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Frequency Distribution and Antibiotics Sensitivity of E. *coli* from Local Muturu Raw Cow Milk

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Abstract: *Milk is widely consumed by almost all households in Nigeria and cases of manifestation of health–related problems arising from the consumption of milk are not uncommon to this study. In this study, frequency distribution and antibiotic sensitivity of Escherichia coli from raw muturu cow milk were studied and the results showed the mean standard deviation of E. coli count was highest in MC 3 with 3.63 ±0.77 (P<0.05). This shows that there was no significant difference (P<0.05) in the total E. coli count among the muturu cows. The frequency occurrence of E. coli was highest (23.3%) in MC 1 and MC 6 followed by MC4 (16.6%) while the least occurrence was seen in MC5(6.6%). The antibiotic susceptibility profile studied shows that ciprofloxacin had the highest sensitivity pattern of (93.3%) followed by Augmentin (83.3%) while gentamycin and tarivid had (80.0%) respectively. The isolate showed resistance to streptomycin, septum and ampicillin at (43.3%), (33.3%) and (30.0%) respectively. The study reveals the high presence of E. coli in muturu cow milk with little resistance to some antibiotics which should not be overlooked while cow herders and milk collectors should pay close attention to personal and environmental hygiene during the processing of raw cow milk.*

Keyword: *Antibiotic sensitivity, frequency, muturu milk*

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1.0 Introduction

Raw cow milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections/intoxications in consumers (Murinda *et al*., 2004; Oliver *et al*., 2005). Microbes may gain entry into raw milk directly from dairy cows experiencing subclinical or clinical mastitis from the farm environment particularly the water source and utensils used for the storage of milk on the farm or during transportation. Generally, bacteria in the milk can occur through colonization of the teat canal or an infected udder (clinical and subclinical mastitis) or become contaminated at various stages be it from the animal, milker (manual as well as automated), extraneous dirt or unclean process water (Gruetzmacher, 1999; Hayes *et al*., 2001).

Muturu milk is a product of the breed of cows called Muturu (Ezekwe and Machebe, 2005). Muturu cows are regarded as the most common cows in Nigeria and therefore the major source of cow milk (Tijjani *et al.,* 2019). Many microorganisms can get access to milk and products, but the presence of *Escherichia coli*(*E. coli*) and *S.aureus*are indicator marker organisms for the assessment of the quality of

milk and milk products(Maikai and Madaki,2018; Tijjani *et al.,* 2019). Recovery and counting of *E. coli* are used as a reliable indicator of faecal contamination and the possibility of the presence of a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra-intestinal diseases in men (Kaper *et al*., 2004). For example, Martin *et al*., (2020) reported two cases of hemolytic uraemic syndrome which provide evidence that raw milk may be a vehicle of transmission of *E.coli* O157: H7, both affected persons consumed raw milk.

Escherichia coli also known as *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes (EPEC, ETEC etc.) can cause serious food poisoning in their hosts and are occasionally responsible for food contamination incidents that prompt product recalls (Tenaillon *et al.,* 2010). The harmless strains are part of the normal microbiota of the gut and can benefit their hosts by producing vitamin K2, and preventing colonisation of the intestine with pathogenic bacteria, having a mutualistic relationship. *E. coli* is expelled into the environment within faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for three days, but its numbers decline slowly afterwards (Vogt and Dippold, 2005). *E. coli* and other facultative anaerobes constitute about 0.1% of gut microbiota, andfaecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells can survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination. A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for many days and grow outside a host (Russell and Jarvis, 2001). Milk contains more water than any other element, around 87% for dairy cows. The other elements are dissolved, colloidally dispersed, and emulsified in water. The quantities of the main milk constituents can vary considerably depending on the individual animal, its breed, stage of lactation, age and health status. Herd management practices and environmental conditions also influence raw milk composition and contamination.

The consideration of the management of muturu milk in Nigeria and the consequence tendency toward contamination as well as the threat to public health requires research attention. Therefore, we have designed this study intending to determine the frequency distribution and antibiotics sensitivity of *E. coli* in muturu cow milk. This aim shall be achieved through the following objectives, namely (i) isolate and characterization of *E. coli* and total viable count of other isolates,(ii)analysis of the result for the frequency distribution rate of viable count and *E. coli* in muturu and (iii) antibiotic susceptibility assay for *E. coli*..

2.0 Materials and Method

2.1 Collection of sample

Using sterile universal bottles, raw mature milk samples were obtained from eighteen female cows from Ikot Ebak in Essien Udim Local Government Area after due consultation and informed consent was granted by the owners of the cows, the samples were aseptically transported to Microbiology Laboratory in the Biological Sciences Department in Akwa Ibom State Polytechnic.

Eosin methylene blue agar (EMB), nutrient agar and Muller Hinton Agar were prepared according to the manufacturer's instruction and were sterilized in the autoclave at 121 ^{0}C for 15

minutes. After preparation, it was allowed to cool to about 45 ^{0}C before use.

2.2 Serial dilution of the samples

A tenfold serial dilution was carried out on the milk samples according to the method described by Cheesbrough (2006). Aseptically, a ten-fold serial dilution was carried out to factor 10^{-3}.

Inoculation of well-labelled Petri dishes with 1ml of dilution factor 10^{-2} and 10^{-3} of each sample was aseptically carried out. About 15ml of the prepared *Eosin methylene* blue agar (EMB) and nutrient agar were aseptically poured into all Petri dishes respectively. The plates were swirled to mix and allowed to solidify appropriately before they were wrapped and incubated for 24 hours using anaerobic conditions.

Enumeration of the total viable count was carried out on all plates by direct plate count technique using a colony counter. The results were used to determine the colony-forming units expressed in ml (Cfu/ml).

Purification of *E. coli* isolates with discrete colonies of metallic green sheen growth from all primary culture plates was picked and subcultured into already prepared and labelled EMB agar plates by streaking method and also kept in an anaerobic jar for 24 hours. After this, all pure isolates were stocked on agar slants in McCartney bottles and preserved in the refrigerator at 4^{O}C for further identification (Cheesbrough, 2006). Pure culture of the bacterial isolates was subjected to standard morphological, physiological and biochemical analysis and tubes by using the procedures and taxonomic scheme described by Cheesbrough (2006).

2.3 *Antibiotic Susceptibility Test*

This test was carried out to determine the antibiotic sensitivity profile of *Escherichia coli* isolated from the muturu raw cow milk samples on the following antibiotics, ciprofloxacin (CPX 10mg), septum (SXT 30mg), streptomycin (S 30mg), ampicillin (PN 30mg), ceporex (CEP 10mg), derived (OFX 10mg), Nalidixic Acid (NA 30 mg), reflacine (REF 10mg), gentamycin (CN 10mg) and augmentin (AU 30g). Mueller Hinton agar was prepared according to the manufacturers' instructions and was poured into sterile dishes. The plates were inoculated with sterile cultures of *E. coli* by streaking technique and commercially prepared antibiotic discs- listed above were then placed aseptically on the surface of the dried agar and pressed to have contact with the isolate. After 24 hours of incubation, the zones of inhibition observed were measured in millimetres (mm) and recorded.

2.4 *Statistical analysis of the sample*

Data obtained from the analysis were subjected to a one-way analysis of variance (ANOVA). Significant differences were obtained at $P<0.05$ by the least standard deviation (LSD) multiple range test, using the statistical package for Social Sciences (SPSS) version 26.

Table 1: Mean Standard Deviation of Total Viable Count and Total *Escherichia coli* Count

Muturu Cows sample	TVC (X10^4 Cfu/ml)	TEcoC (x10^{4}Cfu/ml)
MC 1	6.97±1.93	3.10±1.58
MC 2	4.50±0.72	1.53±0.77
MC 3	7.13±0.59	3.63±0.44
MC 4	4.73±0.79	1.83±0.95
MC 5	5.63±0.98	3.33±0.23
MC 6	9.00±0.27	3.20±0.29

**Values are expressed as mean ± standard error of the mean (SEM), MC - Muturu Cow, TVC = total viable count, TEcoC - Total *Escherichia coli* count

This study examined milk samples from eighteen female muturu cows, about five samples were collected from each animal. In agreement with the results of several previous investigations (Tabaran *et al.,* 2017; Brooks *et al.,* 2012; Costanzo *et al.,*2020), this work reveals high microbial contamination of raw

muturu cow milk, various microorganisms were found present in the raw cow milk and one of the most prevalent organisms in raw cow milk is *E. coli*.

The mean standard deviation of *E. Coli* count shows that MC 3 had the highest cfu/ml with 3.63 ± 0.44 ($P<0.05$). Alaku *et al.* (2004) and Arena, (2004) observed that the total viable count in raw milk could be due to cross-contamination of milk with faeces or lack of hygienic measures during handling and storage of milk. If proper hygienic practices in milk collection with equipment and marketing are not followed, it might be a source of zoonotic disease and serious health hazards to humans (Ray and Bhuriia, 2017).

The result of the Total viable count (TVC) showed a significant ($P<0.05$) increase in sample 6 when compared with samples 1, 3, and 5. The total *E. coli* count (TEcoC) (Table 1) revealed a non-significant difference ($P<0.05$) in mc 1, 3, 5 and 6, but was observed to be significantly lower in MC 2 and 4 respectively when compared to other samples that were analyzed. Such observation agrees with the work of Bandri *et al*., (2017) who reported a non-significant increase in sample among the analysed samples.

Table 2: Summary of cultural morphology

Cultural Characteristics Of EMB	Metallic green sheen
Gram Reaction	-
Cell Shape	Rod in chain
Coagulase	-
Spore	-
Catalasse	+
Oxidase	--
Citrate	-
Urease	-
MB	+
VP	-
Maltose	AG
Mannitol	AG
Lactose	AG
Glucose	AG
Probable Organism	*Escherichia coli*

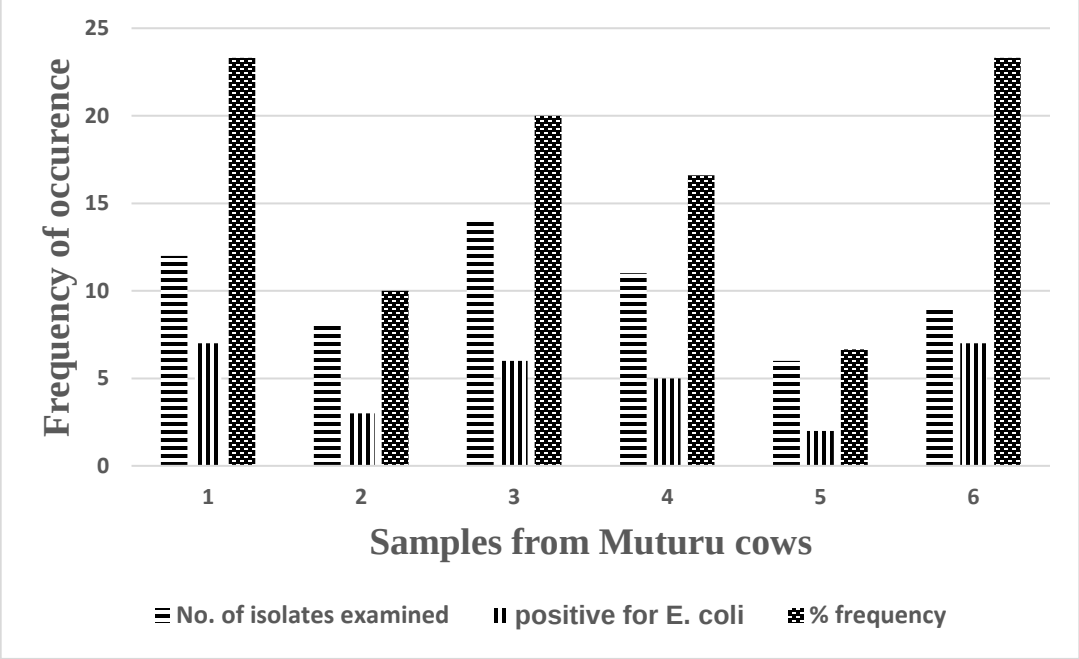

Fig. 1: A plot of frequency occurrence of *E. coli* on samples

Table 3: Percentage of Antibiotic susceptibility to *E. coli*

Organism	E-coli
No. of isolates	30
CPX	28(93.3)
SXT	10(33.3)
S	13(43.3)
PN	9(30.0)
CEPe	10(33.3)
OFX	24(80.0)
NA	23 (76.0)
REF	22(73.0)
CN	24(80.0)-
AU	25 (83.3)

There are some reports on the isolation of *E. coli* from rectal swabs of bovine animals throughout the world. Ogunleye *et al.* (2013) reported a prevalence of 80% *E. coli* in apparently healthy cattle in Nigeria. Wang *et al.* (2013) reported 75% prevalence of *E. coli*in bovine faeces collected from Japan. In Bangladesh, the prevalence of *E. coli* was 23.21% in apparently healthy cattle (Masud *et al.,* 2012). This pathogen has also been isolated from different sources in the country by other researchers like water (Naair *et al.,* 2005), and broiler birds (Mamun *et al.,* 2016). Faecal contamination of milk could also bring about a serious public health problem (Singh *et al.,* 2018).

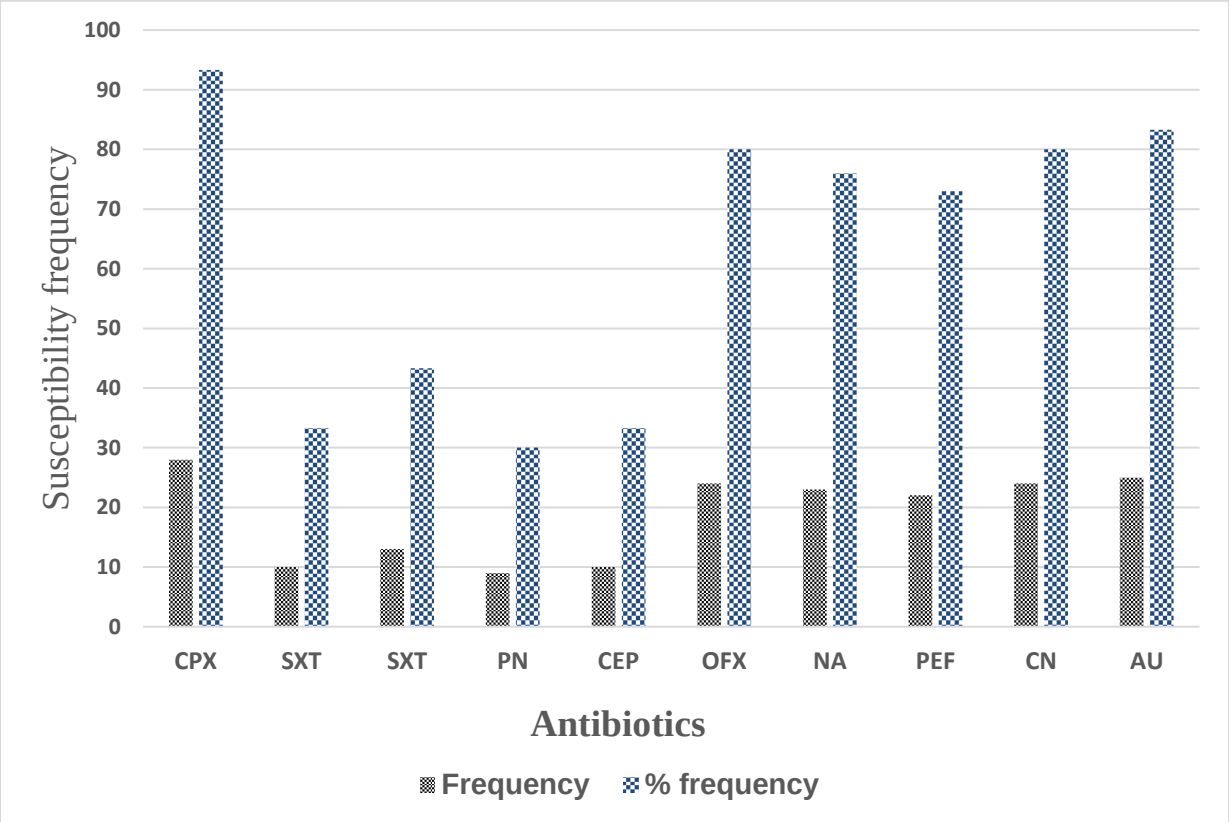

Fig. 2: A plot of the antibiotics susceptibility profile of *E. coli*

This study showed that of the 60 isolates examined 30 isolates were identified as *E. coli.*A high incidence of *E. coli* was found in different types of milk by many researchers (Naqvi, 2020; Martin *et al.,* 2020; Hanjra and Khan, 2017). Adesiyun (2014) found that 63% of examined milk samples were contaminated with *E. coli.* Zeinhom and Abdel-Latef (2014) also observed that *E. coli* was detected in 26.7% and 16% of the milk sampled from markets and farms in Egypt respectively. Alam *et al.,* (2017) isolated *E. coli* from 18% and 12% of market milk and farm milk in the Chittagong region of Bangladesh respectively. Hossain *et al.,* (2017) isolated *E. coli* from 32% milk samples in the Mymenssingh region of Bangladesh. Naqvi, (2020) reported that 13

out of 20 samples (65%) of raw milk obtained from milk vending shops and houses in Pakistan and another 10 out of 20 samples (50%) of raw milk collected from dairy farms in Pakistan were contaminated with *E. coli*. The variation might be due to different geographical locations and hygienic conditions followed by farmers and all personnel in the milk marketing chain, different study methods also can differ with this result. *E. coli* is normally a ubiquitous organism, yet the pathogenic strains if present in milk can be harmful to consumers. Enteropathogenic *E. coli* can cause severe diarrhoea and vomiting in infants and young children. It can also lead to hemorrhagic colitis (bloody diarrhoea) and haemolytic uremic syndrome in humans (Hahn, 2006).

The antibiotics susceptibility test of *E. coli* shows that 28 out of 30 (93.3%) isolates were susceptible to ciprofloxacin following the findings of Bandri *et al.,* (2017) and Hossain *et al.,* 2017 who reported strains of *E. coli* to be susceptible to ciprofloxacin at 87% and 94% respectively. The result of this study shows that 25 out of 30 (83.3%) isolates were susceptible to Augmentin which was not different from the findings of Mamun *et al.* (2016) who reported that isolates from broiler birds were susceptible to Augmentin at 89.5%. Ogunleye *et al.,* (2013) reported that 75% of isolates from apparently healthy cattle in Nigeria were susceptible to Augmentin. In this work, about 24 out of 30 (80.0%) isolates were susceptible to Gentamycin and Travid respectively. This was in consonant with the work of Adesiyun (2014) who reported that there was no resistance to Gentamycin in his work. In 2012, Brooks *et al.,*(2012) who isolated *E. coli* from raw cow milk Cheese found it to be susceptible to Gentamycin, Travid and Ciprofloxacin at 76%, 85% and 92% respectively. The *E. Coli* strains in this work were also susceptible to Nalidixic acid and Reflacine at 76.0% and 73.0% respectively. This result accords with the work of Bandri *et al.,* (2017) who isolated *E. coli* from raw milk samples in Al-Jazirah state, Sudan and reported the *E. coli*strains to be susceptible to Nalidixic acid and tetracycline at 78.13% and 81.25% respectively. Singh *et al.,* (2018) isolated *E. coli* from Bovine nastitis and checked their antibiotic sensitivity to ciprofloxacin at 89.5%, Nalidixic acid at 85%, Tetracyclin at 80.5% and Gentamycin at 92%.

Uddin *et al.* (2011) isolated *E. coli* from raw milk in Dhaka city of Bangladesh and reported that the isolates were 85% resistant to streptomycin. Hossain *et al.,* (2017) also reported *E. coli* to be resistant to streptomycin. The isolates in this study were susceptible to streptomycin in a very descending order indicating resistance (43.3%)

Tanzin *et al.,*(2016) isolated resistant *E. coli* against Gatifloxacin, Septrin and Ceporex from milk samples in the Mymensingh region of Bangladesh while Hossain *et al.,* (2017) reported Erythromycin, Septrin, Amoxicillin and Ceporex resistant *E. coli* from milk samples in the same region. The isolates in this study exhibited susceptibility to Septrim and Ceporex at 33.3% respectively. Mamun *et al.,* (2016) isolated ampicillin-resistant *E. coli* from broiler bird sample.Tabaran *et al.,*(2017) reported that *E. coli* strains (n=43) exhibited a wide variability or resistance against Ampicillin at 39.5% and Streptomycin at 23.3%. The isolates in this study also exhibited resistance to Ampicillin which is supported by the previous studies of other researchers. Therefore, proper care and good manufacturing practice(GMP) have to be observed by the cattle rearers during the milking process by observing the necessary precautionary measures to avoid contaminating the delicate product with *Escherichia coli* which can impact negatively on the health of the consumers.

4.0 Conclusion

The study showed that muturu milk do harbour antibiotic-resistant strains of *E.coli* that can be circulated within the ecosystem through the food chain. This observation is contrary to some public views that accepted that the milk is free from microbial

contamination. Therefore, there is need to implement hygienic practices and examination on the milk before consumption.

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Declarations

The authors declare that they have no conflict of interest.

Data availability

All data used in this study will be readily available to the public.

Consent for publication

Not Applicable.

Availability of data and materials

The publisher has the right to make the data public.

Competing interests

The authors declared no conflict of interest

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Authors' contribution

ITA and EAU designed the work and wrote the manuscript while ADE carried out the bench work