Available online at sjuoz.uoz.edu.krd

Science Journal of University of Zakho

Vol. 5, No. 2, pp. 154 –157, June-2017

p-ISSN: 2410-7549
e-ISSN: 2414-6943

ISOLATION AND IDENTIFICATION OF YEASTS FROM INDOOR SWIMMING POOLS IN DUHOK CITY WITH SPECIAL CONCERN TO THE EFFECT OF SOME DISINFECTANT AGENTS

Berivan A. Abdullah*, and Asia A.M.Saadullah

Dept. of Biology, College of Science, University of Duhok, Duhok, Kurdistan Region, Iraq

Received: Sept. 2016 / Accepted: Mar. 2017 / Published: Jun. 2017 ***https://doi.org/10.25271/2017.5.2.360***

ABSTRACT:
The present study was conducted to study the level of yeast contamination in indoor public swimming pools in Duhok city. A total of 230 samples (50 water sample) were taken from swimming pool water and (180 swab samples) were taken from walls, flour of bathrooms, dressing rooms and flour around the pool in five indoor public swimming pools from September 2014 to February 2015. All samples were examined for the presence of yeasts using three culture media (Potato Dextrose Agar, Malt Extract Agar and Sabouraud's Dextrose Agar). The highest number of Yeast genera was in November (6) followed by October (5), December and January (4) while the lowest number was in September (3). Eleven (11) isolates yeasts were selected randomly for *in vitro* sensitivity to the disinfectant agents **{**Chlorine, Chloroxylenol and Hydrogen peroxide (H_2O_2)}. All tested isolates were highly sensitive to chlorine, Chloroxylenol and H_2O_2. The MIC value of chlorine for most of them was 0.01 % and for Chloroxylenol was 0.1 % except *Candida tropicalis* and *C. glabrata*. Regarding the effect of H_2O_2 the MIC value ranged from 0.1% - 12.8 %.

KEYWORDS: Isolation, Yeast, Duhok City, Chlorine, Chloroxylenol.

1. INTERODCUTION

Yeasts are single celled microorganisms they reproduce by the asexual process of budding or fission. They are ubiquitous and exist as saprobes, mutualisms or parasites in their life styles. (Hawksworth, 1991; Cannon and Hawksworth, 1995).

Swimming considered as one of the most popular sport for both young and old people of both sexes. It has many physical and mental benefits, it is advised as the most appropriate way to keep fit, stay healthy, having fun and for social life.

Numerous infectious agents of bacteria, fungi, viruses and protozoa may be brought into a pool from the bather's skin, in their saliva, urine, feces, also from dust, bird droppings and soil carried on bather's feet, which threatens the health of pool users. Fungi including yeasts grow very easily in damp and wet conditions inducing acute health effects (Robins and Morell, 2007). In recent years the resistance of yeasts toward many disinfectant agents has been increased dramatically, this problem derives the important current role of the *in vitro* disinfectant susceptibility testing to determine which agents are effective for a given infection. Disinfectants are chemical compounds commonly added to water and other domestic activities such as toilet and general house cleaning; they are used to control or reduce the growth of pathogenic microbes (Fraise, 2002). The aim of study is to determine the level of yeast contamination and the effect of some disinfectant agents on some yeast isolates in public swimming pools in Duhok city, Kurdistan Region of Iraq.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fifty water samples and 180 indoor samples were collected from five indoor public swimming pools in Duhok city, Kurdistan Region of Iraq from September 2014 to January 2015. Sampling was carried out twice per month for each swimming pool. Water samples were collected in sterilized bottles and 1ml of Sodium thiosulfate was added to neutralize the chlorine residual in each sample (Bello *et al.*, 2012). Indoor samples from bathrooms, dressing rooms, dried sauna rooms, walls and floor around the pools were taken using sterile cotton swabs. All samples were transferred immediately to the Faculty of Science/ Mycological Research Laboratory and were processed immediately upon arrival.

2.2 Isolation of yeasts from water samples by pouring method:

In this method 1ml of each water sample was added to six empty petridishes: three for Potato Dextrose Agar (PDA), two for Malt Extract Agar (MEA) and one for (SDA) and poured with 20 ml of specified type of molten culture media. Then the plates (3PDA, 1MEA) were shaken gently and incubated at 25°C for 5-7 days and the (1 SDA, 1 MEA) plates were incubated at 37°C for 1 week, and they were checked at frequent intervals.

2.3 Isolation of yeasts from indoor samples by Swab method:

The spreading method was used for isolating fungi from indoor environments according to the method described by Viegas *et al.*, (2011). Each swab was spread over six petridishes containing three types of culture media (PDA, SDA and MEA). Three plates

* Corresponding author

of PDA and one of MEA were incubated at 25°C for 5-7 days; whereas the plates of SDA and MEA were incubated at 37°C for 1 week and were checked at frequent intervals.

2.4 Yeasts Identification

Identification for yeast species other than *Candida* was done by colony characterization and microscopic examination according to the keys and descriptions provided by de Hoog and Guarro, (1995), Ellis *et al*., (2007), Larone, (2011).

2.5 Identification of Candida species

2.5.1 Chromogenic Candida Agar (Rapid Labs Ltd., Essex, UK): Clinically important *Candida* species is identified using this selective medium which contains Chromogenic substrates that detect specific enzymes of some *Candida* species. This selective medium is light sensitive and prepared by dissolving 45.5 g of it in 1 Liter of distilled water; stirred until completely dissolved then autoclaved at 121ºC for 15 minutes and poured in Petridishes and kept in refrigerator. The color of *Candida* sp. colonies grown on chromogenic Candida agar are shown below:

1. *Candida albicans*: emerald with metallic shine color.
2. *Candida dubliensis*: dark green color.
3. *Candida krusei*: light pink color.
4. *Candida tropicalis*: red purple color.
5. *Candida glabrata*: shiny white color.

2.6 Disinfectant agents Sensitivity Test:

2.6.1 Yeast Isolates: Eleven (11) yeast isolates were tested for Disinfectants sensitivity test, each isolate was subcultured before testing in a Sabouraud's Dextrose broth and incubated at 37º C for 24-48 hr.

2.6.2 Media: Yeast isolates were cultured on a Sabouraud's Dextrose Agar (SDA) medium for the Disinfectants sensitivity test. The SDA ((Lab M limited Co. UK) was prepared by dissolving 65 g in 1 liter of D.W., stirred until it completely dissolved and was then autoclaved at 121º C for 15 min. and then poured onto petridishes.

2.6.3 Disinfectant Agents: Three commonly used disinfectants agents were chosen, namely:

1- Chlorine (BKG water solution company, Germany), with the following dilutions: 0.01, 0.04, 0.08, 0.16, 0.32, and 0.64 %.

2- Hydrogen peroxide (H2O2), (30%, M.W 34.01, Alpha chemika, Mumbai, India), with the following dilutions: 0.1, 0.4, 0.8, 1.6, 3.2, and 12.8%.

3- Chloroxylenol (Dettol), (4.85% w/w, Green Planet Industries, United Arab Emirates), with the following dilutions: 0.025, 0.1, 0.2, 0.4, 0.8, and 3.2 %.

2.7 Susceptibility Test (method):

2.7.1 Well Diffusion Method: Disinfectant agent's sensitivity test was performed using the well diffusion method which is simple, inexpensive and easy to read (Magaldi *et al*., 2004). For each yeast isolate, 6 plates of SDA were used. 0.1 ml of yeast isolate was spreaded over 6 SDA plates using a sterile cotton swab. For each plate, 3 wells 6 mm in diameter were cut out of the agar using cork borer and filled with 0.15 ml of the desired disinfectant agents, and incubated at 37º C for 24-48 hr.

3. RESULTS AND DISCUSSION

3.1 Yeasts Genera Associated with Indoor Swimming Pools

Table 1 represents the Yeasts genera isolated during five months of the study, the highest number of Yeast genera was in November (6) followed by October (5), December and January (4) while the lowest number was in September (3).

Candida species were also isolated from water of swimming pools in other countries such as Greece, Nigeria and Iran (Papadopulou *et al*., 2008; Itah and Ekpomhok, 2004; Azizi-far *et al*., 2006). *Candida* species are true opportunistic pathogen which constitutes the normal flora of the skin, oral cavity, gastrointestinal tract, virginal and the urinary tract of humans. They are inhabiting different environments particularly in water and soil (Ellis, 1994). Species of *Candida* are important opportunistic pathogens especially in immunocompromized individuals. *Candida* is the cause of candidiasis involving any part of the body; mucocutaneous, viginitis, bronchial and pulmonary paronchia and onychomycosis (Aronson and Soltani, 1976). *Rhodotorula* sp. isolated during this study commonly known as contaminants, but in recent years they have increasingly regarded as agents of severe infection. *Aurobasidium* sp. known as black yeast is ubiquitous in different environments such as water, air and soil. It causes allergy after exposure via humidity and the condition is characterized by cough, fever and chest infiltrates. *Geotrichum* sp. was found as normal flora in human and seems to cause diseases only in severely compromised hosts. *Trichosporon* sp. has been increasingly involved in disseminated diseases and related with infections in debilitated hosts (Odd, 1988).

Most of the above Yeasts (*Candida*, *Rhodotorula*, *Geotrichum* and *Trichosporon*) have previously been reported from swimming pools (Hosinzadeh *et al*., 2013; Viegas *et al*., 2010; Mohammed and Habeb, 2014; Nanbakhsh *et al*., 2004). Isolation of these Yeasts might due to continuous contamination of the swimming pool water by fungi through air, soil and human bodies. Opportunistic pathogenic Yeasts *Candida*, *Rhodotorula* and *Trichosporon* are abundant in the air and have been isolated from different locations because they reproduce by fragmentation and distribute in air (Aidoo *et al*., 1995).

Table 1. Distribution of Yeasts during different months of Year.

No.	Yeast isolates	Sep.	Oct.	Nov.	Dec.	Jan.
1.	*Aurobasidium* sp.	+	+	+	+	+
2.	*Candida* sp.	+	+	+	+	+
3.	*Geotrichum* sp.	—	+	+	—	—
4.	*Rhodotorula* sp.	—	+	+	+	+
5.	*Trichosporon* sp.	—	—	+	—	—
6.	*Yeasts* (non-identified)	+	+	+	+	+
Total		3	5	6	4	4

3.2 Disinfectants agents Susceptibility Test:

3.2.1 Chlorine: Table 2 shows the result of susceptibility testing of Yeasts against the disinfectant chlorine. The results indicated that all isolates of *Candida* sp., *Geotrichum* sp. and *Rhodotorula* sp. were highly sensitive to chlorine. The MIC value for all tested strains was 0.01 % except for *Geotrichum* sp. was 0.08 %. No resistance was observed among the tested strains. These results are in line with Bianchi *et al*., (1989), who found that two chlorine derivatives were active against the *Aspergillus* and *Candida* species tested.

Table 2. MIC of Chlorine against yeasts.

		Chlorine	
No.	Fungal isolates	MIC (%)	No. of strain inhibited
1.	*Candida albicans* (2)	0.01	2
2.	*C. tropicalis*(2)	0.01	2
3.	*C. krusei* (2)	0.01	2
4.	*C. glabrata*(2)	0.01	2
5.	*Geotrichum* sp. (1)	0.08	1
6.	*Rhodotorula* sp. (2)	0.01	2

3.2.2 Chloroxylenol: Table 3 shows the result of *in vitro* susceptibility testing of Chloroxylenol against yeasts isolates. Chloroxylenol (4chloro 3, 5-dimethyphenol) **(**Detol) is a broad spectrum antimicrobial chemical compound used to control fungi, bacteria, algae and viruses. It is not significantly toxic to humans. All tested yeasts showed high sensitivity to this disinfectant. According to the *in vitro* results of dettol on Yeasts, the results revealed that the concentration of 0.1 % was the MIC for all isolates except for *Candida tropicalis* the MIC was 0.025 %. This result was in line with (Atayese *et al.,* 2010) who studied the effect of dettol on *Candida albicans* and non *albicans* species.

Table 3. MIC of Chloroxylenol against yeasts.

		Chloroxylenol	
No.	Yeasts isolates	MIC (%)	No. of strain inhibited
1.	*Candida albicans* (2)	0.1	2
2.	*C. tropicalis*(2)	0.025	2
3.	*C. krusei* (2)	0.1	2
4.	*C. glabrata*(2)	0.2	2
5.	*Geotrichum* sp. (1)	0.1	1
6.	*Rhodotorula* sp. (2)	0.1	2

3.2.3 Hydrogen Peroxides: Table 4 shows the results of in vitro susceptibility testing of Hydrogen peroxides against yeasts isolates. Hydrogen peroxide (H2O2) is bleach and an oxidizing agent that is active against a variety of microorganisms. At low concentrations, it has a variety of medicinal and domestic uses; at higher concentrations it has many commercial and industrial uses. It is also used for foot and toenail fungus infection (onychomycosis).

Regarding the effect of hydrogen peroxide on yeasts, all isolates were sensitive with different MIC values. *Candida albicans* was more sensitive than others and has MIC value 0.1 %, *Candida tropicalis* and *Rhodotorula* sp. have MIC value 0.4 % while for *Candida glabrata* it was 0.8 %. *Geotrichum sp*. had MIC value 3.2%. *Candida krusei* was more resistant than other and had MIC value of 12.8 %.

Table 4. MIC of Hydrogen peroxides against yeasts.

		H_2O_2	
No.	Yeasts isolates	MIC (%)	No. of strain inhibited
1.	*Candida albicans* (2)	0.1	2
2.	*C. tropicalis*(2)	0.4	2
3.	*C. krusei* (2)	12.8	1
4.	*C. glabrata*(2)	0.8	2
5.	*Geotrichum* sp. (1)	3.2	1
6.	*Rhodotorula* sp. (2)	0.4	2

4. CONCLUSION

It is concluded from the present study that:

1. Indoor public swimming pools are suitable environments for hosting numerous potentially pathogenic yeasts.
2. Yeasts are highly sensitive to disinfectant agents used.

REFERENCES

Aidoo , K.E. , Anderton, A. and Milligan, K.A. (1995) . Airborne mycoflora in a hospital environment. Int. J. Environ. Health Res. 5:223-228.

Aronson, I.K. and Soltani, K. (1976). Chronic mucocutaneous candidias. Mycopathologia, 60: 17–25.

Atayese, A. O., Effedua, H. I., Oritogun, K. S. , Kareem K. T. and Ogunledun A. (2010). Comparative Study of the Antimicrobial Activity of Chlorinated and Non-chlorinated Antiseptics against C. albicans. Academia Arena. 2(9).

Azizi-far, M., Kazemi-fard H., Jandaghi GhR. and Safdari M., (2006). The study of dermatophytic infections in public swimming pools of Qom city during 2004. Rah. Dan. J. Arak Uni.Med. Sci., 3(9): 67-72.

Bello, O., Mabekoje, O., Egberongbe, O. and Bello, K.(2012). Microbial Qualities of Swimming Pools in Lagos, Nigeria. Int. J.App. Sci. and Tech. 2(8):89-96.

Bianchi, P., Repetto, A., Bulletti, S., Mattiacci, P., Rossi, J., Pagiotti, R.,Ribaldi, M. and Schiaffella, F. (1989). In vitro study of the antifungal activity of two chlorine derivatives to be used in antisepsis. Ann. Ig. ;1(3-4):827-40.

Cannon, P. F. and Hawksworth, D. L. (1995). The Diversity of Fungi Associated with Vascular Plants: the known, the unknown and the need to bridge knowledge gap. Adv. in Pla. Sci. 11: 277-302.

de Hoog, G.S. and Guarro, J. (1995). Atlas of clinical fungi. Centraalbureau Schimmelcultures, The Netherlands and Universitat Rovira in Virgili, Spain.
Ellis, D.H. (1994) Clinical mycology. The human opportunistic mycosis. Pfzor. New York 166 pp.
Ellis, D., Davis, S. Alexrou, H., Handke, R. and Bartly, R.(2007). Description of medical fungi. Adeleide, Australia.
Fraise, A. P (2002) Biocide abuse and antimicrobial resistance—a cause for concern? J. Antimicrob. Chemother. 49:11-12.
Hawksworth, D. L. (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycolo.Reas. 95: 641-655.
Hoseinzadeh, E., Mohammady, F., Shokouhi, R., Ghiasian, S. A., Roshanaie, G., Toolabi, A. and Azizi, S. (2013). Evaluation of biological and physico-chemical quality of public swimming pools, Hamadan (Iran). Int. J. Env. Health. Eng., 2(1), 21.
Itah, A. Y. and Ekpombok, M. U. E. (2004). Pollution status of swimming pools in south-south zone of south-eastern Nigeria using microbiological and physicochemical indices. Southeast Asian J. Trop. Med. Public Health, 35, 488- 493.
Larone, D. (2011). Medically Important Fungi: A Guide to Identification. Ed. (5). Washington, DC: ASM Press.
Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M.T., Olaizola, C., and Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. Inter. J. Infec. Dis. 8:39-45.
Mohammad, T.H. and Habeb, K.A. (2014). Epidemiological Study of Keratinophilic Fungi in Baghdad Swimming Pools. J. B. f. S.11(3).
Nanbakhsh, H., Diba, K., and Hazarti, K. (2004). Study of fungal contamination of indoor public swimming pools. Iran. J. Pub. Heal. 33(1), 60-65.
Odds F., (1988). (2nd ed). *Candida* and Candidiasis. Baillière Tindall.
Papadopoulou, C., Economou, V., Sakkas, H., Gousia, P., Giannakopoulos, X., Dontorou, C., and Leveidiotou, S. (2008). Microbiological quality of indoor and outdoor swimming pools in Greece: investigation of the antibiotic resistance of the bacterial isolates. Int. J. Hyg. Environ., 211, 385-397.
Robins, C. and Morell, J. (2007). Mold Housing and Wood. In: Western Wood Products Association.
Viegas, C., Alves, C, Carolino, E., Pinheiro, C., Rosado, L. and Santos, C.S.(2011). Assessment of fungal contamination in a group of Lisbon's Gymnasiums with a swimming Pool. Ita. J. Occup. Env. Hyg. 2(1):15 – 20.
Viegas, C., Alves, C., Carolino, E., Rosado, L. and Silva Santos, C. (2010). Prevalence of fungi in indoor air with reference to gymnasiums with swimming pools. Ind. Bu.env. 19(5):555–561.

کورتیا لێکولینێ:

ئەڤ لێکولینا ناڤبری هاتە ئەنجامدان ئارمانجێت سەرەکی ل ڤێ ڤەکولینێ دا بریتی بوون ل دویف چون لسەر ریژا پیسبونێ ب کەرووان د برگێن مەلەڤانگەهێن گشتی دا ل باژیرێ دهوکێ و لێکولین لسەر کاریگەریا هندەك دەرمانێن پاقژکەر لسەر هندەك جورێت کەرووا. برکێن مەلەڤانگەهێن گشتی د ماوێ بینج هەیڤادا (ژ هەیڤا ایلون ٢٠١٤ هەتا کانونا دو ٢٠١٥ .٢٣٠ نموونە ٥٠ ژ ئافا برکا مەلەڤانگەها و ١٨٠ ل جهێن جوراوجور هاتنە وەرگرتن ل پێنج برکێ مەلەڤانگەهێن گشتی هەمی جورە تاقیکرنێت تایبەت ب کەرووا بۆ هەمی نموونا هاتنە ئەنجامدان ب پشت بەستن لسەر سێ تەکنیکا و سێ جورە ناوەندێن خوراکی تایبەت بە کەرووا Chlorine, Chloroxylenol و (H_2O_2)). سێ تەکنیکا و سێ جورە ناوەندێن خوراکی تایبەت بە کەرووا (Potato Dextrose Agar, Malt Extract Agar و Sabouraud's Dextrose Agar . هەمی جورە تاقیکرنێت تایبەت ب کەرووا بۆ هەمی نموونا هاتنە ئەنجامدان ب پشت بەستن لسەر و سێ جو پاقژکەرا هەمی جورە کەرووێن هاتینە تاقیکرن یێت هەستیاربوون ب ریژەیەکا بلند بەرامبەر chlorine, Chloroxylenol و H_2O_2. ریژا چریا هەستیاریێ یا دەرمانێ chlorine بۆ زوربەی نموونەیا ٠,٠١% بوو. وبو Chloroxylenol لسەر کەرووا ل ٠,٠٢٥ % بوو , لێ ریژا چریا ٠,١% یا کاریگەربوو لسەر هەمی جورێت کەروا تنێ *Candida tropicalis* ریژا چریا وێ یا بریتی بوو ل ٠,٠٢٥ %. دەرمانێ H_2O_2 یێ کاریگەربوو لسەر هەمی جورێت کەرووێن هاتینە تاقیکرن ب ریژا ٠,١ % تنێ هەر ئێ ژ و *Candida glabrata* هەستیار بوون هەتا ریژا ١٢,٨ %.

خلاصة البحث:

أجريت الدراسة الحالية لدراسة مستوى تلوث الخمائر في احواض السباحة العامة المغلقة في مدينة دهوك. تم جمع ٢٣٠ عينة (عينات المياه ٥٠) مأخوذة من مياه احواض السباحة وأخذت (١٨٠ مسحه عينات) من الجدران، وحمامات وغرف تبديل الملابس في أحواض السباحة العامة المغلقة من أيلول ٢٠١٤ إلى شباط٢٠١٥. تم فحص جميع عينات لوجود الخمائر استخدام ثلاث تقنيات عزل اختيارية (أجار أجار سكر العنب البطاطا والشعير استخراج أجار سابورو لسكر العنب). وكان أعلى عدد من أجناس الخميرة في تشرين الثاني (٦) تليها في تشرين الأول (٥)، كانون الأول وكانون الثاني (٤) بينما كان أقل عدد في أيلول (٣). أحد عشرمن الخمائر اختيرت عشوائياً للحساسية ضد المعقمات أظهرت جميع العزلات المختبرة حساسية عالية للمعقمات (chlorine, ChloroxylenolوH_2O_2) التركيز المثبط الأدنى للكلورين ٠,١ بينما كان التركيز المثبط الادنى للكلوروزيلينول ٠,٠١ ما عدا ل *Candida tropicalis and C.glabrata* ، بينما للمعقم الاخير تراوحت بين ٠,١ الى ١٢,٨%.