

# Prevalence and Antibacterial Susceptibility Pattern of *Escherichia Coli* in Fermented Cow Milk Collected from Selected Markets in Maiduguri, Borno State.

<sup>1</sup>Jarafu Umar Ngunupu, <sup>2</sup>Nasiru Adoyiza Usman, <sup>3</sup>Emeka Walter Ndubuisi, <sup>4</sup>Wadai Smith, <sup>5</sup>Isaac Geoffrey Gwary, <sup>6</sup>Apeh Enoch Odekina, <sup>7</sup>Uchechukwu Chisom Chimezie, <sup>8</sup>Desmond Senguro

<sup>1,2</sup> Department of Pharmaceutical Microbiology, University of Maiduguri, Maiduguri

<sup>3,4,5,7</sup> Department of Pharmacology and Toxicology, University of Maiduguri, Maiduguri

<sup>6</sup> Department of Pharmacology and Toxicology University of Igbinedion, Okada.

<sup>8</sup> Department of Pharmaceutical Chemistry, University of Maiduguri, Maiduguri.

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## ABSTRACT

*Escherichia coli* is an enteric food borne pathogen associated with life-threatening disease conditions. The enterobacteria are frequently found in the cattle gastrointestinal tract with a high potential of contaminated animal products such as milk, meat, and cheese. A cross-sectional study was conducted to investigate the presence of *Escherichia coli* in fermented milk products sold within Maiduguri, Borno State. Thirty (30) samples were collected from different sources. Bacteriological isolation and biochemical characterization yielded *Escherichia coli* and the prevalence of *E. coli* was 3.33 % (2/60). Antibacterial susceptibility test was carried out and the bacteria were sensitive to ciprofloxacin (17 mm) and gentamycin (21 mm), while the remaining antibiotics were non sensitive, i.e. cephalixin, cefoxitin and suphamethoxazole-trimethoprim. Based on EUCAST the isolates were resistant to all the antibiotics except gentamycin. The result revealed the presence of *Escherichia coli* in the fermented cow milk samples. The finding indicates possible faecal contamination of the milk product with serious public health consequences. This necessitates the need to screen other milk products in the area. Health authorities in the state need to enlighten dairy product farmers on the zoonotic potential of *Escherichia coli*.

**Key words:** *Escherichia coli*, Fermented milk, Antimicrobial susceptibility, Foodborne pathogens, Public health

## INTRODUCTION

Foods made from or containing the milk of mammals are referred to as dairy goods or milk products. Mammals like cattle, water buffaloes, goats, sheep, camels, and humans are the main source of them. Dairy goods include foods like cheese, butter, and yogurt. A dairy, or dairy factory, is a facility that manufactures dairy products. (Joseph F. Sullivan Center, 2020). Except for regions of central Africa and the majority of East and Southeast Asia, dairy products are consumed all over the world.

Since milk contains significant macro- and micronutrients, it is a popular beverage that is vital to the diets of millions of people worldwide. Due to its composition, milk is known to be beneficial during children and adolescence; yet, its comparatively large concentration of saturated fat raises concerns about possible negative consequences, including on the cardiovascular system. (Visioli & Strata, 2014).

Milk contains all of the necessary nutrients and several anabolic hormones because it is naturally used to feed and support the growth of newborn mammals. In order to meet nutritional needs for calcium and lower the risk of bone fractures, the recommended consumption level has been justified. However, there are worries about the potential negative health effects and the lack of evidence supporting the health benefits of consuming large

amounts of milk products. Consequently, a thorough evaluation of the contribution of dairy consumption to human nutrition and disease prevention is necessary (Haug et al., 2007). In addition to lactose, the primary milk sugar, milk also includes vital amino acids, lipids, vitamins, minerals, and proteins (caseins, whey proteins, and minor proteins). Thus, its use may have played a role in the centuries-long success of human evolution. But in addition to being extremely nourishing for people, milk is also a great place for microbes to develop (Fasce et al., 2020). A vast and diverse variety of bacteria may grow in milk because of its high nutritional content. As a result, in addition to its natural microbiota, a wide variety of additional microorganisms that come from the udder skin, teat canal, milking equipment, tanks, and storage containers all of which represent the farm and pasture environments may colonize the milk as soon as it is milked.

Scientific classification of *Escherichia coli*:

Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	<i>Escherichia</i>
Species:	<i>E. coli</i>

Gram-negative, rod-shaped, flagellated, facultative anaerobic, and nonsporulating, *Escherichia coli* is a member of the enterobacteriaceae family of bacteria. Enterotoxigenic *Escherichia coli* (ETEC), attaching and effacing *Escherichia coli* (AEEC), enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *Escherichia coli* (EHEC), and Shiga-toxin producing *Escherichia coli* (STEC or VTEC) are some of the classifications for this bacterium based on its virulence factor, (Holko et al., 2006; karch et al., 2005; Frohlicher et al., 2008; Solomakos et al., 2009; Wang et al., 2010;).

The bacteria has been the subject of much research for more than 60 years and is simple and affordable to grow and cultivate in a lab setting. The majority of *Escherichia coli*'s chemically defined mediums use carbon as an energy source, making them chemoheterotrophs (Tortora et al., 2010).

*Escherichia coli* is a member of the Enterobacteriaceae, a broad family of gram-negative rods (Cheesebrough, 2005). German pediatrician Theodor Escherichia initially identified this species in the feces of healthy people in 1885. Because it is found in the colon, he named it bacterium coli commune. It was reclassified as *Bacillus coli* after bacterial taxonomy was revised (Migula, 1985).

They naturally occur in warm-blooded creatures' lower gastrointestinal tracts (Singleton et al., 1999). Many of the more well-known diseases, including *Salmonella*, *Yersinia pestis*, *Klebsiella*, and *Shigella*, are members of the *Enterobacteriaceae*, a broad family of Gram-negative bacteria, along with numerous innocuous symbionts. *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter* are some of the other bacteria in this family that cause illness. In the phylum *Proteobacteria*, this family is the sole representative of the class *Gammaproteobacteria* within the order *Enterobacteriales* (Brenner et al., 2005). The innocuous strains, which are a typical component of the gut's flora, can help their hosts by generating vitamin K2 (Bentley et al., 1982) and preventing colonization of the intestine with pathogenic bacteria (Hudault et al., 2001). About 0.1% of the gut flora is made up of facultative anaerobes like *Escherichia coli*, (Russell et al., 2001) and the main way that harmful forms of the bacterium spread illness is by fecal oral transfer. Because cells have a short half-life outside the body, they can be used as indicator organisms to check environmental samples for fecal contamination (Feng et al., 2002).

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## Strains of *Escherichia coli*

One of the most researched bacterial species, *Escherichia coli* (*E. coli*), is well-known for its wide range of genetic and phenotypic variations (Kaper et al., 2004; Croxen et al., 2013). It can be found in a variety of settings, including soil, water, and the intestines of animals, including people. While commensal strains aid in digestion and the production of vitamin K, pathogenic strains are linked to illnesses such as intestinal infections, newborn meningitis, and urinary tract infections (Todar, 2020; Leimbach et al., 2013). Globally, foodborne outbreaks brought on by toxic *E. coli* have become more significant for public health (CDC, 2024). Diarrhea is a common sign of intestinal infections, and symptom profiles frequently reveal the type of pathogenic strain (WHO, 2023).

Intestinal *E. coli* infections are typically categorized into five major pathotypes:

- Enterotoxigenic (*E. coli*, ETEC)
- Enteropathogenic (*E. coli*, EPEC)
- Enteroinvasive (*E. coli*, EIEC)
- Enterohemorrhagic (*E. coli*, EHEC)
- Enteroaggregative (*E. coli*, EAEC)

(Kaper et al., 2004; Nataro & Kaper, 1998).

### Enterotoxigenic (ETEC) Strains

In underdeveloped nations, ETEC is a leading cause of infantile and traveler's diarrhea (Qadri et al., 2005). Contaminated food or water is the means of transmission, and after repeated exposure, immunity is developed. Secretory diarrhea is caused by ETEC's expression of colonization factor antigens (CFAs), which mediate adhesion to intestinal epithelial cells and release heat-labile (LT) and heat-stable (ST) enterotoxins (Gaastra & Svennerholm, 1996).

### Enteropathogenic (EPEC) Strains

In low-income nations, infantile diarrhea is still frequently caused by EPEC, which is transferred through contaminated water or fecal-oral pathways (Nataro & Kaper, 1998). Despite lacking LT and ST toxins, EPEC produces distinctive attaching-and-effacing (AE) lesions by forming localized microcolonies on intestinal epithelium (Dean et al., 2005). Watery diarrhea is brought on by these lesions, which interfere with intestinal absorption.

### Enteroinvasive (EIEC) Strains

Similar to *Shigella* species, EIEC causes sickness akin to dysentery by entering intestinal epithelial cells, proliferating intracellularly, and damaging host cells (Lan et al., 2004). Children in developing nations are primarily affected by infections, while tainted food has been implicated in outbreaks in developed areas (Sansone, 2001).

### Enterohemorrhagic (EHEC) Strains

One of the deadliest strains of *E. coli* is EHEC, which includes *E. coli* O157:H7. It is spread by contaminated vegetables, unpasteurized milk, and undercooked meat, and its infectious dose is minimal (CDC, 2024). Shiga toxins (Stx1, Stx2), which are produced by EHEC, prevent protein synthesis, kill epithelial cells, and cause hemolytic uremic syndrome (HUS) and bloody diarrhea (Tarr et al., 2005). Because *E. coli* O157:H7 cannot ferment sorbitol, it produces colorless colonies on sorbitol-MacConkey agar, allowing for quick laboratory screening (March & Ratnam, 1986).

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## Enteroaggregative (EAEC) Strains

EAEC strains generate biofilms without causing noticeable lesions by adhering to intestinal mucosa in a stacked-brick pattern (Nataro *et al.*, 1995). In children and immunocompromised patients, they are associated with chronic watery or mucoid diarrhea (Harrington *et al.*, 2006).

Uropathogenic (*E. coli*, UPEC) and verotoxin-producing (*E. coli*, VTEC) strains are two more noteworthy *E. coli* pathotypes that are important contributors to systemic and urinary tract infections (Johnson & Russo, 2005).

## Characteristics of *Escherichia coli*

*Escherichia coli* is a facultatively anaerobic, Gram-negative rod that is a member of the Enterobacteriaceae family (Madigan *et al.*, 2022). Usually found as solitary rods, cells are 1.1–1.5 µm wide and 2–6 µm long (Tortora *et al.*, 2021). They exhibit both fermentative and respiratory metabolisms and are oxidase-negative but catalase-positive. Peritrichous (lateral) flagella allow many strains to move, although there are other non-motile varieties (Madigan *et al.*, 2022).

Furthermore, *E. coli* expresses proteinaceous appendages called fimbriae and pili, which facilitate adherence to host tissues and surfaces and aid in colonization and pathogenicity (Proft & Baker, 2009). *E. coli* is a pathogen of significant medicinal importance as well as a model organism in biotechnology due to these structural characteristics and metabolic adaptability (Leimbach *et al.*, 2013).

The cell wall of *Escherichia coli* contains strain-specific O lipopolysaccharide antigens (175 O antigens are now known), as well as flagella or H antigens, if they are present (56 H kinds are known). Additionally, there are eighty distinct capsular polysaccharide (K) antigens. Although only the O and H types are typically given, such as *E. coli* O157:H7, *Escherichia coli* are serotyped depending on the combination of O, H, and K antigens. *E. Coli* serotyping is a valuable epidemiological tool when combined with molecular and phage typing (Bat, 2014).

Lactate, succinate, ethanol, acetate, and carbon dioxide are all products of mixed-acid fermentation, which *Escherichia coli* employs in anaerobic environments and can survive on a broad range of substrates. When *Escherichia coli* coexists alongside hydrogen-consuming species like methanogens or sulphate-reducing bacteria, the hydrogen levels must be low since several routes in mixed-acid fermentation produce hydrogen gas. (Madigan *et al.*, 2006).

*Escherichia coli* grows best around 37 °C (98.6 °F), while certain lab strains can grow at temperatures as high as 49 °C (120 °F). Any medium that contains glucose, ammonium phosphate, monobasic, sodium chloride, magnesium sulfate, potassium phosphate, dibasic, and water is suitable for *Escherichia coli* growth, as is lysogeny broth. Using a wide range of redox pairs, including as the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of substrates like oxygen, nitrate, fumarate, dimethylsulfoxide, and trimethylamine N-oxide, growth can be fueled by either aerobic or anaerobic respiration (Fortadar *et al.*, 2005).

## Isolation of *Escherichia coli*

Numerous clinical specimens, such as urine, pus, feces, cerebral fluid (especially in newborns), endocervical swabs, high vaginal swabs, and blood cultures, can be used to isolate *Escherichia coli* (Cheesbrough, 2010; Forbes *et al.*, 2022). The presence of *E. coli* in sterile bodily locations like blood, cerebrospinal fluid, or urine usually signifies infection because it is a frequent commensal bacterium in the digestive system (WHO, 2023). To avoid contamination and preserve bacterial viability during isolation, aseptic sample collection and suitable transport media are essential (Collee *et al.*, 1996). To differentiate *E. coli* from other Enterobacteriaceae, selective and differential media are used for fecal and enteric samples (Cappuccino & Sherman, 2018).

### Identification of *Escherichia coli*

Based on its biochemical and cultural traits, *Escherichia coli* is initially identified. Blood agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar are the first media on which clinical specimens suspected of containing enteric Gram-negative rods are inoculated (Forbes et al., 2022).

Lactose fermentation and acid generation cause *E. coli* to develop pink to crimson colonies on MacConkey agar, but non-lactose fermenters show up colorless (Madigan *et al.*, 2022). Strong acid production causes the dyes eosin and methylene blue to precipitate, giving *E. coli* colonies on EMB agar a characteristic metallic green sheen (Tortora *et al.*, 2021). In clinical microbiology labs, these differential reactions serve as important markers for presumptive identification.

Biochemical identification further confirms the organism. Distinguishing features of *E. coli* include:

1. Production of indole from tryptophan, detected by Kovac's reagent (Cappuccino & Sherman, 2018).
2. Positive lysine decarboxylase activity, differentiating *E. coli* from other coliforms (Forbes et al., 2022).
3. Ability to utilize acetate as a sole carbon source, reflecting its metabolic versatility (Madigan et al., 2022).
4. Motility due to peritrichous flagella, although some clinical strains may be non-motile (Tortora et al., 2021).

### Pathogenicity of *Escherichia coli*

Viral strains of *Escherichia coli* can cause a variety of illnesses in both domestic animals and people. UTIs, newborn meningitis, and gastroenteritis are common human infection manifestations (Todar, 2007; Kaper *et al.*, 2004). Furthermore, especially in immunocompromised people, *E. coli* is linked to more serious systemic infections like gram-negative pneumonia, septicemia, peritonitis, and mastitis (Forbes et al., 2022; Donnenberg, 2015).

Because they produce strong toxins or have virulence characteristics that improve adhesion, invasion, or immune evasion, certain strains of *Escherichia coli* are extremely harmful. Hemorrhagic colitis and hemolytic uremic syndrome (HUS), a dangerous illness marked by hemolytic anemia, thrombocytopenia, and renal failure, can be brought on by Shiga toxin-producing *E. coli* (STEC), which includes serotypes O157:H7, O104:H4, O26, O103, O111, O121, O145, and O104:H21 (Kaper *et al.*, 2004; Tarr *et al.*, 2005; WHO, 2023).

The *E. coli* O157:H7 strain is a prominent example; it caused the June 2011 outbreak in Europe, which resulted in hundreds of illnesses and multiple deaths from complications mediated by Shiga toxin (Frank *et al.*, 2011). The outbreak brought attention to the dangers that contaminated *E. coli* poses to public health and emphasized the significance of food safety protocols and surveillance (WHO, 2023).

The acquisition of virulence genes through horizontal gene transfer is a major factor in the pathogenic potential of *E. coli* strains. This allows the bacteria to colonize a variety of host niches and cause illnesses ranging from mild diarrhea to infections that can be fatal (Nataro & Kaper, 1998; Todar, 2007).

### Medical importance of *Escherichia coli*

In medicine, probiotics such as the non-pathogenic *Escherichia coli* strain Mutaflor and the *E. coli* O83:K24:H31 strain Colinfant are used mostly to treat inflammatory bowel disease and other gastroenterological conditions (Grozdanov *et al.*, 2004). In contemporary industrial microbiology and biological engineering, *Escherichia coli* is crucial (Lee, 1996). Biotechnology was founded on the work of Stanley Norman Cohen and Herbert Boyes in *Escherichia coli*, who used plasmids and restriction enzymes to produce recombinant DNA (Russo, 2003). The production of human insulin by manipulating *Escherichia coli* was one of the earliest practical uses of recombinant DNA technology (Cornelis, 2000). In microbiology research, *Escherichia coli* is also employed as a model organism. Using *Escherichia coli* as a model bacterium, Joshua Lederberg and Edward Tatum initially



identified the phenomenon known as bacterial conjugation in 1946 (Lederberg *et al.*, 1946). It continues to be the major paradigm for conjugation research.

### **Disease by *Escherichia coli***

*Escherichia coli* is a rod-shaped, flagellated, gram-negative bacterium. Even though it is a vital part of the bacterial gut flora, illnesses can be brought on by intestinal bacteria spreading to other organs (cystitis, pneumonia) or by direct ingestion of pathogenic *Escherichia coli* subtypes (contaminated food, for example). For example, enterohemorrhagic *Escherichia coli* (EHEC) can cause hemolytic-uremic syndrome (HUS) and severe colitis, especially in young children and newborns.

### **Genetic basis of *Escherichia coli* resistance to antibiotics**

The issue of antibiotic resistance is getting worse. The use of antibiotics as growth promoters in animal feeds is likely responsible for some of this, but human abuse of antibiotics is also a contributing factor (Johnson *et al.*, 2006). The rate of adaptative mutations in *Escherichia coli* is "on the order of 10<sup>-5</sup> per genome per generation, which is 1,000 times as high as previous estimates," according to a study published in the Journal Science in August 2007. This finding may be important for the investigation and treatment of bacterial antibiotic resistance (Perfeito *et al.*, 2007). Through a mechanism known as horizontal gene transfer, antibiotic-resistant *Escherichia coli* can also transfer the genes that cause antibiotic resistance to other bacterial species, including *Staphylococcus aureus*. *Escherichia coli* frequently possess several drug-resistant plasmids, which they can easily spread to other species when they are under stress. Plasmids from and to other bacteria can be accepted and transferred by *Escherichia coli* due to species mixing in the intestines. Accordingly, *Escherichia coli* and other Enterobacteria are significant sources of antibiotic resistance that can be transferred (Salyers *et al.*, 2004).

Since types of bacteria that manufacture extended-spectrum beta-lactamases have become more prevalent in recent decades, resistance to beta-lactam antibiotics has become a specific concern. Many, if not all, of the cephalosporins and penicillin's are rendered ineffective as treatments by these beta-lactamase enzymes (Paterson *et al.*, 2005).

### **Genetic Origin of Drug Resistance**

#### **(i) Chromosomal Resistance**

Spontaneous mutations at chromosomal regions that control bacterial susceptibility to antimicrobial drugs give rise to chromosome-mediated resistance (Brooks *et al.*, 2022; Forbes *et al.*, 2022). In the presence of the antimicrobial medication, which inhibits susceptible bacterial populations and promotes the growth of resistant mutants, these mutations are chosen for (Prescott *et al.*, 2020).

The *rpoB* gene, which codes for the  $\beta$ -subunit of RNA polymerase, is mutated in spontaneous chromosomal mutations, which contribute significantly to resistance to several medications, including rifampicin, even if they are not the most common source of clinical drug resistance (Davies & Davies, 2010). Furthermore, bacteria may become resistant to  $\beta$ -lactam antibiotics like cephalosporins and penicillin's due to chromosomal abnormalities that cause the loss or modification of penicillin-binding proteins (PBPs) (Leclercq & Courvalin, 2002).

#### **(ii) Extrachromosomal Resistance**

##### **(a) Plasmid-Mediated Resistance**

Extra-chromosomal genetic elements called plasmids, which frequently contain genes encoding antimicrobial resistance, are involved in plasmid-mediated resistance (Davies & Davies, 2010). In clinical practice, plasmid-mediated resistance is more prevalent than chromosomal resistance (Prescott *et al.*, 2020). Multi-drug resistance (MDR) is a condition whereby resistance plasmids, or R factors, carry numerous genes that give resistance to multiple medications at the same time (Todar, 2020).

For instance, genes conferring resistance to tetracyclines, sulfonamides, streptomycin, and chloramphenicol may be found on a single plasmid (Levin *et al.*, 2017). Enzymes like  $\beta$ -lactamases, which hydrolyze penicillins and cephalosporins'  $\beta$ -lactam ring and eliminate their antibacterial properties, can be encoded via plasmid-borne genes (Forbes *et al.*, 2022).

### **(b) Transposon-Mediated Resistance**

The use of transposons, or mobile genetic elements, is another extrachromosomal resistance strategy. These DNA segments have the ability to transfer resistance genes between bacteria by moving between plasmid and chromosomal DNA (Frost *et al.*, 2005). Thus, resistance features may spread when the genetic material causing antimicrobial resistance in a donor cell is transferred to a susceptible recipient cell (Brooks *et al.*, 2022).

The intercellular transfer of resistance genes occurs through several mechanisms:

- **Conjugation:** The direct, frequently plasmid-mediated transfer of genetic material between bacterial cells through physical contact. Antimicrobial resistance spreads primarily through this route (Levin *et al.*, 2017).
- **Transduction:** The process by which bacteriophages (phages) move bacterial DNA from one cell to another. The transfer of resistance genes, like those encoding  $\beta$ -lactamase, may be mediated via this pathway (Todar, 2020).
- **Transformation:** A spontaneous process wherein a bacterial cell integrates donor genetic material, possibly including resistance determinants, by directly absorbing free DNA from its surroundings (Prescott *et al.*, 2020).

## **Non-Genetic Origin of Drug Resistance**

### **(i) Metabolic Inactivity**

Due to metabolic inactivity rather than genetic changes, several microbes display resistance to antimicrobial medications (Brooks *et al.*, 2022). Since many antibacterial medications only work on cells that are actively dividing, latent or non-replicating cells develop temporary resistance (Levin *et al.*, 2017). For instance, Mycobacterium TB "persister cells" can endure in host tissues for years without proliferating. Antimicrobials have no effect on them while they are in this dormant state, but once their metabolic activity resumes, they become susceptible again (Zhang *et al.*, 2012).

### **(ii) Loss of Specific Target Structures**

The loss or modification of target structures that the antimicrobial drug works upon can also result in microbial resistance (Prescott *et al.*, 2020). For example, certain susceptible bacteria may change into cell wall-deficient forms (L-forms) during penicillin therapy, making them resistant to inhibitors of cell wall synthesis such as cephalosporins and penicillin's (Brooks *et al.*, 2022). Susceptibility to these antibiotics is regained after the bacteria return to their typical shape.

## **Antibiotic used against *Escherichia coli***

Bacterial infections are frequently treated with antibiotics or antimicrobial medicines. Penicillin's (such as ampicillin and amoxicillin), cephalosporins (such as cefuroxime, cefotaxime, and ceftriaxone), aminoglycosides (such as streptomycin and gentamycin), fluoroquinolones (such as ciprofloxacin, levofloxacin, and norfloxacin), nitrofurantoin, and cotrimoxazole are among the classes of antibiotics frequently used to treat infections brought on by *Escherichia coli*.

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## Penicillin

A class of bactericidal antibiotics known as penicillin's prevents the production of cell walls. Certain penicillin's have a limited range of action (i.e. Penicillin's work well against gram-positive bacteria like staphylococci and streptococci. Additionally, they exhibit efficacy against gram-negative bacteria, including salmonella, Shigella, hemophilia, protease, and pseudomonas (Prescott, 2005).

## Cephalosporin

Similar to penicillin's, cephalosporins are a class of antibiotics. Additionally, during the formation of peptidoglycans, cephalosporin suppresses the transpeptidase process. They are extensively categorized into generations and have a wide range of characteristics. Cefazolin, cephalexin, and cefadroxil are examples of first-generation cephalosporins that work better against gram-positive bacteria than gram-negative ones. Cefuroxime, cefprozil, and cefoxitin are examples of second-generation cephalosporins that exhibit enhanced effectiveness against gram-negative bacteria with some anaerobic coverage. With increased action against Enterobacteria and pseudomonas, the third generation is more powerful than the second (Prescott, 2005).

## Aminoglycosides

Gram-negative rods are specifically targeted by aminoglycosides, which are bactericidal antibiotics. Some aminoglycosides are used to treat other organisms; for example, gentamicin is used in conjunction with penicillin G to treat enterococci, while streptomycin is used to treat tuberculosis. Aminoglycosides work through two key mechanisms: misreading of messenger RNA (mRNA) and suppression of the initiation complex. Gentamycin, Streptomycin, Amikacin, and Neomycin are among the aminoglycosides. When treating confirmed or suspected gram-negative infections, particularly those caused by *P. aeruginosa*, *Enterobacter*, *Klebsiella*, *Serratia*, and other species resistant to less toxic antibiotics, such as urinary tract infections, bacteremia, infected burns, osteomyelitis, pneumonia, peritonitis, and otitis, aminoglycosides are commonly used in conjunction with penicillin's or cephalosporins (Prescott, 2005).

## Fluoroquinolones

Nucleic acid synthesis is inhibited by these significant antibiotics. A growing number of infections are being treated with them. Topoisomerase II and bacterial DNA gyrase are inhibited by quinolones. They are antibiotics with a broad spectrum. Enteric bacteria like *Klebsiella pneumoniae* and *Escherichia coli* are effectively combatted by fluoroquinolones. They can be applied to gram-negative infections such as *Haemophilus* and *pseudomonas aeruginosa*. *Staphylococcus aureus* and *Streptococcus pyogenes* are two gram-positive bacteria that they are also effective against. Fluoroquinolones include, for example, pefloxacin, ciprofloxacin, levofloxacin, norfloxacin, and nalidixic acid (Prescott, 2005).

## Chloramphenicol

As a bacteriostatic antibiotic, chloramphenicol works by preventing the creation of new proteins. By blocking the bacterial ribosome's peptidyl transferase function, they stop proteins from elongating. A broad-spectrum antibiotic, chloramphenicol is effective in treating a variety of bacterial infections, particularly those caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Hemophilus influenzae* (Prescott, 2005).

## Nitrofurantoin

Since 1953, nitrofurantoin has been used to treat urinary tract infections (UTI). Currently, it is used to treat simple UTIs and prevent UTIs in those who are at risk of getting them again. Interest in employing nitrofurantoin has grown as a result of growing bacterial antibiotic resistance to other widely used drugs, including fluoroquinolones and trimethoprim/-sulfamethoxazole (Garau, 2008). Nitrofurantoin is one of the first-line treatments for simple UTIs due to its effectiveness in treating the condition and the low rate of bacterial resistance to it (Gupta *et al.*, 2011).



One of the few medications frequently used to treat UTIs during pregnancy is nitrofurantoin (Lee *et al.*, 2008). The medication should not be administered to women in the latter stages of pregnancy because it may cause oxidative damage to the red blood cells and increase the risk of hemolytic anemia in the unborn child, who does not yet have the enzyme pathways required for glutathione metabolism. Neonatal jaundice was more likely to occur in babies whose mothers took this medication late in pregnancy (Nordeng *et al.*, 2013).

### **Cotrimoxazole**

Co-trimoxazole, also known as trimethoprim/sulfamethoxazole (TMP/SMX), is an antibiotic used to treat a range of bacterial and protozoal illnesses. It works as a folate antagonist by preventing the synthesis and metabolism of folate. Trimethoprim's effects result in a backlog of dihydrofolate (DHF), which can counteract the drug's inhibitory effect on tetrahydrofolate biosynthesis. Sulfamethoxazole is used to get rid of the excess DHF by stopping its synthesis in the first place (Wormser *et al.*, 1982).

In treating bacterial infections, co-trimoxazole was said to be more successful than each of its constituents alone; however, this was later called into question. In many countries, its usage has been limited to relatively specific situations where its greater efficacy has been established because to its higher prevalence of adverse effects, particularly allergic responses (Brumfit *et al.*, 1993).

## **MATERIALS AND METHOD**

### **Culture media**

- Peptone saline diluents (PSD)
- Nutrient agar
- MacConkey agar
- Eosin methylene blue (EMB)
- Moeller Hinton agar.

### **Reagents used**

Indole, urease, citrate, Normal saline.

### **Solvents and disinfectants used**

Dettol soap, Dettol liquid, distilled water.

### **Antibiotics used**

The panel of five antimicrobial agents [Ciprofloxacin, Gentamycin, Cephalothin, Cefoxitin, Sulphamethoxazole/Trimethoprim]—was strategically selected to represent the different mechanistic classes of antibiotics, including agents commonly used for the empirical treatment of gastrointestinal and diarrheal infections associated with *E. coli* in both veterinary and human public health sectors within the Maiduguri region.

## **METHODOLOGY**

### **Collection and labelling of samples**

A total of thirty (60) Dairy product (nono) samples were collected from different vendors across Maiduguri metropolis. These were labelled accordingly.

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## Serial dilution

A serial dilution is any dilution in which the concentration decreases by the same factor in each successive step. In this research work, peptone saline diluents (PSD) was prepared by autoclaving a mixture of 1g of peptone water and 8.5g of NaCl dissolved in 1L of distilled water. 45ml of the diluent was pipetted into each of thirty sterilized bottles each containing 5ml of the nono samples, mixed thoroughly to form homogenates 1 to 20 (of 1:10 dilutions). Next, 5 ml of each of the homogenates was measured into another thirty sterile bottles each containing 45 ml of peptone saline diluent, mixed thoroughly to form the second set of homogenates (of 1:100 dilutions).

## Media preparation

Each medium was accurately weighted and dissolved in an appropriate quantity of distilled water, according to the manufacturer's specification; heated on a hot plate till the agar powder melted, and the medium was sterilized in an autoclave at 121°C for 15 minutes,

## Plating

On freshly prepared nutrient agars, the plating (inoculation) of the homogenates, both 1:10 and 1:100 was carried out by using a sterilized wire loop to pick drops of the homogenates and carefully streaking on the surfaces of the agar. The plates were labelled accordingly and incubated at 37°C for 24 hours.

## Isolation of *Escherichia coli*

On freshly prepared MacConkey agar plates, colonies from the nutrient agar plates were inoculated by using a sterilized wire loop. These were also labelled accordingly and incubated at 37°C for 24 hours. Then the growth on MacConkey agar which shows pinkish reddish colouration which signifies that gram negative organism is present, was then inoculated on freshly prepared EMB (eosin methylene blue) to identify *Escherichia coli* from the colonies present. *Escherichia coli* will give a greenish metallic sheen of the plate.

## Biochemical test

Biochemical tests were carried out based on Gram reactions. Among the tests carried out were indole, citrate, and methyl red.

### Indole test

Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have tryptophanase. The test was carried out by inoculating one loopful of each test isolate separately into pre-sterilized Bijou bottles containing 3 ml of tryptone water. These were incubated at 37°C for 48 h after which 0.5 ml Kovac's reagent was added. The set up was examined by shaking after 1 min. A red colour at the interphase was indicative of indole production.

### Citrate test

This test uses a media, in which sodium citrate is the only source of carbon and energy. The medium used was Simmon's medium. The medium was prepared according to the manufacturer's instruction into Bijou bottles and autoclaved at 120°C for 15 minutes. The bottles are allowed to solidify as slanting slopes. They were inoculated with cultures of the isolates and incubated for 24 h at 37°C. It is positive when it is blue and negative when it appears green.

### Methyl red

The methyl red (MR) test detects the production of sufficient acid during the fermentation of glucose and some bacteria have the ability to utilize glucose and convert it to stable acid like lactic acid, acetate or formic acid as the end product. Inside a test tube the microorganism to be tested was inoculated inside the test tube containing

5ml distilled water and peptone water, after 24 hours when the organism grows inside the tube, 2-3 drop of methyl red was drop inside the tube and it form a reddish ring which indicate positive

### Antibiotic Susceptibility Test

The antibiotic susceptibility of isolates to commonly used antibiotics was determined using modified Kirby-Bauer technique as modified by Clinical and laboratory standard institute, (CLSI, 2014). The cultures were standardized by transferring four to five similar colonies of overnight culture with sterile wire loop into separate test tubes containing 5ml normal saline. The turbidity was adjusted to match that of McFarland 0.5, sterile plates of Mueller-Hinton agar (MHA) were prepared according to the manufacturer's instruction. Surface of dried agar media were inoculated with the standardized cultures. After letting the plates dry for five minutes at room temperature, forceps were used to equally put antibiotic sensitivity discs on the plates' dried surface. The plates were incubated for eighteen hours at 37°C in order to stop the development of bacteria while the antibiotics diffused. The CLSI (2014) guideline was used to measure and interpret the zones of inhibition's sizes (Cheesbrough, 2005).

The five different antibiotics used for the sensitivity testing include:

1-Gentamycin (30µg)

2-Ciprofloxacin (5µg)

3-Cefoxitin (30µg)

4-Cephalothin(30µg)

5-Sulphamethoxazole/Trimethoprim(25µg)

### Determination of MAR (Multiple Antibiotic Resistance) Index

The MAR Index of an isolate is defined as  $a/b$ , where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the number of antibiotics to which the isolate was subjected (Jayaraman *et al.*, 2012). This was calculated for isolates that tested positive for *Escherichia coli*.

## RESULT, DISCUSSION AND CONCLUSION

### Characteristic appearance of the isolated *Escherichia coli*

Out of the 60 nono samples dilutions plated on the nutrient agar plates all plates yielded bacterial growth. On inoculation onto MacConkey agar, a total of 21 plates yielded pinkish colony formation, suggesting the possible presence of *Escherichia coli* or other coliforms. The colonies were inoculated on freshly EMB (eosin methylene blue) on which 2 plate shows greenish sheen which indicate the present of *Escherichia coli* and the rest showed mucoid jelly pinkish and turbid growth which indicate the present of *klebsiella spp.*

### Microbiological analysis conducted on nono samples

Below highlight the observations made from the isolation and biochemical tests conducted on the nono samples  $H_1^1-H_1^{30}$  and  $H_2^1-H_2^{30}$ . On nutrient agar which is a general purpose media, in all the sixty plate, they were distinctive colony growth on both the first and second homogenate. From there selected sample were inoculated on macconkey agar on which 21 plate shows pinkish reddish colouration. To further confirm e coli, the growth was inoculated on eosin methylene blue selective media to confirm e coli. Of which only two sample showed greenish metallic sheen which indicated that e coli is present, the reset of the plate showed pinkish mucoid jelly like, which indicate the presence of *klebsiella spp.*

Table 1: Appearance of various media used in the isolation and confirmation of *Escherichia coli*

NONO SAMPLE S	MACCONKE Y	EOSIN METHYLENE BLUE	BIOCHEMICAL TEST			INFERENCE
			INDOLE	CITRATE	METHYL RED	
H1 <sup>27</sup>	PINK COLONIES	GREENISH SHEEN COLOURATIO N	PINK RING FORMATIO N	NO COLOR CHANG E	REDISH RING FORMATIO N	PRESENCE OF <i>ESCHERICHIA COLI</i>
H2 <sup>27B</sup>	PINK COLONIES	GREENISH SHEEN COLOURATIO N	PINK RING FORMATIO N	NO COLOR CHANG E	REDISH RING FORMATIO N	PRESENCE OF <i>ESCHERICHIA COLI</i>
H2 <sup>26</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H1 <sup>30</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H1 <sup>3</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H2 <sup>3</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H1 <sup>26</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H1 <sup>17</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H1 <sup>17B</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H1 <sup>19</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS
H2 <sup>19</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATIO N	COLOR CHANG E	NO RING FORMATIO N	PRESENCE OF COLIFORMS

H1 <sup>6</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H2 <sup>6</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H2 <sup>22</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H2 <sup>24</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H1 <sup>24</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H1 <sup>25</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H2 <sup>25</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H1 <sup>20</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H1 <sup>28</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS
H1 <sup>29</sup>	PINK COLONIES	PINKISH MUCOID COLOUR	NO RING FORMATION	COLOR CHANGE	NO RING FORMATION	PRESENCE OF COLIFORMS

### Result for antibiotic susceptibility test

Table 2 shows the zones of inhibition (mm) by the various antibiotics used against the *Escherichia coli* A

ANTIBIOTIC (WITH ITS CONCENTRATION)	ZONE OF INHIBITION (mm)
CIPROFLOXACIN (5 µg)	17
CEPHALOTIN (30 µg)	0
GENTAMYCIN (30 µg)	21
SUPHAMETHOXAZOLE-TRIMETHOPRIM (25 µg)	0



CEFOXITIN (30 µg)	0
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As shown above, the isolate *Escherichia coli* A was resistant to three out of the five antibiotics used in test. The Multiple Antibiotic Resistance Index was determined using the formula,  $MARI = a/b$  Where, a is the number of antibiotics to which the isolate is resistant and b is the total number of antibiotics to which the test isolates have been evaluated for sensitivity. Therefore, the MARI of the *Escherichia coli* A is 0.6.

## DISCUSSION

Only 2 out of a total of 60 nono samples tested positive for *Escherichia coli*. And the rest of the sample were positive to klebsiella. This poses danger or danger signal to the public. It is noteworthy that the isolated *Escherichia coli* was susceptible to two out of the five antibiotics used in this work. These include Ciprofloxacin, Gentamycin and. High sensitivity to ciprofloxacin and gentamycin have been recorded from previous studies conducted in Nigeria (Wariso *et al.*, 2006) and India (Bharathi *et al.*, 2002). The presence of coliforms indicated contamination and the poor level of hygiene after processing, Coliforms are not supposed to be present in nono because of high temperature short time pasteurization and effective cleaning and good hygienic procedures. The presence of coliforms from this pose's great danger to the health of the consumers and suggest neglect on the part of the processors or the nono vendors. Coliforms are considered as normal flora of the intestinal tract of human and animals and their presence indicates direct faecal contamination. They have been used as indicator organisms for bacteriological quality of milk and its products (ICMSF). The level of presence of coliform and indicator organisms has been described as index of food hygiene. *Klebsiella spp.*, has been related to bacterial pneumonia cases more severe than those produced by *Streptococcus pneumonia* and urinary tract infection.

A key finding of our study is the high prevalence of resistance to sulphamethoxazole-trimethoprim (SXT) among the *E. coli* isolates from fermented cow milk. This pattern is particularly concerning given that SXT is an affordable and widely used first-line drug for treating various bacterial infections, including diarrheal diseases, in settings like Maiduguri. This widespread resistance, therefore, carries significant public health implications, suggesting that empirical SXT treatment for infections potentially originating from contaminated food sources, such as this locally fermented milk, may face high rates of treatment failure. From a mechanistic standpoint, this resistance is often attributable to the acquisition of mobile genetic elements, such as plasmid-mediated resistance genes (*sul* and *dfrA*) that encode for drug-resistant enzymes. Alternatively, while less characterized in environmental isolates, treatment survival *in vivo* could also involve phenotypic mechanisms like the formation of 'persister' cells—a transient, non-growing subpopulation of bacteria that can survive high antibiotic concentrations and later revert to a susceptible state. Further molecular studies are necessary to confirm the underlying genetic basis (plasmid vs. chromosomal) of the SXT resistance observed in this region.

In most foods, the total bacterial count is often an indication for the sanitary quality, safety and utility of foods. It may reflect the conditions under which the product is manufactured such as contamination of raw materials and ingredients, the effectiveness of processing and the sanitary conditions of equipment and utensils at the processing plants

## CONCLUSION

From the available result, it can be inferred from this study that there is a form of compromise in the good manufacturing practice of dairy products (particularly nono) being consumed in Maiduguri Metropolis.

The efficacy of some commonly used antibiotics has been confirmed.

## RECOMMENDATION

- There is need to supervise the diary product, starting from the farm, how it was processed and transported to the local vendor.
- Vendors need to properly take good hygiene methods in order to prevent cross contaminations of the products.

- The public should be educated on the danger of consuming dairy products from unreliable/ unhygienic sources.
- The vendors need to be educated on proper storage of the dairy product.

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