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Full Length Research Paper

Efficacy of three disinfectant agents against contaminating pathogens isolated from public toilets

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The control of infections in public toilets is a matter of great concern and a major challenge specially places of mass gathering. The study aimed to evaluate the antibacterial activity of three disinfectant agents against contaminating pathogens isolated from public toilets surfaces in Makkah city, Saudi Arabia. Hydrogen peroxide, Polyhexamethylene guanidine (PHMGH) and Sodium hypochloride were tested toward selected bacteria isolated from toilets surfaces using MIC technique. The test organisms were *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*. The results showed that MIC values of PHMGH toward isolates were 0.047%, 0.047%, 0.047% and 0.0934% respectively while the MIC values of hydrogen peroxide were 0.0934%, 0.0934%, 0.186% and 0.0934% respectively whereas the MIC values of sodium hypochlorite for all these bacteria was 0.0934%. It was concluded that the PHMGH was the most effective disinfectant agent against tested bacteria and Gram positive bacteria were more susceptible than Gram negative bacteria while *P.aeruginosa* was the most resistant isolate.

Keywords: Public toilets, Disinfectant, Polyhexamethylene guanidine, Hypochlorite, Hydorogen peroxide.

INTRODUCTION

The control of infections in public toilets specially during mass gathering is a matter of great concern and a major challenge. Public toilets can be potential sources of pathogenic microorganisms because fecal material contains large numbers of microorganisms that can be introduced to surfaces upon excretion. One of the main means of transmission of many classic diseases and many urinary, vaginal and anal infections is from human faeces (Hawker et al., 2001); therefore it is extremely important to provide adequate, hygienic public toilets with a set of guidelines of cleaning and disinfections. Microorganisms should be eliminated by the disinfection process, which involves the use of chemical agents. There is a variety of products available on the markets with moderate or even insufficient antimicrobial action (Pitten et al., 2003). The most cost-effective home disinfectant is the commonly used chlorine bleach (a 5-6% solution of sodium hypochlorite), which is effective against most common pathogens. Hydrogen peroxide is used as a medical sterilant and as surface disinfectant but this agent is irritant and its vapor is hazardous to the respiratory system (CDC, 2012). New products and technologies with 'permanent' antimicrobial activity without the health risk or generating resistant microorganisms are needed. Inappropriate choices and inadequate protocols for the disinfection of inanimate surfaces have been a constant and major source of outbreaks of community infections. Cationic biocides have been prominent among other agents used to

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Table 1. The MIC values of PHMGH on different bacterial isolates

Serial dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Concentration (%)	6	3	1.5	0.75	0.375	0.186	0.0934	0.047	0.024
S. aureus,	-	-	-	-	-	-	-	-	+
E. faecalis,	-	-	-	-	-	-	-	-	+
E. coli	-	-	-	-	-	-	-	-	+
P. aeruginosa	-	-	-	-	-	-	-	+	+

(+) = growth, (-) = no growth

Table 2. The MIC values of Sodium hypochlorite on different bacterial isolates.

Serial dilution	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**
Concentration (%)	**6**	**3**	**1.5**	**0.75**	**0.375**	**0.186**	**0.0934**	**0.047**	**0.024**
S. aureus,	-	-	-	-	-	-	-	+	+
E. faecalis,	-	-	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	-	-	+	+
P. aeruginosa	-	-	-	-	-	-	-	+	+

(+) = growth, (-) = no growth

combat cross-infections and have contributed to the overall reduction in infections (Gilbert and Moore, 2005). Correct application of these biocides plays a very effective role in the elimination of infection in public toilet, dental, domestic and hospital settings (McDonnell and Russell, 1999). Polyhexamethylene guanidine (PHMG) derivates are members of the polymeric guanidine family that have been widely used as a disinfectant in different applications such as medicine, food industry and for a variety of solid surfaces (Rosin et al., 2001; Hiti et al., 2002). PHMG and its derivatives exhibit extensive and excellent antimicrobial activity, even against antibiotics resistant bacteria (Zhou et al., 2011). The present study aimed to evaluate the antibacterial activity of PHMGH versus hydrogen peroxide (H2O2) and sodium hypochlorite (NaOCl) using different strains isolated from public toilets surfaces in Makkah city, Saudi Arabia.

MATERIALS AND METHODS

Bacterial isolates

The evaluation of bacterial contamination in toilets surfaces was performed by swab method. All the samples were labeled properly and immediately transported to the of microbiology laboratory at the Department of Environmental and Health Research, the Custodian of the Two Holy Mosques Institute for Hajj and Umrah, Umm Al-Qura University, Makkah, Saudi Arabia**.** Swabs taken from different places from public toilets surfaces were streaked on Blood and MacConkey's agar plates. After incubation the colonies were identified using standard biochemical and microbiological methods. Bacterial isolates chosen for the present study were *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecalis (E. faecalis*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa (P. aeruginosa).* They were maintained on nutrient agar slants for periodic transfers. Disinfectant agents used were; Hydrogen peroxide (H2O2) (6%), Sodium hypochlorite (bleach chlorox) (6%) and PHMGH (6%).

The Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) test was determined according to the method followed by Mazzola *et al.,* (2009) with little modification. In 10 numbered glass tubes (10 x 100 mm), 1 mL of TSB (trypticase soy broth) medium was distributed for every tube, except for the tube number 1. The tubes were submitted to autoclave (constant pressure and 121 °C for 15 minutes). For the first and the second tubes of the series, 1 mL of tested disinfectant agent was added; tube 2 was stirred and 1 mL was withdrawn and transferred for tube 3. This successive transference was repeated until tube 9. Then 0.1 mL of inoculation (tested microorganism) with bacterial populations' ≥ 10^6 CFU/mL, was added to all flasks, except for flask number 9. The MIC was identified as the lowest concentration of the disinfectant agent, which resulted in confirmed inhibition of the growth of the tested microorganism, after 24 h of optimal incubation conditions.

RESULTS

The MIC values of PHMGH toward *S. aureus* , *E. faecalis*, *E. coli* and *P. aeruginosa.* were 0.047%, 0.047%, 0.047% and 0.0934% respectively while the MIC

Table 3. The MIC values of Hydrogen peroxide on different bacterial isolates.

Serial dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Concentration (%)	6	3	1.5	0.75	0.375	0.186	0.0934	0.047	0.024
S. aureus,	-	-	-	-	-	-	-	+	+
E. faecalis,	-	-	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	-	-	+	+
P. aeruginosa	-	-	-	-	-	-	+	+	+

(+) = growth, (-) = no growth

values of hydrogen peroxide were 0.0934%, 0.0934%, 0.186% and 0.0934% respectively whereas the MIC values of sodium hypochlorite for all these bacteria was 0.0934% as shown in tables 1, 2 and 3. Gram positive bacteria are more susceptible than Gram negative bacteria and *P.aeruginosa* was the most resistant isolate**.**

DISCUSSION

Public toilets can result in a buildup of pathogens within surfaces and in the bowl water (Barker and Jones, 2005; Best et al., 2011). Surface contamination could occur within short time of flushing (Best et al., 2011). So the disinfecting of these surfaces using chemical and physical processes is essential. The efficiency of disinfectants vary greatly depending on a various factors, some are specific for each disinfectant while others depends on the type of the organism. Awareness of these factors should lead to better use of disinfection and sterilization processes. The three disinfectants agents were tested toward selected bacteria using MIC technique. These agents were hydrogen peroxide, PHMGH and Sodium hypochloride. The active ingredients concentration in these disinfectants was (6.0% w/v). The test organisms were *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* (Table 1). The MIC values of PHMGH toward these bacteria were found 0.047%, 0.047%, 0.047% and 0.0934% respectively as show in table (1) while the MIC values of hydrogen peroxide were 0.0934%, 0.0934%, 0.186% and 0.0934% respectively as shown in table (3) whereas the MIC values of sodium hypochlorite for all these bacteria was 0.0934% as shown in table (2). The results of the present study showed that PHMGH was the best acting agent as it showed the lowest concentrations and the highest activity against all studied bacteria. Previously it has been proven that PHMGH had broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, yeasts and viruses, including human immunodeficiency virus (Muller, and Kramer, 2005; Krebs et al., 2005). Our result very close to Oule´et al., (2008) who found that antibacterial activity of PHMGH against methicillin-resistant *S. aureus* and *E. coli* concentrations was lower than 0.04%. Hypochlorites are inexpensive, fast acting and have a low incidence of serious toxicity (Yazd, 2008). Sodium hypochlorite showed in this study equal antimicrobial activity toward most types of bacteria and was the second effective antimicrobial agent. This is may be due to the fact that hypochlorites preparations behave as wide spectrum with non-specific killing effects on bacteria even spores and virus (Yazd, 2008; McDonnell and Russell, 1999). Many findings reported near findings of sodium hypochlorite toward *E.coli* and *S.aureus* (Penna et al., 2001; Saleh et al., 2012). Although hydrogen peroxide is oxidising agent active against a wide range of microorganisms (Tasić et al., 2009), it acted as the least effective against bacteria. That may be due to the production of catalase enzyme by aerobic organisms possessing cytochrome systems, which can degrade hydrogen peroxide to water and oxygen (Turner, 1983; Block, 2001). In the present study it was noticed that Gram positive bacteria are more susceptible to the disinfectants agents. This can be explained by the fact that the cell wall of Gram positive bacteria is composed of peptidoglycan and teichonic acid which are very weak barriers to the entry of antiseptics and disinfectants while in Gram–negative bacteria, the outer membrane acts as a selective permeability barrier in limiting the entry of harmful chemical compounds into the bacterial cell, therefore preventing high molecular weight substance to pass in Gram positive bacteria (Saleh et al., 2012; Russell, 1998). *P.aeruginosa* was found to be the highest resistant isolate. This is for many reasons; the differences in Lipopolysaccharides (Lps) composition from Gram negative bacteria, the cation content of the outer membrane and the possession of active efflux pump system acts as wide transporters for disinfectants (Schweizer, 1998). It was concluded that the PHMGH was the most effective disinfectant agent against tested bacteria. Gram positive bacteria are more susceptible than Gram negative bacteria, *P. aeruginosa* showed high resistance to the chemical disinfectants.

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