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Involvement of Certain Genes in the Mechanism of Acquisition of Antibiotic Resistance in *Salmonella* Typhi After Exposure to Medicinal Plant Extracts

Fabrice Ezo'o Mengo[1], Jean Paul Assam Assam[1,2], Sylvain Leroy Sado Kamdem[1], Jean Justin Essia Ngang[1,*]

[1]Department of Microbiology, Faculty of Science, University of Yaounde I, Yaounde, Cameroon
[2]Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang, Cameroon
*Corresponding author: essia.ngang1@gmail.com

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Abstract **Introduction:** In Cameroon, many medicinal plants are used to treat typhoid fever. However, studies have shown that some of these plants can induce resistance to antibiotics used against *Salmonella* Typhi (*S.* Typhi). The mechanisms by which this resistance is acquired are not clear. This article aims to determine the mechanisms of antibiotic resistance acquisition in *S.* Typhi strains exposed to medicinal plant extracts. **Methods:** Two plant extracts, *Enantia chlorantha* and *Irvingia gabonensis*, were used to induce antibiotic resistance in *S.* Typhi. The antibiotics tested were: Ciprofloxacin, Amoxicillin, Chloramphenicol, Gentamicin, and Cotrimoxazole. Five genes were searched for by PCR in the genome of *S.* Typhi samples obtained after exposure to plant extracts. These were: *bla*TEM and *bla*SHV, coding for resistance to β-lactam antibiotics; *sul*1, coding for resistance to sulfonamides; *flo*R, coding for resistance to phenicols; and *int*1, coding for resistance to multiple antibiotics. **Results:** The results demonstrated that, *S.* Typhi exposed to the plant extracts *Enantia chlorantha* and *Irvingia gabonensis* exhibited the presence of genes *sul*1, *flo*R, and *int*1. However, the absence of these genes in the unexposed control strain indicated that plant extracts are able to induce antibiotic resistance through genetic mutation. The *bla*SHV gene was not detected in any of the *S.* Typhi samples, in contrast to the *bla*TEM gene, which was present in all samples, including the control. **Conclusion:** Mechanisms other than genetic mutations need to be assessed to better understand how medicinal plants induce antibiotic resistance.

Keywords: *Salmonella* Typhi, *medicinal plants, antibiotics, mechanisms, resistance, genes*

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1. Introduction

Salmonella enterica serotype Typhi is a Gram-negative bacterium that causes typhoid fever, a potentially fatal disease. The bacterium is typically transmitted via contaminated food or water. Once ingested, the bacteria multiply and spread through the bloodstream. The clinical picture of typhoid fever includes a high fever, nausea, abdominal pain, abnormal stools, headache and, in some cases, a cough [1,2]. The disease can result in serious complications, including intestinal perforation and internal bleeding [3].

The WHO estimates that each year, there are 11 to 20 million cases of typhoid fever, resulting in 128,000 to 161,000 deaths worldwide [4]. The majority of these deaths occur in South Asia and sub-Saharan Africa [5]. A global study has demonstrated that the incidence of typhoid fever in low and middle-income countries in South Asia and Africa is higher than in developed countries [6].

Antibiotics represent a crucial element in the arsenal employed in the fight against typhoid fever. Nevertheless, the spread and use of antibiotics in the medical field have unquestionably contributed to the emergence and rapid spread of antimicrobial resistance in *S.* Typhi strains [7]. The emergence of this phenomenon can occur spontaneously as a result of mutations [8] or by the acquisition of genes that confer antimicrobial resistance to *S.* Typhi [9]. Consequently, this strain harbours a plethora of genes linked to antibiotic resistance, including *bla*TEM, *bla*SHV, *sul*1, *flo*R, and *int*1 [10]. Since *S.* Typhi has developed resistance to Ampicillin, Chloramphenicol, and Cotrimoxazole, Ciprofloxacin has been the drug of choice for the treatment of this infection. However, instances of treatment failure with this antibiotic have been documented [11].

In light with this growing phenomenon, traditional medicine using medicinal plants is emerging as an alternative to fight antibiotic-resistant and multi-resistant strains [12]. However, work carried out by Ezo'o *et al.* in 2018 showed that medicinal plants used in traditional

medicine in Cameroon are capable of inducing antimicrobial resistance in *S.* Typhi and *S. aureus* strains. Therefore, the researchers demonstrated that the phenomenon of antimicrobial resistance acquisition is not only linked to the overuse of antibiotics but also to the mixture of plant extracts [13]. However, the mechanisms by which this resistance is acquired remain unclear. Consequently, this study aimed to verify the hypothesis that gene mutation is one of the explanations for *S.* Typhi resistance to some antibiotics after exposure to medicinal plants.

2. Materials and Methods

2.1. Plant Material

Two plant extracts *Enantia chlorantha* and *Irvingia gabonensis* were used. Besides these two extracts, a mixture consisting of a plant extract (*Enantia chlorantha*) and an antibiotic (Chloramphenicol) was also tested. This mixture was chosen because typhoid fever patients in Cameroon often combine modern and traditional treatments. The parts used, the reference number, and the collection area of the plants used are presented in Table 1.

Table 1. Medicinal plants tested against *S.* Typhi

N°	Scientific name	Part used	Reference	Collection area
1	*Enantia chlorantia*	Barks	2133/SRF/CAM	Mount Eloumden
2	*Irvingia gabonensis*	Barks	48383/HNC	Mount Eloumden

2.2. Antibiotics

Five antibiotics from different families, whose modes of action are shown in Table 2, were used.

Table 2. Mode of action of antibiotics tested against *S.* Typhi

Antibiotics (abbreviation)	Antibiotics families	Mode of action
Amoxicillin (Amx	Béta-lactamine	Inhibition of cell wall synthesis
Cotrimoxazol (SXT/TSU)	Sulfamides	Metabolic antagonism
Chloramphenicol (C)	Phenicols	Inhibition of protein synthesis
Gentamicin (GM)	Aminosides	Inhibition of protein synthesis
Ciprofloxacin (Cip)	Quinolones	Inhibition of nucleic acid synthesis

2.3. Preparation of Aqueous Plant Extracts

To prepare the macerates, the barks of each harvested plant were washed with water, dried, and finely ground using an electric grinder. The resulting powders were macerated in water at a ratio of 10% (w/v) for 48 hours at 25°C. The solutions were filtered on Wattman N°1 filter paper, and the filtrates collected were oven-dried at 45°C. The dry extracts obtained were stored in a refrigerator at 4° C for future use.

2.4. Continuous exposure to the *S.* Typhi Strain

S. Typhi were grown for cultured every 24 hours for 14 days in a renewed nutrient broth containing the plant extracts at a fixed concentration at 0.5 mg/mL for each extract by inoculating 100µL of a subculture to the new culture [13]. The control strain was grown in antimicrobial-free nutrient broth using the same procedure. The various *S.* Typhi samples tested were coded as follows: St_c (initial *S.* Typhi sample unexposed as control), St_{Ec} (*S.* Typhi sample exposed to *Enantia chlorantha*), St_{Ig} (S. Typhi sample exposed to *Irvingia gabonensis*) and St_{Ep+A} (*S.* Typhi sample exposed to the *Enantia chlorantha* plant extract and Chloramphenicol antibiotic mixture). To evaluate the resistance induction in these samples, the MICs of the various antibiotics were assessed on the various exposed strains before and after exposure [14].

2.5. Genes Searched

As bacterial resistance to antibiotics is associated with the presence of antibiotic resistance genes in the bacterial genome, five of these genes were screened by PCR in the genome of *S.* Typhi exposed to plant extracts. These were: the *bla*TEM and *bla*SHV genes, coding for resistance to all β-lactams except cephamycins and carbapenems [15]; the *sul*1 gene, coding for resistance to sulfonamides (Cotrimoxazole) [16]; the *flo*R gene coding for resistance to phenicols (Chloramphenicol) [16]; and the *int*1 gene coding for resistance to several antibiotics [17].

Table 3. Primer sequences used to detect antibiotic resistance genes

Genes	Primer	Primer sequences	Genes size (pb)
TEM	TEM-F	ATAAAATTCTTGAAGACGAAA	1080
	TEM-R	GACAGTTACCAATGCTTAATCA	
SHV	SHV-INT-F	TTATCTCCCTGTTAGCCACC	650
	SHV-INT-R	GATTTGCTGATTTCGCTCGG	
Sul1	Sul-F	TTTCCTGACCCTGCGCTCTAT	793
	Sul-R	GTGCGGACGTAGTCAGCGCCA	
floR	floR-F	ATGACCACCACACGCCCCG	198
	floR-R	AGACGACTGGCGACTTCTTCG	
*Int*1	INT-3'-CS	GGCATCCAAGCAGCAAGC	1950
	INT-5'-CS	AAGCAGACTTGACCTGAT	

2.5.1. Extraction and Purification of the Genomes of Different *S.* Typhi Samples

The DNA of each sample was extracted and purified using the ZymoBIOMICS™ DNA Miniprep Kit, in accordance with the manufacturer's instructions. In order to achieve this, a bacterial suspension was prepared from pure cultures of the various *S.* Typhi samples, which had been grown for 18 to 24 hours on nutrient agar. This was done by transferring the samples to a 1.5 mL Eppendorf tube containing 200 µL of molecular biology water. The protocol was then followed for extraction and purification. To verify the purity of the total DNA collected, part of it was revealed by electrophoresis on a 1.5% concentrated agarose gel prepared with 1X Tris Borate EDTA (TBE) buffer. The remainder was stored at -20°C. Before each use, the DNA extract was thawed at room temperature [18].

2.5.2. Amplification

1.5 µL of DNA extract added to 48.5 µL of the reaction mixture presented in the table and containing primer

described in Table 4. A negative control (a tube in which the DNA extract is replaced by molecular biology water), necessary to detect possible contamination, was introduced into each amplification series.

In parallel, the PCR was performed following the amplification program described in Table 5 using the thermocycler (gen Ampi 9700) [15,16,17].

Table 4. Reaction mixture composition

Component (Initial concentration)		Final concentration	Volume per sample (µL)
Water		/	28,75
Q Solution		5X	5
10X Buffer		1X	5
$MgCl_2$ (25 mM)		2 mM	4
dNTP (20 mM)		200 µM	0,5
Primers (10µM)	Forward	50 pM	2,5
	Reverse	50 pM	2,5
Taq polymerase (5U/µL)		1,25 U	0,25

Table 5. PCR amplification programs

Gene	Denaturation Initial	35 cycles			Terminal elongation
		Denaturation	Hybridation	Elongation	
*Bla*SHV	10 min at 94°C	30 s at 94°C	30 s at 47°C	1 min at 72°C	10 min at 72°C
*Bla*TEM	10 min at 94°C	30 s at 94°C	30 s at 47°C	1 min at 72°C	10 min at 72°C
Int1	10 min at 94°C	30 s at 94°C	30 s at 55°C	2 min at 72°C	10 min at 72°C
Sul1	5 min at 95°C	30 s at 94°C	30 s at 55°C	30 s at 72°C	10 min at 72°C
floR	5 min at 95°C	30 s at 94°C	30 s at 55°C	30 s at 72°C	10 min at 72°C

Table 6. MIC of antibiotics (µg/mL) and Antibiotics resistance phenotype of *S.* Typhi after exposure to different antibacterial agents

ATB	St_C	St_{Ec}	St_{Ig}	St_{Ep+A}
AMX	(2) S	(15) R	(15) R	(>500) R
SXT/TSU	(62) S	(125) R	(125) R	(>500) R
C	(4) S	(15) R	(15) R	(250) R
CIP	(62) S	(62) S	(15) S	(500) R
GM	(8) S	(62) R	(62) R	(15) R

S: strain is sensitive; R: strain has developed resistance; St_C: non exposed *S.* typhi control sample; St_{Ec}: sample of *S.* Typhi exposed to *Enantia chlorantha*; St_{Ig}: sample of *S.* Typhi exposed to *Irvingia gabonensis*; St_{Ep+A}: sample of *S.* Typhi exposed to a plant extract+antibiotic mixture. CIP: Ciprofloxacine, AMX: Amoxicilline, C: Chloramphénicol, GM: Gentamicine, SXT/TSU: Cotrimoxazole.

2.5.3. Revelation of Amplification Products

The PCR products (10µl) were electrophoresed in a 1.5% agarose gel stained with ethidium bromide fluorescence in 1X TBE buffer at 80V for 30 minutes and visualised using under UV light in a transluminator. The size of the DNA fragments seen on an electronic monitor was estimated by comparison with the bands of the molecular weight marker.

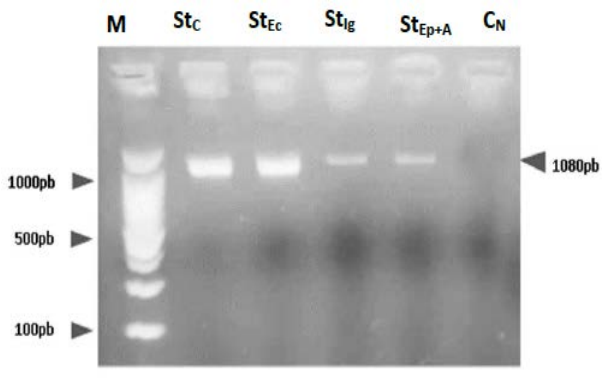
3. Results

3.1. Phenotypes of *S.* Typhi to Antibiotics after Exposure to Antibacterial Agents

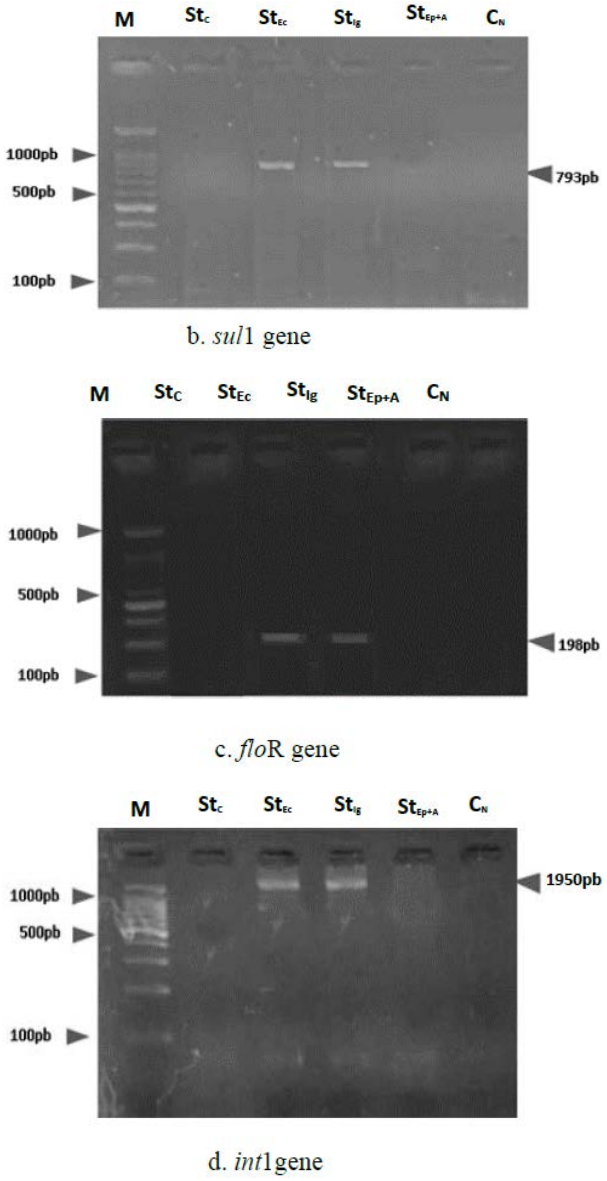
The continued exposure of *S.* Typhi to plant extracts resulted in a reduction in its sensitivity to antibiotics. The MICs values obtained confirmed this phenomenon. Indeed, all the samples of *S.* Typhi exposed successively 14 days to plant extracts developed resistance to the majority of the antibiotics tested, whereas the unexposed control sample (St_C) remained sensitive. Ciprofloxacin was the only antibiotic that retained its efficacy against *S.* typhi despite exposure to *Enantia chlorantha* and *Irvingia gabonensis* plant extracts used alone (Table 6).

3.2. Distribution of Antibiotic Resistance Genes

Figure 1 illustrates the distribution of genes across the various *S.* Typhi samples. The DNA fragments, observed as bands on the agarose gel following electrophoresis, were employed to detect the presence of these genes according to their sizes, as detailed in Table 3. Of the five resistance genes tested, four were detected (*bla*TEM, *sul*1, *flo*R and *int*1), while only one was absent (*bla*SHV).



a. *bla*TEM gene



M: molecular weight marker (100 bp); St_C: sample of initial *S.* Typhi control; St_{Ec}: sample of *S.* Typhi exposed to *Enantia chlorantha*; St_{Ig}: sample of *S.* Typhi exposed to *Irvingia gabonensis*; St_{Ep+A}: sample of *S.* Typhi exposed to a plant extract+antibiotic mixture; C_N: negative control with no strain.

Figure 1. Gene distribution in different *S.* Typhi samples

The control *S.* Typhi sample (St_C) not exposed to antibacterials was sensitive to all antibiotics tested (Table 6). However, *bla*TEM which confers resistance to β-lactam antibiotics, was detected in this sample. Therefore, the presence of this gene would have no influence on the sensitivity of *S.* Typhi to amoxicillin, the β-lactam antibiotic tested.

S. Typhi samples (St_{Ec} and St_{Ig}) exposed to *Enantia chlorantha* and *Irvingia gabonensis* plant extracts respectively developed resistance to all the antibiotics tested except Ciprofloxacin. The resistance genes *sul*1, *flo*R, and *int*1 detected in these samples are thought to be responsible for the acquired resistance. This result shows that the two plants used during exposure induced the expression of these genes. Thus, the resistance observed to Cotrimoxazole would be due to the *sul*1 gene and that observed for Chloramphenicol would be due to the *flo*R gene. The *int*1 gene, which is not specific to one antibiotic, confers resistance to several antibiotics. Thus, the resistance of St_{Ec} and St_{Ig} to amoxicillin and gentamicin could be justified by the presence of specific sequences contained in this gene coding for resistance to these two antibiotics.

The results for *S.* Typhi exposed to a plant extract (*Enantia chlorantha*) combined with an antibiotic (Chloramphenicol) showed that this sample (St_{Ep+A}) developed resistance to all antibiotics tested (Table 6). However, only one of the five tested resistance genes was detected in this sample. This is the *bla*TEM gene, which confers resistance to β-lactam antibiotics. It can therefore be concluded that the presence of the antibiotic at the time of exposure influenced the ability of the plant extract to induce the expression of the other genes. This is because when the *S.* Typhi strain was exposed only to the plant (*Enantia chlorantha*), four of the five genes were detected. Thus, the resistance of St_{Ep+A} to antibiotics (Cotrimoxazole, Chloramphénicol, Ciprofloxacine, and Gentamicine) is the result of a resistance mechanism that does not involve any of the genes that were being sought.

4. Discussion

Results showed that all *S.* Typhi samples developed resistance to the majority of antibiotics tested. This resistance was linked to the presence of the *bla*TEM, *sul*1, *flo*R, and *int*1 genes detected in samples of *S.* Typhi exposed to *Enantia chlorantha* and *Irvingia gabonensis* plant extracts. Indeed, Piekarska in 2023 sequencing of the whole genome of quinolone-resistant *Salmonella* Typhi demonstrated that some of these genes were mutated [19]. A study conducted by Jian *et al.* (2021) demonstrated that under conditions of antibiotic selection pressure, antibiotic-sensitive bacteria can adapt to antibiotics by mutating genes and inactivating them through the processes of destruction or alteration of their structure. These processes are regulated by resistance genes, which are the primary cause of antibiotic resistance in antibiotic-resistant strains [32]. Nevertheless, despite the continuous exposure of *S.* typhi to the plant extracts *Enantia chlorantha* and *Irvingia gabonensis*, this strain remained sensitive to Ciprofloxacin. This explains why this antibiotic is one of the three preferred antibiotics for the first-line treatment of typhoid fever.

The *bla*TEM gene was detected in all *S.* Typhi samples, including the control sample (St_C) not exposed to antibacterials. This gene codes for β-lactamases that are continuously produced by the bacteria in the absence of any antibacterial substance [20]. However, despite the presence of this gene, the St_C sample remained sensitive to amoxicillin, a β-lactam antibiotic. This may be due to the fact that the *bla*TEM gene present in the *S.* Typhi genome is 'silent' in this sample [21]. These results are similar to those of Clemente *et al.* (2015), who found resistance genes in bacteria from a community of individuals with low exposure to antibiotics. These genes were 'silent' to antibiotics, but could be expressed under pressure if these populations were repeatedly exposed to antibiotics [22].

The remaining resistance genes, *sul*1, *flo*R and *int*1, were only identified in St_{EC} and St_{IG} samples following exposure to plant extracts *Enantia chlorantha* and *Irvingia*

gabonensis, respectively. This suggests that these genes were expressed as a result of the active phytochemicals present in the plant extracts, namely *Enantia chlorantha* and *Irvingia gabonensis*. Therefore, the resistance exhibited by this strain to Cotrimoxazole, Chloramphenicol and Gentamicin (Table 6) can be attributed to the expression of these genes, which is induced by the activity of the plant extracts [22]. The *sul*1 gene, which confers resistance to sulphonamides (Cotrimoxazole) [16], has been identified as capable of producing dihydropteroate synthetase, which is involved in nucleic acid synthesis [23]. Sulphonamides act as competitive inhibitors of para-aminobenzoic acid in relation to dihydropteroate synthetase. The bacteria were thus able to evade the effects of Cotrimoxazole by increasing the synthesis of dihydropteroate synthetase and, consequently, the production of para-aminobenzoic acid [23]. The *flo*R gene is capable of conferring resistance to the bacterium through the non-enzymatic mechanism of efflux pumps, which it encodes with a high degree of homology [25]. The presence of the *int*1 gene, which confers resistance to a number of antibiotics, provides an explanation for the multi-resistance observed in *S.* Typhi following exposure to the plant extract. Indeed, the presence of this group of genes in bacteria is typically associated with a multi-resistant phenotype [26,27]. It has been demonstrated that class 1 integrons (*int*1) are resistance integrons that can carry up to 10 resistance genes, which code for resistance to antibiotics or disinfectants [28,29].

However, the absence of these genes (*sul*1, *flo*R and *int*1) in *S.* Typhi exposed to a plant extract combined with an antibiotic demonstrates the diversity of mechanisms involved in the acquisition of resistance described by several authors [30,31]. Indeed, even in the absence of these genes, the St_{EP+A} sample developed resistance to all the antibiotics tested. These modifications involve several resistance mechanisms depending on the mode of action of the bioactive substances contained in these plant extracts [24].

5. Conclusion

The detection of *sul*1, *flo*R and *int*1 resistance genes in *S.* Typhi exposed to *Enantia chlorantha* and *Irvingia gabonensis* plant extracts showed that plant extracts are involved in the expression of antibiotic target genes. However, these genes were not detected in *S.* Typhi exposed to the plant extract antibiotic mixture. The absence of these genes in the S. Typhi sample exposed to the plant extract antibiotic mixture indicates that alternative genes may be involved in this resistance. Furthermore, the combined use of plant extract and antibiotic may have altered the quantity of genes involved in the acquisition of antibiotic resistance. These results show that plant extracts are capable of inducing bacterial resistance to antibiotics by a variety of mechanisms involving resistance genes. To better understand the mechanisms by which bacterial strains acquire resistance to antibiotics following exposure to medicinal plants, this work should be extended to other mechanisms.

Conflict of Interest

The authors assert that they do not possess any conflicts of interest.

References

[1] Kumar P, Kumar R., Fièvre entérique, *Indian Journal of Pediatrics*, 2017, 84: 227-230.

[2] Masuet-Aumatell, C., and Atouguia, J., (2021) Typhoid fever infection—antibiotic resistance and vaccination strategies: a narrative review, *Travel Medicine and Infectious Disease*, 2021, 40, 101946.

[3] Mweu E, Mike English, Typhoid fever in children in Africa, *Tropical Medicine and International Health*, 2008, 13(4), 532-540.

[4] World Health Organization, Media Center, questions and answers, Typhoid fever, 2019, 12 May 2020.

[5] Lancet Global Health, A bright future in typhoid vaccines. *Lancet Global Health*, 2021, 9, 1623.

[6] Bhandari, J., Thada, P.K., DeVos, E., Typhoid Fever, *National Library of Medicine*, 2022, accessed on 28 May 2022.

[7] Ramatla, T., Tawana, M., Onyiche, T.E., Lekota, K.E., Thekisoe, O., Prevalence of antibiotic resistance in *salmonella* serotypes concurrently isolated from the environment, animals, and humans in South Africa: A systematic review and meta-analysis, *Antibiotics* 2021, 10, 1435.

[8] Ventola, C.L., The antibiotic resistance crisis: Part 1: Causes and threats, *Pharmacology and Therapeutics*, 2015, 40, 277–283.

[9] Munita, J.M., Arias, C.A., Mechanisms of Antibiotic Resistance, *Microbiology Spectrum*, 2016, 4, 119–127.

[10] Makhtar, W., Bharudin, I., Samsulrizal, N.H., Yusof, N.Y., Whole Genome Sequencing Analysis of *Salmonella enterica* Serovar Typhi: History and Current Approaches. *Microorganisms*, 2021, 9, 2155.

[11] Harish B.N., Menezes G.A., Sarangapani K., Parija S.C., A case report and review of the literature: Ciprofloxacin resistant *Salmonella enterica* serovar Typhi in India, *Journal of Infection in Developing Countries*, 2008, 2: 324–7.

[12] Abdallah E.M., Plants: An alternative source for antimicrobials, *Journal of Applied Pharmaceutical Science*, 2011, 1: 16–20.

[13] Ezo'o Mengo F., Stéphanie Claire Tchonang, Hermann Ludovic Kemaleu, Sylvain Leroy Sado Kamdem and Jean Justin Essia Ngang, Exposure to Plant Extract Causes the Variation of Antibiotic Susceptibility of Two Bacterial Strains (*Salmonella* Serotype Typhi and *Staphylococcus aureus*), *Journal of Advances in Microbiology*, 2018, 12(2): 1-14.

[14] CLSI (Clinical and Laboratory Standards Institute), "Performance standards for antimicrobial susceptibility testing", *CLSI Supplement* M100, 2021, 31: 100-121.

[15] Weill X., Demartin M., Tandé D., Espié E., Rakotoarivony I., Grimont, *SHV*-12-like extended-spectrum-β-lactamase-producing strains of *Salmonella enterica* serotypes Babelsberg and Enteritidis isolated in France among infants adopted from Mali. *Journal of Clinical Microbiology*, 2004, 42: 2432-2437.

[16] Toleman M., Peter B., Bennett, Jones R., Timothy R., Global emergence of trimethroprim/sulfamethoxazole resistance in Stenotrophomonas maltophilia mediated by acquisition of *sul* genes, *Emerging infectious diseases*, 2007, 13: 559.

[17] Lévesque C., Piché L., Larose C., Roy P., PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother*, 1995, 39: 185-191.

[18] Ecker, David A. Sinclair, Jonathan Eisen, Peter Jones. ZymoBIOMICS™ DNA Miniprep Kit DNA for microbiome or metagenome analyses, 2021, Catalog Numbers: D4300T, D4300, D4304.

[19] Piekarska, K., Wołkowicz, T., Zacharczuk, K., Stepuch, A., Gierczyński, R., The mechanisms involved in the fluoroquinolone resistance of *Salmonella enterica* strains isolated from humans in Poland, 2018-2019: the prediction of antimicrobial genes by in silico whole genome sequencing. *Pathogens*, 2023, 12(2), 193.

[20] Korzeniewska E., Harnisz M., extended-spectrum beta-lactamase positive Enterobacteriaceae in municipal sewage and their emission to the environment, *Journal of Environmental*

Management, 2013, 128: 904-911.

[21] Kumar and Kumar, A., Antibiotic resistome of *Salmonella typhi*: molecular determinants for the emergence of drug resistance. *Frontiers in Medicine*, 2021, 15(5), 693–703.

[22] Clemente C., Erica C., Martin J., Kuldip S., Zhan G., Bin W., Magda M., Glida H., Monica C., Óscar N., Orlana L., Jeremy M., Mike C., Jens W., Phaik L., Jean F., Selena R., Nan S., Se J., Jessica M., Rob K., Gautam D., Gloria D., The microbiome of uncontacted Amerindians. *Advances.sciencemag.org*, 2015, 1 (3).

[23] Peyret M., Mécanismes de résistance aux antibiotiques, *Manuel de bactériologie clinique*, 1994, 1: 209-226.

[24] Yala D., Merad A., Mohamedi D., Ouar Korich M., Résistance bactérienne aux antibiotiques, *Médecine du Maghreb*, 2001, 13-14p.

[25] Arcangioli A., Leroy-Setrin S., Martel J.L., Chaslus-Dancla E., Evolution of chloramphenicol resistance to florfenicol, in bovine *Salmonella Typhimurium* strains implicates definitive phage type (DT) 104, *Journal Medicinal of Microbiology*, 2000, 49: 103-110.

[26] Gillings M., Boucher Y., Labbate M., Holmes A., Krishnan S., Holley M., and Stokes W., Recovery of diverse genes for class 1 integron-integrases from environmental DNA samples, *FEMS microbiology letters*, 2008, 287: 56-62.

[27] Koczura R., Przyszlakowska B., Mokracka J., and Kaznowski A., Class 1 Integrons and Antibiotic Resistance of Clinical Acinetobactercalcoaceticus-baumannii Complex in Poznań, Poland, *Current microbiology*, 2014, 69: 258-262.

[28] Gassama-Sow A., Diallo H., Boye S., Garin B., Sire M., Sow I. and Aïdara-Kane A., Class 2 integron-associated antibiotic resistance in Shigella sonnei isolates in Dakar, Senegal. *International journal of antimicrobial agents*, 2006, 27: 267-270.

[29] An T., Duijkeren V., Fluit and Gaastra W., Characterization of resistance genes associated with class 1 integrons in non-typhoid *Salmonella*. In Proceedings of International Workshop on Biotechnology in Agriculture, 92-95.

[30] Samuelsen, Hanne H., Havard J., Manuela K., Kjersti S., Hilde U., Lars Vorland, Induced resistance to the antimicrobial peptide lactoferricin B in *Staphylococcus aureus*, *Federation of European Biochemical Societies*, 2005, 579: 3421–3426.

[31] Nikaido H., Multidrug resistance in bacteria, *Annual Review of Biochemistry*, 2009, 78: 119 - 146.

[32] Jian Z, Zeng L, Xu T, Sun S, Yan S, Yang L, Antibiotic resistance genes in bacteria: occurrence, spread, and control, *Journal of Basic Microbiology*, 2021; 61: 1049-1070.