 0% **(**table-3**).**

The result showed that found contaminants in solid soap samples which were collected in spring season where it appeared *Aspergillus niger* by 10%, *A.flavus* 8% , *A.terreus* 6% , *A.fumigatus* 2%, *Alternaria alternate* 2%, *Penicillium* spp. 2% , and *Fusarium* spp. 2% **(**table-4**).**

Table (3) fungi isolated from solid soap in Winter Season of special medical labs.

	Isolated fungus	Number of isolates in Winter Season		Sum	%
		solid soap	liquid soap		
1	*Aspergillus niger*	2	0	2	4
2	*A.flavus*	1	0	1	2
3	*A.terreus*	0	0	0	0
4	*A.fumigatus*	0	0	0	0
5	*Alternaria alternata*	0	0	0	0
6	*Penicillium* spp.	0	0	0	0
7	*Fusarium* spp.	0	0	0	0
	Sum.	3	0	3	6
	L.S.D.	0.3		0.3	

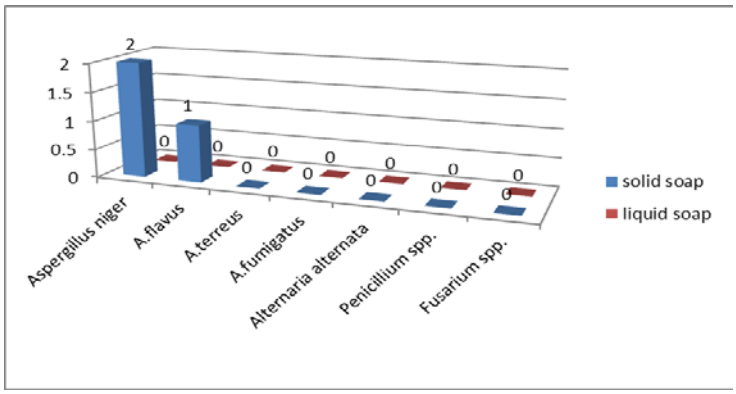


Figure (3) fungi isolated from solid soap in Winter Season of special medical labs.

Table (4) fungi isolated from solid soap in spring Season of special medical labs.

No.	Isolated fungus	Number of isolates in Spring Season		Sum	%
		solid soap	liquid soap		
1	*Aspergillus niger*	5	0	5	10
2	*A.flavus*	4	0	4	8
3	*A.terreus*	3	0	3	6
4	*A.fumigatus*	1	0	1	2
5	*Alternaria alternata*	1	0	1	2
6	*Penicillium* spp.	1	0	1	2
7	*Fusarium* spp.	1	0	1	2
	Sum.	16	0	16	32
	L.S.D.	0.9		0.9	

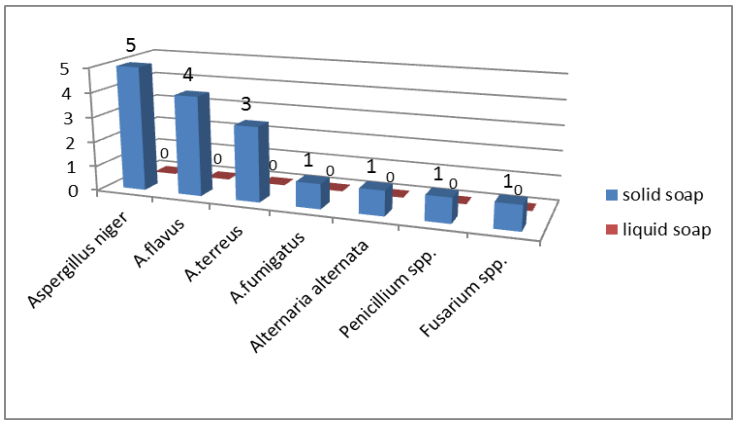


Figure (4) fungi isolated from solid soap in spring Season of special medical labs.

The result showed that found contaminants in solid soap samples which were collected in summer season where it appeared *Aspergillus niger* by 10%, *A.flavus* 8% , *A.terreus* 4% , *A.fumigatus* 2%, *Alternaria alternate* 0%, *Penicillium* spp. 2% , and *Fusarium* spp. 0% **(**table-5**).**

The result showed that found contaminants in solid soap samples which were collected in autumn season where it appeared *Aspergillus niger* by 6%, *A.flavus* 2% , *A.terreus* 2% , *A.fumigatus* 2%, *Alternaria alternate* 2%, *Penicillium* spp. 0% , and *Fusarium* spp. 0% **(**table-6**).**

Table (5) fungi isolated from solid soap in summer Season of special medical labs.

No.	Isolated fungus	Number of isolates in Summer Season		Sum	%
		solid soap	liquid soap		
1	*Aspergillus niger*	5	0	5	10
2	*A.flavus*	4	0	4	8
3	*A.terreus*	2	0	2	4
4	*A.fumigatus*	1	0	1	2
5	*Alternaria alternata*	0	0	0	0
6	*Penicillium* spp.	1	0	1	2
7	*Fusarium* spp.	0	0	0	0
	Sum.	13	0	13	26
	L.S.D.	0.8		0.8	

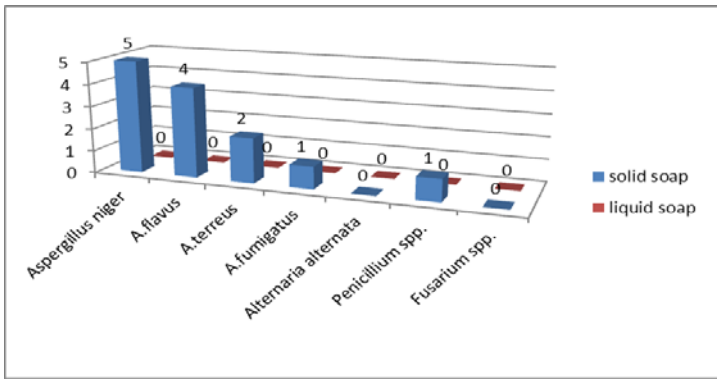


Figure (5) fungi isolated from solid soap in summer Season of special medical labs.

Table (6) fungi isolated from solid soap in autumn Season of special medical labs.

No.	Isolated fungus	Number of isolates in Autumn Season		Sum	%
		solid soap	liquid soap		
1	*Aspergillus niger*	3	0	3	6
2	*A.flavus*	1	0	1	2
3	*A.terreus*	1	0	1	2
4	*A.fumigatus*	1	0	1	2
5	*Alternaria alternata*	1	0	1	2
6	*Penicillium* spp.	0	0	0	0
7	*Fusarium* spp.	0	0	0	0
	Sum.	7	0	7	14
	L.S.D.	0.7		0.7	

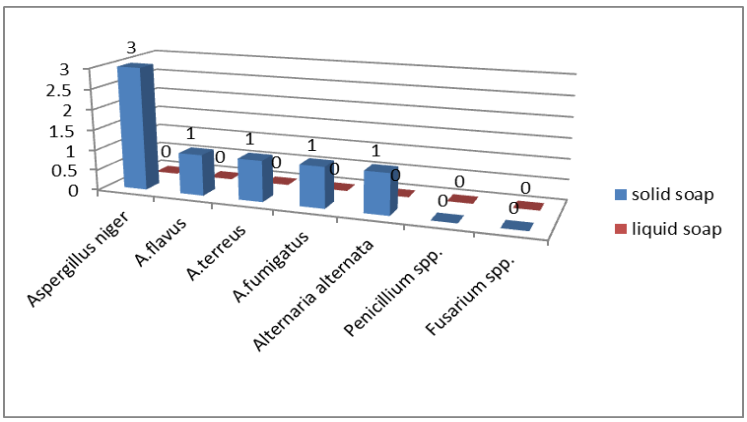


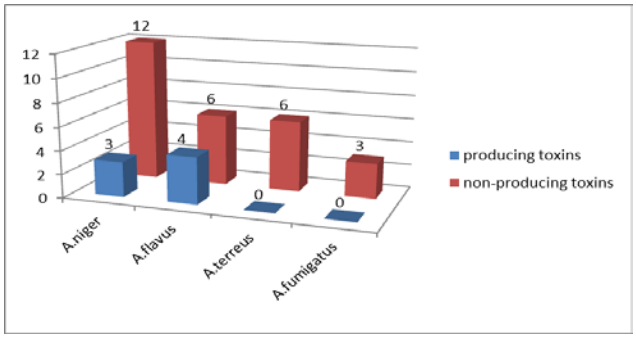
Figure (6) fungi isolated from solid soap in autumn Season of special medical labs.

ELISA examination :

The result showed 3 isolates from *A.niger* were it ability for production of toxin , and 4 isolates from *A.flavu*s it another *ability for production of toxin* **(**table-7**).**

Table(7) ELISA examination for isolates to detect of toxin production .

No.	Fungus isolated	Isolates of producing toxins	Isolates of non-producing toxins	Sum.	%
1	*A.niger*	3	12	15	20
2	*A.flavus*	4	6	10	40
3	*A.terreus*	0	6	6	0
4	*A.fumigatus*	0	3	3	0
	Sum.	7	27	34	60
	L.S.D.	1.6			



Figure(7) ELISA examination for isolates to detect of toxin production .

DISCUSSION :

The most common soap hand-cleaning agents are solid and liquid soap in disposable plastic containers[9]. When in use solid soaps are frequently used because they are typically stored in contact with moisture and remain moist for long periods of time[13]. This supplies an environment which provides the perfect opportunity for fungi to grow[15]. Most solid of soap in communal areas are used by a number of different people[17]. This means that one solid of soap can be in direct contact with skin fungi from one to another to more persons , and may transfer live pathogenic opportunistic fungi which cause infection [19].When using solid of soap, the Centre for Disease Control international recommends placement on a drainable rack between uses[21]. Soap racks that promote drainage of all water from the bar should be installed[1]. In addition, there should be easy access to replacements when soap is lost, dropped, melted, or consumed. Small soap were also recommended that can changed and used in preference to larger solid that are more likely to melt or become contaminated[2] . Liquid soap on the other hand is much better to use. Liquid soap is dispensed straight from a container. It has not been exposed to contaminants[5]. As a result, cross contamination is not happen to occur, that it is use a more cleaning and more hygienic alternative[10] .The reported of 6 which indicated that in solid soaps samples were more contaminates after use than liquid soaps. In another study,[8] indicated that samples from solid and liquid soaps from 26 bath room public were contaminates , while in liquid soaps samples were not found of microorganism[12]. Despite of CDC recommendations most health care center like toilets in lab are using liquid soap container instead of solid soap[3] . In our study, solid soap because wetting , and adhesion of contaminants from people who carry them to other people were found heavily contaminated[7] . Thisstudy revealed quite lower contamination rate in liquid soaps compared with solid soaps[11]. The result showed a high rate of pollution in fungi in the spring season where it reached 32% followed by the summer by 26% , because of the high prosperity of the spread of fungi in this seasons and increased of temperatures ,which is an important factor for the high incidence and spread of fungi[17] .

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