Int.J.Curr.Microbiol.App.Sci (2016) *5*(1): 796-801

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 1(2016) pp. 796-801**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.501.081

Incidence of Broad Spectrum Resistance in *E .coli* Isolated from Zoborodo sold in Samaru, Zaria, Nigeria

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K e y w o r d s

Ampicillin, Antibiogram, Cephalothin, Zoborodo

Article Info

Accepted:
22 December 2015
Available Online:
10 January 2016

A B S T R A C T

Antibiogram of twenty six (26) *E.coli* strains obtained from a hundred and fifty satchets of zoborodo drink against eight antibiotics were carried out to investigate incidence of broad spectrum resistance. Broad spectrum resistance was taken as resistance to ampicillin and cephalothin. Resistance of 69 and 92% of the *E.coli* to ampicillin and cephalothin respectively was obtained, amounting to 92% broad spectrum resistance. *E.coli* exhibiting single antibiotic resistance were all resistant to only cephalothin while all the multiple antibiotic resistance *E.coli* were resistant to both ampicillin and cephalothin. Measures should be taken to increase the hygienic conditions of zoborodo preparation.

Introduction

'Zoborodo' drink today has become popular, with much acceptance in Nigeria and commonly referred to as 'zobo'. It is prepared by boiling the dry calyces of *Hibiscus sabdariffa* in water for about 10-15 min from which the pigment or flavour embedded is extracted. After extraction the filtrate may be taken hot as tea but most times allowed to cool and packaged in plastic sachet containers or bottles then taken as a refreshing drink when chilled.

The sharp sour taste of the raw extract is usually sweetened with sugar cane or granulated sugar, pineapple, orange or other fruits depending on choice. The sweetness of 'zobo' drink does not last long due to spoilage by microbial activities (Nwachukwu *et al.*, 2007). Economically, zobo is cheap and has been shown to be good source of natural carbohydrates, protein and vitamin C which constitute a major reason for consuming the soft drink by the increasing population (Braide *et al*., 2012). However, the greatest limitation for production of 'zobo' drink is the rapid deterioration of the drink. Its shell-life is approximately twenty-four hours following

production if not refrigerated. Microorganisms associated with the dried calyx and the processing for the production of 'zobo' drink and other factors may contribute to its spoilage (Nwachukwu *et al.*, 2007**)**. Production and sale of 'zoborodo' is still at the local level. The low sanitary practice during production and sales accounts for the poor quality and a cause for concern (Ayo *et al*., 2004). The production process of 'zobo' is neither standardized nor mechanised. This allows proliferation of the associated micro-organisms which potentiates spoilage and the short shelf-life associated with this sorrel beverage. Some of these organisms have been found to pose serious health risks to consumers as they are associated with food spoilage and intoxication (Braide *et al*., 2012).

It has been reported that Gram negative bacteria account for approximately 69% of the cases of bacterial food borne disease. Members of the gram negative bacteria mostly *E. coli* are widely distributed in the environment. Contaminated food and water are the major sources by which the bacteria are spread. Selected strains can cause a wide variety of infections in hospitals and community setting. *Escherichia coli* therefore, is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination. Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Okonko *et al*., 2010).

In the past 60 years, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the twentieth century. However, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs (Christopher *et a*l., 2013). Previous studies have shown that *E. coli* is highly resistant to ampicillin, cephalothin, tetracycline and trimethoprim – sulfamethoxazole (Adenaike *et al*., 2013). The aim of this work is to investigate broad spectrum resistance (resistance to ampicillin or cephalothin) in *Escherichia coli* isolated from 'zoborodo' drink.

Materials and Methods

Sample Collection, Identification and Characterisation

A hundred and fifty samples of 'zoborodo' drinks in satchets were purchased from sellers in stores and markets within Ahmadu Bello University, Zaria main campus, Samaru and its environs. They were immediately transported to the laboratory for microbiological analysis. An amount 25ml of each 'zoborodo' sample was homogenized in 225ml of 1% buffered peptone water. The homogenate was pre-enriched by incubating for 18-24hr at 37^{o}C. Ten fold serial dilutions were prepared at the end of the enrichment using 1% buffered peptone water as diluent. Identification and characterisation were carried out using conventional methods and confirmed using Microgen Gram negative Identification kit (Adenaike *et al*., 2013).

Antimicrobial Susceptibility Testing

For susceptibility testing, *E.coli* strains were tested for their susceptibility to eight

antimicrobial agents using the disk diffusion method on Mueller Hinton agar. The inocula were standardise by adjusting the turbidity of the culture to match that of 0.5 MacFarland standard from which 0.1ml of the cultures were plated on Mueller Hinton agar plates, and spread evenly using a sterile spreader (Adenaike *et al*., 2013).

This was allowed to dry for 5 min and antibiotic discs were dispensed on the surface of the media and incubated aerobically at 37^{o}C for 18 h. The following antibiotic discs; Ampicillin 10µg, cephalothin 30µg, Cefpodoxime 10µg, Ceftriaxone 30µg, Ciprofloxacin 5µg, trimethoprim- sulfamethoxazole 25µg, tetracycline 30µg and amikacin 30µg (Oxoid Ltd., Basingstoke, Hampshire, England) were placed gently and allowed to stand for 5minutes to diffuse. After incubation at 37°C for 24 hours the inhibition zone (in diameters) were measured and compared with CLSI zone diameter interpretative standard breakpoints (CLSI, 2008). Intermediate isolates were counted as resistant in all the agents tested (Christopher *et a*l., 2013). *E.coli* ATCC 25922 was used as quality control.

Results and Discussion

Resistance profile of antibiotic susceptibility testing carried out in Plate 1 is shown in Fig. 1. Highest resistance was found in cephalothin and ampicillin with 92 and 69% respectively. A high resistance is also found in tetracycline with 54% resistance. This is probably because tetracycline is one of the older drugs (Introduced in 1948). Surveillance data show that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use for a long time in human and veterinary medicine (Tadesse *et al*., 2012). Gaetti-Jardim *et al*. (2010) also states that resistance to tetracycline could be related to its extensive use in medicine, veterinary and dentistry. High percentage of broad resistance (92%) [Fig. 2] indicates the need for newer and less competitive drugs. The single antibiotic resistance *E.coli* were resistant to only cephalothin while the phenotypes of the multiple antibiotic resistance were all resistant to both ampicillin and cephalothin. As such all the *E.coli* resistant to ampicillin were equally resistant to cephalothin (Table 1). Cephalothin, a prototype of first generation cephalosporins, are narrow spectrum drugs, which have their best activity against Gram-positive pathogens except methicillin-resistant S. aureus (MRSA), and are active against some Gram-negative organisms (Walsh, 2003). Increasing resistance in Gram-negative organisms is likely rendering cephalothin improper for therapy of infections caused by Gram-negative organisms.

Ampicillin on the other hand is a beta-lactam antibiotic. It has had increased resistance due to the ability of some bacteria to produce enzymes (β-lactamase) which hydrolyses the β-lactam ring rendering the antibiotic inactive against the bacteria. This has led to the introduction of newer drugs with higher stability against β-lactamases. Up to 90% of ampicillin resistance in *E.coli* is due to the production of TEM-1 (Lim *et al*., 2009). This enzyme is able to hydrolyze penicillins and early cephalosporins such as cephalothin and cephaloridine. TEM-type β-lactamases are most often found in *E. coli* and *Klebsiella pneumonia* and also in other species of Gram-negative bacteria with increasing frequency (Bradford, 2001).

There is no resistance to ciprofloxacin and Amikacin. Several studies have established that susceptibility to ciprofloxacin or other fluoroquinolones are quite high among *Enterobacteriaceae* (Hassan *et al*., 2011).

Table.1 Resistance Pattern of *E. coli* Isolated from 'Zoborodo' Drink

Single antibiotic Resistance		**Multiple antibiotic resistance**		
Number of isolates (%) in the category	Resistance phenotype	Number of antibiotic combinations	Number of isolates (%) with the pattern	Resistance Phenotype
6 (23)	KF	**2**	2 (8)	AMP, KF
		3	8(31)	AMP, KF, TE
			2(8)	AMP, KF, CPD
		5	2(8)	AMP, KF, CPD, CRO, TE
			4 (8)	AMP, KF, CPD, SXT, TE

Key: AMP-Ampicillin; KF- Cephalothin; CPD- Cefpodoxime; CRO-Ceftriaxone; SXT- Sulphamethoxazole-trimethoprim (Co-trimethoprim); TE-Tetracycline

Fig.1 Resistance Profile of *E. coli* Isolated from 'Zoborodo' Drink

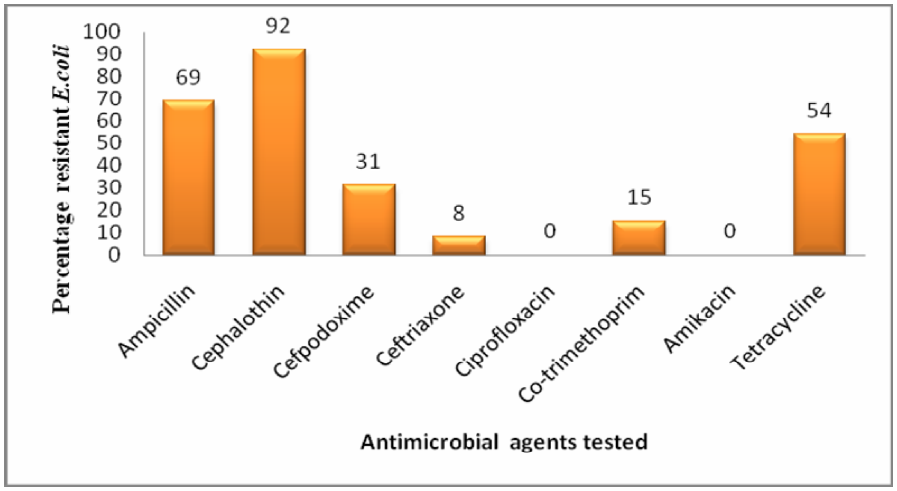


Fig.2 Broad Spectrum resistance

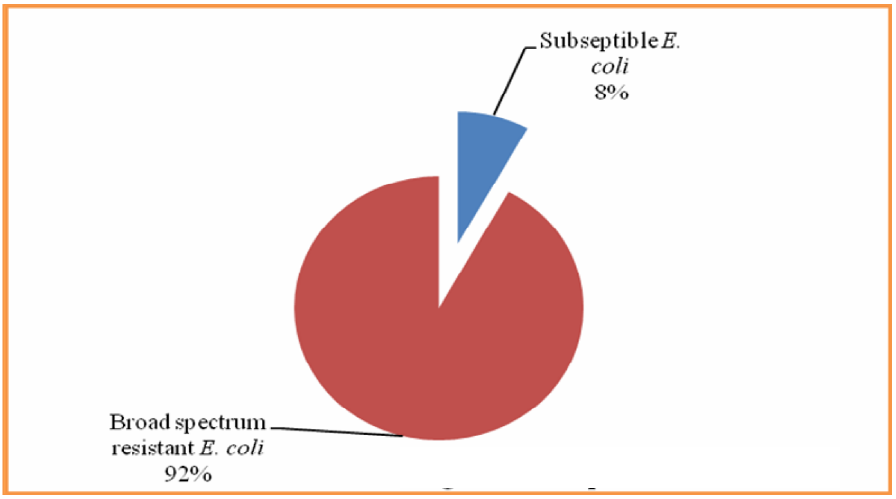
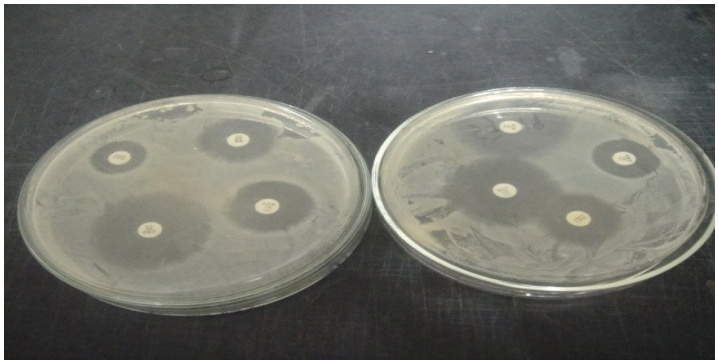


Plate.1 Antibiotic Susceptibility Testing



In conclusion, the presence of *E. coli* in 'zoborodo' provides undeniable evidence of the poor microbiological quality of the sorrel drink. Contamination of food by enteric pathogens can occur from the farm if human sewage is used to fertilize the soils or if sewage water is used to irrigate the plant, worst still as these pathogens exhibit resistance to commonly used antibiotics. Such risks are further increased if the food or drink is mishandled during processing and preparations where pathogens could multiply exponentially under favourable conditions (Nyenje *et al*., 2012). It is mandatory that foods must be free from contaminants as much as possible. The presence of *E.coli*, *S. aureus* and/or *B.cereus* demonstrates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases. Foodborne illness can be prevented by good hygiene practices (Oranusi *et al*., 2013).

Available data suggest that food can contribute to the dissemination of resistant *Enterobacteriaceae* in the community. To ensure that 'zoborodo' drink is microbiologically safe, both the manipulators and the food need to be continually monitored. The commercial manufacture of 'zoborodo' drink consist of a small number of the operations, but this critical process can lead to the introduction of the microorganisms or the proliferation of those already present. Possible sources of the microbial contamination have been identified as a) unhygienic handling; b) raw material; c) inadequate cleaning of the machines used, contact surfaces, clothes and manipulators' hands and d) airborne contamination (de Sousa, 2008).

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How to cite this article:

O. Adenaike, O.S. Olonitola, J.B. Ameh and C.M.Z. Whong. 2016. Incidence of Broad Spectrum Resistance in *E.coli* Isolated from Zoborodo sold in Samaru, Zaria. *Int.J.Curr.Microbiol.App.Sci*. 5(1): 796-801. http://dx.doi.org/10.20546/ijcmas.2016.501.081