

Multidrug resistance of *Salmonella enterica* serovars Typhi and Typhimurium isolated from clinical samples at two rural hospitals in Western Kenya

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Abstract

Background: The threat to human health posed by antibiotic resistance is of growing concern. Many commensals and pathogenic organisms have developed resistance to well established and newer antibiotics. This is a cross-sectional study within two hospital settings to determine in vitro antibiotic susceptibilities of *Salmonella* species isolated in blood, cerebral spinal fluid, pus and stool collected from in- and out-patients. The inclusion criteria was non restrictive to in- and out-patient but preference to severe diarrhea cases with negligible changes to previous treatment regimen was observed. The study was carried out from February 2004 - June 2005. Fifty-three diarrhea patients within the hospital who were chosen by convenient sampling and consented to participate in the study were considered.

Methodology: Either blood or pus was collected using vacutainer tubes and syringe, swabs respectively, and cerebral spinal fluid by lumbar puncture from patients who had fever (temp $\geq 38^{\circ}\text{C}$) and diarrhea. Stool samples were also collected and all specimens analyzed for the presence of *Salmonella* by routine microbiological procedures. The isolates were subjected to antibiotic susceptibility testing using disc diffusion technique.

Results: In St. Elizabeth Mukumu Mission Hospital, *Salmonella enterica* serovar Typhi was most common (56.6%, n=33), followed by *S. typhimurium* (34%, n=18), while in Maseno Mission Hospital only *S. typhimurium* was isolated. Whereas *S. typhi* was more commonly isolated in male adults and female children (P = 0.9), *S. typhimurium* was more common in female and male children (P=0.1). All the isolates were sensitive to ciprofloxacin. However, *S. typhi* was resistant to streptomycin, ampicillin, chloramphenicol and cotrimoxazole; *S. typhimurium* to tetracycline, sulfamethoxazole, cotrimoxazole, ampicillin, chloramphenicol and streptomycin.

Conclusions: *S. typhi* displayed a high resistance pattern to most antibiotic screened than *S. typhimurium*.

Key Words: *Salmonella* infections, *Salmonella typhimurium*, *Salmonella typhi*, drug resistance.

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Introduction

Morbidity and mortality due to bacterial infections is a major public health problem in developing countries including Kenya. This is partly as a result of bacterial resistance to antimicrobial drugs used for therapeutic purposes. Data on the antimicrobial susceptibility pattern is very inadequate in developing countries with very few studies done in Africa [1]. Most of the studies have been done in the main urban cities and their outskirts [1,2,3,4]. Data from rural communities on antibiotic susceptibility is scanty due to scarcity of well-equipped laboratory facilities to perform culture and sensitivity testing [5,6]. However, in developed countries, the incidence of cases and

death has been greatly decreased by a combination of improved sanitation and hygiene, vaccine, and effective antimicrobial chemotherapy [7].

The prevalence of Salmonellosis in Kenya has been of great concern. *Salmonellae* cause a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia [1,8]. Between 1988 and 1990, ninety-seven *S. typhimurium* strains were isolated from patients at Kenyatta National Hospital [3]. In a community based study, bacterial isolates included non-typhoid *Salmonella* (25.5%), *S. typhi* (10.6%), and *S. typhimurium* (10.6%) [1]. One of the bacteria that exhibit antimicrobial resistance is *Salmonella*. Resistance

is a natural biological defense mechanism used by bacteria to survive in the presence of a threatening antibiotic. Antibiotic-resistant genes can be acquired by susceptible strains from resistant strains, thus allowing more bacteria to become resistant to the antibiotic [9]. Alternatively, some bacteria will survive after being exposed to an antibiotic because they have a beneficial mutation that allows them to survive. Among *Salmonella* isolated in Spain from natural water reservoirs, *S. blockley* and *S. typhimurium* had the highest rates of multidrug resistance [10].

Early administration of antibiotic treatment is highly effective in eliminating infections but indiscriminate use of antibiotics has led to the emergence of multidrug-resistant strains [10]. Molecular and epidemiological studies have revealed a tremendous increase in incidence of *Salmonella* resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of *Salmonella* serovar typhimurium in Spain [11]. Similar results have been reported in some parts of Kenya [12]. Although quinolones provide a good therapeutic alternative, multiresistance of *S. typhimurium* is of public health concern and it is important to continue surveillance of resistance levels and its mechanisms [11]. Due to widespread acquisition of resistance, it is important that susceptibility tests are done to guide antibiotic treatment and policy [5,11,13]. It was therefore imperative to determine *S. typhi* and *S. typhimurium* prevalence and drug sensitivity and this was carried out in two health centers in Western Kenya, namely the St Elizabeth Mukumu and the Maseno Mission rural hospitals.

Materials and Methods

Study design

This was a cross-sectional convenience sampling; i.e. diarrhoea patients who were within reach in the hospital and who consented to participate in the study between February 2004 and June 2005 were considered.

Consent to collect samples from the hospital was obtained from relevant authorities. The university ethical board gave permission to conduct the study within the institutional research mandate as stipulated by the National Ethical Board. Specimens were collected from patients who gave oral consent to participate in the study.

The inclusion criteria of the study was non restrictive to either in-patient or out-patient. Preference was given to patients who consented to the study and had both fever (temperature $\geq 38^{\circ}\text{C}$) and diarrhoea. Severe diarrhoea cases with minimal or no changes even after previous treatment regimens were also recruited in the study.

A total of 68 patients with symptoms of fever and diarrhoea from St Elizabeth Mukumu Hospital and Maseno Mission Hospital were clinically sampled. Out of these, 53 patients from St Elizabeth Mukumu Hospital and 15 from Maseno Mission Hospital satisfied the inclusion criteria and were included in the study within the study period. Blood, pus, cerebral spinal fluid and stool samples were collected from patients who had fever and diarrhoea. Standard sampling procedures and processing were applied equally to every sample to avoid bias.

Data was entered into a computerized database using the Statistical Package for Social Sciences (SPSS version 10.0 for Windows) and analyzed by Chi-square test. Further analysis involved frequencies and cross-tabulations with their associations.

Sample collection and Salmonella detection

Thirty peripheral blood and twenty stool samples were collected from patients during the study period. Cerebral Spinal Fluid (CSF) was collected from three patients who were anemic and convulsing. Pus samples from peritoneum tissue were also collected from four of the patients at the St Elizabeth Mukumu Mission Hospital whose blood had been collected and who were also experiencing swollen abdomen and flatulence; a total of 15 samples (5 blood and 10 stool) were collected from Maseno Mission Hospital and processed following standard microbiology procedures.

Following medical examination, samples were collected from the patients based on the clinical judgment of the attending clinician. The samples were then analyzed in the hospital laboratory for the presence of pathogens, and the isolates subjected to antibiotic susceptibility testing. The remaining portion of the samples were then cultured in Selenite-F enrichment and transported in a cool box to the Maseno University Zoology

laboratory within 2 hours from collection for further analysis.

Results obtained at the university laboratory were compared to those obtained from the hospital.

Isolation and identification of Salmonella species in blood, CSF and stool

The collected samples were inoculated in Selenite-F medium and incubated at 37°C for 18 hours for maximum recovery of the isolates. To analyze for the presence of *Salmonella*, inocula were obtained from Selenite-F medium using a sterile cotton swab onto MacConkey agar media and incubated for 18 hours at 37°C. These were then subcultured into Deoxychocolate Citrate Agar (DCA). The isolates were then subcultured in Kligler Iron Agar (KIA) and Simmon Iron Medium (SIM). These steps were followed by biochemical and serotyping identification. The *Salmonella* positive specimens were then subcultured in nutrient broth and stored in the refrigerator at 8°C for antibiotic susceptibility testing.

Antibiotic susceptibility testing

The control bacteria isolates (ATCC 14028-American Type Culture Collection) were obtained from the Kenya Medical Research Institute/Center for Disease Control–Microbiology Unit. The antimicrobial drugs used were obtained from Sigma (United Kingdom. Culture media was prepared by reconstituting commercial powder in distilled water and sterilized at 121°C for 15 minutes in an autoclave per the manufacturer’s instructions. The isolated *S. typhimurium*, *S. typhi*, and other *Salmonella* species were cultured on Mueller-Hinton agar (HIMEDA M173). The isolates were tested by Kirby – Bauer disc diffusion method for drug susceptibility according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines [14]. The Mueller-Hinton Agar plates were smeared evenly using a sterilized swab with *Salmonella* isolates. This was then impregnated with antimicrobial sensitivity discs using sterile forceps and then gently pressed down onto the agar. The antibiotic disc sensitivity was done using ampicillin (10 µg), chloramphenicol (30 µg), cotrimoxazole (25µg), streptomycin (10 µg), tetracycline (30 µg), sulfamethoxazole-trimethoprim (25 µg), and ciprofloxacin (5 µg) (16).

Results

Out of 33 specimens, (62.3%: 33/53) yielded *S. typhi*. Ten (30%) of these were isolated from male adults; 7 (21.2%) from female adults; 2 (6.1%) from a male child; and 8 (24.2%) from female children. The gender for 6 (18.2%) children was not recorded. The species of 2 (3.8% n = 53) *Salmonella* isolates from male adults were not determined (Table 1). Of the isolated 33 *Salmonella typhi*, 20 (60.6%) were from stool and 12 (39.4%) from blood. Four pus samples analyzed from patients with swollen abdomen and flatulence yielded 2 (6.6%) *S.typhi* (Table 2). In St Elizabeth Mukumu Mission Hospital, *S. typhimurium* was isolated in stool from 18 (33% n=53) patients, out of which 2 (11.1%) were from male adults, 3 (16.7%) from female adults, 6 (33.3%) from girls, and 4 (22.2%) from boys. The gender of 2 (11.1%) adults and 1 (5.6%) child was not recorded.

Table 1. Prevalence of *Salmonella* isolates in St Elizabeth Mukumu Mission Hospital.

Bacteria	Number (%) of <i>Salmonella</i> isolates among:					
	Adults			Children		
	Male	Female	Unknown	Male	Female	Unknown
<i>S. typhi</i> (n = 33)	10 (30)	7 (21.2)	0 (0)	2 (6.1)	8 (24.2)	6 (18.2)
<i>S. typhimurium</i> (n = 18)	2 (11.1)	3 (16.7)	2 (11.1)	6 (33.3)	4 (22.2)	1 (5.6)
<i>Salmonella spp.</i> (n=2)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)
Total (53)	13	11	2	8	12	7

Unknown= gender was not recorded.

Fifteen samples (5 blood samples from in-patients and 10 stool samples from out-patients) were collected in Maseno Mission Hospital and analyzed for bacteria isolates. Five samples (2 blood and 3 stool) were negative of *Salmonella* but positive of *Shigella* spp and *Escherichia coli* respectively. The remaining ten samples (3 blood

and 7 stool), were *Salmonella typhimurium* positive. One *Salmonella typhimurium* isolate (10%) was from male adults; 4 (40%) from female adults; 2 (20%) from female children and 3 (30%) from male children. No other species of *Salmonella* were isolated.

Table 2. Number of *Salmonella* isolates collected from various samples both in Maseno and St Elizabeth Mukumu Mission Hospitals.

Collected specimen	<i>S. typhi</i> (n=33)	<i>S. typhimurium</i> (n=18)	Unknown (n=2)
Stool (n=20)	20 (60.6)	18 (100)	1 (50)
Blood (n =30)	13 (39.4)	0 (0)	0 (0)
Cerebral Spinal Fluid (n=3)	0 (0)	0 (0)	1 (50)
Pus (n=4)	2	0 (0)	0 (0)

Antimicrobial drug sensitivity results for Mukumu and Maseno Hospitals are shown in Table 3. In Mukumu, over 90% of *S. typhi* isolates were resistant to ampicillin and streptomycin. Resistance to chloramphenicol was 78.8% followed by cotrimoxazole. No resistance was observed towards tetracycline, sulfamethoxazole and ciprofloxacin. In the same hospital, about 100% of *S. typhimurium* were resistant to ampicillin; 94% to streptomycin; 83% to chloramphenicol; 77% to cotrimoxazole; and 55% to tetracycline and sulfamethoxazole respectively. Other unknown *Salmonella* species were sensitive to tetracycline, sulfamethoxazole, and ciprofloxacin among the screened antibiotics. However, in Maseno, all the *Salmonella typhimurium* isolates were 100% resistant to all the antibiotics tested except ciprofloxacin. Thus in both Mukumu and Maseno, all *S. typhimurium* isolates were sensitive to ciprofloxacin. No *S. typhi* isolated from pus samples were screened for antibiotic resistance since this had been done on blood samples collected from the same patients.

Table 3. Sensitivity pattern of antibiotics tested against *Salmonella* in St Elizabeth Mukumu and Maseno Mission Hospitals.

St. Elizabeth Mukumu Mission Hospital				
Number (%) of <i>Salmonella</i> isolates resistant to antibiotics:				
Antibiotic screened	<i>S. typhi</i> (n=33)	<i>S. typhimurium</i> (n=18)	<i>Salmonella spp</i> (n=2)	Total (n = 53)
Amp (10µg/ml)	30 (90.9)	18 (100)	2 (100)	50
Chlor (30µg/ml)	26 (78.8)	15 (83.3)	2 (100)	43
Cot (25µg/ml)	22 (66.7)	14 (77.8)	2 (100)	38
Strep (10µg/ml)	31 (93.9)	17 (94.4)	2 (100)	50
Tet (30µg/ml)	0 (0)	10 (55.6)	0 (0)	10
Smx (25 µg/ml)	0 (0)	10 (55.6)	0 (0)	10
Cip (5 µg/ml)	0 (0)	0 (0)	0 (0)	0
Maseno Mission Hospital				
Number (%) of <i>Salmonella</i> isolates resistant to antibiotics:				
Antibiotic screened	<i>S. typhi</i> (n=33)	<i>S. typhimurium</i> (n=18)	<i>Salmonella spp</i> (n=2)	Total (n = 10)
Amp (10µg/ml)	N.I	10 (100)	N.I	10
Chlor (30µg/ml)	0 (0)	10 (100)	0 (0)	10
Cot (25µg/ml)	0 (0)	10 (100)	0 (0)	10
Strip (10µg/ml)	0 (0)	10 (100)	0 (0)	10
Tet (30µg/ml)	0 (0)	10 (100)	0 (0)	10
Smx (25 g/ml)	0 (0)	10 (100)	0 (0)	10
Cip (5 µg/ml)	0 (0)	0 (0)	0 (0)	0

Key: µg/ml = micrograms per millilitre, % = percentage, Amp = Ampicillin, Chlor = Chloramphenicol, Cot = Cotrimoxazole, Strep = Streptomycin, Tet = Tetracycline, Smx = Sulfamethoxazole, Cip = Ciprofloxacin. N.I. = Not isolated.
Note - only *S. typhimurium* was isolated from Maseno Mission Hospital patients.

Discussion

Emergence of bacterial resistance to well-known and trusted antibiotics is widely recognized as one of the greatest challenges that physicians face in the management of adult and pediatrics infections [17,18]. It has negatively affected the population by increasing morbidity and mortality rates, reducing desired treatment outcomes, and increasing the need for hospital admission while increasing the cost of patient care. There is, therefore, need for more community-based antimicrobial susceptibility surveys. In the present study, it was observed that *S. typhi* was more prevalent compared to *S. typhimurium* and other *Salmonella* species. It was noted that *S. typhimurium* was more prevalent in female adults and male children while *S. typhi* was more prevalent in male adults and female children. Although these preliminary results were not significant, the study calls for more research to be done to determine the gender disparity to infection by the two bacteria. Other studies in children have

found that for unknown reasons, diarrhoea is more prevalent in boys than in girls. In a cross-sectional study by Onyango, 23.3% and 60% of *S. typhimurium* was isolated among immunocompromised and immunocompetent children attending five health centers within the Kisumu district in Western Kenya respectively [19].

All the patients with Salmonellosis in both health centers were started on ampicillin for a week; however, most patients (n = 45) did not respond and were given other available antibiotics (Table 3). Resistance pattern was observed in more than 45 patients to chloramphenicol, cotrimoxazole, and streptomycin in Mukumu Hospital. *S. typhimurium* showed higher resistance than *S. typhi*. The same resistance pattern was observed in Maseno Mission Hospital for *S. typhimurium*. No *S. typhi* was isolated for antimicrobial comparison purposes. All the isolates in both hospitals were sensitive to ciprofloxacin. In Kenyatta National Hospital, a similar trend of resistance has been reported but not on *S. typhi* [20]. Antibiotics are included in the drug kit supplied to rural health facilities in Kenya. Although antibiotic treatment is not indicated except for severe cases, more often than not antibiotics are administered in *Salmonella* infections, sometimes without the isolation of the causative agent. It is possible that lack of culture and sensitivity diagnostic services in most laboratories in the Western region could have contributed to the spread of resistance due to indiscriminate prescription of antimicrobial [6,21]. In addition, plasmid encoded antimicrobial resistance is also likely to spread to other pathogenic organisms and reduce the ability to treat the infection as well as increase the cost and duration of treatment [20].

Antibiotics play a key role in treating diseases of bacterial origin, a major cause of morbidity and mortality in the developing world. The much needed data on antibiotic susceptibility in rural sites is not available and this study contributes towards the provision of such information. This study provides valuable information to agencies and legislators involved in making policy decisions about the use of antimicrobials. Antimicrobial resistance continues to be experienced world wide within hospitals. This phenomenon is observed in the representative population data discussed herein and it is thought that it may be due to the

need for antibiotics, which is driven by the high incidence of infectious diseases [20]. The reason is that most patients were not responding to the most available antibiotics of choice. Low antimicrobial response observed in this study could be due to unrestricted prescription and use of antibiotics within the study population. *S. typhi* was the most prevalent isolate followed by *S. typhimurium*. Finding that all the *Salmonella* isolates in this study were sensitive to ciprofloxacin suggests that this drug should be protected against emergence of resistant strains. Laboratories in Kenya should perform surveillance by routinely testing *S. typhi* and *S. typhimurium* for susceptibility to first-line treatment drugs, e.g. ciprofloxacin and nalidixic acid, to detect quinolone resistance. Effective surveillance for this drug-resistant *S. typhi* and *S. typhimurium* in our study sites and other developing countries where drug resistance has not yet emerged would ensure prompt diagnosis, susceptibility testing, and appropriate antimicrobial chemotherapy. Although patients' response to antibiotics does not always correspond to *in vitro* susceptibility test results, efficient laboratory diagnosis should be enhanced before prescription of antimicrobials [22]. Furthermore, studies should be conducted to determine why there is gender disparity in *S. typhi*, and *S. typhimurium* infection as seen in this study.

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