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Isolation and Identification of Some Enterobacteria from Retailed Convenience Foods

A. Abiodun Onilude[1], C. Felicia Adesina[2*], A. Omolola Oluboyede[2], A. Abimbola Olajide[1] and Olajumoke Alao Nwaechefu[3]

[1]Department of Microbiology, University of Ibadan, Oyo State, Nigeria.
[2]Department of Microbiology, Lead City University, Ibadan, Nigeria.
[3]Department of Biochemistry, Lead City University, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author A. Abiodun Onilude designed the study. Authors AOO and A. Abimbola Olajide carried out bench work. Author CFA wrote the manuscript while author OAN did the statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Meat pie and Fish roll samples from five different local government areas (LGAs) in Ibadan, Nigeria were analysed microbiologically for presence of Enterobacteriaceae and other common food borne pathogens. Sampling was done twice for three months and plating of food samples was carried out by pour plate and membrane filter techniques on different bacteriological media for Total plate and Coliform counts on samples. Identification was done based on colonial morphology, Gram's reaction and biochemical and sugar fermentation characteristics of isolates. 1483 bacterial counts were obtained from samples evaluated. Identification of isolates showed that 14 genera of microorganisms were represented out of which *Salmonella* spp and *Proteus vulgaris* from the Family Enterobacteriaceae represented about 12% of total number obtained. Others included *Flavobacterium* spp, *Pseudomonas* spp*, Aeromonas* spp *and Moraxella* spp*.* Gram positive bacteria among isolates were: *Staphylococcus epidermidis, Micrococcus* spp*, Lactobacillus* spp*., Norcadia* spp*, Microbacterium* spp, *Bacillus* spp, and *Streptococcus* spp*.* Yeast isolates among the

**Corresponding author: E-mail: feliciadesinac@gmail.com;*

microorganisms were identified as *Rhodotorulla* spp. and *Saccharomyces cerevisiae.* Percentage occurrences of isolates from road side samples were higher than that of Eatery samples in all the LGAs, roadside Fish roll from Oluyole had highest percentage of *Salmonea* spp (42.2%), while that from Ibadan North-East had highest percentage of *Proteus* spp. (28.11%). Roadside Meat pies obtained from Lagelu showed highest percentage of *Salmonella* spp (34.3%) and those from Ibadan South-west showed highest percentage of *Proteus* spp (31%). High occurrence of microorganisms in some of the convenience food samples requires urgent attention by health policy makers and all stakeholders.

Keywords: Bacterial isolates; coliform; eatery; local governments; street foods.

1. INTRODUCTION

Convenience foods or Ready to eat foods are often prepared food stuffs that can be sold as hot, ready-to-eat dishes; as room-temperature, shelf-stable products; or as refrigerated or frozen products that require minimal preparation (typically just heating) [1]. Notable examples of convenience foods are street foods and fast foods; street foods are low in cost compared with restaurant meals and offer an attractive alternative to home-cooked food [1] and they exist in an endless variety. A seller's store is located outdoor or under an easily accessible roof from the street with low cost seating facilities and the marketing success depends exclusively on location and word-of-mouth promotion. Such stores are usually owned by individuals. By contrast, fast food outlets specialize in fewer foods which are usually prepared by frying and baking. Hamburgers, chicken, chips and pizza often predominate. These enterprises, which are usually indoors, invest heavily in seating, air conditioning and bright decor. Marketing strategies are almost exclusively dependent on advertising, sponsorship and special offers which aim to create brand loyalty. Owners usually have a franchise arrangement with a transnational company which also controls the provision of raw materials, the menu and the mode of preparation [2].

The busy and hectic life schedule has opened the way for the fast food industry in most parts of the world to meet food requirements of commuters and urban dwellers [2]. Consumption of roadside foods potentially increases the risk of food borne diseases caused by a wide variety of pathogens. Street foods have been reported to be contaminated by pathogens and have also been implicated in food borne epidemics, particularly in developing countries [3,4,5]. The risk of contamination varies greatly with the type of street food, processing methods, handling practices, storage facilities, personal hygiene of vendors and refuse disposal facility [6]. Generally, cereal and bakery products with low moisture content, adequately sugared, salted, or acidulated products and some fermented products support less bacterial load than dairy, egg and meat products. Foods cooked immediately prior to consumption are safer than those which have been cooked and stored at ambient temperature before being eaten [7]. Dishes containing raw ingredients or made with ice are also high risk items [6]. It is estimated that each year in the United States, approximately 76 million cases of food borne illnesses occur [8] however such data in Nigeria is not documented. Food borne illnesses may be caused by *Campylobacter* spp. non typhoidal *Salmonella* spp, pathogenic *Escherichia coli* and some other pathogens, some of which colonize the gastrointestinal tracts of a wide range of domestic animals, especially animals raised for human consumption [9]. The most common convenience foods found in the eatery and street vendors are meat pie, fish rolls and meat rolls. Convenience foods hawked on the road side can easily get contaminated by these bacteria because of the poor hygienic and handling practices during their sale. Their presence in such foods can lead to the incidence and spread of enteric diseases like diarrhea, dysentery, cholera, typhoid, etc, which can pose health problems to a community, especially in children, the immunocompromised and the society at large.

This research work will assess the safety level of road side foods in five LGAs in Ibadan with emphasis on the enterobacteriaceae load as compared with Eatery produced foods, since there are no quality control measures in place in Nigeria to check their production.

2. MATERIALS AND METHODS

Already made meat pie and Fish rolls were purchased from snack providers, hawkers and

some big eateries located within Ibadan South-West, Ibadan North, Oluyole, Ibadan North-East and Lagelu Local government areas (LGAs) in Ibadan metropolis. Three different sampling points were selected for each local government area and sampling was done at two different periods within a duration of three months.

2.1 Culture Media

All the culture media used in this study were of analytical grade and they include Nutrient Agar, MacConkey Agar, Eosin Methylene Blue Agar, Blood Agar, Potato dextrose agar and Nutrient Broth. Others include Lactose Broth, Simmon's Citrate Agar, Trypticase-Nitrate broth, Peptone Iron Agar, Tomato Juice Medium, Starch Agar Medium, and Christiansen's Urea Agar Medium. Each medium was prepared according to manufacturers' specification for the purpose it was needed, homogenized where necessary and sterilized in an autoclave (Model YX400AJ) at a temperature of 121℃ and pressure of 15 psi for 15 min [10,11]. Each was allowed to cool to room temperature or 40℃ as the case may be before it was used. Incubation was at 37℃ for bacteria and 28±2℃ for fungi.

2.2 Isolation of Microorganisms from Samples

Pour plate and membrane filter techniques were used to plate out each of the samples on the culture media (Nutrient agar and MacConkey agar) at each sampling stage/period. Fungal isolates were cultured on potato dextrose agar. Isolates obtained at each sampling point were subcultured onto appropriate agar plates severally until pure culture of each isolate was obtained. The pure Culture of each bacteria isolate was subcultured onto agar slants incubated and after 24 h they were kept in the refrigerator.

2.3 Identification of Isolates

Isolates were identified based on their colonial and cellular morphology, Gram stain reaction biochemical characteristics, sugar fermentation and acid production [10,11] (Harrigan and McCane, 1986; Fawole and Osho, 1989).

2.4 Statistical Analysis

The results were analysed using Duncan [12] Multiple Range Test. The differences between groups were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

According to Health Protection Agency [13] draft guidelines on assessing the Microbiological safety of Ready to Eat (RTE) foods, the presence of foodborne pathogenic agents in RTE foods is significant and their absence is of paramount importance. With the exception of the aerobic and anaerobic spores, detection of foodborne pathogenic agents at any level is of concern and should be investigated with an urgent response proportional to the level of contamination and risk to consumers [13]. A total of twelve bacterial genera were obtained from the meat pie and fish roll samples (Fig. 1). Out of these, *Salmonella* spp. and *Proteus* spp, which are members of the Family Enterobacteriaceae, made up about 12%. Enterobacteriaceae count may be an indicator of possible enteric contamination in the absence of Coliforms [14].

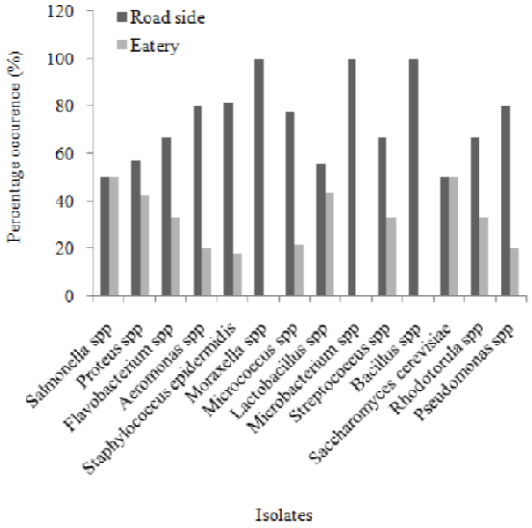

Fig. 1. Comparative percentage of isolates in food samples from roadside and eatries

Absence of coliforms from the samples analysed as shown in Fig. 1 contrasts the result obtained by Mohammed [15], who found high numbers of coliforms in sausage and sharwama samples. Total number of colony forming units obtained in this study was 1483 (obtained from Table 1) out of which *Salmonella* constituted about 1%. This is relatively similar to what was obtained by investigators such as El-Mossalami [16], 5% and Siriken et al. [17] 7%. On the contrary, very high incidence of *Salmonella* in sausage was recorded by Banks and Board [18] (55%); Moreno et al. [19] (18.2%) and Abrahim et al. [20] (20%). However, some investigators such as

Vazgecer et al. [21] and Ismail [22] failed to detect *Salmonella* in meat products. *Salmonella* contamination of moist food which has been stored for 24 h in a warm environment or several days in a cool larder, allowing heavy growth of this bacterium is sufficient to cause food poisoning [15]. *Salmonella* is one of the microorganisms most frequently associated with outbreaks of food-borne illness and food poisoning cases in meat and fish products [15]. *Pseudomonas* spp which was obtained in almost all the samples evaluated in this study (Table 1 to 4), is the quintessential opportunistic pathogen of humans. It is a leading cause of hospital acquired infections (nosocomial infections) and it is difficult to eradicate due to its resistance to most antimicrobial agents [23]. Presence of *Staphylococcus* contamination in some of the samples examined in this study as shown on Tables 1 to 4 might have resulted from man's respiratory passages, skin and superficial wounds which are common sources of *Staphylococcus epidermidis* [24]. When allowed to grow in foods, *Staphylococcus epidermidis* can produce a toxin that causes serious illness. Although, cooking destroys the bacteria, the toxin produced is heat stable and may not be destroyed even by heating [25].

Staphylococcus epidermidis and *Pseudomonas* spp occurred in very large numbers in the food items analysed. This agrees with previous reports by Clarence et al. [26] who reported *S. aureus*, *Escherichia coli, Klebsiella* spp and *Pseudomonas* spp in meat pie. *Salmonella* spp another potential toxin producing bacteria was obtained in samples obtained from two out of all the local government areas in which sampling was done (Figs. 2 and 3). *Proteus* spp was found in both Meat pie and Fish roll samples (Fig. 1) with an occurrence of about 1% (obtained from Tables 1 to 4) of the total number of isolates obtained from all the samples. This isolate was observed to occur in samples obtained from four out of the five LGAs in which sampling was done as shown on Figs. 2 and 3. Their presence in meat products was reported by some investigators but in higher proportion. *Proteus* spp is considered as an indicator of contamination of meat product during any of the processing and storage stages. If the optimal condition for the isolated *Proteus* spp existed, typical cases of food poisoning, urinary infection and other *Proteus* spp related human illnesses could occur due to rapid proliferation of the pathogen. The result from the statistical analysis of the samples of Meat pie and Fish rolls in this study shows that the Microbial content of the isolates were higher in all of the Road side samples as compared with Eatery bought samples (Fig. 1). In Nigeria, there are no guidelines for food hawkers, hence there is a need to come up with rules that will govern the sale of street foods and to ensure the safety and quality of these foods**.** It is recommended that proper facilities and training be given to food vendors. In the meantime, proper sanitary conditions must be practiced by food vendors while Critical control points are properly monitored. Local Governments and the health ministry should consider the establishment of adequate facilities and utility services as well as provision of necessary information, education and training seminars for vendors and consumers.

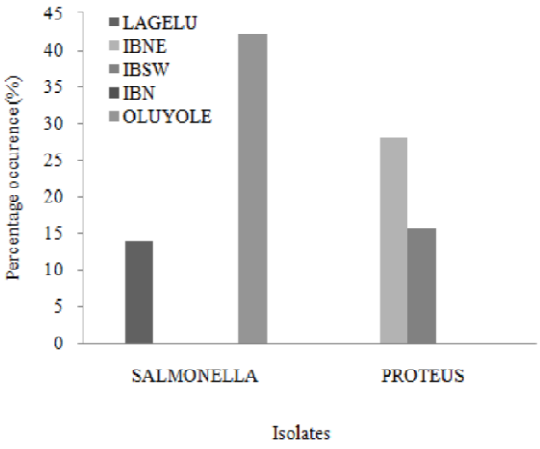

Fig. 2. Percentage occurrence of *Salmonella* and *Proteus* spp in fish roll samples from LGAs

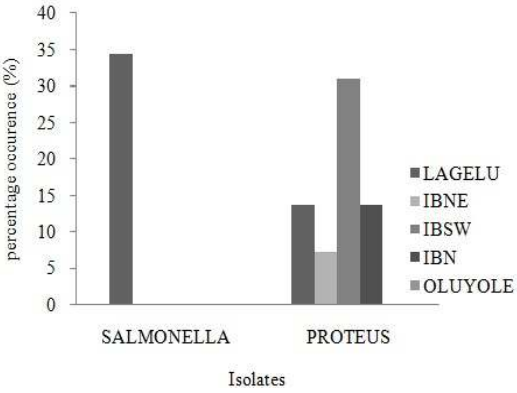

Fig. 3. Percentage occurrence of *Salmonella* spp and *Proteus* spp in meat pie samples from LGAs

Table 1. Microbial load of fish rolls samples from roadside shops

Suspected organisms	Location/ Bacterial load (cfu/mL)										Total bacterial load (cfu/mL)
	Lagelu		Oluyole		IBNE		IBSW		IBN		
Salmonella sp	+ve	1×10^{8}	+ve	3×10^{8}	-		-		-		4×10^{8}
Proteus sp		-	-		-		+ve	1×10^{8}	-		1×10^{8}
Flavobacterium sp		-	+ve	2×10^{8}	+ve	6×10^{8}	-		-		8×10^{8}
Pseudomonas sp	+ve	101×10^{1}	+ve	2×10^{8}	+ve	1×10^{8}	+ve	5×10^{8}	-		101×10^{1}
Aeromonas sp		-	+ve	1×10^{8}	-		+ve	$1v10^{8}$	-		2×10^{8}
Moraxella sp		-	+ve	1×10^{8}	+ve	11×10^{8}	-		-		12×10^{8}
Staphylococcus epidermidis	+ve	1.9×10^{8}	+ve	0.3×10^{8}	-		+ve	2.5×10^{8}	+ve	0.2×10^{8}	4.9×10^{8}
Micrococcus sp	+ve	43×10^{1}	+ve	1×10^{8}	+ve	1×10^{8}	+ve	4×10^{1}	-		49×10^{1}
Lactobacillus sp		-	-		+ve	3×10^{8}	+ve	17×10^{8}	-		20×10^{8}
Microbacterium spp	+ve	101×10^{1}	-		-		-		+ve	48×10^{1}	149×10^{1}
Bacilllus sp		-	+ve	2×10^{8}	-		-		-		2×10^{8}
Streptococcus sp		-	+ve	7×10^{8}	+ve	4×10^{8}	-		-		11×10^{8}
Rhodotorula sp		-	+ve	3×10^{8}	-		+ve	2×10^{8}	-		5×10^{8}
Saccharomyces cerevisiae		-	+ve	12×10^{8}	-		+ve	57×10^{1}	-		69×10^{8}

Table 2. Microbial load of meat pies samples from eateries

Suspected organisms	Location/ Bacterial load (cfu/mL)								T.B.L (cfu/mL)
	Lagelu		Oluyole	IBNE	IBSW		IBN		
Salmonella sp	+ve	2.0×10^{2}	-	-		-		-	
Proteus sp	+ve	2.0×10^{3}	-	-		-		+ve	1.0×10^{3}
Flavobacterium sp		-	-	-		-		-	
Pseudomonas sp		-	-	-		+ve	2×10^{1}	+ve	48×10^{1}
Aeromonas sp		-	-	+ve	1×10^{8}	-		-	
Moraxella sp		-	-	-		-		-	
Staphylococcus epidermidis		-	-	+ve	3×10^{8}	-		-	
Micrococcus sp	+ve	1×10^{8}	-	-		-		-	
Lactobacillus sp		-	-	-		+ve	1×10^{1}	+ve	145×10^{1}
Microbacterium spp		-	-	-		-		-	
Bacilllus sp		-	-	-		-		-	
Streptococcus sp	+ve	2×10^{8}	-	-		-		-	
Rhodotorula sp		-	-	-		+ve	1×10^{8}	-	
Saccharomyces cerevisiae	+ve	2×10^{8}	-	+ve	3×10^{8}	-		-	

TBL - Total bacterial load

Table 3. Microbial load of meat pie samples from roadsides

Suspected organisms	Location/Bacterial load (cfu/mL)										Total Bacterial load (cfu/mL)
	Lagelu		Oluyole		IBNE		IBSW		IBN		
Salmonella sp		-	-	-	-	-	-	-	-	-	-
Proteus sp		-	-	-	+ve	1×10^{8}	+ve	9×10^{8}	+ve	1×10^{8}	11×10^{8}
Flavobacterium sp		-	+ve	1×10^{8}	+ve	5×10^{8}	+ve	1×10^{8}	-	-	7×10^{8}
Pseudomonas sp	+ve	116×10^{1}	-	-	+ve	1×10^{1}	+ve	2×10^{1}	+ve	12×10^{1}	131×10^{1}
Aeromonas sp		-	-	-	+ve	13×10^{8}	+ve	1×10^{8}	-	-	14×10^{8}
Moraxella sp		-	+ve	2×10^{8}	-	-	-	-	-	-	2×10^{8}
Staphylococcus epidermidis	+ve	10.4×10^{8}	+ve	1.6×10^{8}	+ve	0.5×10^{8}	+ve	6.3×10^{8}	+ve	3.6×10^{8}	22.4×10^{8}
Micrococcus sp	+ve	22×10^{8}	+ve	1×10^{8}	-	-	+ve	1×10^{8}	-	-	24×10^{8}
Lactobacillus sp		-	+ve	68×10^{1}	-	-	+ve	1×10^{1}	-	-	69×10^{1}
Nocardia sp		-	+ve	3×10^{8}	-	-	-	-	-	-	3×10^{8}
Microbacterium spp	+ve	62×10^{1}	-	-	-	-	-	-	+ve	76×10^{1}	138×10^{1}
Bacilllus sp	-	-	-	-	-	-	-	-	-	-	-
Streptococcus sp	-	-	-	-	-	-	-	-	-	-	-
Rhodotorula sp	-	-	+ve	7×10^{8}	-	-	+ve	1×10^{8}	-	-	8×10^{8}
Saccharomyces cerevisiae	-	-	+ve	38×10^{1}	-	-	+ve	4×10^{1}			42×10^{1}

Table 4. Microbial load of fish roll samples from eateries

Suspected organisms	Location/Bacterial load (cfu/mL)										Total
	Lagelu		Oluyole		IBNE		IBSW			IBN	bacterial load (cfu/mL)
Salmonella sp	+ve	$3x10^1$	-	-	-		-	-	-	-	$3x10^1$
Proteus sp	-	-	-	-	+ve	$2x10^3$	-	-	-	-	$2x10^3$
Flavobacterium sp	-	-	-	-	-	-	+ve	$1x10^8$	+ve	$3x10^8$	$4x10^8$
Pseudomonas sp	+ve	$1×10^8$	-	-	-	-	-	-	+ve	$2×10^8$	$3×10^8$
Aeromonas sp	-	-	-	-	-	-	-	-	-	-	-
Moraxella sp	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus epidermidis	+ve	$2×10^8$	-	-	-	-	-	-	-	-	$2×10^8$
Micrococcus sp	-	-	-	-	+ve	$6×10^8$	-	-	-	-	$6×10^8$
Lactobacillus sp	-	-	-	-	+ve	$1×10^8$	+ve	$3×10^8$	+ve	$16×10^8$	$20×10^8$
Microbacterium spp	-	-	-	-	-	-	-	-	-	-	-
Bacilllus sp	-	-	-	-	-	-	-	-	-	-	-
Streptococcus sp	-	-	-	-	-	-	-	-	-	-	-
Rhodotorula sp	-	-	-	-	-	-	-	-	+ve	$2×10^8$	$2×10^8$
Saccharomyces cerevisiae	-	-	+ve	$7×10^8$	+ve	$4×10^8$	-	-	-	-	$11×10^8$

4. CONCLUSION

High occurrence of the targeted microorganisms in convenience foods especially the street hawked samples is of serious concern, more so since some of them are opportunistic pathogens. This further corroborates the need for Nigerian government to enforce policies and laws that guide the practice of street hawking of food especially ready to eat foods or develop one if there is no such law at present.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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