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Antibiogram and Microbial Carriage of Campus Buses and Keke Napep Door Handles in Joseph Sarwuan Tarka University Makurdi

Joel Inya Odo[1], Paulyn Tracy Aernan[2] and Rose Doowuese Toryila[2]

[1]Departments of Fisheries and Aquaculture Joseph Sarwuan Tarka University, Makurdi, Nigeria
[2]Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi, Nigeria
Correspondence and requests for materials should be addressed to JIO (email: odojoel@gmail.com)

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Abstract

As people come in contact with surfaces like keyboards, office furniture, toilet doors, buses and keke door handles, there is a probability of picking up microbes deposited on them. This study is aimed at evaluating the antibiogram and microbial carriage of campus buses and keke napep door handles. Thirty swab samples were obtained from buses and keke napep door handles. The samples were taken to the laboratory where they were serially diluted and inoculated. Identification, characterization and biochemical analysis were done using standard microbiological methods. Fungal colonies were macroscopically and microscopically examined for morphology and appearance. Bacterial isolated were identified to be *Staphylococcus spp., Proteus spp., Escherichia coli* and *Shigella spp.* Fungi isolated were identified to be *Aspergillus niger, Mucor spp., Aspergillus fumigates, Rhizopus spp.* and *Yeast spp*. This study has shown that for both bacterial and fungal species, S*taphylococcus spp.*5(33.33%) and *Aspergillus niger* 2(50%) had the highest occurrence while *Proteus spp.* 1(6.67%), *Aspergillus fumigates* and *Mucor spp.* 1(14.29%) had the least occurrence. Results of susceptibility test carried out on bacteria isolates show S*taphylococcus spp.* was most susceptible to levofloxacin having 21.67 ± 2.89 mean zone of inhibition and was resistant to rifampicin which had no zone of inhibition. Furthermore, using Gram-negative disc, all the isolates were susceptible to the antibiotics used except *Proteus spp.* which showed resistance to ciporex and nalidixic acid. Data was further analyzed at $P<0.05$ using ANOVA. The analysis shows that there was no significant difference ($P>0.05$) in the susceptibility analysis. This study has shown that Campus Buses door handles have higher microbial carriage than keke door handles though both can serve as a means of microbial carriage and vehicle for the transmission of disease since they are associated with pathogenic organisms thus, hygienic measures should be taken to avoid the transmission of diseases by these public surfaces in which contact is constantly made with.

Keywords: Campus bus; Keke Napep; Microbes; Contamination

Introduction

Many factors have been shown to influence the bacterial transfer between surfaces, including bacterial species involved, moisture levels, pressure and friction between the contact surfaces and inoculum size on surfaces (Bashir et al., 2016; Zakaria, 2023) Studies have also shown that household surfaces can easily be contaminated with bacteria and that viruses can easily be transferred to hands and from hands to mouth (Rusin et al., 2002). The risk of spreading of *Salmonella* infection to other family members via the environment, including contaminated hands, door handles and surfaces in the toilet areas had been highlighted (Bloomfield et al., 2007). As people come in contact with surfaces such as desks, keyboards and office furniture, toilet lock handles, there is a possibility of picking up microbes deposited on them (Odo et al., 2023).

The toilet and office lock handles are contacted more frequently by their users and visitors, especially the door handles of public buses and offices. The hazards associated with door handles and other fomites had been established but less attention had been directed to campus bus door handles as inanimate objects which could harbour and transmit infectious agents (Amala and Ade, 2015).

Various bacteria have been isolated from public surfaces thereby providing information on the relative hygiene of commonly encountered public surfaces, identifying the environments with contaminants and risk of exposure (Reynolds and Hurst, 2005). The United States (US) Centre for Disease Control (CDC) and Prevention indicate that contaminated public surfaces most of which are of microorganisms are perhaps the most widely spread problems in the contemporary world and is responsible for about one-third of death worldwide, through infections with adverse effects which can reduce economic productivity (WHO, 2002). Human carriers are the main reservoir host of infections according to (El-Ghwas et al., 2023; Bashir et al., 2016). Micro-organisms can be found in any environment including soil, air, water and food as well as on environmental surfaces or objects. The infection can spread to humans in different ways; directly or indirectly via inanimate objects called vectors (Tagoe et al., 2011). The presence of pathogenic bacteria on environmental surfaces such as bus door handles, and plastic items poses a potential risk to vulnerable, immune–compromised individuals (Tayal et al., 2023). It has been shown that hard, non-porous surfaces, such as door handles, have the highest bacterial transfer rates to hands (Rusin et al., 2002; Zakaria, 2022). The microorganisms may find their way on the contact surfaces of the door handles via a hand touch since the hand is the major point/part serving as the vehicle of transmission of common human disease to a susceptible host.

The objective of this study is to evaluate the antibiogram and microbial carriage of campus buses and keke NAPEP door handles in the Federal University of Agriculture Makurdi.

Materials and methods

Study Area

This research was carried out in Makurdi, the capital of Benue state, Nigeria, West Africa. Its geographical coordinates are 7° 14′0 North, 8° 32′0″ East and its original name is Makurdi. The area is characterized by two seasons, the dry season (October to April) and the rainy season (April to October) about 45% of the total population are civil servants and business people. While about 25% are farmers, 30% are vocational workers and students

Sample collection

Swap samples were collected from buses and Keke NAPEP door handles randomly from the south core and north core of the Federal University of Agriculture Makurdi, they were transported to the Laboratory, Microbiology Department and analyzed.

Materials and Reagents

The materials used in this research were; Swap sticks, Nutrient agar (NA), Potato Dextrose Agar (PDA) Petri dishes, wire loops, measuring cylinders, conical flask, and spatula, Microscope, Weighing balance, incubator, autoclave, and refrigerator thermometer, pH meter, Gram reagents, oxidase reagents, urease reagents, Kovac's reagent, oil immersion, distilled water, citrate and 90% ethanol.

Media Preparation

The different media used in this study include Nutrient agar (NA), Potato Dextrose Agar (PDA), Eosin Methylene Blue Agar (EMB), Blood Agar, Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), CLED Agar and Muller Hinton Agar were both prepared in accordance with the manufacturer's specification as follows:

Nutrient agar

5.6g of the nutrient agar powder was weighed and suspended in 200 ml of sterile distilled water. It was then shaken to mix properly and heated for 5 minutes to dissolve the powder completely. After heating, the mouth of the flask was plugged with cotton wool and then wrapped with aluminium foil. The medium was then sterilized in the autoclave at a temperature of 121 °C for 15 minutes. The sterilized medium was poured aseptically into sterile Petri dishes at 45 °C.

Potato Dextrose Agar

3.9 g of the potato dextrose agar powder was weighed and dissolved into 100 ml of distilled water. It was then shaken to mix properly and heated for 5 minutes to dissolve the powder completely. The flask was plugged with cotton wool and then wrapped with aluminium foil. The medium was autoclaved at 121°C for 15 minutes and then poured aseptically into sterile Petri dishes at 45 °C.

Salmonella Shigella agar

6g of the Salmonella-shigella agar powder was weighed and suspended in 100 ml of distilled water. It was thoroughly mixed to ensure that the powder dissolved completely. It was then heated with frequent agitation and boiled for one minute. The medium was not sterilized in an autoclave. After cooling the medium was poured aseptically sterile into petri dishes (Cheesbrough, (2009).

Total viable count

Total viable count was carried out according to (Fawole, and Osho, 2007) using the pour plating method. Swab samples were initially soaked properly in 1 ml of 0.1% peptone water. The sample was serially diluted to 6^{th} dilution. 1ml of 10^4and10^5 dilutions was inoculated in the sterilized Petri dish. 15 ml of sterilized nutrient agar maintained at 45°C was then poured into the Petri dish, mixed uniformly and allowed to solidify. The plates were incubated at 37 °C for 24 hours. Bacterial colonies were counted after the incubation period.

Purification of Bacterial Isolates

Discrete bacterial colonies were further purified by streak plating according to (Fawole, and Osho, 2007). Distinct colonies were examined and enumerated. They were then subcultured aseptically onto well-labelled Eosin Methylene Blue Agar and Blood Agar for Gram-negative enteric bacteria and Gram-positive cocci bacteria respectively. A sterile inoculating loop was used to pick discrete bacterial colonies and streaked onto the surface of the medium. Thereafter, they were incubated at 37°C for 24 hours. The pure cultures of the bacterial isolates were stored on nutrient agar slants at 4°C.

Cultural and Morphological Identification of Bacteria

The Gram-negative enteric bacteria and the Gram-negative aerobic bacterial isolates from the Eosin Methylene Blue Agar were identified by the appearance of the colony on the plates. Each of the isolates has distinct colonies on the media which are depicted by the degree of their ability to ferment lactose for acid production which are characterized by their colour, formation of green metallic sheen as a result of vigorous acid production and the intensity of growth. Isolates suspected to be *Salmonella* were sub-cultured on Salmonella -Shigella Agar. Isolates suspected to be *Escherichia coli* were confirmed by indole test, Urease test and Oxidaze test were used to confirm isolates suspected to be *Proteus spp.* respectively.

Gram-positive cocci isolates from blood agar were identified by their haemolytic reaction depicted by the appearance of their colony. This includes alpha haemolytic reaction which results in incomplete lysis, a greenish cloudy zone around the colonies; beta haemolytic reaction which results in complete lysis, a clear zone with a clean edge around the colony; and gamma haemolytic reaction in which no lysis nor colour change occur around the colony. Suspected bacteria isolates in this category were confirmed by biochemical tests which include catalase test and coagulase test. All the bacterial isolates were subjected to Grams staining to ascertain the morphology and Gram's reaction of the isolates.

Gram Staining Technique

A thin smear of each of the 24-hour old cultures was prepared on clean grease free slides; they were fixed by passing over a flame gently. They were stained by the addition of 2 drops of crystal violet solution for 60 seconds and were rinsed with water. The smears were flooded again with Lugols' iodine for 30 seconds and were rinsed with water. They were decolourized with 70% alcohol for 15 seconds and were rinsed with distilled water. They were counter-stained with two drops of safranin for 60 seconds and finally were rinsed with water, they were then allowed to air dry. The smears were mounted on a microscope and were observed under an oil immersion objective lens. The result was read according to colour appearance. Gram-negative cells appeared pink/red while Gram-positive organisms appeared purple (Fawole and Osho, 2007).

Biochemical Tests

Catalase test

A small quantity of the 24-hour-old culture was transferred into a drop of 3% hydrogen peroxide solution on a clean slide with the aid of a sterile inoculating loop. The result was interpreted based on the appearance of gas seen as white froth (Cheesbrough, 2009).

Coagulase Test

A loopful of the isolates was emulsified with normal saline solution on a microscopic slide. A drop of undiluted plasma was added to the suspension and stirred for five seconds. A coagulase-positive enzyme is indicated by the clumping of colonies together.

Indole Test

Tryptone broth (5mL) was placed into different test tubes, after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes inoculated to serve as the control. The test tubes were then incubated at 37°C for 48 hours, after incubation, 0.5 mL of Kovacs' reagent was added and shaken gently, it was allowed to stand for 20min to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicates a positive result, while yellow colouration indicates a negative result (Cheesbrough, 2009).

Citrate Test

This test detects the ability of an organism to utilize citrate as a sole source of carbon and energy. About 2.4g of citrate agar was dissolved in 100 ml of distilled water. About 10ml of citrate medium was dispensed into each tube and covered; it was sterilized and allowed to cool in a slanted form. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates the utilization of citrate (Cheesbrough, 2009).

Urease Test

The surfaces of the urea agar slant were streaked with a portion of a well-isolated colony. The caps were leaved on loosely and the tubes were incubated at 35°-37°C in ambient air for 48 hours to 7 days. The development of pink color was examined for as long as 7 days.

Oxidase Test

Procedure: 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-p-phenylenediaminedihydrochloride) were added to a piece of filter paper, and then a sterile wire loop was used to take out a colony of the test organisms that were grown in pure culture and was streaked on the filter paper, a positive result was indicated by a change to a deep blue or purple colour which appears after a few seconds (Cheesbrough, 2009).

Identification and Characterization of Fungal Isolates

Characteristics of fungal isolates such as the texture of colonies, spores or conidia-producing structures and spore shapes were obtained and recorded. The characteristics were observed from fungal tissues grown on PDA for three days, spore and mycelium characteristics were examined using ×10 and ×40 objective lens of the microscope. Parameters such as colony, characteristics of

the submerged hyphae, and characteristic shape of matured fruiting bodies were all observed. These characteristics were used in identifying the fungal organisms.

Antibiotics sensitivity test

This test was carried out to determine the resistance and susceptibility of the isolated bacteria to commercial antibiotics, this test was carried out using a disc impregnated with antibiotics, the antibiotics disc used consisted of two types, one type is specific for Gram-positive bacteria and the other one for Gram-negative bacteria. The various antibiotics and their corresponding concentration impregnated in the gram-positive and the gram-negative disc is as follows:

***Gram-positive disc*:** Ciproflox (10 mcg), Norfloxacin (10 mcg), Gentamycin (10 mcg), Amoxil (20 mcg), Streptomycin (30 mcg), Rifampicin (20 mcg), Erythromycin (30mcg), Chloramphenicol (30 mcg), Ampiclox (20 mcg), Levofloxacin(20mcg).

***Gram-negative disc*:** Tarivid (10 mcg), Reflacine (10 mcg), Ciproflox (10 mcg), Augmentin (30 mcg), Gentamycin (10 mcg), Streptomycin (30 mcg), Ciporex (10 mcg), Nalidixic acid (30 mcg), Septrin (10 mcg), Amplicin (30mcg).

The standardization of the inocula was done according to the method of (Fawole and Osho, 2007), while the antibiotic susceptibility testing was carried out using disc-diffusion technique (Kritu et al., 2013). Fresh culture (18-hour culture) was used for this test; bacteria isolates previously preserved on nutrient agar slant were sub-cultured on freshly prepared nutrient agar medium and incubated for 18 hours before the sensitivity test was carried out. After the incubation period, the test was carried out by using an inoculating loop to pick a bacteria colony and emulsified it in a Bijou bottle containing 3.0ml of normal saline. A cotton swab was dipped into the suspension and the swab was pressed against the side of the bottle to remove excess fluid. The inoculated swab was then streaked across the surface of Mueller Hinton agar and allowed to dry for five minutes after which sterile forceps were used to carefully remove the disc from its pack and gently pressed onto the agar surface. The plates were finally incubated at 37 °C for 24 hours. After the incubation period, the diameter of zone of inhibition (clearance) was measured using a millimetre rule from the centre of the disc to the edge of the circumference of the clearance zone and recorded to the nearest millimetre. The results were recorded and interpreted based on Clinical and Laboratory Standard Institute guidelines (CLSI, 2005).

Data Analysis

Data were analyzed for mean and standard deviation. The difference in parameter was tested for statistical difference at $P<0.05$ using ANOVA. All the analysis was done using a Statistical Package for Social Science (SPSS) version 21.

Results

Table 1 shows the occurrence of bacteria isolates from door handles of Buses. *Staphylococcus spp.* had the highest occurrence of 5 (33.33%) while *Escherichia coli* and *Shigella spp.* had the least occurrence of 3 (20%). The frequencies of bacterial isolates from Keke NAPEP door handles as presented in Table 2 from which *Staphylococcus spp.* and *Shigella spp.* had the highest occurrence with 5(33.33%) while *Proteus spp.* had the least occurrence of 1(6.67%) respectively.

Table 1. Occurrence of bacteria isolated from door handles of buses

Organisms	**n (%)**
Staphylococcus spp.	5(33.33)
E. coli	3(20)
Proteus spp.	4(26.67)
Shigella spp.	3(20)
Total	**15(100)**

In Table 3, the occurrence of fungi isolates from bus door handles is presented, *Aspergillus niger* had the highest occurrence of 3(42.85%) while *Aspergillus fumigates* and *Mucor spp.* had the least

occurrences both with 1(14.26%). Among the samples of Keke NAPEP door handles, *Aspergillus niger* also had the highest occurrence of 2(50%) while Yeast and *Mucor spp.* had the least occurrence of 1(25%) respectively as shown in Table 4. Table 5 shows the antibiotic susceptibility analysis of selected organisms from door handles of buses and keke obtained from the Federal University of Agriculture Makurdi. The results were recorded in the form of mean zones of inhibitions of the antibiotics on test isolates. *Staphylococcus spp.* was most susceptible to Levofloxacin with 21.67 ± 2.89 mean zone of inhibition and resistant to Rifampicin with 0.00 ± 00 mean zone of inhibition.

Table 2. Occurrence of Bacteria Isolated from Door Handles of Keke Napep Samples

Organisms	**n (%)**
Staphylococcus spp.	5(33.33)
E. coli	4(26.67)
Proteus spp.	1(6.67)
Shigella spp.	5(33.33)
Total	**15(100)**

Table 3. Occurrence of Fungi Isolated from Door Handles of Buses

Fungal species	**n (%)**
Aspergillus niger	3(42.85)
Aspergillus fumigates	1(14.29)
Mucor spp.	1(14.29)
Rhizopus spp.	2(28.57)
Total	**7(100)**

Table 6 presents the antibiotic susceptibility analysis of *E. coli*, *Shigella spp.* and *Proteus spp.* using Gram-negative disc. The results were recorded in the form of mean zones of inhibition of the antibiotics on the Gram-negative isolates with *E. coli* being more susceptible to streptomycin with mean inhibition of 30.00 ± 0.00 and least sensitive to Ampicillin having the least mean zone of inhibition of 3.33 ± 5.78. *Shigella spp.* was more susceptible to ciprofloxacin and Augmentin both having a mean zone of inhibition of 28.33 ± 2.89 and was least sensitive to streptomycin and Nalidixic acid both having an inhibition of 3.33 ± 5.78 respectively. *Proteus sps*. was more susceptible to Reflacin with a mean zone of inhibition of 30.00 ± 0.00 and was resistant to Ceporex and nalidixic acid both having 0.00 ± 0.00 mean zone of inhibition. Thus, there was a significant difference (P< 0.05) between the measured mean zones of inhibition of the isolates.

Discussion

All over the world, microbiology standards in hygiene are prerequisites for healthy living. It is not uncommon however to observe shifts in hygiene practices that deviate from standards in the developing and developed world. This investigation confirms such deviation, as arrays of microorganisms are found associated with the door handles of campus shuttle buses. This is in conformation with the study of Pinner et al. (1996) which indicated that the regular handling of surfaces creates a prime breeding ground for all sorts of microorganisms that are normally found on the human skin and the environment.

The analysis of the microbial contamination and antibiogram of isolates from bus and keke door handles was carried out in this study. The isolates identified to be associated with bus and keke door handles were both of Gram-positive and Gram-negative origin and fungi. The microorganism found to have a higher occurrence in both the bus and keke samples was *Staphylococcus spp.* This could be due to the fact that *Staphylococcus spp*. is a normal flora of the skin and therefore contact with bus and keke door handles can allow easy transfer of *Staphylococcus* from the skin to these surfaces. This agrees with the study of Miller and Diep (2008) who stated that the ecological niche for *Staphylococcus aureus* is in the anterior nares and also the study of Kluytmans et al., (1997) which indicated that one-quarter to one-third of healthy persons harbour *Staphylococcus aureus* in the nose at any time which can easily be transferred to hands by simply rubbing the nose. This study reveals higher frequencies of Gram-negative organisms compared to Gram-positive organisms as *Staphylococcus spp.* was the only isolated Gram-positive organism from both the

keke and bus handles. This is in disagreement with the work of Bloomfield et al., (2007) who stated that Gram-positive bacteria are mostly resident flora which is attached to the deeper layers of the skin and are more resistant to removal by routine washing while the Gram-negative bacteria on the other hand constitute the transient flora which colonise the superficial layers of the skin and are more amenable to removal by routine washing.

Table 4. Occurrence of Fungi Isolated from Door Handles of Keke NAPEP Samples

Fungal species	**n (%)**
Aspergillus niger	2(50)
Yeast	1(25)
Mucor spp.	1(25)
Total	**4(100)**

The susceptibility test on the bacterial isolates showed that some of the bacteria were resistant to some antibiotics. This is in agreement with the study of Courvalin (2008) who stated that the emergence of antibiotics resistance is an evolutionary process that is based on the selection of microorganisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal.

Table 5. Antibiotic susceptibility pattern of selected organisms from bus and keke handles using gram-positive disc

Antibiotics	**Disc content(*µg*)**	***Staphylococcus aureus***
Ciprofloxacin	10	20.00 ± 0.00
Norefloxacin	10	17.00 ± 1.00
Gentamicin	10	19.67 ± 0.58
Amoxil	20	19.67 ± 2.52
Streptomycin	30	16.33 ± 0.58
Rifampicin	20	00.00 ± 00
Erythromicin	30	19.67 ± 0.58
Chloramphenicol	30	17.33 ± 1.53
Ampiclox	20	19.33 ± 2.52
Levofloxacin	20	21.67 ± 2.89

Table 6. Antibiotic Susceptibility pattern of selected organisms from bus and keke Door handles using Gram-negative disc

Antibiotic	**Disc content(µg)**	***E. coli***	***Shigella spp.***	***Proteus spp.***
Taravid	10	28.33 ± 2.89	22.00 ± 2.00	28.33 ± 2.89
Reflacine	10	28.33 ± 2.89	18.67 ± 4.16	30.00 ± 0.00
Ciproflox	10	28.67 ± 2.31	28.33 ± 2.89	25.00 ± 0.00
Augmentin	30	23.33 ± 2.89	28.33 ± 2.89	18.00 ± 1.73
Gentamycin	30	24.00 ± 1.73	26.33 ± 1.53	21.33 ± 1.16
Streptomycin	30	30.00 ± 0.00	3.33 ± 5.78	19.00 ± 1-73
Ceporex	10	6.67 ± 5.78	7.33 ± 6.43	0.00 ± 0.00
Nalidixic Acid	30	4.00 ± 6.93	3.33 ± 5.78	0.00 ± 0.00
Septrin	30	23.33 ± 2.89	27.67 ± 2.52	21.00 ± 3.62
Amplicin	30	3.33 ± 5.78	24.00 ± 3.45	6.67 ± 11.55

P<0.05
Key: Resistant: 0.00

Conclusion

Microorganisms associated with campus bus and keke door handles in the Federal University of Agriculture Makurdi were isolated and identified to be; *Staphylococcus spp.*, *E. coli*, *Shigella spp.*, *Proteus spp.*, *Aspergillus niger*, *Aspergillus fumigates*, *Mucor spp.*, *Rhizopus spp. and Yeast.* The

antibiogram analysis carried out on some selected isolates reveals that isolates were highly susceptible to antibiotics as just a few antibiotics showed minimal or no inhibitory activity as in the case of *Proteus spp.* which was highly resistant to Ceporex and Nalidixic acid. The overall implication of this result is that the buses and keke which were made to be used as a means of transportation by students and other members of the university community also served as a means of microbial carriage, the consequences of this are a threat to the health of students.

Recommendations

1. Hygienic measures should be maintained especially hand washing, when coming in contact with public places to minimize the rate of microbial transfer through these surfaces.
2. The university authority should take up the task of educating the bus and keke drivers on the need to regularly wash their vehicles and disinfect the door handles of the bus and keke.
3. An in-vivo treatment of isolates on experimental animals like mice should be carried out to know the efficacy of the antibiotics on the isolates.

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JIO, PTA and RDT conceived the concept, wrote and approved the manuscript.

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Competing interest
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