

# Identification Of Resistance Genes and Antibigram Profile of Pathogens from Locally Produced Foods and Drinks in Umudi.

UMEH, CHIJOKE C.<sup>1</sup>, NWANZE, CHIDINMA F.<sup>2</sup>, ELUU, SUNDAY E.<sup>3</sup>, NNAGBO PAULINE A.<sup>4</sup>

<sup>1,2,3</sup>Department of Microbiology, Hezekiah University Umudi Nkwere, Imo State.

<sup>4</sup>Department of Microbiology, Imo State University Owerri, Imo State.

**Abstract-** Foodborne diseases pose a major global public health challenge, particularly in developing areas where food hygiene and handling practices are often inadequate. Locally produced foods and traditional drinks are widely consumed in rural communities, but their preparation and safety remain largely unregulated. This study was designed to isolate and identify pathogenic bacteria from locally produced foods and traditional drinks in Umudi, Nkwere Local Government Area, and to determine their antibiogram profiles and resistance genes. Bacteria were isolated and identified using standard morphological, cultural, and biochemical tests. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion technique following guidelines from the Clinical and Laboratory Standards Institute, and the World Health Organization. Molecular identification for resistance genes was also conducted. Pathogens including *Staphylococcus aureus*, *Shigella* spp., and *Salmonella* spp. were isolated from Bread, Zobo, Agidi, Meat pie, Akara, and Soya milk, whereas no pathogens were found in Akpu. The isolates harbored Extended-spectrum  $\beta$ -lactamases (ESBLs) resistance genes, specifically *bla*CTX-M and *bla*TEM. *Staphylococcus aureus* isolates in Bread and Zobo were highly sensitive to Fluoroquinolones (Ofloxacin, Peflacin, Ciprofloxacin, Levofloxacin) and Amoxicillin, but exhibited resistance to Gentamycin, Augmentin, and Azithromycin. *Shigella* spp. in Akara and Agidi were sensitive to Ofloxacin, Ciprofloxacin, Peflacin, Levofloxacin, and Amoxicillin, while resistant to Gentamycin and Augmentin. Bacteria isolated from Meat pie and Soya milk were sensitive to most antibiotics tested, including Cefuroxime, but resistant to Augmentin and Azithromycin. The isolation of multi-drug resistant pathogens carrying Extended-spectrum  $\beta$ -lactamases genes from ready-to-eat foods indicates a significant health risk. Strict regulatory oversight, improved food handling hygiene, and public health awareness are critical in Umudi to prevent foodborne disease outbreaks and the spread of antibiotic resistance.

## I. INTRODUCTION

Foodborne diseases remain a major public health challenge globally, especially in poor nations with poor food safety and foodborne diseases result from the consumption of food contaminated or infected by pathogens. Rural areas often eat local foods and traditional drinks because they cost less, are easy to find, and fit their culture (Getie et al., 2019). However, food products are often prepared under unhygienic conditions, which increase the risk of microbial contamination. Consumption of foods contaminated with resistant bacteria can lead to infections that are difficult to treat and may result in increased morbidity, mortality, and healthcare costs. Resistance genes carried by foodborne pathogens can also be transferred to other bacteria via gene transfer (Haliu, 2018).

The epidemiology of foodborne diseases has changed rapidly due to changes in the environment and the ability of pathogens to adapt to a new environment (Ababu et al., 2020). Every year, food poisoning causes millions of sicknesses and thousands of deaths, mostly in developing nations. Poor environmental sanitation, changes in eating habits, and lack of hygienic practice during production, packaging, transportation, storage and retail of food are contributing factors that leads to contamination of dairy products (Wallace et al., 2020). In dairy product, the primary materials for the production of dairy products such as ice cream, yoghurt, butter and cheese are water, condensed and dried milk and the contamination of these primary materials affects the quality of the dairy products. However, Harmful germs can contaminate milk and cheese through dirty air, bad worker hygiene, unsafe water, or poor

storage (Labacz and Zulewisk, 2021). Pathogenic bacteria from the Enterobacteriaceae family pose a major global public health threat and are frequently linked to foodborne infections (Manishman et al., 2021). *Salmonella* spp., *Shigella* spp., *Listeria* spp., *Escherichia coli*, *Enterobacter* spp., *Campylobacter* spp. and *Yersinia* spp. are among public health threat pathogenic microorganisms commonly implicated in foodborne diseases (Mariam, 2021).

Antibiotics are vital for modern medicine. Since their discovery, they have helped treat infections and made advanced medical procedures possible, including surgeries, organ transplants, premature baby care, and cancer treatments (Cecchini and Lee, 2017). The improper and excessive use of antibiotics in human medicine and food production, together with bacteria's innate ability to adapt and become resistant over time, are some of the causes of antibiotic resistance in foodborne pathogens (Bilal et al., 2017). The widespread transmission of antibiotic-resistance genes throughout the food chain acts as a key driver of resistance in foodborne bacteria, making pathogens increasingly difficult to treat with conventional antibiotics (Rafiq et al., 2022). Fighting drug resistance diseases needs a wide plan at global, regional, and local levels. Global action plans, funding to low and middle income countries, research collaborations, and policy frameworks to support international collaboration and standardized approaches are the main goals of global efforts to prevent antibiotic resistance.

Cross-contamination may contribute to spread of antibiotic resistance. Cross-contamination can occur when microorganisms harboring antibiotic resistance genes, especially those carried by phages, are purposefully added to food during processing. Cross-contamination can also result from inappropriate food storage practices and the usage of chopping boards and utensils for raw meat. Foodborne bacteria may develop antibiotic resistance due to cross-contamination during manufacturing and handling. Microorganisms that may possess antimicrobial resistance genes are purposely introduced to certain food items during the manufacturing process for technical purposes (Verraes et al., 2013).

One of the ruling impediments to tackling antibiotic resistance, according to the World Health Organization (WHO), is the lack of creativity in fostering the development of novel antibacterial medicines. The World Health Organization states that new antibiotic creation is very low and the overall development of antibacterial medicines is stuck (WHO, 2022). Hence, antibiotic resistance causes more sickness and death because new and useful drugs are hard to find, as research and development has slowed down.

## II. STATEMENT OF THE PROBLEM

Every year, roughly 1 in 9 people worldwide get sick from eating contaminated food. These meals harbor dangerous bacteria, parasites, viruses or toxins that trigger more than 200 health conditions. The severe toll includes million fatalities and a staggering loss of 57.1 million healthy life years globally (WHO, 2024). However, through the oral interview conducted during this study, safety of locally produced foods and drinks in Umudi is largely unregulated. This is because the producers often lack adequate knowledge of food hygiene and safety standards, increasing the risk of contamination with pathogenic microorganisms. Pathogenic contamination is often intensified at the producer level by inadequate implementation of sanitation procedures and improper handling.

There is insufficient information on the antibiogram profiles and resistance genes of pathogens from locally produced foods and drinks in Umudi.

## III. SIGNIFICANCE OF STUDY

By conducting this study, I hope to:

- Enhance understanding of the presence of pathogenic bacteria in locally produced foods and show risks that stem from unhygienic handling. Share the essential details regarding antibiotic resistance genes of the isolated pathogens.
- The results of this study will help raise awareness about food safety and antimicrobial resistance in rural communities especially in Nkwere Local government as well as Imo

State in general. . It will also provide scientific evidence that can be used to improve food handling practices, guide antibiotic use policies, and protect public health.

#### IV. AIM

To identify resistance genes and determine the antibiogram profile of pathogens isolated from locally produced foods and drinks in Umudi, Nkwere Local Government Area.

#### V. OBJECTIVES

The objectives of this study include:

- To isolate bacteria associated with locally produced foods and drinks from Umudi community using standard microbiological methods..
- To identify the isolated bacteria using standard biochemical method.
- To identify the pathogenic isolates at molecular level.
- To determine the antibiotics resistance pattern of the isolates.
- To identify the resistance genes of the isolated pathogens.

#### VI. SCOPE OF THE STUDY

This study will focus on locally produced foods and drinks sold and consumed within Umudi community, Nkwere Local Government Area. The study will be limited to bacteria pathogens and commonly used antibiotics for the resistance pattern and gene identification.

#### VII. METHODOLOGY

##### Media Preparation

The media (Nutrient agar and MacConkey medium) used for the research was sterilized in an autoclave at 121oC for 15 mins at Psi. All glassware was also sterilized in hot air oven at 160oC for 1 hour. Noteworthy, all sterilization was done based on producer's specifications.

##### Sample Collection

The sample collections were performed according to WHO guidelines. The samples were collected randomly from different collection areas in Umudi.

##### Bacteriological Analysis of Samples

The collected water samples were allowed to settle at room temperature at 25oC on the bench before bacterial isolation. One gram of each food sample were mixed into 9.0ml of sterile distil water and was serially diluted in 10 folds. After serial dilution, 1ml aliquot of water were taken from the 3rd fold samples diluents (10<sup>-3</sup>, and aseptically introduced into sterile petri plates using micropipettes (1000µl micropipettes). Pour plate method was aseptically used to pour well-prepared sterile nutrient agar and MacCokey, and incubated at 37oC for 24 hours.

##### Identification of Bacterial Isolates

The bacteria colonies were identified using standard cultural and morphological test on Nutrient agar and MacConkey medium. Additionally, identification were accomplished using biochemical tests which include Gram staining, Catalase test, Indole test, Motility test, Tripple sugar Iron Test, Methyl red test and Oxidase test. The identification of isolates were confirmed by comparing the results of the standard tests to the taxonomic scheme of the Bergey's Manual of Determinative Bacteriology.

##### Bacterial Genomic DNA Extraction of the Isolates

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube was centrifuged at 10,000xg for 1 minute (Zhenyu, 2025).

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 xg for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer

were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-Wash buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000xg for 1 minute (Singh et al., 2022).

The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000xg microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

#### DNA Quantification of the Isolates

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer at 260nm wavelength according to manufacturer's instructions. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

#### 16S rRNA Amplification of the Isolates

The 16s rRNA region of the rRNA genes of the isolates were amplified using forward and reverse specific primers for the isolated pathogens on an Applied Biosystems (thermal cycler ABI 9700) at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for

30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on ultraviolet transilluminator.

#### DNA Sequencing of the Isolates

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min. all these were carried out base on manufacturer's guidelines.

#### Amplification of blactx-M genes from the isolates

CTX-M genes from the isolates were amplified using the CTX-M F: 5- CGCTTTGCGATGTGCAG -3' and CTX-M R: 5'-ACCGCGATATCGTTGGT -3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 94°C for 5 minutes; denaturation, 94°C for 40 seconds; annealing, 52C for 45 seconds; extension, 68°C for 45 seconds for 35 cycles and final extension, 68°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on blue light imaging system for a 550bp product size.

#### blaTEM Amplification of the isolates

TEM genes from the isolates were amplified using the TEM F: 5-TTTCGTGTCGCCCTTATTCC-3' and TEM R: 5'-ATCGTTGTCAGAAAGTAAGTTGG-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template.

The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 55°C for 40 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 200V for 15 minutes and visualized on blue light imaging system for but no product size was seen.

#### Antibiotic Resistance Pattern Screening using the Disk Diffusion Method

The disc diffusion method was used to determine the resistant patterns of the probable bacteria isolates to selected antibiotics. The antibiotics sensitivity disc containing Ceftriaxone (30µg), Cefuroxime (30µg), Ceporex(30µg), Ceftazidime(30µg), Streptomycin(10µg), Gentamycin (10µg), Augmentin(30µg), Ofloxacin (5µg), Ciprofloxacin (10µg), Peflacinen(5µg) and Nalidixic acid (30µg) were used. The tests were carried out according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI, 2018) and Kirby-Bauer disc diffusion technique as recommended by the World Health Organization (WHO). Prepared nutrient agar for each

isolate was incubated for 24 hour at 37oC. The inocula were spread evenly on culture plates with a glass rod and placing of antibiotics discs. The plates were incubated at 37oC for 24 hours, following the measurement of zones of inhibition with the standard of Clinical and Laboratory Standard Institute(CLSI, 2018). The zones of inhibition was measured using a meter rule recorded as milliliters(mm) and compared with CLSI guidelines to their susceptibility, intermediate and resistant level to the antimicrobial agents.

### VIII. RESULTS

Food samples cultured for 24hours has different colony size, shape, color, and number of bacteria pathogens in Nutrient agar measured in colony-forming units per milliliter (cfu/ml). Akara (Ak) has the highest germ count at 102 X 10<sup>3</sup>, while Fufu, Burns, and Moi-moi show no growth. The colonial characteristics of the pathogens from the food samples are shown in the table below:

| Cultural Characteristics of the Isolates |                   |        |                     |                           |  |
|------------------------------------------|-------------------|--------|---------------------|---------------------------|--|
| Samples                                  | Colony morphology |        |                     | Number of colony (cfu/ml) |  |
|                                          | Size              | Type   | Shape               | Color                     |  |
| Br                                       | Large(4mm)        | Smooth | Circular and raised | Golden yellow             |  |
| Zo                                       | Large(3mm)        |        | Entire edge         | Milky                     |  |
| Ag                                       | Large(3mm)        | Smooth | Circular and convex | Milky                     |  |
| Fu                                       | -                 | -      | -                   | -                         |  |
| Mp                                       | Small(2mm))       | Smooth | Circular            | Cream                     |  |
| Bn                                       | -                 | -      | -                   | -                         |  |
| Mm                                       | -                 | -      | -                   | -                         |  |
| Ak                                       | Small(1mm))       | Smooth | Circular            | White                     |  |
| Sm                                       | Small(2mm))       | Smooth | Convex              | Colorless                 |  |

KEY: Br= Bread, Zo= Zobo, Ag= Agidi, Fu=Fufu, Mp=Meat pie, Bn= Burns, Mm= Moi-moi, Ak= Akara, Sm= Soya milk

The biochemical characteristics of the isolates from Bread, Zobo, Agidi, Meat pie, Akara and Soya milk is shown in the below:

Biochemical Characterizations of the isolates

| Samples   | Gram Stain | Catalase | Oxidase | VP | Coagulase | Indole | MR | Motility | TSI | H2S | Probable organism            |
|-----------|------------|----------|---------|----|-----------|--------|----|----------|-----|-----|------------------------------|
| Bread     | + cocci    | +        | -       | +  | +         | -      | +  | -        | -   | -   | <i>Staphylococcus aureus</i> |
| Zobo      | + cocci    | +        | -       | +  | +         | -      | +  | -        | -   | -   | <i>Staphylococcus aureus</i> |
| Agidi     | - rod      | +        | -       | -  | -         | -      | +  | -        | -   | -   | <i>Shigella</i>              |
| Meat pie  | - rod      | +        | -       | -  | -         | -      | +  | +        | -   | +   | <i>Salmonella</i>            |
| Akara     | - rod      | +        | -       | -  | -         | -      | +  | -        | -   | -   | <i>Shigella</i>              |
| Soya milk | - rod      | +        | -       | -  | -         | -      | +  | +        | -   | +   | <i>Salmonella</i>            |

KEY: +rod= positive rod (Gram staining), -rod= negative rod (Gram staining), +rod= positive cocci (Gram staining), += positive, -=negative, TSI= Triple sugar indole, H2s= Hydrogen sulphide, MR= Methyl red, VP= Voges-Proskauer.

The resistant pattern prior to the resistance gene identification of the pathogens were carried out according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI, 2018) and Kirby-Bauer disc diffusion technique as recommended by the World Health

Organization(WHO). Result of the test with the diffusion disc containing popularly use antibiotics in Umudi, Nkwere Local Government, as well as Imo State is shown in the table below:

Sensitivity pattern of the isolates

| Isolates       | OFX      | CN       | PEF      | CPX      | AU      | AZ       | LEV      | CF      | AM       | CTZ      |
|----------------|----------|----------|----------|----------|---------|----------|----------|---------|----------|----------|
| Salmonella spp | 5(17.9%) | 2(7.1%)  | 5(17.9%) | 4(14.3%) | 0(0%)   | 1(3.6%)  | 5(17.9%) | 0(0%)   | 4(14.5%) | 2(7.1%)  |
| Shigella spp   | 5(16.2%) | 1(3.2%)  | 5(16.2%) | 5(16.2%) | 0(0%)   | 1(3.2%)  | 5(16.2%) | 1(3.2%) | 3(9.7%)  | 5(16.2%) |
| Staph. aureus  | 5(15.2%) | 4(12.1%) | 5(15.2%) | 5(15.2%) | 1(2.8%) | 5(14.3%) | 5(14.3%) | 1(3.0%) | 1(3.0%)  | 1(3.0%)  |

CF = Cefuroxime, CTZ= Ceftazidime, CN = Gentamycin, AU = Augmentin, OFX = Ofloxacin, CPX = Ciprofloxacin, PEF = Peflacinen, LEV= Levofloxacin, AZ= Azithromycin, AM= Amoxicillin

The isolates contains the Extended-spectrum B lactamases (ESBLs) resistant genes, blaCTX-M and blaTEM. The Agarose gel electrophoresis showing the blaCTX-M gene bands. Lanes 1, 3, 4, 6, and 7

showing the blaCTX-M gene bands at 500bp while lane L represents the 100bp molecular ladder is shown in figure 1

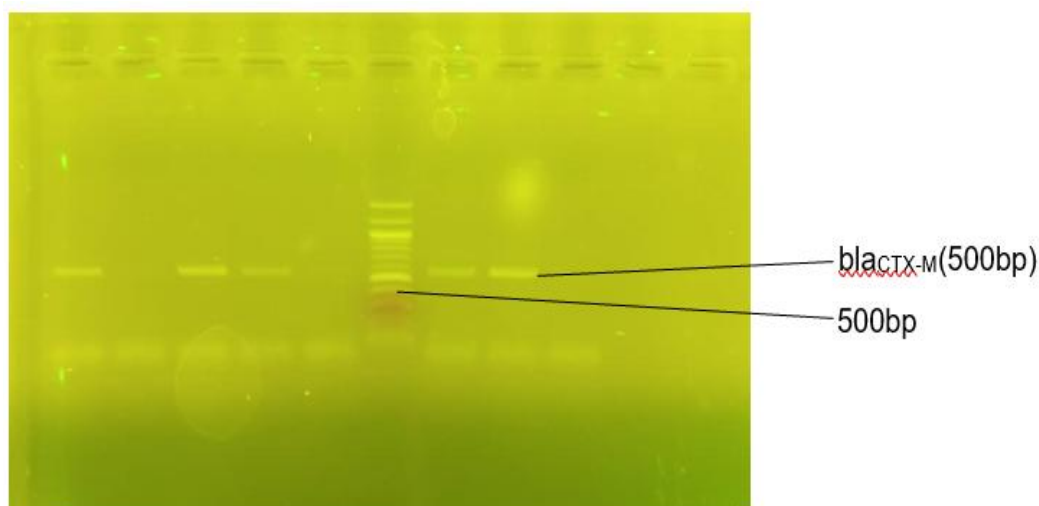


Figure 1: Agarose gel electrophoresis showing the blaCTX-M gene bands.

Agarose gel electrophoresis of PCR-amplified blaTEM gene of Lane L: 100 bp DNA ladder; Lanes 1, 2, 3, 6, 7, and 8: Positive samples showing

blaTEM gene bands at 600 bp; Lanes 4 and 5 is shown in figure 2

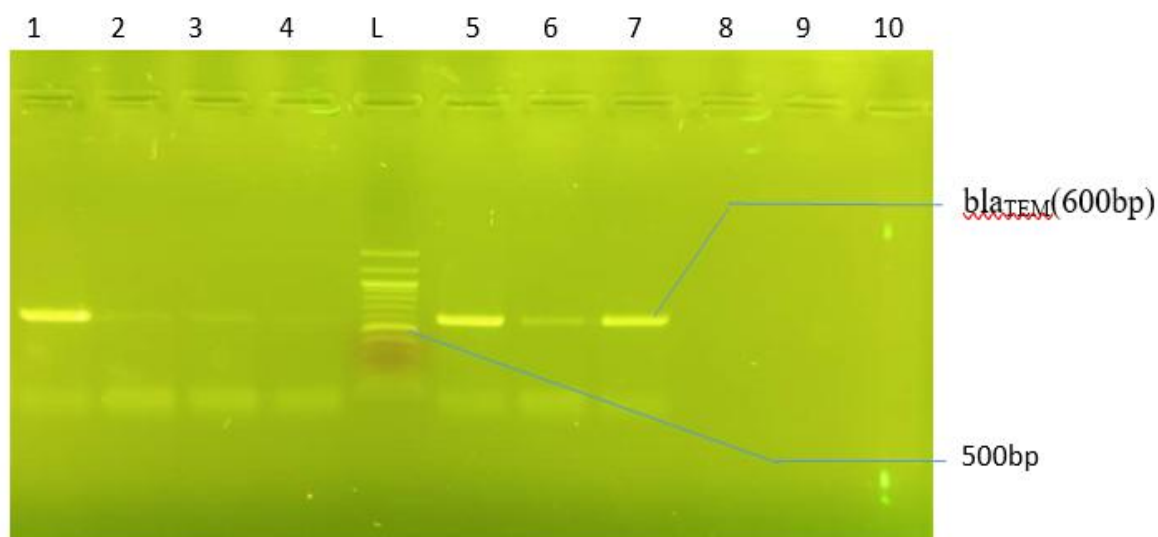


Figure 2: Agarose gel electrophoresis showing the blaTEM gene bands.

## IX. DISCUSSION

The result of the study shows growth of Pathogenic bacteria in locally produced Bread, Zobo, Agidi, Meat pie, Akara, and Soya milk in Umudi Nkwere. Globally, regulatory agencies (such as the US FDA, USDA, EU, and WHO) enforce strict microbiological criteria to manage Salmonella risk and other pathogens. According to National Center for Biotechnology Information, the universal regulatory

standard for pathogens in foods is zero tolerance in Ready-to-Eat (RTE) foods, therefore all the food in this study is unhealthy except common Nigeria food called Akpu..

The pathogenic bacteria isolated in this study are Staphylococcus aureus, Shigella spp and Salmonella spp from Bread, Zobo, Agidi, Meat pie, Akara and Soya milk, but none were isolated from Akpu. According to Ezech-Ernest et al., (2017), the presence

of the pathogens in human food is predominantly a public health hazard caused by exogenous contamination, poor processing hygiene, and inadequate temperature control. The whole result in this study displayed great support to Akeem et al., (2012) that nutrient-rich, baked, or fried snacks are frequently contaminated with *S. aureus* and other pathogenic bacteria. According to Chigekwu Adanna in Online Repository of Michael Okpara University of Agriculture Umudike states despite Zobo drink being moderately acidic (which restricts some growth), *Bacillus cereus* and coliforms thrive in this drink. This usually results from unhygienic preparation water, unwashed raw materials, or prolonged storage at ambient temperatures.

The findings shows a classic Antimicrobial Susceptibility Profile, the isolated bacteria demonstrate sensitivity to powerful broad-spectrum drugs (especially cephalosporin and fluoroquinolones) but show resistance to several common penicillin-derivatives, macrolides, and aminoglycosides in table 4.3. Bacteria in Bread and Zobo (*Staphylococcus aureus*) are very sensitive to Ofloxacin, Peflacinen, Ciprofloxacin, Levofloxacin Fluoroquinolones class of antibiotics, Amoxicillin (Penicillin-class antibiotic) and resistant to Gentamycin, Augmentin and Azithromycin antibiotics. The bacteria in Akara and Agidi (*Shigella* spp) are sensitive to Ofloxacin, Ciprofloxacin, Peflacinen, Levofloxacin, Amoxicillin and resistant to Gentamycin and Augmentin antibiotics. Bacteria in Meat pie and Soya milk are sensitive to Ofloxacin, Gentamycin, Peflacinen, Levofloxacin, Amoxicillin, Ciprofloxacin, Levofloxacin, Cefuroxime and only resistance to Augmentin and Azithromycin. The Antimicrobial Susceptibility Profile in this study examplified Idise et al., (2021), bacteria that show both resistance and sensitivity, but resistant to two or more antibiotics are categorized as Multi-Drug Resistant (MDR). In food hygiene studies (such as those testing cooked beans pudding/Moi moi), Multi-Drug Resistant (MDR) isolates often mean that the food was prepared or stored in unhygienic conditions. When Multi-Drug Resistant (MDR) bacteria are found in locally produced foods, it generally points to poor hygiene during preparation or storage. Okafor et al., (2023) made the same observation and suggested

that the presence of multi-drug resistant (MDR) strains, likely acquired through poor hygiene, contaminated water, or food handling. The two resistance genes possess by the isolates are blaTEM gene and blaCTX-M. The blaTEM gene is a broad family of genes encoding beta-lactamase enzymes, which destroy the chemical structure of beta-lactam antibiotics (like penicillin and cephalosporins). The blaCTX-M gene is a specific, highly prevalent subfamily of bla genes that produces extended-spectrum beta-lactamases (ESBLs), conferring dangerous multidrug resistance. According to Shirin et al., (2021) many studies across various regions find blaTEM to be highly prevalent alongside blaCTX-M often co-existing within the same multidrug-resistant (MDR) isolates. The resistant genes detected in this study correlate with Maha et al., (2020) ‘that finding both blaTEM and blaCTX-M in the same bacterial isolates is a standard observation in clinical and environmental microbiology. This study and other studies show that a majority of ESBL-producing bacteria harbor multiple resistance genes simultaneously, making empirical treatment highly difficult without molecular testing’.

Food processors and handlers should note that safe food handling prevents foodborne illnesses by controlling bacteria and cross-contamination.

## X. CONCLUSION

Pathogens associated to locally produced foods and drinks in Umudi Nkwere in Imo State are *Salmonella* spp, *Shigella* spp and *Staphylococcus aureus*. They are all multi-drug resistant pathogens. The pathogens contain blaCTX-M and blaTEM resistant genes making them resistant to cefotaxime, ceftraxone, penicillin and early generation cephalosporin group of antibiotics.

## REFERENCE

- [1] Ababu, A., Endashaw, D., & Fesseha, H. (2020). Isolation and antimicrobial susceptibility profile of *Escherichia coli* O157:H7 from raw milk of dairy cattle in Holeta District, Central Ethiopia. *International*



- Journal of Microbiology*, 2020, 6626488. [Crossref](#)
- [2] Bilal, M., Mehmood, S., Rasheed, T., & Iqbal, H. (2020). Antibiotics traces in the aquatic environment: Persistence and adverse environmental impact. *Current Opinion in Environmental Science & Health*, 13, 68–74. [Crossref](#)
- [3] Bilal, M., Munir, H., & Iqbal, H. (2020). Potentialities of medicinal plant extracts against biofilm-forming bacteria. In A. Bakrudeen (Ed.), *Microbial Biofilms* (pp. 187–203). CRC Press, Boca Raton, FL, USA. [Crossref](#)
- [4] Bilal, M., Rasheed, T., Iqbal, H., Hu, H., Wang, W., & Zhang, X. (2017). Macromolecular agents with antimicrobial potentialities: A drive to combat antimicrobial resistance. *International Journal of Biological Macromolecules*, 103, 554–574. [Crossref](#)
- [5] Cecchini, M., & Lee, G. (2017). The healthcare and economic burden of antimicrobial resistance. *OECD Health Working Papers*. OECD Publishing.
- [6] Ndukwe, C. A. (2020). *Microbial contamination of locally prepared beverages sold in Umuahia (Zobo, Kunun and Soya Milk)*. Michael Okpara University of Agriculture, Umudike.
- [7] El-Baz, A., El-Sherbini, M., Abdelkhalek, A., & Al-Ashmawy, M. (2017). Prevalence and molecular characterization of *Salmonella* serovars in milk and cheese in Mansoura city, Egypt. *Journal of Advanced Veterinary and Animal Research*, 4(1), 45–51. [Crossref](#)
- [8] Ezech, E., Okeke, O., Ozuah, A. C., & Agbanelo, D. (2017). Bacteriological assessment of meat pie sold at Ochanja Market, Onitsha, Anambra State. *International Journal of Environment, Agriculture and Biotechnology*, 2(2), 767–770. [Crossref](#)
- [9] Getie, M., Abebe, W., & Tessema, B. (2019). Prevalence of enteric bacteria and their antimicrobial susceptibility patterns among food handlers in Gondar Town, Northwest Ethiopia. *Antimicrobial Resistance & Infection Control*, 8, 111. [Crossref](#)
- [10] Haile, A. F., Alonso, S., Berhe, N., Atoma, T. B., Boyaka, P. N., & Grace, D. (2022). Prevalence, antibiogram, and multidrug-resistant profile of *E. coli* O157:H7 in retail raw beef in Addis Ababa, Ethiopia. *Frontiers in Veterinary Science*, 9, 734896. [Crossref](#)
- [11] Haliu, D., Gelaw, A., Molla, W., Garedew, L., Cole, L., & Johnson, R. (2015). Prevalence and antibiotic resistance patterns of *Salmonella* isolates from lactating cows and in-contact humans in dairy farms, Northwest Ethiopia. *Journal of Environmental and Occupational Science*, 4(4), 171–178.
- [12] Lobacz, A., & Zulewska, J. (2021). Fate of *Salmonella* spp. in the fresh soft raw milk cheese during storage at different temperatures. *Microorganisms*, 9(5), 938. [Crossref](#)
- [13] Manishimwe, R., Moncada, P., Bugarel, M., Scott, H., & Loneragan, G. (2021). Antibiotic resistance among *Escherichia coli* and *Salmonella* isolated from dairy cattle feces in Texas. *PLoS ONE*, 16(5), e0242390. [Crossref](#)
- [14] Mariam, S. H. (2021). Isolation and characterization of Gram-negative bacterial species from pasteurized dairy products: Potential risk to consumer health. *Journal of Food Quality*, 2021, 8876926. [Crossref](#)
- [15] Okafor, A., Aleruchi, O., & Robinson, V. (2023). Antibiotic susceptibility pattern of bacteria isolated from stored water in some residential homes. *International Journal of Pathogen Research*, 12(3), 27–34. [Crossref](#)
- [16] Rafiq, A. (2022). Emergence and spread of antibiotic-resistant foodborne pathogens along the food chain: Trends, mechanisms, and global implications. *Journal of Food Safety and Foodborne Diseases*, 14(3), 210–225.
- [17] Rahman, M. A., Rahman, A. K., Islam, M. A., & Alam, M. M. (2018). Detection of multi-drug resistant *Salmonella* from milk and meat in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 16(1), 115–120. [Crossref](#)

- [18] Reta, M. A., Bereda, T. W., & Alemu, A. N. (2016). Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *International Journal of Food Contamination*, 3, 4. [Crossref](#)
- [19] Singh, A., & Zymo Research. (2022). *Quick-DNA Fungal/Bacterial Miniprep Kit instruction manual* (Version 1.2). Zymo Research.
- [20] Verraes, C., Van Boxtael, S., Van Meervenne, E., Van Coillie, E., Butaye, P., Catry, B., de Schaetzen, M. A., Van Huffel, X., Imberechts, H., Dierick, K., Daube, G., Saegerman, C., De Block, J., Dewulf, J., & Herman, L. (2013). Antimicrobial resistance in the food chain: A review. *International Journal of Environmental Research and Public Health*, 10(7), 2643–2669. [Crossref](#)
- [21] World Health Organization. (2022). *2021 Antibacterial agents in clinical and preclinical development: An overview and analysis*. World Health Organization.
- [22] World Health Organization. (2024). *The burden of foodborne diseases worldwide*. World Health Organization.
- [23] Shen, Z. (2025). DNA extraction with Zymo Quick-DNA™ Fungal/Bacterial Miniprep Kit v1. *Protocols.io*. [Crossref](#)