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ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *SALMONELLA TYPHI* AMONG PATIENTS IN THREE HOSPITALS IN KUMASI, GHANA

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ABSTRACT: *Salmonella typhi*, causative agent of typhoid fever, a predominantly human communicable disease, is endemic in Africa and Asia. *S. typhi* is of clinical importance partly due to its mode of infection and partly due to the fact that human activities such as poor hygienic conditions are essential in the transmission and perpetuation of the pathogen. More so antibiotic resistant strains of *S. typhi* have emerged, increasing cost of treatment, morbidity and mortality. Meanwhile, *S. typhi* isolation and sensitivity testing to antibiotics are rarely practiced in the hospitals in and around Kumasi. This study sought to determine the extent of antibiotic susceptibility of *S. typhi* isolated from patients in Kumasi south, North Suntreso and Tafo hospitals in the Ashanti region of Ghana. The isolates were identified using morphological and biochemical means. The isolates were then tested for their sensitivity to some reference antibiotics using the Kirby-Bauer disc diffusion method. One hundred and twenty-eight (128) *S. typhi* were isolated from 900 clinical samples. A total of 52.3% of the *S. typhi* isolates exhibited resistance to ampicillin whereas 32.8, 25.0, 17.2 and 14.9% of the isolates were resistant to co-trimoxazole, chloramphenicol, ciprofloxacin and ceftriaxone respectively. About 19.5% of the *S. typhi* isolates were multidrug resistant. The *S. typhi* isolates were most sensitive to ciprofloxacin and ceftriaxone whilst majority of the *S. typhi* tested were resistant to ampicillin. Ampicillin is therefore no longer suitable for the treatment of *S. typhi* infections in these hospitals.

INTRODUCTION: Typhoid fever caused by *S. typhi*, though curable, tends to be chronic due to the nature of its pathogenesis and its symptomatic similarity with malaria [1]. Although typhoid fever is a rare disease in developed countries [2], poor safe water supply, environmental sanitation and food hygiene in developing countries make typhoid fever a major problem [3].

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In Ghana it is estimated that typhoid fever cases account for 3.2% of all infections recorded in the hospitals [4], as against an annual global estimate of 2.1 million episodes with 216, 000 deaths [5].

In developing countries, great reliance is placed on antibiotic chemotherapy in the treatment of typhoid fever because of the difficulties in preventing typhoid fever by public health measures. The mortality rate of untreated typhoid can be as high as 30%, whereas with appropriate antibiotic chemotherapy it is less than 1% [6]. Attempts to control diseases caused by *S. typhi* through the use of antibiotics have resulted in increased prevalence of resistant strains of this organism [7].

Therefore, in order to effectively treat typhoid fever, culture and antibiotic sensitivity tests must first be determined. Once culture and sensitivity results confirm the type of bacterial infection and sensitivity pattern, treatment may be modified [8]. However, most health facilities in Ghana lack adequately resourced laboratories to culture and test the antibiotic sensitivity of bacteria causing infections. Even, where laboratory facilities are available, culture and sensitivity test results take 48 to 72 hours to be ready [9]. In many cases sensitivity test may not be carried out at all due to lack of microbiologists and the extra cost it constitutes for the patient [10].

As a result of some these problems enumerated, there is very little information on the antimicrobial susceptibility patterns of *S. typhi* in Ghana. This has led to the situation whereby Ghanaian prescribers usually administer broad spectrum antibiotics to treat suspected typhoid fever without diagnostic evidence of antimicrobial susceptibility. This study sought to isolate *S. typhi* from patients in three hospitals (Kumasi South, North Suntreso and Tafo hospitals) in the Ashanti region of Ghana and to determine their susceptibility patterns to antibiotics commonly used to treat typhoid fever in these hospitals.

MATERIALS AND METHODS: The protocols for the study were approved by the ethics committees of the various hospitals. The samples were collected from Kumasi south, Tafo and Suntreso hospitals in Kumasi, Ghana. A total of 900 urine, stool and blood samples were collected between December, 2009 and August, 2010. All the samples were inoculated into 10ml cooked meat broth media and coded appropriately. These were then transported to the microbiology laboratory of the Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology and incubated at 37^{o}C for 72 hours. All the culture media, antibiotic discs and reagents for this study were purchased from Oxoid, Basingstoke, United Kingdom unless otherwise stated.

Isolation of *S. typhi*: Using a calibrated platinum loop, 2µl of each culture was streaked on the surface of blood agar plates, labelled and incubated for 24 hours. The colonies that were greyish, transparent or opaque on blood agar were carefully picked using a sterile inoculating wire and streaked on Bismuth sulphite agar plates. The plates were then incubated at 37^{o}C for 24 hours. Black colonies surrounded by a metallic sheen were picked and streaked on MacConkey agar plates. The MacConkey agar plates were also incubated at 37^{o}C for 24 hours. Colourless colonies on the MacConkey agar plates were fished out and using a straightened inoculating wire, the butt of already prepared Triple Sugar Iron agar was stabbed and the slant streaked. After incubating for 24 hours at 37^{o}C, the inoculated test tubes were observed for characteristic reactions of *S. typhi* which included a red slant, yellow butt, a small amount of blackening of the agar and absence of gas bubbles [11].

Identification and Confirmation of *S. typhi*: The *S. typhi* isolates were screened through various biochemical reactions to confirm their identities. These included urease production test, citrate utilisation test and serological test [12].

Antibiotic sensitivity test: The *in vitro* sensitivity testing of the *S. typhi* isolates to different classes of antibiotics [ampicillin (10µg), chloramphenicol (30µg), ceftriaxone (30µg), co-trimoxazole (25µg) and ciprofloxacin (5µg)] were carried out as recommended by the Clinical and Laboratory Standards Institute [13] using the Kirby-Bauer agar disc diffusion method.

A Suspension of the isolated *S. typhi* was prepared by inoculating colonies into 10ml peptone water and incubated at 37^{o}C for 24 hours. The suspension was then diluted to 0.5 MacFarland turbidity standards. A sterile swab was dipped into the standardized inoculum and used to inoculate the entire surfaces of already prepared Mueller-Hinton agar (Oxoid Basingstoke, UK). The agar plates were left to stand for 15 minutes to allow the surface moisture to dry.

Antibiotic discs were applied to the surfaces of the inoculated agar plates using a disc dispenser (Oxoid 6-place, 90mm) and incubated for 18 hours at 37^{o}C. The diameter of zones of inhibition for each antibiotic was measured (in millimetres) and compared with values provided by the Clinical and Laboratory Standards Institute [13]. *Escherichia coli* (ATCC 25922) served as a control in testing the sensitivity of *S. typhi.*

Data obtained from this study were statistically analyzed using Microsoft excel 2010 edition.

RESULTS AND DISCUSSION: A total of 128 isolates of *S. typhi* was obtained from the various (900) samples collected. *S. typhi* isolates were obtained from 49 (16.3%) of the 300 urine samples and 79 (26.3%) of the 300 stool samples (**Table 1**). No *S. typhi* was isolated from the blood samples collected. The presence of *S. typhi* in urine and stool samples and the absence of the bacterium in blood samples depicted a carrier state among the patients [14]. According to Gonzalez-Escobedo *et al*, [15] carriers may shed *S. typhi* either through their stool or urine continuously or intermittently and the carrier state is usually the source of contamination. Carriers could therefore serve as reservoirs for the dissemination of *S. typhi*, causing serious public health problems. The high stool yield of *S. typhi* observed in this study as illustrated in Table 1 confirms an earlier report by Gonzalez-Escobedo *et al* [15] that carriers of *S. typhi* mainly shed the organism through their faeces more than urine.

Gordon and colleagues [16] asserted that counts of *S. typhi* in blood of patients with typhoid fever mostly indicate a median concentration of one bacterium per millilitre during the first and second weeks of the illness. Thus, *S. typhi* is most frequently isolated during this time. The failure to isolate the bacterium from blood samples during this study could be attributed to late reporting of typhoid fever cases to health facilities for treatment.

TABLE 1: DISTRIBUTION OF *S. TYPHI* AMONG MALES AND FEMALES IN RELATION TO SPECIMEN TYPES

Samples	Hospitals	No. of *S. typhi*	Total No. of *S. typhi*
Stool	Tafo	30	**79**
	North Suntreso	19	
	Kumasi south	30	
Urine	Tafo	14	**49**
	North Suntreso	15	
	Kumasi south	20	
Total			**128**

Antimicrobial susceptibility of *S. typhi*: All the *S. typhi* isolates screened showed high susceptibility to ciprofloxacin (76.5%), cotrimoxazole (60.9%), ceftriaxone (70.3%) and chloramphenicol (58.6%) as shown in **figure 1**. The overall rate of resistance among the 128 *S. typhi* isolates was 52.3% to ampicillin, 32.8% to cotrimoxazole, 25% to chloramphenicol, 17.2% to ciprofloxacin and 14.9% to ceftriaxone. The isolates also showed intermediate resistance of 6.3% to ciprofloxacin and cotrimoxazole. Other intermediate resistance patterns as summarized in figure 1 were 12.5% to ampicillin, 14.8% to ceftriaxone and 16.4% to chloramphenicol.

The susceptibility patterns of the *S. typhi* isolates to ciprofloxacin (76.5%) and ceftriaxone (70.3%) are similar to a research conducted by Kasper and co-workers [17] in Cambodia, where *S. typhi* susceptibility to ciprofloxacin and ceftriaxone were 79% and 81% respectively. Similarly, a study on ciprofloxacin resistant *S. typhi* and treatment failure in the Kuwait by Dimitrov *et al* [18] also revealed susceptibility of 63.3% and 67.3% for ciprofloxacin and ceftriaxone respectively, which were considered to be high. The high susceptibility of *S. typhi* to ciprofloxacin and ceftriaxone recorded at the three hospitals may be due to the fact that ciprofloxacin and ceftriaxone have recently been introduced on the Ghanaian market and so might not have been subjected to abuse as compared to ampicillin [9]. More so, ceftriaxone is expensive and usually administered parenterally, thus limiting self-medication and abuse.

From figure 1, it was observed that the *S. typhi* showed susceptibilities of 60.9% and 58.6% to cotrimoxazole and chloramphenicol respectively. These susceptibilities however, differed from a report by Donkor and colleagues [19] who recorded lower susceptibilities of *S. typhi* to these antibiotics.

Chloramphenicol is a broad spectrum but toxic antibiotic and only used in life-threatening situations when no other antibiotic is effective [20]. Therefore, the reduced susceptibility (58.6%) of the *S. typhi* isolates to chloramphenicol recorded in this study could be as a result of noncompliance with prescription regulations.

In many developing countries including Ghana, people have access to this antibiotic without any prescription, which may lead to improper use [21]. As shown in Figure 1, intermediate resistant strains of *S. typhi* were found to have emerged against all the five antibiotics namely, ciprofloxacin (6.3%), co-trimoxazole (6.3%), ceftriaxone (14.8%), ampicillin (12.5%) and chloramphenicol (16.4%). This trend shows an increasing minimum inhibitory concentration (MIC) of these antibiotics against these strains [17] and higher doses of the antibiotics may therefore be needed in the treatment of typhoid fever caused by these intermediate resistant strains. Intermediate resistance occurs when patients fail to complete the recommended dose of prescription and so the bacteria are exposed to concentrations of the antibiotic that are lower than the minimum inhibitory concentration [22]. The exposure of the bacteria to the antibiotic may lead to gradual development of resistance.

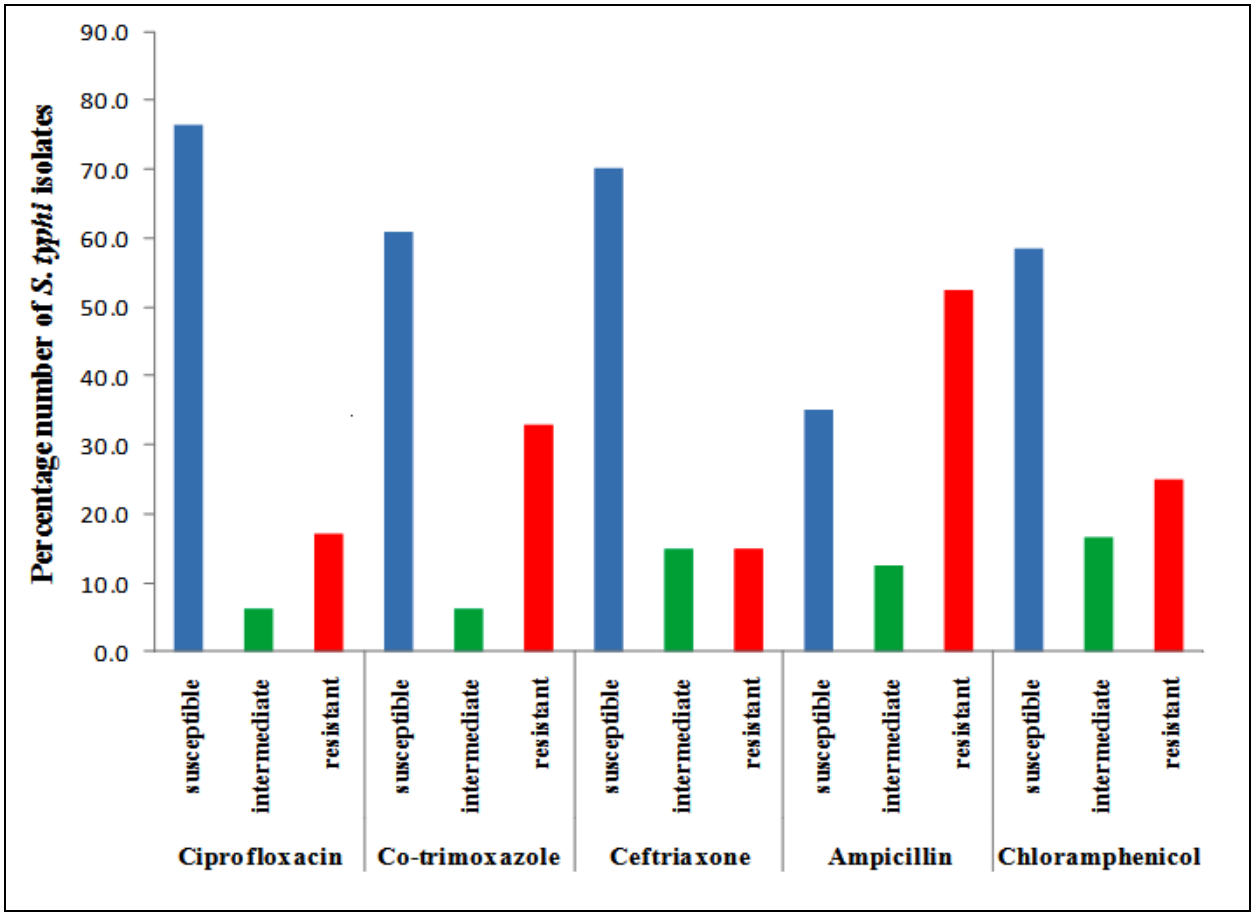

FIGURE 1: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *S. TYPHI* TO DIFFERENT ANTIBIOTICS

Susceptibility patterns of S. typhi isolates from the various hospitals: The *S. typhi* isolates from the various hospitals were tested against the antibiotics and their antibiograms determined. As presented in **figure 2**, the *S. typhi* isolated from Kumasi South hospital produced high susceptibilities to chloramphenicol (88.0%), ciprofloxacin (80.0%) and ceftriaxone (78.0%). Unlike Kumasi South hospital, the *S. typhi* isolates displayed a susceptibility of 38.6% to chloramphenicol in both North Suntreso and Tafo hospitals indicating that a significant number of the isolates were resistant to chloramphenicol (Figure 2). At least 77.3% of *S. typhi* isolates from Tafo hospital were susceptible to cotrimoxazole as compared to 58.8% in North Suntreso hospital and 48.0% in Kumasi South hospital (Figure 2). On the other hand, North Suntreso hospital recorded the highest number of resistant *S. typhi* (70.6%) to ampicillin when compared with 58.0% and 31.8% of the *S. typhi* isolates being resistant to ampicillin in Kumasi South and Tafo hospitals respectively (Figure 2). The differences in the susceptibility patterns from the various hospitals could be a reflection of the lack of a coordinated national pharmaceutical policy. In such circumstances, prescribers rely on prescribing habit as they do not have time to update their knowledge of medicines.

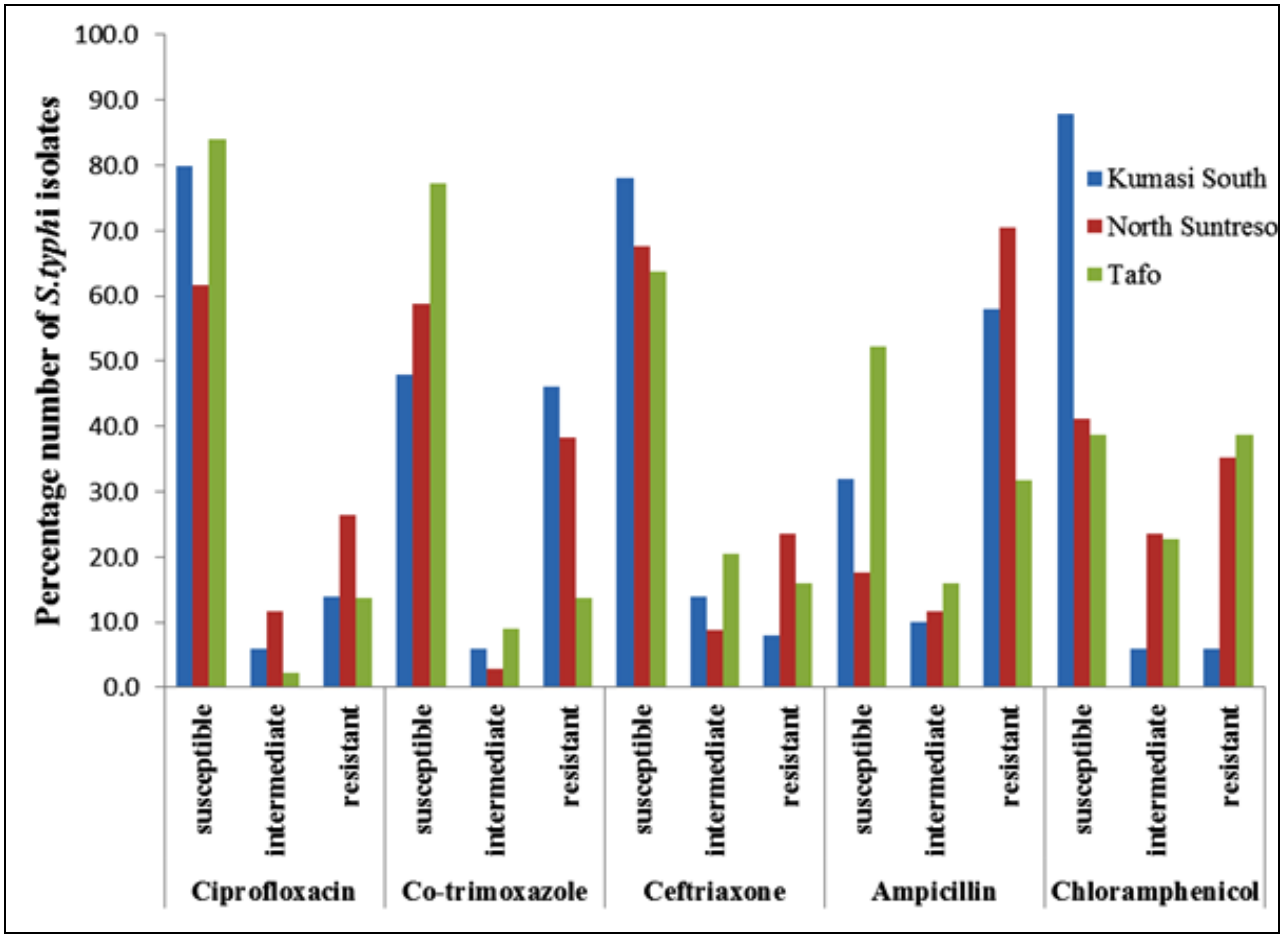

FIGURE 2: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *S. TYPHI* ISOLATED FROM THE THREE HOSPITALS

A total of 19.5% of *S. typhi* were resistant to at least three different antibiotics and were thus, multi-drug resistant (Table 2). The extent of multiple-drug resistant *S. typhi* observed in Tafo hospital was 13.6%. Kumasi South and North Suntreso hospitals registered 20.0% and 26.5% of multiple-drug resistant *S. typhi* respectively (**Table 2**). The presence of multiple-drug resistant strains of *S. typhi* among the isolates may be attributed to antibiotic misuse arising from self-medication in suspected bacterial infections [9]. Self-medication prevents early reporting of patients to hospitals at the onset of disease until complications have occurred [23].

The cumulative effect arising from antibiotic misuse is the proliferation of extrachromosomal resistant genes in the environment and may be potential mechanisms for the level of multiple-drug resistance as noticed in this study. Some other factors identified in literature as the drivers of bacterial resistance to antibiotics such as unnecessary prescriptions and substandard antibiotics could also be the cause of the *S. typhi* resistance observed against the antibiotics in this study [17, 18]. The usefulness of these antibiotics will therefore depend on the effective interventions put in place by health authorities to curb the spread of resistance among bacterial strains to antibiotics [7].

TABLE 2: MULTIPLE-DRUG RESISTANT (MDR) *S. TYPHI*

HOSPITAL	No. of MDR *S. Typhi*		Total No. of MDR *S. typhi*	Total No. of *S. typhi*	Percentage MDR
	STOOL	URINE			
Kumasi south	3	7	10	50	20.0
North Suntreso	3	6	9	34	26.5
Tafo	5	1	6	44	13.6
Total	**11**	**14**	**25**	**128**	**19.5**

CONCLUSION: The study demonstrates that many patients visiting Kumasi South, North Suntreso and Tafo hospitals are chronic carriers of *S. typhi*. The *S. typhi* isolates were most sensitive to ciprofloxacin and ceftriaxone whilst majority of the *S. typhi* tested were resistant to ampicillin. Ampicillin is therefore no longer suitable for the treatment of *S. typhi* infections in these hospitals. These susceptibility patterns exhibited by the *S. typhi* isolates to the antibiotics tested indicate that the efficacy of the relatively cheap empirical therapy for *S. typhi* infections could be jeopardized.

Competing interests: The authors declare that they have no competing interests.

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