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Original article

Bacteriological quality and occurrence of *Escherichia coli* and *Staphylococcus aureus* in ice cream sold in Zaria, Kaduna State Nigeria

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ABSTRACT

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A cross-sectional study was conducted across Zaria metropolis to determine the bacteriological quality and occurrence of *Escherichia coli* and *Staphylococcus aureus* in ice cream sold to consumers by supermarkets and street traffickers. A total of 240 samples comprising 111 industrially and 129 locally produced ice creams were examined for the Total Aerobic Plate and Coliform Counts for *S. aureus* and *E. coli* and identification of the isolates were done using conventional biochemical characterization and Microbact test. The isolate were further subjected to antimicrobial susceptibility test by disk diffusion method. Of the 240 samples, 19 (8.0%) were positive for *S. aureus* and 5 (2.0%) were positive for *E. coli*; 5 (26.3%) of the *S. aureus* isolates were from industrially produced ice cream while 14 (73.6%) were from locally made ice cream with a statistically significant ($P < 0.05$). Overall, the mean Total Aerobic Plate Counts (TAPC) per ml of industrial and locally produced ice cream were 1.14×10^7 and 2.02×10^7 respectively with a statistically significant difference ($P < 0.05$) and the mean counts for Total Coliform Count (TCC) per ml for industrial ice cream and local ice cream were 5.69×10^3 and 4.73×10^3 respectively. Brands of ice cream of industrially produced origin had significantly lower of *S. aureus* and *E. coli* load than those of local sources. Antibiotic

susceptibility profile for *S. aureus* showed that all the isolates were resistant to multiple antibiotics. Antibiotic susceptibility profile for *E. coli* showed that all the isolates were resistant to one or more antibiotics except for Nitrofurantoin and Ciprofloxacin. Locally produced ice creams sold in Zaria appear to pose a higher health risk to consumers compared to industrially produced ice cream. It is imperative that bacteriological standards be enforced in order to prevent ice cream borne infections in humans. There is a need also to control antibiotic usage among dairy farmers and continuous surveillance of antimicrobial resistance trends on animals from which ice cream is sourced.

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1. Introduction

Ice cream is one of the food products of milk consisting of a mix of milk, sweetening, emulsifiers, and stabilizing agents together with flavouring, colouring agents, ice crystals and air bubbles (Graff-Johnson, 1974; Kocak et al., 1998). The nutritional content of an ice cream include; 12% fat, 11% non - fat milk solids, 15% carbohydrate, protein and vitamins (especially vitamin A, B and C) and 1% mineral salts especially calcium and phosphorus salts (Brain and Allan, 1982).

Although ice cream is mostly eaten as a dessert, its significance in human nutrition is unquestionable. It could also be said to be a frozen dairy food made by freezing the processed mix (Kocak et al., 1998). Ice cream is a palatable, nutritive and healthy food, as a result of its palatability, the production and consumption of the product among the populace in Nigeria is largely increasing. Several brands of ice cream with a variety of flavours are being marketed in Nigeria. The Ice creams are usually named by the dominant additive or flavouring agent added to them, natural flavours may be used such as real fruit, cocoa, coffee, ginger and vanilla. All these allow great variations in ice cream flavour, taste and way of structure formation (Vica et al., 2010).

Based on its constituent, ice cream is a nutritious food for man and also an excellent medium for the growth of many microorganisms, some of which may be pathogenic to man. Possible sources of these micro organisms in ice cream have been reported to include raw materials used for the composition of ice cream mix such as separated milk and milk powder, cream, flavouring and colouring substances and stabilizers (Verma, 1972; Bathla and Rao, 1973) and from air during processing (Gomez, 1969). The methods employ in the sales of ice cream could also be a source of contamination, which could be, sells in open containers at retail outlets or in packages which may then be distributed manually in scoops, cones, or sundaes across the counter (Marshall and Arbuckle, 1996; Warke et al., 2000; Champagne et al., 1994; Daniels et al., 2002). Hence, handling during production, storage and distribution condition of ice cream and some of the variables that are used during processing of ice cream can affect the physical, chemical and microbial quality of product.

The presence of some of these organisms in pasteurized ice cream could be due to their ability to survive the pasteurization process as in the case with spore formers, (Aaku, 2000; Osamwonyi et al., 2011) and they may persist in ice cream product thereafter. Psychrotrophic microorganisms are therefore the major contaminants and pathogens associated with ice cream and other foods that are served in frozen or chilled state (Arnaut-Rollier et al., 1999; Caglayanlar et al., 2009). Some of the major psychrotrophic bacteria found in milk and milk products include species of *Bacillus* spp, *Citrobacter* spp, *Clostridium* spp, *Corynebacterium* spp, *Lactobacillus* spp, *Streptococcus* spp, *Enterobacter* spp, *Klebsiella* spp, *Staphylococcus* spp, *Listeria* spp and *Escherichia* spp (Schroder, 1984; Sinell, 1989; Eneroth et al., 1999).

Psychrotrophic bacterial pathogens isolated from dairy products such as ice creams and sherbets have been found to be one of the causes of food poisoning and food borne disease- related outbreaks in different parts of the world (Warke et al., 2000). As most of the ice cream consumers are children of vulnerable age groups and young adults, it is required to be microbiologically safe (Warke et al., 2000; Caglayanlar et al., 2009). Hence, the aim of this study is to determine the bacteriological quality and the occurrence of *E. coli* and *S. aureus* in ice cream sold in Zaria metropolis.

E. coli frequently contaminates food and it is a good indicator of fecal contamination (Diliello, 1982; Soomro et al., 2002; Benkerroum et al., 2004). It is a species of the genus *Escherichia* under the family *Enterobacteriaceae*. Presence of *E. coli* in milk products designates the presence of enteropathogenic microorganisms, which constitute a public health hazard. A subgroup called Enterohaemorrhagic *E. coli* (EHEC) can cause food borne illness as the *E. coli* O157:H7 strain which causes severe and potentially fatal illness known as hemorrhagic colitis, characterized by bloody diarrhea and severe abdominal pain (Dolores and Doyle, 2001).

Staphylococcus aureus is a gram positive coccus, resistant to heat, drying and radiation. They produce enzymes, endotoxins 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). Illness associated with *S. aureus* include, toxic shock syndrome, pneumonia, meningitis, endocarditis, and septicemia (Soomro et al., 2003; Masud et al., 1988). Methicillin Resistant *Staphylococcus aureus* (MRSA) is a critically important human pathogen and attention has been given recently to the potential role of food animals in human MRSA infection and colonization (Khanna et al., 2007; De Neeling et al., 2007) and identification.

2. Materials and methods

2.1. Study design

The study was carried out in Zaria metropolis, Kaduna State. A total of 240 Ice cream, involving the 129 locally produced ice cream (ice cream produced on small scale and hawked on bicycles or sold at sale outlets by street vendors) and 111 industrially produced ice cream (ice cream branded and produced in large scale, packaged or dispensed in scoops or cones across the counter and usually sold at supermarkets) were sampled. The samples were collected based at convenience from strategic locations of Zaria metropolis including Zaria city and Tudun Wada in Zaria Local Government Area and Samaru, Kwangila and Sabon Gari in Sabon Gari LGA. All the ice cream Samples were collected twice weekly within a period of 3 months (April-July, 2013).

2.2. Total aerobic plate and coliform count

The samples collected were placed in sterile polythene bags, appropriately labeled and transported to the appropriate laboratory in an ice pack. The samples were then placed in water bath at 45°C to thaw (Harrigan and McCance, 1976). After thawing, an aliquot of 15 ml of liquid ice cream transferred into a sterile glass bottle and covered. 1 ml of ice cream was transferred into a test tube containing 9 ml of sterile 0.1% peptone water to give a 1:10 dilution. Further dilutions were prepared according to standard method by American Public Health Association (1953).

For the determination of total aerobic plate count, 0.1ml diluted sample was spread over duplicate Nutrient Agar (NA) plates and incubated at 37°C for 24 hours. After incubation, all colonies on the plates were counted and expressed in cfu/ml of ice cream. For the determination of total coliform counts, 0.1ml of dilution was spread evenly on a duplicate MacConkey Agar (MCA), after which all the plates were incubated at 37°C for 24 hours. The total coliform counts were then expressed as the number of coliform or colony forming units per ml (cfu/ml).

2.3. Isolation and identification of *S. aureus* and *E. coli*

For the isolation of *S. aureus*, Brain heart infusion broth supplemented with NaCl (6.5%) and Baird Parker agar (BPA) were used. Colonies that appeared black or greyish-black were Gram-stained and subjected to biochemical tests (McFaddin, 2000). For the isolation of *E. coli*, Eosin Methylene Blue (EMB) agar was used; colonies with greenish metallic sheen were subjected to biochemical tests for *E. coli*. Conventional biochemical test carried out for presumptive identification of *S. aureus* isolates included catalase, coagulase, sugar fermentation (xylose, lactose, sucrose and mannitol), haemolysis on 5% sheep blood agar, pigmentation (Mannitol salt agar) and DNase activity (Barrow and Feltham, 1995). While conventional biochemical tests carried out for presumptive identification of *E. coli* isolates included IMViC, namely: Indole, Methyl red, Voges-Proskauer and citrate utilization and Triple sugar Iron (TSI) and Urease tests.

Further identification of isolates was carried out by the use of Microbact 12S and 12E test systems used for identification of *S. aureus* and *E. coli* respectively. It is a standardized micro-substrate systems designed to emulate conventional biochemical substrates in which isolates with 75% probability for particular species were considered positive after been fed into a computer aided identification package for interpretation. The substrates for

Microbact 12S were: maltose, mannitol, mannose, sucrose, trehalose, n-acetyl glucosamine, arginine, urease, beta-glucosidase, alkaline phosphatase, beta-glucuronidase and beta-46 galactosidase, while the substrates for Microbact 12E were: lysine, ornithine, H₂S, glucose, mannitol, xylose, ONPG, indole, urease, Voges-Proskauer, citrate, and TDA.

2.4. Antimicrobial sensitivity test

The disk diffusion method was used for antimicrobial sensitivity test. Susceptibility patterns of the isolated organisms were tested against a wide range of antibiotics and the test was conducted on the isolated or identified bacteria of *S. aureus* and *E. coli* recovered during the study period. The isolates were tested for ten antimicrobials using the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Verma, 1992; Pelzer et al., 2000). The inhibition zones were reported as the diameter of the zone inhibition surrounding the individual disk in which bacterial growth was absent and the interpretation was made as per the zone inhibition size interpretation chart provided by CLSI (2004).

2.5. Data analysis

Prevalence of *S. aureus* and *E. coli* species in ice cream were processed using the Statistical Package for Social Sciences (SPSS), version 20. All statistical tests were performed and interpreted at an alpha of 0.05. Student t-test was carried out to compare the total aerobic plate counts in ice cream of various origins.

3. Results and discussion

All 240 ice cream samples examined showed positive growth for Total Aerobic Plate Count (TAPC) and 150 (62.5%) samples showed positive growth for total coliform counts (TCC). A total of nineteen (19) *S. aureus* and five (5) *E. coli* were isolated from all the 240 samples analyzed in the study. Out of the 111 industrially produced ice creams examined, *S. aureus* was present in 5 (4.5%) and *E. coli* was present in 2 (1.8%), while of the 129 locally produced ice cream examined, *S. aureus* occurred in 14 (10.8%) and *E. coli* occurred in 3 (2.3%). Five (26.3%) of the 19 *S. aureus* isolated were obtained from industrially produce ice creams while 14 (73.6%) of the *S. aureus* were from locally produced ice cream (Table 1). Also, 2 (40%) of the five *E. coli* isolated were from industrially produced ice creams while the remaining 3 (60%) were from local source (Table 1). Though there was a significant difference between the *S. aureus* from locally and industrially produced ice cream, (p- value of 0.000) there was no significant difference in *E. coli* from both sources p = 0.374).

Results of bacterial counts revealed a mean Aerobic Plate Count of 2.015×10^7 cfu/ml (range; 4.4×10^5 to 7.84×10^7 cfu/ml) for locally produced ice cream and a mean count of 1.14×10^7 cfu/ml (range; 2.0×10^4 to 5.56×10^7 cfu/ml) for industrially produced ice cream. Results for total coliform counts revealed a mean value of 4.73×10^3 cfu/ml for locally produced ice cream (range, 1.0×10^3 to 3.00×10^4 cfu/ml) and mean count of 5.69×10^3 CFU/ml for industrially produced ice cream (range; 1.0×10^3 to 3.5×10^4 cfu/ml), with no statistical significant difference (p = 0.311; CI = 0.05) between the two (Table 2).

All the 19 *S. aureus* and 5 *E. coli* isolates were subjected to antibiotic susceptibility tests with a panel of 13 and 10 antimicrobial agents respectively. The antibiotic susceptibility profile of *S. aureus* shows that all the isolates were resistant to one or multiple antibiotics (Fig. 1). and the Multiple Antibiotic Resistance (MAR) index of *S. aureus* isolates range from 0.46 to 0.84. The antimicrobial resistance pattern for 5 *E. coli* isolated from the ice cream samples showed that each of the isolates had a different pattern, with MAR index range of 0.3-0.7.

Table 1

Frequency and percentage occurrence of *S. aureus* and *E. coli* isolates from industrially and locally produced ice-cream sold in Zaria metropolis.

Organisms	Total No. of ice cream samples	No. of samples positive for <i>S. aureus</i> (%)*	No. of samples positive for <i>E. coli</i> (%)**
Industrial ice cream	111	5 (4.50)	2 (1.80)
Local ice cream	129	14 (10.8)	3 (2.30)
Total No. of samples	240	19 (7.91)	5 (2.08)

* p-value= 0.000, ** p-value= 0.374

Table 2

Mean Total Aerobic Plate and Coliform count obtained from locally and industrially produced ice cream sold in Zaria metropolis.

Variables	Industrial ice cream (range)	Local ice cream (range)	p-value
Mean Total Coliform count (cfu/ml)	5.69×10^3 (1.0×10^3 - 3.5×10^4)	4.73×10^3 (1.0×10^3 - 3.00×10^4)	0.311
Mean Total Aerobic Plate count (cfu/ml)	1.14×10^7 (2.0×10^4 - 5.56×10^7)	2.02×10^7 (4.4×10^5 - 7.84×10^7)	0.034

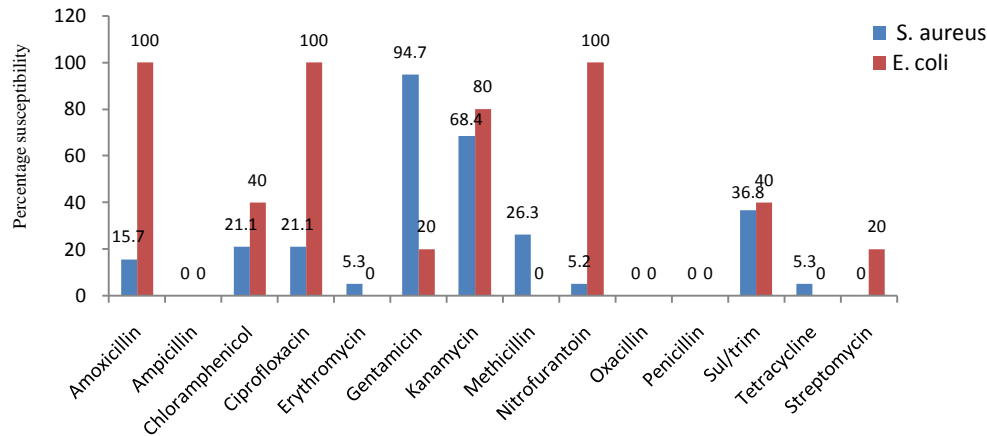


Fig. 1. Percentage antibiotic susceptibility of *E. coli* and *S. aureus* isolates obtained from of ice cream sold in Zaria metropolis.

The microbiological standards for ice cream as put together by International Dairy Federation in 1982 are as follows; 2.5×10^4 - 2×10^5 cfu/ml for Mesophilic organisms and 10 – 100cfu/ml for coliform. While the Total Aerobic Plate Count (TAPC) was within the recommended limit for some of the industrially produced ice cream 69 (62.1%) with a mean value of 1.14×10^7 cfu/ml, none of the locally produced ice cream was within the recommended range (mean value of 2.015×10^7 cfu/ml). This was also observed by Ikenebomeh and Ogaguvia (1993) and Ambili and Beena (2012). The high counts in locally produced ice cream might have originated from the initial micro flora in the raw milk and other ingredients used in the mix, insufficient heat treatment and poor personal hygiene and sanitary practices during processing and at the sale outlets.

The mean total coliform counts obtained from industrially and locally produced ice cream were 5.69×10^3 cfu/ml and 4.73×10^3 cfu/ml respectively ($p = 0.311$) and the frequency of occurrence of total coliform count from the sampled ice creams were 39.7% and 79.9% respectively which were above the recommended limits of 10 – 100cfu/ml (James and Jay, 1978). In previous studies, varying results which do not tally with the recommended standard were reported including 88.4% by Toklu and Yaygin (2000) and 96.4% by Kanbakan et al. (2004). High coliform counts in the ice cream samples may be due insufficient heat treatment, use of contaminated raw materials and tools, failure to adhere to standard ice cream manufacturing practices and poor personnel hygiene (Warke et al., 2000). Kanbakan et al. (2004) reported that inadequate cleaning of the hands, same person selling ice cream and collecting money, open cones and unclean cloth for cleaning the scoops can contribute to high coliform count. Warke et al. (2000), stated that coliform contamination of the hands of personnel in ice cream sales department were higher than on the hands of factory workers.

Higher incidence rates of *E. coli* had been reported in previous studies than the present study, Yusuf et al. (2013) reported a prevalence of 35% *E. coli* in Bauchi; Osamwonyi (2011) reported 30% and Masud (1989) found that 46% of the samples were contaminated with *E. coli* in Pakistan. The levels of coliform found and the presence of *E. coli* in ice cream samples may indicate a lack of good manufacturing practice during the production, hence ice

cream produced in domestic or catering premises may be relatively an important vehicle for the transmission of pathogenic gastrointestinal organisms.

Out of the 240 ice cream samples examined, 19 (8%) were positive for *S. aureus*, with 14 (73.6%) of the isolates from locally produced ice cream while 5 (26.6%) were from industrially produced ice cream ($p = 0.000$). Varying frequency of occurrence of *S. aureus* isolation have been reported; in Ankara, Turkey, *S. aureus* was present in 10% of ice cream sampled (Kocak, 1998) also Warke et al. (2000) reported a rate of 100% in India. The presence of *S. aureus* may be due either to insufficient pasteurization of milk or contamination due to human exposure from the hands, skin and clothing of the handlers. Coughing, talking and sneezing openly produce droplets which could settle on ice cream during processing, transportation, storage and retailing of the product (Warke et al., 2000).

There were 18 antimicrobial resistance patterns observed with *S. aureus* isolates which showed multiple resistances. All the *S. aureus* isolates were resistant to Penicillin, Ampicillin and Oxacillin, which appeared in all the patterns and a high percentage were resistant to Tetracycline and Erythromycin which appeared in 17 of the 18 patterns. The frequency of resistance to Penicillin and Ampicillin were similar to the reports of Kwaga and Adesiyun (1984) and Umoh (1990) who reported widespread resistance of *Staphylococcus* to Ampicillin and Penicillin. The resistance of all the isolates to Penicillin, Oxacillin and Ampicillin is in accordance with the known natural resistance of *Staphylococcus* spp to the β - lactams. In another research, a high level of resistance to Tetracycline was established which was attributed to the excessive and indiscriminate use of the drug in Nigeria (Kabir et al., 2005).

Antimicrobial susceptibility of *E. coli* to 10 antimicrobial agents showed all the isolates to be susceptible to ciprofloxacin and Nitrofurantoin and high percentage (80%) were susceptible to Gentamicin and Kanamycin. However, all the isolates were resistant to 7 out of 10 antimicrobial agents. This resistance may be due to indiscriminate use of antimicrobial agents. There were 5 antimicrobial resistance patterns observed for *E. coli*. All the resistance patterns showed multiple resistances with high frequency of resistance to Ampicillin, Erythromycin and Tetracycline.

4. Conclusion

Overall 198 (82.5%) and 147 (61.3%) of ice cream samples analyzed from Zaria metropolis were contaminated with aerobic bacteria and coliform respectively above limit prescribed by ISO (1995). Also, significantly higher Total Aerobic Counts were more commonly present in local ice creams than industrial ice creams. Ice cream sold in Zaria metropolis especially from local manufacturers were found to be more commonly contaminated with significant levels *S. aureus* and *E. coli*, some of which displayed resistance to several antimicrobial agents. The findings in this study implicated ice cream sold in Zaria metropolis as a vehicle for transmission of food borne disease and specifically, multiple drug resistant *S. aureus* and *E. coli*.

Following our findings, we recommend that, good sanitary practices and Hazard Analysis and Critical Control Points (HACCP) principles be adopted in the production, distribution and sales of ice cream in Zaria and its environs. Also production workers and vendors selling ice cream should be educated on personal hygiene and proper storage of ice cream to further improve the quality of ice cream sold to the public. There is need for continuous surveillance and enforcement of international standard for ice cream by the regulatory authorities and also periodic monitoring of operations to ensure that they maintain proper and hygienic production, storage and distribution of ice cream. Indiscriminate use of antibiotics on animals should be discouraged to reduce spread of multi-drug resistant *Staphylococcus* spp and coliform.

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