Injured coliforms in swimming pools : How big a threat?

Original Article

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ABSTRACT

Background: A swimming pool is an important leisure facility, but it can harbor injured cells creating potential health hazards. Disinfection of swimming pools can cause bacterial injury, when cells are exposed to a suboptimal concentration of disinfectants. Possible pathogenic bacteria can enter into an injured state, for example, Escherichia coli, Klebsiella spp, and Enterococcus faecalis. Injured bacteria can retain their pathogenicity and virulence and they may recover causing diseases.

Aim: To assess the presence of injured coliforms in swimming pools based on differential plating media.

Materials and Methods: This study compared the difference in recovery of coliforms between two differential media, one designed for recovery of injured coliforms (HiCrome ECC selective agar and the other is CHROMagar ECC). A total of 120 samples were collected from 10 semi-public swimming pools with sporadic distribution around Alexandria, Egypt, and included in this study. Five pools were used for swim training, 4 were used for both training and recreational swimming and one was used for children only.

Results: The recovery medium (HiCrome ECC selective agar) detected 1.47 and 2.54 times total coliforms and E. coli, respectively, as CHROMagar ECC. The compliance of samples per fresh water swimming pool Egyptian standard in total coliforms and E. coli fell from 54.10% when examined by CHROMagar ECC medium to 35% by HiCrome ECC selective medium.

Conclusion: The current findings may not be universal to all swimming pools but may be applicable to ones where the physicochemical properties of their water induce coliform injury. Results suggest that the use of media that detect injured yet viable coliforms will give a more sensitive and representative guidance about the quality of examined water and will assist in the treatment and decontamination of swimming pools.

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INTRODUCTION

Bacterial injury results from exposure of bacterial cells to different factors that cause sub-lethal damage to the cell. There are many factors that can cause cell injury including environmental, biological, or human factors. Environmental factors include natural factors such as sunlight, salinity, and temperature. Biological interactions and competition between bacterial species are also documented[1].

A swimming pool is an important leisure facility for a large segment of the population, and it can harbor injured cells creating potential health hazards[2]. Disinfection of swimming pools can cause bacterial injury, when cells are exposed to a suboptimal concentration of disinfectants. Previous studies have shown chlorine and similar disinfectants used in swimming pools to cause injury in Escherichia coli that could not be detected on routine media used for water analysis[3]. Laboratory handling of samples can also lead to cell injury like prolonged storage time and exposure to hot agar media. Injured bacteria require more nutrition for regrowth and can regain their normal phenotype under favorable growth conditions[4,5].

The problem with injured bacteria is that they can retain their pathogenicity and virulence and they may also recover in the host's gastrointestinal tract[6]. Possible pathogenic bacteria can enter into an injured state, for example, E. coli, Klebsiella spp, Enterococcus faecalis, Salmonella spp, and Staphylococcus aureus[4,7]. Pathogenic bacteria may be more resistant than indicator bacteria to chlorine and may even overgrow indicator bacteria, creating a veiled threat[8]. Moreover, a previous study revealed that injured bacteria can also harbor antimicrobial resistance[9].

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Indicator bacteria used for routine examination of swimming pools are exposed to stressors that may introduce them to an injured state. Routine media may fail to detect these injured bacteria and they might go undetected causing a possible threat to pool users[3,10].

This study aimed to assess the presence of injured bacteria in swimming pools based on differential plating media. Water samples were examined for simultaneous detection of total coliforms (TC) and E. coli using selective recovery differential media (HiCrome ECC Selective Agar) and selective differential media (CHROMagar ECC), denoting occurrence of injured coliforms.

MATERIALS AND METHODS

Sample collection

The present study was carried out during a 2-month period from the beginning of June 2014 till the end of July 2014. A total of 10 swimming pools designated A–H (three indoor and seven outdoor) with sporadic distribution around Alexandria, Egypt, were included in this study. Five pools were used to swim training, four were used for both training and recreational swimming, and one was used for children only. All pools were semipublic swimming pools that have specific entry restrictions. In total, 120 samples were collected, 12 from each one. Using EPINFO version 6, using a prevalence of TC of 7% and allowing for a permissible error of 5% around the expected prevalence and using a 5% level of significance and a 95% confidence limit, the minimum required sample size amounted to 101 and was rounded to 120 for increased accuracy.

The study was approved by the Ethics Committee of the High Institute of Public Health, and a written consent was taken from each swimming pool manager. Water samples were collected in the area of, and during the time of maximum bather density, away from fresh water supply. Collection was performed according to standard guidelines [11,12].

Microbial examination

Enumeration of TC and E. coli using both CHROMagar ECC (CHROMagar, Paris, France) and HiCrome ECC selective agar (HiMedia, Mumbai, Maharashtra, India) was done by membrane filtration technique. The plates were inverted and incubated at 37°C for 24 h. Plates showing 20–80 coliform colonies and not more than 200 colonies of all types per membrane were counted using Quebec colony counter. On CHROMagar ECC, typical TC colonies were mauve and typical E. coli were blue, whereas on HiCrome ECC agar, typical TC colonies were salmon to red and typical E. coli colonies were dark-blue to violet. E. coli colonies on HiCrome ECC selective agar were verified by adding a drop of Kovac's reagent on colonies that were dark-blue to violet in color. Formation of cherry-red color indicated a positive reaction for indole reaction[12,13].

According to Egyptian standards regarding fresh water swimming pool standard, samples were recorded as complying when neither TC nor E. coli were isolated from 100 ml of water sample[14].

A colorimetric method designed for field determination of free residual chlorine in water using orthotolidine as the color indicator was used for chlorine measurement as well as pH.

Calculations

Recovery ratio

The recovery ratio was represented by mean count of indicator CFU/100 ml on HiCrome ECC selective medium/ mean count of indicator CFU/100 ml on CHROMagar ECC medium.

Injured total coliforms count

It was represented by subtraction of the total count on CHROMagar ECC medium from total count on HiCrome ECC selective medium.

Percent of injured total coliforms

It was obtained by dividing injured TC count on HiCrome ECC selective medium count times 100.

Injured E. coli count

It was obtained through subtraction of the E. coli count on CHROMagar ECC medium from E. coli count on HiCrome ECC selective medium.

Percent of injured E. coli

It was obtained through dividing injured E. coli count on HiCrome ECC selective medium count times 100.

RESULTS

The HiCrome ECC selective medium recovered 1.47 times TC as CHROMagar ECC medium and 2.54 times E. coli as CHROMagar ECC medium, where the mean counts of TC were 212.35 and 312.98 CFU/100 ml, and E. coli mean counts were 10.08 and 25.65 CFU/100 ml, respectively (Table 1).

Table 1: Recovery ratio of total coliforms on CHROMagar ECC and HiCrome ECC media from swimming pools, Alexandria, Egypt, 2014

Indicator	Mean value of CFU/100 ml on CHROMagar ECC medium	Mean value of CFU/100 ml on HiCrome ECC selective medium	Recovery ratioa
TC	212.35	312.98	1.47
E. coli	10.08	25.65	2.54

The compliance of samples to fresh water swimming pool Egyptian standard (both should be not detectable/100 ml) was lower when examined with HiCrome ECC selective medium. CHROMagar ECC medium showed that 65 (54.1%) samples complied with standards when measuring either of TC or E. coli, whereas with HiCrome ECC selective medium, only 42 (35%) were complying. These differences were statistically significant (P=0.003). When considering both indicators, 30 (25.0%) samples were not complying by HiCrome ECC selective but were complying by CHROMagar ECC, whereas only seven (5.9%) were noncomplying by CHROMagar ECC but complying by HiCrome ECC. The two tested media showed 69.2% agreement (Table 2).

Table 2: Agreement between the yield of the two used media in relation to the microbiological parameter limits of the Egyptian Decree No. 418/1995 for Fresh Water Swimming Pool Standards

	CHROMagar ECC [n (%)]			
HiCrome ECC selective	Complying samples	Noncomplying samples	Total	% Agreement
Noncomplying samples	30 (25.0)	48 (40.0)	78 (65.0)	69.2
Complying samples	35 (29.1)	7 (5.9)	42 (35.0)	
Total	65 (54.1)	55 (45.9)	120 (100)	

Table 3 shows that there was a strong negative relation between residual chlorine and the recovery rates of TC on CHROMagar ECC and HiCrome ECC selective media (P=0.001 and 0.002), whereas a strong positive relation was found between recovery rates of both media with pH (P=0.002 and 0.030).

Tables 4 and 5 show injured TC and E. coli in individual swimming pools. The maximum percent of injured TC was found in swimming pool B (92.8%) and the minimum in swimming pool A (8.7%), and the overall injured TC in the study was 30.4%. The maximum percent of injured E. coli was found in swimming pools C, E, F and G (100%) and the minimum in swimming pool H (18.82%), and the overall injured E coli in the study was 60.7%.

Residual chlorine increased the percentage of injury among TC. Samples with residual chlorine at least 1.5 showed the highest percentage of TC with at least 75% injury (45.71%) (Table 6).

Table 3: Correlation between total coliforms recovery rates of CHROMagar ECC and HiCrome ECC selective media with residual chlorine and pH

	Residual chlorine		pH	
Total coliforms	rs	*P*	rs	*P*
CHROMagar ECC	-0.403	0.001*	0.281	0.002*
HiCrome ECC selective	0.282	0.002*	0.198	0.030*

rs: Spearman coefficient, *$P<0.05$, significant.

Table 4: Distribution of injured total coliforms in the 120 studied swimming pool water samples, Alexandria, Egypt, 2014

Swimming pools	Injured TC CFU/100 ml[a]	%Injury[b]
A	4.90x10	8.70
B	$3.50x10^2$	92.80
Cc	$-6.50x10^2$	-29.80
D	$3.00x10^2$	46.60
E	2.60x10	31.30
F	8.30x10	85.50
G	4.50x10	11.50
H	$17.20x10^2$	27.90
I	4.20x10	11.20
J	$63.00x10^2$	38.80
All swimming pools	8265	30.40

TC, total coliforms.
[a]Injured TC count calculated as subtraction of the total count on CHROMagar ECC medium from total count on HiCrome ECC selective medium.
[b]Percent of injured TC calculated by dividing injured TC count on HiCrome ECC selective medium count times 100.
[c]Fungi were frequently isolated from this pool on HiCrome ECC agar medium.

Table 5: Distribution of injured E. coli in the 120 studied swimming pool water samples, Alexandria, Egypt, 2014

Swimming pool	Injured E. coli CFU/100 ml[a]	%Injury[b]
A	5.30x10	20.54
B	$4.35x10^2$	99.08
C	2.26	100.00
D	2.80x10	75.67
E	3	100.00
F	4.00x10	100.00
G	1	100.00
H	11.0x10	18.82
I	3.20x10	30.47
J	9.40x10	67.69
All swimming pools	798.26	60.7

E. coli, Escherichia coli.
[a]Injured E. coli count calculated as subtraction of the E. coli count on CHROMagar ECC medium from E. coli count on HiCrome ECC selective medium.
[b]Percent of injured E. coli calculated by dividing injured E. coli count on HiCrome ECC selective medium count times 100.

Table 6: Relation between residual chlorine and percent of injured total coliforms in 113 studied swimming pool water samples[a]

	Residual chlorine [n (%)] (ppm)			
TC injury (%)	<1	1	>1.5	Total
No injury	16 (30.76)	22 (42.30)	14 (26.92)	52 (46.01)
Injury				
5 (%)	2 (20.00)	4 (40.00)	4 (40.44)	10 (8.85)
25 (%)	3 (37.50)	5 (62.50)	0 (0.00)	8 (7.07)
50 (%)	4 (50.00)	4 (50.00)	0 (0.00)	8 (7.07)
75+ (%)	5 (14.30)	14 (40.00)	16 (45.70)	35 (31.00)
Subtotal	14 (22.95)	27 (44.26)	20 (32.79)	61 (53.99)
Total	30 (26.50)	49 (43.40)	34 (30.10)	113 (100)

TC, total coliforms.
[a]Seven samples were omitted because CHROMagar ECC showed more yield than HiCrome ECC selective agar.

DISCUSSION

The present study investigated the presence of injured coliforms in swimming pools as one of the chlorinated aquatic systems. To our knowledge, most previous studies, concerned with injured coliforms in aquatic systems, examined the recovery of injured cells from sources of water other than swimming pools.

Microbiological assessment of swimming pools depends on coliforms as indicator bacteria whose presence indicates fecal contaminations. The current study used HiCrome ECC as selective agar that recovers injured TC and E. coli, where it recovered 1.47 and 2.54 times as CHROMagar ECC, respectively, and their results agreed in 69.2% of the samples. Similar recovery media were previously used to detect injured coliforms in previous work[15,16].

Several studies surveyed swimming pools water quality based on their countries standards' or WHO standards; most of them, including Egypt, adopted zero tolerance to TC and E. coli[17–20]. By studying the microbiological quality, according to presence/absence of TC and E. coli, HiCrome ECC selective agar revealed that 78 (65%) of 120 studied samples were noncomplying, and the CHROMagar ECC revealed that only 55 (45.9%) samples were noncomplying. CHROMagar ECC failed in detecting 23 samples, which could present public health threat. Both media gave a higher percentage than results previously reported in Egypt, where 43.3% of samples were noncomplying[17].

However, in one of the examined pools, seven samples of the total 12, yielded fungal colonies on HiCrome ECC agar and not CHROMagar ECC. This may be attributed to the following factors: Tergitol 7 (contained in HiCrome ECC agar) supports the growth of fungi[21]. Moreover, 11 samples of this pool had a pH. more than 7.8. Some investigators discovered types of fungi tolerant to this alkaline environment[22].

Almost 61 (53.99%) of the studied samples showed injured coliforms, with 35 (31%) yielding 75–100% injured cells. This result nearly matched that of Du Preez *et al.*[23], in South Africa, who found that 56% of the chlorinated potable water samples revealed 100% injury. Indeed, the present results showed samples that yielded 75–100% injured cells and were directly proportional with residual chlorine and inversely proportional with pH, where of these 35 samples, 16 (45.7%) had residual chlorine more than 1.5 ppm and 21 (60%) had pH less than 7.2. The problem is that these cells can regrow in case the chlorine level decreases[5].

CONCLUSION

The current findings may not be universal to all swimming pools in Alexandria but may be applicable to the ones where the physicochemical properties of their water induce coliform injury. Results suggest that the use of media that detect, injured yet viable, coliforms will give a more sensitive and representative guidance about the quality of examined water. Hence, the detection of injured bacteria provides an added measure of accuracy to assist the treatment and decontamination of swimming pools.

CONFLICT OF INTEREST

There are no conflicts of interest.

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