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Prevalence and antibioresistance of *Escherichia coli* and *Salmonella* isolated from lettuce and irrigation water in Ouagadougou, Burkina Faso

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ABSTRACT

Introduction. The use of partially or untreated wastewater in the irrigation of vegetable crops constitutes a risk of microbiological contamination that can cause diarrhea in the consumer. Aim. This study mainly aimed to assess the level of contamination of irrigation water and lettuce as well as to determine the antibiotic resistance profile of isolated bacteria. Methods. A total of eighty samples of lettuce and ten irrigation waters were collected from the market gardening sites of Boulmiougou, Bissigin, the National School of Public Health and Tanghin. The research of thermotolerant coliforms, *Escherichia coli* and *Salmonella* was done following ISO standard methods. Isolated *Escherichia coli* and *Salmonella* strains were tested for their resistance profile by using the Kirby-Bauer method with a panel of twenty different antibiotics. Results. Out of the eighty lettuce samples, 68.75% (55) contained thermotolerant coliforms with an average load of 8.75 ×105 CFU g^{-1}. The prevalence of *Escherichia coli* was 57.5% (46) and *Salmonella* prevalence was 11.25% (9) including eight strains of *Salmonella enterica spp.* and one strain of *Salmonella enterica arizonea*. All irrigation waters were contaminated by thermotolerant coliforms at an average concentration of 3.11×10^4 CFU/100mL. The prevalence of *Escherichia coli* in the irrigation waters was 40% (4/10) while *Salmonella enterica spp.* was 20% (2/10). The highest antibiotic resistance was found in *Escherichia coli* strains with ampicillin 28.26%, amoxicillin + clavulanic acid 26.09% and tetracycline 19.57%. Conclusion. The water used to irrigate vegetable crops in Ouagadougou could be a major source of contamination of vegetables by microorganisms. In addition, the consumption of lettuce would constitute a risk of propagation of salmonellosis among the population.

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INTRODUCTION

In recent decades, West African countries have been confronted with a growing urban population [1]. This galloping urbanization and high economic concentration have favored the development of urban and peri-urban agriculture [2]. This agricultural activity, dominated by market gardening, is today confronted with enormous difficulties, including the thorny problem of pollution, due to the use of wastewater for irrigation and the proximity of production sites to major urban traffic routes, industries and public dumps [3, 4]. In Burkina Faso, the reuse of partially or untreated wastewater in market gardening is a common practice. These practices could induce a high contamination of vegetables by microorganisms, some of which are dangerous for the consumer [5]. Indeed, the contamination of fruits and vegetables by enteropathogenic bacteria such as *Salmonella*, *Escherichia coli* O157:H7 and *Vibrio cholerae* is a major public health problem [5, 6]. Moreover, many vegetable products including lettuce are often eaten raw; which amplifies the dangers to which the consumers of these foods. Several cases of intoxication food related to vegetable ingestion

Contaminated have been identified almost everywhere in the world [7]. This contamination can be of environmental, animal or human origin during the cultivation, harvesting or handling of plants before

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consumption [8]. Most of the time, it is a surface contamination [5]. However, recent studies show that *Salmonella* are able to infect and multiply in the mesophyll of some plants such as lettuce [9, 10].

In Ouagadougou, the practice of using all kinds of wastewater (urban or industrial wastewater) is carried out in an unplanned and uncontrolled manner in urban and peri-urban agriculture activities, including the irrigation of vegetables that are consumed in their uncooked state (lettuce in particular), ignoring the standards of water intended for irrigation and the guidelines of the World Health Organization (WHO). Faced with this problem, the present study was conducted to respond to the problems of reusing untreated wastewater in agriculture in the case of Ouagadougou, particularly in the irrigation of vegetable crops, especially raw vegetables, of which we chose lettuce as the study plant, which is widely consumed by the population of Ouagadougou.

This study made it possible to assess the level of microbial contamination of irrigation water and lettuce; to determine the prevalence of *Escherichia coli* and *Salmonella* isolated as well as their antibiotic resistance profiles. The study involved the market garden sites of Tanghin, Boulmiougou, Bissigin and the National School of Public Health of Ouagadougou.

MATERIALS AND METHODS

Study sites

This descriptive and analytical study was carried out on urban market gardening sites in the city of Ouagadougou (Figure 1). Our study was conducted in the market garden sites of Tanghin dams 1 and 2, Boulmiougou, the sites of Bissigin, in and behind the National School of Public Health as shown in Figure 1

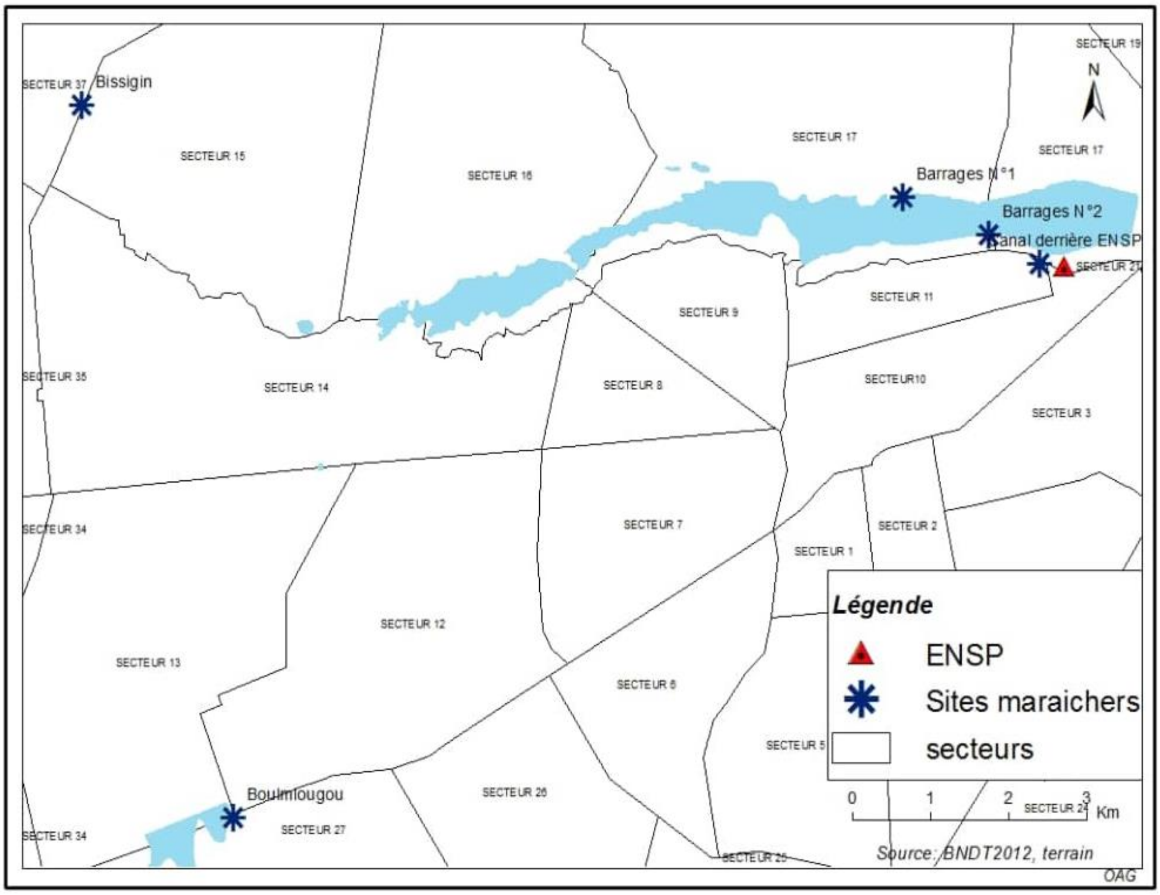

Figure 1. Market gardening sites

Collection of irrigation water and lettuce samples

Lettuce samples were collected at the usual phenological stage of harvest (at maturity) under aseptic conditions using sterile collection bags. Irrigation waters were collected under aseptic conditions using 500 mL glass bottles previously rinsed with the water to be collected. The samples were placed in a cooler containing ice accumulators to ensure the transport of the samples at a temperature close to +4°C. In total, 80 lettuce samples and 10 irrigation water samples including 6 well water, 3 dam water and 1 canal water were collected.

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Microbiological analysis of irrigation water

Cellulose nitrate membrane filters with a pore size of 0.47 µm (Sartorius AG, Göttingen, Germany) were used in combination with a Sartorius Combisart® system to filter the serial dilutions of the collected water samples. Filters were placed on the selective medium, Chromocult, to cultivate total coliforms and *Escherichia coli*. To identify the counts of total coliforms, plates were incubated at for 24 h at 37°C; for *Escherichia coli*, the incubation temperature was 44°C for 24h. For *Salmonella spp*. identification, 2 L of the samples were filtered and the membranes were placed in 90 mL of buffered peptone water BPW for 24 h at 37° C. Thereafter, 1 mL was taken from the pre-enrichment and added to 9 mL of the selective enrichment broth, Rappaport Vassiliadis Soya broth (RVS, Oxoid Ltd., Hampshire, UK), and incubated at 44°C overnight. One µL of enriched broth was streaked onto the XLD agar (Oxoid Ltd., Hampshire, UK) and incubated at 37°C for 24 h. Identification of the red colonies with a black center was confirmed biochemically by API 20E strips [6].

Microbiological analysis of lettuce

For laboratory analysis, 10 g of each lettuce sample were mixed and homogenized into 90 mL of sterile buffered peptone water (BPW, Liofilchem S.r.l., Teramo, Italy) and shaken gently by hand for 2 minutes. Further tenfold serial dilutions were made with in sterile buffered peptone water. Duplicate plates were made for each sample at each dilution under ISO 6887 standard methods. Bacteria counts were expressed as Colony Forming Units per gram of lettuce (CFU g^{-1}).

Thermotolerant coliforms known to be an indicator of fecal contamination were counted onto standard violet red bile lactose (VRBL) agar (Conda Pronadisa, Spain) incubated at 44.5 ± 0.5° C for 24 to 48 h under NF V08–017:1980. Only the Petri dish containing less than 150 colonies were considered. *Escherichia coli* was identified through the Indole, Methyl red, Vogues-Proskauer, Citrate (IMViC) test from thermotolerant coliforms. Suspected colonies were selected and subcultured on Nutrient Agar at 37° C for 24 h. Pure cultures grown on Nutrient Agar were used for Oxidase test and determination of IMViC pattern (indole, methyl red, Voges Proskauer and citrate utilization test) following Standard Procedures for food Analysis. Positive clones were transferred into Levine BBL™ Eosin Methylene Blue Agar (EMB) Agar France, which was incubated at 37 ± 1° C for 24 h. *Escherichia coli* ATCC 8739 was uses as positive control for all samples.

Salmonella was investigated according to the standard - Horizontal method for detection of *Salmonella spp.* ISO 6579:2007. Briefly, the non-selective enrichment was done by adding 25 g of each lettuce sample into 225 mL buffered peptone water incubated at 37°C for 18 to 20 h. The selective enrichment step was performed onto both tetrathionate (Müller-Kauffman) (Liofilchem diagnostic, Italy) and Rappaport Vassiliadis Soy (Difco laboratories) broths incubated respectively at 37 ± 1° C and 42 ± 1°C for 18 to 20 h. A brilliant green at 0.95% was added to the selective media Tetrathionate broth in order to inhibit the growth of Gram-positive bacteria. Isolations were performed onto Xylose Lysine Deoxycholate (HiMedia Laboaratories, India) and *Salmonella-Shigella* (HiMedia Laboaratories, India) agars. Suspected colonies were purified on nutrient agar and then submitted to API 20E (BioMérieux) test for biochemical confirmation. *Salmonella typhimurium* (ATCC 14028) and *Salmonella enteritidis* (ATCC 13076) were used as positive control. The Key biochemical tests including the fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H_2S production, and fermentation of dulcitol [11].

Study of the sensitivity to antibiotics of isolated strains of Escherichia coli and salmonella

All isolates were also tested for susceptibility to 14 different antimicrobial agents using the disk diffusion method on Mueller Hinton II agar (Bio-Rad France) following the European Committee on Antimicrobial Susceptibility Instructions (EUCAST) guidelines [12]. *Escherichia coli* ATCC 25922 and ATCC 35218 were used as a control. The antimicrobial disks (Himedia, India) used were ampicillin (10µg), amoxicillin/clavulanic-acid (30µg+10µg), Cefoxitin (30 µg), cefotaxime (30 µg), cefepime (30 µg), ceftriaxone (30µg), ceftazidime (30 µg), ceftazidime/clavulanic-acid (30 µg + 10 µg), aztreonam (30 µg), imipenem (10µg), meropenem (10µg), amikacin (30 µg), gentamicin (10µg), tobramycin (10 µg), tetracycline (30µg), nalidixic-acid (30µg), ciprofloxacin (5µg), norfloxacin (10µg), cefotaxime (30µg), chloramphenicol (30µg), and trimethoprime/sulfamexazol (25µg). Inhibition diameters of the antibiotics were interpreted according to the EUCAST [12]. The multiresistant is defined as the resistance to at least three different antibiotics family [13]. Extended-spectrum β-lactamases (ESBL) activity was carried out by using amoxicillin/clavulanic-acid against cefotaxime, ceftriaxone.

Statistical analysis of data

Statistical analysis was done using XLSTAT 2021.2 software. Comparison test were done to determine whenever there were significant differences ($P \leq 0.05$).

RESULTS

Contamination of lettuce and irrigation water by *Escherichia coli*

Although thermotolerant coliforms were detected in 55 samples of lettuce (68.75%). The means concentration was 8.75 x10^5 CFU g^{-1}.The prevalence of *Escherichia coli* were 57.5% (46/80). All irrigation water samples were contaminated with thermotelerant coliforms. The means concentration were 3.11×10^4 CFU.100mL^{-1}. The prevalence of *Escherichia coli* in irrigation water were 40% (4/10). However, there is no statistically associated relationship between the presence of *Escherichia coli* in irrigation water and the contamination of lettuce watered by these waters (*p value* = 0.29)

Prevalence of *Salmonella* Isolates from lettuce and irrigation water

A total of 9 *Salmonella* strains were isolated from lettuce with a prevalence of 11.25 % (9/80). These strains belonged to 2 subspecies of the *Salmonella enterica* species including *Salmonella enterica spp* (8/9) and *Salmonella enterica arizonae* (1/9). The prevalence of *Salmonella* in the irrigation water was 20% (2/10). These were 2 well waters contaminated with *Salmonella enterica spp*. The results did not show a statistical link between irrigation water contaminated by *Salmonella* and contamination of lettuce (*p value* = 0.42).

Antimicrobial susceptibility testing of lettuce isolates

All 46 strains of *Escherichia coli* isolated from lettuce were susceptible to imipenem, meropenem, gentamicin, amikacin, tobramycin, ceftazidin, chloramphenicol, doxicyclin, norfloxacin and ciprofloxacin. We observed a low antimicrobial resistance of *Escherichia coli* to ampicillin 28.26 %, amoxicillin/clavulanic-acid 26.09 %, tetracyclin 19.57 %, trimethoprime/sulfamexazol 17.39 %, cefoxitin 4.35 % and 2.17 % resistance to ceftazidime, cefotaxime, cefepime, ceftriaxone, aztreonam and nalidixic-acid (Table 1). The results showed a resistance of 22.22% (2/9) of *Salmonella enterica spp.* to ampicillin, 11.11% (1/9) to nalidixic-acid, amoxicillin/clavulanic-acid and tetracycline respectively (table 1). However, no resistance to the tested antibiotics was obtained with *salmonella enterica arizonae.*

Antimicrobial susceptibility testing of irrigation water isolates

Of the 4 strains of *Escherichia coli* isolated from irrigation water, 2 showed resistance to ampicillin and 1 strain to trimethoprim/sulfamexazol, tetracycline and nalidixic acid (Table 1).

Multi-drugs resistance

This study revealed multidrugs resistance with *Escherichia coli* 07/46 and *Salmonella enterica spp.* 02/9 from lettuce isolates.

Table 1. Antibiogram of strains of *Escherichia coli* and *Salmonella enterica spp.* Isolated from irrigation water and lettuce

Antibiotics	Bacterial strains			
	Escherichia Coli		*Salmonella spp.*	
	Lettuce n=46 (%)	Irrigation water n=4 (%)	Lettuce n=9 (%)	Irrigation water n=2 (%)
AMP	13 (28.26%)	02 (50%)	02 (22.22%)	00
AMC	12 (26.09%)	01 (25%)	01 (11.11%)	00
FOX	02 (4.35%)	00	00	00
CTX	01 (2.17%)	00	00	00
FEP	01 (2.17%)	00	00	00
CRO	01 (2.17%)	00	00	00
CAZ	01 (2.17%)	00	00	00
CAL	01 (2.17%)	00	00	00
ATM	01 (2.17%)	00	00	00
IPM	00	00	00	00
MEM	00	00	00	00
AK	00	00	00	00
GEN	00	00	00	00
TOB	00	00	00	00
TE	09 (19.57%)	01 (25%)	01 (11.11%)	00
NA	01 (2.17%)	01 (25%)	02 (22.22%)	00
CIP	00	00	00	00
NX	00	00	00	00
C	00	00	00	00
SXT	08	02 (50%)	02 (22.22%)	00

AMP = ampicillin, AMC = amoxicillin/cluvulanic-acid, CAZ = ceftazidime, CAL = ceftazidime/cluvulanic-acid, FOX = cefoxitin, ATM = aztreonam, CRO = ceftriaxone, CTX= cefotaxime, FEP = cefepim, IPM = imipenem, MEM= meropenem, GEN = gentamicin, TE = tetracycline, TOB = tobramycin, NA = nalidixicacid, CIP = ciprofloxacin, NX= norfloxacin, AK = amikacin, SXT= trimithoprime/sulfamexazol, C = chloramphenicol, % = percentage, n=number.

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DISCUSSION

The water sources used for irrigation of lettuce in Ouagadougou were above the WHO threshold of 3 log units of *Escherichia coli* per 100 ml [14]. The urban canals of Ouagadougou drain the combined waters of rain, domestic and industrial effluents. Contamination varies considerably not only due to dilution effects but also depending to locality and season [6]. Fecal pollution of irrigation water could be due to unsanitary cultivation areas, located near garbage dumps and gutters. Similar observations were reported by Koffi-Nevry et al. [15]. The agricultural practices of market gardeners also contribute significantly to the contamination of irrigation water. Indeed, market gardeners use large quantities of domestic animal faeces (sometimes fresh) as fertilizer for soil enrichment. This agricultural practice could result in permanent fecal contamination of irrigation water from shallow, uncovered wells. Indeed, Kagambèga et al. [16] reported the presence of *Salmonella* in the faeces of cattle, poultry, pigs and hedgehogs. These waters could thus disseminate numerous pathogenic microorganisms such as *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, *Shigella* or the Norwalk and hepatitis A viruses [17, 18].

The results showed variability in susceptibility to the tested antibiotics. In general, bacterial strains isolated from lettuce showed more resistance to the tested antibiotics than those obtained from irrigation water. In addition, *Salmonella* strains were less resistant than *Escherichia coli* strains. The large majority of bacterial isolates were susceptible to imipenem, meropenem, gentamicin, amikacin, tobramycin, ceftazidine, chloramphenicol, doxicyclin, norfloxacin, and ciprofloxacin. However, low resistance was observed with ampicillin, amoxicillin/clavulanic acid, tetracycline, trimethoprim/sulfamexazol, cefoxitin, ceftazidime, cefotaxime, cefepime, ceftriaxone, aztreonam and nalidixic acid. Previous research has obtained similar results in Burkina Faso on *Salmonella* strains isolated from lettuce [19] and from the environment [20]. The work of Sana et al. [21] has revealed that these antibiotics were the most frequently prescribed by health facilities in Burkina Faso (more than 84%) and this from the first contact with the patient. This syndromic approach to antibiotic prescription could contribute to the increase in antibiotic resistance. In addition, the uncontrolled use of antibiotics through self-medication reported by Somda et al. [19]. These same researchers had already found strains of *salmonella* and *E. coli* resistant to amoxicillin, amoxicillin/clavulanic acid, trimethroprim sulfamethoxazol and erythromycin [22]. There is an increase in the use of antibiotics in the rapidly growing livestock sector in Burkina Faso. Indeed, traditional livestock farming in Burkina does not generally follow good veterinary practices.This results in anarchic use of antibiotics and non-compliance with waiting periods [23]. Thus, Bagré et al. [23] found beta-lactams, sulfonamides, quinolones, aminoglycosides, macrolides and tetracyclines in the milk and products of cows. This overuse of antiobics in veterinary medicine may contribute to the development of antibiotic resistance.

CONCLUSION

The wastewater used to irrigate market gardening crops is not suitable for the irrigation of vegetables. It could be a source of contamination of vegetables by the microorganisms it contains. Thus, the adoption of good farming practices would contribute to avoiding the proliferation of infectious diarrheal diseases linked to the consumption of these vegetable products. Finally, measures should also be taken by the authorities to reduce the overconsumption of antibiotics in human and veterinary medicine in order to protect antibiotic molecules against the increasing phenomenon of resistance.

DECLARATIONS

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Authors' contributions

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Competing interests
The authors declare that they have no competing interests.

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