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Public Health Implications of *Escherichia coli* Contamination in Powdered Soybean

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KEYWORDS

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ABSTRACT:

In Nnewi South, Anambra State, a concerning health issue arises from the consumption of powdered soybean, a staple food in the region, which may be contaminated with Escherichia coli (E. coli). This bacterium can cause a spectrum of health problems, ranging from mild gastrointestinal symptoms to severe life-threatening conditions. This study focused on the health impact of ES isolated from powdered soybean in Nnewi South. In Nnewi South, Anambra State, powdered soybean, a staple food, poses a health risk due to potential E. coli contamination, which can cause mild gastrointestinal symptoms to life-threatening conditions, prompting this study on its health impact. This study investigated the presence and pathogenic potential of E. coli in soybean samples. 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 identified to the molecular level. The pathogenic potential of each strain was assessed in mice, which were orally administered 0.5 mL of inoculum (10^8 cells/mL). The study revealed significant pathological signs, gross lesions, and decreased organ-body weight ratio in infected mice, particularly those infected with ECOHSS52 (E. coli O157:H7 strain SS52). The isolated E. coli strains exhibited reasonable pathogenic features, with ECOHSS52 being the most pathogenic. The findings highlight the potential health risk associated with consuming contaminated soybean products and emphasize the need for proper food handling and safety measures to prevent E. coli infections.

INTRODUCTION

The discovery of *Escherichia coli* in soybean powder poses significant public health concerns, particularly in regions where soy-based foods are staples. Soybean powder, derived from Glycine max, is valued for its high protein content and nutritional benefits. However, contamination can occur during handling, processing, or storage, compromising its safety [1]. *Escherichia coli,* a gram-negative bacterium found in the environment and intestines of humans and animals, can cause gastrointestinal infections and systemic disease [2]. Its presence in food indicates fecal contamination, rendering the product unsafe for consumption.

Soybeans are cultivated for their nutrient-rich seeds, which are rich in B vitamins, phytic acid, and essential minerals. Soybean processing not only produces oil for industrial and culinary uses but also yields a significant amount of protein for animal feed, indirectly supporting human nutrition [3]. Research suggests that consuming soybean milk can lower low-density lipoprotein and total

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cholesterol levels, reducing the risk of cardiovascular disease [4]. The production of soybean powder involves several steps, including cleaning, cracking, grinding, oil extraction, and contaminant removal [3]. However, *E. coli* can form biofilms that enhance its resilience to heat and desiccation, allowing it to persist in powdered foods [5, 6] Since soybean powder is often consumed without further heat treatment, contamination poses a risk to consumer health, as pathogens can survive and cause illness [7].

Pathogenic *E. coli* strains, including Shiga toxin-producing (STEC), enterotoxigenic (ETEC), and enteropathogenic (EPEC), can cause foodborne illnesses. Symptoms may range from diarrhea and urinary tract infections (UTIs) to severe conditions like renal failure or irritable bowel syndrome. To ensure the safety of soybean powder and protect public health, strict controls are necessary throughout the manufacturing, processing, handling, and storage stages to prevent contamination.

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 method of Iheukwumere *et al*. [8]. 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 pour plate technique. All the plates in triplicates 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 inverted position at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions [9], biochemical reactions [9, 10, 11] and molecular characterization [12, 13, 14, 15] The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates [16]

Preparation of the test Isolates: This was carried out using the methods of Wafaa *et al*. [17] and Iheukwumere *et al*. [18]. 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 $\times 10^8$ 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.

Pathological Potential: This was carried out using Albino Wistar rats. The rats were grouped into six groups and each group contained four rats. The first-five groups were challenged with 0.5 mL of each of the test isolate whereas the sixth group was giving only feeds and water as control group. These were allowed and monitored for one month [19, 20]. The infected mice were carefully observed for obvious pathological signs such as anorexia (loss of appetite), weakness (lack of physical strength), simple diarrhea, whitish diarrhea, bloody diarrhea and respiratory distress (difficulty in breathing) for a period of 2 weeks. The number of deaths was also observed. After 2 weeks, some of the infected mice were sacrificed and gross examination of the morphologies of the internal organs forair sacculitis (inflamed air sac), pericarditis (inflamed pericardium), perihepatitis (inflamed peritoneal covering the liver), hepatomegaly (liver enlargement), liver congestion, lung haemorrhage, eroded intestine, bloody intestine and fluid accumulation in the intestine was carried out [17]

Statistical Analysis: The results of the data generated were expressed in Tables. The significance of the pathogenic potential of the isolates were determined using Analysis of variance (ANOVA) at 95% confidence level. Pairwise comparison was carried out in an Excel sheet using student "t" test [21]

RESULTS

The study showed that the quality of nucleic acids (DNA) extracted from the isolates were 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 (ECOHSS52) (isolateY1) and *Escherichia coli* 0157:H7 strain Sakai (ECOH Sakai) (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

Pathological signs and symptoms in the mice infected with *E. coli* are shown in Table 3. From the study, weakness, anorexia and diarrhoea were common in all the infected mice. Bloody diarrhea was significantly ($p \leq 0.05$) recorded among those mice infected by ECOHSS52 and ECOH Sakai, and ECOHSS52 was mostly affected. Death was recorded among the infected mice, and those mice infected by EC0HSS52 and ECOH Sakai were significant ($p \leq 0.05$), and recorded the most fatal cases, and EC0HSS52 killed all the infected mice. Generally, the obvious pathological features were significant ($p \leq 0.05$) among the infected mice when compared to control.

Table 3: Obvious pathological signs and symptoms of the isolates in the experimented mice

	N = 5					
Pathological Feature	EC SEC470	EC V266	EC SUS9EC	ECOH SS52	ECOH Sakai	Control
Anorexia	4	3	3	5	5	0
Weakness	3	4	4	5	5	0
Diarrhoea	5	5	5	5	5	0
Bloody diarrhea	0	0	2	5	4	0
Respiratory distress	3	3	4	5	5	0
Alopecia	2	3	2	2	3	0

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Death	0	1	2	5	4	0

ECSEC470—*Escherichia coli* strain SEC470, ECV266--- *Escherichia coli* strain V266

ECSUS9EC-- *Escherichia coli* strain SUS9EC, ECOHSS52-- *Escherichia coli*

O157:H7strain SS52, ECOH Sakai-- *Escherichia coli* O157:H7 strain Sakai

Characteristics of the visceral organs of the infected mice are shown in Table 4. These organs were noted to be the predilection sites of *E. coli* in living hosts. Pericarditis, perihepatitis, lung haemorrhage, liver congestion and enterocolitis were significant ($p<0.05$) observed in these organs harvested from the mice infected by *E. coli,* and these lesions were more severe in ECOHSS52 and ECOH Sakai. Liver congestion, perihepatitis and enterocolitis occurred in all the mice. Lung haemorrhage and bloody intestine were seen only in those mice infected by EC0HSS52 and ECOH Sakai, and ECOHSS52 proved to be more virulent.

Table 4: Gross pathological lesions on the internal organs of the experimented mice

	N= 5					
Gross lesion	EC SEC470	EC V266	EC SUS9EC	ECOH SS52	ECOH Sakai	Control
Hepatomegaly	3	2	3	5	5	0
Liver Oedema	3	2	3	5	5	0
Perihepatitis	4	4	5	5	5	0
Liver Haemorrhage	0	0	1	3	2	0
Lung Oedema	3	3	3	4	3	0
Lung Haemorrhage	0	0	0	4	4	0
Air Sacculitis	3	3	3	4	5	0
Heart Haemorrhage	0	0	0	3	2	0
Pericaritis	1	2	2	3	3	0
Bloody Intestine	0	0	2	5	4	0
Fluid Accumulation in the Intestine	4	5	5	5	5	0

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

ECSUS9EC- *Escherichia coli* strain SUS9EC, ECOHSS52- *Escherichia coli* 0157:H7strain SS52, EC0H Sakai- *Escherichia coli* 0157:H7 strain Sakai

The mean organ- body weight ratios of the experimented mice are shown in Table 8. There were significant ($P< 0.05$) increased in organ- body weight ratios of the infected mice when compared to the non- infected (control) mice. These anomalies were seen most on ECO157:H7SS52 and ECO157:H7Sakai of which ECO157:H7SS52 was mostly affected (Table 5).

Table 5: Mean organ- body weight ratio of the experimented mice

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Organs	EC SEC470	EC V266	EC SUS9EC	ECOH SS52	ECOH Sakai	Control
Liver(g)	0.027±0.002	0.030±0.001	0.031±0.001	0.034±0.001	0.033±0.002	0.024±0.001
Heart(g)	0.008±0.001	0.009±0.002	0.013±0.001	0.028±0.001	0.027±0.001	0.007±0.001
Lungs(g)	0.051±0.002	0.052±0.001	0.057±0.001	0.061±0.002	0.059±0.002	0.035±0.001

ECSEC470 - *Escherichia coli* strain SEC470, ECV266- *Escherichia coli* strain V266
ECSUS9EC- *Escherichia coli* strain SUS9EC, ECOHSS52- *Escherichia coli*
O157:H7strain SS52, ECOH Sakai- *Escherichia coli* O157:H7 strain Sakai

DISCUSSION

The substantial morbidity and fatality rates in both adults and children demonstrate the well-established significance of E. coli in human illness. Toxins produced by these bacteria cause net fluid leakage into the lumen after colonising the small-intestinal epithelium [22]

[23], who carried out biochemical characterisation of *E. coli*, concur with the traits and identities of the isolates from roasted meat in this investigation. In soybean samples, the presence of strains SEC470, V266, SUS9EC, O157:H7 SS52, and O157:H7 Sakai provides further evidence of *E. coli* contamination. Culture on selective medium after non-selective or selective enrichment has been the standard method for detecting *E. coli*. More speed and sensitivity are now available with molecular techniques. Primers that targeted the sequences surrounding the universal stress protein gene (uspA), a marker that has been shown to be highly specific for *E. coli*, were used in this work to identify isolates; no amplification took place in Gram-negative controls that were not *E. coli*. In a similar vein, [24] reported the identification of uspA in food-borne *E. coli.*

Similar to Mohawk *et al*. [25], infected mice showed pathological symptoms, including weakness, diarrhoea, dysentery, and anorexia, as well as death rates. Lesions such as pericarditis, perihepatitis, pulmonary haemorrhage, liver congestion, and enterocolitis are indicative of the organism's capacity to infiltrate the heart, liver, and lungs [8, 14, 26]. The outer-membrane adhesin intimin and phage-encoded Shiga toxins 1 and 2 (stx1, stx2) are the sources of sorbitol-negative *E. coli* O157:H7's pathogenicity. The release of toxins destroys the intestinal lining as bacterial populations rise, leading to severe cramping and diarrhoea [10, 20].

CONCLUSION

This study has revealed the presence of five *E. coli* strains (SEC470, V266, SUS9EC, O157:H7 SS52, and O157:H7 Sakai) in soybean samples. Notably, O157:H7 SS52 was the most prevalent strain and exhibited the highest virulence in mice, inducing significant pathogenic effects and internal organ lesions. These findings highlight the potential health risk associated with consuming contaminated soybean products within this region.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval

Authors hereby declare that "Principles of Animal Care" (NCARE with Ref No FPSRA/UNN/24/0114), certified on 12th November, 2024 at University of Nigeria,

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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.

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