*Original Research Article*

# Biochemical and resistance profile of antibiotics to strains of *Salmonella spp*. isolated from leafy vegetables in the market garden perimeters in N'Djamena, Chad

## (Biochemical and AntiBiogramic study for resistance strains of *Salmonella spp*. isolated from leafy vegetables in the market garden in N'Djamena, Chad)




**ABSTRACT**

Salmonellosis is one of the most frequently reported foodborne illnesses and the majority of salmonellosis in humans comesfrom fresh fruits and vegetables. The objective of this work is to assessthe level of contamination and to determine the biochemical profile and susceptibility to antibiotics of the strains of *Salmonella spp.* isolated from leafy vegetables produced in market garden farms in N'Djamena in Chad. 144 samples from six (6) different types of leafy vegetables were collected and analyzed according tostandard food microbiology methods. ISO 6579 (2002) was used for microbiological analysis. The classical identification gallery and the API 20E gallery allowed the biochemical identification of isolated strains. The Kirby-Bauer method was used to test the antibiotic susceptibility of strains of *Salmonella spp.* The results obtained show microbiological contamination of vegetables analyzed in particular rocket and lettuce with a proportion of *Salmonella spp.* of 12.50% and 8.33% respectively. However, contamination varies according to the sites and is intimately linkedto the poor bacteriological quality of irrigation water. The study determined the biochemical characteristics of each isolate. On the other hand, the susceptibility profiles of strains to antibiotics were varied. Indeed, the antibiotic sensitivity test of the isolates showed that the strains of *Salmonella spp*isolated are much more resistant to ceftriaxone, ampicillin and chloramphenicol. The most active antibiotics were amoxicillin/ clavulanic acid, nalidixicic acid, ciprofloxacin and imipenem with a sensitivity rate above 80%. However, the resistance of *Salmonella spp* to certain antibiotics is a real public health problem that challenges the authorities incharge of the issue.


**Keywords: leafy vegetables, *Salmonella spp.*, bacterial resistance, biochemical profile, Chad.**

## 1. INTRODUCTION

Food-borne diseases are an important cause of morbidity, mortality and economic losses around the worldso that ensuring food safety to protect public health remains an important challenge in both developing and developed countries. Almost 1/10 of people fall ill each yearfrom foodborne illnesses, resulting in 420,000 deaths per year [1]. Enteric pathogenic bacteria, such as *Salmonella spp*.and some enterohemorragic serotypes of *Escherichia coli*, are implicated in an increasing number of collective foodborne illnesses[2; 3; 4; 5; 6]. Salmonellosis is one of the most frequently reported foodborne diseases in the world [7]. Scientific research in recent decades has shown that the vast majority of salmonellosis in humans comes from fresh fruits and vegetables. Africa's urban and peri-urban areas support fruits and vegetables cultivation, contributing to the food security of populations and the creation of jobs for many low-income households [8].

In N'Djamena, market gardening has become an important element [9]. The market gardening sector plays an important role in its supply of fresh vegetables, where several types of vegetables are produced and contribute enormously to food and nutritional security in households. In order to increase production to meet growing demand, urban gardeners are using unorthodox practices that can promote high contamination of vegetables produced by pathogenic microorganisms: use of sewage for irrigation, untreated manure and non-compliance with basic hygiene rules. Consumption of such products is a risk factor for food poisoning.

Although most of this is surface contamination, recent work shows that salmonella are capable of infecting and multiplying in the mesophyl of certain plants such as lettuce [10; 11]. The alert generated by these studies on the potential risk of *Salmonella spp* infecting and colonizing plants justifies the need to assess the level of contamination of fresh vegetables produced in urban market gardening in N'Djamena. In addition, these pathogenic bacteria carry a significant number of antibiotic resistance genes. The increaseand accumulation of antibiotic resistance is another aspect of the public health problem of salmonellosis [12]. Antimicrobial resistance is a public health concern around the world. Salmonella is one of the microorganisms in which resistant serotypes have emerged, affecting the food chain [13]. Antibiotic resistance leads to the use of more expensive drugs, increasing health carecosts [14].

The objective of this study is to isolate *Salmonella spp* strains from leafy vegetables produced in market gardening farms in N'Djamena and to assess the susceptibility profile to commonly used antibiotics.

## 2. MATERIAL AND METHODS

### 2.1 Study Sites and Sampling

The study was carried out in the city of N'Djamena and more specifically in fivevegetable production sites: DjambaNgato Airport (site 1), DjambaNgato Base (site 2), Sabangali (site 3), Habena Double Lane (site 4) and HabenaKomé (site 5). The biological material is made up of the most cultivated species in these different market gardeningsites at all times. These are:*Lactuca sativa* L. (Lettuce), *Eruca sativa* Mill. (Rocket), *Petroselinum crispum* Mill. (Parsley), *Apium graveolens* L. (Celery), *Brassica oleracea* L. (Cabbage) and *Phaseolus vulgaris* L. (Bean). Study site characteristics were summarized in Table 1.

Table 1: Study site characteristics

| Site of sampling | Code | Location of the site | Source of water | Type of watering |
|---|---|---|---|---|
| Djamba Ngato Aéroport | Site 1 | 2nd arrondissement | Sewers | Watering can (sprinkling) |
| Djamba Ngato Base | Site 2 | 2ndarrondissement | Open drilling | Watering can (sprinkling) |
| Sabangali | Site 3 | 3ndarrondissement | Closeddrilling | Watering can (sprinkling) |
| Habena Double lane | Site 4 | 7ndarrondissement | River | Watering can (sprinkling) |

The overall sample size was 144 (Table 2). The distribution was made taking into account the number of types of market garden products (n = 6) and the number of samples per market garden product (p = 24). Sampling was conducted consecutively, randomly and reasonably within the sites.

With the help of sterile pliers, the samples were removed on different feet, placed in sterile plastic bags and then stored in an ice cooler and sent to the laboratory for analysis within hours. The manipulations were carried out under conditions of total asepsis.

Table 2: Sampling by Production Site

| Site / Specie | Site 1 | Site 2 | Site 3 | Site 4 | TOTAL |
|---|---|---|---|---|---|
| *Lactuca sativa* L. | 6 | 6 | 6 | 6 | 24 |
| *Eruca sativa* Mill. | 6 | 6 | 6 | 6 | 24 |
| *Brassicaoleracea* L. | 9 | 9 | 3 | 3 | 24 |
| *Petroselinumcrispum*Mill. | 9 | 9 | 3 | 3 | 24 |
| *Apiumgraveolens* L. | 9 | 9 | 3 | 3 | 24 |
| *Phaseolus vulgaris* L. | 9 | 9 | 3 | 3 | 24 |
| **TOTAL** | **48** | **48** | **24** | **24** | **144** |

## 2.2 Studyframework

Microbiological analyzes and susceptibility tests of the different bacterial strains took place at the Food Sciences and Nutrition Research Unit of the Laboratory of Research, Diagnosis and Scientific Expertise (Labo-ReDES) of the University of N'Djamena (Chad).

## 2.3 Microbiological Analyzes

In order to determine the sensitivity profile of Salmonella circulating in leafy vegetables produced in market gardening farms in N'Djamena, the microbiological analysis was carried out in accordance with ISO 6579 (2002).It consists of four steps: pre-enrichment, enrichment, isolation and biochemical identification.

1. Sample pre-enrichment: The stock solution which was prepared from 25g of the sample and 225 ml of buffered peptonal water (EPT) was incubated at 37°C for 18h± 2h.

2. Enrichment: Using pipettes, a 1 ml volume of pre-enrichment was transferred for enrichment in 9 ml of Muller-Kauffmann broth with TetraThionate-novobiocine (MKTTn) and Rappaport-Vassiliadis broth with soy (RVS broth). These nutrient media contain germ-active inhibitors that compete with Salmonella. In the MKTn tube, 1ml of pre-enriched culture is transferred and incubated at 37°C for 24h±3h. In the tube containing the RVS, 0.1 ml of the same culture that istransferred and incubated at 41.5°C for 24 hours± 3 hours.

3. Culture:The isolation of Salmonella was carried out from the broth after enrichment, by inoculation by the streak method on the selective media Xylose Lysine Desoxycholate (XLD) and Hektoen which inhibit the growth of competing bacteria and allow visual examination of suspected*Salmonella spp.* colonies. Two (2) boxes of XLD and two(2) boxes of Hektoen are used for each broth. The Petri dishes are then incubated at 37°C for 24h ± 3h for XLD and 24 to 48h for Hektoen.

Typical colonies of Salmonella spp are red with black centre on XLD agar and green with black centre on Hektoen agar. These colonies were retained for biochemical identification.

**2.4 Biochemical Identification**

Firstly,a primary identification of suspect strains by the classical identification gallery which uses the following medium: Simmons citrate (Liofilchem, Italy), KliglerHajna (Liofilchem, Italy), Mannitol-mobility-nitrate (Liofilchem, Italy), ONPG Test (Ortho NitroPhenyl-b-D-Galactopyranoside), Peptone water, Urea-Indole (tryptophan urea).

If the orientation tests are those of the bacteria sought, the last confirmation step is taken. It requires the use of the API 20E gallery (Biomerieux, Marcy l'Étoile, France). Its principle is based on the inoculum of microtubules with a bacterial suspension thatrehydrates the medium. Incubation is done at 37°C for 24 hours during which biochemical reactions (decarboxylation, fermentation, and deamination) are carried out which result in spontaneous colored products revealed by the addition of reagents. Reactions are read using the reading table.

**2.5 Antibiotic Sensitivity Tests (Antibiogram)**

Sensitivity testing of the antibiotic-isolated strain of Salmonella was conducted on the Mueller-Hinton (EUGON) medium known as Kirby-Bauer. The agar thickness was 4 mm, which corresponds to a volume of 25 mL for a Petri dish (Ø 90 mm). The sensitivity test method used is that of diffusion in agar (disc method). The antibiogram was made from a pure strain, isolated on agar or in pure culture. This method is based on the fact that when an antibiotic is deposited on the surface of an agar medium at a given point, it diffuses by establishing a gradient of concentration inversely proportional to the diameter of the antibiotic. The inoculum was sufficiently diluted and compared with the turbidity standard Mc Farland 0.5, which is the optimal density so that after cultivation on Mueller – Hinton medium it has the formation of all isolated but joined colonies.

10 antibiotics from 4 families have been tested: AMX: Amoxicillin (25 µg); AMC: Amoxicillin / Clavulanic acid (20-10 µg); AMP: Ampicillin (10 µg); CEF: Cephaline (30 µg); CRO: Triacefxone (30 µg); IMP: Imipenem (10 µg); NAL: Nalidixic acid (30 µg) CIP: Ciprofloxacin (5 µg); SXT = sulfamethoxazole trimethoprimes (25 µg); CHL: Chloramphenicol (30 µg).

### 2.6 Method of interpreting results

A two-class sampling plan was used for the search for *Salmonella spp*. The limits are its absence or its presence in 25 g of sample. If not present, the sample was of satisfactory microbiological quality and if present, the sample was found to be unsatisfactory or corrupt.

## 3. RESULTS AND DISCUSSION

### 3.1 Distribution of Salmonella spp according to the market garden products analyzed

The proportion of salmonella in the samples of leafy vegetables produced in market gardening sites in N'Djamena was 4.86%. *Salmonella spp* is presentin four (4) speciesnamely: lettuce, rockets, bean and cabbage. In the case of rockets, more than 87.50% of the samples are satisfactory (absence of*Salmonella spp.*in 25 g), fornearly 12.50% of the samples are unsatisfactory (presence in 25 g). 91.66%, 95.83% and 95.83% of lettuce, bean and cabbage samples are satisfactory respectively, while all celery and parsley samples are satisfactory (total absence of salmonella in 25 g). Table 3 showsthe results of the *Salmonella spp.* countsin fresh vegetables at the production sites.

Table 3: Research(%) of Salmonella in leafy vegetables at production sites

| leafy vegetables | *Salmonella spp*(%) | |
|---|---|---|
| | ***Absence in 25 g (satisfactory)*** | **Presence in 25 g (unsatisfactory)** |
| Lettuce | 22 (91,66) | 2 (8,33) |
| Rocket | 21 (87,50) | 3 (12,50) |
| Celery | 24 (100) | 0 (0) |
| Parsley | 24 (100) | 0 (0) |
| Cabbage | 23 (95,83) | 1 (4,16) |
| Bean | 23 (95,83) | 1 (4,16) |
| **TOTAL** | **137 (95,13)** | **07 (4,86)** |

Regarding the identification of Salmonella by site, *Salmonella spp* is present in two vegetable production sites: Site 1 and Site 2. All samples from the other sites are 100% satisfactory (total absence of salmonella in 25 g), Microbiological analysis results for the distribution of isolated *Salmonella spp.* by production site are presented in Table 4.

Table 4: Distribution of isolated *Salmonella spp* by production site (%)

| **Research site** | ***Salmonella spp (%)*** |
|---|---|
| Site 1 (48) | 5 (10,41) |
| Site 2 (48) | 2 (4,16) |
| Site 3 (24) | 0 (0) |
| Site 4 (24) | 0 (0) |
| **TOTAL (144)** | **07 (4,86)** |

At site 1, more than 89.59% of the samples are satisfactory (absence of *Salmonella* in 25 g) for 10.41% of the samples are unsatisfactory (presence in 25 g). While at the site 2, 4.16% of the samples were unsatisfactory.

Isolated *Salmonella spp* had common biochemical traits: ONPG -, Urea -, TDA -, Simmons Citrate +, Indole -, ADH+, ODC+ and LDC. We noted that Salmonella spp were often H2S + low after 24 hours of cultivation and more pronounced 48 hours to72 hours. They all produce glucose gases.

### 3.2 Susceptibility of *Salmonella spp* strains isolated to antibiotics

The strain resistance was tested against 10 antibiotics and the proportions of resistant isolates are shown in Table 5.

At site 1, the isolated *Salmonella spp* strain is completelysensitiveto Imipenem (IMP) and Amoxicillin/clavulanic acid (AMC). The antibiotic resistance rate of the strains isolated in this site is less than 50% except for chloramphenicol (CHL) where the resistance rate is 80%.

At site 2, where irrigation is done with open drilling, apart from Ampicillin (AMP), AMC and Ceftriaxone (CRO), the isolated strains are almost all 100% sensitive to the antibiotics tested. These strains showed 100% resistance to CRO.

All of thestudy sites, apart from chloramphenicol (CHL), the resistance rate of the Salmonella spp strain to the antibiotics tested is less than 50% while the sensitivity rate is greater than 50%. High sensitivities wereobserved with ciprofloxacin (CIP), nalidixic acid (NAL) and Amoxicillin/clavulanic acid (AMC) up to 85.71%, Amoxicillin (AMX), Cephalothin(CEF) and Trimethoprim-Sulfamethooxazole (SXT) are sensitive to 71.42%. Isolated strains of *Salmonella spp* showed total sensitivity (100%) to Imipenem (IMP).

A resistance rate of more than50% was observed with AMP and AMX. However, the isolated *Salmonella spp* strains showed a total sensitivity (100%) to CRO, IMP, NAL and Trimethoprim-Sulfamethoxazole (SXT).

Table 5: Sensitivity test for *Salmonella spp* antibiotics isolated from market garden produce

| SITE | n | ATB | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AMP | | AMX | | AMC | | CEF | | CRO | | IMP | | CHL | | SXT | | NAL | | CIP | |
| | | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) | (R +I) (%) | S (%) |
| **Site 1** | 5 | 40 | 60 | 40 | 60 | 0 | 100 | 40 | 60 | 20 | 80 | 0 | 100 | 80 | 20 | 40 | 60 | 20 | 80 | 20 | 80 |
| **Site 2** | 2 | 50 | 50 | 0 | 100 | 50 | 50 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| **Site 3** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Site 4** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **TOTAL** | 7 | 42,85 | 57, 14 | 28,57 | 71,4 2 | 14,28 | 85,71 | 28,57 | 71,42 | 42,85 | 57,14 | 0 | 100 | 57,14 | 42,85 | 28,57 | 71,42 | 14,28 | 85,71 | 14,28 | 85,71 |

ATB = antibiotic, n = number, (R +I)% = resistance rate + intermediate, S% = sensitivity rate,

AMP = ampicillin; AMX = amoxicillin; AMC = amoxicillin + clavulanic acid; CEF = cephalothin ; CRO = ceftriaxone; IMP = imipenem;

CHL = chloramphenicol; SXT = sulfamethoxazole trimethoprimes; NAL = nalidixic acid; CIP = ciprofloxacin.

### 3.3 Discussion

The results of the *Salmonella spp* research revealed a contamination rate of 4.86%, which shows that some samples of the leafy vegetables produced were of microbiological quality that did not comply with the defined standard. This detection of *Salmonella spp* is a serious health concern.

*Salmonellaspp* are present in two (2) production sites (sites 1 and 2) with a non-satisfaction rate of 10.41% and 4.16% respectively. The rate of *Salmonella spp*contamination in this study is relatively low compared to those reported in other studies [15, 16, 17 and 18]. The identification of *Salmonella spp* confirms the direct contamination of these leafy vegetables by irrigation water. This showsthat the source of water for irrigation has an influence on the microbiological quality of the vegetables produced. Indeed, on these two sites, the market gardeners use for irrigation of plots wastewater either(sewage) or drilling waters exposed to the open air before their use.The study conducted in the urban and peri-urban area of Bamako (Mali) detected salmonella on all plants from all sites studied with a level of 47 ± 4.3 to 4 ± 2.8 depending on the location [19]. In Niger, the prevalence of Salmonella found in lettuce was in descending order 56% in Niamey, 33% in Gaya, 27% in Zinder, 13% in Maradi and Agadez, 10% in Tillaberi and finally 0% in Tahoua respectively [20]. Other studies in lettuce found prevalence of 50% in Ouagadougou in Burkina Faso [21], 22% in Sokoto in Nigeria [22] and 16% in Maiduguri, Nigeria [23] respectively.The results of the antibiogram showed that *Salmonella spp* developed various resistance to beta-lactams. When Penicillins (AMP, AMX and AMC) were switched to Carbapenems (IMP), the proportion of resistant strains gradually decreased from 42.85% to 0%. This finding could be explained by the fact that Penicillins had appeared a long time ago and were much prescribed and sold without laboratory evidence. Such resistance when observed would be related to the production of ExpandedSpectrum Betalactamase (BLSE) by the strain in question [24].Results of Salmonella antibiotic sensitivities tests produced in seven (7) major cities of Niger show that the resistance most frequently encountered are resistance to the penicillin family A (Ampicillin (69.70%), amoxicillin (27.97%) ; amoxicillin + clavulanic acid (19.17%)) [20].Contrary to the emergence of Ciprofloxacin-resistant strains found in Asia by several authors [25; 26], the identified *Salmonellespp* still retains a good sensitivity to second-generation ciprofloxacin. Decreased sensitivity to chloramphenicol was found in France [27] and Dakar [28].

## 4. CONCLUSION

The presence of *Salmonella spp* in leafy vegetables grown in urban areas of N'Djamena is low, which does not prevent a risk of contamination. Thus, despite the benefits of eating these leafy vegetables, they pose a health safety problem in that most of these foods are consumed raw and therefore carry active pathogens - which are generally no eliminated in the washing, thereby increasing the health risks to the consumer. In addition to the danger posed by its presence in leafy vegetables, this pathogen carries a large number of antibiotic resistance genes. The results of the antibiotic sensitivity assessment showed resistance to β-lactams and phenicols, indicating a disturbingcirculation of drug-resistant strains. In order to protect the health of the consumer, measures must be taken at all stages of the production chain. Preventive measures must be taken and sensitivities awareness-raising on Good Hygiene Practices (BPH), Good Production Practices (BPP), Good Distribution Practices (BPD) and

Sales Practices (BPV) to the actors in the food chain to avoidthe proliferation of food-borne diseasesamong city dwellers.

## REFERENCES


1. World HealthOrganization. Food safety. Reminder. December 2015 No. 399. 5p.

2. Deering AJ, Mauer LJ, Pruitt RE. Internalization of *E. coli* O157:H7 and *Salmonella spp*. in plants. *Food Research International* 2012; 45:567–75

3. Van der Linden I, Cottyn B, Uyttendaele M, Vlaemynck G, Maes M, Heyndrickx M. Long-term survival of *Escherichia coli* O157:H7 and *Salmonella enterica* on butterhead lettuce seeds, and their subsequent survival and growth on the seedlings. *International Journal of Food Microbiology* 2013; 161:214–19

4. van Overbeek LS, van Doorn J, Wichers JH, van Amerongen A, van Roermund HJW, Willemsen PTJ. The arable ecosystem as battleground for emergence of new human pathogens. *Frontiers in Microbiology.* 2014; 5:104

5. Martínez-Vaz BM, Fink RC, Diez-Gonzalez F, Sadowsky MJ. Enteric Pathogen-Plant Interactions: Molecular Connections Leading to Colonization and Growth and Implications for Food Safety. *Microbes and Environments.* 2014; ME13139

6. Jang H, Matthews KR. Survival and interaction of *Escherichia coli* O104:H4 on Arabidopsis thaliana and lettuce (*Lactuca sativa*) in comparison to *E. coli* O157:H7: Influence of plant defence response and bacterial capsular polysaccharide. *Food Research International.*2018 ; 108:35–41

7. FAO/WHO. Food safetyriskanalysis. Guide for national foodsafetyauthorities. FAO Food and Nutrition Study Rome - Italy. 2007; 87: 120 p.

8. Koffi-Nevry R, Assi-Clair BJ, Assemand EF, Wognin AS and Koussemon M. Origin of witnesses of faecal contamination of wateringlettuce (Lactuca sativa) grown in the peri-urban area of Abidjan. Journal of Applied Biosciences. 2012; 52: 3669–75.

9. Nazal A.M., Tidjani A., Doudoua Y. et Balla A. Le maraichage en milieu urbain et périurbain : cas de la ville de N'Djaména au Tchad. *Journal of UNiversities and international development COoperation* (JUNCO). Turin, Italie. 2017; 269-81.

10. Kroupitski Y, Golberg D, Belausov E, Pinto R, Swartzberg D, David Granot D, Sela S. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Appl Environ Microbiol.* 2009; 75: 6076–86.

11. Schikora A, Virlogeux-Payant I, Bueso E, Garcia AV, Nilau T, Garcia A V, Nilau T, Charrier A, Pelletier S, Menanteau P , Velge P, Hirt H. Conservation of Salmonella Infection Mechanisms in Plants and Animals. PLoS ONE. 2011 ; 6(9) : e24112. doi:10.1371/journal.pone. 0024112.

12. Courvalin P. Resistance of bacteria to antibiotics: combination of biochemical and geneticmechanisms. Bull. Acad. Vet. 2008; 161:7-12. Available: http://academieveterinaire.free.fr/bulletin/pdf/2008/numero01/07.pdf

13. OMS. Salmonella (infections à, non typhiques). 2018. Retrieved, December11 2021. Available: https://www.who.int/fr/news-room/fact-sheets/detail/salmonella-(non-typhoidal)

14. WHO. Antimicrobialresistant salmonella. INFOSAN. 2005; 3. Retrieved, December 11 2021. Available: http://www.who.int/foodsafety/fs_management/No_03_Salmonella_Apr05_en.pdf

15. Sanda A.A., Inoussa M., Soumana O.S. and Bakasso Y. Diversity and dynamics of Salmonella isolatedfromlettuce (Lactuca sativa L.) in vegetablecrops in Niger (West Africa). Journal of Applied Biosciences. 2017; 119(1), 11917-28.

16. Somda S.N., Isidore B.O.J., Oumar T., Nestor B.I.H., Yves T., Nicolas B., and Aly S. Serotyping and antimicrobial drug resistance of Salmonella isolated from lettuce and human diarrhea samples in Burkina Faso. *African journal of infectious diseases*, 2017; *11*(2), 24-30.

17. Nazal AM, Tidjani A, Doutoum AA, Doungous DM, Amoukou I, Balla A. Analysis of health risk factors in the vegetable production chain in the city of N'Djaména-Chad. *Journal of Food Research*. 2019 ; *8(3) :* 111-21.

18. Nazal A.M. Quality assurance approach in the microbiologicalrisk control plan related to the production of vegetablesfromurban and peri-urban agriculture in the city of N'Djamena. Unique doctoral thesisfrom the Abdou MoumouniUniversity of Niamey (Niger). 2021; 177p.

19. Samaké F., Babana A H, Yaro F K, Cissé D., Traoré I., Kanté F, Koné S., Diallo A., Touré H., Touré O., Sako M. and Ag Iknane A. Relatedhealthriskconsumption of marketgardenproductsgrown in the urban and peri-urban area of Bamako. Mali Public Health. 2011 Volume 1 No. 001: 27-31

20. AlioSanda A., Inoussa M. M., SamnaSoumana O. and Bakasso Y. Diversity and dynamics of Salmonella isolatedfromlettuce (Lactuca sativa L.) in vegetablecrops in Niger (West Africa). Journal of Applied Biosciences. 2017; 119(1), 11917-28

21. Traoré O., Nyholm, O., Siitonen, A., Bonkoungou, O.J.I., Traoré, S.A., Barro, N. and Haukka, K. Prevalence and diversity of *Salmonella enterica* in water, fish and lettuce in Ouagadougou, Burkina Faso. BMC Microbiology. 2015; 15:151.

22. Bagudo A. I., Tambuwal F. M., Faleke O. O., Egwu O. O.andAliero A. A. Prevalence of salmonella serotypes in Sokoto abattoir effluents and vegetables cultivated around the abattoir Microbiology. *Research International*. 2014; 2(2), 13-17.

23. Raufu IA, Zongur L, Lawan FA, Bello HS, Adamu MS, JA Ameh J A., Ambali A G. Prevalence and antimicrobial profiles of *Salmonella serovars* from vegetables in Maiduguri, North eastern Nigeria Sokoto. *Journal of Veterinary Sciences*. 2014 ; 12 (1).

24. Uzunovic-Kamberovic S, Saric D, Sestic S. Community-acquired urinary tract infections by extended-spectrum betalactamase- producing Enterobacteriaceae in Zenica-Doboj Canton, Bosnia and Herzegovina. Med. Glasn. 2006; 3(2): 47- 52.

25. Cooke FJ, Day M, Wain J, Ward LR, Threlfall EJ. Cases of typhoid fever imported into England, Scotland and Wales (2000-2003) *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2007; 101(4):398–404.

26. Lynch MF, Blanton EM, Bulens S, Polyak C, Vojdani J, Stevenson J, Mintz ED. Typhoidfever in the United States, 1999-2006. *JAMA*. 2009 ; 302(8) : 859–65.

27. Weill FX. Salmonella: epidemiology, typing and resistance to antibiotics. Francophone Journal of Laboratories. 2008; 38(400): 37–47.

28. Lefebvre N, Gning SB, Nabeth P, Ka S, Ba-Fall K, Rique M, Debonne J. Clinical and biological aspect of typhoidfever in Senegal: study of 70 cases. Tropical Medicine. 2005; 65(6): 543–48.