

Original Research Article

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Isolation, Enumeration and Antibiotic Sensitivity Pattern of Bacteria from the Hands of Commuters in Akure Metropolis

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ABSTRACT

This study was designed to isolate and determine the antibiotic sensitivity pattern of bacteria associated with the hands of commuters in Akure metropolis. The samples were collected using sterile swab sticks at different motor parks (Obaile, Oke-Ijebu, Benin, FUTA and Road block), then transported to Microbiology laboratory for further analyses. Isolation of microorganisms was carried by direct plating on the bacteriological media. The plates were incubated at 37°C for 24 hours. Enumeration of the bacterial isolates was done by direct count of the colonies formed on the plates. Pure bacterial isolates was obtained by repeated streaking on the fresh media. Different bacteria were isolated. *Staphylococcus aureus* recorded high frequency (90%) while *Escherichia coli* recorded least frequency (12%). All the Gram negative bacterial isolates were susceptible to some of the antibiotics with varied zone of inhibition. *Shigella* spp was susceptible to ciprofloxacin with zone of inhibition 5.0mm. *Escherichia coli* and *Staphylococcus aureus* were resistant to pefloxacin. High microbial loads recorded from the commuters indicated the level of contamination. Therefore, hand sanitization, proper hygiene and the use of antibiotics could help in the control and spread of antibiotic resistant bacteria from fecal origin.

Keywords

Fecal bacteria, Pathogenic microorganisms, Proper hygiene, Antibiotic susceptibility, Hand-contamination

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Introduction

Human hands perform many functions on the human body. Due to involvement of humans in various activities, it is easily exposed to environmental contaminants such as dust, body fluids, surfaces, pollutants, wastes, spoilt materials and during personal hygiene (Ahmad *et al.*, 2014).

Human hands have been implicated as the major transmitter of microorganisms among individual or from one person to another. It is

often act as vectors in the spread of pathogenic microorganisms such as bacteria and viruses from person to person, either through direct contact or indirectly via surfaces (Oranusi *et al.*, 2013). Lack of personal hygiene such as poor sanitary conditions can facilitate the transmission of some of these pathogenic bacteria found in the environment to human hands (Curtis and Carncross, 2003). Proper hand washing in public places as best traditional first line of defense can help in preventing the spread of disease and confer healthy living (Patel *et al.*, 2010). Though lack

of water source such as borehole in public places is one of the major challenges facing the populace (Lau *et al.*, 2012). Improper hand washing without using soap after leaving the restroom contribute greatly to the spread of fecal microorganisms (Barker and Bloomfield, 2000). Human hand to hand contact serves as express means of fecal-oral transmission of various enteric microorganisms capable of causing illness (Prescott *et al.*, 2005).

The skin covering the outermost parts of human hands acts as barrier for the invasion of pathogenic microorganisms and unwanted materials into the body system. Skin is one of the major sense organs covering various parts of the body. It acts as physical barrier, preventing infections of subcutaneous and systemic tissues through the production of sweat and sebaceous secretions containing fatty acids and lysozyme with antimicrobial properties (Ray *et al.*, 2011). Human skin supports the growth of different commensal bacteria as resident candidate on the skin which protect the host from pathogens. Microorganisms are ubiquitous, they can be found on surfaces, air, water, foods, dust and human skin (Rote *et al.*, 2010). Generally, human hands are the major source of microbial contamination mostly acquired by contact with objects or with the environment. Contamination of human hands can occurred as a result of various activities involving hand shaking, touching of contaminated surfaces and farming which principally play a key role in the spread of infectious diseases causing mortality if left untreated (Johnson *et al.*, 2002). Human hands play a crucial role in most human movements and activities. Human hands serve as a reservoir for large number of culturable microorganisms, considerably capable of causing diseases (Dodrill *et al.*, 2011). Environmental and local factors, host immunity, and microorganism adherence and virulence are intricately related to skin infection. Resident bacteria such as

Micrococcus, *Corynebacterium*, *Staphylococcus*, *Enterobacteria*, *Salmonella*, *Shigella*, *Escherichia*, *Proteus*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Clostridium*, *Streptococcus* and *Pseudomonas* are notoriously pathogenic microorganisms found to be transient on the human skin (WHO, 2009).

Environment plays a critical roles in the transmission of infectious diseases either directly through hand to hand contact, or indirectly, via food or other inanimate objects like fomites (AlGhamdi *et al.*, 2011). Human beings serve as a major vehicle in the transmission of fecal bacteria from one place to another as a result of hand washing after toileting or direct contact with the fecal droplet. Human hands usually harbors microorganisms both as part of person's normal microbial flora as well as transient microbes acquired from the environment (Lindberg *et al.*, 2004).

Fecal bacteria are naturally found in faeces of humans and animals. Contamination of hands is one of the important route by which humans are exposed to pathogenic organisms (Langford, 2009). They are usually transmitted by faeco-oral route, usually causes gastrointestinal infections with the symptoms including diarrhea, food poisoning caused by *Staphylococcus aureus*, enteric fever caused by *Salmonella typhi*, Shigellosis caused by *Shigella dysenteriae* (Boschi-Pinto *et al.*, 2008).

Diarrhea is a serious global public health problem. The World Health Organization estimated that over 2.2 million deaths occurred due to diarrhea infections occur annually, especially among children less than five years of age (WHO, 2002).

Bacteria of fecal origin can be found on the hands of commuters (passengers) that move

from one area to the other within the town. The presence of these bacteria on their hands could be an indication of lack of personal hygiene and inadequate hand washing after contact with fecal material from the environment.

Different microorganisms found in the human hands can be transferred to other parts of the body such as nose, skin, eyes, mouth, intestinal tract where they cause infections. Most of these microorganisms are capable of causing gastrointestinal diseases when accidentally ingested by the faeco-oral route (Tambekar *et al.*, 2007).

The faeco-oral route transmission of bacteria is highly favored occurring mostly through unapparent fecal contamination of food, water and hands (Curtis *et al.*, 2000).

Epidemiological evidence have shown that the most important risk factors are behavior's that encourage human contact with fecal matter, including improper disposal of faeces and lack of hand washing after defecation, after handling faeces and before handling food. Mostly, hand contact with ready to eat food represent a potentially important mechanism by which fecal microorganisms contaminate food and water (Yeager *et al.*, 1999).

Many food borne diseases and pathogenic microorganisms are spread by contaminated hands (Curtis *et al.*, 2003). Infants' exposure to faeces form greater risks of diarrhea disease by consuming contaminated water and food (WHO, 2006).

Hand hygiene has a measurable impact on reducing the burden of infection in the developing world (Aiello and Larson, 2002). Proper and good hand washing is the single most effective way to prevent the spread of communicable and infectious diseases among children and adults (Ejemot *et al.*, 2008).

Hand washing is one of a range of hygiene promotion interventions that can interrupt the transmission of bacteria of faecal origin present on the hands of commuters and to determine the level of contamination caused by this bacteria in order to gauge the importance of contaminated hands as route of transmission of bacteria of faecal origin (Curtis and Carncross, 2003). Therefore, this study was carried out to isolate faeca-bacterial pathogens associated with the hands of commuters and to possible control and hygienic measures in preventing the spread of infectious diseases.

Materials and Methods

Sample collection

Approximately 100 swab samples were collected from the hands of commuters at different motor parks (Ilesha garage, Oke-ijebu Park, Obaile Park, Benin Park, FUTA Park and Road-block Park) in Akure metropolis and then transported to the Microbiology laboratory for the microbiological analysis.

Microbiological analysis

The swab sticks were gently placed inside MacCartney bottle containing nine milliliter (9.0 ml) sterile distilled water to form stock culture. For the isolation, the samples were further serially diluted to appropriate dilutions and pour plated. Enumeration of fecal-bacteria was carried out using MacConkey agar, Eosin-methylene blue agar, Deoxychocolate citrate agar and Mannitol salt agar. The agar plates were incubated at 37°C for 24 hours. The isolates were sub-cultured by repeated streaking on their respective media until pure cultures were obtained. The isolates were characterized based on cultural, morphological and biochemical tests, as well as sugar fermentation test (Olutiola *et al.*, 2000).

Antibiotic sensitivity test

The antibiotic sensitivity test of the bacterial isolates were carried out using disc diffusion in order to compare the sensitivity of the bacteria to different commercial available antibiotics according to the method described by CLSI (2009). The antibiotic sensitivity test was investigated using GBMTS discs (Abtek biological Ltd.) containing the following antibiotics: gentamycin – GN, contrimoxazole – COT, erythromycin – E, pefloxacin - PEF, ciprofloxacin – CPX, augmentin – AU, streptomycin – S, chloramphenicol – CH and zinnacef – Z, amoxicillin – AMX, ofloxacin – OFL, ceftriaxone – CEF, rifampin – R, lexoflacin – L, rifampin – R. The commercial antibiotics were placed on nutrient agar plates previously seeded separately with 18 - 24 hours aged culture of each test microorganisms using sterile glass spreader. The plates were incubated at 37°C for 48 hours. After the incubation, the clear zone around each of the disc was measured as diameter zone of inhibition and interpreted accordingly. Unseeded agar plate with antibiotics served as the control. The isolates were scored either sensitive or resistant to antibiotics depending with diameter zone of inhibition.

Results and Discussion

Total bacterial loads

The total fecal-bacteria loads associated with the hands of commuters at various motor park in Akure metropolis is represented (Table 1). Highest bacterial loads (140 cfu/ml) from Obaile motor-park was recorded on Mannitol salt agar. Sample collected from Benin-park recorded high fecal-bacterial counts (106 cfu/ml) on MacConkey agar. On Eosin methylene blue agar, highest fecal-bacterial loads was obtained from Oke-Ijebu-park. On Deoxycholate agar, sample from Obaile motor

park recorded high fecal-bacterial loads (92 cfu/ml) while the least counts (16 cfu/ml) was recorded from Benin motor park. There was no significant different ($p < 0.05$) in the fecal-bacterial loads obtained from road block park on MacConkey, Eosin methylene blue and Deoxycholate agars. The fecal-bacteria isolated include *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhimorium*, *Escherichia coli* and *Klebsiella cruxii* (Table 2). *Staphylococcus aureus* recorded high total and percentage frequency (90) from the sample locations (motor parks) followed by *Escherichia coli* (53) while *Klebsiella cruxii* had the least value (12) (Tables 3 and 4).

Antibiotic susceptibility pattern on the fecal-bacteria

Figures 1 and 2 show the antibiotic sensitivity pattern of both Gram negative and Gram positive bacteria. All the fecal Gram negative bacterial isolates were susceptible to some of the antibiotics used with varied diameter zone of inhibition. *Shigella* spp showed high sensitivity to ciprofloxacin with diameter zone of inhibition 5.0mm. *Escherichia coli* was resistant to pefloxacin and lexoflacin (Figure 1). *Staphylococcus aureus* showed resistant to erythromycin, pefloxacin, zinnacef, amoxicillin and lexoflacin with high sensitivity to gentamycin – GN, contrimoxazole – COT, ciprofloxacin – CPX, augmentin – AU, streptomycin – S, chloramphenicol – CH, ofloxacin – OFL, ceftriaxone – CEF and rifampin – R (Figure 2).

Human hygiene play significant roles in the bacterial contamination. Commuters in the same vehicle can transmit faecal organisms to one another and to other commuters entering the same vehicle through touch contamination. The percentage of bacteria isolated from the hands of the commuters at different motor packs varied with respect to the level of contamination (Lynn *et al.*, 2013). Different

microorganisms have been found to associate with the hands of humans selling along the road sides and commercial places. The presence of these microorganisms indicates the level of unhygiene by the commuters which could pose threat to human health. The increase in total bacterial counts from Obaile Motor Park may be due poor hygienic conditions of the commuters or direct contact with fecal contaminating surfaces and materials. The prevalence of microbiota in the various motor parks could be attributed to the poor hygienic associated with the low level of education of the commuters attending the parks at the time of the study. The spread of infectious disease by the commuters could be attributed to the improper hand washing after defecating, touch of contaminated surfaces such as seats, stirring, walls, tires, luggage and soils (Chinakwe *et al.*, 2012). Human habit such as washing hands with contaminated or dirty water might be a source of fecal-bacteria and other pathogenic microorganisms (Rather, 2009). Isolation of *Staphylococcus aureus* from the human skin has been reported (Willey *et al.*, 2008). Ray *et al.*, (2011) had reported isolation of *S. aureus*, *E. coli*, *Enterococcus faecalis*, *Klebsiella* spp from the hands of the students. Similarly, Chinakwe *et al.*, (2012) had reported the presence of *E. coli*, *Pseudomonas* spp, *Proteus* spp, *Citrobacter* spp, *Klebsiella* spp, *Salmonella* spp, *Enterobacter* spp. and *S. aureus* from the hand wash and swabs of students. The

percentage contamination of *Staphylococcus aureus* on the hands of commuters at the motor parks was found to be high, above 80% compared to other bacteria. The presence of *Staphylococcus aureus* could be a normal flora of the skin and not necessarily an indicator of fecal contamination. High occurrence of *Escherichia coli*, *Shigella* and *Salmonella* from Okejebu, Obaile and Benin motor parks might be due to poor hygienic condition, inadequate hand washing, lack of access to water, high population of illiteracy and uneducated people in the motor parks. The prevalence and highest percentage of bacterial contamination causing diarrhea among poor people and middle economic status has been reported (Itah and Ben, 2004; Tambekar *et al.*, 2009). The low bacterial counts recorded among the commuters from FUTA car park might be attributed to the good hygiene practices among the students and the drivers. The reduction in the number of pathogens after hand washing was also reported by Tambekar *et al.*, (2009). Hygiene promotion may be cost effective intervention of preventing infectious diseases in developing countries (Curtis *et al.*, 2003). Isolation of transmissible microorganisms through handshake has been reported (Oniya *et al.*, 2006). The results obtained were similar to the findings of Mohsen (2017) who reported isolation of some enteric microorganisms from under the fingernails.

Table.1 Total fecal-bacterial loads

| Sample location | Fecal-bacterial loads (cfu/ml) | | | |
|-----------------------|--------------------------------|-----|-----|-----|
| | McA | EMB | DCA | MSA |
| Obaile motor park | 80 | 68 | 92 | 140 |
| F.U.T.A Cab park | 20 | 66 | 18 | 11 |
| Benin motor park | 106 | 40 | 16 | 17 |
| Oke-Ijebu motor park | 48 | 88 | 58 | 18 |
| Road-block motor park | 54 | 50 | 52 | 92 |

Key: McA - MacConkey agar, EMB - Eosin-methylene blue agar, DCA - Deoxychocolate citrate agar, MSA - Mannitol salt agar.

Table.2 Morphological and biochemical characteristics of the fecal-bacteria isolated from the hands of the commuters in Akure metropolis

| Isolate code | G. reaction | Cell shape | Cell colour | CATALASE TEST | OXIDASE TEST | COAGULASE TEST | CITRATE TEST | MOTILITY | INDOLE | GLUCOSE | GALACTOSE | LACTOSE | SUCROSE | MALTOSE | PROBABLE ISOLATES |
|--------------|-------------|------------|-------------|---------------|--------------|----------------|--------------|----------|--------|---------|-----------|---------|---------|---------|-------------------------------|
| P1 | + | C | YL | + | - | + | + | - | - | A | AG | A | A | A | <i>Staphylococcus aureus</i> |
| P2 | - | R | BGM | + | - | - | + | + | - | A | A | AG | A | A | <i>Escherichia coli</i> |
| P3 | - | R | MT | + | - | - | + | - | - | - | A | AG | A | A | <i>Salmonella typhimorium</i> |
| P4 | - | C | CR | + | - | + | + | - | - | AG | A | A | AG | A | <i>Klebsiella cruxii</i> |
| P5 | - | C | CR | + | - | - | + | + | - | - | A | A | A | A | <i>Shigella dysenteriae</i> |

Key: + = Positive/absent, - = negative/present, C = cocci, R = rod, YL = yellowish, MGM = brilliant green metallic, MT = metallic green, A = acid production, AG = acid and gas production

Table.3 Determination of frequency of fecal-bacteria

| Sample location | Sample size | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> | <i>Salmonella typhimorium</i> | <i>Shigella dysenteriae</i> | <i>Klebsiella cruxii</i> |
|-----------------|-------------|-------------------------|------------------------------|-------------------------------|-----------------------------|--------------------------|
| Obaile park | 20 | 14 | 17 | 8 | 7 | 4 |
| Benin park | 20 | 13 | 20 | 4 | 1 | 1 |
| Road block | 20 | 7 | 17 | 2 | 1 | 2 |
| F.U.T.A park | 20 | 10 | 16 | 1 | 1 | 3 |
| Oke-ijebu park | 20 | 9 | 20 | 5 | 4 | 2 |
| Total | 100 | 53 | 90 | 20 | 14 | 12 |

Table.4 Percentage frequency of the fecal-bacteria

| Sample location | Sample size | <i>E. coli</i> | <i>Staph. aureus</i> | <i>Salmonellaspp</i> | <i>Shigellaspp</i> | <i>Klebsiellaspp</i> |
|------------------|-------------|----------------|----------------------|----------------------|--------------------|----------------------|
| Obaile park | 20 | 14(70%) | 17(85%) | 8(40%) | 7(35%) | 4(20%) |
| Benin | 20 | 13(65%) | 20(100%) | 4(20%) | 1(5%) | 1(5%) |
| Road block park | 20 | 7(35%) | 17(85%) | 2(10%) | 1(5%) | 2(10%) |
| F.U.T.A Cab park | 20 | 10(50%) | 16(80%) | 1(5%) | 1(5%) | 3(15%) |
| Oke-ijebu park | 20 | 9(45%) | 20(100%) | 5(25%) | 4(20%) | 2(10%) |
| Total | 100 | 53(53%) | 90(90%) | 20(20%) | 14(14%) | 12(12%) |

Fig.1 Antibiotic sensitivity pattern of Gram negative bacteria

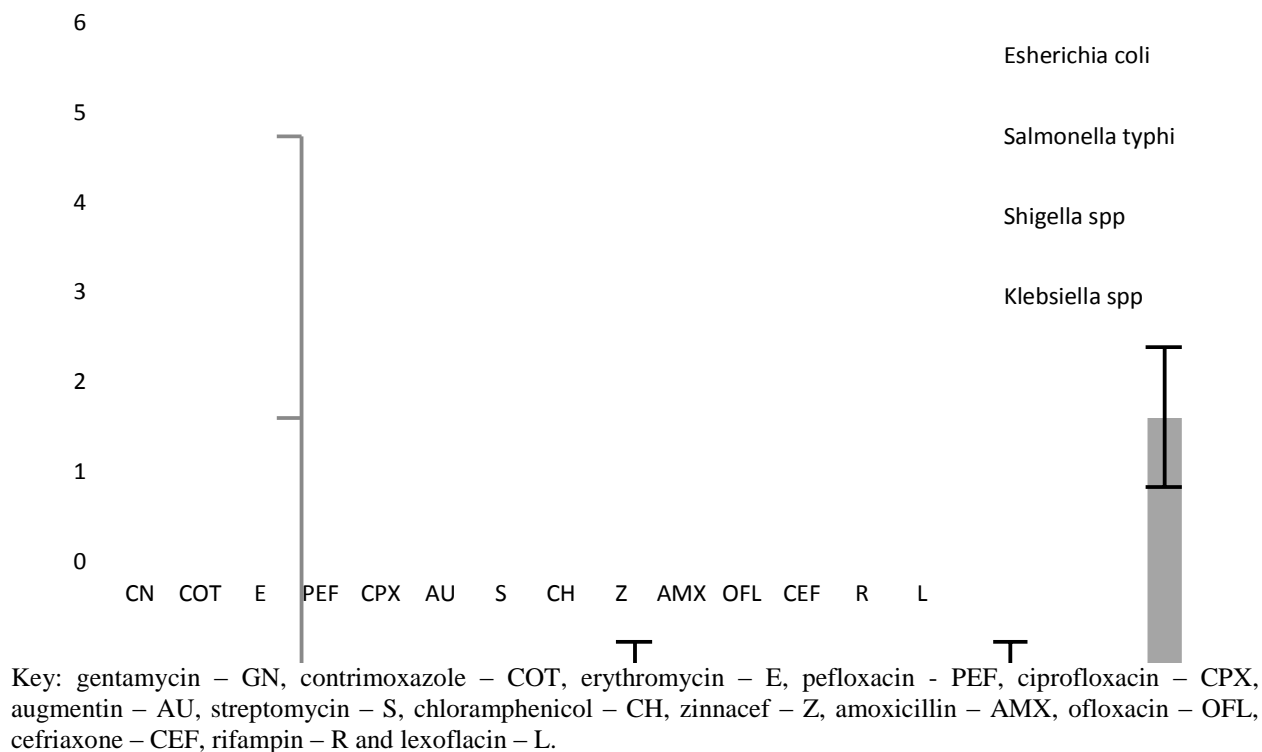
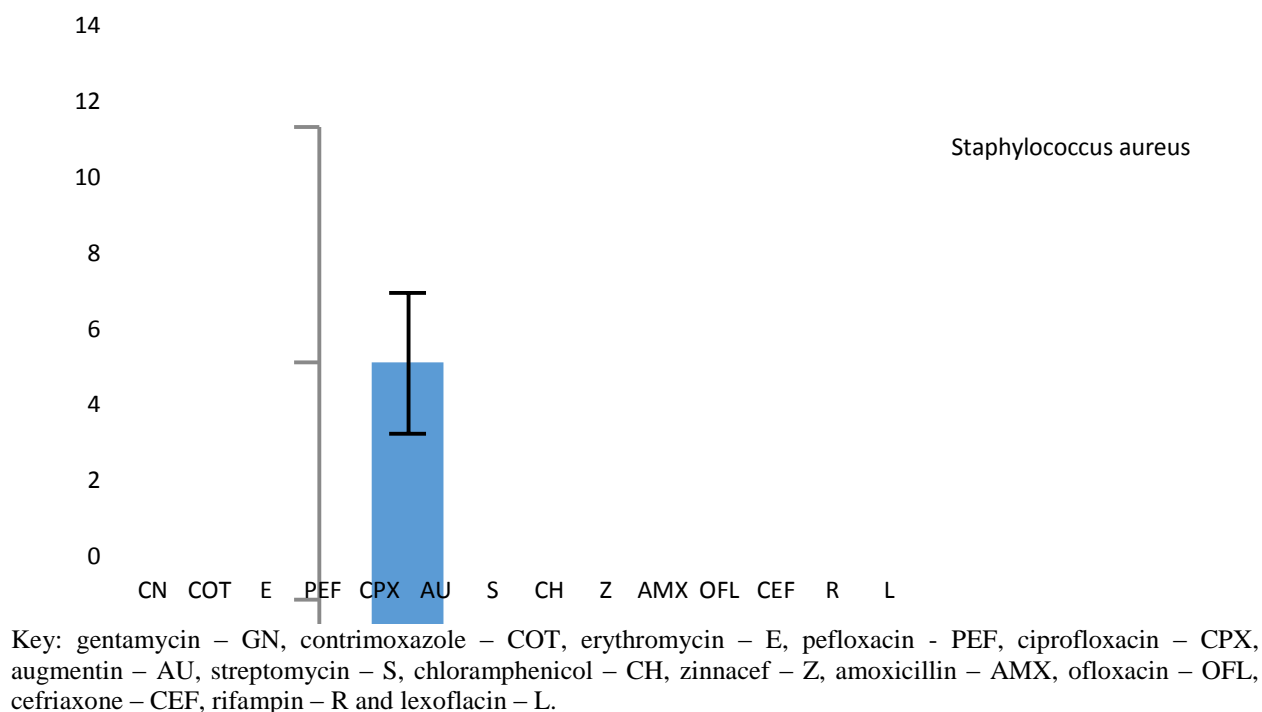


Fig.2 Antibiotic sensitivity pattern of Gram positive bacteria



Lack of uniform information on the use of antibiotics may have led to the emergence of resistant bacterial strains. The presence of multidrug resistant pathogens in the hand of commuters at various motor parks in Akure presents a major threat to human life. Though little information are available on the resistant of microorganisms isolated from the hands of commuters in Akure metropolis.

Nevertheless, adequate policy on the use of antibiotics could effectively address the curative measure in the control and prevention of infectious diseases associated with humans (Klutmans *et al.*, 1997). The presence of multiple drug resistant pathogens from the hands of the commuters at various park present a major threat to human life since different group of commuters come in contact with each either going on transit or for commercial purpose at the various parks. The emergence of resistant bacteria to some antibiotics might be attributed to the genetic manipulation and environmental stress. *Staphylococcus* spp is known as medically important pathogens of great health concern (Martin *et al.*, 2017). The resistance of *Staphylococcus aureus* to erythromycin, pefloxacin, zinnacef and amoxicillin might be due to the presence of peptidoglycans in the cell wall and certain enzymes among other virulence factors (Daka *et al.*, 2012). Aiello *et al.*, (2003) reported occurrence of antibiotic-resistant microorganisms from the hands of individuals. The resistance of Gram negative strains to most antibiotics more than Gram positive due to the intrinsic nature of their cell wall has been reported (Agersew *et al.*, 2015). The result obtained from this study was in agreement with the findings of Ogundare and Ekundayo (2016) who reported the resistant of *S. aureus* to pefloxacin.

In conclusion, this study revealed the microbial loads on the hygiene level among the commuters at various motor parks in

Akure metropolis. Regular surface washing of hands with disinfectant can contribute to the reduction of human hands contamination and the transmission of pathogenic microorganisms among the commuters. The types of microorganisms isolated could induce the attention of epidemiologists and health policy makers to the emergence of the antibiotic resistant bacteria. Proactive measures through compliance to hand washing practices and disinfection of personal items can be taken in the prevention of an outbreak of infectious diseases that could be difficult to handle. More also, periodic bacteriological monitoring, appropriate antimicrobial resistance surveillance and prevention approach should be adopted in curtailing the spread of fecal-contamination among the commuters. Therefore, multiple drug resistant *Staphylococcus aureus* isolated from the hands of the commuters should be critically address by the government by ensuring hand washing strategy at various parks in Akure metropolis and adequate health services in order to ensure safety and healthy living of the commuters.

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