

Full Length Research Paper

Occurrence of *Staphylococcus aureus* in meat pie and eggroll sold in Umuahia Metropolis, Nigeria

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Occurrence of *Staphylococcus aureus* in meat pie and eggroll sold in Umuahia metropolis was investigated. The samples were analyzed according to standard bacteriological methods. Total viable count of meat pie ranged from 1.2×10^3 to 3.4×10^4 cfu/g. Total coliform count ranged from 1.8×10^3 to 4.2×10^3 cfu/g and *Staphylococcus* count ranged from 1.0×10^3 to 4.1×10^3 cfu/g. Analysis of variance showed that total viable count and *Staphylococcus* count were significant at 0.05 level. The total viable count for eggroll ranged from 3.4×10^3 to 3.8×10^4 cfu/g. Total coliform count ranged from 1.7×10^3 to 2.3×10^3 cfu/g. *Staphylococcus* count ranged from 1.5×10^3 to 4.8×10^3 cfu/g. The antibiotic susceptibility of *S. aureus* indicated various patterns. Some were resistant while some were sensitive to the antibiotics tested. The organism was highly sensitive to gentamycin. In order to prevent outbreak of food poisoning, public health establishments should enforce proper handling of foods such as meat pie and eggroll. Proper hygiene should be practiced by handlers of ready to eat foods.

Key words: Meat pie, eggroll, bacterial quality, antibiotic susceptibility.

INTRODUCTION

Meat pie and eggroll are ready to eat food that is widely consumed by children and adults especially the working class of the population during recess or recreational official hours. Meat pie and eggroll are usually placed in cases specially designed with transparent glasses and electric bulbs to provide light and warmth during retail. This method provides condition for increased microbial load and spoilage and poses threat to public health especially the consumption of the leftovers. However, these products can be contaminated during processing from the raw materials, soil, food handlers and utensils which may lead to food borne pathogens such as *Staphylococcus aureus* (Christiansen and King, 2004; Colombari et al., 2007).

S. aureus is responsible for food borne illness (Letertre et al., 2003; Normanno et al., 2005; Prescott et al., 2005). The incidence of food-borne illness is increasing worldwide (Nguz, 2007), possibly due to a change in commercial food production involving minimal processing and consumer demands for ready to eat meals. Contamination of ready to eat foods during processing in factories, domestic kitchens, canteens and on street corners by street vendors had been investigated (Kaneko

et al., 1996; Cogan et al., 2002; Beumer and Kusumaningrum, 2003; Ayeieek et al., 2004; Bas et al., 2006). The knowledge of initial microbiological load of ingredients for food preparation is important, however factors such as handling, processing, storage and display may influence the microbiological load of ready to eat foods at the point of sale (Beuchat and Ryu, 1997; Angelides et al., 2006). Ready to eat foods include pastries, meat pie, sausage, eggrolls, burger, moi-moi, salad, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 2010). Food vendor services are on the increase and many people because of the less home centered activities resort to eating foods outside. Good sanitary measures and proper food handling practices are usually not followed by the food vendors (Bryan et al., 2002).

A number of foods have been reported to have high incidence of bacteria but there is limited information on the health challenges from food borne disease from

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Table 1. Bacterial counts in meat pie samples.

Sample	Total bacterial counts (cfu/g)		
	TVC	TCC	<i>Staphylococcus</i> count
M ₁	9.9 × 10 ^{4a}	4.2 × 10 ^{3a}	4.1 × 10 ^{3a}
M ₂	1.7 × 10 ^{4b}	2.5 × 10 ^{3b}	2.3 × 10 ^{3a}
M ₃	1.2 × 10 ^{3a}	1.0 × 10 ^{3b}	1.0 × 10 ^{3c}
M ₄	2.4 × 10 ^{3a}	1.8 × 10 ^{3a}	1.0 × 10 ^{3b}
M ₅	3.5 × 10 ^{3a}	2.6 × 10 ^{3a}	1.0 × 10 ^{3c}
M ₆	2.3 × 10 ^{4b}	2.1 × 10 ^{4b}	1.5 × 10 ^{3a}
M ₇	4.2 × 10 ^{3a}	3.1 × 10 ^{3a}	2.5 × 10 ^{3a}
M ₈	2.0 × 10 ^{4a}	4.1 × 10 ^{3b}	2.0 × 10 ^{3a}
M ₉	2.5 × 10 ^{4b}	2.7 × 10 ^{3a}	3.0 × 10 ^{3b}
M ₁₀	2.6 × 10 ^{4b}	2.8 × 10 ^{3a}	2.2 × 10 ^{3c}

Means in the same columns with the same alphabets are significantly different ($p < 0.05$). Those with different alphabets are not significantly different.

retailed meat pie and eggroll. Therefore the purpose of this study is to determine the microbial quality of meat pie and eggroll with special interest on the occurrence of *S. aureus* and their antimicrobial susceptibility.

MATERIALS AND METHODS

Sample collection

Fifty samples of meat pie and fifty samples of eggroll were randomly collected from different vendors in Umuahia metropolis, Nigeria. Each sample was placed separately in a sterile polythene bag (sterilized with 70% ethanol) and transported to the laboratory for analysis within 2 h of collection.

Sample preparation

Twenty five grams of each of the samples was homogenized in 225 ml of distilled water in a blender. Thereafter, tenfold serial dilution of the homogenized sample was carried out in sterile distilled water. From the appropriate dilutions, 1 ml was inoculated on Mannitol salt agar, Nutrient agar and MacConkey agar in triplicates. The pour plate method was employed. It was incubated for 24 to 48 h at 37°C (Harrigan and McCance, 2005).

Enumeration of microbial population

After incubation at 37°C for 24 to 48 h, colonies on Nutrient agar plates were counted using the colony counter and recorded as total viable count (TVC). Enumeration of *Staphylococcus* was performed on mannitol salt agar plates. Total coliforms were determined on MacConkey agar plates. Coliform was used as an index of sanitary quality of meat pie and eggroll.

Antibiotic sensitivity test

The susceptibility of *Staphylococcus aureus* to antimicrobial agents was determined by agar diffusion method using Mueller-Hinton agar and antibiotic discs (Abtek Biologicals LTD, UK). A sterile wire loop was used to pick colonies of *S. aureus* and emulsified in 3 ml of sterile physiological saline. The turbidity of the bacterial suspension was matched to the McFarland 0.5 standard (1.5×10^8 cfu/ml). Thereafter, a sterile swab stick soaked in the (10 µg) suspension was used to inoculate the Mueller-Hinton agar by streaking evenly over the surface of the medium. Using a sterile forceps, the antibiotic discs were placed on the inoculated plate. The plates were incubated for 24 h aerobically at 37°C. After incubation, the zone of inhibition was measured in mm using a transparent ruler. The antibiotics tested were ampicillin (10 µg), penicillin (10 µg), cloxacillin (5 µg), erythromycin (5 µg), gentamycin (10 µg), chloramphenicol (25 µg), streptomycin (10 µg), and tetracycline (10 µg). Interpretation of result was done according to the "Manual for Laboratory Investigation of Acute enteric infections" (WHO, 2002). The quality control susceptibility of *S. aureus* (ATCC 2923) to the antibiotics was 19-27 mm for gentamycin, 14-22 mm for streptomycin and 24-35 mm for ampicillin.

RESULTS

The bacterial counts in meat pie is shown in Table 1 which has values that are significantly different at $p < 0.05$ and values that are not significantly different in the same columns. The bacterial counts were total viable count (TVC), total coliform count (TCC) and *Staphylococcus* count. Table 2 shows the bacterial counts in eggroll samples that are statistically different between the columns at < 0.05 and values that are not significantly

Table 2. Total bacterial counts in eggroll samples.

Sample	Total bacterial counts (cfu/g)		
	TVC	TCC	<i>Staphylococcus</i> count
E ₁	9.5 × 10 ^{2a}	4.0 × 10 ^{3a}	2.4 × 10 ^{3d}
E ₂	3.8 × 10 ^{4a}	2.3 × 10 ^{4a}	2.8 × 10 ^{3b}
E ₃	5.6 × 10 ^{3a}	1.7 × 10 ^{3c}	2.5 × 10 ^{3a}
E ₄	9.7 × 10 ^{2a}	3.6 × 10 ^{3c}	2.8 × 10 ^{3b}
E ₅	9.2 × 10 ^{3a}	4.7 × 10 ^{3a}	1.1 × 10 ^{3c}
E ₆	9.2 × 10 ^{3b}	6.2 × 10 ^{3b}	4.8 × 10 ^{3b}
E ₇	9.4 × 10 ^{3a}	5.8 × 10 ^{3b}	4.7 × 10 ^{3a}
E ₈	9.6 × 10 ^{2a}	1.3 × 10 ^{3b}	1.6 × 10 ^{3c}
E ₉	8.6 × 10 ^{3a}	6.4 × 10 ^{3a}	2.2 × 10 ^{3a}
E ₁₀	3.4 × 10 ^{3a}	2.0 × 10 ^{3b}	1.5 × 10 ^{3a}

Means in the same columns with the same alphabets are significantly different ($p < 0.05$). Those with different alphabets are not significantly different.

Table 3. Antibiotic sensitivity of *Staphylococcus aureus*.

Antibiotics	Antibiotic pattern		
	Resistance (%)	Moderately sensitive (%)	Highly sensitive (%)
Tetracycline	90	10	0
Ampicillin	90	10	0
Chloramphenicol	100	0	0
Cloxacillin	50	30	20
Erythromycin	0	50	50
Gentamycin	0	0	100
Penicillin	70	30	0
Streptomycin	30	70	0

different.

The antibiotic sensitivity pattern for *S. aureus* is shown in Table 3. Some isolates were resistant while some were moderately or highly sensitive to the antibiotics tested. The isolates were highly sensitive to gentamycin.

DISCUSSION

The occurrence of *S. aureus* in meat pie and eggroll was investigated using standard methods. The total bacterial count in meat pie ranged from 1.0×10^3 to 9.9×10^4 cfu/g. Analysis of variance at 0.05 level for the total viable count, total coliforms and *Staphylococcus* count indicated significance. The reason for these contaminations may be due to the meat or vegetable inside the pie as well as the nutritional content of the meat which enable these organisms to survive. The total bacterial counts in eggroll as shown in Table 2 indicated a high level of contamination. Eggroll has the tendency to spoil quickly because of the egg which supports microorganisms to thrive easily in the products.

Microorganisms in the foods may be linked to a number of factors such as improper handling and processing, use of contaminated water during washing, cross contamination from other raw materials or the use of dirty processing utensils like knives and trays (Bryan et al., 2002; Khali and Mazhar, 2005). Contamination of meat pie and eggroll could occur during the peeling of the egg shell with bare hands and cutting of the meat into pieces with knife. The handling of the meat pie and eggroll with bare hands, absence of hair covering and handling of money during serving or selling of the products might contribute to the high level of microbial load. Also, the heavy vehicular traffic condition might increase the airborne particles which ultimately contaminate the food. Coliforms are indicator of water or food quality and their presence may be an indication of unhygienic condition. *Staphylococcus* sp is a common organism found on skin, nose or any part of the body (Nester et al., 2001) and can be introduced into the food during handling or processing. The organism can produce toxin in foods (Doyle and Evans, 1999).

Eleanor (2007) reported that the major outbreaks associated with baked products were mostly caused by *S. aureus* and *E. coli*. The antibiotic sensitivity pattern of the *Staphylococcus* sp showed resistance of the isolates to some antibiotics like cloxacillin and tetracycline. This could pose a public health problem to consumers and may result in abuse of these drugs in the treatment of illness. Gentamycin and streptomycin were highly sensitive to *Staphylococcus* sp. This may be attributed to the fact that these antibiotics were able to penetrate the cell wall membrane and damage the nucleic acid of the isolates (Prescott et al., 2005). In order to prevent outbreak of food poisoning, public health establishments should enforce proper handling of foods such as meat pie and eggroll, and proper hygiene should be practiced by production personnel and food vendors.

REFERENCES

- Angelides AS, Chronis EN, Papageorgio DK, Kazakis II, Arsenoglou KC, Stathopoulos GA (2006). Non lactic and contaminating flora in ready to eat foods: A potential food quality index. *Food Microb.*, 23: 95-100.
- Ayeieek HI, Sarimchmetoghi B, Cakiroghu S, (2004). Assessment of the microbiological quality of meals sampled at the meal serving units of a military hospital in Ankara, Turkey. *Food Contr.*, 15: 379-384.
- Bas M, Ersun AS, Kivanc G, (2006). The evaluation of food hygiene knowledge, attitudes and practice of food handlers in food business in Turkey. *Food Contr.*, 17: 317-322.
- Beuchat LR, Ryu JH (1997). Procedure handling and process practices. *Emerging infectious diseases*, 3: 459-465.
- Beumer RE, Kusumanaingrum H (2003). Kitchen hygiene in daily life. *International biodeterioration and biodegradation*, 51: 299-302.
- Bryan FL, Tuefel P, Rooh S, Qadar F, Riaz S, Malik Z, (2002). Hazards and critical control points of food preparation and storage in homes in a village and a town in Pakistan. *J Food Protection*, 3: 301-333.
- Caserani V, Kinston R (2010). *Practical cookery*, 4th edition, Edward Arnold publishers London. pp. 1-10.
- Christiansan LN, King NS (2004). The microbial content of some salads and sandwiches at retail outlets. *J. Milk Food Technol.*, 34: 289-293.
- Cogan TA, Slander J, Bloomfield SF and Humphery TJ (2002). Achieving hygiene in the domestic kitchen: The effectiveness of commonly used cleaning procedures. *J. Appl. Microbiol.*, 92: 885-892.
- Colombari V, Mayer MD, Laieini ZM Mamizuka E, Franco BD, Destro MT (2007). Food borne outbreak caused by *Staphylococcus aureus*: Phenotypic and genotypic characterization of strains of food and human sources. *J. Food Protection*, 70: 488-493.
- Doyle MP and Evans PD. (1999). Food borne pathogens of recent concern. *Ann. Rev. Nutri.*, 6: 25-41.
- Eleanor S (2007). Report on baked food associated outbreak. Food policy and programs. A J of the Hlth Services Dept of Australian government, 7: 1
- Harrigan WF, McCance MF (2005). *Laboratory Methods in Food and Dairy Microbiology*, 2nd edition. Academic press, London. pp. 20-22.
- Kaneko KI, Hayashidani H, Ohtomo V, Kosuge J, Kato M, Takahashi K (1996). Bacterial contamination of ready to eat foods and fresh products in retail shops and food factories. *J. Food Protection*, 62: 644-649.
- Khali LGB, Mazhar KB (2005). Flies and water as reservoirs for bacterial pathogens in urban and rural areas in and around Lahore, Pakistan. *Epidemiol. Infect.*, 113: 435-444.
- Letertre C, Perelle S, Dilasser F, Fach P (2003). Identification of a new putative enterotoxin SEU encoded by the egc cluster of *Staphylococcus aureus*. *J. Appl. Microbiol.*, 95: 38-43.
- Nguz K (2007). Assessing food safety system in sub-saharan countries: An overview of key issues. *Food Contr.*, 18: 131-134.
- Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, Poggiu A, Decastelli L, Mioni R, Scuota S, Bartoli M, Zuccon F, Pirino T, Sias S, Paris A, Quaglia NC, Celano GV (2005). Coagulase positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. *Intern J. Food Microbiol.*, 98: 73-79.
- Prescott M, Harley P, Kian D (2005). *Microbiology*, 6th edition McGraw hill New York publishers USA. Pp910.
- World Health Organisation (2002). *Manuel for laboratory investigations of acute enteric infections*. CDD/83.3 REV.1. pp. 30-35.