Nigerian Journal of Pharmaceutical Research
Vol. 8 No 1 pp. 281 - 287 (September 2010)

MICROBIOLOGICAL EVALUATION OF PACKAGED PINEAPPLE JUICE MARKETED IN KADUNA METROPOLIS

*G. O. Adeshina, T. S. Tahir and J. A. Onaolapo
Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

*Corresponding author: E-mail: dotunkele@yahoo.comTelephone number: 08037880000

Abstract
The microbial quality of ten brands of pineapple drinks sold in retail outlets in Kaduna, Nigeria was assessed by microbiological routine methods. All the pineapple drinks tested had high level of microbial contamination. The bacteria count was between
1.5 x 10^5 and 5.3 x10^6 colony forming units (cfu) ml^{-1}, while the mould/ yeast count was between 2.2 x 10^4 and 5.2 x 10^5 spore forming units (sfu) ml^{-1}. The bacteria species detected consisted of *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Bacillus subtilis,* and *Pseudomonas aeruginosa*. The mould/yeast includes *Penicillum spp., Candida sp, Aspergillus niger* and *Aspergillus flavus*. The susceptibility pattern of *Staph. aureus, E. coli, Enterobacter aerogenes* and *Ps. aeruginosa* isolated from the pineapple drinks showed that *Ps. aeruginosa* showed complete resistance to some of the antibiotics. The results are discussed in the light of health hazards posed by microbiologically poor pineapple drinks.

Key words: Microbiological evaluation, packaged pineapple drinks, marketed,

INTRODUCTION

Pineapple is the leading edible member of the family Bromeliaceae which embraces about 2,000 species mostly epiphytic and many strikingly ornamental. The pineapple fruit contains vitamins, minerals, fiber and enzymes that are good for digestive system and help in maintaining ideal weight and balanced nutrition. They are a good source of manganese, Vitamins B1, B6 and C (Borade, 2009) and can be eaten raw or used in cooking meals. They also have minimal fat and sodium with no cholesterol (PMH, 2009). The phenomena of processing the pineapple fruit in various packaging materials became necessary for ease of consumption due to the perishable nature of the fruit, the nutritional value and the fact that large quantity is consumed at juice stands and at home.

Packaged pineapple juice is consumed throughout the world and today there is a growing demand for it as beverage. There are quite a number of companies that produce packaged pineapple drinks in Nigeria and these companies use different types of packaging materials such as plastics, glass and waxed paperboard. The choice is dependent upon consumer acceptance, anticipated shelf life and cost factors (Ashurst, 2005). It has been reported that these packaging materials serve as sources of contamination to

*Corresponding author
ISSN 0189-8434

pharmaceutical products (Olutimayin and Onaolapo, 1997).

Preservatives such as benzoic acid, sodium benzoate, ascorbic acid, sugar (at least 65% ^{w}/v) and methyl-p-hydroxybenzoate are commonly used in the preservation of packaged fruit juices because they serve as good media for the growth of many different microorganisms and the processing steps can also be a source of contamination to the drinks. Various contaminants have been isolated from packaged fruit drinks despite the addition of these preservatives (Baird, 2004).

The official limit recommended for microbial contamination of drinking water, which also includes fruit drinks, requires the complete absence of some pathogens like *E. coli* and other coliforms, *Pseudomonas aeruginosa* and other organisms like *Staph. aureus*. Other permissible bacteria should not be more than 10^3 colony forming unit (cfu)/ml while mould/yeast should not exceed 10^2 spore forming unit (sfu)/ml (WHO, 2006).

The need for Good Manufacturing Practice, which consists in quality assurance that is aimed at ensuring that product is consistently manufactured to a quality appropriate for its intended use (Baird, 2004) cannot be overlooked in the preparation of packaged fruit juice due to the fact that the general populace depends on these drinks as ready-to-drink items at fast food centers or outlets.

The goal of this study was to provide information on the microbiological quality of some brands of packaged pineapple drinks; identify the microbial contaminants; and test the susceptibility of the contaminants to some commonly prescribed antibiotics.

MATERIALS AND METHODS

Sampling

A total of eight (8) samples each of ten (10) brands of packaged pineapple drinks were purchased from open markets and departmental stores in Kaduna metropolis over a period of three (3) months. Different batches of each brand were picked. All the brands carry production and expiry dates; National Agency for Food, Drug Administration and Control (NAFDAC) registration number and batch number. Samples B, G and H were packed in plastic containers, samples C, E, F and I in glass containers and samples A, D and J in foil paperboard containers. The samples were processed immediately or kept at 4^{o}C in a refrigerator.

Culture media: Nutrient broth (NB), Nutrient agar (NA), Sabouraud's Dextrose Agar (SDA), Sabouraud's Dextrose Broth (SDB), Mannitol Salt Agar (MSA), MacConkey Agar (MA), Centrimide Agar (CA); all Oxoid.

Standard antibiotics: Ampiclox (ACL), ampicillin (AMP)**, g**entamicin (GEN), **o**floxacin (OFL), cotrimoxazole (CTM), and amoxycillin (AMX).

Methods

Each sample of the packaged pineapple drink was shaken vigorously and diluted appropriately. One (1.0) ml of each dilution was aseptically obtained in triplicate from the container and plated on the different agar (MA for coliforms; MSA for *Staph. aureus*; CA for *Ps. aeruginosa* and SDA for moulds/yeasts) and nutrient agar plates which contained an in-activator to neutralize the preservatives. The bacterial plates were incubated at 37^{o}C for 24 – 48 hours while fungal plates were incubated at 25^{o}C for 7 days. All the experiments were carried out in an aseptic screen and repeated three times. The total microbial load was

counted in the various agar plates and the average viable count was recorded. Colonies were selected using their morphological characteristics (size, pigmentation, elevation and consistency). Gram staining methods and further biochemical tests were carried out to identify the organisms that were isolated from the packaged pineapple drinks (Cowan, 1974; Hendrie and Shewan, 1979; Collee and Miles, 1989). The susceptibility pattern of the selected isolates (*E. coli, En. aerogenes, Ps. aeruginosa* and *Staph. aureus*) to the antibiotics was determined using agar diffusion method (Cheesbrough, 1993). The 24-hour broth culture of the isolates was diluted to McFarland standard (10^5 – 10^6cfu/ml) and 2.0ml of the standardized inocula spread on the surface of the previously prepared sterile nutrient agar plates by flooding. Excess were drained off and allowed to dry in a warm incubator for about 15 – 20 minutes.

Using sterile forceps, antibiotic discs were placed on the dried nutrient agar plate and left at room temperature for about 25 min to allow the antibiotics to diffuse in the agar medium. Standard organisms were also used. The diameter of the zones of inhibition of the organisms was measured to the nearest millimeter.

RESULTS

The microbial load of the packaged pineapple drinks was high and varied between the different brands. The bacterial load ranged from$1.5x10^5$ cfu/ml to 5.3 $x10^6$ cfu/ml while the mould/yeast count ranged from $2.2x10^4$ sfu/ml to $5.2x10^5$ sfu/ml (Tables 1 and 2).

Staphylococcus aureus was isolated from samples A, F, H, I and J; *Ps. aeruginosa* was isolated from samples A and B. Samples F, I and J were contaminated with *E. coli* and B, C, D, E, G, H, I and J contained *En. aerogenes. Bacillus subtilis* was isolated from all the brands of the packaged pineapple drinks forming 100% of the contaminants (Table1). Samples B, D and J were contaminated with *Penicillium* sp.; A, D and J were contaminated with *Aspergillus niger* and B, E, F and H contained *Aspergillus flavus.* The yeast, *Candida* sp. was persistently isolated from all the brands (Table 2).

The microbial load was found to vary with the type of packaging material used. Pineapple drinks packaged in plastic containers showed the highest bacterial load of 3.7 x 10^6 - 5.3 x 10^6cfu/ml followed by those in glass which had 2.1 x10^6cfu/ml - 3.4 x 10^6cfu/ml while the drinks in foil paperboards showed the least colony count of 1.5 x 10^5cfu/ml/ - 2.7 x 10^5cfu/ml (Table 1). The highest fungal count of 1.9 x 10^5sfu/ml- 5.2 x 10^5 was however found in the pineapple drink packaged in foil paperboard followed by the ones in glass containers with 4.0 x 10^4sfu/ml – 6.8 x 10^4sfu/ml and the least fungal count was from the drinks packaged in plastic containers which ranged between 2.2 x 10^4sfu/ml – 3.1 x 10^4sfu/ml (Table 2). The susceptibility pattern of the selected isolates showed that the coliforms were more susceptible to the commonly used antibiotics. *Pseudomonas aeruginosa* showed resistance to some of the antibiotics (Table 3).

DISCUSSION

The study of the various packaged pineapple drinks showed extensive microbial contamination characterized with high microbial load and the presence of non-permissible bacterial species such as *E. coli, En. aerogenes, Ps. aeruginosa* and *Staph. aureus* in the drinks. The microbiological quality

Table 1: Bacterial viable count and distribution of bacteria isolated from the various packaged pineapple drinks

Brand	Sample	Packaging material	Bacterial viable count (cfu/ml)	*Staph. aureus*	*E. coli*	*En. aerogenes*	*B. subtilis*	*Ps. aeruginosa*
A	8	Foil	1.5×10^5	+	-	-	+	+
B	8	Plastics	5.3×10^6	-	-	+	+	+
C	8	Glass	3.4×10^6	-	-	+	+	-
D	8	Foil	2.3×10^5	-	-	+	+	-
E	8	Plastics	4.0×10^6	-	-	+	+	-
F	8	Glass	2.1×10^6	+	+	-	+	-
G	8	Plastics	4.8×10^6	-	-	+	+	-
H	8	Plastics	3.7×10^6	+	-	+	+	-
I	8	Glass	3.7×10^5	+	+	+	+	-
J	8	Foil	2.7×10^5	+	+	+	+	-

Key: + = present, - = absent

Table 2: Fungal count and distribution of fungi isolated from the various packaged pineapple drinks

Brand	Sample	Packaging material	Fungal count (sfu/ml)	*Penicillium sp.*	*Asp. niger*	*Asp. flavus*	*Candida sp*
A	8	Foil	5.2×10^5	-	+	-	+
B	8	Plastics	3.1×10^4	+	-	+	+
C	8	Glass	5.6×10^4	-	-	-	+
D	8	Foil	1.9×10^5	+	+	-	+
E	8	Plastics	2.3×10^4	-	-	+	+
F	8	Glass	4.0×10^4	-	-	+	+
G	8	Plastics	2.9×10^4	-	-	-	+
H	8	Plastics	2.2×10^4	-	-	+	+
I	8	Glass	6.8×10^4	-	-	-	+
J	8	Foil	2.0×10^5	+	+	-	+

Key: + = present, - = absent

*Corresponding author
ISSN 0189-8434

Table 3: Antibiotic sensitivity pattern of the bacteria isolated from the packaged pineapple drinks

Organism	Zones of Inhibition (mm)						
	AMC (10µg)	NFT (10µg)	OFL (10µg)	AMP (10µg)	CTZ (10µg)	GTC (10µg)	AML (10µg)
Staph. aureus	26	17	27	30	20	26	19
Staph. aureus NCTC 6571	28	18	29	30	23	30	21
E. coli	19	22	21	19	18	25	21
E. coli NCTC 10413	21	30	21	23	20	26	25
En. aerogenes	19	25	20	22	21	24	23
Ps. aeruginosa	00	00	16	00	00	17	10
Ps. aeruginosa NCTC 6750	00	00	18	00	00	16	00

Key: AMC – Ampiclox; NFT – Nitrofurantoin; OFL - Ofloxacin; AMP - Ampicilin; CTZ – Cotrimoxazole; GTC – Gentamicin; AML – Amoxicillin

of packaged drinks is determined by the presence or absence of indicator organism for faecal pollution, *E. coli*, faecal *Streptococci*, sporulating bacteria, surface water contaminants and pathogenic bacteria such as *Ps. aeruginosa*. Other revivable microorganisms should not be more than between 10^3-10^4cfu/ml for aerobic bacteria and 10^2cfu/ml for mould/yeast (FIP, 1975).

The type of contaminants isolated suggests the route of contamination. The presence of the *E. coli*, *En. aerogenes*, *Staph. aureus* and *Ps. aeruginosa* in some of the samples is of great public health importance. The presence of *E. coli* and *En. aerogenes* indicates faecal contamination, which may be principally from the workers producing the juice and possibly from the water used as vehicle. *Escherichia coli* is not always confined to the intestine and their ability to survive for brief periods outside the body makes them an ideal indicator organism to

test environmental sample for faecal contamination (Willey *et al*., 2008). *Enterobacter aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections in skin and other tissues. It has also been reported that the personnel are the most probable cause of contamination with *En. aerogenes* (Willey *et al*., 2008).

Pseudomonas aeruginosa was isolated from some of the packaged pineapple drinks. The water quality legislation recommended the complete absence of this organism. The presence of *Ps. aeruginosa* in the tested drinks could have resulted from contamination of the water and the processing personnel. *Ps. aeruginosa* is supported in growth by wounds showing signs of infection (Skinner and Carr, 1974). *Ps. aeruginosa* was isolated from industrial water (Chambers and Clarke, 1968). *Ps. aeruginosa* is an opportunistic pathogen which causes complications in immune-compromised patients.

The contamination of the packaged pineapple drinks with *Staph. aureus* can be attributed to poor sanitary habit of the operators who serve as reservoir. *Staphylococcus aureus* has been reported to be one of the commonest bacterial pathogens encountered in the community and *Staph. aureus* infections range in severity from food poisoning or minor skin infections to severe life threatening infections (Brooks *et al*., 2004).

All the tested packaged pineapple drinks were contaminated with *Bacillus subtilis*. This result is in agreement with that obtained by Olutimayin and Onaolapo (1997) who reported the presence of *B. subtilis* in all the samples of analyzed packaged orange drinks. The persistent isolation of *B. subtilis* in all the analyzed samples can be due to poor manufacturing environment and the sporing nature of the organism (Olutimayin, 1995).

The results of the susceptibility testing of the bacteria isolated from analyzed packaged pineapple drinks that are officially non-permissible in drinks to various antibiotics revealed that *Ps. aeruginosa* was resistant to some of the antibiotics. This is of great importance to public health as there is the risk of transferring the resistance to other bacteria present in human body including some pathogenic ones (Olutimayin and Onaolapo, 1997). *Pseudomonas aeruginosa* isolated from bottled water and packaged orange drinks were found to be resistant to several antibiotics (Kailis *et al*., 1991; Olutimayin and Onaolapo, 1997). *Pseudomonas aeruginosa* has been reported to be resistant to many antibacterial agents (Smith, 2004).

From the results of this study it can be concluded that all the analyzed packaged pineapple drinks are not suitable for human consumption because of the presence of various microbial contaminants ranging from pathogenic to non-pathogenic bacteria, mould/yeast in high cell counts which did not meet the official requirements for packaged drinks.

REFERENCES

Ashurst, P.R. (2005): Chemistry and technology of soft drinks and fruit juices. 2nd edition. Philip R. Ashurst (ed). Blackwell Publishing Ltd. UK. p. 203.

Borade, G. (2009): Unique facts about pineapple. Published 8 June, 2009. [Cited 2009 Nov 30]. Available from: http://www.buzzle.com/article/unique-facts-about-pineapple.html.

Baird, R. M. (2004): Microbial spoilage, infection risk and contamination control. In: Hugo and Russell's Pharmaceutical Microbiology, Stephen, P.D., Norman A. H. and Sean P. G. (eds). 7th edition.

Blackwell Scientific Publications, Oxford. pp.263-283.

Brooks, G.F., Butel, J.S. and Morse, S.A. (2004): Pathogenesis of bacteria infection. In; Jawetz, Melnick and Adelberg's Medical Microbiology. 21st edition. Lange Med. Brooks/McGraw-.Hill.] 286 235.

Chambers, C.W. and Clarke, N.A. (1998): Control of bacteria in non-domestic water. Adv. Appl. Microbiol. 8: 105-143.

Cheesbrough M. District laboratory practice in tropical countries (Part 11). Cambridge, University Press UK, 2000; p. 134-143.

Collee, J. and Miles, R.S.(1998): Tests for identification of bacteria. In: Mackie and McCartney's Practical Medical Microbiology. 13th ed. Collee, J.R., Duguid, J.P., Fraser, A.G. and Marmon, B.P. (ed). Churchill Livingstone/Longman, London. pp. 141160.

Cowan, S.T. (1974): Cowan and Steel's manual for the identification of medical bacteria. 2nd ed. Cambridge University Press, Cambridge.

FIP Working Party (2nd report, 1975). Microbiological purity of non compulsory sterile pharmaceutical preparations. Pharm. Acta. Helv. 51: 33-40.

Hendrie, M.S. and Shewan, J.M. (1979): The identification methods of Pseudomonas. In : Identification methods for microbiologist. Skinner. F.A. and Lovelock, D.W. (eds). Society for Applied Bacteriology Technical Series. No.14. Academic Press, London. pp 1-14.

Jones, R. (2004): Factory and hospital hygiene. In: Hugo and Russell's Pharmaceutical Microbiology, Stephen, P.D., Norman A. H. and Sean P. G. (eds). 7th edition. Blackwell Scientific Publications, Oxford. pp.376-386.

Kailis E, Papandreon S, Papa Petropoulou M. (1991). Susceptibility pattern of *Pseudomonas* species isolated from bottled drinking water. Microbio. Ecol. Hlth. Dis.14: 243-244.

Olutimayin G.O. (1995). Microbiological quality of packaged orange drinks marketed in Kaduna metropolis. M.Sc. Thesis. Ahmadu Bello University, Zaria. p. 60.

Olutimayin B. and Onaolapo JA. (1997). Microbiological quality of packaged orange drinks sold in retail outlets in Kaduna. *Nig J Pharm Res Dev* 2(1): 35-43.

Philippine Herbal Medicine. (2009). Pineapple. [Cited 2009 Nov 30]. Available from: http://www.philippineherbalmedicine.org/pineapple.htm.

Skinner, F.A. and Carr, F.G. (1974): The normal microbial flora of man. Society for Applied Bacteriology Symposium No.5. Academy Press, London. pp 30-38.

Smith, A. (2004): Bacterial resistance to antibiotics. In: Hugo and Russell's Pharmaceutical Microbiology. Stephen, P.D., Norman A. H. and Sean P. G. (eds). 7th edition. Blackwell Scientific Publications, Oxford. pp.220-232.

Willey JM, Sherwood LM, Woolverton CJ. (2008). Prescott, Harley and Klein's Microbiology. 7th ed. McGrawHill Company, America. p. 578.