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ORIGINAL ARTICLE

Aetiology and Antimicrobial Susceptibility Pattern of Bacteria Pathogens from Hospitalised Adult Patients at a Tertiary Care Hospital in North Eastern Tanzania

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a dynamic and a rapidly increasing health concern worldwide. However, it is unevenly distributed with limited data from the developing countries. In Tanzania, it is estimated that there is a higher prevalence of AMR pathogens among hospitalised patients in tertiary care hospitals than in lower level health facilities. This is associated with longer hospitalisation, increased health care costs and higher mortality rates. The aim of this study was to determine the aetiology and AMR pattern of bacteria isolates from adult patients admitted at Kilimanjaro Christian Medical Centre.

Methodology: A total of 487 participants were enrolled in a cross sectional study conducted from April 2018 to March 2019. Bacteria isolates were from blood 262 (52.4%), urine 147 (29.4%) and wounds 91 (18.2%). Conventional methods were used to determine bacteria species while antimicrobial susceptibility was determined by using the disc diffusion method.

Results: The isolates were predominantly Gram-negative bacteria with Escherichia coli, the most common pathogen in blood 55 (21%) and urine 45 (30.6%) while Pseudomonas aureginosa18 (19.8%) was the most common isolate from wounds. There was 100% resistance to Ampicilin among E.coli, Klebsiella spp and Proteus spp.. Gentamycin resistance was high in E.coli 57/90 (56.7%), Klebsiella spp 27/58 (46.6%) and P. aureginosa 24/54 (44.4%) while resistance to Amikacin was low.There was high resistance to Ceftriaxone in E.coli 44/62 (70.9%) and Klebsiella spp21/36 (58.3%) and resistance to Ciprofloxacin was 67/92 (72.8%) and 26/55 (47.3%) in E.coli and Klebsiella spp respectively. A relatively lower Carbapenem resistance was observed.

Conclusion: There is an alarming high AMR to commonly used antibiotics, leaving a few available options, which are more expensive and not easily available. Therefore there is an urgent need to strengthen efforts to curb AMR in this setting while focusing treatment on the local culture and sensitivity pattern.

INTRODUCTION

Antimicrobial Resistance (AMR) is a rapidly increasing health problem worldwide. In September 2016, the UN declared AMR 'the greatest and most urgent global risk'[1]. Antibiotics have extensively been misused in both humans and food-producing animals and this accelerated the selection and spread of resistant bacteria.[2,3] The misuse of antibiotics includes; using antibiotics to treat non-bacterial infections, self-medication and using incorrect doses.[3] The Global Health Security Agenda assessment concluded that Tanzania has high levels of inappropriate use of antimicrobials both in humans and animals without proper systems in place to collect data on the prevalence of antibiotic resistance in common pathogens.[4] The prevalence of self-medication was at 58% and 76.3% in community-based studies conducted in rural and urban settings in north-eastern Tanzania.[5,6]

Currently, AMR causes over 700,000 deaths per year worldwide. In USA alone, 35,000 people die each year due to AMR while in the European Union AMR causes 25,000 deaths annually.[7,8,9] It is estimated that by 2050, 10 million deaths will be attributed to AMR every year.[7] In Africa, the available data indicates that the region shares the worldwide trend; however information concerning the extent of the problem is limited because effective surveillance of drug resistance is carried out in only a few areas.[2] A recent systematic review on Africa found that AMR data is not available for more than 40% of the countries.[10]

Although increasing AMR affects both developed and developing countries, these settings face different challenges.[3] Sub-Saharan Africa (SSA) including Tanzania has a higher burden of infectious diseases with less effective active surveillance systems.[11] Moreover, the resistance pattern in these settings may not be comparable to developed countries and -

hence the need for local data.[3] This may be due to lack of appropriate functioning drug regulatory mechanisms, limited diagnostic facilities, unauthorised sale of antimicrobials, inappropriate prescriptions and lack of patient education in developing countries as compared to developed countries.[3]

In a systematic review of antimicrobial resistance in Africa, *Streptococcus pneumoniae* resistance to Penicillin was reported in 14 out of 144 studies with a Median Resistance (MR) of 26.7%. Amoxicillin resistance in *Haemophilus* influenza isolates was 18 out of 53 (34.0%) while MR of *Escherichia coli* to amoxicillin and *gentamycin* was 88.1% and 29.8% respectively. Although *Carbapenem* resistance was uncommon in *Enterobacteriaceae* it was common in *Acinetobacter* and *Pseudomonas aureginosa*.[10]

Ideally, the choice of antibiotic should be guided by AMR surveillance data and treatment guidelines. Contrary to this, patients with bacterial infections in developing countries are mainly treated empirically. This underscores the need for timely and regular updates of the constantly changing drug resistance patterns.[11] Failure to address this poses several detrimental effects in terms of increasing health-care costs, length of hospital stay, as well as increasing morbidity and mortality rate and further likelihood of accelerating development of resistance.[7,12,13] In Tanzania, there is a recommended/ standard guideline for treatment of different bacterial infections. However, several studies have reported different AMR patterns across the country.[14, 15, 16]

This study aimed to determine the current aetiological agents with their antimicrobial susceptibility patterns among hospitalised adult patients to guide antimicrobial stewardship as well as infection prevention and control programs, and improve patients' care. The study also aimed to heighten the awareness of policy-makers, health care workers, and the general population on the extent of AMR in this setting.

MATERIALS AND METHODS

Study Area and Population

Across sectional record based study which included patients admitted at Kilimanjaro Christian Medical Centre (KCMC) between April 2018 to March 2019 was conducted. KCMC is a zonal referral hospital (level 3 health facility) in North-eastern Tanzania with an official bed capacity of 630 serving over 15 million people. In Tanzania, it is estimated that there is a higher prevalence of AMR pathogens among hospitalised patients in tertiary care hospitals than in lower level health facilities.[11] The inclusion criteria was; all hospitalised patients aged 14 years and above who had their blood, urine or wound swab culture taken during the study period. These included patients admitted in the Medical, General Surgery, Urology and Orthopedics wards. It also included patients admitted in Medical and Surgical intensive care units. Patients whose cultures had no growth or yielded growth of contaminants were excluded from the study.

Sample Size and Sampling Procedures

Purposeful sampling method was used where patients whose culture grew bacteria isolates from blood, urine and wound samples (positive culture results) were identified from the laboratory registry.

Data Collection Tools and Data Collection Procedures

Data was collected from the KCMC microbiology laboratory registry. Selected patients with positive culture results and susceptibility patterns were then reviewed and extracted as the primary data source. These were linked with patients' information in the patients' files from medical records through a data extraction sheet. Species were identified using selective culture media and biochemical identification methods. Antimicrobial susceptibility testing was performed using disc diffusion on Muller–Hinton II Agar (MHA) according to Clinical Laboratory Standards Institute (CLSI, 2013) guidelines. Gram-Negative (GN) bacteria isolates were tested for various antibiotics examples, i.e., Ampicillin, Amoxicillin-clavulanic, Ceftriaxone, Ciprofloxacin and Gentamicin. Gram-Positive (GP) bacterial isolates were tested for drugs such as Ceftriaxone, Trimethoprim-sulfamethoxazole, Erythromycin and Vancomycin. The choice of antibiotic agents varied depending on the range of antibiotics available to the laboratory.

Data Analysis

The data was entered and analysed using Statistical Package for Social Sciences (SPSS) version 23 developed by International Business Machine Corporation (IBM). Categorical data was summarised in percentages. Continuous variables were summarised using median with their respective measures of dispersion. Descriptive statistics was used to determine patterns of antibiotic resistant isolates among patients with positive blood, urine and wound swabs culture.

Ethical Clearance

Ethical clearance was obtained from the Institution Review Board at KCMU-College Tumaini University, Ethical clearance certificate number: 2368. Privacy and confidentiality was adhered to by using code numbers as opposed to using patients' names.

RESULTS

Socio-Demographic and Clinical Characteristics of Study Participants

A total of 2,934 cultures from wound, blood and urine specimen were done at KCMC, and out of these 717 (24%) cultures had bacteria growth. A total of 487 patients with positive culture results met the inclusion criteria and therefore were enrolled in the study. 13 specimens had 2 isolates each; making a total of 500 bacteria isolates that were analysed. (Figure 1)

Participants' age ranged from 14 to 110 years with median age (IQR) of 60 (41-73) years. Most of the patients 341 (70%) were males and majority were admitted in Urology 223 (45.8%) and Medical wards 104 (21.4%). The most prevalent disease condition was urinary tract infections (UTIs) 143 (29.4 %), while the main comorbidity was Diabetes Mellitus 89 (18.3%) (Table 1)

Aetiology of Infections

The distribution of pathogens from blood, urine and wounds specimens was 262 (52.4%), 147 (29.4%) and 91 (18.2%) respectively. The majority, 436 (87.2%) were Gram-negative (GN) with *Escherichia Coli* being the most frequent GN 113 (22.6%) while *Staphylococcusaureu s*41 (8.2%) was the most predominant Gram-positive (GP) isolate. The *E.coli* was the most common isolate from both blood and urine samples; 55 (21%) and 45 (30.6%) respectively. *Pseudomonas aeruginosa* was the predominant isolate from wounds 18 (19.8%) (Table 2)

TABLE 1: Socio-Demographics and Clinical Characteristics of the Study Participants (N=487)

Characteristics	Frequency	Percentage (%)
Median age in years (IQR)	60 (41-73)	
Age(Years)		
14-34	103	21.1
35-54	108	22.1
55-64	67	13.9
65+	209	42.9
Sex		
Male	341	70.0
Female	146	30.0
Ward		
Surgical	91	18.7
Medical	104	21.4
ICU	47	9.6
Burn	18	3.7
Urology	223	45.8
Obstetric	4	0.8
Referral		
Dispensary	14	2.9
Health centre	35	7.2
District hospital	101	20.7
Regional hospital	76	15.6
Zonal hospital	2	0.4
Self referral	259	53.2
Disease condition		
Wounds *	120	24.6
UTI	143	29.4
Pneumonia	18	3.7
URTI	9	1.8
Intestinal obstruction	10	2.1
Peritonitis	6	1.2
Comorbidities		
Diabetes mellitus	89	18.3
Renal Failure	49	10.1
Cancer	32	6.6
Stroke	18	3.7
Obstructive Uropathy	72	14.8

IQR - Interquartile range, ICU- Intensive Care Unit (Medical and Surgical) URTI - Upper Respiratory Tract Infection, UTI - Urinary Tract Infection, * Wounds - Diabetic wounds, traumatic wounds, bed sores, chronic ulcerative wounds, burn wound, cellulitis, post-surgical wounds, Obstructive Uropathy - Prostate enlargement and urethral stricture.

Antimicrobial Susceptibility

Gram-Negative Bacteria

Penicillin resistance was high with 100% resistance to - ampicillin among *E. coli*, *Klebsiella Spp* and *Proteus Spp* isolates tested. A relatively high resistance rate to Gentamicin was noted across the spectra of the GN; *E. coli* 57/90(56.7%), *Klebsiella Spp* 27/58(46.6%) and *Pseudomonas Spp* 24/54 (44.4%) in contrast to Amikacin which showed low rates of resistance (17.6%) (Table 3).

High AMR was also observed to Fluoroquinolones in *E. Coli* 67/92 (72.8%) and *Klebsiella Spp* 26/55(47.3%) showing resistance to Ciprofloxacin. Resistance to third-generation Cephalosporins was also high across all commonly isolated GN bacteria. *E. Coli* were resistant to Ceftriaxone, Cefotaxime and Ceftazidime by 44/62 (70.9%), 34/49 (69.4%) and 9/13 (69.2%) respectively. Relatively high sensitivity was observed to Carbapenems (Table 3).

Gram-Positive Bacteria

There were lower AMR rates in GP as compared to GN bacteria. *S. aureus* demonstrated high resistance to Erythromycin 21/35 (60.0%), Clindamycin 13/25 (52.0%), Ciprofloxacin 11/24 (45.8%) and Meropenem 3/7 (42.9 %). (Table 4)

DISCUSSION

Socio-Demographic Characteristics

The study was conducted at a tertiary care hospital in the North-eastern part of Tanzania, from April 2018 to March 2019 on bacterial isolates from blood, wound and urine cultures among hospitalised patients. The study showed high AMR pattern to commonly isolated pathogens. This is consistent with similar studies conducted in other developing countries.[17,18,19,20]

The majority of the participants were males. This is similar to reports from other studies within Sub-Saharan Africa (SSA), countries like; Tanzania, Ethiopia and Rwanda which registered 61%, 51.3% and 54.3% male participants respectively.[14,17,21] A relatively higher proportion of male participant in this study can be due to the fact that a significant number of patients 223 (45.8%) where from the Urology ward, which admits more male than female patients.

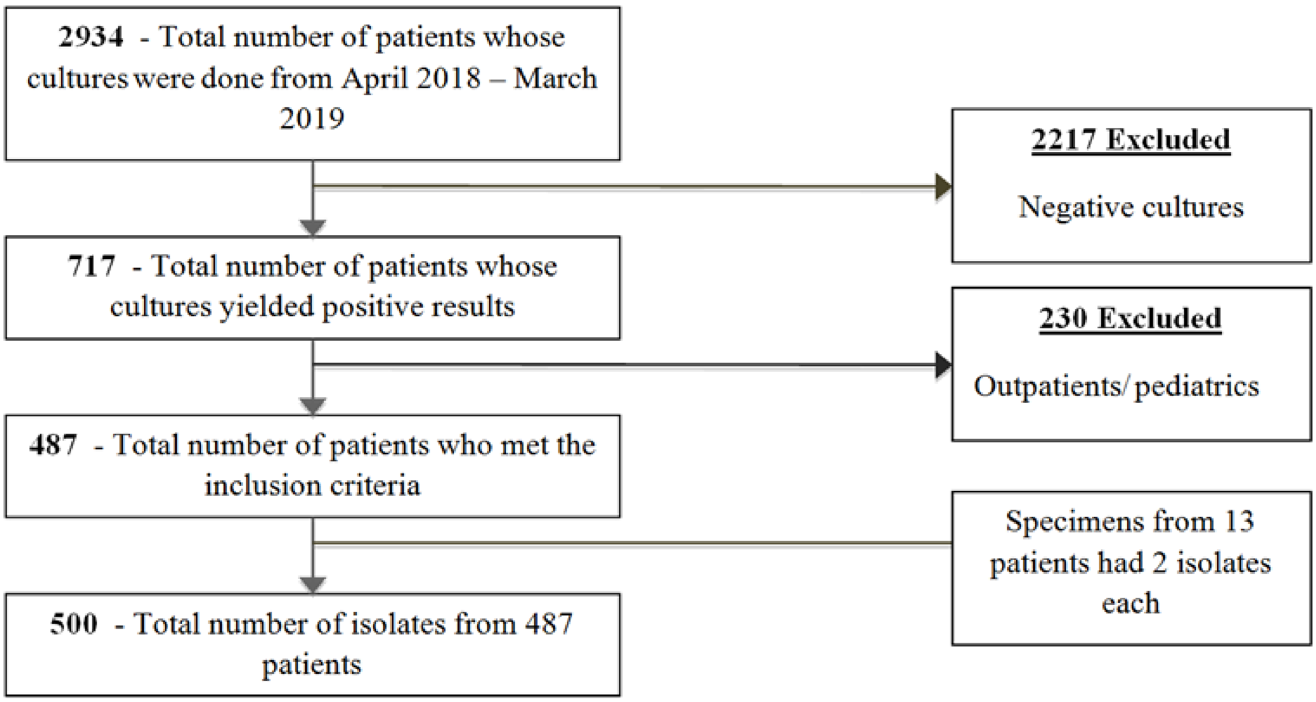
A similar study that was conducted in Rwanda among hospitalised adult patients reported a significant proportion of participants being above 65 years of age.[21] The possible reason for this finding could be due to the association between comorbidities and old age. More than a third of participants in this study had either Diabetes Mellitus, Renal failure, Cancer or Stroke. These comorbidities, together with old age cause immune suppression thus affecting the body's ability to fight infections.[22,23] The study also shows the double burden of communicable and non-communicable diseases in this setting.

Aetiology of Infections:

The dominant pathogens were GN bacteria. This is in agreement with other studies conducted in hospital settings in Tanzania, Ghana, Lebanon and Ethiopia.[14,18,24,25]

This can be contributed to the fact that majority of these isolates are normal flora on skin and in the gastro intestinal system of healthy individuals, hence they can easily be disseminated to other areas to cause infection.

FIGURE 1: Flowchart for Study Participants (487) and Culture Isolates (500)



Some can also be found in the hospital environment, in instruments such as ventilators, linens and can easily be transmitted through the hands of healthcare workers. As a result, GN bacteria have emerged as a hospital acquired pathogens.[8]

In a systematic review on antimicrobial resistance in West Africa, *E Coli* was found to be the most commonly reported bacterium (60.4%).[18] It was a predominant isolate in both blood and urine cultures. Kumburu et al and Musicha et al, found *E.coli* as the most common cause of blood stream infections by 28.4% and 8.8% in Tanzania and Malawi respectively.[14,26] Furthermore, findings from other hospital based studies in Tanzania, some European countries, Gabon, and India reported that *E. coli* was the most common isolate in UTIs by 26.2%, 52%, 36.3% and 52% respectively.[15,19,27,28] GN facultative anaerobe, a colonizer of the gastrointestinal tract is well known to be the most frequent cause of community and hospital acquired UTI and pyelonephritis.[13] On the other hand, *Pseudomonas Spp* which was the the predominant isolates from wounds in this study was also a predominant isolate from surgical site infections at Bugando Medical Centre (BMC) 14 (15.6%) and Muhimbili National Hospital 24 (16.3%) in Tanzania as well as isolates from burn wounds in a Ghanaian hospital 26 (30.2%).[15,16, 29] It was also the most frequent isolate from wounds of patients with Diabetes Mellitus[30]. Contrarily, some studies in Tanzania, Ghana and Bangladesh have reported *S. aureus* as the most common isolate from wound infections by 16 %, 46.3%, and 40.4% respectively.[14,31,32] The hospital environment might have contributed to the observed difference. It is possible that there was cross contamination among admitted patients since *S. aureus* are prominent nosocomial pathogens commonly found in the hospital environment.[8]

Klebsiella Spp were the most common isolates among patients with Stroke. This is in agreement with a study conducted in Taiwani (23.5%).[33] Stroke patients have stroke-induced immune suppression with prolonged hospital stay making them susceptible to infections. *Klebsiella Spp* are one of the major causes of nosocomial infections.[34]

Antimicrobial Susceptibility

There was a higher AMR rate among GN bacteria as compared to GP bacteria. We also found alarming and high rates of AMR among common isolates against commonly used antibiotics, higher than findings from a previous study done at KCMC 5 years prior. However, unlike in our study, the previous study considered both adult and paediatric -patients and samples collected included; blood, wound swabs, sputum and stool cultures.[14] A good example is on resistance to penicillin whereby; *E.coli* resistance to Ampicilin was 13/19 (68.4%) while in *Klebsiella Spp* resistance was 24/26 (92.3%).[14] Similarly for Cephalosporins, *E. coli* resistance to Ceftriaxone was 8/19 (42.1%), while *P. aureginosa* resistance to Ceftazidime was 4/22 (18.2%).[14] High AMR

TABLE 2: Bacteria Isolates Obtained from all Culture Positive Specimens (N500)

Bacterial isolated	Type of clinical specimens			
	Blood n (%)	Wound n (%)	Urine n (%)	Total N (%)
Gram-positive				
S. aureus	31(11.8)	9(9.9)	1(0.6)	41 (8.2)
S. pyogenes	10(3.8)	1(1.1)	0(0.0)	11 (2.2)
Enterococcus pp	5(1.9)	0(0.0)	5(3.4)	10 (2)
Bacillus spp	0(0.0)	0(0.0)	2(1.4)	2 (0.4)
Gram-negative				
E. coli	55(21.0)	13(14.3)	45(30.6)	113 (22.6)
Coliform spp	36(13.7)	12(13.2)	23(15.6)	71 (14.2)
Klebsiella spp	32(12.2)	12(13.2)	26(17.7)	70 (14)
P. aeruginosa	34(13.0)	18(19.8)	16(10.9)	68 (13.6)
Proteu spp	22(8.4)	16(17.6)	7(4.8)	45 (9)
Citrobacter spp	15(5.7)	4(4.4)	14(9.5)	33 (6.6)
Enterobacter spp	6(2.3)	1(1.1)	6(4.1)	13 (2.6)
Acinetobacter spp	9(3.4)	3(3.3)	0(0.0)	12 (2.4)
Serratia spp	2(0.8)	1(1.1)	2(1.4)	5 (1)
Morganella spp	3(1.1)	0(0.0)	0(0.0)	3 (0.6)
Providencia spp	1(0.4)	1(1.1)	0(0.0)	2 (0.4)
Shigella spp	1(0.4)	0(0.0)	0(0.0)	1 (0.2)
Total	**262 (100)**	**91(100)**	**147(100)**	**500 (100)**

rates were also observed for other most commonly used and easily available antibiotics including Gentamycin and Ciprofloxacin.[14]

These high rates of resistance are in agreement with other similar reports, which showed 90% and 96% *E.coli* resistance to Ampicilin in Tanzania and Rwanda respectively.[11,21] Moreover, at BMC, *E.coli* resistance to 3rd generation Cephalosporins and Ciprofloxacin was found to be as high as 12/19 (63%) and 16/24 (66.7%) respectively with even a higher resistance of *P. aureginosa* to third generation Cephalosporins 14/16 (87.5%).[27] This observation might be contributed by the irrational use of antibiotics. Antibiotics are widely used in the community either without prescription or guidance of culture and sensitivity results. Mboya et al., found that the use of most antibiotics 135 (88.8%) bought from the community pharmacies in the municipality where KCMC is located were irrational. The most bought antibiotics were; Ampicilin-cloxacilin 41 (27%), Amoxycylin 29 (18.4%), Metronidazole 14 (8.7%) and Ciprofloxacin 13 (8.1%).[31] Injectables, especially Ceftriaxone are commonly used in hospital settings as shown at BMC.[15] The emergency of AMR as a result of selection pressure can explain the phenomenon observed.[35] Due to lack of resources, empiric treatment without adequate antimicrobial susceptibility evidence is usually given in limited resources settings, which may be entirely ineffective or foster further resistance.[36, 37]

On the other hand, overall lower AMR rates were observed against Amikacin and the lowest against Carbapenems. In Rwanda, *E.coli* sensitivity to Imepenem was exactly the same as reported in this study at 92% while at BMC, *Klebsiella Spp* were 100% sensitive to Carbapenems.[14,27] Amikacin and Carbapenems are relatively less used antibiotics but are increasingly becoming the preferred treatment options.

CONCLUSION

There is an alarming and increasing AMR from isolates among patients admitted at KCMC. AMR was more observed among GN bacteria with resistance to commonly used antibiotics. This is of a major concern in a region that is highly burdened with both communicable and non-communicable diseases, with limited supply of more expensive and more effective drugs.

We recommend the study findings to be used by the antimicrobial stewardship programs to assist clinicians in selecting appropriate antibiotics against various infections in different disease conditions. The local health authorities should also be prompted to step up infection control programs in health facilities. Efforts to curb AMR should also be directed towards the community as witnessed by the fact that most of the patients were self referral from the community, however they presented with high levels of antimicrobial resistant pathogens at the time of hospital admission.

Study Limitations:

Some patients' record files had missing data and thus some detailed information on patient profiles was not available. It was also difficult to distinguish community from hospital-associated infections for some patients due inadequate documentation. Some samples were taken after starting antibiotic treatment, which may have led to

TABLE 3: Antimicrobial Resistance Pattern of Common Gram Negative Isolates to Commonly Used Antibiotics

Antimicrobials	E. coli		Coliform spp		Klebsiella spp		Pseudomonas spp		Proteus spp	
	T	R(%)	T	R(%)	T	R(%)	T	R(%)	T	R(%)
*Amoxy-clav	105	61(58.1)	62	37(59.7)	62	12(19.4)	9	7(77.8)	39	18(46.2)
Amikacin	74	10(13.5)	53	8(15.1)	55	1(1.8)	51	9(17.6)	31	5(16.1)
Ampicillin	19	19(100.0)	13	11(84.6)	8	8(100.0)	2	1(50.0)	2	2(100.0)
Ceftriaxone	62	44(70.9)	39	24(61.5)	36	21(58.3)	20	16(80.0)	30	13(43.3)
Ceftazidime	13	9(69.2)	8	6(75.0)	13	8(61.5)	33	11(33.3)	5	2(40.0)
Ciprofloxacin	92	67(72.8)	65	27(41.5)	55	26(47.3)	63	21(33.3)	38	3(7.9)
Cefotaxime	49	34(69.4)	16	10(62.5)	24	16(66.7)	6	5(83.3)	16	3(18.8)
Gentamycin	90	57(56.7)	61	28(45.9)	58	27(46.6)	54	24(44.4)	39	18(46.2)
Nitrofurantoin	61	18(29.5)	30	13(43.3)	41	22(56.7)	3	2(66.7)	16	11(68.8)
Meropenem	38	2(5.2)	27	5(18.5)	0	0(0.0)	19	4(26.7)	9	1(11.1)
Tetracycline	7	5(85.7)	1	1(100.0)	5	4(80.0)	1	1(100.0)	0	0(0.0)
*Trime-sulf	7	5(71.4)	1	1(100.0)	4	1(25.0)	1	1(100.0)	2	1(50.0)
Piperacillin	5	0(0.0)	6	2(33.3)	5	0(0.0)	50	7(14.0)	3	1(33.3)
Imepenem	37	3(8.1)	16	1(6.3)	19	0(0.0)	38	3(7.9)	7	0(0.0)

T - total number of isolates tested against antimicrobial agent, R - percentage of isolate resistance to antimicrobial agent, *Amoxy-clav: Amoxicillin–Clavulanic Acid, *Trime-sulf: Trimethoprim-sulfamethoxazole (co-trimoxazole) selection and over-representation of resistant isolates.

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