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ANALYSIS AND ANTIBIOGRAM STUDY OF MICROORGANISMS ASSOCIATED WITH FISH AND MEAT RETAIL SURFACES IN EBELLE, NIGERIA

*** Omotade Richard OGUNREMI [1], Enobong Sunday UKPONG [2], Aima Airenobuwa NOSA [2]**
[1]*Department of Biological Sciences, First Technical University, Ibadan, Nigeria, tadeogunremi@yahoo.com*
[2]*Department of Biological Sciences, Samuel Adegboyega University, Ogwa, Nigeria*
*Corresponding author
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Abstract: *Education and regulations of hygienic practices are poorly carried out in the informal sector of food supply chain in developing countries, especially rural communities. Therefore, this study was carried out to investigate microbiological status of fish and meat retail surfaces in Ebelle market. Four swab samples of fish and meat retail surfaces were collected and examined for microbiological load and profile. Safety of selected isolates was assessed by haemolytic analysis and antibiotic susceptibility profile tests. The total bacterial counts from the surfaces were within the range of 5.06 - 6.40 Log_{10} CFU/cm^2. Coliforms were detected only in fish retail surface swab samples; EF1 (3.96 Log_{10} CFU/cm^2) and EF2 (5.58 Log_{10} CFU/cm^2). EF2 had a total E. coli count of 4.78 Log_{10} CFU/cm^2. The identified species include Bacillus sp. (23.08%), Escherichia coli (7.69%), Proteus sp. (15.38%), Pseudomonas sp. (7.69%), Salmonella sp. (97.69%), Staphylococcus aureus (15.38%) and Streptococcus sp. (23.08%). The total heterotrophic fungal counts were between 2.27 and 3.29 Log_{10} cfu/cm^2. β-, α- and γ-haemolysis was exhibited by 9, 2 and 2 isolates respectively. Antibiotic susceptibility test showed that some isolates were multidrug resistant. The results obtained in this study indicate the retail surfaces are potential sources of food contamination. Therefore it is pertinent for regulatory and public health authorities to establish cleaning and sanitation guidelines and train rural food vendor and retailers on the steps and importance of cleaning and sanitation.*

Keywords: *Food safety, Food contact surface, Pathogens, Antibiotics resistance, Sanitation.*

1. Introduction

Microorganisms have evolved mechanisms of adhering and surviving on relatively dry surfaces, especially food contact surfaces [1, 2]. The nature of surfaces used in meat and fish retailing, storage and distribution include stainless steel, wood, plastic, concrete, and glass. These surfaces are subject to contamination by microorganisms and following failure to implement or comply with cleaning and sanitation guidelines, these surfaces are typically conditioned with nutrients and other organic materials that are sufficient for the attachment, viability and growth of microorganisms [2]. They develop microbial communities known as biofilms; microbial aggregates that are firmly attached to surfaces and embedded in exopolymers [3]. Biofilms are difficult to detach and resistant to biocidal agents. Therefore, conventional cleaning and sanitation regimes have not effectively decontaminated affected surfaces [3, 4].
Biofilm-laden processing, storage and distribution surfaces represent an important source of contamination for a wide variety of food materials with pathogenic and spoilage microorganisms [4]. Incidentaly, proper regulations and adherence to adequate hygienic and sanitation practices remains a challenge in the informal sector of the food supply chain in developing countries, especially the rural communities. This could be implicated in

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the high incidence of foodborne infectious diseases in Africa. The World Health Organization (WHO) estimates that 3 million infants die annually from the consumption of contaminated foods [2]. A recent estimate of global foodborne diseases with respect to incidence, mortality and Disability Adjusted Life Years (DALYs) showed that Africa has the highest burden of foodborne diseases per population [5]. DALYs were attributed to foodborne diarrhoeal disease agents, particularly *Salmonella* sp., *Shigella* spp., *Escherichia coli* and *Vibrio cholerae*.

There is a paucity of information on the microbiological status of food retailing surfaces in a typical Nigerian rural community. It is imperative to generate the baseline data for increased awareness of public health inspectors and food supply regulators about the need to train rural food handlers on the guidelines for cleaning and sanitizing food contact surfaces and good hygienic practices (GHP) and for effective monitoring of activities in the food supply chain. Therefore, this study was carried out to assess the burden and potential pathogenicity of microorganisms on fish and meat retail surfaces in Ebelle market, Nigeria.

2 Materials and Methods

2.1 *Samples*

Swab samples were aseptically taken from a defined area (25 cm^3) of fish and meat retail surfaces in Ebelle market, Igueben local government area, Edo state, Nigeria. The descriptions of the surfaces are presented in Table 1. The samples were collected using sterile cotton swabs, immediately shaken and sealed in tube containing 10 ml of 0.85% physiological saline solution according to NSW Food Authority [6]. The swab samples were immediately transported to the Microbiology Laboratory, Department of Biological Sciences, Samuel Adegboyega University, Edo state for microbiological analysis.

2.2 *Enumeration and isolation of microorganisms*

A 1 ml of appropriate serial dilutions of each swab solution was pour-plated in appropriate media and incubated at appropriate condition to obtain the counts of different groups of microorganisms on the surfaces in CFU/cm^2. Total aerobic bacteria, coliform and *Escherichia coli* counts were determined after incubation for 24 hours at 37°C, using nutirent agar, MacConkey agar and Eosine Methylene Blue agar respectively. Fungal counts were determined after incubation for 72 hours at 30°C, using Potatoes Dextrose agar, supplemented with streptomycin. Pure cultures of randomly selected bacterial colonies were obtained after repeated streaking on nutrient agar plates. The pure cultures were maintained on nutrient agar slants at 5°C and renewed at two weekly intervals.

2.3 *Characterization and identification of bacterial isolates*

Morphological examinations and biochemical tests were carried to determine the characteristics of pure bacterial cultures. They include; colony and cell morphology and Gram reaction, catalase, oxidase, indole and sugar fermentation tests [7]. The isolates were identified by comparing their morphological and biochemical characteristics with the characteristics of reference organisms as described in Bergey's Manual for Determinative Bacteriology.

2.4 *Haemolytic test*

Fresh bacterial cultures were streaked in triplicate on blood agar plates (5% (w/v) defibrinated sheep blood) and incubated at 37 °C for 24 hours. The plates were

examined for β-haemolysis (clear zones around colonies), α-haemolysis (green zone around colonies), and γ-haemolysis (no zones around colonies) [8].

2.5 *Antibiotic susceptibility profile*

The antibiotic sensitivity profile of each bacterial isolate was determined using the agar disc diffusion method of Kirby Bauer. Fresh culture of bacterial isolate was suspended in 0.85% sterilized physiological saline solution and adjusted to 0.5 McFarland turbidity standard, equivalents to 1.5 X 10^8 CFU/mL. Mueller-Hinton agar plates were seeded with 0.2 mL of bacterial suspension by spread plate method with the aid of sterilized cotton swab and left to dry for 15 minutes at room temperature. Commercially available antibiotics discs were aseptically placed on each seeded agar plates. The antibiotic discs were; Ampiclox (APX-30 µg), Amoxicillin (AMX) (25µg), Augmentin (AU) (30µg), Chloramphenicol (CH) (30µg), Ciprofloxacin (CPX) (5µg), Erythromycin (E-15 µg), Gentamycin (GEN-10 µg), Ofloxacin (OFX) (5µg), Perfloxacin (PEF-5 µg), Rifampicin (RA-5 µg), Septrin (SXT) (25µg), Sparfloxacin (SFX) (5µg), Streptomycin (STR) (10µg) and Zinnacef (Z-25 µg) for Gram-positive and/or Gram-negative bacteria. The plates were incubated for 24 hours at 37°C. The resultant diameter of visible zones of inhibition were measured in millimeters (mm) and classified as resistant (R), intermediate (I) or sensitive (S) in accordance to the guidelines of the Clinical and Laboratory Standard Institute [9].

2.6 *Statistical analysis*

Data was collected in duplicate and presented in mean ± standard deviation of replicate values. Counts were expressed in logarithmic units of microorganisms per centimeter square (log_{10} CFU cm^{-2})

3. Results and Discussion

3.1 *Nature of fish and meat retail surfaces*

Food contact surfaces in informal sectors of food supply chain are major concerns as significant risk factors for foodborne diseases [10]. With reference to microbiological perspective, the nature of food contact surfaces can help prevent accumulation of microorganisms and potential contamination of foods. Food contact surfaces should be smooth, non-absorbent and easy to clean and sanitize. In Ebelle market, the surfaces used for displaying and chopping fishes and meats were bare wooden or laid with papers or polyethylene (Table 1). Concerns with wooden and paper laid surfaces is the perviousness to moisture, roughness and difficulty cleaning them. Thereby creating conditions that promote microbial growth and contamination of foods.

Table 1
Characteristics of sampled retail surfaces in Ebelle market

Retail surface name	Surface code	Description of surface
Ebelle meat retail surface 1	EM1	Bare wooden surface
Ebelle meat retail surface 2	EM2	Bare wooden surface
Ebelle fish retail surface 1	EF1	Wooden surface covered with paper
Ebelle fish retail surface 2	EF2	Wooded surface covered with polyethylene

3.2 *Analysis of microorganisms*

The total bacterial counts on the different sampled surfaces, including fish and meat retail surfaces ranged from 5.06 to 6.40 Log_{10} CFU/cm^2. There were no significant differences in the total bacteria counts for different types of retail surfaces. The highest total bacteria count was recorded for EM2, a meat retail surface. Coliforms were detected only on fish retail surfaces, with counts ranging from 3.96 to 5.58 Log_{10} CFU/cm^2. Out of the retail surfaces

examined, only EF2, a fish retail surface was contaminated with *Escherichia coli*. Heterotrophic fungi were recorded for all the sampled surfaces, with counts ranging from 2.85 to 3.29 Log_{10} CFU/cm^2 (Table 2). The bacterial counts on fish and meat contact surfaces reported in this study are considered to be unacceptable, being significantly above the acceptable level of 3.0 CFU/cm^2 as recommended by US Public Health Service and The Public Health Laboratory Service (PHLS) in the UK [11, 12]. Consistent with studies of food contact surfaces in meat and fish processing facilities by Gounadaki *et al*. [13] and Lani *et al.* [14] respectively, our results showed >5 Log_{10} CFU/cm^2 and ~3 Log_{10} CFU/cm^2 of bacteria and fungi respectively on fish and meat retailing surfaces in Ebelle market. In a related study Uzendu *et al.* [15] reported >5 Log_{10} CFU/cm^2 total heterotrophic bacterial count before and after beef processing on slaughter slabs and evisceration tables. Besides, bacteria and molds greater than 4 Log_{10} CFU/cm^2 were reported on all surfaces sampled in some butchers' cold chain [16]. In agreement with this study, food contact surfaces in a child care center had generally low in *E. coli* and coliform counts [10].

Table 2

Microbial counts on meat and fish retail surfaces

SURFACE ID	Counts (Log_{10} CFU/$cm^{2)}$			
	Total Bacteria	**Total coliform**	***E. coli***	**Total heterotrophic fungi**
EM1	5.46	NIL	NIL	2.85
EM2	6.40	NIL	NIL	2.92
EF1	5.06	3.96	NIL	2.27
EF2	5.51	5.58	4.78	3.29

Analysis of morphological and biochemical features of 13 bacterial isolates recovered from the enumeration agar plates and comparison with published reference databases revealed 7 species on fish and meat retail surfaces. The identified species include *Bacillus* sp. (23.08%), *Escherichia coli* (7.69%), *Proteus* sp. (15.38%), *Pseudomonas* sp. (7.69%), *Salmonella* sp. (97.69%), *Staphylococcus aureus* (15.38%) and *Streptococcus* sp. (23.08%) (Fig. 1). Three of the 7 species, were found on fish retail surface, including *Bacillus* sp., *Proteus* sp. and *Staphylococcus aureus.* This indicates poor hygienic practice and ineffective sanitation and that the surfaces used to retail fish and meat are sources of contamination with pathogenic and spoilage microorganisms. Similar to this study, the reported bacterial pathogens and spoilers were previously reported from several food contact surfaces, including knives, chopping boards, holding tanks, pipes and milling, mixing, mincing and stuffing machines [2, 3, 16, 17, 18]. Mohammed *et al.* [19] reported the occurrence of *E. coli* on food contact surfaces in a restaurant. *Pseudomonas* spp., a prominent food spoilage microorganism was isolated from the surface of mixing machine used to produce keropok lekor', a traditional processed fish product in Malaysia [14] and meat cutting board in a Brazilian hospital [20].

The most predominant bacterial species on fish and meat retail surfaces are *Bacillus* sp. and *Streptococcus* sp. respectively. The persistence of *Bacillus* spp. on food contact surfaces is due to their resistance to desiccation, either in vegetative or spore form and biofilm production potentials

[21]. The predominance of *Bacillus* spp. was previously reported for different meat contact surfaces of two abattoirs in Ibadan, Nigeria [15].

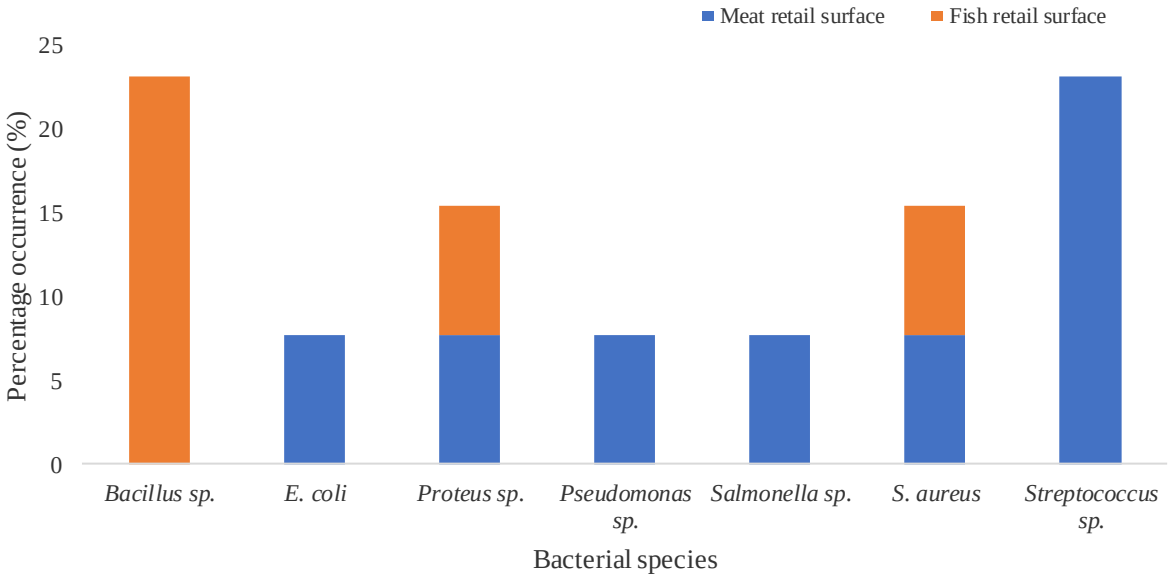

Fig. 1 Percentage occurrence of bacterial species isolated from meat and fish retail surfaces

3.3 *Haemolytic properties of isolates*

Nine isolates demonstrated β-haemolytic activities on blood agar, including *Bacillus* sp. (2 strains), *Proteus* sp. (1), *Pseudomonas* sp. (1), *Salmonella* sp. (1), *Staphylococcus aureus* (2) and *Streptococcus* sp. (2). *Escherichia coli* EM152 and *Proteus mirabilis* EM252 showed α-haemolysis while *Bacillus* sp. EF141 and *Streptococcus* sp. EM261 exhibited γ-haemolysis.

3.4 *Antibiotic resistance pattern of isolates*

Antibiotics susceptibility profiling of the 13 bacterial isolates revealed that 7, 5 and 2 out of 8 Gram-positive bacterial strains and 0, 3 and 1 out of 5 Gram-negative bacterial strains were resistant to amoxicillin, streptomycin and ciprofloxacin respectively (Table 4 and 5).

Table 3
Haemolytic activity of bacterial isolate isolated from meat and fish retail surfaces

Isolate code	Haemolysis
Staphylococcus aureus EM151	β
Escherichia coli EM152	α
Salmonella sp. EM161	β
Pseudomonas sp. EM241	β
Streptococcus sp. EM251	β
Proteus mirabilis EM252	α
Streptococcus sp. EM261	γ
Streptococcus sp. EM262	β
Bacillus sp. EF141	γ
Staphylococcus aureus EF143	β
Proteus vulgaris EF161	β
Bacillus sp. EF252	β
Bacillus sp. EF261	β

Key: β-haemolysis: clear zones around colonies, α-haemolysis: green zone around colonies, γ –haemolysis: no zones around colonies.

Resistance was also recorded by 5 and 6 Gram-positive bacterial strain against ampiclox and rifampicin. Significant resistance to septrin and chloramphenicol were each found in 3 Gram-negative bacterial strains. Predominant proportions of the Gram-positive and -negative strains were sensitive to ciprofloxacin, perfloxacin and gentamycin while exclusive sensitivity to amoxicillin was exhibited by Gram-negative strains (4/5) (Table 5). Antibiogram of the bacterial strains to 10 antibiotics revealed multidrug antibiotic resistance (MAR) index ranging from 0.1 to 0.5 (Table 4 and 5). The highest level of MAR index (0.5) was recorded for *Bacillus* sp. EF141, having multidrug resistance pattern against AMX^R, APX^R, CPX^R, RA^R and STR^R and *Salmonella* sp. EM161 against CH^R, CPX^R, SFX^R, STR^R and SXT^R.

Besides the implications of food spoilages and infections by microbial contaminants from the environment, microbial resistance to antibiotics is an important threat to human health, where treatment failures of foodborne infections are imminent. Food contact surfaces are potential sources of resistant bacteria or genes, where contributory factors such as sub-optimal application of biocides and genetic exchanges within biofilm communities prevails [2]. The resistance to ampiclox, amoxicillin, chloramphenicol, rifampicin, septrin or streptomycin reported in this study was previously reported for bacterial isolates from food contact surfaces by Lani *et al.* [14] and Mohammed *et al.* [19].

Table 4

Antibiotics susceptibility and multidrug resistance profile of Gram-positive bacterial isolate from meat and fish retail surfaces

Strain (8)	Antibiotics										MAR index
	PEF	GEN	APX	Z	AMX	RA	CPX	STR	SXT	E	
S. aureus EM151	S	S	I	I	I	I	S	R	S	I	0.1
Streptococcus sp. EM151	I	I	R	I	R	R	S	R	I	I	0.4
Streptococcus sp. EM261	S	S	R	S	R	I	S	I	I	I	0.2
Streptococcus sp. EM262	S	S	R	R	R	R	S	I	I	I	0.4
Bacillus sp. EF141	I	I	R	I	R	R	R	R	I	S	0.5
S. aureus EF143	S	S	I	I	R	R	S	R	I	S	0.3
Bacillus sp. EF252	I	S	I	I	R	R	S	R	S	S	0.3
Bacillus sp. EF262	S	S	R	I	R	R	R	S	S	S	0.4
Resistant	-	-	5	1	7	6	2	5	-	-	
Intermediate	3	2	3	6	1	2		2	5	4	
Susceptible	5	6	-	1	-	-	6	1	3	4	

Key: Perfloxacin (PEF-5 µg), Gentamycin (GEN-10 µg), Ampiclox (APX-30 µg), Zinnacef (Z-25 µg), Amoxicillin (AMX-25 µg), Rifampicin (RA-5 µg) Ciprofloxacin (CPX-5 µg), Streptomycin (STR-10 µg), Septrin (SXT-25 µg), Erythromycin (E-15 µg), R- Resistant, I- Intermediate, S- Susceptible, MAR- Multiple Antibiotics Resistance

Table 5

Antibiotics susceptibility and multidrug resistance profile of Gram-negative bacterial isolate from meat and fish retail surfaces

Strains (5)	Antibiotics										MAR index
	SXT	CH	SFX	CPX	AMX	AUG	GEN	PEF	OFX	STR	
E. coli EM152	I	I	S	S	S	R	S	I	S	S	0.1
Salmonella sp. EM161	R	R	R	R	I	S	S	I	I	R	0.5
Pseudomonas sp. EM241	R	R	I	S	S	I	I	S	S	R	0.3
Proteus mirabilis EM252	R	R	S	S	S	I	R	S	S	R	0.4
Proteus vulgaris EF161	I	S	S	S	S	S	S	S	S	S	0
Resistant	3	3	1	1	-	1	1	-	-	3	
Intermediate	2	1	1	-	1	2	1	2	1	-	
Susceptible	-	1	3	4	4	2	3	3	4	2	

Key: Septrin (SXT-25 µg), Chloramphenicol (CH-30 µg), Sparfloxacin (SFX-5 µg), Ciprofloxacin (CPX-5 µg), Amoxicillin (AMX-25 µg), Augmentin (AU-30 µg), Gentamycin (GEN-10 µg), Perfloxacin (PEF-5 µg), Ofloxacin (OFX-5 µg), Streptomycin (STR-10 µg), R- Resistant, I- Intermediate, S- Susceptible, MAR- Multiple Antibiotics Resistance

4. Conclusion

This study revealed high microbial load and the presence of microbial species of public health and economic importance on the surfaces used to display and chop fish and meat in a Nigerian rural market. This indicates the significance of these surfaces as sources of food contamination. Therefore it is pertinent for regulatory and public health authorities to establish cleaning and sanitation guidlines for food contact surfaces in rural markets. Rural food vendor and retailers should be adequately trained on the steps and importance of cleaning and sanitation. Public health authorities should implement an effective monitoring programme, where routine verification for cleanliness and sanitation, including visual inspection and microbiological analysis of surface that come in contact with foods that are meant for pulic consumption are carried out.

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