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Biochemical and Molecular Identification of *Escherichia coli* and *Salmonella* spp. Isolated from Milk and Fermented Milk Products Vended in Sabon-Gari and Zaria Local Government Area, Kaduna State, Nigeria

Fathuddin, M. M.[1*], Ado, S. A.[1], Tijjani, M. B.[1], Kazeem H. M.[2], Obidah J. S.[3], Fathuddin R.[4] and Musa A. I.[5]

[1] Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria – 810107, Kaduna State, Nigeria
[2] Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – 810107, Kaduna State, Nigeria
[3] Department of Microbiology, Faculty of Life Sciences, Modibbo Adama University, Yola – 640231, Adamawa State, Nigeria
[4] School of Life Sciences (SLS), B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai – 600048, Tamil Nādu, India
[5] Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria – 810107, Kaduna State, Nigeria

Abstract
***Salmonella* spp. and *Escherichia coli* are two of the most prevalent food-poisoning organisms. As part of this investigation, *Escherichia coli* and *Salmonella* spp. were isolated from some fresh milk and fermented milk products in Zaria. This study aimed to investigate the prevalence and molecular characterisation of *Salmonella* spp. and *Escherichia coli* in fresh milk and fermented milk products vended Sabon-Gari and Zaria Local Government Area, Kaduna State, Nigeria. Four hundred (400) samples were used consisting of one hundred (100) samples of milk, 100 samples of Kindirimo, 100 samples of Nono, and 100 samples of yoghurt. In addition, a routine microbiological isolation detected 11% of the samples positive for *Escherichia coli* and 16% for *Salmonella* spp. All isolates were biochemically characterised and identified; however, none of the selected isolates of *Escherichia coli* or *Salmonella* spp. possessed the virulence genes *rfbE* or *invA.* Thus, 16S ribotyping was utilised to authenticate the isolates' identities as *Escherichia coli* strain NCCP 14540, *Escherichia coli* strain E57, *Salmonella bongori* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium. However, more molecular research is required to determine which other *Salmonella* and *Escherichia coli* serotypes were present in the study region.**
Keywords: *Escherichia coli, Salmonella* spp., *rfb*E, *invA,* Milk, Fermented Milk Products, Zaria, Sabon-gari

* **- Corresponding Author:**

Introduction

The following terms have been defined by the National Agency for Food and Drug Administration and Control (NAFDAC) in Milk and Dairy Products Regulations (2021): "Milk" means a natural, unprocessed mammary secretion of an animal, whether it is collected from a single milking or multiple and is either consumed as a liquid or used for further processing. In this context, "milk product" means the result of processing milk, which may include added ingredients for food or other components necessary for processing. ´Yoghurt" is a milk product pasteurized and fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. However, Indigenous fermented milk products (FMPs), such as *Nono* and *Kindirimo*, are not defined by NAFDAC.

Oyeyinka et al. (2023) reported that Nigeria's Hausa people's beloved fermented milk products are made by the Fulani people, who often make cultured milk products for subsistence. This milk can be either full-fat (*Kindirimo*) or partially cultured skimmed (*Nono*). It is made by collecting unpasteurised cow milk in a *kwarya* (calabash) container and letting it ferment spontaneously for one day at room temperature.

The *Enterobacteriaceae* family includes the facultative anaerobic Garm-negative bacterium *E. coli*, characterised by its rod-shaped, non-spore-forming, and flagellated bacteria (Sarba et al., 2023). Pathogenic *E. coli* is serotyped using surface antigen profiles of K [capsular], H [flagellar], and O [somatic] (Denamur et al., 2021). While other animals, such as sheep and goats, could harbour *E. coli* O_{157}, cattle are the most common hosts. While *E. coli* O_{157} is a severe foodborne disease, most *E. coli* strains are innocuous (Sarba et al., 2023).

Salmonella species are members of the facultative intracellular bacteria, are gram-negative and rod-shaped, and may infect many hosts. *Salmonellae* are classified into two species: *Salmonella enterica* and *Salmonella bongori*. (Gebeyehu et al., 2022). By analyzing the bacterial outer membrane's flagellar 'H' antigen and the somatic 'O' antigen, more than 2700 unique Salmonella serovars have been found (Chatterjee et al., 2023). Abey et al. (2024) found that out of 2700 serovars, roughly 1500 are Salmonella enterica subsp. enterica, making it the most frequent zoonotic pathogen causing salmonellosis in humans and other animals. The finding of *Salmonella* spp. and *E. coli* O_{157} in milk and FMPs thus suggests a potential public health issue.

Ribotyping is a technique for identifying and characterising organisms from distinct species, such as bacteria with 16S ribotyping and fungi with 23S ribotyping (Qurban and Ameen, 2020). However, this method often finds microbes in every part of the body (Hatzenpichler et al., 2020). According to Regueira-Iglesias et al. (2023), the 16S ribosomal RNA gene is a popular tool in bacterial phylogenetic study because of its large size (about 1500 base pairs), prevalence in bacteria, and consistent activity.

The significance of this research lies in its potential to improve public health and food safety by identifying and characterising *E. coli* and *Salmonella* spp. gotten from milk, *Nono, Kindirimo*, and yoghurt.

Materials and Methods

Sample collection

The study utilised four hundred (400) samples—100 of each kind of milk and FMPs—that were marketed in Sabon Gari and Zaria. To isolate *E. coli* and *Salmonella* spp., 50 mL of milk and FMPs were collected from various sampling points, placed in a sterile, transparent zip-lock bag, labelled, and quickly sent to the Industrial Microbiology Laboratory at Ahmadu Bello University for analysis.

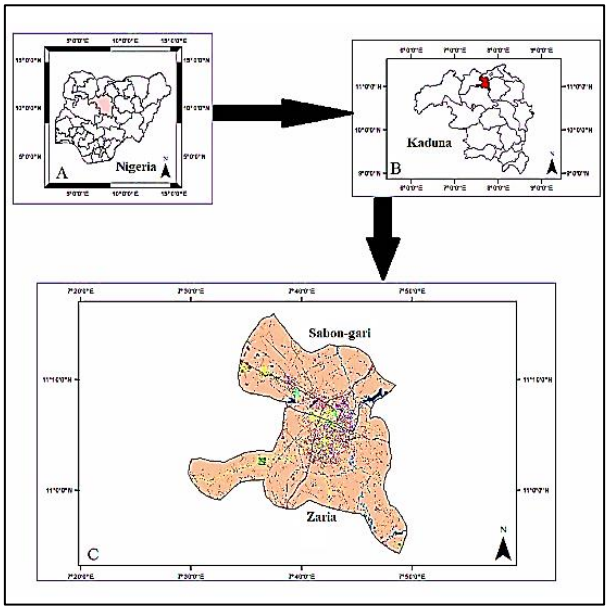


Fig 1: Map of Sabon-gari and Zaria (Department of Geomatics, Ahmadu Bello University, Zaria)

Isolation of Escherichia coli

The isolation followed the method employed by Naratama and Santoso (2020) with

modifications. After mixing 25 mL of the sample with buffered peptone water 225 mL, the mixture was grown for 24 h at 37°C. A loopful was streaked onto Chromocult Coliform Agar Enhanced Selectivity (CCAES) plates, then incubated for 24 h at 37°C. Afterwards, positive colonies (purple) were subcultured onto Eosin Methylene Blue (EMB) Agar and incubated for 24 h at 37°C. The Green-metallic Sheen growth will be subcultured and stored on nutrient agar slants for Biochemical Identification.

Isolation of Salmonella spp.

The isolation procedure followed the method described by Fathuddin and Obidah (2024). In the pre-enrichment culture, 25 mL of the sample and 225 mL of buffered peptone water were mixed and grown for 24 h at 37 °C. Next, the pre-enrichment culture underwent centrifugation at 5000 rpm for 5 minutes to separate the pellets. The sediment was placed into 9.0 mL of a selective enrichment medium Rappaport-Vassiliadis Broth (RV) and then incubated at 42°C for seven days. After incubation, subculture onto Xylose Lysine Deoxycholate (XLD) agar was incubated at 37°C for 48 h. Plates were observed for growth. Positive growth was then transferred to nutrient agar slants for biochemical identification.

Biochemical Identification of Isolates

The isolates were revived from the Nutrient Agar Slant by subculturing them on nutrient agar and incubating them for 24 h at 37°C. Then, the biochemical test carried out was Gram's reaction, Morphology, Indole, Methyl Red, Voges-Proskauer, Citrate, Triple Sugar Iron, Catalase, Motility, Blood Agar (β-haemolysis), DNase Production, Glucose, Lactose, Mannitol, Oxidase, Sucrose, Xylose, and Urease as per the protocol described Chauhan and Jindal (2020).

Detection of Virulence Gene in the Isolates

To detect the distinctive *rfbE* and *invA* genes in five (*E. coli*) and four (*Salmonella* spp.) biochemically identified isolates, they were evaluated using a Polymerase Chain Reaction (PCR) technique. Table 1 gives details of the primers used. The study was carried out at the Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology and the Department of Veterinary Public Health at Ahmadu Bello University.

Table 01: Sequences and parameters of primers

Microorganism	Target gene	Primer sequence (5'–3')	Amplicon length (bp)	References
E. coli	*rfbE*$_{O157}$	F: GCCACCCCCATTTTCGTTG R: TCCTCTCTTTCCTCTGCGGT	601	Wei et al., (2018)
Salmonella spp.	*inv*A	F: CTTTAGCCAAGCCTTGACGAAC R: AAAGGCAATACGCAAAGAGGT	284	

16s Ribotyping of the Isolates

The isolate was subjected to identification using 16S rRNA. At the Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, the genome DNA was amplified using universal primers 27F (Table 2). Later, at the Inqaba Biotec West Africa, the samples were further amplified using universal primers 907R and 1492R (Table 2). The nucleotide sequences obtained were subjected to a BLAST search in the NCBI database to identify similarities with known sequences.

Table 02: Sequences of 16S rRNA primers

Target gene		Primer sequence (5'–3')	Amplicon length (bp)	Reference
16S rRNA	27F	F: AGAGTTTGATCCTGGCTCAG	1500	Indraswari et al., (2021) Sichangi et al., (2020)
	907R	R: GGTTACCTTGTTACGACTT		
	1492R	R: CCGTCAATTCCTTTAGAGTTT R: GG(CT)TACCTTGTTACGACTT		

Results and Discussion

Isolation of Escherichia coli and Salmonella spp.

Forty-four suspected *E. coli* were isolated from the four hundred samples. Meanwhile, sixty-five suspected *Salmonella* spp. were isolated.

Biochemical Characterisation of Isolates

The biochemical characterisation, as presented in Table 3, shows that both organisms were gram-negative rods, with *E. coli* being positive for indole, methyl-red, catalase, motility, and

urease. They could also utilise Triple Sugar Iron and produce acid and gas and ferment sugars such as glucose, lactose and mannitol. And *Salmonella* spp. being positive for methyl-red, catalase, and motility. Also, utilise Triple Sugar Iron and produce alkaline slant, acid butt, gas and hydrogen sulphide and ferment sugars such as glucose, mannitol and xylose. Our findings conform with the description by Ghali-Mohammed et al. (2023) and Yakubu et al. (2020), who isolated and characterised *E. coli,* as Naik et al. (2015) and Nair et al. (2015) for *Salmonella* spp.

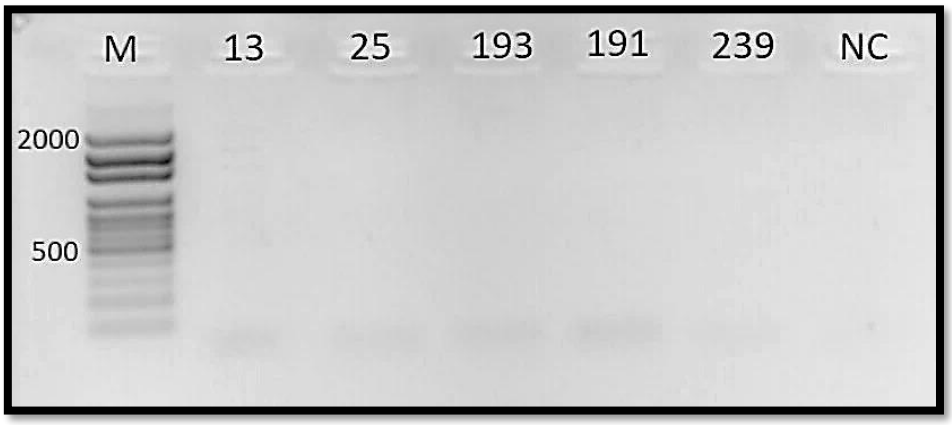
Table 03: Biochemical identification test

Gram	Morphology	Indole	Methyl Red	Voges Proskauer	Citrate	Triple Sugar Iron	Catalase	Motility	Glucose	Lactose	Mannose	Xylose	Urease	Probable Bacteria
-	Rod	+	+	-	-	$^{A}/_{A}$+ G	+	+	+	+	+	-	+	*Escherichia coli*
-	Rod	-	+	-	-	$^{K}/_{A}$+ G+H_2S	+	+	+	-	+	+	-	*Salmonella* spp.

Key: A = Acid, K = Alkaline, $^{-}/_{-}$ = $^{Slant}/_{Butt}$, G = Gas, H_2S = Hydrogen Sulphide Production, + = Positive Reaction, - = Negative Reaction.

Detection of rfbE Virulence Gene

Plate 1 displays the PCR amplification product for *E. coli*'s *rfb*E gene on an Agarose gel. The negative control was only normal Nuclease-free water. There was no amplification among the chosen isolates, indicating that the selected virulence gene does not exist but does not rule out the presence of other virulence genes.



Key: M – Molecular Ladder, (13, 25,193, 191, 239) – Wells containing samples, NC – Negative control (Nuclease-Free Water)

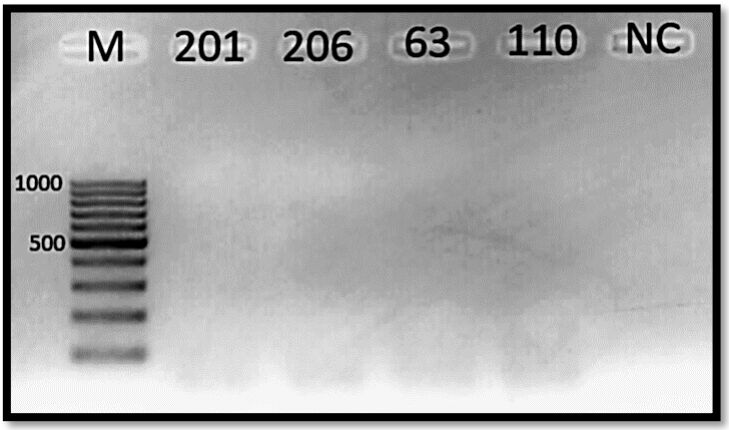
Plate 01: Agarose gel post-electrophoresis showing PCR amplification product of *rfb*E gene for *Escherichia coli* at 601 bp.

Barbour et al. (2015) reported that 0.0% of *E. coli* had the *rfbE* gene, which was isolated from milk from five provinces of Lebanon. Elafify et al. (2020) showed that in Egypt, out of thirty-six (36) isolates were only three (3) *E. coli* isolates which harboured the *rfb*E gene, which indicates the rarity of the *E. coli* O_{157}: H_7 in nature. Sobeih et al. (2023) studied milk and FMPs from Kafr El-Sheikh governorate in Egypt; out of twenty-one (21) *E. coli* isolates, thirteen (13) had the *rfb*E genes, which indicates an increase in the *E. coli* O_{157}:H_7 population.

Detection of invA Virulence Gene

Plate 2 shows the post-electrophoresis agarose gel showing the PCR amplification product for

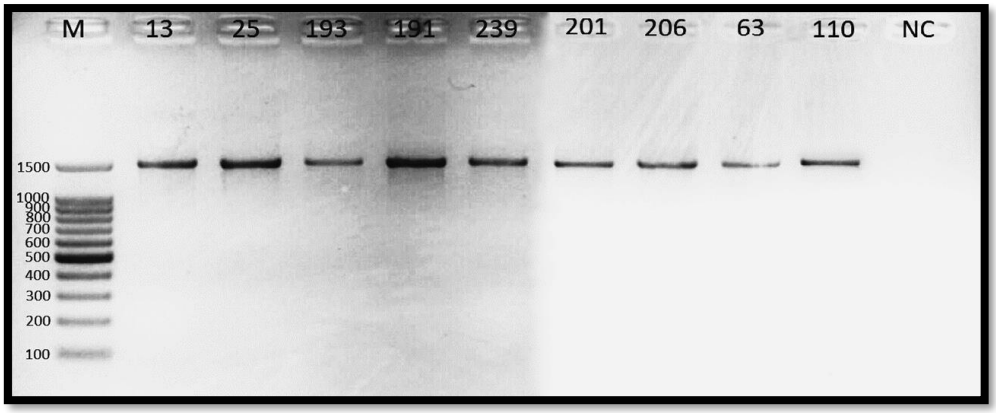
Salmonella spp.'s *invA* gene. Normal Nuclease-free water was the negative control.



Key: M – Molecular Ladder, (201, 206, 63, 110) – Wells containing samples, NC – Negative control (Nuclease-Free Water)
Plate 02: Agarose gel post-electrophoresis showing PCR amplification product of *inv*A gene at 284 bp for *Salmonella* spp.

Akinyemi et al. (2021) detected the *invA* gene among 75% of the isolated *Salmonella* spp. in Lagos. Almas et al. (2021) reported that 70% of isolated *Salmonella* spp. in Lahore had confirmed the presence of *invA* genes. Kanteh et al. (2021) reported that in the Gambia, invasive *Salmonella* spp. constituted about 10% of the total *Salmonella* spp. isolates.

16S Ribotyping of Escherichia coli and Salmonella spp.
Plate 3 shows the amplified 16S rRNA at 1500 bp.



Key: M – Molecular Ladder, (13, 25,193, 191, 239, 201, 206, 63, 110) – Wells containing samples, NC – Negative control (Nuclease-Free Water)

Plate 03: DNA band from 16S rRNA gene amplification

As shown in Tale 4, the sequencing results confirm the selected organisms to be *Escherichia coli* (193 and 239) and *Salmonella* spp. (110 and 201).

Table 04: 16S Ribotyping of the selected *E. coli* isolates

Name of Sample	Sequence Name	Sequence Size (bp)	Query Cover (%)	Percent identity (%)	GenBank Accession	Organism Description
193	193_16S-907R-0823_G08_3730XL	888	98	97.83	CP042982.1	*Escherichia coli* strain NCCP 14540 chromosome,

						complete genome
	193_16S-1492R-1023_E09_3730XL	1180	92	94.91	CP142557.1	*Escherichia coli* strain ExPEC_A351 chromosome, complete genome
239	239_16S-907R-0823_H08_3730XL	890	97	98.50	CP121153.1	*Escherichia coli* strain E57 chromosome, complete genome
	239_16S-1492R-1023_F09_3730XL	1197	87	95.53	MT878606.1	*Escherichia coli* strain L-1 16S ribosomal RNA gene, partial sequence
110	110_16S-907R-0823_D09_3730XL	890	97	98.96	MG663487.1	*Salmonella bongori*
	110_16S-1492R-1023_B10_3730XL	1103	74	92.14	MH255592.1	*Salmonella* sp.
206	206_16S-907R-0823_A09_3730XL	889	91	99.14	MH356711.1	*Salmonella enterica* subsp. *enterica* serovar Typhimurium
	206_16S-1492R-1023_G09_3730XL	1194	92	92.44	MH255592.1	*Salmonella* sp.

The nucleotide sequences of the two strains were found to align with the rRNA gene sequences of several species of *Escherichia coli* strain [NCCP 14540 (ExPEC_A351) and E57 (L-1 16S)] and *Salmonella* spp. [*S. bongori* and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*] respectively, with identities of over 92.14%. The percentage identity confirms the molecular identity of our isolates, which were also identified in other related studies as described by Neamah et al. (2022), in molecular-identified *E. coli* obtained from milk affected by mastitis with a 99% homology identity, higher than ours (94.91-98.50%). Also, Indraswari et al. (2021), molecular-identified pathogenic *E. coli* (*E. coli* Sakai and *E. coli* O_{104}:H_4) obtained from cow meat in Yogyakarta, Indonesia, did not give similarity percentages. Bano et al. (2020) identified *Salmonella* sp. from raw milk samples sold in Pakistan using 16S amplification and got a similarity percentage of 98%, similar to ours. Also, in the studies conducted by Rashad et al. (2023), molecular-characterised *Salmonella* spp. isolated from raw and heat-treated milk from the local markets in Egypt had similarity identities of over 97.86%, close to ours at 92.14-99.14%. The implication of the presence of *E. coli* and *Salmonella* in milk and milk products poses a serious public health risk, as these pathogens can cause severe foodborne illnesses that may lead to hospitalization, long-term health consequences, and even death if proper food safety measures are not followed. Rigorous food safety practices throughout the food production and preparation chain are critical to mitigate these risks.

Conclusion

This study detected and characterised *E. coli* and *Salmonella* spp. gotten from milk and FMPs vended around Sabon-gari and Zaria Local government areas. The current study shows that *E. coli* and *Salmonella* spp. are influential milk contaminations regardless of the locations sampled, year's season and source of milk. Based on biochemical characterisation, the total number of *E. coli* and *Salmonella* spp. isolated were forty-four and sixty-five, respectively, which consisted of 11% and 16% of the sample collected, which was low considering the total samples collected. Furthermore, the *rfbE* and *invA* genes were not detected; however, the 16S ribotyping did confirm the isolates to be *E.*

coli and *Salmonella* spp. with a similarity of 92.14-98.50%. Additional molecular studies must determine the serotypes of *E. coli* and *Salmonella* spp. circulating in the research area. Our results highlight the need to promote hygienic food handling and proper cooking practices to reduce or eliminate the risk of pathogenic bacteria from these foods.

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REFERENCE

Abey, S. L., Gedefaw, M., Molla, W., Dagnaw, G. G., Mengistu, B. A., Kinde, M. Z., Nigatu, S., Jemberu, W.T., Adugna, T., Berju, A., Dagnachew, S., Birhanu, K., Ejo, M., and Bitew, A. B. (2024). Molecular Detection and Antimicrobial Resistance Profile of Salmonella isolated from Humans, Animals, Abattoir Equipment and Environment. *App. Food Res.*, 100437. DOI: https://doi.org/10.1016/j.afres.2024.100437

Akinyemi, K. O., Fakorede, C. O., Abegunrin, R. O., Ajoseh, S. O., Anjorin, A. A., Amisu, K. O., Opere, B. O., and Moro, D. D. (2021). Detection of *invA* and *blaCTM*-genes in *Salmonella* spp. isolated from febrile patients in Lagos hospitals, Nigeria. *Ger. J. Microbiol.* 1: 1–10. DOI: https://doi.org/10.51585/gjm.2021.3.0008

Almas, M., Malik, A., Ali, Q., Muhammad, T., Hadi, F., Gill, S. A., Mazhar, M., Zahra, N., Farrukh, S. Y., Shafiq, M. I., and Zameer, M. (2021). Detection of *INVA* gene and cytotoxin of *Salmonella enteritidis* in food samples using molecular methods. *J. Pharm. Res. Int.* 33(12): 20-28. DOI: https://doi.org/10.9734/jpri/2021/v33i1231249

Bano, S. A., Hayat, M., Samreen, T., Asif, M., Habiba, U., and Uzair, B. (2020). Detection of pathogenic bacteria *Staphylococcus aureus* and *Salmonella* sp. from raw milk samples of different cities of Pakistan. *Nat. Sci.* 12(05): 295-306. DOI: https://doi.org/10.4236/ns.2020. 125026

Barbour, E. K., Kassabian, T. J., Shaib, H., Kassaify, Z., Iyer, A., Azhar, E., Harakeh, S., and Kumosani, T. (2015). The significance of *Escherichia coli*-induced mastitis in cows is associated with the presence of virulence genes and wide-range resistance to twenty antimicrobials. Intern. *J. Appl. Res. Vet. Med.*, 13(1): 51-63. Retrieved from: http://www.jarvm.com/articles/Vol13Iss1/Vol13%20Iss1Barbour.pdf

Chatterjee, R., Chowdhury, A. R., Mukherjee, D., and Chakravortty, D. (2023). From Eberthella typhi to Salmonella Typhi: the fascinating journey of the virulence and pathogenicity of Salmonella Typhi. *ACS omega*, 8(29), 25674-25697. DOI: https://doi.org/10.1021/acsomega.3c02386

Chauhan, A., and Jindal, T. (2020). Microbiological Methods for Environment, Food and Pharmaceutical Analysis, 1st Ed. Switzerland. *Springer Nature*. p.425-468.

Denamur, E., Clermont, O., Bonacorsi, S., and Gordon, D. (2021). The population genetics of pathogenic Escherichia coli. *Nat. Rev. Microbiol.* 19(1): 37-54. DOI: https://doi.org/10.1038/s41579-020-0416-x

Elafify, M., Khalifa, H. O., Al-Ashmawy, M., Elsherbini, M., El Latif, A. A., Okanda, T., Matsumoto, T., Koseki, S., and Abdelkhalek, A. (2020). Prevalence and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* in milk and dairy products in Egypt. *J. Environ. Sci. Health B*. 55(3): 265-272. DOI: https://doi.org/10.1080/03601234.2019.1686312

Fathuddin, M. M. and Obidah S. J. (2024). Concentration technique for Viable but Non-Culturable organisms. *protocols.io* DOI: dx.doi.org/10.17504/protocols.io.261ge53x7g47/v1

Gebeyehu, A., Taye, M., and Abebe, R. (2022). Isolation, molecular detection and antimicrobial susceptibility profile of *Salmonella* from raw cow milk collected from dairy farms and households in southern Ethiopia. *BMC Microbiol.* 22(1): 84. DOI: https://doi.org/10.1186/s12866-022-02504-2

Ghali-Mohammed, I., Odetokun, I. A., Raufu, I. A., Alhaji, N. B., and Adetunji, V. O. (2023). Prevalence of *Escherichia coli* O157 isolated

from marketed raw cow milk in Kwara State, Nigeria. *Sci. Afr.* 19: e01469. DOI: https://doi.org/10.1016/j.sciaf.2022.e01469

Hatzenpichler, R., Krukenberg, V., Spietz, R. L., & Jay, Z. J. (2020). Next-generation physiology approaches to study microbiome function at single cell level. *Nat. Rev. Microbiol.*, 18(4), 241-256. DOI: https://doi.org/10.1038/s41579-020-0323-1

Indraswari, A., Suardana, I. W., Haryanto, A., and Widiasih, D. A. (2021). Molecular analysis of pathogenic Escherichia coli isolated from cow meat in Yogyakarta, Indonesia using 16S rRNA gene. *Biodiversitas J. Bio. Diver.*, *22*(10).

Kanteh, A., Sesay, A. K., Alikhan, N. F., Ikumapayi, U. N., Salaudeen, R., Manneh, J., Olatunji, Y., Page A. J. and Mackenzie, G. (2021). Invasive atypical non-typhoidal *Salmonella* serovars in The Gambia. *Microb. Genom.* 7(11): 000677. DOI: https://doi.org/10.1099/ mgen.0.000677

Naik, V. K., Shakya, S., Patyal, A., and Gade, N. E. (2015). Isolation and molecular characterization of Salmonella spp. from chevon and chicken meat collected from different districts of Chhattisgarh, India. *Vet. World*, *8*(6), 702. DOI: https://doi.org/ 10.14202/vetworld.2015.702-706

Nair, A., Balasaravanan, T., Malik, S. S., Mohan, V., Kumar, M., Vergis, J., and Rawool, D. B. (2015). Isolation and identification of *Salmonella* from diarrheagenic infants and young animals, sewage waste and fresh vegetables. *Vet. World*. 8(5): 669. DOI: https://doi.org /10.14202/vetworld.2015.669-673

Naratama, M. R., and Santoso, I. (2020). Non-fecal and fecal coliform tests of ready-to-eat food and drinks using fluorogenic and chromogenic media. J. Phys.: Conf. Ser. 1442: 012064. Basic and Applied Sciences Interdisciplinary Conference 2017 18–19 August 2017, UI Depok Campus, Indonesia DOI: https://doi.org/10.1088/1742-6596/1442/1/ 012064

National Agency for Food and Drug Administration and Control [NAFDAC] (2021) Milk and Dairy Products Regulations. *Fed. Rep. Nig Off Gazette* Vol 108 Available from: https://faolex.fao.org/docs/pdf/nig188321.pdf

Neamah, A. A., Fahad, K. H., Sadeq, J. N., and Al-Fatlawi, M. A. (2022). Molecular characterisation and phylogenetic analysis of *Escherichia coli* isolated from milk of cattle affected by mastitis. *Iraqi J. Vet. Sci.* 36(1): 251-254. https://doi.org/10.33899/IJVS.2021.129934.1702

Oyeyinka, A. T., Makhuvele, R., Olatoye, K. K., and Oyeyinka, S. A. (2023). African fermented dairy-based products. In: Indigenous Fermented Foods for the Tropics 1st Ed. London. *Academic Press*. p.169-188

Qurban, A., and Ameen, A. (2020). Bacterial identification by 16S Ribotyping, A Review. *Biotechnol. Ind. J.* 16(2): 204. DOI: https://doi.org/10.37532/0974-7435.2020.16(2).204

Rashad, N. S., Refaat, M. H., Ismail, E. E. A., and Soudy, F. A. (2023). Molecular Identification and Characterisation of *Salmonella* Sp. In Raw and Heat-Treated Milks from Local Market. *Ann. Agric. Sci. Moshtohor.* 61(3): 611-630. DOI: https://doi.org/10.21608/ASSJM.2024.253296.1259

Regueira-Iglesias, A., Balsa-Castro, C., Blanco-Pintos, T., & Tomás, I. (2023). Critical review of 16S rRNA gene sequencing workflow in microbiome studies: From primer selection to advanced data analysis. *Mol. Oral Microbiol.*, 38(5), 347-399. DOI: https://doi.org/10.1111/omi.12434

Sarba, E. J., Wirtu, W., Gebremedhin, E. Z., Borena, B. M., and Marami, L. M. (2023). Occurrence and antimicrobial susceptibility patterns of *Escherichia coli* and *Escherichia coli* O157 isolated from cow milk and milk products, *Ethiopia. Sci. Rep.* 13(1): 16018. DOI: https://doi.org/10.1038/s41598-023-43043-8

Sichangi, M. B., Nduko, J. M., and Matofari, J. W. (2020). Molecular investigation of potential lactic acid bacteria starter culture organisms/probiotics in the Kenyan spontaneously fermented milk, amabere amaruranu. *Pakistan J. Nutr.* 19: 132-145. DOI: https://doi.org/10.3923/pjn.2020.132.145

Sobeih, A. M. K., Moawad, A. A., and Sharshar, T. (2023). Antibiotic-resistant Shiga toxin-producing Escherichia coli isolates from milk

and milk products. *J. Hellenic Vet. Med. Soc.*, *74*(1), 5283-5288.

Wei, C., Zhong, J., Hu, T., and Zhao, X. (2018). Simultaneous detection of *Escherichia coli* O157: H7, *Staphylococcus aureus* and *Salmonella* by multiplex PCR in milk. *3 Biotech*, 8(1), 1-7. DOI: https://doi.org/10.1007/s13205-018-1086-5

Yakubu, R. O., Lawan, M. K., Kwaga, J. K. P., and Kabir, J. (2020). Isolation, Molecular Detection and Antimicrobial Susceptibility Profile of *Escherichia coli* O157: H7 in Household-reared Small Ruminants in Zaria Metropolis, Kaduna State, Nigeria*. Sahel J. Vet. Sci.* 17(4): 16-23. DOI: https://doi.org/10.54058/saheljvs.v17i4.196