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Technical Report

Microbial contaminants of commercially bottled non-alcoholic drinks produced in Nigeria

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When microbiological analyses were conducted on 90 samples of soft drinks representing 30 different products commercially available in Nigeria, contaminants were detected in 50% of them. The isolates were mainly saprophytic and non-pathogenic: *Bacillus* spp. (35%), *Lactobacillus* spp. (26%), *Pediococcus* spp. (6%), *Staphylococcus epidermidis* (6%) and *Micrococcus* spp. (3%) accounted for the bacterial isolates while *Aspergillus niger* (6%) and *Saccharomyces* spp. (16%) accounted for the fungal isolates.

Key words: Bacteria, microbial load, moulds, non-alcoholic drinks, yeast.

Non-alcoholic beverages are highly prone to microbial contamination (Odunfa 1987). While high-level microbial contamination can cause economic loss through product spoilage and consumer rejection, lower and usually inconspicuous levels may, if uncontrolled, pose grave human health problems (Frazier & Westhoff 1978).

The recent economic hardship in Nigeria has led to an outright ban on the import of the raw materials traditionally used for the production of various beverages, including non-alcoholic. This has led to the use of local and unconventional raw materials for the purpose (Ogbonna & Obi 1991) and to changes in production practice, product quality and shelf-life. The present report is on the microbiological profile of the entire spectrum of the bottled non-alcoholic beverages now available in Nigeria.

Materials and Methods

Sampling Method

Non-alcoholic beverages (three bottles each) were purchased from retail outlets across Nigeria. The bottles were cleaned externally with absolute ethanol to disinfect and sampled using two distinct sampling methods.

Method I. From each alcohol-cleaned bottle, 100 ml were removed and filtered either through a 0.4-μm pore membrane-filter for bacteria or a 0.8-μm pore filter for yeasts and moulds (Odunfa 1987). The membranes were then placed on appropriate nutrient media and incubated at 30°C for 48 h.

Method II. Triplicate aliquots of beverage samples (100 ml each) were placed in sterile centrifuge tubes, centrifuged at $15,000 \times g$ for 30 min, and supernatants decanted aseptically, leaving about 0.5 ml in each tube. After vigorous agitation, these residues were used to inoculate appropriate nutrient media by spreading on agar plates and were then incubated at 30°C for 48 h.

Identification of Isolates

Bacterial colonies from Methods I and II were streaked on standard plate count agar, tomato juice agar or MacConkey agar (all Oxoid) plates while yeasts and moulds were inoculated on Sabouraud's dextrose agar. The bacteria were identified microscopically and by biochemical and physiological tests, with reference to standard identification manuals (Buchanan & Gibbons 1974). The yeasts and moulds were identified by microscopy and sugar assimilation and oxidation tests (Kreger van Rij 1984).

Statistical Analyses

All statistical analyses were by Student's *t*-tests.

Results

Mean c.f.u. values of 19.7 and 31.0/100 ml sample were recorded using Methods I and II, respectively. Statistical analyses of the rates of microbial isolation showed that Method II gave significantly more isolates ($P = 0.001$) than Method I. The different microbial contaminants isolated from the non-alcoholic beverages (listed in Table 1) included bacterial mould and yeast species. The order of occurrence of specific bacterial

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Table 1. Frequency of occurrence of microorganisms in 90 non-alcoholic beverages commercially available in Nigeria.

Isolate	Frequency (%)
Bacteria	77
Bacillus spp.	36
B. sphaericus	10
B. megaterium	13
B. subtilis	13
Lactobacillus spp.	26
L. fructivorans	10
L. brevis	6.5
L. delbrueckii	6.5
L. kafir	3
Pediococcus pentosaceus	7
Staphylococcus epidermidis	7
Micrococcus cycae	3
Fungi	23
Saccharomyces cerevisiae	16
Aspergillus niger	7

ages (listed in Table 1) included bacterial mould and yeast species. The order of occurrence of specific bacterial genera was as follows: *Bacillus* > *Lactobacillus* > *Pediococcus* = *Staphylococcus* > *Micrococcus*. Bacteria accounted for 77% of the total microbial isolations while fungal isolates (*Saccharomyces* spp. and *Aspergillus niger*) accounted for the remaining 23%. The rates of isolation of the different bacterial and fungal genera from individual non-alcoholic beverage groups are shown in Table 2. The trend for microbial isolation was as follows: soda drinks > cola drinks = orange drinks > lemon/miscellaneous drinks = malt drinks.

Discussion

Most of the isolates from the non-alcoholic drinks were saprophytic. Except for *Staphylococcus epidermidis*, which is sometimes an opportunistic pathogen (Buchanan & Gibbons 1974), no pathogen was isolated. The non-malt drink categories (i.e. cola, orange, soda, and lemon/miscellaneous) together accounted for > 90% of all the microbial isolations, with the soda group having the highest value of 32% (Table 2). This situation may not be unconnected with the pH values of the soda group (Table 2), which were generally > 3.5 and therefore judged microbiologically unsafe for this category of products (Frazier & Westhoff 1978). The malt drink category presented one of the lowest rates of microbial isolation (10%) and this may be attributed to the more rigorous process of malt drink preparation, which includes hop addition and boiling, together with final pasteurization. This ensures that any microbial contaminants introduced at the various earlier stages of production are eliminated. The resinous components of hops are known to possess broad spectrum anti-microbial potency. The malt drinks examined gave bitterness values, due to hops, of between 12 and 14 E.B.U.

Critical study of the isolates shows that the osmo- and thermo-tolerant *Bacillus* spp. (Frazier & Westhoff 1978) predominated. These organisms, which are also salt-tolerant and produce thermo-resistant spores, can survive preservative concentrations much higher than those normally used in non-alcoholic beverages (Odunfa 1987). More importantly, being environmental in occurrence, they may have been introduced into these drinks from either the environment and/or the raw materials (Frazier & Westhoff 1978). The *Lactobacillus* spp. were the next major group of isolates. Their widespread occurrence in soft drinks has been widely established, being associ-

Table 2. Rates of microbial isolation from the different groups of non-alcoholic beverages examined.

Microbial genus	Occurrence (%)				
	Malt	Cola	Orange	Soda	Lemon/Miscellaneous
Bacillus	9	36	18	27	9
Lactobacillus	25	0	37	25	12
Pediococcus	–	100	–	–	–
Staphylococcus	–	100	–	–	–
Micrococcus	–	–	100	–	–
Saccharomyces	–	–	40	60	–
Aspergillus	–	–	–	100	–
Totals	10 (35)	26 (20)	26 (66)	32 (75)	6.5 (29)
pH range	5.1 to 5.5	2.9 to 3.5	3.3 to 3.7	3.4 to 3.7	3.4 to 3.7

* Values represent the total rates of isolation of microbial contaminants from the different groups of beverages followed, in parenthesis, by the percentage of non-alcoholic drinks within each category giving positive microbial cultures.

ated with their 'special adaptative properties' (Odunfa 1987). As common food contaminants, they are very acid-, CO_2- and salt-tolerant, and can grow well in the presence of 30% (w/w) sugar (Frazier & Westhoff 1978). Other common contaminants of food and beverages that were isolated include: *Pediococcus* spp. *Aspergillus niger* and *Saccharomyces cerevisiae*.

Their isolation is in agreement with earlier reports linking them with beverage and soft-drink factory environments (Odunfa 1987). *Staphylococcus epidermidis* and *Micrococcus cycae* are osmophilic bacterial members of the normal human flora (Buchanan & Gibbons 1974). Their presence in the non-alcoholic drinks may have been the result of contamination from factory personnel.

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