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Isolation and molecular identification of *Salmonella* with high multidrug resistance to first line typhoid antibiotics in Southwest Cameroon

Rita Ayuk Ndip¹, Laupy Anne Awah¹, Stephen Mbigba Ghogomu¹, Fidelis Cho-Ngwa^{1,2}, Moses Njutain Ngemenya^{*1,3}

1- Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon.

2- National Higher Polytechnic Institute, University of Bamenda, Cameroon.

3- Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Buea, P.O. Box, 63 Buea, South West Region, Cameroon.

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ABSTRACT

Background: The prevalence of typhoid fever in Cameroon is high. However, studies on susceptibility of *Salmonella* to the recommended antibiotics are few and not extensive. This study investigated the susceptibility of *Salmonella* isolated from clinical specimens in the South West region of Cameroon and the genes responsible for their antibiotic resistance. **Methods:** *Salmonella* was isolated from clinical specimens collected from some medical laboratories in the study area by culture on *Salmonella* Shigella agar. Identification and resistance genes analysis were done using biochemical tests, PCR and gene sequencing. Disc diffusion test was done using 11 commercial antibiotics and minimum inhibitory concentrations determined by microdilution test to assess antibiotic susceptibility profile. The Multi-Antibiotic Resistance (MAR) index values were calculated. **Results:** *Salmonella* was isolated from 50 out of 115 specimens, of which 46 (92 %) were multidrug resistant (MDR) with MAR indices > 0.2. The MDR isolates showed high resistance to penicillins and low resistance to ciprofloxacin. PCR revealed that antibiotic resistance was largely due to the *tem-1*, *Sul 1*, *dfrA1*, *floR* genes. Sequencing revealed double mutations at codons 83 (Ser83-Phe) and 87 (Asp87-Gly) and one mutation at codon 83 (Ser83-Trp) in the quinolone resistance determining regions of the *gyrA* gene in two isolates. **Conclusion:** This study has revealed presence of MDR *Salmonella enterica* isolates in Southwest Cameroon with several resistance genes. This finding will guide use of antibiotics in treatment of *Salmonella* infections in the study area and justifies the search for new efficacious antibacterials against the MDR strains.

Introduction

Salmonella enterica causes gastroenteritis, enteric (typhoid and paratyphoid) fevers in humans and animals [1]. *Salmonella* infections were initially treated with first line drugs ampicillin, sulphamethoxazole-trimethoprim, and chloramphenicol. Due to emergence of multidrug resistant (MDR) *Salmonella* strains, these drugs have become less effective [2]. This led to a shift to third generation cephalosporins (ceftriaxone) and

fluoroquinolones (ciprofloxacin). However, resistance to ciprofloxacin has also emerged with reported decreased susceptibility to the drug [3]. The World Health Organisation recommends use of fluoroquinolones as optimal treatment choice in areas showing resistance to first line drugs and use of third generation cephalosporins in severe MDR cases [4,5]. The prevalence of MDR bacteria continues to rise worldwide leading to increased incidence of treatment

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* Corresponding author: Moses Njutain Ngemenya

E-mail address: njutain.moses@ubuea.cm

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failure, increased morbidity, mortality as well as increased healthcare cost [6].

In Cameroon, typhoid fever is highly prevalent due to poor sanitation and fecal contamination of water sources [7]. Also, increasing levels of antimicrobial resistance in *Salmonella* has been reported. A study in the Northwest region of Cameroon showed a significant decrease in sensitivity to ciprofloxacin and all first line antibiotics [8]. Also, a five years retrospective study of medical records reported a high degree of multidrug resistance in *Salmonella* in Buea health district in the Southwest region [9]. This has been attributed to indiscriminate use of antibiotics, inadequate dosing and poor compliance to treatment regimens, circulation of low quality drugs, no susceptibility testing in some health facilities and irrational use of the antibiotics. Studies on antibiotic susceptibility of *Salmonella* in Cameroon have been few, limited to culture and susceptibility testing without molecular characterization of resistant isolates and have not been extensive in geographical coverage. Determination of resistant genes would reveal the mechanisms of antimicrobial resistance and is vital in guiding treatment and monitoring evolution of resistance. Increasing resistance justifies the search for new efficacious antibacterials and alternative treatments. This study aimed to investigate the antibiotic susceptibility and the implicated genes of clinical *Salmonella* isolates collected in the South West region of Cameroon using susceptibility testing and molecular techniques.

Materials and Methods

Purification and identification of *Salmonella* isolates

Culture media comprising *Salmonella* Shigella (SS) agar, Nutrient agar, Mueller Hinton agar and broth (Liofilchem, Italy) were prepared strictly according to manufacturer's instructions. A total of 115 clinical samples were obtained from medical laboratories in the District hospital and JET Medicare in Kumba; Regional Hospital Buea; the Regional Hospital, District Hospital and Grosself Laboratory in Limbe, and District Hospital Tiko) in the South West Region. The specimens were sub-cultured 2-3 times on SS agar to obtain uniform colonies prior to characterization. Colonies were subjected to a series of biochemical tests using the API (Analytical Profile Index) 20E test kit (Biomérieux, France).

Antibiotics susceptibility testing

Antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method [1], following the

Clinical and Laboratory Standards guidelines [10]. All 50 clinical *Salmonella* isolates (identified from culture and biochemical tests) and two American Type Culture Collection control strains, *Salmonella typhimurium* 14028 and *Salmonella enterica* 13076, were tested. All procedures were carried out under sterile conditions. Briefly, 50 µL of 0.5 McFarland standard turbidity (1×10^8 CFUs/mL) was prepared and uniformly spread over freshly prepared Mueller Hinton agar. Commercial antibiotic (Liofilchem, Italy) discs were carefully placed on the inoculated plates with 5 discs per plate. Eleven antibiotic discs from 8 antibiotic classes were used. Plates were incubated at 37 °C (DHP-9052, B. Bran Scientific and Instrument Company, England) for 24 hours. Diameters of inhibition zones were measured in millimetres (mm) and data were interpreted based on reference values [10].

Determination of minimum inhibitory concentration and minimum bactericidal concentration values

The minimum inhibitory concentration (MIC) was determined on selected isolates that displayed MDR in the susceptibility test. This was done for the antibiotics recommended by WHO for treatment of *Salmonella* infections [4], using the broth microdilution method [11]. Antibiotics were purchased from a certified pharmacy in Buea, capital city of the South West region and all tablets ground to obtain fine powder. A stock solution of 1024 µg/mL was prepared (in broth) and diluted serially two-fold giving concentrations from 1 to 512 µg/mL. The assay was set up in a 96 well microtiter plate in duplicate. In wells containing antibiotic (100 µL), 100 µL of inoculum (5×10^5 CFUs/well) was added giving final concentrations of 0.5 to 256 µg/mL. Negative (bacteria, no antibiotic) and positive (25 µg/mL gentamycin) control wells were included. The plates were incubated at 37 °C for 24 hours. Then 20 µL bromothymol blue was added and incubated for an additional 4 hours to indicate bacterial growth by a colour change from blue to yellowish. The minimum bactericidal concentration (MBC) was determined by sub-culturing all wells with inhibited growth on nutrient agar using a sterile wire loop. Plates were incubated at 37 °C for 24 hours and observed for any growth and MBC was considered as the lowest concentration that showed no bacterial colonies [1].

Extraction of genomic DNA

Bacterial genomic DNA was isolated from all 50 resistant *Salmonella* isolates by conventional boiling centrifugation as described [12]. Briefly, a single

colony from a pure nutrient agar culture was grown overnight at 37 °C in 1mL Luria Bertani (LB) broth. Bacterial cells were pelleted by centrifugation at 14,000 rpm for 5mins in a microcentrifuge (Eppendorf centrifuge 5415D, Germany). The supernatant was discarded and the pellet re-suspended in 500µL deionized distilled water by vortexing (Vorte-2 Genie, Scientific Industries, Inc, USA) and centrifuged for 5mins at 14,000rpm. The supernatant was discarded, 200µL of sterile deionized distilled water was added and boiled for 10 mins in a heating block at 100 °C (Lab exchange, Germany), then immediately chilled on ice for 5 mins. The sample was then centrifuged for 5 mins at 14,000rpm at 4 °C (Eppendorf centrifuge, 5415D). The resulting supernatant was carefully transferred to a new Eppendorf tube. The DNA quality was determined by running a 1% gel and stored at -20°C for further use.

Molecular characterization of clinical isolates of *Salmonella*

Confirmation of *Salmonella* species was done using primers specific to *invA* gene by polymerase chain reaction (PCR) amplification as described [13]. *Salmonella* isolates (n = 50) were further subjected to molecular characterization for the identification of resistance-associated genes (n = 16) according to reported method [14]. The isolates were tested for the presence of genes encoding resistance to beta-lactams, chloramphenicol, sulphonamides, trimethoprim and mutation in *gyrA* and *parC* for fluoroquinolone resistance using specific primers (Table 1). The PCR was performed in 25 µL volumes containing 12.5µL One Taq Quickload 2x Master mix (New England Biolabs, UK), 0.5µL each of forward and reverse primers, 9.5µL distilled water and 2µL template DNA. DNA amplifications were carried out using an Eppendorf 2720 PCR thermocycler (Applied BioSystems, USA) under the following reaction conditions: initial denaturation at 95 °C for 5 mins followed by 35 cycles of enaturation at 95 °C for 2mins, annealing at various primer annealing temperatures for 30s, extension at 68 °C for 1.5min and final extension at 68°C for 5 mins. The amplified products were resolved on 1.5 or 2% agarose gel stained with ethidium bromide (0.5µg/mL) and visualized using a photogel documentation system (BIORAD Molecular Imager[®]GelDoc[™]XR,) with Image Lab[™] Software.

Sequencing of DNA

Polymerase chain reaction amplification was carried out to detect the presence of gyrase and topoisomerase IV genes in *Salmonella* using primers (Table 1). DNA

sequencing was performed by automated sequencer services (Genewiz, Germany) to detect mutations in the quinolone resistance determining regions (QRDRs) of *gyrA* and *parC*. The results were analyzed using Bioedit software version 7.0 where the nucleotides of *gyrA* and *parC* from QRDR were compared to reference nucleotides of *Salmonella* isolates available in National Center for Biotechnology Information (NCBI) GenBank with accession nos. X78977 and AE008878, respectively.

Data analysis

Diameters of zones of inhibition and MIC values were interpreted based on reference values [11]. Proportions of resistant isolates were reported as percentage of total resistant isolates. Multi-antibiotic resistance (MAR) indices were determined using the formula $MAR\ index = \frac{A}{B}$

where, A = number of antibiotics that each isolate was resistant to, B = total number of antibiotics tested [15]. Indices > 0.2 were considered as high risk (indicate MDR).

Results

Antibiotic susceptibility of *Salmonella* isolates

All 50 isolates showed resistance to at least one antibiotic. Norfloxacin, imipenem and ceftriaxone displayed high activity while high resistance was detected to methicillin and ampicillin (Table 2). Multi drug resistance was recorded in 36 (72%) isolates, (resistant to at least one antibiotic in three of the chemical classes), (Table 2), while 7 (14%) isolates showed extensive drug resistance (> 5 antibiotic classes), [16]. In terms of the MAR index, 45 (90%) isolates had values > 0.2 indicating multidrug resistance. The 6 isolates that displayed MDR in the diffusion test were used for the MIC assay for antibiotics of four classes (Table 3). The clinical isolates were most sensitive to ciprofloxacin with an average MIC of 63.79 µg/mL, and resistant to all first line antibiotics with MIC values several folds above their MIC breakpoints for resistance which further confirmed the isolates were MDR.

PCR detection of *Salmonella* and resistance-associated genes

All 50 isolates were confirmed as *Salmonella* by the amplification of *invA* gene by PCR, which generated an amplicon of 284 base pairs (Figure 1). From the PCR, the detection frequency of the resistance genes ranged from 2 to 96 % (Table 4) with the highest being 48 (96.0 %) of the 50 isolates positive for the *tem-1* gene (Figure 2), followed by *sulI* and *dfrA1* genes in

40 strains each (80.0%) while the least was the *cat2* gene in one isolate (2.0 %). Meanwhile *oxa-1*, *sul2*, *sul3*, *dfra14*, *dfxB*, *cat1* and *cat3* genes were not detected in any isolate.

Detection of gene mutations

The isolated Salmonella strains were analysed for mutations in *gyrA* and *parC* genes for fluoroquinolone resistance. Of the 50 isolates, only 12 revealed presence of *gyrA* and *parC* genes. Seven of them were *Salmonella typhimurium* (BU03, BU04, BU06, BU13, BU15, BU53 and BU54), four *Salmonella typhi* (BU05, BU07, BU11 and BU70) and one *Salmonella*

paratyphi A (BU10). Following sequencing of ten isolates and alignment of sequences to the references in the NCBI GenBank, point mutations were found in two isolates. *S. typhimurium* (BU03) had double mutations at codon 83 (Ser83-Phe) and codon 87 (Asp87-Gly) while *S. typhimurium* (BU06) had one mutation at codon 83 (Ser83-Trp) with no mutation detected among the *parC* genes. BU03 and BU06 contained no mutation at codon 119 of *gyrA* whereas no non synonymous mutations and some synonymous mutations were detected in *parC* genes of all ten isolates.

Table 1. Primers sequence specific to different antibiotic resistant genes.

SN	Antibiotics	Gene(s)	Primer Sequences 5'-3'	Amplicon size (bp)	Annealing temperature (°C)	Reference(s)
1.	β-Lactamase inhibitors	<i>tem</i>	F:TTGGGTGCACGAGTGGGTTA R: GACAGTTACCAATGCTTAATCA	503	55	[14]
		<i>oxa-1</i>	F-ACCAGATTCAACTTTCAA R-TCTTGGCTTTTATGCTTG	598	55	
2.	Sulphonamides	<i>Sul1</i>	F-TTCCTGACCCGCGCTCTAT R-GTGCGGACGTAGTCAGCGCCA	793	55	[13]
		<i>Sul2</i>	F-CCTGTTTCGTCGACACAGA R-GAAGCGCAGCCGCAATTCAT	667	55	
		<i>Sul3</i>	F-ATGAGCAAGATTTTTGGAATCGTAA R-CTAACCTAGGGCTTTGGTATTT	792	55	
3.	Trimethoprim	<i>dfxA1</i>	F-GTGAAACTATCACTAATGG R-TTAACCCTTTTGCCAGATTT	474	50	[14]
		<i>dfxB</i>	F-GATCACGTGCGCAAGAATC R-AAGCGCAGCCACAGGATAAAT	141	60	
		<i>dfxA14</i>	F-GAGCAGCTICTITTTIAAAGC R-TTAGCCCTTTIICCAATTTT	393	58	
4.	Chloramphenicol	<i>cat1</i>	F-AACCAGACCGTTCAGCTGGAT R-CCTGCCACTCATCGCAGTAC	549	55	[14]
		<i>cat2</i>	F-AACGGCATGAACCTGAA R-ATCCCAATGGCATCGTAAAG	547	55	
		<i>cat3</i>	F-ATCGGCATCGGTTACCATGT R-ATCCCCTTCTTGCTGATATT	310	55	
		<i>cmlA</i>	F-GGCCTCGCTCTTACGTCATC R-GCGACACCAATACCCACTAGC	662	55	
		<i>cmlB</i>	F-ACTCGGCATGGACATGTACT R-ACGGACTGCGGAATCCATAG	840	55	
		<i>floR</i>	F-ATGACCACCACACGCCCCG R-AGACGACTGGCGACTTCTTCG	198	55	
5.	Fluoroquinolone	<i>gyrA</i>	F-AAATCTGCCCGTGTCTGTTGGT R-GCCATACCTACGGCGATACC	343	55	[14]
		<i>parC</i>	F-CTATGCGATGTCAGAGCTGG R-TAACAGCAGCTCGGCGTATT	270	62	
6.	Invasion A gene (Genus-specific primer)	<i>invA</i>	F-GTGAAATTATCGCCACGTTCCGGC AA R-TCA TCG CAC CGT CAA AGG AAC C	284	55	[13]
7.		16S <i>rDNA</i>	F-AGAGTTTGATCMTGGCTCAG R-CCGTCAATTCMTTTRAGTTT	907	55	[13]

Internal amplification control (IAC)	<i>BlaTE M-1</i>	F-CAGCGGTAAGATCCTTGAGA R-ACTCCCCGTCGTGTAGATAA	643	55	[13]
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F: forward primers; R: reverse primer

Table 2. Antibiotic susceptibility profile of *Salmonella enterica* isolates based on disc diffusion method.

Antibiotic class	Antibiotic	Antibiotic Susceptibility		
		Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Penicillins	Ampicillin	5 (10)	4 (8)	41 (82)
	Methicillin	0 (0)	0 (0)	50 (100)
Fluoroquinolones	Ciprofloxacin	15 (30)	17 (34)	18 (36)
	Norfloxacin	41 (82)	9 (18)	0 (0)
Cephalosporins	Ceftriaxone	23 (46)	0 (0)	27 (54)
	Ceftazidime	6 (12)	5 (10)	39 (78)
Antifolates	Sulfamethoxazole /Trimethoprim	14 (28)	4 (8)	32 (64)
Tetracyclines	Tetracycline	2 (4)	7 (14)	41(42)
Phenicols	Chloramphenicol	23 (46)	8 (16)	19 (38)
B-Lactams	Amoxicillin/Clavulanate	14 (28)	18 (36)	18 (36)
Carbapenems	Imipenem	45 (90)	-	5 (10)

Where n= number of isolates that were susceptible, intermediate or resistant to an antibiotic

Table 3. Antibiotic susceptibility based on minimum inhibitory concentration (MIC).

No.	Bacterial Isolates / Control strain*	MIC values (µg/mL)			
		AMP	CHL	SXT	CIP
1	<i>S. typhi</i>	256	>256	256	16
2	<i>S. typhi</i>	128	>256	256	0.75
3	<i>S. typhi</i>	>256	>256	>256	94
4	<i>S. typhi</i>	128	160	>256	128
5	<i>S. typhimurium</i>	192	> 256	128	16
6	<i>S. typhimurium</i>	256	128	256	128
7	<i>S. typhimurium</i> *	>256	256	>256	>256
8	<i>S. enteriditis</i> *	256	96	256	256

Antibiotics and corresponding MIC breakpoints in µg/mL: AMP = Ampicillin = ≥ 32 ,
 CHL = Chloramphenicol = ≥ 32 , SXT = Sulphamethoxazole-Trimethoprim = $\geq 4/76$,
 CIP = Ciprofloxacin = ≥ 4 [11].

Table 4. Distribution of antimicrobial resistance genes in Salmonella clinical isolates.

Antibiotic class	Target gene	n	%
β-Lactamase	<i>Tem-1</i>	48	96
	<i>Oxa-1</i>	ND	0
Sulphonamides	<i>Sul 1</i>	40	80
	<i>Sul 2</i>	ND	0
	<i>Sul 3</i>	ND	0
Trimethoprim	<i>dfrA1</i>	40	80
	<i>dfrA14</i>	ND	0
	<i>dfrB</i>	ND	0
Phenicols	<i>Cat 1</i>	ND	0
	<i>Cat 2</i>	1	2
	<i>Cat 3</i>	ND	0
	<i>cmlA</i>	5	10
	<i>cmlB</i>	3	6
	<i>floR</i>	40	80
Fluoroquinolones	<i>gyrA</i>	12	24
	<i>parC</i>	12	24

ND: gene not detected

Figure 1. PCR amplification of *invA* gene Lane MM: Molecular weight markers 100 bp DNA ladder; Lane PC: Positive control (ATCC 14028); Lanes 1-4: Samples positive for Salmonella spp.

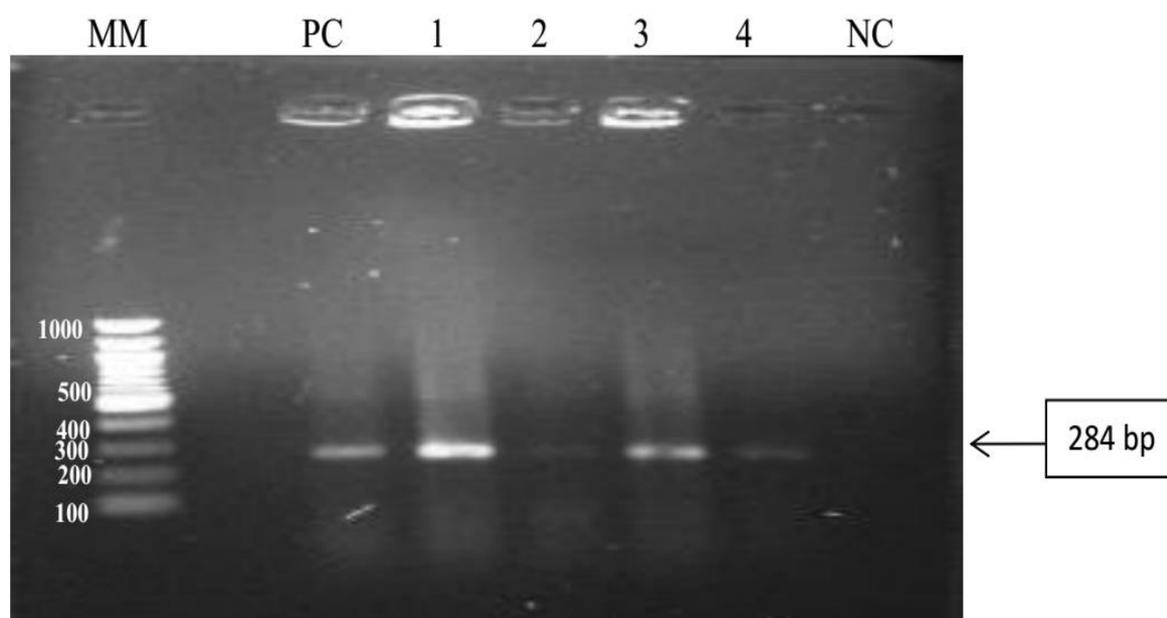
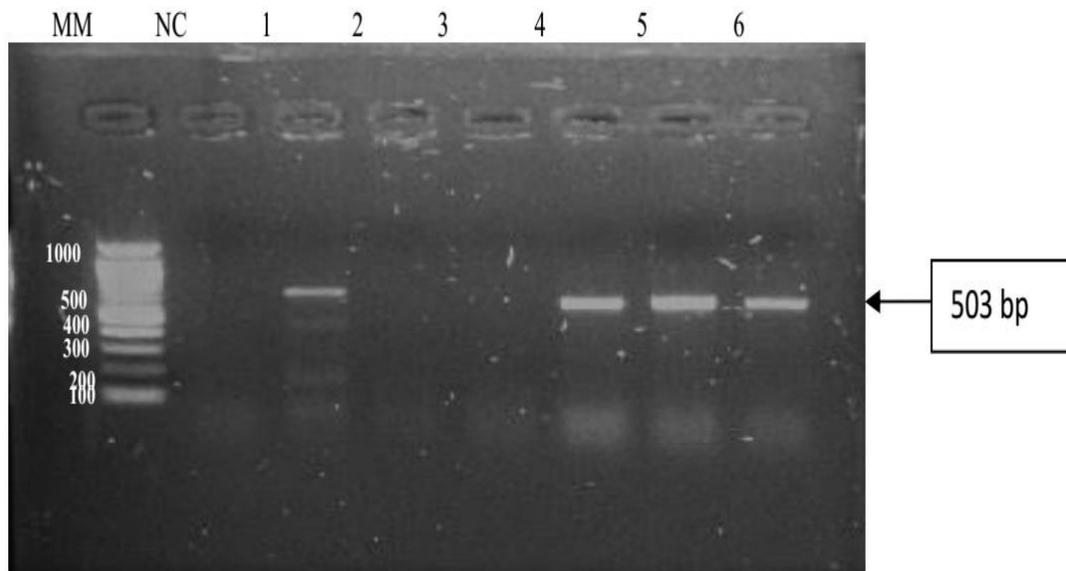


Figure 2. PCR amplification of *tem* gene (503 bp). Lane MM: Molecular weight markers 100 bp DNA ladder; Lane NC: Negative control; Lane 1, 4- 6: Positive samples; Lanes 2 & 3: Negative samples



Discussion

Recent increases in antibiotic-resistant bacteria have prompted the scientific community to perform routine surveillance of microbial populations to determine the extent of the resistance [1]. Previously, typhoid fever was treated successfully with inexpensive drugs (ampicillin, chloramphenicol, sulphamethoxazole-trimethoprim), but the emergence of antibiotic resistance to these first line drugs have made treatment ineffective and very costly [9]. Routine antibiotic susceptibility testing is necessary for effective treatment of typhoid fever to determine which antibiotics effectively kill the bacterium [2].

In this study, 50 clinical *Salmonella* isolates were examined for their susceptibility to various antibiotics and the presence of drug resistance-associated genes. Eleven antibiotics belonging to 8 different antibiotic classes and 17 genes were tested in this study. For the antibiogram results, 48 (96%) *Salmonella* isolates were multidrug resistant. The penicillins displayed the highest level of resistance hence should be excluded in the treatment of typhoid fever. Other first line antibiotics recorded lower but considerable levels of resistance (**Table 2**).

The two fluoroquinolones (ciprofloxacin and norfloxacin) showed percentage resistance of 36% and 0% respectively. Interestingly, **Khadka et al.** [1] recorded no resistance for co-trimoxazole (sulphamethoxazole-trimethoprim) compared to 64% in this study. This difference could be due to variations

in the factors accounting for emergence of resistance such as misuse of antibiotics among others.

The lower resistance to fluoroquinolones, amoxicillin-clavulanic acid and imipenem supports their continued use in the treatment of typhoid fever. The MIC values confirmed the resistance recorded in the disc diffusion test with values several folds above the standard breakpoints except ciprofloxacin which showed sensitivity to one isolate and values close the its breakpoint [11]. These findings confirm earlier reports and constitute a strong basis to search for more efficacious antibacterials.

Analysis of resistance genes in the clinical isolates of *Salmonella* strains (n= 50) revealed the presence of the *tem* gene in almost all the isolates. This suggests that resistance to β -lactamase antibiotics is mediated by the *tem* gene only which encodes a β -lactamase enzyme. The *oxa-1* gene was not detected in any isolate whereas β -lactamase resistance in *Salmonella* isolates were attributed to *bla*_{TEM-1} and *bla*_{oxa-1} genes [17,18] and **El-Tayeb et al.** [14], revealed the presence of *carb*-like gene (carbenicillinase) in isolates that exhibited resistance to β -lactam antibiotics.

It was found that isolates that exhibited resistance to sulphamethoxazole- trimethoprim were associated with presence of *dfrA1* and *sul1* genes only, indicating both genes are responsible for the resistance. This report was supported by another study

which revealed presence of *dfrA1* only [14] but in contrast to the study by **Zishiri et al.** [19], which revealed the presence of *sul1* and *sul2* genes. The resistance to chloramphenicol is highly associated with the acquisition and expression of efflux pumps that reduce toxic levels of the drug in bacterial cells. The resistance to phenicols in this study was associated with the presence of *cat2*, *cmlA*, *cmIB* and *floR* genes. However, *floR* gene was most prevalent in isolates (n=40) suggesting that this gene is responsible for chloramphenicol resistance. This finding is similar to other studies that reported the presence of *floR* gene in various Salmonella Pathogenicity island-1 [14,20].

The main mechanism of resistance to fluoroquinolones in Gram-negative bacteria is point mutations in genes coding for DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*). These mutations alter the binding sites of gyrase or topoisomerase which are targets of antimicrobial agents [21,22]. In the present study, both *gyrA* and *parC* genes were detected in 12 Salmonella isolates, indicating emerging resistance to fluoroquinolones in the study area. This finding is in contrast to a study in Saudi Arabia by **El-Tayeb et al.** which detected few isolates with *gyrA* and *parC* genes [14].

Sequencing detected the QRDR of *gyrA* gene in two Salmonella isolates which has been reported in other studies [17,23,24]. Results of this study suggest that *parC* mutations may not be necessary to generate high level resistance to ciprofloxacin. However, **Eaves et al.** [25], found that isolates with mutation in both *gyrA* and *parC* were more susceptible to ciprofloxacin than isolates with a mutation in *gyrA* alone. Findings of this study differ from those of **El-Tayeb et al.** [14], who reported mutations at positions 13 and 24 in *gyrA* gene and high variation of mutation in *parC* gene, **Murgia et al.** [18] who reported one mutation in *gyrA* (Asp87Asn) and one mutation in *parC* (Thr54Ser) and **Eaves et al.** [26] who reported a novel mutation inside the QRDR of *gyrA* at codon Asp72, Asp 82 and Ala 119.

Double mutations at both codon 83 and 87 have been identified in clinical isolates of *S. typhimurium* DT204 showing high level resistance to fluoroquinolones [26]. In this study, it was found that mutations in *gyrA* gene led to subsequent changes in amino acids at codon 83 from serine to phenylalanine or serine to tryptophan and codon 87 from aspartic acid to glycine. These findings could explain the mechanism of resistance to fluoroquinolones associated with changes in DNA gyrase. The rest of the nucleotide substitutions resulted in no changes in

amino acid sequence thus being synonymous mutations since they do not cause a change in amino acid.

Conclusion

This study has revealed considerable presence of multidrug and extensively resistant *Salmonella enterica* isolates in Southwest Cameroon, with increasing resistance to fluoroquinolone and cephalosporin antibiotics. This resistance is largely due to the *Tem-1*, *Sul 1*, *dfrA1*, *floR* and to a lesser extent *gyrA* genes. This finding justifies the exploration of alternative approaches in the use of available antibiotics and the search for new efficacious antibacterials for treatment of Salmonella infections.

Conflict of interest and funding information

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References

- 1-**Khadka P, Thapaliya J, Thapa S.** Susceptibility pattern of *Salmonella enterica* against commonly prescribed antibiotics, to febrile-paediatric cases, in low-income countries. BMC Pediatrics 2021; 21:38.
- 2-**Paul UK, Bandyopadhyay A.** Typhoid fever: a review. Int J Adv Med 2017; 4(2):300-306.
- 3-**Khanal PR, Satyal D, Bhetwal A, Maharjan A, Shakya S, Tandukar S, et al.** Renaissance of conventional first-line antibiotics in *Salmonella enterica* clinical isolates: assessment of MICs for therapeutic antimicrobials in enteric fever cases from Nepal. BioMed Res Int 2017; 6 pages.
- 4-**World Health Organization.** Antimicrobial resistance: global report on surveillance. Geneva:World Health Organization; 2014. Available at: <https://apps.who.int/iris/handle/10665/112642>. Accessed January 25, 2020.
- 5-**Dyson ZA, Klemm EJ, Palmer S, Dougan G.** Antibiotic Resistance and Typhoid. Clinical

- Infectious Diseases 2019;68 (Suppl 2): S165 – S170.
- 6-**Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee LH.** Salmonella: a review on Pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science* 2015; 8(3):284-293.
- 7-**Akoachere J-FTK, Tanih NF, Ndip LM, Ndip RN.** Phenotypic characterization of *Salmonella typhimurium* isolates from food-animals and abattoir drains in Buea, Cameroon. *Health Popul Nutr* 2009; 27(5): 612-618.
- 8-**Nkeza K, Michel K, Nwobegahay JM, Goon DT, Njukeng PA.** The susceptibility pattern of salmonella species to commonly used antibiotics in the Bamenda district health area, Cameroon. *Afr J Pathol Microbiol* 2016; 5: 4 pages
- 9-**Amin ET, Njumkeng C, Fualefac A, Fondugallah, JA, Njukeng PA.** Trends of Salmonellosis and Its Resistance to Antimicrobials in the Buea Health District, Cameroon. *International Journal of Medical Science and Current Research* 2019; 2, (4): 456-465.
- 10-**Clinical and Laboratory Standards Institute.** Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S. West Valley Road, Suite 2500; 2016.
- 11-**Clinical and Laboratory Standards Institute.** Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition. CLSI document M07-A9. Wayne, PA; 2012.
- 12-**Medici DD, Croci L, Delibato E, Pasquale SD, Filetici E, Toti L.** Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect *Salmonella enterica* serotype enteritidis in poultry. *Appl Environ Microbiol* 2003; 69(6):3456-3461.
- 13-**Adesiji YO, Deekshit VK, Karunasagar I.** Antimicrobial-resistant genes associated with *Salmonella* spp. isolated from human, poultry, and seafood sources. *Food Sci Nutr* 2014; 2(4):436–442.
- 14-**El-Tayeb MA, Ibrahim AS, Al-Salamah AA, Almaary KS, Elbadawi, YB.** Prevalence, serotyping and antimicrobials resistance mechanism of *Salmonella enterica* isolated from clinical and environmental samples in Saudi Arabia. *Braz J Microbiol* 2017; 48(3):499–508.
- 15-**Mthembu TP, Zishiri OT, El Zowalaty ME.** Molecular detection of Multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. *Infect Resist* 2019; 12:3537-3548.
- 16-**Magiorakos AP, Srinivaran A, Carey RB, Carmeli Y, Falagas ME, Giske CG et al.** Multidrug-resistant, extensively drug-resistant and Pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18:268-281.
- 17-**Ceysens PJ, Matheus W, Vanhoof R and Bertrand S.** Trends in serotype distribution and antimicrobial susceptibility in *Salmonella enterica* isolates from humans in Belgium, 2009 to 2013. *Antimicrob Agents Chemother* 2015; 59: 544-552.
- 18-**Murgia M, Bouchrif B, Timinouni M, Al-Qahtani A, Al-Ahdal MN, Cappuccinelli P, et al.** Antibiotic resistance determinants and genetic analysis of *Salmonella enterica* isolated from food in Morocco. *Int J Food Microbiol* 2015; 215:31-39.
- 19-**Zishiri OT, Mkhize N, Mukaratinwa S.** Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. Isolated from commercial chickens and human clinical isolates

- from South Africa and Brazil. Onderstepoort J Vet Res 2016; 83(1): a1067
- 20-**Hsu YM, Tang CY, Lin H, Chen YH, Chen YL, Su YH, et al.** Comparative study of class integron, ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline (ACSSuT) and fluoroquinolone resistance in various *Salmonella* serovars from humans and animals. Comp Immunol Microbiol Infect Dis 2013; 36(1): 9-16.
- 21-**Neupane GP, Kim DM, Kim SM, Lee BK.** In vitro synergism of ciprofloxacin and cefotaxime against nalidixic acid-resistant *Salmonella enterica* serotypes Paratyphi A and Paratyphi B. Antimicrob Agents Chemother 2010, 54:3696-3701.
- 22-**Hassing RJ, Menezes GA, Pelt WV, Petit PL, Genderen V, Goessens WF.** Analysis of mechanisms involved in reduced susceptibility to ciprofloxacin in *Salmonella enterica* serotypes Typhi and Paratyphi A isolates from travellers to Southeast Asia. Int J Antimicrob Agents 2011; 37(3):240-243.
- 23-**Marimón JO, Gomariz M, Zigorraga C, Cilla G, Pérez-Trallero E.** Increasing Prevalence of Quinolone Resistance in human nontyphoid *Salmonella* isolates obtained in Spain from 1981 to 2003. Antimicrob Agents Chemother 2004; 48(10):3789-3793.
- 24-**Chen S, Cui S, McDermott PF, Zhao S, White DG, Paulsen I, et al.** Contribution of Target Gene Mutations and Efflux to Decreased Susceptibility of *Salmonella enterica* Serovar Typhimurium to Fluoroquinolones and other Antimicrobials. Antimicrob Agents Chemother 2007; 51(2):535-542.
- 25-**Eaves DJ, Randall L, Gray DT, Buckley A, Woodward MJ, White AP.** Prevalence of mutations within the quinolone resistance determining region of *gyrA*, *gyrB*, *parC*, and *parE* and association with antibiotic resistance in quinolone resistant *Salmonella enterica*. Antimicrob Agents Chemother 2004;48:4012-4015.
- 26-**Eaves DJ, Liebana E, Woodward M.J, Piddock LJV.** Detection of *gyrA* mutation in quinolone resistant *Salmonella enterica* by denaturing high performance liquid chromatography. J Clin Microbiol 2002; 40:4121-4125.