Determination of Antibiotic Resistance Patterns of Beta-Lactamase-Producing *Escherichia coli* Strains from Kindirmo in Nasarawa Town, Nasarawa State, Nigeria

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Abstract

This study investigates the antibiotic resistance patterns of beta-lactamase-producing Escherichia coli in Kindirmo in Nasarawa town, Nasarawa State, Nigeria. Over five months, 169 samples were collected from four sampling points. Escherichia coli was isolated using the streak-plating method and identified through biochemical tests. All isolates tested positive for beta-lactamase production. The agar-disc diffusion technique assessed the beta-lactamase-producing E. coli strains' susceptibility to 10 antibiotics. Out of 169 samples, 10 were contaminated with E. coli, resulting in a prevalence of 5.92%. The isolates showed complete resistance to six antibiotics, while 70% and 50% demonstrated susceptibility to ofloxacin, pefloxacin, and gentamicin, respectively. Six antibiotic-resistance phenotypes were identified among the E. coli isolates. The presence of beta-lactamase-producing E. coli in Kindirmo suggests that consuming the product in the area poses a risk to consumers. Authorities should enforce basic hygiene requirements during production and sales to safeguard public health.

Keywords: Antibiotic Resistance, Beta-lactamase, E. coli, Kindirmo, Nasarawa State, Nigeria

INTRODUCTION

Traditional foods, such as milk and its derivatives, constitute vital sources of essential nutrients like high-quality proteins, carbohydrates, fats, B vitamins (B2 and B12), and minerals (iodine, potassium, and phosphorus) (Lucey, 2015). Additionally, milk contains dissolved gases, predominantly carbon dioxide, nitrogen, and oxygen (Lucey, 2015). While milk and its products offer significant nutritional benefits, they can also harbour harmful microorganisms like *Salmonella*, *Escherichia coli*, *Listeria*, *Brucella*, and *Mycobacterium* species, posing considerable health risks, especially to vulnerable populations such as individuals with compromised immune systems, older people, pregnant women, and children (FDA, 2020).

Escherichia coli, a notable Gram-negative bacterium, is linked to hospital and community-acquired infections (Wu *et al.*, 2013). The rise of beta-lactamase-producing bacteria, including *E. coli*, is a significant concern due to the widespread use of cephalosporins. Initially observed in clinical settings, these bacteria are increasingly identified in food-producing animals, contributing to food safety issues with broader environmental ramifications (Agersøn and Aarestrup, 2013; Gandra *et al.*, 2014). Reports suggest that food-producing animals are potential reservoirs for beta-lactamase-producing bacterial pathogens, facilitating their community spread (Veenemans *et al.*, 2014; Dohmen *et al.*, 2015). The escalation of resistance in beta-lactamase-producing bacteria to multiple antibiotics has led to heightened usage of carbapenems (Dahm *et al.*, 2015), which, in turn, has spurred the emergence of carbapenemase-producing bacteria (Liu *et al.*, 2018). Carbapenemase enzymes produced by these bacteria can hydrolyze all beta-lactam antibiotics, posing significant challenges in managing infectious diseases (Badri *et al.*, 2018).

Food safety concerns are particularly complex in many developing countries, characterized by numerous informal markets where a substantial portion of food is sold, making enforcing food safety regulations difficult and often avoided. In Nigeria, hygiene levels and food safety practices

among milk and milk product vendors are frequently inadequate. Many rural, suburban, and urban areas lack essential infrastructure and food safety education, leading to compromised sanitary measures, food contamination, and spreading pathogens harboring antibiotic resistance determinants such as beta-lactamase-producing bacteria. The increasing prevalence of beta-lactamase-producing bacteria in communities significantly contributes to the overall burden of antimicrobial resistance (van den Bunt *et al.*, 2017). To our knowledge, no published articles exist on the occurrence and antibiotic resistance patterns of beta-lactamase-producing *E. coli* strains in milk and milk products from Nasarawa town. In the light of these considerations, this study aimed to determine the antibiotic resistance patterns of beta-lactamase-producing *E. coli* strains from *Kindirmo* in Nasarawa town, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Study Area

The research was conducted in Nasarawa town, Nasarawa State, Northern Nigeria. Nasarawa covers an area of approximately 570.4 square kilometers, with a population of 189,835, according to the 2006 census (NBS, 2009). This town is about 10.5 kilometers from Abuja, the Federal Capital Territory, 37 kilometers from Keffi, and 165 kilometres from Lafia, the Nasarawa state capital. The geographical coordinates of the study location are around 8°21'58" N of the equator and 7°5'58" E of the Greenwich Meridian (NBS, 2009).

Sample size

The sample size was determined using the 12.6% prevalence of *Escherichia coli* in milk, as reported by Reuben *et al.* (2013). The sample size was determined by using the equation described by Naing *et al.* (2006):

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where n= is the sample size;

P is the prevalence from a previous study= 12.6% = 0.126;

Z is the standard normal distribution at 95% confidence interval = 1.96;

d is the absolute desired precision at 5%= 0.05

Therefore, n = $\frac{(1.96)^2 \times 0.126 \times (1-0.126)}{(0.05)^2}$

$$= \frac{0.4231}{0.0025}$$

n = 169 samples

Collections of samples

Traditionally pasteurised milk (*Kindirmo*) samples were collected from various locations: Tammah, Gunki, Federal Polytechnic, Nasarawa campus, and Nasarawa main market. Purposive sampling was used to collect the samples based on the concentration of vendors at the different sampling points. The milk samples were collected in sterile, screw-capped containers and transported in an ice-packed cooler to the Microbiology Laboratory of the Department of Applied Biology/Microbiology, Federal Polytechnic, Nasarawa, for analysis.

Isolation of *Escherichia coli*

A 10 ml aliquot of each milk sample was mixed with 90 ml of buffered peptone water and incubated at 37°C for 24 hours under aerobic conditions. After incubation, a loopful of the enriched culture was streaked on Eosin-methylene blue (EMB) agar plates and incubated for another 24 hours at 37°C. Dark-red colonies with a green metallic sheen were considered presumptive *E. coli* colonies. These presumptive colonies were then sub-cultured onto freshly prepared EMB plates to obtain pure colonies. Purified colonies were streaked onto nutrient agar slants and stored at refrigeration temperature for further identification and characterisation (Cheesbrough, 2010).

Identification and Characterisation of the *Escherichia coli* Isolates

The presumptive *E. coli* isolates were identified using Gram reaction and conventional biochemical tests: indole, methyl-red-Voges-Proskauer, and citrate utilisation tests. Presumptive *E. coli* colonies were inoculated into nutrient broth and incubated at 37°C for 24 hours. Thereafter, 3 drops of Kovac's reagent were added and shaken gently. Positive reactions were indicated by the development of a red colour in the reagent layer above the broth within 1 minute. In negative reactions, the indole reagent retained its yellow colour. Five (5ml) of methyl red - Voges Proskauer (MRVP) broth was inoculated with presumptive *E. coli* colonies and incubated at 37°C

for 48-72 hours. After this incubation period, 1 ml of the broth was transferred to small serological tubes. To this small quantity, 3 drops of methyl red were added. The development of red colouration on adding the indicator signified a positive result, while a yellow colour showed a negative result. To the rest of the broth in the original tube, 15 drops of 5% - naphthol in ethanol were added, followed by 5 drops of 40% potassium hydroxide. These were mixed by shaking. The caps were loosened, and the bottles were placed in a slanting position. The development of a red colour starting from the liquid-air interface within 1 hour indicated VP positive tests. No colour development occurred in VP negative tests. Simmon's citrate agar slants were prepared in Bijou bottles in accordance with the manufacturer's instructions, and positive colonies obtained from the presumptive tests above were inoculated onto the slants and incubated at 37°C for 24-48 hours with the Bijou bottles loosely capped. A deep blue colour's development indicates a citrate-positive result (Cheesbrough, 2010).

Assessment of Beta-Lactamase Production in the *E. coli* Isolates

The iodometric method was used to assess the beta-lactamase production in the isolates. This test involves preparing a starch indicator by dissolving starch powder in boiling water and an iodine reagent by mixing potassium iodide and iodine in distilled water. A benzylpenicillin solution in phosphate buffer saline is also prepared and sterilised (Bush and Jacoby, 2010).

To perform the test, a loopful of an overnight-grown beta-lactamase-producing *E. coli* strain is added to the penicillin G solution and incubated at 37°C for 30 minutes. Two drops of starch solution and one drop of iodine reagent are added to the mixture. Colour changes from dark blue to colourless within 10 minutes, indicating a positive beta-lactamase production test (Bush and Jacoby, 2010).

This method helps identify the presence of beta-lactamase-producing *E. coli* strains, which play a significant role in antibiotic resistance development, especially to beta-lactam antibiotics.

Standardisation of inocula

To prepare the concentration of the suspension for both the test bacterium and the standard strain (*E. coli* ATCC 25922 obtained from the National Veterinary Research Institute, Vom, Nigeria), a 0.5 scale of McFarland's standard (1.5 × 10^8 cells/mL) in 0.8% normal saline was used.

This was carried out as follows: purified beta-lactamase-producing *E. coli* colonies were picked using a sterile wire loop. A suspension was prepared by mixing the colonies with normal saline in small tubes. The density of the prepared suspension was then compared with that of the 0.5 McFarland standard. The suspension density of the test organism was matched with that of the standard by adding more *E. coli* colonies or more sterile saline as needed. This was done to ensure that the bacterial suspensions had the appropriate density for the antibiotic susceptibility test, allowing for accurate and reliable results (Cheesbrough, 2010).

Antibiotic Susceptibility Test of the Beta-Lactamase-Producing *Escherichia coli* Strains

The antibiotic susceptibility test of the beta-lactamase-producing *Escherichia coli* strains was performed using the agar-disc diffusion method, per the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2016). A panel of ten antibiotics was tested on these strains, which included ofloxacin (10 μg), pefloxacin (10 μg), ciprofloxacin (10 μg), augmentin (30 μg), gentamicin (10 μg), streptomycin (30 μg), ceporex (10 μg), nalidixic acid (30 μg), cotrimoxazole (30 μg), and ampicillin (10 μg). To conduct the test, 0.5 ml of the suspension of the test organism was placed in the middle of prepared Mueller-Hinton agar plates and spread evenly using a sterile cotton swab stick. Subsequently, a ring of each disc containing single concentrations of each antibiotic was placed on the inoculated surface using sterile forceps. The plates were then incubated at 37°C for 24 hours. After incubation, the zones of growth inhibition created by each antibiotic against the test organism were measured using a transparent metre rule, and the results were compared to the CLSI breakpoints (CLSI, 2016) to determine susceptibility, intermediate, or resistance levels.

RESULTS

A statistically significant relationship (P = 0.000) between the presence of beta-lactamase-producing *E. coli* strains in *Kindirmo*, Nasarawa town, and the different sampled areas using chi-square. The findings are detailed in Table 1.

The antibiotic susceptibility test results for the ten (10) beta-lactamase-producing *E. coli* strains are presented in Table 2. Among these strains, 70% were susceptible to ofloxacin, while 30%

were susceptible to pefloxacin. However, all the beta-lactamase-producing *E. coli* strains were completely resistant to streptomycin, ceporex, nalidixic acid, cotrimoxazole, augmentin, and ampicillin.

The antibiotic resistance patterns of these ten beta-lactamase-producing *E. coli* strains are displayed in Table 3, with six different patterns observed, including combinations of 6, 7, and 8 antibiotics. No resistance pattern was found with a single, two, three, four, or five antibiotics.

Table 1: Occurrence of Beta-Lactamase-Producing *Escherichia coli* Strains in *Kindirmo* in Nasarawa Town, Nasarawa State, Nigeria

Location	No. Collected	No. Positive	(%) Occurrence	P-value
TMH	60	5	8.3	
GNK	50	3	6.0	
FPC	30	1	3.3	0.000
NSM	29	1	3.4	
Total	169	10	5.92	

No. = Number, TMH= Tammah, GNK= Gunki, NSM= Nasarawa Market, FPC= Federal Polytechnic, Nasarawa Campus

Table 2: Antibiogram Susceptibility Profile of Beta-Lactamase-Producing *Escherichia coli* isolated from *Kindirmo* in Nasarawa Town, Nasarawa State

	N = 10			
Antibiotic	Conc. (µg)	R (%)	I (%)	S (%)
Ofloxacin	10	0(0)	3(30)	7(70)
Pefloxacin	10	5(50)	2(20)	3(30)
Ciprofloxacin	10	0(0)	10(100)	0(0)
Augmentin	30	10(100)	0(0)	0(0)
Gentamicin	10	5(50)	4(40)	1(10)
Streptomycin	30	10(100)	0(0)	0(0)
Ceporex	10	10(100)	0(0)	0(0)
Nalidixic acid	30	10(100)	0(0)	0(0)
Cotrimoxazole	30	10(100)	0(0)	0(0)
Ampicillin	30	10(100)	0(0)	0(0)

N = number, Conc. = Concentration, R= Resistance, I= Intermediate, S= Susceptible

Table 3: The antibiotic Resistance Patterns of beta-lactamase-producing *Escherichia coli* from *Kindirmo* in Nasarawa Town, Nasarawa State

No. of Antibiotics	Resistance Pattern	No. (%) of Strain
6	AUG, STR, COT, NAX. CEP	1(10)
6	GEN, STR, CEP, NAX, COT, AMP	1(10)
7	PEF, GEN, STR, CEP, NAX, COT, AMP	1(10)
7	PEF, AUG, STR, CEP, NAX, COT, AMP	3(30)
7	AUG, GEN, STR, CEP, NAX, AMP	2(20)
8	PEF, AUG, GEN, STR, CEP, NAX, COT, AMP	2(20)

PEF= Pefloxacin, AUG= Augmentin, GEN= Gentamicin, STR= Streptomycin, CEP= Ceprorex, NAX= Nalidixic acid, COT= Cotrimoxazole, and AMP= Ampicillin

DISCUSSION

The study found that 10 (5.92%) of the 169 *Kindirmo* samples examined were contaminated with beta-lactamase-producing *Escherichia coli* strains. This could reflect poor sanitary practices employed during milking, milk handling, processing, and distribution. This percentage occurrence is lower than that recorded in some studies in India and Osun State, Nigeria (Khan *et al.,* 2014; Abike *et al.,* 2015).

They recorded 20 and 9.2% occurrence of beta-lactamase-producing *Escherichia coli* strains in milk, respectively. The lower occurrence of beta-lactamase-producing *E. coli* strains in *Kindirmo* in this study compared to other studies could be attributed to the traditional methods of producing the product, which might have exposed it to fewer contaminants. The occurrence of beta-lactamase-producing *E. coli* in *Kindirmo* in this study reflects poor sanitary practices during milking, milk handling, and

processing. However, this study emphasises the need for improved sanitary practices to further reduce bacterial contamination in *Kindirmo*.

As observed in this study, the complete resistance of the beta-lactamase-producing E. coli strains to several antibiotics is a significant concern. This resistance could be due to contamination from human sources or the environment, as these antibiotics are not commonly used in livestock management in the study area. The multidrug-resistant nature of the BLPEC strains is worrisome, as it can lead to the transmission of antibiotic-resistant bacterial pathogens to humans via the food chain (Badri *et al.,* 2017; Akarsh *et al.,* 2019; Kamaruzzaman *et al.,* 2020; Nahar *et al.,* 2023).

The six (6) antibiotic resistance patterns recorded in this study disagree with the three (17) and thirteen (13) patterns recorded among *E. coli* isolated from milk in Osun state, Nigeria (Abike *et al.,* 2015). Differences in resistance patterns between this study and the others could be attributed to varying antibiotic use and misuse practices in different areas.

CONCLUSION

A 5.92% occurrence of beta-lactamase-producing *E. coli* strains was recorded in this study. This highlights the potential public health risk associated with the consumption of *Kindirmo* in the study areas. The beta-lactamase-producing *E. coli* strains isolated in this study were completely resistant (100%) to ampicillin, augmentin, cotrimoxazole, nalidixic acid, streptomycin, and ceporex. This is a cause for serious public health concern as these multidrug-resistant *E. coli* strains can be transmitted to humans through the food chain and cause infections that would not respond to chemotherapy, leading to an increased risk of death among affected individuals. It is recommended that basic hygiene requirements be met in preparing and selling dairy products. This will greatly enhance the overall quality standards and safety of the product. Also, relevant authorities should raise awareness among the general public about the dangers of indiscriminate antibiotic use. This will help reduce the emergence of antibiotic-resistant bacteria and promote responsible antibiotic consumption practices.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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