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Research Article

Identification of Bacteria from Public Toilets of Modern Market (Goico) in Musanze Town, Rwanda

MUKANDAYISHIMIYE Julienne *, IZERE Cedrick, ISHIMWE Alain Prudence, MUKASHEMA Hyacinthe, NZABANTERURA Innocent, UWIHANGANYE Jean Chrysostome, HABANABAKIZE Jean Baptiste and KORINEZA Dary Lorraine

Ines-Ruhengeri, Faculty of Applied Fundamental Sciences, Department of Biomedical Laboratory Sciences, Rwanda.

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*Address for Correspondence:

MUKANDAYISHIMIYE Julienne, Ines-Ruhengeri, Faculty of Applied Fundamental Sciences, Department of Biomedical Laboratory Sciences, Rwanda.

Abstract

Background: Bacteria are present on all surfaces, to which they were carried by many direct and indirect methods of transmission. A toilet is simply a receptacle into which both solid and liquid waste of human origin, in the form of urine and excreta are discharged. In public toilets, complete strangers mix and use the same sanitary facilities, with all the related risks of bodily fluid exchange, contamination and organism transmission. Unhygienic use of the toilet's facilities may cause urine and fecal residues after use to serve as a major reservoir or source of human pathogen, which may in turn bring about disease outbreak.

Aim: This study aimed at Identification of Bacteria from Public Toilets of Modern Market (Goico) In Musanze Town, Rwanda

Method: This cross-sectional study was conducted to identify bacteria from GOICO market toilets. About 40 samples; 20 from male and 20 from female toilets were collected and transported to INES-clinical microbiology laboratory for microbiological analysis. Laboratory techniques including culture, gram stain, biochemical tests and antibiogram were performed.

Results: The most predominant bacteria isolated was *S. aureus* (31.43%), followed by *S. typhi* (20%), *Streptococcus* (15.71%), *E. coli* (12.86%), *P. aeruginosa* (10%), *P. mirabilis* (7.14%), *C. freundii* (1.43%), *S. epidermidis* (1.43%). It was noted that female toilets were more contaminated (51.43%) than male toilets (48.57%). All gram positive and gram-negative bacteria were sensitive to Gentamicin. Almost all bacteria except *P. mirabilis* were sensitive to Norfloxacin, gram negative showed a resistance to Vancomycin and Novobiocin.

Conclusion: These results should alert public toilets users that extreme caution must be executed while using these toilets, as they may be a source of pathogenic bacteria. The best way to keep a toilet and its seat free of bacteria is to clean it with bleach. Otherwise, keep the bacteria at bay by washing your hands, and if you just don't trust that toilet seat, wipe it down with a disinfectant or antibacterial wipe before you sit.

Keywords: Bacteria, Public toilets, *E. coli*, *S. typhi*, Urinary tract

INTRODUCTION

Bacteria are found everywhere and constitute a major part of ecosystem. They live either freely or as parasites. In some cases, they live as transient contaminants in fomites where they constitute a major health hazard as sources of community acquired infections. Fomite can be described as any non-living object or substance that is capable of carrying infectious organism hence transmitting them from one individual to another. There are several factors that affects the contamination of fomites such as presence of moisture, frequency of use and general cleanliness. It is a well-known fact that fomites are one of the major sources of spread of community-acquired infections and they provide a possible route for pathogen to pass between communities. Fomites includes among others door handles, toilets seats, chairs, and these are especially found in public places¹.

A toilet is simply a receptacle into which both solid and liquid waste of human origin, in the form of urine and excreta are discharged. A public toilet may therefore be defined as a facility shared or used by a group of persons in a public setting or environment. They may be situated in the markets, and transport centers, schools, hospitals, factories, etc. The role of

public toilets serving as vehicle for continuous source of epidemics has made research in this area very valuable. Shared toilets can also provide an ideal condition for the spread of pathogens from person to person especially urinary tract infections^{2,3}.

In general, the risk of disease transmission through fomites is determined by the frequency of site contamination and exposure; level of pathogen excreted by the host; likelihood of transfer of the infectious agent to a susceptible individual; virulence of the organism; immuno-competence of the persons in contact; the practice of control measure such as disinfectant use and personal hygiene. It is estimated that 2.5 or more billion globally lack access to improved sanitation facilities. This sanitation deficit continues to leave the public exposed to a wide range of fecal contaminants responsible for a multitude of diseases⁴.

Using a dirty a toilet exposes a user to the risk of contracting diseases such as diarrhea, other intestinal and respiratory infections. According to a recent poll conducted by George Pacific corps, Atlanta, nearly 30 percent (30%) of Americans avoid public toilets out of fear of germs and infections. Some organisms could be acquired by contact with toilet. A survey by

the Union housing ministry showed that 55% of public washrooms were either dirty or completely unusable in Delhi. Toilet disease can be caused by bacteria, yeast, viruses and other parasites⁵.

According to WHO, plenty of people's developed diseases caused by contaminated public toilets that may results in severe health problems such as genitourinary tract infections as well as gastrointestinal diseases which can be found in children and adults by using contaminated services. In females, some genitourinary disorders such as vulvovaginitis, acute and chronic pregnancy, premature rupture of membrane and acute pyelonephritis would arise mostly due to *E. coli*. In males, acute and chronic urethritis, cystitis, and prostatitis are mostly likely. In addition, *E coli* contamination is principally qualified to lead on infertility in males and females. Acute cystitis, urethritis, and vaginal discharges are the main problems that occur in children are affected by these bacteria⁶.

In public toilets, complete strangers mix and use the same sanitary facilities, with all the related risks of bodily fluid exchange, contamination and organism transmission. Research has shown that public toilets are vital components for breeding places for disease causing bacteria. Sanitary conditions in public places have always been a major problem, especially restrooms. It is estimated that 2.5 or more billion globally lack access to improved sanitation facilities. This sanitation deficit continues to leave the public exposed to a wide range of fecal contaminants responsible for a multitude of diseases⁵.

Using a dirty a toilet exposes a user to the risk of contracting diseases such as diarrhea, other intestinal and respiratory infections. Public toilets represent a suitable environment for development of pathogenic microbes especially such as *Enterobacter*, *Klebsiella*, *CA-MRSA* and enteropathogens such as *Salmonella enteritis*, and *Escherichia coli*, and unfortunately, plenty of people's developed diseases caused by contaminated public toilets that may results in severe health problems such as genitourinary tract infections as well as gastrointestinal diseases which can be found in children and adults by using contaminated services⁷.

In females, some genitourinary disorders such as vulvovaginitis, acute and chronic pregnancy, premature rupture of membrane and acute pyelonephritis would arise mostly due to *E. coli*. In males, acute and chronic urethritis, cystitis, and prostatitis are mostly likely. In addition, *E coli* contamination is principally qualified to lead on infertility in males and females. Acute cystitis, urethritis, and vaginal discharges are the main problems that occur in children are affected by these bacteria⁹.

Therefore, enhancing personal hygiene, sanitizing public toilets regularly and correctly and using public toilets safely can prevent the transmission, diffusion, and spread of bacterial infections. Fixing this problem should be a priority. Proper and adequate toilets and sanitation facilities are key to preventing waterborne diseases like cholera and diarrhea. For the cleanliness of the shared toilets to be guaranteed, it is not enough to keep the toilet seat clean, disinfecting is also needs as it has been found that disinfecting a toilet seat can eliminate pathogenic bacteria¹⁰. Therefore, this study aimed at identification of bacteria from public toilets of modern market GOICO, in Musanze town, Rwanda.

METHODOLOGY

Study area

The study was conducted at GOICO market located in Musanze district, Northern Province of Rwanda. This area was chosen because it has a large population and limited number of toilets.

The shared toilets are used by individuals of different backgrounds with different hygienic habits and thus makes it difficult to maintain cleanliness at all times.

Study design and sample size

This was cross-sectional study. The study included 40 samples from toilet seats of GOICO market, which were in use during the time of sample collection.

Sample collection

Samples were collected on public toilets by adopting the cotton swab rinse technique to pick up bacteria with aseptic precaution, and transferred to a sterile screw-capped tube containing 5ml of peptone water. Samples were then transported to INES Microbiology lab for analysis with help of appropriate transport media with optimum temperature.

Antibiotic susceptibility test

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines on MHA. The suspension of 3–5 colonies of freshly grown test organisms was prepared to the equivalent of 0.5 McFarland standard. The MHA surface was covered by swirling the swab with the suspension. The plates were exposed to room temperature to dry for 5 min: Then, the discs were applied over the inoculated plates using sterile forceps and incubated at 37°C for 24 hours. The diameter of the clear zone around the disc was measured using a ruler. Results were interpreted as sensitive, intermediate, and resistant according to CLSI 2022 recommendation.

Statistical analysis

Data were entered into MS excel spreadsheet and analyzed using SPSS statistical software (version 22.0). Study findings were explained in words, chart, figures and tables.

RESULTS

Identified pathogenic microorganisms from public toilets

Toilet seats from GOICO were sampled for bacterial contamination. A total of 40 swab samples were collected of which; 20 were from male toilet seats, and 20 were from female toilet seats. The most predominant isolated bacteria was *S.aureus* (31.4%) and the least were *C.freundi* and *S.epidermidis* (1.43% both).

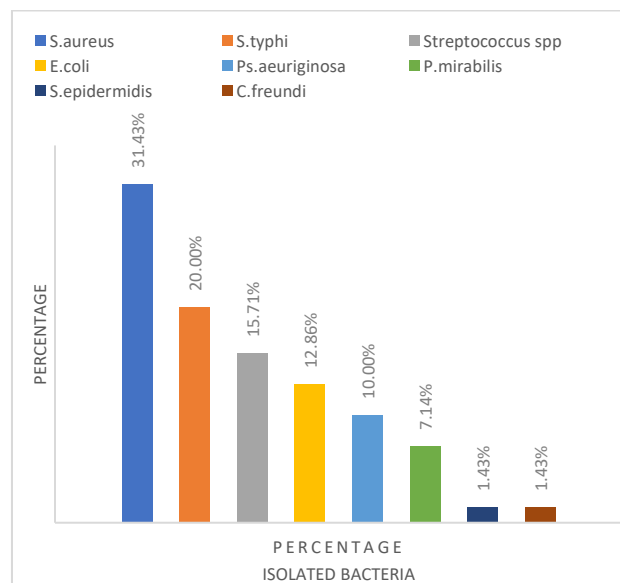


Figure 1. Isolated bacteria from GOICO public toilets seat.

Comparison of isolated bacteria between male and female toilets

Table 1: Comparison of isolated bacteria between male and female toilets

Bacteria	Female toilets		Male toilets	
	Frequency	Percentage	Frequency	Percentage
<i>C.frendi</i>	1	3%	0	0%
<i>S. epidermitis</i>	1	3%	0	0%
<i>E. coli</i>	5	14%	4	12%
<i>P. mirabilis</i>	3	8%	2	6%
<i>P. aeruginosa</i>	5	14%	2	6%
<i>S. aureus</i>	9	25%	13	38%
<i>S. typhi</i>	5	14%	9	26%
<i>Streptococcus spp</i>	7	19%	4	12%
Total	36	51.43%	34	48.57%

Table 1 represents bacteria identified from female and male toilets. The results showed that almost all bacteria have a high frequency in female toilets compared to those from male expect for *S.aureus* (25%; 38%) and *S.typhi* (14%; 26%). *C.freundi* and

S.epidermidis were found only in female toilets with 3%, *E.coli* (14%; 12%), *P.mirabilis* (8%; 6%), *Ps.aeruginosa* (14%; 6%), *Streptococcus spp* (19%; 12%).

Antimicrobial susceptibility pattern of selected antibiotics

Table 2: Antibiotics susceptibility test of isolated bacteria

Antibiotics	Bacteria with inhibition zone(mm)																							
	<i>C. frendi</i>			<i>Strep.spp</i>			<i>S. epidermitis</i>			<i>E. coli</i>			<i>S. aureus</i>			<i>S. typhi</i>			<i>P. mirabilis</i>			<i>P. aeruginosa</i>		
	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
Gentamicin	-	-	1 3	-	-	2 0	-	2 5	-	-	2 2	-	-	2 8	-	-	3 1	-	-	1 1	-	2 8	-	
Rifampicin	-	2 1	-	-	2 3	-	-	2 4	-	-	2 6	-	-	3 0	-	7	-	-	5	-	-	6	-	
Chloramphenicol	-	1 7	-	-	2 3	-	-	-	1 7	-	-	1 5	-	3 0	-	8	-	-	-	-	1 4	-	2 5	
Norfloxacin	-	2 4	-	-	2 2	-	-	2 5	-	-	2 5	-	-	3 2	-	-	3 2	-	8	-	-	-	1 5	
Novobiocin	1 2	-	2 0	-	-	1 5	-	2 9	-	1 3	-	-	-	1 6	8	-	-	1 1	-	-	-	-	1 4	
Vancomycin	6	-	-	-	2 2	-	3	-	-	8	-	-	-	2 9	-	1 0	-	-	1 0	-	-	6	-	

The table 2 illustrates antimicrobial activity of six antibiotics on isolated bacteria. Those antibiotics are: Gentamicin, Rifampicin, Chloramphenicol, Norfloxacin, Novobiocin, and Vancomycin. All gram positive and gram-negative bacteria were sensitive to Gentamicin. All identified gram-negative showed resistance to Vancomycin and Novobiocin

DISCUSSION

Improper sanitation of toilet seats has been found to favor pathogenic and nonpathogenic bacteria colonization and human being can acquire them either via direct contact with the human body especially with broken skin or indirectly as a toilet is flushed and water splashes or is aerosolized¹¹. However, not much has done to address epidemics associated with shared

toilet seats even though some dangerous pathogens can be transmitted in that way.

In this study, GOICO toilet seats were evaluated by isolating and identifying the bacteria contaminants. Out of the 40 samples assessed, the results showed that all toilets were contaminated with potentially pathogenic and nonpathogenic bacteria since so many different people use them. The most predominant bacteria isolated were *Staphylococcus aureus* (31.43%), this is because they are major components of the normal flora of the skin and nose, which probably explains its high prevalence as contaminant as it can easily be discharged by several human activities. This observation is in conformity with the finding of other researchers *Staphylococcus aureus* is the most important potential pathogen that cause boils, abscesses, wound infections toxic shock syndrome and pimples³.

S. typhi, *Streptococcus spp*, *E. coli*, *Ps aeruginosa* and *P. mirabilis* were other major contaminants, this is almost similar to the study conducted by Marineli (2013) where *Ps.aeruginosa* and *E.coli* as well as other *Enterobacteriaceae* frequently on sites such as the toilet seat and handle. Gram-negative rods isolated in this study indicate the possibility of the presence of fecal contamination on toilets. This might be due to the fact that the toilet was used by a person infected, also most people go to toilet and end up contaminating their hands with fecal and urinal material and fail to wash their hand because they take the issue of hygiene with levity, they also lack the concept of hand washing as a simple means of stopping and this spread of infectious agents, this correspond with the work of¹², who reported that the high rate of isolation of these organisms is only achieved during epidemics in which human hands serve as the vehicle of transmission.

Proteus mirabilis has been found to be a common cause of UTIs associated with the presence of renal stones³. *Proteus* is often the second invader of ulcers, pressure, sores, burns and damaged tissues. Furthermore, it can cause septicaemia occasionally meningitis and chest infections¹³. The high rate of toilet seats, colonization by bacterial and presence of pathogenic bacteria such as *Salmonella spp*, *E coli*, *S aureus* and others observed in this study had mostly found in developing countries like ours where safe drinking water and basic sanitation is lacking with high morbidity and mortality rate. The occurrences of *Salmonella spp* on toilets was in accordance with the work of¹⁴, who reported the isolation of *Salmonella spp* from offices, toilets seat and door handles.

According to Table 2, female toilets seats were more contaminated with 51.43% compared to male with 48.57%. According to¹⁵, women's restrooms actually contain more faecal bacteria than men's. Faecal bacteria, found in the intestinal tract, are an indicator of the potential presence of other disease-causing organisms that shed from the intestinal tract. Among identified bacteria, *C .freundi* and *S.epidermidis* were only found on female toilets, a high frequency of *E coli* and *P mirabilis* were isolated from female toilets.

Female are known to be colonized by those bacteria in their urogenital tract which may lead to UTI. 75%-90% of acute UTI are caused by uropathogenic strains of *E coli*, 10-20% of acute UTI are caused by coagulase negative *Staphylococcus* and 5% or less are caused by other gram-negative rods (*Proteus*, *Citrobacter*). Female get UTIs more often because a female's urethra is shorter than a man's. This makes it easier for bacteria to get into the bladder. *E. coli*, happen when an infected person's feces contaminates a surface, those surfaces include toilets, toilet handles and toilet seats^{3,16}.

Antibiotic resistance is a naturally occurring process. However, increases in antibiotic resistance are driven by a combination of germs exposed to antibiotics, and the spread of those germs and their resistance mechanisms. Antibiotics save lives but their use can contribute to the development of resistant germs. Antibiotic resistance is accelerated when the presence of antibiotics pressure bacteria to adapt. In this study *P.mirabilis* was resistant to almost all antibiotics except Gentamicin and Chloramphenicol (intermediate). All gram-negative bacteria were resistant to Vancomycin, the efficacy of Vancomycin to gram positive bacteria was shown in another study³.

All gram positive and gram-negative bacteria were sensitive to Gentamicin, almost all bacteria except *P.mirabilis* were sensitive to Norfloxacin. All identified gram-negative showed resistance to Vancomycin and Novobiocin [Table 3]. Vancomycin is active only with respect to Gram-positive bacteria. It is the most powerful of all of the known antibiotics with respect to *S. aureus* including methicillin- and cephalosporin-resistant strains.

CONCLUSION

In conclusion, it is important to note that there is high level of bacterial contamination in GOICO toilet seats. Higher level of contamination was found in female toilets with 51.43% compared to 48.57% of male. The most predominant bacteria isolated was *S.aureus* (31.43%), followed by *S. typhi*(20%), *Streptococcus spp*(15.71%), *E.coli*(12.86%), *Ps.aeruginosa*(10%), *P.mirabilis*(7.14%), *S.epidermidis* and *C.freundi*(1.43% each). Various contaminated toilets outcomes can include UTIs mostly in female, skin, and community associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Gentamicin, Norfloxacin, Novobiocin, Chloramphenicol, Vancomycin, and Rifampicin were used in antibiogram. All gram positive and gram-negative bacteria were sensitive to Gentamicin, All bacteria except *P.mirabilis* were sensitive to Norfloxacin. All identified gram-negative showed resistance to Vancomycin and Novobiocin

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Conflict of interests

Authors declare no conflict of interests

Ethical consideration

The ethical clearance form was granted by INES-Ruhengeri research committee. A letter from Ines Ruhengeri administration was presented to the Director of GOICO asking for the permission before carrying out research.

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