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Abdul-Halim Osman
[1] Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana
[2] Department of Medical Microbiology, University of Ghana Medical School, Ghana

Christian Bonsu
Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

Stephen Osei-Wusu
Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

Isaac Darko Otchere
Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

Adwoa Asante-Poku
Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

Correspondence Author;
Adwoa Asante-Poku
Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana

Isolation and characterization of bacteria contaminants from doorhandles of a research institution and a teaching facility at a tertiary institution in Ghana

Abdul-Halim Osman, Christian Bonsu, Stephen Osei-Wusu, Isaac Darko Otchere and Adwoa Asante-Poku

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Abstract
The rising frequency of epidemic breakouts of certain diseases and their rapid transfer from one community to another has become a serious public health concern. Surfaces in public contact, like doorknobs, that are routinely handled by numerous hands can potentially spread illness. This study aimed to isolate, identify, and quantify the bacteria load on the door handles of a research facility and a teaching facility. The analysis included sixty samples. Using standard microbiological methods and the MALDI-TOF biotyper, each sample was identified. Bacterial contamination in the samples collected on Friday was higher than it was on Monday. The research faculty were the most contaminated on Monday, whereas the teaching facility had the highest count. *Serratia rubidea*, *Bacillus species*, and *Klebsiella* species were the least bacteria observed, with *Acinetorbacter* and *Staphylococcus* species being the most frequently found.
Doorhandle is a strong hotspot for transmissible diseases. Our findings revealed a moderate degree of bacterial contamination on door handles, characterized by a notable presence of potentially disease-causing bacteria with a high bacterial load. It is necessary to perform research to minimize the emergence of pandemics. but little attention is paid to it, especially in sub-Saharan Africa.

Keywords: Doorhandle, fomite, bacteria, public health, contaminant, pathogenic, *Staphylococcus*

Introduction
Microorganisms, existin g ubiquitously, play a vital role in the functioning of every ecosystem. They inhabit diverse environments, either as free-living organisms or as parasites, contributing to the balance of nature [1, 2]. However, some microorganisms assume the role of transient contaminants, colonizing inanimate objects (Fomites) and hands, thereby posing significant health hazards as potential sources of community and hospital-acquired infections [3-5]. The adaptability of microbes to thrive on human hands as well as fomites, have made public places, including transportation hubs, restaurants, and educational facilities, as conduits for microorganism transmission among individuals [2, 6]. The escalating incidence of epidemic outbreaks and their rapid spread among individuals in settings or the spread from one community to the other have emerged as pressing concerns in public health [6, 7].

Exposure and contact with contaminated inanimate surfaces play a significant role in the spread of infectious diseases. These surfaces, known as fomites, encompass a range of objects such as door handles, sinks, faucets, sphygmomanometers, thermometers, lockers, tables, and chairs [8]. Particularly, those encountered in public offices, restaurants, hotels, hospitals, and restrooms have been identified as significant sources of concern [9]. One of the most implicated probable sources of infections is doorhandles at public places as they have been identified to be a potential reservoir and a vehicle of infectious disease transmission among individuals [6, 10-12]. These doorhandles in the public sectors serve as gateways, connecting countless individuals on daily basis as they are frequently touched. However, beneath their smooth and nondescript exteriors sometimes lies a hidden menace-microbe contaminants, including bacteria [11, 13]. These bacterial contaminants can live as transient contaminants on the handles or hands where they constitute a major health hazards.

Several factors influence the residence and transfer of bacteria between these surfaces, including the size of the inoculum deposited on the surfaces, pressure, moisture levels, friction between contact surfaces, and the specific bacterial species involved [3, 4]. Unfortunately, many doorhandles in public settings fulfill multiple of these conditions that contribute to their susceptibility to bacterial contamination, and thereby putting frequent users of the doorhandles at risk of bacterial infections. This concern is further compounded when pathogens become resistant to disinfectants or develop antibiotic resistance, amplifying the severity of the issue [14, 15]. One contributing factor to the transmission of these pathogens to their preferred sites of infection is the potential transfer of bacteria through food consumption with contaminated hands [16, 17].

Bacterial infection has recently been estimated to become the leading cause of deaths by 2050, causing 10 million deaths across the globe [18, 19]. Further, it is predicted that, there will be lesser treatment options to some bacterial infections as they are increasingly becoming multidrug-resistant to the current antibiotics [20, 21]. However, research has rarely been conducted in Teaching Facilities and Research institutions, where clinical bacterial isolates and other pathogenic bacteria are commonly used for research purposes. As a result, little information and data exist to inform how doorhandles of Teaching and Research Institutions pose threat to individuals and random visitors. This study therefore sort to isolate, quantify and characterize bacterial contaminants from doorhandles in a Research Institute and a Teaching Facility in the University of Ghana to provide scientific insights with policy implications.

Materials and Methods

Study site and Sample collection

The tertiary institution's teaching and research facilities served as the sites for this investigation. Samples were taken from doorknobs in the following areas: lecture rooms, offices, labs, libraries, and restrooms. Identification numbers were affixed to collected samples to guarantee their appropriate arrangement. Samples were collected from doorhandles during the busiest time of day (11:00 AM–1:00 PM) and transported to the Lab for analysis. A total of sixty samples were analysed in this study.

Sample collection

The American Public Health Association's swab-rinse procedure was used to collect the samples. Door handles were thoroughly swabbed with sterile swabs saturated with regular saline and placed into a small sterile bottle filled with the same sterile saline. The tiny bottles were wrapped in cellophane and brought to the laboratory for analysis.

Sample processing

Each sample was processed to identify the bacteria in the sample. Using pour-plate methods, each sample was serially diluted and aseptically inoculated on Plate Count Agar plates (PCA) to enable the enumeration of viable bacterial colonies, MacConkey agar (Oxoid) for the recovery of any possible Gram-negative bacteria and some Gram-positives in the sample. The remaining part of the samples was enriched in alkaline peptone water and selenite broth before culture unto Salmonella Shigella agar and Thiosulfate–citrate–bile salts–sucrose (TCBS) agar respectively for the recovery of any possible *V. Cholera*. All incubations were done aseptically, and plates observed for growth for an extra 24 hours if growth was not seen during first incubation. All distinct individual colonies on the different agar were purified on nutrient agar prior to identification. Bacterial and fungal colonies were identified using Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). Manufacturer's instructions on extended direct Transfer method of identification were followed. This generates a unique spectrum of the relative abundances of ribosomal proteins and compared to the reference from the database of the MALDI-TOF to identify organism based on score of similarity as previously described by Guo *et al*. [22]. Further, the quantification of the bacteria on the positive PCA plates were determined in CFU/mL by the aid of colony counter as described and done by others [2, 23] and the equation:

$$\frac{Number\ of\ colonies\ formed\ \times dilution\ factor\ of\ plate}{Volume\ of\ inoculum\ plated}$$

Statistical Analysis

The obtained data was summarized and analyzed by Microsoft Excel 2013 version. Among the other statistical analysis, the calculation and interpretation of the viable colonies were done by this tool.

Results

On average, 35% of the doorhandles across both study sites were contaminated. There was no difference ($p > 0.05$, CI=0.35-3.83, at 95% CL) between the contaminated doorhandles seen at the teaching facility (36.7%) and contaminated doorhandles observed at the Research Institute (33.3%). Furthermore, 16.6% of the total doorhandles were consistently contaminated with bacteria each day the samples were collected in the week (Table 1).

Table 1: Summary of doorhandle contaminations from the two study sites

Study Site	Number of Samples	Number of Positive Samples	Percentage Positive
Teaching Facility	30	11	36.7%
Research Institute	30	10	33.3%
Total Average	60	21	35%
Consistently Contaminated within the Week	30	5	16.7%

Microbial Load Estimates on the Doorhandles

The bacteria load from the doorhandles ranged from 3.7×10^3 on Monday to 2.6×10^6 CFU/ml on Friday (Table 2). Averagely, the samples collected from doorhandles of the teaching facility on Friday of the week had a higher number of viable bacteria colonies (Approximately 543,428 colonies) compared to samples collected on Monday (Approximately 162,500 colonies). Contrastingly, samples sampled doorhandles from the research institute were more contaminated on Monday (Approximately 100,666 colonies) compared to Friday (Approximately 33,666 colonies). The most contaminated doorhandle from the teaching facility

was Lab/Lecture 5b on Friday compared to Entrance Door 1a of the research institute on Monday with 190,000 colonies. Interestingly, whereas every doorhandle sampled at the research facility irrespective of day of sampling was contaminated, there were three (3) and two (2) doorhandles on Monday and Friday respectively from the teaching facility that were sterile (That is no bacteria were isolated from these samples with zero colony count) by culture (Table 2).

Table 2: Total bacteria counts of doorhandles on different days at the Teaching Facility and the Research Institute

Sample: Monday	Number of viable bacterial colony	Sample: Friday	Number of viable bacterial colony
Teaching Facility			
Office 1a	0	Office 1b	2.5×10^4
Office 2a	3.8×10^3	Office 2b	2.4×10^4
Lab/Lecture 5a	1.1×10^6	Lab/Lecture 5b	2.6×10^6
Lab/Lecture 6a	3.7×10^3	Lab/Lecture 6b	0
Washroom 8a	0	Washroom 8b	7.8×10^4
Lab 9a	0	Lab 9b	1.0×10^6
Library 10a	3.0×10^4	Library 10b	0
Research Institute			
Entry Door 1a	1.9×10^5	Entry Door 1b	2.0×10^4
Entry Door 2a	9.8×10^4	Entry Door 2b	2.1×10^4
Washroom 8a	1.4×10^4	Washroom 8b	6.0×10^4

Abbreviations: a= sampled on Monday. b= sampled on Friday

Distribution of Bacteria Isolates at Both Sites

In total, 11 different bacteria species were identified from the positive cultures using the MALDI-TOF mass spectrometry bio-typing. Whiles 9(81.8%) of the identified bacteria (Including *Serratia rubidea*, *Psychrobacter maritimus*, *Arthrobacter koreensis* & *Pseudomonas montelli* were restricted to the teaching facility, *Pseudomonas stutzeri*, *Acinetobacter quillouiae*, *Bacillus cereus*, *Stapylococcus warneri* & *Klebsilla viricola* were restricted to the research institute) were study site-specific only two (*Staphylococcus cohnii* and *Acinetorbacter schindleri*) were isolated from both study sites as shown below (Table 3).

Table 3: Bacterial isolates on the door handles across both study sites

Study Site		Bacteria										
		Serratia rubidea	***Psychrobacter maritimus***	***Acinetobacter schindleri***	***Arthrobacter koreensis***	***Staphylococcus cohnii***	***Pseudomonas montelli***	***Pseudomonas stutzeri***	***Acinetobacter quillouiae***	***Bacillus cereus***	***Staphylococcus warneri***	***Klebsiella viricola***
Teaching facility	Office 1b	√										
	Office 2a		√									
	Lab/ Lecture 5a			√								
	Lab /Lecture 5b				√							
	Washroom 8a					√						
	Washroom 8b			√								
	Lab 9b						√					
	Library 10a		√									
	Washroom 15b					√						
Research facility	Entrance Door 1a							∇				
	Entrance Door 1b			∇					∇			
	Entrance Door 2a								∇			
	Entrance Door 2b			∇						∇		
	Washroom 7a										∇	
	Washroom 8a					∇						
	Washroom 8b					∇						
	Office 3b											∇

√ Isolated from doorhandle at teaching facility, ∇ Isolated from doorhandle at research facility. Abbreviations: a= sampled on Monday. b= sampled on Friday

Discussion

In recent times, compelling evidences are amassing supporting that, doorhandles are potential fomites for bacterial transmissions, establishing them as significant

fomites capable of facilitating cross-infections and recontamination among individuals in a public setting. This is further substantiated by the increasing evidence demonstrating the ability of various bacteria to colonize and strive on inanimate surfaces, including doorhandles [3, 4]. The general findings of our study revealed moderate level of bacteria contaminants on doorhandles but high number of viable bacteria colonies across the Teaching and Research Facilities. The presence of larger numbers of bacterial colonies is attributed to several factors. Principally the doorhandles in the Research Institute and the Teaching Facility are made of nonporous materials, providing good surfaces for microbial attachments because of their coarse nature [13, 24]. They also retain some moisture and comparatively moderate heat which are conducive for microbial proliferation.

Our study reports 35% bacteria contamination of doorhandles in the two study sites, significantly less than the findings of Nabawanuka *et al.* [21] who reported 86.0% contamination on doorhandles of a tertiary institution in Uganda, 60.9% bacterial contamination of office door handles in a tertiary institution in Nigeria [25], 86.7% by Nworie *et al.* [6] on doorhandles of public conveniences in Abuja, 64.5% bacterial contamination on toilet doorhandles reported by Abios *et al.* [26] in a University campus in Nigeria. The relatively moderate contaminated door handles in this study may be attributed to the improvement of hand hygiene resulting from the COVID-19 pandemic preventive measures. This moderate number is similar to the 40% bacterial cultures in Al-Najaf Province [27] but higher than the 20.3% reported by Omololu-Aso *et al.* [28] on door-knobs in Obafemi Awolowo University Teaching Hospital Complex in Nigeria. Further, the high number of viable bacteria colony across the two institutions is similar to the reported figure of 9.6×10^6 CFU/mL on door-nob of wards [29] and corroborates the findings of Odigie *et al.* [12], who observed a low (0.03×10^4 CFU/mL) to high viable colony of 4.17×10^4 CFU/mL on a doorhandle of a tertiary institution. The relatively higher number of viable colonies in the Teaching facility was expected considering the high number of staff and student population engaging with the doorhandles [30, 31]. Nevertheless, the observed microbial loads varied depending on the day and location. For instance, while the teaching facility saw higher contamination levels on Friday, the research institute had higher levels on Monday. This variation may be attributed to differences in usage patterns and cleaning protocols between the two sites. The identification of 11 distinct bacterial species, some unique to each location, further supports the influence of environmental factors on contamination profiles [32]. Moreso, the higher average bacterial colony counts recorded on door handles in the Teaching facility compared to the research facility may be attributed to of the significantly higher door contact due to the relatively high number of students in the teaching facility compared to staff of the research facility. Nevertheless, the 36.7% recorded value of the Teaching Facility in our study is less than the 75.29% bacteria contamination of doorhandles reported by Tiku *et al.* [33] in the University of Calabar, and Al-Harmoosh *et al.* [10] who reported 80% in Classrooms doors handles and 75% in offices doors handles. Further, The 33.3% recorded value of the Research Institute is also less than the findings of Al-Harmoosh *et al.* who reported 90% bacteria growth in laboratories doors handles [10]. These findings in comparison with other reports suggests that both the research and teaching facilities of the University of Ghana may have better decontamination culture.

We observed dominance of *Staphylococcus species* among the characterized isolates irrespective of study stie. This aligns with the report of Alothaim *et al.* on bacterial contaminants on doorhandles of community pharmacies [13]. The common distribution of *staphylococcus* on the handles can be ascribed to its pronounced existence within the skin and nasal microbiota, thus underscoring its elevated incidence as a contaminant and enabling its easy dissemination through various human activities [10]. The result of this present study further shows that *P. maritimus*, *B. cereus*, *S. rubidea*, *A. schindleri*, *A. koreensis*, *P. stutzeri*, *K. viricola*, and *S. cohnii* are the common doorhandle contaminants in the institutions in agreement with earlier reports [6, 10, 13, 34]. With the exception of *A. koreensis*, all the identified bacterial species have been associated with human infections [35-42].

Moreover, we observed that, the structural location of the doorhandles plays critical role in their contamination with viable bacteria. as doorhandles that are situated on main entrances and doorhandles that had higher human contacts in these two institutions recorded the highest viable bacterial counts in CFU. This observed trend agrees with the findings of Wojgani *et al.* [43] who found that, door location had an impact on its handles` contamination. Moreover, the specific doorhandles with the highest colony count appeared to be lecture room, laboratory, and washroom doorhandles this is similar to the findings of Odigie *et al.* [12], who also saw toilet doorhandle to be the most contaminated doorhandle in a tertiary institution. Suggesting that, there might be fecal contaminants on the door handles [26]. This may be attributed to the high levels of exposure to individuals who throng in and out without using appropriate hand hygiene, and disseminating contaminants and their flora to the doorhandles [12, 34], this is in agreement with the findings of Nworie *et al.*, [6] who reported that the levels of contamination vary depending on traffic exposure and the specific characteristics of the surrounding environment.

Conclusion

Doorhandles of Research Institutes and Teaching Facilities are considerably contaminated with potential disease-causing bacteria and can therefore serve as fomite for the transmission of infectious pathogens among individual. This study reinforces the importance of routine cleaning and disinfection of high-touch surfaces, like door handles, to minimize the risk of bacterial transmission. The diverse bacterial communities found on door handles, along with the potential for cross-contamination, underscore the need for context-specific hygiene practices and further research to better understand the dynamics of microbial contamination in different environments

Limitations and Recommendations

This study is limited by the small sample size and focus on only two locations within the bigger university of Ghana community. Future research could expand to a wider range of environments and investigate the factors influencing the temporal dynamics of door handle contamination. Additionally, incorporating molecular methods that do not require culturing may be of help in determining total

contaminations and not only culturable microbes. We recommend installation of automated doors at main entrances to curb the mass human contacts on a doorhandle. Furthermore, following the presence of these bacteria on the handles, further research should be carried out to assess their antibiotic resistance patterns. Finally, research should be carried out particularly on the risk of other potential pathogens including viral and fungal contamination on doorhandles of Teaching Facilities and Research Institutes.

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