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Microbiological Assessment of Car Doors and Steering Wheels at Benue State University, Makurdi: Public Health Implications

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Abstract

Microbiological assessments of car surfaces remain a fundamental approach to control hotspots of microbial contamination. This study was aimed at assessing the level microbial contaminations associated with car doors and steering wheel of cars within the faculty of science, Benue state university, Makurdi. A total of forty (40) samples were collected in duplicates. These included twenty duplicate samples from car door handles and twenty duplicate samples from car steering wheels respectively using sterile swab sticks and transported to Charis Research and Diagnostic laboratory for analysis. The samples were analysed using cultural, biochemical and morphological techniques. The results revealed that the heterotrophic bacterial count range from 1.97 x 10^4 to 2.41 x 10^4 CFU/cm^2 while the fungi count range from 1.9 x 10^3 to 3.7 x 10^3 CFU/cm^2. *Staphylococcus* spp. had the highest occurrence of 14(70%) and 9(45%), *Proteus* spp. had an occurrence of 6(30%) and 2(10%) for car door handles and car steering wheels while there was no detection of *Salmonella* in all the samples assessed. The fungi occurrence rate observed was *Aspergillus* spp. [7(35%)] for car door handle and 3(15%) for car steering wheel while *Rhizopus* spp. had a prevalence rate of 4(20%) for car door handle and 1(5%) for car steering wheel. This study affirmed that car surfaces could serve as a reservoir of potential pathogens. Hence, routine disinfection of these surfaces is very important.

Keywords: Microbiological assessment, Car doors, Steering wheels, Nigeria

1. Introduction

Human microbiome is influenced by several factors including environmental exposure to microorganisms encountered via physical contacts (Stephenson, Gutierrez, Peters, Nichols, & Boles, 2014). Several microorganisms can survive on inanimate objects long enough to be transmitted within human population having contact with the contaminated surfaces (Osei, Nyarko, & Atter, 2021). Microbiological investigation of frequently touched surfaces has become an interest to researchers because contaminated surfaces could serve as reservoir of potential pathogens (Maori, Agbor, & Ahmed, 2013; Nworie, Ayeni, Eze, & Azi, 2012). Public door handles are often hotspots for microbial contamination especially because of the frequent and inevitable use of most door handles by different individual (Ikede Rex et al., 2022).

The major source of the spread of community acquired infections are fomites; such fomites include door handles of convenience, showers toilet seats and faucets, sinks lockers, chairs, and tables especially those found in public places such as markets, banks, dormitories, schools, churches, public offices, hospitals, hotels, restaurants and rest rooms (Bright, Boone, & Gerba, 2010). The handle of car doors and steering wheel are one of the most important reservoirs of potential pathogens that human have direct contact with on daily basis (Al-Harmoosh, Eidan, Al-Hadrawy, Mohammed, & Hamed, 2018). Previous investigations have reported that

commonly used surfaces such as door handles, car steering, desk and other items can be a major source of potentially pathogenic microbial contaminants (Nwankwo, Okey-kalu, & Eze, 2022, Stephenson et al., 2014).

Several microorganisms have been associated with public surfaces including *Staphylococcus*, *Micrococcus,* Enterobacteriaceae and several fungi genera (Al-Ghamdi et al., 2011; Oluyemi, Oluyemi, & Omonike, 2018; Zenbaba et al., 2023). *Staphylococcus* species are notably a commensal microorganism that colonizes the human population but could simultaneously trigger opportunistic infections of soft tissues, skin, blood, septic arthritis, sepsis and pneumonia (Kozajda, Jeżak, & Kapsa, 2019; Nadimpalli et al., 2018). Similarly, *Proteus* spp. of the Enterobacteriaceae family is also usually classified a natural microflora of the gut and skin (Drzewiecka, 2016) but has been implicated as potential pathogen associated with Crohn's disease (Hamilton, Kamm, Ng, & Morrison, 2018). Fungal-linked infections are relatively not frequent in immunocompetent and healthy individuals like the bacterial infections, although some ubiquitous fungal can still cause lethal diseases in immunocompromised individuals (Gnat, Łagowski, Nowakiewicz, & Dylag, 2021). Some common invasive infection-inducible fungal include the *Aspergillus* species, *Candida* species, *Pneumocystis* species and *Cryptococcus* species (Fang et al., 2023). Exposure of food materials to unclean hand surfaces colonized with these groups of microorganisms could influence food contamination (Nworie et al., 2012). Foodborne diseases are a widespread and growing public health concern globally because of the health hazards they constitute. Benue State University, Makurdi is an institution situated in the capital city of Benue state and also abode a large population of individuals being the only state-owned university in Benue state, Nigeria. Hence, the study aimed at assessing the microorganisms associated with car doors and steering wheel of cars at Benue State University, Makurdi.

2. Materials and Methods

2.1 Study area

This is a cross-sectional study carried out in the faculty of science, Benue State University, Makurdi. The investigation was conducted across four departments which include biology, chemistry, physics and maths/computer department. Benue state university is a state-owned university situated in Makurdi, Benue state, Nigeria.

2.2 Bacterial and fungal isolation

A total of twenty car door handles and twenty steering wheels swab samples were collected randomly across departments at the early hours of the day and investigated in this study using sterile swab sticks that were pre-moistened using sterile normal saline water based on ISO/DIS 14698-1 recommendation (International Organization for Standardization [ISO], 2003), then transported to Charis Rhema Research and Diagnostic Laboratory immediately on ice-packs for microbiological analysis in accordance with the protocols of Cheesbrough (2006). The samples were collected by swabbing the surfaces of car door handles and steering wheels with properly labeled sterile swab sticks aseptically. The swab samples were soaked in test tubes containing 9mL of normal saline and serially diluted to get diluent 10^1, 10^2, 10^3, 10^4 and 10^5 respectively. Using serial dilution techniques, 100 µL of diluents was inoculated into Nutrient Agar (NA) (Lab M, Lancashire, United Kingdom) plates in triplicates for heterotrophic bacteria enumeration, Eosin methylene blue agar (EMBA) (HiMedia) for enumeration of *Proteus* spp., Mannitol salt agar (MSA) (HiMedia) for enumeration of *Staphylococcus* spp. and Xylose lysine deoxycholate agar (XLDA) (HiMedia) for enumeration of *Salmonella* spp. then incubated at 37^{o}C for 24 hours. The detection of fungal spores was investigated using pour plate method on Potato dextrose agar (HiMedia) and incubated at 25±2^{o}C for 5-7 days. After incubation, the agar culture plates were observed and recorded. Based on phenotypic characterization, distinct colorless and transparent colonies on EMBA are to be considered as presumptive *Proteus* spp. isolates, distinct yellow colonies on MSA are to be considered as presumptive *Staphylococcus* spp. isolates, distinct black colonies on XLDA are to be considered as presumptive *Salmonella* spp. The presumptive isolates were purified on nutrient agar and subsequently stored on nutrient agar slants at 4^{o}C for further analysis.

2.3 Morphological and biochemical characterization of bacterial and fungal isolates

The characteristic growth of the isolates obtained from EMBA, MSA and XLDA medium were further characterized by Gram staining, motility test, coagulase test, indole test, catalase test, oxidase test, hydrogen sulphide test, urease test and sugar fermentation test. The cultural characteristics of fungi isolates indicated the presence of *Rhizopus* spp. with white cottony mycelia with black dots. The presence of *Aspergillus* spp. was characterized by white to brown/black colonial characteristics and conidiospores arising from a foot cell with conidia produced in chains. Microscopic identification of fungal isolates was carried out using wet mount preparation which was examined using x40 objective lens. There is the production of sporangiospores in a spherical sporangium.

2.4 Data analysis

All data in this study were analysed using the statistical package (SPSS) version 21.0 and Microsoft Excel 2013. Descriptive statistics were used to analyze the enumeration of the respective microorganisms.

3. Results

The result of this study shows that the total microbial load ranges between 1.97 x 10^4 - 2.41 x 10^4 CFU/cm^2 for bacteria and 1.9 x 10^3 - 3.7x10^3 CFU/cm^2 for fungi. This study also reveals that the total fungi count from the car steering and car door handle of cars in the department of biology, physics, chemistry and mathematics/computer were higher with 3.7 x 10^3, 2.9 x 10^3, 3.4 x 10^3 and 1.9 x 10^3 CFU/cm^2 respectively as compared to the total bacterial count with 2.1 x 10^4, 1.9 x 10^4, 2.4 x 10^4 and 2.2 x 10^4 CFU/cm^2 for biology, physics, chemistry and mathematics/computer department.

Table 1. Total bacteria and fungi count.

Location	Number of Samples	TBC (CFU/cm^2 x 10^4)	TFC (CFU/cm^2 x 10^3)
Biology Dept.	10	2.11±1.02	3.7±2.05
Physics Dept.	10	1.97±0.14	2.9±1.31
Maths/Computer Dept.	10	2.23±1.21	1.9±0.87
Chemistry Dept.	10	2.41±0.90	3.4±0.11

Key: TBC = Total Heterotrophic Bacterial Count, TFC = Total Fungi Count, Dept. = Department.

Staphylococcus spp. had the highest occurrence of 14(70%) for the car door handle and 9(45%) for car steering wheel as compared to *Proteus* spp. with 6(30%) for car door handle and 2(18.2%) for car steering wheel. Car door handle had a higher contamination rate of 20% while car steering wheel had a least contamination rate of 11%.

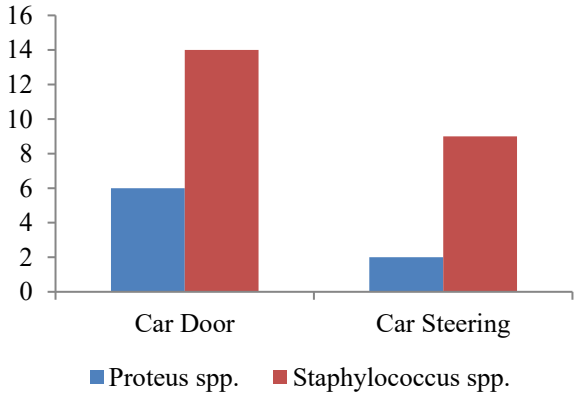

Figure 1. Occurrence of bacterial isolate of car door handle and car steering wheel.

The prevalence of fungi isolate was highest for *Aspergillus* spp. with 7(35%) for car door handle and 3(15%) for car steering wheel while *Rhizopus* spp. had a prevalence rate of 4(20%) for car door handle and 1(5%) for car steering wheel. The highest prevalence rate of fungi was recorded in physics department 6(40%). Chemistry and

Mathematics and Computer had a prevalence rate of 4(26.7%) respectively while Biology department had the least prevalence of 1(6.6%).

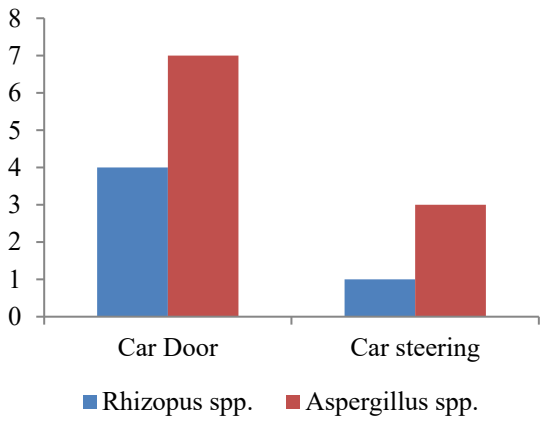

Figure 2. Occurrence of fungal isolate of car door handle and car steering wheel.

4. Discussion

Public surfaces such as door handles are very prone to microbial contamination and these surfaces are potential reservoirs of pathogens which subsequently encourage cross contamination and infection spread (Bright et al., 2010). In this study, the total microbial load range observed range from 1.97 x 10^4 - 2.41 x 10^4 CFU/cm^2 for heterotrophic bacteria and 1.9 x 10^3 - 3.7x10^3 CFU/cm^2 for fungi. This is lower in comparison to total heterotrophic bacterial counts range of 1.04±0.06 – 7.04±0.05 CFU/cm^2 observed on assessment of toilet door handles of schools in Nigeria. This study also reveals that the total fungi count from the car steering and car door handle in the department of biology, physics, chemistry, mathematics/computer were higher with 3.7 x 10^3, 2.9 x 10^3, 3.4 x 10^3 and 1.9 x 10^3 CFU/cm^2 respectively as compared to the total bacterial count with 2.1 x 10^4, 1.9 X 10^4, 2.4 x 10^4 and 2.2 x 10^4 CFU/cm^2 for biology, physics, chemistry and mathematics/computer department. The result of this study revealed a higher heterotrophic bacterial count across all the departments as compared to the total fungi count. In this study, the detection of *Staphylococcus* spp. and *Proteus* spp. was observed while *Salmonella* spp. was absent. Several studies have also reported the presence of *Staphylococcus* in bacteriological investigation door handles (Bashir, Muhammed, Sani, & Kawo, 2016; Ikede Rex et al., 2022). The presence of *Staphylococcus* in door handles and surface exposed to frequent human touch could be attributed to its ubiquitous presence on human skin. Al-Harmoosh et al. (2018) have also reported the detection of *Proteus* in comparable study. In contrary to this study, Al-Harmoosh et al. (2018) and Dawodu and Akanbi (2021) reported the detection of *Salmonella* in car door handles and automated teller machines respectively which was absent in this study. The disparity in rate of occurrence could be attributed differences in bacteria adaptive and physiological properties as these factors influences microbial tolerance to environmental factors. However, the ability of pathogenic strains of *Staphylococcus* species to survive unfavourable conditions for a long period of time and could still be contagious to exposed humans have been previously reported (Hübner, Hübner, Kramer, & Assadian, 2011). Furthermore, *Staphylococcus* species have also been linked to several virulence determinants (Conlon, 2014; Igbinosa, Beshiru, Akporehe, & Ogofure, 2016; Oheagbulem, Oche, Akuakolam, & Akinnibosun, 2023). Although, exposure of human to microbial contaminants such as *Staphylococcus* species via mouth or broken skin tissues could induce infections, they are usually minimal in young and healthy human population as they are capable of eliminating the pathogens within few hours or days prior to exposure (Angen et al., 2017). Some staphylococcal strains are capable of producing specific immunomodulatory toxins which may induce toxic shock syndrome (Sergelidis & Angelidis, 2017) and food poisoning (Denayer, Delbrassinne, Nia, & Botteldoorn, 2017). Similarly, several food poisoning outbreaks have been linked to *Proteus* species resulting from unhygienic food handling practices (Drzewiecka, 2016; Ma et al., 2022). *Proteus* species are associated with diverse virulence factors potentially relevant to gastrointestinal pathogenicity including the production of hemolysins, urease and IgA proteases (Hamilton et al., 2018).

The detection of *Aspergillus* spp. and *Rhizopus* spp. was observed in this study. Similar microbiological investigation has equally reported the presence of fungal spores in air microflora (Oluyemi et al., 2018). Other microbiological investigations have also reported the ubiquitous presence of fungi in other environmental samples (Akinnibosun, Beshiru, & Igbinosa, 2021; Ohagim, Ikon, Matthew, & Ohagim 2017; Onwubiko & Chinyeaka, 2015). The ubiquitous presence of these spores in air microflora could be implicated as the source of contamination in car door handles and other open surfaces. Clinical investigations have revealed that prolonged exposure to fungal spores elevates the risk diseased conditions such pneumonitis, hypersensitivity, allergic alveolitis and allergic fungal sinusitis (WHO, 2009), which could subsequently cause deterioration of kidney or/and liver especially when ingested via food (Ji et al., 2019). *Aspergillus* spp. is one of the most frequently found fungal genera in the environment and several species of this genus are producer of mycotoxin which triggers mycotoxicosis in human (Nielsen, 2003). Several other fungal genera including *Rhizopus* spp., *Mucor* spp. and *Lichtheimia* spp. have been implicated in disease conditions of individuals with metabolic disorders and immunocompromised patients (Gnat et al., 2021). Contamination of food materials by mycotoxins could promote the manifestation of chronic or acute health related problems including carcinogenic, estrogenic and immunosuppressive related health challenges as influenced by factors such as time of exposure, exposure dosage, individual health conditions, age and sex (Afsah-Hejri, Jinap, Hajeb, Radu, & Shakibazadeh, 2013; Awuchi et al., 2022).

The findings of this research revealed variation in microbial load from various car surfaces and this result is consistent with the reports of Nworie et al. (2012) and Al-Harmoosh et al. (2018). This report also shows that the car door handle had more contamination rate of 11 (55%) than the car steering wheel 4 (20%) and this contrasts with the result of Osei et al. (2021) who reported a higher contamination rate of the car steering wheel. In agreement with this study, in has been previously observed that concentration of fungal spores is usually higher in outdoor surfaces, especially in relatively cool and humid conditions (Pavan & Manjunath, 2014). Previous studies have affirmed the influence of meteorological and climate factors such as temperature and relative humidity on the proliferation and dispersion of fungal spores in the environment (Zingales, Taroncher, Martino, Ruiz, & Caloni, 2022). These reports demonstrated affirmed the reservoir capacity of car door handles and steering wheels and their contributing influence to contamination of hand surfaces. Previous studies have affirmed that human hands are major route that influence the transmission and spread of disease causing microorganisms and the surge of foodborne illnesses (Ahmednur, Esmael, & Feresa, 2022; Oranusi, Akande, & Dahunsi, 2013). Inadequate hand washing practices during food handling have been reported as a prominent cause of foodborne illnesses (Odetokun et al., 2022). Although, there have been significant rise in behavioral changes and awareness towards proper hand washing and other hygienic practices post-COVID-19 pandemic (Bizuneh, Mohammed, & Yesuf, 2022), more public health campaign including improved hand hygiene practices should continually be a routine intervention in both local and urban communities. Proper and routine hand washing practices after exposure to surfaces is therefore important in the mitigation of respiratory infections and diarrheal diseases (Teumta et al., 2019). Nevertheless, further studies that could ascertain the pathogenicity and investigation regarding specific virulence factors that might be associated with these microorganisms will give better insight on their health implication.

5. Conclusions

This study emphasized on the potentials of doors handles and other surfaces to serve as reservoir of environmental and human microflora of potentially opportunistic pathogens including *Staphylococcus* species. Thereby, making it necessary for users to get enlightened on the possible risk associated the activities of these microbial contaminants in disease transmission. Strict compliance to proper hand washing practices with clean water and detergents coupled with adherence to other standard hygienic measures could sustainably curtails the chances of cross-contamination during food handling which could subsequently triggers food borne diseases. Enhanced and more stringent biosecurity measures should be implemented in food handling to control potentially pathogenic and opportunistic pathogens. Therefore adherence to routine disinfection and cleaning of door handles, steering wheel, regular washing of hands and the use of disinfectants in controlling microbial contaminants in frequently touched surfaces is important as it will reduce the disease spread and promote public health.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

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