International Journal of Biomedical and Health Sciences
Vol. 6, No. 1, June 30, 2010
Printed in Nigeria

0794-4748/2010 $12.00 + 0.00

http://www.asopah.org

IJBHS 2010046/6209

Indigenous commercial drinks as potential sources of extended spectrum β-lactamases (ESBLS) producing organisms in Kano, Nigeria

M. Yusha'u[1]*, M. I. Umar and K. Suleiman[2]

[1]Department of Biological Sciences, Bayero University, Kano, Nigeria
[2]Dept. of Biological Sciences, College of Arts, Science and Remedial Studies, Kano, Nigeria

(Received April 2, 2010)

ABSTRACT: Ten samples each of ten different indigenous commercial drinks were processed for bacterial isolation and Gram staining reactions. Gram negative isolates were subjected to biochemical tests for identification using standard procedures and confirmed enterobacteriaceae isolates were screened for extended spectrum β-lactamases (ESBLs) production using Clinical Laboratory Standards Institute (CLSI) breakpoint. Suspected ESBLs producers were subjected to Disc Diffusion Test (DDST) using standard discs of Augmentin {AUG 30µg (Optudisc)} and prepared discs of Ceftriaxone {CXN 30µg (Medireich)} and Ceftazidime {CAZ 30µg (Glaxo-Smithkline)}. Of the 80 samples processed, 11 (13.75%) yielded Gram negative isolates belonging to enterobacteriaceae family. These include; *Citrobacter fruendii* 2, *Escherichia coli* 1, *Klebsiella pneumoniae* 1, *Morganella morganii* 1, *Proteus vulgaris* 1, *Salmonella typhi* 2 and *Serratia mercescens* 3. Among the enterobacteriaceae isolates screened, the results of CLSI breakpoint test showed that 7 (36.36%) were ESBLs producers viz: *Citrobacter fruendii* 1, *Escherichia coli* 1, *Morganella morganii* 1, *Serratia mercescens* 2. On subjecting the CLSI positive isolates to DRM, only 4 (18.75%) were confirmed ESBLs producers. These include; *Citrobacter fruendii* 1, *Escherichia coli* 1 and *Morganella morganii* 1.

Keywords: Water-borne pathogens; Commercial drinks, Extended spectrum β-lactamases (ESBLs).

Introduction

Water borne or related pathogens including bacteria are spread in water either through human ingestion of contaminated water or drinks. The most common and wide spread problem is pathogens from human excreta which contaminate water supplies and subsequently commercial drinks leading to spread of diseases such as typhoid fever, diarrhoeal diseases and cholera (Jorge, 1997).

*To whom correspondence should be addressed.
E-mail: mryushau@gmail.com

There seems to be no standardization and quality control in both processing and delivery of indigenous drinks and the apparent lack of quality control predisposes the consumers to serious health risk. According to Idakwo and Abu (2004) a wide variety of microorganisms pathogenic to human beings are transmitted through contaminated water. Also the world health organization (WHO, 1982), reported that some 300,000 people die every day from water related diseases like typhoid and paratyphoid fevers, cholera, bacillary dysentery and gastroenteritis.

Extended spectrum β-lactamases (ESBLs) are enzymes that confer variable level of resistance to oxyiminocephalosporins and occur predominantly in the family enterobacteriaceae with *Klebsiella pneumoniae* been the most commonly reported worldwide (Kotra, *et al*, 2002).

At present there exist more than 200 different natural variants worldwide which constitute serious threat to current β-lactam therapies and represent major therapeutic challenges for clinicians (Lin *et al.*, 2005).

This research is important at the present situation because of the collapse of primary healthcare system which resulted in most of the people resorting to purchase of drugs over the counters and in some cases from roadside hawkers which led to the use and overuse of drugs that expose them to the danger of acquiring ESBL-producing organisms (Paterson and Bonomo, 2005).

Materials and Methods

Antibiotics

The antibiotics used in this research were; Augmentin (AUG 30µg) (Optudisc), Ceftriaxone (CXN 30µg) (Medireich) and Ceftazidime (CAZ 30µg) (Glaxo-Smithkline).

Sample Collection

Food samples were collected in sterile plastic containers from ten different producers at ten different locations.

Bacterial Analysis of Food Samples

Drinks were prepared for bacterial isolation according to the method described by FAO (1979). One mL of each drink sample was pipetted out into test tubes containing 9mLs of peptone water to make 10^1 dilution which was further serially diluted up to 10^5. The samples were subjected to microbial analysis by streaking on MacConkey agar and the inoculated plates incubated at 37^{o}C for 24 – 48 hours (Nester *et al.*, 2004).

Gram's Staining

Bacterial colonies formed after bacterial analysis of food samples were Gram stained as described by Brooks *et al*. (1989).

Biochemical Tests

Gram negative isolates were further subjected to indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production according to standard procedures described by Cheesbrough (2004).

Disc Preparation

Improvised discs of Whatman No. 1 filter paper (6mm diameter) were punched and sterilized in bijou bottles by autoclaving at 121^{o}C for 15mins. Sensitivity discs were prepared by weighing 3mg of powdered cephalosporin and dissolving in 1mL sterile distilled water followed by placing the improvised paper discs in the solution such that each disc took up 0.01mL to make the disc potency of 30µg.

Inoculum Standardization

The isolates were cultured on prepared Brain Heart Infusion (BHI) Agar (Biotech, England) plates and incubated for at 37°C for 24 hours so as to obtain confluent growth for sensitivity test. Few colonies of isolates from BHI plates were dispensed in sterile normal saline to match the 0.5 McFarland standards for sensitivity tests as described by NCCLS (1999).

CLSI Breakpoint Test For ESBLS Screening

The sensitivity of standard inocula of isolates to Ceftriaxone (CXN 30µg) and Ceftazidime (CAZ 30µg) discs were determined on Mueller Hinton Agar (Biotech, India) using Kirby Baueur method (NCCLS, 1999).

DDST For ESBL Confirmation

The isolates were sub-cultured on Brain Heart Infusion Agar (BHI) {Biotech, England} using streak plate method and the plates incubated at 35°C for 18-24 hours so as to obtain confluent growths. Improved procedure of Jarlier *et al*.; (1998) was employed in the screening of isolates for ESBL production on Mueller Hinton Agar (Biotech, India) using standard inocula from BHI plates. The isolates were inoculated using sterile swab sticks onto the surface of Mueller Hinton agar and discs containing Ceftriaxone and Ceftazidime were placed 20mm center to center from the Augmentin disc. The plates were incubated at 35°C for 18-24 hours after which the plates were read.

Results

Of the 80 samples processed, 11 (13.75%) were positive for members of the enterobacteriaceae family via biochemical characterization. Of the 11 enterobacteriaceae isolates subjected to ESBL detection using CLSI breakpoint, 4(36.36%) were found to be positive (Table 3) which include; *Citrobacter fruendii* (2), *E. coli* (1), *Klebsiella pneumoniae* (1), *Proteus vulgaris* (1) and *Serratia mercescens* (2) with no detection in *Morganella morganii* and *Salmonella typhi*. However, only 4 (18.75%) were confirmed to be ESBL producers based on DDST (Table 4) which include; *Citrobacter fruendii* (1), *Klebsiella pneumoniae* (1) and *Serratia mercescens* (2). Inhibition zones of ≤22mm against ceftazidime disc and ≤25mm against ceftriaxone disc are suspicious for ESBLs production in CLSI method. Positive DDST result is indicated by an enhancement of inhibition zone of cephalosporin discs towards the central Augmentin disc.

Table 1: Occurrence of Gram negative bacterial isolates in Food Samples.

S/No.	Food Samples	Number screened	Number positive	% occurrence
1.	Zobo	10	3	3.75
2.	Koko	10	0	0.0
3.	Kunun aya	10	0	0.0
4.	Kunun zaki	10	6	7.5
5.	Ginger	10	0	0.0
6.	Nono	10	0	0.0
7.	Fura da nono	10	1	1.25
8.	Satchet water	10	1	1.25
	Total	**80**	**11**	**13.75**

Table 2: Occurrence of Gram negative species based on Biochemical Reactions.

S/No.	Isolates	Lac.	Urea	Cit.	Mot.	Ind.	Slope	Butt	H_2S	Gas	No. identified	% occurrence
1.	*Citrobacter fruendii*	….+	+/-	+	+	-	R/Y	Y	+/-	+	2	18.18
2.	*Escherichia coli*	+	-	-	+	+	Y	Y	-	+	1	9.09
3.	*Klebsiella pneumoniae*	+	….+	+	-	-	Y	Y	-	+	1	9.09
4.	*Morganella morganii*	-	+	-	+	+	R	Y	-	-	1	9.09
5.	*Proteus vulgaris*	-	+	+/-	+	+/-	R	Y	+	+	1	9.09
6.	*Salmonella typhi*	-	-	+/-	+	-	R	Y	+	+/-	2	18.18
7.	*Serratia mercescens*	….+	+	+	+	-	R	R	-	-	3	27.27

Key: Lac. = Lactose, Cit. = Citrate, Mot. = Motility, Ind. = Indole, H_2S = Hydrogen Sulphide production, R = Alkaline reaction, Y = Acid reaction, ….+ = Late positive reaction, +/- = Few species give negative reactions

Table 3: Screening for ESBLs among the isolates based on CLSI breakpoint.

S/No.	Isolates	Number screened	Number positive	% occurrence
1	*Citrobacter fruendii*	2	1	9.09
2	*Escherichia coli*	1	1	9.09
3	*Klebsiella pneumoniae*	1	1	9.09
4	*Morganella morganii*	1	0	0.0
5	*Proteus vulgaris*	1	1	9.09
6	*Salmonella typhii*	2	1	9.09
7	*Serratia mercescens*	3	1	9.09
	Total	**11**	**7**	**63.64**

Table 4: Confirmation of ESBLs among the isolates based on DDST.

S/No.	Isolates	Number screened	Number positive	% occurrence
1	*Citrobacter fruendii*	1	0	0.0
2	*Escherichia coli*	1	0	0.0
3	*Klebsiella pneumoniae*	1	1	14.29
4	*Proteus vulgaris*	1	1	14.29
5	*Salmonella typhi*	1	0	0.0
6	*Serratia mercescens*	2	2	28.57
	Total	**7**	**4**	**57.14**

Discussion

Of the 80 food drink samples processed for bacterial analysis and subjected to Gram's staining technique, 11 (13.75%) were Gram negative. High occurrence of Gram negative organisms among the isolates is an indication of possible outbreaks of infection since the organisms are pathogenic once found living outside their natural habitat i.e. gastrointestinal tract.

On subjecting the Gram negative isolates to biochemical tests, all were identified as members of the enterobacteriaceae family (Table 2). The high occurrence of enterobacteriaceae may be due to poor hygienic practices which may result in some of the ESBLs non-producing isolates acquiring plasmids responsible for ESBLs production since plasmids can easily be transferred between organisms living in the same environment and replicate alongside the bacterial chromosome (Gunseren *et al.*, 1999).

Of the 11 enterobacteriaceae isolates subjected to ESBL detection using CLSI breakpoint, 7 (63.64%) were found to be positive (Table 3) while only 4 (57.14%) were positively confirmed to be ESBL producers based on DDST (Table 4). The variation in ESBLs positive results between the CLSI and DRM procedures may be due to false positive results caused in organisms with multiple β –lactamases that interfere with the test results which can only be detected using iso-electric focusing and DNA sequencing (CLSI, 1999).

In general, the percentage prevalence of ESBLs producers among the different species of enterobacterial isolates screened was higher in *Klebsiella pneumoniae* (100%) and Proteus vulgaris (100%) followed by *Serratia mercescens* (66.66%). There exist significant differences in ESBLs production among the isolates when the results were subjected to chi-square statistical analysis at 5% confidence level and 5 degree of freedom.

Recommendations

In view of the worldwide occurrence and quick spread of ESBLs among bacterial pathogens and the problems that may be caused by treatment failure due to infections with ESBLs producing organisms, it could be recommended that;

Government should strengthen awareness campaigns on improved hygienic practices so as to reduce the rate of microbial infections as well as spread of ESBLs among both enterobacteriaceae and other bacterial pathogens.

People should ensure proper processing and handling of these products before selling to consumers.

References

Anderson, J. G., Rowan, N. J., MacGregor, S. J., Fouracre, R. A. and Farish, . (2000): Inactivation of food-borne entero-pathogenic bacteria and spoilage fungi using pulsed-light. *Plasma science* **28(1):** 83-88.

Brooks, G.F., Butel, J.S. and Ornston, N.L. (1989): Medical Microbiology. 19th edition. Prentice – Hall International (UK) Limited, London. P588.

Casals, I. and Pringler, J. (1990): Meterranean Congress of Chemotherapy, Bacelona, Spain, 20-25 May, 1990.

Cheesbrough, M. (2004): District Laboratory practice for tropical countries, Part 2. Cambridge University Press, UK. Pp. 180 – 197.

Clinical and Laboratory Standards Institute (Formerly National Committee for Clinical Laboratory Standards) (1999): Performance standard for antimicrobial susceptibility testings. NCCL approved standard M100-59.

Clinical and Laboratory Standards Institute (2005): Performance standards for antimicrobial susceptibility testing; 15th informational supplement (M100-S15).

Food and Agriculture Organization of the United Nations FAO (1979): Manuals of food quality control 4. Microbiological analysis.

Gunseren, F., Mamikoglu, L., Ozturk, S., Yucesoy, M., Biberoglu, K., Yulug, N., Doganay, M., Sumerkan, B., Kocagoz, S., Unal, S., Cetin, S., Calangu, S., Koksal, I.,

Leblebicioglu, H. and Gunaydin, M. (1999): A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey. *J. Antimicrob. Chemother.* **43:** 373-378.

Idakwo, P. Y. and Abu, G. O. (2004): Distribution and statistical analysis of Bacteria in Lake alau in the arid Northern Nigeria. *Nig. J. Appl. Sci. Env. Mgt*, **8(1):**5-9.

Jorge E. H, Diana M., and David S. (1997): Environmental problems in Third World Cities Earth Scan Publication Ltd. London Pp 72-75.

Kotra, L. P., Samama, J. and Mobashery, S. (2002): β-lactamases and resistance to β-lactam antibiotics. In: Lewis. K., Salyers, A., Tabar, H. W. and Wax, R. G. edn. Bacterial resistance to antibiotics. New York, Marcel Decker, 123-60.

Lin, R., Chin, Y. and Lee, M. (2005): Antimicrobial activity of Antibiotics in Combination with Natural flavonoids against extended spectrum beta-lactamase producing Klebsiella pneumoniae. *Phytotherapy Research* **19:** 612-617.

Nester, E. (2004): A Human Perspective of Biotechnology. 4th ed., McGraw Hill, New York. Pp.102; 12338 – 12343.

Paterson, D. L. and Bonomo, R. A. (2005): Extended spectrum β-lactamases: Clinical update. *Clin. Microbiol. Rev*. **18(4)**: 657-686.

Sanders, C. C. (1992): β-lactamases of gram negative bacteria; new challenges for new drugs. *Clin. Infect. Dis.* **14:**1089-1099.

World Health Organization WHO (1982): Guide lines for drinking water quality Vol. EFP82:39.