

Unravelling Shiga toxin-producing and enteropathogenic serotypes of *Escherichia coli* in dried fish sold in markets in Bobo-Dioulasso, Burkina Faso

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Abstract

Dried fish is widely consumed in Burkina Faso, yet its microbial safety remains insufficiently documented. *Escherichia coli*, including Shiga toxin-producing strains (STEC) and enteropathogenic strains (EPEC), represents a major public health concern. This a prospective descriptive study. A prospective descriptive study was performed from July 2024 to September 2025 across ten markets in Bobo-Dioulasso. The Methodology applied is based on the collection of thirty dried-fish samples and the application of standard microbiological methods for the isolation and biochemical identification of *E. coli*. Antibiotic susceptibility testing was performed according to EUCAST/CA-SFM (2024) guidelines. Molecular screening for virulence genes (*stx1*, *stx2*, *eaeA*) was performed using PCR. The results show that contamination was highest in the *Colma*, *Belle-Ville*, and *Koko* markets (100%). All isolates were resistant to amoxicillin and fosfomycin, while sensitivity remained high for meropenem (95%) and amikacin (75%). Three isolates carried virulence genes: *stx1* (11.1%), *stx2* (5.6%). No strain was positive for *eaeA*. Molecular clustering revealed distinct clusters, with STEC strains forming a separate group. The genetic distance shows the degree of similarity between strains. Three main clusters are clearly distinguishable. The first one comprising isolates that lack virulence genes or 16S RNA and have been excluded from further analysis. The second cluster represent shigatoxigenic strains. All these strains carry the *stx1* gene. The third cluster isolates represent strains that possessed 16S rRNA gene only. This study highlighted a high prevalence of *E. coli* contamination in dried fish in Bobo-Dioulasso, including STEC strains carrying *stx1* and *stx2*. The presence of multidrug-resistant isolates highlights the need for improved hygiene practices, routine surveillance, and reinforced regulatory measures to minimize foodborne risks.

Keywords: Dried fish; *E. coli*; Multiresistant; Virulence genes; Bobo-Dioulasso

1. Introduction

Aquaculture and fisheries play a major role in global food security, providing approximately 143 million tons of fish intended for human consumption (Hilborn, 2012). As in many African countries, these two sectors also represent an important source of animal protein, which is essential for local diets (Abdoulahi et al, 2021). Globally, nearly one billion people rely on fish as their primary source of animal protein (FAO, 2007). This high level of consumption is largely attributed to its nutritional benefits and relatively affordable cost (Bardoe et al, 2023). In Africa, fish accounts for an average of 22% of animal protein intake and may exceed 50% in West Africa (FAO, 2022). In Burkina Faso, per capita

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fish consumption is estimated at 8.2 kg/year (FAO, 2022), and national fishery production could reach up to 20,000 tons per year (Coulibaly, 2022).

Due to the lack of cold storage facilities and the high cost of energy, fishermen rely on processing techniques such as smoking and, more commonly, drying, which is particularly widespread in Burkina Faso (Anihouvi et al, 2019). Although these processes extend the shelf life of fish products, they do not completely eliminate microbiological risks. Processed products may be exposed to multiple sources of contamination including work surfaces, utensils, handlers, and the environment facilitating the introduction of microorganisms such as coliforms, *staphylococci*, *clostridia*, and *Salmonella* (Micha et al, 2018). Contaminated dried fish can therefore cause foodborne illnesses, sometimes of severe clinical significance (Abotchi, 2010).

Coliform bacteria constitute a heterogeneous group of Gram-negative, aerobic or facultatively anaerobic bacilli that include the genera *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, and *Serratia* (Health Canada, 2012). Among them, *Escherichia coli* is of particular importance, as it is widely used as an indicator of hygiene in the fish-processing industry (Thibautheon et al, 2021). During handling, fish, whose natural defenses are weakened, can become more susceptible to contamination (Micha et al, 2018).

Some *E. coli* strains are pathogenic and produce Shiga toxins (STEC), responsible for gastrointestinal diseases that may develop into hemorrhagic colitis and hemolytic uremic syndrome the leading cause of acute renal failure in children and can even be fatal (Mommeja, 2004; FAO, 2022). STEC infections are mainly associated with the consumption of contaminated food, animal contact (particularly cattle), person-to-person transmission, or ingestion of polluted water (Bouvier, 2017).

Despite the significance of these risks, data on the presence and diversity of *E. coli* strains in dried fish marketed in Burkina Faso remain limited. This study, entitled "Identification and Characterization of Shiga Toxin-Producing and Enteropathogenic *Escherichia coli* Serotypes in Dried Fish Sold in Markets in Bobo-Dioulasso".

2. Material and methods

2.1. Study area, design and period

The study was conducted in Bobo-Dioulasso, located in western Burkina Faso, within the Hauts-Bassins region. The municipality covers approximately 160,000 hectares and is administratively divided into seven districts. With an estimated population of 903,887 inhabitants in 2021, it is the second largest city of the country. The area is characterized by a tropical climate with an average annual temperature of 26 °C (INSD, 2020). This prospective descriptive study was carried out from July 2024 to September 2025 in three main phases: sample collection across local markets; microbiological analyses, performed at the Laboratory of Applied Biological Sciences, Université Aube Nouvelle (Bobo-Dioulasso) and molecular characterization (PCR) carried out at the Molecular Biology and Biotechnology Laboratory of Centre Muraz.

2.2. Biological material and sampling

The study was based on samples of dried fish collected from various merchants in the municipality of Bobo-Dioulasso. The samples were stored and managed in sterile bags, placed in the refrigerator, and kept cool. Sampling was carried out randomly. These samples were collected from ten (10) markets in Bobo. A total of 30 samples were taken from three merchants in each market. These samples were placed in sterile bags and then stored in a refrigerator at the analysis laboratory of the Université Aube-Nouvelle in Bobo.

2.3. Preparation of the stock suspension and strains isolation

The stock suspension was prepared in accordance with the standard (ISO 6887-1(2017)), and buffered peptone water (BPW) was first prepared according to the manufacturer's instructions. For each sample, 10 g of fish crushed using a mortar were weighed on a precision scale into a beaker, to which 90 ml of diluent corresponding to a 10⁻¹ dilution was added and incubated at room temperature for 20 minutes. A homogeneous solution was obtained following homogenization. This stock suspension was used to perform a series of successive decimal dilutions (ISO 6887, 2017). Isolation was performed in accordance with NF V08-054 (2009). Aliquots from the 10⁻³ dilution were streaked onto Violet Red Bile Lactose agar (VRBL) and incubated at 37 °C for 24 h (AFNOR, 2009).

2.4. Morphological and microscopic characterization

Colonies were examined macroscopically (shape, size, color, and margin). Typical lactose-fermenting colonies were selected for further analysis. Gram staining was performed according to the standard protocol following the manufacturer's instructions. Smears were heat-fixed, stained with crystal violet, treated with Lugol's iodine, decolorized with ethanol, and counterstained with safranin. Observation under oil immersion ($\times 100$) allowed confirmation of Gram-negative bacilli.

2.5. Biochemical characterisation

Biochemical characterisation consists of oxidase and catalase tests. Oxidase activity was evaluated using oxidase-impregnated discs (Figure 2). The absence of a purple coloration within seconds indicated a negative oxidase reaction, consistent with *E. coli* (Shields et al., 2013). Concerning the catalase test, a loopful of a 24 h culture was mixed with 3% hydrogen peroxide (Figure 3). Immediate bubble formation indicated a positive catalase reaction (Reiner, 2013).

2.6. Antibiotic Susceptibility Testing

Antibiotic susceptibility was tested using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following the recommendations of EUCAST (2024). After that, the inoculum was prepared using one to two identical colonies from a 24 h pure culture, which were suspended in sterile 0.9% saline and adjusted to 0.5 McFarland. This step was followed by diffusion assay. At this step, the inoculum was streaked onto MH agar, and antibiotic discs were placed at least 30 mm apart. Plates were incubated at 37 °C for 24 h. Results interpretation consists of measuring inhibition zone diameters using a digital calliper and classifying them as Susceptible (S), Intermediate (I), or Resistant (R) according to EUCAST breakpoints. Antibiotics Tested: Meropenem (MRP), Amoxicillin (AMX), Amoxicillin/Clavulanic acid (AMC), Fosfomycin (FOS), Amikacin (AK) and Tobramycin (TOB).

2.7. Molecular Detection of Virulence Genes

Molecular detection is followed in four steps. The first one is based on DNA extraction. DNA was extracted by Chelex lysis. A mixture of 100 μ L Chelex and 100 μ L bacterial suspension was incubated at 56 °C (30 – 60 min), vortexed, heated at 90 °C (10 min), then centrifuged (13,000 rpm, 5 min). Supernatants were used as templates. After that, the second step is preparing the reaction medium. It is prepared at the start of each PCR run and used immediately. The volume to be prepared depends on the number of samples to be tested. The mix is obtained by combining the master mix, PCR water, F primers, and R primers. After preparation, the mix is thoroughly vortexed and used immediately, with 19 μ L of the mixture and 1 μ L of bacterial DNA in each well (Table 1). The third step concerning the PCR amplification program follow-up. Amplification conditions followed Paton & Paton (1998), with cycles including denaturation (95 °C), annealing (60 – 65 °C), and extension (72 °C) (Table 2). The last step is based on electrophoresis and visualization. At this step a 2% agarose gel in TBE buffer was prepared. PCR products and molecular weight marker were loaded and run for 30 – 40 min. Visualization was done under UV transillumination. Samples were considered positive when a clear band corresponding to the expected size was observed.

2.8. Statistical Analysis

Data were encoded in Microsoft Excel and analyzed using R software (version 4.5.0) for descriptive and inferential statistics. The Fisher's exact test ($\alpha = 5\%$) was used to assess associations between categorical variables, particularly in cases of small sample size. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Prevalence of *E. coli* Contamination

Among the 30 dried fish samples analyzed, 20 isolates of *Escherichia coli* were obtained, corresponding to a contamination rate of 66.7%.

3.2. Prevalence of strains by sampling site

The results reveal that markets such as Colma, Belle Ville, and Koko provided the highest number of strains, while Market 22, Accart Ville, and Nineta had lower numbers (Figure 1). This distribution indicates that certain sites pose a risk due to the presence of *E. coli* strains.

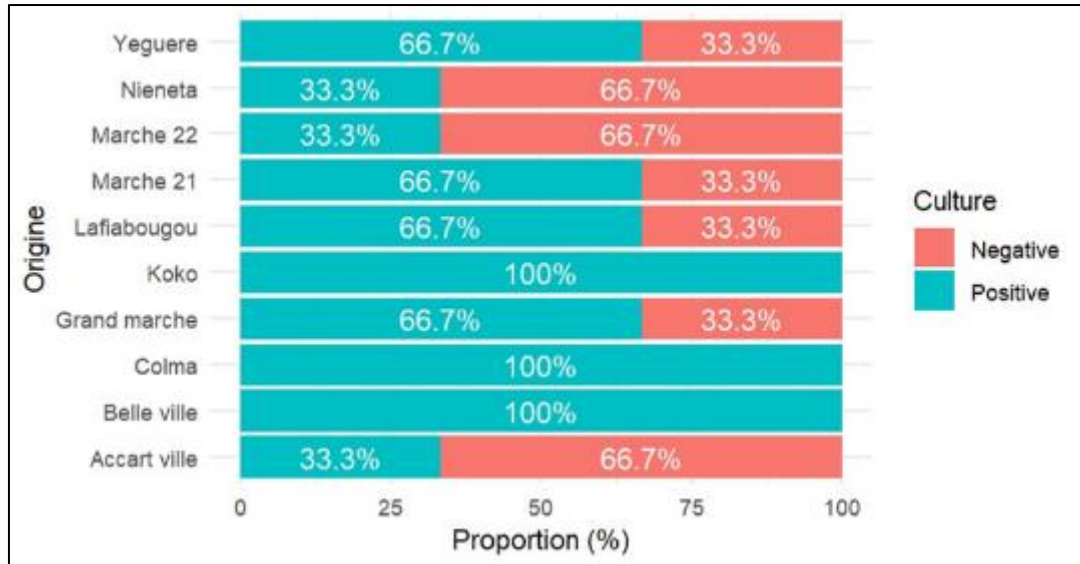


Figure 1 Distribution of *E. coli* strains by market

3.3. Antibiotic susceptibility

These different strains of *E. coli* were subjected to an antibiogram and showed total resistance to Amoxicillin and Fosfomycin, followed by moderate resistance to Amoxicillin + Clavulanic Acid (Figure 2). Low resistance was observed to Amikacin (25%), Meropenem (5%), and Tobramycin (45%), compared to sensitivity rates of 75%, 95%, and 55%, respectively. With regard to the resistance index (RI) of the isolated strains, a slight variation was observed depending on the site, as shown in Figure 3. The median RI ranges from 0.33 to 0.68 depending on the site, with individual values. The Accart ville, Belle ville, Colma, and market 22 sites had the highest median (0.68), while the Nieneta and Koko sites had the lowest median of 0.33.

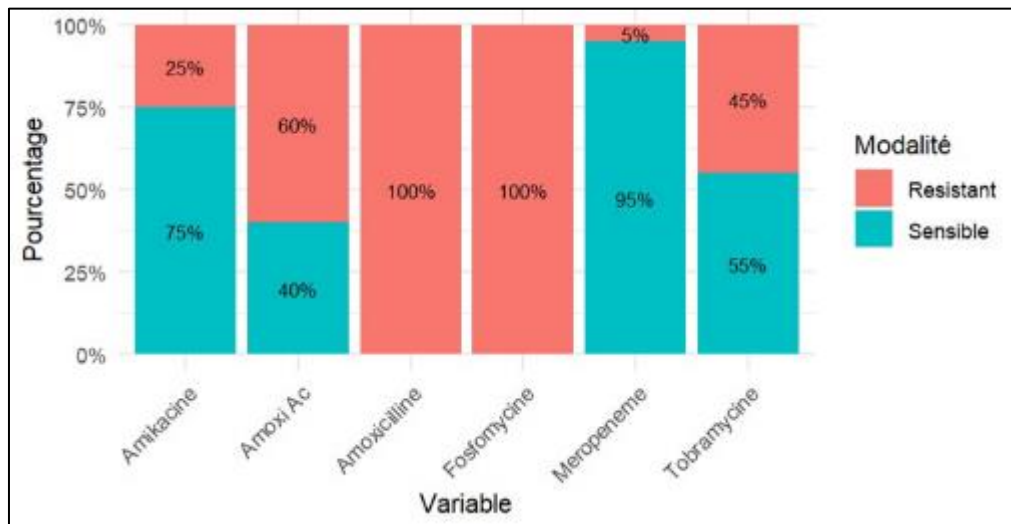


Figure 2 Susceptibility profile of *E. coli* strains to antibiotics

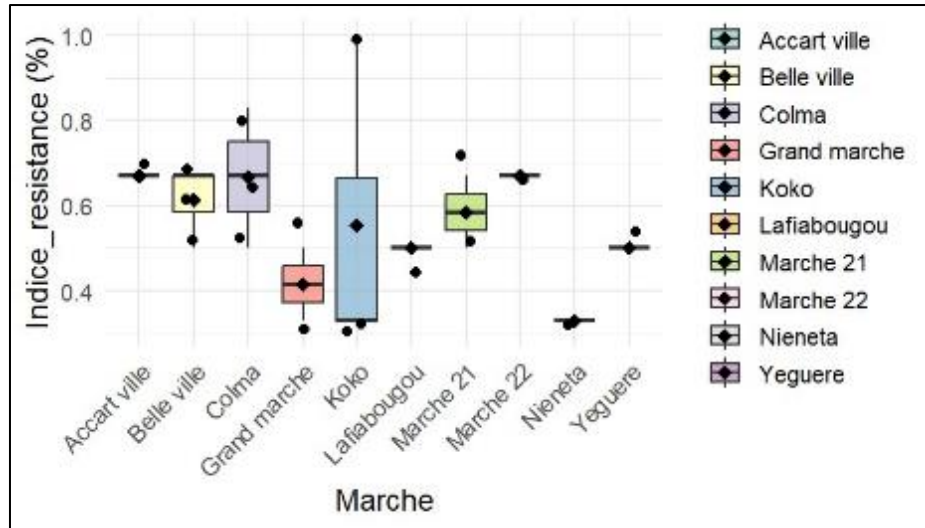


Figure 3 Resistance index of strains according to sampling sites

3.4. Molecular characteristics of strains

Four genes of interest were amplified. The 16S RNA gene was used as a molecular marker to confirm the presence of the bacterium. The *eaeA* gene was used to identify enteropathogenic *E. coli* (EPEC), while the *stx1* and *stx2* genes were used to detect Shiga toxins from *E. coli* (STEC). **Figure 4** shows that 18 strains were positive for 16S RNA. Regarding virulence genes, our results indicate that 11.1%, 5.6%, and 0% possess the *stx1* gene (Ni1 and Col3), the *stx2* gene (KOK3), and the *eaeA* gene, respectively. The genetic distance (vertical axis) shows the degree of similarity between strains: the smaller the distance, the more similar the profiles are (**Figure 5**). Three main groups (clusters) are clearly distinguishable. The purple cluster and green isolates are strains that lack virulence genes or 16S RNA and have been excluded from further analysis. The green cluster and red isolates represent shigatoxigenic strains. All these strains carry the *stx1* gene. The blue cluster and blue isolates all possess 16S rRNA. The KOK 3 strain carries the *stx2* gene, making it shigatoxigenic.

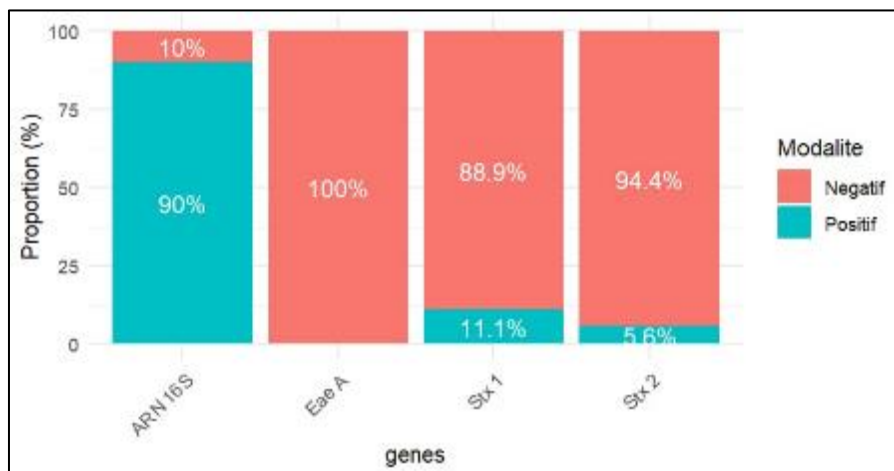


Figure 4 Result of amplification of different genes

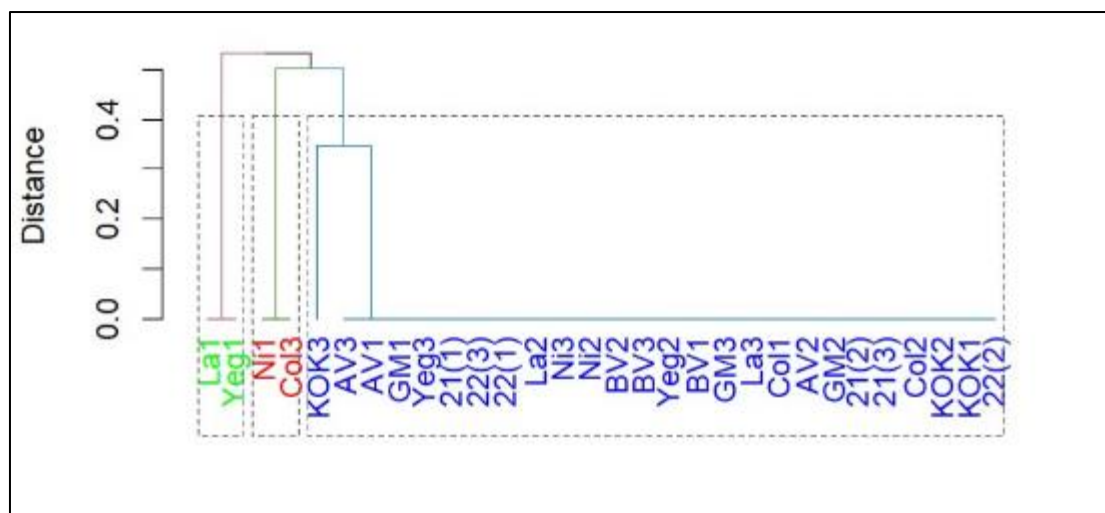


Figure 5 Dendrogram of isolates according to gene profile

4. Discussion

The objectives of this study were to identify and characterise the level of *E. coli* contamination in dried fish sold in markets in the city of Bobo-Dioulasso. These strains were characterised to assess their pathogenic potential and antibiotic susceptibility. A total of 30 dried fish samples were analysed, with 66.7% (20/30) contaminated with *E. coli*. This high rate could be explained by poor hygiene and storage conditions for dried fish. However, this rate is lower than that reported in a study conducted in Yaounde, Cameroon, by Ziem et al (2023), who observed a prevalence of 80% (76/95), but higher than that of de Abeid et al (2015), who obtained 15.38% (39/95). This could be explained by differences in sample size, study areas, and hygiene conditions.

The analysis was conducted between the different markets in the city of Bobo Dioulasso, and the results showed that the highest frequency of dry fish contamination was 100% in Koko, Belle Ville, and Colma. This could be explained by the fact that these are the most densely populated neighbourhoods, where compliance with good processing practices is often difficult, leading to a very high risk of contamination. In addition, it was noted that no neighborhoods showed *E. coli* contamination.

The antibiogram results enabled us to establish the precise sensitivity and resistance profiles of the *E. coli* strains isolated from dried fish. The detection of resistance to antibiotics commonly used in clinical practice or aquaculture raises concerns about public health and microbial risk management. The antibiotics tested were chosen based on the recommendations of EUCAST, (2024). It was found that 100% of our strains were resistant to two antibiotics, namely Amoxicillin and Fosfomycin. This high rate of resistance could be explained by phenomena such as self-medication and the transfer of resistance genes between bacteria. Our results are consistent with those reported by Acasio et al, (2022), who found 100% resistance to amoxicillin, and by Ziem et al, (2023), who reported 80.6% resistance. As for amoxicillin-clavulanic acid, 60% of strains were resistant, which differs from the results reported by Ziem et al, (2023), who found 100% resistance, and Acasio et al, (2022), who found 87.1% resistance. As for Tobramycin, 45% of isolates are resistant, which is higher than the rate of 27.3% reported by Ziem et al, (2023). In our study, amikacin (75%) and meropenem (95%) have a high sensitivity rate, which contrasts with the work of Ziem et al, (2023), which found a rate of 81.8% for AK. This difference could be explained by the selective pressure exerted locally by the irregular or inappropriate use of certain antibiotics in aquaculture practices (Miranda et al, 2018). In his study, the relatively high efficacy of Amikacin remains encouraging, but the decrease compared to our study could signal the emergence of acquired resistance, as has already been described in certain environmental strains (Machado et al, 2021). The observed efficacy of Meropenem could reflect its rare use in aquaculture (Wang et al., 2021). These results show that, despite the high resistance rates observed with certain antibiotics, molecules such as Meropenem and Amikacin retain therapeutic potential (Balkhy et al., 2023). The treatment of pathogens and the promotion of growth in livestock, aquaculture, and agricultural production could contribute to the increase of antibiotic-resistant strains in the environment (Hounmanou et al, 2016). Multidrug-resistant Gram-negative bacteria pose a significant threat to global public health (Bennini et al, 2017). The strains' multidrug resistance was assessed by calculating resistance indices. All indices were above 0.2, indicating a high-risk source of contamination (Woh et al, 2023). The widespread multidrug resistance of *E. coli* poses significant

risks to public health. The high prevalence of resistance in food isolates suggests potential challenges in treating foodborne infections (Farina et al., 2024).

After analyzing antibiotic susceptibility profiles, the rest of our results focused on the molecular characterization of the virulence of *E. coli* strains. In our study, 3/18 strains produced the *stx* gene (Ni1, Col3, and KOK3) responsible for shigatoxins production. These results differ from those of Acasio et al, (2022), who recorded 5/31 of these *E. coli* strains isolated from various foodstuffs. STEC are considered a major threat in foodborne diseases. For example, *E. coli* O157:H7 has become the first of several STEC called enterohaemorrhagic *E. coli* (EHEC), which can produce one or more Shiga toxins. The main reservoirs of STEC are ruminants, which continuously spread bacteria into the environment, contaminating food and water (Possa et al, 2025). These strains can survive in fresh ground beef and on fresh leafy vegetables (Possa et al, 2025). The *eaeA* gene was not detected in any of our strains. Our results differ from those obtained by Sarr (2012), who found (2/268). Among the two strains that tested negative for the ARN 16S gene and therefore cannot be considered *E. coli*. This observed difference could be explained by the diversity of methods used to preserve and store dried fish.

A Fisher's test was performed to assess the possible relationship between the presence of virulence genes (*stx1*, *stx2*, and *eaeA*) and the antibiotic Amikamycin, resulting in a *p-value* = 1. This means there is no significant difference between the *eaeA* gene and the antibiotic Meropenem (*p-value* = 0.33).

5. Conclusion

This study demonstrated that dried fish sold in Bobo-Dioulasso's main markets is significantly contaminated with *Escherichia coli*, a concern given Burkina Faso's high consumption of fish products. Microbiological and molecular analyses confirmed the presence of *E. coli*, including strains harbouring *stx1* and *stx2* genes, which are associated with Shiga toxin production and severe foodborne illness. Although some isolates were susceptible to Amikacin and Meropenem, the overall level of antimicrobial resistance was high, highlighting the potential for the dissemination of resistant strains through widely consumed food products. These findings reinforce the need to improve hygiene practices throughout the artisanal processing chain and to implement routine microbiological monitoring to safeguard public health.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declare that they did not use any generative AI technologies, such as Large Language Models (e.g., ChatGPT, Copilot) or text-to-image generators, for writing or editing this manuscript.

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