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REVIEW ARTICLE

MICROBIAL CONTAMINATION OF DOORKNOBS IN PUBLIC TOILETS DURING HAJJ

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ABSTRACT

Although there are plenty public toilet facilities in the holy sites during Hajj, the presence of the pathogenic bacteria on the doorknobs poses a potential risk to the pilgrims**.** The present study aimed to identify microbial contaminants and evaluate the prevalence of MRSA and PVL strains from doorknobs surfaces of the toilets in Arafat, Muzdalifah and Mina places**.** Bacterial contamination was performed by swab method from 224 randomly selected toilets. Identification was done using standard microbiological methods and further confirmed using the API 20E and VITEK 2 Compact 15 identification system. The mecA and PVL genes of Staphylococcus isolates were detected by PCR. Contamination was detected in (78.3%) of doorknobs. The highest number of contamination was in Muzdalifah (100%) followed by Arafat (73.3%). The total number of Gram positive and Gram negative bacteria was (49.2%) and (35.0%) respectively. High rate of isolates identified was Staphylococcus *aureus* (*S. aureus)*(22%) followed by Coagulase negative Staphylococci (CoNS) **(**17.3%) and *acinetobacter* (10%). Out of 42 *S. aureus* isolates (16.7%) were found to be MRSA (positive for mecA genes) and (31 %) were positive for PVL**.** The study highlights the fact that bacterial contamination on doorknobs of toilets serve as a source for potential community infections. Therefore proper cleaning and effectiveness of hand washing hygiene during hajj are essential.

INTRODUCTION

During Hajj, millions of Muslim pilgrims gather in the holy sites (Arafat, Muzdalifah and Mina) in Makkah. Although there are plenty public toilet facilities in the holy sites, these toilets are extremely crowded. If not regularly decontaminated, however, residual organisms from an infected person can survive in significant populations. Toilets can provide an ideal environment for spread of pathogens from gut, respiratory tract and skin via hands and surfaces from one person to another (Gerhardts *et al*., 2012). Toilet handles contamination is one of the common ways by which organisms that are not resident in the hand are picked up by contact with surfaces. Due to the unhygienic use of the toilet facilities, faecal matter remains a major reservoir source of human pathogens, which in adverse situation may bring about outbreaks of infection (Maori *et al*., 2013). The presence of the pathogenic bacteria on door knobs poses a potential risk to the pilgrims. It has been shown that hard, non-porous surfaces, such as door handles, have the highest bacterial transfer rates to hands (Rusin *et al*., 2002).

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The hands serve as a medium for the propagation of microorganisms from place to place and from person to person. Although it is nearly impossible for the hand to be free of microorganisms, the presence of pathogenic bacteria may lead to chronic or acute illness. Human hands usually harbor microorganisms both as part of the body normal flora as well as transient microbes contracted from the environment (Dodrill *et al*., 2011). *S. aureus* including methicillin resistant *S. aureus* (MRSA), from the skin, or gut microbes removed from the body during bathing or handwashing, can survive on the surfaces of the doorknobs. These organisms may be transmitted to others by direct contact, or by transfer via hands to other surfaces. The emerging "community-associated" strains of *S. aureus*, some of which are methicillin resistant, whilst others can carry the Panton–Valentine leukocidin (PVL), are of increasing concern. PVL is a toxin produced by some strains of *S. aureus*. These strains can cause cellulitis, abscesses, boils, carbuncles. The dominant resident microbes are *Staphylococcus epidermidis, Diphtheroids, Micrococcus* etc (Elizabeth *et al*., 2006). *Staphylococcus* epiderdimis which is found on almost every hand (Larson *et al*., 1992), in addition to certain members of Enterobacteriaceae family (Leyden *et al*., 1991; Scott and Bloomfield, 1990). Pathogens that may be present on the hand include *Escherichia coli*,

Salmonella typhi, *Shigella* spp *Clostridium perfringes*, *Giardia lamblia*, Norwalk virus and Hepatitis A virus; *Pseudomonas aeruginosa*, *S. aureus*, *Proteus mirabilis*, *Citrobacter freundii*, *Enterobacter spp*; *Streptococcus spp*, *Klebsiella spp*. (Orskov *et al*., 1997). The present study aimed to identify microbial contaminants and evaluate the prevalence of MRSA and PVL strains from doorknobs surfaces of the toilets during Hajj season in Makkah, Saudi Arabia.

MATERIAL AND METHODS

During Hajj 1436 (2015), the evaluation of bacterial contamination in door knobs surfaces was performed by swab method from 224 randomly selected toilets at the holy sites (Arafat, Muzdalifah and Mina). All the samples were labeled properly and immediately transported to the 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. Swabs taken from doorknobs surfaces were streaked on Blood and MacConkey's agar plates. After incubation the colonies were identified using standard biochemical and microbiological methods. Identification of *S. aureus* was based on the colony morphology, Gram staining, catalase (Sigma), coagulase tests and latex slide agglutination Staphytect Plus test (Oxoid). Gram negative isolates were further confirmed using the API 20E test strips following the manufacturer's instructions (Biomérieux, France). The identification of the isolates was also assessed using a VITEK 2 Compact 15 identification system (bioMérieux, USA) following manufacturer instructions. *S. aureus* clinical isolates were detected as MRSA (oxacillin-resistant) using the oxacillin-salt agar screening test, according to NCCLS guidelines (CLSI, 2004).

PCR reaction

The mecA and PVL genes of Staphylococcus isolates were detected by PCR. For mecA gene, the primers used were mecAP4 (TCCAGATTACAACTTCACCAGG) and mecAP7 (CCACTTCATATCTTGTAACG) which amplify 162 bp fragment (Milheiriç *et al*., 2007). For PVL gene the primers used were Luk-pvl- F (ATCATTAGGTAAAATGT CTGGACATGATCCA) and Luk-pvl- R (GCATCAAGTG TATTGGATAGCAAAAGC) which amplify 433 bp fragment (Badiou *et al*., 2010). DNA was isolated from overnight cultures grown on blood agar at 37°C. Genomic DNA was extracted by using microwave method (Ahmed *et al*., 2014) with some modification. Briefly, cell pellets were incubated for 30 min at 65°C, after washing with TE and addition of 50 µl of 10% SDS (Sigma). The micro-tubes were then placed in a microwave oven and heated three times were extracted with an equal volume of chloroform/isoamyl alcohol (24:1) for 15 min. The aqueous phase was recovered by centrifugation for 20 min and precipitated with ethanol. A 50 µl PCR mixture containing 3 µl of DNA template, 1 µl (100 pmol) of each primer and a 25 µl of Taq PCR Master Mix polymerase containing 100 mM Tris-HCl, 500 mM KCl at pH 8.3 at 20°C, 1.5 mM MgCl2, 200 M of each of deoxyribonucleoside triphosphate and 0.025U Taq polymerase (Qiagen, USA) was prepared. The thermal cycling conditions consisted of initial denaturation for 5 minutes at, followed by 35 cycles (40 s at 95°C, 40 s at 53°C, and 60 s at 72°C), and an elongation step of 10 min at 72°C. Ten microliters of each PCR product was mixed with 2 µl loading buffer and separated on a 2% agarose gel (Sigma) in TBE buffer. Amplified products were visualized under UVP BioDoct-It digital imaging system (UVP, Inc., Cambridge, UK) after staining with ethidium bromide (Sigma).

RESULTS

The study has isolated and identified the bacterial isolates from the different surfaces of doorknobs of toilets in the holy sites at Makkah. Contamination was detected in 191 out of 244 (78.3%) doorknobs (table1). The highest number of contamination was in Muzdalifah (100%) followed by Arafat (73.3%) as shown in table1. The total number of Gram positive and Gram negative bacteria was 94 (49.2%) and 67 (35.0%) respectively, while the total number of fungi (including yeasts) was 23(12.1%) as shown in table 2. High rate of isolates identified was *S. aureus* 42 (22%) followed by CoN *S.* 33(17.3%) and *acinetobacter* 19 (10%), while less rate identified 1(0.5%) was *Ralstonia pickettii*, *Serratia odorifera*, *Yersinia aldovae* and *Raoultella ornithinolytica* (table 2). Out of 42 *S. aureus* isolates, 7 (16.7%) were found to be MRSA (positive for mecA genes) and 13 (31 %) were positive for PVL as shown in table 3 and Figure 1.

Table 1. Incidence of positive specimens from toilets of holy sites

Holy site	Total examined	Total positive	% of positive samples
Arafat	71	52	73.3
Muzdalifah	66	66	100
Mina	107	73	68.2
Total	244	191	78.3

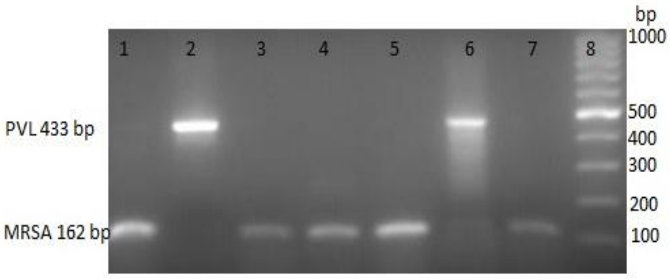

Figure 1. Multiplex PCR assay for mecA and PVL gene detection. Lane1; positive control for mecA; Lane 2; positive control for PVL, Lane 3,4,5 and 7; positive mecA gene fragment (162 bp), Lane 6; positive PVL gene fragment (433 bp), Lane 8; 100-bp DNA ladder

DISCUSSION

This study revealed the level of bacterial contaminants on door handles surfaces of toilets during hajj season (2015) at the holy sites (Arafat, Muzdalifah and Mina). Contamination was detected in 191 (78.3%). The toilets can serves as a serious source on infection because every time a toilet is flushed, it releases high numbers of aerosolized droplets that can carry bacteria to other surfaces such as doorknobs (Barker and Jones, 2005). In a similar study, Nworie *et al*., (2012) reported higher rate of contamination in door handles of toilets with (87.2%) Nworie *et al*. (2012). The highest number of

contamination was in Muzdalifah (100%) followed by Arafat (73.3%). Muzdalifah is an open, level area lies just southeast of Mina on the route between Mina and Arafat.

Table 2. Differentiation of isolates from study by Gram staining reaction

Sources	Bacterial isolates	Number isolated	Percentage
Gram positive	*S. aureus*	42	22.0
94 (49.2%)	CoN *S.*	33	17.3
	Streptococcus faecalis	10	5.2
	Bacillus spp	9	4.7
Gram negative	*Acinetobacter* spp	19	10.0
67 (35%)	*Enterobacter cloacae*	12	6.4
	Klebsiella spp	12	6.4
	Pseudomonas spp	6	3.1
	E. coli	2	1.0
	Ralstonia pickettii	1	0.5
	Sphingomonas paucimobilis	2	1.0
	Pantoea spp	4	2.1
	Leclercia adecarboxylata	4	2.1
	Serratia odorifera	1	0.5
	Yersinia aldovae	1	0.5
	Cronobacter sakazakii	2	1.0
	Raoultella ornithinolytica	1	0.5
Fungi and yeasts 23	*Aspergillus species*	10	5.2
(12.1%)	*Candida specie*	13	6.8
Unidentified 7 (3.7%)	*Unknown*	7	3.7
Total		191	100

Table 3. Incidence of positive MRSA and PVL among S. aureus isolates

S. aureus	PVL	%
MRSA 7 (16.7%)	4	57.1
MSSA 35 (83.3%)	9	25.7
Total 42 (100%)	13	31%

At Muzdalifah, contamination is expected to be higher, due to the lack of hand hygiene compliance as pilgrims are busy in collecting pebbles which will be thrown in the Stoning ritual in Mina. That would likely increase the opportunities for cross contamination. In addition, pilgrims spend the night at Muzdalifah, often sleeping in the open air, before leaving for Mina the next morning. In the present study, the total number of Gram positive bacteria (49.2%) was higher than Gram negative bacteria (35.0%). That is may be due to the fact that most skin flora bacteria are Gram positive, which would account for their predominance on doorknobs (Nwankwo and Afuruobi, 2015). *S. aureus* was of the highest rate (22%) followed by CoNS (17.3%). Both of them have been implicated in a variety of infections, wound and septicemia (Komolade and Adegoke, 2008).

The result of the present study agrees with that reported (Nworie *et al*. 2012; Nwankwo and Afuruobi, 2015) who found that the prevalent bacterium in similar studies was *S. aureus*. Staphylococci are a major cause of nosocomial and community acquired infections and they can have a high intrinsic resistance to antimicrobials (Nelson *et al*., 2006). Our results showed that (16.7%) of *S. aureus* isolated from doorknobs samples were carrying the mecA gene and 13 (31%) were positive for PVL. A recent conference abstract from Arizona reports MRSA contamination from many of the offices and public toilets sampled suggesting widespread contamination with CA-MRSA (Otter and French, 2009). PVL can also cause invasive infections, including necrotising haemorrhagic pneumonia in the community (Rojo *et al*., 2010). Community *S. aureus* strains are usually more likely to produce PVL than hospital-acquired *S. aureus* (Holmes *et al*., 2005). In the present study, *Acinetobacter* spp. was the prevalent Gram-negative bacterium (10 %) followed by *Klebsiella* spp *and Enterobacter cloacae* (6.4%) while less rate of identification (0.5%) was seen in *Ralstonia pickettii*, *Serratia odorifera*, *Yersinia aldovae* and *Raoultella ornithinolytica*. Although *Acinetobacter* can be found normally in many sources in the environment, some strains can cause infections. This pathogen is mainly spread via hands and through contact with equipment therefore cleaning and hand washing with soap and water is very important. Also the presence of enteric pathogens may be due to fecal contamination resulting of improper hand washing after the use of toilet.

This is evident in its isolation from doorknobs which are usually held and touched by hands. Previous studies have investigated enteric pathogens contamination in public and homes toilets (Mkrtchyan *et al*., 2013; Barker and Bloomfield, 2000; Scott *et al*., 1982). The rate (6.4%) of *Klebsiella* spp of isolated during this study, is less than the finding of Nworie *et al*., (2012) who reported the percentage of the same organism as 25.7% Nworie *et al*., (2012). On the other hand, presence of *E. coli* (1.0%) (in the door handles is less than that obtained by Sabra, 2013 (22.5%). In a similar study, door handles sampling demonstrated that contact surfaces are potential contaminants of *Pseudomona*s spp, *Klebsiella* spp, *E.coli* spp, *Enterobacter* spp, *Streptococcus* (Soom and Atu, 2015). In a similar study, Flores *et al*., 2011 determined the bacterial contamination in toilets. They found that most of these organisms were skin flora belonging to such phyla; *Propionibacteriaceae*, *Corynebacteriaceae*, *Staphylococcaceae* and *Streptococcaceae*. The result of this study gives fungal species such as Candida spp (6.8%) and Aspergillus. These results

indicate that handle knobs surfaces at those three holy places may increase the population of fungal infections. It could be concluded that bacterial contamination in the doorknobs of toilets during Hajj is high seasons is high especially in Muzdalifah. The mostly isolated bacteria are Gram positive bacteria mainly Staphylococcus species. The study highlights the fact that the public toilets could provide a reservoir for CA-MRSA and serve as a source for potential community infections. Therefore proper cleaning and effectiveness of hand washing hygiene during hajj and community settings are essential.

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