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Assessment of Potential for Cross Contamination by Shopping Carts and Baskets in Akure, South-West, Nigeria in Pandemic Period

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Abstract: At supermarkets and grocery stores (modern retail system), people are exposed to many surfaces, such as refrigerator, door handles and shopping carts. This study evaluates microbial contamination of shopping carts and baskets at different shopping malls in Akure, Nigeria. The swab samples of carts and baskets handles were collected using sterile swab sticks, bacterial and fungi were isolated and identified using standard microbiological methods. Antimicrobial susceptibility of all bacterial isolates was carried out using disc diffusion test. The bacteria counts ranges between 50 cfu/cm^2 to 242 cfu/cm^2 while the number of fungi range between 1.8 sfu/cm^2 to 2.0 sfu/cm^2. The identity of bacterial isolates were confirmed as *Staphylococcus aureus, Micrococcus* species, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* and while *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* are the fungi identified. The most occurred bacteria and fungi were *Staphylococcus aureus* 5(35.71%) and *Aspergillus niger* 5(41.67%) respectively. All bacterial isolates showed least susceptibility to amoxicillin and ceftriaxone and highest against erythromycin. The presence of high microbial load, pathogenic bacteria and fungi in shopping basket and carts indicated that they could act as source of transmitting pathogens from person to person and a vehicle for the dissemination of antibiotic resistant gene in the community. Hence there should be awareness towards the public health implications of poorly sanitized shopping baskets and carts.
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Key words: baskets, carts, microbial load, antibiotics

Introduction

Retail in Nigeria was once confined to traditional open markets and small local storekeepers loosely referred to as the informal retail sector of the Nigerian economy which serviced communities. The old or traditional retail system which is adjudged to account for almost 90 percent of retail activity in Nigeria has continued to decline because of government's policy, changes in the composition of Nigeria's population, rising income level and increasing sophistication of the Nigerian consumer. Today, more families shop together and organised facilities which meet their need are attracting more shoppers. Therefore, the old or traditional structure of retail continues to give way to the new (NRSR, 2014).

At supermarkets and grocery stores (modern retail system), people are exposed to many surfaces, such as refrigerator, door handles and shopping carts. With every touch, these surfaces become contaminated with more and more potentially harmful microorganisms. However, measures were barely put in place by theses supermarkets and grocery stores to minimize the spread of these microbes (Duberg, 2011). A shopping cart or trolley and basket are used by customers inside the shop for transport of merchandise to the checkout counter and parking lot during shopping (Patrick *et al.,* 2010). People believe that bacteria are only present in research laboratories, hospitals and clinics and thus they have a misleading feeling of security in other places. Lack of knowledge about where germs prowl could be the cause of health problems, in fact 80% of infections are spread through hand contact with hands or other objects (Duberg, 2011). Shopping carts and handheld shopping baskets in supermarkets are subject to accidental bacterial contamination through contacts with a variety of food and also placement of children in grocery shopping carts has recently been implicated as a source of infection (Barzani, 2016).

The reservoir of any organism, which may be animate or inanimate objects, in the epidemiology of any bacterial disease is very important (Al-Ghamdi *et al.,* 2011). The pathogens live and or multiply in the reservoir on which their survival depends. Many epidemiological studies have confirmed that contaminated surfaces played a major role in the spread of infectious diseases (Anwar *et al.,* 2007; Al-Ghamdi *et al.,* 2011). Most people do not know that bacteria are found on many common objects outdoors, in their offices, and even in their homes. Such objects include; playground equipment, kitchen sinks, computer keyboards, escalator

handrails, elevator buttons, the shopping baskets and carts handles. The latter objects are places that are most touched by the bare hands of people who are in various hygienic conditions.

According to Fullerton *et al.* (2007), shopping cart handles was identified as one of the most biologically contaminated public surfaces, it has also been implicated that riding in shopping carts as a risk factor for food-borne pathogen infection in infants. More importantly, previous studies revealed that shopping cart handles are among the leading sources of germs and bacteria in public facilities such as shopping malls, airports and bus stations (Reynolds *et al.,* 2005; Ghamdi *et al.,* 2011; Irshaid *et al.,* 2014). According to these two studies (Reynolds *et al.,* 2005; Ghamdi *et al.,* 2011), the total number of bacteria appeared to be higher on SC handles compared to that found in other public objects such as restaurant tabletops, escalators, chair arm rests, public restrooms and playground equipment, to name a few. In addition, it was also reported that some of these bacterial species can survive for up to several days on nonporous surfaces and are infectious at very low doses.

Also other study found that shopping carts and baskets are contaminate with Coagulase negative staphylococci, *Staphylococcus aureus*, *Pseudomonas* spp. and Gram negative bacilli (Mizumachi *et al.,* 2010). Mizumachi *et al.* (2010), also reported frequent exposure to pathogenic *Staphylococcus aureus* on shopping cart handles and suggested that this was a hidden reservoir of this organism that points to a need for shopping basket sanitation. Occurrence of coliform and *E. coli* bacteria on shopping carts was reported by Gerba and Maxwell (2012), they stated the implications that the consumer is exposed to enteric bacteria on a regular basis when using grocery shopping carts. Several factors have been identified to affect the transfer rate of bacteria from surface to another surface. These include bacteria type, source and destination surfaces, time post inoculation, and moisture level (Al-Harbi *et al.,* 2017).

More than ever, the Nigerian consumer is interested in a decent shopping environment, neatly-arranged and labeled commodities and the experience that goes with buying at an organised outlet (NRSR, 2014). However, the level of bacterial contamination on shopping baskets and carts is not a subject of public health concern because of the limited availability of information (Barzani, 2016). Despite the great expansion in trade, the spread of malls and supermarkets in recent years in Akure metropolis, much attention has not been paid to the possibility of the presence of bacterial contamination on the trolley and basket used for shopping by different customers. Therefore, the aim of this study is to assess the microorganisms associated with shopping basket and carts used in Akure supermarkets and to determine the antimicrobial property of pure honey and morringa leaf extract on the pathogenic bacteria isolated from the shopping carts and basket.

Materials and Methods

Study area

This study was conducted to assess the bacterial contamination on shopping carts and baskets in shopping malls in Akure metropolis. The laboratory investigation was carried out in the Research Laboratory of The Federal University of Technology, Akure. This study was carried out between February and May, 2016. A total of twenty five (25) basket and carts were tested for contamination in five (5) most popular and visited shopping malls in Akure metropolis, these stores were assigned A, B, C, D and E letters for anonymity.

Collection of swab samples from shopping carts and baskets

Shopping carts and baskets were selected randomly from each store for sampling from the customer cart return corral to capture the recent usage ones. The shopping carts and baskets were swabbed with a sterile cotton wool swabs moistened in sterile normal saline. A single sterile swab was wiped firmly over the entire a surface area of each shopping cart and baskets handle, from end-to-end firmly over a selected surface area (per square centimeter) of each shopping base. The surface area was recorded for each sampling area. The swab was then return back into the corresponding labeled bag. All swab samples were immediately transported to laboratory within 60 minutes of collection at ambient temperature and processed for cultivation of bacteria.

Bacterial and fungal growth counting

Samples were cultured within one hour of collection by spreading the swab bacterial load on nutrient and potato dextrose agar plate surfaces. All plates were incubated at 37 °C for 48 hours for bacteria and 27 for 72 hours for fungi. The resulting colony/spore forming units (CFUs/SFUs) were counted for each plate and the number of colonies/spores in each plate was then converted to CFU/SFU per cm^2.

Isolation and identification of isolates

The grown bacterial colonies were morphologically compared based on size, color, margins, and elevation. Morphologically different colonies were considered as different colonies and then transferred to new nutrient agar plates to get pure cultures. The pure cultures were stored in double strength nutrient agar slant stock cultures and stored at -4 °C for further studies. While the fungi spores were compared based on size, color, margins, and mycelia, spores were then viewed under microscope for differentiations. Different fungi were

transferred to double strength slant containing PDA stock cultures and stored at -4 °C for further studies. Grown isolates were identified by their colony morphology, Gram staining reaction and biochemical tests including catalase test, citrate utilization test, motility test, indole test, urease test, sugar fermentation test and coagulase test. The fungal isolates were identified based on morphology and microscopic characteristics (Cheesbrough, 2006).

Antibiotic susceptibility test

The modified Kirby-Bauer method was carried out to determine the susceptibility of obtained isolates to some antibiotics such as OFL- Ofloxacin 5µg, STR- Streptomycin 10µg, CHL- Chloramphenicol 30µg, CRO- Ceftriaxone 30µg, GEN- Gentamycin 10µg, PFX- Pefloxacin 5µg, COT- Cotrimoxazole 10µg, CPX- Ciprofloxacin 5µg, ERY- Erythromycin 20µg, AMX- Amoxicillin 25µg, NIT- Nitrofuranton 200µg, AUG- Augmentin 30µg, TET- Tetracycline 30µg antibiotics as described by (Benson, 2005).

Results

A total of twenty five (25) samples were collected from five (5) selected shopping malls (designated A, B, C, D and E) located in Akure, Ondo state, Nigeria, results of the average number of bacteria (cfu/cm^2) and fungi (sfu/cm^2) were presented in Table 1. The number of heterotrophic bacteria ranges between 50.43 cfu/cm^2 in shopping mall A to 257.177 cfu/cm^2 in shopping mall B while the number of fungi range between 1.60 sfu/cm^2 in shopping mall E to 3.00 sfu/cm^2 in shopping mall C.

Biochemical and morphological characteristics of bacterial isolates confirmed the presence of four bacteria species namely; *Staphylococcus aureus, Micrococcus* species, *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. However, three fungi species were identified as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*.

Table 1: Total bacterial count (cfu/ cm^2) and fungi count (sfu/ cm^2) of shopping carts and baskets

Sample point	Total Bacterial count (cfu/cm^2)	Total Fungi count (sfu/ cm^2)
A	50.43±0.54^a	2.42±0.31^b
B	257.17±0.36^d	1.90±0.06^a
C	114.03±0.81^b	3.00±0.11^b
D	135.01±0.43^c	1.80±0.05^a
E	243.33±0.73^d	1.60±0.02^a

Values are presented as mean ± standard error of mean, values carrying same superscript on the same column are not significantly different at $p<0.05$ using new DMRT.

Percentage Occurrence of bacteria in samples

As shown in Table 2, the percentage occurrence of the bacteria isolates from the samples indicated that *Staphylococcus aureus* has the highest percentage of 5(35.71%), followed by *Micrococcus* species 4(28.57%) and *Staphylococcus epidermidis* 3(21.43%), while the least was observed for *Klebsiella pnemoniae* 2(14.29%). Shopping mall A has the least bacterial diversity 2(14.29%) while others had higher and the same bacterial diversity 3(21.43%). The most occurred fungi is *Aspergillus niger* 5(41.67%) followed by *Aspergillus flavus* 4(33.33%) and *Aspergillus fumigatus* 3(25.00%), the fungal diversity of cart and basket in shopping mall A and C were the same 3(25.00%) while B, D and E has lower diversity 2(16.67%) (Table 3).

Table 2: Percentage Occurrence of bacteria isolated from shopping carts and baskets

Bacterial isolates	A	B	C	D	E	Percentage occurrence (%)
Staphylococcus aureus	+	+	+	+	+	5(35.71)
Staphylococcus epidermidis	-	+	+	-	+	3(21.43)
Mirococcus species	+	-	+	+	+	4(28.57)
Klebsiella pneumonia	-	+	-	+	-	2(14.29)
Total (%)	2(14.29)	3(21.43)	3(21.43)	3(21.43)	3(21.43)	14(100)

Key: + = present, - = not present

Table 3: Percentage Occurrence of fungi isolated from shopping carts and baskets

Bacterial isolates	A	B	C	D	E	Percentage occurrence (%)
Aspergillus niger	+	+	+	+	+	5(41.67)
Aspergillus flavus	+	+	+	-	+	4(33.33)
Aspergillus fumigatus	+	-	+	+	-	3(25.00)
Total (%)	3(25.00)	2(16.67)	3(25.00)	2(16.67)	2(16.67)	12(100)

Key: + = present, - = not present

Antibiotic sensitivity for bacterial isolates using commercial antibiotics

The antibiotic sensitivity for bacterial isolates using commercial antibiotics revealed that the highest zone of inhibition was observed against all gram positive isolates in erythromycin. Amoxicillin and ceftriaxone showed least zone of inhibition against all bacterial isolates. All bacterial isolates showed zone of inhibition against gentamycin with the highest zone of inhibition recorded against *Staphylococcus aureus* with a zone diameter of 18 mm Table 4.

Table 4: Antibiotics sensitivity (mm) determination for bacteria isolates

Bacterial isolates	OFL	STR	CHL	CRO	GEN	PFX	COT	CPX	ERY	AMX	NIT	AUG	TET
Staphylococcus aureus	15	3	0	0	18	15	0	14	21	0	ND	ND	ND
Staphylococcus epidermidis	8	0	7	4	14	6	8	7	16	0	ND	ND	ND
Micrococcus species	1.8	1.0	0	0	10	18	12	0	17	0	ND	ND	ND
Klebsiella pneumoniae	15	ND	ND	0	15	0	0	16	ND	0	0	15	0

KEY: OFL- Ofloxacin 5µg, STR- Streptomycin 10µg, CHL- Chloramphenicol 30µg, CRO- Ceftriaxone 30µg, GEN- Gentamycin 10µg, PFX- Pefloxacin 5µg, COT- Cotrimoxazole 10µg, CPX- Ciprofloxacin 5µg, ERY- Erythromycin 20µg, AMX- Amoxicillin 25µg, NIT- Nitrofuranton 200µg, AUG- Augmentin 30µg, TET- Tetracycline 30µg, ND – Not determined

DISCUSSION

Epidemiological studies have reported fomites in the transmission of human pathogens within high-exposure in hospitals, child-care facilities, long-term care facilities and sports facilities (Bures *et al.,* 2000; Al-Harbi *et al.,* 2017) however, much attention has not been paid on materials that are been constantly used in shopping environment. According to the data obtained from this study, the presence of bacteria and fungi on the handles of shopping baskets/carts were confirmed in all the supermarkets. The occurrence of microorganisms in shopping carts/basket have been reported in the study of Reynold *et al.* (2005), in their study, shopping cart handles was ranked third among the surfaces sampled for contamination. The range of bacterial counts observed in this study is smaller compared with study of Gerba and Mawell (2012), who recorded bacteria counts per shopping basket/cart ranged between 110 and 11,000,000 CFU, the differences could be due to frequency of contact, time of the day and humidity. The presence of bacteria and fungi in shopping carts/ baskets suggest that they are sites where microorganisms can be found, the handles could act as a mechanical means by which microorganisms move from one point to another. This can be attributed to the fact that humans, who can be either active or passive carriers get in contact with shopping baskets/carts during shopping activities and therefore help in the distribution of these microorganisms. These microorganisms present could spread through hand contact with hands or other objects (Duberg, 2011). Shopping carts/baskets in supermarkets are subject to accidental microbial contamination through contacts and has been implicated as a source of infection (Barzani, 2016).

The reservoir of any organism, which may be animate or inanimate objects, in the epidemiology of disease is very important (Al-Ghamdi *et al.,*

2011). The pathogens live and or multiply in the reservoir on which their survival depends. Many epidemiological studies have confirmed that contaminated surfaces played a major role in the spread of infectious diseases (Anwar *et al.,* 2007; Al-Ghamdi *et al.,* 2011). However, most people do not know that microorganisms are found on many common objects, *Staphylococcus aureus, Micrococcus* species, *Klebsiella pneumoniae, Staphylococcus epidermidis Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* were recovered as a microbial contaminants of shopping carts/baskets in this study. The presence of organisms of the micro flora of the skin such as *Staphylococcus aureus, Micrococcus* species, and *Staphylococcus epidermidis* found on the handles of shopping baskets/carts shows that these bacteria are of the microbiota of the skin and are always on the skin to prevent the possibility of attack from pathogenic organisms and thus transmitted to shopping baskets/carts on handling (Beaugerie and Petit, 2004). *Staphylococcus* species are found in all individuals and usually expelled from the respiratory tract through the nose and mouth (Al-Harbi *et al.,* 2017).

Pathogenic bacteria like *Klebsiella* specie, was also present and this can be said to be due to the handling of the shopping baskets/carts by different customers who may be already immuno-compromised or immunosuppresed by the pathogen. The results from this study is in agreement with previous reports which revealed that shopping carts/baskets are among the leading sources of germs and bacteria in various public facilities, including shopping malls, airports and bus stations (Reynolds *et al*., 2005; Ghamdi *et al*., 2011). The fungal isolates; *Aspergillus fumigatus, Aspergillus niger, and Aspergillus flavus* obtained in this study are of opportunistic mycotic importance and have been implicated to cause human mucor mycosis in immunocompromised adults and HIV patients (Aina *et al.,* 2011). Thus, if these shopping baskets/carts are left without sanitization or disinfection, they may play a key role in the transmission of these bacteria and fungi from person to person, and can cause serious diseases to human being. Several factors may have contributed to the survival and establishment of bacterial cells and fungi on surfaces of shopping carts/basket; these include characteristics of an organism and its surrounding environment. The existence of specific surface structures such as pili, flagella, and extracellular polysaccharides have been suggested to affect the adhesion and survival of bacteria (Peng *et al.,* 2001) and spore formation for the survival of fungi.

All the bacterial isolates showed a reduced zone of inhibition to amoxicillin a penicillin group and ceftriaxone cephalosporin antibiotics, these two antibiotics are commonly used for the treatment of infections. This reduction in zone of inhibition could as a result of bacteria have developed resistance against the tested antibiotics. As stated in the report of CLSI (2017), a reduced zone of inhibition in antibiotic susceptibility assay is an indication for antibiotic resistance. Therefore shopping carts/basket could be a vehicle for the dissemination of antibiotic resistant gene in the community.

Conclusion

The study confirmed the presence of high microbial load, pathogenic bacteria and fungi in shopping basket/carts. These findings confirm the notion that the shopping baskets/carts might act as source of transmitting pathogens from person to person. The results of this study indicated that poor sanitation and hygiene conditions exist among these tested shopping baskets/carts of the five selected stores. The findings from the study also recognize the need to take urgent measures to improve the sanitary conditions of these shopping baskets/carts.

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