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

OPEN ACCESS
SCICELL

REGULAR ARTICLE

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

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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 $3.67 \pm 0.33 \times 10^2$ cfu/g - $2.71 \pm 0.05 \times 10^4$ cfu/g; $3.33 \pm 0.33 \times 10^2$ cfu/g - $2.39 \pm 0.04 \times 10^4$ cfu/g and 0.00 ± 0.00 cfu/g - $3.70 \pm 0.21 \times 10^3$ cfu/g respectively. The concentration of microbes on the food contact surfaces from ready-to-use serving plates were 0.00 ± 0.00 cfu/cm^2 - 14.67 ± 0.33 cfu/cm^2 and 0.00 ± 0.00 cfu/cm^2 - 22.67 ± 0.33 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 middle-income 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 food-borne 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 cm^2 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 ºC 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:

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

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

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:

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

Where: Microbial count was expressed as colony forming units per square centimeter of contact surface (cfu/cm^2).

$$Area\ equivalent\ of\ volume\ of\ inoculated\ sample = \frac{Volume\ of\ inoculated\ sample\ X\ Total\ area\ of\ sampled\ surface}{Total\ volume\ equivalent\ of\ swabbed\ area}$$

$$Area\ equivalent\ of\ volume\ of\ inoculated\ sample = \frac{Volume\ of\ inoculated\ sample\ X\ Total\ area\ of\ sampled\ surface}{Total\ volume\ equivalent\ of\ swabbed\ area} \quad (3)$$

Given that: Volume of inoculated sample = 0.1 ml
Total area of sampled surface = 25 cm^2
Total volume equivalent of swabbed area = 10 ml

RESULTS

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

Sample locations	Sources of Microbes	Isolates	Cultural Examinations		
			NA	**MA**	**MSA**
Restaurant 1	Ready-to- eat fried rice	IU1	Non-pigmented mucoid colonies	Lactose fermenting colonies	No growth of Bacteria
Restaurant 2	Ready-to- eat fried rice	EF1	Non-pigmented colonies	Lactose fermenting colonies	No growth of Bacteria
Restaurant 3	Ready-to- eat soup	MC1	Non-pigmented large mucoid colonies	Lactose fermenting colonies	No growth of Bacteria
Restaurant 4	Ready-to- eat soup	FB1	Non-pigmented mucoid colonies	NP	Non-mannitol fermenting colonies
		FB2	Translucent patchy colonies	Lactose fermenting colonies	NP
Restaurant 5	Ready-to- eat Jollof rice	MB1	Non-pigmented mucoid colonies	NP	Mannitol fermenting colonies
		MB2	Non-pigmented mucoid colonies	Lactose fermenting colonies	NP
Restaurant 6	Ready-to- eat fried rice	AG1	Non-pigmented mucoid colonies	Lactose fermenting colonies	No growth of 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 locations	Isolates	Gram staining of Colonies	Biochemical Examinations								Probable Microbes
			Co	**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
Restaurant 4	FB1	Gram positive cocci	-	+	Np	Np	Np	Np	Np	γ	*Micrococcus* species
	FB2	Gram negative rods	Np	+	+	-	-	-	-	γ	*Enterobacter* species
Restaurant 5	MB1	Gram positive cocci in bunches	+	+	Np	Np	Np	Np	Np	β	*Staphylococcus* species
	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

Sample locations	**Isolates**	**Cultural Examinations**		
		NA	**MA**	**MSA**
Restaurant 1	IUH1	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP
	IUH2	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies
Restaurant 2	EFH1	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies
	EFH2	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP
Restaurant 3	MCH1	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP
	MCH1	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies
Restaurant 4	FBH1	Non- pigmented pinpoint colonies	NP	Mannitol fermenting colonies
	FBH2	Non- pigmented mucoid colonies	Lactose fermenting colonies	NP
Restaurant 5	MBH1	Yellow pigmented colonies	NP	Non- mannitol fermenting colonies
Restaurant 6	AGH1	Large mucoid colonies	Lactose fermenting colonies	NP
	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 locations	Isolates	Gram staining of Colonies	Biochemical Examinations								Probable Microbes
			Co	**Ca**	**Ur**	**Ci**	**Mr**	**Vp**	**In**	**Hm**	
Restaurant 1	IUH1	Gram negative rods	Np	+	+	+	-	-	-	γ	*Enterobacter* species
	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	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*
	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

Sample locations	Isolates	Cultural examinations		
		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 locations	Isolates	Gram staining of Colonies	Biochemical examinations								Probable Microbes
			Co	Ca	Ur	Ci	Mr	Vp	In	Hm	
Restaurant 1	IUP1	Gram negative rods	Np	+	+	+	-	-	-	γ	*Enterobacter* species
Restaurant 2	EFP1	Gram negative rods	Np	+	+	+	-	-	-	γ	*Enterobacter* species
Restaurant 3	MCP1	Gram negative rods	Np	+	+	+	-	-	-	γ	*Enterobacter* species
Restaurant 4	FBP1	Gram positive oval shaped cells mainly in pairs	Np	Np	Np	Np	Np	Np	Np	γ	*Saccharomyces* species
Restaurant 5	MBP1	Gram positive cocci in pairs	-	-	Np	Np	Np	Np	Np	β	*Streptococcus* species
Restaurant 6	AGP1	Gram positive cocci in pairs	-	-	Np	Np	Np	Np	Np	β	*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 locations	Concentration of Microflora		
		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; cm^2: square centimeter.

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

Sources of Microbes	Sample Locations	Concentration of microflora		
		Mean TAVC (cfu/cm^2)	Mean TCC (cfu/cm^2)	Mean TSC (cfu/cm^2)
Hands of food handlers	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
	Restaurant 3	22.67 ± 0.33	0.00 ± 0.00	20.33 ± 0.33
	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
Ready-to-Use serving plates	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
	Restaurant 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	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 ± SE (Standard error); cfu: colony forming units; cm^2: 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 $3.67 \pm 0.33 \times 10^2$ cfu/g to $2.71 \pm 0.05 \times 10^4$ cfu/g. The mean TCC ranged between $3.33 \pm 0.33 \times 10^2$ cfu/g to $2.39 \pm 0.04 \times 10^4$ cfu/g, while the mean TSC were between 0.00 ± 0.00 cfu/g and $6.60 \pm 0.29 \times 10^3$ 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/cm^2 (as recorded in the hands of the food handlers). Mean TCC were between 0.00 ± 0.00 cfu/cm^2 and 1.33 ± 0.33 cfu/cm^2, while the values of the mean TSC ranged from 0.00 ± 0.00 cfu/cm^2 to 20.33 ± 0.33 cfu/cm^2.

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., *Streptococcu*s 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. *Streptococc*i 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.

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BACTERIAL EMPIRE
2019, VOL. 2, NO. 3, 64-69

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REGULAR ARTICLE

ASSESSMENT OF 16S rRNA SEQUENCE AND HETEROTROPHIC PLATE COUNT (HPC) METHODS OF IDENTIFYING BACTERIA FROM DRINKING WATER SYSTEMS IN BENIN CITY METROPOLIS

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ABSTRACT

A variety of simple culture-based tests which are proposed to recover a wide range of microorganisms from water are collectively known as heterotrophic plate count (HPC) and used as an indirect indicator to give information about water quality. The aim of this study was to assess the heterotrophic plate count (HPC) culture-based dependent and 16S rRNA independent techniques of identifying bacteria. The HPC was conducted by incubating a filtered sample of water on R2A agar plates and enumerating the number of resultant bacterial colonies that grow on each plate. The molecular analysis was performed by extracting the deoxyribonucleic acid (DNA) from bacterial isolate and polymerase chain reaction (PCR) was done to obtain the amplicons (PCR products). Purified PCR products were sequenced by ABI V3.1 Big dye kit and the analysis of sequence was conducted by the basic local alignment search tool (BLAST) to identify their closest relatives. A total number of 17 isolates of *Pseudomonas*, *Bacillus* and *Proteus* were phenotypically identified, while the nucleotide sequence revealed the presence of 59 diverse strains with distribution (percentage) of *Pseudomonas* (25) 42.2 %, *Bacillus* 17 (28.8 %) and *Proteus* (17) 28.8 %. The investigated isolates when compared from gene data-base recorded genetic relatedness with similarity index of 61 to 100 %. The 16S rRNA gene sequences from DNA extractions showed microbial consortia in drinking water to comprise of a broad array of bacterial diversity, including those of critical concern to human health such as *Pseudomonas* and *Bacillus.* Finally, there was no significant correlation between the HPC test and 16S rRNA sequence analysis.

Keywords: HPC, DNA extraction, 16S rRNA gene, PCR, Sequencing and Blast

INTRODUCTION

The basic duty of the Water Authority is to evaluate the challenge of microbial contamination for drinking water in relation to Total Coliform Rule (TCR). This requires the assessment of coliform group of bacteria in drinking water distribution system (**WHO, 2002**). The heterotrophic plate count (HPC) test is applied as indirect indicator of water safety and consists of a variety of simple culture-based approaches that are intended to obtain a wide range of bacteria from water consortia and pollutant sources.

The HPC is also employed as a marker for investigating aesthetic quality causes and challenges and provide data concerning water quality by estimating the levels of heterotrophic bacteria in water samples (**Bartram, Cotruvo, Exner, Fricker & Glasmacher, 2003).** However, the HPC is limited in spectrum and accuracy of information it reveals because, the test does not virtually detect all virulent bacteria. More so, the use of bacterial inactivation (chlorination and chemotherapeutic agents) seldom permits the growth of some potentially pathogenic species not observed by total coliform and HPC tests, such as *Pseudomonas*, *Bacillus* and *Mycobacterium***.**

Consequently, improvements in culture-based independent sequencing techniques now allows the evaluation of microbial consortia in much greater extent than was earlier performed from culture-based dependent approach, such as practiced in HPC (**Farcas, 2012**). The previous understanding of bacterial identification relied on growth in enrichment culture should be accepted with caution. Nevertheless, there is currently a paradigm shift whereby, the identity of a microbe in an environmental sample can be inferred by the sequences of its 16S ribosomal ribonucleic acid (rRNA) genes (**Medlar *et al*., 2014**).

The ribosome is found in all living organisms and the genetic sequences that encode the rRNA are relatively preserved in the domains of life. The multiple nucleotide sequences of gene can exist in a single bacterium, from which specific section can be targeted for replication. The 16S rRNA gene plays structural role in protein synthesis and annealing capacity of universal primers. Applying the molecular approach, it is now possible to investigate the microbial load and conduct detailed evaluation of broad network of environments, determine greater spectra of bacterial phylogeny, genetic similarity and identify uncommon bacterial in samples (**Stamatakis, 2014**).

MATERIALS AND METHODS

Sample Collection

Drinking water samples were collected thrice from different storage tanks from four locations in Benin City metropolis (Sapele, Auchi, Oko Central and Ugbowo Roads axis) in the months of March to May 2018. Samples were also obtained from the end-user point (taps) by allowing the taps to run for I minute before capturing 1Litre of the water sample in sterile containers. Forty milliliters of water were transferred into 50 ml conical tube prior to performing the heterotrophic plate count (HPC) test and deoxyribonucleic acid (DNA) extraction.

Heterotrophic plate counts (HPC)

The HPC tests were conducted according to protocols described in standard methods for the examination of water and waste water (**APHA, 2005**). Water samples were diluted in sterile peptone water (Zymo Research, Johannesburg, S/A) and filtered through 0.45 µm diameter Millipore membrane filters. The filters were then transferred onto R2A agar plates and incubated at 28 ºC for 48 hr. The number of colonies on each plate was then counted prior to the colour and morphology of each colony.

Identification and characterization of bacteria

Three bacterial colonies were picked based on their different colonial morphologies and each of them was phenotypically characterized with prescribed standard methods (**Barrow and Feltham, 2003**).

16S rRNA gene sequencing from HPC colonies

Further identification of the pure culture of the bacterial isolates from the biofilm water samples was achieved by polymerase chain reaction (PCR), amplification, purification and sequencing of 16S rRNA gene (**Ghatak, Muthukumara & Nachimuthu, 2013**). The following outlines the key steps in the methods taken.

DNA extraction

The chromosomal DNA was extracted using Zymo Pure Miniprep Kit, as prescribed by the manufacturer (Zymo Research Centre, Johannesburg, South Africa). The DNA extract was purified by adding 5.0 µL nuclease-free water and

incubated for 30 min. The extracted DNA product was eluted in 25 µL DNA elution buffer and stored at -20 ºC as DNA template, ready for use in PCR process.

Amplification of 16S rRNA gene

The protocol for DNA amplification using the genomic guilded sequence method was employed.
The 16S rRNA gene from the chromosomal DNA was PCR amplified using universal primer sets 27F (5 AGA GTT TGA TCC TGG CTC AG-3) and 1492R 5 TAC GGT CTA CTT GTT ACG TA-3). The PCR master mix contained the following components of up to 25 µL: One taq master mix, 12.5 µL; Forward and Reverse primers, 1.25 µL; Nuclease free water, 5.0 µL and DNA template, 5.0 µL. The process was performed in Gene PCR Thermo Cycler with the recommended guidelines: Initial denaturation at 94 ºC for 30 min; denaturation at 94 ºC for 1 min; annealing at 50 ºC for 1 min, extension at 72 ºC for 1 min; final extension at 72 ºC for 7 min and hold at 4 ºC.

Purification of the PCR products

The PCR products were cleaned using Exo SAP PCR master mix (South Africa) as per manufacturer protocol: PCR Mixture 10.0 µL; Exo SAP Mix (Exonuclease 50.0 µl and Shrimp Alkaline Phosphate 200 µl). The mixture was incubated at 37 ºC for 30 min and the reaction was stopped by heating the mixture at 95 ºC for 5 min. The purified PCR products were eluted in 5 µL nuclease free water for 30 min and stored at -20 ºC until used for sequencing.

Sequencing of the 16SrRNA gene

The purified PCR products were sequenced by using universal primers 27F and 1492R. To obtain the full-length sequence of the 16S rRNA gene, the sequencing was done by the ABI V3.1 Big dye kit according to manufacturer's instructions.

Analysis of sequences by Basic Local Alignment Search Tool (BLAST) and phylogenetic analysis

The sequences were compared using the BLAST to identify their closest relatives. A detailed phylogenetic analysis was conducted using the Geneious package (version 9.0.5) program (South Africa). The sequences were aligned by importing closely related sequences from GeneBank (htpp;/ncbi.nlm.nih.gov/genebank) and the aligned sequences were subjected to maximum likelihood and Neighbour-joining analyses. The bootstrap analysis was performed to estimate the confidence of the 16S rRNA gene-tree topology.

RESULTS

The bacterial isolates phenotypically identified were, *Bacillus*, *Pseudomonas* and *Proteus* species.

Table Phenotypic characterization of isolates obtained from biofilm water samples

Morphological Examination			**Biochemical Examination**							**Isolates**
Colonial Characteristics on nutrient agar (NA)	**Colonial Characteristics on MacConkey agar (MA)**	**Gram Staining**	**Ca**	**Ox**	**In**	**Mr**	**Ci**	**Vp**	**La**	
Greenish pigmented colony with an entire margin	Colourless colony with an entire margin	negative rods	+	+	-	-	+	-	-	Pseudomonas sp.
Mucoid colony with entire margin	Colourless colony with entire margin	positive rods	+	+	-		+	+	+	*Bacillus* sp.
Mucoid swarming colony with an entire margin	Colourless colony with an entire margin	negative rods	+	-	-	+	+	-	-	*Proteus* sp.

Legend: Ca: Catalase test; Ox: Oxidase test; In: Indole test; Mr: Methyl red test; Vp: Voges Proskauer test; Ci: Citrate test; La: Lactase test; -: negative reaction; +: positive reaction; sp. represents species.

Table 2 Distribution Percentage of bacterial isolates by the corresponding methods of identification

Isolates	**Phenotypic: Frequency (%)**	**Sequencing: Frequency (%)**
Pseudomonas	7 (41.2)	25 (42.2)
Bacillus	6 (35.3)	17 (28.8)
Proteus	4 (23.5)	17 (28.8)
Total	17	59

The phenotypic distribution of bacterial isolates recorded the highest number (7) for *Pseudomonas* and the least (4) was reported for *Proteus*

Table 3 Identification of bacterial isolates using 16S rRNA gene sequencing

Samples	**Isolates**	**Closest relative in gene data base**	**Similarity (%)**	**Accession number**
SA-R-1	*Pseudomonas aeruginosa* strain 907-R-AO8-02	*Pseudomonas aeruginosa*	80	KX860116
OK-T-4	*Proteus penneri* strain 907-R – B08-05	*Proteus penneri* strain FFL8	100	JN092595
AC-R-8	*Pseudomonas aeruginosa* strain 907-R-F07-16	*Pseudomonas aeruginosa* strain KCTC 23843	61	MG009256
OK-R-9	*Proteus* sp. strain 907-R-GO7-19	*Proteus* sp. strain KL11	62	KP313867
UB-R-12	*Bacillus* sp. strain 907-H07-22	*Bacillus* sp. strain B-3-35	100	KT583528
UB-T-13	*Bacillus cereus* strain 907-R-G07-19	*Bacillus cereus* strain D12-2	100	CP016315
AC-T-15	*Pseudomonas hibiscicola* strain 907-R- F06-24	*Pseudomonas hibiscicola strain IHBB 6867*	61	KF668478

Legend: OK (Oko Central road), UB (Ugbowo Road), SA (Sapele Road), AC (Auchi Road), R (Reservoir), ne), T (Tap)

The results of the nucleotide sequence of part of 16S rRNA gene analysis of bacterial isolates revealed other strains with closest identity with their accession numbers when compared from gene data-base by Neighbour joining method proframme to include: *Pseudomonas aeruginosa* (KX860116), *Proteus penneri* strain FFL8 (JN092595), *Pseudomonas aeruginosa* strain KCTC 23843 (MG009256), Proteus sp. strain KL11 (KP313867), *Bacillus* sp. strain 3-35 (KT583528) *Bacillus cereus* strain D12-2 (CP016315), *Pseudomonas hibiscicola* strain IHBB 6867 (KF668478),

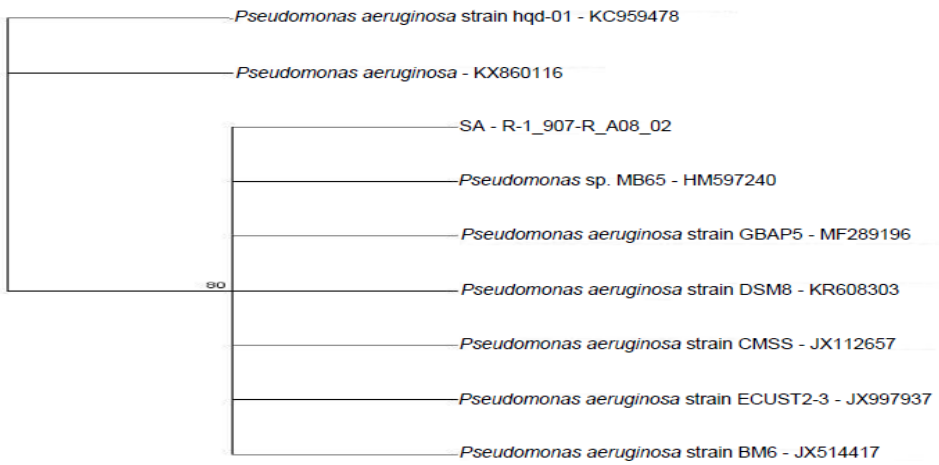

Figure 1.1 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **SA- R- 1** has similar sequence with *Pseudomonas aeruginosa* with accession number KX860116

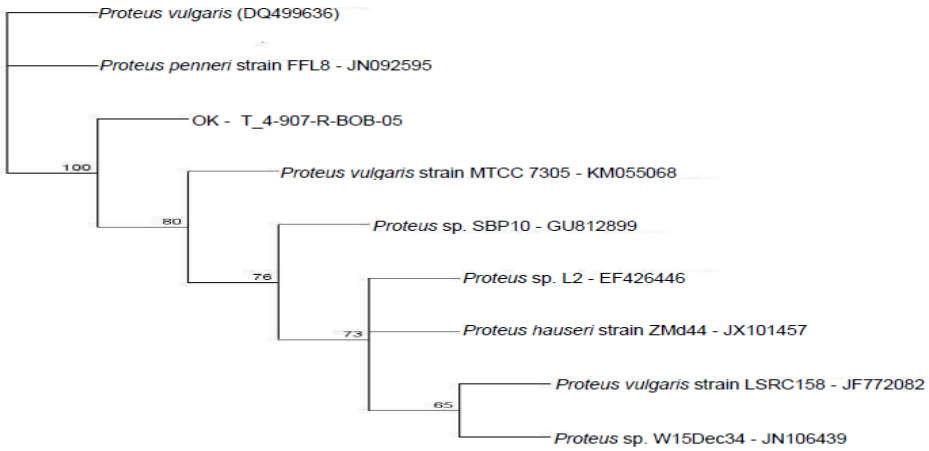

Figure 1.2 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **OK - T-4** has similar sequence with *Proteus penneri* strain FFL8 with accession number JN092595

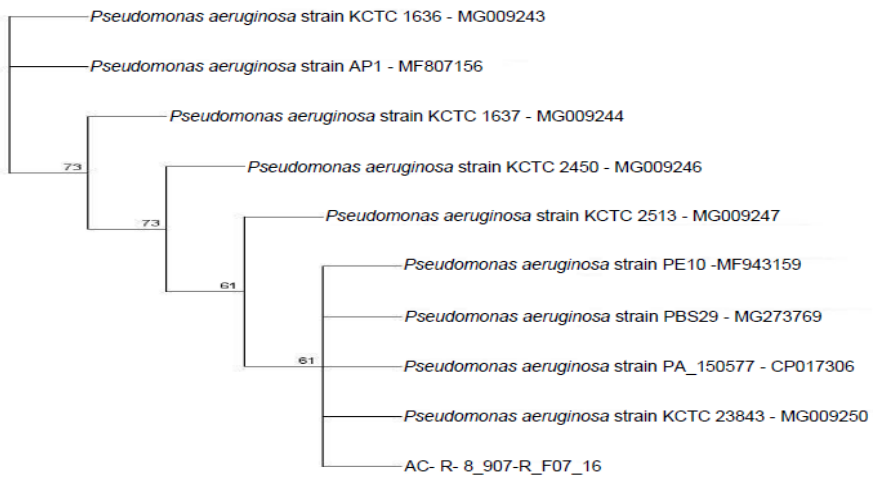

Figure 1.3 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **AC- R-8** has similar sequence with *Pseudomonas aeruginosa* strain KCTC 23843\ with accession number MG009250

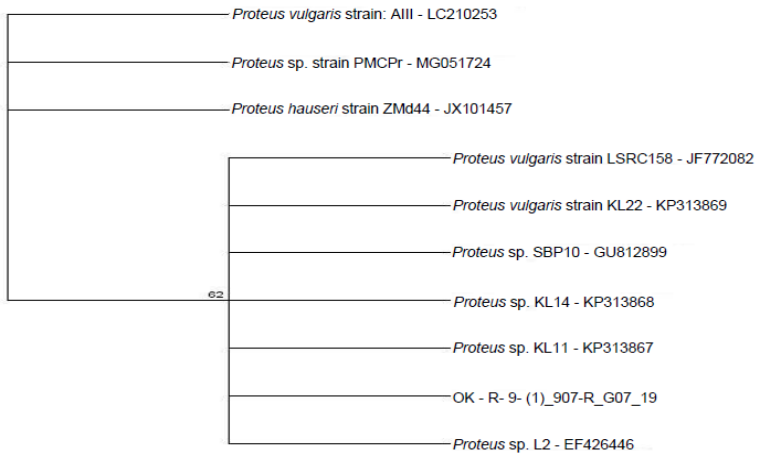

Figure 1.4 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **OK- R- 9** has similar sequence with *Proteus sp*. KL11 with accession number KP313867

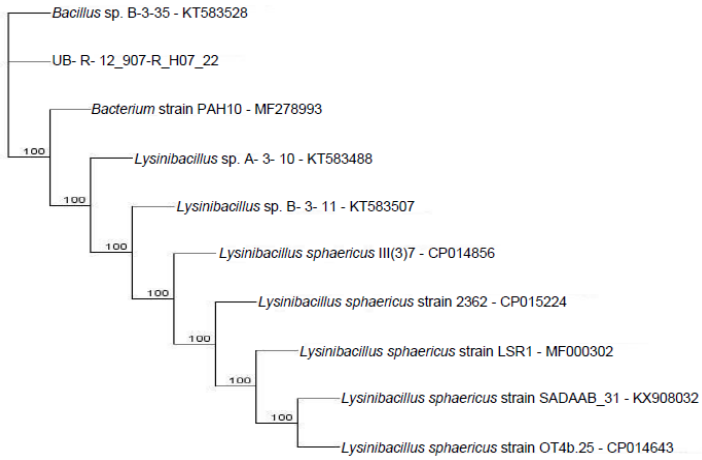

Figure 1.5 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **UB- R- 12** has similar sequence with *Bacillus sp.* B-3-35 with accession number KT583528

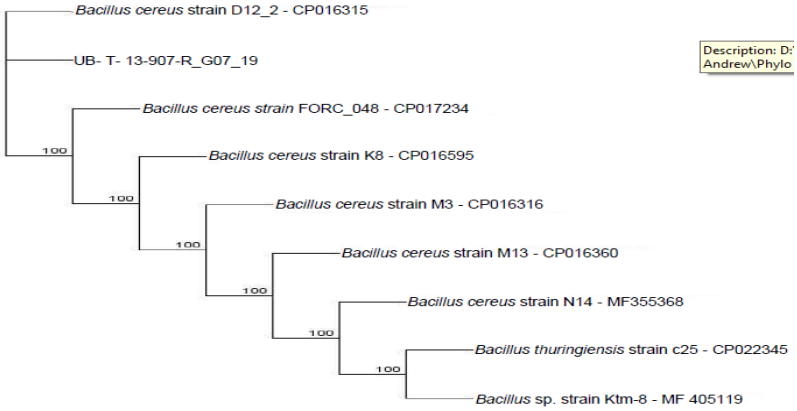

Figure 1.6 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **UB- T- 13** has similar sequence with *Bacillus cereus* strain D12_2 with accession number CP016315

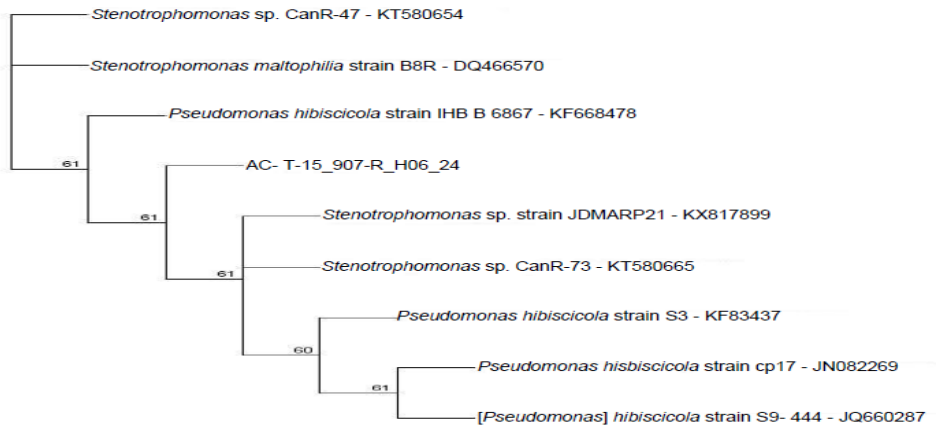

Figure 1.7 Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate **AC- T-15** has similar sequence with *Pseudomonas hibiscicola* strain IHB B 6867 with accession number KF668478

DISCUSSION

The data collected in this study indicated no significant relationship between the heterotrophic plate counts and sequences, with the bacterial isolates phenotypically identified were *Bacillus, Pseudomonas and Proteus.* The highest frequency (percentage) was recorded for *Pseudomonas* (41.2%) and *Bacillus* 35.3% and *Proteus* 23.5% (Table 2). The genus *Pseudomonas* is of particular concern because of the diversity of obligate and opportunistic pathogens contained within the genus that caused nosocomial infections in susceptible patients and their high intrinsic resistance to a variety of antibiotics, including β - lactams, aminoglycosides and fluroquinolones that made them very difficult to eliminate. The B*acillus* produced putative virulent factors capable for triggering infections, responsible for endocarditis and neurological cases (**Farcas, 2012**).

In the phylogenetic analyses of biofilm water samples, all the isolates had similar sequences when compared with those from gene data-base which include: *Pseudomonas aeruginosa* (KX860116), *Proteus penneri* strain FFL8 (JN092595), *Pseudomonas aeruginosa* strain KCTC 23843 (MG009256), Proteus sp. strain KL11 (KP313867), *Bacillus* sp. strain 3-35 (KT583528) *Bacillus cereus* strain D12-2 (CP016315), *Pseudomonas hibiscicola* strain IHBB 6867 (KF668478).

The phylogenetic analysis of strains examined in this study, illustrated many similarities to previous studies pertaining to drinking water biofilms. The 16s rRNA sequences affiliated to *Pseudomonas*, *Bacillus* and *Proteus*-like organisms have been previously shown to be present in drinking water biofilms (**Ghatak *et al*., 2013**). In general, the results from sequences, found significant levels of *Stenotrophomonas maltophila, Pseudomonas hibiscicola, Pseudomonas aerugniosa, Bacillus cereus, Bacillus thuringienis and Lysinbacillus sphaericus.* Others *were proteus penneri, Proteus vulgaris and Proteus hauseri.* In addition, all sequences were closely related and numerically more than the cultured bacteria thereby, supported the notion that culture-based methods can under-estimate the bacteria diversity of drinking water systems. Lavender and kinzelman compared agar-based count of *Escherichia coli* in water samples to detection by quantitative polymerase chain reaction (qPCR), wherein an *E. coli*-specific gene was amplified to quantify the *E. coli* in the sample and discovered qPCR to be more sensitive than culture-based methods (**Lavender & Kinzelman, 2009**). Approximately, nearly a third of sequences analyzed in this study showed 100 % homologous similarity with sequences in the currently available data bases (Table 3). This suggested that some drinking water bacteria represent novel bacterial species.

The phylogenetic analysis further revealed other sequences which were closely related to *Sternotrophomonas maltophila* such as, *Pseudomonas hibiscicola,* which has been implicated in production of Laccase, an oxidase enzyme useful in Environmental pollution Control Programmes. The *Lysinbacillus sphaericus* identified, also produces α and β proteins (Binary toxins) that act following ingestions. These toxins can be used in insect control programs to reduce the population of disease vector species. More so, the bioremediation potential of the identified strain A-3-10 (Fig. 1.6) is able to reversibly bind heavy metals such as lead, cadmium, uranium due to the presence of the proteinaceous surface (**USEPA, 2012**).

The alignment with sequences available within gene data-base showed that, there was no significant correlation between the sequences and heterotrophic bacterial counts (HBC) and revealed little similarity in over-all community diversity, as well as significant distortion in relative abundance particularly for *Pseudomonas spp.* (25) 42.2 %, B*acillus* (17) 28.8 % and *Proteus (*17*)* 28.8 % (Table 2). It should be noted that the percentage (frequency) of *Pseudomonas, Bacillus* and *Proteus* obtained in sequencing were relatively higher than bacterial distribution from HPC. Comparatively, the sequencing revealed total number of 59 homologous isolates, while 17 was recorded from the HPC (thrice the value of HPC). This further confirmed that molecular approach gives more detail and broad spectrum of bacterial investigation than standard methods.

These results implied that, heterotrophic bacterial count (HBC) has little relevance for determining parameters of drinking water quality regarding microbial communities. This is particularly striking given that HBC may not detect the potential presence of pathogenic microbes of concern to human health. Furthermore, that drinking water ecosystem is much more microbiologically complex than culture-based surveys would suggest. Therefore, such a determination would infer that low HBC imply the presence of microbes that are not detected by conventional (HPC) monitoring technique, but which might be of potential human concern (**APHA, 2005**).

CONCLUSION

The obtained results in this study reveal a high possibility of cross contamination of food from food handlers and contact surfaces sampled. Organisms like *Enterobacter* sp., *Bacillus* sp, *Streptococcus* sp., *Micrococcus* sp., *Staphylococcus aureus*, *Streptococcus* sp. were isolated from the food and contact surfaces. It is recommended that some preventive measures be adopted to avoid contamination of cooked food like the routine examination of cooked food in restaurants by the relevant authorities and consequent sanctions if minimum standard are violated, hygiene awareness for personnel who prepare and handle ready to eat cooked food, training on the adoption on the integration of Hazard Analysis Critical Control Point (HACCP) procedures into the food preparation and production process.

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