

*Full Length Research Paper*

## **Prevalence and characterization of *Salmonella* isolated from vegetables salads and ready to eat raw mixed vegetable salads in Abidjan, Côte d'Ivoire**

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Raw vegetables have been linked to many outbreaks of *Salmonella* foodborne; however there is few data on the presence of this bacteria in raw vegetables in Côte d'Ivoire. The objective of this study is to determine the prevalence, diversity and antibiotic resistance level of *Salmonella* strains in vegetables salads and ready-to-eat raw mixed vegetable salads in Abidjan. From a total of 552 samples, *Salmonella* strains were biochemically and molecularly identified by detection of the 16S rRNA gene and serotyping with specific antisera. The antibiotic resistance level was phenotypically determined by disc diffusion method and the presence of the gene encoding for resistance was determined by PCR. The prevalence of *Salmonella* spp in vegetables salads and ready-to-eat raw mixed vegetable salads was 8.54 and 2.61%, respectively. The serotypes identified were *S. typhimurium*, *S. enteritidis*, *S. selby*, *S. hadar*, *S. typhy*, *S. paratyphi* C and *S. adamstown*. It was observed that there were non-resistant (tetracycline and streptomycin) and multiresistant (nalidixic acid and ciprofloxacin) strains. Genes, such as *tetA*, *tetB*, *aaa* [3] -IV and *QnrA* were highlighted at different proportion. Vegetable's salads and ready-to-eat raw mixed vegetable salads in Abidjan contain various serotypes of *Salmonella* spp. displaying resistant to antibiotics and harboring the genes encoding for resistance. It is important to make subsequent risk control to evaluate and prevent possible food poisoning.

**Key words:** *Salmonella*, vegetables salads, Abidjan, prevalence, antibiotic resistance.

## INTRODUCTION

*Salmonella* is a gram-negative rod-shaped bacterium, part of the *Enterobacteriaceae* family whose ecological niche is the intestinal tract of animals and humans (CDC, 2015). They are mainly spread in environment from excreta (Delahoy et al., 2018). The genus *Salmonella* has two distinct species (*Salmonella enterica* and *Salmonella bongori*) and includes over 2,500 known serotypes, which are considered potential human pathogens (Bharmoria et al., 2017).

*Salmonella* spp are a common and important cause of infectious disease in humans worldwide (WHO, 2017). Those bacteria include typhoid serotypes (*S. Typhi*) causing human typhoid fever, para-typhoid (*S. paratyphi* A) and No Typhoid *Salmonella* (NTS) serotypes. The NTS have generally a wide range of vertebrate hosts and cause various clinical presentations that usually include diarrhea self-limiting (Bharmoria et al., 2017). Typhoid fever is estimated to be responsible for 26.9 million illnesses and 269,000 deaths per year worldwide whereas NTS cause about 93.8 million illnesses and 155 000 deaths per year (Bharmoria et al., 2017).

The widespread distribution of *Salmonella* in the environment, their increasing prevalence in the global food chain, and their virulence and adaptability cause enormous medical, health and economic impact worldwide. The mortality rate from *Salmonella* spp infections ranges from 1 to 30% depending on the serotype, region, stage of disease and antibiotic therapy (U.S. DAERS, 2014). Thus, according to statistics on foodborne diseases, *Salmonella* almost always ranks first for the number of cases of hospital visits, premature death and lost productivity in the US. Each year, *Salmonella* contributes to 1 million illnesses, 19,000 hospitalizations and 380 deaths in the United States (CDC, 2014). In the European Union, nearly one in three food-borne outbreaks in 2018 was caused by *Salmonella* with 91,000 cases. European Food Safety Authority (EFSA) has estimated that the overall cost of human salmonellosis could reach up to 3 billion euros per year (EFSA, 2019).

Most *Salmonella* spp infections are caused by consuming contaminated food, usually of animal origin, such as eggs, pork and poultry meat, and dairy products (WHO, 2017). However, a study by the Centers for Disease Control and Prevention (CDC) showed that different types of fresh produce are increasingly involved and can account for 46% of illnesses (Painter et al., 2013). Consistent with this, a recent source attribution study estimated that fruits and vegetables were involved in around 50% of salmonellosis (CDC, 2015).

*Salmonella* spp. was reported as an etiologic agent for

a total of 56 outbreaks in several states associated with fresh produce between 2010 and 2017 with a total of 3778 diseases, hospitalization rates experienced by 28.3% and 16 known deaths. Among this fresh produce responsible for outbreaks are tomatoes, onions, lettuce, cucumbers and vegetable salads (Carstens et al., 2019). Outbreaks of food poisoning outbreaks have also been reported worldwide. Factors influencing the increase of *Salmonella* outbreaks associated with vegetables include changes in agricultural practices and eating habits, as well as increased global trade of fresh produce (Collins, 1997).

In the Ivory Coast, as in the countries of sub-Saharan Africa, salmonella infections are frequent. In this part of Africa, typhoid fever caused by *Salmonella Typhi* is endemic and is a real public health problem because of the very inadequate hygiene (WHO, 2010). Non-typhoidal *Salmonella* has also become a major cause of blood infections, causing thousands of deaths each year, especially in young children with a 20-25% untreated death rate. Diagnosis is difficult due to the clinical picture which merges with that of other febrile conditions, and increased resistance to antibiotics is a real problem (WHO, 2010). However, *Salmonella* spp has been reported in various food matrices (Yao et al., 2017; Koffi et al., 2014; Karou et al., 2013), but little data is available on the role that can be played by vegetables salads and ready to eat raw mixed vegetables in the transmission of *Salmonella* to populations. Thus, the aim of this study is to make the microbial and molecular characterization of *Salmonella* strains isolated in vegetables salads and some ready to eat raw mixed vegetables salads sold in Abidjan (Cote d'Ivoire).

## MATERIALS AND METHODS

### Sampling of vegetables salads and ready to eat raw mixed vegetable salads

A total of 552 samples including 246 vegetable salads and 306 ready to eat raw mixed vegetable salad were taken respectively from the fields, markets and from collective catering in Abidjan. Vegetables including tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*) and onion (*Allium cepa*) were randomly collected i) on field and ii) from the different lots of same sellers in the markets. The vegetable salads which are prepared directly by the vendors at the points of sale in the traditional way, by cutting and mixing different types of raw vegetables salads (generally onions, tomatoes, cucumbers and/or lettuce) were collected directly from these saleswomen in restaurants. Two samples respectively by fields and restaurant and one sample per vendor in the market were collected. After collecting, all the samples were transported to the laboratory in a cooler with ice pack that maintained low temperature at about 4°C for analysis.

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## Detection, isolation and serotyping of *Salmonella*

The detection of *Salmonella* spp was carried out according to standard NF EN ISO 6579: 2002. From each sample of vegetable and vegetable salad previously crushed, 25 g were weighed aseptically and added to 225 ml of Peptone water (Bio-Rad, France) then homogenized. Each suspension was incubated at 37°C for 18 h for the pre-enrichment step. After incubation, 0.1 ml of this suspension was added to 10 ml of RVS broth (Bio-Rad, France); in parallel, a series of two drops was placed in the center of two Petri dishes containing a Rappaport Vassiliadis Semi-solid Medium (MSRV) supplemented with novobiocin at 20 mg/L (Lyofilichen, France). RVS and MSRV broths were incubated at 42°C for 24 h for the first and 24 to 48 h for the second. Both media were then inoculated by streaking on agar Hektoen (Bio-Rad, France) and *Salmonella*-Shigella agar (Bio-Rad, France) and incubated at 37°C for 24 h. Only the discolored RVS tubes and the discolored MSRV dishes which migrated (therefore exhibiting a growth halo which moved away from the point of deposition) were the subject of inoculation. For MSRV boxes, seeding was done from the end of the growth halo. On *Salmonella*-Shigella agars, *Salmonella* shows colorless and transparent colonies (due to lactose fermentation), with or without black center (production of H<sub>2</sub>S) and on Hektoen, blue to green colonies with or without black center. Five characteristic colonies on each box were subcultured on nutrient agar and incubated at 37°C for 24 h. Serotyping of *Salmonella* spp. was carried out according to the Kauffman-White scheme, using specific antiserum (Grimont and Weill, 2007).

## Molecular confirmation of *Salmonella* strains

The genotypic identification of *Salmonella* strains and the detection of the genetic support for antibiotic resistance were made by polymerase chain reaction (PCR) respectively according to the protocols of Smith et al. (2015) and Shahrani et al. (2014). PCR was performed according to the steps of heat shock DNA extraction, amplification and detection of amplification products. The genotypic identification of *Salmonella* strains consisted of the detection of the *16S rRNA* gene common to all strains of *Salmonella* spp. The primers used to target genes encoding for the *16S rRNA* were those designed by Smith et al. (2015).

The amplifications were performed using a thermal cycler (Techne Genius, USA) in a final reaction volume of 25 µl containing different reagents (Sigma Aldrich, St. Louis, USA). This is a solution 10x buffer, MgCl<sub>2</sub> (1.5 mM and 2.5 mM), dNTPs (200 µM), of each primer (0.8 µM F-5'TGTTGTGGTTAATAACCGCA-3' and 0.5 µM R-5'CACAAATCCATCTCTGGA-3'), Taq DNA polymerase (1.25U and 0.5U) and 1 µl of DNA.

Amplification was performed following an initial denaturation at 95°C for 3 min, followed by 30 cycles of 94°C for 45 s, 54.1°C for 45 s and 72°C for 1 min and a final step of 72°C for 5 min, then storage at 4°C.

Visualization of amplification products was made by electrophoresis in an agarose gel (Invitrogen, Carlsbad, CA, USA) at 1.5% with 0.5 mg/ml of ethidium bromide (Sigma Aldrich, Canada). Migration was performed at 100 volts for 45 min and the gels were visualized under UV light. The sizes of the amplification products were estimated by comparison with a molecular weight marker (Sigma Aldrich, Saint Louis, USA) used as a standard.

## Susceptibility to antibiotic

The phenotypic antibiotic resistance of isolated *Salmonella* spp. was determined using the disk diffusion method (Bauer et al., 1966). The interpretation was made according to the recommendations of the Antibiotic Committee of the French Society

of Microbiology (CA-SFM) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (CA-SFM/EUCAST, 2019).

Pure colonies, cultivated the day before at 37°C. on trypticase casein soya agar (BBL, Canada), were used. Thirteen discs (13) impregnated with antibiotics (Bio-Rad, Manes, France) belonging to different families of antibiotics were tested. These are Beta-lactams [ampicillin (25 µg), amoxicillin + acid clavulanic (10 µg), cefuroxime (10 µg), cefotaxime (10 µg), cefepime (30 µg), aztreonam (30 µg)], quinolones [nalidixic acid (30 µg), ciprofloxacin (5 µg)], aminoglycosides [streptomycin (10 µg), gentamicin (15 µg), tetracyclines (tetracycline 30 µg)], phenicol's [chloramphenicol (30 µg)] and sulfonamides [cotrimoxazole (30 µg)]

These antibiotic discs were conventionally arranged on the surface of the agar. Incubation was carried out for 24 h at 37°C. The inhibition diameters around the antibiotic discs were estimated and the interpretation in sensitive (S) or resistant (R) categories was performed according to the reference CASFM/EUCAST (2019) standard. The *E. coli* ATCC 25922 strain was used for the quality control of the method.

## Molecular detection of gene encoding for antibiotic resistance

For the detection of resistance genes, only strains with phenotypic resistance were taken into account. The resistance genes sought are the genes conferring resistance to ampicillin (*CITM*), tetracycline (*tetA*, *tetB*), chloramphenicol (*cat 1*, *cmlA*), quinolones (*Qnr*) and gentamicin (*aaa/3j-IV*).

The amplifications were performed using a thermal cycler (Techne Genius, USA) in a final reaction volume of 25 µl containing different reagents (Sigma Aldrich, St. Louis, USA). This is a solution 10x buffer (10 mM Tris-HCl, pH 8.3 at 25°C, 50 mM KCl), MgCl<sub>2</sub> (1.5 mM and 2.5 mM), deoxyribonucleotides (dNTPs) (200 µM), of each primer (0.8 µM of F and 0.5 µM of R) (Table 1), Taq DNA polymerase (1.25 U and 0.5 U) and extracted DNA (1 µl).

For the detection of antibiotic resistance genes, the amplification program consisted of an initial denaturation at 95°C for 8 min, followed by 32 cycles of 94°C for 60 s, 55°C for 70 s and 72°C for 2 min and a final step of 72°C for 5 min then storage at 4°C.

Visualization of amplification products was made by electrophoresis in an agarose gel (Invitrogen, Carlsbad, CA, USA) at 1.5 and 2% depending on the size of the desired gene, with 0.5 mg/ml of ethidium bromide (Sigma Aldrich, Canada). Migration was performed at 100 volts for 45 min and the gels were visualized under UV light. The sizes of the amplification products were estimated by comparison with a molecular weight marker used as a standard.

## Statistical analysis

Statistical analyzes were performed with the IBM SPSS statistical program for Windows version 20. Descriptive statistics were used to determine the percentages of sensitivities to different antibiotics. Descriptive statistics (frequency, mean) were used for quantitative variables.

## RESULTS

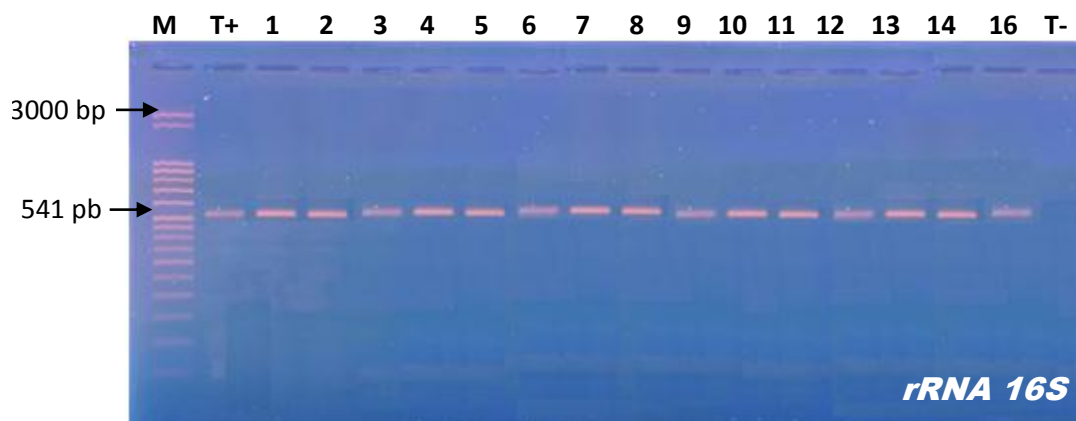
### Electrophoretic profile of amplification products of the *16S rRNA* gene of *Salmonella* spp. isolated from samples

All the isolated *Salmonella* spp during this study, were

**Table 1.** Primers used for confirmation of strains and detection of antibiotic resistance genes.

Gene	Sequence 5' to 3'	References	Size (pb)	Type of PCR	T (°C)
<i>TetA</i>	(F)-GGTTCACTCGAACGACGTCA (R)-CTGTCCGACAAGTTGCATGA	Randall et al. (2014)	577	mPCR	55
<i>Tet B</i>	(F)-CCTCAGCTTCTCAACGCGTG (R)-GCACCTTGCTGATGACTCTT		634		
<i>QnrA</i>	(F)-GGGTATGGATATTATTGATAAAG (R)-CTAATCCGGCAGCACTATTTA	Mammeri et al. (2005)	670	Spcr	55
<i>aac[3]-IV</i>	(F)-CTTCAGGATGGCAAGTTGGT (R)-TCATCTCGTTCTCCGCTCAT		286	sPCR	55
<i>CITM</i>	(F)-TGGCCAGAAGTACAGGCAAA (R)-TTTCTCCTGAACGTGGCTGGC	Van et al. (2008)	462	sPCR	55
<i>cat1</i>	(F)-AGTTGCTCAATGTACCTATAACC (R)-TTGTAATTCATTAAGCATTCTGCC		547		
<i>cmlA</i>	(F)-CCGCCACGGTGTGTTGTTATC (R)-CACCTTGCCTGCCATCATTAG		698	mPCR	55

bp: Base pairs; sPCR: Simple PCR; mPCR: Multiplex PCR; C: Concentration.



**Figure 1.** Electrophoretic profile of *16S rRNA* gene amplification products in *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads. M: Molecular marker of 50bp (Sigma Aldrich, Saint Louis, USA); T+: positive control *16 S rARN* (541pb); Lines 1 to 16: strains tested positive by the presence of *ARN 16 S* gene (541pb); T-: negative control.

confirmed to be *Salmonella* spp. by the presence of the *16S rRNA* gene. Figure 1 shows the electrophoretic profile of the *16S rRNA* gene amplification product of 541 base pairs of *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads in Abidjan.

#### Prevalence and frequency of *Salmonella* serotypes in samples

The prevalence of *Salmonella* spp. is 8.54% in vegetables salads and 2.61% in ready to eat raw mixed vegetable salads. The serotyping of these *Salmonella*

spp., revealed seven (07) serotypes, with variable frequencies. The prevalence of each of these serotypes is summarized in Table 2.

#### Antibiotic resistance level of *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads

The investigation of the susceptibility of isolated *Salmonella* spp. strains shows resistant to at least one antibiotic variable resistance level for vegetables salads isolates (81%) and ready to eat raw mixed vegetable salads' (62.5%). Resistance rates to at least one

**Table 2.** Frequencies of *Salmonella* serotypes isolated from vegetables salads and ready to eat raw mixed vegetable salads.

<i>Salmonella</i> serotypes	Frequencies of <i>Salmonella</i> serotypes (%)	
	Vegetable's salads (N=246)	Ready to eat raw mixed vegetable salads (N=306)
<i>S. enteritidis</i>	6 (2.44)	2 (0.65)
<i>S. typhimurium</i>	6 (2.44)	1 (0.33)
<i>S. hadar</i>	6 (2.44)	2 (0.65)
<i>S. selby</i>	1 (0.41)	1 (0.33)
<i>S. typhi</i>	-	1 (0.33)-
<i>S. paratyphi c</i>	-	1 (0.33)
<i>S. adamstown</i>	2 (0.81)	-
<b>Total</b>	<b>21 (8.54)</b>	<b>8 (2.61)</b>

(-): non detected.

**Table 3.** Resistance rate to at least one antibiotic of *Salmonella*.

<i>Salmonella</i> serotypes	Resistance rate to at least one antibiotic of <i>Salmonella</i> serotypes (%)	
	Vegetable's salads (N=21)	Ready to eat raw mixed vegetable salads (N= 8)
<i>S. enteritidis</i>	5 (83.3)	1 (50)
<i>S. typhimurium</i>	5 (83.3)	1 (100)
<i>S. hadar</i>	5 (83.3)	1 (50)
<i>S. selby</i>	1 (100)	1 (100)
<i>S. typhi</i>	-	1 (100)
<i>S. paratyphi c</i>	-	0
<i>S. adamstown</i>	1 (100)	-
<b>Total</b>	<b>17 (81)</b>	<b>5 (62.5)</b>

antibiotic vary from 0 to 100% depending on *Salmonella* serotypes (Table 3).

The multidrug resistance (resistance to at least three families of antibiotics) concerned respectively 4.8 and 25% of *Salmonella* spp isolated from vegetables salads and ready to eat raw mixed vegetable salads. In vegetables salads it was a strain of *S. typhimurium* (simultaneous resistance to ampicillin, streptomycin and tetracycline) and in ready to eat raw mixed vegetable salads a strain also of *S. typhimurium* (simultaneous resistance to ampicillin, gentamycin, ciprofloxacin and tetracycline) and *S. hadar* (simultaneous resistance to gentamycin, nalidixic acid and tetracycline).

The resistance levels observed in *Salmonella* serotypes from vegetables salads and ready to eat raw mixed vegetable salads varied from antibiotic to another (Table 4). Resistances by decreasing manner have concerned tetracycline (61.9% for vegetables salads strains and 62.5% for ready to eat raw mixed vegetable salads isolates), streptomycin (57.1% for vegetables salads strains and 37.5% for ready to eat raw mixed vegetable salads isolates), gentamycin (9.5% for vegetables salads strains and 25% for ready to eat raw mixed vegetable salads isolates), acid nalidixic (4.8% for vegetables salads strains and 25% for ready to eat raw mixed vegetable salads isolates), cotrimoxazole (4.8% for

vegetables salads strains and 12.5% for ready to eat raw mixed vegetable salads isolates), ampicillin (4.8% for vegetables salads strains and 12.5% for ready to eat raw mixed vegetable salads isolates), and ciprofloxacin (0% for vegetables salads strains and 12.5% for ready to eat raw mixed vegetable salads isolates). No resistance to beta-lactams and chloramphenicol has been observed.

### Antibiotic resistance genes of *Salmonella*

The *QnrA* gene 670 bp (Figure 2), conferring resistance to quinolones, *aac [3] -IV* of 286pb (Figure 2) conferring resistance to gentamycin and *tetA* 577 bp and 634 bp of *tetB* (Figure 3) conferring tetracycline resistance have been identified in *Salmonella* spp.

The *CIMT*, *cat1* and *cmlA* gene conferring resistance to ampicillin and chloramphenicol have not been detected. Tables 5 and 6 shows the frequency of antibiotic resistance genes of *Salmonella* spp in vegetables salads and ready to eat raw mixed vegetables salads.

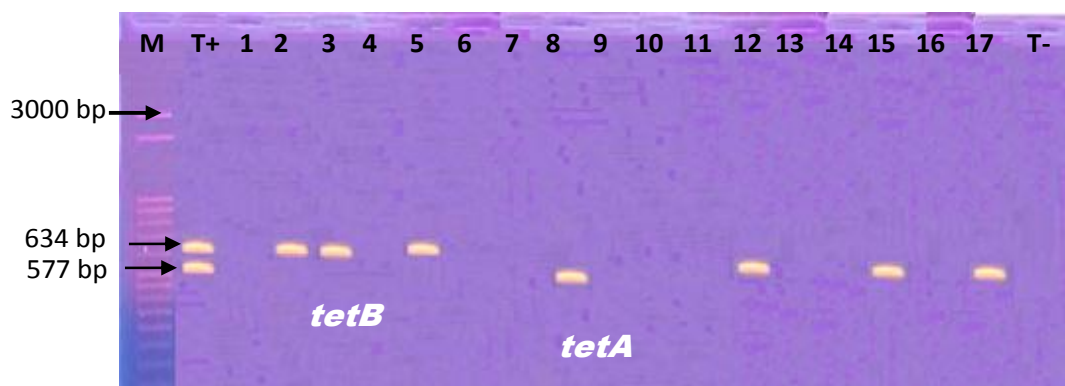
## DISCUSSION

This study highlighted the presence of *Salmonella* spp in

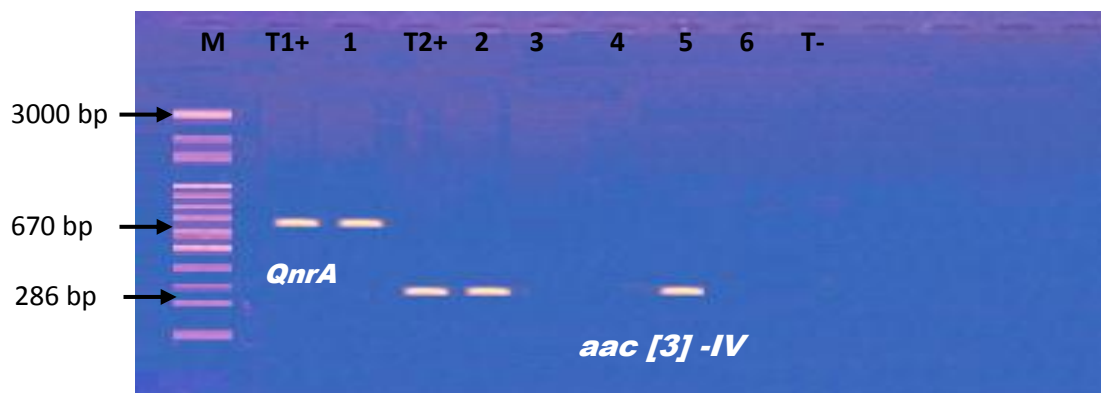
**Table 4.** Antibiotic resistance levels of *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads.

Type of food	<i>Salmonella</i> Serotypes	Antibiotic resistance levels of <i>Salmonella</i> isolated from vegetable salads and ready to eat raw mixed vegetable salads N (%)												
		AM	AMC	CFX	CTM	ATM	FEP	C	TE	GM	S	NA	CIP	SXT
Vegetables salads	<i>S. enteritidis</i>	0	0	0	0	0	0	0	4 (66.7)	1 (16,7)	3 (50)	0	0	0
	<i>S. typhimurium</i>	1(16,7)	0	0	0	0	0	0	3 (50)	0	5 (83.3)	1 (16.7)	0	0
	<i>S. hadar</i>	0	0	0	0	0	0	0	4 (66,7)	0	3 (50.0)	0	0	1 (16.7)
	<i>S. selby</i>	0	0	0	0	0	0	0	1 (100)	1(100)	1 (100)	0	0	0
	<i>S. adamstown</i>	0	0	0	0	0	0	0	1 (50)	0	0	0	0	0
	<b>Total</b>	<b>1 (4.8)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>13(61.9)</b>	<b>2 (9.5)</b>	<b>12(57.1)</b>	<b>1 (4.8)</b>	<b>1 (4.8)</b>	<b>1 (4.8)</b>
Ready to eat raw mixed vegetables salads	<i>S. enteritidis</i>	0	0	0	0	0	0	0	1(50)	0	0	0	0	0
	<i>S. typhimurium</i>	0	0	0	0	0	0	0	1 (100)	1(100)	1 (100)	1 (100)	0	0
	<i>S. hadar</i>	1 (50)	0	0	0	0	0	0	1 (50)	1 (50)	0	1 (50)	1 (50)	0
	<i>S. selby</i>	0	0	0	0	0	0	0	1(100)	0	1(100)	0	0	1(100)
	<i>S. Typhi</i>	0	0	0	0	0	0	0	1(100)	0	1(100)	0	0	0
	<i>S. paratyphi c</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Total</b>	<b>1 (12,5)</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>5 (62.5)</b>	<b>2(25.0)</b>	<b>3 (37.5)</b>	<b>2 (25.0)</b>	<b>1 (12.5)</b>	<b>1 (12.5)</b>	

AM: ampicillin; AMC: Amoxicillin + clavulanic acid; CFX: cefuroxime; ATM: aztreonam; FEP: cefepime; chloramphenicol, TE: tetracycline; GM: gentamicin; S: streptomycin; NA: Acid nalidixic; CIP: ciprofloxacin; SXT: Cotrimoxazole



**Figure 2.** Electrophoretic profile of the amplification products of tetracycline resistance gene (*tetA*, *tetB*) in *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads. M: Molecular marker of 50 bp (Sigma Aldrich, Saint Louis, USA); T+: positive control *tetA* (577 bp) and *tetB* (634 bp); Lines 2, 3 and 5: strains tested positive for the presence of *tetB* (634 pb); Lines 8; 12; 15 and 17: strains tested positive by the presence of *tetA* gene (577 pb); Lines 1, 4, 6, 7, 9, 10, 11, 13, 14 and 16: strains tested negative by the absence of *tetB* gene (634 pb) and *tetA* gene (577 pb); T-: negative control.



**Figure 3.** Electrophoretic profile of the amplification products of quinolone (*qnrA*) and to gentamicin (*aac[3]-IV*) resistance gene in *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads. M: Molecular marker of 50 bp (Sigma Aldrich, Saint Louis, USA); T1+: positive control *QnrA* (670 bp); Lines 1: strain tested positive by the presence of *QnrA* gene (670 bp); T2+: positive control *aac[3]-IV* (286 bp); Lines 2 and 5: strains tested positive by the presence *aac[3]-IV* gene (286 bp); Lines 3, 4 and 6: strains tested negative by the absence of *aac[3]-IV* gene (286 bp). T-: negative control.

**Table 5.** Antibiotic resistance genes of *Salmonella* serotypes isolated from vegetables salads in Abidjan.

Serotypes of <i>Salmonella</i>	Antibiotic resistance genes of <i>Salmonella</i> isolated from vegetables salad (%)						
	CIMT (N=1)	Aaa(3)-IV (N=2)	QnrA (N=0)	TetA (N=13)	TetB (N=13)	cat1 (N=0)	CmlIA (N=0)
<i>S. enteritidis</i>	0	1 (50)	0	1 (7.7)	0	0	0
<i>S. typhimurium</i>	0	0	0	0	1 (7.7)	0	0
<i>S. hadar</i>	0	0	0	1 (7.7)	1 (7.7)	0	0
<i>S. selby</i>	0	0	0	1 (7.7)	0	0	0
<i>S. adamstown</i>	0	0	0	0	0	0	0
<b>Total</b>	<b>0</b>	<b>1 (50)</b>	<b>0</b>	<b>3 (23,1)</b>	<b>2 (20)</b>	<b>0</b>	<b>0</b>

N: number of strains tested.

**Table 6.** Antibiotic resistance genes of *Salmonella* serotypes isolated from ready to eat raw mixed vegetables salads in Abidjan.

<i>Salmonella</i> serotypes	Antibiotic resistance genes of <i>Salmonella</i> isolated from ready to eat raw mixed vegetables salad (%)						
	CIMT (N=1)	Aaa(3)-IV (N=2)	QnrA (N=1)	TetA (N=5)	TetB (N=5)	cat1 (N=0)	CmlIA (N=0)
<i>S. enteritidis</i>	0	0	0	1 (20)	0	0	0
<i>S. typhimurium</i>	0	1 (50)	0	0	0	0	0
<i>S. hadar</i>	0	0	1 (100)	0	0	0	0
<i>S. selby</i>	0	0	0	0	0	0	0
<i>S. Typhi</i>	0	0	0	0	0	0	0
<i>S. paratyphi C</i>	0	0	0	0	1 (20)	0	0
<b>Total</b>	<b>0</b>	<b>1 (50)</b>	<b>1 (100)</b>	<b>1 (20)</b>	<b>1 (20)</b>	<b>0</b>	<b>0</b>

N: number of strains tested.

vegetables and ready to eat raw mixed vegetable salads in Abidjan. The contamination of vegetables by *Salmonella* strains is very common in gardening products. This presence could be due to agricultural practices using irrigation water and untreated animal manure (shallow artificial wells) for soil fertilization and

watering of crops in Abidjan. Contact of vegetables with these elements could therefore be at the origin of the contamination. Indeed, pathogenic strains have been isolated from manure, irrigation water and crop soils in Abidjan (Wognin, 2014). Studies on environmental sources of *Salmonella* contamination indicate that water

is an important source, especially irrigation water containing manure, wild faces or sewage effluent (Islam et al., 2005) and its quality is a product safety indicator. Also, domestic, wild and farm animals present in and near fields are carriers of *Salmonella* (Yao et al., 2017; Koffi et al., 2014; Toe, 2013). Thus, *Salmonella* strains can spread in soil, water, crops or other animals and survive there for several months; the environment can thus become a source of danger. Vegetable's contamination could also be explained by the precarious conditions of harvest, transport, marketing on the markets and preparation of salads in collection restaurants due to non-compliance with basic food safety and hygiene measures. To this must be added a lack of disinfection of vegetables before preparing ready to eat raw mixed vegetable salads (Toe et al., 2017). Thus, when considering the vegetable food chain, from farm to fork, contamination of vegetables can occur at several stages of this chain and even at the final stage of the preparation of restaurant salads (Matthews, 2013).

In accordance with our results, studies carried out around the world have revealed the presence of *Salmonella* in these food matrices (Yang et al., 2020; Abakari et al., 2018; Maysa and Abd-Elall, 2015; Abakpa et al., 2015; Raufu et al., 2014; Guchi and Ashenafi, 2010). On the other hand, in South Africa (Van Dyk, 2016), in the United States (Pagadala et al., 2015; Bohaychuk et al., 2009), and in Canada (Leang, 2013), an absence of *Salmonella* has been noted. These authors explained this absence by low exposure to contamination of vegetables. The prevalence of 2.6% of *Salmonella* spp. in vegetable salad is lower than those obtained by Yang et al. (2020) China (3.4%), Azimirad et al. (2021) in Iran (19.44%), and Abakari et al. (2018) in Ghana (73.3%). In vegetables, the prevalence of 8.4% is close to those obtained in Nigeria (6.3% and 8%) by Abakpa et al. (2015) and Raufu et al. (2014) and lower than those obtained by Guchi and Ashenafi (2010) in Ethiopia (10%). The differences observed in the different studies regarding the prevalence of *Salmonella* can be attributed to the specificity of each country and the implementation of good hygiene practices and the culture conditions, sales and vegetable preparations are not always the same. According Ogundipe et al. (2012), in developing countries where sales conditions remain precarious, the conception of food security differs considerably from that of industrialized countries. Also, according to these authors, in these countries, traditional methods, the temperature of storage and inadequate personal hygiene of the handlers that promote contamination are still observed during the marketing of fresh products (Ogundipe et al., 2012). The results of the serotyping revealed the presence of seven serotypes which are *S. enteritidis*, *S. typhimurium*, *S. hadar*, *S. selby*, *S. typhi*, *S. paratyphi C* and *S. adamstown* in vegetables and salads of vegetables. In agreement with our results, *S. enteritidis*, *S. typhimurium*, *S. hadar* and *S.*

*typhi* were also isolated from vegetables and vegetable salad in other studies. Indeed, they have been identified in Iran (*S. typhimurium*: 4.44%) per Kochakkhani et al. (2018), in Egypt (*S. typhimurium*: 3.3%) by Maysa and Abd-Elall (2015), in Nigeria (*S. typhi*: 7.7%; *S. hadar*: 4.3%; *S. typhimurium*: 4.1%; *S. paratyphi*: 2.0%) by Abakpa et al. (2015) and Raufu et al. (2014), in Mexico (*S. enteritidis* 2.81%; *S. typhi* 1.4%) by Quiroz-Santiago et al. (2009). The presence of these serotypes strictly adapted to humans and ubiquitously reflects the fact that vegetables salads can be contaminated by humans as well as by animals through their excreta during the cultivation, handling and preparation of these products. The presence of these animals through their droppings and the use of animal manure, together with human contamination, significantly contribute to the spread of these *Salmonella* serotypes. Note that *S. typhimurium* and *S. enteritidis* were generally detected in the majority. This result is not surprising given their ubiquitous character and their predominance in vegetables and vegetable salads in several other studies (Abakpa et al., 2015; Raufu et al., 2014; Quiroz-Santiago et al., 2009). Also, these two serotypes are most involved in collective food poisoning in the world (Muvhali et al., 2018). This result thus highlights the risk of collective food poisoning incurred by the populations in Abidjan.

Resistances to various classes of antibiotics have been observed. These include tetracycline, streptomycin, cotrimoxazole, ampicillin, gentamicin, ciprofloxacin and nalidixic acid. These resistances could be due to use of antibiotics for the treatment of animals whose droppings (usually from chickens) through the litter is used without prior treatment as manure for the fertilization of cultivated soils in Abidjan and also the water used for irrigation. Strains resistant to various classes of antibiotics have already been observed in chicken droppings, manure, irrigation water and crop soil in Abidjan (Wognin, 2014). These resistances can also potentially be resulting from the contaminations of food chain by human. In accordance with our results, more or less significant resistances to these different classes of antibiotics has been observed in vegetables salads and ready to eat raw mixed vegetable salad around the world (Adzitey, 2018; Kemajou et al., 2017). The authors justified the presence of these resistant strains by agricultural practices and the lack of hygiene during the preparation of salads as well as an absence or insufficient decontamination.

Resistance was particularly high against tetracycline. The high resistance levels for tetracycline are explained by its extensive use in livestock farms in Abidjan due to its affordable cost, its broad spectrum and the ease of obtaining the product and also because it is in addition the component of several veterinary products sold in the city (Toe, 2013). A study by Toe (2013) showed that tetracycline is the antibiotic most used in chicken farms in Abidjan. It also showed a predominance of resistance to tetracycline in strains isolated from chicken droppings;



which droppings through the litter is used as manure for soil fertilization of crops vegetable growers in Abidjan. The absence of chloramphenicol resistance is explained by the fact that it no longer or rarely used in farms in Abidjan because of its ban because of serious risks to human health.

The resistant nature of *Salmonella* isolates to ciprofloxacin was highlighted in ready to eat raw mixed vegetable salads. This presence may be due to the use of ciprofloxacin in both human medicine and veterinary medicine in Abidjan (Ouattara et al., 2013). Resistance to ciprofloxacin may affect the treatment of certain infections, particularly typhoid fever. Indeed, ciprofloxacin is one of the latest alternatives for the treatment of *Salmonella* causing typhoid fever (Chattaway et al., 2016). In agreement with our results, no resistance to ciprofloxacin was observed in *Salmonella* isolates in vegetables in Japan (Nawas et al., 2012) and Nigeria (Kemajou et al., 2017). In Nigeria, these authors explain this absence by the controlled use of ciprofloxacin in human medicine as in animal medicine. Indeed, for these authors, the absence of resistance to ciprofloxacin is due to the reduction in its prescription by doctors and its high cost in Nigeria. These factors have been limited to the supply and misuse of ciprofloxacin, reducing the emergence of the resistance in most bacterial isolates to the antibiotic.

Resistance to tetracycline, quinolones and gentamicin has been associated with the presence of the *tetA* and *tetB*, *Qnr* and *aaa [3] -IV* genes, respectively, according to other studies performed in vegetables and raw vegetable salads (Sobur et al., 2019; Shakerian et al., 2016). This result is not surprising as more and more resistance genes to antibiotics are found in strains isolated from vegetables (Yang et al., 2020; Zahras et al., 2019; Shakerian et al., 2016). These genes could be acquired both through exchanges with other enteric bacteria and through the growing environment of vegetables, including manure, soil and irrigation water.

In Mexico, the results of a study by Lugo-Melchor et al. (2010) show the presence of the *tetA* gene among the tetracycline resistant *S. typhimurium* strains isolated from irrigation water used for culture. Indeed, the *tetA* and *tetB* genes are generally found and maintained in soil and water for a long time and diffuse rapidly due to their localization on plasmids, transposons and integrons (Börjesson et al., 2010; Sengeløv et al., 2010; Sengeløv et al., 2003). The presence of resistance carried by genes in vegetables and raw vegetable salads poses a risk to the consumer. Indeed, once ingested, the *Salmonella* carrying the antibiotic resistance genes can transmit these genes by vertical transfer (is transmitted within the same species) or horizontal (is transmitted from one bacterial species to another) on the bacteria commensal flora or other pathogenic or opportunistic bacteria and in case of infection, the use of these antibiotics will no longer be effective for the treatment of the infection

(Founou et al., 2016).

## Conclusion

This study shows that vegetables salads and ready to eat raw mixed vegetable salad are not clean as regards the presence of *Salmonella* species. Thus, serotypes including *S. enteritidis*, *S. typhimurium*, *S. hadar*, *S. selby*, *S. adamstown*, *S. typhi* and *S. paratyphi C* are isolated and resistant to various classes of antibiotics. Such pathogens can be implicated in food poisoning among consumers. Thus, vegetables and vegetable salads consumed in Abidjan could then be vectors of transmission of these *Salmonella* serotypes and this situation could negatively affect their state of health. In developing countries where food safety remains a problem, it therefore seems more than important to minimize contamination at each level of the vegetable food chain through the application of good cultivation, sales and handling practices of these foods; added to that is an effective decontamination of vegetables with disinfectants before any consumption in raw form.

This situation could negatively affect their health. It then seems more than important to minimize contamination at each level through the application of good hygiene, culture, handling measures of these foods and effective decontamination.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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