

The Microbiological Quality of Ice Used to Cool Drinks and Foods in Ogbomoso Metropolis, Southwest, Nigeria

Agbaje Lateef^{1*}, Julius K. Oloke¹, Evariste B. Gueguim Kana¹, and Esther Pacheco²

¹Departments of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria, ²Science Laboratory Technology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria.

The microbiological safety of commercial ice used to refrigerate drinks and fish was evaluated using 40 ice samples collected from four ice manufacturing factories in Ogbomoso, Nigeria. All the samples were contaminated by bacteria and the microbial load ranged from 1.88 to 3.20×10^4 cfu/ml which is largely above the recommended loads of <500 and <1000 cfu/ml for ice obtained from manufacturing plants and retail outlets, respectively. The bacterial isolates obtained from the ice samples include *Pediococcus cerevisiae*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Bacillus firmus*, *Pseudomonas aeruginosa*, *Streptococcus equi*, *Staphylococcus epidermidis*, and *Micrococcus luteus*. The degree of resistance shown by the isolates to the antibiotics differs ranging from 50-87.5%, with multiple-drug resistance to 4-7 antibiotics. The isolates showed 100% resistance to Cotrimoxazole, Ampicillin, Cefotaxine and Cephalexin, while none of them was resistant to Gentamicin. The resistance to other antibiotics ranged from 26.67% for Ofloxacin, 66.67% for Erythromycin to 86.67% for Tetracycline. The present study reveals that ice may represent novel route of spread of antibiotic-resistant bacteria especially in developing countries. In view of the results herein reported, it is highly recommended that national regulatory guidelines should be established for the production of ice.

Commercial ice should be safe to consume and be of the same quality as drinking water because it is ingested directly when added to juices and soft drinks or indirectly when used to refrigerate foods such as fish and seafoods (Falcão *et al.*, 2002). Ice is sometimes contaminated with pathogenic microorganisms where a contaminated water source is used in its production or where there are lapses in hygiene in their handling. Outbreaks of gastroenteritis due to contaminated ice have been reported (Quick *et al.*, 1992; Khan *et al.*, 1994; Pedalino *et al.*, 2003) in other parts of the world. The possible causes of these outbreaks were due to the consumption of ice contaminated with pathogens such as Norovirus and *Giardia lamblia*. An investigation revealed that a server's hands might have contaminated ice machines with Norovirus and there was direct transfer from the hands of a *Giardia lamblia* carrier who scooped up ice for restaurant customers with her contaminated bare hands (Quick *et al.*, 1992). Recently, a major outbreak of hepatitis A in Lampang and Chiang Rai, Thailand, affecting about nine hundred people, was also reportedly due to contaminated ice (APEC, 2005). Initial investigations pointed to an ice factory in Chiang Rai Province which drew its water from contaminated artesian wells.

A number of studies from different countries have shown that the microbiological quality of ice manufactured for use to cool foods and drinks could be a cause for concern (Moyer *et al.*, 1993; Wilson *et al.*, 1997; Vieira *et al.*, 1997; Nichols *et al.*, 2000). These studies showed that *E. coli*, coliforms and a variety of microorganisms could be present in ice demonstrating either the poor quality of source water used or a lack of hygiene in production or handling or both. If the quality of source water is not good, harmful microorganisms may be present and since the process of freezing cannot destroy them, many of them can survive in ice, although their numbers reduce gradually with time. Although when ice is thawed the microorganisms remaining may be injured, but they tend to recover their viability so that when the ice melts into drinks, they may be able to survive there too (FEHD, 2005). This means that if harmful microorganisms are present in the source water from which the ice is made, they may also be viable in the ice when it is used, and capable of causing infection in the customer. Therefore the relationship between contaminated water and human diseases emphasizes the importance of a study to gain information about the hygienic conditions of commercial ice. In Nigeria, there is dearth of information on the microbiological contamination of commercial ice used to refrigerate drinks and foods, and the attendant public health implications.

*Corresponding author, mailing address: Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria. Phone: +234-8037400520. E-mail: agbaje72@yahoo.com

The purpose of the present study is to determine the microbiological quality of edible ice from ice manufacturing plants in Ogbomoso metropolis, Southwest Nigeria. The bacterial isolates were evaluated to determine their resistances to the commonly recommended antibiotics in Nigeria. The results will provide scientific information to assess the risk of edible ice to public health in a developing nation such as Nigeria, and assist in setting guidelines for the hygienic production of ice as it is presently obtainable in the production of sachet water in the country.

MATERIALS AND METHODS

Sampling. A total of 40 ice samples were aseptically collected from four ice manufacturing factories in Ogbomoso, Oyo state, Nigeria. The ice samples were prepared from water obtained from deep wells. The sampling period was between August and November, 2005. The samples were kept below 5°C during transportation to the laboratory and were analyzed within 4h of collection.

Microbiological analysis. The samples were allowed to melt at 5°C under aseptic condition, after which they were used for the analysis. The total colony count was done by pour plate method using nutrient agar (Lateef, 2004; Lateef *et al.*, 2005). The ice water samples were serially diluted using sterile distilled water, and 0.2 ml of appropriate dilution was used to inoculate the plate in duplicate. The plates were incubated at 37°C for 24 h, after which the total colony count was determined. Distinct colonies based on colonial morphology were purified to obtain pure cultures that were subjected to routine primary and biochemical tests. The isolates were identified according to the scheme of Buchanan and Gibbons (1974). The three-tube procedure using lactose broth (Hammad and Dirar, 1982; Fawole *et al.*, 2002; Bakare *et al.*, 2003) was used to detect the coliform and determine the most probable number (MPN) of coliform bacilli using McCrady table. A 0.1 ml, 1 ml, and 10 ml of each sample were used to inoculate the lactose broth in five replicates. Tubes were incubated at 37°C for 48 h and the MPN was determined in accordance with standard method (APHA, 1985). For the detection of fecal coliform bacteria, production of acid and gas was taken as positive indication (D'Auriac *et al.*, 2000).

Antibiotic sensitivity test. The bacterial isolates were tested for their sensitivity to antibiotics by means of M2-A6 disc diffusion method recommended by the National Committee for Clinical Laboratory Standards, NCCLS (NCCLS 1997) using nutrient agar. The commercial discs used contained the following: Gentamicin (Gen) 10 µg; Tetracycline (Tet), 30 µg; Cotrimoxazole (Cot), 25 µg; Nalidix acid (Nal), 30 µg; Ampicillin (Amp), 25 µg; Cefotaxime (Cro), 30 µg; Ofloxacin (Of), 10 µg; Cephalexin (Crl), 30 µg; and Erythromycin (Ery), 5 µg. The plates were incubated at 37°C for 24 h, after which the zones of inhibition were examined and interpreted accordingly (Chortyk *et al.*, 1993) considering the appropriate breakpoints (Andrews, 2005). Earlier, the potencies of all the antibiotics used in the study were confirmed using susceptible *E. coli* strains.

RESULTS

The attributes of the ice samples depicting the source, type, use, the microbial load of heterotrophic bacteria and the bacterial isolates are as shown in Table 1. The ice samples were produced by the factories for onward use by consumers in different forms. They are used for diverse purposes, which include cooling of water and drinks that are directly consumed by humans without further treatment. Also, a sizeable amount of the ice is used to refrigerate fish. The water samples used to produce these ice samples were obtained from deep wells. In Nigeria, the National agency for food and drug administration and Control (NAFDAC) in its guidelines listed well and deep well water as unacceptable sources of water for the production of package water (NAFDAC, 2004). This is because in most Nigerian cities, the general mode of disposal of sewage is by the use of the cesspools, septic tanks and pit latrines. And except for very few factories now, there are no sewer and modern sewage treatment plants. Consequently, ground water is polluted to high degree by seepage from various sources (sewage, ponds, refuse dumps, leaching of fertilizers, pesticides from agriculture, detergent, radioactive wastes, etc.). In our previous studies, we have investigated the effect of disposal of untreated wastes from a pharmaceutical, and soap and detergent industries on the emergence of resistant bacteria in Nigeria (Lateef, 2004; Lateef and Adewoye, 2004).

Table 1. The attributes of the commercial ice samples

Source	Type of ice	Uses	Microbial load (cfu/ml) ^a	Isolates ^b
Factory A	Bars	Drinks	1.88 × 10 ⁴	<i>Pediococcus cerevisiae</i> , <i>Bacillus subtilis</i> , <i>Streptococcus pyogenes</i> , <i>Bacillus firmus</i> , and <i>Pseudomonas aeruginosa</i> .
Factory B	Shaved	Fish refrigeration	2.19 × 10 ⁴	<i>Streptococcus equi</i> and <i>Bacillus firmus</i> .
Factory C	Cubes	Drinks	3.10 × 10 ⁴	<i>Streptococcus equi</i> , <i>Staphylococcus epidermidis</i> , <i>Streptococcus pyogenes</i> , and <i>Micrococcus luteus</i> .
Factory D	Shaved	General refrigeration	3.20 × 10 ⁴	<i>Micrococcus luteus</i> and <i>Pseudomonas aeruginosa</i> .

^a Mean value of duplicate of 10 samples obtained from each factory within sampling period of four months.

^b Distinct bacterial isolates.

Table 2: Resistance of the bacterial isolates and their patterns

Bacterial isolate	Source (Ice samples from different factories)	% resistance to the antibiotics ^a	Resistance pattern ^b
<i>M. luteus</i>	C	87.5	Tet Cot Ery Amp Cro Ofx Crl
<i>M. luteus</i>	D	75.0	Tet Cot Ery Amp Cro Cfl
<i>P. cerevisiae</i>	A	50.0	Cot Amp Cro Crl
<i>B. subtilis</i>	A	75.0	Tet Cot Amp Cro Ofx Crl
<i>B. subtilis</i>	A	75.0	Tet Cot Ery Amp Cro Crl
<i>S. pyogenes</i>	A	87.5	Tet Cot Ery Amp Cro Ofx Crl
<i>S. pyogenes</i>	A	75.0	Tet Cot Ery Amp Cro Crl
<i>S. equi</i>	B	75.0	Tet Cot Ery Amp Cro Crl
<i>S. equi</i>	C	62.5	Tet Cot Amp Cro Cfl
<i>S. pyogenes</i>	C	62.5	Tet Cot Amp Cro Cfl
<i>S. epidermidis</i>	C	87.5	Tet Cot Ery Amp Cro Ofx Crl
<i>B. firmus</i>	A	62.5	Tet Cot Amp Cro Cfl
<i>B. firmus</i>	B	75.0	Tet Cot Ery Amp Cro Cfl
<i>P. aeruginosa</i>	A	75.0	Tet Cot Nal Amp Cro Crl
<i>P. aeruginosa</i>	D	62.5	Cot Nal Amp Cro Crl

^a% Resistance obtained from the antibiogram.

^bResistance pattern constructed from the antibiogram; antibiotic codes as defined under materials and methods.

The mean counts of heterotrophic bacteria (microbial load) showed high presence of bacteria in all the ice samples in the range of $1.88-3.20 \times 10^4$ cfu/ml. However, none of the ice samples showed the presence of coliform as MPN of 0 was obtained for all of them. The microbial loads fall within the range reported by (Falcão *et al.*, 2002) for some Brazilian ice, but fall short of the quality reported for packaged ice obtained in Florida, USA (Schmidt and Rodrick, 1999). The microbial quality is also lower than the ice samples analyzed in Hong Kong whereby only 6 out of 12 samples obtained from ice manufacturing plants had aerobic plate count (APC) of <10 cfu/ml, while only 3 out of 89 ice samples obtained from retail outlets had aerobic plate count of ≥ 1000 cfu/ml (FEHD, 2005). The permissible levels of aerobic plate counts of ice from manufacturing plants and retail outlets are <500 and <1000 cfu/ml respectively (Wilson *et al.*, 1997, Nichols *et al.*, 2000).

The presence of high APC counts in ice is an indication of unsanitary conditions or poor hygiene practices during or after production. In many previous studies on the quality of ice, microbiological criteria for drinking water, similar to those recommended by World Health Organization (WHO, 2004), were usually applied. This is because many countries do not have specific national microbiological guidelines for ice. However in the United States, the International Packaged Ice Association (IPIA) produced guidelines (IPIA, 1989) for the industry aiming at assuring the microbiological quality of packaged ice. These guidelines require that ice must not contain coliforms and APC should be <500 cfu per ml. These guidelines have been criticized to be unrealistic for all types of ice particularly the loose ice that have undergone handling

process (Wilson *et al.*, 1997, Nichols *et al.*, 2000). In this regard, it has been suggested that the APC of acceptable loose ice should be <1000 cfu/ml. It is evident from this study that all the ice samples analyzed, despite being obtained from factories did not meet any of the microbiological criteria for ice. This may not be unrelated with the source of water used in the production of ice, aside the unsanitary hygiene practices. We have earlier reported high microbial load ($8.0 \times 10^2-2.5 \times 10^5$) of bacteria from well water samples (Fawole *et al.*, 2002; Lateef *et al.*, 2005) in Ogbomoso metropolis. Evidence from this study suggests that the microbiological quality of the ice samples is a cause for concern. The bacterial isolates obtained from the ice samples include *Pediococcus cerevisiae*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Bacillus firmus*, *Pseudomonas aeruginosa*, *Streptococcus equi*, *Staphylococcus epidermidis*, and *Micrococcus luteus*. It is known that most of these organisms are pathogenic in man and capable of causing wide range of diseases.

The resistances of the bacterial isolates to different antibiotics and their patterns are as shown in Table 2. The degree of resistance shown by the isolates differs ranging from 50-87.5%, with multiple-drug resistance to 4-7 antibiotics. The cumulative resistance of all the bacterial isolates to each antibiotic showed that all of them were resistant to Cotrimoxazole, Ampicillin, Cefotaxime and Cephalexin, while none was resistant to Gentamicin. The resistance to other antibiotics ranged from 26.67% for Ofloxacin, 66.67% for Erythromycin to 86.67% for

Tetracycline. The multiple-drug resistance obtained in this study falls within the range that we have reported for bacterial isolates obtained from diverse clinical, food, water, effluents and fish samples in Nigeria (Lateef, 2004; Adewoye and Lateef, 2004; Lateef *et al.*, 2004; Lateef *et al.*, 2005). The emergence of bacteria resistant to most of the commonly used antibiotics is of considerable medical significance (Khan and Malik, 2001) because of the public health implications, and there are several reports on the incidence of bacterial resistance among bacterial isolates obtained from food materials (Grewal and Tiwari, 1990; Singh *et al.*, 1995; Desai and Kamat, 1998; Khan and Malik, 2001).

As far as we know, this is the first report of work to evaluate the antibiotic sensitivity of bacterial isolates from edible ice, with the view of determining their public health implications. Although consumption of ice may not in itself represent immediate threat to personal or public health since it is not consumed in large quantities like packaged or bottled water, the potential for transmission of disease exists in ice industry that is not regulated. The present study reveals that ice may represent novel route of spread of antibiotic-resistant bacteria especially in developing countries. As a manufactured food, production of ice is covered by the regulations of Good Manufacturing Practices (GMP) which address the facilities where it is manufactured, quality of source of water and sanitary practices during ice production. There should also be performance of analytical tests and establishment of HACCP to ensure microbiological safety of ice.

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