Contribution of Dug-Out Wells to *Salmonella* Dissemination in Kwaebibirem District of Ghana

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Abstract

Typhoid fever is rare in the developed world, but in Kwaebibirem District of Ghana, Salmonella infections are very common. Typhoid and paratyphoid fevers in addition to gastroenteritis are frequently reported. The reservoir, prevailing *Salmonella species* and their antimicrobial susceptibility patterns are not known, but in Ghana treatment of these infections are mostly empirical. 464 samples (270 stool and 194 blood) were collected from patients and 188 water samples were collected from different water sources in Kwaebibirem District and cultured for *Salmonella* at St. Dominic Hospital, Akwatia. *Salmonella* prevalence of 11.6% (54/464) among patients and 2.7% (5/188) from dug-out wells were obtained. Total viable bacterial count in the water samples averaged 2.56 $x10^3$-1.2 x 10^{13}per milliliter. Five (5) out of 51 (9.8%) dug-out wells yielded *Salmonellae* upon culture. Typhoidal *Salmonellae* [11% (6/54)] and 68.6% (38/54) non-typhoidal *Salmonellae* were isolated from patients. The most affected age group ranged 6-15years with prevalence of 42.6% (23/54). The most frequent isolated was *Salmonella Typhi* 20% (11/54) followed by *Salmonella Enterica,* 29.6% (16/54). The *Salmonella* isolates were all susceptible to the cephalosporins (cefoxitin, cefotaxime, cefepime) the carbapenems (imipenem and meropenem) the quinolones (norfloxacin and ciprofloxacin) and the aminoglycoside (amikacin). Their resistant proportions to other drugs were ampicillin (69.5%), piperacillin (69.5%) and co-trimoxazole (76.3%). *Salmonella* infections were common in Kwaebibirem District, and home owned dug-out wells posed risk of *Salmonella* transmission to the people.

Keywords: Salmonella infection, typhoid fever, dug-out well, well water, antimicrobial resistance

Introduction

Typhoid and paratyphoid infections are common in Kwaebibirem District in Ghana, where it is among the top ten (10) and accounts for about 1054 admissions and more than 200 deaths annually as a result of these infections (CHIM/PPME-GHS, 2014). Meanwhile, the *Salmonella* types causing the infections and the disease burden are not known. So also the sources of *Salmonella* spread in Kwaebibirem District are not known. This study was therefore conducted to determine the prevalence of *Salmonella* infection, the antimicrobial susceptibility patterns of the isolates and to determine whether water constitutes a major source of *Salmonella* infection to the people of Kwaebibirem District.

This study was conducted after obtaining ethical clearance from the joint committee on Human research, publication and ethics of School of Medical Sciences, KNUST, Kumasi.

Materials and Methods

This study was conducted at Akwatia in the Kwaebibirem District of the Eastern Region of Ghana from September 2012 to August 2014. The Kwaebibirem District with a land size of 1230 km^2, is located on the South-Western part of the Eastern region. The District lies in the west semi-equatorial climatic zone with bi-modal rainfall around 1120 mm^3. The average temperature ranges between 26.5^oC in July and 37^oC in January. Treated water supply to the inhabitants of the district comes from varied sources: Pipe borne water supply is about (37%), River/stream (22.3%), Wells (21.6%), Boreholes (14.9%) and Tanker supply services (0.5%). The district has a population of 192,562 (a population density of 154/km2). The inhabitants mostly are farmers, but some engage in small scale mining and petty trading. There are also artisans, government and company workers and petty traders (2010 Population & Housing Census, Summary Report of Final Results, Ghana Statistical Service, 2012). Patients were received from the various parts of the district. Then individuals from whom *Salmonella* was isolated were traced to their communities. Water samples were collected from the communities, transported to the laboratory in cold box and tested for Salmonella. This was done in an attempt to identify the source of the infection to these individuals.

Culture procedures

***Blood*:** Non duplicate 194 blood samples were inoculated into trypticase soy broth (Liofilchem, Italy) and incubated at 37^oC overnight, and then sub-cultured on blood agar and MacConkey agar (Liofilchem, Italy).

***Stool*:** 270 Stool samples were cultured for Salmonella using Selenite F broth and Salmonella-Shigella agar (Liofilchem, Italy).

***Water*:** About 500ml of portable water taken from wells, standpipes, ponds and sachet (commercially packaged water in sachets) were filtered through sterile Whatman filter paper into a sterile vacuum flask. The whatman filter paper was then immersed in Selenite-F broth and incubated overnight at 37 ^{o}C. It was then sub-cultured onto Salmonella-Shigella agar (Liofilchem, Italy) and incubated overnight.

Identification and isolation of Salmonella

Growths that occurred in the cultural processes were identified by biochemical tests and confirmed by Vitek 2. The antimicrobial susceptibility of the isolates were determined by the Kirby-Bauer disc diffusion method and the Minimum inhibitory (MIC) was determined by Vitek 2. Extended spectrum B-lactamase (ESBL) production of the isolates was determined using the double disc synergy test on Muller-Hinton agar.

Results

Fifty four (54) *Salmonella* strains were isolated from 464 samples of blood and stool tested, giving *Salmonella* prevalence of 11.6%. Typhoidal and Non-typhoidal *Salmonellae* were isolated with *Salmonella Enterica* (30%) being the most common isolate, followed by *Salmonella Typhimurium* (24%) and (15%) for *Salmonella Enteritidis* as indicated in Figure 1.

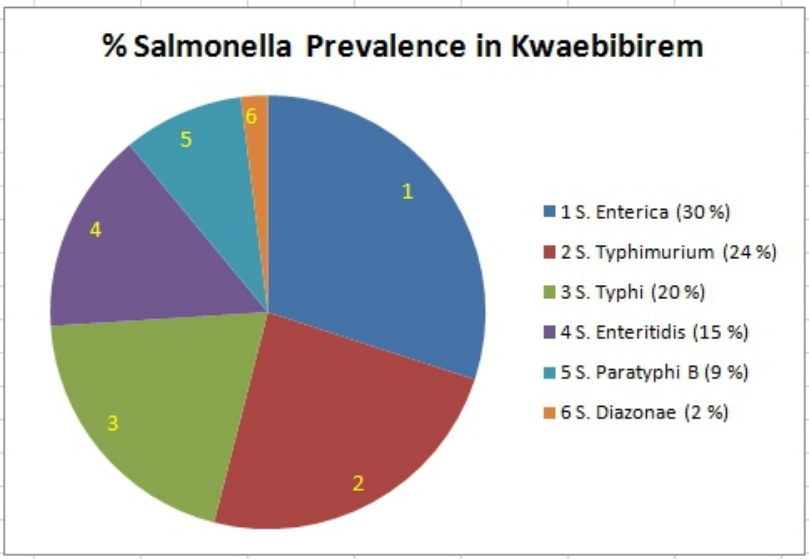

Figure 1 Species prevalence of Salmonella in Kwaebibirem district

The results indicate that Akwatia recorded the highest number of isolates 15 (27.8%). This was followed by Asamankese with eight isolates (14.9%) and Kade with four isolates (7.4%). Of the 15 isolates obtained from

humans in Akwatia, 5 were *S. Enterica*, 3 were *S. Enteritidis* and 3 for *S. Typhimurium* and 2 each of *S.Paratyphi B* and *S. Typhi* were isolated. Five isolates were obtained from dug-out wells sampled. No isolates were obtained from pipe-borne water, sachets water, and streams sampled. *Salmonella* isolates were obtained from many more communities where one isolate each was obtained from Abenaso Nkwanta, Abomasu, Adwafoakwa, Afosu, Akroso and many other towns indicating wide spread of the various *Salmonellae* in the area of study.

Table 1 Locality from which *Salmonella* was isolated and number isolated

Locality	*S. Diarizonae*	*S.Enterica*	*S.Enteritidis*	*S.Paratyphi B*	*S.Typhi*	*S.Typhimurium*	Total	%
Akwatia		5	3	2	3	7	15(5)	33.9%
Asamankese		3	3		1	1	8	13.6%
Kade		3				1	4	6.8%
Boadua		1	1			1	3	5.1%
Abaam					1	1	2	3.4%
Akwatia Manso					1	1	2	3.4%
Okumaning		1			1		2	3.4%
Pramkese	1			1			2	3.4%
Abenaso Nkwanta		1					1	1.7%
Abomosu			1				1	1.7%
Adwafoakwa					1		1	1.7%
Afosu					1		1	1.7%
Akroso		1					1	1.7%
Akyem Tweapease				1			1	1.7%
Akyinso					1		1	1.7%
Amanfrom					1		1	1.7%
Amantem Nkwanta						1	1	1.7%
Amonom				1			1	1.7%

Apinamang						1	1	1.7 %
Brekumanso Zongo						1	1	1.7 %
Dwenase		1					1	1.7 %
Osenase					1		1	1.7 %
Staff Village						1	1	1.7 %
Topremang						1	1	1.7 %
TOTAL	**1**	**16**	**8**	**5**	**12**	**17**	**59**	**100 %**

( )= number of organisms isolated from water

Patients in the 6-15 year age group were most affected followed by 16-30year age group. The mean age of affected patients was 18years in an age range of 3-74 years. More females (55.6%) were infected than males (44.4%) with *Salmonella*. Details are shown in Table 2.

Table 2 Distribution of *Salmonella* species isolates in relation to demographic characteristics of infected patients in Kwaebibirem District.

Isolates / Parameters	Typhoidal Salmonellae		Non-Typhoidal Salmonellae				Total 54(100 %)
	S. Typhi 11(20 %)	S. Paratyphi B 5(9%)	S. Typhimurium 13(24%)	S. Enterica 16(30 %)	S. Enteritidis 8(15%)	S. Diarizonae 1(2%)	
Age groups							
0-5	1	0	1	1	2	0	5(9.3%)
6-15	7	2	6	6	1	1	23(42.6 %)
16-30	1	2	4	5	4	0	16(29.6 %)
31-50	1	1	1	3	1	0	7(13.0 %)
51+	1	0	1	1	0	0	3(5.6%)
Sex							
Male	5	1	5	9	3	1	24(44.4 %)
Female	6	4	8	7	5	0	30(55.6 %)
Sample type							

Blood	11	4	0	0	0	0	15(27.8 %)
Stool	0	1	13	16	8	1	39(72.2 %)
Water	1	0	4	0	0	0	5(9.3%)

Antimicrobial susceptibility patterns of the isolates

The isolates exhibited high resistance to some antibiotics tested ranging from 69.5% to 90% against Ampicillin, Piperacillin and co-trimoxazole. Isolate of *Salmonella Paratyphi B* and *Salmonella Diarizonae* were most resistant, where none at all was susceptible to Ampicillin, Piperacillin and co-trimoxazole.

Resistance results were varied against amikacin and gentamycin, the amoniglycosides tested. Thirty one (52.5%) isolates were susceptible to amikacin at MIC of <=2 ug/ml and 51 (86%) isolates were sensitive to Gentamycin at <=1 ug/ml. Similar results were obtained against ciprofloxacin and norfloxacin the quinolones tested, where 56 (95%) isolates were sensitive to ciprofloxacin at MIC of <=0.25 ug/m. Varied sensitivity results were obtained against the cephalosporins tested, but no isolate was resistant to meropenem and imipenem the cabapenems. The detailed results of the resistant proportions are in figure 2 and the MIC results are in table 3.

Figure 2 Antimicrobial susceptibility patterns of Salmonella isolates in Kwaebibirem District

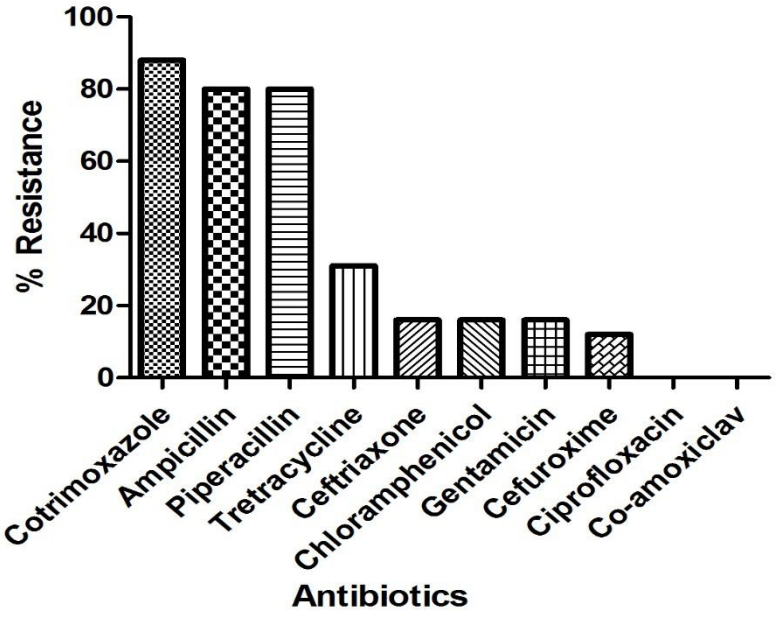

Table 3 Minimum inhibitory concentrations (MICs) of 59 Salmonella isolates in Kwaebibirem

Antibiotics	MIC(μg)	Interpretation	No of isolates (%)
Ampicillin	≥32	R	41 (69)
	16	I	2(3)
Piperacillin	≥128	R	41 (69)
	64	I	2(3)
Piperacillin/tazobactam	≤128	R	43 (73)
	32	I	5(8)
Amoxicillin/clavulanic acid	≤2	S	18 (31)
	4	S	9 (16)
	8	S	29 (49)
	16	I	3(5)
Cefazolin	≤4	S	30 (51)
	8(19)	S	19(32)
Cefoxitin	≤4(55)	S	55(93)
Cefotaxime	≤1(53)	S	53(90)
Ceftazidime	≤1(58)	S	58(98)
Ceftriaxone	≤1	S	43(73)
Cefuroxime	≤1	S	47(80)
	16	I	6(10)
Cefepime	≤1	S	59(100)
Imipenem	≤1	S	59(100)
Meropenem	≤1	S	59(100)
Amikacin	≤2	S	31(53)
	4(27)	S	27(46)
	8(1)	I	1(2)
Gentamycin	≤1	S	51(86)
	≥16	R	8(13)
Ciprofloxacin	≤0.25	S	56(95)
	0.5(3)	S	3(5)
Norfloxacin	0.5	S	46(78)
	2	S	13(22)
Tetracycline	≤1(41)	S	41(69)
	4(2)	I	2(3)
	≥16(16)	R	16(27)
Trimethoprim/sulfamethoxazole	≤20(14)	S	14(24)
	≥320	R	45(76)

S=Susceptible; I= intermediate; R=Resistance

Discussions

Human *Salmonella* carriers are often sources of human *Salmonella* spread, so also are contaminated water and food. The patients and carriers spread the disease through the contamination of food and water they handle (Gupta and Garg, 2014).

This study attempted to establish the prevalence of Salmonella infection among patients attending St. Dominic Hospital, the sources of the infection and the antimicrobial susceptibility patterns of the isolates. High

prevalence of salmonella infection was detected among patients attending the St Dominic Hospital and also in well-water. There were 54 *Salmonella* isolates obtained from different samples of patients, where 15 (28 %) came from blood samples and 39 (72 %) were isolated from stool samples. Five (5) *Salmonella* isolates were isolated from dug-out well water samples. Salmonella prevalence was 11.0% among the patients and 2.7 % in water samples. The high prevalence of *Salmonellla* among patients in the district perpetuate the disease in the district through indiscriminate defaecation in the environment (Levantes *et al.*, 2012). Rain water washes the faeces from upland into the low lying open du-out wells (Abdulkadir *et al.*, 2014). Some wells too are sited close to latrines and seepage from the latrines contaminates these well waters with faecal microbes including *Salmonella (Abdulkadir et al., 2014)*. The majority of inhabitants in Kwaebibirem district depend on communal Kumasi Ventilated Improved Pit (KVIP) type latrines whiles some inhabitants depend on household pit latrines. The availability of toilets is about 60% (http://kwaebibirem.ghanadistricts.gov.gh/). Provision of communal KVIP has its access restricted by the payment of a token, a measure which has reduced its usage, so some of the populace defaecate indiscrimately in the nearby bushes. Gold and diamond mining in addition to sand and stone quarrying activities lead to silting of many streams and rivers, (especially the Birim River), which formerly were sources of drinking water for the inhabitants but these rivers are now polluted leading to the dependence of dug-out wells. Urbanization has hampered the district's ability to control solid and liquid waste disposal leading to the creation of large garbage hills which also serve as sites for defaecation in the towns (Addo *et al.*, 2014). Rain water flows from these garbage sites and the neighbouring thickets into low lying rivers and wells contaminating them with enteric bacteria. These activities disseminate *Salmonella* causing gastroenteritis and typhoid in the district (Attua *et al.*, 2015). Not all *Salmonellae* serotypes found in the environments are the same as those isolated from humans (Setti *et al.*, 2009) because some isolates are associated with animals (Levantes *et al.*, 2012; Andoh *et al.*, 2016). In this present study well water samples yielded *Salmonella* serovars commonly associated with human clinical disease (Abdulkadir *et al.*, 2014). This was observed probably because pit latrines sited in close proximity to dug-out wells leak into the water (Karkey *et al.*, 2016) creating a source of infection to the people who drink from them. It was unclear when *Salmonella* was not isolated from the River waters sampled. But it is perhaps river water runs and the contaminants are washed downstream away from the point of contamination. Dug-out wells retain the contaminants and also serve as culture medium which increase them to infectious dosages (Nwaiwu and Nwachukwu, 2016).

Studies in Nigeria found well-water contaminated with bacteria with counts ranging from 100 to 800 col/100ml of water (Olabisi *et al.*, 2008) and in Tanzania surface waters contaminated with Salmonella (Lyimo *et al.*, 2016) had results similar to the Kwaebibirem study.

Salmonella infection is treated with an appropriate antibiotic (de Jong *et al.*, 2014), but recent increases in antibiotic-resistant bacteria have prompted routine surveillance of microbial populations to determine the appropriate antibiotic to choose (de Jong *et al.*, 2014; Eze *et al.*, 2015). The 46 *Salmonella* isolates of clinical origin and five (5) from water in this present study were examined for their susceptibility to various antibiotics and extended spectrum beta-lactamase production (Tansarli *et al.*, 2014; Harish and Menezes, 2015). There were varied susceptibility results to the antibiotics tested as presented in figure 2 and the MIC in Table 2. However, no strain was found to produce extended spectrum beta lactamase (ESBL), perhaps because ESBLs are associated with nosocomial infections contrary to *Salmonella* infections which are community acquired (Pitout *et al.*, 2005). High proportions of *Salmonella* were susceptible to ciprofloxacin and norfloxacin posing no resistant problems to Quinolone in the Kwaebibirem district as was obtained elsewhere (Humphries and Schuetz, 2015; Kariuki *et al.*, 2015; Rodriguez-Martinez *et al.*, 2015; Lyimo *et al.*, 2016). So also the susceptibility of the isolates to Carbapenems (Imepenem and Meropenem) tested had no resistant strains. These antibiotics are not common in the Kwaebibirem district community because they are relatively more expensive and beyond the reach of most inhabitants. Chloramphenicol is still a drug to consider in the treatment of *Salmonella* infections as the resistance proportion was less than 20% in the study area. Pharmacy and chemical shops would normally not stock such drugs because they would not sell out quickly so would not be abused because many people cannot afford to purchase them.

Conclusion

Prevalence of *Salmonella* infection in Kwabibirem District is high recording about 12%. The disease affects both male and females of all ages, but the youth (average age of 18years) are most affected. Well-waters taken from the communities were contaminated with *Salmonella*, along with a high total viable enteric bacterial counts, suggesting fecal contamination of the Wells.

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