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Bacterial Contamination Of Frequently Used Fomites In Public Transport Facilities In Imo State, Nigeria

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Abstract

The increase in the transmission of infection by pathogens through contaminated fomites in public places has become a significant concern for public health. This study was designed to assess bacterial contamination of frequently used fomites in public transport facilities in Imo State, Nigeria. A total of 105 Swab samples from frequently used fomites (countertops, doorknobs, toilet basins, seats, faucets and walls) in Sam Mbakwe Cargo Airport and four selected bus terminals in Imo State were analyzed using standard microbiological procedures. The study reveals high contamination in fomites from bus terminals of Imo Transport Company and Young shall grow motors with the toilet basin in the bus terminal of Imo Transport Company having the highest total heterotrophic count of 8.0 X106 CFU/m2. Samples from Sam Mbakwe cargo airport and God is good motors were the least contaminated. A total of 54 isolates comprising 28(51.9%) gram-positive and 26(48.1%) gram-negative bacteria were identified. Isolates identified were Streptococcus sp, Pseudomonas sp, Staphylococcus aureus, Escherichia coli, Bacillus sp, Proteus sp, Klebsiella sp, Shigella sp and Providencia sp, with Staphylococcus aureus (20.4%) as the most predominant organism. In comparison, Shigella (3.7%) and Providencia sp (1.9%) were the least isolated organisms. Antibiotic sensitivity profile of Gram positive isolates revealed highest sensitivity (88.5%) to ofloxacin and least sensitivity (30.8%) to ceftazidime while Gram negative isolates showed highest (78.9%) and least (31.6%) to ceftazidime and ciprofloxacin respectively. This study has shown that frequently used fomites can serve as vehicles for the transmission of potential pathogens. The authors, therefore, recommend maintenance of effective and regular cleaning and sanitation methods by management and users at these places to prevent the spread of infectious agents

Keywords: *fomites, contamination, bacteria, pathogens, antibiotics.*

1. Introduction

Mobility of individuals and goods is essential for economic and social activities such as commuting, manufacturing, the delivery of consumer products or energy supplies. Transport systems are the driving force behind this versatility and consist of infrastructures, modes and terminals. The make it possible for people, organizations and businesses to live socio-economically (Jean-Paul, 2017). The transport network is continuously growing to meet the need for ever-increasing passengers and goods. Bus terminals and airports are now principally used in major cities worldwide. The growth of global travel facilitates the rapid and sometimes uncontrollable transmission of infectious agents, which are accidentally or purposely released, from person to person worldwide (Kassem, 2009; Mendes et al., 2015). These transport systems with their numerous hand touch objects and surfaces serve as means for the possible spread of disease due to the large population of people coming together in confined spaces from around the world. (Kulmala, 2017). Currently, contamination of inanimate objects (fomites) and surfaces have become a significant phenomenon. Fomites can transmit human pathogens directly, through the surface to mouth contact or indirectly through the finger and hand to mouth contact. (Butz et al., 2013). Transmission of human

pathogens by formites could also come from exposure to the eyes, nose, or the skin (especially when abraded) (Hall and Douglas 2011; Beltrami *et al.,* 2013).The significant formites for transmission of pathogens in public transport facilities include doorknobs, toilet basin, countertops, waiting seats, faucets. According to Keplies et al. (2001), these inanimate objects serve a host to a large community of microbes comprising a full spectrum of bacteria, viruses, fungi, archea, and other potential pathogens and microbial metabolites that may be harmful to man. Fomites have been implicated in the spread of many viral infections, including SARS virus. (Gwaltney and Hendley, 2002; Hendley et al., 2013). SARS virus is reported to remain infectious for 96 hours on inanimate materials (Duan et al., 2013). The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is causing the coronavirus pandemic can remain active in aerosols for about three hours, up to four hours on copper, about 24 hours on cardboard and up to two to three days on plastic and stainless steel (Van Doremalen, 2020).
Additionally, bacteria have also been isolated on fomite surfaces, including those with pathogenic capacity and antibiotic-resistant ability (Kiepeis et al., 2001). Among the bacterial pathogens isolated from formites and surfaces in public facilities include Escherichia coli, Vibrio cholera, Streptococcus sp, Proteus sp, Pseudomonas aeruginosa, Staphylococcus sp E. *faecalis*, and Salmonella choleraesius. Klebsiella sp. There are few reports on bacterial contamination of hand touch surfaces in Uyo, Nigeria (Ofonime and Adegoke, 2018), London (Otter and French, 2009); USA (Reynolds et al., 2005); Chittagong City, Bangladesh (Chowdhury et al., 2016); Oregon, USA (Yeh et al., 2011) Tigrey, Ethiopia (Kahsay et al., 2019). There is a lack of information on the bacterial contamination of public transport facilities in Owerri. This work was therefore aimed at assessing bacterial contamination of fomites at Sam Mbakwe cargo airport and selected bus terminals in Owerri, Imo State.

2. Methods, Study Design and Sampling Technique

This study was conducted from May-November 2018 at Imo State, Nigeria. A total of 105 swabs samples were collected from toilet basin, windows, seat, departure floors, doorknobs, countertops, faucets (taps) in Sam Mbakwe Cargo Airport and four selected bus terminals in Owerri, Imo state. The selected transport companies have the biggest and busiest terminals in Imo State. The companies include God is Good Motors, ABC transport company, Young Shall grow motors, God is good motors and Imo transport company Ltd. Samples were randomly collected by swiping a 4 cm^2 section using swab stick moistened in sterile normal saline. Samples were collected in duplicate. They were placed in well-labelled swab caps kept in a cold box containing ice and transported to the microbiology laboratory at Imo State University Owerri within 30 minutes for microbiological analysis.

Isolation of bacteria

Isolation was done following standard microbiological techniques. The swabs were inoculated onto plate count agar and McConkey agar. Inoculated plates were incubated aerobically for 24 h at 37 °C. After incubation, isolates were streaked on plate count agar and Macconkey agar using known loop size diameter to enable calculation of colony forming unit (CFU). Pure culture of each isolate was identified based on cellular morphology and biochemical characteristics. Gram reaction, motility, indole test, coagulase, TSI, urease, oxidase, Simon's Citrate agar and carbohydrate utilization tests were carried out.

Antibiotic Sensitivity Test

The Antibiotic sensitivity test was done using disc diffusion, according to Bauer *et al.,* (1966) on Muller-Hinton agar medium. The following antibiotic were used: Ceftazidime (30mg), cefuroxime (30mg), gentamycin (10mg), Erythromycin (5mg), cloxacillin (5mg), ofloxacin (30mg), augmentin (30mg), cefixime (5mg), nitrofurantoin (300mg) and ciprofloxacin (5mg) (Abtek biological Ltd, UK). Pure culture of each isolate was incubated in nutrient broth at 370C for 24hrs. The inoculum for each isolate was prepared by dilution with normal saline to the turbidity of 1.5x108 CFU/ml (0.5McFarland standards). 0.1ml was spread inoculated on already solidified Muller Hinton agar (Oxoid ltd Basingstoke, UK) Using sterile glass rod. The antibiotic disc was positioned carefully on the agar surface to prevent the zone of

inhibition from overlapping each other. The plates were incubated at 37^0C for 24hrs. Results were recorded by measuring the zone of incubation and comparing with the CLSI susceptibility (CLSI, 2010).

3. Result and Discussion

Enumeration of bacterial from fomites from different locations

Our study sampled a total of 105 swabs from Sam Mbakwe Cargo Airport and the terminals of the four major transport company located in Owerri. The total heterotrophic bacteria count enumerated from the samples is shown in figure 1. The result indicates that fomites in all locations were contaminated with bacteria. Among the fomites, toilet basins were found to be the most contaminated in all locations with Imo transport company facility having the highest heterotrophic count of 8.0 X 10^5 CFU/m^2. On the other hand, countertops and seats from Sam Mbakwe airport had the lowest heterotrophic count. Several researchers have reported a preponderance of bacteria on toilet seats in public facilities. (Ogba and Obio, Fankem et al. 2012; Sampson et al., 2019; Ngonda, 2017) This high bacteria count observed in the toilet seats corresponds with the low level of cleaning and sanitary conditions as seen during the sampling. Flores et al. (2011) opine that the microbial diversity of public toilets is abundant due to the high rate of individual activities with different hygienic practices. Public restrooms must be kept clean for the convenience of users, but when not cleaned regularly, they are critical sources of microbial transmission and disease (Barker and Boone, 2013).

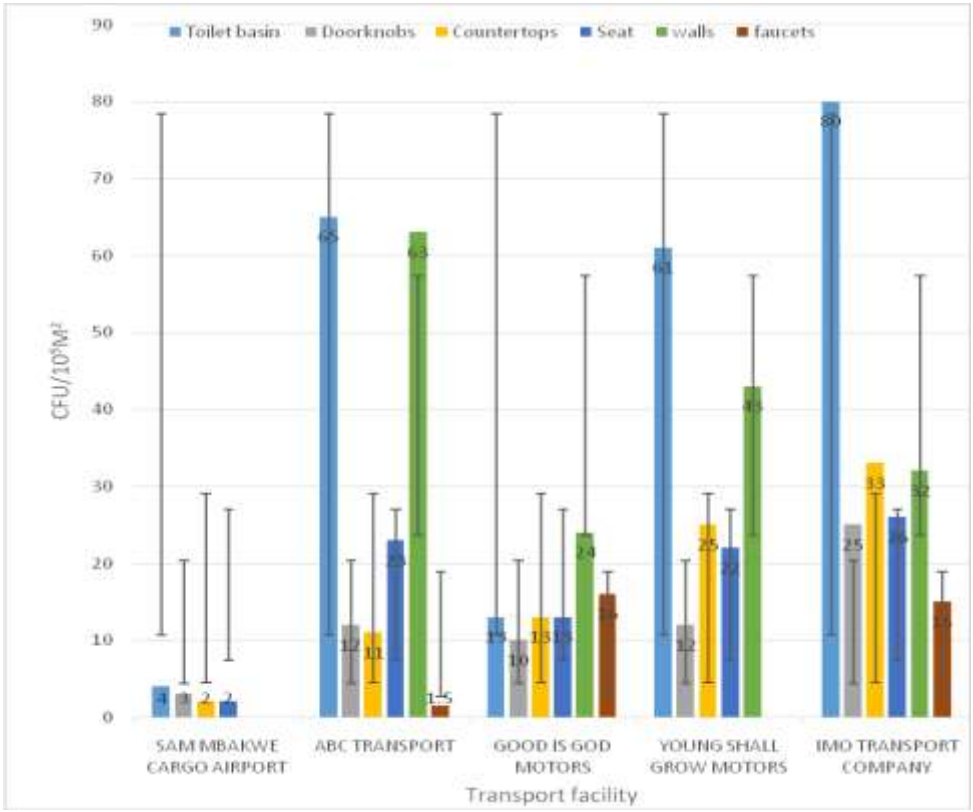

Fig 1: Enumeration of total heterotrophic count from fomites in different transport facilities

IDENTIFICATION AND DISTRIBUTION OF BACTERIAL ISOLATES FROM FORMITES

Bacterial distribution profile of swabbed fomites

A total of 54 bacterial isolates were identified from the swabbed items in all location. 28 (51.9%) were gram-positive bacteria, while 26 (48.1%) were gram-negative bacteria. Isolates identified include *Streptococcus* sp (18.5%), *Pseudomonas* sp (9.3%), *Staphylococcus aureus* (20.4%), *Escherichia coli* (18.5%), *Bacillus sp* (13%), *Proteus sp* (5.6%), *Klebsiella sp*. (9.3%), *Shigella sp* (3.7%) and *Providencia* sp (1.9%). Fig 2.

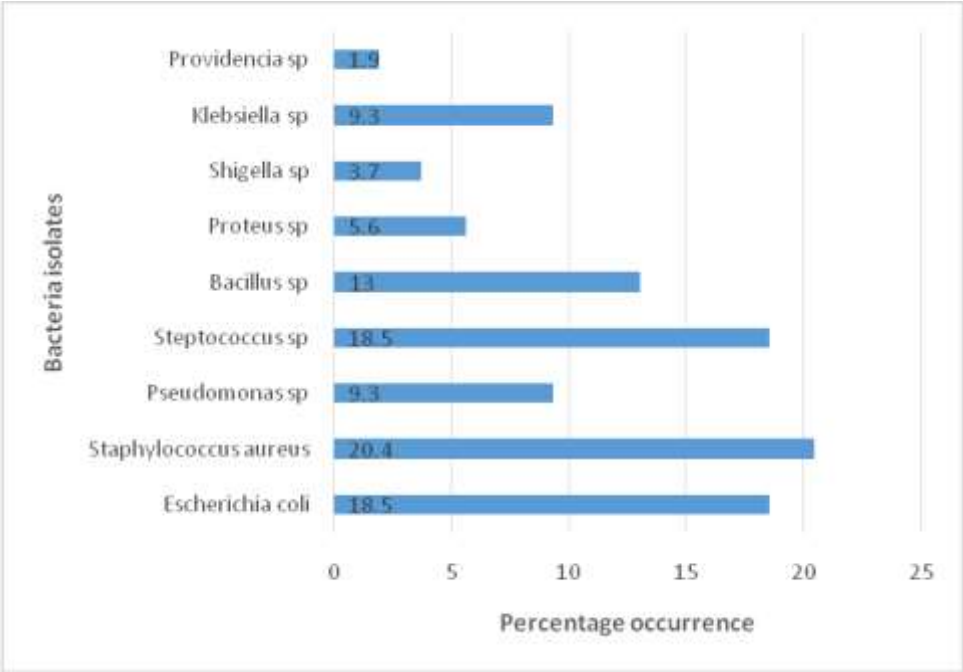

Fig 2: Percentage occurrence of bacteria isolates from fomites in different facilities

These organisms have been reported by several other researchers from hand touch surfaces in public places. Ofonime et al. (2018) reported the prevalence of *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, and *species of Micrococcus, Bacillus, Streptococcus, Pseudomonas, Proteus, Klebsiella* and *Serratia* from hand touch surfaces at bus terminals in Uyo, Nigeria. Al-Harbi (2017), implicated *Staphylococcus epidermidis, Enterococcus faecalis, Staphylococcus aureus,Escherichia coli, Streptococcus pyogenes, Pseudomonas alcaligenes, Streptococcusagalactiae, Klebsiellaoxytoca, Klebsiellapneumoniae*, and *Pseudomonas aeruginosa* from frequently used fomites in Kuwait. Other reports have also reported similar organisms from hand touch surfaces in public places. (Nworie et al., 2012; Alonge et al., 2018; Ngoda 2017). Fomites from Imo transport company was the most contaminated with about 38.9% of total bacterial isolates while fomites from Sam Mbakwe airport was the lowest contaminated (9.3%) followed by ABC transport (14.8%). This result was, however, expected judging from the sanitary condition of these surfaces at the time of sampling. At Sam Mbakwe airport, we discovered that fomites were clean and better maintained than those we met at Imo Transport Company. We also observed that the Imo transport company terminal was the busiest with a lot of users. The Imo transport company terminal is a state-owned public corporation that charges lower fares than the other privately-owned companies. This lower fares charged by Imo Transport Company was attributed to its busyness and may have also contributed to the contamination rate of the frequently used fomites in her terminal. The highest number of bacteria were identified from swabs from toilet basins and doorknobs (Fig. 3)

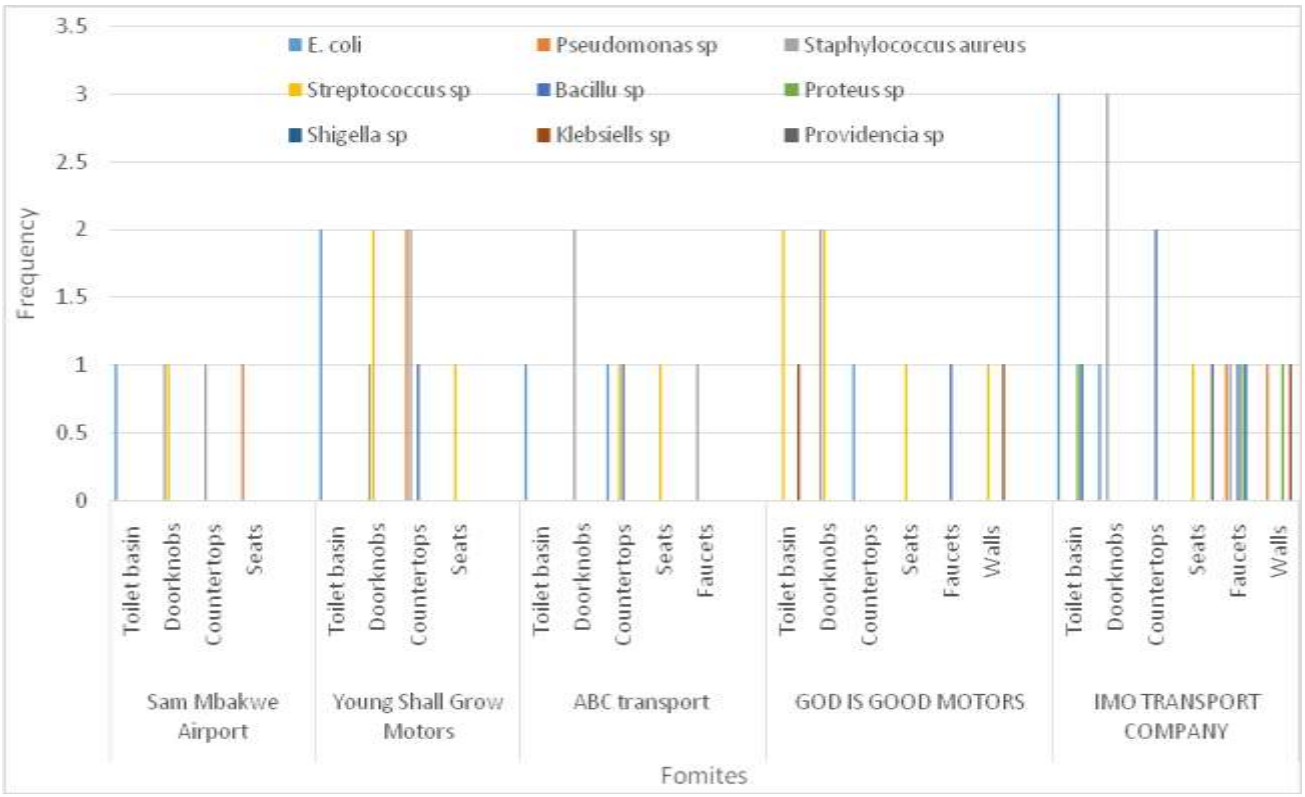

Fig 3: Distribution of bacteria isolates in fomites from different facilities

Toilet seats and doorknobs are among the reported sources of infections in public facilities. Nworie et al. (2012) reported a high infection rate (87.1%) from toilet door handles in their study. The result also agrees with the findings of Ngonda (2017), who reported a high prevalence of bacteria in toilet door handles. Boone and Gerba, (2010) recorded similar rates of contamination of public conveniences and opined that contamination levels are directly proportional to the frequency of usage, exposure and nature of the environment. This result was, however, contrary to the reports of Ofonime and Adegoke, 2018, who recorded the highest prevalence of bacteria from countertop surfaces. *Staphylococcus aureus* was the most predominant bacteria from our study. *Staphylococcus sp* inhabits the skin and nasal surfaces of a healthy individual. This may account for its high prevalence as it can easily contaminate fomites through contact with humans. This observation conforms to the finding of other researchers (Brooks et al., 2007; Nworie et al., 2012). *Staphylococcus aureus* is the most significant possible pathogen causing burns, abscesses, wound infection and toxic shock syndrome (Brooks et al., 2007; Nworie et al., 2012)

E. coli and *Streptococcus* sp were the second most predominant organism isolated from this study. The presence of *Streptococcus* sp. indicate the possibility of mouth or nasal contamination (aerosol discharge from mouth and nose), i.e. body flora might have been shed to those surfaces by the passengers (Adegoke and Komolafe, 2009; Komolafe and Adegoke, 2008). *Streptococcus* can cause a wide range of diseases ranging from pharyngitis to more severe and life-threatening infections such as acute rheumatic fever. The identification of *E. coli, Klebsiella pneumonia, Shigella sp and Proteus sp* may be an indication of faecal contamination of the fomites. *Klebsiella pneumoniae* is predominant in the environment and is a commensal of the gastrointestinal tract of humans. It has a considerable capacity to cause a variety of human diseases, from urinary tract infections to pneumonia. (Podschun and Ullmann, 1998).The presence of *Bacillus* sp in the fomites may be due to its spore-forming ability, which is likely to cause it to spread into the air and thus be able to settle on the fomites surface (Adegoke and Okoh, 2011). Antimicrobial resistance represents one of the biggest health challenges, especially in developing countries. Indeed, the

antimicrobial resistance study of the WHO (2015) illustrated related global mortality of nearly 700,000 deaths per year with an estimated 10 million deaths by 2050. In this study, Ofloxacin (88.5%) and gentamycin (73.1%) recorded the highest sensitivity against Gram positive organisms. Resistance to Gram positive isolates was highest with Ceftazidime (69.2%) (Fig 4)

For Gram negative isolates, highest sensitivity was observed with ceftazidime (79%) while 68.4% of isolates were resistant to ciprofloxacin, making it the least effective antibiotics against Gram negative isolates.

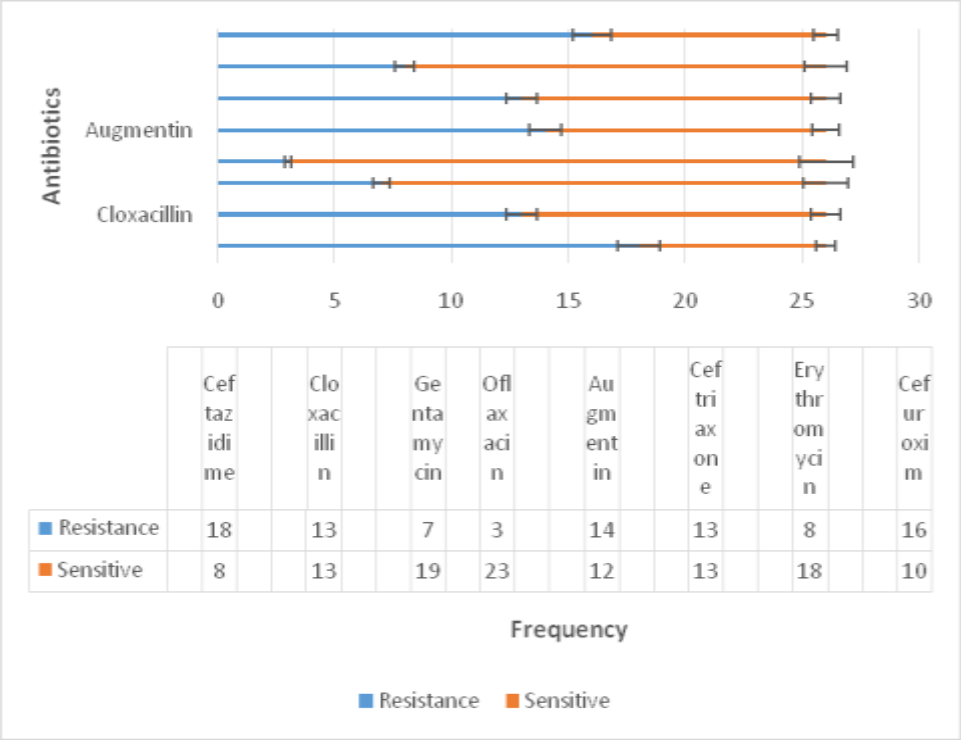

Fig 4: Gram positive antibiotic sensitivity profile

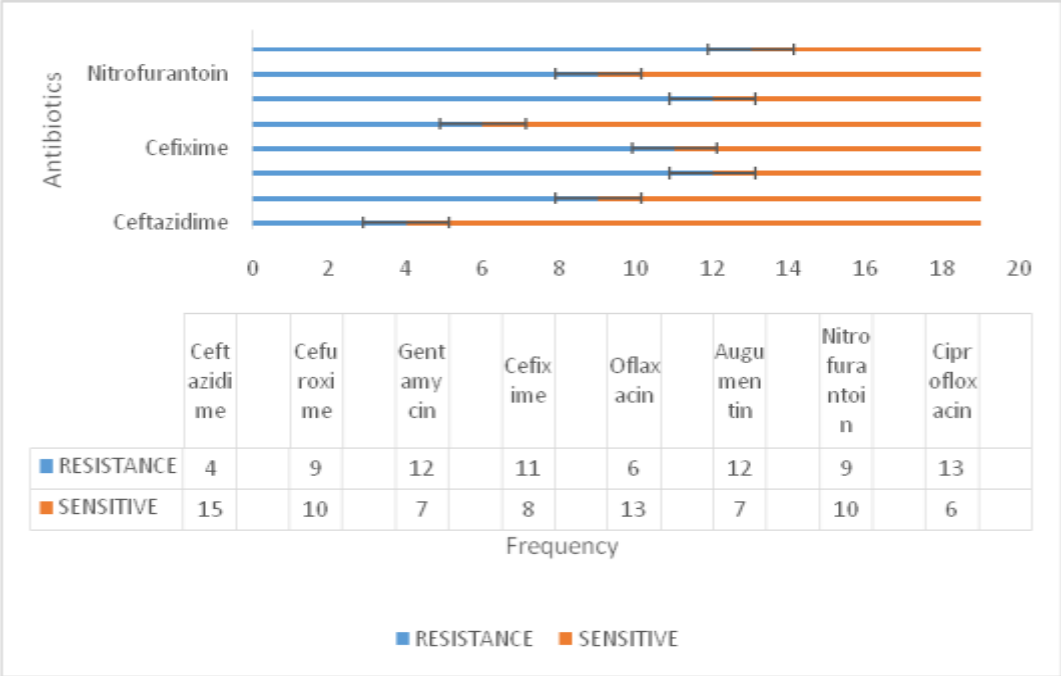

Fig 5: Gram negative antibiotic sensitivity profile

John and Adegoke (2018) have also reported similar antibiotic spectrum against gram-positive and gram-negative bacteria from hand contact Surfaces at bus Terminals in Uyo Metropolis. Boma and Olieme (2011), Akubuenyi et al., (2011); Ezeonu and Ugwu, (2011) and David et al. (2011) have also reported resistance profile of bacteria associated with contact surfaces. This present study, however, contrasts with the report of Jombo et al. (2010).

4. Conclusion

This study investigated the bacterial contamination of fomites in public transport facilities. Several pathogens reported in previous epidemiological studies were isolated in this study. These include *Staphylococcus aureus,Escherichia coli,Pseudomonas sp*, *Klebsiellapneumonia*, *Streptococcus* sp, *Proteus* sp, *Shigella* sp and *Bacillus* sp. This study shows that frequently or heavily used fomites harbor highly pathogenic bacteria with a potential of causing infection at an epidemic level. Therefore, adequate measures for the regular cleaning and disinfection of all frequently used fomites in these public transport facilities should be adopted. The author also recommends maintenance of good personal hygiene by users to prevent the transmission of pathogens from these fomites.

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