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Bacteriological Profile of Bacterial Isolates from the Hands and Nasal Swabs of Food Vendors in University of Benin, Nigeria

Aziegbemhin A.S[1]. and Dunkwu-Okafor, A[1]*

[1]Department of Microbiology,
Faculty of Life Sciences,
University of Benin
P.M.B1154 Benin City,
Edo State,
Nigeria.

Email: afamefuna.dunkwu-okafor@uniben.edu

Abstract

This research was undertaken to investigate the bacteriological profile of bacterial isolates from the hands and nasal swabs of food vendors at the University of Benin, Benin City, Edo State. A total of 20 different samples were randomly collected from ten (10) different food vendors at the University of Benin Food Court, Benin City, Nigeria. Swab samples were inoculated using streak plate and incubated at 24 hours at 37°C. The samples were sub-cultured after 24 hours and identified based on cultural characteristics, morphology and biochemical tests. The bacteria isolated include Staphylococcus epidermidis, Staphylococcus aureus, and Bacillus cereus from nasal swab samples while Staphylococcus epidermidis and Staphylococcus aureus were isolated from hand swab samples. The frequency of occurrence of the bacteria recovered from hand was Staphylococcus epidermidis 7(70%), Staphylococcus aureus 3(30%) while Staphylococcus aureus 5(50%), Staphylococcus epidermidis 2(20%) and Bacillus cereus 3(30%) were recovered from nasal swab respectively. The antibiotic susceptibility and resistance pattern exhibited by the recovered isolates from the nasal swab showed that 10(100%) of all the isolates were susceptible to Pefloxacin, Ciprofloxacin, Streptomycin and Co-trimoxazole(septrin) respectively while 0(0%) of the recovered isolates were resistant to Cefuroxime respectively whereas the recovered Staphylococcus epidermidis from the hand swab had 5 (71.4%) which were susceptible to Gentamycin, 3(42.9%) which were susceptible to Ampiclox, 2(28.6%) which were susceptible to Rocephin, and 5(57.1%) were susceptible to Erythromycin. Consequently, it is suggested that the food vendors should adhere strictly to good hygiene practices and health practices in line with regulatory standards.

Keywords: Food, Vendors, Swab, Hands, Resistance

INTRODUCTION

In tertiary institutions in Nigeria, small restaurants and canteens in addition to street vendors provides people with meals outside their homes. Street vended foods are popular because of their unique flavours and the convenience of obtaining those (Nyenje *et al*., 2012). The large number of people involved in the preparation and serving of meals presents possibilities for the risk of microbial contamination (Abdu *et al*., 2017). The ready-to-eat food is the food that

Author for Correspondence

is ready for immediate consumption at the points of sale (Ochei, 2014). Studies reveal that the hands of ready-to-eat food service employees spread foodborne disease, mainly because of poor personal hygiene (Cruickshank, 1990). Food-borne diseases are diseases which arise from ingesting bacteria, toxins and as well as cells produced by microorganisms present in the food (Doyle and Evans, 1999).

Food vendors play an important role in ensuring food safety throughout the chain of production, processing, storage, and preparation (Ochei, 2014). Approximately 10 to 20% of food-borne disease outbreaks are due to contamination by the food vendors. Abdu et al. (1996) stated that improper food handling practices contributed to approximately 97% of foodborne illnesses in food service establishments and homes. Statistical evidence indicates that food poisoning caused by the catering industry is 70% higher than that caused by any other sector (Lambrechts *et al*., 2014).

There has been an increase in antibiotic resistance in Nigerian populace and University community in particular. Hand washing has been seen as an essential measure to protect against the spread of disease and is one of the main practices to reduce the transfer of bacteria between individuals and from individuals to food surfaces (Lambrechts *et al*., 2014).

However in Nigerian Universities, a number of foods related disease outbreaks have been reported (Ochei *et al*., 2014). But there is limited information on the health challenges from food borne diseases from ready-to-eat food retailed (canteens and restaurants) within a highly populous community like the University of Benin, Benin City, Edo state, Nigeria with over seventy thousand persons. It is in view of this, that this study was undertaken to assess the bacterial burden of the nasal swabs and hand-swabs of food vendors in University of Benin, Benin-City, Edo State, Nigeria.

MATERIALS AND METHODS

Study Population and Sample Collection: A total of twenty samples, ten each in the form of swabs were collected from the hands and nostrils of food vendors working at different food buka at the Food Court in the University of Benin, Benin City, Nigeria. Nose swabs were self-collected by participants.

Ethical Consideration: Informed consent was obtained from the food vendors before collecting the samples.

Preparation of Culture Media: All media were prepared according to the manufacturer's instruction.

Collection of Samples: The hand swab and nasal swab samples were collected by swabbing the palms and nostrils of food vendors with a sterile swab stick moistened with 1ml sterile water respectively (Ochei et al., 2014) The samples were labelled, packaged and transported to the laboratory for immediate microbiological analysis.

Inoculation of the Plates: Following collection of samples, the swab sticks were streaked aseptically on the surface of already solidified Nutrient agar and MacConkey agar (HiMedia, India) which were prepared according to the manufacturer's instructions for 24hr at 37 ^{0}C in

an incubator and grown aerobically and also anaerobically by placing in an anaerobic jar. After the incubation time, the different cultured plates were examined for microbial growth. All colonies that were cultured from the swab culture were subcultured for pure isolates and the isolates were identified by cultural characteristics (Abdu *et al*., 2017).

Sub Culturing of Bacterial Isolates: A single isolated colony of the bacteria was picked up aseptically with the aid of a sterilized wire loop and were streaked on a freshly prepared nutrient agar plate. The nutrient agar (HiMedia, India) plates were incubated at 37^{o}C for 24hr. The pure bacterial isolates were stored as slants in a refrigerator (Abdu *et al*., 2017).

Characterization and Identification of Bacterial Isolates: Identification and characterization of the bacterial isolates was performed on the basis of cultural characteristics, morphological characteristics and biochemical characteristics (Cheesebrough 2006; Cowan *et al*., 2003).

Cultural Characteristics: Cultural characteristics of the colony of the bacterial isolates were observed on the nutrient agar plate after 24 hr of incubation at 37^{o}C and were recorded. These characteristics include: size, shape, transparency, elevation and colour (Okareh and Erhahon, 2015).

Morphological Characteristics: This was carried out using Gram staining reaction. Gram staining is a common technique used to differentiate between Gram positive bacteria and Gram negative bacteria based on their shapes, arrangement and Gram reaction. Smears were prepared from the cultured plates aseptically on a clean glass slides and the slides were properly labeled. A drop of sterile water was emulsified on the smear and was dried, heat fixed. The smear was then stained with crystal violet for 1 min and then washed off with water. Lugol iodine was applied to the smear for 1 min and then washed off with water. The smear was decolourized with few drops of alcohol for 30 sec and then washed off immediately. Then the smear was counterstain with safranin for 1 minute and then washed off with water for 1 min. The slide was then air dried and then blotted with cotton wool. A drop of immersion oil was dropped on the smear and was examined under the oil immersion lens of the binocular microscope. Gram positive bacterial cells appear purple under the microscope while Gram negative bacteria appear red (Abdu *et al*., 2017).

Biochemical Characteristics: Specific biochemical tests were carried out to further identify the organism and they include: catalase test, oxidase test, citrate utilization test, indole test, sugar fermentation test and urease test (Abdu *et al*., 2017).

Tests for Virulence Factor: Specific tests were carried out to ascertain their virulence potential and they include: biofilm formation and capsule stain (Abdu *et al*., 2017).

Biofilm Formation: This was carried out using the tube adherence method which was suggested by Christensen *et al*. (1982) which had little modification. Mueller Hinton medium was inoculated with 24-hour bacterial culture in test tubes and incubated aerobically at 37^{o}C for 48 hr. The contents of the test tubes were discarded and the tubes were stained with 0.1% safranine and washed with sterile water thrice and allowed to dry. A positive result was taken as the presence of a layer of the stained material adhered to the inner wall of the tubes but if only a stained ring was seen at the liquid-air interface, it was considered as no biofilm formation.

Capsule Staining: A smear of the test organism is made on a clean microscopic slide using a sterilized inoculating loop and is stained with crystal violet and the dye is spread across the smear and allowed to dry for 5-7 min. The slide is then rinsed with copper sulphate solution air dried and observed under oil immersion at ×100 magnification of the microscope. A Positive result was indicated by a clear or faint blue halo on a transparent background while its absence was regarded as a negative result (Hughes and Smith, 2007).

Antibiotic Susceptibility Pattern: This was carried out using the Disk Diffusion (Kirby-Baucer) method. Mueller Hinton agar plates were prepared and appropriately labeled according to the manufacturer's specifications. The bacterial isolates were inoculated into 1ml sterile water and the turbidity of the test bacterial suspension was compared with that of 0.5ml MacFarland standard. High bacterial suspension was reduced by adding sterile while low bacterial suspension was increased by adding more bacterial isolate. The bacterial suspension was inoculated into the Mueller Hinton plates by dipping a sterile swab stick into the standardized bacterial suspension using spread plate technique and the plates were left to dry for 15 minutes. Antibiotic disk containing varying concentration of various types of antibiotics was placed using a sterilized forceps on the surface of the dried plates aseptically and the disk was pressed down lightly to the agar plates to ensure complete contact between the disk and agar surface. The antibiotics used include: pefloxacin (PEF) 10µg, gentamycin (CN) 10µg, ampicillin and cloxacillin (APX) 30µg, zinnacef (Z) 20µg, amoxacillin (AM) 30µg, rocephin (R) 25µg, ciprofloxacin (CPX) 10µg, streptomycin (S) 30µg, co-trimoxazole (SXT) 30µg, erythromycin (E) 10µg. The plates were incubated for 24 hr and visible zones of inhibition were measured and calculated in millimetres and Multiple Antibiotic Resistance (MAR) indexes was interpreted (Baucer *et al*., 1996; Clinical and Laboratory Standards Institue, 2014).

RESULTS

A total of twenty individuals participated in this study. Among them, sixteen were female and four were male. The age of participants ranged from 18-45. Among the samples collected from the individuals, of whom ten were nasal swabs and the other ten, were hand swabs. The result obtained from the nasal swab and hand swab of food vendors is represented below: The cultural, morphological and biochemical characteristics of the isolates recovered from the nasal swab are represented in Table 1a. A total of three (3) bacteria were isolated from the nasal swab of food vendors. All were Gram-positive and the bacteria isolated were: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus*. Table 1b shows the cultural, morphological and biochemical characteristics of the isolates recovered from the hand swab where two (2) bacteria were isolated from the hand swab of food vendors. All were Gram-positive and the bacteria isolated are: *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Table 1a: Cultural, Morphological and Biochemical Characterization of Isolated Bacteria from the Nasal swab of Food Vendors

ISOLATES	A_N	B_N	C_N
Cultural Characteristics			
Shape	Circular	Circular	Irregular
Colour	White	Golden-yellow	Grey-white
Size	Small	Small	Large
Elevation	Raised	Convex	Convex
Transparency	Translucent	Opaque	Opaque
Morphological Characteristics			
Gram Stain	+	+	+

Cell type	Cocci	Cocci	Rod
Biochemical Characteristics			
Catalase	+	+	+
Oxidase	-	-	-
Indole	-	-	-
Citrate	-	+	+
Urease	-	-	-
Coagulase	-	+	-
Sugar Fermentation			
Glucose	+	+	+
Lactose	+	+	-
Probable Identity:	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Bacillus cereus*

Key points: (+) = Positive (-) = Negative (N) = Nasal Swab

Table 1b: Cultural, Morphological and Biochemical Characterization of Isolated Bacteria from the Hand swab of Food Vendors

ISOLATES	**A_H**	**B_H**
Cultural Characteristics		
Shape	Circular	Circular
Colour	White	Golden-yellow
Size	Small	Small
Elevation	Raised	Convex
Transparency	Translucent	Opaque
Morphological Characteristics		
Gram Stain	+	+
Cell type	Cocci	Cocci
Biochemical Characteristics		
Catalase	+	+
Oxidase	-	-
Indole	-	-
Citrate	-	+
Urease	-	-
Coagulase	-	+
Sugar Fermentation		
Glucose	+	+
Lactose	+	+
Probable Identity:	*Staphylococcus epidermidis*	*Staphylococcus aureus*

Key points: (+) = Positive (-) = Negative (H) = Hand Swab

The different bacteria isolated which are represented in Table 1a and Table 1b respectively above occurred in varied frequency where one was the most prevalent than the others. Figure 1a and Figure 1b below shows the frequency (%) of the different isolates as they occurred in the nasal swab and hand swab sample respectively. From Figure 1a chart, *Staphylococcus aureus* (50%) had the highest frequency of occurrence while the other two (2) isolates had 20% and 30% respectively while Figure 1b chart, *Staphylococcus epidermidis* (70%) had the highest frequency of occurrence and *Staphylococcus aureus* (30 %) had the lowest frequency of occurrence.

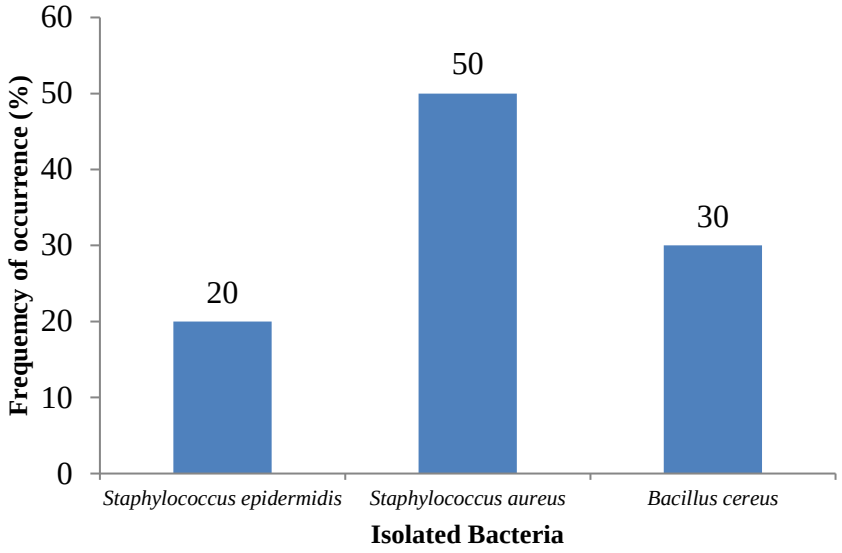

Figure 1a: Frequency of Occurrence (%) of Bacteria Isolated from Nasal swab of Food vendors

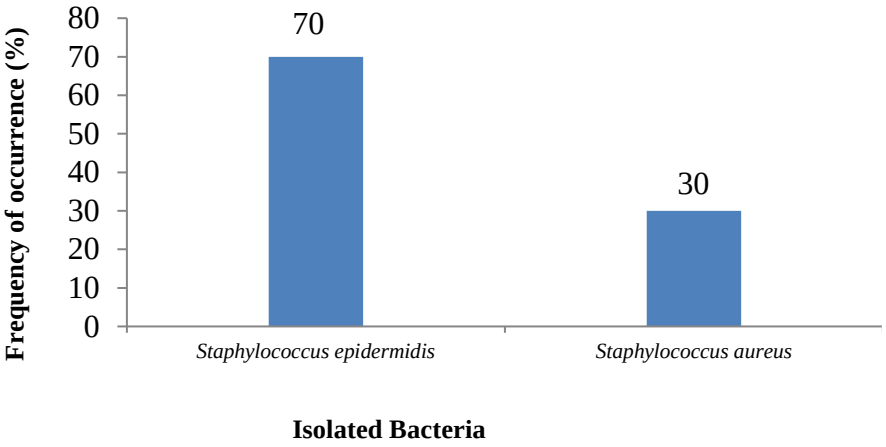

Figure 1b: Frequency of Occurrence (%) of Bacteria Isolated from Hand swab of Food vendors

The antibiotic susceptibility and resistance pattern exhibited by the bacterial isolates from the nasal swabs and hand swab are represented in Table 2a and Table 2b respectively. Table 2a and Table 2b shows that 100% of all the bacteria were susceptible to Pefloxacin, Ciprofloxacin, Streptomycin and Septrin respectively while all the bacteria isolated were resistant to Zinnacef respectively. In Table 2a, *Bacillus cereus* were susceptible to all seven (7) antibiotics used (100%) while in Table 2b, 71.4% of *Staphylococcus epidermidis* were susceptible to Gentamycin. 42.9% were susceptible to Ampiclox, 28.6% were susceptible to Rocephin, and 57.1% were susceptible to Erythromycin.

Table 2a: Antibiotic Susceptibility and Resistance Pattern of Bacteria Isolated from Nasal Swabs

Isolates (mm)		Susceptibility of isolates (%)									
	No of isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
Staphylococcus aureus	5(50)	5(100)	5(100)	2(40)	0(0)	2(40)	2(40)	5(100)	5(100)	3(60)	1(20)
Staphylococcus epidermidis	2(20)	2(100)	2(100)	0(0)	0(0)	0(0)	0(0)	2(100)	2(100)	2(100)	0(0)
Bacillus cereus	3(30)	3(100)	0(0)	3(100)	0(0)	0(0)	3(100)	3(100)	3(100)	3(100)	3(100)

Key points: (PEF)= Pefloxacin,10µg (CN)=Gentamycin,10µg (APX)=Ampiclox,30µg (Z)=Zinnacef,20µg (AM)=Amoxacillin,3oµg (R)=Rocephin,25µg (CPX)=Ciprofloxacin,10µg (S)=Streptomycin,30µg (SXT)=Septrin,30µg (E)=Erythromycin,10µg

Table 2b: Antibiotic Susceptibility and Resistance Pattern of Bacteria Isolated from Hand Swabs

Isolates (mm)		Susceptibility of isolates (%)									
	No of isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
Staphylococcus aureus	3(30)	3(100)	3(100)	1(33.3)	0(0)	1(33.3)	1(33.3)	3(100)	3(100)	2(66.7)	1(33.3)
Staphylococcus epidermidis	7(70)	6(85.7)	5(71.4)	3(42.9)	0(0)	0(0)	2(28.6)	5(71.4)	7(100)	7(100)	4(57.1)

Key points: (PEF)= Pefloxacin,10µg (CN)=Gentamycin,10µg (APX)=Ampiclox,30µg (Z)=Zinnacef,20µg (AM)=Amoxacillin,3oµg (R)=Rocephin,25µg (CPX)=Ciprofloxacin,10µg (S)=Streptomycin,30µg (SXT)=Septrin,30µg (E)=Erythromycin,10µg

Figure 2a and Figure 2b shows the multiple antibiotic resistance (MAR) index of the isolated bacteria.

$$\text{MAR index} = \frac{\text{Number of resistant organism}}{\text{Total number of antibiotics used}}$$

As shown in the charts below, in figure 2a, all the bacterial isolates except S.A N1, S.A N2, S.A N4, B.C N1, B.C N1 and B.C N3 were above the permissible limit which is 0.2. S.A N2, S.A N4 had a MAR index 0.1 which is the lowest and S.A N3 having a MAR index of 0.5 which is the highest while in figure 2b, all the bacterial isolates except S.E H1, S.E H3, S.E H 7, S.E H 8, S.E H5 and S.E H9 were above the permissible limit which is 0.2. This indicates that all except S.E H1, S.E H3, S.E H 7, S.E H 8, S.E H5 and S.E H9 are pathogenic. S.E H1, S.E H3, S.E H 7, S.E H 8, S.E H5 and S.E H9 had a MAR index of 0.2 which is the lowest and S.E H6 having a MAR index of 0.5 which is the highest which is seen in Figure 2b

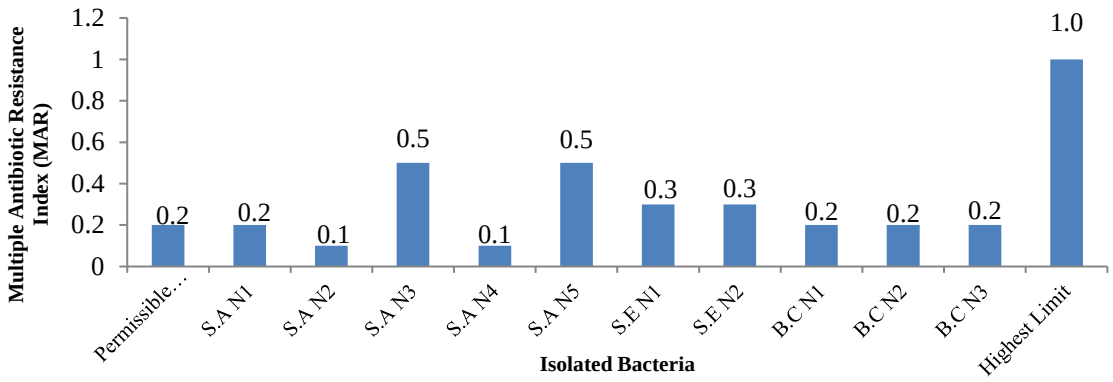

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Figure 2a: Multiple Antibiotic Resistance (MAR) Index of bacteria isolated from the nasal swab of food vendors

Key points: S.A N= *Staphylococcus aureus* isolated from nasal swab
S.E N= *Staphylococcus epidermidis* isolated from nasal swab
B.C N= *Bacillus cereus* isolated from nasal swab

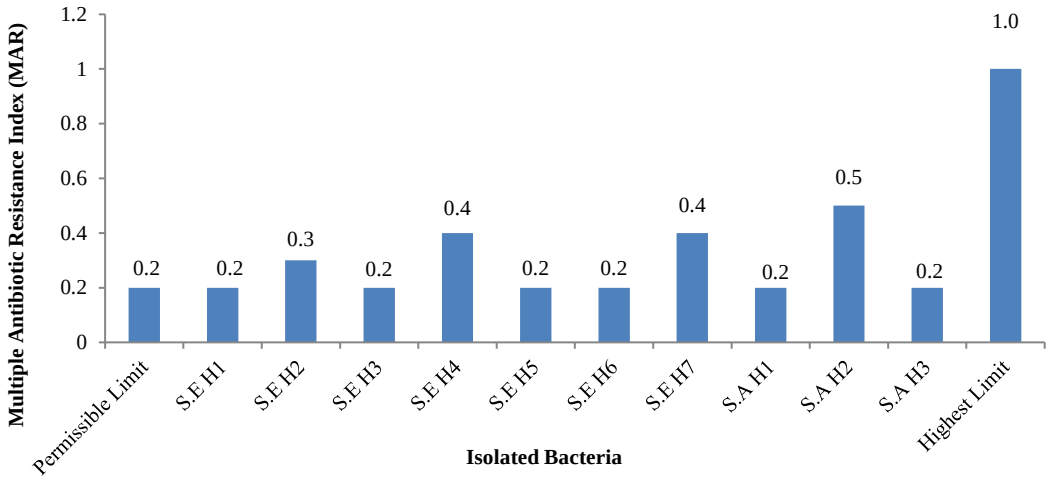

Figure 2b: Multiple Antibiotic Resistance (MAR) Index of bacteria isolated from the hand swab of food vendors

Key points: S.E H= *Staphylococcus epidermidis* isolated from hand swab ; S.A H= *Staphylococcus aureus* isolated from hand swab

Table 3a: Virulence Factors of Isolated Bacteria from the Nasal swab of Food Vendors

Isolates (n)	Biofilm Formation	Capsule Staining
Staphylococcus epidermidis (2)	Strong	+
Staphylococcus aureus (5)	Strong	-
Bacillus cereus (3)	Moderate	-

Key point: (+) = Positive (-) = Negative (n) = Number of isolates

Table 3b: Virulence Factors of Isolated Bacteria from the Hand swab of Food Vendors

Isolates (n)	Biofilm Formation	Capsule Staining
Staphylococcus epidermidis (7)	Strong	+
Staphylococcus aureus (3)	Strong	-

Key points: (+) = Positive (-) = Negative (n) = Number of isolates

The virulence factors exhibited by the bacterial isolates from the nasal swabs and hand swab are represented in Table 3a and Table 3b respectively. In Table 3a, *Staphylococcus epidermidis* and *Staphylococcus aureus* formed a strong biofilm and *S. epidermidis* was found to be capsulated having a smooth appearance on nutrient agar but the other two isolates were non-capsulated while in table 3b, *Staphylococcus epidermidis* was found to be capsulated and also produce a strong biofilm adherence.

DISCUSSION

The importance of the nose and hands to human existence cannot be over-emphasized where it functions primarily as an organ of respiration and the hand as the main body organ by which work is carried out and maneuvered through our everyday activities. This study reveals the bacteriological profile of bacteria recovered from the hands and nasal swabs of food vendors at the University of Benin. The positive growth of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus* was confirmed by the cultural, morphological and biochemical characterization as shown in Table 1a and Table 1b. This is in agreement with the findings of Rodríguez-Vicente (2016). The recovery of *Staphylococcus aureus* and *Staphylococcus epidermidis* could be due to their presence as normal flora of the nose and human skin. These micro-flora are potential human pathogens but the presence of *B. cereus* among the nasal swab samples isolated demonstrates potential health risks as these organisms are implicated in food-borne diseases. The presence of these bacteria could be as a result of direct or cross-contamination from the open-air, utensils, vendor's hand or skin or water used (Rodríguez-Vicente, 2016). Several official reports have stated that *Bacillus cereus* and *Escherichia coli* are major causes of diarrhoea (Wagner, 2009).

S. aureus was found to be the most prevalent microorganism in the nasal swab and occurred at a frequency of 5(50%) while *S. epidermidis* occurred in hand swab at a frequency of 7(70%) corroborating earlier reports from Rodríguez-Vicente (2016). The least prevalent microorganisms occurred at a frequency of 2(20%) and 3(30%) which were *S. epidermidis* for nasal swab *and S. aureus* for hand swab respectively as shown in Figure 1a and Figure 1b.

Almost all isolated organisms except *Bacillus cereus* identified in this study were 17(100%) susceptible to gentamycin, ciprofloxacin, pefloxacin, septrin and streptomycin. *S. epidermidis* recovered from hand swab showed 6(85.7%) to pefloxacin, 5(71.4%) to gentamycin, 3(42.9%) to ampiclox, 2(28.6%) to rocephin as shown in Table 2a and Table 2b. This means that these antimicrobials are still the drug of choice for the management of food-borne illnesses in the locality which is inclined to the work of Temesgen *et al.* (2016). On the other hand, *S. aureus* 0(0%) evolved resistance to ampiclox, zinnacef and other tested antimicrobial drugs which would make the treatment of *S. aureus* infection difficult and this is also is in agreement with the findings of Temesgen *et al.* (2016). These resistant organisms may have been introduced through natural or anthropogenic causes within the natural environments and could have been attributed to poor sanitary and inadequate hand sanitation conditions as reported by Ibekwe *et al*. (2008). It is significantly notable that some of the resistant organisms recovered during this study may have transferred their resistance to recipient organisms supporting the earlier reports stated by Abdu *et al*. (2013) that even if these organisms are non- pathogenic like *Staphylococcus epidermidis*, they are easily capable of transferring their resistances to true pathogens within the gastrointestinal tract, this may be regarded as one way of acquiring antibiotic resistance.

Figure 2a and Figure 2b shows that the majority of the isolates were resistant to more than two of antibiotics where a high percentage of isolates had a MAR index greater than 0.2 which suggested that the isolates originated from a high-risk source of contamination which is in agreement with the findings of Onaolapo *et al*. (2016). The highest MAR index of 0.5 was seen in S.A H2 and S.A N3 isolates from *S. aureus* which were recovered from hand and nasal swabs respectively. This indicated that the isolates do not respond to the effect of the antibiotics. This could be attributed to the possession of multiple antibiotic genes as stated by Kaplan *et al*. (2005). In this respect, the detection of different virulence factors such as biofilm formation and capsule staining produced by *S. aureus*, *S. epidermidis* and *B. cereus* isolates were recorded. Biofilm formation is an important factor in the pathogenesis of *S. aureus*. In this study, 20(100%) of the total isolates showed biofilm formation whereas higher percentage of the isolate from the nasal swab 7(70%) presented strong adherence compared to other results reported by Khan *et al.* (2011) who showed that only 14.5% of isolates were strong biofilm producers. *S. epidermidis* showed positive capsulation to capsule staining technique which was seen as smooth colonies on nutrient agar correlating with the findings of Hughes and Smith (2007) of forming capsule which serves as a protective structure from phagocytosis and promotes the attachment of cells to surfaces seen in Table 3a and Table 3b.

CONCLUSION

This study demonstrated that resident bacteria are readily present in the hands and noses of food vendors but pathogenic bacteria are also present. Most of the bacteria showed multi-resistance to the antibiotics used, which is of public health concern. The results also buttressed the need for education of these food vendors on the health risks involved in poor personal hygiene. Therefore it is recommended that the food vendors should maintain standard environmental and personal hygiene while preparing and packaging or serving the food to the consumers which is essential to reduce the contamination rate. In addition, regular inspection of food vending practices and the safety of foods sold in the university is required to improve the health of consumers.

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