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Susan Kavai

Kenya Medical Research Institute

Cecilia Mbae

Kenya Medical Research Institute

Celestine Wanjiku

Kenya Medical Research Institute

Ronald Ngetich

Kenya Medical Research Institute

Zillah Wakio

Kenya Medical Research Institute

Robert Onsare

Kenya Medical Research Institute

Samuel Kariuki (✉ samkariuki2@gmail.com)

Kenya Medical Research Institute

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Antimicrobial resistance and sickle cell protein analysis in invasive non-typhoidal salmonella isolated from outpatient and hospitalized children below 16 years in informal settlements in Nairobi, Kenya

Susan Mutile Kawai^{1*}, Cecilia Mbae¹, Celestine Wanjiku¹, Ronald Ngetich¹, Zillah Wakio¹, Robert Onsare¹, Samuel Kariuki^{1,2}

¹Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya

²Wellcome Sanger Institute, Hinxton, Cambridge CA10 1SA, United Kingdom

Correspondence: S. Kariuki, Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840-00200, Nairobi, Kenya (samkariuki2@gmail.com, Skariuki@kemri.org)

ABSTRACT

Background:

Invasive Non-typhoidal *Salmonella* (iNTS) disease continues to be a major public health problem, especially in sub Saharan Africa where incidence rates are 227 cases [range 152-341] per 100,000 population. Populations at risk of iNTS include adults with HIV infection, malnourished children, those with recent malaria or sickle-cell anaemia (SCA). Individuals with SCA are at an increased risk of invasive bacterial infections with the proportion of deaths from infection reported to be as high as 38% in the United States and 29% in Jamaica.

In Kenya, iNTS disease is particularly a major challenge in poor informal settlements with infants and young children less than 5 years of age being the most affected; mortality rates can be 20-25% unless prompt treatment is administered.

Methods

Our study was conducted in 3 outpatient sites and 1 inpatient site, the outpatient sites were all located within Mukuru informal settlement, a densely populated slum, 15km East of Nairobi City. Blood and stool samples from children with fever alone and with fever and diarrhea were collected for processing for presence of iNTS using basic microbiology procedures. Dry blood spots were also taken and processed for sickle cell protein markers using High performance liquid chromatography (HPLC).

Results

A total of 22,246 blood and stool samples were collected from children < 16 years of age with fever/with or without diarrhea, for a period of 6 years and subjected to microbiological culture and detection of bacterial pathogens. Out of these 741 (3.3%) tested positive for *Salmonella* species. A total of 338/741(41%) NTS were isolated across all the sites; these consisted of 158/741(21%) *Salmonella* Enteritidis and 180/741 (24%) *Salmonella* Typhimurium.

The most common resistance phenotype was ampicillin, cotrimoxazole and chloramphenicol (35.03%). We had 12/338 (3.6%) isolates (11 of them being *Salmonella* Typhimurium) that were ESBL producers conferring resistance to 3rd generation cephalosporins (Amp C β -lactamases) while only 0.3% were resistant to ciprofloxacin. A total of 118 (35.03%) isolates were MDR.

Out of 2684 dry blood samples subjected to HPLC for investigation of sickle cell disease traits, 1820/2684 (67%) had normal hemoglobin (Hb AA/ Hb AF); (162/2684 (6%) tested positive for Sickle Cell Traits (Hb AS/Hb AFS); while 4/2684 (0.2%) tested positive for Sickle cell disease (Hb FS).

Conclusion

The high MDR resistance phenotype in iNTS isolates and emerging resistance to third generation cephalosporins is of great concern in management of iNTS in our settings. Sickle cell disease was not a major factor among children with iNTS disease and no significant association with iNTS was observed.

Keywords: Sickle Cell Disease, Non Typhoidal Salmonella, phenotypic AMR profiles, outpatient and hospitalized, Children, below 16 years, informal settlements, Nairobi, Kenya.

Background

Globally Non-typhoidal *Salmonella* (NTS) disease is associated with an estimated 3.4 (range 2.1-6.5) million cases annually (overall incidence 49 cases [range 30-94] per 100,000 population). Sub-Saharan Africa (SSA) bears the highest burden with incidence (227 cases [range 152-341] per 100,000 population) and number of cases (1.9 [range 1.3-2.9] million cases); infants, young children, and young adults were most affected, with with a case fatality rate between 20 to 25% [1, 2, 3, 4, 5].

In SSA, invasive non-typhoidal Salmonella (iNTS) disease is caused majorly by two serovars; *Salmonella* serovars Typhimurium and Enteritidis. It is a major challenge in SSA as it is responsible for increased cases of childhood morbidity and mortality. Serovar *Salmonella* Typhimurium is more common than *Salmonella* Enteritidis [6].

In most SSA countries, empirical treatment is often administered due to lack of strengthened laboratory systems in the health care facilities [7; 8]. This greatly contributes to inability to detect antimicrobial resistance which can persist in the patient's body system for many months [9, 10].

Invasive NTS disease (iNTS) in children in SSA has been associated with malaria infection, malnourishment as well as sickle cell disease [11,12]. Besides iNTS infections, patients with sickle cell disease (SCD) are susceptible to a variety of other bacterial infections, which are a major cause of morbidity and mortality [13]. This increased susceptibility to infections is related to abnormalities in the defence mechanisms of these patients, including functional hypo-splenism, an abnormality in the alternative pathway of complement activities, and defective neutrophil function [13,14].

Devitalisation of the gut and bone due to repetitive vaso-occlusive crises, macrophage saturation with red cell breakdown products as a result of chronic haemolysis, and underlying splenic and hepatic dysfunction, are known to predispose patients with SCD to *Salmonella* infection [13]. Reduction of iNTS disease incidence has been reported following an improvement of effective malaria control measures [15].

Increasing antimicrobial resistance (AMR) in iNTS is of great global concern, and the situation is even more serious in low and middle- income countries where empiric treatment options for effective treatment of life-threatening invasive disease are limited. Several studies have shown that strains of NTS that are multidrug resistant to recommended first-line antibiotics, including ampicillin, Trimethoprim-sulfamethoxazole, chloramphenicol and kanamycin have emerged in several African countries over the past 20 years. Multi-drug resistance in iNTS has previously been reported in Kenya and Malawi (4,10,16) posing a major challenge to treatment and management options [17].

Salmonella Typhimurium, sequence type 313 (ST313) is a distinct phylogenetic lineage that has emerged in Africa. It is now a significant cause of iNTS disease in Africa. A recent study (18) reported isolating ST313 with both lineages I and II, but fewer ST19 strains, an important cause of iNTS disease as well as asymptomatic carriage.

Treatment failure and complications are associated with lack of proper diagnostic capacity that can aid in management of these multi-drug resistant strains [15,16]. We report on MDR iNTS from patients treated at outpatient clinics showing limited prevalence of sickle cell disease or sickle cell trait.

METHODS

Study Site

We studied patients attending three outpatient facilities and one inpatient facility; in Nairobi County namely, Mukuru kwa Njenga clinic (MMM), Municipal county council clinic (Mukuru health centre) (MCC), and Mukuru kwa Reuben clinic (MR) and Mbagathi district hospital clinic (MB) (inpatient facility). Mukuru informal settlement is situated in the eastern side of Nairobi about 15 Kilometres from the city centre. It is one of the largest slums in the city with a population of around 150,000 people (KNBS, 2011) in an area of 5km². Mbagathi district hospital mostly serves patients from Kibera slum which is also located within Nairobi County.

These informal settlements are densely populated and characterized by limited basic services and infrastructure for providing clean water, sanitation facilities, solid-waste management, roads, drainage, and electricity [8]

Study Design

The study utilized a prospective longitudinal study design

Study Population

The study sought to recruit suspected cases of children < 16 years of age with bloodstream infections in Mukuru and Kibera informal settlement. We recruited patients presenting for care at 3 outpatient medical facilities and 1 inpatient facility that serves the informal settlements. These patients were approached for participation in the study if they were; residents in the Mukuru and Kibera slum; presented with a subjective history of at least 3 days of fever and have an axillary temperature of at least 37.5 °C or they presented with a history of fever of any duration and have an axillary temperature of at least 37.5 °C; or they reported having had three or more loose or liquid stools (children > 2 years) or 8 or more for infants in the 24 hours before presentation, or one or more loose or liquid stool with visible blood. We also included controls for our study, these were individuals (children) who presented themselves to the clinic to attend the mother child clinic. The cases were therefore age matched with the controls. For every case, we sort for 2 controls. The controls ideally were healthy individuals recruited from our sites and residing in Mukuru or Kibera slums [8].

Detection of bacterial pathogens

Blood for culture

For blood culture 1-3 ml for children < 5 years of age and 5-10 ml for 5-16 years of age was collected in syringe, placed into TSB media in Bactec bottles, and transported daily to and analyzed at the KEMRI laboratory. Blood cultures were incubated at 37°C in a computerized BACTEC™ 9050 Blood Culture System (BD, Franklin Lakes, New Jersey, USA), and sub cultured after 24 h onto blood,

chocolate and MacConkey agar plates. The blood cultures were subsequently observed for a further 7 days for signs of bacterial growth (auto-detection). A final subculture was performed for all blood cultures on the 8th day regardless of the state of bacterial growth. From the subcultures, bacterial isolates were identified using biochemical tests on API20E strips (API System, Montalieu Vercieu, France) and further typed by species-specific serological tests for *Salmonella* species [8].

Stool cultures

The rectal swab or loopful of the stool specimen was transported to KEMRI laboratory and initially cultured on selenite F (Oxoid, Basingstoke, UK) broth aerobically at 37°C overnight. Broth cultures were then sub cultured on MacConkey agar and *Salmonella-Shigella* agar (Oxoid, UK) and incubated at 37°C overnight. To identify suspect *Salmonella* bacteria, non-lactose fermenting colonies were biochemically tested using triple sugar iron (TSI) slants. From the subcultures, bacterial isolates were identified using biochemical tests on API20E strips and further typed by species-specific serological tests [8].

Malaria Test (Rapid Detection Test)

Using a sterile lancet, a gentle prick was made towards the pulp of the 4th finger at the disinfected site. The first drop of blood was expressed by applying gentle pressure to the finger and wiped away with a dry piece of cotton wool. Gentle pressure was applied to the same finger until a new blood drop appeared. Using the blood collection device provided in the RDT kit, the open end was gently immersed in the blood drop. The required volume of blood was collected as per manufacturer's instructions. The collected blood was transferred to the sample well. Holding the buffer bottle vertically, a drop of the buffer was added into the buffer well. Interpretation of the results was done according to the manufacturer's instructions.

Processing of dried blood spots for sickle cell diagnosis

High Performance Liquid Chromatography (HPLC)

Diagnosis of SCD was done using HPLC technique with samples run against a known range of SC protein standards. The HPLC was connected to the computer, which shows separation of the various haemoglobin types on a screen. HPLC offers the advantages such as full automation of the entire procedure and accurate quantification of the levels of haemoglobin present as long a variant or known Hb in the patient sample does not interfere with the interpretation [14,19].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the disk diffusion technique for all commonly used antimicrobials in Kenya on Mueller-Hinton agar (Oxoid, Basingstoke, UK). For Gram negative enteric bacterial species this includes ampicillin 10µg, tetracycline 30µg, gentamicin 10µg, trimethoprim 5µg, sulfamethoxazole 100µg, chloramphenicol 30µg, co-amoxiclav 20:10µg, cefuroxime 30µg, ceftazidime 30µg, ceftriaxone 30µg, cefotaxime 30µg, ciprofloxacin 5µg and nalidixic acid 10µg, MICs were performed using the E-test strips (AB BIODISK, Solna, Sweden). Results were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (2017). MDR prevalence was defined as resistance to ampicillin, chloramphenicol and co-trimoxazole [8].

Ethical Considerations

The study was approved by the Scientific and Ethics Review Unit (SERU) of KEMRI (SSC No. 2076). All parents and/or guardians of participating children were informed of the study objectives and voluntary written consent was sought and obtained before inclusion. A copy of the signed consent was filed and stored in

password protected cabinets at KEMRI. All methods were carried out in accordance with relevant guidelines and regulations.

Results

Out of 22,246 patient samples collected, 741 (3.3%) were positive for *Salmonella* spp. From the 741 *Salmonella* spp, 338 (41%) were iNTS, 220/338 (65%) were cases who presented to the clinic with diarrhoea. 8/338 (2.4%) were patients that had NTS as well as tested positive for Malaria.

NTS AMR profiles from 2013-2018

Resistance trends for *S. Typhimurium* against the first line antibiotics were as follows: Ampicillin (54.48%), sulfamethoxazole-trimethoprim (44.7%), tetracycline (22.75%), chloramphenicol and (22.57%). The resistance for the cephalosporins (Ceftazidime {CAZ}, ceftriaxone {CRO}, Cefotaxime {CTX}, cefpodoxime {CPD}) was generally low over the six-year period (**Figure 1**).

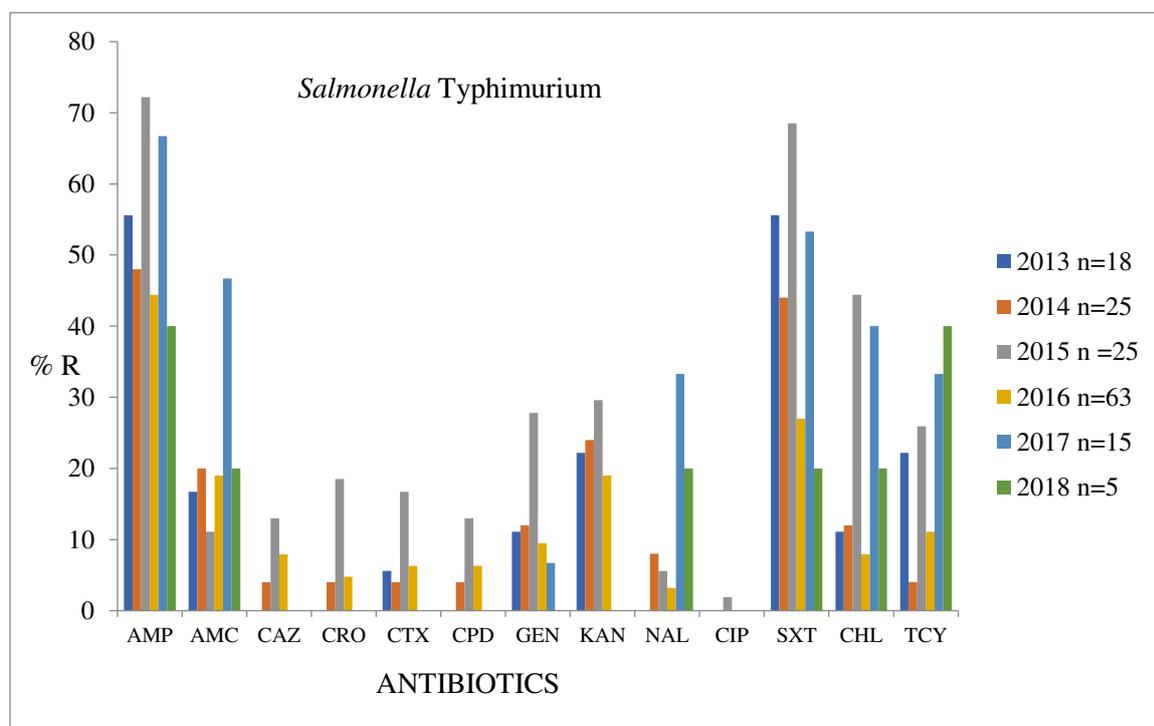


Figure 1: Resistance Trends of *S. Typhimurium* against a Panel of 13 antibiotics from 2013-2018

1 isolates subjected to a panel of 14 selected antibiotics which include Ampicillin (AMP), Amoxicillin Clavulanate (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefpodoxime (CPD), Gentamicin (CN), Kanamycin (K), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Sulfamethoxazole-trimethoprim (SXT), Azithromycin (AZM), Chloramphenicol (C), and Tetracycline (TCY). No of isolates annually is represented by letter n.

Resistance trends for *S. Enteritidis* against the first line antibiotics were also quite high throughout the six-year period. Their average resistance was as follows: Ampicillin (25.8%), sulfamethoxazole-trimethoprim (22.4%), chloramphenicol (20.8%), and tetracycline (18.83%). The resistance for the cephalosporins (Ceftazidime {CAZ}, ceftriaxone {CRO}, Cefotaxime {CTX}, cefpodoxime {CPD}) was generally low over the six-year period (**Figure 2**).

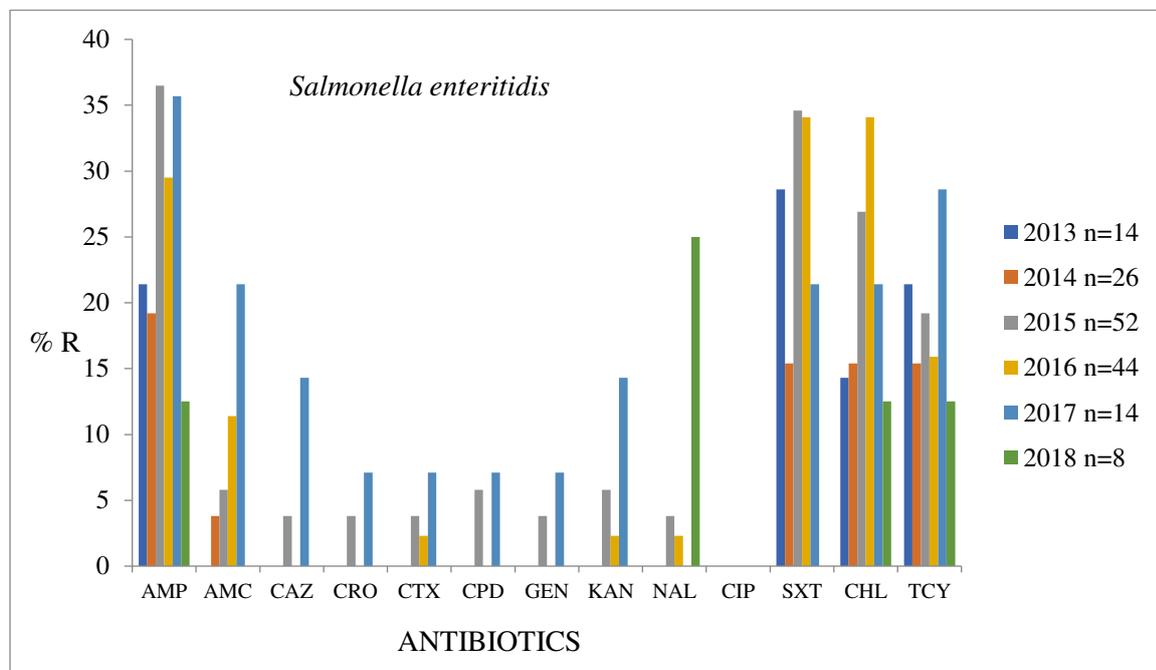


Figure 2: Resistance Trends of *S. enteritidis* against a Panel of 13 antibiotics from 2013-2018

1 isolates subjected to a panel of 14 selected antibiotics which include Ampicillin (AMP), Amoxicillin Clavulanate (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefpodoxime (CPD), Gentamicin (CN), Kanamycin (K), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Sulfamethoxazole-trimethoprim (SXT), Azithromycin (AZM), Chloramphenicol (C), and Tetracycline (TCY). No of isolates annually is represented by letter n.

Occurrence of NTS and social demographic factors

A total of 338 (100%) were isolated. 180/338 (53.25%) were *S. Typhimurium* while 158/338 (46.74%) were *S. Enteritidis*. The P value was < 0.05 indicating that there was a significant association between the iNTS and the age groups isolated from. Age group 0-5 years had most NTS isolated. Across all age groups females were more affected than males. It was also observed that stool samples had more NTS isolated as compared to blood samples **Table 1**.

Table 1: NTS Verses Social Demographic Factors Distribution

Serotype	Age	No of NTS	Odds	P-	Sex	Sample Type	Status
	Groups	Isolated	Ratio	Value			
S. Typhimurium	0-5 yrs	112/180	6.5882	0.001	52 males/60 females	34 blood/78 Stool	42 controls/70 cases
	6-10 yrs	51/180	3.0	0.002	25males/26 females	28 blood/23 stool	15 control/26 cases
	11-16yrs	17/180	R		6males/11 females	7 blood/10 stool	4 controls/13 cases
S. Enteritidis	0-5 yrs	103/158	7.9231	0.0001	59 males/54 females	30 blood/73 Stool	37 controls/66 cases
	6-10 yrs	42/158	3.2308	0.0005	15 males/27 females	15 blood/27 stool	14 control/ 28cases
	11-16yrs	13/158	R		6 males/7 females	6 blood/7 stool	5 controls/8 cases

Occurrence of NTS and Sickle cell disease/Trait

A total of 2684 samples from both blood and stool samples were subjected to a HPLC for processing of sickle cell disease. A total of 1820/2684(67.8%) tested normal haemoglobin, 162/2684(6.03%) were confirmed to have sickle cell trait while 4/2684(0.2%) tested positive for sickle cell disease. Interestingly 4/2684(0.2%) samples that tested positive for SCD tested negative for NTS in both blood and stool samples (**Table 2**).

Table 2: iNTS and fecal NTS in SCD

SAMPLE ID	RESULT	COMMENT	Status	<i>Salmonella</i> in Blood	<i>Salmonella</i> in Stool
MCC1129	Hb AFS	Sickle cell trait	Positive	None	S. Enteritidis
MR1369	Hb AFS	Sickle cell trait	Positive	None	S. Typhimurium
MCC1996	Hb AS	Sickle cell trait	Positive	None	S. Typhimurium
MB355	Hb AS	Sickle cell trait	Positive	None	S. Enteritidis
MB511	Hb AFS	Sickle cell trait	Positive	None	S. Enteritidis
MMM2516	Hb AFS	Sickle cell trait	Positive	S. Enteritidis	None
MR2220	Hb FS	Sickle cell disease	Negative	None	None
MR2097	Hb FS	Sickle cell disease	Negative	None	None
MB234	Hb FS	Sickle cell disease	Negative	None	None

MB1521	Hb FS	Sickle cell disease	Negative	None	None
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Key: MCC-Municipal County Council, MR-Mukuru kwa Reuben, MMM-Mukuru kwa Njenga, MB-Mbagathi district hospital.

Occurrence of NTS bacteremia and Sickle cell disease

A total of 47 NTS were isolated from the blood samples subjected for SCD test and only 1/47(2.13%) was positive for sickle cell trait Hb AFS. 46/47(97.87%) were found to have Normal haemoglobin 45(Hb AA) and 1(Hb AF). In addition, a total of 51 NTS were isolated from the stool samples and 5/51(9.80%) were positive for sickle cell trait 3 (Hb AFS) and 2(Hb AS) while 46/51(90.20%) were found to have normal haemoglobin 41(Hb AA)5(Hb AF) **Table 3.**

Table 3: Distribution of NTS in blood and stool in samples subjected to HPLC

	No. of NTS in blood	No. of NTS in stool	Normal haemoglobin	Sickle cell disease	Sickle cell trait
S. Typhimurium	19	34	45 Hb AA/ 1 Hb AF	0	1 Hb AFS
S. Enteritidis	28	17	41 Hb AA/5 Hb AF	0	3Hb AFS/2 Hb AS
Total	47	51			

Occurrence of NTS in Malaria and HIV

A total of 8/338 (2.4%) children positive for NTS were also positive for Malaria but negative for HIV. These children came from a population of both cases and controls. S. Typhimurium was isolated in 7 malaria positive patients; 5 cases and 2 controls while S. Enteritidis was isolated in one malaria positive patient. A total

of 5/338 (1.5%) patients positive for NTS tested positive for HIV. Among these 3 were cases while 2 were controls (healthy individuals) **Table 4.**

Table 4: Distribution of Malaria and HIV in Patients with INTS Disease

NTS isolated	Malaria Positive	HIV Positive
S. Typhimurium	7/338 (5 cases, 2 controls)	2/338 (1 case, 1 control)
S. Enteritidis	1/338 (1 case)	3/338 (2 cases, 1 control)
Total	8/338	5/338

Discussion

The most common resistance phenotypes in this study were ampicillin 147/338(43.5%), chloramphenicol 80/338(23.7%) and co-trimoxazole 128/338 (37.9%). On average, the resistance trends from highest to lowest were observed from 2015, followed by 2016, 2013, 2017, 2014 then finally 2018. iNTS species such as *S. Enteritidis* and *S. Typhimurium* continue to pose a global burden of disease[6,20] hence causing a serious challenge in the management of infections especially in LMICs such as Kenya. These iNTS infections occur in urban informal settlements due to several factors such as crowding, street foods, poor drainage systems, poor hygiene and sanitation [8,21].

The burden of iNTS disease in Kenya still remains a challenge with large numbers of morbidity and mortalities among children below five years [8]. In a previous study, it was estimated that among children younger than 5 years, diarrhoeal

diseases were responsible for 499 000 deaths (447 000–558 000 ;), representing 8.6% (7.7–9.5) of the 5.82 million deaths in this age group globally [22]. In a previous GEMS Study, it was estimated that 223 (2.0%) of 11 108 children with moderate to severe diarrhoea and 43 (0.3%) of 16369 matched controls died between study enrolment and the follow-up visit at about 60 days (hazard ratio [HR] 8.16, 95% CI 5.69–11.68, $p < 0.0001$) [23].

A previous study reported that the impact of iNTS on childhood mortality exceeds malaria in some African communities [24]. This study estimated mortality rates for iNTS among hospitalized patients in Africa to range from 4.4 to 27% for children [6;25] and 22 to 47% for adults [9,26].

The burden of disease due to iNTS is significant in sub Saharan Africa and South East Asia. [27]. It was reported that iNTS occurred in an estimated 88 cases per 100,000 person-years in the age group of 5 years old in rural Kenya, while in Mozambique, NTS was estimated to account for 120 cases per 100,000 person-years [27].

These incidences are likely to be under-estimates as many children with iNTS die before reaching the health facilities [25,28].

In SSA, risk factors for iNTS disease in children include HIV infection; malnutrition; [6] and malaria [29]. SCD is also an important risk factor in African children [30]. NTS bacteraemia overlaps significantly with malaria in Africa, both in terms of seasonality and affected age groups [31].

Many attempts have been made to explain how malaria causes susceptibility to NTS. The most consistent and likely evidence is that malarial haemolysis of the red blood cells creates favourable conditions for bacterial growth [32] This haemolysis prevents the effective eradication of NTS that successfully enter the

systemic blood stream via the intestines. It remains inconclusive whether or not malaria infections facilitate the entry of NTS into the blood stream. Our study reported no significant association of iNTS with HIV, Malaria or even Sickle cell disease This is in contrast to studies done in the western part of Kenya where the malaria is endemic, and SCD and HIV are risk factors to iNTS disease [33].

The age group 0 to 5 years across all the sites had the highest number of iNTS infections A similar study in Kenya [34] revealed similar findings to our study that infants (below 1 year) were more likely to be infected with enteric pathogenic bacterial infection (OR 0.3, 95% CI 0.1-0.8) than the older ones. A recent study done in Lusaka Zambia also implicated a high prevalence of infection on the ages of 0-12 months (61.3%) [35].

Salmonella species were once susceptible to a broad range of affordable and effective antimicrobial drugs, but multidrug-resistant strains [9;36] have emerged in Africa. *S* Typhimurium was shown to contain a composite element encoding multidrug-resistant genes located on a virulence-associated plasmid (pSLT-BT), thus potentially linking resistance to antimicrobial drugs with virulence.

Resistance has necessitated the widespread use of expensive drugs for empirical management of sepsis, such as third generation cephalosporins and fluoroquinolones (eg, ciprofloxacin), which are majorly unaffordable in our settings, and which could promote the development of further resistance [36, 37].

Conclusion

It is evident that public health education on improved WASH techniques is essential to control sanitation-related infections. However, as WASH infrastructure will be long in coming especially in overcrowded living conditions in informal settlements, introduction of a bivalent efficacious vaccine that would

target Salmonella serovars Typhimurium and Enteritidis would significantly lower the disease burden in high-risk populations.

Abbreviations

MMM-Mukuru Kwa Njenga

MCC- Municipal County Council

MR-Mukuru kwa Reuben

KNBS-Kenya National Bureau of Statistics

SSA-Sub Saharan Africa

DALYS-Daily Adjusted Life Years

SAC-School Age Children

NTDS-Neglected Tropical Diseases

TSI-Triple Sugar Iron

API-Analytical Profile Indexing

KEMRI-Kenya Medical Research Institute

SERU-Scientific Ethics Review Unit

WASH-Water Hygiene and Sanitation

SCD-Sickle cell disease

INTS -Invasive non typhoidal salmonella

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Declarations

Ethics approval and consent to participate

The study was approved by the Scientific and Ethics Review Unit (SERU) of KEMRI (SSC No. 2076). All parents and/or guardians of participating children were informed of the study objectives and voluntary written consent was sought and obtained before inclusion.

Consent for publication

All authors participated in the project leading to this manuscript and contributed significantly to its preparation.

Availability of data and materials

The data analysed and used for this study are available from the corresponding author on reasonable request and will be on open source-access.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SK designed the study; CM coordinated the collection and processing of the samples. RO supervised the processing of samples, RN, conducted the receiving

and processing of the samples, SMK, ZW, and CW analysed the data. SMK and SK drafted the manuscript. All reviewed the draft manuscript. SK approved the final manuscript.

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Figures

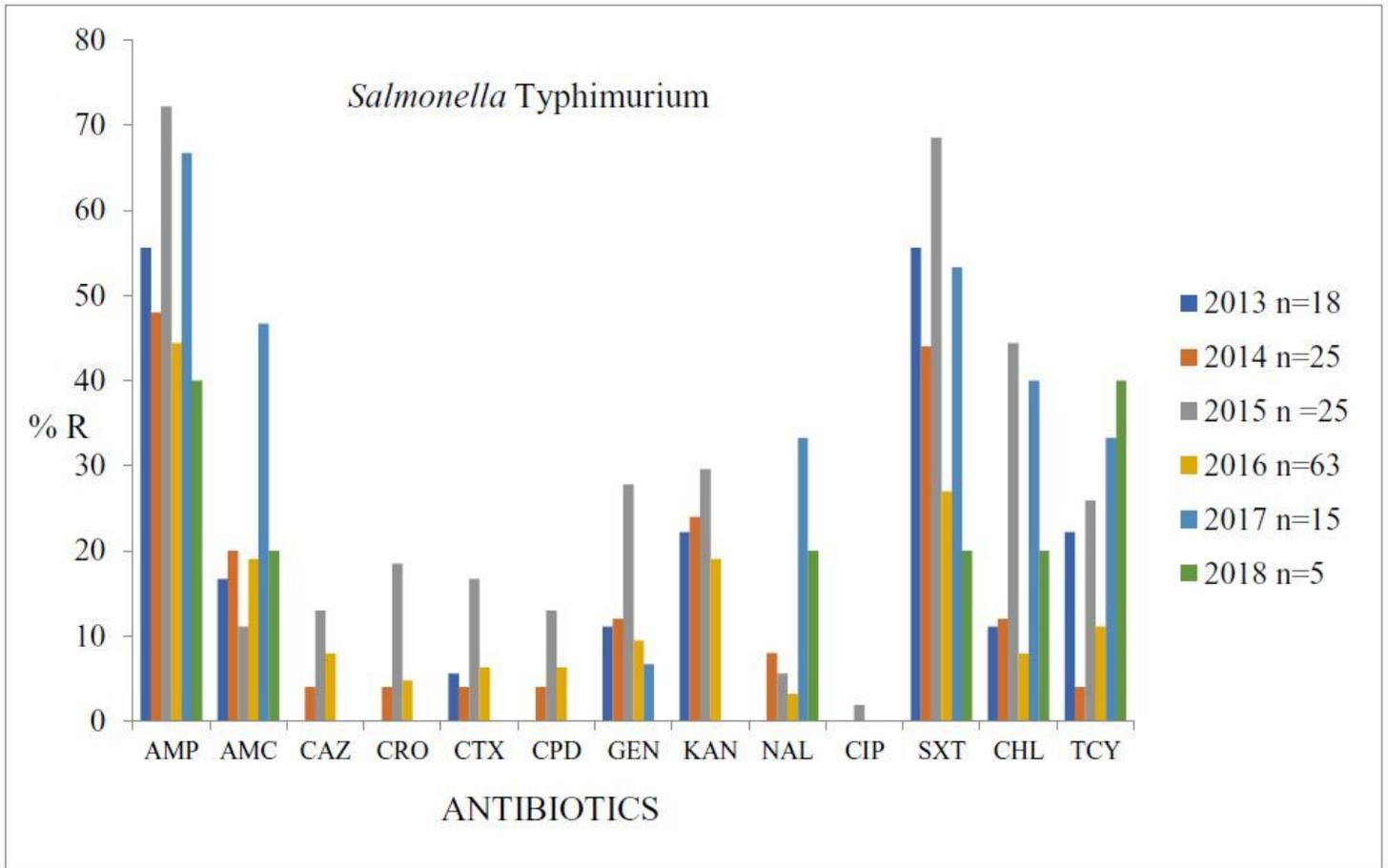


Figure 1

Resistance Trends of *S. Typhimurium* against a Panel of 13 antibiotics from 2013-2018. 15 isolates subjected to a panel of 14 selected antibiotics which include Ampicillin (AMP), Amoxicillin Clavulanate (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefpodoxime (CPD), Gentamicin (GN), Kanamycin (K), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Sulfamethoxazole-trimethoprim (SXT), Azithromycin (AZM), Chloramphenicol (C), and Tetracycline (TCY). No of isolates annually is represented by letter n.

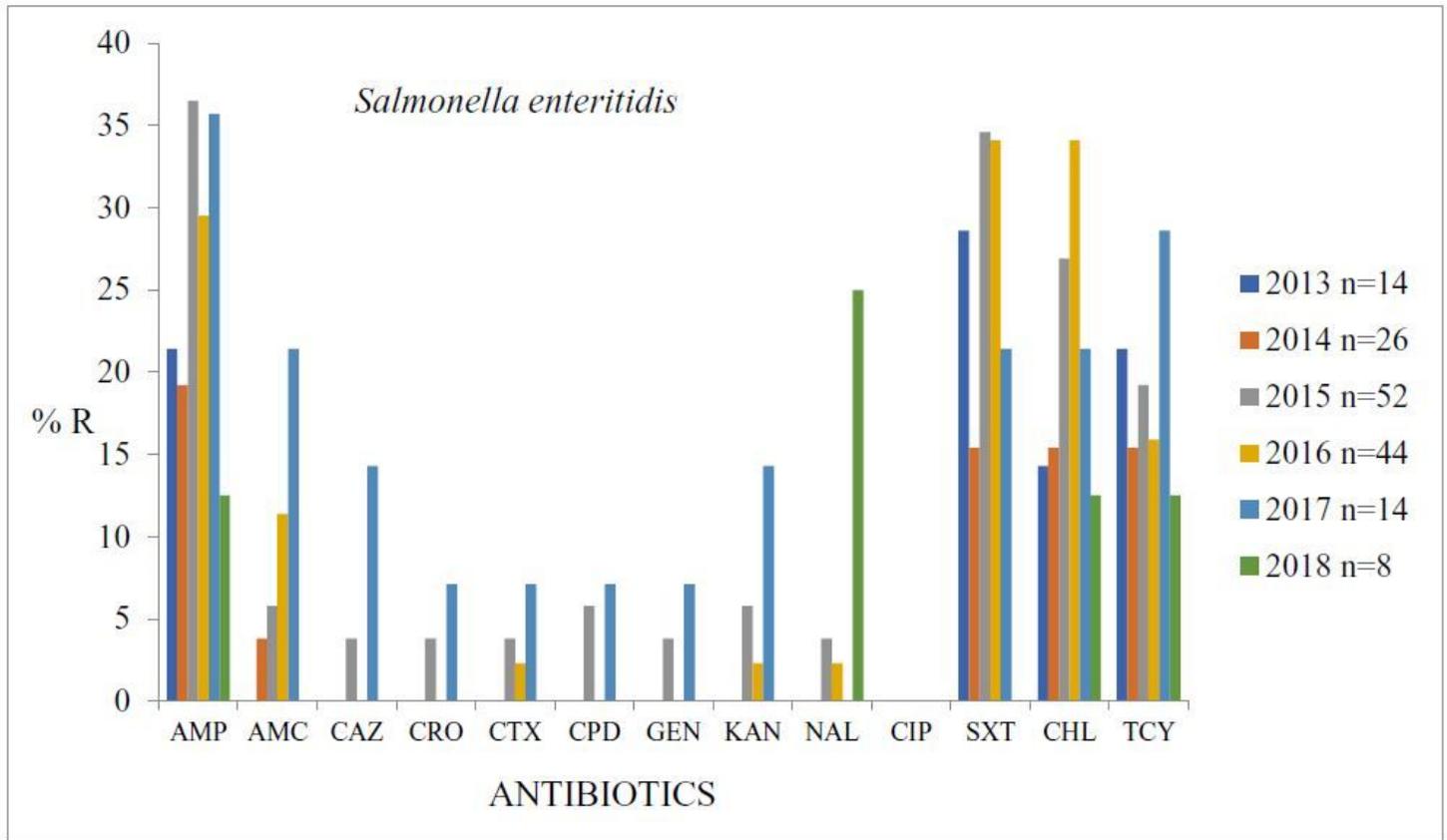


Figure 2

Resistance Trends of *S. enteritidis* against a Panel of 13 antibiotics from 2013-2018. 1 isolates subjected to a panel of 14 selected antibiotics which include Ampicillin (AMP), Amoxicillin Clavulanate (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefpodoxime (CPD), Gentamicin (CN), Kanamycin (K), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Sulfamethoxazole-trimethoprim (SXT), Azithromycin (AZM), Chloramphenicol (C), and Tetracycline (TCY). No of isolates annually is represented by letter n.