MICROBIAL ANALYSIS AND ANTIBIOGRAM OF CAR INTERIORS OF HEALTH WORKERS IN AKWA IBOM STATE POLYTECHNIC MEDICAL CENTRE

www.akwapolyjournal.org
P-ISSN: 2536-6733 | E-ISSN: 2616-0773

Supported by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/DESS / POLY / IKOT-OSURUA/ ARJ /1-2)

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ABSTRACT

This study explores the microbial ecosystem within car interiors, specifically targeting vehicles owned by healthcare workers at the Akwa Ibom State Polytechnic Medical Center. The investigation employed standard microbiological techniques to assess microbial presence and diversity by collecting 20 swabs from various surfaces, such as the steering wheel, dashboard, gear handle, and driver's seats across five vehicles. Notably, the analysis revealed significant variations in microbial load across different surfaces, with the driver's seat and gear handle in particular vehicles exhibiting the highest bacterial colony counts. The results revealed varying levels of microbial contamination in different areas. For instance, the driver's seat in sample A exhibited the highest total colony count at 120 Cfu/m2, followed by the steering wheel with 41 Cfu/m2.

Similar findings were observed in the other samples, highlighting the presence of potentially harmful microorganisms. The study observed that fungal species identified varied in prevalence, with *Aspergillus* sp.

The most common are *Fusarium* sp., *Candida* sp., and *Penicillium* sp., in descending order of frequency. Furthermore, the study assessed the antimicrobial susceptibility of the bacterial isolates, uncovering a spectrum of sensitivity and resistance to various antibiotics. The findings underscore the car interior as a potential reservoir for transmitting pathogenic microorganisms to occupants, posing public health implications, particularly for healthcare workers who may inadvertently contribute to disseminating these organisms. This research highlights the need for regular microbial surveillance and the implementation of effective sanitation practices within personal and professional vehicles to mitigate health risks.

Keywords: *Microorganisms, antibiogram, bacterial isolates, fungal isolates, car interior.*

INTRODUCTION

Cars are the most widely used mode of transportation for most people. People spend much time traveling in cars. It is become a vital transportation system that is widely used. These vehicles come in various designs and comfort levels, ranging from air-conditioned (A/C) cars to non-air-conditioned (non-A/C) cars with lower comfort standards.

In particular, air-conditioned cars (A/C) are known for their sealed environments, minimizing external air exchange when the doors are closed (Rose *et al*., 2000).

Consequently, passengers in air-conditioned cars are exposed to a specific microenvironment throughout their journeys, which may contain various microorganisms with associated health risks.

A typical passenger compartment in a car commonly comprises upholding, steering wheel, dashboard, ceiling, floor, doors, and various fittings. Pollutants in the car are significantly associated with emissions from interior materials used to equip the compartment, including leather, plastics, fabrics, carpets, sealants, adhesives, paints, foam cushions, etc. (Zhang *et al*., 2008). People need to keep their

cars cleaner than their homes. Eating food, drinking, pouring water, not washing or cleaning hands, and non-sweeping the carpets are all factors that result in an unhygienic environment within cars (Kosek *et al.,* 2003).

A dirty car due to spillages of food items may become the breeding ground for microorganisms, resulting in health problems. Microorganisms, without difficulty, obtain a place to remain viable in various inanimate objects like automobile cars. The microbes in the soil and dust may enter the vehicle through animals or passengers' footwear (Kosek *et al.*, 2003).

Maintaining good car hygiene is crucial, especially for people traveling with small children. The dashboard, the steering wheel, and the gear are the most commonly touched areas within the cars, and they must be kept clean. Very few studies have been conducted on the pathogens within the cars. It has yet to be given due importance.

A typical steering wheel had an average of 700 bacteria compared to the 60 types on a public toilet seat. A significant source of bacteria comes from food spills in various locations (Saxena *et al.*, 2013).

Microbes can also enter the vehicle through the air and heating vents. The footwear of passengers also plays an essential role in contamination (Saxene *et al.*, 2013). The areas touched with the hands harbor the most germs. The top spots for germs are dashboards, gear handles, cup holders, and children's car seats (Saxena *et al.*, 2013). While most bacteria are unlikely to cause health problems, some cars harbor potentially harmful bacteria species.

Staphylococcus and *Propionibacterium* are the predominant organisms.

Staphylococcus sp. are ubiquitous in the environment, and strains in the nose often contaminate hands and fingers, leading to skin carriers; hence, the car surface could serve as a reservoir for pathogenic staphylococci. They are essential in human colonization and infection (Gosa *et al.*, 2014; Kumar *et al.*, 2009).

Another standard organism isolated from cars is *Bacillus cereus*, a vital source of food poisoning. Harmful bacteria like *Escherichia coli*, *Salmonella, and Campylobacter*,

all of which can cause illness, are also isolated. Enteric pathogens are the most frequent cause of diarrhea and account for an annual mortality rate of about five million people worldwide (Kosek *et al.,* 2003). Each can survive for as long as one month inside a car.

Airborne microbe pollution is always an occupational and public health concern associated with lung impairment, respiratory allergies, infections, and other health problems. Microbial exposure inside a vehicle has attracted significant attention in recent years (Jo & Lee, 2008; Wang *et al.*, 2010; Li *et al.*, 2013).

For example, *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria were* found to be the dominant fungal genera in vehicles, and the maximum bacterial aerosol concentration was 2530 Cfu/m3 (Jo & Lee, 2008).

Moreover, the average concentrations of bacteria and fungi in commuting trains were 417 and 413 Cfu/m3, respectively, and the combined maximum level of bacterial and fungal aerosols was 1000 Cfu/m3 in public buses and passenger cars (Jo & Lee, 2008). In addition, the in-vehicle bacterial concentrations were significantly higher in summer in public buses than in passenger cars, and the in-vehicle fungal concentrations were generally higher in summer than in winter (Wang *et al.*, 2010).

As for microbial pollution control, the use of cleaning air conditioners in vehicle cabins was shown to have the capacity to reduce the total number of microorganisms, such as bacterial and fungal spores, by over 80 % (Jo & Lee, 2008).

However, (Li *et al.*, 2013) proved that automobile air conditioning filters are often heavily contaminated with microbial agents, including human opportunistic pathogens and high endotoxin levels. The air conditioner-filtered bacteria and fungi from the air stream could proliferate under high humidity conditions such as rain or snowing (Li *et al.*, 2013).

Other factors that could lead to the spread of microorganisms in the car interior are temperature, warmth and moisture, nutrients, environmental pH, etc. Therefore, the study was aimed at the microbial analysis and antibiogram of car interiors of health care

workers in Akwa Ibom State Polytechnic Medical Centre.

MATERIALS AND METHODS

Methods

Sample collection

A total number of 5 cars were sampled in this study from health workers at Akwa Ibom State Polytechnic Medical Center. Four(4) swabs were collected from each vehicle, and 20 swabs were obtained. The researchers labeled the swab sticks accordingly and dipped them in peptone water before using them to swab the car's interior parts. Swabs were collected from the driver's seat, handle, steering wheel, and dashboard. The researchers sealed the swab sticks carefully and took them to the laboratory for microbial analysis.

PREPARATION OF CULTURE MEDIA

According to the manufacturer's instructions, the researchers prepared the culture media (nutrient agar and Sabouraud Dextrose Agar) and autoclaved it at 121 ^{0}C for 15 minutes. After autoclaving, 15 ml of molten nutrient agar and Sabouraud Dextrose Agar were aseptically poured into disposable sterile plates and allowed to set.

MICROBIAL ANALYSIS

Cultivation of microorganisms

The prepared agar plates were carefully labeled using masking tape according to the label on the swabs. The swabs were then inoculated onto the appropriate plates containing nutrient agar. The plates were incubated invertedly at 37 ^{0}C for 24 hours for bacterial growth, while the Sabouraud Dextrose Agar (SDA) plates were incubated invertedly at room temperature for 3-5 days for fungal growth according to Cheesbrough (2006).

ENUMERATION OF BACTERIAL ISOLATES

Bacterial isolates were characterized based on their morphological and cultural characteristics. The researchers carried out a Visual inspection of the appearance of colonies to identify their characteristics. The researchers observed the surface, appearance, shape form, elevation, margin edges, and optical appearance according to the methods described by Cheesbrough (2006).

The isolates were subcultured, and the following biochemical tests were carried out

for further characterization: gram staining, spore staining, catalase test, coagulase test, motility test,

urease test, oxidase test, citrate test, sugar fermentation test according to the methods described by Cheesbrough (2006).

Presumptive identification was done by comparing each isolate's colonial and biochemical features to that of a standard identification manual. The microorganism population was obtained in Cfu/cm2 by counting the number of colonies of microbes seen in the Petri dishes.

ANTI-MICROBIAL SUSCEPTIBILITY TESTING

The disk diffusion technique determined the antibiotic susceptibility profile. The test was carried out to determine the antibiotic susceptibility profile of bacteria isolated from different car swabs. The researchers used Kirby Baver's disc diffusion method on Muller Hinton agar plates to prepare Muller Hinton agar according to the manufacturer's instructions.

After sterilization, the agar was allowed to cool to 45 ^{0}C and then poured into labeled sterile petri dishes and allowed to solidify.
The bacterial isolates were aseptically inoculated onto the agar plates, respectively. Different antibiotics were placed gently on the inoculated plates using sterile forceps and covered immediately.

All plates were incubated invertedly at 37 ^{0}C for 24 hours. The gram-positive antibiotics used include Streptomycin (30 mcg), Gentamycin (10 mg), Ciprofloxacin (10 mcg), Ofloxacin (10 mcg), Erythromycin (30 mcg), Levofloxacin (20 mcg), Rifampicin (20 mcg). The gram-adverse antibiotics include Augmentin (20 mcg), Gentamycin (10 mcg), Peflacine (10 mcg), Ceporex (10 mcg), and Streptomycin (30 mcg).

The study noted Zones of inhibition around each antibiotic dice, and the zones' diameters were measured with the meter rule in mm for the interpretation of the zone of inhibition as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2012).

Characterization and Identification of Fungal Isolates

The fungal growths that appeared were identified using cultural and morphological features. The fungal isolates were identified by staining with lacto-phenol cotton blue. The researchers placed two drops of lacto phenol cotton blue reagent on

a clean grease complimentary glass slide. Then, a small tuff of the fungus was obtained using an inoculating needle transferred to the glass slide. A cover slip was placed over the preparation and examined under x 40.

RESULTS AND DISCUSSION

Results

Total Heterotrophic Bacterial Colony Counts

The total bacterial colony counts of all the samples are shown in Table 4.1. In sample A, the driver's seat recorded the highest total colony count of 120 Cfu/m2, followed by the steering wheel, which recorded 41 Cfu/m2. Sample B had the highest total colony count of 159 Cfu/m2 and 139 Cfu/m2 total colony count in the gear handle.

In sample C, the highest total colony count of 104 Cfu/m2 was recorded on the dashboard, while 14 Cfu/m2 was recorded on the steering wheel. In comparison, sample D had 60 Cfu/each in both the dashboard and the driver's seat, respectively, while sample E had the highest colony count of 155 Cfu/m2 in the dashboard and 35 Cfu/m2 in the gear handle.

CULTURAL, MICROSCOPIC, AND BIOCHEMICAL CHARACTERIZATION OF THE BACTERIAL ISOLATES

Table 4.3 shows the morphological, cultural, and biochemical identification of the bacterial isolates, which revealed bacterial genera of the species *Staphylococcus sp*., *Propionibacterium* sp., *Escherichia* sp., *Pseudomonas* sp., *Corynebacterium sp*., and *Bacillus* sp.

Fig.1.1. Percentage frequency of occurrence of bacterial isolates

Figure 1 shows the percentage/ frequency occurrence of bacterial isolates obtained from car interior samples. The highest percentage frequency of occurrence was recorded for *Staphylococcus sp*.(18.5%), *Bacillus* sp (17.2%), *Escherichia* sp (16.9 %), *Propionibacterium* sp (16.7%), *Pseudomonas* sp (16.6%) while *Corynebacterium* recorded the minor percentage of occurrence of (14.1%).

TOTAL HETEROTROPHIC FUNGAL COLONY COUNTS

Table 4.2 shows the samples' total heterotrophic fungal colony counts per surface area (cfu/cm2). The highest fungal count was recorded in sample A, which had 4 Cfu/m2 in the dashboard and 3 Cfu/m2 in the car seat and steering wheel, respectively. The sample B dashboard recorded a total fungal

count of 2 Cfu/m2 and 1 Cfu/m2 in the car seat. A total fungal count of 6 Cfu/m2 was recorded in the dashboard, and 5 Cfu/m2 in a car seat in sample c. Sample D had a total fungal count of 3 Cfu/m2 and 1 Cfu/m2 in the steering wheel, while sample E recorded no count

Cultural and microscopic characteristics of the fungal isolates

Table 4.4 shows the cultural and microscopic characteristics of the fungal isolates, which revealed fungal genera of the species *Candida* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium sp*.

Fig.2.1.Percentage frequency of occurrence of Fungal isolates

Figure 2 shows fungal isolates' frequency/percentage distribution obtained from car interior samples.

The highest percentage of occurrence was recorded for Aspergillus sp (37.1 %), *Fusarium* sp (31.4 %), *and Candida* sp (20 %), while *Penicillium* sp recorded the lowest percentage of (11.4 %).

ANTIBIOGRAM OF THE BACTERIAL ISOLATES

Table 4.5 shows antibiogram patterns of Gram-positive and Gram-negative bacteria isolates in car interior samples. From the result, *Staphylococcus* sp was sensitive to Ceftazidime (23 mm), Streptomycin (16mm), and Levofloxacin (18mm) and resistant to Erythromycin (11mm).

Propionibacterium sp was sensitive to Ciprofloxacin (29mm), Ceftazidime (29mm), Streptomycin (28mm), Erythromycin (27mm), Azithromycin (27mm), and Levofloxacin (22mm). *Escherichia* sp was sensitive to Peflacine (34mm), Ciprofloxacin (24mm) and Ofloxacin (19mm).

Pseudomonas sp showed no zone of inhibition in all the antibiotics. *Corynebacterium* sp was sensitive to Gentamycin (28mm), Ceftazidime (27mm), Erythromycin (25mm), Streptomycin (23mm), Ciprofloxacin (22mm), Levofloxacin (22mm), Amoxil (20mm), Azithromycin (20mm) and was resistant to Rifampicin (13mm). *Bacillus* sp was sensitive to Erythromycin (29mm), Ciprofloxacin (28mm), Levofloxacin (28mm), Ceftazidime (25 mm), Azithromycin (24 mm), Gentamycin (22mm), Streptomycin (22mm), Amoxil (18mm), and was resistant to Rifampicin (14mm) and Cefuroxime (14 mm).

Table 4.1: Heterotrophic Bacterial Colony Counts

z	**Interior parts of the cars sampled**	**Total Colony Count (Cfu/cm^2).**
A	Driver's seat	120 colonies
	Dash board	12
	Gear handle	09
	Steering wheel	41
B	Driver's seat	44
	Dash board	159
	Gear handle	139
	Steering wheel	67
C	Driver's seat	10
	Dash board	104
	Gear handle	09
	Steering wheel	14
D	Driver's seat	60
	Dash board	60
	Gear handle	31
	Steering wheel	09
E	Driver's seat	20
	Dash board	155
	Gear handle	35
	Steering wheel	12

Table 4.2. Characterization and identification of bacterial isolates

S/N	**Biochemical Tests**	**Test Organisms**					
		***Bacillus* sp**	***E.coli.* sp**	***Staphylococcus* sp**	***Propionibacterium* sp**	***Pseudomonas* sp**	***Corynebacterium* sp**
1	Gram reaction	+	+	+	+	-	+
2	Citrate	+	+	+		+	-
3	Oxidase	-	+	-	+	+	-

4	Catalase	+	+	+	+	+	+
5	VP	+	+	+	+	-	+
6	Urease	-	+	+	+	-	-
7	Indole	-	-	-	+	-	-
8	Spore	+	-	-	-	-	-
9	motility	+	-	-	-	-	-
10	MR	+	+	+	+	-	+

Key: + = positive , - = negative .

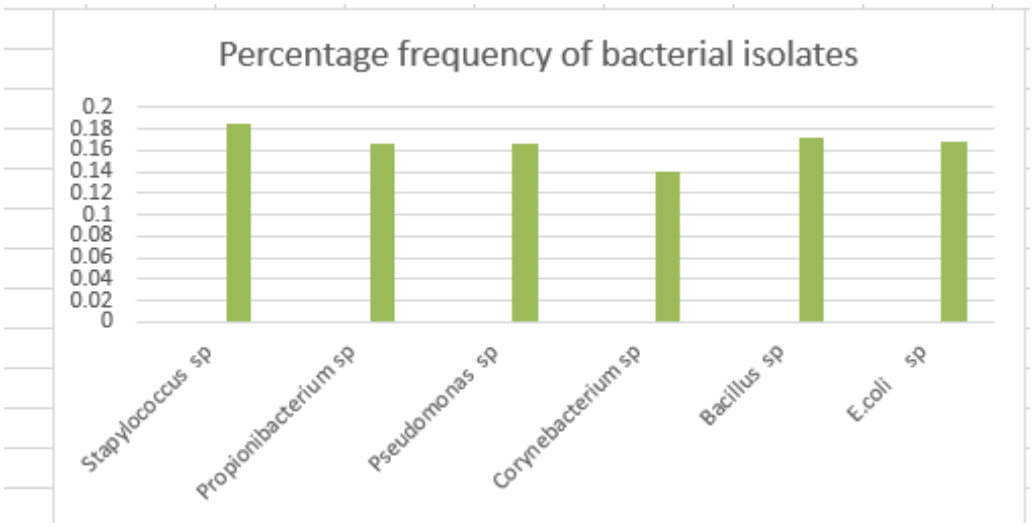

Fig. 1. Percentage frequency of bacterial isolates

Table 4.3: Heterotrophic fungal colony counts

No. of Cars sampled	Interior parts of the cars sampled	Total Colony Count (Cfu/cm^2).
A	Driver's seat	03
	Dash board	04
	Gear handle	01
	Steering wheel	03
B	Driver's seat	01
	Dash board	02
	Gear handle	-
	Steering wheel	-

C	Driver's seat	05
	Dash board	06
	Gear handle	03
	Steering wheel	02
D	Driver's seat	01
	Dash board	03
	Gear handle	-
	Steering wheel	01
E	Driver's seat	00
	Dash board	-
	Gear handle	-
	Steering wheel	-

Fig.2. Percentage frequency of fungal isolates

Table 4.4: Cultural and Microscopically Characterization of the fungal isolates

Colony Appearance	**Surface colour**	**Reverse side colour**	**Microscopy**	**Most probable Organisms**
Moist	Creamy	Creamy	Small, oval ,unicellular, pseudo- hyphae	*Candida* sp
Powdery	Brown	Dark-brown	Non-septate hyphae with stalk-like conidiophores expanding at the top to a swollen vesicle in a clome-shaped structure being round conidia in chains	*Aspergillus* sp
Cottony	Red	White	septate hyphae with canoe-shaped, multi-septate macroconidia on branched conidiophores	*Fusarium* sp
Velvety	Bluish green with white boarder	White	Septate hyphae with branched conidiophores having flask-shaped sterigmata bearing unbranched chains of round conidia	*Penicillium* sp

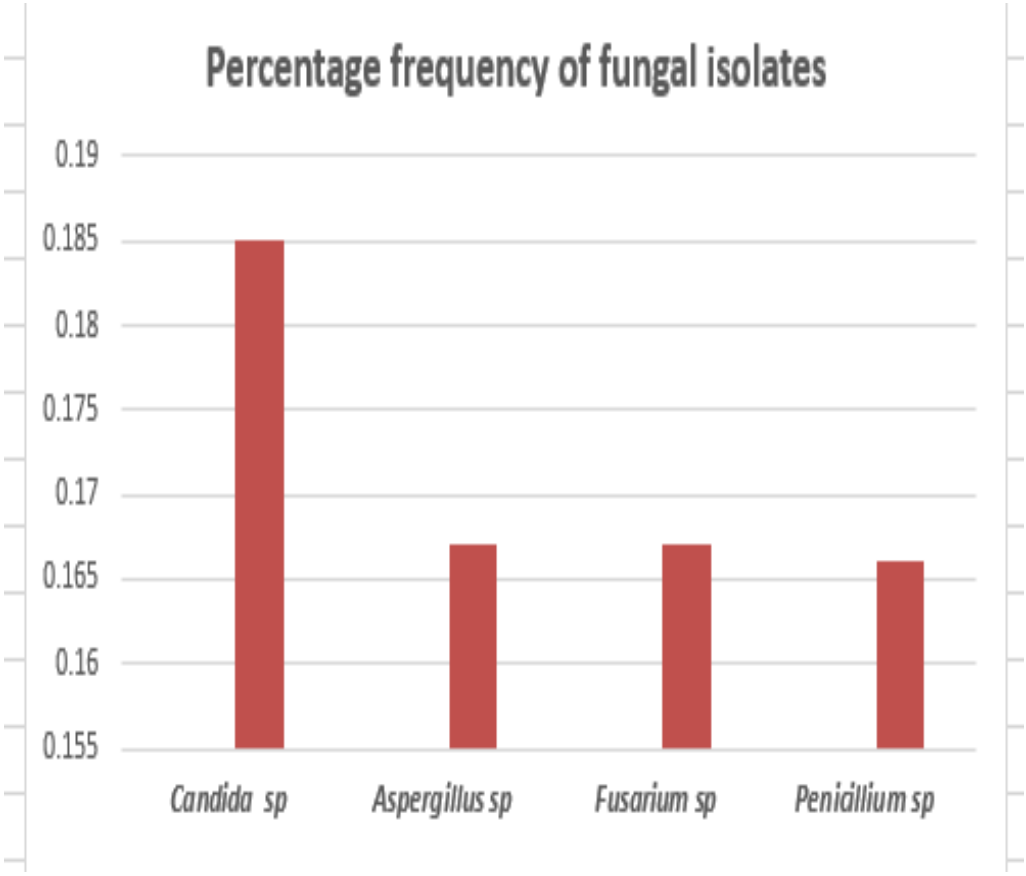

Table 4.5: Antibiogram of the bacterial isolates

Isolates	Drugs and their zones of inhibition in millimeters (mm)																	
	CN	S	AML	CPX	E	RD	CFT	CEF	AZM	LEV	CEP	OFX	AU	PEF	SXT	CH	APX	PN
Staphylococcus sp	25	18			11					18								
Propionibacterium sp	24	28		29	27		29		27	22								
Escherichia sp				24								19		34				
Pseudomonas sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
Corynebacterium sp	28	23	20	22	25	13	27		20	22					vi			
Bacillus sp	22	22	18	28	29	14	25	14	24	28								

Key: Code for interpretation of antibiotics of susceptibility test. 18 mm is Sensitive (S) 13 mm is Resistant (R) 12.0 mm - 17.9 mm is Intermediate (1)

Symbols	Antibiotics
CN	PEFGentamycin
S	Streptomycin
AML	Amoxil
CPX	Ciprofloxacin
E	Erythromycin
RD	Rifampicin
CFT	Ceftazidime
CEF	Cefuroxime
AZM	Azithromycin
LEV	Levofloxacin
OFX	Ofloxacin
PEF	Reflacin

4.2 DISCUSSION

Even though humans spend a significant amount of time inside automobiles, very little is known about the microbial ecology of car interiors and how this might impact the occupant's microbiome. This study enumerated and analyzed bacterial communities of frequently touched car interior surfaces. These results indicated that the most highly colonized locations were areas the researchers suspected to have frequent touching by the occupants, including locations on the steering wheel, gear handle, dashboard, and driver's seat. The study reported six species of bacteria and four species of fungi.

In sample A, the driver's seat recorded the highest total colony count of 120 Cfu/m2, followed by the steering wheel, which recorded 41 Cfu/m2. Sample B had the highest total colony count of 159 Cfu/m2 on the dashboard and 139 Cfu/m2 total colony count in the Gear handle. In sample C, the highest total colony count of 104 Cfu/m2 was recorded on the dashboard, while 14 Cfu/m2 was recorded on the steering wheel. Sample D had 60 Cfu/m2 each in both the dashboard and the driver's seat, respectively, while sample E had the highest colony count of 155 Cfu/m2 in the dashboard and 35 Cfu/m2 in the gear handle.

Microorganisms isolated were *Staphylococcus* sp, *Escherichia*, *Pseudomonas* sp, *Bacillus* sp, *Propionibacterium* sp, and *Corynebacterium* sp. *Bacillus* are spore formers and can resist environmental pressures and microenvironment. Of particular concern is *the Staphylococcus* species, which colonies the human skin and is likely to be transmitted to inanimate surfaces in a hospital environment and, hence, indirectly to the patients. They are capable of causing a variety of nosocomial infections.

The percentage frequency of occurrences of the isolated bacteria were *Staphylococcus* sp (18.5 %), *Bacillus* sp (17.2%), *Escherichia* sp (16.9 %), *Propionibacterium* sp (16.7%), *Pseudomonas* sp (16.6%) while *Corynebacterium* sp recorded the minor percentage of occurrence of (14.1%).

The highest fungal count was recorded in sample A, which had 4 Cfu/m2 in the dashboard and 3 Cfu/m2 in the car seat and steering wheel, respectively. The sample B

dashboard recorded a total fungal count of 2 Cfu/m2 and 1 Cfu/m2 in the car seat. A total fungal count of 6 Cfu/m2 was recorded on the dashboard and 5 Cfu/m2 in the car seat in sample c. Sample D had a total fungal count of 3 Cfu/m2 and 1 Cfu/m2 in the steering wheel, while sample E recorded no count.

The fungal isolates and their percentage frequency of occurrence were *Aspergillus* sp (37.1 %), *Fusarium* sp (31.4 %), *and Candida* sp (20 %), while *Penicillium* sp recorded the most negligible percentage of (11.4 %).

In this study, Aspergillus sp**.** was the most prevalent fungus at 37.1 % prevalence in the car interior of health workers in Akwapoly. *Aspergillus* sp are commonly associated with allergies and respiratory ailments when inhaled (Rose *et al*., 2000; Raper & Fennell, 1973). The antibiotic susceptibility pattern showed that some isolates were sensitive to a wide range of bacteria while some were resistant to the isolates. This information emphasizes the diversity and prevalence of potentially harmful microorganisms within cars. The concentration of microbes in the studied cars exceeded recommended limits for indoor environments, posing a potential health risk to commuters.

CONCLUSION AND RECOMMENDATION

CONCLUSION

This study concludes that cars' interiors are a potential contamination source with many commensally harmful microorganisms. These colonized microorganisms are a source of infection for drivers and those traveling in cars. It can also become a potential hazard to the person driving for a long time and needing to care more to clean the interior.

This suggests the potential of the steering wheels and palms as fomites, which can result in community-acquired diseases and infectious diseases since most of the microbial contaminants found were pathogenic. Measures to reduce microbial growth and exposure within cars are warranted to protect passengers from potential health. The study's findings suggest that the microenvironment within cars can harbor a diverse range of potentially harmful microorganisms, which can pose health risks to passengers, especially those with compromised immune systems.

RECOMMENDATIONS

The results of this study emphasize the significance of adhering to regular cleaning and disinfection protocols for car surfaces to uphold a hygienic environment. It is highly recommended that individuals, particularly those in the healthcare profession, observe rigorous hand hygiene practices by washing their hands before and after any patient interaction to minimize the risk of transmitting infections.

Moreover, it is advisable to abstain from consuming food in the car, as recent studies have indicated that this behavior can significantly compromise the cleanliness of the car environment, mirroring the unhygienic conditions commonly found in restrooms. Additionally, it is essential to keep car windows and vents closed to reduce the circulation of potentially harmful microorganisms.

Lastly, there is a critical need for further research to comprehensively understand the effects of different disinfectants on the microbial communities present in cars, specifically those used by healthcare professionals and individuals in commercial settings.

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