Antibiogram and Resistant Gene Profile of *E. coli* Isolated from Yoghurt

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Abstract

The antibiotic susceptibility profile of *Escherichia coli* isolated from yoghurt samples within the Port Harcourt metropolis was investigated. Yoghurt samples (sachets and bottled yoghurts) were randomly bought from vendors within Port Harcourt and were transferred in an ice-pack container to the microbiology laboratory, at Rivers State University where they were analysed. Enumeration and isolation of faecal coliform were done using Eosin methylene Blue Agar (EMB). Aliquot from 10^{-1} dilution of the sample was aseptically transferred into dried EMB agar plates in duplicates, spread evenly using a sterile bent glass rod and incubated at 45℃ for 24-48 hours. After incubation, plates were observed for growth and counts were recorded for the determination of colony forming unit while colonies on the plates were subcultured on freshly prepared nutrient agar plates. Morphological, gram reaction and biochemical tests were used for identification. The Kirby Bauer disk diffusion test on Mueller-Hinton agar using commercially prepared antibiotics was used in determining the antibiotic susceptibility profile of the isolates. Results showed that the mean range of the faecal coliform load of the yoghurts was $0.0\text{-}6.0\times10^{2}$ CFU/mL. More so, out of the samples analyzed, only two samples were positive for *E. coli*. Results of the antibiotics susceptibility showed that all five *E. coli* isolates were resistant to more than two antibiotics and exhibited multi-drug resistance. Some of the isolates possessed Inc-P and TEM-resistant genes. Although they were completely susceptible to Nalidixic acid, Levofloxacin, Ceftriaxone and Ofloxacin antibiotics. More so, 40% resistant isolates had a MAR index of 0.2, while the other 40 and 20% resistant isolates had a MAR index of 0.5 and 0.3, respectively. Contamination of the samples could be through manufacturing or packaging and distribution processes. Thus, good manufacturing practice is highly recommended.

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Keywords: *faecal coliform, gentamycin resistant gene, ESBL gene, yoghurt*

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Introduction

Yoghurt is a sour milk beverage made by blending fermented milk with various ingredients that provide flavour and colour. Although it is a traditional beverage in the Balkans and Middle East (Ghandge *et al.*, 2008), yoghurt is consumed by all people of all nations. Yoghurt is produced by the symbiotic actions of two lactic acid bacteria, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus* which ferment lactose to lactic acid giving it its soured taste (Dirisu *et al.*, 2015). Yoghurt can serve as food and plays an important role in human nutrition, health maintenance, and therapeutic and dietetic functions (Khan *et al.*, 2008). The nutritional quality of yoghurt has been reported and is known to contain high-quality protein, calcium and phosphorous. Its carbohydrate can be utilized easily by those intolerants to lactose (Alakali *et al*., 2008; Ghandge *et al*., 2008). It is also believed that yoghurt has valuable therapeutic properties and helps in curing gastrointestinal disorders (Vasiljevic and Shah, 2008). Yoghurt also serves as a medium for the growth of microorganisms due to its high nutritional content hence it is liable to contamination. Moulds and yeast are the primary contaminants in yoghurt. Fungi growing in yoghurt utilize some of the acids, which will invariably reduce the acidity and hence favour the growth of putrefactive bacteria (Oyeleke, 2009) or other pathogenic organisms such as *Staphylococcus aureus* (Ifeanyi *et al*., 2013; De *et al*., 2014; Makut *et al*., 2014).

E. coli is a widespread species found in the intestines of farm animals, poultry, and humans. The majority of *E. coli* strains are non-pathogenic, but a few are very pathogenic, causing watery and bloody diarrhea; *E. coli* 0157:H7 has been linked to life-threatening diseases such as haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (Rahman *et al.*, 2017). *E. coli* have been found to contaminate ready-to-eat (RTE) foods (Abebe *et al*., 2020). This species can

survive on hands and other surfaces and is readily transferred to foods (Ayamah *et al.*, 2021). As people consume more poultry, milk, and beef, the risk of contracting diseases of animal origin, such as pathogenic *E. coli*, has increased. The major inhabitant of human and animal guts is *E. coli*, a member of the Enterobacteriaceae family. *E. coli* has been identified as indicator species of fecal and enteric pathogen contamination. Shiga toxins (stx1 and stx2), enterohemolysin (hlyA), and intimin (eaeA) are virulence factors that play a key role in the development of these disorders (Bruyand *et al.*, 2018). There are additional studies focusing on *E. coli* and other pathogens in RTE foods (Secim and Ucar, 2017).

Antibiotics have been used in human and veterinary medicine for many years to minimize morbidity and mortality and the economic effect of bacterial infections. However, *E. coli* has developed resistance to one or more antibiotics, which has raised public health concerns. The indiscriminate and rising use of antibiotics is linked to the high occurrence of resistant bacteria. Antimicrobials are used in the food production process to prevent and control illnesses, improve growth, and increase feed efficiency in food-producing animals (CDC, 2022). The use of these antibiotics at low doses for long periods of time to feed animals, for example, can result in the selection and spread of antibiotic resistance to other microbes in the food chain (Lima *et al.*, 2017). The challenge of yoghurt production in Nigeria is characterized by inadequate housing facilities, lack of bacteriologically free water, poor waste management, and lack of adequate environmental sanitation in areas of high population density and people with low income (Taiwo *et al.*, 2018). Thus, this study is aimed at investigating the antibiogram of *E. coli* in yoghurts sold in Port Harcourt metropolis.

Materials and Method

Collection of Sample

Forty yoghurt samples comprising of sachets and canned packaged yoghurts were randomly bought from different vendors within Port Harcourt. The samples were placed in an ice-packed container and were quickly transferred to the Microbiology laboratory, at Rivers State University for immediate analysis.

Microbiological Analysis

The ten-fold serial dilution method as described by Prescott *et al.*, (2011) was used. In this method, 1 mL from the stock solution of the sample was withdrawn using a sterile 1mL pipette and transferred into another test tube containing 9mL sterile normal. The dilution was carried out serially in a stepwise fashion until a dilution of 10^{-6} was reached. An aliquot (0.1mL) from 10^{-1} and 10^{-3} dilutions was withdrawn with a sterile 1mL pipette and inoculated directly on dried Eosin methylene Blue Agar (EMB) and Nutrient agar plates in duplicates for enumeration and isolation of faecal coliform and total heterotrophic bacteria from the samples. Also, an aliquot of 1mL of the yoghurt sample was withdrawn and inoculated on the surface of the dried EMB plates in duplicates without dilution. The inoculated plates were spread evenly using a sterile bent glass rod and were incubated at 45℃ for 24-48 hours.

Isolation and Preservation of Pure Cultures

The suspected *E. coli* isolates that grew on the respective EMB plates after 48 hours of incubation were subcultured on freshly prepared nutrient agar plates. The subcultures were incubated at 37℃ for 24 hours. These were then preserved refrigerated in nutrient agar slant and used for identification, antibiotic susceptibility test and plasmid profiling.

Identification of Bacterial Isolates

Identification of bacterial isolates were done using the methods described by Cheesbrough (2006). The biochemical tests adopted were the indole test, catalase test, oxidase test, Methyl Red test, Voges Proskauer test, citrate test, motility and sugar fermentation test.

Preparation of 0.5 McFarland Standards

This was prepared by dissolving 0.5ml of 0.048 $BaCl_2$ (1.175%$BaCl_2$-$2H_2O$) in 99.4ml of 0.18M H_2SO_4 (1%v/v). The turbidity was obtained by checking the absorbance in a spectrophotometer with 1-cm light path. The absorbance was seen to be between 0.08 and 0.1 at 625nm. The 0.5 McFarland was agitated before each use to standardize the turbidity of the test organism (CLSI, 2020).

Standardization of Bacterial Isolates for Antibiotics Sensitivity

About 5ml of normal saline was dispensed into test tubes and sterilized by autoclaving at 121ºC, 15Psi for 15 minutes. After sterilization, test tubes were cooled in a cold water bath. The test bacterial isolates (18-24 hours old culture) were transferred aseptically into labelled test tubes containing the normal saline using a sterile wire loop. The turbidity of the normal saline was compared with the turbidity of the McFarland Standard (CLSI, 2020).

Antibiotic Susceptibility Test

The Kirby Bauer disk diffusion method was carried out as described by Wemedo and Robinson (2018). In this method, a sterile solid Muller Hinton agar, each of the test organisms from the already prepared McFarland standard was aseptically inoculated using a sterile swab stick. The test isolates were seeded horizontally and vertically and allowed to dry for 5 minutes before the antibiotic disc was aseptically placed on the solid media using sterile forceps. The plates were incubated at 37°C for 24 hours after which the diameter of the zones of inhibition was measured to the nearest millimetre and the readings were interpreted as resistant, susceptible or intermediate CLSI (2020). The antibiotics used were Augmentin (30 µg), Amoxicillin and Clavulanic Acid (25/10µg), Imipenem (10 µg), Norfloxacin (30 µg), Nalidixic acid (30 µg), Levofloxacin (5 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Cefixime (5 µg), Ofloxacin (5 µg), Gentamycin (30 µg), and Zenacef (30 µg).

Amplification of Gentamycin Resistant Gene (Gmr)]

The IncP-1genes from the isolates were amplified using the IncP-1F: 5'- GCCGTTAAAATTAAGCCC-3' and IncP-1R: 5'-CTTGATTGAAGGGTTGGGCG-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 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, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 47ºC for 30 seconds; extension, 72ºC for 40 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25mins

Amplification of TEM gene

The TEM genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTGAG-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 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, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 58ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes

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Statistical Analysis

The faecal load of the yoghurt samples was presented in Microsoft Excel, the mean and standard deviations of the faecal load were determined, and the measured zones from the antibiotic susceptibility test were used in calculating the percentage resistant, susceptible and intermediate of the isolates. All Analysis was done using the Statistical Package for Social Science software (SPSS version 25).

Results

The results of the bacterial load of the yoghurt samples are presented in Table 1. Results showed that the mean range of the total heterotrophic bacterial load and the faecal coliform load of the yoghurts was 0.0-1.6×10^3 and 0.0-6.0×10^2 CFU/mL, respectively. More so, the results showed a disparity in the aerobic bacterial and faecal coliforms as some samples were void of aerobic and coliform bacterial load while some yoghurt samples (SFY and JY) had visible growth of faecal coliform (Fig 1).

Table 1: Bacterial Load of the Yoghurt Samples

Samples	Total Heterotrophic Bacterial Count	Total faecal coliform
Fy	$1.6\pm0.8\times10^3$	$0.0\pm0.0\times10^2$
Gly	$6.5\pm0.3\times10^2$	$0.0\pm0.0\times10^2$
glys	$4.0\pm0.1\times10^2$	$0.0\pm0.0\times10^2$
Hy	$0.0\pm0.0\times10^2$	$0.0\pm0.0\times10^2$
Jy	$5.5\pm0.3\times10^2$	$2.0\pm0.1\times10^2$
Ky	$0.0\pm0.0\times10^2$	$0.0\pm0.0\times10^2$
Sby	$0.0\pm0.0\times10^2$	$0.0\pm0.0\times10^2$
Sfy	$0.0\pm0.0\times10^2$	$6.0\pm0.3\times10^2$
Sy	$1.1\pm0.7\times10^3$	$0.0\pm0.0\times10^2$

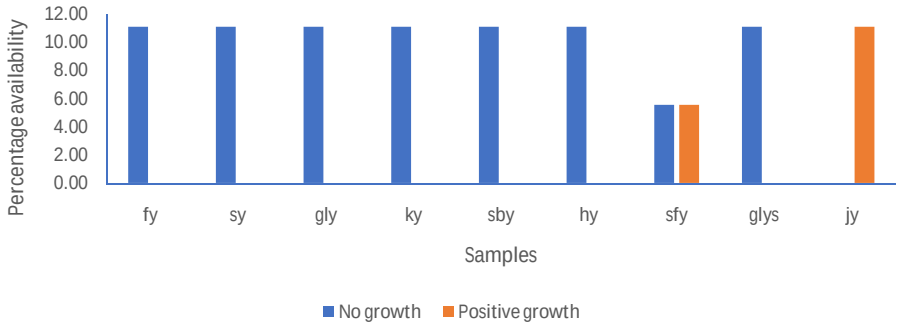

Fig. 1: Percentage growth of faecal coliform in Yoghurt samples

Results showing the phenotypic and morphological characteristics of the various *E. coli* isolates are presented in Table 2. Results showed that isolates E1, E2, E3, E4, and E5 which displayed green metallic sheen on EMB plates were all Gram-negative rods. They were positive for catalase, indole methyl red and

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motility while being negative for oxidase, citrate and Voges Proskauer. There was a variable response to sucrose as E3 and E5 fermented sucrose while E1, E2 and E4 did not ferment sucrose. All the isolates fermented mannitol, lactose and glucose as well as produced gas from glucose fermentation. All these are known responses of *E. coli*, thus, were classified as *E. coli.*

Results of the distribution of faecal coliforms across the samples showed that *E. coli* were only isolated in SFY and JY yoghurt samples (Fig. 2).

Results showing the antibiogram of *E. coli* isolates are presented in Table 3 while the MAR index and types of antibiotics to which the isolates were resistant to is presented in Table 4. Results showed that all five *E. coli* isolates were resistant to more than two antibiotics and exhibited multi-drug resistance. Isolate E1 and E4 were resistant to three antibiotics, isolates E2 and E3 were resistant to seven different antibiotics and E5 was resistant to four different antibiotics. Plate 1 shows the antibiotic susceptibility test of *E. coli*.

Plate 1. Antibiotic susceptibility of isolate E3

Table 2: Morphology and Biochemical Response of *E. coli*

Isolate Code	Macroscopy						Gram's Reaction	Biochemical Test												Probable Identity
	Shape	Elevation	Opacity	Edge	Size	Colour	Gram Reaction	Catalase	Oxidase	Citrate	Indole	Methyl Red	Vogues Proskauer	Motility	Sucrose	Glucose	Mannitol	Lactose	Growth at 45°C	
E1	round	convex	opaque	smooth	1mm	metallic sheen	-ve rods	+	-	-	+	+	-	+	--	AG	A	AG	+	*E. coli*
E2	round	convex	translucent	smooth	2mm	metallic sheen	-ve rods	+	-	-	+	+	-	+	-	AG	A	AG	+	*Escherichia coli*
E3	round	convex	translucent	smooth	2mm	metallic sheen	-ve rods	+	-	-	+	+	+	+	+	AG	A	AG	+	*E. coli*
E4	round	convex	opaque	smooth	1mm	metallic sheen	-ve rods	+	-	-	+	+	-	+	-	AG	A	AG	+	*Escherichia coli*
E5	round	convex	opaque	smooth	1mm	metallic sheen	-ve rods	+	-	-	+	+	-	+	+	AG	A	AG	+	*Escherichia coli*

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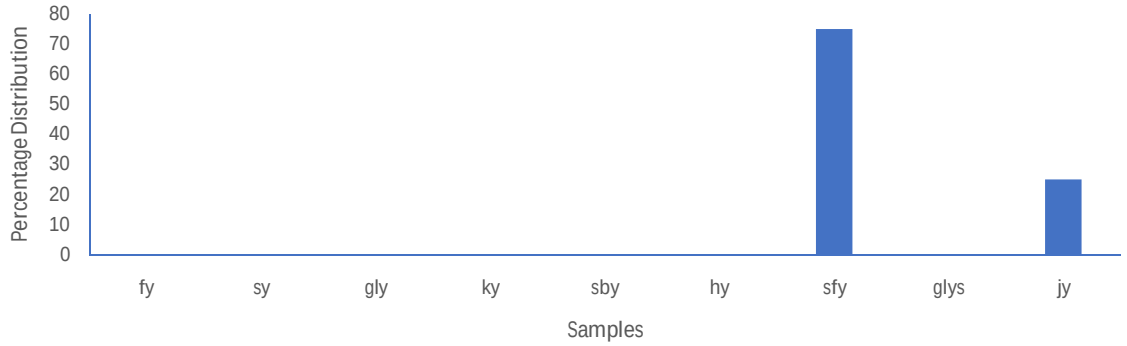

Fig. 2: Percentage Distribution of faecal coliform in Yoghurt samples

Table 3: Antibiotic Susceptibility Pattern of *E. coli* (n = 5)

Antibiotics	Susceptible [n (%)]	Resistant [n (%)]	Intermediate [n (%)]
Augmentin (30 µg)	0	4 (80)	1 (20)
Amoxicillin and Clavulanic Acid (25/10µg)	1 (20)	4 (80)	
Imipenem (10 µg)	1 (20)	4 (80)	0
Norfloxacin (30 µg)	0	5 (100)	0
Nalixidic acid (30 µg)	5 (100)	0	0
Levofloxacin (5 µg)	5 (100)	0	0
Cefuroxime (30 µg)	2 (40)	2 (40)	1 (20)
Ceftriaxone (30 µg)	5 (100)	0	0
Cefixime (5 µg)	3 (60)	2 (40)	0
Ofloxacin (5 µg)	5 (100)	0	0
Gentamycin (30 µg)	3 (60)	1 (20)	1 (20)
Zenacef (30 µg)	2 (40)	2 (40)	1 (20)

Table 4: MAR Index of *E. coli*

Antibiotics Resistant to	MAR Index	% Resistant
AUG, NF, CXM	0.2	2 (40%)
AUG, ACX, IMP, NF, CTX, GEN, ZEN	0.5	2 (40%)
AUG, ACX, IMP, NF	0.3	1 (20%)

Amplified *E. coli* gene

Plate 2 showed the amplified Gentamycin resistance gene band. Lane L represents the 100bp molecular ladder while lanes E1 and E2 represent the IncP-1 bands at 200bp. Plate 3 showed the amplified TEM gene. Lanes E1 and E3 represent the TEM band at 700bp while L represents the 100bp molecular ladder.

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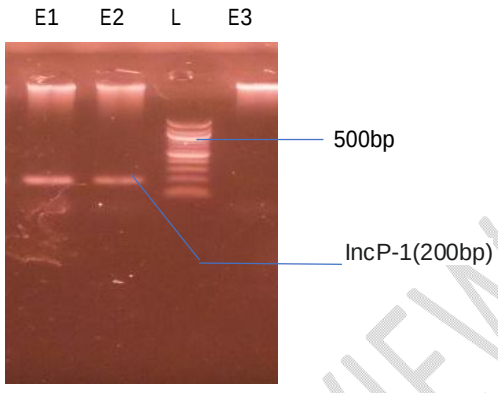

Plate 2: Agarose gel electrophoresis showing the amplified Gentamycin resistance gene band

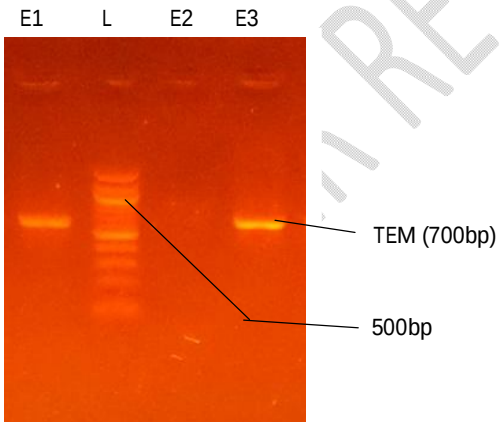

Plate 3: Agarose gel electrophoresis showing the amplified TEM gene

Discussion

The antibiotics susceptibility profile of *E. coli* isolated from yoghurt samples sold within the Port Harcourt metropolis was investigated. The total heterotrophic bacterial load in the present study is less than the count of 2.0×10^3 reported by Taiwo *et al.* (2018). The counts of the yoghurt samples also vary from those reported in previous studies (Oyeleke *et al.*, 2009, Nwamaka and Chike, 2010). The disparity observed in the total heterotrophic bacterial and faecal coliform load of the various yoghurt samples could be a reflection of the manufacturing, packaging and storage processes employed by the respective

industries. This statement agreed with Previous studies (Afolabi *et al*., 2017; Kisanthini and Kavitha, 2019). The presence of faecal coliform in some of the yoghurt samples could be a reflection of poor hygienic conditions during the manufacturing, packaging and storage process especially if the materials used in processing the samples were contaminated with faecal matter. This agreed with Matin*et al.* (2018) who also reported the presence of coliforms in all yoghurt samples studied. The absence of coliform growth in this study agreed with Afolabi*et al.* (2017) who reported no coliform in yoghurt samples and this is a pointer to the health status of the yoghurt samples as it serves as a parameter usually employed to check the yoghurt quality in many countries (Tamine and Robinson 2007). In addition, the absence of coliform in these samples could largely be attributed to the pasteurisation process of the premix used as well as good processing hygiene.

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The presence of *E. coli* in some of the yoghurt samples indicates that the samples are not fit for consumption since *E. coli* which represent an indicator organism could be an alarm for the presence of pathogens especially intestinal organisms in the yoghurt samples (WHO, 2012). More so, *Escherichia coli* has frequently been associated with foodborne illness. Particularly, over the past decade, it has been reportedly increasing from all parts of the world and in the worst case, a strain *E. coli* O157:H7 has been regarded as one of the most serious foodborne pathogens leading to severe illnesses and high mortality rates in humans (Bedasa *et al*., 2018).

Antibiotic Susceptibility Profile

The high rate of resistance to antibiotics illustrates increased antibiotic resistance among *E. coli* isolates. The emergence of multidrug resistance in *E. coli* has highlighted the need to raise public awareness, educate physicians and veterinarians, and take appropriate actions to curb indiscriminate antibiotic use. In the present study, all five *E. coli* isolates were susceptible to nalidixic acid, LBC, Ceftriaxone and Ofloxacin while susceptibility to Cefuroxime, Cefotaxime, Gentamycin and Zenacef was 40%, 60%, 60% and 40%, respectively. Thus, higher susceptibility to gentamycin was recorded with only one (20%) exhibiting resistance while the other 20% exhibited intermediate response. Geletu*et al.* (2022) in their study have reported the susceptibility of *E. coli* isolates to gentamycin and ciprofloxacin which agreed with the present study. Although, in the present study, sensitivity to gentamycin was low as compared to their study. Furthermore, 80% resistance was observed in Augmentin, Amoxicillin/Clavulanic Acid and Imipenem while 100% resistance to Norfloxacin was recorded. About 40% of the isolates were resistant to ceftazidime, Zenacef and Cefotaxime. Susceptibility of *E. coli* isolates to nalidixic acid has been reported by Sultana *et al.* (2021) although the level of susceptibility which was recorded as 60% is lower than the 100% sensitivity of the *E. coli* isolates in the present study. The multi-drug resistance of *E. coli* to the antibiotics showed that 40% were resistant to three and seven different antibiotics while 20% were resistant to four different antibiotics. A high MAR index of 0.2 and above was observed and this could be attributed to the overuse of antibiotics.

Antibiotics have been used in the dairy industry for more than five decades to treat or prevent disease and to increase milk production or improve feed efficiency (Riediker *et al*., 2004). Food products such as milk, cheese, yoghurt, and other dairy products have been implicated as potential sources for the transmission of the pathogen to humans (Normanno *et al.,* 2007). Furthermore, foods, which may be contaminated with antibiotic-resistant bacteria, represent ideal vehicles for the transmission of antibiotic-resistant strains (Phillips *et al*., 2004). So, food is an important vehicle for the transfer of AMR factor to the intestinal tract of consumers very efficiently (Spanu *et al.,* 2012). Thus, the presence of these resistant isolates in the present study could be a problem since AMR could be transferred to the consumers. However, the mechanism of spreading antibiotic-resistant bacteria from food animals to humans remains controversial (Adeleke *et al*., 2000).

The presence of IncP-1 which represent the gentamycin-resistant gene and the TEM gene in the plasmids of these isolates could have also influenced the observed resistance in the present study. The plasmid of the incompactibility (Inc) group, also called the IncP, can transfer and replicate in all gram-negative

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bacteria including *E. coli*. They are composed of backbone genes that encode a variety of essential functions and accessory genes that have implications for human health. These genes often code for resistance to broad-spectrum antibiotics. The plasmids of the subgroup IncP-1 are of considerable interest to both molecular biologists and environmentalists due to their highly efficient conjugative transfer and ability to replicate in a broad range of hosts (Shintani *et al*., 2010). The TEM gene is one of the Extended Spectrum Beta-Lactamases (ESBL) that encodes β-lactam resistance in Gram-negative bacilli (Zeynudin *et al.*, 2018). Thus, this could be why some isolates showed resistance to the beta-lactam drugs. The non-detection of beta-lactam and gentamycin-resistant genes in some isolates could be that these genes were not responsible for the observed resistance. It could also be that other genes which were not assayed for were responsible or other methods of resistance such as efflux pump or mutations of the antibiotic binding site could be responsible (Prescott *et al.*, 2011). The increasing use of antibiotics in human and veterinary medicine and agriculture production system has caused the increasing development of high levels of antibiotic resistance and the rapid global spread of antibiotic-resistant genes (Aminov, 2009).

Conclusion

The presence of faecal coliform in some of the yoghurt samples is a clear indication that they could harbour pathogenic microorganisms that could pose serious health problems especially gastroenteritis to immunocompromised persons. Furthermore, the high level of antibiotic resistance observed in this study could mean the high use of antibiotics in animal farming as well as the ingestion of resistant isolates from the environment, especially during the feeding of the animals. Although the mechanisms of how antimicrobial resistance is transmitted from animals or animal products to man are still not well understood, the presence of resistant isolates in the intestines of consumers could contribute to or breed resistant isolates which could increase the antibiotic menace globally. Thus, strict hygiene should be followed and enforced both from raw material acquisition to the dispatch of the products to consumers. Proper pasteurization should be carried out and water used for production and processing should be void of microbial contaminants.

References

Ghandge, P. N., Prasad, K. and Kadam, P. S. (2008). Effect of fortification on the physicochemical and sensory properties of Buffalo milk yoghurt. Electronic *J. Environ. Agric. Food Chem*. 7(5), 2890-2899

Dirisu, C. G., Lily, G., and Igwe, E. (2015). Microbiological load of yoghurt sold in Omoku schools, Rivers State, Nigeria. *African Journal of Microbiology Research*, 9(34), 1960-1963

Khan, K., Rehman, S. U., Khan, M. A., Anwar, F. and Bhadar, S. (2008). Physical and chemical quality appraisal of commercial yoghurt brands sold at Lahore. ARPN *J. Agric. Biol. Sci*. 3(3), 14 -21

Alakali, J. S., Okonkwo, T. M. and Lordye, E. M. (2008). Effect of stabilizers on the physicochemical and sensory attributes of thermized yoghurt. *Afr. J. Biotechnol*. 7(2), 158-63.

Vasiljevic, T. and Shah, N. P. (2008). Probiotics- from metchnicoff to bioactives. Int. Diary J. 18:714-728.

Oyeleke, S. B. (2009). Microbial assessment of some commercially prepared yoghurt retailed in Minna, Niger State. *Afr. J. Microbiol. Res.* 3, 245-248.

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Ifeanyi, V. O., Ihesiaba, E. O., Muomaife, O. M. and Ikenga, C. (2013). Assessment of Microbiological Quality of Yogurt Sold by Street Vendors in Onitsha Metropolis, Anambra State, Nigeria. *Br. Microbiol. Res. J.* 3(2), 198-205

De, N., Goodluck, T. M. and Bobai, M. (2014). Microbiological quality assessment of bottled yogurt of different brands sold in Central Market, Kaduna Metropolis, Kaduna, Nigeria. *Int. J. Curr. Microbiol. Appl. Sci.* 3(2), 20-27.

Makut, D., Ogbonna, A. I. and Dalami, H. (2014). An Assessment of the Bacteriological Quality of Different Brands of Yoghurt Sold in Keffi, Nasarawa State, Nigeria. *J. Nat. Sci. Res.* 4 (4), 19-22.

Rahman, M.A., Rahman, A.K.M., Islam, M.A. and Alam, M.M. (2017) Antimicrobial resistance of Escherichia coli isolated from milk, beef and chicken meat in Bangladesh. Bangladesh *J. Vet. Med*., 15 (2), 141–146

Abebe, E., Gugsa, G. and Ahmed, M. (2020) Review on major food-borne zoonotic bacterial pathogens. *J. Trop. Med*., 467-4235.

Ayamah, A., Sylverken, A.A. and Ofori, L.A. (2021). Microbial load and antibiotic resistance of Escherichia coli and Staphylococcus aureus isolated from ready-to-eat (RTE) kebab sold on a University campus and its environs in Ghana. *J. Food Qual*., 862-2903.

Bruyand, M., Mariani-Kurkdjian, P., Gouali, M., de Valk, H., King, L.A., Hello, S.L., Bonacorsi, S. and Loirat, C. (2018). Haemolytic uremic syndrome due to Shiga toxin-producing Escherichia coli infection. *Med. Mal. Infect.*, 48 (3), 167–174.

Secim, Y. and Ucar, G. (2017). Evaluation of the desserts, which are hosmerim, cheese halva, Kunafah produced in Turkish cuisine-in aspect of tourism. *Int. J. Soc. Econ. Res*., 3 (5), 1478–1484.

CDC. (2022) What is an antibiotic? In: National Antimicrobial Resistance Monitoring System (NARMS), Department of Health and Human Services. (Centers for Disease Control and Prevention). Frequently Asked Questions about Antibiotic Resistance. CDC, Atlanta. Available from: http://www.cdc.gov/narms/faq_pages Retrieved on 6-10-2022

Lima, C.M., Souza, I.E.G., dos Santos Alves, T. and Leite, C.C. (2017) Antimicrobial resistance in diarrheagenic Escherichia coli from ready-to-eat foods. *J. Food Sci. Technol*., 54 (11), 3612–3619.

Taiwo, O. S., Afolabi, R. O., Oranusi, S. U., Owolabi, J. B., Oloyede, A. R., Isibor, P. O. and Ige, O. J. (2018). Microbiological Assessment of Commercial Yogurt Sold in Ota Metropolis, Ogun State, Nigeria. IOP Conference Series: *Earth and Environmental Science*, 210, 012019

Prescott, L.M., Harley, J. and klein, D.A. (2011). Microbiology 8th.ed, McGraw-Hill New York. pp. 809-811.

Cheesebrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, London, UK; 143 – 156.

Clinical and Laboratory Standard Institutes (2022). Performance standards for antimicrobial disk susceptibility tests. CLSI document M100. Clinical and Laboratory Standard Institutes, 30th edition.

Wemedo, S. A. and Robinson, V. K. (2018). Evaluation of Indoor Air for Bacteria Organisms and their Antimicrobial Susceptibility Profiles in a Government Health Institution. *Journal of Advances in Microbiology;* 11(3): 1-7.

Nwamaka, T.N. and Chike, A.E. (2010) Bacteria Population of Some Commercially Prepared Yoghurt Sold in Enugu State, Eastern Nigeria. African Journal of Microbiology Research, 4, 984-988.

Microbial Contamination of Yoghurt-An Overview

Kisanthini, S. and Kavitha, M. B. (2019). Microbial Contamination of Yoghurt-An Overview. *International Journal of Science and Research (IJSR)*, 7, 583

Matin, A., Banik, T., Badsha, M. R., Hossain, A., Haque, M. M. and Ahmad, M. (2018). Microbiological quality analysis of yoghurt in some selected areas of Bangladesh. *International Journal of Natural and Social Sciences*, 5(4), 82-86

Afolabi, L. O., Adelowo, M. O., and Onilude, H. A. (2017). Assessmentof the microbiological qualities of locally and industrially producedyoghurt in Lagos. *Dutse Journal of Pure and Applied Sciences*, 3(1), 2017

Tamine, A. Y. and Robinson, R. K. (2007). Yoghurt: Science and Technology. 3rd ed. Cambridge,Woodhead Publishing Limited. 808.

World Health Organization. (2012). General information related to microbiological risks in food.Available online http://www.who.int/foodsafety/micro/general/en/index.html Accessedon 19.07.12. Accessed 19 July 2016: 7pm.

Bedasa, S., Shiferaw, D., Abraha, A. (2018). Occurrence and antimicrobial susceptibility profile of Escherichia coli O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia. *Food Contamination*, 5, 2.

Geletu, U. S., Usmael, M. A., & Ibrahim, A. M. (2022). Isolation, Identification, and Susceptibility Profile of E. coli, Salmonella, and S. aureus in Dairy Farm and Their Public Health Implication in Central Ethiopia. *Veterinary medicine international*, 2022, 1887977.

Sultana, T., Rabbi, M. F., Sarke, B. R., Islam, M. S., Begum, and Hessian, K. M. M. (2021). Prevalence and antibiotic resistance patters of Escherichia coli isolation from milk and product. *J. Bio-Sci.* 29(2): 81-91

Riediker, S., Rytz, A. and Stadler, R. H. (2004). "Cold-temperature stability of five β-lactam antibiotics in bovine milk and milk extracts prepared for liquid chromatography–electrospray ionization tandem mass spectrometry analysis. *Journal of Chromatography A*, 1054, 359–363

Comment [M17]: Check as per other reference pattern?? Italics

Comment [M18]: Check this - Is this related for reference (This is a part of Kisanthini S et al 2019 reference)

Comment [M19]: Check as per other reference pattern and detail

Normanno, G., La Salandra, G. and Dambrosio, A. (2007). "Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products," *International Journal of Food Microbiology*, 115, 290–296.

Phillips, I., Casewell, M. and Cox, T. (2004). "Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data," *Journal of Antimicrobial Chemotherapy*, 53, 28–52.

Spanu, V., Spanu, C., Virdis, S., Cossu, F., Scarano, C. and De Santis, E. P. L. (2012). "Virulence factors and genetic variability of Staphylococcus aureus strains isolated from raw sheep's milk cheese," *International Journal of Food Microbiology*, 153, 53–57.

Adeleke, O. E., Adeniyi, B. A. and Akinrinmisi, A. A. (2000). Microbiological quality of local soymilk: a public health appraisal. *African Journal of Biochemical Research*, 3, 89-92.

Shintani, M., Takahashi, Y., Yamane, H., and Nojiri, H. (2010). The behavior and significance of degradative plasmids belonging to Inc groups in Pseudomonas within natural environments and microcosms. *Microbes Environ.* 25, 253–265.

Zeynudin, A., Pritsch, M., Schubert, S., Messerer, M., Liegl, G., Hoelscher, M., Belachew, T., and Wieser, A. (2018). Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β -lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. 1–10.

Aminov, R.I. (2009), The role of antibiotics and antibiotic resistance in nature. *Environmental Microbiology*, 11, 2970-2988

Comment [M20]: Check as per other reference pattern and detail