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Bacteriological Tracing of *Haemophilus influenzae* in Some Public Toilet Seat Bowls of Port Harcourt

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The study aimed at tracing *Haemophilus influenzae* on toilet seat bowls of some public toilets in Port Harcourt. The toilets were coded DER, TYU, AFR for park, market and hospital toilets respectively, for the purpose of the study. Swabs were collected aseptically from the toilet seat bowls, with a moistened swab stick, and then introduced into a freshly prepared sterile peptone water for viability of the *Haemophilus influenza* isolates. Thereafter, the spread plate technique was employed in which the swabbed samples were inoculated into Chocolate agar. Result showed toilet seat bowl coded DER had a *Haemophilus influenzae* mean count of 5 x 10^2CFU/ml while codes TYU and AFR had a mean *Haemophilus influenzae* counts of 6 x 10^2 and 9 x 10^2 CFU/ml respectively. A percentage frequency occurrence of *Haemophilus influenzae* on the toilet bowl showed DER coded toilets had 17% frequency of occurrence, while codes TYU and AFR had 25 and 58% frequency of occurrence respectively. A total of 49 isolates of *Haemophilus influenzae* were identified macroscopically and biochemically. The presence of *Haemophilus influenzae* on surface of toilet seat bowl may have resulted from aerosols generated during

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flushing. Thus, influenza infection (respiratory tract infection) may affect users. Hence, toilet seats, specifically, codes AFR class of toilets should be closed before and after flushing at all times.

Keywords: Haemophilus influenza; seat bowls; public toilet; Port Harcourt.

1. INTRODUCTION

Fecal defecation in flush toilet bowls comes up with numerous challenges as microbes are expelled out, specifically, bacteria after flushing [1]. According to Palmer [2], the bacterium, *Haemophilus influenzae* is amongst many bacteria that are expelled in human waste. The *Haemophilus influenza,* bacterium is a normal flora of the human body were they colonize the upper respiratory tract (nose and mouth) as commensals. *Haemophilus influenzae* are implicated in respiratory tract infection as it could be transmitted from person to person by inhalation of droplets [3]. The presence of *Haemophilus influenzae* on toilet seat may spark up respiratory challenges considering the bacterium aerosolized property and the high magnitude of aerosols in the toilet [3]. Abney [4] stated that toilet as a receptacle were humans urinate or defecate. It consists of bowl, seat cover, water tank, shower points etc. The toilet bowl has a lid cover which covers the bowl seat when the toilet is not used, to prevent certain items from falling into it. In most toilets the lids are absent and even when present, users leave it open [4]. Public toilets which are available for public use are essential and available to address the sanitation-related target of the Millennium Development Goals [5]. Public toilet may be located in the markets, schools, eateries, offices, factories, hospitals, factories etc. Lack of public toilets in a public vicinity calls for open defecation which is associated with offensive odor from feces and urine. Open defecation in-turn disperse fecal matter, degrading air quality for diseases emergence via inhalation [5]. Large concentrations of bacteria and virus are found in stool after defecation and in the process of flushing, these bacteria disperse onto external parts of the toilet, such as the seat bowl sides, bowl rim and bowl seat [6]. Bacteria such as *Escherichia coli, Haemophilus influenzae Salmonella* spp. and *Shigella* spp*.* of less than 10^6 coliform forming units are often dispersed and remain under conditions of desiccation on toilet seats for an optimum nine (9) days [7]. According to Knowlton et al. [8] there have been reports of outbreaks of diseases associated with flush toilets. Flush toilets have been implicated in the spread of diseases via aerosols generated. Outbreak such as Shigellosis amongst many others have been reported. Although measures adopted to reduce the incident of pathogen in toilets have been adopted. The presence of *Haemophilus influenzae* on toilet seat other than inside the toilet bowl may pose respiratory challenges and direct transmission into human body. Thus, the study aimed at tracing of *Haemophilus influenzae* on toilet seat bowls.

2. MATERIALS AND METHODS

2.1 Study Area

Three classes of public toilets (hospital, market and park toilets) were chosen for the study. The toilets were coded AFR, TYU and DER for the purpose of the study for toilets located in hospitals, markets and parks respectively. The toilets code AFR are unique, with patients and out-door patients while toilet code TYU and DER users are largely for the general public use. All servicing public toilet points are located in Port Harcourt, Nigeria.

2.2 Collection of Swab from the Seats of Toilet Bowl

Swabs from the toilet seat bowl were collected using a swab sticks in the mornings when the toilets have been sanitized for early users. The swabbed samples were collected aseptically, with a moistened swap stick, and then introduced into a sterile prepared peptone water in a tube for increased visibility of *Haemophilus influenzae* [9]. The swabbed samples after collection were then taken to the Biology Laboratory of Ignatius Ajuru University of Education, Rumuolumeni for Microbiological analysis.

2.3 Determination of *Haemophilus influenzae* Load

Enumeration of *Haemophilus influenzae* load on the toilet seat bowl involved employing the spread plate technique as adopted by Cheesebrough [10]. The technique involved the spread of an aliquot of 10^{-1} obtained from the dilution of the swabbed sample introduced in

peptone water. An aliquot of the 10^{-1} sample was inoculated on a freshly prepared chocolate media. This was then followed by spreading, with a glass spreader and incubatting at 37°C for 24 hours. Growth observed were identified and counted or enumerated as colony forming unit per ml. Chocolate media used was prepared by the addition of five mill (5ml) blood into a freshly prepared Nutrient agar, that was not allowed. Prior to the addition of the blood to the freshly prepared nutrient agar, the Nutrient agar was prepared by dissolving the required quantity needed into distilled water and autoclaved as instructed by manufacturer before use.

2.4 Biochemical Characterization of *Haemophilus influenzae*

In presumptive test to characterize *Haemophilus influenzae*, certain morphological features were considered, with respect to size, shape, colour, elevation and opacity of the colonies on the Nutrient media plate. In further test to confirm the identity of *Haemophilus influenzae,* Some key biochemical reactions were adopted such as the Methyl red test, Citrate test, Voges-Proskauer test, indole test, catalase test, sugar fermentation test and motility test.

2.5 Methyl Red/ Voges-Proskauer (MR/VP) Test

In carrying out this test as adopted by Cheesebrough [10], a loopful of the probable *Haemophilus influenzae* cell was inoculated into 10ml freshly prepared MR/VP broth medium, that was prepared according to manufacturer's instructions. The tube was then incubated at 35 - 37^{0}C for 48 hours, after incubation, the broth culture was shared into two parts (5ml) each, one part denoted the methyl red potion while the other part denoted the Voges Proskauer potion. To the part with methyl red, 5-6 drops of methyl red reagent was added and to the part with Voges Proskauer, 0.6ml (6 drops) of 5% a – naphthol and 0.2ml (2 drops) of 40% KOH reagent were added. Development of a bright red coloration is indicative of positive MR/VP test, thus confirms *Haemophilus influenzae* and the reverse indicative of a non-*Haemophilus influenzae*.

2.6 Citrate Test

This test was done to determine the ability of probable *Haemophilus influenzae* cell to utilize Sodium Citrate as its source of carbon and inorganic ammonium salt as its source of nitrogen [10]. Simmon citrate agar was prepared in a capped tube and a sterile wire loop used to pick a loopful of the presumed *Haemophilus influenzae* and streaked on slant surface. The tube was then incubated at 37^{0}C for 24hrs. Change in colour from green to blue indicated a positive result for *Haemophilus influenzae* cell while no change in colour indicated a negative *Haemophilus influenzae* result [10].

2.7 Indole Test

The test was carried out to ascertain the ability of presumed *Haemophilus influenzae* cell to breakdown the amino acid tryptophan in the medium into indole in the presence of the enzyme tryptophanase. The presumed *Haemophilus influenzae* cells were inoculated into test tubes containing 10ml of sterile tryptone broth and the tubes incubated for 24 hours at 37°C. Thereafter, 0.5ml of Kovac's reagent was added to the media and shaked gently and examined for red colour in the surface layer which indicates a positive result for indole utilization of the cell. While the absence of a colour change, indicated a negative result (Cheesebrough, 2000).

2.8 Catalase Test

In a follow up to this text, the presumed *Haemophilus influenzae* cell was tested for catalase property.. The test was done to determine the ability of *Haemophilus influenzae* to breakdown Hydrogen Peroxide into Oxygen and water. In achieving this, a visible effervescent denoted *Haemophilus influenzae* positive reaction when a small inoculum was introduced into a 3% hydrogen Peroxide solution placed on a slide, while the absence of catalase is evident by a lack of or weak production of effervescence or non-*Haemophilus influenzae* (Cheesebrough, 2000).

2.9 Sugar Fermentation Test

The sugar fermentation test was done to evaluate the ability of the presumed *Haemophilus influenzae* cell to break-down sugar (lactose and maltose) to yield acid and gas. Peptone broth incorporated with 1% sugar was used to constitute the sugar broth test. An indicator was thereafter added to the sugar broth with Durham tube added to the tube in an inverted position. After sterilization, a loopful of the presumed *Haemophilus influenzae* colony

was introduced into the medium and then incubated at 35 -37^{0}C for 24 - 48 hrs. Change in color of the medium from purple to yellow and gas production indicated a positive sugar fermentation test for *Haemophilus influenzae* while no change in color depicted no sugar utilization by the cell (Cheesebrough, 2000).

2.10 Motility Test

This test was used to determine the motility of the presumed *Haemophilus influenza* cell Motility of the cell is determined by the presence of flagella (a locomotory organelle). Semi solid Nutrient agar was used for this test. The agar media was prepared and the isolate picked with a sterile straight wire and by stabbing into the media, the cell was inoculated. Thereafter, the medium in the tube was incubated at 37^{0}C for 24 - 48 hrs. Growth in diffuse form, from the line of stab into the medium indicated a positive result for motility, whereas growth only along the line of stab indicated a negative result (Cheesebrough, 2000).

2.11 Morphological Characterization

2.11.1 Gram staining

Gram staining reaction was done as carried out by Cheesebrough (2000) to confirm the status of the *Haemophilus influenza* cell. A smear was made by placing a drop of distilled water on a clean grease-free slide using sterilized wire loop. Thereafter a loopful of the presumed *Haemophilus influenza* was mixed with the distilled water on the slide and stirred evenly. The smear was air-dried for twenty (20) minutes to allow the cell glue to the slide. Thereafter, crystal violet stain was applied on the smear and allowed to remain on the slide for 1 min. The crystal violet was rinsed off under slow running tap water and again Lugol's iodine (a mordant) was used for 1min. Distilled water was again used to rinse off the Ligol's iodine. Alcohol was used to flood the smear for 30 second and again rinsed with water. The smear was then counter stained with safranin for 30 seconds and again rinsed with distilled water. Finally, the smeared slide was dried and examined under a x100 objective lens microscope. Gram positive cell was indicated by a purple appearance while Gram negative cell appeared pink

3. RESULTS

3.1 Enumeration of *Haemophilus influenzae* Load on Toilet Seat Bowl

Table 1, showed the mean load of *Haemophilus influenzae* on the toilet seat bowl. Toilets coded DER had a *Haemophilus influenzae* mean count of 5 x 10^2CFU/ml while toilets coded TYU and AFR had a mean counts of 6 x 10^2 and 9 x 10^2 CFU/ml respectively.

3.2 Percentage Frequency of Occurrence of *Haemophilus influenzae*

Table 2, showed the percentage frequency occurrence of *Haemophilus influenzae* on the toilet bowl. Sample point coded DER had 17 percentage occurrence while sample points coded TYU and AFR had 25 and 58 percentage occurrence respectively.

3.3 Macroscopic Feature of *Haemophilus influenzae*

Table 3, showed the macroscopic features of the *Haemophilus influenzae* colony on Chocolate agar media as seen on plates after inoculation and incubation. The colonies were noted cream white, with a very small sized colony. The colonies were also noted oval in shape, opaqued with low elevation.

Table 1. Mean Load of *Haemophilus influenzae*

Bacteria	DER (CFU/ml)	TYU(CFU/ml)	AFR(CFU/ml)	T-test
Haemophilus influenza	5 x 10^2	6 x 10^2	9 x 10^2	*P*>0.05

Keys; (CFU/ml) = Coliform Forming Unit Per Mill, DER=Park, TYU=Market, AFR=Hospital

Table 2. Percentage Frequency of Occurrence of *Haemophilus influenzae*

Toilet Codes	Frequency of Occurrence (n)	% Frequency of Occurrence
DER	2	17
TYU	3	25
AFR	7	58

Keys; DER=Park, TYU=Market, AFR=Hospital

Table 3. Macroscopic Feature of *Haemophilus influenzae*

GramReaction	Colour	Size	Shape	Opacity	Edge	Elevation
	White	Small	Oval	Opaque	Curved	Low

Table 4. Biochemical Feature of *Haemophilus influenzae*

lactose	oxidase	catalase	coagulase	indole	urease	glucose	citrate	Bacteria
+	-	-	-	+	+	+	-	*Heamophilus influenzae*

3.4 Biochemical Feature of *Haemophilus influenzae*

Table 4, showed the biochemical characterization of *Haemophilus influenzae* as expressed. *Haemophilus influenzae* utilized lactose, glucose, and also indole and urease. The enzymes coagulase and catalase properties failed to feature. Citrate utilization and oxidase were also negative.

4. DISCUSSION

An account of a significant difference in counts of *Haemophilus influenzae* within Park, Market and Hospital toilets seat bowls is quite usual and dissimilar to studies carried out by Matini et al. [11]. Matini et al. [11] carried out a survey on public restroom and noted that public restrooms have too many counts of bacteria whereas this study failed to confirm that report. Matini et al. [11] pointed out that the standard count for bacteria is 1000 CFU/ml. However, it was not stated if samples from seat swab were considered. Sampson et al. [12], who worked on the toilet seats failed to identify counts for *Haemophilus influenzae*, but noted counts of heterotrophic bacteria at 2.7 x 10^5 CFU/ml. Heterotrophic bacteria counts noted by Sampson et al. [12] were however, greater than counts of *Haemophilus influenzae* obtained in all sampling points in this study. The insignificant difference of *Haemophilus influenzae* counts recorded amongst the sampling point is also not in consonant with studies carried out by Archibald [13], who in their study noted the highest bacteria population in public restroom is 1468 CFU/m^3 as against the lowest, of 480 CFU/m^3. The counts derived from seats of these public restroom although are not detrimental as pointed out by Matini et al. [11] who pointed detrimental effects fall between 10^4 and 10^5 CFU/g giving that the toilets are less intended to have greater loads of *Haemophilus influenzae* or any other bacteria as the sampling points under study have high public usage. The advent of bacteria on toilet seat bowl and other surface is due to aerosolization during flushing of feces (biomatter) [14]. Aerosols desiccate and remain adrift in the air currents [15] and later fall on surfaces. Thus, as a result of this, the identification of *Haemophilus influenzae* in this study is significant as the bacteria implication in respiratory tract infection can be fatal [3]. Studies by Matini [11] failed to identify *Haemophilus influenzae* in restrooms, but *Escherichia coli, Staphylococcus aureus* and *Salmonella* spp.. Similarly, Archibald [13], identified *Staphylococcus aureus, Micrococcus, Bacillus* spp,, *Klebsiella* spp. and *Cronobacter* isolated from restroom with exception of *Haemophilus influenzae.* Sampson et al. [12] who worked on toilet seat did not identify *Haemophilus influenzae* but *Staphylococcus aureus, Bacillus, Klebsiella* spp., and *Escherichia coli.* The greater prevalence of *Haemophilus influenzae* in hospital above the park and market restroom may have been pointed out by Best et al. [16], where they pointed out that toilets in hospitals do not have lids, reason for high contamination of the toilet air environment immediately after flushing lidless toilet. However, studies of toilets in markets and park have not been specifically considered probably due to less provision of toilet facilities.

5. CONCLUSION

The study traced the presence of *Haemophilus influenzae* on some public toilet seats. The presence of *Haemophilus influenzae* on surface of toilet seat bowl resulted from aerosols generated during flushing. Hence, influenza infection (respiratory tract infection) may emerge as these toilets may serve as a source of disease transmission via inhalation.

RECOMMENDATION

Due to aerosilization after flushing feces, toilet seats should be closed before and after flushing at all times.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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