**BACTERIOLOGICAL ANALYSIS OF MINI BUSES IN EFFURUN DELTA STATE**

**ABSTRACT**

Transportation is vital to every human community. One means of transportation in most parts of the world are the buses. As part of living in society, many common spaces are shared with other people; this makes transmission of diverse microorganisms that can lead to infections possible. People who use public transport can pass bacteriological infections to other people. Bacteriological analysis of mini buses in Effurun was carried out to isolate, identify and carryout an antibiotic sensitivity test on bacteria associated with public transport vehicles and door handles. A total of eighty (80) samples were collected from different car parks in Effurun. *Staphylococcus aureus, Bacillus species, Escherichia coli and Salmonella species* were the bacteria isolated with *S. aureus* having the most percentage occurrence of 40% and *Bacillus species* having the least percentage occurrence of 10%. Ciprofloxacin was the antibiotic with the highest sensitivity and Augumentin had the lowest sensitivity. It is presumed, however, that the bacterial contamination of these door handles is changeable as passengers use them. The organisms that can tolerate a dry environment, like the ones isolated in this study, are the ones that are most likely to persist more on these handles. Regular cleaning practices may be a good strategy to limit colonization by potentially pathogenic microbes.

**Keywords; Bacteriological, Analysis, Mini, Buses, Effurun**

**Introduction**

Transportation is vital to every human community. One means of transportation in most part of the world are the public transport vehicles (Adamu *et al*., 2017). People who use public transport The handles of car doors are one of the most important environmental places for microbial infection and disease transmissions through a direct contact on a daily basis (Al-Harmoosh *et al*., 2018). Both faeces and urine may find their ways on the contact surfaces of the door handles through a hand touch since the hand is the major public serving as the vehicle of transmission of common human disease to a susceptible host (Bashir *et al*., 2016).

Transportation networking system is expanding continually to meet up the need of huge load of passengers and goods carried. At the same time this networking has drawn great attention from public health scientists as pathogenic microbes have now achieved a better way of amplification that is faster and in extensive number than before. Several studies have already been conducted throughout the world focusing on presence and abundance of microbial contamination on public hand touch surfaces of bus, train, mobile phone, hand knob, ATM booth, hospital, shopping cart etc (Chowdhury *et al*., 2016). Bacteria that can cause severe gastroenteritis have been found on public contact surfaces, and handles (Rusin, 2002) which demonstrate that germs that can be readily transferred from your hands to almost any frequently used surface. Other studies have implicated environmental surfaces in the transmission of bacteria (Manning *et al*., 2001). The hands are the chief organs for physical manipulation of the environment. As a paired organ, the hand is controlled by the opposing brain hemisphere (Maria and Eliane, 2004) and enables one to do all manner of things. They serve as a medium for the propagation of microorganisms from place to place and from person to person (Aliyu *et al*., 2018). Although it is nearly impossible for the hand to be free of microorganisms, the presence of pathogenic bacteria may lead to chronic or acute illness. Human hands usually harbor microorganisms both as part of the body normal flora as well as transient microbes contracted from the environment (Dodrill *et al*., 2011). One common way by which organisms that are not resident in the hand are picked up is by contact with surfaces such as table tops, door knob or handles, banisters, toilet handles and taps in restrooms.

Microbes in public area such as public transport, restaurants, schools, day care centers can be a critical issue in public health, since they can bring a large number of people together which facilitate the transmission of microbes (Stepanovic *et al.* 2006; Kassem *et al.* 2007). This becomes a subject of prime concern when microbes are drug resistant and pathogenic. Therefore, increased attention has been paid to environmental microbes, to the numbers and strains of bacteria found in public places (Reynolds *et al.* 2005; Kassem *et al.* 2007; Otter and French, 2009). Public transportation system has become increasingly important in urban areas due to mass transit and increased awareness to energy-saving methods of transportation (Barrero, 2008). During the travel, various components of the vehicle such as seats, handle, door handle are frequently encountered and may act as the important reservoir for transmission of different pathogenic and non-pathogenic microbes (Oranusi *et al.* 2016).

The normal microbiota of the skin includes among others, coagulase negative *Staphylococcus diptheroides, Staphylococcus aureus, Streptococcus* (various species*), Bacillus spp, Mallassessia furfur, Candida spp* and occasionally, *Mycobacterium* spp are found on the skin (Roth and Jenner, 1998). However, this normal microbiota can produce disease condition if introduced into foreign locations or compromised hosts. For this reason, detailed handle cleaning of public transportation vehicles which thousands of people use every day is an important issue. In Effurun, public transport services are mainly run by touts and civilians in the title of commercial bus drivers.

**Materials and Methods**

 **Study Area**

This research was conducted in Effurun, Uvwie L.G. A. of Delta state in Nigeria. Effurun is one of the major commercial towns in Delta State. Is a fast-growing community with high social activities being boosted by the presence of Industries, shopping malls, tertiary institution (Federal University of Petroleum Resources and Petroleum Training Institute) It is densely populated.

 **Sample Collection**

A total of eighty (80) samples from handles of mini buses doors were collected using sterile swabs that were transferred to the laboratory within half an hour. The mini buses were chosen to work in different parts of the city. The samples were collected using sterile cotton swabs moistened with normal saline. The samples were collected by rubbing the swab, on the door handles, in a tri-directional manner: up/down, left/right and diagonally, while rotating the swab, to ensure that each door handle was properly sampled. The swabs were then reinserted in the tube and immediately transported to the microbiology laboratory for microbiological analysis to commence. (Cheesbrough, 2004).

 **Materials**

The materials used for this research work are electric thermostatic incubator, microscope, weighing balance, conical flasks. Gas cylinder, pressure pot, Petri dishes, Methylene, wire loops, Bunsen burner, microscopic slide, masking tape, spatula, methylated spirit, normal saline, nutrient agar, oil immersion, measuring cylinder.

 **Methods**

 **Media Preparation**

Sterilization of Conical Flask, test tubes and all the equipment needed for the experiment was carried out in the laboratory. The glassware’s were Sterilized in the oven at 120°C for an hour after washing with Soap; While other equipment was surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The Media; Nutrient Agar, MacConkey Agar, *Salmonella-Shigella* Agar and Mannitol Salt Agar were Prepared following the manufacturers guide.

**Preparation of MacConkey Agar**

MacConkey agar was prepared by suspending 48.5 grams of agar powder in 1000 ml of distilled water. The media was then placed in a water bath for 10 minutes to homogenize and sterilized by autoclaving at 121°C for 15 minutes. (Cheesbrough, 2004).

**Preparation of Mannitol Salt Agar**

Mannitol agar was prepared by suspending 111 grams of agar powder in 1000 ml of distilled water. The media was then sterilized by autoclaving at 121°C for 15 minutes (Cheesbrough, 2004).

**Preparation of Nutrient Agar**

Nutrient agar was prepared by suspending 28 grams of agar powder in 1000 ml of distilled water. The media was then placed in a water bath for 10 minutes to homogenize and sterilized by autoclaving at 121°C for 15 minutes (Cheesbrough, 2004).

**Preparation of *Salmonella-Shigella* Agar**

*Salmonella-Shigella* agar was prepared by suspending 60.0 grams of agar powder in 1000 ml of distilled water. The media was then placed in a water bath for 10 minutes to homogenize and sterilized by autoclaving at 121°C for 15 minutes (Cheesbrough, 2004).

 **Samples Processing**

In this study, the microbiological burden of public transport such as trams metro buses and buses, which are frequently used in daily life several samples from door handles of commercial buses from different towns or regions in Effurun is taken. Samples were taken from vehicles morning and evening by swab method. Upon arrival to the microbiology laboratory, each swab was immediately inoculated on culture media: *Salmonella Shigella* agar (SSA), MacConkey Agar (MA) and Mannitol salt agar (MSA) plates by streaking. The inoculated plates were incubated for 24-48 hours at 37°C. After 24 hours, each type of colony growing on any of the inoculated plates was then subcultured on Nutrient agar, SSA, MacConkey agar and Mannitol Salt agar to obtain a pure culture. (Cheesbrough, 2004).

**Identification of the Isolates**

Discrete colonies on Nutrient agar, *Salmonella-Shigella* agar, Mannitol Salt agar and MacConkey agar were carefully examined macroscopically for cultural characteristics such as shape, size, and colour. Gram staining as well as biochemical test was also carried out. The organisms were identified using Bergy’s Manual of Systematic bacteriology (Sneath *et al.,* 1986).

**Gram Staining**

The method of Onyeagba (2004) was adopted in identification of bacteria Gram reaction. Bacteria smear from fresh cultures were made on a clean grease free slide, air dried and heat fixed. The slide was flooded with crystal violet for one minute, decanted and rinsed with water. Lugol’s iodine (mordant) was applied for 60 seconds and rinsed. Acetone was used in decolorizing and washed immediately then counter stained with neutral red for 1 minute. It was then rinsed with water, blotted carefully and air dried. Finally, the slides were observed under the microscope using oil immersion objectives (x100).

**Biochemical Tests**

**Catalase Test**

The test for catalase production was done using 3% hydrogen perioxide solution in drops in a clean grease slide and a 24 hours’ culture (a loopful) was added/ the production of effervescence (bubbles) with the H2O2 gives a positive test, while the absence of effervescence indicates negative result. (Cheesbrough, 2004)

**Coagulase Test**

This test is used to identify *Staphylococcus aureus* which produces enzyme coagulase. A drop of distilled water was placed on the two separate glass slide and the colony of the test was emulsified on each of the slide. A loopful of plasma was introduced on the slide and gently rocked. Clumping within 10 seconds indicate a positive coagulase test (Cheesbrough, 2004)..

**Indole Test**

This test is important in the identification of *Enterobacteria*. Most strains of *Escherichia coli* and *Proteus vulgaris* breaks down the amino acid tryptophan with the release of insole. The organism was inoculated in bijou bottle containing 3ml of sterile peptone water and incubated at 35-37c for up to 48 hours. Kovac's reagent (0.5ml) was added and shake gently. A red colour in the surface layer within 10minutes shows positive indole test, no red colour on the surface layers shows negative indie test (Cheesbrough, 2004).

**Citrate Ultilization Test**

This test is one of several techniques used occasionally to assist in the identification of *Enterobacteria*. The test is based on the ability of an organism to use citrate as its only source of carbon. Slope of Simon's citrate were prepared in bijou bottles according to manufacturer's instruction. A sterile straight wire loop was used to streak the slope with a saline suspension of the test organism and incubated at 35c for 48hours. Alright blue colour in the medium indicates positive citrate but no change in colour shows negative citrate test (Cheesbrough,2004).

**Urease Test**

The test is used to determine the ability of the organism to produce the enzyme urease, which hydrolyzed urea. Hydrolysis of urea produces ammonia and CO2, the formation of ammonia of alkalinizes the medium and the pH shift was detected by the colour change of phenol red from light orange to magenta which indicated a positive result. A well-isolated colony was picked from the surface of the medium and inoculated as single streak on the slant surface of Christensen's urea agar (Cheesbrough,2004).

**Hydrogen Sulphide Production Test**

This test is used to determine whether the microbe reduces sulphur containing compounds to sulphides to produce hydrogen sulphide gas. A well isolated colony was picked from the surface of the medium and inoculated into the bijou bottle containing 3ml of protons water and incubated at 35-37ºC. A blackening on the medium indicate a positive result of H2S production test while the absence of the blackening colour on the medium indicate a negative result (Cheesbrough, 2004).

**Motility Test**

This test is used to determine if an organism is motile or non-motile. A straight sterilized needle to collect a colony of an 18- to 24-hour old culture growing on agar medium and stabbed to depth of about half inch in the middle of a bijou bottle containing solidified nutrient agar. The needle was kept in the same line it entered as it was removed and incubated for 24 hours at 37oC. A diffuse zone of growth flaring out from the line of inoculation indicates a positive result. (Cheesbrough, 2004).

**Antibiotic Susceptibility** **Test**

Distinct colonies from a 24 hours’ nutrient agar plate were suspended into sterile normal saline in a tube to achieve a bacteria suspension equivalent to 0.5 McFarland turbidity standards. A cotton swab was dipped into the bacterial suspension and the swab pressed against the side of the tube to drain off excess fluid. The active surface of the agar plate was then inoculated with the swab of inoculums ensuing confluent growth of the bacteria.

Antibiotic discs (CPX, CN, S, CH, AUG, OFL, AMX and APX) were placed onto the inoculated plates with a flame sterilised forceps and the plates incubated at 37oC for 18-24 hours. After incubation, the diameter zones of inhibition produced by each antibiotic against the isolates were measured with a metre rule. The drugs were interpreted as sensitive, intermediate or resistant following the direction of the CLSI, (2015)

 **Results and Discussion**

**Result**

**Identification of Bacteria Isolated from the Samples**

The bacteria isolated from the samples were *Staphylococcus aureus*, *Bacillus species*, *Escherichia* *coli* and *Salmonella* *species*. They are identified using their colonial characteristics gram staining and biochemical tests.

**The Antibiogram of the Isolated Bacteria**

The *Staphylococcus aureus* isolates were 100% resistant to augmentin and susceptible to ofloxacin, ciprofloxacin and gentamicin, *Bacillus* species and *Escherichia coli* were 100% resistant to augmentin and ofloxacin and susceptible to ciprofloxacin although they displayed intermediate resistance to gentamicin, streptomycin, amoxicillin and chloramphenicol while Salmonella was 100% resistant to Streptomycin, amoxicillin and ampiclox and susceptible to augmentin, and ciprofloxacin. The outcome of the experiment is documented in the table 2.

**Percentage Occurrence of the Bacterial Isolates**

The number and percentage occurrences of the isolated bacteria were *Staphylococcus aureus*, 16 (40%), *Bacillus* species, 4 (10%), *Escherichia coli*, 6 (15%) and *Salmonella* *species*, 14 (35%). These are shown in table 3.

**Table 1:** **Identification of Bacteria Isolated from the Samples**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |
| **Isolate code** | **Cellular morphology** | **Indole test** | **Citrate test** | **H2S test** | **Urease test** | **Catalase test** | **Motility test** | **Coagulase test** | **Probable organism** |
| AH01 | Purple rods  | + | + | - | - | + | + |  | *Bacillus* species |
| AH02 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| AH03 | Red rods  | + | - | - | - | + | + |  | *Escherichia coli* |
| AH04 | Pink rods | - | + | - | - | + | + | - | *Salmonella* species |
| SP01 | Pink rods | - | + | - | - | + | + | - | *Salmonella* species |
| SP02 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| SP03 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| SP04 | Red rods  | + | - | - | - | + | + |  | *Escherichia coli* |
| UB01 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| UB02 | Purple rods | + | + | - | - | + | + |  | *Bacillus* species |
| UB03 | Pink rods | - | + | - | - | + | + | - | *Salmonella* species |
| UB04 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| OR01 | Red rods  | + | - | - | - | + | + |  | *Escherichia coli* |
| OR02 | Pink rods | - | + | - | - | + | + | - | *Salmonella* species |
| OR03 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| OR04 | Pink rods | - | + | - | - | + | + | - | *Salmonella* species |
| UT01 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |
| UT02 | Pink rods | - | + | - | - | + | + | - | *Salmonella* species |
| UT03 | Red rods  | + | - | - | - | + | + |  | *Escherichia coli* |
| UT04 | Purple cocci in clusters | - | - | + | - | + | - | + | *S. aureus* |

**KEY;**

**+ = Positive -= Negative**

**Table 2: Antibiotics Profile of Isolated Bacteria**

|  |  |  |
| --- | --- | --- |
| **Bacteria** |  | **Zones of Inhibition (in milimeters)** |
|  | **CPX** | **CN** |  **S** | **CH** | **AUG** | **OFL** | **AMX** | **APX** |
| *Staphylococcus aureus* |  18 |  20 |  14 |  12 |  0 |  24 | 10 | 8 |
| *Bacillus* *species* | 22 | 18 | 18  | 16 | 0 |  0 | 18 | 14 |
| *Escherichia coli* | 20 | 14 | 8 |  10 | 0 | 0 | 16 | 12 |
| *Salmonella* *species* | 18 | 10 |  0 | 12 | 20 | 8 |  0 | 0 |

**KEY;**

**CPX- ciprofloxacin, CN- gentamicin, S- streptomycin, CH- chloramphenicol, AUG- augmentin , OFX- Ofloxacin, AMX- amoxicillin, APX- ampiclox.**

**18mm- 24mm = sensitive**

**13mm – 17mm = intermediate**

**0mm – 12mm = resistant**

**Table 3: Showing the Percentage Occurrence of the Bacteria Isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| **S/N** | **Isolates** | **Number of occurrence** | **Percentage occurrence** |
| 1 | *S. aureus* | 16 | 40% |
| 2 | *Bacillus species* | 4 | 10% |
| 3 | *Escherichia coli* | 6 | 15% |
| 4 | *Salmonella species* | 14 | 35% |
|  |  | 40 | 100% |

 **Discussion**

This study unveiled an obvious bacterial contamination of the mini buses’ door handles with a considerable number of organisms considered to be potential pathogens. This could be attributed to the fact that these door handles are frequently touched by the big number of passengers that depend on these public transport vehicles for transportation within the city. As a considerable number of the microbial skin flora is of Gram-positive bacteria, it would be logical to find them predominant on door handles as previously reported (Nwankwo and Chinyeaka, 2015).Out of 80 samples processed, all samples (100%) showed bacterial contamination, a result which is very comparable to that reported by Otter and French in 2009, who observed that 90% of the hand-touch surfaces in the public transport system and in public areas of a hospital in central London were contaminated by bacteria. The predominant bacteria that were isolated and characterized from the door handle surface samples included two Gram-positives (*Staphylococcus aureus* and *Bacillus species*) and two Gram-negatives (*Escherichia coli* and *Salmonella* species). The most prevalent bacterium was *S. aureus* (40%), followed by *Salmonella* specie (35%).

In this study, the most frequently isolated bacterium was *Staphylococcus aureus*. This result is in full agreement with previous studies as it was also the most frequently isolated bacterium from door handles/knobs of public conveniences (Nworie *et al*., 2012), door handles of a university (Nwankwo and Chinyeaka., 2015) and internal door handles/knobs of public restrooms (Orogu and Okobia, 2021). However, the rate of isolation of this organism (40% of the samples), in this study, was lower than those previously reported even for the handles/knobs of public restrooms. It was not unexpected to isolate this organism from the door handles of public transport vehicles, as it can be part of the normal flora of the skin and nostrils of a good proportion of the population and can easily be discharged by several human activities (Orogu *et al*., 2018), however, the rate was definitely higher than expected. *Staphylococcus aureus* is a potentially pathogenic organism that can cause different types of infections ranging from local infections like abscesses to life threatening systemic infections like meningitis, endocarditis, osteomyelitis, pneumonia and others (Tong *et al*., 2015).

The second most frequent isolates from the door handles, in this study, was *Salmonella* species isolated from 35% of the samples while *Escherichia coli* from 15% of the samples. Although, these isolates are members of the normal flora of the intestinal flora, they have been a cause of many nosocomial and community acquired infection. (Darouiche, 2004) *Bacillus* *species* are non-pathogenic but they can contaminate food and are usually dispersed and transmitted through aerosols (Ziegler and Perkins, 2008). Some *Bacillus* *species* can cause food poisoning and result in food intoxication. It can either cause nausea, vomiting, abdominal cramps and diarrhoea (EMBL).

 **Conclusion**

The results of this study, which demonstrated the presence of potentially pathogenic bacteria on the door handles of public transport vehicles should be taken seriously as all types of passengers use these cars, some who may be the carriers of multi-resistant strains of organisms, and others who may be immunocompromised. The organisms contaminating these door handles can colonize individuals and through them be transmitted to others. It is presumed, however, that the bacterial contamination of these door handles is changeable as passengers use them. The organisms that can tolerate a dry environment, like the ones isolated in this study, are the ones that are most likely to persist more on these handles.

It is therefore recommended that Regular cleaning practices may be a good strategy to limit colonization by potentially pathogenic microbes; Adopting and monitoring more frequent cleaning using effective detergents; Users of public transport vehicles should observe healthy practices such as washing hands after using the vehicles.

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