

BACTERIAL EMPIRE

2019, VOL. 2, NO. 3, 58-63



REGULAR ARTICLE

MICROBIAL ASSESSMENT OF READY-TO-EAT FOOD AND FOOD CONTACT SURFACES IN SELECTED RESTAURANTS IN OKADA, SOUTH -SOUTH NIGERIA

Akpoka, A.O. 1, Okwu, M.U. 1, Imade, O.S. 1, Enaighe, A.A. 1, Solanke, E.O. 1, Erifeta, G.O. 1, Izevbuwa, E.O. 1,

Address (es): Akpoka Augustine Obhioze,

- ¹Department of Biological Science, College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria.
- ² Department of Biochemistry, College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria.

*Corresponding author: ausbones@gmail.com

ABSTRACT

Good food hygiene ensures that the preparation and preservation of foods are safe for human consumption. It ensures that food is prevented from microbial contamination at the levels of production to consumption. The aim of this study was to determine the microbial content of ready-to-eat cooked food, ready-to-use serving plates and hands of food handlers in six selected restaurants in Okada, Edo State, Nigeria. The samples were aseptically collected from the restaurants and taken to the microbiology laboratory of Igbinedion University, Okada for analysis. The microbial content of the samples was identified by standard microbiological methods. The microorganisms isolated were *Enterobacter* species, *Streptococcus* species, *Micrococcus* species, *Bacillus* species, *Staphylococcus aureus* and *Saccharomyces* species. The total aerobic viable counts, total coliform counts and total *Staphylococcus* counts in the ready-to-eat cooked food from the six restaurants were $3.67 \pm 0.33 \times 10^2 \text{ cfu/g} - 2.71 \pm 0.05 \times 10^4 \text{ cfu/g}; 3.33 \pm 0.33 \times 10^2 \text{ cfu/g} - 2.39 \pm 0.04 \times 10^4 \text{ cfu/g}$ and $0.00 \pm 0.00 \text{ cfu/g} - 3.70 \pm 0.21 \times 10^3 \text{ cfu/g}$ respectively. The concentration of microbes on the food contact surfaces from ready-to-use serving plates were $0.00 \pm 0.00 \text{ cfu/cm}^2 - 14.67 \pm 0.33 \text{ cfu/cm}^2$ and $0.00 \pm 0.00 \text{ cfu/cm}^2 - 22.67 \pm 0.33 \text{ cfu/cm}^2 - 22.67 \pm 0.33 \text{ cfu/cm}^2$ in the hands of the food handlers. Therefore, foods provided to consumers at these restaurants are not of acceptable microbiological quality.

Keywords: Foodborne disease, Food intoxication, Food poisoning, Food Hygiene practice, Public health, Exposure Measurement

INTRODUCTION

Food hygiene is an important public health problem and as such has drawn the attention from several sectors with efforts being intensified to improve food safety. These efforts became necessary as a response to the increasing number of food safety problems and rising consumer concerns with food and waterborne diseases which has been reported to kill about 2.2 million people annually (Akonor and Akonor, 2010). Food hygiene practices describe the preservation and preparation of food in a way that ensures the food is safe for human consumption. It also encompasses the prevention of contamination of food at the level of production, collection, transportation, storage, preparation, sale and consumption (CAC/RCP, 1997). The World Health Organization (WHO) defines foodborne illness as a disease, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food. It has a high incidence rate, is widespread, endemic and an increasing public health concern in both developed and developing countries (World Health Organization, 2011). The WHO general principle of food hygiene basically includes: Preventing the contamination of food with pathogens spreading from people, pets and pests by separating raw foods from cooked foods; cooking food within the appropriate duration of time and temperature to kill most pathogens; and the storage of food under appropriate conditions (WHO, 2010). Kitchen safety includes proper storage of food items prior to use, maintaining a clean environment when preparing the food and ensuring all serving dishes are free of any type of contamination. As a scientific discipline, food safety describes the handling, preparation and storage of food in ways that prevent foodborne illnesses (CAC/RCP, 1997).

Food handling personnel play an important role in ensuring food safety throughout the chain of food production, processing, storage and preparation. The mishandling and disregard to hygienic measures on the part of food vendors have been reported to introduce contaminants and pathogens that survive and multiply in sufficient numbers to cause illness in the consumer (WHO, 1989; Greig et al., 2007; Todd et al., 2007a; 2007b). Although, food contamination may occur at any point of processing or preparation, food handlers play a critical role in the occurrence and spread of foodborne illnesses as their hands and other body parts may harbor microorganisms. Their actions may also compromise the chain of safety. Food handlers and other factors like improper food preparation practices, contaminated equipment, unsafe keeping of food (temperature and time), poor personal hygiene and food from unsafe sources are major causes of foodborne illnesses (Akonor and Akonor, 2010). A food handler refers to anyone who works in a food business and either handles food or surfaces that are likely to be in contact with food such as cutlery, plates and bowls. They may be involved in one or more activities that includes making, cooking, preparing, serving, packing,

displaying and storing food. Food handlers can as well be involved in manufacturing, producing, collecting, extracting, processing, transporting, delivering, thawing or preserving food (FSANZ, 2019). Food, when not properly handled can serve as good growth medium and depending on the type could support a broad range of pathogenic microorganisms capable of causing potentially severe health hazards like food poisoning, intoxication or death. Food sanitation, which involves the keeping of the food preparation area clean and relatively germ-free, when properly executed significantly reduce the chances of foodborne infection. This is possible because the contamination of food most commonly occurs via faeco-oral transmission by mechanical (biological) vectors or contaminated hands. The origin of microbial contaminants may either be environmental, natural or technological (Cowan, 2016; Tortora, 2016).

Ready-to-eat food is not a nominated food or class of food within Standard. This Product group is defined as: Food that is ordinarily consumed in the same state as that in which it is sold and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer (Australia New Zealand Food Standards Code, 2017). Ready-to-eat foods usually include several ingredients which may or may not be cooked. Some ready-to-eat foods are regarded as 'potentially hazardous'. Such foods can support the growth of pathogenic (food poisoning) bacteria and must be kept at certain temperatures to minimize the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food (NSW, 2009). Consumers purchase ready-to-eat food with a rational expectation that they were prepared, produced, processed or packaged under hygienic conditions and not contaminated or adulterated by any biological, chemical or physical hazards. These expectations are 'supposed' to be regularly enforced by regulations that govern production, processing, distribution and retailing of food and drugs in any country. In Nigeria, this task is performed by National Agency for Food Drug Administration and Control (NAFDAC) and the hygiene aspect handled at the local levels by the Public Health Care (PHC) and Environmental Health units of the various Local Government Councils. Governments all over the world have intensified their efforts to improve food safety in response to the increasing number of foodborne diseases. Due to the variety of ready-to-eat foods, the interpretation of microbiological results obtained from testing must account for the method of processing and the individual components of the food. To assist with interpreting the microbiological analyses of such foods as part of our monitoring and surveillance program (i.e. surveys), the NSW Food Authority uses criteria that are based on interpretive guides published by the United Kingdom's Health Protection Agency and by Food Standards of Australia, New Zealand (FSANZ, 2001; NSW, 2009).

In Nigeria, as in other developing countries, most foodborne disease outbreaks are either underreported, underestimated or unreported. Despite the data gaps and

limitations of these initial estimates, it is apparent that the global burden of foodborne diseases is considerable, and affects individuals of all ages. The impact of unsafe food is estimated to cost low and middle-income economies about \$110bn in lost productivity and medical expenses each year. An estimated 600 million, (almost 1 in 10 people in the world) fall ill after eating contaminated food and with a mortality of 420,000 annually, resulting in the loss of 33 million healthy life years (WHO, 2019). This global burden of food-borne disease is unequally distributed globally. Relative to their population, low- and middleincome countries in South Asia, Southeast Asia, and sub-Saharan Africa bear a proportionately high burden. Although the aforementioned regions account for 41 per cent of the global population they account for 53 and 75 percent of all foodborne illnesses and related deaths globally. Young children are most susceptible to unsafe food. Children under five make up only nine per cent of the world's population but account for almost 40 per cent of food-borne disease and 30 percent of related deaths (The World Bank, 2018). The total productivity loss associated with food-borne diseases and the annual cost of treating food-borne illnesses in low and middle-income countries are estimated at \$95.2bn per year, is \$15bn respectively (The World Bank, 2018). This work, therefore, is aimed at carrying out a microbial assessment of the ready-to-eat cooked food and their contact surfaces in selected restaurants in the study area.

MATERIALS AND METHODS

Study Sites

Six restaurants mostly patronized by consumers in Okada, Edo State, Nigeria were selected for this study. Okada is the headquarters of Ovia North-East Local Government Area of Edo State, Nigeria. It has an area of 2,301 km², geographically located on Latitude 6.73469° and Longitude 5.39083° with a population of 153,849 according to the 2006 census. It is home to Igbinedion University whose student population contributes a sizeable percentage of the population. The occupation in Okada is predominantly farming.

Collection of Samples

Three category of samples were collected for the analysis viz: Ready-to-eat cooked food, particularly rice and soup, herein referred to as 'ready-to-eat food', washed serving plates herein referred to as 'ready-to-use serving plates' and from the hands of food handlers/servers in the selected restaurants.

The serving plates' and hands of the food handlers/servers was done by swabbing 25 cm² area of their contact surfaces with 5 sterile swab sticks according to the method specified by ISO 18593:2018 (ISO, 2018). The area of sampling was delimited by sterile templates (an improvised wire that was used to properly define the area of sampling).

After swabbing, the swab sticks were put into a sterile container containing 10 ml of 2 % W/V sterile peptone water.

These samples were stored in sterile containers from the sampled restaurants and were immediately transported to the microbiology laboratory of Igbinedion University where they were analyzed within 6 hours of collection. One-gram portion of each sample was used to prepare 10-fold serial dilutions to 10⁻⁵ in 1.5 % W/V sterile peptone water, after which 0.1 ml of each diluted suspension was subsequently spread on triplicate petri-plates containing sterile solidified media.

Isolation of Microbes

Isolation of microbes from food samples and contact surfaces of food was performed by spread plating method (APHA, 1998) using both general purpose medium (nutrient agar) and selective/differential media (MacConkey agar complemented with crystal violet, and mannitol salt agar). After incubation of agar plates at 37 $^{\circ}$ C for 18 to 24 hours, distinct colonies seen on the plates were then enumerated and identified.

Identification and Characterization of Microbes

Phenotypic identification of microbes was performed according to standard methods (Barrow and Feltham, 2003). Morphological traits examined include

the orientation, size, and pigmentation which were performed by visual inspection of microbial isolates on petri-plates, as well as cell wall characteristics which was performed by Gram staining of the isolates. Biochemical traits examined include: the production of coagulase enzyme (coagulase test); the production of catalase enzyme (catalase test); the production of urease enzyme (urease test); biodegradation of tryptophan to produce indole (indole test); utilization of citrate as a sole carbon source (citrate test); production of stable acids from glucose fermentation (methyl red test); production of acetoin as the main end product with small quantities of mixed acids from glucose metabolism (Voges Proskauer test); and production of haemolysins (hydrolysis test).

Exposure Assessment

Exposure assessment was used to quantitatively evaluate the impact of hygiene practices by restaurants situated in Okada, Edo state, Nigeria (Cassini et al., 2016). The probability of exposure of consumers to pathogenic microbial species isolated from ready-to-eat food samples and food contact surfaces such as ready-to-use serving plates and hands of food handlers was deduced from the prevalence of pathogenic species present in these samples; while the concentration of the microbial isolates in the examined samples was used to deduce the extent of contamination in the restaurants. Parameters such as total aerobic viable counts (indicator of the overall hygiene status in the restaurants), total coliform counts (indicator of potential faecal contamination in the restaurants), and total presumptive Staphylococcus counts (indicator of unsanitary food handling in the restaurants) were used to extensively measure the extent of contamination in the restaurants.

The microbial counts were performed using the spread plate method (**APHA**, **1998**). Total aerobic viable counts (TAVC), total coliform counts (TCC), and total presumptive *Staphylococcus* counts (TSC) were carried out by spread plating of the samples on sterile nutrient agar, MacConkey agar supplemented with crystal violet, and mannitol salt agar respectively. The microbial counts of the food samples were then deduced using the following:

$$Microbial counts = \frac{number of colonles counted}{volume of sample inoculated} \times dilution factor$$

$$Microbial counts = \frac{number of colonies counted}{volume of sample inoculated} \times$$
(1)

dilution factor

Where: Microbial counts were expressed as colony forming units per gram of food samples (cfu/g). Dilution factor is expressed as the reciprocal of specific dilution counted $\frac{1}{d}$.

The microbial counts in the food contact surfaces were deduced using the following equation:

$$Microbial\ count = \frac{Number\ of\ colonies\ counted}{Area\ equivalent\ of\ the\ volume\ of\ inoculated\ sample} \tag{2}$$

Where: Microbial count was expressed as colony forming units per square centimeter of contact surface (cfu/cm²).

Area equivalent of volume of inoculated sample = volume of inoculated sample X Total area of sampled surface

Total volume equivalent of swabbed area
Area equivalent of volume of inoculated sample =
Volume of inoculated sample X Total area of sampled surface

Total volume equivalent of swabbed area

Given that: Volume of inoculated sample = 0.1 ml Total area of sampled surface = 25 cm² Total volume equivalent of swabbed area = 10 ml

RESULTS

(3)

Table 1a Phenotypic characterization of microbial isolates obtained from Ready-to-eat food sold in selected restaurants in Okada

Sample	Sources of Microbes	Isolates	Cultural Examinations						
locations	Sources of Microbes	isorates	NA		MA		MSA		
Restaurant 1	Ready-to- eat fried rice	IU1	Non-pigmented colonies	mucoid	Lactose colonies	fermenting	No growth of I	Bacteria	
Restaurant 2	Ready-to- eat fried rice	EF1	Non-pigmented colon	ies	Lactose colonies	fermenting	No growth of I	Bacteria	
Restaurant 3	Ready-to- eat soup	MC1	Non-pigmented large colonies	mucoid	Lactose colonies	fermenting	No growth of I	Bacteria	
D 4	Decide to set com	FB1	Non-pigmented colonies	mucoid	NP		Non-mannitol colonies	fermenting	
Restaurant 4	Ready-to- eat soup	FB2	Translucent patchy co	olonies	Lactose colonies	fermenting	NP		
Restaurant 5	Ready-to- eat Jollof	MB1	Non-pigmented colonies	mucoid	NP		Mannitol colonies	fermenting	
Restaufallt 3	rice	MB2	Non-pigmented colonies	mucoid	Lactose colonies	fermenting	NP		
Restaurant 6	Ready-to- eat fried rice	AG1	Non-pigmented colonies	mucoid	Lactose colonies	fermenting	No growth of I	Bacteria	

Legend: NA: nutrient agar; MA: MacConkey agar; MSA: mannitol salt agar; NP: Not performed;

Table 1b Phenotypic characterization of microbial isolates obtained from Ready-to-eat foods sold in selected restaurants in Okada

Sample		C	Biochemical Examinations								Probable Microbes
locations	Isolates	Gram staining of Colonies	Со	Ca	Ur	Ci	Mr	Vp	In	Hm	
Restaurant 1	IU1	Gram positive rods in chains	Np	+	+	+	Np	Np	Np	γ	Bacillus species
Restaurant 2	EF1	Gram negative rods	Np	+	+	-	-	-	-	γ	Enterobacter species
Restaurant 3	MC1	Gram positive cocci in pairs	-	-	Np	Np	Np	Np	Np	β	Streptococcus species
D 4 44	FB1	Gram positive cocci	-	+	Np	Np	Np	Np	Np	γ	Micrococcus species
Restaurant 4 FB2	FB2	Gram negative rods	Np	+	+	-	-	-		γ	Enterobacter species
Restaurant 5	MB1	Gram positive cocci in bunches	+	+	Np	Np	Np	Np	Np	β	Staphylococcus species
M	MB2	Gram negative rods	Np	+	+	-	-	-	-	γ	Enterobacter species
Restaurant 6	AG1	Gram positive cocci in pairs	Np	+	+	-	-	-	-	γ	Enterobacter species

Legend: Co: Coagulase test; Ca: Catalase test; Ur: Urease test; In: Indole test; Mr: Methyl red test; Vp: Voges Proskauer test; Ci: Citrate test, Hm: Haemolysis test; -: negative reaction; +: positive reaction; NP: Not performed; β represents complete haemolysis; γ represents no haemolysis; sp. represents species.

Table 2a Phenotypic characterization of microbial isolates obtained from the hands of Ready-to-eat food handlers in selected restaurants in Okada

Comple leastions	Isolates	Cultural Examinations				
Sample locations	isolates	NA	MA	MSA		
Restaurant 1	IUH1	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP		
Restaurant 1	IUH2	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies		
Restaurant 2	EFH1	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies		
Restaurant 2	EFH2	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP		
Restaurant 3	MCH1	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP		
Restaurant 5	MCH1	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies		
Restaurant 4	FBH1	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies		
Restaurant 4	FBH2	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP		
Restaurant 5	MBH1	Yellow pigmented colonies	NP	Non- mannitol fermenting		
		1 &	·	colonies		
Restaurant 6	AGH1	Large mucoid colonies	Lactose fermenting colonies	NP		
Restaurant 6	AGH2	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies		

Legend: NA: nutrient agar; MA: MacConkey agar; MSA: mannitol salt agar; NP: Not performed

Table 2b Phenotypic characterization of microbial isolates obtained from the hands of Ready-to-eat food handlers in selected restaurants in Okada

Sample Isolates		Gram staining of Colonies -		hemic	al Exai	Duckahla Mianakaa					
locations	Ca			Ur	Ci	Mr	Vp	In	Hm	 Probable Microbes 	
Restaurant 1	IUH1	Gram negative rods	Np	+	+	+	-	-	-	γ	Enterobacter species
Restaurant 1	IUH2	Gram positive cocci in clusters	+	+	Np	Np	Np	Np	Np	β	Staphylococcus aureus
Restaurant 2	EFH1	Gram positive cocci in clusters	+	+	Np	Np	Np	Np	Np	β	Staphylococcus aureus
EFH2	Gram negative rods	Np	+	+	+	-	-	-	γ	Enterobacter species	
Restaurant 3	MCH1	Gram negative rods	Np	+	+	+	-	-	-	γ	Enterobacter species
MCH1	MCH1	Gram positive cocci in clusters	+	+	Np	Np	Np	Np	Np	β	Staphylococcus aureus
Restaurant 4	FBH1	Gram positive cocci in clusters	+	+	Np	Np	Np	Np	Np	β	Staphylococcus aureus
Restaurant 4	FBH2	Gram negative rods	Np	+	+	+	-	-	-	γ	Enterobacter species
Restaurant 5	MBH1	Gram positive cocci		+	Np	Np	Np	Np	Np	γ	Micrococcus species
Restaurant 6	AGH1	Gram positive cocci in pairs	-	-	Np	Np	Np	Np	Np	β	Streptococcus species
	AGH2	Gram positive cocci in clusters	+	+	Np	Np	Np	Np	Np	β	Staphylococcus aureus

Legend: Co: Coagulase test; Ca: Catalase test; Ur: Urease test; In: Indole test; Mr: Methyl red test; Vp: Voges Proskauer test; Ci: Citrate test, Hm: Haemolysis test; -: negative reaction; +: positive reaction; NP: Not performed; β represents complete haemolysis; γ represents no haemolysis; sp. represents species.

Table 3a Phenotypic characterization of microbial isolates obtained from Ready-to-eat food serving plates used in restaurants in Okada

Comple leastions	Isolates	Cultural examinations						
Sample locations	isolates	NA	MA	MSA				
Restaurant 1	IUP1	Non- pigmented mucoid colonies	Lactose fermenting colonies	No bacteria growth				
Restaurant 2	EFP1	Non- pigmented mucoid colonies	Lactose fermenting colonies	No bacteria growth				
Restaurant 3	MCP1	Non- pigmented mucoid colonies	Lactose fermenting colonies	No bacteria growth				
Restaurant 4	FBP1	Non- pigmented large mucoid colonies	Lactose fermenting colonies	No bacteria growth				
Restaurant 5	MBP1	Large mucoid colonies	Lactose fermenting colonies	No bacteria growth				
Restaurant 6	AGP1	Large mucoid colonies	Lactose fermenting colonies	No bacteria growth				

Legend: NA: nutrient agar; MA: MacConkey agar; MSA: mannitol salt agar.

Table 3b Phenotypic characterization of microbial isolates obtained from Ready-to-eat food serving plates used in restaurants in Okada

Sample				chem	ical e	xami	_					
locations	Isolates	Gram staining of Colonies	C	C	\mathbf{U}	C	\mathbf{M}	\mathbf{V}	I	H	Probable Microbes	
locations				a	r	i	r	p	n	m		
Restaurant 1	IUP1	Gram negative rods	N p	+	+	+	-	-	-	γ	Enterobacter species	
Restaurant 2	EFP1	Gram negative rods	N p	+	+	+	-	-	-	γ	Enterobacter species	
Restaurant 3	MCP1	Gram negative rods	N p	+	+	+	-	-	-	γ	Enterobacter species	
Restaurant 4	FBP1	Gram positive oval shaped cells mainly in	N	N	N	N	Np	N	N		Saccharomyces	
Kestaurant 4	LDLI	pairs		p	p	p	мр	р	p	γ	species	
Restaurant 5	MBP1	Crom mositive essei in mains			N	N	Np	N	N	O	Ctuanta a a a aug anasia a	
Kestaurant 5	MDP1	Gram positive cocci in pairs	-		p	p	мр	p	p	Р	Streptococcus species	
Restaurant 6	Restaurant 6 AGP1 Gram positive cocci in pairs	_		N	N	NIm	N	N	ß	Stuanta a a a aug an a aiga		
Acstaurant 0	AUFI	Grain positive cocci in pans			p	p	Np	p	p	Р	Streptococcus species	

Legend: Co: Coagulase test; Ca: Catalase test; Ur: Urease test; In: Indole test; Mr: Methyl red test; Vp: Voges Proskauer test; Ci: Citrate test, Hm: Haemolysis test; -: negative reaction; +: positive reaction; Np: Not performed; β represents complete haemolysis; γ represents no haemolysis; sp. represents species.

Results of the phenotypic characterization of microbes isolated from the ready-to-eat food samples, the of the food handlers and the ready-to-use serving plates are presented in Tables 1, 2, and 3. *Enterobacter* and *Streptococcus* species were found in all the samples examined. *Staphylococcus* and *Micrococcus* species were.

Table 4 Concentration of microbes in ready-to-eat cooked foods sold in some restaurants in Okada

Sources of microbes	Sample	Concentration of Microf	lora	
Sources of microbes	locations	Mean TAVC (cfu/g)	Mean TCC (cfu/g)	Mean TSC (cfu/g)
Ready-to-Eat fried rice	Restaurant 1	$2.71 \pm 0.05 \times 10^4$	$2.39 \pm 0.04 \times 10^4$	0.00 ± 0.00
Ready-to-Eat fried rice	Restaurant 2	$3.67 \pm 0.33 \times 10^{2}$	$3.33 \pm 0.33 \times 10^{2}$	0.00 ± 0.00
Ready-to-Eat soup	Restaurant 3	$4.80 \pm 0.15 \times 10^3$	$1.00 \pm 0.17 \times 10^3$	0.00 ± 0.00
Ready-to-Eat soup	Restaurant 4	$2.60 \pm 0.12 \times 10^3$	$8.67 \pm 1.20 \times 10^2$	$3.70 \pm 0.21 \times 10^3$
Ready-to-Eat 'Jollof' rice	Restaurant 5	$1.15 \pm 0.03 \times 10^4$	$7.33 \pm 1.20 \times 10^2$	$6.60 \pm 0.29 \times 10^3$
Ready-to-Eat fried rice	Restaurant 6	$4.67 \pm 0.67 \times 10^{2}$	$3.33 \pm 0.33 \times 10^{2}$	0.00 ± 0.00

TAVC: Total aerobic viable counts; TCC: Total coliform counts; TSC: Total *Staphylococcus* counts; Mean ± SE (Standard error); cfu: colony forming units; cm²: square centimeter.

Table 5 Concentration of microbes in the food contact surfaces of some restaurants in Okada

Sources of	Sample	Concentration of microflora	a	
Microbes	Locations	Mean TAVC (cfu/cm²)	Mean TCC (cfu/cm²)	Mean TSC (cfu/cm²)
	Restaurant 1	12.33 ± 0.33	1.33 ± 0.33	5.00 ± 0.58
	Restaurant 2	3.33 ± 0.33	1.00 ± 0.00	3.00 ± 0.00
Hands of	Restaurant 3	22.67 ± 0.33	0.00 ± 0.00	20.33 ± 0.33
food handlers	Restaurant 4	10.67 ± 0.33	0.00 ± 0.00	8.00 ± 0.00
	Restaurant 5	6.67 ± 0.33	0.00 ± 0.00	5.00 ± 0.00
	Restaurant 6	14.33 ± 0.33	0.00 ± 0.00	12.33 ± 0.33
	Restaurant 1	14.67 ± 0.33	0.67 ± 0.33	0.00 ± 0.00
	Restaurant 2	5.67 ± 0.33	1.00 ± 0.00	2.33 ± 0.33
Ready-to-Use	Restaurant 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
serving plates	Restaurant 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Restaurant 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Restaurant 6	12.67 ± 0.33	1.33 ± 0.33	0.00 ± 0.00

TAVC: Total aerobic viable counts; TCC: Total coliform counts; TSC: Total Staphylococcus counts; Mean \pm SE (Standard error); cfu: colony forming units; cm²: square centimeter.

The concentration of microbes in the ready-to-eat food and food contact surfaces (the hands of the food processors and the ready-to-use serving plates) is presented in Tables 4 and 5. The mean TAVC of microbes in the ready-to-eat food samples ranged between $3.67\pm0.33\times10^2$ cfu/g to $2.71\pm0.05\times10^4$ cfu/g. The mean TCC ranged between $3.33\pm0.33\times10^2$ cfu/g to $2.39\pm0.04\times10^4$ cfu/g, while the mean TSC were between 0.00 ± 0.00 cfu/g and $6.60\pm0.29\times10^3$ cfu/g. In the food contact surfaces, the mean TAVC ranged between 0.00 ± 0.00 cfu/g (as recorded in the ready-to-use serving plates) to 22.67 ± 0.33 cfu/cm² (as recorded in the hands of the food handlers). Mean TCC were between 0.00 ± 0.00 cfu/cm² and 1.33 ± 0.33 cfu/cm², while the values of the mean TSC ranged from 0.00 ± 0.00 cfu/cm² to 20.33 ± 0.33 cfu/cm².

DISCUSSION

The study was designed to determine the microbial quality of ready-to-eat cooked foods in some selected restaurants in Okada. The main aim was to use this means to evaluate the level of personal and environmental hygiene in the selected restaurants. The organisms isolated were *Enterobacter* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Saccharomyces* sp. and *Staphylococcus aureus* (Tables 1, 2, and 3). The bacterial load in some of the ready-to-eat foods and some ready-to-use serving plates (Tables 4 and 5) indicated that the ready-to-eat foods served to consumers and the plates used in serving in some of the restaurants were of unacceptable quality (ICMSF, 1996). The isolation of *Bacillus* sp, and *S. aureus* corroborates the findings of Nichols *et al.* (1999); Mensah *et al.* (2002); Idowu (2006); Taulo *et al.* (2008); Oranusi *et al.* (2013) that these organisms amongst others are routinely isolated from ready-to-eat foods prepared under similar conditions.

Bacillus species, one of the isolated organisms is widely found in soil with some strains as spores in carcasses and animal products. It can produce toxins capable of causing two types of illness characterized by diarrhea, nausea and vomiting. However, the strain isolated from the restaurant was γ (gamma) hemolytic which indicates a likelihood of not being pathogenic. The occurrence of Bacillus sp. in the foods could be as a result of it being a spore former. Their heat-resistant spores may have survived processing while vegetative cells were eliminated. Contamination of foods could have resulted from inappropriate processing, inadequate heating, or secondary contamination via contact with contaminated equipment and utensils (Gopal et al., 2015).

Enterobacter was also isolated and can be found on the human skin, plants, soil, water, sewage, intestinal tracts of humans and animals, and some dairy products. Isolating this from food or contact surface indicates faecal contamination or at least confirms poor hygiene practice.

Streptococcus sp. has been implicated in many food poisonings with associated symptoms manifesting 12 to 72 hours with symptoms like sore throat, fever, nausea, vomiting, stuffy nose and a rash. Streptococci sp. are widely distributed in nature and frequently form part of the normal human flora (WHO, 2010).

Micrococcus is a relatively harmless bacteria found in soil, water, and meat products and capable of causing food poisoning through its enterotoxins. On the other hand, Staphylococcus is ubiquitous. It is very common on skin, and can also be found in the nasal passages, throats, etc. Virulent strains produce enterotoxins that can cause staphylococcal food poisoning, usually transmitted by the ingestion of toxins in contaminated food. It is characterized by a short latency period and resolution of symptoms after 24–48 hours. The incubation period is 1 – 4 hours. Clinical symptoms include nausea, vomiting, abdominal discomfort and diarrhea (CDC, 2018). Saccharomyces sp. was also isolated but not known to cause food poisoning.

High concentrations of coliforms in food is usually associated with food poisoning and corresponding symptoms (WHO, 1993). Consequently, the mere presence of coliforms at high concentrations even without the associated presence of well-known enteropathogens is enough to establish poor hygiene practices in the sampled restaurants.

Although *E. coli* was not detected, the presence of *Enterobacter* could also be an indication of possible faecal contamination of food, water or food handlers and poor hygienic processing practices (Little *et al.*, 1998; Tambekar *et al.*, 2007). The presence of *S. aureus* is largely as a result of human contact and this suggests poor hygiene practices of the operators since this organism is a normal flora of the skin and nasal passage (Garret, 1988; Nichols *et al.*, 1999). Akpoka *et al.* (2019) reported the following prevalence of microbial isolates from ready to eat foods (*Bacillus* species (12.50 %), *Enterobacter* species (50 %), *Streptococcus* species (12.50 %), *Micrococcus* species (12.50 %), *Staphylococcus aureus* (12.50 %)); the hands of ready to eat food servers (*Enterobacter* sp. (36.1 %),

Streptococcus sp. (9.09%), Micrococcus sp. (9.09%), Staphylococcus aureus (45.46%)); The ready-to-use serving plates (Streptococcus (33.3%), Enterobacter (50%) and Saccharomyces (16.1%) in the same study area.

It is mandatory that foods must be free from contaminants as much as possible. The presence of these isolates demonstrates a potential health risk as these organisms are pathogenic and have been implicated in foodborne diseases (Granum, 2005; Wagner, 2009; CFIA, 2009).

Foodborne illness can be prevented by good hygiene practices such as the use of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) application in the chain of food production and processing. Education of the food handlers/food vendors on food safety practices and a close and stringent supervision of ready-to-eat foods sold in these restaurants by relevant authorities are required to prevent foodborne illness and the authors confirmed that these measures are not presently in place or enforced in the study area.

CONCLUSION

The presence of any of these isolates establish a potential health risk as these organisms are pathogenic and are often implicated in foodborne diseases. The Ready-to-eat foods, the hands of food handlers and utensils must be free from these microorganisms as well as other contaminants as much as possible. The organisms isolated from the various sample types indicates a high possibility of cross contamination of the food from the hands of food handlers and 'ready-to-use' utensils. The ready to eat food, ultimately presented the greatest potential hazards to the consumer in the study area. However, the other contact surfaces contributed significantly to that hazard because the proper food hygiene practices and kitchen safety were not in place.

Acknowledgement: We gratefully thank the Department of Biological Sciences, Igbinedion University, Okada for making their laboratory facilities available to us. We also thank Suru Micheal for assisting in the samples collection.

Funding Information: The author(s) received no specific funding for this work.

REFERENCES

Akonor, P. T. & Akonor, M. A. (2013). Food safety knowledge: the case of domestic food handlers in Accra. *Eur. J. of Nutri. & Food safety*, 3(3), 99-111. Doi: https://doi.org/10.9734/EJNFS/2013/3227.

APHA (2005). Standard Methods for Examination of Water and Wastewater, 21st ed., American Public Health Association WWA, Washington, D.C. p. 38. https://ajph.aphapublications.org/doi/pdf/10.2105/AJPH.85.8_

Akpoka, A. O., Imade, O. S., Okwu, M. U. & Solanke, E. O. (2019). The Prevalence of foodborne pathogens recovered from ready-to-eat food from restaurants in Okada, Edo state, Nigeria, *Bacteria Empire*, 2 (2), 37-4. https://office.scicell.org/index.php/BE/article/view/6

Australia New Zealand Food Standards Code (2017). Standard 1.1.2. Standard under the Food Standards Australia New Zealand Act 1991 https://www.legislation.gov.au/Details/F2017C00715

Barrow, G. I. & Feltham, R. K. A. (2003). Cowan and Steel's Manual of Medical Bacteria. 3rd ed. p. 352. Cambridge University Press. Doi: https://doi.org/10.1017/CBO9780511527104

CAC/RCP (1999). Recommended international code of practice general principles of food hygiene. 1-1969, Rev. 3, 1997. Amended 1999. http://www.fao.org/3/y1579e/y1579e02.htm

Cassini, A., Hathaway, S., Havelaar, A., Koopmans, M., Koutsoumanis, K., Messens, W. & Scheutz, F. (2016). Microbiological risk assessment. *EFSA Journal*, 4, 1 - 10. Doi: https://doi:10.2903/j.efsa.2016.s0507

CDC (2018). Staphylococcal Food Poisoning. Retrieved from https://www.cdc.gov/foodsafety/diseases/staphylococcal.html

CFIA (2009). Canadian Food Inspection Agency, Salmonella *Food* Safety Facts. Preventing foodborne illness. Retrieved from https://www.canada.ca/en/public-health/services/food-safety.html

Chakravarty, I. & Canet, C. (2002). Street foods in Calcutta. *Pakistan Journal of Nutrition*, 8 (10), 1657-1660. Doi: https://doi.org/10.14687/jhs.v13i3.3925.

Cowan, M. K. (2016). Microbiology fundamentals a clinical approach, 2nd Ed, McGraw-Hill Education, New York. Retrieved from https://www.mheducation.com/highered/product/microbiology-fundamentals-clinical-approach-cowan-bunn/M9780078021046.html

Flint, S. (1998). Formation and Control of Biofilms of Thermo-Resistant Streptococci on Stainless Steel thesis Department of Food Technology, Massey University; Palmerston North.

FSANZ. (2019).

 $\frac{http://www.foodstandards.gov.au/consumer/safety/faqsafety/pages/foodsafetyfactsheets/healthandhygieneresp101.aspx}{Accessed July 1st 2019}.$

Gopal, N., Colin, H., Ross, P. R., Beresford, T. P., Fenelon, M. A. & Cotter, P. D. (2015). The Prevalence and Control of Bacillus and Related Spore-Forming Bacteria in the Dairy Industry. *Frontiers in Microbiology*, 6,1418. Doi: https://doi:10.3389/fmicb.2015.01418

Granum, P.E. (2005). *Bacillus cereus as a Food-borne Pathogen*. In: Microbiology and Molecular Biology. Pp. 409 - 420. Great Britain. Caister Academic Press. Retrieved from https://www.caister.com/fbp

Greig, J. D., Todd, E. C. D., Bartleson, C. A. & Micheals, B. S. (2007). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 1. Description of the problem, methods and agents involved. *Journal of Food Protection*, 70, 1752 -1761. https://jfoodprotection.org/doi/pdf/10.4315/0362-028X-70.9.2199

Idowu, O. A. (2006). Oral faecal parasites and personal hygiene of food handlers in Abeokuta, Nigeria. *Africa Health Science*, 6, 160 - 164. Doi: https://doi:10.5555/AFHS.2006.6.3.160

ISO (2018). Horizontal methods for sampling techniques from surfaces using contact plates and swabs. In: Microbiology of Food and Animal Feeding Stuffs. (ISO 18593:2018). British Standards Institution, London. p. 14. Retrieved from https://www.iso.org/standard/64950.html

Little, C. L., Monsey, H. A., Nichols, G. L. & de Louvois, J. (1998). The microbiological quality of ready-to-eat dried and fermented meat and meat products. *International Journal of Environmental Health Research*, 8, 277-284. Doi: https://doi:10.1080/09603129873381

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. & Ablordey, A. (2002). Street foods in Accra, Ghana: How safe are they? *Bulletin: World Health Organization*, **80** (7), 546 - 554. Doi: https://doi: 10.1590/S0042-96862002000700006

Nichols, S. L., Little, C. L., Mithani, V. & De Louvois, J. (1999). The microbiological quality of cooked rice from restaurants and take-away premises in the United Kingdom. *Journal of Food Protection*, 62: 877 - 882. https://doi.org/10.4315/0362-028X-62.8.877

Olumide A. (2016). Public health implications of microbial food safety and foodborne diseases in developing countries. Food Nutr Res., 60. 29819. DOI: https://doi.org/10.3402/fnr.v60.29819

Oranusi, S. U., Oguoma, O. I. & Agusi, E. (2013). Microbiological quality assessment of foods sold in student's cafeterias. Global Research *Journal of Microbiology*, **3**(1), 1 - 7. Doi: https://doi:w2010.4236/oalib.1101541

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J. & Lyng, J. G. (2010). A comparison of selected quality characteristics of foods prepared from thermo sonicated and conventionally heated milks, *Food Chemistry*, 119 (3), 1108 - 1113. Doi: doi: 10.1016/j.foodchem.2009.08.025

Tambekar, D. H., Shirsat, S. D., Suradkar, S. B., Rajankar, P. N. & Bangin-War, Y. S. (2007). Prevention of transmission of infectious disease: Studies on hand hygiene in healthcare among students. *Continental Journal of Biomedical Sciences*, **1**, 6-10. Doi: https://doi.10.1007/s11947-010-0365-x

Taulo, S., Wetlesen, A., Abrahamsen, R., Mkakosya, R. & Kululanga, G. (2008). Microbiological quality of water associated management practices and risks at source transport and storage points in a rural community of Lungwena, Malawi. *Africa Journal of Microbiologica Research*, 7(2), 131-137. Available online http://www.academicjournals.org/ajm

Todd, E. C. D., Greig, J. D., Bartleson, C. A. & Michaels, B. S. (2007a). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 2- Description of outbreaks by size, severity, and settings. *Journal of Food Protection*, **70**, 1975. Doi: https://doi.org/10.4315/0362-028X-70.8.1975 Todd, E. C. D., Greig, J. D., Bartleson, C. A. & Michaels, B. S. (2007b). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3- Factors contributing to the outbreaks and description of outbreak categories. *Journal of Food Protection*, 70, 2199 - 2217. https://www.ncbi.nlm.nih.gov/pubmed/17900100

Tortora, G. J., Funke, B. R. & Case, C. L. (2016). *Microbiology an introduction* 12th Ed, Pearson Education, Inc. Retrieved from https://trove.nla.gov.au/work/6856000?q&sort=date+desc&=1556715020972&versionId=209469624

Wagner, A. B. (2009). Epidemiology of food poisoning outbreaks in Singapore, 2001-2005. Epidemiological News Bulletin, *Journal of Microbiology*, 31, 68-72. Doi: https://doi.org/10.9734/jamb/2016/30948

WHO (2008). Foodborne Disease Outbreaks. Guidelines for Investigation and Control. Retrieved from https://www.who.int/iris/handle/10665/43771

WHO (2010). Prevention of foodborne disease: Five keys to safer food World Health Organization. Retrieved from https://www.who.int/foodsafety/consumer/5keys/en/

WHO (2011). Food Safety and foodborne illness. https://www.who.int/news-room/fact-sheets/detail/food-safety

WHO (1989). Programme for Control of Diarrhoeal Diseases. Manual for Laboratory Investigations of Acute Enteric Infections. CDD/83.3, *Review* 1. Geneva. whqlibdoc.who.int/whf/1989/vol10-no1/WHF 1989 10(1) p110-115.pdf

WHO (1993). World Health Organization Food Safety Unit. Contaminated food: a major cause of diarrhoea and associated malnutrition among infants and young children. Facts Infant Feed, 1, 1–4. https://www.ncbi.nlm.nih.gov/pubmed/1234 World Bank (October 25, 2018). Nigeria, others lose \$110bn yearly to foodborne diseases. World Bank Press Release, NO: 2019/072/AGR. https://www.worldbank.org/en/news/press-release/2018/10/23/food-borne-illnesses-cost-us-110-billion-per-year-in-low-and-middle-income-countries