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

**ABSTRACT**

Every human society depends on transportation. In most regions of the world, buses are a mode of transportation. Living in a society involves sharing numerous common areas, which allows a variety of germs that might cause illnesses to spread. Public transportation users have the potential to spread bacterial illnesses to others. To isolate, identify, and conduct an antibiotic sensitivity test on bacteria linked to public transportation vehicles and door handles, a bacteriological investigation of minibuses in Effurun was conducted. Eighty (80) samples in all were gathered from several Effurun parking lots. *Salmonella species, Bacillus species*, *Escherichia coli,* and *Staphylococcus aureus* were the bacteria that were isolated; *S. au*reus was the most common, occurring 40% of the time, while Bacillus species were the least common, occurring 10% of the time. The antibiotic with the highest sensitivity was Ciprofloxacin, whereas the one with the lowest sensitivity was Augmentin. However, it is assumed that as passengers use these door handles, the bacterial contamination changes. The organisms that are most likely to survive longer on these handles are those that can withstand a dry environment, such as the ones identified in our study. Frequent cleaning procedures could be an effective way to reduce the number of potentially harmful microorganisms that colonize.

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

**Introduction**

Every human society depends on transportation. Public transit vehicles are a mode of mobility in the majority of the world (Adamu *et al*., 2017). Car door handles are among the most significant environmental locations for the everyday direct contact that causes microbial infection and illness transmission (Al-Harmoosh *et al*., 2018). Given that the hand is the primary public site for the transmission of common human diseases to a vulnerable host, both urine and feces may end up on the contact surfaces of door handles through hand contact (Bashir *et al*., 2016).

In order to accommodate the massive volume of passengers and cargo being transported, the transportation networking infrastructure is constantly growing. Public health specialists have also taken a keen interest in this networking because pathogenic bacteria have improved their amplification methods, making them faster and more numerous than previously. Numerous research on the prevalence and quantity of microbiological contamination on public hand-touch surfaces, such as those of buses, trains, mobile phones, hand knobs, ATM booths, hospitals, shopping carts, etc., have already been carried out worldwide (Chowdhury *et al*., 2016). Handles other public touch surfaces have been discovered to harbor bacteria that can cause severe gastroenteritis (Rusin, 2002), proving that germs can easily spread from your hands to nearly any surface you use on a regular basis. Environmental surfaces have been linked in other research to the spread of microorganisms (Manning *et al*., 2001). The primary organs for physically modifying the surroundings are the hands. The hand is a paired organ that allows one to accomplish a wide range of tasks and is controlled by the opposite hemisphere of the brain (Maria and Eliane, 2004). They act as a conduit for the spread of germs between individuals and between locations (Aliyu *et al*., 2018). Even while a hand can never be completely devoid of microorganisms, the presence of harmful bacteria can cause either acute or chronic sickness. Both temporary bacteria acquired from the environment and natural flora found in the body are typically found on human hands (Dodrill *et al*., 2011). Contact with surfaces like table tops, door knobs or handles, banisters, toilet handles, and bathroom taps is a frequent way for organisms that are not found in the hand to be acquired.

Since they can bring a lot of people together, which makes it easier for germs to spread, microorganisms in public places like restaurants, daycare centers, public transportation, and schools can be a serious public health concern (Stepanovic *et al*., 2006; Kassem *et al*., 2007). When bacteria are harmful and resistant to drugs, this becomes a major worry. As a result, environmental microorganisms and the types and quantities of bacteria present in public areas have received more attention (Reynolds *et al*., 2005; Kassem *et al*., 2007; Otter and French, 2009). Because of mass transit and growing awareness of energy-efficient transportation options, public transportation has grown in importance in urban areas (Barrero, 2008). Numerous vehicle components, including seats, handles, and door handles, are commonly encountered during travel and may serve as significant reservoirs for the transmission of numerous pathogenic and non-pathogenic bacteria (Oranusi *et al*., 2016).

Coagulase negative bacteria are among the components of the skin's natural microbiota. The skin contains Streptococcus (different species), *Staphylococcus diptheroides*, *Staphylococcus aureus*, *Bacillus species*, *Mallassessia furfur, Candida species*, and rarely *Mycobacterium specie*s (Roth and Jenner, 1998). However, if brought into unfamiliar environments or damaged hosts, this typical microbiota might result in disease conditions. Because of this, it is crucial to thoroughly clean the public transit vehicles that thousands of people use on a daily basis. The majority of people who operate public transportation in Effurun are touts and civilians who work as 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**

Sterile swabs were used to gather eighty (80) samples from the door handles of minibuses, which were then brought to the lab in less than 30 minutes. The minibuses were selected to operate around the city. Sterile cotton swabs wet with regular saline were used to gather the samples. To guarantee that every door handle was accurately sampled, the swab was rotated while stroking the handles in three different directions: up/down, left/right, and diagonally. After that, the swabs were put back in the tube and taken right away to the microbiology lab so that the microbiological analysis could start (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**

Conical flasks, test tubes, and all other necessary equipment for the experiment were sterilized in the lab. After being cleaned with soap, the glassware was sterilized in an oven set at 120°C for an hour. To lessen microbiological contamination, other equipment was surface sterilized using 70% ethanol (Agrios, 2005). Scalpels and inoculating loops were sterilized by heating them to red hot and immersing them in 70% ethanol for 20 seconds. Following the manufacturer's instructions, the media—Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar, and Mannitol Salt Agar—were prepared.

**Preparation of MacConkey Agar**

48.5 grams of agar powder were suspended in 1000 milliliters of distilled water to create MacConkey agar. After 10 minutes of homogenization in a water bath, the media was autoclaved for 15 minutes at 121°C to sterilize it. (2004, Cheesebrough).

**Preparation of Mannitol Salt Agar**

111 grams of agar powder were suspended in 1000 milliliters of distilled water to create mannitol agar. After that, the material was autoclaved for 15 minutes at 121°C to disinfect it (Cheesbrough, 2004).

**Preparation of Nutrient Agar**

28 grams of agar powder were suspended in 1000 milliliters of distilled water to create nutrient agar. After ten minutes of homogenization in a water bath, the media was autoclaved for 15 minutes at 121°C to sterilize it (Cheesbrough, 2004).

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

To make Salmonella-Shigella agar, 60.0 grams of agar powder were suspended in 1000 milliliters of distilled water. After 10 minutes of homogenization in a water bath, the media was autoclaved for 15 minutes at 121°C to sanitize it (Cheesbrough, 2004).

 **Samples Processing**

Several samples from the door handles of commercial buses from various towns or districts in Effurun are taken for this study, which examines the microbiological load of public transportation, including trams, metro buses, and buses, which are commonly utilized in daily life. Using the swab approach, samples were collected from cars in the morning and evening. Each swab was streaked onto culture media, including Salmonella Shigella agar (SSA), MacConkey Agar (MA), and Mannitol salt agar (MSA) plates, as soon as it arrived at the microbiology lab. The inoculation plates were incubated at 37°C for 24 to 48 hours. A pure culture was obtained by sub culturing each type of colony developing on any of the infected plates on nutrient agar, SSA, MacConkey agar, and mannitol salt agar after a 24-hour period. (2004, Cheesebrough).

**Identification of the Isolates**

Discrete colonies on Salmonella-Shigella agar, Mannitol Salt agar, MacConkey agar, and Nutrient agar were meticulously inspected macroscopically for cultural traits such size, shape, and color. Both a biochemical test and Gram staining were performed. Bergy's Manual of Systematic Bacteriology was used to identify the organisms (Sneath *et al*., 1986).

**Gram Staining**

The Gram reaction method of Onyeagba (2004) was used to identify the bacteria. Fresh cultures of bacteria were smeared on a sanitized, grease-free slide, allowed to air dry, and then heated to fix it. After a minute of crystal violet flooding, the slide was decanted and given a water cleaning. After applying Lugol's iodine (mordant) for 60 seconds, the area was washed. After decolorizing with acetone and washing right away, neutral red was employed as a counterstain for one minute. After that, it was thoroughly blotted, rinsed with water, and allowed to air dry. Lastly, oil immersion objectives (x100) were used to view the slides under a microscope.

**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**

The coagulase-producing *Staphylococcus aureus* is identified with this assay. On two different glass slides, a drop of distilled water was applied, and the test colony was emulsified on each slide. The slide was filled with a loopful of plasma and rocked gently. A positive coagulase test is indicated by clumping within 10 seconds (Cheesbrough, 2004).

**Indole Test**

When identifying Enterobacteria, this test is crucial. The majority of *Proteus vulgaris* and *Escherichia coli* bacteria produce indole when they break down the amino acid tryptophan. Three milliliters of sterile peptone water were added to a bijou bottle to inoculate the organism, which was then cultured for up to 48 hours at 35–37 degrees Celsius. After adding 0.5 ml of Kovac's reagent, a gentle shake was given. If the surface layer turns red after 10 minutes, the indole test is positive; if it doesn't turn red, the indole test is negative (Cheesbrough, 2004).

**Citrate Ultilization Test**

This test is one of a number of methods that are occasionally employed to help identify Enterobacteria. The ability of an organism to use citrate as its only carbon source forms the basis of the test. As directed by the manufacturer, slope of Simon's citrate was produced in bijou bottles. The slope was streaked with a saline suspension of the test organism using a sterile straight wire loop, and it was then incubated for 48 hours at 350C. The medium's blue color indicates a positive citrate test, while the absence of color change indicates a negative citrate test (Cheesbrough, 2004).

**Urease Test**

 The purpose of the test is to ascertain whether the organism can manufacture the urease enzyme, which hydrolyzes urea. The solution becomes alkalinized when urea is hydrolyzed, producing ammonia and CO2. The pH shift was identified by the color of phenol red changing from light orange to magenta, indicating a successful outcome. In order to inoculate a single streak on the sloping surface of Christensen's urea agar, a well-isolated colony was selected from the medium's surface (Cheesbrough, 2004).

**Hydrogen Sulphide Production Test**

This test determines whether the microorganism produces hydrogen sulfide gas by reducing sulfur-containing chemicals to sulfides. After selecting a well-isolated colony from the medium's surface, it was inoculated into a bijou bottle with three milliliters of proton water and incubated at 35 to 370C. A positive H2S production test result is indicated by a blackening of the medium, whereas a negative result is indicated by the absence of the blackening color on the medium (Cheesbrough, 2004).

**Motility Test**

The purpose of this test is to identify if an organism is motile or not. An 18- to 24-hour-old culture colony developing on agar medium is collected using a straight, sterile needle that is poked to a depth of around half an inch in the center of a small container filled with solidified nutritional agar. After being withdrawn, the needle was left in the same line and incubated at 37°C for a whole day. A favorable outcome is indicated by a diffuse zone of growth that flares out from the inoculation line. (Cheesebrough,2004).

**Antibiotic Susceptibility** **Test**

To create a bacterial suspension that met 0.5 McFarland turbidity standards, distinct colonies from a 24-hour nutrient agar plate were suspended in sterile normal saline in a tube. To remove extra fluid, a cotton swab was dipped in the bacterial mixture and rubbed against the tube's side. The swab of inoculums was then used to inoculate the agar plate's active surface, causing the bacteria to proliferate confluently.

Using flame-sterilized forceps, antibiotic discs (CPX, CN, S, CH, AUG, OFL, AMX, and APX) were deposited onto the inoculation plates, which were then incubated for 18 to 24 hours at 370C.Following incubation, a metre rule was used to measure the diameter zones of inhibition that each antibiotic produced against the isolates. According to the CLSI's guidelines, the medications were classified as sensitive, moderate, or resistant (2015).

 **Results and Discussion**

**Result**

**Identification of Bacteria Isolated from the Samples**

*Salmonella species, Bacillus species, Escherichia coli*, and *Staphylococcus aureus* were among the microorganisms that were isolated from the samples. Gram staining and biochemical tests are used to identify them based on their colonial characteristics.

**The Antibiogram of the Isolated Bacteria**

While *Bacillus species* and *Escherichia coli* showed intermediate resistance to Gentamicin, Streptomycin, Amoxicillin, and Chloramphenicol, their isolates of *Staphylococcus aureus* were 100% resistant to Augmentin and susceptible to Ofloxacin, Ciprofloxacin, and Gentamicin. On the other hand, *Salmonella species* 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 investigation revealed a clear bacterial contamination of the door handles of the minibuses, with a significant number of organisms thought to be possible diseases. This may be explained by the fact that many passengers who rely on these public transit vehicles for city transportation frequently touch these door handles. Gram-positive bacteria make up a significant portion of the microbial skin flora, so it makes sense that they would be more common on door handles, as has been documented earlier (Nwankwo and Chinyeaka, 2015).

All (100%) of the 80 samples that were processed had bacterial contamination. This finding is very similar to that of Otter and French (2009), who found that 90% of the hand-touch surfaces in a central London hospital's public areas and public transportation system were contaminated by bacteria. Two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus species*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella species*) were the most common bacteria isolated and described from the door handle surface samples This result is in line with the works of Orogu and Ehiwario (Orogu *et al.,*2017; Ehiwario *et al.,*2025). *Salmonella specie* (35%) and *S. aureus* (40%) were the most common bacteria.

*Staphylococcus aureus* was the most commonly isolated bacterium in this investigation. Since it was the most commonly isolated bacterium from university door handles (Nwankwo and Chinyeaka, 2015), public convenience door handles (Nworie *et al*., 2012), and public restroom door handles (Fakhoury and Nawas, 2018), this result is completely consistent with earlier research. However, even for the handles and knobs of public restrooms, the isolation rate of this organism (40 percent of the samples) in our investigation was lower than those previously reported. Since it can be found in the normal flora of the skin and nostrils of a significant portion of the population and is readily released by a variety of human activities, the isolation of this organism from the door handles of public transportation vehicles was not surprising (Orogu *et al*., 2018). Nevertheless, the rate was unquestionably higher than anticipated. According to Tong *et al*. (2015), *Staphylococcus aureus* is a potentially harmful organism that can cause a variety of infections, from minor ones like abscesses to serious systemic infections including meningitis, endocarditis, osteomyelitis, pneumonia, and others.

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**

Since all kinds of passengers use public transportation vehicles, including those who may be immunocompromised and others who may be carriers of multi-resistant strains of organisms, the findings of this study, which showed the presence of potentially harmful bacteria on the door handles of these vehicles, should be taken seriously. The microorganisms that contaminate these door knobs have the ability to colonize people and spread to other people. However, it is assumed that as passengers use these door handles, the bacterial contamination changes. The organisms that are most likely to survive longer on these handles are those that can withstand a dry environment, such as the ones identified in our study.

Thus, it is advised that regular cleaning procedures could be a useful tactic to prevent the colonization of potentially harmful microorganisms; increasing the frequency of cleaning and keeping an eye on it with efficient detergents; Washing their hands after taking public transportation is one of the healthy habits that users should follow.

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