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Diversity of Commercial Intercity Vehicles (Buses and Tricycle) Microbiome

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Abstract

The isolation of microbes from frequently touched inner surfaces of intercity vehicles in Owerri was carried out to evaluate the diversity of microorganisms that are associated with this transport means and their health implications. One hundred and twenty (120) samples were collected; Sixty (60) each from twenty (20) different Buses and Tricycles respectively. Samples were collected with sterile swab sticks moistened in normal saline and cultured on Nutrient Agar, MacConkey Agar, Eosin Methylene Blue Agar and Potato Dextrose Agar. Isolation and characterization of isolates were done using standard microbiological methods and with reference to standard identification manuals. Bacterial and fungal species isolated included Bacillus, Staphylococci, Pseudomonas, Klebsiella, Enterococci and Aspergillus, Penicillium, Mucor, Yeasts, Alternaria, Cladosporium, Trichoderma and Fusarium. Some of the bacteria and fungi isolates are potential pathogens which have been implicated in diseases. Therefore, regular washing of vehicles, good hygiene and sanitary measures are recommended to keep the vehicles neat and safe.

Keywords: *Built environment, good hygiene, microbiome, sanitary measures, vehicles*

1.0 Introduction

The Commission of the European Communities (1985) defines commercial motor vehicle as any motorised road vehicle, which by its type of construction and equipment is designed for, and capable of transporting, whether for payment or not: (i) not more than nine persons, including the driver (ii) goods and standard fuel tanks (EC Council Directive 68/297, substituted by EC Council Directive 85/347). Examples of commercial vehicles include: truck, van, coach, bus, taxicab, semi-truck, trailers, box truck and in Nigeria and few other countries tricycle has been added to this list.

Microorganisms are ubiquitous in nature, being present in the environments, that human encounter daily and some are of potential concern to man's health. Humans spend a significant amount of time indoor and thus the microbial ecology of indoor environment likely impacts the human microbiome (Klepies *et al.*, 2001; Kembel *et al.*, 2012). Studies on the microbial communities of built environment suggest that indoor microbiomes originate mainly from outside air or the human skin. (Pakarinen *et al.*, 2008; Rintala *et al.*, 2008; Grice & Segre, 2011).

The microbial species present in a built environment are predominantly determined by exposure to microbes and selection of certain microbial types by the environment (Martiny *et al.*, 2006). Most studies examining the microbiome of built environment have concentrated on buildings, with particular focus on healthcare settings (Rintala *et al.*, 2008; Tringe *et al.*,2008; Amend *et al.*, 2010; Kembel *et al.*, 2012). There is paucity of information on the microbial ecology of the automobile built environment and its health

implication. Commercial vehicles provide favourable environments for communicable diseases transmission and are potentially important fomite for exposure of microbes. Microorganisms spread through direct contact (by touching someone else), through the air (coughing, sneezing, talking or singing) and through indirect contact (touching objects and surfaces that are contaminated). All these conditions for easy spread of microbes, are provided in the built environment of commercial vehicles.

The *Staphylococcus* genus is of particular importance concerning fomite colonization and transmission to human. Staphylococci frequently colonize human skin and mucosal surfaces, and these are likely to be transmitted to inanimate surface that humans come into contact with (Payne *et al.*, 2003; Safdar & Bradley, 2008; Foster, 2009; Pynnonen *et al.*, 2011). Of particular concern is *Staphylococcus aureus*, which has the capacity to cause a variety of devastating infectious diseases (Lowly, 1998; Klevens *et al.*, 2007; Otto, 2012). *S.aureus* infections and outbreaks have previously been associated with exposures to a multitude of contaminated fomites including whirlpools, towels, handrails and razors (Miller & Diep, 2008; Kassem, 2011). Because pathogens/potentially pathogenic microbes can colonize commonly touched inanimate objects, it is feasible that surfaces of automobiles could serve as reservoirs for pathogens and may play an important role in human colonization and infection. In this study, the microbial communities (microbiome) of the vehicle (Buses and Tricycles) built environments a potentially important fomite for exposure of microbes to man was investigated.

2.0 Materials and Methods

2.1 Sample collection

A total of 120 samples were collected from 20 each of buses and tricycles respectively. Three samples (in duplicates) were collected from each bus and tricycle to include swabs from approximately two square inch area of the steering, seats, and inner surfaces specifically the door and window handles in buses and surfaces commonly held by passengers in the tricycles. The swab sticks were moistened in normal saline, the vehicles surfaces were swabbed and the swabs shielded back in the sterile container. The samples were appropriately labelled and transported to the Department of Microbiology laboratory Federal University of Technology, Owerri (FUTO) for analysis within one hour of sample collection

2.2 Media and Sample Preparation

All the media used [Nutrient Agar (NA), MacConkey Agar (MA), Eosin Methylene Blue (EMB) Agar, Nutrient Broth (NB), and Potato Dextrose Agar (PDA)] were from MERCK, Germany and HiMedia, India. EMB Broth (EMBB) (Sigma-Aldrich, USA) was employed for coliform test. The media were all prepared following the manufacturer's instructions and were incubated overnight to check for sterility before use.NA was used for total heterotrophic bacteria count and isolation, MA and EMB agar were used for coliforms count and isolation, while PDA was used for fungal count and isolation. The swabs were sluiced out in two (2.0) ml NB to produce working stock.

2.3 Analysis of Specimen

The spread plate technique was adopted for inoculation of the plates ((Jolt *et al*., 1994). Aliquot 0.1 ml of the working stock was spread inoculated onto the different media in duplicate; in addition, the cotton wool end of the swab stick was broken into EMB Broth (Sigma-Aldrich, USA) with inverted Durham tubes for coliform test. NA, MA, EMB and EMBB were incubated for 24-48h at 37 °C while PDA was left at ambient room temperature of 28± 3 °C for 3 - 5 days. At the expiration of incubation period, plates were examined; microbial colonies enumerated using colony counter (Gallenkamp, England), total counts was expressed as colony forming units per ml (cfu/ml), (equivalent to colony forming units per square inch swabbed surfaces)

of Buses/Tricycle. Characteristic colonies were isolated and purified by repeated sub culturing on freshly prepared nutrient agar. Pure cultures were stored on slants for further characterization and identification.

2.4 Coliform Test

The method as described by Oranusi *et al.,* (2003) was adopted. Samples with gas formation indicated by the Durham tubes and/ or colour change of dye in the EMB medium were reported as positive for presumptive coliform test. Confirmatory coliform test was carried out by plating out positive presumptive test cultures on EMB agar plates and incubating overnight at 37°C. A plate of the EMB cultures was however, incubated at 44°C for faecal coliform *E. coli* isolation. The presence of characteristic greenish metallic sheen black colonies typical of *E. coli* or brown mucoid colonies characteristics of *E. aerogenese* is a positive confirmatory test. The colonial growths were treated for completed test and stored at 4°C for further characterization.

2.5 Identification of Isolates

Pure cultures of isolates stored on slants were sub-cultured on appropriate medium and incubated for 24-48 h to check for viability and also to reconfirm purity. Identification of bacterial isolates was based on morphological characteristics of colonies, microscopy and biochemical tests including catalase production, indole test, methyl red, Voges-Proskauer, citrate utilization, coagulase, oxidase and urease production, gelatin liquefaction, starch hydrolysis, fermentation of sugars, temperature and salt tolerance tests and motility test. The Analytical Profile Index (API) system [Biomerieux© sa] with reference to standard identification data base was employed in the further identification of the bacterial isolates (Jolt *et al*., 1994; MacFaddin, 2000). Fungal isolates were identified based on morphological characteristics and microscopy with reference to standard identification keys and atlas (Hanlin & Ulloa, 1988; Samson & Reenen-Hoekstra, 1988; Tsuneo, 2010).

2.6 Statistical Analysis

All data from colony counts are presented as mean and standard deviation. The level of significance in differences of means was determined by Duncan's Multiple Range (DMR) test using SPSS 20.0 software for windows. (SPSS, 2011)

3.0 Results

Table 1 shows the microbial counts from the swabbed surfaces of vehicles (Buses and Tricycles) investigated. The Table reveals that the microbial counts from the swabs of inner surfaces of the vehicles were significantly higher ($p \leq 0.05$) than counts obtained from the seats and steering. The table also shows that the microbial counts from the swabs of seats were lower than counts from the steering and inner surfaces of the vehicles except for total aerobic plate count (TAPC) from tricycles.

Table 2 presents the predominant microbial species isolated from swabs of vehicle surfaces. The major bacterial species as shown on the table included *Bacillus, Staphylococcus, Klebsiella* and *Pseudomonas*. The predominant fungal isolates include *Aspergillus, Penicillium, Yeasts* and *Rhizopus*. Other fungal species isolated include *Paecilomyces, Fusarium* and *Alternaria*.

Table 3 shows the susceptibility of bacteria isolates from swabs of vehicles surfaces to commonly used antibiotics. All the isolates (100 %) were multi-resistant, having resistance to at least three of the ten antibiotics tested. Seven out of the eight tested isolates (87.5 %) were resistant to four antibiotics, 62.5 % were resistant to 5 of the antibiotics while 37.5 % and 25.0 % were respectively resistant to 6 and 7 out of

the ten commercial antibiotics tested. The Table 3 also reveals that the tested isolates were mostly susceptible to Ofloxacillin, Gentamycin and Ciprofloxacin.

4.0 Discussion

Human development and its attendant modernization and revolution in every sphere of human endeavours in terms of commerce and trade, industry, life style, job specifications and general life aspirations and goals, has made it such that humans spend a significant amount of their time and life inside vehicles. However, very little is known about the microbial ecology of vehicles and how this might impact the occupants micro biome. The results of this study indicate that the most frequently touched areas of the vehicles; the steering, seats, inner surfaces-door and window handles and iron rails constantly held by passengers were all highly contaminated.

The significantly high microbial load recorded for swabs of inner surfaces of the vehicles could be a reflection of the diversity of microbial loads of the different passengers and the bus conductor that have to touch the door and window handles and or hold the iron rails in the tricycles and buses in order to maintain balance on the journey which is often through rough and pothole-filed roads. The breathing, talking, singing, shouting and even quarrelling and in some cases fighting in the vehicles would have contributed immensely to microbial load of the inner surfaces of the vehicles. The commercial buses are generally not fitted with air conditioning system, thus the windows and often the doors are left open for ventilation. The Tricycle by its type of construction allows for air and dust flow on transit. These increase the dust particle and microbial deposits inside the vehicles.

The significantly lower microbial counts recorded for the vehicle seats when compared to counts from the inner surfaces and steering could be explained by the fact that passengers that board the vehicle contribute to cleaning the vehicle seats indirectly by using his or her clothes as they sit, shift and change positions in the vehicles. That the steering was highly contaminated could be explained by the microbial load on the driver's hands, deposits from dust particles and passengers sitting close to the driver. Some of the drivers do not have conductors (bus boy, thus the driver serves to load passenger's goods into the vehicle, frequently open the door for the passenger to board and alight, and with the same hands handle the steering. Similarly, faults in the vehicles are often fixed quickly by the driver and with hands not washed, the journey continues.

The diversity of bacterial and fungal isolates recorded in this work could be a reflection of the diverse sources of contamination of the swabbed surfaces; the passengers (commuters), conductor, driver, dust and even from the goods. In Nigeria, vehicles are not strictly designated for specific commercial activity; for passengers only or for goods: life animals, slaughtered animal, vegetables, fruits, timber only. A particular vehicle can be used for all of the above services in the course of a day's business. Vehicle operators just catch in on any available business at any point in time. This attitude of the vehicle operators though not monitored for the vehicles used in this research, could contribute to the microbial load and diversity of vehicles.

The isolation from the swabbed surfaces of the vehicles of bacterial and fungal species such as *Staphylococcus*, *Klebsiella*, *Streptococcus*, *Pseudomonas*, *Penicillium*, *Aspergillus*, *Rhizopus* and *Yeasts* calls for concern. Members of these genera commonly colonize human skin, mouth and respiratory tract, which suggests that the prevalent genera on vehicles were deposited there by skin to surface contact, from the mouth or respiratory tracts of commuters, *Staphylococcus aureus* has been reported for its role in human infections (Balaban & Rasooly, 2000; Hidroin *et al.*, 2008; Lowly, 1998; Klevens *et al.*, 2007; Otto, 2012; Phillsbury *et al.*, 2013).

The fungal species have been reported to be pathogens or opportunistic pathogen specifically in the immune-compromised and are implicated in mycoses, ear and eye infections and mycotoxicoses (Alberti *et al.*, 2001*;* Lillard-Roberts, 2006., DiSalvo, 2008; Khan & Karuppayil, 2012; Hymery *et al.*, 2014; Miller, 2014, Benedict & Park, 2014).The presence of *Bacillus* spp and the moulds in the swab samples conform to the fact that bacillus and mould species are spore formers and ubiquitous. They have been reported as contaminants of built environments (Kembel *et al*., 2012).

Bacterial isolates of this study were multi-resistant to the common antibiotics in use. These calls for concern, and also points to the fact that, some of the isolates could be environmental contaminants which have been reported to be highly resistant to antimicrobial agents. Poor regulations, inappropriate use of antibiotics, poor quality drugs and its availability as over the counter drugs, have been reported to contribute to drug abuse and multi-drug resistance (Yah *et al.,* 2007). It is pertinent to note that commuters can easily be infected with multidrug resistant microorganisms that can be life threatening and because of lack of awareness to the fact that the vehicle built environment could be a veritable source for disseminating pathogenic organisms, epidemiological surveillance and provision of preventive measures and cure will be adversely affected. This study reveals that commercial vehicles (buses and tricycles) could be a source of health hazard for transmitting pathogens/potential pathogens to the commuters. It is therefore recommended that commercial vehicles should be washed regularly to reduce or be free from pathogen contamination and the provision of air conditioning system will improve aeration and thus reduce the level of dust associated with open windows and door when the vehicle is in motion.

Table 1: Microbial Counts (cfu/square surface) of Swabbed Surface of Vehicles

Swabbed surfaces	**Buses**			**Tricycles**		
	TAPC	Coliform count	Fungal count	TAPC	Coliform count	Fungal count
Steering	$7.6 \times 10^{7} \pm 0.02^{b}$	$1.3 \times 10^{2} \pm 0.06^{d}$	$2.4 \times 10^{6} \pm 0.60^{a}$	$5.6 \times 10^{4} \pm 0.00^{c}$	$1.5 \times 10^{2} \pm 0.00^{d}$	$3.5 \times 10^{5} \pm 0.40^{f}$
Seat	$8.5 \times 10^{6} \pm 0.00^{c}$	$1.0 \times 10^{2} \pm 0.04^{d}$	$3.2 \times 10^{4} \pm 0.02^{b}$	$6.8 \times 10^{7} \pm 0.40^{b}$	$2.0 \times 10^{2} \pm 0.02^{d}$	$2.4 \times 10^{5} \pm 0.06^{f}$
Inner surfaces	$5.3 \times 10^{9} \pm 1.03^{a}$	$2.3 \times 10^{3} \pm 0.82^{a}$	$3.6 \times 10^{6} \pm 1.02^{a}$	$4.5 \times 10^{9} \pm 1.01^{a}$	$1.4 \times 10^{2} \pm 0.01^{d}$	$6.2 \times 10^{7} \pm 0.80^{e}$

Values are mean ±SD of duplicate counts. Values with different alphabet superscript down the column and across the row for same count are significantly ($P < 0.05$) different
TAPC = total aerobic plate count?

Table 2: Predominant Microorganisms Isolated from Swabs of Buses and Tricycles

Vehicle	**Swabbed surfaces**	**Species of organisms isolated**	
		Bacteria	Fungi
Buses	Steering	*Staphylococcus, Bacillus, Klebsiella, Streptococcus, Pseudomonas,*	*Penicillium, Aspergillus, Rhizopus, Yeasts, Alternaria, Curvularia*
	Seat	*Pseudomonas, Bacillus, E. coli, Klebsiella, Staphylococcus, Micrococcus,*	*Yeasts, Fusarium, Paecilomyces, Verticillium, Aspergillus, Penicillium*
	Inner surfaces	*Bacillus, Staphylococcus, Pseudomonas, Corynebacterium, E. coli, Klebsiella, Proteus,*	*Mucor, Cladosporium, Aspergillus, Myrotechium, Stachybotrys, Rhizopus, Scopulariopsis*
Tricycles	Steering	*Bacillus, Enterococcus, Staphylococcus,*	*Aspergillus, Mucor, Penicillum*
	Seat	*Klebsiella, Bacillus, Paecilomyces,*	*Yeasts, Trichothecium, Aspergillus*
	Inner surfaces	*Bacillus, Klebsiella, Staphylococcus, Pseudomonas,*	*Geotricum, Mucor, Ulocladium, Aspergillus, Trichoderma*

Table 3: Susceptibility of Bacterial Isolates from Swabs of Buses and Tricycles to Commonly used Antibiotics

Antibiotics	**Conc. (µg)**	***P. aeruginosa***	***S. aureus***	***E. coli***	***K. Pneumonia***	***S. epidermidis***	***Proteus spp***	***Bacillus cereus***	***Micrococcus spp***	**Control Standard virgin *S. aureus***
AMX	25	0.0	8.0	8.0	6.5	8.0	8.0	7.0	12.0	10.0
OFL	5	15.0	16.0	18.0	15.0	20.0	16.0	18.0	18.0	15.0
STR	10	10.0	11.0	13.0	14.0	12.0	15.0	11.0	13.0	12.0
CHL	30	11.0	12.0	15.0	10.0	16.0	12.0	10.0	11.0	14.0
CEF	30	0.0	8.0	9.0	0.0	6.0	6.0	11.0	0.0	9.0
GEN	10	16.0	15.0	17.0	16.0	18.0	18.0	12.0	18.0	14.0
PEF	5	9.0	13.0	10.0	10.0	12.0	12.0	11.0	11.0	12.0
COT	25	0.0	6.0	8.0	9.0	8.0	5.0	10.0	9.0	10.0
CPX	10	16.0	18.0	16.0	18.0	16.0	20.0	13.0	20.0	15.0
ERX	5	0.0	8.0	10.0	11.0	12.0	8.0	0.0	14.0	11.0

Key: AMX = Amoxicillin; OFL = Ofloxacillin ; STR = Streptomycin ; CHL = Chloramphenicol ; CEF = Ceftriazone ; GEN = Gentamycin; PEF = Pefloxacin ; COT = Cotrimazole; CPX = Ciprofloxacin; ERX = Erythromycin

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