Original Research Article

ISOLATION AND ANTIBIOTICS SUSCEPTIBILITY PATTERN ASSOCIATED WITH SHOPPING TROLLEYS AND BASKETS FROM SUPERMARKETS IN AKURE, ONDO STATE, NIGERIA

ABSTRACT

Pathogens on public shared objects among people have generated a lot of public health concerns. People are brought together and thus facilitate transmission of pathogens either directly or indirectly within the supermarkets. This study was aimed at isolating bacteria from items carriage from supermarkets and assesses the antibiotic susceptibility pattern of the isolates. A total of 253 swab samples were obtained from handles of trolleys and baskets used by customers from four major supermarkets in Akure. Each sample was immediately transferred into 5ml of peptone water. The samples were processed using a standard microbiological method. Bacteria growths were identified based on cultural, morphological and biochemical characteristics. Susceptibility test of the isolates was done on Mueller-Hinton agar. The study shows that out of the 353 isolates identified, 37.1% were *S. aureus*, 49.6% were *S. epidermidis,* 5.7% were *Klebsiella* sp and 7.6% were *E. coli*. Results showed that Ampiclox and Cotrimoxazole (*S. aureus* in Trolleys), Ampiclox, Zinnacef, Amoxacillin, Ciprofloxacin and Streptomycin (*S. aureus* in baskets), Rocephin (*S. epidermidis* in trolleys) and Amoxacillin (*S. epidermidis* in baskets) are ineffective. Regular disinfection of items carriage and educating people to improve hand washing habits are recommended as measures to decreasing transmission of pathogens and their infections.

Keywords: *S. aureus*, *S. epidermidis*, Antibiotics, Shopping Trolleys and Baskets.

INTRODUCTION

From day to day activities of human both indoors and outdoors, there has been increase in the spread of infectious organisms and thus imposing dangerous health issues [3]. Increased attention has been paid to contamination of objects within clinical settings because most people believe that microbial load are much more heavy in research laboratories, hospitals and therefore consider other places safe [2]. Recently, commonly shared objects from different public places such as parks, daycare centres, restaurants and university settings, were examined and discovered that there is need to pay much attention to microbes found on daily used objects in public places to reduce the risk of disease transmission [1,2]. With the spread of supermarket and hypermarket across the world, large number of people are brought together and thus facilitate transmission of microbes either directly [hand with hand contact] or indirectly [involving inanimate objects or fomites such as shopping baskets, shopping trolleys, supermarkets shelves and some other objects within the supermarkets] [6].

Some pathogenic organisms such as *Staphylococcus aureus, Staphylococcus, epidermidis, Escherichia coli, Enterococcus faecalis, Bacillus* sp, *Micrococcus* sp and some other fungal species have been reported on computer keyboards, door handles, tap heads, elevator buttons, ATM machines and shopping carts [1]. Information on the microbial contamination in communal areas of a workplace or in carriage items in supermarkets is of great importance as this would be of great help in identifying sources of infection with the view of taking preventive measures [5]. The current study was conducted to isolate contaminating bacteria from shopping trolleys, shopping baskets, and to assess the antibiotic susceptibility pattern of the isolates. Pathogenic microorganisms found on objects can live for a long period of time owing to microbial characteristics, fomites characteristics, and environmental factors such as temperature and relative air humidity. Therefore, there is increasing public health concern in investigation of fomites in public settings.

MATERIALS AND METHODS

Sample collection

A total number of 253 samples [157 shopping trolleys and 96 shopping baskets] were randomly collected from four different supermarkets in Akure, Ondo state, Nigeria from January to April, 2016. 2cm X 2cm area on handles of shopping trolleys and shopping baskets was swabbed using sterile peptone water moistened swabs and immediately transferred into 5ml of peptone water. Collected samples were kept in cold box and were transported to the Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko for further evaluation.

Culture and isolation of contaminating bacteria

Isolation of different bacterial contaminants from numerous handles of carriage items was performed through standard techniques. The media used include MacConkey agar, Blood agar, Mannitol salt agar, Nutrient agar, peptone water and Mueller-Hinton agar. All samples were plated within 2-4 hours of specimen collection the plates were incubated aerobically at 37^{o}C for 24 hours. After incubation, pure isolates based on cultural, morphological and biochemical characteristics were identified [7]. *S. aureus* was differentiated from *S. epidermidis* with the help of biochemical tests (Catalase and coagulase tests). Antibiotic susceptibility test was carried out using Mueller-Hinton agar prepared according to manufacturer's specification and sterilized at 121^{o}C for 15 minutes. The medium was then poured into appropriate Petri dishes aseptically. Antibiotics used include; Pefloxacin (Pef, 10 µg), Gentamycin (CN, 10 µg), Ampiclox (APX, 30 µg), Zinnacef (Z, 20 µg), Amoxicilin (AM, 30µg), Rocephin (R, 25 µg), Ciprofloxacin (CPX, 10 µg), Streptomycin (S, 30 µg), Septrin (SXT, 30 µg), Erythomycin (E, 10 µg), Chloramphenicol (CH, 30 µg), Spafloxacin (SP, 10 µg), Augmentin (AU, 30 µg), Tarivid (OFX, 10 µg) produced by Maxicare medical laboratory Nigeria. Antibiotic susceptibilities for Gram positive and Gram negative bacterial isolates were determined according to clinical and laboratory standard institute [CLSI] using the disc diffusion method [8].

RESULTS

The four different supermarkets were designated as A, B, C and D. Table 1 showed the distribution of samples from shopping trolleys and baskets from each supermarket of which 173, 46, 19 and 15 carriage items were from supermarkets A,B,C and D respectively. Figure 1 shows the percentage distribution of shopping trolleys and baskets from each supermarket. Out of 253 examined trolleys and baskets, 155(61.3%) showed bacterial contamination and 2(0.8%) trolley showed no bacterial growth while all the 96(37.9%) examined shopping baskets showed bacterial contamination.

The distribution of bacterial isolates from shopping trolleys and baskets in the sampled supermarkets are reported in Table 2. Total number of bacterial isolates obtained from carriage items from the four sampled supermarkets were 159(45.04) and 90(25.50) from trolleys and baskets examined in supermarkets A, 35(9.91) and 27(7.65) bacteria isolated from trolleys and baskets in supermarket B while 29(8.22) and 13(3.68) isolates were obtained from baskets examined in supermarkets C and D as there were no trolleys found in these supermarkets.

Out of 353 isolates identified, qualitative bacterial analysis of the isolates obtained from the handles of all examined objects revealed that *S .epidermidis* (49.6%) had the highest prevalence followed by *S. aureus* (37.1%), *E. coli* (7.6%) while *Klebsiella* sp (5.7%) was the least found isolates.

Resistance patterns of both Gram positive and Gram negative isolates obtained from shopping trolleys and shopping baskets were presented in Table 3. Gram positive isolates (*S.aureus* and *S. epidermidis*) were tested against 10 antibiotics. *S. aureus* (72) obtained from trolleys was resistant to Ampiclox 39 (31.9) and Cotrimoxazole 37 (51.4 while *S.aureus* from baskets was resistant to Amoxacilin 38(64.4), Ampiclox 34(57.6), Zinnaccef 32(54.2), Ciprofloxacin 31(52.5) and Streptomycin 30(50.8). It was observed that less than half of *S.epidermidis* from trolleys (n=96) and baskets (n=79) were resistant to antibiotics used.

Gram negative bacteria obtained showed different level of resistance to all antibiotics used. 50% of *Klebsiella* sp isolated from trolleys and baskets were resistant to

Sparfloxacin, Augmentin, Cotrimoxazole and Chloramphenicol. It is also observed that *Klebsiella* sp from trolleys were all susceptible to Streptomycin. *Klebsiella* sp from baskets were all susceptible to Gentamycin and Streptomycin. Of the total number of 11 *E. coli* that were isolated from all trolleys examined, 6(54.5) were resistant to Amoxacillin, Augmentin and 1(9.1) was resistant to Ciprofolxacin, Pefloxacin and streptomycin. *E.coli* from basket showed multi-resistant to almost all antibiotics used.

Table1: Distribution of shopping trolleys and baskets from each supermarket

Supermarkets	Shopping baskets n=96	Shopping trolleys n=157	Total
A	45	128	173
B	17	29	46
C	19	-	19
D	15	-	15
Total	**96**	**157**	**253**

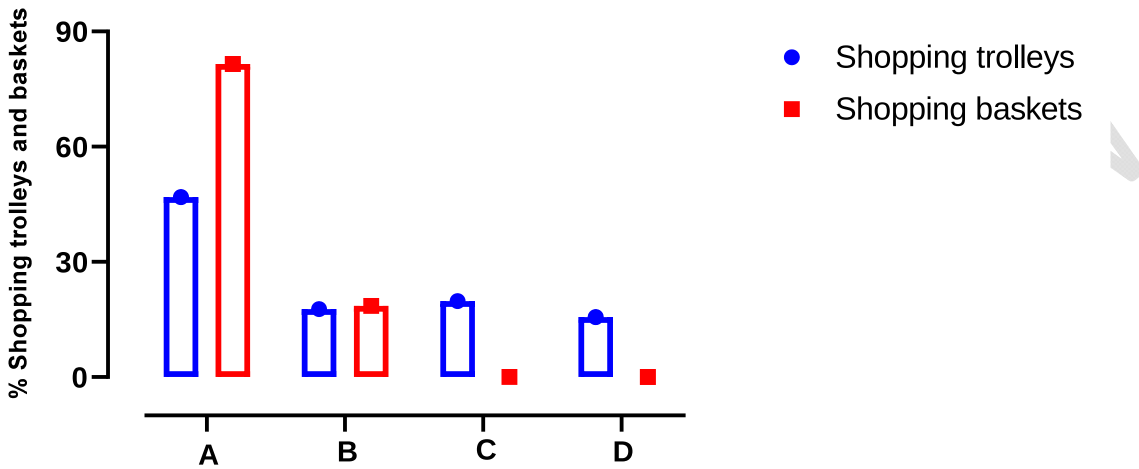

Figure 1: Percentage distribution of shopping trolleys and baskets from each supermarket

Table 2: Prevalence of bacterial isolates from shopping trolleys and baskets in each supermarkets

Bacteria Isolates	A (T) (%)	A (B) (%)	B (T)(%)	B (B)(%)	C (T)(%)	C (B)(%)	D (T)(%)	D (B)(%)	Total Isolates on trolleys	Total Isolates on baskets	Total (%)
S. aureus	53 (73.6)	26 (44.1)	19 (26.4)	13 (22.0)	-	15 (25.4)	-	5 (8.5)	72 (37.1)	59 (37.1)	131 (37.1)
S.epidermidis	82 (85.4)	52 (65.8)	14 (14.6)	11 (13.9)	-	9 (11.4)	-	7 (8.9)	96 (49.5)	79 (49.7)	175 (49.6)
***Klebsiella* sp**	13 (86.7)	4 (80)	2 (13.3)	-	-	1 (20)	-	-	15 (7.7)	5 (3.1)	20 (5.7)
E.coli	11 (100)	8 (50)	-	3 (18.75)	-	4 (25)	-	1 (6.25)	11 (5.7)	16 (10.1)	27 (7.6)
Total	159 (45.04)	90 (25.50)	35 (9.91)	27 (7.65)	-	29 (8.22)	-	13 (3.68)	194	159	353

Table 3: Resistance pattern of *S. aureus* and *S. epidermidis* from shopping trolleys and

Antibiotics	Trolleys *S.aureus*(n=72)	Baskets *S.aureus*(n=59)	Trolleys *S.epidermidis*(n=96)	Baskets *S.epidermidis*(n=79)
Pefloxacin	28 (38.9)	29 (49.2)	32 (33.3)	21 (26.6)
Gentamycin	26 (36.1)	23 (40.0)	21 (21.9)	18 (22.8)
Ampiclox	39 (31.9)	34 (57.6)	30 (31.3)	20 (25.3)
Zinnacef	23 (48.6)	32 (54.2)	34 (35.4)	28 (35.4)
Amoxacillin	20 (27.8)	38 (64.4)	34 (35.4)	34 (43.0)
Rocephin	29 (40.3)	24 (40.7)	37 (38.5)	29 (36.7)
Ciprofloxacin	18 (25)	31 (52.5)	28 (29.2)	30 (38.0)
Streptomycin	28 (38.9)	30 (50.8)	25 (26)	31 (39.2)
Cotrimoxazole	37 (51.4)	23 (40.0)	29 (30.2)	29 (36.7)
Erythromycin	16 (22.2)	29 (49.2)	24 (25)	24 (30.4)

Table 4: Resistance pattern of Gram negative isolates from shopping trolleys and baskets

Antibiotics	Trolleys *Klebsiella* sp. (n=15)	Baskets *Klebsiella* sp. (n=5)	Trolleys *Escherichia coli* (n=11)	Baskets *Escherichia coli* (n=11)
Chloramphenicol	5 (33.3)	4 (80.0)	2 (18.2)	5 (31.3)
Sparfloxacin	10 (66.7)	1 (20.0)	2 (18.2)	3 (18.8)
Ciprofloxacin	4 (26.7)	1 (20.0)	1 (9.1)	6 (37.5)
Cotrimoxazole	8 (53.3)	2 (40.0)	5 (45.5)	6 (37.5)
Amoxacillin	6 (40.0)	2 (40.0)	6 (54.5)	8 (50)
Augmentin	9 (60)	2 (40.0)	6 (54.5)	7 (43.8)
Gentamycin	4 (26.7)	0 (0.0)	3 (27.3)	3 (18.8)
Pefloxacin	6 (40.0)	1 (20.0)	1 (9.1)	3 (18.8)
Ofloxacin	4 (26.7)	2 (40.0)	1 (9.1)	4 (25)
Streptomycin	0 (0.0)	0 (0.0)	1 (9.1)	2 (12.5)

DISCUSSION

This study presents the isolation and antibiotics susceptibility pattern of some Gram positive and Gram negative bacteria associated with items carriage from supermarkets. The number of bacteria isolates obtained varies from one supermarket to the other (Table 2). This could be attributed to level of hygiene with the sanitary condition of the supermarkets environment and how frequent the carriage items are being used by customers. The users of these items carriage also vary in their hygienic status and could thus facilitate transmission of these pathogens directly or indirectly when their hands come in contact with these objects. The atmospheric condition and temperature in Nigeria could be conducive for the persistence of the bacteria from handles of shopping trolleys and baskets which is in agreement with the report of [14] and [6] who reported that the humid tropical conditions of Portugal and Malaysia respectively may have contributed to the survival of bacteria on public objects.

The isolated bacteria namely; *S. aureus*, *S. epidermidis*, *Klebsiella* sp and *E.coli* from these items carriage are of great medical importance. They are known as human pathogens, causing infections and resulting in life threatening diseases. *S. aureus* is often considered to be the greatest challenging of the three pathogens *S. aureus* can cause a range of illnesses, from minor skin infections, such as such as pimples, impetigo, boils, cellulitis, folliculitis, pimples (Hammadi *et al.,* 2017). *S. epidermidis* is much less commonly found as pathogens but are occasionally associated with endocarditis, wound infections and prosthetic joint infections. *Klebsiella* sp is vital opportunistic pathogens, mainly among the immunocompromised. They can also cause bacteremia and hepatic infections, and have been isolated from a number of unusual infections, including endocarditis, primary gas-containing mediastinal abscess, etc.

Fomites consist of either porous or non-porous surfaces that when contaminated with pathogenic microbes, they can be transferred to a new host, thus serving as vehicles in transmission. It is being speculated that non-porous fomites allow bacteria localization only along the surface, allowing increased exposure during contact with the skin. The colonization of inanimate objects by viable pathogenic microorganisms has been reported by [12] and [4].

The study shows that out of 353 isolates identified 49.6% were *S. epidermidis*, 37.1% were *S. aureus*, 5.7% were *Klebsiella* sp and 7.6% were *E.coli* (Table 2). It is being demonstrated from this study that Gram positive bacteria are the most common contaminating microbes and are thereby transmitted readily from examined objects. This is because they are major components of normal flora of the skin and nose and this could explain their prevalence on fomites. Of the 253 samples obtained and processed, the results shows that *S. epidermidis* and *S. aureus* were more frequently isolated compared to *Klebsiella* sp and *E.coli* due to their ability to survive on objects for a long period of time, this is in accordance with [11] and [6]. The high numbers of *S. epidermidis* showed how poor the items carriage are been cared for when it comes to washing and disinfection. This increase the risk of coming into contact with a disease causing organisms. According to [9] and [13]. It is revealed that placement of small children in shopping trolleys exposed them to infection from common enteric bacteria such as *E.coli* and the likes. The bacteria isolates obtained in this study cause several infections when they find their way to lymphatic channels or blood [17]

Observations have been made that the resistance of bacteria isolates to antibiotics is not constant but varies with time and environment [10]. Screening of bacteria pathogens for their antibiotics resistance pattern is needed in different communities [15]. The resistance pattern of the bacterial isolates to antibiotics used reveals *S. aureus* and *S. epidermidis* were highly resistant to applied antibiotics followed by *Klebsiella* and *E.coli*. This indicates that most of antibiotics used were ineffective. This result is similar to the result obtained by [16].

CONCLUSION

This study has investigated the resistance pattern of bacteria sp on items carriage in supermarkets. The presence of pathogens like *S.aureus*, *S. epidermidis*, *Klebsiella* sp and *E.coli* were revealed. However, there is need for consistent antimicrobial resistant surveillance for important and commonly isolated pathogens on inanimate objects for development and implementation of measures that can reduce the spread of antimicrobial resistance and prevent arising public health problem. This study recommends routine disinfection of public shared objects, hand washing habit, provision of disposable hand gloves to customers and proper use of antibiotics.

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