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*Full Length Research Paper*

# Microbiological evaluation and possible origins of the microbial contamination of vegetables in Ouagadougou (Burkina Faso)

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This study aimed to contribute to the health safety of widely consumed vegetables in Ouagadougou by investigating the potential contamination of onion, tomato, cucumber and lettuce with pathogenic microorganisms. A survey was conducted, involving 102 producers in fields, 102 vendors in markets, and 205 consumers. The most commonly consumed vegetables were identified as onion, tomato, cucumber and lettuce. A total of 264 samples, comprising 80 from fields and 184 from markets, were subjected to microbiological analysis, focusing on the isolation and identification of *Escherichia coli* and *Salmonella* spp. In market samples, onions exhibited the highest contamination with *E. coli*, registering a value of  $316.2 \times 10^3$  CFU/g, while tomatoes showed the least contamination with *E. coli* at a load of  $6.7 \times 10^3$  CFU/g. Lettuce had the highest prevalence of *Salmonella* at 20.31%, while onions had the lowest prevalence at 2.38%. For field samples, cucumbers demonstrated the highest contamination with *E. coli* at  $10 \times 10^3$  CFU/g, whereas onions had the least contamination at  $2.4 \times 10^3$  CFU/g. *Salmonella* was only detected in lettuces, with a prevalence of 4.76%.

**Key words:** Convenience vegetables, *Escherichia coli*, evaluation, microbial contamination, *Salmonella* spp.

## INTRODUCTION

Fruit and vegetables constitute a crucial component of a healthy diet, serving as vital sources of nutrients, vitamins, and fiber. They play a significant role in promoting human health and well-being, with a particular emphasis on preventing vitamin C and A deficiencies (Gomes and Reynolds, 2021; Kumar et al., 2013). According to the Food and Agriculture

Organization/World Health Organization (FAO/WHO), a daily intake of at least 400 g of fruits and vegetables is recommended to prevent chronic diseases such as heart disease, cancer, diabetes, and obesity. This intake is also effective in preventing or alleviating various micronutrient deficiencies, especially in developing countries. Despite the well-established health benefits

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associated with fruit and vegetable consumption, an unfortunate correlation exists between increased consumption of raw vegetables and a rise in the frequency of diseases linked to raw fruits and vegetables (Adjrah et al., 2011).

Vegetables commonly consumed in the sub-region include lettuce, cucumber, carrot, tomato, onion, parsley, and garlic (Toe et al., 2017). However, these vegetables are also recognized as a significant source of infection by various pathogens, including fungi, bacteria, viruses, and parasites (FAO/WHO, 2004). Incidents of food poisoning associated with the ingestion of contaminated vegetables have been documented worldwide (Panisset et al., 2003; Alegbeleye et al., 2018). Concerns about the safety of fresh fruits and vegetables have escalated as food-borne illnesses linked to these products continue to rise (EFSA, 2013).

Bacterial pathogens are identified as the most common agents causing foodborne illnesses, with viruses following closely (Phoeurk et al., 2019). Many of these enteric bacteria are implicated in an increasing number of collective foodborne infections (Martínez-Vaz et al., 2014). The consumption of raw vegetables heightens the risk of transmitting infectious diseases such as cholera, typhoid fever, and gastroenteritis, particularly if consumers neglect to observe proper hygiene rules (FAO/WHO, 2004).

Contamination of vegetables by *Salmonella* and *Escherichia coli* poses a global public health concern, particularly in developing countries (FAO/WHO, 2007). However, key participants in the vegetable value chain, including producers, processors, traders, and consumers, often prioritize the health benefits of vegetables over considerations of quality and hygiene (Adjrah et al., 2011; Antwi-Agyei et al., 2015).

Despite the nutritional and health advantages of vegetables, there has been a rise in human infections linked to the consumption of prepared fresh fruits and vegetables in recent years (Anin et al., 2016). Diseases associated with the consumption of contaminated fruit and vegetables are prevalent in various regions of developing countries, yet they are often underestimated due to a lack of reliable survey and surveillance data (Traoré et al., 2015). In Burkina Faso, there is a dearth of data on the hygienic quality of mass-market vegetables.

The objective of this study is to evaluate the microbiological quality and identify the source of contamination in mass-market vegetables in Ouagadougou.

## MATERIALS AND METHODS

### Study site and sampling

The study was conducted in Ouagadougou, as shown in Figure 1. Microbiological analyses were performed at the Laboratory of

Molecular Biology, Epidemiology and Surveillance of Food-Transmissible Bacteria and Viruses (LaBESTA) at the Joseph KI-Zerbo University. Survey sites were chosen based on literature data, which helped identify key areas for vegetable production and sales, whether wholesale or retail, in Ouagadougou.

The selected production areas included Boulmiougou, Tanghin, Loubila, Basky, and Ouaga 2000. Sales sites encompassed markets in Zone 1, Rayongo, Cité An II, El Nour, Gounghin, Zogona, Dassasgho, and Nabi Yaar. Actors present at these sales sites were interviewed as part of the survey. To ensure a representative sample of consumers, individuals were randomly selected from the streets of various neighborhoods for inclusion in the survey.

### Survey carried out

A comprehensive survey was conducted in Ouagadougou, encompassing fields (102 growers), markets (102 market women), and consumers (205 individuals). The primary objectives were to identify widely consumed vegetables in the city and assess practices posing a risk of biocontamination in both cultivation fields and public markets. Three distinct types of cards were employed for consumers, producers, and vendors to gather information relevant to each group.

The field survey specifically focused on pinpointing cultivation practices that could contribute to vegetable biocontamination. The investigation utilized the five key principles for growing safe fruits and vegetables, as outlined by the World Health Organization (FAO/WHO, 2007). These key areas included the quality of water used for irrigation, the use of manure as fertilizer, maintenance and hygiene of tools and storage areas, personal hygiene practices of producers, and control over animal access to cultivation fields.

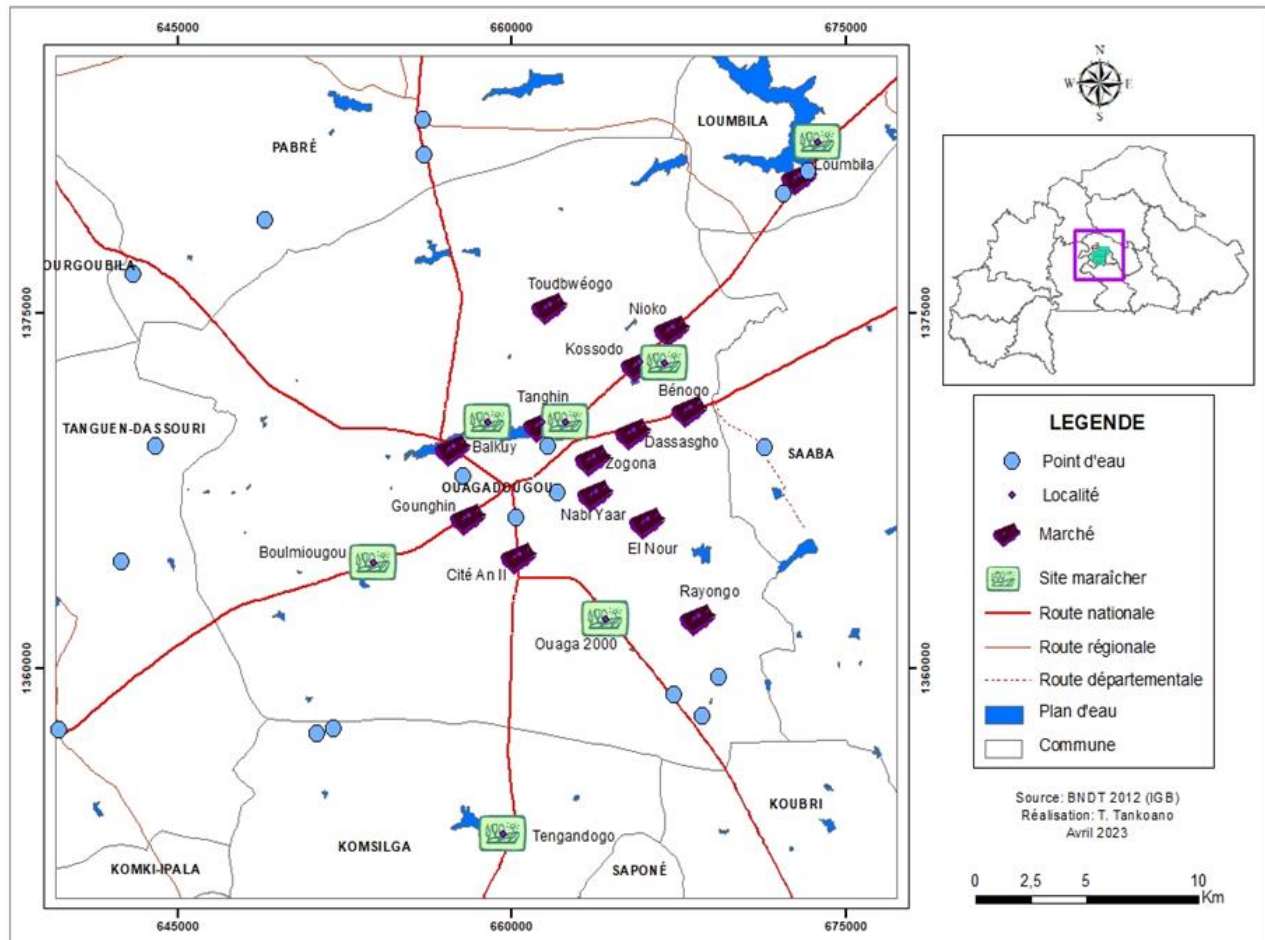
At the market level, the survey aimed to elucidate the behavior and practices of women vendors, as well as the hygiene conditions within markets that might impact the microbiological quality of vegetables. Information was sought regarding the conditions under which vegetables are sold, the personal hygiene practices of female vendors, the overall hygiene of sales premises and the market environment, and the storage conditions for unsold vegetables at the end of the day.

The consumer survey gathered information on vegetable consumption patterns, including details about where, when, and how often vegetables are consumed. Additionally, consumers' perceptions of the hygiene practices of female vendors and the potential association of vegetables with instances of illness were documented.

### Sampling

Fresh vegetable samples were systematically obtained from the surveyed production areas and markets. Each sample, comprising 59 tomato samples, 49 cucumber samples, 106 lettuce samples, and 50 onion samples, was carefully collected in sterile bags and meticulously labeled with a unique identification number. To maintain sample integrity, they were promptly transported to the laboratory in a cooler equipped with ice.

The sampling strategy encompassed firm tomatoes without visible damage or cracks, damaged tomatoes, whole cucumbers, onions with their outer shells, and bunch of lettuce. Sampling occurred in both the fields for tomatoes, cucumbers, and lettuce, and in the markets for onions. Due to the limited availability of data on the prevalences of *Salmonella* and *E. coli* strains specific to the tested vegetables, namely tomatoes, cucumbers, onions, and lettuce, the sample size required for representativeness was determined using the formula provided by the World Health Organization (WHO, 1991) for probability sampling (Formula 1).



**Figure 1.** Map of the city of Ouagadougou with surveyed and sampled sites

$$N = PQ/(E/L)^2$$

Where N is the minimum sample size, P is the estimate of the expected proportion (prevalence rate), Q is the value of (1-P), E is the tolerated margin of error (statistical risk in %) and L is the smallest deviation for the accepted statistical risk (1.96 for the 5% risk). The above relationship for p equals 0.8 gives a minimum of 246 samples.

A lettuce sample consisted of two lettuce plants taken at random from different parts of the same field or from the same vendor's lots. For tomatoes, a sample consisted of 10 balls. For cucumbers, a sample was made up of 1 to 2 depending on size, and an onion sample was made up of 5 whole bulbs. These vegetables were taken from different parts of the same field, or from different lots held by the same vendor. All the onions were collected only in the markets, with the exception of one batch of 8 samples taken in the Boulmiougou market garden. In the fields, only market-ready vegetables were taken into account. Samples were taken in both fields and markets in a single pass (Figure 2).

### Microbiological analysis

*Salmonella* detection was conducted using a method adapted from NF EN ISO 6579:2002. Initially, a predetermined quantity of Eau Peptonnée Tamponnée (EPT) was added to 225 ml vials, and the

contents of the sachets were mixed. Following this, 25 g of each sample, contained in sterile sachets, was added to 225 ml of Buffered Peptone Water and homogenized using a stomacher. The mixture underwent incubation at 37°C for 18 to 24 h, constituting the pre-enrichment stage. Subsequently, 0.01 ml of the pre-enriched suspension was added to 10 ml of Rappaport Vassiliadis Soja (RVS) broth and incubated at 42°C for 24 h. After incubation, 10 µl of Rappaport Vassiliadis Soja (RVS) was streaked onto XLD agar, followed by incubation at 37°C for 24 h. *Salmonella* strains on XLD agar exhibited black colonies with a red background, consistent with the color of the XLD medium. Three characteristic colonies from each XLD medium were transferred to XLD agar for colony purification and then incubated at 37°C for 24 h. Subsequently, three characteristic colonies from the XLD medium were transferred to Muller Hilton (MH) agar and incubated at 37°C for 24 h for phenotypic identification. Phenotypic identification involved determining biochemical characteristics such as glucose fermentation, lactose oxidation, gas and hydrogen sulfide (H<sub>2</sub>S) production on Kligler-Hajna agar, urease and indole production, and citrate utilization as the sole carbon source on Simmons citrate agar.

For *E. coli* enumeration, 25 g of each vegetable sample was added to a vial containing 225 ml of peptone-buffered water and subjected to stomaching for 5 min to prepare the stock solution. This stock solution was used to prepare cascade dilutions in tubes containing 9 ml of buffered peptone water. For plating, 100 µl of the 10 to 2 and 10 to 3 dilutions were spread directly onto



Tomato



Onion



Lettuce



Cucumber

Figure 2. Vegetables.

Methylene Blue Eosin agar (EMB) and incubated at 44°C for 24 h. Characteristic *E. coli* colonies on EMB (dark purple with or without metallic sheen and dark center) were counted. These colonies were then transferred to Mueller-Hinton (MH) agar and incubated at 37°C for 24 h. Suspected *E. coli* colonies underwent identification through biochemical tests, including glucose fermentation, lactose oxidation, gas and hydrogen sulfide (H<sub>2</sub>S) production on Kligler-Hajna agar, and urease, indole, and citrate production as the sole carbon source on Simmons citrate agar. Results were expressed as colony-forming units (CFU) per plate.

## RESULTS

### Microbial loads in field vegetables

Bacteriological analysis revealed varying *E. coli* loads in different vegetables, with successive high levels observed in cucumbers, tomatoes, lettuces, and onions, ranging from  $2.4 \times 10^3$  to  $10 \times 10^3$  CFU/g. Cucumbers exhibited the highest contamination with *E. coli*, while onions showed the least contamination. Notably, onions, tomatoes, and cucumbers did not show contamination

with *Salmonella*. Conversely, two lettuce samples were found to be contaminated with *Salmonella*, resulting in a prevalence of 4.76% (Table 1).

The microbiological analysis of samples collected from markets revealed varying *E. coli* loads in different vegetables, with successive high levels observed in onions, cucumbers, lettuces, and tomatoes, ranging from  $6.7 \times 10^3$  to  $316.2 \times 10^3$  CFU/g. Onions exhibited the highest contamination with *E. coli*, while tomatoes showed the least. Notably, onions, tomatoes, lettuces, and cucumbers were found to be contaminated with *Salmonella*. Lettuces demonstrated a notably high presence of *Salmonella* with a prevalence of 20.31%, followed by tomatoes at 11.36%, cucumbers at 2.94%, and onions at 2.38% (Table 2).

### Vegetables producers' practices

Analysis of our survey data revealed that: 100% of fields visited had no sanitary facilities. In the event of injuries or lesions to market gardeners, protective measures

**Table 1.** Average *E. coli* load (CFU/g) and *Salmonella spp* prevalence in vegetables collected from field.

Microorganism	Tomato (n=15)	Onion (n=8)	Cucumber (n=15)	Lettuce (n=42)
<i>E. coli</i>	6.6 × 10 <sup>3</sup>	2.4 × 10 <sup>3</sup>	10 × 10 <sup>3</sup>	3.5 × 10 <sup>3</sup>
<i>Salmonella spp.</i>	Absent (0%)	Absent (0%)	Absent (0%)	2 (4.76%)

**Table 2.** Average load of *E. coli* (CFU/g) and presence of *Salmonella* in staple vegetables sampled in Ouagadougou markets.

Microorganism	Tomato (n= 44)	Onion (n= 42)	Cucumber (n=34)	Lettuce (n=64)
<i>E. coli</i>	6.7 × 10 <sup>3</sup>	316.2 × 10 <sup>3</sup>	34.3 × 10 <sup>3</sup>	13.8 × 10 <sup>3</sup>
<i>Salmonella spp.</i>	5 (11.36%)	1(2.38%)	1(2.94%)	13 (20.31%)

were not applied (39%). Most of the fields were not fenced (72%), and there were piles of garbage (33%), domestic animals (14%) or livestock close to the fields (51%). Most farmers (94%) use animal manure to improve the soil, and 45% admit that they do not treat it before use. Water from wells and dams is used mainly (75%) for watering vegetables, and (100%) of this water was untreated. When harvested, vegetables are placed in contact with the soil (21.6%), packed in plastic bags (56%) and transported to markets by motorcycle (80%).

### Vegetables vending conditions in markets

Vegetables are mostly washed at reception with dirty water (83%). Vendors say they use public toilets for their needs (78.4%) and wash their hands afterwards with soap and water (85%). Vegetables are mostly sold in open-air markets (65%) and in unsanitary conditions (35%). The rest of the vegetables not sold during the day are kept at room temperature (100%).

### Knowledge about vegetable-related illnesses

More than half (75%) of the consumers questioned said that vegetables can carry diseases. Consumer perceptions differed significantly according to the level of education. The main reasons given by consumers for the involvement of vegetables in cases of disease transmission were the absence or inadequate cleaning of vegetables (44.3%), lack of hygiene during preparation (26.9%) and poor storage conditions (19.9%).

### Knowledge of the main place of vegetable consumption

Vegetables are eaten exclusively in restaurants in 28% of cases, at home in 48% and in other places in 24% of

cases. Vegetables are mainly eaten for dinner (61%) and lunch (32%), with 77% consuming them at least once a day.

## DISCUSSION

The surveys conducted revealed that the production of staple vegetables in Ouagadougou is exclusively carried out by men. These findings align with previous studies by Alio Sanda et al. (2017) and Toe et al. (2017), which demonstrated that in Niger and Abidjan, respectively, market gardening is predominantly practiced by men (100 and 73.3%). In Ouagadougou, growers are primarily aged between 30 and 45 (69.5%) and operate medium-sized fields (63.7%). The absence of female representation and the average size of farms may be attributed to cultural practices in the country and the demanding nature of irrigation work.

The study suggests that the presence of *enterobacteria*, such as *Salmonella* and *E. coli*, in vegetables is likely due to the precarious hygienic conditions under which they are cultivated and sold, including equipment contact and the immediate environment of the products. The high microbial load observed in vegetables from both markets and fields could be linked to inadequate storage facilities, poor personal hygiene among vendors and growers, insufficient waste disposal and sanitation facilities, the use of water for plant irrigation, animal manures for soil amendment, and a lack of sanitary facilities.

In onion samples from both fields and markets, the microbial load of *Escherichia coli* was successively observed (2.4 × 10<sup>3</sup> CFU/g and 316.2 × 10<sup>3</sup> CFU/g). In contrast, Anin et al. (2016) found no *Escherichia coli* in their onion samples from Abidjan (0 CFU/g). The *Escherichia coli* microbial load (9.2 × 10<sup>2</sup> CFU/g) reported by Anin et al. (2016) in tomato purée in Abidjan is higher than the levels found in the present study for tomato samples (6.6 × 10<sup>3</sup> CFU/g for fields and 6.7 × 10<sup>3</sup> CFU/g for markets).

*Salmonella* prevalences of 0% in onions, tomatoes, and field cucumbers align with the guidelines set by the WHO, which recommend no presence of *Salmonella* in any raw vegetable intended for consumption (WHO, 2012). However, the prevalences of tomatoes, onions, cucumbers, and lettuces from markets did not meet this recommendation (Prevalence over 0%). The prevalence of *Salmonella* in lettuce samples from markets (20.31%) and fields (4.76%) is lower than the 50% reported by Traoré et al. (2015) in lettuce from fields in Burkina Faso. Additionally, the prevalence of *Salmonella* in tomato samples (5.3%) reported by Toé et al. (2017) in Abidjan is lower than the levels found in tomato samples collected from markets in this study (11.36%).

The disparity in *Salmonella* prevalence between field and market lettuces could be attributed to the practice in the field where vegetables remain unwashed, coming into contact with potentially contaminated water and manure used as fertilizer. Some authors in Burkina Faso have noted that fertilizers utilized by farmers are often untreated and may contain pathogenic bacteria detected in animals' feces (Bako et al., 2018; Kagambèga et al., 2013). However, the *Salmonella* prevalence in vegetables from the fields reported in this study is lower than the levels reported by Toe et al. (2017) in lettuce (16.1%) and cucumbers (4.8%), but similar in onions (0%).

The prevalence of *Salmonella* in onions found in our study from the fields (0%) aligns with the findings by Anin et al. (2016). Conversely, we observed a prevalence of *Salmonella* in market tomato samples (11.36%), while Anin et al. (2016) found no *Salmonella* in their tomato purées. Alio Sanda et al. (2017) in Niger reported higher *Salmonella* prevalence (36.94%) than that found in lettuce from fields (4.76%) and markets (20.31%) in our study. In Malaysia, Saw et al. (2020) found a *Salmonella* prevalence of 0% in tomato samples, which is consistent with the present results for tomatoes from fields but lower than that found in tomatoes from markets (11.36%). The cucumber prevalence reported by Saw et al. (2020) at 10% was higher than that in our market and field samples, respectively (2.94% and 0%).

Additionally, Saw et al. (2020) found a prevalence of 0% in their lettuce samples, which is lower than the levels observed in our field and market lettuces (4.76 and 20.31%, respectively). These differences in *Salmonella* prevalence in vegetables across different countries could be explained by variations in climate, production practices, and the level of hygiene control in each country.

## Conclusion

A significant portion of our diet includes raw foods, and depending on their nature and production methods, these raw foods can carry germs, some of which may be

pathogenic for humans. These germs are typically susceptible to cooking. However, in the absence of cooking, the risks associated with consuming raw food must be addressed by controlling the microbiological quality of raw materials. The contamination of staple vegetables in Ouagadougou is attributed to factors such as ignorance, inadequate, or poorly applied good hygiene practices. Notably, the vegetables consumed in Ouagadougou are more commonly contaminated by *E. coli* than *Salmonella*, posing a risk of food poisoning. Raising awareness among various stakeholders in this sector, including producers, vendors, and consumers, has the potential to significantly reduce contamination issues associated with these products, which are an important source of nutrients.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Antibiotic resistance profile of *Escherichia coli* isolates among patients suspected of urinary tract infections at the Sourou Sanou University Hospital Centre of Bobo-Dioulasso, Burkina Faso

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This study aims to describe the antibiotics resistance (antimicrobial resistance [AMR]) profile of *Escherichia coli* isolates among patients suspected of urinary tract infections (UTIs) at the Sourou Sanou University Hospital Center (SSHUC) of Bobo-Dioulasso in Burkina Faso. A descriptive cross-sectional study was conducted from June 1 to August 31, 2021 at SSUHC. An exhaustive sampling was performed and the urine samples of patients suspected of UTIs were collected and subjected to urine analysis and culture. The culture consisted of bacterial isolation, identification, and Antibiotic Susceptibility testing (AST). In this study, 41.88% (178/425) patients were confirmed cases of UTIs. *E. coli* was involved in 43.8% (78/178) of the UTIs. The frequency of extended-spectrum beta-lactamase producing *E. coli* isolates was 23% (18/178). The resistance profile of *E. coli* was 93% for Amoxicillin, 79% for Amoxicillin + Clavulanic acid, 75% for Gentamicin, 70% for Ciprofloxacin, and 53% for Cefotaxime. The study confirmed the predominance of *E. coli* isolates in ITUs at the referral hospital of Bobo-Dioulasso with high levels of resistance to beta-lactams. There is a need to reduce AMR progression in Burkina Faso by strengthening the AMR surveillance system.

**Key words:** Resistance, antibiotics, *Escherichia coli*, urinary tract infections, Bobo-Dioulasso.

## INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infectious diseases, accounting for the majority of hospital visits in medicine (Emonet et al., 2011). These

infections can start from asymptomatic bacteriuria and lead to severe renal infection (Thirion and Williamson, 2003). They may be categorized by some urinary tract



abnormalities such as vesicoureteral reflux, presence of kidney stones, prostatic hypertrophy or contribute to the complication of another condition (Herinirina, 2009).

It is estimated that 3% of girls and 1% of boys would develop symptomatic UTIs during their childhood (Iacobelli et al., 2009) while 20% of the adult female population would present an episode of UTIs during their lifetime (Caillaud et al., 2017). According to data reported in 2006 in the United States, UTIs were responsible for 11,000,000 hospital visits and 500,000 hospitalizations (Nielubowicz and Mobley, 2010). In Africa, particularly in Burkina Faso, the prevalence of UTIs was estimated at 69.7% (Kafando et al., 2023).

Generally, Enterobacteriaceae species are the main pathogens responsible for UTIs, and *Escherichia coli* is the most common isolates found in UTIs, particularly in mild cystitis cases (Kafando et al., 2021).

UTIs caused by *E. coli* are considered as priority infections for disease surveillance system due to the high frequency of these bacterial isolates, their virulence and resistance to antibiotics (Hassaine and Boulanoir, 2019). Given the frequency of these infections and possible overdiagnosis, there is a significant risk of antibiotics overuse (Foxman, 2010). The latter can lead to the selection of single or multi-drug resistant bacterial strains. Steady increase in antibiotic resistance in UTIs led to the realization of many studies that attempted to determine the most appropriate treatment through identification of the most active antimicrobial agent and adequate treatment duration for mild cystitis (Zalmanovici et al., 2010). It is in this perspective that this study was conducted to describe the antibiotic resistance profile of *E. coli* isolates among patients suspected of UTIs at the Souro Sanou University Hospital Center (SSUHC) of Bobo-Dioulasso from June to August, 2021.

## MATERIALS AND METHODS

### Type, period, and site of the study

This was a descriptive cross-sectional study that was conducted over a period of three months (June 1 to August 31, 2021) at the Souro Sanou University Hospital Center (SSUHC) in Bobo-Dioulasso. The SSUHC is located in Bobo Dioulasso, capital of the Hauts-Bassins region and the economic capital of Burkina Faso. The health coverage regions of the SSUHC, which include the Haut-Bassins, the Cascades, Boucle du Mouhoun, and Southwest regions with a total population estimated at 6,555,016 inhabitants in 2022. The hospital has a capacity of 656 beds, and six departments namely medicine, pediatrics, surgery, obstetrics and reproductive medicine, pharmacy, and laboratory. Bacteriological analyses were performed in the Laboratory Department of the Bacteriology-Virology Service of the SSUHC, which hosts the National Reference Laboratory for antimicrobial resistance (AMR) surveillance in

Burkina Faso.

### Sampling and data collection

An exhaustive sampling was performed by involving hospitalized patients in the different hospital wards and patients from the community who were suspected of UTIs during their stay at the SSUHC in Bobo-Dioulasso.

Urine samples were collected using the clean-catch technique. Briefly, patients were recommended to disinfect their hands before opening the container and collect the midstream urine that had been in the bladder for at least 4 h in the morning. Socio-demographic and clinical data were collected using laboratory requisition forms.

### Inclusion criteria

All patients having one of the symptoms of an infection of the urinary tract such as dysuria, pollakiuria, urinary incontinence, lumbar pain, macroscopic hematuria, and were referred to the Laboratory of Bacteriology for urine analysis and culture.

### Cytology

Cytological analysis was carried out on all urine samples using KOVA Glass cell. Prior to this analysis, the pellet of each urine sample was used for Gram staining. The type of Gram staining guided the choice of the culture medium for bacterial isolation.

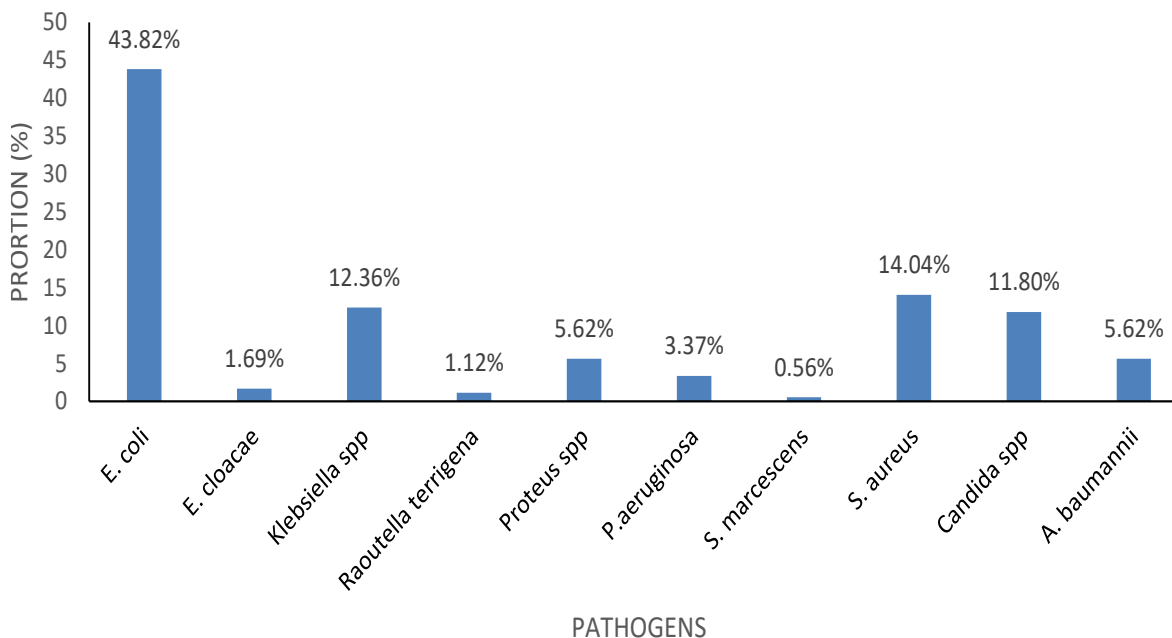
### Isolation and identification of bacteria

Bacterial count was performed by plating the urine on cystine lactose electrolyte deficient (CLED) culture media using the top-down tight striations method 10 µl inoculating loop. Eosin Methylene Blue (EMB) agar medium was used to isolate pathogenic *E. coli* from urine samples. The plates were incubated at 35 ± 37°C for 18 to 24 h. Urine samples were considered positive when the leukocyturia was higher than 10<sup>4</sup> ml<sup>-1</sup> and bacteriuria higher than 10<sup>3</sup> Colony Forming Units (CFU)/ml (Nielubowicz and Mobley, 2010). API 20E (bioMerieux SA, France) was used for the identification of *E. coli* strains.

### Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out using the Kirby-Bauer agar diffusion technique according to the recommendations of the French Society of Microbiology (CA-SFM, 2015). The bacterial suspension was prepared with sterile physiological water from a pure strain and compared to Mac Farland 0.5. Bacterial suspension was inoculated on Muller Hinton medium (Iiofilchem diagnostic) by swabbing. Antibiotic discs were deposited with sterilized metallic forceps on the surface of Mueller-Hinton agar. Strains were tested for antibiotic susceptibility testing using cefotaxime 30 µg (CTX), the amoxicillin/clavulanic 20/10 µg (AMC), imipenem 10 µg (IPM), gentamicin 10 µg (GEN), ciprofloxacin 5 µg (CIP), amikacin 30 µg (AK) (Iiofilchem diagnostic, Italy). The culture

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**Figure 1.** Distribution of pathogens in patients suspected of urinary tract infections at the Sourô Sanou University Hospital Center. n= Total number as 178.

plates were incubated at  $35 \pm 37^{\circ}\text{C}$  for 18 to 24 h. After incubation, the inhibition diameters were measured using a caliper and their interpretation was done according to the recommendations of the CA-SFM (CA-SFM, 2015).

#### Detection of extended spectrum beta-lactamases (ESBL)

ESBL detection was performed by the so-called "champagne cork" synergy image search between the beta-lactamase inhibitor discs AMC 20/10  $\mu\text{g}$  brought together with a CTX 30  $\mu\text{g}$  disc according to CA-SFM recommendations.

#### Quality control

The quality control of the antibiotic disks and culture media was done using the reference strains *E. coli* ATCC 25922 for susceptible bacterial strains and *Klebsiella pneumoniae* ATCC700603 for resistant bacterial strains according to the recommendations of the CA-SFM.

#### Ethical considerations

The authorization of the Director General of the SSUHC was obtained for data collection. Anonymity and confidentiality with respect to the data collected from the patients were observed throughout and after this study.

#### Data analysis

Data analysis was performed with Microsoft Excel 2016. Quantitative variables were expressed as median and qualitative variables as proportions.

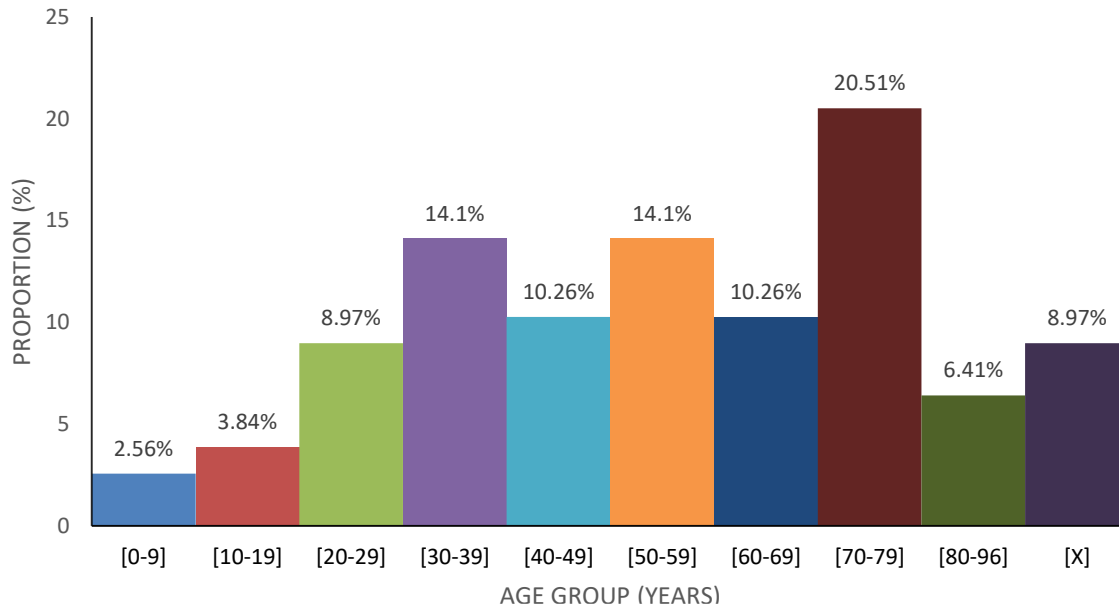
## RESULTS

### Socio-demographic characteristics of UTI caused by *E. coli*

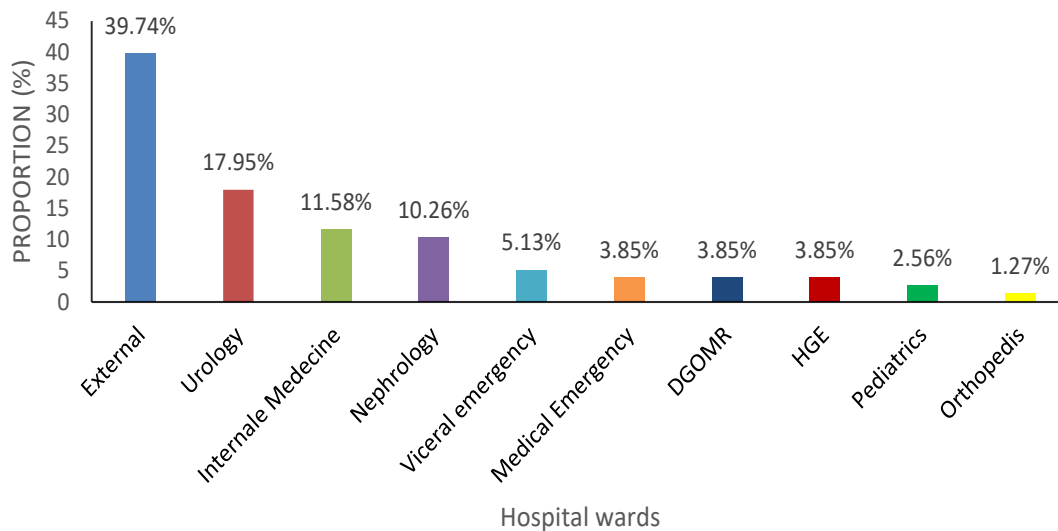
Out of the 424 patients included in this study, UTI was confirmed in 178 patients. Uropathogenic *E. coli* (Figure 1) was found in urine samples from 51% female patients. The age group between 70 and 79 years old was the most affected by uropathogenic *E. coli*, representing 20.51% (Figure 2). Regarding the hospital wards, Uropathogenic UTI caused by *E. coli* was more frequent in patients living in community settings, giving a proportion of 39.74% (Figure 3). Other identified species were as follows: *Enterobacter cloacae* (n=3), *Klebsiella* species (n=26), *Raoutella terrigena* (n=2), *Proteus* species (n=10), *Pseudomonas aeruginosa* (n=2), *Serratia marcescens* (n=1), *Staphylococcus aureus* (n=25), *Candida* species (n=21), and *Acinetobacter baumannii* (n=10).

### Antibiotic resistance profile of uropathogenic *E. coli* isolates

A total of 78 *E. coli* isolates showed a high level of resistance to beta-lactams, including amoxicillin (93%), amoxicillin + clavulanic acid (79%), cefotaxime (53%), gentamicin (25%), and ciprofloxacin (70%) whereas imipenem and amikacin were still active on uropathogenic *E. coli* isolates with an estimated resistance rate of 5% (Table 1).



**Figure 2.** Distribution of uropathogenic *E. coli* by age group in patients suspected of UTI at the Sourou Sanou University Hospital Center. X: unknown age group. n= Total number as 78.



**Figure 3.** Distribution of uropathogenic *E. coli* by hospital wards in patients suspected of UTI at the Sourou Sanou University Hospital Center. DGOMR: Department of Gyneco-Obstetrics and Reproductive Medicine, HGE: Hepato-Gastroenterology. n= Total number as 78.

### Phenotype of resistance *E. coli* strains

As regards resistance to beta-lactam antibiotics, the penicillinase phenotype was the most common at 93%, followed by ESBL at 23% as shown in Figure 4. Aminoglycoside resistance in *E. coli* strains showed a predominance of the gentamicin-resistant phenotype at 31%. As for resistance to quinolones, 70% of strains were resistant to ciprofloxacin (Table 2).

### DISCUSSION

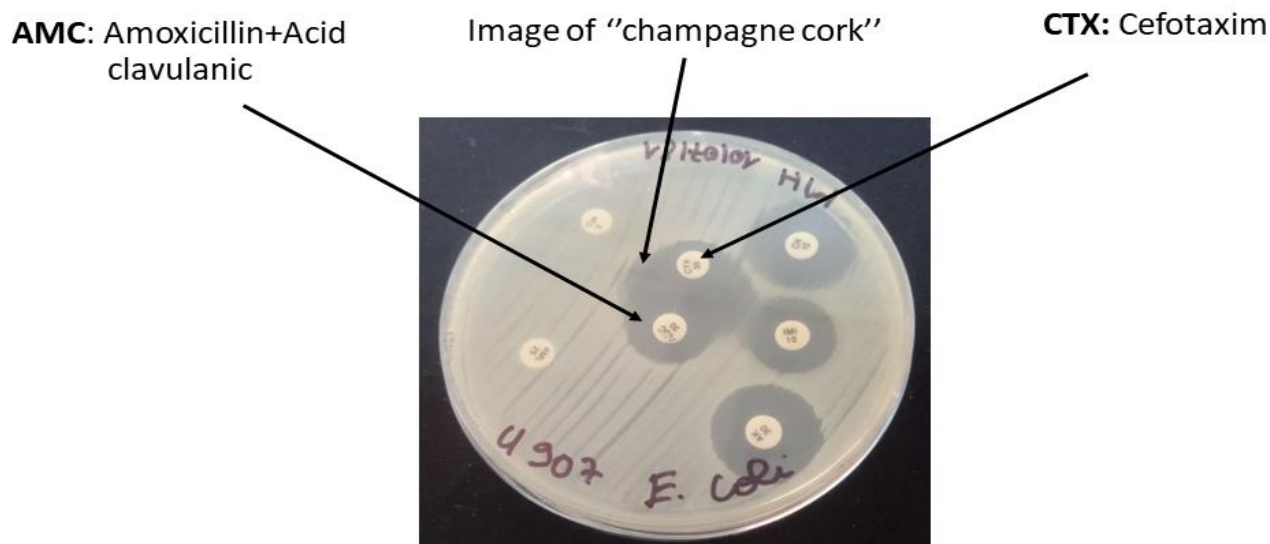
A description of the antibiotic resistance profile of *E. coli* isolates among patients suspected of UTIs at the Sourou Sanou University Hospital Center of Bobo-Dioulasso was shown in this study.

In this study, 178 (178/425) patients were UTI confirmed cases. The distribution of pathogens by species showed that *E. coli* was the most represented uropathogen

**Table 1.** Antibiotic resistance profile of uropathogenic *E. coli* isolates according to the activity of antibiotics.

Antibiotic resistance profile of <i>E. coli</i> isolates	Antibiotics						
	AX (%)	AMC (%)	CTX (%)	IMP (%)	AK (%)	CN (%)	CIP (%)
Rate of antibiotic susceptibility	00 (00)	14.1 (11)	35.8 (28)	89.7 (70)	89.7 (70)	64.1 (50)	25.6 (20)
Rate of intermediate antibiotic resistance	6.4 (05)	7.6 (05)	10.2 (08)	5.0 (04)	5.0 (04)	10.2 (08)	03.8 (03)
Rate of total antibiotic resistance	93 (73)	79 (62)	53 (42)	5.0 (04)	5.0 (04)	25 (20)	70 (55)

AX: Amoxicillin, AMC: Amoxicillin-clavulanic acid, CTX: Cefotaxime, IMP: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, ESBL: Extended spectrum beta-lactamase.



**Figure 4.** Production of Extended-spectrum beta-lactamase by an *E. coli* isolated from urine sample of a patient at the Sourou Sanou University Hospital Center of Bobo-Dioulasso, Burkina Faso. There is a clear representation of 'champagne cork' between CTX and AMC.

(43.82%). Indeed, *E. coli* is the species most commonly isolated in UTIs. This can be explained by the pathophysiology of UTIs, which generally occurs through ascending pathway with colonization of the perineum by Enterobacteriaceae species from gastric intestinal tract, in particular *E. coli* (Dalal et al., 2009; Bruyère et al., 2013). This finding correlates with the study of Bounemour (2018) on the bacteria responsible for UTIs in Algeria in 2018, in which *E. coli* was the predominant species causing UTIs (44.01%). Lobel and Soussy (2007) reported high prevalence of *E. coli* (70-95%) in UTIs. *S. aureus* was the second most prevalent species after *E. coli*, with a frequency of 14.04% in this study. *S. aureus* is a relatively rare cause of UTI in the general population (Demuth et al., 1979). Although isolation of *S. aureus* from urine samples is often secondary to staphylococcal bacteremia occurring elsewhere (e.g., in endocarditis) in some patients, *S. aureus* causes ascending colonization and infection of the urinary tract (Lee et al., 1978).

Regarding the distribution of uropathogenic *E. coli* by age group, the age group between 70 and 79 years old

was the most affected by UTIs caused by *E. coli* (20.51%). This could be due to the high frequency of underlying diseases such as diabetes, prostate disease, indwelling catheters, bladder hypoactivity, and weakened immune system that may favor the occurrence of opportunistic infections in this age group representing the elderly patients (Gonthier, 2000; Bally and Troillet, 2008). This finding correlates with the study of Doumia (2019) on the antibiotic resistant profile of uropathogens in Mali. In this study, 60 year-old patients and above were the most affected by UTI and this accounted for 30.1% (Doumia, 2019). Other authors also confirmed the predominance of UTIs among elderly patients (Gonthier, 2000; Durand-Gasselien and Haber, 2001). It was rationalized that decrease in functional autonomy of bladder is associated with the occurrence of UTIs in elderly patients (Falcou et al., 2018).

The distribution of uropathogenic *E. coli* by age gender in patients suspected of UTIs at the Sourou Sanou University Hospital Centre indicated that women were mainly affected (51%) by UTIs caused by *E. coli* isolates.

**Table 2.** Multidrug resistance (MDR) profile of *Escherichia coli* isolates.

Phenotype	Number of <i>E. coli</i> isolates	Proportion of <i>E. coli</i> isolates (%)
Penicillinase	73	93
ESBL	18	23
Resistance to Imipenem (Carbapenem)	04	05
Resistance to Amikacin and Gentamicin (Aminoglycosides)	24	31
Resistance to Ciprofloxacin (quinolone)	55	70

This female predominance could be due to the anatomy of their urinary tract, which is characterized by the shortness of urethra and the proximity between their anus and urethra leading to frequent UTIs in women (Barrier Letertre Clémence, 2013). Indeed, it is reported that more than 30% of women and nearly 10% of men suffer from UTIs at least once in their lifetime (Zalmanovici et al., 2010). Furthermore, factors such as bacterial infection of the periurethral glands, the turbulent flow of urine over the surface of the urethra, the contiguity of the perineal orifices and sexual intercourse favor the occurrence of UTI in women (Zalmanovici et al., 2010).

The distribution of uropathogenic *E. coli* isolates by hospital wards at the Souro Sanou University Hospital Center showed a high frequency of uropathogenic *E. coli* (39.74%) which was found in patients from community settings. Indeed, *E. coli* is an ubiquitous microorganism found in both hospital and community settings (Pitout et al., 2005; Sabir et al., 2014). It is the most common species causing UTIs in community settings, and is involved in 85% of UTIs cases in some cases (Naber et al., 2008). Lack of sanitation in developing countries could contribute to the spread of uropathogenic *E. coli* strains. In addition, poor management of biomedical waste in resource-constrained countries could also be a key driver of UTIs in the community settings.

Regarding the antibiotic resistance profile of *E. coli* isolates, there was high resistance to beta-lactams at the proportions of 93% for Amoxicillin, 79% for Amoxicillin + Clavulanic acid. Similar observation was made by Bounemour (2018) in Algeria who found a resistance profile of uropathogenic *E. coli* strains at 95% for Amoxicillin.

Penicillinase resistance in *E. coli* has long been described, and is most often associated with TEM penicillinases (Rakotovao-Ravahatra et al., 2017; Abraham and Chain, 1988). This high rate of resistance is the result of the selection pressure due to the excessive prescription and sometimes misuse of these antibiotics both in hospitals and primary care centers (Sbiti et al., 2017). As for resistance to third-generation cephalosporins, around 55% of *E. coli* isolates were resistant to Cefotaxime, and in 18% of cases, this resistance was associated with ESBL production. Yandai et al. (2019) and Amady et al. (2021) reported higher rates than the present study, with 33.33 and 25.4% of

ESBL phenotypes, respectively (Yandai et al., 2019; Amady et al., 2021). Resistance to third-generation cephalosporins (3GCs) considerably reduces therapeutic options and contributes to a continued increase in carbapenem prescribing which retains good activity in most cases (Forestier et al., 2012). This high rate of resistance highlights the extent of the antibiotic resistance phenomenon, which could be favored not only by the abusive use of antibiotics in our healthcare settings but also self-medication. Uropathogenic *E. coli* strains were also producers of ESBL. ESBL-producing bacteria are a major concern in the hospital setting due to their epidemic spread and multiple antibiotic resistance (Kalambry et al., 2019). Hospital-acquired infections caused by ESBL-producing Enterobacteriaceae represent a real therapeutic problem due to multidrug resistance, and hence, the limited choice of antibiotic molecules available on the market (Paterson et al., 2000).

Resistance of *E. coli* strains to aminoglycosides was 5% for Amikacin and 25% for Gentamicin. Ouattara (2022) found 20.69% resistance to Amikacin and 41.43% to Gentamicin. The same is true of those described by Yandai et al. (2019) who found a resistance rate of 33% for Gentamycin and 15% for Amikacin. The disparity in *E. coli* resistance to gentamycin and amikacin may be linked to enzyme activity. *AAC(3)-II* spares amikacin and hydrolyzes gentamicin, while *AAC(6)-I* hydrolyzes amikacin and *AAC(6)-II* hydrolyzes gentamicin. The predominance of *AAC(3)-II* and *AAC(6)-II* enzymes is thought to be responsible for the high level of gentamycin resistance, as these are their preferred substrates (Xiao and Hu, 2012). Plasmids encoding ESBL genes often result in resistance to other antibiotics such as aminoglycosides, chloramphenicol, sulfonamide/trimethoprim combination, cyclins, and fluoroquinolones (Ouedraogo et al., 2016; Sanou et al., 2021).

As for quinolone resistance, 70% of the strains were resistant to ciprofloxacin. Similar results were reported by Sbiti et al. (2017) and El Bouamri et al. (2014) who reported in their studies, rates of ciprofloxacin co-resistance ranging from 82%. This resistance is thought to be a consequence of the high prescription of fluoroquinolones in the treatment of UTIs caused by enterobacteria, especially *E. coli* (Lahlou et al., 2009). In addition, the resistance associated with fluoroquinolones is linked to the presence of genes *qnr* (alleles A, B, S)

and in particular the *aac(6')-Ib-cr* gene which most often coexists with BLSE genes and confers dual aminoglycoside-piperazine-quinolone (Coque et al., 2008). This high rate of antibiotic resistance highlights the extent of the antimicrobial resistance phenomenon, which could be favored not only by the abusive use of antibiotics in our healthcare settings, but also self-medication (Sbiti et al., 2017).

Imipenem and Amikacin were still active on the majority of uropathogenic *E. coli* isolates in this study. It is suggested that Imipenem remains the most active antibiotic molecule on *E. coli* isolates because it is only prescribed in hospital settings and used as a last therapeutic option for UTIs treatment in our context.

## Conclusion

The objective of this study was to describe the resistance profile of *E. coli* isolates among patients suspected of UTIs at the Souro Sanou University Hospital Center of Bobo-Dioulasso from June to August, 2021. This study finding underscored that the bacterial ecology has not changed much in recent years with predominance of *E. coli* among the uropathogenic bacteria in this study. In addition, some antibiotics such as amoxicillin, amoxicillin + clavulanic acid (beta-lactams) and ciprofloxacin (fluroquinolone) are no longer required for the treatment of UTIs.

These data show the emergence of resistance to beta-lactam antibiotics, often combining several mechanisms, including ESBL production, carbapenemases, resistance, quinolones, and antibiotic resistance. In view of these findings on the resistance profiles of uropathogenic bacteria, it is therefore necessary to strengthen the AMR surveillance in order to define better therapeutic strategies adapted to the local epidemiology, optimize the probabilistic antibiotic therapy of ITU, and reduce the progression of AMR.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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