ORIGINAL RESEARCH

Microbiological Characteristics and Resistance Profile of Isolated Bacteria in Market Garden Products in N'Djamena, Chad

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Abstract

The phenomenon of antimicrobial resistance is a threat for public health. The aim of this study was firstly to assess the microbiological quality of fresh vegetables and fruits produced in the market gardens of N'Djamena-Chad; and secondly to study the antibiotic resistance profile of isolated strains. In order to achieve these objectives, data were collected through field surveys and the fresh fruits and vegetables harvested. The collected samples were microbiologically characterized using standardized methods. A total number of 180 fresh vegetables and fruits were analyzed. The results obtained on the prevalence of isolated pathogens showed a high microbial diversity (*Aeromonas hydrophila, Aeromonas sobria, Escherichia coli*, *Salmonella spp., Staphylococcus aureus*) and a high microbiological contamination of vegetables analysed. Lettuce was the most contaminated sample with about 46.7% of *Escherichia coli* and 7% *Salmonella spp.* However, contamination varied according to the site and was closely related to the poor quality of irrigation water. The study determined the biochemical characteristics of each isolate. The antibiotic sensitivity test of the isolates showed that the isolated pathogenic bacteria are all resistant to the antibiotics tested. A significant difference (p = 0.02) was observed between percentages mean of penicillin resistance (67%) and cephalosporins (29%). The consequences of this contamination are health risks for consumers and producers.

Practical Applications
Awareness on good farming practices for producers of vegetable and fruits is necessary to preserve consumers' health.

Keywords: vegetables, fruit, bacterial resistance, health, pathogen.

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1. Introduction

Market gardening is an intensive vegetable and fruit crop cultivation most often practiced by vulnerable sections of urban and peri-urban areas (Yehouenou *et al.*, 2013). As estimated by the Food and Agriculture Organization of the United Nations, about 800 million people depend on urban agriculture (FAO, 2012). Chad's agriculture employs more than 80 percent of the labor force. The dryness of the climate limits crop diversity, and the primary sector relies mainly on the production of fruits and vegetables. In N'Djamena, there are several sites for vegetables production, the largest of is found along the Chari's and Logone's rivers. Part of the population engaged in this urban agriculture consists of a variety of socio-cultural groups among which rural exodus citizens, laborers, "Byke riders" and students. Market gardeners use untreated household waste, cow dung, faeces from pigs grown on the site and inappropriate pesticides especially those for cotton treatment and as well as contaminated water. All these factors do not guarantee the quality of the vegetables produced (Assogba-Komlan *et al.*, 2002). Urban proximity is likely to create specific risks for urban agricultural products (Aubry *et al.*, 2013). Thus, unhygienic conditions under which some crops are produced; especially leafy vegetables represent a danger for public health both in N'Djamena and other African cities such as Dakar and Yaoundé (Kenmogne *et al.*, 2010). Many of the diseases that affect the urban population are related in part to the use of domestic and industrial wastewater discharged in the community (Agossou *et al.*, 2014). Such discharges are hazardous for the population due to their toxic effects (Agueh *et al.*, 2015). In Chad, certain digestive infections are attributed rightly or wrongly to the consumption of market garden produces. Because of these side effects and in order to create awareness and reduce the risk for people to be contaminated or sick we found important to conduct this study with the objective to evaluate the microbiological quality and antibiotic resistance profile of isolated bacteria in market garden products from vegetable farms in the city of N'Djamena. Specifically, to determine the prevalence in some pathogenic bacteria found on crops produced in market gardens and responsible of digestive disorders ; to link the contamination level of the water used for watering and the crops; to determine the biochemical characteristics of each isolate and finally to perform a sensitivity test of antibiotics against isolated microbes. This study concerns the sanitary measures required for Good Hygienic Practice in food production that can reduce the incidence of microbiological contamination of market garden products.

2. Material and methods

2.1. Type of study, sites and period

This is a prospective and analytical study carried-out in 5 market gardening sites in N'Djamena: "Djamba Ngato Airport, Djamba Ngato Base, Sabangali, Habena-lined path and finally Habena-Kome". These are the best organized and dynamic sites on which market gardening is practiced almost permanently. The research was conducted at the Food and Nutrition Sciences Laboratory (LARSAN) from January 2017 to July 2018.

2.2. Characteristics of vegetable products and sampling

The harvest of fresh vegetables, at maturity, within the selected sites was carried out randomly. With this empirical choice it was possible to have a more diversified sample representation of market gardening products. The sample's size was estimated by taking into account the total number of crop (n = 12) and the number of samples per market garden (p = 15). A total of 180 (n x p = 12 x 15= 180) samples was collected. Fresh vegetables and fruits were harvested in the morning and evening after watering. Samples were collected in sterile forceps, placed in sterile plastic bags and kept in an insulated cooler with ice. The samples (leaves, fruits, roots) were collected in sterile bags or bottles, preserved at 4 °C and transported to the Research Laboratory of Food Science and Nutrition (LARSAN), Faculty of Human Health, University of N'Djamena-Chad for analysis. Once in the laboratory, the samples were weighed, ground and homogenized using Stomacher 400 homogenizer, which is a dual-action kneading and stirring mill for homogenizing samples in sterile pouches.

2.3. Bacteriological analyzes

This was carried out on the samples collected using the standard microbiological methods as described by AFNOR (2002). Two methods were used for the isolation and identification of pathogens: the manual method and the automated method with the Vitek 2 Compact (Biomérieux, Marcy l'étoile, France). The automated method was used each time to confirm the results of the manual method.

2.3.1. Sample preparation

Ten (10) g of leaves, roots or fruits were ground with a mortar or stomacher to prepare the stock solution. This test sample was introduced into a sterile dilution bag (plastic bag). The stock solutions were cascaded from 10^{-1} to 10^{-6} by adding 1 ml of the dilution in 9 ml of buffered peptone water. 0.1 ml of 10^{-6} dilutions was seeded in the various culture media for the detection of pathogenic microbe in vegetables.

2.3.2. Isolation and identification the pathogens

Escherichia coli

The bacteriological analysis of *E. coli* in fresh vegetables was carried out according to the methods described by ISO 16649-2 (2001). The stock solution was incubated at 37 °C for 24 and 1 ml of the enriched mixture innoculated in duplicate in petri dishes (90 mm in diameter) containing Eosine Methylene Blue agar (Liofilchem, Roseto degli Abruzzi, Italy) using Pasteur pipettes. The inoculated dishes were incubated at 37°C for 24h; suspicious colonies of *E. coli* (dark purple, curved, metallic-on-EMB) were subjected to biochemical tests.

Staphylococcus aureus and *Aeromonas spp*

Isolation of staphylococci was performed using the Chapman selective medium. Seeding of the medium was done with 0.1 ml of the solutions chosen on the surface of Petri dishes previously cast and solidified and the inoculum was spread using a curved Pasteur pipette. The dishes were incubated for 24 to 48 hours at 37 °C. At the end of this period, colonies of *Staphylococcus aureus* appeared in yellow. For

Aeromonas spp, medium M17 or polyvitex agar was used for their isolation.

Salmonella spp.

Salmonella detection was carried as described in ISO 6579 (ISO, 2002) following the steps below:

i. Pre-enrichment of the samples: The stock solution previously prepared was homogenized by stirring the sample for one minute and incubating it at 37 °C for 6 to 12 hours.

ii. Enrichment: A volume of 0.1 ml of the pre-enrichment was transferred for enrichment into 10 ml Rappaport-Vassiliadis broth (RVS broth) (Liofilchem, Italy) and incubated at 37 °C for 24 h.

iii. Isolation: The isolation was carried out from the broth after enrichment, by streaks of 1 ml broth on Hektoen agar (Bio-Rad). The dishes were therefore incubated for 24 hours at 37 °C.

iv. Identification: Green colonies with black spots at the center were characterizing Salmonella which were then selected for biochemical identification.

2.3.3. Biochemical identification

The biochemical identification and antibiogram of the bacteria were carried out with the Vitek 2 compact automaton (Biomérieux, Marcy l'étoile, France). Vitek 2 determined the minimum inhibitory concentration (MIC) of antimicrobians according to the European Antibiogram Committee (EAC). To measure the magnitude of the bacterial resistance phenotypes, three Gram- bacilli (AST N103, AST N222, AST N233) antibiogram cards and one Gram + bacilli antibacterial card (AST GP67) were used. The antibiotic tested were: penicillin, ampicillin, amoxicillin + clavulanic acid, cefalotin, ceftriaxone imipenem, nalidixic acid, ciprofloxacin, gentamicin, tobramycine, doxycycline, tetracycline, vancomycin, trimethoprim-sulfamethoxazole, fusidic acid, rifampicin, Chloramphenicol.

Strains of *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control of the Viteck 2 automated system.

2.3.4. Antibiotic resistance profile of the identified strains

The sensitivity test method used is that of agar diffusion (disk method). Antibiotic sensitivity assay was done using disk diffusion method on agar plates. Antibiotics were chosen based on their current and abusive use in human, animal and agricultural health. Twenty-four antibiotics (BioRad, Marnes-la-coquette, France) belonging to 10 different families were tested: beta-lactam, Aminoglycosides, Cyclins, Phenicolates, Sulfamides, Quinolones, Fluoroquinolones, Rifampicin, Glycopeptides and fusidic acid.

2.5. Data processing

The data were analyzed using Excel software 2013. They were treated taking into consideration the bacterial etiology, the type of vegetables and the resistance to antibiotics. The chi-square test (χ^2) was used to compare the qualitative variables with a threshold of significance set at 5%.

3. Results and Discussion

3.1. Prevalence of isolated pathogens in market garden products

Table 1: Prevalence of isolated bacteria in market garden produce

Part Analyzed	Market garden products	Number of germs per sample (%)			
		E. coli	*Aeromonas spp*	*Salmonella sp.*	*S. aureus*
Leaf	Lettuce	7 (46.7)	2 (13.3)	1 (7)	3 (20)
	Rocket	1 (7)	0 (0)	0 (0)	0 (0)
	Celery	2 (13.3)	1 (7)	0 (0)	1 (7)
	Parsley	5 (33.3)	0 (0)	0 (0)	0 (0)
	sorrel	7 (46.7)	0 (0)	1 (7)	0 (0)
	Spinach	1 (7)	0 (0)	0 (0)	0 (0)
	Cabbage	6 (40)	0 (0)	1 (7)	0 (0)
Fruit	Okra	2 (13.3)	0 (0)	0 (0)	1 (7)
	Green beans	3 (20)	0 (0)	0 (0)	3 (20)
	Pepper	2 (13.3)	0 (0)	0 (0)	2 (13.3)
Root / bulb	Carrot	3 (20)	0 (0)	0 (0)	3 (20)
	Green onion	3 (20)	0 (0)	0 (0)	5 (33.3)
	Total	42 (23.3)	3 (1.66)	3 (1.66)	18 (10)

The bacteriological analyzes of vegetable products from farms in the city of N'Djamena showed an important charge of pathogenic bacteria. The massive contamination of garden product by pathogenic microbes evidence faecal contamination. Lettuce had a high microbial load with proportions of *Escherichia coli* and *Salmonella spp.* of 46.7% and 7% respectively ($x^2 = 39.459$, ddl = 1, p = 0.001).

Table 1 shows the prevalence of pathogens isolated in vegetable products. Among the *Aeromonas* isolated, two *Aeromonas hydrophila* (13.3%) and *Aeromonas sobria* (7%) were identified. In the city of N'Djamena, vegetable crops are grown at various sites along the Chari and Logone river side and around stagnant waters of some neighborhoods commonly known as "Bouta". The inhabitants of neighborhoods that do not have restrooms defecate in the rivers and "Bouta".

On the other hand, they drop daily garbages composed of corpses (cats, dogs, chickens, mice, food remains …). The hospitals of the place, the factories release untreated wastes into the two rivers. The use of irrigation water, which is generally infected with faeces, urines (human and animal), immature compost and phytosanitary products, lead to the contamination of market garden crops (Agbossou *et al.*, 2003).

The identification of *Salmonella* confirms the direct contamination of leafy vegetables by irrigation wastewater. This detection of *Salmonella* in lettuce, cabbage, rocket and sorrel samples is a serious health concern. The prevalence of *Salmonella spp.* in this study is relatively low compared to those reported in other studies in West Africa (Sanda et *al.*, 2017, Somda *et al.*, 2017).

Table 2: Distribution of pathogenic bacteria according to the harvesting sites of market garden product

Search site	E. coli n (%)	Aeromonas spp. n (%)	Salmonella spp n (%)	S. aureus n (%)
Habena	3 (20)	0 (0)	0 (0)	2 (20)
Moursal	4 (27)	1 (0)	0 (0)	2 (20)
Sabangali	5 (33.3)	0 (0)	0 (0)	2 (20)
Djamba Ngato Aéroport	5 (33.3)	2 (0)	1 (7)	3 (20)
Djamba Ngato Base	2 (13.3)	0 (0)	0 (0)	2 (20)
Total	**19**	**3**	**1**	**11**

On the other hand, other authors have reported the presence of salmonella in food (Wahome *et al*., 2014; Gamané *et al*., 2018).

3.2. Distribution of isolated bacteria according to the sampling sites of vegetable products

High levels of faecal contamination indicator bacteria coincide with the sites where producers irrigate contaminated water. The site with the highest pathogenic bacteria content was that of Djamba Ngato Airport, where bacterial species were isolated from samples with a high level of contamination: *Escherichia coli* (33.3%) and *Staphylococcus aureus* (20%) with a significant difference $x^2 = 38.496$ (dd1 = 1, p = 0.001). This high prevalence of bacteria in this site can be explained by the fact that this location is a dump of the remains of meals, corpses of animals and all kinds of droppings.

The presence of these bacteria also meant faecal-oral contamination of water used for watering vegetable crops. Water of poor physicochemical and especially bacteriological quality can effectively promote contamination of vegetable crops (Koffi-Nevry *et al.*, 2012)**.** Under these conditions, the consumption of fresh vegetables from these crops could constitute a high risk for public health.

Table 2 shows the distribution of bacteria according to the sampling sites.

3.3. Biochemical characteristics of isolates

Escherichia coli- presented the following biochemical characteristics: Lactose + sometimes with delay, but some appear Lactose - on the isolation medium (Hektoen). These are *Escherichia coli* always having an action on ONPG, indole + and Urea -.

Aeromonas exhibited the following biochemical characteristics: oxidase +, ODC +, LDC +, gelatin + and sucrose +.

Isolated *Salmonella spp.* had common biochemical characteristics: ONPG -, Urea -, TDA -, Simmons + Citrate, Indole -, ADH +, ODC + and LDC. It was noticed that *Salmonella* was often weak H_2S^+ after 24 hours of culture and more pronounced 48 hours to 72 hours. They all produce gases in glucose. Seventy-five percent (75%) of the isolated *Staphylococcus aureus* were coagulase positive, catalase + and fermentated many sugars except inositol.

Table 3: Antibiotic sensitivity testing of antibacterial agents isolated from market garden products

	Bacterial							
ATB	*E. coli* (n = 42)		*Aeromonas spp.* (n = 3)		*Salmonella spp* (n = 3)		*Staphylococcus aureus* (n = 18)	
	n (% R)	n (% S)	n (% R)	n (% S)	n (% R)	n (% S)	n (% R)	n (% S)
PEN	-	-	-	-	-		16 (83.3)	2 (11.1)
AMP		-	-	-	1 (33.3)	2 (66.7)	-	-
AMX	35 (83.3)	7 (17)	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	-	-
AMC	21 (50)	21 (50)	0 (0)	3 (100)	1 (33.3)	2 (66.7)	-	-
OXA	-	-	-	-	-	-	12 (66.7)	6 (33.3)
CEF	10 (24)	31 (74)	-	-	0 (0)	3 (100)	5 (28)	13 (72.2)
CRO	15 (36)	27 (64.3)	-	-	0 (0)	3 (100)	7 (39)	11 (61.1)
IPM	5 (12)	37 (88.1)	-	-	0 (0)	3 (100)	1 (5.6)	17 (94.4)
GMN	8 (19)	34 (81)	-	-	-	-	9 (50)	9 (50)
TOB	7 (17)	35 (83.3)	-	-	-	-	11 (61.1)	7 (39)
DO	-	-	0 (0)	3 (100)	-	-	-	-
CHL	19 (45.2)	23 (54.8)	-	-	1 (33.3)	2 (66.7)	10 (55.6)	8 (44.4)
VCN	-	-	-	-	-	-	10 (55.6)	8 (44.4)
SXT	37 (88.1)	5 (12)	1 (33.3)	2 (66.7)	0 (0)	3 (100)	-	-
FAD	-	-	-	-	-	-	0 (0)	18 (100)
NAL	11 (26.2)	31 (74)	2 (55.7)	1 (33.3)	0 (0)	3 (100)	7 (39)	11 (61.1)
CIP	6 (14.3)	36 (85.7)	0 (0)	3 (100)	1 (33.3)	2 (66.7))	3 (17)	15 (83.3)

ATB = antibiotic, SXT = sulfamethoxazole trimethoprim, DO = doxycycline, CEF = cephalothin, AMP = ampicillin, AMX = amoxicillin, AMC = amoxicillin + clavulanic acid, CRO = ceftriaxone, IMP = imipenem, OXA = oxacillin, CIP = ciprofloxacin, FAD = fusidic acid, TET = tetracycline, TOB = tobramicyne, GMN = gentamycin, NAL = nalidixic acid, CHL = chloramphenicol, VCN = vancomycin, PEN = penicillin, (-) = ATB not required for the test, R = Resistance, S = Sensitivity

3.4. Sensitivity test of antibacterial agents isolated from vegetable products with antibiotics

Isolated strains were most resistant to beta-lactamines (penicillin, ampicillin, amoxicillin, amoxicillin + clavulanic acid, and oxacillin) with an average order of 60%. Such resistance can be related to the production of Beta-lactamases by the studied strains (Bessimbaye et al., 2015). Similar results were reported by Sanda *et al.* (2017).

The third (CRO) and fourth (CAZ) generations cephalosporins were about 85% sensitive. Carbapenems (IPM) were also sensitive at a percentage of 94%. The isolated strains developed various resistances to beta-lactamines. It was found that the proportion of resistant strains gradually decreased (Table 3). The resistance of *Staphylococcus aureus* strains and *E. coli* were thought to be related to the production of beta-lactamases inactivating antibiotics of penicillin and cephalosporin families. A significant difference ($x^2 = 33.478$,

dof = 1, p = 0.001) was observed between the mean proportions of penicillin resistance (67%) and cephalosporins (29%). All isolated *Staphylococcus aureus* had a 100% sensitivity to fusidic acid (FAD). In contrast, *Staphylococcus aureus* developed a 55.6% resistance to vancomycin (glycopeptides). Cross-resistance has been observed in most *E. coli* strains to antibiotics of the beta-lactam family. Aminoglycosides (GMN, TOB) were sensitive to the average order of 80% for all isolated strains. However other works (Ndoutamia *et al.*, 2014. Toudji *et al.*, 2017; Ranjbar et *al.*, 2018) have been done on antibiotic resistance. In contrast, all isolates were sensitive to the average order of 84% quinolone (NAL) and the average order of 87% fluoroquinolone (CIP).

4. Conclusion

The study of microbiological and hygienic characteristics of fresh vegetables revealed a considerable microbial diversity (*Aeromonas hydrophila, Aeromonas sobria, Escherichia coli*, *Salmonella spp. and Staphylococcus aureus*) and an important level of contamination especially of lettuce by *Escherichia coli*. This contamination of market gardening products is more intense in Djamba Ngato. The presence of pathogenic bacteria seems to be related to the use by market gardeners of contaminated water for crop irrigation. The use of contaminated water as an irrigation source in urban market gardening has an influence on the microbiological and hygienic quality of vegetable products. Market gardening products, especially those irrigated with contaminated water is a real risk to public health. As result, the risk to the health of the consumer is intensified when raw leaves, fruits or roots are rinsed or not properly washed. Added to this is the antibiotics resistance of bacterial pathogens isolated garden produce , clearly leading to high toxicity vegetables speculations. Indeed, the sensitivity test of bacterial agents isolated from vegetable products to antibiotics showed that all isolated strains were most resistant to Penicillins (PEN, AMP, AMX, AMC, OXA). In order to better protect consumers, it is urgent to monitor the quality of agricultural inputs used in market gardening and the hygiene of producers and market gardening environment. This involves producers' supervision and relocation of producers to safe sites and sensitization of the population on the risks.

Acknowledgement

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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