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Occurrence and Rapid Enzymatic Detection of *Candida* spp. in Drinking Water; A Correlation with Bacterial Indicators and Physicochemical Parameters.

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ABSTRACT

Drinking water is essential for life of humans and other creatures on the earth. Drinking water can be an important carrier for pathogenic microorganisms and chemicals which led to dangerous human diseases. Correct, rapid, sensitive and continuous monitoring of microbial and physicochemical drinking water quality is essential for keeping safe drinking water. This study investigates the rapid enzymatic detection and occurrence of *Candida* spp. and bacterial indicators (total coliforms, *E. coli*, enterococci) using chromogenic media in distribution system drinking water samples. Also, physicochemical quality of drinking water was examined. The correlation between microbial and physicochemical parameters was studied. Results showed occurrence of *Candida* spp and bacterial indicators in four seasons with higher average counts (CFU/100 ml) in summer. Physicochemical quality of drinking water was complied with permissible limits. Chromogenic media proved rapid monitoring tool for detection of *Candida* spp. and bacterial indicators without confirmatory tests required. Significant correlation (*r*) observed between average counts of *Candida* spp. and both bacterial indicators and most of examined physicochemical parameters.

Keywords: Chromogenic media, Candida, physicochemical quality, drinking water.

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INTRODUCTION

Yeasts, including *Candida* spp., are a significant component of the microbiota of most natural aquatic ecosystems and also can occur in drinking water distribution systems as a result of their ability to survive treatment practices and become incorporated into biofilms [1-3].Most of these microorganisms have no known human health effect. However, a small number of species, especially the anamorphic genus *Candida*, are important opportunistic pathogens [3]. Infections due to *Candida* spp. have increased in recent years and are of particular importance due to rising numbers of immunocompromised and hospitalized patients. *Candida albicans*is the main cause of approximately 90% of candidiasis; however, other Candida species including; *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*are also have the ability to cause candidiasis [4-6]. Direct monitoring of pathogenic microorganisms in drinking water sometimes is difficult because (i) pathogens have low concentrations in source and treated water (ii) detection methods are time-consuming and have a low recovery efficiency. The microbiological quality of drinking water is assessed by monitoring of non-pathogenic bacteria (bacterial indicators). An indicator organism is an organism that can provide information about the health of a water body through the organism's presence, condition, or numbers. These bacterial indicator include total coliforms, *Escherichia coli*, enterococci and *Candida* spp. The ideal microbial indicator organism should occur where pathogens do, occur in greater quantity, be more resistant to disinfection than pathogens and should be easily isolated and enumerated. In drinking water treatment plants, total coliforms, *Escherichia coli* and enterococci are widely used as indicators of fecal contaminations of water [7-10]. Application of enzyme-based detection technology (chromogenic media) facilitates a rapid and sensitive detection and enumeration tool of *Candida* spp. and bacterial indicators, compared to standard cultivation-based methods. These media contain chromogenic substrates which react with enzymes secreted by targeted microorganisms producing colonies with various pigmentations. These enzymes are species specific, allowing organisms to be identified to the species level by their color and colony characteristics [11]. The aim of the present work is to study the occurrence and rapid enzymatic detection of four *Candida* spp. (*Candida albicans*, *Candidaglabrata*, *Candida krusei* and *Candida tropicalis*) in drinking water distribution system, and to study the relationship between occurrence of *Candida* spp. and other bacterial indicators (total coliforms, *E. coli* and enterococci) and some physicochemical quality of drinking water.

MATERIALS AND METHODS

Samples and sampling

A 456 drinking water samples from distribution system were collected according to standard guidelines [12] for one year (four seasons), Egypt. All samples were collected in sterile 1L polypropylene bottle (Thermo Scientific®, USA) and stored at 4°C for transportation to the laboratory. Samples were examined within 6h after collection.

Microbiological examination

Collected drinking water samples were examined for detection and enumeration of *Candida* spp., total coliforms, *E. coli* and enterococci using rapid enzymatic media. Membrane filtration method using sterile cellulose nitrate membrane filter (Whatman®, 47mm diameter, 0.45µm pore size) was used according to (APHA) [12]as follow:

A- *Candida* spp.:

The chromogenic medium, HiCrome Candida Differential Agar (CDA) (Himedia®, India), was used for detection and enumeration of *Candida* spp. in drinking water samples. CDA has the ability to detect and enumerate four species of Candia including; *Candida albicans*, *Candidaglabrata*, *Candida krusei* and *Candida tropicalis*. Each *Candida* spp. appear with different morphology (appearance and color) as following; *C. albicans* (light green smooth), *C. glabrata* (cream to white smooth), *C. krusei* (purple fuzzy) and *C. tropicalis* (blue to metallic blue raised). A 42.72 grams of dehydrated CDA medium was suspended in 1000 ml distilled water and brought to boiling to dissolve the medium completely. CDA media does not require autoclaving. The medium then cooled to 50°C and poured into sterile Petri dishes and left to solidify. A 100 ml of each drinking water sample was filtered. After filtration, membrane filters were placed on the surface of solidified CDA Petri dishes avoiding air bubbles under membrane filter and then incubated at 30°C for 40-48 hours.

B- Total coliforms and *Escherichia coli*:

Rapid Hicoliform agar (RHA) (Himedia®, India) was used as a rapid enzymatic medium for detection and enumeration of both total coliforms and *E. coli* at the same Petri dish. Both yellow and blue-green colonies are counted as total coliforms, while the blue-green colonies with blue fluorescence light using UV lamp (366 nm) are considered as *E. coli*. A 31.03 g of the dehydrated medium was suspended in 1000 mL distilled water, heated to boiling to dissolve the media completely, then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Inoculated Petri dishes were incubated at 37°C for 24 h.

C- Enterococci:

The chromogenic Rapid Hi-Enterococci agar (RHE) (Himedia, India) was used for rapid detection and enumeration of Enterococci from water samples. Enterococci colonies appear as blue green colonies. A 33.61 g of the dehydrated medium was suspended in 1000 ml distilled water, heated to boiling to dissolve the medium completely, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes, cooled to 50°C and poured into sterilized Petri dishes. Inoculated Petri dishes were incubated at 37°C for 24 h.

Physicochemical analysis:

Some physicochemical parameters were measured in drinking water samples. All parameters were measured according to (APHA) [12]. The measured parameters and measurement methods were summarized in Table (1).

Table 1: Measured physicochemical parameters of drinking water samples.

Parameters	Unit	Methodology
Residual chlorine.	mgL^{-1}	N,N-diethyl-p-phenylenediamine (DPD).
Turbidity.	NTU	Nephelometric method.
Temperature.	°C	Non-depth temperature measurement.
Conductivity.	µs/cm	Nonplatinum-electrode.
Total Dissolved Solids (TDS).	mgL^{-1}	APHA, 2005
Ammonia (NH_4).	mgL^{-1}	Spectrophotometeric.
Nitrite (NO_2)	mgL^{-1}	Spectrophotometeric.
Nitrate (NO_3)	mgL^{-1}	Ultraviolet spectrophotometric screening.
Total alkalinity	mgL^{-1}	Titration.
pH	-	Electrometric pH measurement.
Sulfate (SO_4)	mgL^{-1}	Turbidimetric.
Phosphate (PO_4)	mgL^{-1}	Spectrophotometeric.
Dissolved Oxygen (DO)	mgL^{-1}	Electrometric.

Statistical analysis:

Pearson's correlation coefficient (*r*) test was carried out for studying the correlation between occurrence of the four *Candida* spp., and both of bacterial indicators and measured physicochemical parameters, in drinking water samples. Statistical analysis was carried out using Microsoft Excel® (2013) program.

RESULTS AND DISCUSSION

Microbial analysis

One of the most important parameters for better quality of drinking water is to be safe from microbial contamination, especially, pathogenic microorganisms such as bacteria, viruses, parasites and yeasts. These pathogens have the ability to cause waterborne diseases and outbreaks.

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Obtained results in Figure (1), indicated the occurrence of the four *Candida* spp. (*Candida albicans*, *Candidaglabrata*, *Candida krusei* and *Candida tropicalis*) in drinking water samples. Distribution system samples showed variations in counts of the four *Candida* spp., *C. albicans* was the highest occurrence, followed by *C. glabrata*, *C. krusei* and finally *C. tropicalis* which showed the lowest occurrence. Summer season showed the highest counts of *Candida* spp., while winter season showed the lowest counts. Muittari [13] and Hageskal [14], reported outbreaks of symptoms similar to hypersensitivity pneumonitis after saunas, baths, showers, laundering and dishwashing. These symptoms were related to fungal contamination of water such as *Candida* spp. Servais [15]and Yamaguchi [16], proved the appearance and colonization of drinking water distribution systems by different saprophytic microorganisms such as yeasts and *Candida* spp., bacteria and fungi, which were able to grow using biodegradable organic materials inside distribution system pipes. Yamaguchi [16], in his work, discovered that *Candida* spp. was the most prevalent yeast isolates from tap and mineral drinking water samples, *C. parapsilosis* was the predominant yeast identified, followed by *C. glabrata* and *C.albicans*. Candida Differential Agar (CDA) medium appeared as a promising medium not only, for direct and rapid detection and enumeration of *Candida* spp. from drinking water, but also, as a differential tool between four *Candida* spp. depending on specific enzyme theory which led to different morphological characteristics of the four species without any confirmatory tests. Many authors [6,11,17,18] used different commercial chromogenic media and reported suitability of enzymatic based methods (chromogenic media) for rapid detection, enumeration, differentiation and identification of *Candida* spp. from water, food, blood and other clinical samples.

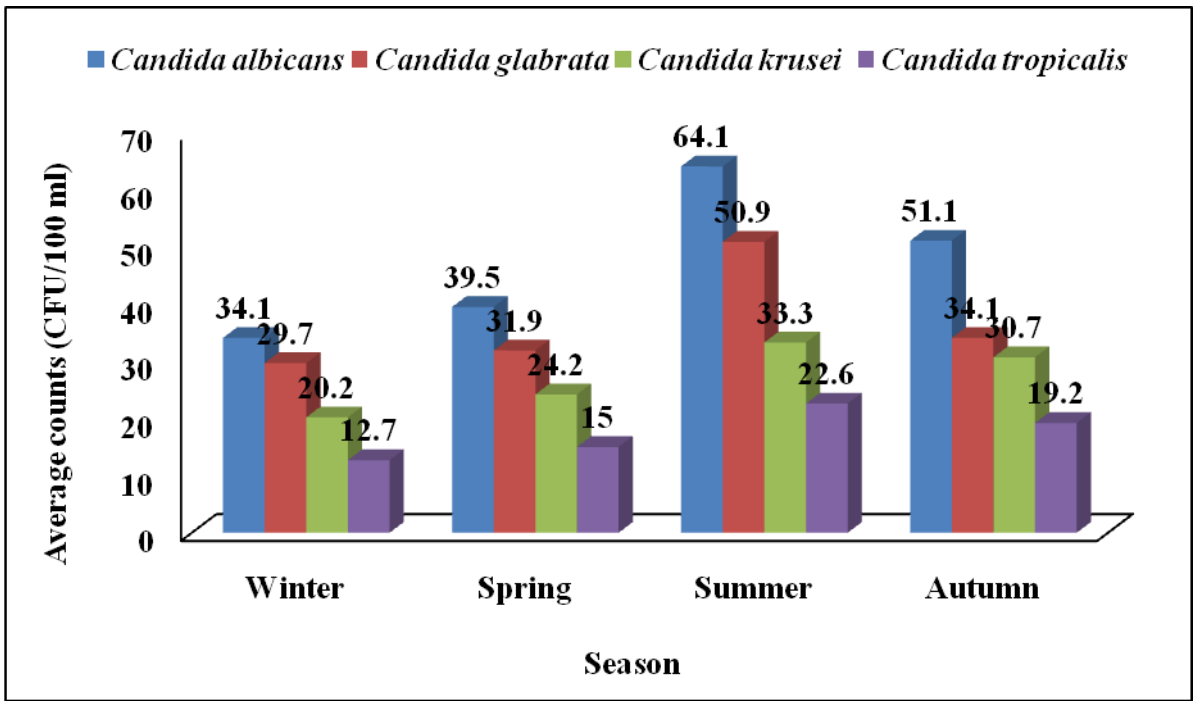

Fig 1: Occurrence of four *Candida* spp. in drinking water.

Classical bacterial indicators such as total coliforms, *E. coli* and enterococci have long been used as certified and routine indicators for fecal pollution of drinking water and food. Presence of such indicators in drinking water considered as an index of the presence of fecal materials and the possibility of presence of pathogenic microorganisms [8,19]. All studied bacterial indicators were observed in most of collected drinking water samples from distribution system during four seasons. From Figure (2), it can be observed that, average counts of these bacterial indicators behave the same behavior such as *Candida* spp., since, higher counts of total coliforms, *E. coli* and enterococci were observed during summer season and lowest counts were during winter season. This may be attributed to the high temperature of drinking water during summer season in Egypt, which encourage growth and reproduction of bacteria in water. Occurrence of such bacterial indicators in the distribution system indicate the presence of fecal pollution in drinking water, this may be due to, cracks in the old pipes which allow contamination with soil and neighbor sewerage system pipes, or un regularly distribution system washing and disinfection which allow occurrence of biofilm inside pipes. The ability of

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chromogenic media (called defined substrate technology) for detection and enumeration of total coliforms and *E. coli* from water and different types of samples such as food and soil were reported by many authors [7,8,19-23].In addition, Al-wasify [18], demonstrated the high sensitivity and specificity of chromogenic media for detection and enumeration of some pathogens in water. Pearson's correlation coefficient (*r*) statistical analysis results proved the significant correlation between average counts of both studied *Candida* spp. (*Candida albicans*, *Candidaglabrata*, *Candida krusei* and *Candida tropicalis*) and other bacterial indicators (total coliforms, *E. coli* and enterococci) in distribution system drinking water (Table 3). Also, Figures (1 & 2) showed the same behavior for *Candida* spp. and bacterial indicators in relation to seasonal variations.

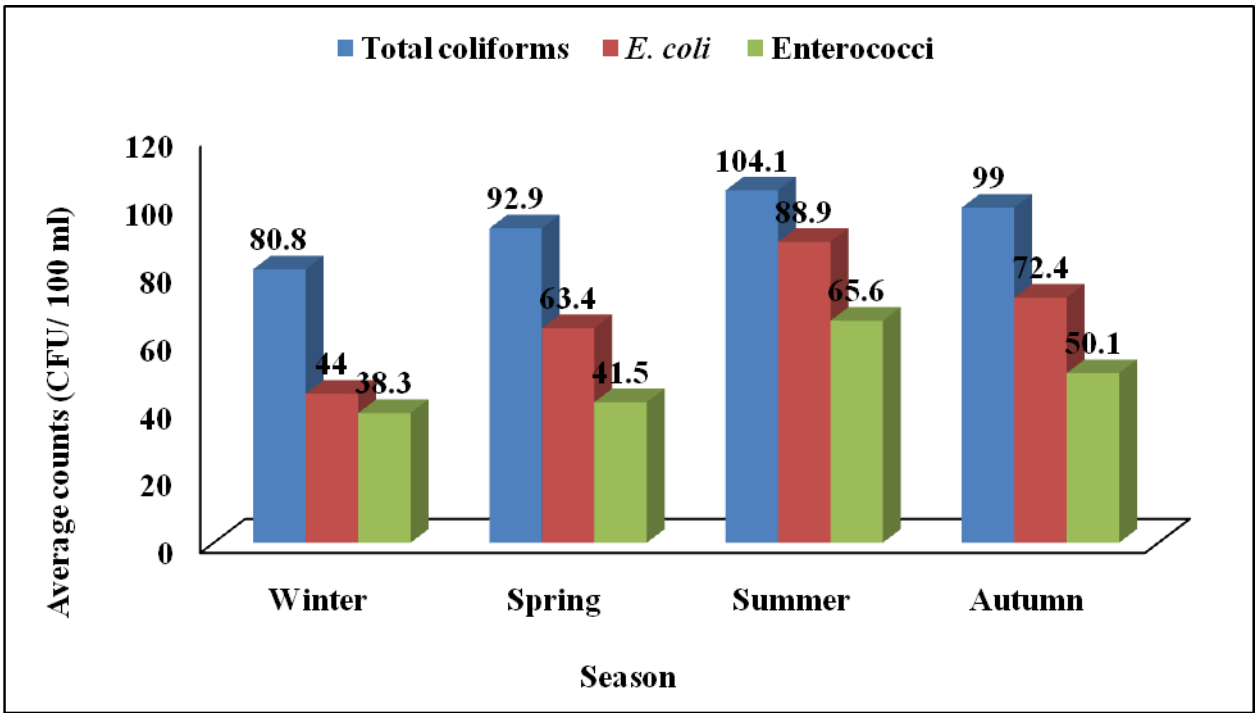

Fig 2: Occurrence of bacterial indicators in drinking water.

Physicochemical analysis:

Physicochemical quality of drinking water is one of the most important criteria for safe human consumption of water for drinking and other daily activities such as cooking and bathing. Average values of some measured physicochemical parameters of distribution system drinking water were summarized in Table (2). Averages of all physicochemical parameters were within the permissible limits according to Egyptian standard methods for drinking water (2007). Statistical analysis results using Pearson's correlation coefficient (*r*) between occurrences of *Candida albicans*, *Candidaglabrata*, *Candida krusei* and *Candida tropicalis*, and measured physicochemical parameters of distribution system drinking water samples were summarized in Table (4). Results showed significant correlation between occurrence of *Candida* spp. and some parameters including; temperature, conductivity, total dissolved solids (TDS), sulfate and dissolved oxygen (DO). However, there was weak and moderate correlation with the rest of measured parameters. Habuda-Stanić [24] and Mostafa [25], proved that, there was correlation between microbiological and physicochemical quality of drinking water. They reported that, there was an observed relationship between average values of some microbial parameters such as total coliform, *Escherichia coli*, total bacterial count at 37°C and 22°C, enterococci and *Clostridium perfringens* and the average values of five physicochemical parameters including temperature, turbidity, pH, free residual chlorine and ammonia, in public and municipal drinking water supply in Croatia.

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Table 2: Average values of measured physicochemical parameters in drinking water samples

Parameters	Unit	Average values per season				Permissible limits
		Winter	Spring	Summer	Autumn	
Residual chlorine	mgL^{-1}	1.33	1.58	1.28	1.31	5.0
Turbidity	NTU	0.56	0.53	0.57	0.97	1.0
Temperature	°C	19.64	23.45	25.82	21.95	-
Conductivity	µs/cm	471.73	416.31	361.34	451.45	-
Total Dissolved Solids (TDS)	mgL^{-1}	311.35	274.77	238.49	297.95	1000
Ammonia	mgL^{-1}	0.23	1.61	0.13	0.35	0.5
Nitrite (NO_2)	mgL^{-1}	0.04	0.02	0.02	0.06	0.2
Nitrate (NO_3)	mgL^{-1}	0.11	0.02	0.08	0.09	45
Total alkalinity	mgL^{-1}	129.45	122.38	117.98	130.3	-
pH	-	7.29	7.28	7.27	7.3	6.5-8.5
Sulfate (SO_4)	mgL^{-1}	49.68	43.38	35.82	42.06	250
Phosphate (PO_4)	mgL^{-1}	0.01	0.03	0.03	0.06	-
Dissolved Oxygen (DO)	mgL^{-1}	8.64	8.36	7.82	8.4	-

Table 3: Statistical analysis between *Candida* spp. and bacterial indicators

Candida spp. / Bacterial Indicators	Pearson's correlation coefficient (*r*)			
	Candida albicans	*Candidaglabrata*	*Candida krusei*	*Candida tropicalis*
Total coliforms	0.9268	0.7835	0.9705	0.9521
E. coli	0.9635	0.8755	0.9655	0.9726
Enterococci	0.9897	0.9703	0.9234	0.9708

Table 4: Statistical analysis between *Candida* spp. and physicochemical parameters

Candida spp. / Parameters	Pearson's correlation coefficient (*r*)			
	Candida albicans	*Candidaglabrata*	*Candida krusei*	*Candida tropicalis*
Residual chlorine	-0.5159	-0.4612	-0.4462	-0.4884
Turbidity	0.2394	-0.1254	0.4330	0.3158
Temperature	0.8111	0.8508	0.7634	0.8008
Conductivity	-0.7861	-0.8972	-0.6875	-0.7553
Total Dissolved Solids (TDS)	-0.7862	-0.8972	-0.6877	-0.7554
Ammonia	-0.4283	-0.4125	-0.3370	-0.3915
Nitrite (NO_2)	-0.1386	-0.4339	0.0174	-0.0810
Nitrate (NO_3)	0.0698	0.0454	0.0043	0.0400
Total alkalinity	-0.5786	-0.773	-0.4498	-0.5348
pH	-0.4305	-0.7023	-0.2545	-0.3666
Sulfate (SO_4)	-0.9490	-0.8986	-0.9319	-0.9507
Phosphate (PO_4)	0.4603	0.1045	0.6647	0.5445
Dissolved Oxygen (DO)	-0.9140	-0.9703	-0.8264	-0.8876

CONCLUSION

The obtained results during the present study indicated the ability and suitability of chromogenic media for rapid detection and enumeration of *Candida* spp. in water. Also, there was a correlation among occurrence of *Candida* spp. in water samples and occurrence of both some classical bacterial indicators (total coliforms, *Escherichia coli* and enterococci) and physicochemical quality of water.

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