

# Assessment of Foods and Drinks for the Presence of Extended Spectrum Beta Lactamase (ESBL) Producing Bacteria in Gombe Metropolis, Nigeria

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## Abstract

**Objectives:** To make preliminary screening study for the presence of ESBL producing bacteria, in food and drinks sold in Gombe metropolis. 30 samples of food and drinks were collected and analyzed. **Method:** Bacterial isolation was performed on nutrient agar and identification started from gram staining; the gram negative isolates obtained, among which ESBL producers were found and further subjected to standard biochemical tests and confirmed to be enterobacteriaceae. They were then screened for ESBLs in accordance with Clinical and Laboratory Standard Institute (CLSI) breakpoint, and the suspected ESBLs bacteria were confirmed using double disc synergy testing (DDST) with standard Augmentin (Amoxycillin/Clavulanic Acid, 30µg; Oxoid England, CT0223B), Ceftriaxone (CRO 30µg; Oxoid England, CT0417B) and Ceftazidime (CAZ 30µg; Oxoid England, CT0412B). **Findings:** The fourteen 14(100%) Gram negative bacteria isolated were found to include *Pseudomonasaeruginosa* 2(14.29%) and members of the family Enterobacteriaceae (enteric gram negative rods): *Citrobacterfreundii* 2(14.29%), *Enterobacterspp.* 1(7.14%), *Escherichiacoli* 4(28.57%), *Klebsiellapneumoniae* 3(21.43%), *Proteusvulgaris* 1(7.14%) and *Salmonellatyphi* 1(7.14%). When screened for ESBL production based on CLSI breakpoint, only 6 (49.99%) were positive and included *C.freundii* 1(7.14%), *E.coli* 2(14.29%), *K.pneumoniae* 1(7.14%), *S. typhi* 1(7.14%) and 1(7.14%) for *Ps. aeruginosa*. On subjecting them to DDST, only 2(33.34%) out of them were found to be ESBLs positive by an increase in inhibition zone of 5mm toward the centre disc, Augmentin. They include *E. coli* 1(16.67%) and *S. typhi* 1(16.67%). **Application:** In a nutshell, foods and drinks sold therein could serve as reservoirs for ESBLs producing bacteria.

**Keywords:** ESBL, Foods and Drinks, Gombe Metropolis, Screening

## 1. Introduction

Foods and drinks are substances ingested by all living organisms for the continuance of life. However, foods consumed by humans and animals serve as favourable media for rapid growth of microorganisms because of

the abundant organic matter, moisture and suitable pH contained and because of the fact that the microorganisms are equally living organisms. Presence of these microorganisms in foods and drinks warrants their rapid multiplication which renders the food unfit and dangerous for human consumption<sup>1</sup>.

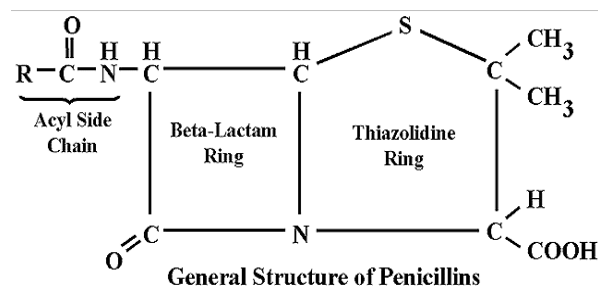
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Food safety doesn't begin at the grocery store or in the kitchen. It begins on the farm. In the developed world of Europe, concerns about food safety, welfare of plants and animal as well as traceability are more preferable than the food products being supplied in plenitude suggested EUROSTAT, (2008). The reverse is the case for developing nations especially in Africa where famine is the order of the day in many countries or many parts of a certain country, Nigeria inclusive. This therefore hampers the need for food safety as the need for the food at any condition is at its peak.

Foods and drinks commercialized in markets have likelihood of being exposed to pathogenic microorganisms because of poor handling by the sellers. Waterborne and other related pathogens including bacteria are spread in water either through human ingestion of contaminated water or drinks. Meanwhile, faecal pathogens present in contaminated water used in the production of some drinks are due to ineffective sanitary measures<sup>2,3</sup>. The pathogens as a result, get into the locally made drinks such as Sorrel juice (Zobo), tiger nut juice (Kununaya), millet juice (Kununzaki) which require water as a growth medium and ingredient. Table water sold in sachets water may equally harbour these pathogenic microorganisms. Water contaminated with these pathogenic microorganisms may be a source of some diseases comprising of typhoid and paratyphoid fevers, dysentery and allied diarrheal infections including cholera. There have been reports by WHO that 300,000 people die daily from water related infections<sup>3</sup>. These microbial food contaminants of food include the enteric gram negative rods (enterobacteriaceae) among which there are indicator organisms, the presence of which indicates possible faecal contamination. Some members of this group produce an enzyme, extended spectrum beta lactamase (ESBLs)<sup>4,5</sup> a typical example of which bacteria, cause disease of public health concern.

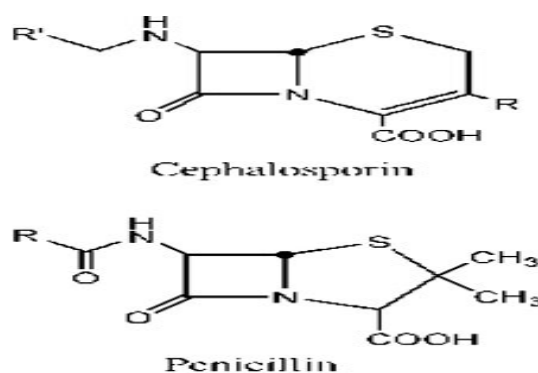
Beta lactamases are enzymes that hydrolyze the beta-lactam antibiotics, mostly penicillins Figure 1. This is achieved when the enzymes break open the  $\beta$ -lactam ring in  $\beta$ -lactam antibiotics through the addition of water molecule to the common  $\beta$ -lactam bond, which renders the beta lactam ring responsible for the antibacterial activity of the antibiotic inactive. The discovery of this hydrolyzation by penicillinases was firstly by Abraham and Chain in 1940 in an *E. coli* strain. The  $\beta$ -lactam ring, a four member cyclic amide, is composed of three car-

bon atoms and a nitrogen atom. It is termed  $\beta$ -lactam ring because the nitrogen atom is attached to the  $\beta$ -carbon relative to the carbonyl. The first beta-lactam antibiotic, penicillin was discovered in 1928 by Alexander Fleming in which case that he found out the mould contaminant *Penicilliumnotatum* inhibited growth of Staphylococcal colonies on a culture plate<sup>6</sup>.



**Figure 1.** General structure of penicillins.

However, another beta-lactam antibiotic, Cephalosporin Figure 2 was further isolated from a fungus called *Cephalosporiummacremonium* by an Italian scientist GiuseppeBrotzu in 1948. It is also composed of 4-membered beta lactam ring and a 6-membered dihydrothiazolidine ring. It bears two side chains, R1 and R2. The cephalosporins are grouped into various generations following modifications in the side chains; first generation (cephalexine, cephalothin, etc), second generation (cefaclor, cefoxitin, etc) third generation (ceftriaxone, cefuroxime, etc) fourth generation (cefepime, cefquinomeetc) and the fifth generation (ceftaroline, ceftobiprole, fosamil)<sup>6,7</sup>.



**Figure 2.** Structure of  $\beta$ -lactam antibiotics: Cephalosporin and penicillin.

The  $\beta$ -lactam antibiotic exerts its bactericidal activity by inhibition the synthesis of cell wall, and this achieved

when it covalently attaches to an enzyme peptidoglycan transpeptidase, a Penicillin-Binding Protein (PBP), that catalyzes cell wall formation in the final steps. Bacterial cell damage by hydroxyl radicals in this process has also been reported. However, the exact mechanism of how this occurs is yet to be clear. Various Penicillin binding proteins unique to bacteria have been identified. In addition, the effects  $\beta$ -lactam antibiotics and their spectrum of activity are dependent on these PBPs which bind through their active sites, to antibiotics<sup>4</sup>.

They are so called beta lactam antibiotics because of the nitrogen atom attached to the beta carbon attached to the carbonyl ring. Enclosed in a circle is the structure of the beta lactam ring<sup>6</sup>.

Extended Spectrum Beta Lactamases (ESBLs) are enzymes produced by bacteria that were previously exposed to penicillins which help confer on the bacteria resistance to penicillins. In other word, the enzymes are produced by bacteria to confer on them the ability to resist the action of penicillins (Beta lactam antibiotics) and their allies, the cephalosporins (Extended spectrum beta lactam antibiotics). Meanwhile, they are equally the widespread major sources of gram negative bacterial multidrug resistance. There exist different families of ESBLs in which the two major ones, Sulfhydryl variable gene (SHV-2), and Temoniera gene (TEM-3) were discovered and described in Germany in 1982 and 1987 respectively. These major families of SHV-2 and TEM-3 expanded to give rise to more subtypes. In addition, there had been discoveries of other families that confer an ESBL phenotype. The cefotaximases of the CTX-M type are the most important of them and they were discovered to have emerged in *Escherichia coli* and are as well, the most dominant throughout the globe. Phylogenetically, there exist five major group of CTX-M. Under these, over 100 different CTX-M types are recognized. The first isolation was achieved in 2014 from livestock<sup>8,9</sup>.

Extended spectrum beta lactamases (ESBLs) have also been discovered from other enteric gram negative rods such as *Morganellamorganii*, *Klebsiella pneumoniae* and also from *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Capnocytophaga ochracea*<sup>10-13</sup>. Their presence can be attributed to their association with community-acquired urinary tract infections by *E. coli*.

The formation of ESBLs is from the transformation of beta lactam enzymes by point mutation in the *bla* gene, which consequently results in amino acid sequence alter-

ations near active site of the enzyme; this finally results in the alteration of substrate specificity. The active site is now modified for accommodating the side chain of cephalosporins. The changes of TEM at positions, Arg164 (-His, -Ser), Gly 238 (-Ser, -Ala) and Glu 240 (-Lys) result in phenotypic variants. Resistance experienced in ESBLs producers is not only from point mutation in the *bla* gene, change in enzyme regulation that leads to increased enzyme production or alteration in outer membrane protein, porin channels could also be contributing factors for increased resistance. Nowadays, the genetic system of pathogenic bacteria in the stress situation created by the presence of microbial control agent is so versatile that the resistance to drugs/antibiotics has become increasingly common<sup>14,15</sup>.

Bacterial mechanism of resistance to antibiotics that lead to the production of ESBLs producers is as a result of their exposure to antibiotics which allows them develop certain special mechanisms to overcome the antimicrobial agents. These mechanisms equally offer for the microorganisms, resistance to other antimicrobials of the same class and even to those of different classes<sup>16</sup>.

There are two main mechanisms involved in inducing antibiotic resistance to microorganism and these include Intrinsic (phenotypic) and Extrinsic (genotypic). The Intrinsic mechanisms are achieved through enzymatic inactivation/modification<sup>14,17</sup>, efflux pumps and outer membrane permeability change<sup>14</sup>, alteration of bacterial target site and intracellular metabolic rearrangement<sup>17,18</sup>. Extrinsic (Genotypic) resistance however, could occur through Horizontal Gene Transfer (HGT); process; a mechanism whereby genetic material is transferred between individual bacteria of the same species or different, or through Vertical Gene Transfer (VGT) such as conjugation, transformation or transduction<sup>14</sup>. These mechanisms alongside unmentioned others are the major cause of threat to human race through imparting resistance to individual bacteria of the same species and even to those of different species. This research therefore aims at preliminarily screening of some foods and drinks sold at Gombe metropolis, Gombe state, Nigeria for the presence of extended spectrum beta lactamase producing bacteria. This is with the objective of assessing the bacteriological quality of the foods and drinks in question as; roasted meat (Balangu), fried fish, sorrel juice (Zobo), tiger nut juice (Kununaya), millet juice (Kununzaki) and cow milk (Nono).

## 2. Methodologies

### 2.1 Study Area

Gombe metropolis is the sampling site. It is the capital city of Gombe state, one of the six states of the Northeastern geopolitical zone of Nigeria. Gombe state consists of eleven local government areas, among which, is Gombe local government area that harbours the sampling site. The study was limited to Gombe metropolis where food and drinks samples for the analysis were collected from different areas locations.

### 2.2 Sample Collection

The samples used for this research work were foods and drinks, and were purchased from six different producers at five different locations in Gombe metropolis namely; Babbarkasuwa (Main market), TsohuwarKasuwa (Old market), Kasuwan Mata (Ladies market), Kasuwar Tumfure (Tumfure market) and Kasuwar Pantami (Pantami market). This made a total of 30 samples. Collection was aseptically enhanced, and the samples transferred to sterile plastic containers. They were labelled and then transported immediately to Microbiology Laboratory Gombe State University for analysis. The samples collected as mentioned earlier were roasted meat (balangu), fried fish, sorrel juice (zobo), tiger nut juice (kununaya), millet juice (kununzaki) and cattle milk (Nono).

### 2.3 Analysis of the Samples

The Food and drinks under investigation were prepared for bacterial isolation by carrying out serial dilution as described<sup>19</sup>. 25 g of the food samples were suspended in 225 ml sterile peptone water and homogenized in a 1-litre Waring Pro Speciality Waterfall Blender (Model PBB25U, 230V 330W, UK) to form a stock solution. The drinks however, were the stock solutions themselves. One millilitre aliquots of each drink and homogenized food sample were pipetted into test tube containing 9ml of peptone water (Oxoid, UK) to make  $10^{-1}$  dilution which was further diluted serially to  $10^{-5}$ . The samples were then inoculated by spread plating on MacConkey agar plates<sup>10</sup> and Eosin Methylene Blue (EMB) and incubated in an incubator (Model M8NI/150/VIS, GENLAB, Cheshire, England) at 37°C for 24-48 hours.

### 2.4 Identification of the Isolates Obtained

Bacterial colonies observed after serial dilution and pour plating of the homogenized foods and drinks samples through observation of growth in the inoculated Petri plates were subjected to Gram staining as described<sup>20</sup>. This is with a view to obtaining the gram negative bacilli among which are found the ESBLs<sup>4,5</sup>.

Biochemical tests were carried out for further identification, on the Gram negative isolates<sup>20</sup>. These include Indole production test, Motility test, Citrate utilization test, Urease production test, Hydrogen sulfide ( $H_2S$ ) production, Lactose fermentation, as well as acid and gas production on Triple Sugar Iron (TSI) or Kligler Iron Agar (KIA). Analytical profile index of enterobacteriaceae (API 20E, Biomerieux, France) was further carried out on the obtained and biochemically analyzed gram negative rods to confirm their identities<sup>21</sup>.

### 2.5 Standardization of Inoculum

The isolates were cultured on prepared Nutrient agar (Oxoid, England) plates and incubated for 24 hours at 37°C to obtain confluent growth for sensitivity. Few colonies of isolates from the nutrient agar plates were then dispensed in sterile normal saline to match the turbidity of 0.5 McFarland standard, which, is a solution of Barium Sulphate obtained from 0.6 ml of 1% Barium Chloride added to 99.4 ml of Sulphuric acid, for sensitivity<sup>20</sup>.

### 2.6 Clinical Laboratory Standard Institute (CLSI) Breakpoint Test for ESBLs Screening

The sensitivity of standard inocula of isolates to Ceftriaxone (CTR 30µg, CT0417B, Oxoid UK) and Ceftazidime (CAZ 30µg, CT0412B, Oxoid, UK) discs was determined on Mueller Hinton Agar (Oxoid, UK) using Kirby-Bauer (1966) method.

The standardized inoculum of the test organism was emulsified on the surface of MHA (Oxoid, England) using sterile cotton swab (220210 BD SWUBE, India). This was followed by the aseptic application of the Ceftriaxone (CTR 30 µg) and Ceftazidime (CAZ 30 µg) on the surface of the inoculated Mueller Hinton Agar (MHA) 20mm from each disc and 15 mm from the edge of the plate using sterile forceps. After 30 minute of disc application, the plates were incubated at 37°C for 24 hours at inverted position<sup>22,23</sup>. After an overnight incubation, the diameters



for inhibition zones were measured in millimetre using a meter rule<sup>24</sup>.

## 2.7 Double Disk Synergy Test (DDST) for ESBLs Confirmation

The isolates were subcultured on nutrient agar by streak plate method and incubated at 35°C for 18-24 hours so as to obtain confluent growth. Improved procedure<sup>25</sup> was employed on screening of isolates for ESBLs production on Mueller-Hinton Agar (MHA) using standard inocula from Nutrient Agar (NA) plates. The isolates were further inoculated using sterile swab stick onto the surface of MHA. The discs containing 2 third generation cephalosporins (Ceftriaxone 30µg and Ceftazidime 30µg) all placed at 20mm distance apart centre to centre from an Augmentin (Amoxycillin/Clavulanic Acid, 30µg, CT0223B Oxoid, UK) disc placed at the centre. The plates were then incubated at 35°C for 18-24 hours after which the plate was read<sup>1,3,10,24</sup>.



**Figure 3.** Pink colonies on MacConkey agar indicating the presence of coliforms.

## 3. Experimental Results

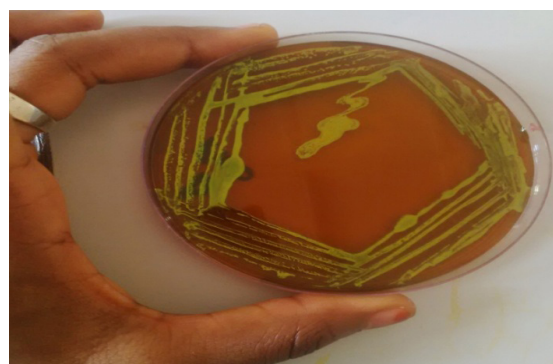
Among the food samples isolated, tiger nut juice, locally called kununaya had the highest number of bacteria isolated. This may not be unconnected by the fact that it is an easy-spoilt juice which cannot stay for up to 12 hours without microbial action even under refrigeration. This is then followed by sorrel juice (Zobo) and Nono (milk). Fried fish recorded only two bacterial isolates and finally millet juice and meat with only 1 bacterial isolate each Table 1. The identity of the isolates obtained by biochemical tests after Gram's staining indicated the presence of

*Pseudomonas* and members of the enteric gram negative rods Table 2. When screened for ESBLs production, *E. coli* still had the highest percentage of occurrence of 14.29% while *Enterobacter spp.* and *Proteus vulgaris* had no occurrence at all Table 4. On the basis of confirmation of ESBLs producers using DDST, only *E. coli* and *S. Typhi* were found to be ESBLs producers Table 5.

**Table 1.** Occurrence of bacterial isolates in food and drinks

S/No.	Food Samples	No. Screened	No. Isolated	% Occurrence
1	Sorrel (Zobo)	5	3	10.00
2	Tigernut Juice (Kunun Aya)	5	4	13.33
3	Millet Juice (Kunun Zaki)	5	1	3.33
4	Cattle Milk (Nono)	5	3	10.00
5	Fried Fish (Kifi)	5	2	6.67
6	Meat (Balangu)	5	1	3.33
Total		30	14	46.66

There have been reports of increasing resistance of enterobacteriaceae to broad spectrum cephalosporins from different countries especially *E. coli*, *Salmonellae* and *Klebsiella species*. This is predominantly due to ESBLs production. There have equally been many reports on ESBL isolates from clinical diagnosis, from food, drinks, environment etc available from different researches and in different parts of the country, Nigeria; Kano, Enugu, Abeokuta, Benin and many more<sup>26</sup>.



**Figure 4.** Green metallic colonies sheen on EMB indicative of *E. coli*.

**Table 2.** Result of biochemical characterization of bacterial isolates. The percentage signifies the percentage occurrence of each organism from among the total isolated

Lact.	Urea	Citr.	Mot.	Ind.	Slope	Butt	H <sub>2</sub> S	Gas	Positive	%	Identity
...+	D	+	+	-	R/Y	Y	d	+	2	14.29	<i>C. freundii</i>
+	-	+	+	-	Y	Y	-	+	1	7.14	<i>Enterobacter spp.</i>
+	-	-	+	+	Y	Y	-	+	4	28.57	<i>E. coli</i>
+	+	+	-	-	Y	Y	-	+	3	21.57	<i>K. pneumoniae</i>
-	+	d	+	+	R	Y	+	d	1	7.14	<i>Proteus vulgaris</i>
-	-	+	+	-	R	Y	-	-	2	14.29	<i>Ps. aeruginosa</i>
-	-	-	+	-	R	Y	+	-	1	7.14	<i>S. typhi</i>
Total									14	100	

**Key:** Lac=Lactose, Urea=Urease production, Citr.=Citrate, Mot.=Motility, Ind.=Indole, H<sub>2</sub>S=Hydrogen Sulphide Production (Blackening), R=Alkaline reaction (Red), Y=Acid reaction (Yellow), d=Different strains produce different results, +=Late positive reaction, +=Positive reaction, -=Negative reaction.

**Table 3.** Zones of Inhibition for Screening for ESBLs Based on CLSI Breakpoint using Ceftriaxone and Ceftazidime Antibiotics

Isolates	Ceftazidime (CAZ) ≤ 22mm	Ceftriaxone (CTR) ≤ 25mm	No.screened
<i>Citrobacterfreundii</i>	30	23	2
	12	10	
<i>Enterobacter spp.</i>	27	23	1
<i>Escherichiacoli</i>	14	11	4
	33	25	
	34	25	
	13	07	
<i>Klebsiella pneumoniae</i>	30	24	3
	14	09	
	29	24	
<i>Proteus vulgaris</i>	12	14	1
<i>Pseudomonasaeruginosa</i>	28	20	2
	26	25	
<i>Salmonellatyphi</i>	00	10	1

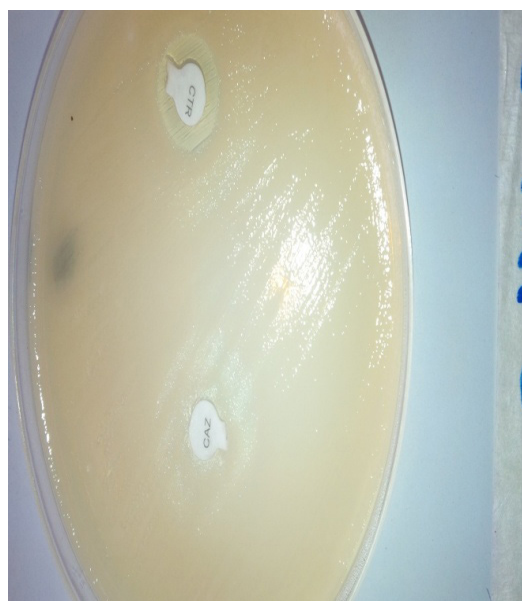
The zones of inhibition for susceptibility to Ceftazidime were recorded highest for *Escherichia coli* and then *Klebsiella pneumoniae* and *Citrobacterfreundii*. *Salmonella typhi* was resistant (Table 3). However, the number of isolates screened was also highest for *E. coli*

Out of the 30 samples of food and drinks collected within Gombe metropolis for this research study and subjected to bacterial analysis, 14 (46.66%) were found to be gram negative following gram staining reaction. The 30 samples of the foods and drinks include; 5 Sorrel juice (Zobo), 5 Kunun aya, 5 Kunun zaki, 5 Nono, 5 Fried Fish and 5 meats, and, were the samples screened. The 14 Gram negative isolates were confirmed to be members of the enterobacteriaceae also, following identification by biochemical tests. A total of 14 bacteria was isolated

from the samples out of which 3(10%) were from Sorrel juice (Zobo), 4(13.33%) from KununAya, 1(3.33%) from Kununzaki, 3(10%) from Nono, 2(6.67%) from fried fish and 1(3.33%) from meat. From the result, Kununaya had the highest prevalence of bacteria isolated from it among the foods and drinks, while kununzaki and meat have the least percentage of occurrences for both foods and drinks. This result does not go in line with the work of <sup>27</sup>, where high number of *Klebsiella* spp. 63(84.0%) and *E. coli* 45(60%) were reported to have been isolated from meat.

This variation may be due to the fact that the food samples used in their work were raw (meat, milk, chicken, minced meat), that had not undergone heat processing while the samples used in this study are heat processed foods that are ready to eat. The heat processing might have led to reduction of microbial load in the food. Although the result in table 1 does not correspond to work of<sup>27</sup>, it goes in line with the work of<sup>1</sup> who reported a total 16 isolates from food. A research conducted in Kano, Nigeria. The presence of this enterobacteriaceae may be as a result of contamination during production process or poor sanitary hygiene of the place of production, utensils used or the handlers of the product because their presence indicates faecal contamination. Table 1 show the number of foods and drinks screened, the number of bacteria isolated and their percentage of occurrence.

Table 2 shows the biochemical reactions of the isolated bacteria. The biochemical tests include; citrate test, motility test, Lactose fermentation test Figure 3 Indole production test, hydrogen sulphide (H<sub>2</sub>S) production, acid and gas production on KIA. This confirmed the bacterial isolates to be members of *Enterobacteriaceae*. Among the bacterial isolates, *Escherichia coli* Figure 4 has the highest percentage of occurrence of 4 (28.57%) followed by *Klebsiella pneumoniae* and *Citrobacter freundii* with 14.29% each, with the least being *Salmonella typhi*, *Pseudomonasaeruginosa* and *Enterobacter spp.* with each having 7.14%.



**Figure 5.** Screening for ESBLs producers based on CLSI breakpoint.

**Table 4.** Screening for ESBLs producers among the bacterial isolates based on CLSI breakpoint

S/No.	Isolates	No. Screened	No. Positive	% Occurrence
1	<i>C. freundii</i>	2	1	7.14
2	<i>Enterobacter spp.</i>	1	0	0.00
3	<i>E. coli</i>	4	2	14.29
4	<i>K. pneumoniae</i>	3	1	7.14
5	<i>P. vulgaris</i>	1	0	0.00
6	<i>Ps. Aeruginosa</i>	2	1	7.14
7	<i>S. typhi</i>	1	1	7.14
	<b>Total</b>	14	6	42.85

The 14 (100%) enterobacteriaceae isolates identified were screened for ESBLs production using CLSI Breakpoint where the zones of inhibition were read for Ceftazidime (CAZ  $\leq 22$ ) and Ceftriaxone (CTR  $\leq 25$ ), or no zone was recorded Table 3. In this table, the minimum zone of inhibition recorded for Ceftazidime (CAZ) antibiotic is 0.00mm and 10mm for Ceftriaxone (CTR) antibiotic was produced by *Salmonella typhi* and the maximum zone of inhibition produced by *E. coli* was 33mm for CAZ and 25mm for CTR. The result obtained in this table is similar to the work of<sup>28</sup> in which non-among the *Salmonella* isolates showed resistance to CAZ and CTR.

**Table 5.** Confirmation of ESBLs producers among the isolates based on DDST

S/No.	Isolates	No. Screened	No. Positive	% Occurrence
1	<i>C. freundii</i>	1	0	0.00
2	<i>E. coli</i>	2	1	16.67
3	<i>K. pneumoniae</i>	1	0	0.00
4	<i>Ps. Aeruginosa</i>	1	0	0.00
5	<i>S. typhi</i>	1	1	16.67
	<b>Total</b>	6	2	33.34

Table 4 shows the number of bacteria subjected to screening for ESBL production using CLSI breakpoint alongside their respective number for positive and percentage of occurrence. The suspected ESBLs producers Figure 5 were only 6 with percentage occurrences of

42.84%. These include *Citrobacter freundii* 1(7.14%), *Escherichia coli* 2(14.29%), *Klebsiella pneumoniae* 1(7.14%), *Salmonella typhi* 1(7.14%) and 1(7.14%) for *Pseudomonas aeruginosa*. *Escherichia coli* had the highest prevalence with 14.29% and the least occurring *Salmonella typhi* with 7.14%. The result is similar to the work of<sup>1</sup> on commercial food sold in Kano, Nigeria where he reported 6.25% of occurrence of *E. coli* and but it goes contrary to his work on commercial drinks in which 9.09% of *Escherichia coli* and *Salmonella typhi*, each were recorded. Modified DDST method was used to confirm for ESBLs production in which among the 6 (42.84%) isolates found positive, 2(28.58%) were ESBLs producers by an increase in inhibition zone of 5mm toward the centre disc Augmentin Table 5, they include: *Escherichia coli* 1(16.67%) and *Salmonella typhi* 1(16.67%).

Although there is limited work done on screening for ESBL producers from food and drinks in Gombe state, Nigeria, there are indeed similar works conducted in other parts of Nigeria. The result obtained from this research work is in line with previous report made by<sup>1</sup> but with higher percentage in *E. coli* isolated from food in Nigeria (Kano). However, it differs from his work made on commercial drinks with 14.29% of ESBLs production by *Klebsiella pneumoniae* and higher percentage of 28.57 in *Serratiamercescens* have higher positive strains than other isolated enterobacteria (*Salmonella* and *E. coli*). Also<sup>22</sup> reports 44.4% of *E. coli*, 38.5% *K. pneumoniae* and *K. oxytoca* (26%). This shows high incidence of ESBLs producing *E. coli*, however high incidence of ESBLs producing *K. pneumoniae* in raw milk has been reported by<sup>29</sup> in India, Doon Valley. Similarly<sup>26</sup>, reported about 20.7% of MDR strains of enterobacteria (*E. coli*, *Salmonella* and *Klebsiella species*) in poultry feed. Variation in the results obtained may also be due to the environment, how populated the environment is, how developed the environment is (rural, urban or city), so also weather may be another factor of variation and the source of the samples used for the research.

## 4. Conclusion

This study suggests that foods and drinks (Sold in Gombe metropolis) subjected to investigation could also serve as reservoirs for ESBLs producing bacteria. Moreover, only *E. coli* and *S. typhi* were found to harbour ESBLs although other pathogens detected may prove deleterious to health if the products are consumed.

## 5. Acknowledgment

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## 6. References

1. Yusha'u M, Abubakar HM, Kawo AH. Commercial foods as potential sources of Extended Spectrum Beta Lactamases (ESBLs) Producers in Kano, Nigeria. International Journal of Pharmaceutical and Applied Sciences. 2010; (1):59–62.
2. George EA, Sankar S, Jesudasan MV, Sudandiradoss C, Nandagopal B. Incidence of extended spectrum beta lactamase producing *Escherichia coli* among patients, healthy individuals and in the environment. Indian Journal of Medical Microbiology. 2014; 32(2):172–4.
3. Yusha'u M, Abubakar HM, Kawo AH. Commercial drinks as potential sources of Extended Spectrum Beta Lactamases (ESBLs) Producers in Kano, Nigeria. International Journal of Biomedical and Health Sciences. 2010; 1:103–8.
4. Johan T. Extended-spectrum beta-lactamase- producing enterobacteriaceae, epidemiology, risk factors, and duration of carriage. Infectious Disease Research Unit. Sweden by Media-Tryck, Lund University; 2012. p. 18–32.
5. Blaak H, de Kruijf P, Hamidjaja RA, van Hoek AH, de Roda Husman AM, Schets FM. Prevalence and characteristics of extended spectrum beta lactamase producing *E. coli* in dutch recreational water influenced by waste water treatment plant. Veterinary Microbiology. 2014; 171(3-4):448–59.
6. Rao PNS. Extended spectrum beta lactamases- A comprehensive review. 2015. p. 1–108.
7. Rawat D, Nair D. Extended-spectrum  $\beta$ -lactamases in Gram negative bacteria. Journal of Global Infectious Diseases. 2010; 2(3):263–74.
8. Herbert H, Roger S. Transfer of Extended-Spectrum B-Lactamase (ESBL)-producers at the human-food chain-environment-wildlife interface in Switzerland. The APUA Newsletter. 2014; 32(2):5–8.
9. Snow LC, Warner RG, Cheney T, Wearing H, Strokes M, Harris K, Teale CJ, Coldham NG. Risk factors associated with extended spectrum beta lactamase producing *Escherichia coli* [CTX-M] on dairy farms in North West England and North Wales. Preventive Veterinary Medicine. 2012; 106(3-4):225–34.
10. Rout S, Dubey D, Panigrahy R, Padhy RN. Surveillance of extended-spectrum b-lactamase producing bacteria in an Indian teaching hospital. Journal of Taibah University Medical Sciences. 2014; 9(4):274–81.



11. Sahu MC, Dubey D, Rath S, Debata NK, Padhy RN. Multidrug resistance of *Pseudomonas aeruginosa* as known from surveillance of nosocomial and community infections in an Indian teaching hospital. *Journal of Public Health*. 2012; 20(4):413–23.
12. Dubey D, Sarangi R, Debata NK, Padhy RN. Detection of metallo- $\beta$ -lactamase producing *Klebsiella pneumoniae* in a neonatal septicemia. *Journal of Acute Disease*. 2013; 2(1):82–4.
13. Rath S, Dubey D, Sahu MC, Padhy RN. Surveillance of ESBL producing multidrug resistant *Escherichia coli* in a teaching hospital in India. *Asian Pacific Journal Tropical Disease*. 2014; 4(2):140–9.
14. Yasmin T. Prevalence of ESBL among *Escherichia coli* and *Klebsiella* spp. in a tertiary care hospital and molecular detection of important ESBL producing genes by multiplex PCR. Department of microbiology and Immunology, Mymensingh Medical College, Mymensingh; 2012. p. 159.
15. Gillespie SH, Hawkey PM. Principles and practice of clinical bacteriology. 2nd ed. Chichester, England, John Wiley and Sons; 2006. p. 620.
16. World Health Organisation WHO. Guide line for drinking water quality; 2001; 82:1–39.
17. Sharma P, Sudhir K, Pawas G, Vikas S, Rameshwar S. Antibiotic resistance among commercially available probiotic. *Food Research International*. 2014; 57:176–95.
18. Mishra MP, Debata NK, Padhy RN. Surveillance of multidrug resistant uropathogenic bacteria in hospitalized patients in India. *Asian Pacific Journal Tropical Biomedical*. 2013; 3(4):315–24.
19. Nester E. A human perspective of biotechnology. 4th edn. New York: McGraw Hill; 2004. p. 12338–43.
20. Cheesbrough M. District laboratory practice in tropical countries. part 2, 2<sup>nd</sup> ed. UK: Cambridge University; 2006. p. 35–197.
21. Awong-Taylor J, Craven KS, Griffiths L, Bass C, Muscarella M. Comparison of biochemical and molecular methods for the identification of bacterial isolates associated with failed loggerhead sea turtle eggs. *Journal of Applied Microbiology*. 2008; 104(5):1244–51.
22. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 12th informational supplement. M100–S12. Wayne PA., National Committee for Clinical Laboratory Standards; 2002.
23. Nakamura A, Komatsu M, Noguchi N, Ohno Y, Hashimoto E, Matsutani H, Abe N, Fukuda S, Kohno H, Nakamura F, Matsuo S, Kawano S. Analysis of molecular epidemiologic characteristics of Extended Spectrum  $\beta$ -Lactamase (ESBL)-producing *Escherichia coli* colonizing faeces in hospital patients and community dwellers in a Japanese city. *Journal of Infection and Chemotherapy*. 2016; 22(2):102–7.
24. Dia ML, Ngom B, Diagne R, Ka R, Lo S, Cisse MF, Arlet G, Sow AI. Molecular detection of CTX-M-15- type  $\beta$ -lactamases in *Escherichia coli* strains from Senegal. *New Microbe and New Infect*. 2016; 9:45–6.
25. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae, hospital prevalence and susceptibility pattern. *Reviews of Infection Disease*. 1988; 10(4):867–78.
26. Oyinloye JMA Jnr, Ezekiel CN. Extended-Spectrum Beta-Lactamase (ESBL)-producing multidrug resistant enterobacteria from commercial poultry feeds in Nigeria. *Annals of Biological Research*. 2011; 2(2):250–4.
27. Gundogan N, Avci E. Prevalence and antibiotic resistance of Extended Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species isolated from foods of animal origin in Turkey. *African Journal of Microbiology Research*. 2013; 7(31):4059–64.
28. Rahmani M. Phenotypic and genotypic studies of Extended Spectrum Beta- Lactamase (ESBL) resistance among salmonella isolates from poultry sources in Iran. *Iranian Journal of Veterinary Medicine*. 2012; 6(4):235–40.
29. Chauhan S, Farooq U, Singh V, Ajay K. Determination of prevalence and antibacterial activity of ESBL (Extended Spectrum Beta-Lactamases) producing *Klebsiella* species isolated from raw milk of doon valley in India. *International Journal of Pharma and Bio Sciences*. 2013; 4(1):417–23.