

Stool Culture Confirmed Salmonella Carriage, Plasmodium Infection, Associated Risk Factors, and Multidrug Resistance Patterns among Widal-Positive Patients in Yaoundé, Cameroon

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Abstract

Typhoid fever and malaria remain major public health concerns in tropical regions where both infections are endemic, largely due to overlapping clinical manifestations such as fever, headache, diarrhoea, vomiting, and chills, which complicate accurate diagnosis. The Widal test, although widely used in resource-limited settings, is limited by poor specificity and frequent cross-reactivity with non-salmonella conditions. This limitation can be attributed to non-specific polyclonal B-cell activation induced by *Plasmodium* spp. infection, which can result in false-positive Widal test outcomes in cases of co-infection with *Salmonella* spp., leading to misdiagnosis, inappropriate treatment, and the emergence of antimicrobial resistance. This study aimed to determine the prevalence of stool culture confirmed *Salmonella* spp. carriage, *Plasmodium* spp. infection and co-infection among Widal-positive patients, identify associated risk factors, and assess multidrug resistance (MDR) patterns of *Salmonella* isolates in Yaoundé. A cross-sectional study was conducted from September 2022 to April 2023 across five hospitals. Participants presenting with symptoms suggestive of typhoid fever and/or malaria who were tested positive to the Widal test were enrolled by convenience sampling. Sociodemographic and clinical data were collected using structured questionnaires. Malaria diagnosis was confirmed by microscopy of thick blood smears, while stool samples were cultured on Hektoen enteric agar for *Salmonella* isolation, followed by identification using the API 20E system and serotyping by

slide agglutination. Antibiotic susceptibility testing was performed using the disk diffusion method (CASFM 2023 guidelines), and MDR indices were calculated. Among 277 Widal-positive participants, stool culture confirmed *Salmonella* spp. carriage was detected in 23 (8.4%), *Plasmodium* spp. in 113 (40.8%), and concurrent carriage of both in 12 (4.4%). Of the 23 *Salmonella* isolates, only 7 (30.4%) were typhoidal serovars (*S. Paratyphi* B); the remaining 16 (69.6%) were non-typhoidal serovars. Notably, *S. Typhi* was not recovered. A significant association was found between water source and *Salmonella* spp. carriage ($p < 0.001$). Among typhoidal isolates, resistance was observed to amoxicillin-clavulanic acid (100%) and piperacillin-tazobactam (71%), with no resistance to third-generation cephalosporins. Non-typhoidal isolates exhibited more complex resistance profiles, including carbapenem resistance. Overall, 73.9% of all isolates were MDR. These findings indicate that malaria is the more common cause of febrile illness among Widal-positive patients in this setting, and that the majority of culture-confirmed *Salmonella* isolates were non-typhoidal, underscoring the need for improved diagnostic strategies beyond the Widal test to reduce misdiagnosis and inappropriate antibiotic use.

Keywords

Salmonella spp., *Plasmodium* spp., Coinfection, Widal Test, Multidrug Resistance, Cameroon

1. Introduction

Typhoid fever is a systemic and prolonged febrile illness caused by typhoidal *Salmonella* serovars, principally *Salmonella* Typhi and *Salmonella* Paratyphi A and B [1]. Humans are the only natural reservoir for these serovars, and transmission occurs through the ingestion of food or water contaminated with human faeces, particularly in endemic areas where carriers may contaminate food during handling [2]. Symptomatic infection manifests as fever, headache, nausea, constipation, diarrhoea, and general weakness. According to the World Health Organization (WHO), approximately 9 million cases of typhoid fever occur annually worldwide, resulting in more than 110,000 deaths [1]. The disease is particularly common in sub-Saharan Africa, where rapid population growth, urbanisation, and limited access to safe water, sanitation, and health infrastructure facilitate transmission. In Cameroon, national prevalence data remain limited; however, regional studies have reported prevalence rates ranging from 9% to 40%, indicating that enteric fever remains a significant public health concern [3]-[5].

Malaria also represents a major public health challenge in many tropical regions where it coexists with enteric fever, further complicating disease diagnosis and management. In Cameroon, several *Plasmodium* species infect humans, including *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*, with *P. falciparum* accounting for more than 95% of cases. According to the WHO, an estimated 249 million malaria cases and 608,000 malaria-related deaths were recorded worldwide in

2022 [6]. The occurrence of *Salmonella* and *Plasmodium* coinfection in sub-Saharan Africa is well documented and is often influenced by shared socio-epidemiological factors such as poor sanitation, inadequate hygiene practices, and high population density [7]-[9].

Accurate and early diagnosis of enteric fever is essential not only for identifying the causative agent but also for detecting potential carriers who may contribute to disease transmission [10]. Blood and stool cultures remain the most reliable diagnostic approaches; however, these techniques are expensive and often unavailable in resource-limited settings. Consequently, the Widal test remains the most commonly used diagnostic method in many countries, including Cameroon, because it is inexpensive and requires minimal laboratory infrastructure [11]. However, the Widal test detects antibodies against *S. Typhi* and *S. Paratyphi* O and H antigens specifically and does not predict the presence of non-typhoidal *Salmonella* (NTS) organisms such as *S. dublin* or *S. typhimurium*, which cause gastroenteritis or invasive NTS disease rather than typhoid fever. Positive Widal results in patients with these NTS infections therefore represent false positives in the context of typhoid diagnosis [12].

The limited specificity of the Widal test is further compounded in malaria-endemic regions. A study by Samal *et al.* on confirmed malaria patients showed positive Widal tests in eight cases, yet blood culture for *Salmonella* was negative in all, and all patients recovered following antimalarial therapy alone [13]. This is consistent with evidence that malaria infection induces polyclonal B-cell activation, resulting in non-specific antibody production that may cross-react with *Salmonella* antigens used in the Widal test, potentially causing false-positive results [14]. Several other conditions, including tuberculosis, dengue fever, endocarditis, chronic liver disease, and brucellosis, have also been associated with Widal cross-reactivity in endemic regions [12] [15]. These phenomena collectively increase the misclassification rate of the Widal test and may contribute to inappropriate antibiotic prescribing.

Historically, *Salmonella* infections were treated with first-line agents such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. However, the emergence of multidrug-resistant (MDR) strains has reduced the effectiveness of these therapies, shifting clinical practice toward third-generation cephalosporins such as ceftriaxone and fluoroquinolones such as ciprofloxacin [16]. The coexistence of MDR *Salmonella* and malaria in Cameroon further complicates appropriate management.

Despite this coexistence, limited data are available on the prevalence of culture-confirmed *Salmonella* carriage stratified by typhoidal and non-typhoidal serovars among Widal-positive patients in Yaoundé, and on the extent of MDR among circulating strains. This study therefore aimed to determine the prevalence of stool culture confirmed *Salmonella* spp. carriage among Widal-positive patients; assess the occurrence of concurrent *Salmonella* spp. and *Plasmodium* spp. coinfection; identify associated risk factors; and evaluate the antibiotic resistance profiles of

typhoidal and non-typhoidal *Salmonella* isolates.

2. Materials and Methods

2.1. Study Design and Setting

Study area

This study was conducted in Yaoundé, the capital city of Cameroon, roughly located at 3° 52' North latitude and 11° 31' East longitude.

Type and period of study

This was a cross-sectional study conducted between September 2022 and April 2023. The study was carried out at five health facilities within the city, including the Yaoundé University Teaching Hospital, Biyem-Assi District Hospital, Efoulan District Hospital, Nkolndongo District Hospital, and Mvog-Ada District Hospital.

Study population

A total of 277 participants age ranged from 1 to 89 years with a positive Widal test were recruited on a convenience base from the five selected health facilities. The participants were enrolled regardless of nationality, social class, or religious background. Participants were distributed across the hospitals as follows: The Yaoundé University Teaching Hospital (n = 102); the Biyem-Assi District Hospital (n = 13), the Efoulan District Hospital (n = 68); the Nkolndongo District Hospital (n = 35) and the Mvog-Ada District Hospital (n = 59). These participants were selected for the study as there were considered to be in a better position to give accurate and reliable information required for the study regarding *Salmonella* spp. and *Plasmodium* spp. infections and on antibiotic use prior to sampling, which was collected through participants interviews; participants were not excluded based on prior antibiotic exposure.

Inclusion Criteria

Participants were eligible if they attended one of the selected hospitals during the study period, had a positive Widal test result (titre > 1:80), and signed the informed consent form.

Exclusion Criteria

Participants who declined to provide informed consent, had a negative Widal test result, or provided insufficient sample volumes for laboratory analysis were excluded.

Sample Size Determination

The minimum sample size was calculated using the Lorentz formula based on an estimated typhoid fever prevalence of 16.2% in Cameroon, as reported by Masumbe *et al.* [17]. With the calculation assumed: by 95% confidence level ($\alpha = 0.05$) and Precision (d) = 0.05. The calculated minimum sample size was 210 participants. To improve statistical power and account for potential data loss, 277 participants were included in the study.

2.2. Data and Sample Collection

Sociodemographic and clinical informations of the study participants were col-

lected using a pretested structured questionnaire administered through face-to-face interviews. After completing the questionnaire, 5 mL of venous blood was collected from each participant into ethylenediaminetetraacetic acid (EDTA) tubes. In addition, stool samples were collected from participants using sterile containers.

2.3. Laboratory Procedures

2.3.1. Conduct of the Survey

The survey was conducted over a period of eight months in five hospitals. It enabled us to collect blood and stool samples, sociodemographic data, risk factors and clinical data from each participant. Specifically, the information that we collected were:

- 1) The socio-demographic aspects that enabled us to obtain information on age, sex, area of residence, etc.
- 2) The quality of the water they drink, the type of toilet they use, the history of *Salmonella* spp. and/or *Plasmodium* spp. infection within the last three months, and the use of mosquito nets in the household.
- 3) The symptoms at the time of diagnosis, treatment taken before arriving at the hospital and the type of treatment.

2.3.2. Typhoid and Malaria Detection

The following tests were performed on biological sample of patients involved in this study.

Widal Slide Agglutination Test

The Widal agglutination test was performed on all blood samples using the rapid slide titration method to detect *Salmonella* antigens; somatic (O) and flagella (H) antigens (Medsorce Ozone Biomedicals, India). According to the manufacturer's instructions, an antibody titer of >1:80 was considered significant and usually suggestive of infection. Only participants with positive Widal test results were further tested for malaria parasites.

Malaria Parasite Detection

Standard size thick and thin blood films were prepared, thoroughly air-dried and the thin films fixed with absolute methanol for malaria species identification. The blood films were stained with 10% (v/v) Giemsa solution for 20 minutes for the detection of *Plasmodium* parasites and speciation, these procedures were carried out in accordance with standard protocols described by the World Health Organization [18].

Stool Culture for *Salmonella* spp. Isolation

Approximately 1 g of stool sample from each participant was enriched in 5 ml of Müller Kauffmann broth solution and cultures of stools were plated onto Hektoen agar. The plate was incubated for 18 - 24 hours at 37°C. Isolation of *Salmonella* in stool culture indicated an infection. If there was no growth, the culture was considerate negative. Presence of growth was followed by Urea test and by mobility test to detect the presence of mobile rods. Then, biochemical test using

Kligler Iron Agar (KIA) to read acid production of the slant, as and H₂S production. The presence of a read slant, yellow butt, weak H₂S reaction (black colony centres) and no gas indicate a positive KIA. Final identification of isolates was performed using the API 20E identification system for Enterobacteriaceae and other Gram-negative bacilli.

Serotyping of *Salmonella* spp. Isolates by Slide Agglutination

All the *Salmonella* spp. isolates were serotyped according to the Kauffmann-White-Le Minor (KW) scheme which is a modification of the original scheme from the 1930s [19]. Serotyping was based on the agglutination of bacteria with specific sera to identify variants of the somatic (O) and flagellar (H) antigens. The anti-*Salmonella* agglutinating serums was from Bio-Rad (Marnes-la-Coquette, France). Briefly, a pure bacterial culture of 18 to 24 hours was added in a sterile saline on a clean glass slide and mixed to obtain a smooth suspension. The suspension was initially tested with polyvalent O antisera for screening purposes. Samples showing agglutination were further tested with monovalent O antisera to identify specific somatic (O) antigens. Subsequently, H antisera were applied to determine flagellar (H) antigens in both phase one and phase 2. The slide was gently mixed, and reactions were observed within 30 - 60 seconds, visible agglutination indicated a positive antigen-antibody reaction. A saline control was included to exclude auto-agglutination. The resulting O and H antigen profiles were recorded and used to assign the corresponding *Salmonella* serotypes.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method [20], following the guidelines of the Antibigram Society of the French Microbiology society (CASFM, 2023) Standards guidelines [21]. All 23 clinical *Salmonella* strains and a reference strains of *Escherichia coli* ATCC 2599 was tested. Briefly, 50 µL of 0.5 McFarland standard turbidity (1×10^6 CFUs/mL) was prepared and uniformly spread over freshly prepared Mueller Hinton agar. Commercial antibiotic (Rapid Lab) discs were carefully placed on the inoculated plates with 14 discs per square plate. Sixteen antibiotics discs; amoxicillin-clavulanic acid (30 µg), amoxicillin (20 µg), piperacillin-tazobactam (36 µg), ticarcillin-acide clavulanique (85 µg), pefloxacin (5 µg), ofloxacin (5 µg), nalixidic acid (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), céfoxitine (10 µg), ceftriaxone (30 µg), cefepime (30 µg), imipénem (10 µg), ertapénem (10 µg), trimethoprim-sulphamethoxazole (25 µg), fosfomycine (200 µg) from five classes of antibiotic were used. Plates were incubated at 37°C for 18 - 24 hours. Diameters of inhibition zones were measured in millimetres (mm) and interpreted according to CASFM reference values. Multidrug resistance index (MDR) was defined as resistance to three or more antibiotics tested, while extensive drug resistance index (XDR) was defined as resistance to more than five antibiotics, as described by Magiorakos *et al.* [22].

Ethical and Legal Considerations

This study received ethical approval from the Ethical Committee of the Delegation of Public Health for the Centre Region Cameroon, under approval number

009702/CRERSHC/2022/09. A research authorization was also obtained from the directors of each participating hospital. Anonymity of participants and confidentiality of results were scrupulously respected.

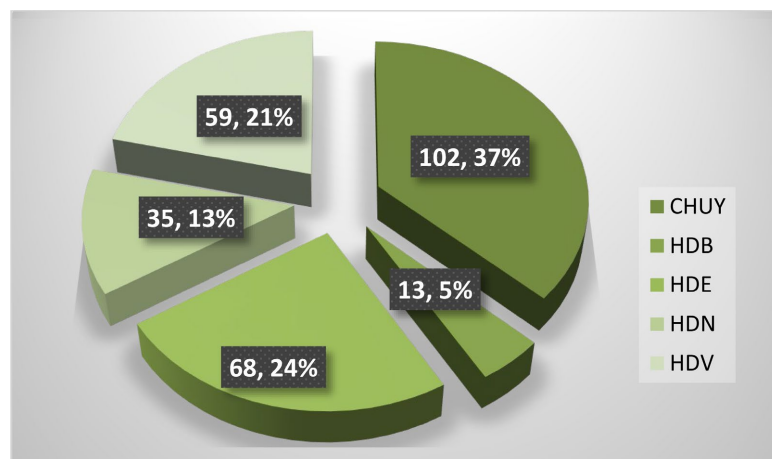
Data Management and Analysis

Data were entered and cleaned in MS Excel before exporting using Statistical Package for Social Sciences (SPSS) version 20 for analysis.

3. Results

3.1. Socio-Demographic Characterization of the Study Population

The study involved 277 participants with positive Widal test. Participant's age ranged from 01 to 89 years with mean age of 33 ± 1.3 years. They were recruited in varied proportion in different hospitals as indicated in **Figure 1**. Majority of the study participants were females ($n = 175$; 63.17%). Most of the participants ($n = 151$, 54.51%) had attained high school and 64 (23.1%) had no educational status (**Table 1**).



Legend: CHUY = Yaounde University teaching Hospital; HDB = Biyem-Assi District Hospital; HDE = Efoulan District Hospital; HDN = Nkoldongo District Hospital; HDV = MvogAda District Hospital.

Figure 1. Proportion of participants according to inclusion side.

Table 1. Proportion of Coinfection, *Salmonella* spp. and *Plasmodium* spp. among positive Widal test patients in relation to socio-demographic characteristics.

Characteristics	Total N = 277	Coinfection ¹ N = 12 ²	95% CI	<i>Salmonella</i> spp. ¹ N = 11 ²	95% CI	<i>Plasmodium</i> spp. ¹ N = 113 ²	95% CI	Négatif N = 141 ²	p-value ³
Sex									0.8
Male	102 (36.82%)	3 (25%)	6.7%, 57%	5 (45%)	18%, 75%	41 (36%)	28%, 46%	53 (38%)	
Females	175 (63.17%)	9 (75%)	43%, 93%	6 (55%)	25%, 82%	72 (64%)	54%, 72%	88 (62%)	
Age Group									0.2
>50	65 (23.46%)	0 (0%)	0.00%, 30%	1 (9.1%)	0.48%, 43%	27 (24%)	17%, 33%	37 (26%)	
1 - 10	34 (12.27%)	1 (8.3%)	0.44%, 40%	1 (9.1%)	0.48%, 43%	11 (9.7%)	5.2%, 17%	21 (15%)	

Continued

11 - 20	33 (11.91%)	2 (17%)	2.9%, 49%	4 (36%)	12%, 68%	13 (12%)	6.5%, 19%	14 (9.9%)
21 - 30	55 (19.85%)	3 (25%)	6.7%, 57%	3 (27%)	7.3%, 61%	25 (22%)	15%, 31%	24 (17%)
31 - 40	52 (18.77%)	2 (17%)	2.9%, 49%	2 (18%)	3.2%, 52%	20 (18%)	11%, 26%	28 (20%)
41 - 50	38 (13.71%)	4 (33%)	11%, 65%	0 (0%)	0.00%, 32%	17 (15%)	9.3%, 23%	17 (12%)
Education								0.7
None	64 (23.1%)	1 (8.3%)	0.44%, 40%	2 (18%)	3.2%, 52%	22 (19%)	13%, 28%	39 (28%)
Primary	21 (7.58%)	1 (8.3%)	0.44%, 40%	0 (0%)	0.00%, 32%	8 (7.1%)	3.3%, 14%	12 (8.5%)
Secondly	41 (14.80%)	1 (8.3%)	0.44%, 40%	2 (18%)	3.2%, 52%	19 (17%)	11%, 25%	19 (13%)
Tertiary	151 (54.51%)	9 (75%)	43%, 93%	7 (64%)	32%, 88%	64 (57%)	47%, 66%	71 (50%)

¹Criteria associated to p-value; ²n (%); ³Fisher's Exact Test for Count Data with simulated p-value; Abbreviation: CI= Confidence interval.

3.2. Proportion of *Salmonella* spp., *Plasmodium* spp. and *Salmonella* spp./*Plasmodium* spp. Coinfection among Positive Widal Patients

Among the 277 participants included in the study, 23 (8.4%) participants were positive for *Salmonella* spp., 113 (40.8%) were *Plasmodium* spp. positive, and 12 (4.4%) participants coinfection *Salmonella* spp. and *Plasmodium* spp. (Table 2). The clinical features of the positive Widal patients are summarized in Table 2. Only vomiting (p = 0.003), diarrhoea (p = 0.008) and fatigue (p = 0.002) of coinfecting patients, showed statistical significant differences when compared with the clinical features with no coinfecting participants.

Concerning the API 20E identification system for Enterobacteriaceae, all the 23 isolates confirm to be *Salmonella* spp.

Table 2. Clinical features of participants evaluated for *Salmonella* spp., *Plasmodium* spp. and coinfection.

Parameter	Coinfection N = 12 ¹	p-value ²	<i>Salmonella</i> spp. N = 11 ¹	p-value ²	<i>Plasmodium</i> spp. N = 113 ¹	p-value ²
Fever	10 (83%)	0.494	11 (100%)	0.226	103 (91%)	0.089
Headache	9 (75%)	0.540	9 (81%)	0.407	90 (79%)	0.695
Diarrhoea	7 (58%)	0.008	7 (63%)	0.001	23 (20%)	0.001
Constipation	5 (41%)	0.209	3 (27%)	0.729	22 (19%)	0.392
Abdominal discomfort	8 (66%)	0.700	8 (72%)	0.020	41 (36%)	0.013
Vomiting	7 (58%)	0.003	7 (63%)	0.001	25 (22%)	0.040
Chills	4 (33%)	0.760	8 (72%)	0.001	5 (4%)	0.001
Fatigue	8 (66%)	0.002	5 (45%)	0.002	25 (22%)	0.003

¹n (%); ²Fisher's Exact Test for Count Data with simulated p-value.

3.3. Factors Associated with *Plasmodium* spp. and *Salmonella* spp. Infections

Salmonella spp. and *Plasmodium* spp. coinfection were usually associated with

poor toilet facilities, consumption of unhygienic water, poor handwashing habits, and non-usage of insecticide treated bed nets. During this study, a statistically significant association was observed between the type of water consumed and the infected groups ($p < 0.001$). Patients who consumed water from combined sources (Forage and Municipal Tap Water) represented a substantial proportion of the coinfection cases.

Handwashing practices were high across all groups, with more than 90% of participants reporting that they washed their hands regularly. No significant association was observed between this practice and the presence of infections. Regarding the use of mosquito nets, approximately 60% of participants reported sleeping under a mosquito net. However, there was no statistically significant difference between the infectious groups ($p = 0.8$), as shown in **Table 3**.

Table 3. Factors associated with *Plasmodium* spp. and *Salmonella* spp. infections.

Characteristic	Coinfection N = 12 ¹	95% CI	<i>Salmonella</i> spp. N = 11 ¹	95% CI	<i>Plasmodium</i> spp. N = 113 ¹	95% CI	Negatif N = 141 ¹	95% CI	p-value ²
Hand washing									0.2
Yes	12 (100%)	70%, 100%	9 (82%)	48%, 97%	109 (96%)	91%, 99%	135 (96%)	91%, 98%	
Often	0 (0%)	0.00%, 30%	2 (18%)	3.2%, 52%	4 (3.5%)	1.1%, 9.4%	6 (4.3%)	1.7%, 9.4%	
Mosquito net use									0.8
No	6 (50%)	25%, 75%	4 (36%)	12%, 68%	42 (37%)	28%, 47%	53 (38%)	30%, 46%	
Yes	6 (50%)	25%, 75%	7 (64%)	32%, 88%	71 (63%)	53%, 72%	88 (62%)	54%, 70%	
Types of water									<0.001
Forage	1 (8.3%)	0.44%, 40%	3 (27%)	7.3%, 61%	24 (21%)	14%, 30%	33 (23%)	17%, 31%	
Forage, Mineral	0 (0%)	0.00%, 30%	0 (0%)	0.00%, 32%	21 (19%)	12%, 27%	15 (11%)	6.3%, 17%	
Forage, MTW	8 (67%)	35%, 89%	2 (18%)	3.2%, 52%	35 (31%)	23%, 40%	43 (30%)	23%, 39%	
Mineral	1 (8.3%)	0.44%, 40%	2 (18%)	3.2%, 52%	18 (16%)	10%, 24%	38 (27%)	20%, 35%	
MTW	2 (17%)	2.9%, 49%	1 (9.1%)	0.48%, 43%	13 (12%)	6.5%, 19%	4 (2.8%)	0.91%, 7.6%	
MTW, weal	0 (0%)	0.00%, 30%	3 (27%)	7.3%, 61%	2 (1.8%)	0.31%, 6.9%	8 (5.7%)	2.7%, 11%	

¹n (%); ²Fisher's exact test; Abbreviation: CI = Confidence Interval; MTW = Municipal Tap Water.

Serotyping of *Salmonella* spp.

The 23 confirmed *Salmonella* isolates were classified into two clinically distinct groups as presented in (**Figure 2**). Typhoidal *Salmonella* (TS) accounted for 7 of 23 isolates (30.4%), represented exclusively by *S. Paratyphi* B; notably, *S. Typhi* was not recovered from any participant. Non-typhoidal *Salmonella* (NTS) comprised the remaining 16 isolates (69.6%), distributed across six serovars: *S. dublin* (n = 5; 21.7%), *S. typhimurium* (n = 5; 21.7%), *S. enteritidis* (n = 3; 13.0%), *S. Kentucky* (n = 1; 4.3%), *S. Arizonae* (n = 1; 4.3%), and *S. Schleissheim* (n = 1; 4.3%).

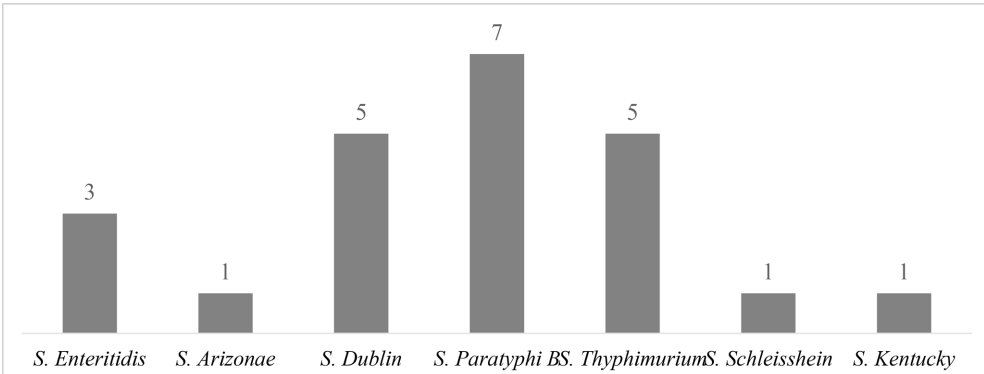


Figure 2. Proportion of Serotypes according to *Salmonella* spp. isolates.

Antimicrobial Susceptibility Profile of *Salmonella* spp. Isolates

The overall analysis shows a heterogeneous distribution of antimicrobial resistance as presented in (Figure 3). The highest resistance rates were observed for amoxicillin-clavulanic acid (AMC; 23/23; 100%), piperacillin-tazobactam (PIT/PTZ; 19/23; 83%), pefloxacin (PEF; 18/23; 82%), as well as ofloxacin (OFX) and nalidixic acid (NA), each with (13/23; 57%). However intermediate levels of resistance were observed for imipenem (IMP; 10/23; 43%), ertapenem (ETP; 5/23; 22%), trimethoprim-sulfamethoxazole (SXT; 5/23; 22%), and ciprofloxacin (CIP; 4/23; 17%). In contrast, low resistance rates were found for amoxicillin (AM; 3/23; 13%), ticarcillin-clavulanic acid (TCC; 2/23; 9%), cefepime (FEP; 2/23; 9%) and levofloxacin (LEV; 2/23; 9%). No resistance was observed for ceftriaxone (CRO), ceftiofloxacin (FOX), or fosfomycin (FOS).

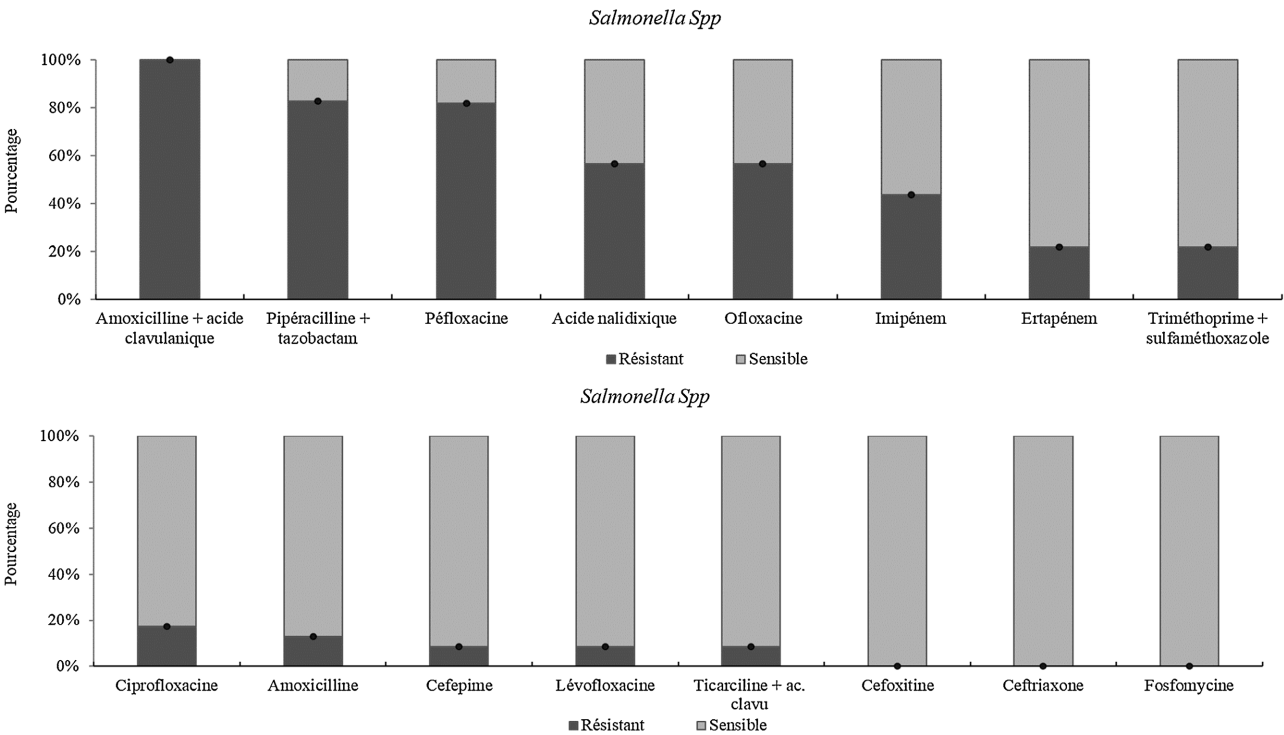


Figure 3. Susceptibility profile of *Salmonella* spp. isolates to selected antibiotics.

Antimicrobial Resistance by Serovar Group: Typhoidal Salmonella vs. Non-Typhoidal Salmonella

Resistance profiles differed substantially between TS and NTS isolates (**Table 4**).

Table 4. Occurrence of MAR index in *Salmonella* spp. isolates.

Hospital	Isolate ID	Type infection	Serotype	Group	Antibiotics Resistance Profile	MAR Index	Resistant Classes
Typhoidal Salmonella (TS): <i>S. Paratyphi B</i> only (n = 7)							
HDB	HDB06	Coinfection	<i>S. Paratyphi B</i>	TS	AMC, PEF	0.125	2
HDV	HDV36	Coinfection	<i>S. Paratyphi B</i>	TS	AMC, PIT, OFX, NA, PEF, IMP	0.375	3
HDN	HDN04	Coinfection	<i>S. Paratyphi B</i>	TS	AMC, PIT, PEF, IMP, ETP, NA	0.375	3
HDB	HDB11	Coinfection	<i>S. Paratyphi B</i>	TS	AMC, PIT, OFX, NA, PEF	0.3125	2
HDN	HDN02	Monoinfection	<i>S. Paratyphi B</i>	TS	AMC, OFX, NA, PEF	0.25	2
CHU	CHU06	Monoinfection	<i>S. Paratyphi B</i>	TS	AMC, AM, PIT, OFX, CIP, NA, PEF	0.4375	2
CHU	CHU05	Monoinfection	<i>S. Paratyphi B</i>	TS	AMC, PIT, FEP, OFX, NA, PEF, IMP, ETP, SXT	0.5625	5
Non-Typhoidal Salmonella (NTS)—<i>S. dublin</i>, <i>S. typhimurium</i>, <i>S. enteritidis</i>, <i>S. kentucky</i>, <i>S. arizonae</i>, <i>S. schleissheim</i> (n = 16)							
HDN	HDN14	Coinfection	<i>S. typhimurium</i>	NTS	AMC, PIT	0.125	2
HDV	HDV05	Coinfection	<i>S. dublin</i>	NTS	AMC, PIT, PEF	0.1875	2
HDN	HDN12	Coinfection	<i>S. dublin</i>	NTS	AMC, PIT, OFX, IMP	0.25	3
HDV	HDV43	Coinfection	<i>S. dublin</i>	NTS	AMC, PIT, PEF, IMP	0.25	3
CHU	CHU02	Coinfection	<i>S. enteritidis</i>	NTS	AMC, PIT, OFX, NA, PEF	0.3125	2
CHU	CHU01	Coinfection	<i>S. enteritidis</i>	NTS	AMC, PIT, NA, PEF, IMP, ETP	0.375	3
HDN	HDN19	Coinfection	<i>S. Kentucky</i>	NTS	AMC, PIT, OFX, CIP, NA, LEV, PEF, IMP, SXT	0.5625	4
HDV	HDV30	Monoinfection	<i>S. typhimurium</i>	NTS	AMC, PIT	0.125	1
HDE	HDE05	Monoinfection	<i>S. enteritidis</i>	NTS	AMC, PIT, FEP	0.1875	2
HDE	HDE40	Monoinfection	<i>S. typhimurium</i>	NTS	AMC, PIT, PEF	0.1875	2
HDN	HDN25	Monoinfection	<i>S. typhimurium</i>	NTS	AMC, AM, PIT, OFX, PEF	0.3125	2
HDE	HDE23	Monoinfection	<i>S. Schleissheim</i>	NTS	AMC, PIT, OFX, NA, PEF	0.3125	2
HDE	HDE01	Monoinfection	<i>S. typhimurium</i>	NTS	AMC, PIT, OFX, NA, PEF, ETP	0.375	2
CHU	CHU03	Monoinfection	<i>S. Arizonae</i>	NTS	AMC, TCC, PIT, LEV, PEF, IMP, SXT	0.4375	4
HDV	HDV09	Monoinfection	<i>S. dublin</i>	NTS	AMC, AM, OFX, CIP, NA, PEF, IMP, SXT	0.5	4
CHU	CHU04	Monoinfection	<i>S. dublin</i>	NTS	AMC, PIT, OFX, NA, PEF, IMP, CIP, ETP, SXT	0.5625	4

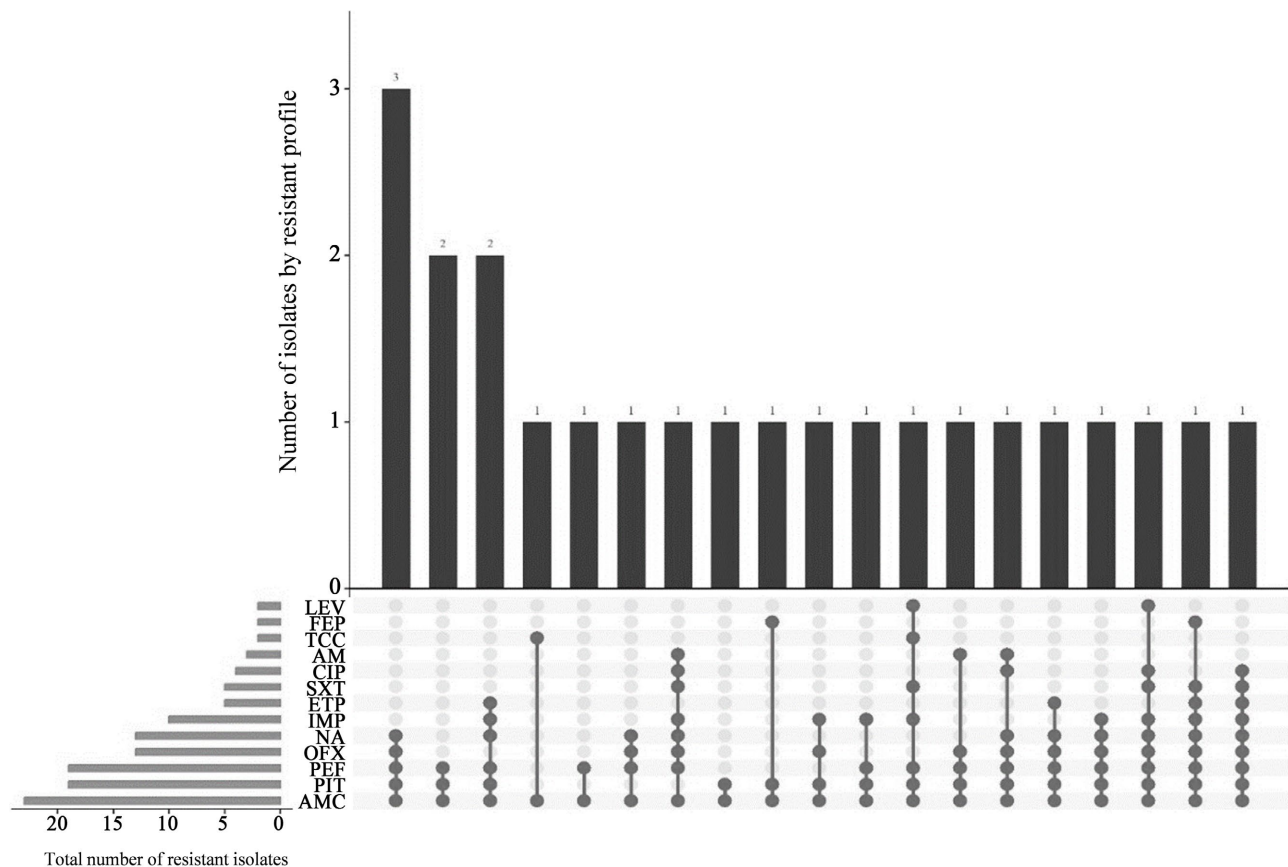
AMC = amoxicillin-clavulanic acid; PIT = piperacillin-tazobactam; PEF = pefloxacin; OFX = ofloxacin; NA = nalidixic acid; IMP = imipenem; ETP = ertapenem; SXT = trimethoprim-sulfamethoxazole; CIP = ciprofloxacin; AM = amoxicillin; TCC = ticarcillin-clavulanic acid; FEP = cefepime; LEV = levofloxacin, CRO = ceftriaxone, FOX = ceftiofur, FOS = fosfomycin; TS = Typhoidal Salmonella; NTS = Non-Typhoidal Salmonella.

Among the 7 typhoidal isolates (*S. Paratyphi* B), all showed resistance to AMC (7/7; 100%), 5 were resistant to PIT (71%), and 5 to PEF (71%). Two isolates were resistant to PIT and OFX/NA combinations, and three additionally showed IMP or ETP resistance. Crucially, no TS isolate showed resistance to third-generation cephalosporins (CRO, FOX) or fosfomycin, supporting the continued utility of these agents for confirmed enteric fever in this setting.

Among the 16 NTS isolates, resistance profiles were more diverse and generally more complex. All 16 showed AMC resistance, and the majority were resistant to PIT, PEF, OFX, and NA. Several NTS isolates exhibited carbapenem resistance: *S. Dublin* (HDV09, CHU04), *S. Enteritidis* (CHU01), and *S. Arizonae* (CHU03) were resistant to IMP or ETP. The *S. Kentucky* isolate (HDN19) displayed the broadest resistance profile, with resistance to AMC, PIT, OFX, CIP, NA, LEV, PEF, IMP, and SXT consistent with a MAR index of 0.5625.

The UpSet plot illustrates the diversity of co-resistance patterns in the 23 *Salmonella* spp. isolates (Figure 4). The most frequent profile corresponds to simultaneous resistance to AMC, NA, and PEF, suggesting substantial selective pressure on β -lactam combinations and quinolones and was observed in three isolates, all NTS.

However, the majority of the resistance combinations were observed only once, indicating a high diversity of resistance profiles among the *Salmonella* spp.



isolates. Some profiles also include resistance to carbapenems (IMP, ETP) and multiple fluoroquinolones (OFX, CIP, PEF), which may reflect the emergence of more complex multidrug-resistant phenotypes.

Occurrence of Multiple Antibiotic Resistance indexes (MAR)

The percentage occurrence of MAR index for *Salmonella* spp. isolates across various side of inclusion is presented in **Table 4**. Overall, 73.9% of all isolates were MAR (17/23). Among TS isolates, 5 of 7 (71.4%) met MDR criteria; among NTS isolates, 12 of 16 (75.0%) were MAR. Multiple antibiotic resistance indices ranged from 0.125 to 0.5625.

4. Discussion

Coinfection is the simultaneous attack of any host by multiple pathogenic species. In the case of this survey, emphasis was laid on *Salmonella* spp. and *Plasmodium* spp. coinfections. These are two endemic infections common in the tropics, and it is frequent to see patients suffering from the two pathologies at once. Importantly, the emergence and spread of multidrug-resistant *Salmonella* strains in such coinfecting patients represent a critical and underexplored threat. *Plasmodium* spp. infection induced immunomodulation that may enhance susceptibility to invasive *Salmonella* infections, while Widal test and overlapping clinical symptoms can lead to misdiagnosis and inappropriate antimicrobial use, thereby accelerating resistance selection. The present study aims to determine the prevalence of stool culture confirmed *Salmonella* spp. carriage among Widal-positive patients; assess the occurrence of concurrent *Salmonella* spp. and *Plasmodium* spp. coinfection; identify associated risk factors; and evaluate the antibiotic resistance profiles of typhoidal and non-typhoidal *Salmonella* isolates.

In this study, among 277 Widal-positive participants, *Salmonella* spp. was confirmed in 23 (8.4%), *Plasmodium* spp. in 113 (40.8%), and concurrent infection in 12 (4.4%). These relatively low prevalences observed in this study may be attributable to the urban setting in which it was conducted, where the population likely has greater awareness of infection risk factors and improved access to preventive measures. These low prevalence's observed in this study are consistent with the findings of Ndip *et al.*, who reported a low prevalence of *Salmonella* spp. and *Plasmodium* spp. coinfection 12 (6.8%), *Salmonella* spp. 14 (7.9%) and *Plasmodium* spp. 146 (82%) using similar methods in Kumba Cameroon [23].

In contrast, several studies conducted in Cameroon using Widal test method to determine the prevalence of malaria-typhoid coinfection, was reported in Yaoundé, Masumbe *et al.* found *Plasmodium* spp. prevalence of 44.8%, *Salmonella* spp. prevalence of 37.2%, and *Salmonella* spp. and *Plasmodium* spp. coinfection prevalence of 19.8% [24]. These differences of prevalences observed in these two methods is likely due to the use of the Widal test, which has lower specificity. Although both diseases are endemic in Cameroon, our findings indicate that malaria is the predominant cause of febrile illness, with a comparatively higher prevalence.

Analysis by sex in this study showed that, the proportion of female with positive culture was higher than that of male. Among the 12 coinfecting participants 9 (75%) were females, while 3 (25%) were males. The high population of females obtained in this study could be explained by the fact that women prefer formal hospital treatment than men. This result is in line with the findings of Ngai and colleagues in Dschang-Cameroon, which revealed an estimated prevalence of 72 (56.31%) females in 2024 [25], stipulating that women usually prefer hospital consultations prior to any medication intake.

Concerning clinical features, among the 12 coinfecting participants, 10 (83.3%) reported fever, while eight (66.7%) experienced abdominal discomfort. Headache was reported by 9 participants (75%), and fatigue by 8 (66.7%). Similar clinical manifestations are documented by (Edet *et al.*, 2016; Masumbe *et al.*, 2025; Rufai *et al.*, 2023). Statistical analysis revealed that vomiting ($p < 0.003$), diarrhoea ($p < 0.008$), and fatigue ($p < 0.002$) were significantly associated with coinfection. This association could be explained by the coexistence of these infections that amplify immune activation, physiological stress and pathophysiological mechanisms, resulting in more pronounced clinical features compared to mono-infections. These findings are consistent with those reported by Nodem *et al.* in Adamawa, who demonstrated that headache, vomiting, and diarrhoea were significantly associated with *Plasmodium* spp. and *Salmonella* spp. coinfection [26].

The risk factors identified in this study included the type of toilet facility, hand-washing habits, bed net usage, and source of drinking water. Among these, the source of drinking water ($p < 0.001$) was the only factor significantly associated with *Salmonella* spp. infection. This can explain the fact that *Salmonella* spp. is primarily transmitted via the faecal-oral route through the ingestion of contaminated water and food; therefore, the source of drinking water constitutes a significant determinant of infection risk. Similar findings were reported by Achonduh-Atijegbe *et al.* [27] observed a significant association between the main source of drinking water and typhoid fever among participants ($p = 0.047$).

A critical finding of this study is the serovar distribution among confirmed isolates. Of the 23 *Salmonella*-positive cases, only 7 (30.4%) were typhoidal serovars and exclusively *S. Paratyphi* B, while 16 (69.6%) were non-typhoidal serovars (NTS) including *S. dublin*, *S. typhimurium*, *S. enteritidis*, *S. Kentucky*, *S. Arizona*, and *S. Schleissheim*. Notably, *S. Typhi* was not recovered from any participant. These NTS serovars do not cause typhoid fever and are not specifically detected by the Widal test, which is specific to *S. Typhi* and *S. Paratyphi* O and H antigens. The positive Widal results in patients harbouring NTS organisms most likely represent false positives, whether arising from malaria-induced polyclonal B-cell activation [13] [14], prior typhoid vaccination, past *S. Typhi* exposure, or cross-reactivity with unrelated pathogens [12] [15]. This reinforces the importance of not conflating Widal test positivity with confirmed typhoidal *Salmonella* infection, and highlights the clinical risk of prescribing antibiotics for presumed typhoid fever in patients who have NTS gastroenteritis or invasive NTS disease instead.

Antimicrobial resistance (AMR) in *Salmonella* spp. is increasingly worsening in low and middle-income countries [28]. Despite the establishment of AMR surveillance systems in Cameroon [29], it remains a significant public health challenge, characterized by a high prevalence of multidrug resistance (MDR) and widespread inappropriate antibiotic use, including self-medication. In the present study, *Salmonella* spp. exhibited variable resistance profiles across the different classes of antibiotics tested. Regarding enteric fever specifically, the 7 TS isolates (*S. Paratyphi* B) all showed resistance to AMC (100%) and the majority to PIT (71%) and PEF (71%); These findings contrast with those reported by Ndima Etouke *et al.* [30], who observed a lower resistance rate of 30% to amoxicillin-clavulanic acid among *Salmonella* isolates in Dschang, Cameroon. However, no TS isolate was resistant to third-generation cephalosporins (ceftriaxone, cefoxitin) or fosfomycin. This is consistent with findings from Nigeria by Ohanu *et al.* [31], Cameroon by Ndima Etouke *et al.* [30], and Ethiopia by Amsalu *et al.* [32], which have also reported absent or very low cephalosporin resistance in typhoidal serovars from stool cultures. The preserved susceptibility to ceftriaxone among TS isolates supports its continued empirical use for confirmed enteric fever in this setting.

In contrast, NTS isolates exhibited substantially more complex and heterogeneous resistance profiles. All 16 NTS isolates were AMC-resistant, and many showed resistance to multiple fluoroquinolone. More concerning, several NTS isolates displayed carbapenem resistance, including *S. dublin*, *S. enteritidis*, *S. Arizonae*, and *S. Kentucky*. The *S. Kentucky* isolate (HDN19) showed resistance to nine antibiotic agents across four classes, meeting the criteria for XDR. This level of resistance is clinically alarming for a pathogen capable of causing invasive bacteraemia, particularly in immunocompromised or malnourished patients. NTS invasive disease represents a distinct syndrome from enteric fever, with different management implications.

Furthermore, the level of resistance to quinolones observed in this study was generally comparable to previous reports, although slightly higher. More than half of the *Salmonella* spp. isolates were resistant to nalidixic acid (57%), a value that is higher compare to those reported in Dschang, Cameroon (34.2%) by Ndima Etouke *et al.* [30] and in Nigeria (37.5%) by Amsalu *et al.* [32]. In contrast, resistance to ciprofloxacin remained relatively low (17%), although still higher than the 12.5% reported in Ethiopia by [33]. The high percentage of resistance to the commonly used antibiotics could be linked to their misuse and abuse in the area under study and in the treatment of other unrelated infections such as malaria [33]; also, clinicians often rely on Widal results to initiate antibiotic therapy, even though the test has limited accuracy [34]. Additionally, incomplete treatment due to many reasons in developing countries may also be the factor contributing to the development of antibiotic resistance. These results are in agreement with previous reports of a worldwide occurrence of MDR *Salmonella* spp.

The overall MDR rate of 73.9% (17/23 isolates) is high, though lower than the 86.2% reported by Ndima Etouke *et al.* [30] in Dschang, Cameroon, and compa-

rable to the 66.8% reported in Ethiopia [33]. When disaggregated, MDR rates were 71.4% (5/7) among TS and 75.0% (12/16) among NTS isolates.

The high overall resistance observed including to quinolones used empirically in febrile illness, likely reflects widespread antibiotic use in this setting. Clinicians frequently initiate antibiotic therapy based on Widal results despite its limited diagnostic accuracy [34], and self-medication with antibiotics is common. Incomplete treatment courses further accelerate resistance selection [33].

5. Conclusion

Plasmodium spp. was the predominant cause of febrile illness among Widal-positive patients in this study, identified in 40.8% of participants. Stool culture confirmed *Salmonella* spp. carriage was detected in 8.4%, and concurrent infection in 4.4%. Critically, the majority of confirmed *Salmonella* isolates (69.6%) were non-typhoidal serovars. Third-generation cephalosporins retained full activity against typhoidal isolates, supporting their use in confirmed enteric fever. Non-typhoidal isolates showed more complex resistance profiles, including carbapenem resistance, representing a distinct clinical and public health threat that warrants separate surveillance. Drinking water source was the only factor significantly associated with *Salmonella* spp. carriage ($p < 0.001$). The overall MDR rate was 73.9%. These findings collectively underscore the inadequacy of the Widal test as a standalone diagnostic tool in malaria-endemic settings and highlight the urgent need for culture-based confirmation to guide appropriate antimicrobial therapy and reduce selection pressure for MDR strains.

6. Limitation of the Study

This study has some limitations. Due to poor lab settings in the hospitals where the work was carried, stool culture was commonly used, although it has relatively low sensitivity with respect to blood culture.

Author Contributions

Guiamdjo Simo, Gonsu and Mbacham designed the work. Guiamdjo Simo collected the samples. Guiamdjo Simo and Chafa performed the analyses and drafted the manuscript. All authors interpreted the results, read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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