ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol.3, Issue, 6, pp.006-009, June, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF ANTIBIOTIC RESISTANT ORGANISMS IN LOCALLY PRODUCED DRINKS

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ARTICLE INFO

Article History:
Received 9th January, 2011
Received in revised form
11th March, 2011
Accepted 17th April, 2011
Published online 2nd June 2011

Key words:
Local drinks,
Microbial quality,
Antibiotic resistance.

ABSTRACT

Five samples each of zobo, yoghurt and kunu were purchased from different hawkers in Umuahia and Okigwe metropolis in the Eastern part of Nigeria. The microbial qualities were determined according to standard methods. The antibiotic sensitive patterns of the bacterial isolates were also determined. Microbial count ranges from 3.5-8.9cfu/ml in zobo, 2.0-8.0 cfu/ml in yoghurt and 1.8-4.2cfu/ml in kunu. Bacteria isolated from the samples included, *Pseudomonas* species, *Streptococcus* species, *Proteus* species, *Klebsiella* species with high occurrence of *Escherichia coli* and *Staphylococcus aureus*. Most of the isolates were resistant to Tetracycline, Ampicillin, Gentamicin, Colistin and Cotrimoxazole. The occurrence of antibiotic resistant organisms in these drinks is of public health importance and may be responsible for prolong treatment of illness. Therefore proper quality control of these products should be encouraged so as to reduce the microbial load as well as antibiotic resistant bacteria.

INTRODUCTION

Locally produced drinks are becoming popular in Nigeria. These local drinks are patronized by people at various social gatherings probably because of their low cost price and the purported medicinal values. Some of the locally produced drinks are zobo, kunu and yoghurt. Zobo is an indigenous non-alcoholic drink processed from hot water extract of dried calyx of Roselle. The Roselle (*Hibiscus sabdariffa*) is a vegetable plant (Ilondu and Iloh, 2007) of West African origin being widely cultivated in India, Australia and Africa. In spite of the increasing popularity of zobo drinks, one of its greatest disadvantage is that it goes bad easily, having a very short shelf life of 24 hours at room temperature (Nwachukwu, *et al*., 2007). It is served chilled at various social gatherings and its popularity has recently spread wide because of its purported medical value, low price and nutritional properties (Oboh and Elusiyan, 2004., Osueke and Ehirim, 2004).

Kunu is a non-alcoholic drink prepared traditionally from millet or sorghum. It is mostly produced in Northern Nigeria (Gaffa *et al*, 2002). The drink is readily consumed by adults and children in the savannah region of Nigeria as a refreshing drink, an appetizer and a food complement. Kunu in the recent time has gained wide acceptance in the southern Nigeria. It is cheap compare with industrially produced drinks thereby making it a drink for the masses especially at time of economic hardship. The general belief that it enhances lactation in nursing mothers further increases its popularity.

Yoghurt is a product made by blending fermented milk with various ingredients that provide flavor and colour and consumed as diet or as a refreshing beverage especially when refrigerated (Shahid *et al*., 2002). Yoghurt is largely consumed and has high nutritional value and a rich source of carbohydrates, proteins, fats, vitamins, calcium and phosphorus. These drinks can be contaminated with micro organisms from unclean equipments, poor hygiene and poor processing procedures. These contaminants may affect the quality of the drinks and be detrimental to human health when consumed. Among these organisms, there may be some that can resist antibiotics. Resistance to antimicrobial agents is an emerging problem worldwide. Therefore the objective of this study is to determine the microbial quality of locally produced drinks and also evaluate the antibiotic resistant organisms in these drinks.

MATERIALS AND METHODS

Collection of samples

Five samples each of zobo, yoghurt and kunu were purchased from different hawkers in Umuahia and Okigwe metropolis in Eastern part of Nigeria. The samples were labeled and carefully transferred into the ice crystals box and transported to the microbiology laboratory. The analyses of the samples were done within 2hr of collection in microbiology research

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Bacteriological analyses

The media used were Nutrient agar, MacConkey agar and Muller-Hinton agar. The preparation of each medium was done based on the manufacturer's instruction. This was done by dissolving a certain weight of powder in appropriate volume of distilled water in a conical flask. The mouth of the flask was well covered with cotton wool wrapped with aluminum foil before autoclaving to sterility at 121^{o}C for 15min. After autoclaving, the flask and their contents were allowed to cool to 45^{o}C before they were disposed into sterile Petri dishes and allowed to gel. Serial dilution of each of the samples was carried out. From the appropriate serial dilution, 0.1ml was inoculated into Petri dishes containing Nutrient agar (for total viable counts) or MacConkey agar (for enumeration of presumptive coliforms) and spread with sterile bent glass rods. Each sample was inoculated in duplicate plates. Thereafter, the plates were incubated for 24-48hours at 37˚C. Then the colonies were counted and recorded as colony forming unit per ml (cfu/ml). Each microbial isolate was sub-cultured on a fresh media using streak techniques to obtain pure isolate and was inoculated into slants and preserved as stock culture for further tests.

The identification of the bacterial isolates was done based on colonial morphology and physiological characteristics, staining and biochemical reaction of each isolate (Cheesbrough, 2002).The colonial morphology and physiology of the isolates in terms of shapes, elevation, edges, colour and pigments were observed on the different culture media. Some of the biochemical tests were catalase, coagulase, oxidase, indole production, citrate utilization, voges proskauer and methyl red.

Antibiotic sensitivity test

The antibiotic sensitivity of isolates was done using the Agar disc diffusion method (Cheesbrough 2002). The bacterial isolates from the drinks were tested for sensitivity to antibiotics (ampicillin, chloramphenicol, cloxacillin, erythromycin, gentamicin, penicillin, streptomycin, tetracycline, colistin, nalidixic acid, nitrofurantoin, cotrimoxazole). The sensitivity test was determined by using Muller-Hinton Agar and gram positive and negative multi-discs (Abtek Biological Ltd. UK). A loopful of confluent growth of a pure culture was inoculated into a sterile test tube of sterile peptone water and standardized by using a turbidity standard. A sterile cotton swab stick was dipped into the inoculum. The excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. Then the swab was streaked all over the surface of the agar plate. The antibiotic disc was aseptically taken with a sterile forceps and placed on the surface of the agar and gently pressed to stick to the agar surface. The plates were incubated at 37˚C for 24hours. After incubation the diameter of each inhibition zone (including the diameter of the disc) was measured and recorded in mm. For organisms to be regarded as been resistant to antibiotics there should be no zone of inhibition or less than 5mm in diameter from the antibiotic disc. The experiments were repeated in duplicates for all of the pure isolates.

RESULTS

The total viable counts in zobo, yoghurt and kunu samples are shown in Tables 1, 2, and 3. The results revealed a range of total viable counts from $3.5x10^5$ to $8.9x10^5$ for zobo samples and $2.0x10^5$ to $8.0x10^5$ for yoghurt while in kunu the total viable count ranges from $1.8x10^5$ to $4.2x10^5$. Microbial counts in kunu were generally low compare with the other drinks. The gram positive discs were ampicillin (10µg), chloramphenicol (10µg), cloxacillin (5µg), erythromycin(5µg), gentamicin (10µg), penicillin (1i.u), streptomycin (10µg), tetracycline (10 µg), while the gram negative discs were ampicillin (25µg), colistin (25µg), gentamicin (10µg), nalidixic acid (30µg), nitrofurantoin (200µg), cotrimoxazole (25µg), streptomycin (25µg), tetracycline (25µg). Tables 4 and 5 showed the percentage antibiotic sensitivity pattern of gram negative and gram positive bacteria. Both gram negative and positive bacteria showed high percentage of resistance to ampicillin.

Table 1: Total microbial counts in zobo samples

Location	Sample	Microbial counts on Nutrient agar plates	MacConkey agar plates
Umuahia	Um 1	5.0×10^5	4.0×10^5
	Um 2	4.0×10^5	5.0×10^5
	Um 3	5.0×10^5	6.0×10^5
	Um 4	5.0×10^5	4.9×10^5
	Um 5	8.0×10^5	8.9×10^5
Okigwe	Ok 1	5.0×10^5	4.2×10^5
	Ok 2	5.0×10^5	3.9×10^5
	Ok 3	4.0×10^5	4.1×10^5
	Ok 4	6.6×10^5	6.8×10^5
	Ok 5	3.5×10^5	4.2×10^5

Table 2: Total microbial counts in yoghurt samples

Location	Sample	Microbial counts on Nutrient agar	MacConkey agar plates
Umuahia	Um 1	4.5×10^5	2.3×10^5
	Um 2	4.1×10^5	3.4×10^5
	Um 3	5.2×10^5	4.5×10^5
	Um 4	2.2×10^5	2.0×10^5
	Um 5	3.2×10^5	2.1×10^5
Okigwe	Ok 1	5.2×10^5	5.0×10^5
	Ok 2	5.4×10^5	4.0×10^5
	Ok 3	7.2×10^5	6.0×10^5
	Ok 4	6.0×10^5	5.0×10^5
	Ok 5	7.0×10^5	8.0×10^5

Table 3: Total microbial counts in kunu samples

Location	Sample	Microbial counts on Nutrient agar	MacConkey agar plates
Umuahia	Um 1	3.7×10^5	3.6×10^5
	Um 2	2.2×10^5	3.5×10^5
	Um 3	1.8×10^5	3.8×10^5
	Um 4	2.9×10^5	2.3×10^5
	Um 5	2.3×10^5	2.3×10^5
Okigwe	Ok 1	3.9×10^5	3.0×10^5
	Ok 2	4.2×10^5	4.1×10^5
	Ok 3	2.3×10^5	3.8×10^5
	Ok 4	2.4×10^5	4.0×10^5
	Ok 5	1.8×10^5	4.1×10^5

Table 4: Antibiotic sensitivity pattern of gram negative bacteria

Antibiotic	% resistance	% sensitive
Ampicillin	80	20
Colistin	75	25
Gentamicin	100	0
Nalidixic acid	20	80
Nitrofurantoin	0	100
Cotrimoxazole	100	0
Streptomycin	15	85
Tetracycline	60	40

Table 5: Antibiotic sensitivity pattern of gram positive bacteria

Antibiotic	% resistance	% sensitive
Ampicillin	100	0
Chloramphenicol	50	50
Cloxacilin	100	0
Erythromycin	50	50
Gentamicin	0	100
Penicillin	50	50
Streptomycin	0	100
Tetracycline	50	50

DISCUSSION

The microbial quality as well as the evaluation of antibiotic resistant organisms in locally produced drinks (zobo kunu, yoghurt) were investigated. The study revealed microbial counts of 3.5 to 8.9 x 10^5 cfu/ml in zobo, 2.0 to 8.0 x 10^5cfu/ml in yoghurt and 1.8 to 4.2 x 10^5cfu/ml in kunu samples. This result supports the report of earlier studies on microbial quality of some these drinks (Amusa *et al*.,2005. Oluwafemi and Da Silva, 2006). Microbial counts recorded in these locally prepared drinks were generally high and should be of public health concern. The microbial load can lead to the reduction of shelf-life and spoilage of the drinks. Presence of organisms could be attributed to the quality of water used in preparing drinks, nature of the environment, equipments or materials and hygienic conditions of the processors (Jay, 1996. Nwachukwu and Otokunefor, 2002). These (could be an indicative of inadequate precautionary measures during processing, production or packaging Hunter, 1993). Different diseases may occur in human (Consumers) as a result of high microbial counts e.g. diarrhea, gastro intestinal infection, urinary tract infection.

The antibiotic susceptibility pattern of the isolates revealed surprising results. Most of the gram negative bacterial isolates in the local drinks showed a higher percentage of resistance to ampicillin, gentamicin, colistin, cotrimoxazole and tetracycline while gram positive bacterial isolates were mainly resistant to ampicillin and cloxacillin. Similarly, antibiotic resistant bacteria have been reported in different drinking water supplies (McKeon *et al*., 1995. Nwachukwu and Otokunefor, 2003, Nwachukwu and Emeruem, 2007). Oyagade and Fasuan (2004) also isolated antibiotic resistant *E. coli* strains from well water. These antibiotics namely ampicillin, erythromycin, gentamicin, penicillin, tetracycline, cotrimoxazole were employed in this study because most of them are readily available and affordable and are used in many health centers in sub-Saharan Africa (Okeke, 2003). Therefore the continued use of these drugs by patients for treatment of any bacterial infections may be inadequate or inappropriate. The potential public health hazard associated with antibiotic resistance in drinks for human consumption should not be overlooked. Among other problems, these resistant organisms in drinks may promote multiple antibiotic organisms (Walter and Vennes, 1985) in human. The widespread of antimicrobial is a serious concern as some of resistant bacteria may be transmitted to humans through foods and drinks. Bacterial strains that are resistant to an antibiotic can produce enzymes that inactivate the drug or alter their own genes through mutation or by acquiring resistant genes from other organisms (Trevors *et al*.1987, Spratt, 1994). This study has provided awareness about the possible presence of antibiotic resistant bacteria in drinks and a focus for monitoring. In order to reduce or eliminate the microbial load or antibiotic resistant bacteria, the production, processing environment and handlers should adopt good hygienic practices. Again, ingredients used for processing of zobo, kunu, yoghurt should be properly washed and water sterilized to prevent contamination.

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