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Isolation and Identification of *Escherichia Coli* from Students' Toilet Seats in the University of Buea Restrooms

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Abstract

Exposure to enteric pathogens through direct contact with contaminated toilet surfaces is one of the major sources of disease transmission in a public setting. Toilet seats are potential carriers of pathogenic microorganisms if used under poor hygienic conditions. The emergence and spread of *Escherichia coli (E. coli)* is a global health concern. To combat the spread of *E. coli*, it is necessary to identify all possible sources and potential pathways by which this occurs. This study aimed at isolating and identifying *E. coli* associated with toilet seats in the university of Buea, as an indication of fecal contamination. Samples were collected from a total of twenty (20) toilet seats in six (6) different campus buildings: U-Block, Classroom block 1, Classroom block 2, Restau, Amphi 250 and G-block. These samples were analysed for the presence of *E. coli* using a combination of cultural, morphological and biochemical methods. Each toilet seat was swabbed with a sterile stick and inoculated on Eosin Methylene Blue (EMB) agar. Then incubated at 37^0C for 24 hours. After 24 hours, growth was observed on the EMB agar plates. A colony was picked from each plate, with unique *E. coli* features and subjected to Gram staining. All isolates were sub-cultured on nutrient agar and identification of *E. coli* was done based on morphology and standard biochemical tests. *E. coli* contamination was observed most in U-block toilets followed by G-block toilets. This study showed the contamination of toilet seats by *E. coli* and how this poses health challenges. Therefore, increase in the number of toilets as well as proper personal hygiene and sanitary practice is recommended to prevent *E. coli* infections amongst students.

Keywords*: Escherichia coli, toilet seats, sanitary practice, university setting*

Introduction

Escherichia coli is a bacterium that can cause severe foodborne diseases.[1] This organism inhabits the lower gastrointestinal tract (GIT) of mammals and is responsible for several gastrointestinal illnesses, including non-bloody and bloody diarrhea.[2] *Escherichia coli* is also a major cause of urinary tract and other extraintestinal infections, resulting in considerable morbidity, mortality.[3] Most of such infections are caused by distinctive *Escherichia coli* strains called Extraintestinal pathogenic *Escherichia coli* (ExPEC) because of their ability to invade and cause disease at extra intestinal sites.[4] Flush toilets create microdroplets containing viable bacteria[5] and both public and

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private restrooms have been shown to be variably contaminated with human source bacteria.[3] The transmission of this bacterial pathogen is usually via fecal-oral route from food materials, water, animals and environment.

A toilet a simply a receptacle in which both solid and liquid waste of human origin, in the form of urine and excreta are discharged.[6] It is referred to as a public toilet when it is open to the public, accessible to a group of individuals. Toilets could be compartmented in a room or small building and demarcated into male and female sections.[7-9] The toilets in the campus of the university of Buea, are more or less public toilets since they are shared by students. A toilet seat is structured for the user to sit on. Students make use of the toilets per day for urination and defecation. As students come in contact with the surfaces of toilet seats, there is a possibility of picking up microbes deposited on them. *Escherichia coli* can spread when an infected person doesn't wash his or her hands after having a bowel movement. The bacteria are then spread when that person touches someone or something, like food. Thus, this study aimed at isolating and identifying *Escherichia coli* and providing useful information on the contamination of toilets seats by *Escherichia coli* in the university of Buea.

Materials and Methods

Study Area
This study was conducted on toilet seats used by students in the university of Buea.

Study design
This study was based on laboratory investigation with toilet seats samples collected from students' toilets in different class buildings of the university of Buea. It is a presumptive study.

Collection of Sample

Sample collection was done on Monday and Thursday of the week. On each sample collection day, commercially marketed sterile swabs and gloves was purchased. Samples were collected at noon, when people had made use of these toilet seats to maximise the chances of isolation. A total of 10 samples were collected on each collection day. Gloves were worn and specimens were collected from toilet seats on UB campus by means of these sterile cotton swabs. The sterile swab sticks were wiped firmly on the entire surface of the toilet seat. Each swab was labelled after collection, immediately placed in a sterile zip lock bag, and was immediately put in a cool box containing ice packs with temperature maintained at +2 to $+6^0$C. Only one sample was collected from each toilet seat. A total of 20 samples from toilet seats were obtained for this study. Samples were transported to the teaching laboratory within 3 hours of collection for processing.

Culture
The ability to ferment lactose gives an option to use EMB agar to discriminate *E. coli* from other non-lactose fermenting coliforms. Each of the swab sticks were dipped in normal saline and inoculated onto already prepared EMB agar plate as above and streaked out by a burner flame with the aid of a sterile wire loop. Then all of these plates were incubated for 24 hours at 37^0C. After 24 hours, growth was examined. A sterile swab stick was inoculated on EMB agar and another on

NA, incubated at 37^0C as controls to check for sterility of the swab stick. This was to ensure that the isolates that was obtained after inoculation come from samples and not due to contamination.

Gram Staining

Colonies on EMB were subjected to Gram staining in order to determine the bacteria morphology. The procedure was carried out according to Cheesbrough.[10] A smear was made by placing a drop of sterile distilled water on a labelled clean glass slide. A single colony was picked from the culture media plate using a sterile inoculating loop and mixed gently to emulsify on the slide. The smear was left to air-dry. Fixation was done by passing the slide over a flame three times. And then allowed to cool before staining. The slide was placed on a staining rack found in the sink. The slide was stained by flooding the smear with crystal-violet stain for 30-60 seconds. The slide was washed with running water. Lugol's iodine was added and left on the slide for 30-60 seconds. The iodine was rinsed off with tap water. 95% alcohol was used to decolorize for 15 seconds and washed immediately with water. The slide was counterstained with safranin for 2 minutes and washed with water. The back of the slide was wiped clean and placed on a drying rack for smear to air-dry. A drop of oil was added on the dried smear and examined at X100 objective of the light microscope.

Purification of presumptive *E. coli* isolates

Purification of *E. coli* colonies was done in a non-differential medium such as nutrient agar. Colonies from the EMB agar plates were sub-cultured on nutrient agar plates by quadrant streaking method. A sample of the bacteria was picked by a sterile loop and a pool of inoculum was made in the nutrient agar plate. The sample was then streaked several times along one edge of the petri dish. The wire loop was re-flamed, cooled, streaked again to a second quadrant. The wire loop was re-flamed, cooled and streaked again to a third quadrant. The loop was re-flamed, cooled and streaked again to a fourth quadrant. The plates were then incubated at 37^0C for 24 hours.

Biochemical identification of *E. coli*

All pure presumptive *E. coli* isolates were further subjected to biochemical tests which include; catalase test and indole test.

Catalase test

E. coli belongs to the Enterobacteriaceae family which are catalase positive. A small amount of the bacterial colony from the nutrient agar plate was transferred to the surface of a clean, dry glass slide using a sterile wire loop. A drop of 3% hydrogen peroxide (H_2O_2) was placed onto the slide and was carefully mixed. The slide was observed immediately for the production of gas bubbles.

Indole test

This test is important in the identification of enterobacteria. This test is particularly useful in differentiating *E. coli* which are indole-positive from some closely related enteric bacteria which are indole-negative. A syringe was used to measure 4ml of tryptophan broth and put in a sterilized test tube. A pure colony from nutrient agar plate was picked and suspended in the medium. The medium was well stoppered incubated at 37^0C for 24 - 28 hours. A few drops of Kovac's reagent were added to the medium. It was observed immediately for a change in color.

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Results

Table 2: The presence or absence of presumptive *E. coli* isolates of toilet seats on EMB agar from UB restroom

S/N	Collection Site	Presence or absence of presumptive *E. coli* isolates
1	U-Block toilet 1	Present
2	U-Block toilet 2	Present
3	U-Block toilet 3	Absent
4	U-Block toilet 4	Present
5	U-Block toilet 5	Present
6	G-Block toilet 1	Present
7	G-Block toilet 2	Present
8	G-Block toilet 3	Absent
9	G-Block toilet 4	Absent

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10	G-Block toilet 5	Absent
11	Amphi 250	Absent
12	Restau	Present
13	Classroom Block 1 toilet 1	Present
14	Classroom Block 1 toilet 2	Absent
15	Classroom Block 1 toilet 3	Absent
16	Classroom Block 1 toilet 4	Present
17	Classroom Block 2 toilet 1	Absent
18	Classroom Block 2 toilet 2	Absent
19	Classroom Block 2 toilet 3	Absent
20	Classroom Block 2 toilet 4	Absent

Each colony was large, circular, low convex, grayish white, moist smooth and opaque after 24 hours of incubation on nutrient agar.

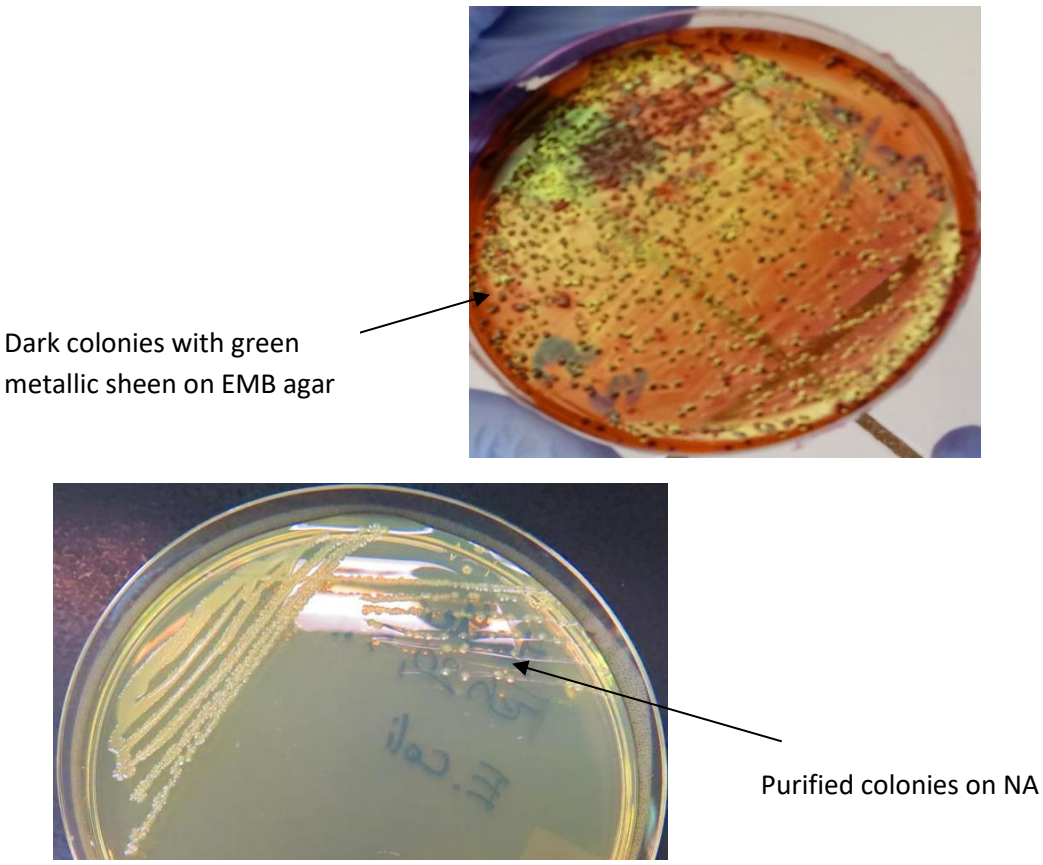

Figure 1: Typical *E. coli*colonies on EMB agar and NA

Each bacterial isolate with the above growth characteristics were gram stained. As expected, each isolate showed a gram reaction typical of E. coli; Gram negative rods in an irregular arrangement and pink in colour under high-power magnification (x1000) (Figure 4).

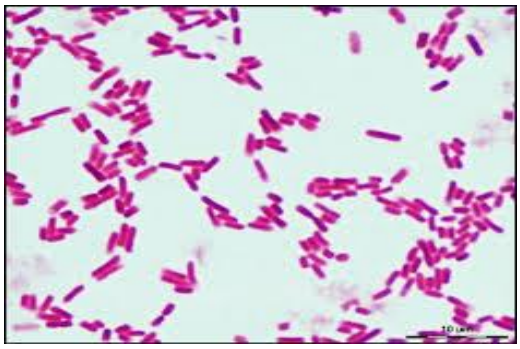

Figure 2: Gram negative rods in an irregular arrangement

All 9 isolates were subjected to biochemical tests including catalase and indole to confirm their identity as *E. coli*. The isolates were tested for catalase production and this was confirmed by the observation of the evolution of oxygen bubbles immediately after the addition of a single drop of 3% hydrogen peroxide. All 9 isolates tested positive for catalase production. All the 9 catalase positive isolates were tested for the presence of indole by means of Kovac's reagent in the test. The isolates tested indole-positive indicated by the change in colour from yellow to cerise red after addition of few drops of the Kovac's reagent.

Of the 20 samples, 9 were positive for *E. coli* according to cultural and biochemical characterization. This included 4 from U-block, 2 from G-block, 1 from restaurant, 1 from classroom block 1 and 1 from classroom block 2 (Table 1).

Table 2: Prevalence of *E. coli* on toilet seats

Site of sample collection	**Number of ample tested each building**	**Number of sample positive for *E. coli***
U-Block	5	4
G-Block	5	2
Restau	1	1

Indole positive

Indole negative

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Amphi 250	1	0
Classroom Block 1	4	1
Classroom Block 2	4	1
Total	20	9

Discussion

This study has isolated and identified *E. coli* from different toilet seats in some UB restrooms. Microorganisms constitute a major part of every ecosystem. The toilet seat is a hard surface not potentially suitable for bacterial growth but some bacteria like *E. coli* can survive on it for up to two weeks, even if the toilet surfaces were cleaned. Toilets are the perfect breeding ground for these bacteria. *E. coli* is found in our intestines but if we are exposed to it from contaminated food, water or nonporous toilet seats, we could suffer from gastrointestinal tract infections characterized by diarrhea, UTIs, pneumonia. It can be passed from person to person especially when people don't wash their hands properly. It can spread from airborne toilet mist. The hands serve a medium for the propagation of microorganisms from place to place and from person to person.

The toilet samples were inoculated on EMB agar which is a selective as well as differential medium for the isolation of fecal coliforms. The differential ingredient is lactose. EMB contains the dye eosin and methylene blue that inhibit the growth of gram-positive bacteria. Therefore, EMB is selective for gram-negative bacteria. In addition, the gram-negative bacteria that grow on it can be differentiated based on their ability to ferment lactose. When bacterial cells ferment lactose, acid is produced that precipitates the dyes in the medium and the colonies develop a green metallic sheen. This medium was chosen for *E. coli* growth because the bacteria have a unique characteristic feature clearly indicative of the bacteria. *E. coli* produces a green metallic sheen on EMB. The samples were swabbed on the EMB plates following strict aseptic techniques to avoid *E. coli* contamination from other sources.

Samples from visibly unclean toilet seats were more contaminated than those visibly clean. Thus, a toilet seat's appearance may predict its likelihood of lower *E. coli* contamination. Likewise, a single toilet in a building such as restaurant where both male and female students make use of one toilet seat was significantly associated with *E. coli*. Since females are especially vulnerable to UTIs[11] these finding suggests that females might benefit from using extra caution when frequenting public restrooms like practicing hand hygiene and using barriers on toilet seats. The

finding that some toilet seats were contaminated with *E. coli* bacteria suggests that human feces are the main source for the bacteria.

Although toilet seats were contaminated, the risk of transmission is not confined to these surfaces. Certain other surfaces such as toilet door handles, sinks, faucets which are touched by hands could also be contaminated. Therefore, individuals could reduce their risk of acquiring *E. coli* from these sites by using hand sanitisers after exiting the restroom. Likewise, the community conceivably could benefit if disinfectant products were used to clean these toilets for greater bio-burden reduction. However, personal hand hygiene remains crucial.[12]

The study revealed variable quantities of contamination of *E. coli* on toilet seats in UB restrooms. A total of 9 samples were contaminated with *E. coli*. Prevalence of *E. coli* was highest in U-block and restaurants toilet seats due to uncleanliness and single use for both male and female students respectively.

Conclusion

The twenty toilet seats from five buildings yielded main findings which have potentially significant public health implications. Some restrooms toilet seats were contaminated with *E. coli*. This contamination occurred in somewhat predictable ways in relation to the cleanliness of the restrooms. Although the presence of *E. coli* corresponded to toilet seats consistent with human fecal source, several other gram-negative bacteria growths were observed. There is the possibility of *E. coli* contamination of other surfaces of the restroom. These surfaces serve as media for infections transfer.

References

1. Lee H, Yoon Y. Etiological agents implicated in foodborne illness world wide. Food science of animal resources. 2021;41(1):1.
2. Naylor SW, Gally DL, Low JC. Enterohaemorrhagic E. coli in veterinary medicine. International journal of medical microbiology. 2005;295(6-7):419-441.
3. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Microbes and infection. 2003;5(5):449-456.
4. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut pathogens. 2019; 11:1-6.
5. Stenström TA, Seidu R, Ekane N, Zurbrügg C. Microbial exposure and health assessments in sanitation technologies and systems. Stockholm: Stockholm Environment Institute; 2011.
6. Faris K, Alemayehu T, Wubshet M, Hailu D. Human and Other Liquid Waste Management. Ethiopian Public Heath Training Initiative (EPHTI). 2002.

Citation: Ngomo SSI, Cho JF. Isolation and Identification of *Escherichia Coli* from Students' Toilet Seats in the University of Buea Restrooms. Elite Journal of Public Health, 2024; 2 (5): 21-31

7. Sheikh S. Public toilets in Delhi: an emphasis on the facilities for women in slum/resettlement areas. CCS Working Paper; 2008.
8. Onyeaghala EO, Maxwel EA, Obeagu EI, Hassan AO. Assessment of the Problems Associated with the use of Public Latrine System in Ife-North Local Government, Osun State, Nigeria. RESEARCH IN MEDICAL SCIENCES (NIJRMS). 2024;5(1).
9. Hasssan AO, Obeagu EI, Onu FU. A Survey of microbial contamination of door handles in various locations in Lokoja metropolis, Kogi state, Nigeria. International Journal of Current Research in biology and Medicine. 2022;7(1):8-16.
10. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, United Kingdom, 2006; 60-64.
11. Vasudevan R. Urinary tract infection: an overview of the infection and the associated risk factors. J Microbiol Exp. 2014;1(2):00008.
12. Mohamed M, Owens K, Gajewski A, Clabots C, Johnston B, Thuras P, Kuskowski MA, Johnson JR. Extraintestinal pathogenic and antimicrobial-resistant Escherichia coli contamination of 56 public restrooms in the greater Minneapolis-St. Paul metropolitan area. Applied and environmental microbiology. 2015;81(13):4498-4506.

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