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Assessing the Burden of *Escherichia coli* Powdered Soybean Consumed in Nnewi South, Anambra State

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KEYWORDS

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ABSTRACT:

In Anambra State, particularly Nnewi South, a concerning trend has emerged, with increasing cases of gastrointestinal illnesses, including diarrhea, bloody diarrhea, abdominal pain, and hemolytic-uremic syndrome (HUS). These health issues are linked to the consumption of powdered soybean. This study investigated the presence of Escherichia coli (E. coli) in powdered soybean samples collected from ten major towns in the region. A total of 200 samples were screened using pour plate technique, and isolates were characterized and identified based on morphological, biochemical, and molecular characteristics. The study revealed the presence of five E. coli strains: SEC470, V266, SUS9EC, O157:H7 strain SS52, and O157:H7 strain Sakai. The distribution of these strains varied significantly, with ECSEC470 being the most predominant (29.19%). Samples collected from Ebenato and open markets had the highest number of E. coli isolates. Notably, sorbitol-negative E. coli were more prevalent in samples collected near hospital settings. The study highlights the presence of diverse E. coli strains in powdered soybean, emphasizing the need for proper handling and storage practices to minimize the risk of contamination and potential health implications.

Introduction

The presence of various *Escherichia coli* strains in ready-to-eat powdered soybeans poses a significant food safety risk, especially in regions with high soybean consumption. Soybeans (Glycine max), a nutrient-rich legume native to East Asia, are widely cultivated for their edible beans. These beans are an excellent source of phytic acid, dietary minerals, and B vitamins. The processing of soybeans also yields soybean oil, a versatile product used in both industrial and culinary applications. Furthermore, soybeans serve as a vital protein source in animal feed, ultimately contributing to the production of animal protein for human consumption [1]. Notably, soybean milk has been found to have medicinal properties, including the ability to reduce low-density lipoproteins and serum cholesterol levels, thereby lowering the risk of heart disease [3], Given the nutritional benefits and widespread use of soybeans, ensuring the safety of soybean products, such as powdered soybeans, is crucial to preventing food-borne illnesses.

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Soybean powder production involves several steps, including cleaning, cracking, grinding, oil extraction, and impurity removal [1]. However, powdered soybeans can pose a food safety risk due to potential contamination with Escherichia coli (*E. coli*), a bacterium that can survive for extended periods in powdered foods and form biofilms, making it resistant to environmental stresses like heat and drying [3, 4]. *E. coli* is a gram-negative, rod-shaped bacterium commonly found in the intestines of humans and animals, as well as in the environment and various foods. While many strains are harmless, some can cause severe food-borne illnesses, including diarrhea and urinary tract infections [5].

Ready-to-eat foods like powdered soybeans are particularly vulnerable to contamination due to inadequate heat treatment. Factors contributing to contamination include poor handling and storage practices, inadequate processing, and cross-contamination. Studies have shown that *E. coli* can survive in powdered soybeans for extended periods and resist environmental stresses [3]. Research has detected *E. coli* in powdered soybean products, highlighting the need for proper handling and processing practices to minimize contamination risks. For example, a Nigerian study found *E. coli* present in 12% of powdered soybean samples, alongside other bacterial isolates [6]. Nnewi is a town in Anambra State, Nigeria, located within the South-East Geopolitical Zone. Ukpor is the administrative headquarters of Nnewi South Local Government Area, which also includes the towns and villages of Ezinifite, Utuh, Ebenator, Osumenyi, Amichi, Unubi, Ekwulumili, and Azigbo. This LGA is highly industrialized, hosting numerous manufacturing firms that produce a wide range of goods. A thriving trade network revolves around markets such as Eke Amichi, Eke Osu, and Abanitor, where a diverse array of products is bought and sold. Nnewi South also supports its economy with multiple banks, hotels, hospitals, rest areas, government agencies, and private enterprises.

Materials and Methods

Study Area: Nnewi comprises two local government areas—Nnewi North and Nnewi South—within Anambra State in southeastern Nigeria. Together they cover approximately 1,076.9 square miles (2,789 km²). As of 2005, the Nnewi metropolitan area and its surrounding satellite towns had an estimated population of 2.5 million. Located at the southernmost edge of Anambra State in the South Senatorial Zone, Nnewi lies near latitude 6°11′ N and longitude 6°55′ E. My study focuses on Nnewi South LGA, which has its own local government administration and an estimated population of around 1 million. Ukpor serves as the administrative headquarters of Nnewi South. Other towns within this LGA include Ekwulumili, Ezinifite, Utuh, Ebenator, Osumenyi, Amichi, Unubi, and Azigbo. Amichi, Azigbo, Unubi, Ezinifite, Osumenyi and Utuh. Others are Akwuihedi, and Ebenato. Nnewi South Local Government Area occupies a total land area of approximately 1,689.1km² and lies in a tropical rainforest of Nigeria which features such vegetation as the Iroko trees, Palm trees, Bamboo trees, Raffia Palms, Coconu trees, Breadfruit trees, Oil Bean trees and other perennial trees. However, due to human activities, particularly in the form deforestation for purposes of farming and construction works, the forest vegetation has largely disappeared giving way to derive savannah vegetation of shrub land and bushes. The climate of the city is typically an equatorial rain forest type characterized by two main seasons: the Rainy season, which lasts between April and October and the Dry season which lasts between November and March. In most part of Nnewi -South Local Government, the temperature is usually high all the year around with average minimum temperature at about 32°C and 24°C respectively.

Collection of Samples: A total of 200 ready-to-eat soyabean samples (100 samples each for soyabean). The soyabean samples were aseptically collected using sterile aluminum foil, from different point where they were sold in different towns in Nnewi South L.G.A by adopting square root of N+1 sampling method. The samples were done in such that samples were randomly collected from the top, middle and below. After collection, the samples were placed in a cooler to maintain the temperature during transportation for laboratory analysis. All samples were analyzed within 4 h of collection and where analysis was to be delayed, the samples were refrigerated at 4°C.

Culture and Isolation of *Escherichia coli:* This was carried out using the modified method of [7]. One gram (1.0 g) of each sample was first measured and dissolved

Journal of Chemical Health Risks
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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*.

Characterization and Identification of the Isolates: 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 [7], biochemical reactions [7, 8] and molecular characterization [9, 10, 11, 12]. 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.

Determination of Prevalence of the Isolates in the Studied Samples: The occurrences of different strains of the bacterial isolates associated with the soybean samples collected from different locations in Nnewi South were counted and recorded according to the method described in the study published by [13], [14] and [15]. The number of the occurrences of the predominate pathogenic bacteria were counted, and their percentages of occurrences were appropriately calculated and recorded

Statistical Analysis: The results of the data generated were expressed in percentage, tables and figures. The significance of the prevalence study were determined using Analysis of variance (ANOVA) at 95% confidence level. Pairwise comparison was carried out in an Excel sheet using student "t" test [16]

Results

The characteristics of the *E. coli* (EC) isolates are summarized in Table 1. Isolates X1, X2, X3, Y1, and Y2 were confirmed as *E. coli* based on their colonial morphology and biochemical properties. The reddish appearance on MacConkey agar and motility with indole utilization are consistent with *E. coli.* Isolates X1, X2, and X3 were sorbitol-fermenting (SOR+), while Y1 and Y2 were non-sorbitol-fermenting (SOR-), a characteristic of Shiga toxin-producing *E. coli* (*E. coli* O157:H7). The isolates' fermentation patterns for inositol, xylitol, dulcitol, and D-arabinose are detailed in Table 1. These biochemical profiles helped identify the strains. The isolates' characteristics are consistent with known properties of *E. coli.*

Table 1: Characteristics and identities of the isolates

Parameter	X1	X2	X3	Y1	Y2
Appearance on Mac Conkey agar	Red	Red	Red	Red	Red
Elevation	Raised	Raised	Raised	Raised	Raised
Size (mm)	2.40	2.30	2.40	2.00	2.10
Edge	Entire	Entire	Entire	Entire	Entire
Motility	+	+	+	+	+
Gram Reactions	–	–	–	–	–
Cell morphology	Rod	Rod	Rod	Rod	Rod
Catalase	+	+	+	+	+
Methyl red	+	+	+	+	+
Voges- Proskaeur	–	–	–	–	–
Indole	+	+	+	+	+
Citrate	–	–	–	–	–
H_2S	–	–	–	–	–
Glucose	+	+	+	+	+
Maltose	+	+	+	+	+
Inositol	+	+/-	–	+	+
Sorbitol	+	+	+	–	–

Journal of Chemical Health Risks
www.jchr.org
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Galactose	+	+	+	+	+
Xylitol	+/-	+	+/-	+	+/-
Dulcitol	+/-	+/-	+	+/-	+
D- Arabinose	-	+	+	-	+
Mannitol	+	+	+/-	+	+

+ = Positive, - = Negative, +/- = partially positive, H_2S = Hydrogen sulfide

The results of the study showed that the quality of nucleic acids (DNA) extracted from the isolates were pure as the ratios of the absorbance A260/A280 were within the stipulated range 1.80-1.90 (Table 2). 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 2: 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 3: 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

Journal of Chemical Health Risks
www.jchr.org
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Y2	2844	2844	100%	0%	100%	CP011428.1	*Escherichia coli* 0157:H7 strain Sakai Complete genome

The study found that ECSEC470 was the most prevalent isolate, followed by ECOHSS2, ECV266, ECSUS9EC, and ECOHSakai. Ebenato had the highest distribution of isolates, while Ukpor had the lowest. The distribution of sorbitol-negative EC was significantly higher in Ebenato compared to other areas. Akwaihedi and Unubi also had a high distribution of isolates, but the difference was not significant. The study also showed that isolates were more commonly found in open markets, with a statistically significant difference compared to shops and supermarkets. However, the difference was not significant when compared to areas near hospitals. Notably, sorbitol-negative EC (ECOHSS2) was more frequently found near hospital settings. ECOHSakai was not detected in supermarkets. Overall, the study highlights the varying distribution of EC isolates across different locations. The findings suggest a significant presence of EC isolates in certain areas. The study's results have implications for public health. Further research is needed to understand the factors contributing to the distribution of EC isolates.

Table 4: Occurrences of the isolates in the studied powdered soybean samples

N=20 Location	ECSEC470	ECV266	ECSUS9EC	ECOHSS2	ECOHSakai	Total
Ebenato	10(3.11)	10(3.11)	14(4.35)	18(5.59)	14(4.35)	66(20.50)
Amichi	6(1.86)	2(0.62)	6(1.86)	2(0.62)	0(0.00)	16(4.96)
Azigbo	8(2.48)	8(2.48)	4(1.24)	6(1.86)	4(1.24)	30(9.32)
Osumenyi	8(2.48)	2(0.62)	0(0.00)	6(1.86)	12(3.73)	28(8.70)
Akwaihedi	12(3.73)	12(3.73)	10(3.11)	12(3.73)	6(1.86)	48(14.91)
Ukpor	6(1.86)	0(0.00)	0(0.00)	6(1.86)	0(0.00)	12(3.73)
Ezinifite	10(3.11)	0(0.00)	8(2.48)	8(2.48)	4(1.24)	30(9.32)
Utuh	12(3.73)	10(3.11)	0(0.00)	8(2.48)	0(0.00)	30(9.32)
Unubi	14(4.35)	6(1.86)	0(0.00)	14(4.35)	0(0.00)	34(10.56)
Ekwulumiri	8(2.48)	2(0.62)	8(2.48)	10(3.11)	0(0.00)	28(8.70)
Total	94(29.19)	52(16.15)	50(15.53)	86(26.71)	40(12.42)	322(100.00)

Table 5: Distribution of the Isolates in the sampling source

Sampling Source	ECSEC470	ECV266	ECSUS9EC	ECOHSS2	ECOHSakai	Total
Open market	29 (9.01)	18 (5.59)	23 (7.14)	18 (5.59)	11 (3.42)	99 (30.74)
Shops	19 (5.90)	7 (2.17)	9 (2.80)	11 (3.42)	7 (2.17)	53 (16.46)
Supermarket	11 (3.42)	3 (0.93)	5 (1.55)	7 (2.17)	0 (0.00)	26 (8.07)
Street	14 (4.35)	11 (3.42)	7 (2.17)	17 (5.28)	5 (1.55)	54 (16.77)
Near Hospitals	21 (6.52)	13 (4.04)	6 (1.86)	33 (10.25)	17 (5.28)	90 (27.95)
Total	94 (29.19)	52 (16.15)	50 (15.53)	86 (26.71)	40 (12.42)	322 (100.00)

Journal of Chemical Health Risks

www.jchr.org

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Discussion

Escherichia coli plays a significant role in various diseases, contributing to substantial morbidity and mortality rates across age groups. This bacterium colonizes the small intestine's epithelial surface, producing toxins that lead to fluid secretion into the lumen [17]. The presence of *E. coli* in soybean samples likely results from cross-contamination, poor handling, and inadequate food preparation practices [18]. Variations in *E. coli* strains across different soybean samples may be attributed to differences in ingredient composition, texture, and the sanitary conditions during preparation. Research suggests that factors such as water activity, oxygen levels, pH, and nutrient content influence microbial growth in food samples [19]. Additionally, protein sources and other ingredients can contribute to variations in microbial populations [20]. These findings highlight the importance of proper food handling and preparation practices to minimize the risk of *E. coli* contamination.

This study's findings on the characteristics and identities of *Escherichia coli* isolates from powdered soybean samples align with [21] report on biochemical characterization of *E. coli.* The detection of specific *E. coli* strains, including SEC470, V266, SUS9EC, O157:H7 SS52, and O157:H7 Sakai, in soybean samples confirms the presence of *E. coli.* Traditionally, laboratory detection of *E. coli* relied on enrichment and culture on selective media. However, molecular techniques have improved detection sensitivity and speed. In this study, *E. coli* strains were identified using primers targeting the universal stress protein (usp A) gene, which is highly specific for *E. coli.* This approach is consistent with Chen and Griffiths [22] report on detecting usp A in *E. coli* isolated from food samples, highlighting the effectiveness of molecular techniques in identifying *E. coli* strains.

*Escherichia col*i O157:H7 SS52 was predominantly isolated from the powdered soybean samples studied. Human activities during processing, transportation, and preparation likely contributed to the high counts of this strain. According to Ali *et al*. [23], pathogenic *E. coli* in food poses a risk of cross-contamination and threatens handlers and consumers. Similarly, Shea *et al*. [1] noted that *E. coli* presence in food indicates potential contamination with other pathogens, highlighting the importance of proper handling and preparation practices.

Conclusion

This study revealed the presence of various *Escherichia coli* strains in powdered soybean samples, with ECSEC470 being the most prevalent. Notably, Hemolytic Uremic Syndrome (HUS)-induced *E. coli* strains accounted for 39.13% of the isolates, highlighting a significant public health concern.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval

Not applicable

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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