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Critical Appraisal of Microbial Load of Food Sold and Consumed by Workers at the Federal Secretariat Abuja Nigeria

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Abstract **Original Research Article**

The aim of the study was to assess microorganisms causing spoilage of food sold in local restaurants in Abuja, Nigeria. The study was done within the context of reducing the prevalence of food borne illnesses caused by microorganisms and to increase reliance of cooked food outside the home. Microorganisms causing spoilage of cooked food in local restaurants in Abuja were investigated. Forty-eight (48) samples were inoculated into three different media (Rice, Beans, Eba, Pounded yam) and analysed biochemically and microbiologically. Four (4) samples each were collected from Omobola Restaurant, Omobokun Restaurant, Mariam Restaurant and Ekiate Restaurant. All the samples were cultured in MacConkey Agar, Nutrient Agar and Potato Dextrose Agar and incubated at 37°C for 24 hours. The forty-eight (48) samples inoculated were all positive for fungi and bacteria isolates, which had the following percentage frequency of occurrence *Salmonella typhi* 37.5% had the highest prevalence, *Staphylococcus* spp. 27.5%, *Escherichia coli* 12.5%, *Shigella* spp 5.00%, *Bacillus* spp 15.00% and the *Streptococcus* spp 2.50% with the lowest prevalence from bacteria isolates. *Aspergillus* spp 37.50% with the highest prevalence from the fungi isolates, *Rhizopus* spp 17.50%, *Mucor* spp 30.00% and *Penicillium* spp 15.00% with the lowest prevalence.

Keywords: Isolates, Microorganisms, Occurrence, Ready-to-eat food, Spoilage, Illnesses.

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1. STUDY BACKGROUND/LITERATURE REVIEW

Approximately 30% of all emerging infections in the World over the past 60 years were caused by pathogens commonly transmitted through food as stated by [1]. Bacteria and viruses such as *Salmonella*, Norovirus, *Campylobacter*, *Listeria*, *Escherichia coli* etc. are a common cause of food borne diseases [2]. In Africa, prevalence of food borne diseases in the region is estimated at 41.6% in 1990, 35.6% in 2011 and 35.0% in 2012 [1]. Food contaminated by harmful bacteria, viruses, parasites or chemical substances can lead to a wide range of health problems. This is responsible for more than 200 diseases, including: typhoid fever, diarrhoea and cancers, among others [2]. Studies have shown that the following pathogens are prevalent: *Campylobacter*, *Salmonella*, *Shigella*, Hepatitis, *Brucella*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and rotavirus [3]. Prevalence of food borne diseases is related to the available environmental risk factors in food services as stipulated by World Health Day 2015, food safety- the global view. These risk factors can be split into two broad categories social, economic, cultural, political and physical, chemical as well as biological factors [3].

Food borne diseases (FBDs) means any diseases of an infectious or toxic nature caused or thought to be caused by consumption of food with micro-organisms and toxic or food borne diseases are transmitted by vectors that carry diseases causing agents from humans. These vectors include: humans, animals, insects and rodents [4].

Types of food borne diseases are: typhoid, cholera, dysentery, staphylococcal, shigellosis,

salmonellosis, campylobacteriosis, enteritis, amoebiasis, giardiasis and viral hepatitis [6]. These foods borne diseases are the result of ingestion of foodstuffs contaminated with microorganisms such as bacteria, fungus, virus, flies, protozoan and chemical hazards or mycotoxins [3].

The contamination of food may occur at any stage in the process from food production to consumption (farm to fork) as related to contaminated environment of water, air and soil (pollution), in daily human activities (food service environment) where micro-organisms live and toxic are produced in to the environment [2]. According to [3], prevalence of food borne diseases are very common among people with poor food education on storing, handling, preparing and processing food specifically in the poor families, low hygienic standard, absence of committed health officers in both developed and developing countries. Also [3] added some risk factors related to prevalence of food borne diseases in Africa such as inadequate fresh water for drinking, poor-sewage treatment, increased level of over population, environmental (air, water, soil) contamination, absence of improved waste management, poor participation of the community and institutions in the environmental hygiene.

Furthermore, outbreaks of food borne diseases in the Africa continent has been reported in a number of countries. These include acute aflatoxicosis in Kenya, in 2004 which was associated with maize, bromine poisoning in Angola in 2007 associated with the use of sodium bromide as salt, anthrax in Zimbabwe [2, 7, 8] mentioned that environmental risk factors such as contaminated environments, consumption of contaminated foods as the source of these food borne diseases outbreaks. Then according to [9], approximately 10 to 20% of food borne diseases outbreaks are due to contamination by the food handler and failure to observe satisfactory standards in the preparation (processing, cooking, storing or retailing of food).

The major food or water-borne diseases in Nigeria include: hepatitis, a viral disease which affects the liver and which is spread through food or water contaminated with faeces causing jaundice, fatigue, abdominal pain, diarrhoeal, and dark coloured urine; and typhoid fever, a bacterial disease which is also spread through food or water contaminated by faeces leading to high fever and death [6].

1.1. LITERATURE REVIEW

In developing countries, the burden of food borne diseases is estimated to be higher due to presence of favourable environment for growth of wide range of food borne diseases microbes [12]. For example, in the year 2000 alone 2.1 million people died from diarrhoeal diseases. reported that 90% of annual death from diarrhoeal were among children particularly in developing countries. This is attributed to contamination of food and drinking water [3]. WHO (2000) reported that the incidence of food borne diseases in Africa is due to environmental risk factors such as lower socio-economic classes with low educational level, rapid staff turnover, high level of seasonal staff literacy, language problems, poor motivation due to low pay and job status are some of the proposed reasons for the lack of applying the acquired knowledge especially in small food businesses as stated by Travis *et al.*, (1986): Burch and Sawyer (1999). According to Walker *et al*., (2003), when food poisoning outbreaks are investigated, it has been established that small and medium sized businesses are often important locations in the transmission of food borne diseases. Scott (2001) observed that approximately 80% of meat samples tested was contaminated with *Salmonella* spp. Contamination rate of vegetable with *Salmonella* spp. was 5% and fermented food at 9%. Six strains of *Cronobacter sakazakii* and two strains of *Yersinia enterocolitica* are known to contaminate food. A substantially higher rate of contamination by *Bacillus cereus* is in fermented food 82%, meat 2% and fish or seafood at 5%. 7% *Listeria* spp. isolates have been found in meat and fish or seafood samples. Approximately, 39% of samples tested were found to be contaminated with *Staphylococcus* spp. as reported by [16], food borne diseases will remain one of the top ten diseases in 2020. Kaferstein [6] stated that the prevalence of food borne diseases in the developing world is even higher, although it is difficult to obtain the data that would support this assumption. There are more than 250 known food borne diseases [1]. These food borne diseases are grouped into bacterial, parasitic, viral, toxic elements, dusts, and suspended elements (risk factors) (WHO, 2015). WHO (2015) categorized food borne diseases according to their source as shown pathogenic bacteria (*Bacillus cereus, Campylobacter* spp., *Mycobacterium* spp, *Salmonella typhi, Salmonella paratyphi, Vibrio cholerae* and *Staphylococcus aureus*); viruses: (Hepatitis A., E. virus and Poliovirus); protozoa (*Cryptosporidium* spp, *Entamoeba histolytica, Toxoplasma gondii)*; Trematodes (*Facsiola hepatica* and *Clonorchis sinensis*), Cestodes (*Taenia solium* and *Taenia saginata*); Nematodes (*Ascaris lumbricoides, Trichuris trichiura*); natural toxins (Mushroom toxins, mycotoxins and plant toxicants); chemicals (pesticides, toxin metals, polychorinated biphyls, fluoride, zinc, nitrites and *Monosodium glutamate*). Environmental risk factors include: biological, social, economic, political and geographical (factors) [7]. A study of 157 outbreaks of *Escherichia coli* in the United States by [22] found that 80% of suspected hamburgers were prepared and eaten at home. In Australia, approximately 90% of *Salmonella* spp. infections are generally thought to be associated with manufactured foods [20]. Data available from Canada covering 1996 and 1997 has identified home prepared foods as the most common exposure setting for cases of *Salmonella* spp.,

Campylobacter spp. and pathogenic *E. coli* infection [24]. Food borne agents that have been introduced into the home via humans include: species of *Salmonella, Shigella sonnei, Staphylococcus aureus*, rotavirus and hepatitis A virus [28]. Programs on diarrhoeal diseases and other food borne diseases should be introduced in the school's curriculum in order to reduce the incidence of food borne diseases [3]. Kaferstein [6] suggested that community members must be educated about a contaminated food product, advise them on proper preparation of foods, disposing foods end products, boiling of microbiological contaminated water, avoidance of chemical contaminated foods and emphasizing personnel hygiene measure and exclusion of infected persons from work or school in the food service during the food borne outbreaks.

2. METHODS

A total of 16 samples were obtained asceptically from the following restaurants: Omobokun, Ekiate, Omobola and Mariam. Each food sample (10 g) was asceptically weighed into a mortar and grounded with a sterile pestle. Then, transferred into a nutrient broth (peptone water). Volume of distilled water (90 ml) was poured into the mortar and the mixture was homogenized. Ten (10) ml of the mixture was then transferred to a test-tube and followed by serial dilutions. Serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} were made. Exactly, 0.1ml of serial dilutions 10^{-2}, 10^{-3} and 10^{-4} were cultured on nutrient agar, Potato Dextrose Agar and MacConkey agar petri dishes. Fungal plate count dishes using Miles and Misra method [23, 31]. The petri dishes were incubated at 37°C. The number of colonies seen were counted using a colony counter and recorded as colony forming unit per gram (cfu g^{-1}). Gram reaction [19]. Coagulase test [15], Catalase test [15], Motility test [21], Oxidase test [18], Indole test [15], were all carried out in appropriate and relevant aspects.

3. RESULTS

Table 1: Identification of Bacteria and Fungi Isolates of Rice in Different Restaurants

Restaurants	Food Sample	Gram Reaction	Catalase Test	Coagulase Test	Oxidase Test	Isolated Microorganisms
OMOBOKUN	Rice	ve Gram negative rod	_	_	_	*Salmonella typhi*
		cocci in clusters	+	+	_	*Staphylococcus spp.*
		Non-septate hyphae with branched sporangiospores	Nil	nil	nil	*Mucor spp.*
MARIAM	Rice	Brown colonies with dark centers	-ve Gram short rods	+	_	_ *E. coli*
		Cream coloured colonies	+ve Gram rods	+	_	+ *Bacillus spp*
		Grey on surface	Septate hyphae	Nil	nil	nil *Aspergillus niger*
EKIATE	Rice	Pink colonies	-ve Gram rod	_	_	_ *Salmonella typhi*
		Creamy white colonies	Positive cocci clusters	+	+	_ *Staphylococcus spp*
		Grey on surface	Septate hyphae	Nil	nil	nil *Aspergillus niger*
OMOBOLA	Rice	Brown colonies with brown centers	Gram negative short rods	_	_	_ *Salmonella typhi*
		Cream colored colonies	Gram positive rods	+	_	+ *Bacillus spp*
		Yellow and fluffy black spores	Non-septate hyphae with branched spores	Nil	nil	nil *Mucor*

Table 2: Identification of Bacteria and Fungi Isolates of Beans in Different Restaurants

Restaurants	Media Used	Cultural Features	Gram Reaction	Catalase Test	Coagulase Test	Oxidase Test	Isolated Microorganisms
OMOBOKUN	MAC	Pink colonies	Gram negative rod	_	_	_	*Salmonella typhi*
	NA	Creamy white colonies	Gram positive rods	+	_	+	*Bacillus spp*
	PDA	Creamy with black spores spread around the surface	Long branched chains of conidiophores septate hyphae	nil	nil	nil	*Penicillium spp*
MARIAM	MAC	Brown colonies	Gram negative short rods	+	_	_	*E. coli*
	NA	Creamy white	Gram positive cocci in clusters	+	+	_	*Staphylococcus spp*
	PDA	White to grey cotton growth surface	Stolon and unbranched sporangiospores	nil	nil	nil	*Rhizopus spp*
EKIATE	MAC	Pink colonies	Gram negative rod	+	+	_	*Shigella spp*
	NA	Creamy white colonies	Gram positive rods	+	_	+	*Bacillus spp*
	PDA	Yellow and fluffy black spores	Non-septate hyphae with branched spores	nil	nil	nil	*Mucor*
OMOBOLA	MAC	Pink colonies	Gram negative rods	+	+	_	*Shigella spp*
	NA	Cream colored colonies	Gram positive rods	+	_	+	*Bacillus spp*
	PDA	Yellow and fluffy black spores	Non-septate hyphae with branched spores	nil	nil	nil	*Mucor*

Table 3: Identification of Bacteria And Fungi Isolates of Eba in Different Restaurants

Restaurants	Cultural Features	Gram Reaction	Catalase Test	Coagulase Test	Oxidase Test	Name of isolates
OMOBOKUN	Pink colonies	Gram negative rod	_	_	_	*Salmonella typhi*
	Creamy white colonies	Gram positive cocci in clusters	+	+	_	*Staphylococcus spp*
	Cream in black spores spread around the surface	Septate hyphae	nil	nil	nil	*Aspergillus spp*
MARIAM	Brown colonies	Gram negative short rods	+	_	_	*E. coli*
	Creamy white	Gram positive cocci in clusters	+	+	_	*Staphylococcus spp*
	White to grey cotton growth surface	Stolon and unbranched sporangiospores septate hyphae	nil	nil	nil	*Rhizopus spp*
EKIATE	Pink colonies	Gram negative rod	_	_	_	*Salmonella typhi*
	Creamy white colonies	Positive cocci in clusters	+	+	-	*Staphylococcus spp*
	Grey on surface	Septate hyphae	nil	nil	nil	*Aspergillus spp*
OMOBOLA	Pink colonies	Gram negative rods	_	_	_	*Salmonella typhi*
	Cream coloured colonies	Gram positive cocci in clusters	+	+	_	*Staphylococcus spp*
	Grey on surface	Septate hyphae	nil	nil	nil	*Aspergillus niger*

"Eba" is a stiff dough made by soaking Garri in hot water.

Table 4: Identification of Bacteria and Fungi Isolates of Pounded Yam in Different Restaurants

Cafeteria	Cultural Features	Gram Reaction	Catalase Test	Coagulase Test	Oxidase Test	
OMOBOKUN	Pink colonies	Gram negative rod	_	_	_	*Salmonella typhi*
	Creamy white colonies	Gram positive cocci in chains	_	_	_	*Streptococcus spp*
	Grey on surface	Septate hyphae	nil	nil	nil	*Aspergillus spp*
MARIAM	Brown colonies	Gram negative short rods	+	_	_	*E. coli*
	Creamy white	Gram positive cocci in clusters	+	+	_	*Staphylococcus spp*
	White to grey cotton growth surface	Stolon and unbranched sporangiospores	nil	nil	nil	*Rhizopus spp*
EKIATE	Pink colonies	Gram negative rod	_	_	_	*Salmonella typhi*
	Creamy white colonies	Gram positive rods	+	_	+	*Bacillus spp*
	Grey on surface	Septate hyphae	nil	nil	nil	*Aspergillus niger*
OMOBOLA	Pink colonies	Gram negative rods	_	_	_	*Salmonella typhi*
	Cream colored colonies	Gram positive cocci in clusters	+	+	_	*Staphylococcus spp*
	Grey on surface	Septate hyphae	nil	nil	nil	*Aspergillus niger*

Table 5: Summary of Bacteria and Fungi Isolates in Various Food Samples Collected From Omobokun, Omobola, Mariam and Ekiate Restaurants

Names of restaurants	Rice	Beans	Eba	Pounded Yam
OMOBOKUN	*Salmonella typhi*, *Staphylococcus* spp, Mucor	*Salmonella typhi*, *Bacillus* spp, *Penicillium* spp	*Salmonella typhi*, *Staphylococcus spp*, *Aspergillus* spp	*Salmonella typhi*, *Aspergillus* spp, *Streptococcus* spp
OMOBOLA	*Salmonella typhi*, *Bacillus* spp, *Mucor*	*Shigella* spp, *Bacillus* spp, *Mucor*	*Salmonella typhi*, *Staphylococcus* spp, *Aspergillus niger*	*Salmonella typhi*, *Staphylococcus* spp, *Aspergillus* niger
MARIAM	*E. coli*, *Bacillus* spp, *Aspergillus niger*	*E.coli*, *Staphylococcus* spp, *Rhizopus* spp	*E.coli*, *Staphylococcus* spp, *Rhizopus* spp	*E.coli*, *Staphylococcus* spp, *Rhizopus* spp
EKIATE	*Salmonella typhi*, Staphylococcus spp, *Aspergillus niger*	*Shigella* spp, *Bacillus* spp, *Mucor*	*Salmonella typhi*, *Staphylococcus* spp, *Aspergillus* spp	*Salmonella typhi*, *Bacillus* spp, *Aspergillus* spp

Table 6: Bacterial Percentage of Occurrence

BACTERIA	FREQUENCY OF OCCURRENCE	PERCENTAGE OF OCCURRENCE (%)
Salmonella typhi	30	37.50
Staphylococcus aureus	22	27.50
Escherichia coli	10	12.50
Shigella spp.	04	5.00
Streptococci spp.	02	2.50
Bacillus spp.	12	15.00
TOTAL	80	100

Table 7: Fungi Percentage of Occurrence

FUNGI	FRQUENCY OF OCCURRENCE	PERCENTAGE OF OCCURRENCE
Aspergillus niger	15	37.50
Rhizopus spp.	07	17.50
Mucor spp.	12	30.00
Penicillium spp.	06	15.00
TOTAL	40	100

ANALYSIS OF RESULTS. (X2) TEST HYPOTHESIS:-

H_A - Microorganisms causing food spoilage infect food sold for human consumption in local restaurants in Abuja.

H_o - Microorganisms causing food spoilage do not infect food sold for human consumption in local restaurants in Abuja.

Table 8: Analysis of Data Using Chi-Square

Microorganisms	Observed frequency (O)	Expected frequency (E)	Obs-Exp (O-E)	$(Obs-Exp)^2$ EXP
Salmonella typhi	30	12.00	+18	27.00
Staphylococcus spp	22	12.00	+10	8.33
Escherichia coli	10	12.00	-2	0.33
Shigella spp	04	12.00	-9	6.75
Streptococcus spp	02	12.00	-10	8.33
Bacillus spp	12	12.00	0	0.00
Aspergillus niger	15	12.00	+3	0.75
Rhizopus spp	07	12.00	-5	2.08
Mucor spp	12	12.00	0	0.00
Penicillium spp	06	12.00	-6	3.00
				$\sum x2 = 56.57$

Degree of freedom = (n-1) = (10-1) =9
Degree of Freedom (df) =9
Probability (P) = 0.01 and 0.05
At 0.01 = 21.67
At 0.05= 16.92
Fcal = 56.57
Since Fcal > Ftab at 0.01 and 0.05 level of significance when df= 9.

Null hypothesis is rejected while the Alternate hypothesis is accepted. Stating that microorganisms significantly cause food spoilage in local restaurants in Abuja.

RESULTS

A total of forty-eight (48) samples showed positive to the growth of microorganisms, 60% of the organisms were bacteria and 40% were fungi. The bacterial isolates, which were *Staphylococcus* species, *Streptococcus* species, *Salmonella* species, *Bacillus* species, *Escherichia coli.* Colonial morphology and microscopic examination were used for identification of fungal isolates which includes: *Mucor* spp, *Aspergillus* spp, *Rhizopus* spp and *Penicillium* spp. Among the bacteria contaminants isolated (Table 6), *Salmonella typhi* had the highest prevalence 37.50%, followed by *Staphylococcus* spp 27.50%, and *Streptococcus* spp with the lowest prevalence 2.50%. For fungi isolates, (Table 7), *Aspergillus* spp 37.50% had the highest prevalence while *Penicillium* spp 15.00% had the lowest prevalence.

4. DISCUSSION

The results obtained showed that microorganisms cause food spoilage. Bacteria isolated from samples collected (Rice, Beans, Eba and Pounded yam) were *Salmonella typhi*, *Staphylococcus spp*, *Streptococcus spp*, *Escherichia coli*, *Bacillus spp* and *Shigella spp*. The percentage occurrence of bacteria isolated were, *Salmonella typhi* 37.5%, *Staphylococcus* spp 27.5%, *Escherichia coli* 12.5%, *Shigella spp* 5.00%, *Streptococcus spp* 2.50% and *Bacillus spp* 15.00% (Table 6). Fungi isolated includes; *Aspergillus spp*, *Rhizopus spp*, *Mucor spp* and *Penicillium spp*. The percentage of occurrence were, *Aspergillus spp* 37.50%, *Rhizopus spp* 17.50%, *Mucor spp* 30.00% and *Penicillium spp* 15.00% (Table 7). The fact that these contaminants were at high level in these environment is of great concern, this shows that food is infected by microorganisms. Some of the isolated microbes are directly or indirectly in the contamination of food causing food borne illnesses. *Salmonella typhi* 37.50% is found to the have the highest occurrence (Table 6) due to poor hygiene is also found in human throat, nose and skin and *Streptococcus spp* 2.50% is the lowest (Table 6) due to proper handling of food. While for fungi *Aspergillus spp* 37.50% is found to have the highest occurrence (Table 7) and *Penicillium spp* 15.00% is the lowest (Table 7).

Microbial growth on food is very high; Salmonella spp can survive improper heating of food leading to contamination. *Staphylococcus spp* have also been found to be relatively resistant to some temperature which is a property that favours their transmission from one host to another [24]. Its occurrence is as a result of forceful release as in sneezing, coughing or talking by food handlers since it is a normal resident in the respiratory tract [24].

Aspergillus spp among the fungi isolates have been associated with common contaminants of starchy foods and grow in or on many plants and trees [29]. Microorganisms can easily contaminate food if bad sanitation measures are observed and the use of unclean fomites and utensils [24]. However, an important factor which contributes greatly to the microbial infection of food is the poor infrastructure of restaurants, making it very easy to harbor them by aerial spores or bacterial spores carried in air and several other insects such a flies which are of high population in restaurants. Generally, the increased level of bacterial and fungi load infecting food observed in this study could also be as a result of contamination arising from the preparation method and the food handlers.

5. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study revealed that different microorganisms that infest food cause food borne illnesses, some of the isolated bacteria are of public importance thus the presence in food can cause health problems and disorders including food borne diseases, food poisoning and food intoxication. Microorganisms capable of endangering human lives were isolated from the food samples, the practice of preparation and selling of food in unclean environments, where there are no emphasis of the hygiene standards leads to increase in proliferation of microorganisms. Provision of education and training is necessary to all participants in food production to consumption for the reduction of microbial infection.

Preventing cross contamination is a key factor in preventing foodborne illness and its associated impacts. However, simple precautions can reduce the risk. Avoiding the consumption (that is, do not eat) certain foods for example, eating raw meats and fish should be avoided and salads prepared in restaurants where meats and vegetables share a common surface during preparation should be avoided. Caution should be taken when serving food and keeping food at room temperature for long period of time should be avoided. Adequate heat treatment and cooking foods properly reduces the risk of food borne illness from foods contaminated by certain pathogens especially food infection causing food borne illnesses.

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