

# Antibiotic Susceptibility Patterns of Bacterial Isolates from Routine Clinical Specimens from Referral Hospitals in Tanzania: A Prospective Hospital-Based Observational Study

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**Introduction:** Antimicrobial resistance is one of the biggest threats of modern public health. Although sub-Saharan Africa is highly burdened with infectious diseases, current data on antimicrobial resistance are sparse.

**Methods:** A prospective study was conducted between October 2018 and September 2019 to assess the antibiotic susceptibility patterns of clinical bacterial isolates obtained from four referral hospitals in Tanzania. We used standard media and Kirby-Bauer disc diffusion methods as per Clinical and Laboratory Standards Institute (CLSI) standards.

**Results:** We processed a total of 2620 specimens of which 388 (14.8%) were culture-positive from patients with a median (IQR) age of 28 (12–44) years. Of the positive cultures, 52.3% (203) were from females. Most collected specimens were ear pus 28.6% (111), urine 24.0% (93), wound pus 20.6% (80), stool 14.9% (58), and blood 8.3% (32). Predominant isolates were *S. aureus* 28.4% (110), *E. coli* 15.2% (59), *P. aeruginosa* 10.6% (41), *P. mirabilis* 7.0% (27), *V. cholerae* 01 Ogawa 6.2% (24), *Klebsiella* spp. 5.2% (20) and *Streptococcus* spp. 4.6% (18). Generally, the isolates exhibited a high level of resistance to commonly used antibiotics such as Ampicillin, Amoxicillin-Clavulanic acid, Erythromycin, Gentamicin, Tetracycline, Trimethoprim, third-generation Cephalosporins (Ceftriaxone and Ceftazidime), and reserved drugs (Clindamycin and Meropenem). *S. aureus* isolates were resistant to most of the antibiotics tested; 66.7% were classified as MRSA infections.

**Conclusion:** Antibiotic resistance to commonly prescribed antibiotics was alarmingly high. Our findings emphasize the need for comprehensive national control programs to combat antibiotic resistance.

**Keywords:** antibiotics, antimicrobial resistance, AMR, antibiotic susceptibility testing, methicillin-resistant *Staphylococcus aureus*, MRSA, bacterial isolates

## Background

Antimicrobial resistance (AMR) has become a severe global health threat, especially in sub-Saharan Africa where the burden of infectious diseases is high.<sup>1</sup> Common illnesses like pneumonia, diarrhea, sexually transmitted infections, post-operative infections, and tuberculosis are increasingly becoming untreatable.<sup>2</sup> Patients infected with drug-resistant pathogens are at increased risk of unfavorable clinical outcomes and consume more health-care resources than patients infected with non-resistant pathogen of the same type.<sup>3</sup> Recent data shows that about

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700,000 death per year are attributable to AMR infections and projected to increase to 10 million annually by 2050 if the present trends persist.<sup>4</sup> Clinically, the emergence of AMR is primarily driven by non-compliance with prescribed antimicrobials therapy, which in turn promotes spontaneous mutations in chromosome or control genes leading to new mutant pathogens with selective pressure in the presence of antimicrobials.<sup>5</sup> Further accumulation of such beneficial mutations catalyzes the evolution of multi-drug-resistant strains,<sup>6</sup> which necessitate the use of broad-spectrum antibiotics as the ultimate choice. Especially alarming is the emergence and spread of multi-drug resistant bacteria which are hard to treat with the available antibiotics.<sup>2</sup> This include the emergence and spread of extended-spectrum beta-lactamases (ESBL), *AmpC* beta-lactamases, and carbapenemase-producing Gram-negative bacteria (ie carbapenem-resistant Enterobacteriaceae-CRE) and *Staphylococcus aureus* (MRSA) which are rapidly increasing worldwide.<sup>7-9</sup>

In sub-Saharan Africa, AMR is mostly exacerbated by limited access to appropriate therapies, irrational use of antibiotics, and lack of clinical microbiology laboratories for drug susceptibility testing.<sup>1</sup> Most infections are managed empirically using antibiotics which are mainly obtained over-the-counter without a proper prescription. At the level of public health systems, the situation is worsened by a lack of coordinated AMR surveillance and weak regulatory frameworks for the access and use of antibiotics.<sup>10</sup> In addition, the emergence of resistant pathogens is aggravated by underlying illnesses, such as human immunodeficiency virus (HIV), which has promoted the utilization of antibiotics against opportunistic infections.<sup>11</sup>

Although bacterial infections particularly Gram-negative bacteria are among major causes of illness and death in sub-Saharan Africa,<sup>12,13</sup> data on their antibiotic resistance remain scant due to limited disease detection and surveillance capacity. Prevalent bacterial infections include lower respiratory infections (pneumonia), diarrheal diseases, urinary tract infections, bloodstream infections (typhoid, sepsis, meningitis, and bacteremia), sexually transmitted infections (eg, gonorrhoea), and healthcare-associated infections (eg, MRSA).<sup>12,13</sup> A recent situation analysis on antibiotic use and resistance in Tanzania revealed a lack of national data representativeness for antibiotic resistance for common bacterial infections.<sup>14</sup> The current study aimed to provide additional evidence

on the resistance pattern of bacterial isolates to widely used antibiotics in Tanzania.

## Methodology

### Study Design and Setting

We conducted a hospital-based observational study between October 2018 -September 2019 involving four cross-border tertiary hospitals from four different regions in Tanzania. Such hospitals included: Maweni Regional Hospital in Kigoma Region, Musoma Regional Hospital in Mara Region, Sumbawanga Regional Hospital in Rukwa Region, and St. Benedict Ndanda Hospital (Masasi) in Mtwara Region (Figure 1). These hospitals were among satellite study sites for the East African Public Health Laboratory Network (EAPHLN) project and health facilities that form a surveillance system for monitoring cross-border disease outbreak dynamics in the country. The majority of inhabitants (80%) reside in rural settings, practicing subsistence farming and informal trade.

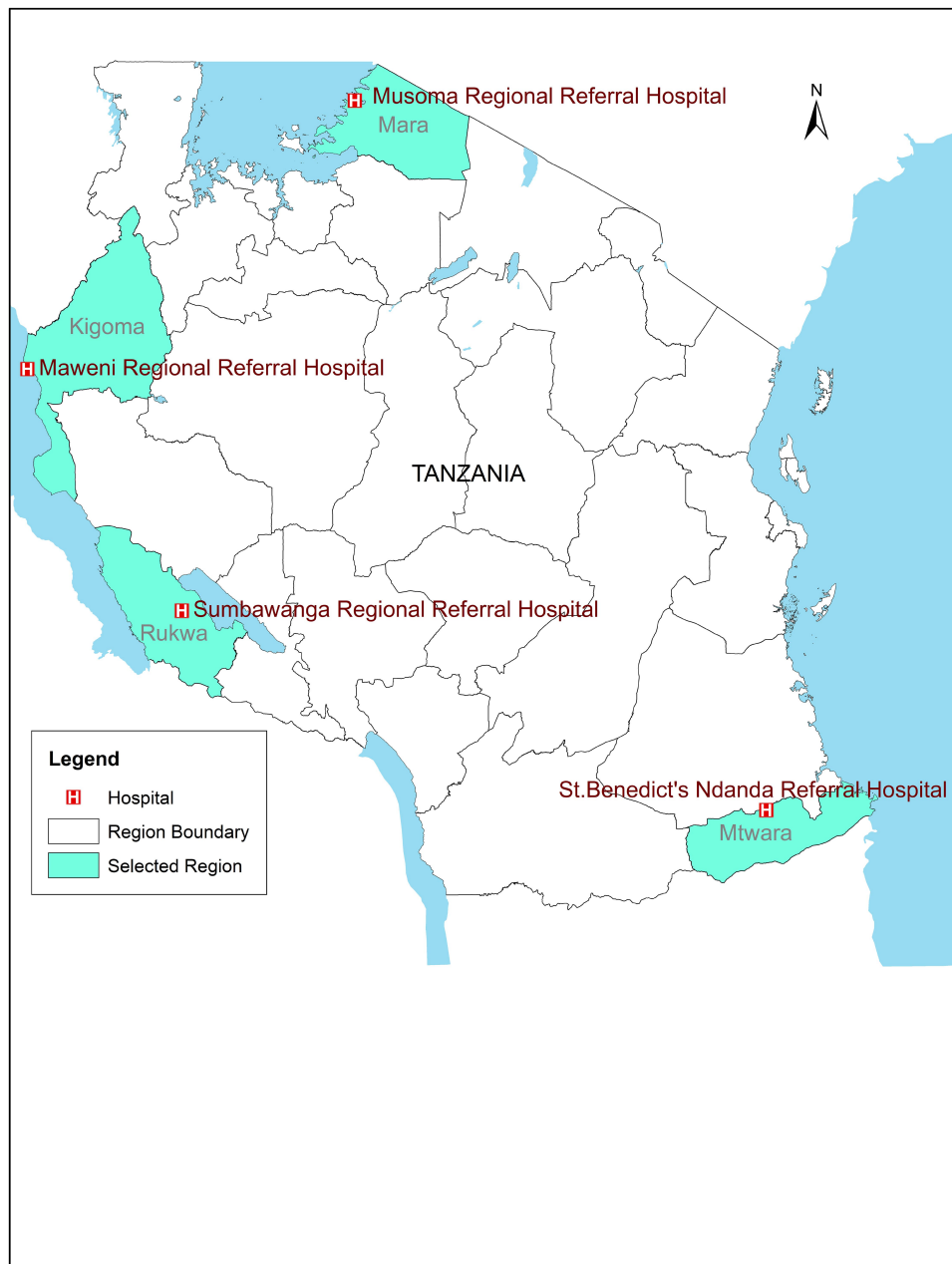
Administrative boundary shapefile was obtained from the Tanzania National Bureau of Statistics.

### Data Collection

Clinical specimens were collected from both outpatients and inpatients as part of each hospital's routine clinical care. Thus, specimens were requested based on clinicians' assessment and then submitted for microbiological tests.

### Laboratory Procedure

Specimen processing, identification of organisms to the genus and/or species level, and in-vitro antibiotic susceptibility testing were performed in accordance with the standard microbiological procedures and the CLSI guidelines.<sup>15</sup> Pathogenic bacteria were identified using standard microbiological methods such as morphology on culture media, gram staining, and conventional biochemical tests. In case of ambiguity, the analytical profile index (API™) biochemical test kit (BioMérieux, France) and/or serological tests (antisera for *V. cholerae*) were used to confirm identification. In-vitro antibiotic susceptibility testing was performed using the Kirby Bauer disc diffusion method on Mueller-Hinton agar medium. The following antibiotic agents were tested: Amoxicillin/Clavulanic acid (20/10µg), Ampicillin (10µg), Ceftriaxone (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Erythromycin (15µg), and Trimethoprim/Sulfamethoxazole (1.25µg). Other antibiotic agents that were tested included:



**Figure 1** Map of the Republic of Tanzania showing geographic locations of the four study sites.

Gentamicin (10 $\mu$ g), Meropenem (10 $\mu$ g), Nitrofurantoin (300 $\mu$ g), and Tetracycline (30 $\mu$ g). Gram-negative bacteria *E. coli* and *Klebsiella species* were screened for ESBL production using Ceftazidime (30 $\mu$ g) and Cefotaxime (30 $\mu$ g). The double-disk synergy test for Ceftazidime (30 $\mu$ g), Cefotaxime (30 $\mu$ g), and Co-amoxicillin /Clavulanate (20/10 $\mu$ g) were used to confirm ESBL production. MRSA was detected using Cefoxitin (30 $\mu$ g) disc and isolates with a zone of inhibition of  $\leq 21$  mm were referred to as phenotypically confirmed MRSA. Reading and interpretation of zone sizes were as per CLSI guidelines. Isolates

with intermediate or resistant results on antibiotic susceptibility were classified as resistant strains during data analysis. Laboratory activities were done at a respective hospital's laboratory as per the routine procedure. Participating laboratories were supported with reagents by the project and regular supportive supervision from the study research team.

### Quality Control

Quality control strains *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative

controls (respectively) for the detection of ESBL. *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and other quality control strains were used for all laboratory methods in accordance with the CLSI guidelines.

## Data Management and Analysis

Demographic information (ie, age, sex, and place of residence) was collected by laboratory technicians from the request forms and entered in an Excel spreadsheet. Data analysis was performed using STATA™ version 14.1 (Stata Corp LP, College Station, Texas, USA). Variables (ie, bacterial isolates, antibiotics susceptibility and demographic characteristics) were summarized as frequencies, percentages, medians, and inter-quartile ranges as deemed appropriate.

## Results

### Patients Demographic Characteristics

We processed a total of 2,620 specimens during the observation period, but only 388 (14.8%) were culture-positive and 19 (0.7%) were discarded due to contamination. The patients' median (IQR) age was 28 (12–44) years and females constituted 203 (52.3%) of the participants. St. Benedict Ndanda Hospital contributed the majority of the specimens (see Table 1).

### Culture Results

Most of the collected specimens were ear pus (28.6%), urine (24.0%), wound pus (20.6%), stool (15.0%), and blood (8.3%). *S. aureus* was highly recovered from wound pus, ear pus, and blood (Table 2).

The most frequent isolate recovered was *S. aureus* (28.3%), followed by *E. coli* (15.2%), *P. aeruginosa* (10.6%), *P. mirabilis*, *V. cholerae* 01 Ogawa, *Klebsiella*

sp. and *Streptococcus* sp. *S. aureus* isolates were prevalent among males (54.5%) and common in individuals aged 16–49 (48.6%) while *E. coli* was predominant in females (79.7%). *P. aeruginosa* was common in specimens from female patients (63.4%). Both *V. cholerae* and *Klebsiella* sp. isolates were more prevalent among males (Table 3).

## Antibiotic Resistance Pattern of the Bacterial Isolates Identified

Table 4 reports the antibiotic resistance patterns of bacterial isolates to commonly used antibiotics tested by the participating laboratories. Gram-negative bacteria were prevalent isolates in this study. Both gram-negative and-positive demonstrated a high level of resistance to widely used antibiotics tested. *E. coli* isolates were highly resistant to Ampicillin (100%), Amoxicillin-Clavulanic Acid (75.0%), Gentamicin (70.2%), Tetracycline (70.2%) and Ciprofloxacin (23 [42.6%]), but least resistant to Ceftriaxone, Meropenem, and Nalidixic Acid. Only two isolates were screened for ESBL and both isolates were susceptible to Ceftazidime, hence no evidence of ESBL in the present study. *Klebsiella* spp. demonstrated resistance to Ampicillin (100%), Amoxicillin-Clavulanic acid (90.9%), Gentamycin (64.7%), and Ceftriaxone (55.6%), Ciprofloxacin (52.6%). Among Gram-positive, the most prevalent *S. aureus* was highly resistant to Erythromycin (76.3%), Gentamycin (54.0%), Ciprofloxacin (40.0%) and Clindamycin (34.9%). In addition, 22/33 (66.7%) of *S. aureus* isolates were resistant to Cefoxitin hence presumed MRSA.

## Discussion

The emergence and pervasiveness of resistant bacteria represent a substantial global public health crisis.<sup>2</sup> In the

**Table 1** Demographic Characteristics of Study Participants (N=388)

Demographic Characteristics	Total	Study Sites			
		Maweni n=86	Musoma n=85	Ndanda n=158	Sumbawanga n=59
<b>Sex</b>					
Male	185 (47.7)	40 (46.5)	44 (51.8)	73 (46.2)	28 (47.5)
Female	203 (52.3)	46 (53.5)	41 (48.2)	85 (53.8)	31 (52.5)
<b>Age</b>					
0–4	46 (12.0)	8 (9.3)	11 (13.8)	23 (14.7)	4 (6.8)
5–15	65 (17.0)	22 (25.6)	10 (12.5)	21 (13.4)	12 (20.3)
16–49	191 (50.0)	43 (50.0)	41 (51.3)	77 (49.0)	30 (50.9)
50+	80 (20.9)	13 (15.1)	18 (22.5)	36 (22.9)	13 (22.0)
Median (IQR)	28 (12, 44)	26 (10,40)	28.5 (13.5, 47)	27 (14, 46)	27 (11,45)

**Notes:** Six participants had age missing; 5 were from Msoma and 1 from Ndanda.

**Table 2** Distribution of Identified Bacterial Isolates by Clinical Specimen

Isolate Type	N	Specimen Type							
		Blood	Ear Pus	Urethral Pus	Vaginal Pus	Wound Pus	Stool	Urine	CSF
<i>Acinetobacter</i> spp.	1	–	1 (100.0)	–	–	–	–	–	–
<i>Citrobacter</i> spp.	10	–	2(20.0)	1(10.0)	–	3(30.0)	3(30.0)	1(10.0)	–
<i>E. coli</i>	59	–	1(1.7)	1(1.7)	1(1.7)	3 (5.1)	1(1.7)	51(86.4)	1(1.7)
<i>Enterobacter</i> spp.	5	–	3(60.0)	–	–	–	2(40.0)	–	–
<i>K. oxytoca</i>	2	–	–	–	–	–	–	2(100)	–
<i>Klebsiella</i> spp.	20	–	5(25.0)	–	–	4(20.0)	–	11(55.0)	–
<i>N. gonorrhoeae</i>	8	–	–	3(37.5)	3(37.5)	–	–	2(25.0)	–
<i>P. mirabilis</i>	27	–	13 (48.2)	–	–	10 (37.0)	–	4(14.8)	–
<i>P. aeruginosa</i>	41	4(9.8)	27(65.9)	–	–	9(22.0)	–	1(2.4)	–
<i>Proteus</i> spp.	3	–	–	–	–	–	–	3(100.0)	–
<i>P. vulgaris</i>	5	–	–	–	–	4(80.0)	–	1(20.0)	–
<i>Pseudomonas</i> spp.	8	–	1(12.5)	–	–	4(50.0)	–	3(37.5)	–
<i>S. typhi</i>	4	1(25.0)	–	–	–	–	3(75.0)	–	–
<i>Salmonella</i> spp.	11	–	–	–	–	–	11(100)	–	–
<i>Serratia</i> spp.	1	–	1(100.0)	–	–	–	–	–	–
<i>S. sonnei</i>	8	–	–	–	–	–	8(100)	–	–
<i>Shigella</i> spp.	4	–	–	–	–	–	4(100)	–	–
<i>S. aureus</i>	110	13(11.8)	46(41.8)	1(0.9)	–	43(39.1)	2(1.8)	5(4.5)	–
<i>S. saprophyticus</i>	4	–	–	–	–	–	–	4(100)	–
Staphylococci (CoNs)	5	–	2(40.0)	–	–	–	–	3(60.0)	–
<i>Staphylococcus</i> spp.	5	1(20.0)	3(60.0)	–	–	–	–	1(20.0)	–
<i>S. pneumoniae</i>	4	1(25.0)	–	–	–	–	–	–	3(75.0)
<i>S. aeruginosa</i>	1	–	1(100)	–	–	–	–	–	–
<i>Streptococcus</i> spp.	18	12(66.7)	5(27.8)	–	–	–	–	1(5.6)	–
<i>V. cholerae</i> O1 ogawa	24	–	–	–	–	–	24(100)	–	–
Total	388	32(8.3)	111(28.6)	6 (1.5)	4(1.0)	80(20.6)	58(14.9)	93(24.0)	4(1.0)

present study, we evaluated the in-vitro susceptibility patterns of bacterial pathogens to widely prescribed antibiotics. Gram-negative bacteria constituted the majority of the isolates, this corroborates with previous studies in Tanzania.<sup>16,17</sup> Predominant bacteria were *S. aureus*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *V. cholerae* O1 Ogawaa, *Klebsiella* sp., *Streptococcus* sp., *Salmonella* sp., *Citrobacter* sp., *N. gonorrhoeae*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella* spp., *Salmonella* spp., and *Citrobacter* spp. Most of these isolates are in the WHO's list of priority bacterial pathogens for research, discovery and development of new antibiotics.<sup>2</sup> The pathogens exhibited a substantial high level of resistance to widely prescribed antibiotics such as Ampicillin, Amoxicillin-Clavulanic Acid, Erythromycin, Trimethoprim, Ceftriaxone, Tetracycline, Nalidixic Acid, and Chloramphenicol. Previous studies have shown a similar pattern of antibiotic resistance in Tanzania<sup>16–18</sup> and other parts of Africa.<sup>19</sup>

Ear pus, urine, wound pus, stool, and blood specimens were the most frequent clinical specimens analyzed. The presence of a large number of ear pus specimen points toward suppurative otitis diseases which are common in children and adolescents below 15 years especially in sub-Saharan Africa,<sup>20</sup> and their transmission is linked to poor hygienic conditions. *P. aeruginosa*, *S. aureus*, *Proteus* spp., and *Klebsiella* spp. are the most cited causative agents for otitis in sub-Sharan Africa and are reported to exhibit a high level of resistance to multiple antibiotics.<sup>20</sup> On the other hand, the majority of the participants were women of reproductive age, which explains the high number of urine specimens and *E. coli* processed in this study.

Resistance was also observed to third-generation cephalosporins (Ceftriaxone and Ceftazidime) and Clindamycin and Meropenem are among essential antibiotics reserved for use in tertiary hospitals listed in the WHO antibiotic stewardship programmes,<sup>21,22</sup> suggesting that we are precariously nearing a point of treatment option

**Table 3** Distribution of Identified Bacterial Pathogens from Different Clinical Samples

Isolate	Sex		Age*			
	Female	Male	0–4	5–15	16–49	50+
<i>Acinetobacter</i> spp.	1(100)	0	0	0	1(100)	0
<i>Citrobacter</i> spp.	3(30.0)	7(70.0)	1(10.0)	0	8(80.0)	1(10)
<i>E. coli</i>	47(79.7)	12(20.3)	4(6.8)	3(5.1)	33(55.9)	19(32.2)
<i>Enterobacter</i> spp.	3(60.0)	2(40.0)	0	0	5(100)	0
<i>K. oxytoca</i>	1(50.0)	1(50.0)	0	0	0	2(100)
<i>Klebsiella</i> spp.	8(40.0)	12(60.0)	0	1(5.6)	10(55.6)	7(38.9)
<i>N. gonorrhoeae</i>	4(50.0)	4(50.0)	0	1(12.5)	6(75.0)	1(12.5)
<i>P. mirabilis</i>	14(51.9)	13(48.2)	2(7.4)	3(11.1)	13(48.2)	9(33.3)
<i>P. aeruginosa</i>	26(63.4)	15(36.6)	6(15.0)	8(20.0)	17(42.5)	9(22.5)
<i>Proteus</i> spp.	1(33.3)	2(66.7)	1(33.3)	0	1(33.3)	1(33.3)
<i>P. vulgaris</i>	3(60.0)	2(40.0)	0	0	3(60.0)	2(40.0)
<i>Pseudomonas</i> spp.	4(50.0)	4(50.0)	2(25.0)	0	4(50.0)	2(25.0)
<i>S. typhi</i>	2(50.0)	2(50.0)	0	2(50.0)	2(50.0)	0
<i>Salmonella</i> spp.	4(36.4)	7(63.6)	0	4(36.4)	6(54.6)	1(9.1)
<i>Serratia</i> spp.	1(100)	0	0	0	1(100)	0
<i>S. sonnei</i>	4(50.0)	4(50.0)	0	0	7(87.5)	1(12.5)
<i>Shigella</i> spp.	1(25.0)	3(75.0)	0(0.0)	0(0.0)	3(1.6)	1(1.3)
<i>S. aureus</i>	50(45.5)	60(54.6)	18(16.8)	22(20.6)	52(48.6)	15(14.0)
<i>S. saprophyticus</i>	3(75.0)	1(25.0)	0	1(25.0)	3(75.0)	0
Staphylococci (CoNs)	2(40.0)	3(60.0)	0	0	3(60.0)	2(40.0)
<i>Staphylococcus</i> spp.	4(80.0)	1(20.0)	2(40.0)	0	3(60.0)	0
<i>S. pneumoniae</i>	1(25.0)	3(75.0)	1(25.0)	3(75.0)	0	0
<i>Streptococcus</i> spp.	7(38.9)	11(61.1)	6(33.3)	9(50.0)	1(5.6)	2(11.1)
<i>V. cholerae</i> 01 Ogawa	9(37.5)	15(62.5)	3(12.5)	8(33.3)	8(33.3)	5(20.8)

**Note:** \*Six participants had missing age response.

failure. The use of third-generation cephalosporins is insufficiently controlled in Tanzania and a high resistance level has been described previously.<sup>16</sup> The observed high rate of resistance is presumably because patients may have constantly pre-exposed to a wide variety of antibiotics including self-prescribing sub-optimal dosages before their referrals to tertiary hospitals.

As this study was conducted in hospitals located in borders, the spread of AMR across neighboring countries such as Kenya, Uganda, and Burundi are highly possible. This highlights the need for coordinated actions among stakeholders including the neighboring countries for effective measures in fighting infectious antibiotic resistance.

Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, *P. mirabilis*, *V. cholerae* 01 Ogawa, *Klebsiella* spp., and *Streptococcus* spp. were prevalent, similar to earlier studies conducted in Tanzania.<sup>16–18</sup> *E. coli*, and *Klebsiella* spp. were frequently found in urine specimens; the two are cited as the leading etiologies of urinary tract infections (UTI) especially among pregnant women.<sup>23,24</sup> *E. coli* and *Klebsiella* spp. are the most important bacteria associated

with ESBL-mediated resistance to multiple antibiotics, including carbapenems and cephalosporins – the most potent agents for treating multi-drug resistant bacteria.<sup>2</sup> In our study, both isolates *E. coli* and *Klebsiella* spp. were highly resistant to many antibiotics tested include Ciprofloxacin, an antibiotic commonly used to treat UTI. *P. mirabilis*, which is also known to cause UTI, was less frequently recovered in urine, but abundant in the ear and wound swabs. The high observation of UTI causing bacteria in the current study can be described as a result of poor sanitation and hygiene practices. For better patient management, clinicians should prescribe antibiotics for UTI depending on whether the infection is uncomplicated (lower-tract UTI) or complicated (associated with a condition, such as a structural/functional abnormality of the genitourinary tract or the presence of an underlying disease).<sup>25</sup>

Cholera remains a major public health problem especially in sub-Saharan Africa,<sup>26,27</sup> and during the implementation of this study, Tanzania was experiencing numerous waves of cholera outbreaks. Like most other

**Table 4** Drug Susceptibility Pattern of Bacterial Isolates Tested in the Laboratories

Isolate	AMP		OXA		FOX		AMC		ERY		CLI		GEN		CIP	
	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)
<i>Citrobacter</i> sp.	9 (100)	0	-	-	-	-	5(50.0)	5(50)	-	-	-	-	5(83.3)	1(16.7)	3(33.3)	2(66.7)
<i>E. coli</i>	45(100)	0	-	0	1(100)	0	27(75.0)	9(25)	6(85.7)	1(14.3)	-	-	33(70.2)	14(29.8)	23(42.6)	31(57.4)
<i>Enterobacter</i> sp.	3(100)	0	-	-	-	-	3(75.0)	1(25)	-	-	-	-	3(100)	0	0	3(100)
<i>K. oxytoca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1(100)
<i>N. gonorrhoeae</i>	-	-	-	-	-	-	10(90.9)	1(9.1)	-	-	13(100)	0	11(64.7)	6(35.3)	10(52.6)	9(47.4)
<i>P. mirabilis</i>	25(96.2)	1(3.8)	-	-	-	-	14(60.9)	9(39.1)	-	-	-	-	25(100)	0	6(25.0)	18(75)
<i>P. aeruginosa</i>	21(100)	0	-	-	-	-	16(88.9)	2(11.1)	-	-	-	-	32(86.5)	5(13.5)	6(16.7)	30(83.3)
<i>Proteus</i> sp.	3(100)	0	-	-	-	-	0	2(100)	-	-	-	-	0	3(100)	1(33.3)	2(66.7)
<i>P. vulgaris</i>	-	-	-	-	-	-	4(100)	0	-	-	-	-	-	-	1(20.0)	4(80.0)
<i>Pseudomonas</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(12.5)	7(87.5)
<i>S. typhi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(25.0)	3(75.0)
<i>Salmonella</i> sp.	11(100)	0	-	-	-	-	-	-	-	-	-	-	-	-	2(18.2)	9(81.8)
<i>Serratia</i> sp.	1(100)	0	-	-	-	-	1(100)	0	-	-	-	-	-	-	0	1(100)
<i>S. sonnei</i>	-	-	-	-	-	-	2(100)	0	-	-	-	-	2(50.0)	2(50.0)	5(83.3)	1(16.7)
<i>Shigella</i> sp.	-	-	-	-	-	-	1(50.0)	1(50.0)	-	-	-	-	-	-	0	4(100)
<i>V. cholerae</i> O1	13(93.9)	1(7.1)	-	8(100)	0	-	-	-	0	11(100)	-	-	0	9(100)	2(15.4)	11(84.6)
<i>S. saprophyticus</i>	-	-	2(66.7)	1(33.3)	1(50.0)	1(50.0)	-	-	3(75.0)	1(25.0)	2(100)	0	0	1(100)	1(50.0)	1(50.0)
<i>Staphylococcus</i> sp.	-	-	1(25.0)	3(75)	3(100)	0	-	-	2(100)	0	1(33.3)	2(66.7)	3(100)	0	1(20.0)	4(80.0)
<i>S. pneumoniae</i>	-	-	0	4(100)	-	-	-	-	1(100)	0	0	3(100)	1(100)	0	0	2(100)
<i>Streptococcus</i> sp.	-	-	-	-	-	-	-	-	12(100)	0	2(14.3)	12(85.7)	9(81.8)	2(18.2)	7(46.7)	8(53.3)
<i>S. aureus</i>	-	-	-	-	-	-	-	-	61(76.3)	19(23.7)	23(34.9)	43(65.1)	27(54.0)	23(46.0)	32(40.0)	48(60)
<i>Staphylococci</i> (CoNs)	1(100)	0	-	-	-	-	-	-	4(100)	0	-	-	1(50.0)	1(50.0)	1(50.0)	1(50.0)
Isolate	N	CRO		TET		SXT		MEM		NAL		NIT		CHL		
		R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	
<b>Gram-negative</b>	10	-	-	-	-	-	-	1(16.7)	6(83.3)	-	-	-	-	-	-	-
		5(23.8)	33(70.2)	14(29.8)	1(33.3)	1(33.3)	1(33.3)	3(30.0)	7(70.0)	5(62.5)	3(37.5)	2(6.1)	31(93.9)	14(50.0)	14(50.0)	
<i>Citrobacter</i> sp.	59	-	16(76.2)	1(50.0)	1(50.0)	0	1(100)	0	1(100)	0	0	2(100)	0	2(100)	2(100)	
<i>E. coli</i>	5	1(50.0)	1(50.0)	1(50.0)	1(50.0)	0	1(100)	0	1(100)	0	0	0	0	0	0	
<i>Enterobacter</i> sp.	2	1(50.0)	1(50.0)	1(50.0)	1(50.0)	0	1(100)	0	1(100)	0	0	0	0	0	0	
<i>K. oxytoca</i>	20	5(55.6)	4(44.4)	4(44.4)	4(44.4)	0	3(15.8)	17(84.2)	3(30.0)	3(30.0)	0	7(70)	4(44.4)	5(53.6)		
<i>N. gonorrhoeae</i>	8	0	6(100)	6(100)	6(100)	0	2(20.0)	8(80.0)	2(50.0)	2(50.0)	2(50.0)	2(50.0)	10(66.7)	5(33.3)		
<i>P. mirabilis</i>	27	9(40.9)	13(59.1)	4(16.0)	1(4.8)	0	18(66.7)	9(33.3)	2(50.0)	2(100)	2(100)	0	12(66.7)	8(33.3)		
<i>P. aeruginosa</i>	41	13(65.0)	7(35)	20(95.2)	1(4.8)	0	2(66.7)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	1(50.0)		
<i>Proteus</i> sp.	3	-	-	2(66.7)	1(33.3)	0	0	0	0	0	0	0	0	0		

(Continued)

Table 4 (Continued).

<i>P. vulgaris</i>	5	2(50.0)	2(50.0)	2(50.0)	2(50.0)	0	3(100)	1(100)	0	0	2(100)	-	-
<i>Pseudomonas</i> sp.	8	0	3(100)	-	-	-	-	0	2(100)	-	-	-	-
<i>S. typhi</i>	4	0	1(100)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	-	-	1(33.3)	2(66.7)	0	4(100)
<i>Salmonella</i> sp.	11	-	-	3(60.0)	2(40)	7(77.8)	3(22.2)	-	-	0	1(100)	2(28.6)	5(71.4)
<i>Serratia</i> sp.	1	0	1(100)	1(100)	0	-	-	-	-	0	-	0	1(100)
<i>S. sonnei</i>	8	2(66.7)	1(33.3)	-	-	-	-	2(100)	-	0	1(100)	2(25.5)	6(75.0)
<i>Shigella</i> sp.	4	-	-	-	-	-	-	-	-	-	-	0	1(100)
<i>V. cholerae</i> O1	24	0	9(100)	2(9.1)	20(90.9)	8(88.9)	2(22.1)	0	2(100)	9(100)	-	8(40.0)	12(60.0)
<b>Gram-positive</b>													
<i>S. saprophyticus</i>	4	-	-	1(33.3)	2(66.7)	-	-	-	-	0	1(100)	2(100)	0
<i>Staphylococcus</i> sp.	4	-	-	2(50.0)	2(50.0)	-	-	-	-	1(100.0)	0	2(66.7)	1(33.3)
<i>S. pneumoniae</i>	4	-	-	0	2(100)	0	3(100)	-	-	0	1(100)	-	-
<i>S. aureus</i>	119	5(45.4)	6(54.6)	51(63.8)	29(36.2)	-	-	-	-	3(12.0)	22(92.0)	15(25.0)	45(75)
<i>Staphylococci</i> (CoNs)	5	-	-	4(100)	0	-	-	-	-	0	4(100)	0	2(100)
<i>Streptococcus</i> sp.	18	8(66.7)	4(33.3)	7(70.0)	3(30.0)	-	-	-	-	-	-	7(50.0)	7(50.0)

Notes: Three *P. aeruginosa* isolates were tested for CAZ. 1 (33.3%) isolate was resistant (results not shown in this Table).

Abbreviations: CAZ, ceftazidime; CRO, ceftriaxone; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; TMP, trimethoprim; MEM, meropenem; NAL, nalidixic acid; NIT, nitrofurantoin; SPT, spectinomycin; CHL, chloramphenicol.

bacteria of clinical relevance, cholera is continuously becoming more resistant to a wide range of antibiotics.<sup>26–28</sup> In the present study, cholera isolates were highly resistant to Ampicillin, Trimethoprim-Sulfamethoxazole, Nalidixic Acid, Chloramphenicol, and least resistant to Ciprofloxacin. The isolates were, however, susceptible to widely used antibiotics for treatment of cholera (Tetracycline), Ceftriaxone, Meropenem, Gentamicin, Erythromycin, and Cefoxitin. As in the case of UTI, availability of clean and safe water sources, proper sanitation and hygiene, and public health education is critical in preventing/controlling the spread of *V. cholerae* in communities.

The majority of *P. aeruginosa* isolates were recovered from wound pus and ear pus and the pathogen was highly resistant to Ampicillin, Amoxicillin-Clavulanic Acid, Gentamicin, Ceftriaxone, Tetracycline, Nalidixic Acid, and Meropenem but more susceptibility to Ciprofloxacin. Resistance to Meropenem was 66.7%, relatively higher than the 55.2% reported by Mikomangwa et al (2020)<sup>18</sup> and 19% in a study conducted at Bugando Medical Centre using isolates collected between 2012 and 2017.<sup>29</sup> This suggests that *P. aeruginosa* is increasingly becoming resistant to antibiotics. *N. gonorrhoeae* was susceptible to Ceftriaxone but exhibited high resistance to Chloramphenicol (100%) and Ciprofloxacin (87.5%). High level of resistance to Azithromycin, Tetracycline, Ciprofloxacin, Penicillin, and to the injectable extended-spectrum cephalosporin Ceftriaxone have been described especially in regions where Gonorrhoea is most prevalent.<sup>30</sup> By 2017, treatment failure to third-generation cephalosporin antibiotics had been confirmed in at least 10 countries worldwide; South Africa, Australia, United Kingdom, Canada, Japan, Norway, France, Slovenia, Sweden, and Northern Ireland.<sup>30</sup>

*S. aureus*, a Gram-positive bacterium was the most common isolate that constituted nearly one-third of all bacterial isolates, mainly recovered from the ear and wound pus and blood specimens. Isolation of *S. aureus* in stool and urine was more likely due to skin contamination picked up during specimen collection. Of the isolates tested, (66.7%) were resistant to Cefoxitin and classified as MRSA infection. This observation corroborates with that recently reported at Muhimbili National Hospital and Bugando Medical Centre (61%),<sup>18</sup> and those from other sub-Saharan Africa countries; Kenya (53.5%),<sup>31</sup> Rwanda (82%)<sup>32</sup> and Eritria (72%).<sup>32</sup> *S. aureus* isolates also exhibited resistance to Erythromycin, Tetracycline, and

Gentamicin and moderate susceptibility to Ciprofloxacin, and Ceftriaxone. *S. aureus* isolates were mainly susceptible to Nalidixic acid, Clindamycin, and Chloramphenicol.

## Limitation

Despite the current findings can be used for operational discussion on possible comprehensive national control programs on antimicrobial resistance, genotypic characterization of antibiotic resistance and virulence genes could have provided more information on the genetic profile and complement phenotypic methods. In addition, in some instances, fewer isolates were tested against specific antibiotics (eg, only two *E. coli* isolates were assessed for the presence of ESBL), hence hindering their generalizability.

## Conclusion

Clinical isolates exhibited high resistance to most of the commonly used antibiotics. This emphasizes the need to strengthen national control programs such as routine antibiotic susceptibility testing and surveillance.

## Data Sharing Statement

All important data and methods are reported in the main text. Additional datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

## Ethical Considerations

The study was granted ethical clearance from the National Health Research Ethics Committee (NathREC) of Tanzania with reference number NIMR/HQ/R.8a/Vol.IX/2706. As this study involved collected specimens from routine clinical practices, informed consent was waived by the NathREC. We obtained the gateway permissions from the administrative authorities of each of the participating hospitals. Data access complied with relevant data protection and privacy regulations.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation; took part in drafting, revising, or critically reviewing the

article. All authors gave final approval of the version to be published and agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare no conflicts of interest for this work.

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