

## Antimicrobial Resistance Patterns of bacterial Septicaemia infecting infants in Mbita Subcounty, Western region of Kenya

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### Abstract

**Background:** Gram positive bacteria such *Escherichia coli*, Group B *Streptococcus* coagulase-negative staphylococci, *Staphylococcus aureus*, and Gram-negative bacteria such as *Klebsiella* and *Pseudomonas* species are listed as some of the bacteria etiologies for pediatric septicemia. These bacteria are rapidly becoming multi drug resistant to penicillin (or aminopenicillin), gentamicin, the pragmatic antibiotic treatment regimens. Further, the ever-increasing burden of bacteria septicemia infection due to extended-spectrum  $\beta$ -lactamase (ESBL) producing Gram negative bacteria cumulatively presents a major health concern in the management and treatment of bacterial septicemia. In this study we present data on the prevalence and type of antimicrobial resistant patterns among children with bacterial septicemia in Mbita Sub county Hospital, Western region of Kenya.

**Methods:** Blood samples were obtained from 248 children whose parents/guardian consented. The bacterial isolation and characterization were done using the automated BACTEC 9240 system, conventional culture using morphology, Gram stain and biochemical identification. Further identification and resistant gene detection were determined using Polymerase Chain Reaction (PCR). Descriptive statistics were used to present data.

**Results:** Eighty-four (33.9%) patients had septicemia where *Staphylococcus epidermidis* (28.6%), *S. aureus* (13.1%), *Escherichia coli* (13.1%) and single *Salmonella Paratyphi B*, *Citrobacter freundii*, *Gemella morbillorum*, *Klebsiella pneumoniae*, *Lactococcus lactis cremoris*, *Pantoea* spp, and *Pseudomonas putida* were implicated. The majority of gram-negative bacteria were resistant to penicillin (Ampicillins) 100%, 96.1% to tetracycline, 84.6% to sulphonamides (Trimethoprim/sulfamethoxazole), 73.1% Aminoglycosides (Gentamicin) 73.1% and 19.2% to Quinolone (Ciprofloxacin). For gram positive bacteria majority 96.7% were resistant to sulphonamides (Trimethoprim/sulfamethoxazole) followed by tetracycline 76.7%, penicillin (Oxacilline) 73.3% and least resistant to Quinolone (Ciprofloxacin) 30%. Various antimicrobial resistant genes *mecA*, *SullI*, *blaTEM*, *TetA aac (3)* were identified.

**Conclusion.** In this geographically defined region of Kenya, of the 33.9% children with septicemia, gram positive bacteria were the leading cause septicemia. High level resistance due to various resistant genes were seen all type of antibiotics by both Gram positive and negative bacteria. Rapid antibiotic resistant testing is encouraged for appropriate treatment and management of septicemia infection.

**Keywords:** bacterial Septicemia, Epidemiology, Children under five, South Nyanza, Kenya

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### Introduction

Globally, bloodstream infections and sepsis are major causes of morbidity and mortality. Epidemiological data from high-income countries reported that 31.5 million cases of sepsis and 5.3 million sepsis attributable deaths occur annually (Fleischmann *et al.*, 2015). Data from low-and-middle-income countries (Reinhart *et al.*, 2017). Previous estimates, shows a range of 380 000–2 000 000 annual cases of neonatal sepsis in sub-Saharan Africa and 270 000 annual associated deaths, highlight the substantial burden of disease (Seale *et al.*, 2010; Sepsis Alliance, 2016). Most studies on sepsis and bloodstream infections report an increasing incidence over the last two decades, particularly among the immunocompromised, multimorbid, and elderly patients, or due to failure of empiric antibiotic regimens as result of antimicrobial resistance (Goto & Al-Hasan, 2013; Cassini *et al.* 2019).

Children in developing countries are the most susceptible segment of the population, laying emphasis on the need for extraordinary consideration given that bloodstream infections and sepsis is the major cause of child mortality and morbidity (Kissoon *et al.*, 2011). Invasive bacteria contribute largely to the etiologies of septicemia in African children (Jacob *et al.*, 2009). Some of the bacteria etiologies for pediatric septicemia reported in varying prevalence in sub-Saharan Africa include *Escherichia coli*, Group B *Streptococcus* coagulase-negative staphylococci, *Staphylococcus aureus*, and Gram-negative organisms such as *Klebsiella* and *Pseudomonas* species (Zaidi *et al.*, 2009). The rapid spread of multi drug resistant bacterial pathogens is likely to complicate treatment of bacteria septicemia (Taxt *et al.*, 2020). The widely embraced pragmatic antibiotic treatment regimens for sepsis based on penicillin (or aminopenicillin) in combination with gentamicin are faced with emergence of multidrug resistance jeopardizing their utility (Fuchs *et al.*, 2018).

Of importance, the ever-increasing burden of bacteria septicemia infection due to extended-spectrum  $\beta$ -lactamase (ESBL) producing Gram negative bacteria represents a major health concern (Taxt *et al.*, 2020). These bacteria, mainly *Escherichia coli* and *Klebsiella pneumoniae*, are not only resistant to all penicillins and third generation cephalosporins, but also frequently express co-resistance to gentamicin (Taxt *et al.*, 2020). Inevitably, treatment failure accompanied by increased treatment cost and fatality are likely to occur especially in developing nations. The increasing prescription of third and fourth generation - last-resort antibiotics such as carbapenems as initial antibiotic treatment of sepsis is likely to worsen sepsis management in developing nations most of which sell these antibiotics without the need for doctors' prescription (Taxt *et al.*, 2020). Increased emergence and spread of AMR are likely to be a big challenge in the near future especially in resource limited nations. Inevitably, rapid diagnosis of bacteria septicemia is crucial in the prescription of appropriate antimicrobial therapy likely to lower morbidity and mortality (Buehler *et al.*, 2016). In this study we present data on the prevalence and type of antimicrobial resistant patterns among children with bacterial septicemia in Mbita Sub county Hospital, Western region of Kenya.

## METHODOLOGY

### Study setting and design

This was a descriptive hospital based cross-sectional study conducted between 2019 and 2020 among children presenting with symptoms suggestive of septicemia as described by the WHO (Bataar *et al.*, 2010), attending Mbita District Hospital in Nyanza Kenya. Using the formula for estimating the population proportion with specified relative precision described by Lemeshow *et al.* (1990) setting the  $\alpha$  at 0.05, and prevalence of bacterial septicemia of 76% (WHO, 2014), a total of 281 patients were recruited to achieve 0.95 power.

### Recruitment and ethical approvals

This study recruited patients if: 1. Had clinical symptoms suggestive of septicemia as defined by the WHO. 2. Attending/admitted at Mbita District Hospital. 3. Age between 1 day to 120 months. 4. Parents/guardian providing informed consent. Study patients were then consecutively enrolled till the desired number was achieved. This study was approved by Ethical Review Committee of Kenyatta University before commencing to field activities. Each participant signed a form of informed consent

### Laboratory analysis

**Blood sample collection and transportation:** From each of the participants enrolled, about 2-5ml (children) blood samples were collected aseptically in aerobic and anaerobic blood culture bottles BD (Becton Dickinson, US). The sampling bottles were appropriately labelled in line with pathological/request forms details i.e. name, sample code, date, time and location of the hospital and patients. The blood samples collected aseptically into appropriate blood culture tubes were packed in primary cases and secondary cases according to the WHO guideline for transporting infectious materials. The samples were maintained in upright position in a cooler box and transported to NUITM-KEMRI Biosafety level 2 laboratory at Mbita for processing.

### Microbiological analysis

Samples were incubated at 37°C for at least 3 weeks in the BACTEC 9270 (Becton Dickinson, US) automated machine. Generally, the BACTEC indicates signals for any positive culture wells. All positive culture bottle was taken to the Biosafety level III laboratory where they were sub cultured using sterile and disposable loops on basic, differential, selective media (Oxoid type) and other appropriate media like blood agar, chocolate blood agar (CBA), Xylose lysine deoxycholate (XLD), DHL, Salmonella–Shigella agar (SS), and Bromol thymol blue (BTB)

for other etiological agents. All plates were incubated at 37°C for 18-24hrs. Blood agar, Brucella agar and CBA were incubated in the presence of 5-10% CO<sub>2</sub>. The suspected colonies were examined and characterized by their morphology, Gram stain, biochemical identification (Sahin, *et al.*, 2008). All the identified and clinically significant isolates were purified, accurately labelled and stored in 15-40% glycerol, at -80°C for any future work or references.

### Antibiotic susceptibility testing

Isolates were tested for their antimicrobial susceptibility by the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). The drugs tested included; cefoxitin (CTX), streptomycin (STR), tetracycline (TET), Ampicillin (AMP), chloramphenicol (CHLO), ciprofloxacin (CIP), Nalidixic acid (NA), gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT). The antimicrobial susceptibility was classified using the CLSI guidelines, as susceptible, intermediate, or resistant to each antibiotic. In addition, isolates were also classified as either non-susceptible (including both intermediate and resistant isolates) or susceptible. Isolates found to be non-susceptible or resistant to three or more antimicrobial categories were classified as multidrug resistant (MDR).

### Detection of resistant genes

**Plasmid DNA extraction:** Profiling for R-plasmids and screening for the presence of previously reported resistance gene *TetA gene*, *StrA gene*, *Str B gene*, *Sul2gene*, *Amp C gene*, *Gyr A gene*, *CatA1 gene* were done with modification as described by Szczepanowski *et al.*, (2009). The representative of all bacterial isolates belonging to various antibiograms were selected and sub-cultured on Mueller-Hinton media overnight at 37°C and the plasmid DNA extracted using the alkaline lysis method described by Sambrook *et al.*, (2001). Briefly, the plasmid DNA was extracted from 2-ml overnight cultures grown in Luria-Bertani (LB) broth (Difco Laboratories, Becton Dickinson, Sparks, MD). Plasmid sizes were determined using electrophoresis in comparison with plasmids from the reference strains *E. coli* V517 and *E. coli* 39R861 (Macrina, 1979).

**PCR and amplicon detection.** The reaction mix of the PCR was composed of about 100 ng total plasmid DNA as template, 2.5 µl PuReTaq Ready-To-Go PCR beads and 0.5 µM of each primer (Table 1) and filled up to 25 µl with sterile double-distilled water. The PCR was performed using Bio-Rad iCycler Thermal Cycler (Champaign, US). The initial step of the reaction was denaturation of DNA at 94 °C for 4 min. This step was followed by 35 cycles composed of 1 min denaturation at 94 °C, 1 min annealing at 58 °C and 45 s polymerization at 72 °C. The final polymerization step was performed for 10 min at 72 °C. The amplicons were analyzed by gel electrophoresis (in 1% agarose in Tris/HCl/acetate buffer), stained with ethidium bromide and visualized under UV light.

### Data analysis

Descriptive statistics frequencies (%), standard deviation, mean, and medians (interquartile ranges at 25% and 27%) were used to present data. Where applicable, test for significance was done using Fisher's exact test or Chi-square at the significance level of P 0.05. All statistical analyses were performed using STATA v 13 (StataCorp LP, College Station, TX, USA).

**Table 1 gene identities, primer sequences, molecular sizes and the source**

Target	Forward	Reverse	Size-bp	Reference
<i>StrA</i>	GCTGGATAGGTTAAGGGCGG	CTCTATGGGCACTGTCCACATTG	383	Hochhut <i>et al.</i> , 2001
<i>Sul2</i>	AGGGGGCAGATGTGATCGAC	TGTGCGGATGAAGTCAGCTCC	625	Hochhut <i>et al.</i> , 2001
<i>TetA</i>	ATAAAATTCTTGAAGACGAAA	GACAGTTACCAATGCTTAATC	1080	Weill <i>et al.</i> , 2004
<i>CTX-M</i>	AAA AAT GAT TGA AAG GTG GT	CAG CGC TTT TGC CGT CTA AG		Karunakaran <i>et al.</i> , 2012
<i>CAtA1</i>	AAGCGAACGA	GGAAGTAAAA		Hochhut <i>et al.</i> , 2001
<i>AmpC</i>	AACACACTGATTGCGTCTGAC	CTGGGCCTCATCGTCAGTTA	1870	peres-peres <i>et al.</i> , 2002
<i>Gyr A</i>	TTAATGATTGCCGCCGTCGG	TACACCGGTCAACATTGAGG	648	Jaktaji & Mohiti, 2010
<i>mecA</i>	GTA GAA ATG ACT GAA CGT CCGATAA	CCA ATT CCA CAT TGT TTC GGTCTA A	533	Wielders <i>et al.</i> , 2002

*StrA* - Streptomycin resistance; *Sul2* - sulfamethoxazole resistance; *TetA* - tetracycline resistance; *CTX-M* – Ceftriaxone; *CAtA1 gene* – Chloramphenicol; *AmpC*- Augmentin; *Gyr A* – Ciprofloxacin; *mecA* Gene - Oxacillin/methicillin

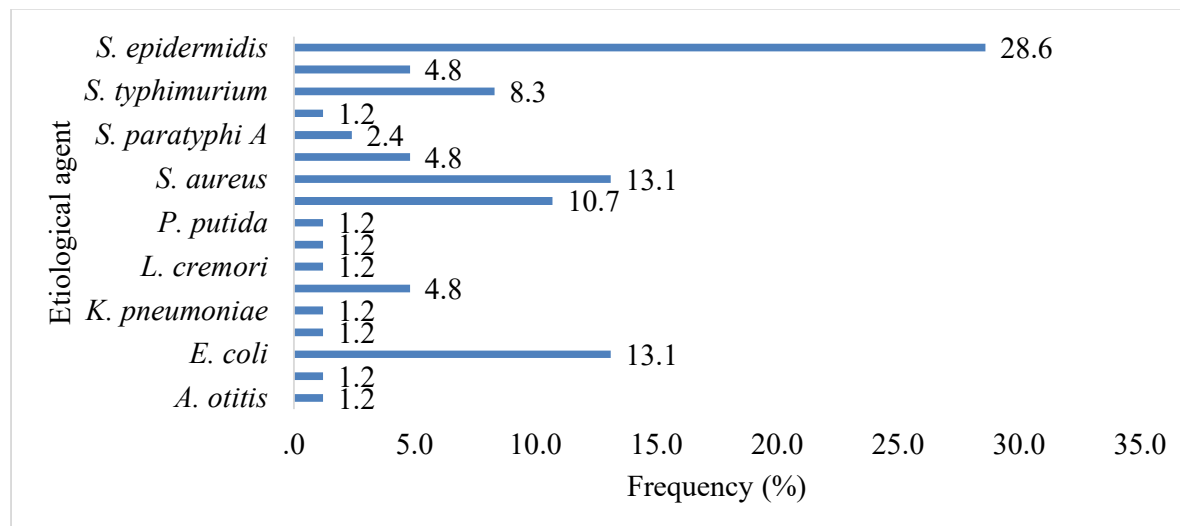
## RESULTS

### Characteristics of study patients

All the data was available for 248 out of recruited 281 children. The mean ( $\pm$  standard deviation - SD) age of the participants was 27.93 ( $\pm$ 20.6) months with 30.6% of them aged between 1 to 12 months. The majority of the patients 50.8% were males, 48% from Rusinga locality and 91.9% HIV negative. The mean body temperature for the patients 38°C ( $\pm$  20.5) ranging between 37 to 40°C. There were 58.9% patients with body temperatures above 37.6 °C. The mean WBC of the patients was 17720.9 Cells/ml ( $\pm$  8929.1) Cells/ml ranging between 12075 to 22450 Cells/ml. about 25.4% of them had WBC above the normal levels of 10501 cells/ml. The mean respiratory rate (RR) of the patients was 30.6 ( $\pm$  10.6) breaths/min ranging between 18 to 96 breaths/min with 71.4% having RR between 20 and 30 breaths/min. Co-infection/complications among the study patients included: malaria reported in 83 (33.5%) of the patients followed by respiratory illnesses 33 (13.3%), Hematologic diseases 31(12.5%), Gastrointestinal disorders 27(10.9%), malnutrition and meningitis in 9 (3.6%) each. There were 6 (2.4%) patients who had Nervous system diseases, 5(2.1%) with Ear nose and throat infections and 2 (0.8%) with HIV. There were 20 (8.1%) patients who reported no other co-infection.

### Etiology of septicemia among study patients

A total of 18 different etiological agents were identified in this study from 84 of the 248 (33.9%) children who had septicemia. The most common causative agent of septicemia was *Staphylococcus epidermidis* (28.6%) followed by *S. aureus* and *E. coli* each at 13.1%. Others included *P. aeruginosa* (10.7%), *S. typhimurium* (8.3%), *S. hemolyticus* (4.8%) among others (Figure 1).



**Figure 1: Frequency of etiological agents of septicemia among the study patients**

#### Antimicrobial resistant patterns of bacterial septicemia etiological agents

Table 1 summarizes the drug susceptibility patterns for bacteria causing septicemia among study participants. Susceptibility testing showed that majority of gram-negative isolates were resistant to penicillin (Ampicillins) 100% followed by tetracycline 96.1%, sulphonamides (Trimethoprim/sulfamethoxazole) 84.6%, Aminoglycosides (Gentamicin) 73.1% while they were least resistant to Quinolone (Ciprofloxacin) 19.2%. For gram positive bacteria majority 96.7% were resistant to sulphonamides (Trimethoprim/sulfamethoxazole) followed by tetracycline 76.7%, penicillin (Oxacilline) 73.3% and least resistant to Quinolone (Ciprofloxacin) 30%.

**Table 1: Distribution of drug susceptibility pattern of bacteria causing septicemia**

Drug susceptibility patterns for gram negative isolates							
Antibiotic class	Antibiotic tested	Susceptibility pattern				Resistant	
		Sensitive		Intermediate		N	%
		N	%	N	%	N	%
Penicillins	Ampicillin	0	0	0	0	26	100
	Amoxicillin/Clavulanate	16	61.5	1	3.9	9	34.6
Cephalosporin	Cefuroxime	7	26.9	1	3.9	18	69.2
	Ceftriaxone	10	38.5	2	7.5	14	53.9
Tetracyclines	Tetracycline	0	0	1	3.9	25	96.1
Quinolone	Ciprofloxacin	20	76.9	1	3.9	5	19.2
Aminoglycosides	Gentamicin	6	23.1	1	3.9	19	73.1
Sulfonamides	Trimethoprim/sulfamethoxazole	3	11.5	1	3.9	22	84.6
Drug susceptibility patterns for gram positive isolates							
Penicillins	Oxacillin	8	26.7	0	0	22	73.3
	Amoxicillin/Clavulanate	14	46.7	0	0	16	53.3
Macrolides	Erythromycin	16	53.3	0	0	14	46.7
Tetracyclines	Tetracycline	6	20	1	3.3	23	76.7
Quinolone	Ciprofloxacin	20	66.7	1	3.3	9	30
Aminoglycosides	Gentamicin	14	46.7	4	13.3	12	40
Sulfonamides	Trimethoprim/sulfamethoxazole	0	0	1	3.3	29	96.7

N - Frequency; % - Percentage

## Drug resistant genes among the multi drugs resistant bacterial etiological agents

The number of multi-drug resistant bacterial isolates included 4 *Staphylococcus epidermidis*, 4 *S. aureus*, 7 *E. coli* (4 Enterotoxigenic and 3 Enteropathogenic *E. coli*), 4 *S. paratyphi* (3 *S. paratyphi* A and 1 *S. paratyphi* B), 3 *S. typhimurium* and 3 *P. aeruginosa*

***S. aureus* and *S. epidermidis*:** Plasmids isolated from both *S. epidermidis* and *S. aureus* species harbored resistance genes, *mecA* and *SullI*, highlighting their role in dissemination of antibiotic resistance. High frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (87.5%), Oxacilin (75.0%), Erythromycin (62.5%) and Amoxyclovanic acid (50.0%). All the isolates were multiply resistant to between two to six antibiotics. One of the *S. epidermidis* did not carry any plasmid but showed phenotypic resistance to Oxacilin, Amoxyclovanic acid, Sulfamethoxazole-Trimethoprim and Tetracycline (Table 2).

***E. coli*:** Plasmids isolated from the *E. coli* isolates studied harbored resistance genes, *blaTEM*, *SullI*, and *TetA*. High frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (100%), Ampicillin (100%), Tetracycline (100%) and Gentamycin (85.7%). All the isolates were multiply resistant to between four and eight antibiotics. However, there was no apparent relationship between carriage of plasmids and antimicrobial resistance (Table 2).

**Table 2. Distribution of resistant genes among MDR *S. epidermidis*, *S. aureus* and pathogenic *E. coli***

Sample	Isolate	No. of plasmids	Approximate sizes of the plasmids (Kb)				Pattern of drug resistance	Antimicrobial genes detected
			1500	3000	4000	>10000		
MDH/BLD/54	<i>S. aureus</i>	2	0	0	0	2	Ox, Amoclav, Sxt, Tet, Ery	<i>mecA</i>
MDH/BLD/55	<i>S. aureus</i>	1	0	0	0	1	Ox, Gen, Tet, Ery	<i>mecA</i>
MDH/BLD/15	<i>S. aureus</i>	1	0	0	0	1	Cip, Tet, Sxt, Ery	<i>SullI</i>
MDH/BLD/135	<i>S. aureus</i>	1	0	0	0	1	Ox, Sxt	<i>mecA</i>
MDH/BLD/157	<i>S. epidermidis</i>	0	0	0	0	0	Ox, Amoclav, Sxt, Tet	none
MDH/BLD/62	<i>S. epidermidis</i>	2	0	0	0	2	Amoclav, Sxt, Ery	<i>mecA</i>
MDH/BLD/216	<i>S. epidermidis</i>	2	0	0	0	2	Ox, Cip, Gen, Tet, Sxt	<i>mecA</i>
MDH/BLD/104	<i>S. epidermidis</i>	2	0	0	0	2	Ox, Amoclav, Cip, Sxt, Tet, Ery	<i>mecA</i>
MDH/BLD/183	<i>E. coli</i> (EPEC)	3	1	1	0	1	Amp, Gen, Sxt, Tet	<i>blaTEM</i> , <i>SullI</i>
MDH/BLD/222	<i>E. coli</i> (EPEC)	5	1	1	0	3	Amp, Amoclav, Gen, Sxt, Tet	<i>SullI</i> , <i>TetA</i>
MDH/BLD/229	<i>E. coli</i> (EPEC)	5	1	1	0	3	Amp, Gen, Sxt, Tet	<i>blaTEM</i> , <i>SullI</i> , <i>TetA</i>
MDH/BLD/142	<i>E. coli</i> (ETEC)	4	1	1	0	2	Amp, Amoclav, Sxt, Tet	<i>blaTEM</i> , <i>SullI</i> , <i>TetA</i>
MDH/BLD/163	<i>E. coli</i> (ETEC)	3	1	1	0	1	Amp, Gen, Sxt, Tet	<i>blaTEM</i> , <i>SullI</i> , <i>TetA</i>
MDH/BLD/215	<i>E. coli</i> (ETEC)	3	1	1	0	1	Amp, Gen, Sxt, Tet	<i>blaTEM</i> , <i>SullI</i>
MDH/BLD/156	<i>E. coli</i> (ETEC)	4	1	1	0	2	Amp, Amoclav, Cfm, Cfx, Gen, Cip, Sxt, Tet	<i>blaTEM</i> , <i>SullI</i> , <i>TetA</i>

Where Ox- Oxacillin; Amoclav-Amoxicillin/Clavulanate; Sxt- Trimethoprim/sulfamethoxazole; Tet- Tetracycline; Ery-Erythromycin; Gen- Gentamicin; Cip- Ciprofloxacin; mec A-Methicillin-resistant *Staphylococcus aureus*; *blaTEM* - Nonspecific TEM  $\beta$ -Lactamase; *TetA*- Tetracycline Resistant gene A; *SullI* - Sulfonamide resistance gene

***Pseudomonas spp* :** The antimicrobial gene, *blaTEM*, was detected in five of the plasmids while four of the plasmids carried *SullI*, *TetA* and *aac (3)* antimicrobial resistance genes. High frequency of resistance was observed for Tetracycline (100%), Ampicillin (100%), Cefixime (80%), Cetriaxone (80%), Amoxyclovanic acid (80.0%), Gentamicin (60.0%) and Sulfamethoxazole-Trimethoprim (60%), All the isolates were multiply resistant to between to two to six antibiotics.

***Salmonella spp*:** Plasmids isolated from the *Salmonella* isolates studied harbored resistance genes, *blaTEM*, *SullI*, *aac (3)* and *TetA* highlighting their role in transmission of resistance. High frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (100%), Ampicillin (100%), Tetracycline (100%), Cefixime (100%), Gentamycin (100%) and Ceftriaxone (57.1%). All the isolates were multiply resistant to between to four to seven antibiotics. One of the *Salmonella* did not carry any plasmid but showed phenotypic resistance to Cefixime, Ampicillin, Sulfamethoxazole-Trimethoprim and Tetracycline. However, there was no apparent relationship between carriage of plasmids and antimicrobial resistance.

**Table 3. Distribution of resistant genes among MDR *S. paratyphi* and *S. typhimurium***

Sample	Isolate	No. of plasmids	Approximate sizes of the plasmids				Pattern of drug resistance	Antimicrobial genes detected
			1500	3000	4000	>10000		
MDH/BLD/093	<i>S. paratyphi</i> B	4	0	1	1	2	Amp, Cfm, Gen, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/219	<i>S. paratyphi</i> A	3	0	1	1	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/238	<i>S. paratyphi</i> A	5	0	1	1	3	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/203	<i>S. paratyphi</i> A	3	0	1	1	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/133	<i>S. Typhimurium</i>	0	0	0	0	0	Amp, Cfm, Gen, Sxt, Tet	-
MDH/BLD/134	<i>S. Typhimurium</i>	5	0	1	1	3	Amp, Cfm, Gen, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/189	<i>S. Typhimurium</i>	3	0	1	1	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	SulIII, TetA, aac(3)
MDH/BLD/11	<i>P. aeruginosa</i>	1	0	0	0	1	Amp, Amoclav, Cfm, Cfx, Cip, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/4	<i>P. aeruginosa</i>	2	0	0	0	2	Amp, Amoclav, Cfx, Gen, Sxt, Tet	blaTEM, SulIII, TetA, aac(3)
MDH/BLD/8	<i>P. aeruginosa</i>	2	0	0	0	2	Amp, Amoclav, Cfm, Cfx, Cip, Tet	blaTEM, SulIII, TetA
MDH/BLD/35	<i>P. aeruginosa</i>	1	0	0	0	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, aac(3)
MDH/BLD/175	<i>P. aeruginosa</i>	1	0	0	0	1	Amp, Amoclav, Cfm, Gen, Cip, Tet	blaTEM, SulIII, TetA, aac(3)

Where: Amp – Ampicillin; Cfm – Cefuroxime; Cfx – Ceftriaxone; Amoclav-Amoxicillin/Clavulanate; Sxt- Trimethoprim/sulfamethoxazole; Tet- Tetracycline; Gen- Gentamicin; Cip- Ciprofloxacin; blaTEM - Nonspecific TEM  $\beta$ -Lactamase; TetA- Tetracycline Resistant gene A; SulIII - Sulfonamide resistance gene; aac(3) - Aminoglycoside acetyltransferase gene

### Discussions

Early diagnosis and early appropriate treatment are crucial in cases of bacterial blood infection. In severe sepsis, the case fatality increases for each hour the antibiotic treatment is delayed (Ferrer *et al.*, 2014). Therefore, empirical antibiotic treatment has to be initiated before the results of blood cultures are available. However, as infections with resistant microbes is an escalating problem worldwide, it is increasingly challenging to maintain appropriate antibiotic regimens for initial empiric therapy (Nathan and Cars, 2014; WHO, 2014). Resistant pathogenic bacteria are found frequently worldwide (WHO, 2014). Studies have shown that most developing countries are home to a number of risk factors for the emergence and spread of antibiotic resistance. Misuse of antibiotics, over-the-counter and parallel market access, and counterfeit or poor-quality drugs, combined with substandard hygiene and living conditions, are the driving forces behind the emergence and spread of antibiotic resistance (Kelesidis *et al.*, 2007). The potential for the development and rapid spread of new forms of resistance is highlighted by the recent worldwide proliferation of NDM-1-producing Enterobacteriaceae. The gene, which confers resistance to carbapenems, originated in India in 2009, and since 2010 NDM-1-producing Enterobacteriaceae have been reported in North America, Europe, and Asia (Kumarasamy *et al.*, 2010). The WHO has recently heightened awareness of this pressing issue with calls for action to contain antibiotic resistance on a global scale (WHO, 2014). In Kenya, a regimen containing penicillin and gentamicin, plus metronidazole if an anaerobic infection is suspected, has been recommended for more than thirty years in sepsis with unknown focus and etiology (Lee *et al.*, 2014). In recent years, however, increasing numbers of infections with methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamase producing Enterobacteriaceae, and vancomycin resistant enterococci have been detected (Lee *et al.*, 2014). Selection of inherently resistant microbes due to antibiotic use is also a challenge. Updated knowledge about the distribution of microbes in serious infection and their resistance against antimicrobial agents is needed to ensure appropriate empiric antimicrobial treatment regimens. It is also important to identify subgroups in which tailored regimens are required. This was the basis important aspect of this study

### *S. aureus*

The three out of four *S. aureus* isolates causing septicemia had the *mecA* antimicrobial gene while one carried *SulIII* antimicrobial gene. One of the four isolates were multi-drug resistant to five different drugs (Oxacillin, Amoxicillin/Clavulanate, Trimethoprim/sulfamethoxazole, Tetracycline and Erythromycin). Two isolates were resistant to four different drugs and one isolate to two different drugs. Our study further shows that 75% of the *S. aureus* were Methicillin resistance. The *S. aureus* with varying antibiotic profiles have been associated with sepsis in other settings. In Kilifi District Hospital on the Kenyan coast Talbert *et al.*, (2010) reported that all the *Staphylococcus aureus* blood culture isolates were susceptible to methicillin. The *S. aureus* causing neonatal sepsis in Tikur Anbessa University Hospital, Ethiopia; showed high-level resistance to ampicillin, ceftriaxone, cephalothin, chloramphenicol, and gentamicin (Shitaye *et al.*, 2010). In Pakistan, Mir *et al.*, (2011) all the 4.2% reported *S. aureus* were methicillin-resistant *S. aureus* (MRSA). Dramowski *et al.*, (2015) the *S. aureus* etiology of septicemia 65% of them were Methicillin-resistant. About 40% of all lethal cases of sepsis caused by *Staphylococcus* spp among children admitted at the Hospital, affiliate of Vilnius University Hospital in in

Lithuania was resistant to Methicillin (Bobelytė, 2017). Our study and other showing significantly high prevalence of multidrug resistant *S. aureus* is worrying given bacteraemias due *S. aureus* are difficult to treat and is associated with 29–63% mortality (Kaasch *et al.*, 2014; Fortuin-de Smidt *et al.*, 2015). The emergence of new CA-MRSA strains in the community has huge implications on patient treatment (David *et al.*, 2010).

### ***S. epidermidis***

There were 3 out of 4 (75%) *S. epidermidis* that had antimicrobial gene, *mecA* and only one isolate did not carry any plasmid. All four *S. epidermidis* were resistant to 3 to 6 different antibiotics. The strain that had no plasmid containing antimicrobial gene, *mecA* showed phenotypic resistance to Oxacilin, Amoxycylavulanic acid, Sulfamethoxazole-Trimethoprim and Tetracycline. Studies continue to report the importance of Coagulase-negative staphylococci as among the leading cause of nosocomial sepsis, especially in neonates (Marchant *et al.*, 2013; Becker *et al.*, 2014). Coagulase-negative staphylococci sepsis most often originates from the infection of indwelling medical devices, such as in catheter-related bloodstream infections or central line-associated blood stream infections (Vassallo *et al.*, 2015). Most prominent among Coagulase-negative staphylococci infections are those due to the skin commensal *S. epidermidis* (Vassallo *et al.*, 2015). However, the bacterial factors contributing to the development of sepsis, in particular in CNS, are poorly understood. Most *S. epidermidis* blood infections are caused by methicillin-resistant strains, with methicillin resistance rates even exceeding those found among *S. aureus* (Qin *et al.*, 2017).

Methicillin-resistant *S. epidermidis* isolates from patients are cross-resistant to all b-lactam antibiotics, even though some might be susceptible to certain b-lactam agents by in vitro testing (Raad *et al.*, 1998). Therefore, this pattern of resistance is similar to what has already been established with methicillin-resistant *S. aureus*. The prevalence of resistance has increased rapidly over the last three decades and has been attributed to the selection effect of the increasing use of b-lactam antibiotics. Studies have demonstrated an increase in the prevalence of resistant *S. epidermidis* in hospitals when isolates from 1964 were compared with isolates from 1986 or resistance and plasmid profiles (Raad *et al.*, 1998). This resistance was plasmid-mediated. Some investigators attributed resistance in *S. epidermidis* to the action of the *mecA* gene (Raad *et al.*, 1998). However, Mempel *et al.*, (1994) demonstrated that *S. epidermidis* isolates could be methicillin resistant and lack *mecA* transcription.

### ***E. coli***

The seven pathogenic *E. coli* species harbored three types of antimicrobial gene, *SullI*, *blaTEM* and *TetA*. One pathogenic ETEC was resistant to 8 different antibiotics, 3 ETEC were multi-drug resistant to 4 different antibiotics. One EPEC was resistant to 5 different antibiotics while the remaining 2 EPEC were resistant to four different antibiotics. Similar studies exist showing high level antibiotic resistance to *E. coli* species isolated from neonatal septicemia cases. Studies showed resistance to penicillin/ampicillin ranging from 55% among *E. coli* isolates in Georgia (Macharashvili *et al.*, 2009) to 100% among *E. coli* isolates in Uganda (Mugalu *et al.*, 2006). Resistance to gentamicin among *E. coli* ranged from 0% in Pakistan (Mir *et al.*, 2011), 21.7% in South Africa (Dramowski *et al.*, 2015), 67 in India (Jyothi *et al.*, 2013). In Lithuania, sepsis-causing pathogens *E. coli* was characterized by the development of increasing antibiotic resistance for which initial empirical antibiotic therapy might fail (Bobelytė *et al.*, 2017). Resistant to third generation cephalosporins ranged from 6% for *E. coli* isolates in Uganda (Mugalu *et al.*, 2006) to 48% in India (Jyothi *et al.*, 2013). Concerning the extended spectrum beta-lactamase production in *Enterobacteriaceae*; one reported extended spectrum beta-lactamase production in 65% of *E. coli* isolates (Jain *et al.*, 2006). In India a study among pediatrics sepsis patients attending Majeedia Hospital of Hamdard University in New Delhi showed various resistance profile of *E. coli* including: ampicillin (92%), amoxicillin (90.9%), amoxiclav (68.4%), cefuroxime (54.5%), cefaclor (84.2%), cefotaxime (52.9%), cefoperazone (36.8%), gentamicin (19%), amikacin (12%), netilmicin (13%), ciprofloxacin (26.1%), chloramphenicol (16.7%) and tetracycline (44.4%) (Alam *et al.*, 2011). In Pakistan Ullah *et al.*, (2016) reported higher level of antibiotic resistance among *E. coli* isolates including third line antibiotics; Amikacin 58%, Ciproflaxacin 67.3%, Enoxacin 83.3%, Imipenem 94.7% and Ofloxacin 77.7%. In Ghana, Obeng-Nkrumah *et al.* (2016) reported various antibiotic resistance among *E. coli* isolates including ampicillin (97.7%), amoxicillin clavulanic acid (53.5%), gentamicin (53.5%) and ciprofloxacin (62.1%).

### ***Salmonella spp***

Six out of seven *Salmonella* species studied were found carrying at least three plasmids of varying sizes. Antimicrobial gene, *SullI*, was detected in the six *Salmonella* isolates while *aac (3)*, *TetA* and *blaTEM* genes were detected in five *Salmonella* isolates. One of the *Salmonella* did not carry any plasmid but showed phenotypic resistance to Cefixime, Ampicillin, Sulfamethoxazole-Trimethoprim and Tetracycline. There was one *Salmonella paratyphi* B that was multi-resistant to five different antibiotics, three *Salmonella paratyphi* A were multi-resistant to six different antibiotics. Further, there were two and one *Salmonella typhimurium* that were multi-resistant to



five and six different antibiotics respectively. Generally high frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (100%), Ampicillin (100%), Tetracycline (100%), Cefixime (100%), Gentamycin (100%) and Ceftriaxone (57.1%). Studies confirms that the invasive forms of *Salmonella* disease include enteric fevers (typhoid and paratyphoid fevers) and NTS bacteraemia and are important causes of morbidity and mortality in Asia and Africa (Smith et al., 2014). In India a study among pediatrics sepsis patients attending Majeedia Hospital of Hamdard University in New Delhi showed various resistance profile in *Salmonella typhi* to various antimicrobials as follows ampicillin (46.4%), amoxicillin (27.3%), amoxiclav (15.4%), cefuroxime (10%), cefotaxime (25%), cefoperazone (10.5%), netilmicin (10.5%), ciprofloxacin (6.3%), chloramphenicol (9.1%) and tetracycline (37%). *Salmonella typhi* did not show resistance to gentamicin and amikacin (Alam et al., 2011). On the other hand, in the same study *Salmonella paratyphi* A did not show resistance to antimicrobials tested (Alam et al., 2011). In Pakistan Ullah et al., (2016) reported higher level of antibiotic resistance among *Salmonella* spp including third line antibiotics Amikacin 100%, Ciproflaxacin 66.7%, Enoxacin 66.7%, Imipenem 66.7% and Ofloxacin 83.3%. In Ghana, Obeng-Nkrumah et al. (2016) reported various antibiotic resistance among *Salmonella* spp including ampicillin (63.9%) and amoxicillin clavulanic acid (23.9%). In Zanzibar, the majority of the *S. Typhi* isolates 6/7 (86%) were multidrug-resistant (i.e. resistant to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol), but susceptible to cefotaxime (Onken et al., 2015).

### ***Pseudomonas* spp**

The antimicrobial gene, *blaTEM*, was detected in five of the *Pseudomonas* plasmids while four of the plasmids carried *SullI*, *TetA* and *aac (3)* antimicrobial resistance genes. High frequency of resistance was observed for Tetracycline (100%), Ampicillin (100%), Cefixime (80%), Cetriaxone (80%), Amoxyclovanic acid (80.0%), Gentamicin (60.0%) and Sulfamethoxazole-Trimethoprim (60%), All the isolates were multiply resistant to between to two to six antibiotics. These results were similar to those in other studies; In India Alam et al., (2011) among pediatrics sepsis patients attending Majeedia Hospital of Hamdard University in New Delhi showed various resistance profile in *Pseudomonas* spp including: ampicillin (93.8%), amoxicillin (93.3%), amoxiclav (90.9%), cefuroxime (75%), cefotaxime (46.2%), cefazidime (38.5%), cefoperazone (42.9%), gentamicin (33.3%), amikacin (11.8%), netilmicin (23.1%), ciprofloxacin (20%), ofloxacin (20%), chloramphenicol (40%) and tetracycline (36.4 %). In Ghana, Obeng-Nkrumah et al. (2016) reported various antibiotic resistance among *Pseudomonas* spp gentamicin (32.8%), ampicillin (13.1%), ciprofloxacin (19.6%). In Pakistan Mir et al., (2011) reported no resistance to antibiotics including gentamicin among Gram-negative bacteria including *Pseudomonas* and *E. coli*. In Vietnam 56% of the sepsis causing *Pseudomonas* isolates were resistant to carbapenem resistant (Le et al., 2016). In Turkey, *Pseudomonas* spp which was the major causative pathogens of sepsis in children was 40.5% resistant to imipenem (Teke et al., 2016). Evaluation of the trends in antimicrobial resistance of bloodstream infections at a general hospital in Mid-Norway, showed, *Pseudomonas* spp were 100% resistant to cefotaxim and 6.9% to Imipenem (Mehl et al., 2017).

In conclusion, in this geographically defined region of Kenya, of the 33.9% children with septicemia, gram positive bacteria were the leading cause septicemia. Specifically, *S. epidermidis* and *S. aureus*. However, several other Gram-negative bacteria were implicated such as *E. coli*, *P. aeruginosa*, *S. typhimurium* and *S. hemolyticus*. It should be noted that gram-negative bacteria have often been implicated in the pathogenesis of severe sepsis and septic shock than the Gram-positive counter parts (Alexandraki and Palacio, 2010). Compared to Gram positive, majority of Gram-negative bacteria were resistant to penicillin (Ampicillins) 100% tetracycline 96.1%, sulphonamides (Trimethoprim/sulfamethoxazole) 84.6%, Aminoglycosides (Gentamicin) 73.1% and least resistant to Quinolone (Ciprofloxacin) 19.2%. The Gram-positive bacteria were resistant to sulphonamides (Trimethoprim/sulfamethoxazole) 96.7%, tetracycline 76.7%, penicillin (Oxacilline) 73.3% and Quinolone (Ciprofloxacin) 30%. The following antimicrobial resistant genes *mecA*, *SullI*, *blaTEM*, *TetA* *aac (3)* were identified However, we did not find apparent relationship between carriage of plasmids and antimicrobial resistance.

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### **Competing interests**

The authors declare no competing interests.

## Authors' contributions

This work was part of Master of Science degree for GHS in infectious diseases of Kenyatta University. GHS, GG, YI and MK conceived the study. CK participated in the laboratory assays while GHS analyzed the data and prepared the draft manuscript. GHS, GG, YI and MK provided guidance and mentorship during the implementation of the study. All authors reviewed and approved the final manuscript.

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