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Research Article

Characterization of Salmonella and other Gram Negative Bacterial Pathogens obtained from Stool and Blood, a Cross-Sectional Study at Cape Coast Teaching Hospital, Ghana

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Abstract

Background: *Salmonella* infections are of serious public health concern since these bacteria frequently cause foodborne illness, human gastroenteritis and bacteremia worldwide. Though invasive *salmonella* are uncommon in developed nations it is still common in developing countries. *Salmonella* is frequently isolated in Cape coast Hospital and are often found to be drug resistant. This study therefore determined the antimicrobial susceptibility and PFGE patterns of *Salmonella* and other gram negative organisms isolated.

Methods: Cross-sectional study involving 971 samples (463 blood and 508 stool) was carried out over 13 month period. Isolates were identified and antimicrobial susceptibility test performed. The Salmonellae were serotyped and ESBL and fingerprinted by PFGE. Data obtained was analyzed with SPSS Version 21.0 taking confidence level of 95% and p values<0.05.

Results: A total of 17 Salmonellae being 3.3% and 3 *Shigella flexneri* recording 0.6% were recovered from the stool samples. The *Salmonella* serovars encountered were *Salmonella Typhi* (13/17) and *Salmonella Typhimurium* (4/17). There was 82% multidrug resistance among the *Salmonellae* with none producing ESBL. The PFGE analysis of the similar *Salmonella* serovars indicated they were different clones. Blood samples yielded different bacteria types (69/463, 14.9%) other than *Salmonella*.

Conclusion: *Salmonella* was not obtained from blood but was obtained from stool with 3.3% prevalence. There was a high prevalence of bacteremia caused by other pathogens recording 14.9%. There was high multidrug resistance rate of 98.6% among the blood isolates and 82% among the *Salmonella* isolates.

Keywords: Drug Resistance; *Salmonella* Serovars; Blood Culture; Stool Culture; Bacteria

Abbreviations

ESBL: Extended-Spectrum Beta-Lactamase; TS: Typhoidal Salmonella; NTS: Non-typhoidal Salmonella; PFGE: Pulsed-Field Gel Electrophoresis; WHO: World Health Organization; API: Analytical Profile Index; CLSI: Clinical and Laboratory Standards Institute.

Background

Salmonellae are enteric gram negative organisms that are widely distributed in nature. They can reside as common commensals in the gastrointestinal tracts of animals and man or cause diseases that range from self-limited diarrhea to bacteremia with enteric fever [1]. They sometimes invade vascular structures, bone or other localized sites [2]. While some *Salmonellae* are ubiquitous, others are highly host adapted, infecting only a limited number of animal types [3]. The most significant human host-adapted Salmonellae are S. Typhi which cause typhoid fever. Human beings remain the only known host or reservoir for *S. Typhi* [4]. Other *Salmonella* serotypes are important zoonotic pathogens in humans [5]. Contaminated food and meat have become a key route of spread for non-typhoidal *Salmonella* because there are a great number of animal reservoirs. Frequent animal reservoirs include turkeys, chickens, cattle and pigs; several other wild and domestic animals also harbour these bacteria [6,7].

Although several reports have it that *Salmonella* infections has declined significantly, particularly in developed world, there are continued difficulties in *Salmonellae* control [8] in the developing countries. Challenges posed include the extensive dissemination of *Salmonellae* in food [9], antibiotics resistance [10,11] and capacity building to improve early epidemic detection via regularly *Salmonellae* subtyping using molecular methods and a regular surveillance report especially in the developing world [12]. In spite of the clinical importance of *Salmonella* diseases, surveillance data globally continue to be inadequate and is more so typified by the deficit of researches and publications from Africa particularly in central, eastern and western Africa [13].

Factual information and statistics on *Salmonella* infection are limited in many African countries, South and Central America as well as Asia where just 1 to 10% of incidences are reported [14]. Currently, there is limited documentation on the incidence, antibiotic sensitivity patterns, ESBL production and genetic characterizations of *Salmonella* species and other gram negative bacterial pathogens in Ghana. No such work has been carried out in Cape Coast. Due to the emerging increase in *Salmonella* infections with a corresponding rise in antibiotic resistance in many parts of Ghana, there was the need to access the state of *Salmonella* infection in Cape Coast. This work helped to establish the prevalence of *Salmonella*, their antibiotic resistance patterns and genetic relatedness as well as whether they produce ESBL or not. It aimed at contributing information to national data on the strains of *Salmonella* in Ghana, their prevalence and antibiotic resistance patterns as well as current surveillance report on the antimicrobial resistance patterns and prevalence of other bacterial pathogens in Cape Coast.

Materials and Methods

This was a cross-sectional study carried out at the Cape Coast Teaching Hospital, which serves as the biggest and the main referral hospital in the Cape Coast Metropolis and the Central Region of Ghana. Participants included patients of all sexes and age groups in both the outpatient and inpatient departments. Samples were collected from 971 participants made up of 508 stool samples and 463 blood sample over a 13 month period (March, 2014 – April, 2015). The whole research was sectioned into two main stages. The first stage involved collection of samples, isolations, identifications and confirmations of Salmonellae and other pathogens based on their colonial morphologies or cultural characteristics, stains reactions, motility and biochemical and serological properties. The second stage involved antibiotics susceptibility testing of all isolates, Salmonellae screening for Extended Spectrum Beta Lactamases (ESBLs) production and genetic analysis employing Pulsed Field Gel Electrophoresis (PFGE) technique.

Sample collection and processing

Stool and blood samples were collected and processed for culture and subsequent bacteria isolation and identification using protocols described in the Guidelines on Standard Operating Procedures for Microbiology by WHO and Identification of Enterobacteriacae, National Standard Method, by Health Protection Agency [15].

Storage and final confirmation

Organisms which depicted all the characteristics of Salmonella according to the identification protocol above were stored for confirmation and further characterization. All the blood isolates and the three Shigella isolates from stool were also stored for confirmation and antimicrobial susceptibility testing. In storing, 850 µl of the bacterial broth (peptone water + pure bacteria culture) was thoroughly mixed 150 µl of sterile glycerol. The mixture was then stored in a regular -20°C freezer. Final confirmation of all isolates was done at the Noguchi Memorial Research Laboratory in Accra, Ghana using the Analytical Profile Index (API) system.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on all isolates using Kirby Bauer disk diffusion method as described by the Clinical Laboratory Standard Institute and the Guidelines on Standard Operating Procedures for Microbiology by WHO [16]. The antimicrobial discs used were ampicillin (10µg), co-trimoxazole (25µg), tetracycline (30µg), cefuroxime (30µg), gentamicin (10µg), levofloxacin (5µg), ceftriaxone (30µg), chloramphenicol (10µg), cefotaxime (30µg), ciprofloxacin (5µg), meropenem (10ug) and amikacin (30µg).

ESBL Screening of *Salmonella* Isolates

The initial screening for ESBL in the *Salmonella* isolates was done using single discs of cefpodoxime (10µg), ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg) at 20mm apart. The outcome of the initial screening was confirmed phenotypically using the combination disc methods consisting of: cefotaxime (30µg); cefotaxime/clavulanic acid (30µg/10µg) and ceftazidime (30µg); ceftazidime/clavulanic acid (30µg/10µg).

Any inhibition zone diameter greater than or equal to 5mm for each antibiotic tested in combination with clavulanic acid versus its inhibition zone when tested alone equals ESBL positive result.

Pulsed field gel electrophoresis (PFGE)

PFGE was carried out on only the *Salmonella* isolates employing the PulseNet international standardized protocol. Analysis of the generated patterns was performed using Molecular Analyst Fingerprinting Plus software with data sharing tools. *Salmonella* serovar *Braenderup* was utilized as a reference standard organism.

Data management and analysis

The data obtained was entered into Microsoft Excel spreadsheet and later imported into SPSS Version 21.0 software (Chicago, Ill, USA) for statistical analysis. Descriptive data analysis was done. Confidence level of 95% was adopted and p values <0.05 were considered significant.

Results

Sociodemographic characteristics of patients and the prevalence of isolates

A total of 971 samples consisting of 463 blood and 508 stool were collected. Females constituted 62.3% of participants. The ages of participants ranged from 1 day to 78 years with a modal age group of 0-20 year (44.7%). The sex and age distribution of participants are shown in Table 1.

		Number	Percentage (%)
Sex	Female	605	62.3
	Male	366	37.7
Total		971	100.0
Age group	0 - 20 years	434	44.7
	21- 40 years	386	39.8
	41 - 60 years	92	9.5
	> 60 years	59	6.0
Total		971	100.0

Table 1: Sex and Age distribution of participants.

The prevalence of bacteremia was 14.9% (69/463) but none of the isolates was *Salmonella*. On the other hand, 17 (3.3%) *Salmonellae* were recovered from the stool samples cultured. The *Salmonella* serotypes were Salmonella Typhi (13/17, 76.5%) and *Salmonella Typhimurium* (4/17, 23.5%) (Table). Other *Salmonella* serotypes were not identified. Three Shigella flexneri isolates (3/508, 0.6%) were also obtained from the stool samples.

With the *Salmonella* isolates, more females (10/17, 59%) were infected than males. The age groups mostly infected were 21-40years (10/17, 59%) and 0-20 (6/10, 35%). The most presented complaints among these patients from whom *Salmonella* was isolated were diarrhoea (n = 6, 35%) and gastroenteritis (n = 6, 35%). The 3 *Shigella flexneri* were isolated from 2 females (2/3, 67%) and 1 male (1/3, 33%).

For the blood pathogens isolated, as high as 65/69 (94%) were from in-patients. The 17 *Salmonellae* isolates were recovered from 11/17 (64.7%) out-patients and 6/17 (35.3) in-patients while all the *Shigella* isolates were recovered from out-patients.

Blood isolates

Staphylococcus aureus was the most prevalent isolate 24/69 (34.8%). This was followed by *Citrobacter freundii* (10/69, 14.5%), *Escherichia coli* (6/69, 8.7%) and *Pseudomonas aeruginosa* (6/69, 8.7%). The isolates obtained from blood are presented in Table 2.

Isolate	Number	Percentage (%)
S. aureus	24	34.8
Citrobacter freundii	*10*	14.5
Escherichia coli	6	8.7
Pseudomonas aeruginosa	6	8.7
Enterobacter sp.	5	7.3
Staphylococcus epidermidis	5	7.3
Streptococcus agalactiae	4	5.8
Klebsiella pneumoniae	3	4.3
Klebsiella oxytoca	2	2.9
Streptococcus pyogenes	2	2.9
Proteus vulgaris	1	1.4
Proteus mirabilis	1	1.4
Total	69	100.0

Table 2: Isolates obtained from blood cultures.

Antimicrobial resistance patterns of isolates

Antimicrobial resistance patterns of blood isolates

Although most of the isolates exhibited high resistance, none of them was resistant to all antibiotics used. Multi-drug resistance was 98.6%. Resistance to ampicillin was the most common (66/69, 96%), followed by tetracycline (63/69, 91%), co-trimoxazole (60/69, 87%) and cefuroxime (49/69, 71%). The antibiotics with least bacterial resistance were amikacin (2/69, 3%), meropenem (5/69, 7%), levofloxacin (7/69, 10%) and ciprofloxacin (12/69, 17%).

	Antibiotics											
Isolates	**CXM**	**TET**	**CHL**	**AMP**	**LEV**	**CTR**	**CIP**	**CTX**	**AMK**	**GEN**	**COT**	**MEM**
Citrobacter freundii (n =10)	60%	100%	60%	100%	0%	60%	10%	80%	10%	10%	100%	20%
Enterobacter sp (n = 5)	60%	80%	40%	80%	0%	80%	0%	80%	0%	40%	80%	20%
E. coli (n = 6)	67%	83%	33%	100%	17%	67%	67%	67%	0%	17%	67%	0%
Kleb. Oxytoca (n = 2)	100%	100%	100%	100%	0%	50%	0%	100%	0%	50%	100%	0%
Kleb. pneumoniae (n = 3)	100%	100%	100%	100%	0%	33%	0%	0%	0%	0%	100%	0%
Proteus mirabilis (n = 1)	100%	100%	0%	100%	0%	100%	0%	100%	0%	0%	100%	0%
Proteus vulgaris (n = 1)	0%	100%	100%	100%	0%	100%	0%	100%	0%	0%	100%	0%
P. aeruginosa (n = 6)	50%	100%	83%	100%	33%	67%	17%	33%	17%	0%	100%	0%
Staph. aureus (n = 24)	79%	83%	46%	96%	8%	83%	13%	58%	0%	38%	88%	4%
Staph. epidermidis (n=5)	80%	100%	80%	100%	20%	80%	40%	80%	0%	40%	100%	20%
Strep. agalatiae (n = 4)	50%	100%	50%	67%	33%	0%	33%	67%	0%	0%	50%	0%
Strep. pyogenes (n = 2)	100%	100%	100%	100%	0%	50%	0%	0%	0%	50%	50%	0%
TOTAL (n = 69)	71%	91%	58%	96%	10%	68%	17%	62%	3%	25%	87%	7%

Table 3: Antibiotics resistance patterns of isolates obtained from blood.

Key: GEN: Gentamycin; COT: Co-Trimoxazole; TET: Tetracycline; CIP: Ciprofloxacin; AMP: Ampicillin; LEV: Levofloxacin; CTX: Cefotaxime; CTR: Ceftriaxone; CHL: Chloramphenicol; AMK: Amikacin; CXM: Cefuroxime; MEM: Meropenem.

Isolates	**Number of Antibiotics**								
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Citrobacter freundii (n = 10)		1	1	3	1	2		2	
Enterobacter sp. (n = 4)				1	1		2		
Escherichia coli (n = 6)		1	1		1	1		2	
Klebsiella oxytoca (n = 2)					1		1		
Klebsiella pneumoniae (n = 3)				2	1				
Proteus mirabilis (n = 1)					1				
Proteus vulgaris (n = 1)					1				
Pseudomonas aeruginosa (n = 6)			1	1	2	1		1	
Staphylococcus aureus (n = 24)		1	3	4	6	5	3		2
Staphylococcus epidermidis (n = 5)					1	2	1	1	
Streptococcus agalactiae (n = 4)	1		1		1	1			
Streptococcus pyogenes (n =2)				1	1				

Table 4. Multidrug resistance pattern of isolates obtained from blood.

Antimicrobial resistance pattern of *Salmonella* isolates

None of the 17 isolates was resistant to all the antibiotics tested. Resistance to ampicillin, co-trimoxazole and tetracycline were 65% (11/17) each while none of the isolates was resistant to cefotaxime, ceftriaxone, gentamycin, amikacin, meropenem, levofloxacin and ciprofloxacin. Multi-drug resistance was 82% with the highest being 6 antibiotics (1/17, 6%), followed by 5 antibiotics (3/17, 18%) and 4 antibiotics (7/17, 41%). Details are presented in the table below.

Antimicrobial resistance pattern of *Shigella* isolates

There was multidrug resistance in all the 3 Shigella isolates with the highest number of antibiotic multiple resistance being 5. Resistance to tetracycline and co-trimoxazole were 100% each. No antimicrobial resistance was recorded against cefuroxime, cefotaxime, ceftriaxone, gentamycin, amikacin, meropenem, levofloxacin and ciprofloxacin.

ESBL test on *Salmonella* isolates

Initial screening showed that all the 17 Salmonella isolates were sensitive to cefpodoxime (10µg), ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). In a phenotypic confirmation with combine discs of cefotaxime (30µg); cefotaxime/clavulanic acid (30µg/10µg) and ceftazidime (30µg); ceftazidime/clavulanic acid (30µg/10µg), none had a zone diameter difference of ≥5mm. Hence none of the Salmonellae was an ESBL producer.

Number (%) of resistant strains							
Type of antibiotic	S. Typhi (N = 13)			S. Typhimurium (N = 4)			Total (N =17)
	R (%)	IS (%)	S (%)	R (%)	IS (%)	S (%)	
Tetracycline	9 (69%)	1 (8%)	3 (23%)	2 (50%)	0 (0%)	2 (50%)	11 (65%)
Co-trimoxazole	11(84%)	1(8%)	1(8%)	0 (0%)	1 (25%)	3 (75%)	11 (65%)
Meropenem	0 (0%)	0 (0%)	13 (100%)	0(0%)	0 (0%)	4(100%)	0 (0%)
Ampicillin	11(85%)	2 (15%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	11 (65%)
Cefuroxime	0 (0%)	3 (23%)	10 (77%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Chloramphenicol	2 (15%)	1 (8%)	10 (77%)	0 (0%)	2 (50%)	2 (50%)	2 (12%)
Cefotaxime	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Gentamycin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Amikacin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Ciprofloxacin	o (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)

Table 5: Antibiotic resistance pattern of *Salmonella* isolates.

Key: S: Susceptible; IS: Intermittently Susceptible; R: Resistant

Pulsed-Field Gel Electrophoresis (PFGE)

In the genetic analysis using pulsed-field gel electrophoresis, there was indistinguishable closeness in the fingerprints among the Salmonella Typhi. No notable difference was also observed among the Salmonella Typhimurium gel pattern analyzed. However, a slight difference was observed in the Salmonella Typhi (Lanes C, F) pattern which suggests the possibility of different clones circulating in Cape Coast Municipality. The details of these observations could only be determined by the BioNumeric software which was not available for this study. The gel patterns are presented in Figures 1 and 2.

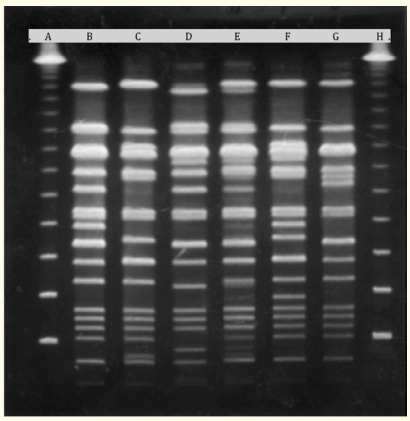

Figure 1: Pulsed-field gel electrophoresis picture showing the genome fingerprint of *S. Typhi*.

A, H – Salmonella Braenderup (control organism)

B, C, D E, F, G – Salmonella Typhi (test organism)

Discussion

Frequency of *Salmonella* and *Shigella* isolation

Invasive serovars of *Salmonellae* (TS and NTS) have emerged as a critical public health issue in sub-Saharan Africa [17-19]. According to studies by Berkley., *et al*. and Gordon., *et al*. NTS have been documented as the most frequent cause of invasive bacterial infections in some parts of Africa including Kenya (170/26986, 0.6%), Ivory Coast (21/319, 6.6%) and Malawi (2439/27581, 8.8% and 2517/35197, 7.2%) [19,20]. However, in this current study, no Salmonella was found amongst the 69/463 (14.9%) blood isolates obtained. A surveillance study carried out by Kwambana-Adams., *et al*. in the Gambia, documented a 0.8% prevalence of Salmonella in blood cultures [21] whilst Mohanty., et al. in India also documented 0.69% prevalence [22]. On the contrary, an earlier report of 6.5%

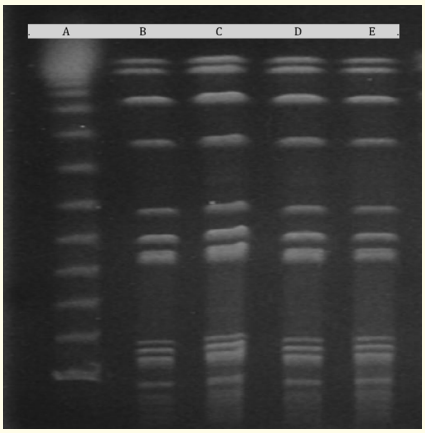

Figure 2: Pulsed-field gel electrophoresis picture showing the genome fingerprint of S. Typhimurium
A –Salmonella Braenderup (control organism)
B, C, D, E – Salmonella Typhimurium (test organism)

(181/2768) prevalence of Salmonella bacteraemia has been documented by a research carried at the Korle-Bu Teaching Hospital, Accra-Ghana [13]. The variations noticed among these studies could be attributed to the relative contributions of non-typhoidal and typhoidal serovars to infections since they vary in places and times [23], even within same countries [19,24].

Frequency of *Salmonella* and *Shigella* isolation

There was *Salmonella* prevalence of 3.3% (17/508) amongst the cultured stool samples. This is similar to the result of a study in India which documented a prevalence of 4.2% [25] and in Ethiopia which reported 1.08% prevalence in stool cultures [26]. Others low prevalence rates documented include: Babylon province 0% [27], 2.5% [28], 1.6% [29] and recently in Kenya 0% [30] by Musyoki., et al. The low prevalence rate witnessed in this study among others, might be the result of increasing awareness of the people with regard to environmental and personal hygiene by the activities of health institutions and other partners. In contrast to this current finding however, researches carried out at different parts of the world have documented higher *Salmonellae* prevalence in stool: Nasarawa State in Nigeria, 16.9% [31], Bangladesh, 16% [32] and Ethiopia, 11.5% [33].

With the background knowledge that typhoidal infections generally occur as a result of ingesting contaminated food or water [31], the low isolation rate of typhoidal Salmonellae in this research, may be attributed to the availability of portable and clean drinking water as well as improved environmental hygiene among the study population.

Frequency of *shigella* isolation from stool cultures

Three *Shigella* flexneri (3/508, 0.6%) were isolated from the stool samples. A similar finding of 0.5% prevalence in rural areas and 0.4% in urban areas has been documented in Korea [34]. Again, lower prevalence rates have been documented in China (11.24 per 100,000 persons) and several other developed countries, like England, USA, Australia and France (1.8–6.5 per 100,000 persons) [35]. However, higher prevalence rate of Shigella have also been reported elsewhere across the globe like Pakistan, 6.2% [36], Bangladesh, 8% [37], Nepal, 4% [38] and Ethiopia, 8.7%, 5.7% in diarrheic children, diarrheic adults respectively [39]. The low rate of isolation observed in this present study could be attributed to a variety of factors including: continuous hygiene educational programs at the community and schools, aggressive infection-control measures in the hospitals and healthcare centres and possible under-testing or investigation of shigellosis cases by general practitioners.

Prevalence of *Salmonella* serovars

In this work, Typhoidal Salmonellae (TS), 76.5% was more prevalent than Non-Typhoidal Salmonellae (NTS) 23.5%. This differs from a work in Gambia, which found NTS prevalence to be as high as 86% as against only 14% of TS [21]. The witnessed differences in Salmonella serovars distribution are well documented in many investigations which concluded that Salmonella serovars differs by geographical region [6,40].

Antibiotic resistance pattern of isolates

Antibiotic resistance pattern of *Salmonellae*

In this present work, all isolated *Salmonella* serotypes were susceptible to levofloxacin, ciprofloxacin, amikacin, meropenem, gentamycin, ceftriaxone and cefotaxime. This agrees to the findings of Groß., *et al*. who also reported similar Salmonella susceptibility pattern to these antimicrobials in Ghana [41]. High susceptibility of Salmonella to ciprofloxacin and norfloxacin have also been documented by Malla., *et al* [42]. The antimicrobial pattern observed in this study agrees with the proposal that in the treatment of gastroenteritis (Salmonella induced), ciprofloxacin should be given at the onset of severe gastroenteritis whilst ceftriaxone is given to children with systemic salmonellosis [43]. The high antimicrobial susceptibility in this study in Cape Coast could be attributed to appropriate drugs prescription and proper usage of these drugs in the management or treatment of salmonellosis. Moreover, it might also be the result of the expensive nature of these antibiotics rendering them less assessable to the community hence less indiscriminate use.

A study in Accra, Ghana, [44] reported high proportions of Salmonellae isolated to be resistant to ciprofloxacin. Again, the rise in occurrence of resistance to extended spectrum cephalosporins as well as the decline in susceptibility to fluoroquinolones in Salmonella isolates causing infections in man, have been documented in Southeast Asian countries [45] and worldwide by WHO [46] and others [12]. Nonetheless, the findings in this study indicates that both groups of drugs still remain drugs of choice in treating salmonellosis in Cape Coast. This agrees to a study by Nata., et al. in Indonesia who conducted a 5 year review on antimicrobial resistance patterns from January 2011 to December 2015 and reported that all the strains were susceptible to ceftriaxone, ciprofloxacin, and levofloxacin [9].

There was high antimicrobial resistance pattern against ampicillin (65%), tetracycline (65%), and co-trimoxazole (65%) in this study. Similar to this outcome, several other studies have also demonstrated high resistance pattern of Salmonellae against these argents [26,47,48]. Moreover, high rates of antimicrobial resistance (>50% to 100%) to chloramphenicol, ampicillin and trimethoprim/sulphamethoxazole have been documented in Africa, South America and Asia [47,49]. In another study in Kenya, Kariuki., et al. demonstrated that the prevalence of multiple drugs resistance of NTS to all the commonly used antibiotics such as chloramphenicol, ampicillin and co-trimoxazole increased from 31% in 1994 to 42% in 2003 [50]. Also, Parry, in his study concluded that antimicrobial resistance in Salmonella to chloramphenicol, ampicillin and co-trimoxazole is common in Africa [51]. The observed trend in this study and elsewhere could be attributed to upsurge and indiscriminate or uncontrolled use as well as easy accessibility to these antimicrobials to communities. Other possible contributing factors may include, prescriptions not taken up to the expected duration of therapy, administration of antimicrobials for viral infections, sales of antimicrobials without medical supervision and the use of antibiotics in foods/agriculture [31,52].

Furthermore, there was high multi-drug resistance among isolates. The percentage of Salmonella Typhi which showed multidrug resistance was 92% (12/13) while that of Salmonella Typhimurium was 50% (2/4), altogether summing to 82% (14/17). Similar results were found earlier in Ghana [13,52] and in Nepal where Salmonella isolates were found to be resistant to at least four antibiotics [38].

Antibiotic Resistance Pattern of *Shigella* Isolates

Recorded resistance to co-trimixazole and tetracycline were 100% each with that of ampicillin being 67%. No antimicrobial resistance was recorded against cefuroxime, ceftriaxone, cefotaxime, gentamycin, levofloxacin, amikacin and ciprofloxacin. Shigella isolates with high resistance to tetracycline (88.43%), ampicillin (88.90%) and sulfamethoxazole (82.92%) have been recorded in China [35]. Similar findings are documented in Bangladesh, where there was increased antimicrobial resistance to tetracycline and co-trimoxazole but no resistance against gentamycin, cefuroxime and ciprofloxacin [37] and in central Israel, where a significantly increased resistance to tetracycline (from 23% to 87%) and ampicillin (85%) and emerging resistance to quinolones including ciprofloxacin (0.5% to 2%) [54].

Antibiotic resistant pattern of blood isolates

Although most of the isolates exhibited high multi-drug resistance, none of them was resistant to all antibiotics used. Resistance to ampicillin was the most common (66/69, 96%), followed by tetracycline (63/69, 91%), co-trimoxazole (60/69, 87%) and cefuroxime (49/69, 71%). The antibiotics which recorded least bacterial resistance were amikacin (2/69, 3%), meropenem (5/69, 7%), levofloxacin (7/69, 10%) and ciprofloxacin (12/69, 17%). There was 98.6% multidrug resistance among isolates with the highest multiple resistance being 10 antibiotics (2/69, 3%), followed by 9 antibiotics (6/69, 9%), 8 antibiotics (8/69, 11%) and 7 antibiotics (13/69, 19%). Similar trends are presented in the sensitivity patterns of the *Salmonella* isolates as discussed above.

Sex and age distribution of patients with *Salmonella* isolates

More females (n = 10, 59%) were infected than males. A 58.0% prevalence of Salmonella Typhi in females is also documented [55]. Again, Dakora in her study in Ghana reported that, of the 54 Salmonella isolated, 55.6% (30/54) were from females and 44.4% (24/54) from males [56]

Some studies have reported that, there is a high Salmonellae infection rates in children of school-going age and young adults [24,57]. This assertion is consistent with this study which recorded a prevalence rate of 59% in participates ≤25 year. This observation could be due to the under developed immune system of this age category and possible first time encounter rendering them more susceptible to enteric pathogenic bacteria [58].

ESBL Prevalence among *Salmonella* Isolates

Finding of ESBLs amongst *Salmonellae* is a newly emergent threat globally. Both healthcare related outbreaks as well as community outbreaks have been documented [59,60]. In this study, no Salmonella serovar was found to produce ESBL. This finding agrees to that of Boni-Cisse., *et al*. who found no ESBL producing

Salmonella species in their study in Cote d'Ivoire [61]. A study in Ghana which screened 54 Salmonella isolates, found none to produce ESBL [56]. On the contrary, since 1988, NTS isolates with resistance to ESBLs have been reported in Northern and Western African countries, Southern America, the Middle-East, Eastern Europe and East Asia, Russia, India, Turkey, Greece and the United States [8,51]. Other countries where ESBL producing Salmonellae have been reported include India [8], Argentina [3], Belarus, Hungary and Latvia [62], Kuwait and United Arab Emirates [63], Lebanon and elsewhere [64,65]. Differences in geographical distribution of ESBLs may be based on different treatment and prophylactic protocols [5].

Genetic relatedness of *Salmonellae* isolated

The pulsed-field gel electrophoresis analysis showed a close genomic relatedness within the Salmonella species analyzed. The genomic fingerprint sequence analysis revealed that all the Salmonella spp. were related within species denoting that they are from a common origin. Further details of these observations could only be determined by the BioNumeric software which was not available for this study.

Conclusion

There is low prevalence of *Salmonella* in Cape Coast; 0% and 3.3% in blood and stool respectively. Among the *Salmonellae* isolates, 13(76.5%) were *Salmonella* Typhi and the remaining 4 (23.5%), *Salmonella Typhimurium*. The *Salmonella* species isolated were distributed among both sexes and age groups with the 1-25years age group recording the highest infection rate. Prevalence of other pathogens isolated were *Staphylococcus aureus* (24/69, 34.8%), Citrobacter freundii (10/69, 14.5%), Escherichia coli (6/69, 8.7%) and *Pseudomonas aeruginosa* (6/69, 8.7%) from blood and Shigella flexneri (3/508, 0.6%) from stool. High percentage of Salmonella isolates, 82% (14/17) exhibited multidrug resistance. In spite of this observed trend, no Salmonellae was resistant to ciprofloxacin, levofloxacin, amikacin, gentamycin, ceftriaxone, meropenem, cefotaxime, cefoxitin and ceftazidime. Among the other blood isolates, resistance to ampicillin, tetracycline, co-trimoxazole and cefuroxime were 96%, 91%, 87% and 71% respectively. The antibiotics with less bacterial resistance were amikacin (2/69, 3%), meropenem (5/69, 7%) levofloxacin (7/69, 10%) and ciprofloxacin (12/69, 17%). There was also 98.6% multidrug resistance among the blood isolates.

Conflict of Interest

The authors declare that there are no conflict of interest.

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