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Bacterial Contamination of Door Handles of Commercial Buses in Umuahia Metropolis Abia State

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Abstract

Commercial vehicles are all kinds of vehicles used to transport goods and people. Microbiological investigation of publicly touchable surfaces has become an interest to researchers because surfaces that are contaminated can function as reservoir of potential pathogens. This study was carried out to determine the levels of bacterial contamination on the surfaces of two door handles on public buses within Umuahia metropolis. A total of 70 samples, 35 each from the passengers and driver's door handles surface were collected and analyzed. The analysis involved bacterial isolation and identification of organisms by standard microbiological procedures. The prevalence and bacteria count were carried out. The antibiotic sensitivity pattern of the isolates were done using the disc diffusion method. A total number of 73 bacterial organisms were isolated. The following potential pathogenic bacteria were observed; *Staphylococcus aureus* (6.8%), *Staphylococcus epidermidis* (5.5%), *Streptococcus faecalis* (2.7%), *Escherichia coli* (6.8%), *Klebsiella* spp. (5.5%), *Enterobacter* spp. (4.1%), *Pseudomonas aeruginosa* (17.8%), *Proteus* spp. (23.3%), Micrococcus spp. (6.8%) and *Bacillus* spp. (20.5%). Ofloxacin, Pefloxacin and Gentamicin are effective and exhibited encouraging results while Ampicillin and Trimethoprim were resistant to most isolates. The result actually shows that the door handles of motor vehicle used for public transportation can be a significant reservoir for spreading potential pathogenic microorganisms.

Keywords: Bacterial contamination, Commercial buses door handles, Antibiogram

1. Introduction

There is a misconception that microbes are only found in research laboratories and healthcare facilities. Causes of health problems could be as a result of lack of knowledge about where germs grow. About 80% of infections are spread through direct contact with hands (Al-Ghamdi et al., 2011; Reynolds, Watt, Boone, & Gerba, 2005). Microorganisms that cause infections can be found in any environment including soil, air, water, food and other organisms as well as on environmental surface or objects. The spread of infectious diseases due to contact with the hands is a major concern. Hand washing that is well known and the first thing that is done in preventing diseases from spreading has been abandoned and must be enhanced vigorously by families, schools and healthcare professionals.

The ability of inanimate objects to support viable microorganisms for a long period of time is well documented (Fierer, Hamady, Lauber, & Knight, 2008) and such environmental surfaces and objects especially those in proximity with persons and frequently touched pose a threat to human health and area cause for concern. Also recent studies have shown the presence of bacterial pathogens on hard, non-porous surfaces such as kitchen surfaces, floor surfaces, toilet surfaces, door handles etc. (Maori, Agbor, & Ahmed, 2013; Nworie, Ayeni, Eze, & Azi,

2012) from which pathogens are easily transmitted to unsuspecting members of the public posing potential risk to vulnerable, immune-compromised individuals (Fierer et al., 2008). Currently, some of these bacterial pathogens have become antibiotic resistant, which is a major public health crisis facing the world today (Ventola, 2015; Voicu et al., 2017). Chittagong and many other cities of the country lack adequate transportation. Exorbitant prices of other forms of private transportation and inadequate provision of railroads in the city have left people with no option than to use public bus. Public buses are rarely cleaned and focus only on sweeping of floors and windows even if cleaned (Yeh, Simon, Millar, Alexander, & Franklin, 2011). Microbial load on touch surfaces are built up by the unhygienic and humid condition of buses and may lead to mild to severe infection in human from such reservoirs through interpersonal transfer of microorganisms.

Nworie et al. (2012) carried out a research on 180 door handles/knobs of public toilets of public offices, motor parks and markets that were selected in Abuja Metropolis, Nigeria. It was discovered that 156 (86.7%) yielded bacterial growth. The bacterial isolates were *Staphylococcus aureus* (30.1%), *Klebsiella pneumoniae* (25.7%), *Escherichia coli* (15.6%), *Enterobacter* spp. (11.2%). *Citrobacter* spp. (7.1%), *Pseudomonas aeruginosa* (5.9%) while *Proteus* species was the least isolated (4.5%).

Sabra (2013) Investigated on public female restrooms at Taif, Kingdom of Saudi Arabia, in order to know the level of contamination and bacterial loads. It was discovered that out of the 260 specimens, 187 (71.9%) turned out to be positive. Toilet handles (91.3%) had a higher percentage followed by room door handles 59 (73.8%), and room sink 38 (63.3%). Bacterial isolates in order of how they were frequently isolated are; *S. aureus* 40.6%, *E. coli* 22.5%, *Bacillus* spp. and *K. pneumoniae* 21.4%, *Enterococcus faecalis* 13.4%, *Citrobacter* spp. 9.6%, *P. aeruginosa* 8.6% and *Proteus mirabilis* 7%.

The aim of this study was to evaluate the potential bacterial pathogens as contaminants on commercial bus door handles.

2. Materials and Methods

2.1 Study Area

This study was conducted in Umuahia metropolis, Abia State in the South-East of Nigeria between April to July 2021. Samples was collected from three different locations as stated below:

Sample A was collected in MOUAU School Park, sample B was collected in Isi-Gate, while sample C was collected at Cooperative. All samples were properly labeled and transported to the Microbiology laboratory for examinations.

2.2 Sample collection and processing

Sample was collected by swabbing the door handle with a sterile cotton swab stick soaked in sterile water from each location aseptically. Each sample was taken twice per bus randomly and were transported to the laboratory for analysis (Cheesbrough, 2006).

Serial dilution was done from the soaked sample collected from the three different locations in Umuahia metropolis. In the procedure, 9ml of normal saline was poured into 5 different sterile test tubes labelled 10-1, 10-2, 10-3, 10-4, and 10-5. For each of the sample collected, 2ml of normal saline was added and shaken, I ml was transferred into the first test tube labeled 10-1 and tilted gradually to mix, after that 1ml from it was taken into the second test tube labeled 10-2 and from it I ml was taken after shaking to mix to the test tube labeled 10-3 until the last dilution factor i.e. 10-5 where after mixing I ml was discarded. Serial dilution was done throughout the project processes for each of the sampled collected. All the available samples were processed for microbial isolation in a sterile atmosphere, by swabbing the work bench and lightning a spirit lamp on the table. The following culture media were used: MacConkey agar, Nutrient agar, Mannitol salt agar and Blood agar were used. The samples were inoculated on the plates of the different prepared media aseptically using streak method and incubated for 24hours at 37°C. Pure colonies of isolate organisms were identified and characterized using standard microbiological techniques (Cheesbrough, 2006).

2.3 Antibiotic susceptibility test

Antibiotic disc sensitivity testing was carried out on each of the bacterial Isolates with the Mueller-Hinton agar. Standard paper discs soaked with adequate amount of antibiotics were placed in the Mueller-Hinton agar in this method. After Incubation for 24 hours, antibiotic activity was determined by zone of Inhibition, (No growth)

around the antibiotic disc and was measured in millimeters (mm) with a transparent meter rule and a protractor. The susceptibility was carried out for the following antibiotics; Ofloxacin (10µg), Peflacine (10µg) Ceftriaxone (10µg), Amoxicillin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg). Amoxicillin/Clavulanate (10µg) and Ampicillin (30µg). With the aid of 0.1ml of 0.5 McFarland's standard as a turbidity check for a semi confluent growth, these antibiotics were tested after an overnight cultures of the isolates on Mueller-Hinton agar. With reference to the Clinical and Laboratory

Standard Institute (CSL1) performance standard for anti-microbial susceptibility, the organism's susceptibility or resistance pattern to the drugs used was done.

3. Results

In the course of this investigation, a total of 70 samples, 35 from the driver's door handle side and 35 from the passenger's door side were aseptically collected with a sterile swab stick, labeled properly and transported to the laboratory for analysis.

Table 1 shows all the bacterial isolates and their percentage. A total of 61 bacterial organisms were isolated. These organisms comprised of nine (9) genera out of which *Proteus* spp. (23.3%) and *Bacillus* spp. (20.5%) ranked the highest in frequency of occurrence.

Table 2 shows the mean bacterial load of isolates from the passenger and driver's side. Antibiotic pattern of the isolates is shown in Table 3. The result indicates that most of the isolates were sensitive to the tested antibiotic.

Table 1. Prevalence of various isolates observed in the study.

Isolates	No isolated	Percentage (%)
Pseudomonas aeruginosa	13	17.8
Proteus species	17	23.3
Klebsiella species	4	5.5
Micrococci	5	6.8
Staphylococcus epidermidis	4	5.5
Bacillus species	15	20.5
Escherichia coli	5	6.8
Staphylococcus aureus	5	6.8
Streptococcus faecalis	2	2.7
Enterobacter spp.	3	4.1
TOTAL	**73**	**100**

Table 2. Mean bacterial load of isolates (cfu/cm²) from the passenger and driver's side.

Isolates	Passenger	Driver
Pseudomonas aeruginosa	$9.0\times10^{3}\pm3.4$	$5.0\times10^{2}\pm1.4$
Proteus species	$4.0\times10^{4}\pm2.3$	$3.0\times10^{3}\pm2$
Klebsiella species	$7.0\times10^{4}\pm1.1$	$4.0\times10^{4}\pm2.3$
Micrococci	$5.0\times10^{3}\pm3.1$	-
Staphylococcus epidermidis	$6.3\times10^{2}\pm3.2$	$2.0\times10^{2}\pm2$
Bacillus species	$4.0\times10^{4}\pm2.1$	$3.4\times10^{3}\pm2.3$
Escherichia coli	$8.0\times10^{4}\pm2.3$	$3.5\times10^{5}\pm3$
Staphylococcus aureus	$6.0\times10^{3}\pm5.2$	$4.I\times10^{3}\pm2.5$
Streptococcus faecalis	$5.0\times10^{2}\pm1.5$	$2.3\times10^{4}\pm2.1$
Enterobacter spp.	$7.0\times10^{3}\pm2.2$	-

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Table 3. Antibiotics susceptibility pattern of the isolates from various motor vehicle door handles.

		Number sensitive (%)								
Organism	**No Tested**	**OFX**	**PEF**	**CN**	**AU**	**S**	**SXT**	**PN**	**CPX**	**CEP**
P. aeruginosa	15	8 (61.5)	7 (53.8)	6 (46.1)	1 (7.7)	6 (46.1)	0 (00)	0 (00)	8 (61.5)	3 (23.0)
Proteus species	17	14 (82.3)	12 (70)	10 (58.8)	4 (23.5)	5 (29.4)	2 (11.8)	2 (11.8)	10 (58.8)	4 (23.5)
Klebsiella species	4	4 (100)	3 (75)	0 (00)	2 (50)	2 (50)	0 (00)	0 (00)	2 (50)	1 (25)
Micrococci	5	-	-	-	-	-	-	-	-	-
S. epidermidis	4	1 (25)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)
Bacillus species	15	-	-	-	-	-	-	-	-	-
Escherichia coli	5	3 (60)	3 (60)	3 (60)	3 (60)	3 (60)	0 (00)	0 (00)	0 (00)	1 (20)
S. aureus	5	3 (60)	3 (60)	2 (40)	1 (20)	2 (40)	0 (00)	0 (00)	1 (20)	1 (20)
S. faecalis	2	0 (00)	1 (50)	0 (00)	1 (50)	0 (00)	1 (50)	0 (00)	0 (00)	0 (00)
Enterobacter spp.	3	0 (00)	0 (00)	2 (66.7)	1 (33.3)	1 (33.3)	0 (00)	0 (00)	0 (00)	0 (00)

Key SXT=Trimethoprim, CN= Gentamicin, AU=Amoxicillin/ Clavulanate, OFX=Ofloxacin, PEF= Pefloxacin, PN=Ampicillin, CEP= Cephalexin, S= Streptomycin, CPX= Ciprofloxacin

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4. Discussions

Bacterial contamination of door handles is well documented and these fomites act as pathway for cross-infections. Some of the contaminants can be highly pathogenic and can be transferred directly or may result in auto-inoculation (Kennedy, Enriquez, & Gerba, 2007).

The surfaces of commercial bus door handles usually come in contact with the palms of various individuals of different hygienic backgrounds. Evaluation of possible bacterial contaminant is targeted at the isolation and identification of potential pathogens which could cause infection in man when the palms are contaminated. A total of 70 door handles swabs from different commercial bus of the passenger and driver side in Umuahia metropolis were investigated to ascertain the level of bacterial contamination and to identify the bacteria contaminants in this study. Out of 70 samples assessed, 62 (88.6%) of them yielded bacterial growth. Some of the positive samples examined had more than one type of bacterial isolate. This result compares favorably with that obtained by Nworie et al. (2012) in Abuja Metropolis, Nigeria, who reported that out of 180 door handle swabs assessed, 156 (86.7%) showed bacterial growth, and also in agreement with reports of Onwubiko and Chinyeaka (2015) who observed 86 (86%) bacterial contamination of door handle out of 100 samples assessed in MOUAU.

The outcome of this research showed that *Staphylococcus aureus*, *Bacillus* spp., *Micrococcus* spp., *Escherichia coli*, *Proteus* spp., *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Enterobacter* spp., *Pseudomonas aeruginosa* and *Klebsiella* spp. are the main bacterial isolates associated with the door handles frequently as also recorded by Bashir, Muhammad, Sani and Kawo (2016) whose result shows to be *Staphylococcus aureus*, *Bacillus* spp., *Micrococcus* spp., *Escherichia coli*, *Salmonella* spp. and *Klebsiella* spp. are the main bacterial isolates always associated with toilet door handles. Through the skin and direct contact, these organisms may probably have their way to the door handles.

In the current study, both Gram negative and Gram positive bacteria were isolated. This is at variance with the reports of Datta, Rani, Chander and Gupta (2009) who isolated only Gram positive bacteria: *Staphylococcus*, *Enterococcus*, *Micrococcus* and *Streptococcus* from mobile phones and in agreement with the studies of Yazah, Yusuf and Agbo, (2012), and Cataño, Echeverri and Szela (2012) who isolated Gram positive and Gram negative bacteria in their research from currency note and computer keyboards, curtains, cellphones, white coats and ties.

The findings from this research reveals *Proteus* spp. and *Bacillus* spp. to be the most frequently bacterial isolates with a percentage occurrence of 23.3% and 20.5% respectively with the viable count being higher in the passenger's side when compared with the driver's side due to the number using the handle is higher. This is in agreement with finding of Onwubiko and Chinyeaka (2015) who reported that in canteens, female restroom, male and female hostels, the level of contamination was higher in that order as compared to classrooms and laboratory that were low. Laboratory and classrooms showed a lower level of contamination and it could be attributed to the fact that they are not always in use as other places studied. This is also in agreement with the findings of Boone and Gerba (2010) and Nworie et al. (2012) who reported that depending on the exposure, environment and traffic, the level of contamination varies.

A high percentage of gram-negative rods isolated in this study which includes *Proteus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* spp. indicate the possibility of the presence of faecal contamination on the door handles. This may be as a result of contaminated hands with fecal and urinary material while using the toilets. The issue of hygiene is taken with levity which result in failure to wash their hands and the concept of hand washing as a common means of stopping the spread of these infectious agents are lacked. This compares favorably with the work of Zhad, Zhad, Doyle and Meng (1998), who reported that the high rate of isolation of these organisms in which human hands serve as means of transmission is only obtained during epidemics. Gram negative sepsis, urinary tract infections are most commonly caused by *E. coli*, *Klebsiella* spp. and *Proteus* spp. Also a high percentage of *Bacillus* spp. a gram positive organism isolated could be explained by the fact that *Bacillus* spp. are everywhere with its ability to form spores that can withstand harsh environmental condition, withstand dry heat and certain chemical disinfectants for extended period of time (Onwubiko & Chinyeaka, 2015). This is also in agreement with the work of

Brooks, Carroll, Butel and Morse (2007) who reported that *Bacillus* spp. was discovered to be a few of the primary organism that was isolated from door handles, Ashgar and El-Said (2012), also reported the isolation of *Bacillus* spp. from environmental sites in Mecca city.

Staphylococcus aureus cause boils, toxic shock syndrome, pneumonia, abscesses, wound infections and other disease (Yusha'u, Bello, & Sule, 2010) while *Staphylococcus epidermidis* is responsible for endocarditic and infections of patients with low immunity. The presence of these organisms indicate that the use of public buses, public motorcycle, ATMs and door handles can lead to circulation of serious skin infections.

Streptococcus A strains are found in the throat and skin and can cause strep throat and impetigo, a common skin infection that primarily affects children. The manifestation of *Enterobacter aerogenes* and *E. coli* shows likely faecal contamination of the ATMs, public toilets and motorcycle and the consequence could be a potential source of food poisoning when eating with infected hands and preparing food without proper hand washing (Yusha'u et al., 2010).

Antibiotics susceptibility studies of the isolates showed a pattern of resistance to commonly used antibiotics such as Trimethoprim, Ampicillin and Gentamicin. This is in agreement with the reports of Onwubiko and Chinyeaka (2015), and Nwankwo and Offiah (2016) who reported that antibiotic resistant microorganism contaminates environmental surfaces such as door handles in a tertiary institution in Umuahia and interface of automated teller machine (ATM) in Umuahia metropolis respectively. The isolates recorded in their research were resistant to commonly used antibiotics such as Cotrimoxazole, Amoxicillin/Clavulanate, Gentamicin and Ampicillin.

5. Conclusion

The results of this study show that several bacteria often occupy public contact area such as door handles and function as a possible source of infections.

Regular cleaning of door handles by drivers and hand washing by the passengers could reduce contamination as a result of transmission of these bacteria organisms.

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Conflict of Interest:

The author's do not report any financial or personal connections with anyone.

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Ethical Approval

This study was approved by the National Union of Road Transport Workers {Umuahia branch} (NURTW/003/05/21). Date: 6th, February, 2021.

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