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Full Length Research Paper

Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria

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Eight triplicate samples of meat pie were randomly sampled from standard eatery and local kiosk in Benin City and analyzed microbiologically for the rates of *Staphylococcus aureus* and *Escherichia coli.* The mean microbial load on the fresh meat pie from the standard eatery ranged from $3x10^3$ – $5x10^3$cfu/g while the air preserved and refrigerated meat pie for (2 days) ranged between 2.3 $x10^4$ -3.8 $x10^4$ cfu/g and 8x 10^3-1.5 $x10^4$ cfu/g respectively. The mean microbial load of the fresh meat pie from the local kiosk ranged between $7x10^3$-$2.8x10^4$ cfu/g while the air preserved and refrigerated meat pie for 2days ranged between $3x10^{-4}$ to too numerous to count (TNTC) and 1.3 $x10^4$ -$2.8x10^4$ cfu/g respectively. Six genera of the isolated bacteria include *Staphylococcus, E. coli, klebsiella, Pseudomonas, Bacillus and Enterococcus*. Statistical analysis of the mean microbial load showed a significant difference ($P<0.05$) between control and air preserved meat pie and no significant difference in the mean microbial load between control and refrigerated meat pie were ($P>0.05$).

Key words: *Escherichia coli*, meat pie, mean microbial load, *Staphylococcus*.

INTRODUCTION

Ready to eat foods can be described as the status of foods being ready for immediate consumption at the point of sale. Ready to eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002). Different terms have been used to describe such ready to eat foods. These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, moin-moin, salad or coleslaw, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 1974). A general observation of our society shows a social pattern characterized by increased mobility, large numbers of itinerary workers and less family or home centered activities. This situation however has resulted in more ready to eat foods taken outside home. Thus food vendor services become on the increase and responsebility for good manufacturing practices of food such as good sanitary measures and proper food handling have been transferred from individuals/families' to the food vendors who rarely enforce such practices (Musa and Akande, 2002). According to Doyle and Evans (1999), Food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. Data on issues of food borne diseases are well documented world wide (Hazariwala et al., 2002). Food borne illnesses is a major international health problem with consequent economic reduction (Duff et al., 2003). In United states, it has been estimated that seven pathogens found in animal products such as *Escherichia coli* 0157 :H7, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringes*, *Salmonella* spp., *Toxoplasma gondii* and *Staphylococcus aureus* account for approximately 3.3 - 12.3 million cases of food borne illnesses and a record of 3900 deaths each year.(Talaro et al., 1996; Buzby et al., 1997). Outbreaks of food borne diseases are caused by foods that are contaminated intrinsically or that become contaminated during harvesting, processing or preparation (Torok et al., 1997). In most countries, the most common food–borne illness is

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Staphylococcus food intoxication (Talaro et al., 1996). Enterotoxigenic *Staphylococcus* strains and *E. coli* strains have been isolated from foods implicated in illnesses (Adeyiwu, 1995, Firstenberg and Sullivan, 1997; Cencil et al; 2003). *E. coli* and *S. aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling (Adamolekun and Adamolekun, 1992). *S. aureus* is a gram positive coccus, resistant to heat, drying and radiation. Its strains can be pathogenic and relatively non pathogenic. They produce disease when the bacteria contaminate food. They produce some enzymes which are implicated with staphylococcal invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal (Prescott et al., 2005). Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxins are gene–based that is carried on plasmids. The intensity of the signs and symptons may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin .Some signs and symptoms of staphylococcal food poisoning include: Nausea, vomiting, abdominal cramp, prostration and diarrhea. *E. coli* a member of the genus *Escherichia* with the family *Enterobacteriaceae*. Members are widely distri-buted in the environment contaminated food and water are the major sources by which the bacteria are spread. Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005). These include diarrheal illnesses, Urinary tract infections, meningitis, sepsis, wound infections, noscomial pneumonia, inflammatory dysentery (Spangler, 1992). A subgroup called Enterohemorrhagic *E. coli* (EHEC) can cause food borne illness as the *E. coli* 0157:H7 strain which causes severe and potentially fatal illness known as hemorrhagic colitis which is characterized by bloody diarrhea and severe abdominal pain(Dolores and Doyle, 2001). *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination. However in Nigeria, a number of foods have been reported to have high incidence of bacteria (Adesiyun, 1995, Okonko et al., 2009). But there is limited information on the health challenges from food borne diseases from meat pie retailed within a highly populous community. The purpose of this study is focused on assessing the bacteriological state (occurrence of *E. coli* and *S. aureus*) in meat pie sold in Benin City ,Edo State Nigeria; characterize other bacterial isolates and highlight the health implications of consuming such contaminated ready to eat food (meat pie).

MATERIALS AND METHODS

Study Area

The study areas are the standard eatery and a local kiosk in Benin City, Edo state. She is one of the most cosmopolitan Cities in Nigeria with a population of over 2 million inhabitants. These study areas was choosen based on their location and large volume of patro-nage per day.

Chemical and reagents

All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England.

Sample collection

Eight samples of fresh meat pie were purchased from a standard eatery and local kiosk. The samples were aseptically collected in a clean polyethylene bag and transferred immediately to the laboratory for further analysis. These samples were all collected in the month of May, 2007. The methods of preservation of the food products by the (standard eatery and local kiosk) were employed in this study.

Sample analysis

Sample analyses of the meat pie were carried out in 2 parts: a) pastry b) Minced meat for aerobic count. This is because of the difference in mode of preparation of the pastry/minced meat before the final meat pie is prepared. 10 g of each food sample was weighed out and homogenized into 90 ml of sterile distilled deionized water using a sterile warring blender. Ten fold dilutions of the homogenates was made; 0.1 ml of 10^{-2} 10^{-3} and 10^{-4} dilutions of the homogenate was plated in replicate on MacConkey agar, Eosin Methylene blue agar, Mueller Hinton Agar, Mannitol salt agar using pour plate method. The plates were then incubated at 37℃ for 24 - 48 h. MacConkey agar was used for coliform enumeration while Mannitol salt agar was used for the isolation of *S. aureus*. Total viable aerobic bacteria count was performed on Mueller Hinton Agar. At end of the incubation periods, colonies were counted using the illuminated colony counter (Gallenkamp, England).The count for each plate were expressed as colony forming unit of the suspension (cfu/ml).

Identification of isolates

Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Gram staining as well as appropriate biochemical tests according to Cowan (1985) and Olutiola et al. (1991) were carried out.

Statistical analysis: Data were analyzed using the general linear model procedure and ANOVA.

RESULTS

Six genera of bacteria were isolated from the meat pie (pastry and Minced meat). The isolates were identified as *S. aureus, E. coli, klebsiella* spp, *Pseudomonas* spp, *Bacillus* spp and *Enterococcus* spp by comparing their morphological and biochemical characteristics (Catalase, Oxidase, Coagulase, Indole, Urease, Sugar tests) with standard reference organisms (Cowan, 1985; Olutiola et al., 1991) (Table 1).

Table 2a represents the mean colony forming unit of the total bacteria per gram of meat pie sample from standard eatery. Fresh meat pie served as control had a growth ranged between 3×10^3 - 5×10^3 cfu/g while th

Table 1. Morphological and biochemical characteristics of bacteria isolates from meat pie.

Parameters	Isolates					
	A	B	C	D	E	F
Grams reaction	+	-	+	-	+	+
Catalase test	+	+	+	+	+	-
Citrate test	-	+	+	+	N/A	+
Oxidase test	-	-	+	+	N/A	-
Coagulase test	+	-	-	-	N/A	-
Indole test	-	+	-	-	-	-
Urease activity	+	-	+	N/A	N/A	N/A
Cellular morphology	cocci	straight rods	rods	rods	rods	cocci
Growth in blood agar (colony)	creamy white	circular	large white	greenish	N/A	creamy
Growth in Mannitol salt agar	bright yellow	N/A	N/A	N/A	N/A	N/A
Growth in MacConkey agar	N/A	red/ pink	mucoid	pale	pink	pink
Glucose	+	+	+	N/A	N/A	+
Lactose	+	+	N/A	N/A	N/A	+
Sucrose	+	+	+	N/A	N/A	+
Mannitol	+	+	-	N/A	N/A	+
Maltose	+	+	N/A	N/A	N/A	+
Most probable organism						

Staphylococcus aureus, E. coli, klebsiella, Pseudomonas, Bacillus, Enterococcus

- (No growth), + (growth), N/A Not applicable.

Table 2a. Total mean colony unit of bacteria per gram of meat pie sample from standard eatery.

Control	Samples	Mean total bacteria count cfu/g
fresh meat pie	Sample A Pastry	5×10^3
	Sample A minced meat	3×10^3
	Sample B Pastry	4×10^3
	Sample B Minced meat	5×10^3
Room air preserved for 2 days	Sample A Pastry	3×10^4
	Sample A minced meat	3.5×10^4
	Sample B Pastry	2.3×10^4
	Sample B Minced meat	3.8×10^4
Refrigerated for 2 days	Sample A Pastry	8×10^3
	Sample A minced meat	1.5×10^4
	Sample B Pastry	9×10^3
	Sample B Minced meat	10×10^3

room air preserved meat pie (pastry /minced meat) for 2 days range between 2.3×10^4 - 3.8×10^4 cfu/g and the refrigerated sample for 2 days ranged between 8×10^3 - 1.5×10^4 cfu/g.

Table 2b shows the mean total colony forming count for *S. aureus* and *E. coli* from standard eatery. Fresh meat pie served as control with no significant growth .The cfu/g was 3×10^3 while the room air preserved for 2 days ranged between 1.0×10^4 - 4.0×10^4 cfu/g for *S. aureus* and 2×10^3 - 4×10^3 cfu/g for *E. coli* and refrigerated sample for 2 days ranged between 2×10^3 - 8×10^3 cfu/g for isolates from the minced meat for *S. aureus* only and 2×10^3 cfu/g from minced meat for *E. coli*.

Table 3a represents the total mean colony unit of bacteria per gram of meat pie sample from local kiosk. Fresh meat pies from the kiosk served as control and have a microbial load of 8×10^3 - 2.8×10^4 cfu/g. While the room air preserved meat pie ranged between 3×10^{-4} to TNTC and refrigerated 1.7×10^4 - 2.8×10^4 cfu/g.

Table 3b shows the mean total colony forming unit of incidence of *S. aureus* and *E. coli* from meat pie from the local kiosk. Fresh meat pie served as control with growth range between 3×10^3 – 8×10^3 cfu/g for *S. aureus* while *E. coli* ranged between 3×10^3 - 5×10^3 cfu/g. The room

Table 2b. Total colony forming count for *Staphylococcus aureus* and *Escherichia coli* from standard eatery.

Control	Samples	*S. aureus* count	Presence of *S. aureus*	*E. coli* count	Presence of *E. coli*
Fresh meat pie	Sample A Pastry	NG	-	NG	-
	Sample A Meat	3×10^3	+	2×10^3	+
	Sample B Pastry	NG	-	NG	-
	Sample B Meat	NG	-	NG	-
Room air preserved for 2 days	Sample A Pastry	1.0×10^4	+	4×10^3	+
	Sample A Meat	4.0×10^4	+	4×10^3	+
	Sample B Pastry	1.7×10^4	+	2×10^3	+
	Sample B Meat	2.5×10^4	+	3×10^3	+
Refrigerated for 2 days	Sample A Pastry	NG	-	NG	-
	Sample A Meat	8×10^3	+	2×10^3	+
	Sample B Pastry	NG	-	NG	-
	Sample B Meat	2×10^3	+	NG	-

(+) present, (-) absent, (NG) No growth.

Table 3a. Total mean colony unit of bacteria per gram of meat pie sample from local kiosk.

control	Samples	Total bacteria count cfu/g
Fresh meat pie	Sample A Pastry	8×10^3
	Sample A minced meat	7×10^3
	Sample B Pastry	2.8×10^4
	Sample B Minced meat	2.3×10^4
Room air preserved for 2 days	Sample A Pastry	3×10^4
	Sample A minced meat	TNTC
	Sample B Pastry	3.8×10^4
	Sample B Minced meat	TNTC
Refrigerated for 2 days	Sample A Pastry	1.7×10^4
	Sample A minced meat	1.5×10^4
	Sample B Pastry	2.8×10^4
	Sample B Minced meat	2.3×10^4

(TNTC) too numerous to count

Table 3b. Represents the mean total colony forming unit for the incidence of *Staphylococcus aureus* and *Escherichia coli* from a local kiosk.

Control	Samples	*S. aureus* count	Presence of S. *aureus*	*E. coli* count	Presence of *E. coli*
Fresh meat pie room air preserved for 2 days	Sample A Pastry	4×10^{-3}	+	3×10^3	+
	Sample A Meat	3×10^3	+	5×10^3	+
	Sample B pastry	NG	-	NG	-
	Sample B Meat	8×10^3	+	3×10^3	+
	Sample A Pastry	1.0×10^4	+	4×10^3	+
	Sample A Meat	1.5×10^4	+	4×10^3	+
	Sample B Pastry	1.9×10^4	+	2×10^3	+
	Sample B Meat	1.8×10^4	+	3×10^3	+
Refrigerated for 2 days	Sample A Pastry	5.0×10^3	+	4×10^3	+
	Sample A Meat	6.0×10^3	+	5×10^3	+
	Sample B Pastry	1.0×10^3	+	NG	-
	Sample B Meat	1.0×10^4	+	4×10^3	+

(+) present, (-) absent, (NG) No growth.

air preserved meat pie sample ranged between 1.0×10^4 - 1.9×10^4 for *S. aureus*, $1 \times 10^3 - 7 \times 10^3$ cfu/g for *E. coli.* The refrigerated meat pie ranged between 1.0×10^3 - 1.0×10^4 cfu/g for *S. aureus*, 4.0×10^3 - 5×10^3 cfu/g for *E. coli*.

DISCUSSION

In this study, the defection of estimation of *Staphylococcus aureus* and *Escherichia coli* in ready to eat food (meat pie) were exceptional. Biological contaminants of bacterial origin presents as major cause of food–borne disease given rise to acute to chronic illnesses such as *E. coli* gastroenteritis, Brucellosis and Camplylobacteriosis (Edema et al., 2005). From the mean total viable count carried out the predominant organisms include *S. aureus, E. coli, Bacillus* spp, *Enterobacter, Pseudomonas* and *Klebsiella*. This agrees to reports of (Oluwafemi and Simisaye, 2005; Okonko et al., 2009), were they isolated almost similar organisms from sausages and seafood processors respectively. The presence of these organisms in ready to eat food (meat pie) depicts a deplorable state of poor hygienic and sanitary practices employed in the processing and packaging of these food products. From the results obtained, meat pie sample (pastry and minced meat) were contaminated with high level of *S. aureus* and *E. coli*. This result agrees to previous reports by El-Gohany (1994) that foods of animal origin (minced meat) either cooked or uncooked were predominantly contaminated with *E. coli* and *S. aureus*. Waites and Arbuthnott *(*1999) reported of *S .aureus* and *E. coli* contamination in minced meat, sausage rolls and pies. They reported 60.9% prevalence of *S. aureus*, 50% *E. coli*, 40% S*higella*, 38% *Morganella morganii*. From recent findings, food mixtures such as pastries, salads, sauces, soups have been frequently incriminated in food poison outbreaks (FSRI, 2003; FDA, 2007 a, b, c, d). The isolate *Bacillus* spp have been incriminated to contribute towards these life threatening illnesses. On comparing the bacterial contamination between the eating outlet (standard eatery and local kiosk), the result obtained is still on the high level 3×10^3 - 2.8×10^4 cfu/g. This is an indication of recontamination in food handling hygiene techniques starting from the processing raw material to the finished product (Ikeme, 1990; Ojeibun, 1994). According to (Edema et al., 2001, Okonko et al., 2008 a, b) the presence of *E. coli* is an indication of fecal contamination of the water sources that were utilized in the processing of these food products. There was a comparable difference between the mean total microbial counts between the normal room air preserved to refrigerated meat pie. The refrigerated samples tend to show reduced growth when compared with the normal room air preserved samples. This could be attributed to the change in temperature which leads normal metabolic activities of these organisms coming to near halt. However, some microorganisms tend to survive such cold environment due to production of cold shock proteins which protect them from the changes that might affect their normal metabolic processes (Prescott et al., 2005). From the results, it is clear that minced meat sample had more bacterial contamination than the pastry.

This may be because meat offers a rich nutrient media for microbial growth (Phillips, 2003). Statistical analysis between the control and room air preserved for 2 days showed $P < 0.05$ and between the control and refrigerated sample $P > 0.05$. From this investigation, the issue of food poisoning is of paramount importance particularly in developing world were there are limited social amenities such as power and access to potable water. This study clearly confirmed the deplorable state of food consumed in such settings. Food poisoning/illnesses are entirely preventable by practicing good sanitation and food handling techniques (Betty and Richard, 1994).Thus to safeguard against the risks of this disease of moderate severity as described by Mossel and Van *(*1990) on incidence of staphylococcal food poisoning and *E. coli* contamination in foods, there is need to educate and advocate for good manufacturing practices among food processors and food vendors. Also relevant agencies in Nigeria such as Consumer protection Rights, NAFDAC and SON need to ensure and enforce strict compliance to Hazard analysis critical control points (HACCP) in all food production sectors in Nigeria.

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