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Full Length Research Paper

In-vitro antibacterial activity of *Pterolobium stellatum* leaves extract against selected standard bacteria

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Traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines. The experimental study was conducted between February and May, 2016 at University of Gondar on antibacterial effect of leaf extract of *Pterolobium stellatum*. The purpose of the present study was to test the antimicrobial effect of *P. stellatum* extracted leaves against some standard pathogenic bacteria. The collected plant leave sample was extracted with the solvent ethanol, methanol, chloroform and distilled water. Finally, the antibacterial effect of the extract was tested with some bacteria species (*Escherichia coli*, *Pseudomonas* species, *Salmonella* species, *Shigella* species, *Staphylococcus aureus* and *Streptococcus pyogenes*) then the inhibition zone; the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. The extract of ethanol and methanol solvents showed high anti-bacterial activity on both Gram negative and Gram positive bacteria. The higher and statistically significant ($P<0.05$) inhibition was seen in ethanol extract for all bacteria and the highest inhibition was shown against *Shigella* spp. (21.33±1.52) whilst the lower inhibition was statistically significant ($P<0.05$) with chloroform extract. Both the MIC and MBC of the test extract were effective at the lowest concentration.

Key words: Antibacterial, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), *Pterolobium stellatum,* sensitivity test.

INTRODUCTION

Pathogenic microorganism borne illnesses are a continuous threat to public health (Yashphe et al., 2006; Araujo et al., 2015). Bacterial species present the genetic ability to acquire and transmit resistance against currently available anti-bacterial since there are frequent reports on the isolation of bacteria that are known to be sensitive to routinely used drugs and became multi-resistant to other medications available on the market (Chandra, 2013; Kouadio et al., 2020). Antimicrobial resistance is a challenge of microorganism against an antimicrobial drug

efficacy that was originally effective for treatment of infections caused by it (Adesokan et al., 2007; Yhiler et al., 2019). Interest in the use of natural plant-derived products versus chemicals as antimicrobials is increasing significantly (Suppakul et al., 2003; Santos et al., 2016). Further, various bacteria have developed resistance to certain antibiotics, and thus, other forms of bactericidal agents are required (Oussalah et al., 2006; Kadaikunnan et al., 2015).

Since the time immemorial medicinal plant as whole or their parts are being used in treating all types' of diseases (Jamuna et al., 2011; Marathe et al., 2013). Medicinal plants are used as natural resources for the treatment of various diseases since a long time ago (Mitiku et al., 2014; Elizabeth et al., 2015) and have been the main source for new drug development (Kumara et al., 2009; Ayandele *et al.,* 2018). The data of plant usage for treatment in many forms provide a major focus in global health care, as well as contributing substantially to the drug development process (Maikai et al., 2009; Mitiku et al., 2014). Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments as they contain anti-microbial properties (Miyakis et al., 2011; Araujo et al., 2015).

It is believed that plants which are rich in a wide variety of secondary metabolites, belonging to chemical classes such as tannins, terpenoids, alkaloids, and polyphenols are generally superior in their anti-microbial activities (Pandian et al., 2006; Hemalatha and Dhasarathan, 2010; Marathe et al., 2013). Therefore, the strength of biological activities of a natural product is dependent on the diversity and quantity of its antimicrobial constituents (Cos et al., 2006; Liu, 2006; Elizabeth et al., 2015). Furthermore, natural products, either pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Chakraborty, 2009; Meenakumari et al., 2011; Idris and Abubakar, 2016). This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Alikhan et al., 2012; Alfatah et al., 2013; Mimura et al., 2020).

Pterolobium stellatum is a tall, scrambling or climbing shrub with woody rope-like stems. Young plants are densely covered with hairs on stem and leaves. The stem has hooking prickles in pairs at the nodes and scattered ones between the nodes. Leaves are compound with 7 to 15 pairs of leaflets. The leaf axis is armed on the lower side with paired reflexed prickles. The flowers are small and sweetly scented with a pale yellowish-white color. The seed pods are broadly winged with a red to scarlet color when young, becoming brown with age (Afolayan and Aliero, 2006). Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agents, a systematic investigation was undertaken to screen the local flora for their antimicrobial activities. Then, this study has investigated *in-vitro* antibacterial activity of *P. stellatum* leaves against some bacterial species.

MATERIALS AND METHODS

Study site description

Gondar, the historical town in the country, is located to the Northern and about 747 km far from Addis Ababa, Capital of Ethiopia. Geographically, Gondar is bounded by 12°35' 07'' North latitude and 37°26' 08'' East longitude and its altitude varies in between 2000 and 2200 m above sea level. Gondar has a humid subtropical mild summer climate that is mild with dry winters, mild rainy summers and moderate seasonality. This climate is usually found in the highlands of some tropical countries. According to the Hold-ridge life zones system of bioclimatic classification Gondar is situated in or near the subtropical dry forest biome. The annual average temperature is 19.1°C. Average monthly temperatures vary by 4°C (7.2°F) (Gondar Agriculture and Rural Development Office).

Plant leaves collection and identification

The medicinal plant *P. stellatum* was selected for this study on the base of data obtained from local people and literatures because this plant is traditionally used for wound treatment. Fresh and healthy leaves of *P. stellatum* were collected from University of Gondar at Atse Tewodros and Maraki campus garden on the winter season. The voucher specimens were identified by Mr. Abiyu Eniyew at University of Gondar, herbarium of botany laboratory and the voucher specimen PLS No. 0113/17 was deposited. Figure 2 shows the plant collected from University of Gondar garden.

Preparation of crude extract of plant materials

All the necessary chemicals, media and equipment for this study were obtained from Microbiology Laboratory of Department of Biology, University of Gondar. The leaf of the plant was washed with running tap water and finally with sterile distilled water. Then, it was dried in an open air, protected from direct exposure to sunlight, to prevent degradation of active ingredients (Girish and Satish, 2008). The plant material was ground using grinding machine (Kika-werke-GM.BH, Germany) and passed through mesh sieve to obtain a fine powder. From the sieved powder sample 50 g of the extract was mixed with 500 ml, that is, 1:10 ratio, of extracting solvent (Chloroform, Ethanol, Methanol, and Water) then shake with mild shaking for 24 h on a shaker. The extract was filtered using filter paper (Whatman Paper No. 1) and the solvent were evaporated on the rotary evaporator under reduced pressure at 78, 61, 65 and 93°C, respectively. The extracts were dried at room temperature (Farrukh et al., 2010).

Standard antibiotics

Gentamicin obtained from University of Gondar teaching hospital pharmacy was used as positive control and distilled water, chloroform, ethanol and methanol solvents were used as negative controls for the anti-bacterial susceptibility test. Since all of the negative controls had 0.00 inhibition zone, all presented as negative control.

Test bacterial strains

Standard *Staphylococcus aureus, Escherichia coli, Shigella* species, *Pseudomonas* species, *Salmonella* species, and *Streptococcus pyogenes* species were obtained from University of Gondar teaching referral hospital. These bacterial strains are isolated and laboratorically identified culture collection for test and researches in the institution. Since all test strains are clinically isolated and identified; they have no identifying codes, that is, ATTCC strains.

Preparation of McFarland and turbidity standard for inoculation

Standardization of the density of isolated inoculums for susceptibility test was done by the methods described in Erturk (2006). In order to determine the active place of test organisms, each isolates was grown in 5 ml of Muller-Hinton broth (MHB) in separate test tube for each bacterial strain for 24 h in incubator. Samples from exponential phase were taken to adjust the inoculums density with 0.5 McFarland and Turbidity prepared by adding a 0.5 ml of $BaCl_2$ solution into 99 to 95 ml of H_2SO_4 (Erturk, 2006). The turbidity of the inoculums was adjusted.

Preparation of culture media

Muller Hinton agar (MHA) media was used for sensitivity. The media was prepared and treated according to manufactures guidelines. 38 g of MHA was mixed with 1 L of distilled water and settled in hot plate then autoclaved separately enclosed under 15 psi pressures at 121°C for 15 min. The medium was later dispensed into 70 mm sterile agar plates and left to set. The agar plates were incubated for 24 h at 37°C confirming their sterility when no growth occurred after 24 h the plates were considered sterile.

Agar well diffusion

Bacterial strains were tested in MHA media by making (6 mm) well in the media using a steric-borer. Inoculums from exponential growth of each bacteria isolates were mixed using vortex. The turbidity of the reconstituted organisms was adjusted to 0.5 McFarland standards. Both the standard and bacterial suspensions were agitated on vortex mixer medially prior to use. From these suspensions a volume of 100 µl bacteria were inoculated by using micro-pipette. After inoculating the bacterial isolates, the plates were allowed to dry for 5 min after which the crude extracts and the controls were dispensed into each well. The plates were incubated at 37°C for 24 h. The inhibition zone sizes were measured in millimeters compared to standard Gentamicin (Theuretzbacher, 2011).

Minimum inhibitory concentration (MIC)

The MIC of the extract was determined by MBH dilution technique (Zied et al., 2011). First the leaves of the crude extracts were prepared in different concentrations (6.25, 12.5, 25 and 50 mg/100 mL). Broth containing test tubes was tightly closed, arranged in test tube rank and autoclaved under 15 psi pressures at 121oC for 15 min. The broths were allowed to cool until the temperature is equitable to room temperature. The extracts with different concentrations (100 mL) and the test bacteria [1×10^{-8} CUF/ml] was aseptically introduced. The inhibition of growth was observed after 24 h incubation at 37°C. The presence of growth was evaluated by comparing the negative control, positive control and culture containing test tubes. The lowest concentration of compound that showed antimicrobial activity against test organisms was recorded as MIC value (Igoji et al., 2005).

Minimum bactericidal concentration (MBC)

Broth containing test tubes that did not show any bacterial growth at MIC was used to determine MBC. Small volumes of these broths are streaked onto the surface of MHA medium by sterile wire loop. The medium was incubated at 37°C for 24 h. The least concentration of plant extracts that effectively inhibit bacterial growth on the agar plate was recorded as MBC of the extracts (Igoji et al., 2005).

Data analysis

The data collection instrument was experimental through basic laboratory technique. Data like susceptibility was analyzed using SPSS software package version 20.00. Microsoft Excel was employed for analysis of MIC and MBC.

RESULTS

Antibacterial sensitivity test

Antimicrobial activity of *P. stellatum* leaves extract was evaluated based on the diameter of clear inhibition zone in millimeters. If there is no inhibition zone, it is assumed that there is no anti-microbial activity. Table 1 shows the diameter of zone of inhibition of bacterial growth at varying concentration of the leaf extract in chloroform, distilled water, ethanol figure and methanol after 24 h incubation. The extract of ethanol and methanol solvents shows high anti-microbial activity on both Gram negative and Gram positive bacteria. Higher inhibition was seen in ethanol extract for all bacteria: *S. pyogenes* (20.67±0.57), *E. coli* (18.67±1.15), *S. aureus (*19.67±0.57), *Pseudomonas* spp*.* (19.33±1.15), *Salmonella* spp*.* (20.33±0.57) and the highest inhibition was seen against *Shigella* spp. (21.33±1.52) and the lower inhibition was with chloroform extract (8.67±1.15), (8.33±1.52), (8±2), (8.67±1.3), respectively and with *Shigella* spp. (6.33±0.57) except *Salmonell*a (9.67±0.57) which was in distilled water (Figure 1).

Minimum inhibitory concentration (MIC in mg/µl)

MIC of all crude extract against the test organisms were performed using broth dilution method. All the test organisms were inhibited by all extract solvents with the range of 6.25 to 50% and the obtained result of MIC ranged from 6.25 to 12.5%. Chloroform extract result showed MIC of 6.25 against *E. coli*, *Pseudomonas* spp*.* and *Salmonella* spp*.* and 12.5 was for the rest tested organisms. The result of chloroform and other solvents are shown in Table 2.

Minimum bactericidal concentration (MBC in mg/µl)

The MBC values were performed using agar well diffusion

Table 1. Antimicrobial sensitivity test (inhibition zone is measured in millimeters).

Solvent	Dose/100 µl	Gram positive bacteria		Gram negative bacteria			
		S. pyogenes	*S. aureus*	*Pseudomonas* spp.	*Shigella* spp.	*Salmonella* spp.	*E. coli*
Chloroform	5 mg	6±1	4±1	6±1	4.33±1.52	6±1	4.6±0.57
	25 mg	7.33±1.52	5.33±1.15	7±1.73	5±1	7±2.51	5±1
	50 mg	8.67±1.55	8±2	8.67±1.3	6.33±0.57	10.33±1.52	8.33±1.52
Ethanol	5 mg	11.67±1.18	11.33±1.30	11±1.73	10.33±0.57	9.67±0.57	10±2
	25 mg	17.33±0.57	15.67±1.15	16±1	15.67±1.08	15.33±0.57	14.67±1.53
	50 mg	20.67±0.57	19.67±0.57	19.33±1.15	21.33±1.52	20.33±0.57	18.67±1.15
Methanol	5 mg	7.33±0.57	7.67±1.52	4.33±0.57	7±1	7.67±1.52	8±1
	25 mg	12.671.52	9.67±0.57	13±1	9.67±1.15	9.67±1.08	11.67±1.15
	50 mg	19±1	12.33±1.52	16.67±1.52	12±1	13±0.40	11.67±1.52
Water	5 mg	5.67±1.52	4±1	4±1	6.67±1.52	7.33±0.57	6±1
	25 mg	8±1	8±1	7.33±0.57	8.66±0.57	8±1	8±1
	50 mg	11±1	10.33±1.52	12.33±0.57	11±1	9.67±0.57	10±2
Positive	P1-P6	12.5±1.23	12.07±1.35	12.1±1.25	12.7±1.9	11.47±1.76	13.37±1.77
Negative	N1-N6	0.0	0.0	0.0	0.0	0.0	0.0

Sensitivity test

Sensitivity test

Sensitivity test

MIC test
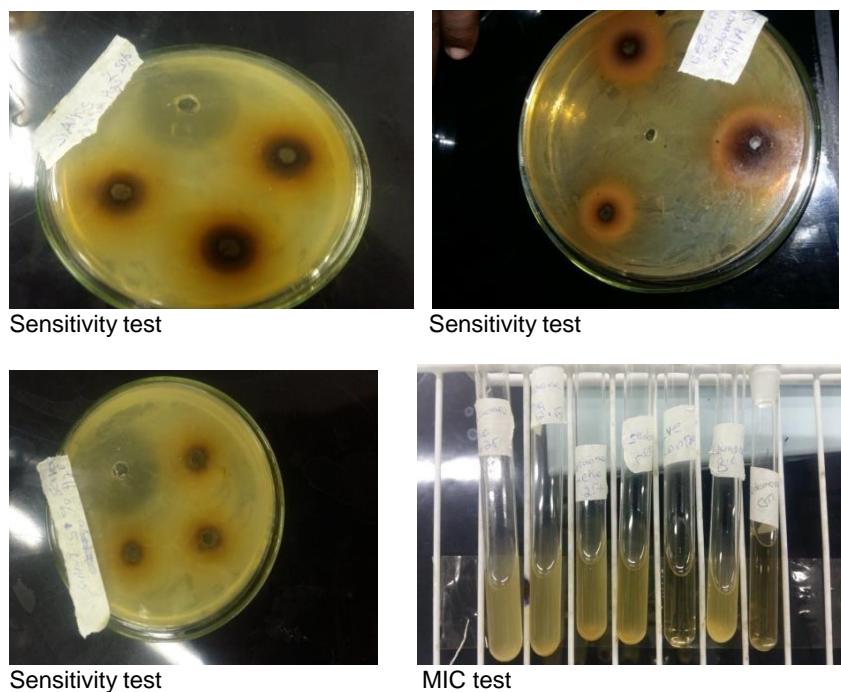
Figure 1. Representative figures for the work.

method from the MIC for all test organisms. The range was obtained from 6.25 to 12.5%. MBC of *S. aureus* for all extracted solvents was 12.5 but the other testes organisms were with different value (Table 3).

DISCUSSION

The bacterial resistances against multiple antibiotic are of great concerns to both veterinary and human medicine

Figure 2. *Pterolobium stellatum*.
Source: University of Gondar Garden.

Table 2. MIC value of *P. stellatum* leaf extract.

Extract	*E. coli*	*S. aureus*	*Shiegella* spp.	*Psudomonas* spp.	*Salmonella* spp.	*S. pyogenes*
Methanol	6.25	12.5	6.25	6.25	6.25	12.5
Ethanol	12.5	12.5	6.25	12.5	12.5	6.25
Chloroform	6.25	12.5	12.5	6.25	6.25	12.5
Water	12.5	6.25	6.25	6.25	12.5	12.5

Table 3. MBC value of *P. stellatum* leaf extract.

Extract	*E. coli*	*S. aureus*	*Sheigella*	*Pseudomonas*	*Salmonella*	*S. pyogenes*
Methanol	12.5	12.5	6.25	6.25	6.25	12.5
Ethanol	12.5	12.5	12.5	12.5	12.5	6.25
Chloroform	6.25	12.5	12.5	12.5	6.25	12.5
Water	12.5	12.5	12.5	6.25	12.5	12.5

worldwide and have been posing serious problems in the treatment of infectious diseases (Madubuike et al., 2018). Antibiotics which are widely used for the treatment of infectious diseases are under constant threat due to the emergence of antibiotic resistant pathogens (Marathe et al., 2013; El-Banna and Qaddoumi, 2016). Many studies were conducted and information is available on *P. stellatum*. In East Africa, fresh leaves of *P. stellatum* were 'chewed or a decoction is drunk to treat tuberculosis and related respiratory diseases (Pandian et al., 2006) used for weight loss or athletic performance enhancement (Girish and Satish, 2008). In Kenya, a root decoction is used by the Maasai against stomach-ache. Juice of the roots is swallowed to treat snake-bites. In Malawi, a root infusion is drunk by women against infertility (Hassan et al., 2007). Tea from the leaves is used to treat fever;

packets of leaves are burned under the bed of colic sufferers (Salatino et al., 2007).

Agar diffusion methods are the highly recommended method in antibacterial testing (El-Banna and Qaddoumi, 2016). In this study, anti-microbial activity of *P. stellatum* was conducted; this antimicrobial activity was recorded and analyzed based on the inhibition zone. The current study clearly indicated that chloroform, distilled water, ethanol, and methanol extract of *P. stellatum* could be able to inhibit the tested bacteria. This result was in line with the previous report by Dhiman et al. (2011). The plant extract shows antibacterial activity against the test organisms due to the plant active ingredients that inhibit bacterial growth (Meenakumari et al., 2011). However, the degree of their inhibition pattern is different; this may be because of the difference in bacterial strain and the

kind of solvent used (Zied et al., 2011; Alikhan et al., 2012). The variation in effectiveness of the extracts' concentration against the isolates under study may be attributed to its phytochemical composition couple with better membrane permeability gradient of the bacterial organisms for chemicals and their possible metabolism (Madubuike et al., 2018).

Antibacterial activity difference between extracts may be attributed to the fact that different compounds from the plant material get extracted in solvents of different polarities (Marathe et al., 2013; Elizabeth et al., 2015). In our study, ethanol extracts of this plant showed highest inhibition zones compared to the positive control (Gentamicin) while the negative control had no antimicrobial activity. The result was in accordance with previous report by Calderon et al. (2012), in which they are widely used to obtain crude extracts of phytochemicals from plant materials in the herbal medicine industry for therapeutic applications. Chloroform extract has shown the lowest inhibition zone against all bacteria's except *Salmonella* spp*.* which was given the lowest inhibition zone with distilled water. All the plant extracts showed minimum inhibition zones at the concentration of 5 mg/ml against all bacteria in agar well diffusion method and the highest was 50 mg/ml. This result was in accordance with the previous results by Biruhalem et al. (2011).

The antimicrobial activity of the crude extract may be attributed to a specific compound or a combination of compounds (Marathe et al., 2013; Elizabeth et al., 2015). These bio-actives can be alkaloids, flavonoids, coumarins, saponins and steroids compounds of plant origin known to have antibacterial activity (Marathe et al., 2013). The obtained data showed that all the solvent plant extracts were active against all the test organisms such as *Staphylococcus aureus, Shigella* spp*., Salmonella* spp*., Pseudomonas* spp*., S. pyogenes* and *E. coli.* The ethanol extract against all test organism (Gram-positive and Gram-negative bacteria) showed maximum inhibition zone than the other. This is due to the antibiotic active compounds of the plant leave extracted by ethanol is highly effective on all tested organisms (Dhiman et al., 2011). The antimicrobial activities analysis of the crude extract revealed the presence of some of phytochemicals active coumpounds including: flavonoids, steroids, triterpenes, tannins, saponins and alkaloids (Madubuike et al., 2018).

Most of the Gram positive bacteria are highly sensitive than Gram negative (Selvamohan et al., 2012; Michael et al., 2013). It is an established fact that the Gram-positive and negative bacteria react differently to antibacterial agents due to the differences in their cell wall component (peptidoglycan) and the ability of these agents to penetrate them (Idris and Abubakar, 2016; Elizabeth et al., 2015; Ko and Stone, 2020). The other possible explanation is that the presence of some bioactive compounds in the extract might be responsible for the

extracts higher effect against Gram-positive than Gram-negative bacterium (Lima et al., 2006 as cited in Santos et al., 2016; Ko and Stone, 2020). But in our results both of them were sensitive; this may be due to the fact that plant extract is active against both Gram negative and positive bacteria.

There is a difference when compared with positive control in 5 mg concentration of chloroform extract. *E. coli* inhibition zone has a significant bacterial difference when compared with other bacteria of 25 mg concentration of chloroform extract. *E. coli* and *S. pyogenes* with 50 mg concentration have a difference when compared with others in chloroform extract. Ethanol with 5 mg concentration has a significant dose difference within the group. *E. coli* has a difference in the positive control in all extract of distilled water.

Determining the appropriate concentration required to inhibit and kill the organism and determination of MIC and MBC is crucial in antibacterial experiment, respectively (Idris and Abubakar, 2016). In this study, the MIC results range from 6.25 to 12.5 mg/µl. Methanol and chloroform in concentration of 6.25 mg/µl inhibit *E. coli* and the other strains with 12.5 mg/µl. Methanol, ethanol and chloroform inhibited *S. aureus* with 12.5 mg/µl, whereas distilled water with 6.25 mg/µl. *Shigella* was inhibited with concentration of 6.25 mg/µl for all extracts except with chloroform (12.5 mg/µl). The MBC results of all extracts against the tested organisms showed similar range with MIC. *S. aureus* of all solvent extract has the range of 12.5% but different in others. *Pseudomonas* spp. has a significant extract difference with 25 mg of all solvent extract except in distilled water. This result was agreed with the results stated by Surjeet et al. (2011).

Active components in plants may provide potential sources of new drugs for the safe and effective treatment of microbial diseases (de Oliveira Santos et al., 2016). Our investigation clearly indicates that leaves of *P. stellatum* contain a great potential of anti-microbial component which has contributed a great role in pharmaceutical industries and healing various disease. The high potency observed in our study is therefore a rapid response call for further analysis of this plant using higher molecular techniques to ascertain its safety in the management of human and animal diseases (Madubuike et al., 2018).

Conclusion

The present work demonstrated that *P. stellatum* leaves extract have the antimicrobial potential on all tested bacteria: *S. aureus, Shigella* spp*., Salmonella* spp*., Pseudomonas* spp*., S. pyogenes* and *E. coli* with various solvents: methanol, ethanol, chloroform and distilled water between 5, 25 and 50 mg dose difference. Ethanol and methanol extracts showed high antimicrobial activities than chloroform and distilled water. Ethanol extract has

high anti-bacterial effect than others extract and chloroform has less effect. Both the MIC and MBC of the test extract were effective with the lowest concentration. Further studies like isolation and analyzing the specific antibacterial principle, effectiveness of other parts of the plant, the toxicity and isolation of the bioactive compounds are needed to better evaluate the antibacterial potential of the plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Prevalence and susceptibility to antibiotics of *Neisseria gonorrhoeae* strains isolated from genital samples in Bangui, Central African Republic

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The frequency and antibiotic resistance of *N. gonorrhoeae* are increasing worldwide. This study aimed to determine the prevalence of *N. gonorrhoeae* in genital swabs. A collaborative cross-sectional study was conducted by a team of Microbiologist, Dermato-Venerologist and Occupational Physician in Bangui, from January 2015 to December 2018. Genital samples were stained by the Gram method, grown on blood agar and the colonies identified according to the biochemical characteristics. Antibiotic sensitivity was determined by the agar diffusion method from disks. The prevalence of *N. gonorrhoeae* was 3.2%, more in males (90.5%), unschooled patients (61.1%), those with a history of STIs (56.8%), and female sex workers (p = 0.006) The strains of *N. gonorrhoeae* isolated were highly resistant to all antibiotics commonly prescribed in proportions from 60.9 to 95.8%. No resistance was observed to Ceftriaxone and Spectinomycin. This study highlights the interest of intensifying the prevention of *N. gonorrhoeae* infection in female sex workers, of the revision of the protocols of management of the *N. gonorrhoeae* infection and of a policy to ensure a better availability of new antibiotics selected. Understanding the mechanisms of resistance will be the subject of a characterization study in molecular biology.

Key words: *N. gonorrhoeae*, STI, prevalence, antibiotic resistance, female sex workers, Central African Republic, Africa.

INTRODUCTION

According to the World Health Organization (WHO), 30.6 million people were infected with *N. gonorrhoeae* in 2016. This number corresponds to a global prevalence of 0.9 and 0.7% respectively in women and men (Kirkcaldy et al., 2019; Rowley et al., 2019). Women in the WHO Africa region were the most affected (1.6%) while the combined prevalence among men and women aged 15-24 was 4.6% in South Africa and 8.2% in East Africa (Kirkcaldy et al., 2019; Torrone et al., 2018). The global incidence in 2016 was 20 per 1,000 women and 26 per 1,000 men, corresponding to 86.9 million new cases. The WHO African Region had the highest incidence (Kirkcaldy et al., 2019; Rowley et al., 2019). The trend is generally increasing in isolation of *N. gonorrhoeae*, which varies from one region to another depending on the diagnostic facilities (Kirby Institute, 2018; Choudhri, 2018). Gonococcal infection is usually asymptomatic in women (> 50%) unlike in men (10%). Complications of gonorrhea in women can be the cause of ectopic pregnancies and in both sexes of infertility (Unemo and Shafer, 2014). In addition, this high prevalence is associated by an increase in antibiotic resistance, with strains producing β-lactamases inactivating 3rd generation cephalosporins (C3G), the last resort for treatment of gonorrhea (WHO, 2016a). Thus, WHO has drawn worldwide attention to the risk of a therapeutic deadlock and the need to develop new drugs (Wi et al., 2017; Weston et al., 2017), especially in countries with limited resources, in sub-Saharan Africa. The Central African Republic (CAR) should not escape this reality because it is part of the Africa WHO region, the most affected. The probabilistic antibiotic therapy for *N. gonorrhoeae* infection in CAR is based on the use of ceftriaxone or/and Ciprofloxacin. However, the latest work on *N. gonorrhoeae* dates back to 1980. They already reported an increase in resistance to penicillins and the production of a β-lactamase (Georges et al., 1982). Thirty years later, these data require updating. In this context, this study aims to determine the prevalence and the antibiotic resistance of *N. gonorrhoeae* strains isolated from genital swab samples as well as the prevalence of sex workers among them at the National Reference Center of IST and Antiretroviral Therapy in Bangui.

METHODS

Study site

This is a cross-sectional study that took place at the National Reference Center of IST and Antiretroviral Therapy in Bangui, Central African Republic, from January 1, 2015 to December 31, 2018, that is a period of 4 years. The National Reference Center of IST and Antiretroviral Therapy is a public health establishment. It includes an Anonymous Screening Unit, Outpatient Treatment Service for HIV and tuberculosis, an STI care service and a medical analysis laboratory. The mission of the National Reference Center of IST and Antiretroviral Therapy in Bangui is to prevent STIs / HIV and provide comprehensive care for people living with STIs / HIV; as well as operational research. In order to promote access to care against STIs for female sex workers, an occupational doctor met in parallel with the female sex workers to make them aware of the systematic use of services and in particular to have access to prophylaxis of Accident of blood exposure of sexual origin (Corevih, 2019).

Study population

The population of our study consisted of patients referred to the National Reference Center of IST and Antiretroviral Therapy laboratory for sampling. Were included in this study, male or female patients, aged 15 years and over, with or without genital discharge, referred to the center's laboratory for a bacteriological study with culture of the urethral or vaginal sample. Any patient taking an antibiotic treatment or even having stopped the treatment less than 72 h before the sample was not included. The inclusion of patients was systematic in order to obtain a sufficient number of strains for the study of their resistance to antibiotics.

Data collection and bacterial analysis of genital samples

A standard data collection sheet containing socio-demographic and biological information (results of the culture and the antibiogram) made it possible to collect the data. *N. gonorrhoeae* has been the subject of a classical bacteriological diagnosis (Unemo and Shafer, 2014). Briefly, the vaginal and urethral samples were stained by the Gram method (Cypress Diagnostics, Hulshout, Belgium), inoculated on cooked and multivitamin blood agar made selective by the addition of vancomycin, colistin and nystatin (Cypress Diagnostics, Hulshout, Belgium). Bacterial identification using biochemical characters by API NH (BioMérieux, Marcy-l'Etoile, France) was completed by the study of antibiotic sensitivity using the agar diffusion method from antibiotic discs (Cypress Diagnostics, Hulshout, Belgium) according to the recommendations of the European Committee on Antimicrobial Susceptibility testing (EUCAST) for each study year (EUCAST, 2022).

Statistical analysis and ethical considerations

The data were entered and analyzed with Epi-Info version 7 software (WHO, Geneva & CDC, Atlanta). The general prevalence of *N. gonorrhoeae* was determined by relating the number of patients carrying *N. gonorrhoeae* to the number of total patients tested. Likewise, the prevalence of *N. gonorrhoeae* in the different groups was determined by relating the number of *N. gonorrhoeae* isolated in the group to the total number of *N. gonorrhoeae* isolated

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Table 1. Characteristics of isolation cases of *N. gonorrhoeae* (N=95).

Characteristics	Number	Percentage (%)
Sex		
Male	86	90.5
Female	9	9.5
History of STIs*		
Yes	54	56.8
No	41	43.2
Educational level		
Unschooled	58	61.1
Primary	17	17.9
Secondary	12	12.6
Superior	8	8.4
Sampling		
Urethral	86	90.5
Vaginal	9	9.5

*STIs. Sexual transmitted diseases.

Table 2. Distribution of the number of samples and the proportion of *N. gonorrhoeae* over time.

Years	Samples number	% *N. gonorrhoeae*
2015	711	2.8
2016	743	3.1
2017	764	3.2
2018	786	3.7
Average	751	3.2

(95). The prevalence of resistance to each antibiotic was determined by relating the number of strains resistant to this antibiotic to the total number of strains tested (95). Fisher's exact test, a parametric test obeying 2 conditions (random distribution of the sample and number in each group <5), was calculated, with online software BiostaTGV online statistic tests (https://biostatgv.sentiweb.fr/?module=tests/fisher), to compare the proportions of categories of qualitative variables with a significance level of 5%. The study received authorization from the Ethics and Scientific Committee of the Faculty of Health Sciences and the Pasteur Institute in Bangui.

RESULTS

During the study period, 3004 patients were received at the laboratory of the National Reference Center of IST and Antiretroviral Therapy for a bacteriological examination of the genital sample. The mean age of the patients was 29.7 years with extremes of 19 and 44 years. Women were more represented with 58.9% (1769); the sex ratio was 0.82. Among women, the female sex workers number was 197 (11.1%). Ninety-five (95) strains of *N. gonorrhoeae* were isolated by bacteriological examinations of genital samples; the prevalence was 3.2%. Among the 95 patients from whom *N. gonorrhoeae* was isolated, 86 or 90.5% were men and 9 women or 9.5%; the sex ratio (M / F) was 10.1. A history of STIs was noted in more than half of the cases, 56.8%. Unschooled patients predominated with 61.1%. *N. gonorrhoeae* was isolated from urethral and vaginal swabs in 90.5 and 9.5% respectively (Table 1). The evolution of the number of patients received at the laboratory for the analysis of genital samples is presented in Table 2 with an average of 751 patients and 3.2% isolation of *N. gonorrhoeae* per year. The patients were classified into different groups: men, women and female sex workers among women. These groups were compared according to Table 3. Among the 9 women, carriers of *N. gonorrhoeae*, 4 were female sex workers (4.2%) of the 3004 identified patients or 44.4% of the infected women. Comparison of female sex workers with other women showed that female sex workers were significantly more carriers of *N. gonorrhoeae* than other women ($p = 0.006$). On the other hand, there is no difference between the female sex workers and the rest of the participants of all sexes (Table 3). The antibiotics tested are those indicated for the antibiogram *of N. gonorrhoeae* according to the recommendations of the European Committee on Antimicrobial Susceptibility testing (EUCAST) for each study years (EUCAST, 2022). Regarding antibiotic resistance, the isolated *N. gonorrhoeae* strains showed a very high rate of resistance to commonly used antibiotics, 95.8% to nalidixic acid, 83.3% to chloramphenicol, 82.6% to penicillin G and 60.9% to ciprofloxacin. The rate of resistance to tetracycline is moderate (34.8%) and low for gentamycin (13.0%) and azythromycin (10.3%). No resistance was observed with ceftriaxone and spectinomycin (Figure 1).

DISCUSSION

This study aimed to determine the prevalence and resistance to antibiotics of *N. gonorrhoeae* strains isolated from samples of genital swabs as well as the prevalence of female sex workers among them at the National Reference Center for STIs and of Antiretroviral Therapy in Bangui. During the study period, 3,004 patients were received at the CNRISTTAR laboratory for a bacteriological analysis of the genital samples. Among them, there were 1769 women (58.9%) including 197 female sex workers (11.1%). The prevalence of *N. gonorrhoeae* was 3.2%. Men were in the majority (90.5%), like unschooled patients (Table 1). Female sex

Table 3. Comparison of the prevalence of *N. gonorrhoeae* in female sex workers and other participants.

	Isolation of *N. gonorrhoeae*		Total	Statistical test
	Yes	No		
Female sex workers	4	193	197	p-value = 0.006;
Other women	5	1567	1572	Odds ratio = 8.1;
Total	9	1760	1769	CI95%: (1.8 – 38.1)*

	Isolation of *N. gonorrhoeae*		Total	Statistical test
	Yes	No		
Female sex workers	4	193	197	p-value = 0.5;
Others (female+male)	91	2716	2807	Odds ratio= 0.6;
Total	95	2909	3004	CI95%: (0.1 – 0.7)*

CI95%: Confidence interval.
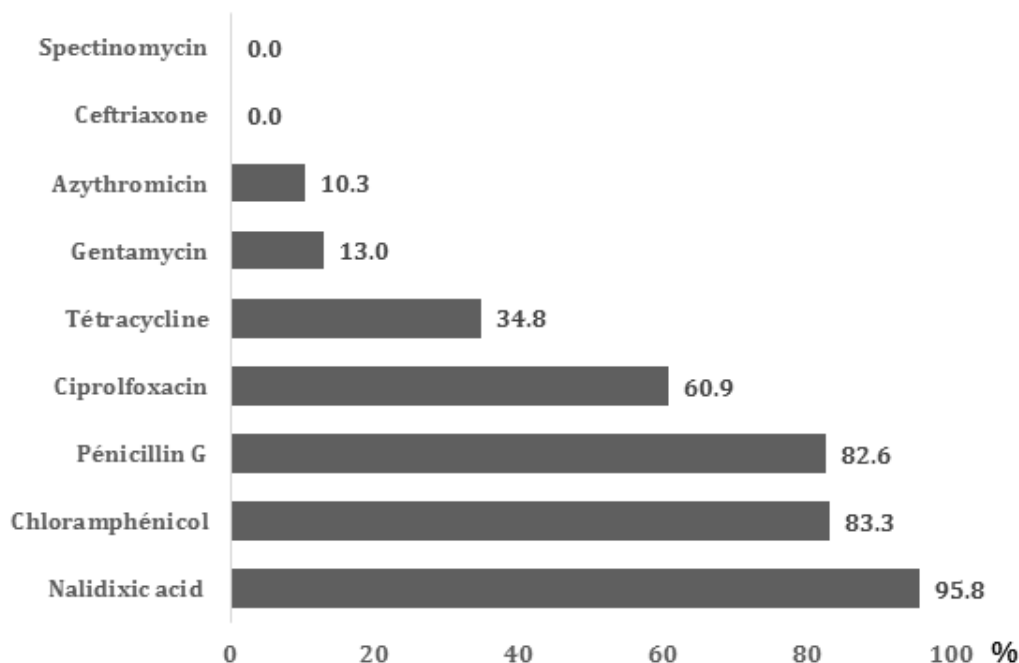
Figure 1. Proportion (%) of *N. gonorrhoeae* strains resistant to antibiotic.

workers were significantly more carriers of *N. gonorrhoeae* than the other women and the rest of the participants, regardless of gender (Table 3). The vast majority of *N. gonorrhoeae* strains were resistant to nalidixic acid (95.8%), chloramphenicol (83.3%), Penicillin G (82.6%) and ciprofloxacin (60.9%). The rate of resistance was moderate to low, 34.8, 13.0 and 10.3%, respectively, to tetracycline, gentamycin and azythromycin.

In contrast, no strain was found to be resistant to ceftriaxone or spectinomycin (Figure 1). This study updates the data on *N. gonorrhoeae* in the CAR, the latest data were published on 1982, ie around thirty years. The prevalence of *N. gonorrhoeae* was 3.2%. This prevalence is higher than the global prevalence of 0.9 and 0.7% respectively among women and men (Kirkcaldy et al., 2019; Rowley et al., 2019) and that observed among women in Ndjamena in Chad (1.2%) (Compain, 2019). The prevalence of our series was lower than that of South Africa (4.6%) and East Africa (8.2%). These data from the CAR and other countries and regions show that the prevalence of *N. gonorrhoeae* varies from one country

or even from one geographic region to another. In this study, the detection of *N. gonorrhoeae* was carried out by classical bacteriological examination with culture. The prevalence would therefore be underestimated because the trend is now to use nucleic acid amplification tests (Poncin and Bercot, 2019) as is the case in the studies by Compain, Kirkcaldy and Torrone (Compain et al., 2019; Kirkcaldy et al., 2019; Torrone et al., 2018), more sensitive than culture.

Strains of *N. gonorrhoeae* were variously distributed by sex. In fact, the majority of men were more affected (90.5%) than women (9.5%). These results confirm the fact that genital *N. gonorrhoeae* infection is noisier in men, leading them to seek health services, unlike women who are often carriers or even asymptomatic. The same observation was noted in France (Poncin and Bercot, 2019). However, WHO data indicates a higher prevalence among women than among men (Kirkcaldy et al., 2019; Rowley et al., 2019). The results of our study were obtained in patients referred to the laboratory. There is a high probability of detection of *N. gonorrhoeae* among them, particularly in men who were most often symptomatic. First, the great majority of women were pregnant, sent from the antenatal consultation, referred to the laboratory for prenatal analyzes, most often asymptomatic with theoretically a low probability of isolation of *N. gonorrhoeae*. On the other hand, the use of molecular tests can reveal *N. gonorrhoeae* even if the culture is negative. However, nucleic acid amplification tests were not used with our series, hence the low proportion of women with a sex ratio of 10.1.

Some women, female sex workers, were more carriers of *N. gonorrhoeae* than other women (p = 0.006). If the investigation of the determination of the behavior and the serological profile among female sex workers in Bangui identified 1,051 PS (CNLS, 2019) with 11.7% of VIH seroprevalence while it was not exhaustive, and that in our series female sex workers identified on a duration of 4 years were 197 but more carriers of *N. gonorrhoeae* than the other women, it is that the female sex workers would not frequent enough the National Reference Center of IST and Antiretroviral Therapy. Female sex workers are one key population for HIV (WHO, 2016b). HIV and *N. gonorrhoeae* are transmitted sexually. Priority prevention actions must be implemented for female sex workers to reduce the HIV seroprevalence and the *N. gonorrhoeae* prevalence too. This is why the female sex workers must be well sensitized and organized around care centers such as the National Reference Center of IST and Antiretroviral Therapy. This organization must involve Dermato-Venerologists and Occupational Safety and Health Doctors since the female sex workers activity is for a several number of women an income-generating activity allowing them to meet their needs (CNLS, 2019).

The high prevalence of *N. gonorrhoeae*, particularly among female sex workers in CAR is not the only

problem encountered in the control of this bacterium in CAR. Our series results showed that the *N. gonorrhoeae* antimicrobial resistance is the second one. *N. gonorrhoeae* strains were highly resistant to nalidixic acid (95.8%), chloramphenicol (83.3%), penicillin G (82.6%) and ciprofloxacin (60.9%). These are the cheapest antibiotics, the most commonly used in Bangui, and inexpensive to cost. However, ciprofloxacin is one of the antibiotics recommended in CAR for the treatment of *N. gonorrhoeae* infection (DGLSIST, 2007). The cost of managing an *N. gonorrhoeae* infection would therefore increase. Only a study of the cost of care could confirm this hypothesis. This strong resistance is linked to self-medication which is a widespread practice in the CAR and especially to the free sale of drugs in the street. Shops called "Minipharma" that sell counterfeit drugs are legion in the streets of Bangui. An increase in resistance to penicillins and a strain producing β-lactamase have already been described in 1982 (Georges et al., 1982). If this trend continues, CAR would in a few years be in a therapeutic stalemate against *N. gonorrhoeae*. This trend of rapid and significant increase in resistance of *N. gonorrhoeae* has been observed in several countries on several continents (Martin et al., 2019; Unemo et al., 2016, Gasser et al., 2018). This is why the antibiotic resistance of *N. gonorrhoeae* has become worrying and the alert is raised for the development of other treatments (Bodie, 2019; Alirol, 2017). The rate of resistance was moderate against tetracycline (34.8%) and low for gentamycin (13.0%) and azthromycin (10.3%). With nearly 35% resistance, tetracycline could not even be prescribed as probabilistic antibiotic therapy because the treatment would only be effective against 2 out of 3 strains. Tetracycline must not be given to pregnant women. Azythromycin and gentamycin, although presenting a low rate of resistance, would hardly be effective. Gentamycin is not applicable in the treatment of *N. gonorrhoeae* infection because of the injectable route for the first and the cost for the other. These 2 antibiotics are therefore not indicated in current practice. The fact that no resistance has been observed with ceftriaxone and spectinomycin shows that these 2 molecules would be the antibiotics of choice for treating *N. gonorrhoeae* infection, this is the protocol in some countries (Pogany et al., 2015).

However, spectinomycin is hardly available in Bangui. The remedy therefore remains ceftriaxone, an antibiotic recommended in the treatment of *N. gonorrhoeae* infection in combination with ciprofloxacin in CAR. The most ceftriaxone advantage is the single dose in treatment of *N. gonorrhoeae* infection. The CAR should therefore revise its management protocol for *N. gonorrhoeae* infections by replacing ciprofloxacin with azythromycin or spectinomycin. A policy must be put in place to ensure good availability of these 2 antibiotics in all Central African Republic territory.

Conclusion

The prevalence of *N gonorrhoeae* was 3.2%; female sex workers were more carriers than the other women. The prevalence of resistance to common antibiotics was very high. These results suggest urgent reinforcement of prevention actions against the transmission of *N gonorrhoeae* to female sex workers, the revision of the protocols for the management of *N gonorrhoeae* infections and a policy to ensure the best availability of the antibiotics indicated in the new therapeutic protocols.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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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 S*almonella* 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_2S) 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, MgCl₂ (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[3]-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), MgCl₂ (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)
TetA	(F)-GGTTCACTCGAACGACGTCA (R)-CTGTCCGACAAGTTGCATGA	Randall et al. (2014)	577	mPCR	55
Tet B	(F)-CCTCAGCTTCTCAACGCGTG (R)-GCACCTTGCTGATGACTCTT		634		
QnrA	(F)-GGGTATGGATATTATTGATAAAG (R)-CTAATCCGGCAGCACTATTTA	Mammeri et al. (2005)	670	Spcr	55
aac[3]-IV	(F)-CTTCAGGATGGCAAGTTGGT (R)-TCATCTCGTTCTCCGCTCAT	Van et al. (2008)	286	sPCR	55
CITM	(F)-TGGCCAGAACTGACAGGCAAA (R)-TTTCTCCTGAACGTGGCTGGC		462	sPCR	55
cat1	(F)-AGTTGCTCAATGTACCTATAACC (R)-TTGTAATTCATTAAGCATTCTGCC		547	mPCR	55
cmlA	(F)-CCGCCACGGTGTTGTTGTTATC (R)-CACCTTGCCTGCCCATCATTAG		698		

bp: Base pairs; sPCR: Simple PCR; mPCR: Multiplex PCR; C: Concentration.
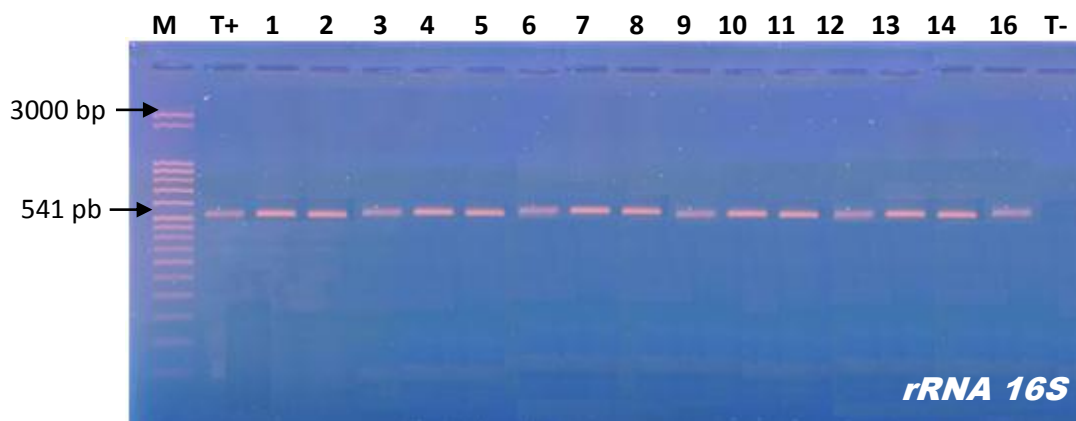
Figure 1. Electrophoretic profile of *16S rRNA* gene amplification products in *Salmonella* spp. isolated from vegetables salads and ready to eat raw mixed vegetable salads. M: Molecular marker of 50bp (Sigma Aldrich, Saint Louis, USA); T+: positive control *16 S rARN* (541pb) ; Lines 1 to 16: strains 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.

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

(-): non detected.

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

Salmonella serotypes	Resistance rate to at least one antibiotic of *Salmonella* serotypes (%)	
	Vegetable's salads (N=21)	Ready to eat raw mixed vegetable salads (N= 8)
S. enteritidis	5 (83.3)	1 (50)
S. typhimurium	5 (83.3)	1 (100)
*S. h*adar	5 (83.3)	1 (50)
S. selby	1 (100)	1 (100)
S. typhi	-	1 (100)
*S. p*aratyphi *c*	-	0
S. adamstown	1 (100)	-
Total	**17 (81)**	**5 (62.5)**

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.
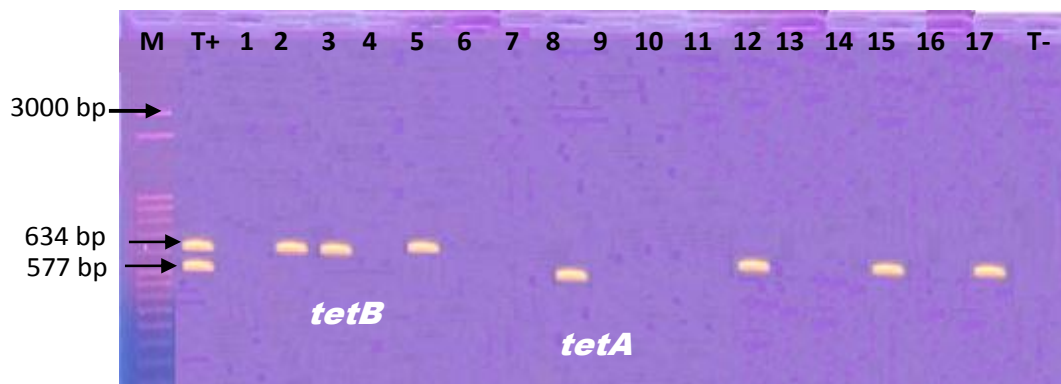
DISCUSSION

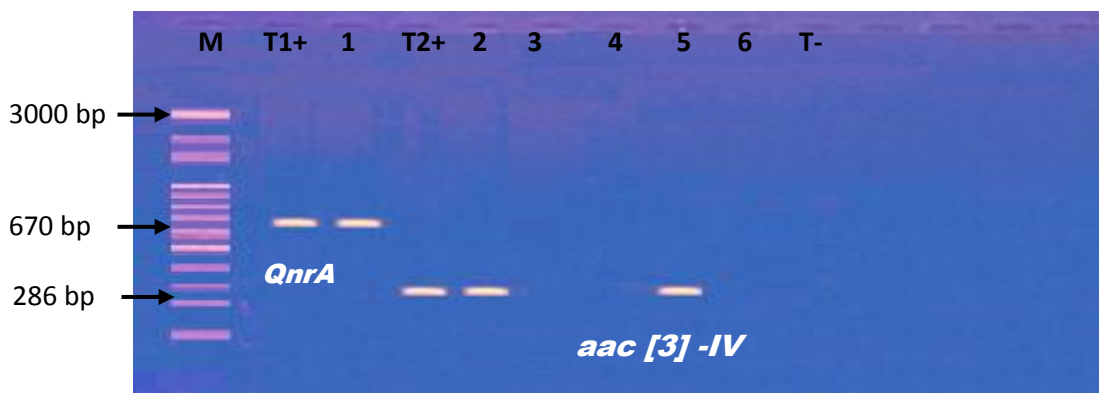This study highlighted the presence of *Salmonella* spp in

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

Type of food	*Salmonella* Serotypes	Antibiotic resistance levels of *Salmonella* 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	*S. enteritidis*	0	0	0	0	0	0	0	4 (66.7)	1 (16,7)	3 (50)	0	0	0
	S. typhimurium	1(16,7)	0	0	0	0	0	0	3 (50)	0	5 (83.3)	1 (16.7)	0	0
	S. hadar	0	0	0	0	0	0	0	4 (66,7)	0	3 (50.0)	0	0	1 (16.7)
	S. selby	0	0	0	0	0	0	0	1 (100)	1(100)	1 (100)	0	0	0
	S. adamstown	0	0	0	0	0	0	0	1 (50)	0	0	0	0	0
	Total	**1 (4.8)**	**0**	**0**	**0**	**0**	**0**	**0**	**13(61.9)**	**2 (9.5)**	**12(57.1)**	**1 (4.8)**	**1 (4.8)**	**1 (4.8)**
Ready to eat raw mixed vegetables salads	*S. enteritidis*	0	0	0	0	0	0	0	1(50)	0	0	0	0	0
	S. typhimurium	0	0	0	0	0	0	0	1 (100)	1(100)	1 (100)	1 (100)	0	0
	S. hadar	1 (50)	0	0	0	0	0	0	1 (50)	1 (50)	0	1 (50)	1 (50)	0
	S. selby	0	0	0	0	0	0	0	1(100)	0	1(100)	0	0	1(100)
	S. Typhi	0	0	0	0	0	0	0	1(100)	0	1(100)	0	0	0
	S. paratyphi c	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**1 (12,5)**	**0**	**0**	**0**	**0**	**0**	**0**	**5 (62.5)**	**2(25.0)**	**3 (37.5)**	**2 (25.0)**	**1 (12.5)**	**1 (12.5)**

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 bp); 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 pb); 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 *Salmonella*	Antibiotic resistance genes of *Salmonella* isolated from vegetables salad (%)						
	CIMT (N=1)	*Aaa(3)-IV* (N=2)	*QnrA* (N=0)	*TetA* (N=13)	*TetB* (N=13)	*cat1* (N=0)	*CmlA* (N=0)
S. enteritidis	0	1 (50)	0	1 (7.7)	0	0	0
S. typhimurium	0	0	0	0	1 (7.7)	0	0
S. hadar	0	0	0	1 (7.7)	1 (7.7)	0	0
S. selby	0	0	0	1 (7.7)	0	0	0
S. adamstown	0	0	0	0	0	0	0
Total	**0**	**1 (50)**	**0**	**3 (23,1)**	**2 (20)**	**0**	**0**

N: number of strains tested.

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

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

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. parathyphi* 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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