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Detection of Enterotoxigenic *Escherichia coli* in Ready-To-Eat Powdered Soybean

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ABSTRACT:
Escherichia coli plays a significant role in various diseases, contributing to substantial morbidity and mortality among children and adults. Its enterotoxins are key virulence factors, highlighting the need for understanding its pathogenic mechanisms and developing effective prevention and treatment strategies. This study investigated the enterotoxigenicity potentials of *Escherichia coli* (*E. coli*) strains isolated from ready-to-eat powdered soybean samples in Nnewi South, Anambra State. Two hundred soybean samples were collected and screened for *E. coli* using microbiological techniques. Five *E. coli* strains (SEC470, V266, SUS9EC, O157:H7 SS52, and O157:H7 Sakai) were isolated and evaluated for heat-stable enterotoxin production using the intragastric method in suckling mice. The study revealed significant differences ($p \leq 0.05$) in gut/body ratio (G/B) and fluid accumulation ratio (FAR) among the isolates. Notably, ECSEC470, ECOHSS52, and ECOH Sakai showed significant ($p \leq 0.05$) production of heat-stable enterotoxins, with G/B ratio and FAR values ≥ 0.065 and ≥ 0.070, respectively. ECOHSS52 recorded the highest values, indicating potent enterotoxin production. The study highlights the presence of enterotoxigenic *E. coli* strains in soybean samples, posing a potential health risk to consumers. These findings emphasize the need for proper handling and processing of soybean products to prevent contamination and ensure food safety.

INTRODUCTION

The consumption of ready-to-eat foods has surged in popularity due to convenience, but this trend has raised concerns regarding food safety [1]. One such food that has gained recognition is powdered soybean, which is utilized in various culinary applications and is rich in protein and nutrients. However, improper handling, inadequate cooking, and improper storage can lead to contamination by pathogenic microorganisms, including E. coli. Prior studies have indicated that soybean products can serve as a vehicle for *E. coli* transmission, especially when produced under unsanitary conditions [2].

Escherichia coli (*E. coli*), a bacterium belonging to the Enterobacteriaceae family, is well-known as a predominant commensal organism that resides in the gastrointestinal tracts of warm-blooded animals and people. In its commensal role, *E. coli* plays a

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beneficial part in digestive processes and contributes to the overall health of its hosts, with a relationship that is generally non-pathogenic [3]. Among the pathogenic strains, enterotoxigenic *E. coli* (ETEC) is recognized as a predominant cause of diarrhoea, particularly in developing countries and among travelers [4]. ETEC produces enterotoxins that irritate the intestinal lining, ultimately leading to fluid secretion and diarrhoea. The public health implications of ETEC infections are particularly acute due to their association with foodborne illnesses, especially from contaminated food products [5].

Soybean powder is made from soybeans (*Glycine max*), a legume native to East Asia known for its edible seeds rich in phytic acid, minerals, and B vitamins. A major byproduct is soybean oil, which is used in culinary and industrial applications. Soybeans are also a key protein source for livestock, contributing to the human food supply [6]. Soybean milk offers health benefits, including reducing cholesterol levels and lowering cardiovascular disease risk [7].

The production process includes cleaning, cracking, grinding, oil extraction, and impurity removal [8]. However, powdered soybeans may carry food safety risks, particularly from contamination with *Escherichia coli* (*E. coli*), which can survive in powdered products and form biofilms resistant to environmental stressors [9, 10].

The enterotoxigenicity of *E. coli* strains isolated from food sources is a critical aspect of understanding the potential health risks associated with consuming these products. The assessment of different *E. coli* strains regarding their enterotoxin production is essential not only for public health surveillance but also for regulatory purposes to ensure food safety [11]. Various serotypes of *E. coli* possess different virulence factors, and the identification of enterotoxigenic strains from ready-to-eat powdered soybean can provide insights into the prevalence and potential outbreak sources of food-borne illnesses.

MATERIALS AND METHODS

Isolation and Characterization of *Escherichia coli* from the Soybean Samples*:* Ready-to-eat soybean samples were randomly collected from different locations in Nnewi South, Anambra State, Nigeria. This was carried out using the modified method of [12]. One gram (1.0 g) of each sample was first measured and dissolved in 10 ml of sterile distilled water. One milliliter of the aliquot was aseptically plated on Eosin Methylene Blue agar (EMBA/Biotech) using the pour plate technique. All the plates in triplicate were incubated inverted at 44.5±2°C for 24 h for *E. coli*. The isolates were sub-cultured on nutrient agar (Biotech), incubated in an inverted position at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions [12], biochemical reactions [12, 13] and molecular characterization [14, 15, 16]. The colonial description aimed to identify the colors of the isolates on agar media plates, as well as their sizes, edges, consistencies, and optical properties.The objective of the colonial description was to determine the isolates' colors on agar medium plates in addition to their sizes, edges, consistencies, and optical characteristics.

Preparation of the test Isolates: This was carried out using the method of [17, 18, 19, 20]. Broth cultures of the isolates were centrifuged at 3000 rpm for 10 minutes. The sediments were diluted with sterile phosphate buffer saline (PBS) and adjusted to 1.50×10^8 cells/ml using 0.5 McFarland matching standard prepared by mixing 0.5 ml of 1.175% $BaCl_2.2H_2O$ and 99.5 ml of 1% concentrated H_2SO4.

Suckling Mice Test for Enterotoxigenicity: This process was carried out using the approach that Asiton et al. [21] outlined. Shortly before to the experiment, six groups of five nursing mice each were created by separating the one- to three-day-olds from their mothers. The mice were intragastrically inoculated with 0.1 ml of the prepared cells using syringes without needles. All the inoculated mice were kept at room temperature (30±2°C) for 4 h, after which they were killed with chloroform. The gut (intestine) was surgically removed from the body, and each was weighed

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separately. The fluid accumulation (FA) ratio (weight of entire intestine/ total body weight-weight of intestine) of each animal was calculated. The gut/body ratio was also calculated.

Statistical Analysis: The results of the data generated were expressed in Tables. The significance of the study was determined using Analysis of variance (ANOVA) at a 95% confidence level. Pairwise comparison was carried out in an Excel sheet using Student "t" test [22]

RESULTS

The results of the study showed that the quality of nucleic acids (DNA) extracted from the isolates was pure as the ratios of the absorbances A260/A280 were within the stipulated range 1.80-1.90 (Table 1). The results of the sequencing of 16s rRNA using universal primer (16s) revealed the presence of *Escherichia coli* strain SEC 470 (ECSEC470) (isolate X1), *Escherichia coli* strain V266 (ECV266) (isolate X2), *Escherichia coli* strain SUS9EC (ECSUS9EC) (isolate X3), *Escherichia coli* 0157:H7 strain SS52 (ECO157:H7SS52) (isolateY1) and *Escherichia coli* 0157:H7 strain Sakai (ECO157:H7Sakai) (isolate Y2) (Table 2).

Table 1: Quality of nucleic acid (DNA) used for the study

Sample	Concentration of Nucleic acid (ng/µL)	A260	A280	260/280
X1	109.80	0.2438	0.1340	1.82
X2	119.70	0.3459	0.1880	1.84
X3	128.40	0.3571	0.1920	1.86
YI	117.10	0.3312	0.1820	1.82
Y2	108.50	0.2239	0.1239	1.85

Table 2: Molecular identities of the isolates

Isolate	Max score	Total score	Query Cover	Gap	Identity	Accession Number	Description
X1	1297	1297	100%	0%	96%	CP007594.1	*Escherichia coli* strain SEC470 Complete genome
X2	1290	1290	100%	0%	99%	LC056477.1	*Escherichia coli* strain V266 Complete genome
X3	1190	1190	100%	0%	99%	KF991482.1	*Escherichia coli* strain SUS9EC Partial genome
Y1	2856	2967	100%	0%	100%	CO010304.1	*Escherichia coli* 0157:H7 strain SS52 Complete genome
Y2	2844	2844	100%	0%	100%	CP011428.1	*Escherichia coli* 0157:H7 strain Sakai Complete genome

The enterotoxigenicity among the isolates using the suckling albino mice is displayed in Table 4.When the gut/body weight (G/B) ratio is greater than 0.065 and above, the isolate is significant for enterotoxin. Also, when the fluid accumulation is greater than 0.070 and above, what was isolated is significant for enterotoxin. The present study showed that ECSEC470, ECOHSS2 and EC0H Sakai were

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significant (P<0.05) for enterotoxin after 4 h (Table 5). ECV266 showed a slight positive result after 4 h of exposure of the mice to the organism. ECSUS9EC and control were non-significant (P>0.05) to enterotoxins after 4 h exposure (Table 4). It was observed that all the SOR-negative strains isolated from the soybean samples were significantly to heat-stable than enterotoxin when compared to SOR-positive strains. Additionally, ECOHSS2 was significantly (P<0.05) the most heat-stable enterotoxin-producing strain compared to other heat-stable enterotoxin-producing strains encountered in this study.

Table 3: Enterotoxigenicity study of the isolates

Parameter	ECSEC470	ECV266	EC SUS9EC	ECOHSS2	ECOH Sakai	Control
BW(g)	1.815	1.840	1.820	1.830	1.820	1.850
GW (g)	0.118	0.117	0.102	0.123	0.119	0.101
FAR	0.070	0.068	0.059	0.072	0.070	0.058
G/B	0.065	0.064	0.056	0.067	0.065	0.055
Inference	+	+/-	-	+	+	-

BW: Body Weight; GW: Gut Weight; FAR: Fluid Accumulation Ratio; G/B: Gut weight/Body weight; FAR GW/BW-GW: FAR is positive when the value is ≥ 0.070, G/B is positive when the value is ≥ 0.065.

ECSEC470-*Escherichia coli* strain SEC470; ECV266- *Escherichia coli* strain V266

ECSUS9EC- *Escherichia coli* strain SUS9EC; ECOHSS52- *Escherichia coli* 0157:H7strain SS52, ECOH Sakai- *Escherichia coli* 0157:H7 strain Sakai

Discussion

The characteristics and identities of the isolates from roasted meat samples in this study are in line with the report of [23], who worked on biochemical characterization for *E. coli*. The presence of *Escherichia coli* SEC470, *Escherichia coli* V266, *Escherichia coli* SUS9EC, *Escherichia coli* O157:H7 SS52 and *Escherichia coli* O157:H7 Sakai from the studied soyabean samples supported the occurrence of *Escherichia coli* in the samples. Traditionally, the laboratory detection of *Escherichia coli* has relied on non-selective and/or selective enrichment and subsequent culture on selective media. The debut of molecular techniques provides a more sensitive and rapid technique for detecting these organisms. *Escherichia coli* strains were initially identified using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein (usp A) and were found to be highly specific for *E. coli* strains. No amplification was observed from any of the non-*E. coli* Gram-negative bacteria were tested. Chen and Griffiths [24] reported the detection of usp A from *E. coli* isolated from food samples.

The enterotoxigenicity study was ascertained using the fluid accumulation ratio (FAR) and gut-body weight ratio (G/B). The presence of enterotoxin among the studied organisms reported the findings of many researchers [21, 25, 26]. The enterotoxin produced by these organisms shows that the organisms have the potential to produce heat-stable enterotoxin [27]. The heat-stable enterotoxin is not destroyed by heating at 100°C for 30 minutes, and it activates guanylate cyclase, causing the increased production of cyclic guanosine monophosphate (cGMP) and subsequent hypersecretion [27]. Also, for organisms to produce heat-stable enterotoxin intragastrically in suckling mice signifies that the heat-stable enterotoxin is truly type 1 (ST-1) [27]. The inability of ECV266 and ECSUS9EC to produce toxins after 4h exposure could be attributed to the difference in the stability of the toxin in solution [21] or the length of time of exposure. It could also be that the mechanisms other than the production of

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enterotoxin are responsible for their pathogenic activity, although the isolates are from food samples which were contaminated either by environmental factors [21].

CONCLUSION

This study has confirmed the presence of various *Escherichia coli* strains, including SEC470, V266, SUS9EC, O157: H7 SS52, and O157:H7 Sakai, in soybean samples. Notably, these strains produced heat-stable enterotoxins in infected rats, with ECO157:H7 SS52 exhibiting the highest enterotoxin production. These findings highlight the potential health risk associated with consuming contaminated soybean products and underscore the importance of proper food safety measures.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval

All authors hereby declare that "Principles animal care" (NCARE with Ref No FPSRA/UNN/24/0114), certified on 12th November, 2024 at University of Nigeria, Nsukka, were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

REFERENCES

1. Santos, J. M., Gonçalves, M. C., Martins, H. A., Pinelli, J. J., Isidoro, S. R., Piccoli, R. H., 2018. Listeria species have adapted both homologously and heterologously to the essential oils of condiment plants. Advances in Microbiology, 8(8), 639-649.

2. Agboke, A. A., Osonwa, U. E., Ibezim, E. C., 2011. Evaluation of microbial content of some soybean milk products consumed in Nigeria. Journal of Pharmacy & Bioresources, 8(1), 34-40

3. Brenner, D. J., Krieg, N. R., Staley, J. R., Garrity, G. M. (Eds.). 2005. Bergey's Manual® of Systematic Bacteriology: Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria. New Delhi: Springer US, 22-- 31

4. Nel, E. D. (2010). Diarrhoea and malnutrition. South African Journal of Clinical Nutrition, 23(sup1), 15-18.

5. Mueller, M., Tainter, C. R. 2023. Escherichia coli infection. In StatPearls [Internet]. StatPearls Publishing.47－53

6. Shea, Z., Singer, W. M., Zhang, B. 2020. Soybean production, versatility, and improvement. Legume crops-prospects, production and uses, 29-50.

7. Messina, M. J. 2016. Soy and health update: Evaluation of the clinical and epidemiologic literature. *Nutrients*, 8(12), 775

8. Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A. ., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., Ilechukwu, C. C. 2025a. Exploring the Phytochemical and Antimicrobial Properties of Fruit Vinegar: A Study on Phoenix Dactylifera and Malus Sylvestris. *IPS Journal of Applied Microbiology and Biotechnology,* **4**(1), 115–122.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 1724-1730 | ISSN:2251-6727

9. Phillips, C. A. 2016. Bacterial biofilms in food processing environments: a review of recent developments in chemical and biological control. *The International Journal of Food Technology and Science, 51*(8), 1731-1743.

10. Reihani, S., Khosravi, K. 2019. Review of parameters influencing submerged fermentation's ability to produce single-cell proteins. *Electronic Journal of Biotechnology, 37*, 34–40

11. Johnson, J. R., Kuskowski, M. A., Smith, K., T., T., Tatini, S. 2005. Antimicrobial-Resistant and Extra-intestinal Pathogenic *Escherichia coli* in Retail Foods. *The Journal of Infectious Diseases*, *191*(7), 1040-1049.

12. Cheesbrough, M. 2010. *District laboratory practice in tropical countries* (2nd ed.). Cambridge University Press, 34－43

13. Iheukwumere, I.H., Olusola, T.O. Chude, C. 2017a. Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. Journal of Natural Sciences Research, 8, 21–33

14. Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Nwike, M. I., Nwakoby, N. E., Ilechukwu, C. C. 2025b. Safety Evaluation of Vinegar from Phoenix Dactylifera and Malus Sylvestris: Toxicity and Acetic Acid Content. *IPS Journal of Applied Microbiology and Biotechnology,* **4**(1), 123–131.

15. Gabriela, I. F., Cecilia, L. E., Teresa, I. C. Maria, E. E. 2014. Detection and characterization of shiga toxinproducing Escherichia coli, Salmonella species and Yersinia strains from human, animal and food samplesin San Luis, Argentina. International Journal of Microbiology, 20, 1–11

16. Iheukwumere, I.H., Olusola, T.O. Chude, C. 2018a. Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research,* **8,** 21–33.

17. Wafaa, A.A., Soumaya, S.A, Hatem, M.E. Rehab, E.D. 2012. A trial to prevent Salmonella Enteritidis infection in broiler chickens using antigenous bacterin compared with probiotic preparation. Journal of Agricultural Science, **4**(5), 91–108.

18. Iheukwumere, I. H., Amadi, R. E. Unaeze, B. C. 2017b. Enterotoxigenicity profile of Salmonella enterica serovar Typhimurium in suckling albino mice. *Journal of Natural Sciences Research* **7** (14) :16–20.

19. Iheukwumere, I.H., Chude, C. Unaeze, B.C. 2018b. Toxicological study and antibacterial activities of effectively validated medicinal plants against enteric bacteria isolated from chicken feeds. *Journal of Health, Medicine and Nursing,* **7,** 19–34.

20. Iheukwumere, I.H., Chukwura, E.I. Chude, C. 2018c. *In vivo* activities of some selected antimicrobial agents against enteric bacteria isolated from chicken feeds on broiler layers. *Journal of Biology, Agriculture and Healthcare,* **9,** 21–36.

21. Asiton, A. D., Gideon, O. A, Soe, N. S. 2012. Enterotoxigenicity profile of *E. coli*, *Vibrio* and *Salmonella* species. Journal of Experimental Biology, **24,**(2), 64–66.

22. Iheukwumere, I.H., Dimejesi, S.A., Iheukwumere,C.M., Chude, C.O., Egbe, P.A., Nwaolisa,C.N., Amutaigwe, E.U., Nwakoby, N.E., Egbuna, C., Olisah, M.C. Ifemeje, J.C.(2020). Plasmid curing potentials of some medicinal plants against citrate negative motile *Salmonella* species. *European Journal of Biomedical and Pharmaceutical Sciences,* **7**(5), 40 -47.

23. Paton, J.C. Paton, A.W. 2012. Pathogenesis and diagnoses of shiga toxin- producing *Escherichia coli* infections. *Journal of Clinical Microbiology,* **11,** 450–479

24. Chen, J. Griffiths, M. W. 1998. PCR differentiation of *Escherichia coli* from other Gram-negative bacteria using primers derived from the nucleotide sequences flanking the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(2), 1724-1730 | ISSN:2251-6727

gene encoding the universal stress protein. *Letters in Applied Microbiology,* **27,** 369–371

25. Murray, R. J. 2006. Recognition and management of Staphylococcus aureusand Salmonella toxin mediated disease. *International Medical Journal,* **2005,**(2), 106–119.
26. Balaban, N., Rasooly, A. 2009. Staphylococcal enterotoxins. International J*ournal of Food Microbiology* , **61,** 1–10.
27. Arora, D.R., Arora, B. B. 2008. Text book of Microbiology, 4th Ed. CBS Publishers and Distributors, New Delhi, India.. 40−49.