ORIGINAL PAPER

Antibiotic Susceptibility Profile of Enterobacteriaceae Isolates from a Locally Preferred Drink – Kunun-Zaki in Kaduna State

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: 'Kunun-zaki' is a local drink made from slightly fermented corn or maize, with water as the medium. It is commonly taken by the people in the northern part of Nigeria. It has high nutritional benefit, even though it can also serve as a potential source of waterborne disease if not prepared under strict hygienic condition.

Objective: This study was carried out to isolate Enterobacteriaceae bacteria from 'kunun-zaki' drinks and to determine their antibiotic susceptibility to commonly prescribed antibiotics within Kaduna metropolis of Kaduna State, Nigeria.

Methods: A total of 20 samples were randomly collected from different locations (within a period of two weeks), analysed and coliform count determined. The bacterial isolates were identified using gram staining, selective media and standard biochemical tests. Antibiotics susceptibility testing was carried out using Kirby-Bauer agar diffusion method.

Results: The following bacteria were isolated: Enterobacteriaceae (50%), *Staphylococcus aureus* (30%), *Pseudomonas aeruginosa* (20%). The Enterobacteriaceae bacteria isolated were: *Klebsiella pneumoniae* 4 (20%), *Escherichia coli* 4, (20%), *Citrobacter freundii* 1 (5%) and *Proteus vulgaris* 1 (5%). The antibiotic susceptibility test revealed that ciprofloxacin, gentamicin, imipenem and ofloxacin (each 100%) chloramphenicol (90%), amoxicillin-clavulanate (80%) and streptomycin (70%) were active against the isolates while the isolates were resistant to ceftazidime (100%), meropenem (80%), cefoxitin and ceftriaxone (70%).

Conclusion: This study showed the presence of Enterobacteriaceae contaminants in locally consumed drink (kunun-zaki) sold within Kaduna metropolis which pose a potential public health risk including the risk of transmission of strains which can lead to the spread of antibiotic resistance in the society.

Keywords: Enterobacteriaceae; Antibiotic resistance; Local drink.

INTRODUCTION

Kunun-zaki (also called kunun) is a non-alcoholic, cereal-based beverage drink common to the people of the northern part of Nigeria. It is produced locally from millet (*Pennisetum typhoideum*), sorghum (*Sorghum vulgare*), maize (*Zea may*), rice (*Oryza sativa*) and acha (*Digitalis exillis*). The grains are either used singly or combined; the most common combination is

sorghum/millet. Steeping is usually done in ordinary water for 12-72 hours depending on the grain type (Gaffa *et al*., 2002). The grains are milled with or without spices such as ginger, red pepper, black pepper, clove and garlic, sweet potatoes are also added. The mixture is divided into two unequal proportions. One of the proportions is mixed up with boiling water and the other smaller portion with malted rice and sweet potato paste. The two

proportions are then mixed together at about 75-80^{0}C this left to undergo fermentation at room temperature for 20-24 hours. The product is thereafter sieved and it is ready for drinking, addition of sugar to taste is optional (Amusa and Ashaye, 2009; Oluwalana and Adedeji, 2013). Some health benefits of kunun include: it quenches thirst and reduce dehydration and its richness in fibres helps in promoting a healthy digestive system (Elmahmood and Doughari, 2007; Maji *et al*., 2011).

Enterobacteriaceae is a family of gram-negative, bacilli that inhabit the large intestine of humans and other mammals as commensals and symbionts (Mackie *et al*., 1997). They are also in abundance in almost any moist environment, notably soil, water, and the domestic environment (Scott, 1982). They are mostly non-pathogenic but some of them may become opportunistic or pathogenic causing diseases such as gastroenteritis, urinary tract infections. Some Enterobacteriaceae like *Escherichia coli*, are always considered to be of faecal origin, and exist only transiently in other environments. Enterobacteriaceae includes the following genera: *Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Salmonella, Serratia, Shigella*.

The high water content (about 85%) coupled with crude methods of preparation and packaging in an unhyigienic environment predispose the drink to microbial contamination (Elmahmood and Doughari, 2007, Ayo *et al*., 2010). Microorganisms such as *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Streptococcus* spp, *Proteus vulgaris* had been isolated from local drinks produced and sold in Nigeria (Makut *et al*., 2013, Aboh and Oladosu, 2014; Ayandele, 2015). This can result to some debilitating disease conditions (Willey *et al*., 2008). Resistance of these microorganisms to commonly used antibiotics can also occur which may lead to high morbidity, mortality and economic losses. A high prevalence of Enterobacteriaceae bacteria resistance to antibiotics had been reported: Iredell *et al*., (2016) in a review on antibiotic resistance in Enterobacteriaceae reported that antibiotic resistance is more available than ever before in *E. coli* and *Klebsiella pneumoniae*. Maku *et al*., (2013), also reported that *Escherichia coli* isolated from kunun drink from Keffi metropolis, Niger State, Nigeria showed high resistance to Chloramphenicol (75%), followed by Cotrimoxazole (68.7%) and Sparfloxacin (68.7%).

This study was aimed at isolating Entereobacteriaceae bacteria from Kunun-zaki sold in Kaduna metropolis and to determine their antibiotic susceptibility profile.

METHODOLOGY

Materials and Methods

Study Area

The study area is Kaduna town, the ancient capital of Northern Nigeria and capital of present Kaduna State, located in the northern part of Nigeria. Kaduna is about 162 km away from the Federal Capital Territory (FCT) Abuja and 234 km away from Kano, the capital of Kano state. Kaduna State is located on latitude 10.52224N and longitude 7.43828N.

Sample Collection

Ten different locations where kunun-zaki is sold in Kaduna metropolis were randomly selected for the purpose of sample collection. Within a period of two weeks, samples of kunun-zaki were aseptically collected in duplicates into a sterile corked tubes from the ten different locations. The samples were labelled appropriately, placed into separate plastic bags and conveyed in an ice packed cooler to the Pharmaceutical Microbiology laboratory of the Kaduna State University, Kaduna. The ten different locations where samples were collected include:

1. Shop No: 5, Kanti kori motor park, Kaduna central market.
2. Shop No: 23, Kano road, Kaduna
3. Opposite Olusegun Obasanjo House Kaduna.
4. Opposite Ungwar rimi Primary Health care centre
5. Layin Nama, Bakin dogo market.
6. Shop No: 13, Kaduna state university school market
7. Shop No: 3, Leventis complex, Kaduna
8. Kaduna State University back gate.
9. Shop No: 7, Layin Ungwar rimi, Bakin dogo market Kaduna
10. No: 1c Korau road Ungwar rimi, Kaduna.

Determination of total coliform count

Standard Plate Count method was used to determine the total coliform count of the samples (Sanders, 2012). A seven-fold serial dilution of each sample was made and plated out on MacConkey agar plates using spread plate technique. The plates were incubated at 37°C for 24 h. The total coliform count of the kunun-zaki samples obtained from the different locations were expressed as Colony Forming Units per millilitre (CFU/mL) of kunun-zaki.

Purification, Gram staining and identification of isolates

Each colony was isolated in a pure form by sub culturing in a fresh MacConkey agar plate and pure cultures were temporarily preserved on nutrient agar slants at 4°C for further work. An overnight activated culture was then used to determine colony morphology and gram staining. The following biochemical tests were carried out according to Cheesbrough, (2005) to identify the isolates: Citrate utilization test, sugar fermentation test, indole test, catalase, oxidase, coagulase, methyl red and Voges-Proskauer tests.

Growth on Selective Media

The Enterobacteriaceae were further identified by growing the isolates on selective media among which were MacConkey agar, Eosin Methylene Blue agar, mannitol salt agar and Salmonella-Shigella agar. All the media were prepared according to the manufacturer's instructions.

Antibiotic Susceptibility Test

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method. A suspension of each isolate was prepared in sterile normal saline. The isolates were standardized by making a turbid suspension of each isolates in sterile normal saline, this was compared with 0.5 McFarland Standard. A sterile swab was dipped into the standardized bacteria suspension (1.5×10^8 cfu/ml), pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar (Oxoid, Basingstoke, England) plate in three directions, rotating the plate approximately 60° to ensure even distribution. With the petri dish lid in place, the surface of the agar was allowed to dry for about 3-5 minutes. Sterile forceps were then used to place the antibiotic discs in a circular pattern on the agar plate, the plate was allowed for 30 minutes prediffusion time and thereafter incubated at 37°C for 16-18 hours. This procedure was carried out for all the isolates. After incubation, the zone of inhibition in diameter for each antibiotic was measured and interpreted as sensitive, intermediate and resistant according to Clinical and Laboratory Standard Institute (CLSI, 2018) guidelines. The following antibiotic discs were tested: Chloramphenicol (30 µg), ceftazidime (30 µg), Ciprofloxacin (5 µg) Amoxicillin-clavulanate (30µg), Gentamicin (10 µg), cefoxitin (30 µg) Pefloxacin (10 µg), Ofloxacin (30 µg), Streptomycin (10 µg), ceftriaxone (30 µg), meropenem (10 µg) and imipenem (10 µg) discs.

Determination of Multiple Antibiotics Resistant Index (MARI) and Multidrug Resistance (MDR)

The MARI for each isolate was determined by dividing the number of antibiotics to which the test isolates were resistant to by the total number of antibiotics tested (Krumperman, 1983). The isolates that showed resistance to three or more classes of antibiotics were classified as multidrug resistant.

RESULTS AND DISCUSSION

A total of 20 samples were collected from ten locations in Kaduna. The mean total coliform count of the samples ranged from 2.0 to 15.0×10^5 cfu/ml (Table 1). Considering the fact that water is the medium for producing kunnu-zaki. this total coliform count is higher than the WHO guidelines, which stated that coliform bacteria must not be detectable in any 100-ml drinking water sample (WHO, 2011). Comparing the findings of this study with previous reports, the total coliform count obtained in this study was lower than that reported by Ayandele, (2015) and Etang *et al.*, (2017) who reported $0.2-5.0 \times 10^5$ cfu/ml from kunun hawked in Ladoke Akintola University of Technology and $1.0-2 \times 10^3$ cfu/ml from kunun drinks locally produced and sold in Calabar, Southern Nigeria respectively. However, a higher value of $5.1 \times 10^2 – 2.0 \times 10^8$ cfu/ml from kunun sold in Abuja Municipal Council in the Federal Capital Territory Nigeria was reported by Aboh and Oladosu (2014).

Table 1: Results of Mean Coliform Count

S/No	Samples	Mean coliform count (cfu/ml)
1	1a	3.0×10^5
2	1b	1.2×10^5
3	2a	1.1×10^5
4	2b	4.0×10^5
5	3a	1.5×10^5
6	3b	6.0×10^5
7	4a	2.2×10^5
8	4b	6.2×10^5
9	5a	2.0×10^5
10	5b	4.0×10^5
11	6a	5.0×10^5
12	6b	3.0×10^5
13	7a	5.0×10^5
14	7b	4.0×10^5
15	8a	7.0×10^5
16	8b	6.0×10^5
17	9a	2.3×10^5
18	9b	7.0×10^5
19	10a	1.5×10^6
20	10b	4.1×10^5

Identification of the Enterobacteriaceae Isolates

The results of the biochemical tests and growth of the isolates on selective media to further identify the Enterobacteriaceae isolates is presented in Table 2. The following bacteria were isolated: Enterobacteriaceae 10 (50%), *Staphylococcus aureus,* 6 (30%), *Pseudomonas aeruginosa,* 4(20%). The Enterobacteriaceae bacteria isolated were: *Klebsiella pneumoniae* 4 (20%)*, Escherichia coli* 4 (20%)*, Citrobacter freundii* 1 (5%) *and Proteus vulgaris* 1 (5%). Kunun-zaki is a very popular and highly consumed local drink, the isolation of these organisms from the drink poses a high risk of potential source of health hazard to the consumers. There were previous studies on the assessment of bacterial contaminants of local drinks in different part of Nigeria where similar bacteria were isolated. Ayandele, (2015) reported on microbiological analysis of hawked kunun and zobo drinks within LAUTECH campus, Ogbomoso, where *Proteus spp* (33.3%) and *E. coli* 33.3%) were isolated. This prevalence was higher than that obtained in this study. Lower prevalence of *E. coli* (4.9%), *Citrobacter spp* (2.4%) and similar prevalence of *Klebsiella spp* (22%) was reported by Aboh and Oladosu, (2014) who analysed kunun sold in Abuja Municipal Area Council in Federal Capital Territory, Abuja. Others were Raimi, (2013) and Bulakarima *et al*., (2017) who isolated *E. coli, Klebsiella spp, Proteus spp* and *Enterobacter spp* from zobo drink sold at Osun and Borno states of Nigeria respectively.

The high level of contamination observed in these drinks may be from the source of water used in processing the drink, and the poor hygiene of the handlers during the processing procedures while transferring from one container to the other.

Table 2: Result of Biochemical tests and growth on selective media

S/No	samples	TSI			CT	CA	CO	OX	MR	VP	IN	EMB	MAC	MAN	SSA	Suspected Organism
		G.U	G.P	H$_2$S												
1	1a	-	-	-	-	+	+	-	+	+	-	Less dark	Pale pink	yellow	pink	*S.aureus*
2	1b	+	+	-	+	+	-	-	+	-	-	Less dark	pink	pink	pink	*Klebsiella pneumoniae*
3	2a	+	+	-	+	+	-	-	+	-	-	Less dark	pink	pink	pink	*Klebsiella pneumonia*
4	2b	-	-	-	+	+	+	-	+	+	-	Less dark	Pale pink	yellow	pink	*S.aureus*
5	3a	+	+	-	+	+	-	-	+	-	-	Less dark	pink	pink	pink	*Klebsiella pneumonia*
6	3b	+	-	+	+	+	+	-	+	+	-	Less dark	Pale pink	yellow	pink	*S.aureus*
7	4a	+	+	-	+	+	+	-	+	+	-	Less dark	Pale pink	yellow	pink	*S.aureus*
8	4b	+	-	+	+	+	+	-	+	+	-	Less dark	Pale pink	yellow	pink	*S.aureus*
9	5a	+	+	-	+	+	-	+	-	-	-	Less dark	green	yellow	Slight growth	*Pseudomonas aerginosa*
10	5b	+	+	+	+	+	-	-	+	-	-	Blue-black	pink	yellow	black	*Citrobacter freundii*
11	6a	+	+	+	+	+	-	-	+	-	-	Less dark	colourless	No growth	black	*Proteus vulgaris*
12	6b	+	+	-	+	+	-	-	+	-	+	Blue-black	pink	pink	pink	*E. coli*
13	7a	+	-	+	+	+	-	+	-	-	-	Less dark	colourless	No growth	black	*Pseudomonas aeruginosa*
14	7b	+	+	-	+	+	-	-	+	-	+	Blue-black	pink	yellow	pink	*E. coli*
15	8a	+	-	-	+	+	-	-	+	-	+	Blue-black	pink	pink	pink	*E.coli*
16	8b	+	+	+	+	+	-	+	-	-	-	Less dark	Green	No growth	black	*Pseudomonas aeruginosa*
17	9a	+	+	-	+	+	-	-	+	-	+	Blue-black	Pink	yellow	pink	*E. coli*
18	9b	+	+	-	+	+	-	+	-	-	-	Less dark	green	yellow	black	*Pseudomonas aeruginosa*
19	10a	+	+	-	+	+	-	-	-	+	-	Less dark	Pink	yellow	pink	*Klebsiella pneumonia*
20	10b	+	+	+	+	+	+	-	+	+	-	Less dark	Pale pink	yellow	pink	*S.aureus*

KEY: += positive, - = negative, IN=indole, CA= catalase, CT=citrate, H$_2$S= hydrogen sulphide, MR= methyl red, VP= Voges-Proskauer, G=gas, S=sugar, OX=oxidase, CO= coagulase, GU= glucose utilisation, GP=Gas production, EMB. = Eosin methylene blue, MAC. = MacConkey agar, MAN. = Mannitol salt agar, SSA = salmonella-Shigella agar, EMB: Blue-black= positive, Less dark = negative

The Enterobacteriaceae which are more of coliform bacteria while present in food or drink is an indication of faecal or environmental contamination. It also indicates possible presence of pathogens (Leclerc, 2001). These microorganisms are versatile in their nutrient requirements and can survive with limited nutrient availability (Berger and Oshiro, 2002). The ingestion of these bacteria with contaminated water constitute public health risks to the immunocompromised members of the population, especially children, elderly and sick people

(LeChevallier *et al.,* 1980). Even though *E. coli* is a normal flora of human intestine, some strains can some strains can cause serious food poisoning in human and are usually transmitted through faecal-oral route. Presence of *E. coli* in other parts of the body can cause serious disease such as urinary tract infections, bacteraemia and meningitis. Some enteropathogenic *E. coli* strains can cause acute diarrhoea (Akyala, 2014). *Citrobacter freundii* isolated from contaminated drinking water had been reported to be responsible for foodborne disease out breaks and gastrointestinal diseases in Mexico City and Pakistan (Steele and Odumeru, 2004; Azizullah *et al.,* 2011).

Antibiotics Susceptibility Testing
The antibiotics susceptibility test revealed that all the Enterobacteriaceae isolates were 100% susceptible to ciprofloxacin, gentamicin, imipenem and ofloxacin (Figure 1). This is similar to Ayandele, (2015) who reported 100% susceptibility to ciprofloxacin, gentamicin and ofloxacin by bacterial isolates from microbiological analysis of kunun and zobo drink sold in LAUTECH, Ogbomoso. In this study, 90%, 80% and 70% of the isolates were susceptible to Chloramphenicol, amoxicillin-clavulanate and streptomycin respectively. All the isolates were resistant to ceftazidime (100%) (Figure 2). Comparing

the susceptibility profile of the isolates *Citrobacter freundii* and *Proteus vulgaris* were the most resistant. Comparing the beta lactam antibiotics tested in this study, the isolates were highly resistant to meropenem, ceftriaxone and cefoxitin. But amoxicillin-clavulanate showed a better activity against *E. coli* (100%), *C. freundii* (100%) and *K. pneumoniae* (75%). The result of the multiple antibiotic resistance index showed that all the Enterobacteriaceae isolates had MARI value greater than 0.2 which showed that all the isolates were resistant to more than two antibiotics irrespective of the antibiotic category (Table 3). It is also an indication that these isolates might have originated from an environment where antibiotics had been abused or that these multidrug resistant strains might have been imported and spread within the community. Only 20% of the isolates were classified as multidrug resistant (Table 4) which implies that only 20% were resistant to at least one agent in three or more antibiotics category. The high level of MARI calls for an awakening to a war against abuse and misuse of antibiotics in our society. Several treatment failure and resistance to antibiotics being experienced in infection treatment and control may be due to abuse of antibiotics in our community.
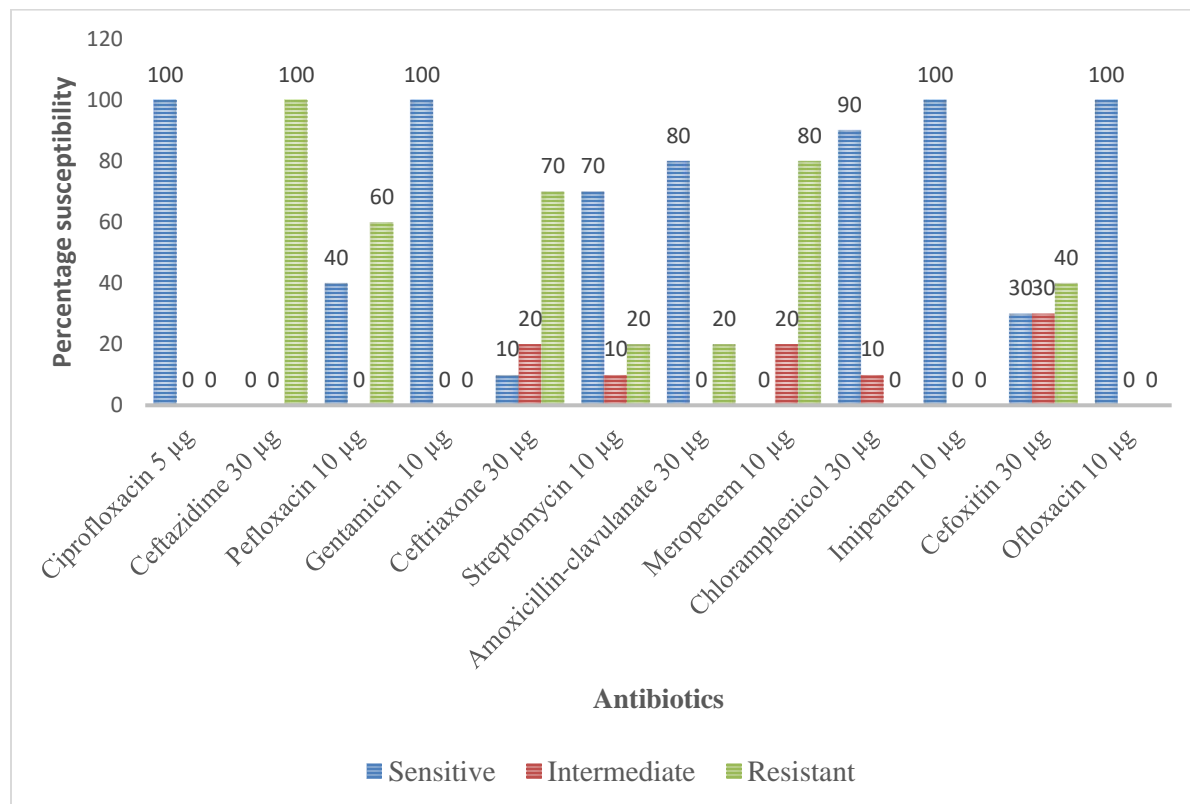

Figure 1: Percentage antibiotics susceptibility of all the Enterobacteriaceae isolates showing the sensitivity, intermediate and resistance
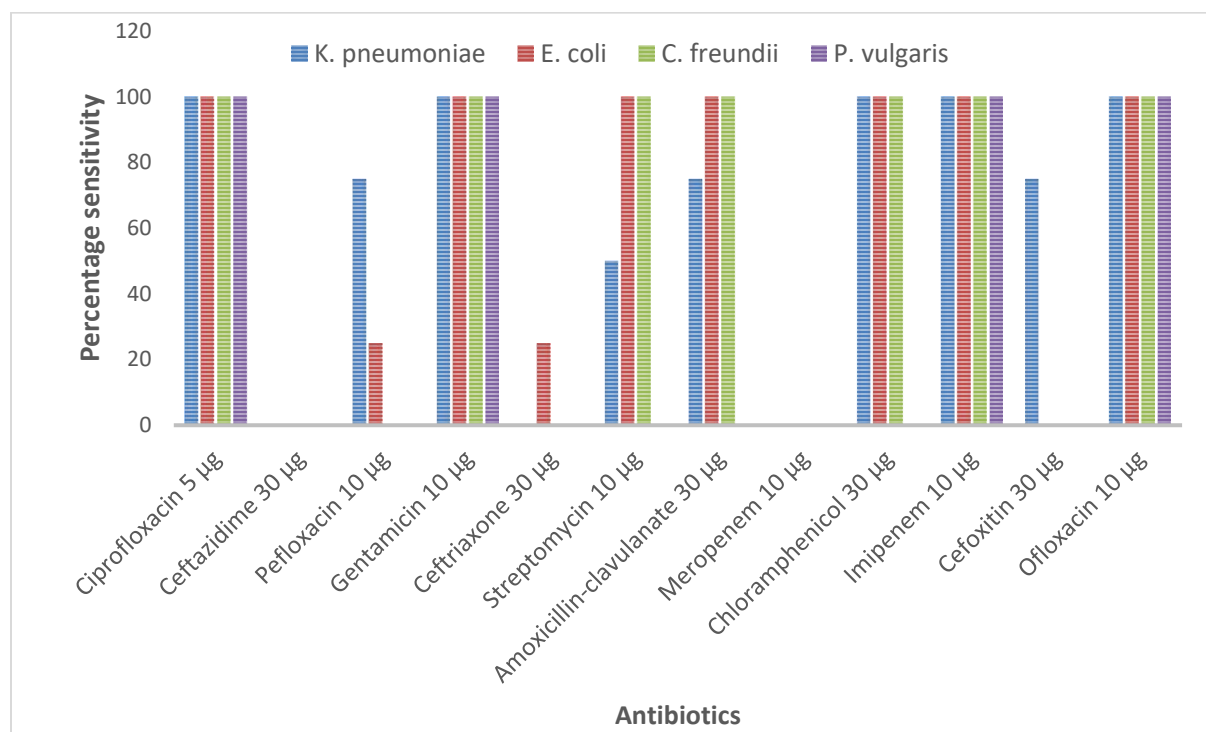
Figure 2: Comparison between the susceptibility pattern of the Enterobacteriaceae isolates

Table 3: Resistance pattern of the Enterobacteriaceae isolates

Isolates		Antibiotics to which resistant	MARI	MDR status
K. pneumonia	1b	CAZ, CTX, MEM,	0.25	NMDR
	2a	CAZ, CTX, MEM	0.25	NMDR
	3a	CAZ, CTX, MEM, S	0.33	NMDR
	10a	CAZ, PER, CTX, MEM, S, AMC, FOX	0.58	MDR
E. coli 6b		CAZ, PER, CTX, MEM, FOX	0.42	NMDR
7b		CAZ, CTX, MEM, FOX	0.33	NMDR
8a		CAZ, PER, CTX, MEM, FOX	0.42	NMDR
9a		CAZ, PER, MEM, FOX	0.33	NMDR
C. ferundii	5b	CAZ, PER, CTX, MEM, FOX	0.42	NMDR
P. vulgaris	6a	CAZ, PER, CTX, S, AMC, MEM, C, FOX	0.67	MDR

Key: CAZ=Ceftazidime, CTX=Ceftriaxone, MEM=Meropenem, S=Streptomycin, PER=Pefloxacin, AMC=Amoxicillin-clavulanate, FOX=Cefoxitin, C=Chloramphenicol, MDR=multidrug resistant, NMDR=not multidrug resistant, MARI=multiple antibiotics resistant index.

In Nigeria today, production and sales of local drinks like kunun-zaki is not directly monitored by any government agency. This has allowed the sales of contaminated drinks to proliferate in our community. This should be of great concern to the government especially because of the public health implication.

CONCLUSION

All the kunun-zaki local drink sampled in this study were contaminated with *Staphylococcus auerus, Pseudomonas aeruginosa*. The Enterobacteriaceae isolated were *Klebsiella pneumonia Escherichia coli, Citrobacter freundii* and *Proteus vulgaris.* The isolates were highly resistant to ceftazidime, meropenem, cefoxitin and ceftriaxone all of which were beta lactam antibiotics. However, the isolates were highly susceptible to ciprofloxacin, ofloxacin, gentamicin and imipenem.

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