



Antimicrobial Resistance Pattern in *Escherichia coli* Isolated from Children Admitted at Selected Health Care Facilities in Moshi Municipality, Tanzania

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Authors' contributions

This work was carried out in collaboration between both authors. Author MD performed all laboratory procedures. Author BN provided advice on how to go about the study including writing research report. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2020/v6i330153

Editor(s):

(1) Dr. Ana Claudia Coelho, University of Tras-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Anubhuti Khare, Pushpagiri Institute of Medical Sciences & RC, India.

(2) Chandra Shekhar, Acharya Narendra Deva University of Agriculture and Technology, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57550>

Received 23 March 2020

Accepted 29 May 2020

Published 09 June 2020

Original Research Article

ABSTRACT

Background: Antimicrobial resistance (AMR) development among Gram negative bacteria has become a significant public health threat worldwide. Currently there are fewer antimicrobials available for treatment and prevention of some of bacterial infections. Antimicrobial resistance development in some of bacterial strains undermines empirical treatment regimens, thereby limiting choice of appropriate antibiotic. As this problem continues to grow, epidemiological surveillance is warranted to generate data that is reliable to understand the distribution of AMR among healthcare settings and geographical regions.

Objective: The main objective of this study was to determine the prevalence and pattern of antibiotic resistance of *Escherichia coli* isolates from children admitted at selected health care facilities in Moshi municipality, Tanzania. This was descriptive cross sectional facility based study conducted from October 2017 to April 2018. The study aimed at answering the following questions; What are the carriage rates of *Escherichia coli* among children admitted at selected health facilities in Moshi municipality, What is the resistance rates of *Escherichia coli* isolated from children against the commonly used antibiotics in Moshi Municipality and What is the prevalence of the Extended spectrum beta lactamase producing *Escherichia coli* isolates from children admitted at health facilities in Moshi municipality?

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Results: *Escherichia coli* were isolated from all 282 cultured archived rectal swabs and all isolates were susceptible to amikacin and meropenem. Extended spectrum beta lactamase producers were 13.1% and 86.9% were nonextended spectrum beta lactamase producers. Antimicrobial resistance was detected in 282 isolates on the commonly used antimicrobial agents: ampicillin (83%), trimethoprim (75.9%), cefuroxime (32.6%), ceftriaxone (30.1%), ceftazidime (29.4%), ciprofloxacin (27.7%), amoxicillin/clavunic (24%), gentamicin (21%), chloramphenicol (16.3%) and piperacillin-tazobactam (8.1%). All Extended spectrum beta lactamase producing isolates were resistant to Ampicillin.

Conclusion: Amikacin and meropenem is still the antibiotic of choice as a second line for treatment of infections caused by *Escherichia coli*. Majority of the isolates were resistant to ampicillin and Trimethoprim. Both ESBL and non ESBL producing *Escherichia coli* showed resistance to commonly used antibiotics, hence, further studies are warranted to understand mechanisms used by non ESBL producing *Escherichia coli* to develop resistance.

Keywords: Antimicrobial resistance; *Escherichia coli*; children; extended spectrum beta lactamase.

1. INTRODUCTION

Antimicrobial resistance (AMR) development among Gram negative bacteria has become a significant public health threat worldwide. Currently there are fewer antimicrobials available for treatment and prevention of some of bacterial infections [1]. Antimicrobial resistance development in some of bacterial strains undermines empirical treatment regimens, thereby limiting choice of appropriate antibiotic [2]. As this problem continues to grow, epidemiological surveillance is warranted to generate data that is reliable to understand the distribution of AMR among healthcare settings and geographical regions [3].

AMR among Gram-negative bacteria poses a challenge in treating and controlling bacterial infectious diseases worldwide [4,5]. The currently available information on the prevalence and patterns of AMR to different geographical regions has demonstrated that it is on rise [6,7,8]. The recommended and available antimicrobials have shown to be losing their ability to kill most of the infectious bacteria by developing resistance and becoming difficult to treat patients [9]. Some bacteria pose ability to produce enzymes such as Extended Spectrum β -Lactamase (ESBL), which is responsible for the resistance to cephalosporin and penicillin [10]. The increase in circulating resistant strains of infectious bacteria make it difficult for the use of current available and recommended antimicrobial drugs [11].

Resistance of Gram negative bacteria to the recommended and available antimicrobial drugs is a growing public health problem in different locations worldwide leading to increased morbidity and mortality to patients [12].

Surveillance report has shown that 500 000 people suffer from antimicrobial resistance worldwide [13]. The report on AMR in Africa revealed varying degree of AMR to the tested antimicrobials like amoxicillin 72.9%, trimethoprim/ sulfamethoxazole 75%, gentamicin 22.1% and ceftriaxone 17.2% [14]. The study on AMR pattern at tertiary hospital in Tanzania showed that resistance of *Klebsiella pneumoniae* and *Escherichia coli* isolates to third generation cephalosporin were 38.5% and 29.3% respectively [15]. Although AMR has been reported worldwide, there is limited information on *Escherichia coli* isolates in children admitted at health facilities in Moshi Municipality, Tanzania. Therefore, this study aimed to determine the *Escherichia coli* carriage and patterns of antibiotic resistance in children admitted at selected health facilities in Moshi Municipality, Tanzania.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was a descriptive cross-sectional facility-based study conducted in Moshi Municipality from October 2017 to April 2018. The archived samples which were preserved at -80°C in teaching laboratory of Kilimanjaro Christian Medical University College (KCMU Co) were obtained from pediatric wards in children admitted with gastrointestinal infection of three purposively sampled health facilities in Moshi municipality, Northern Tanzania; Kilimanjaro Christian Medical centre (KCMC) is located in the Northern part of Tanzania, KCMC is a zonal referral hospital, Mawenzi Regional Referral Hospital is located at the centre of Moshi Town,

Mawenzi Regional Referral Hospital is a referral hospital for Kilimanjaro region and Pasua health centre which is a primary health care facility in Moshi municipality. All identified *Escherichia coli* isolates were included into the study.

2.2 Bacterial Isolates

Archived rectal swabs collected from all enrolled children aged 3 to 168 months admitted at Kilimanjaro Medical Centre, Mawenzi Regional Referral hospital and Pasua Health Centre was cultured for bacterial growth at the teaching laboratory of Kilimanjaro KCMUCo in Moshi. Rectal swabs were first inoculated on MacConkey gar (MCA) plates and incubated at 37°C for 16 hours. Grown colonies were identified by using Kligler Iron Agar (KIA), Sulfide Indole and Motility (SIM), citrate utilization as well as Gram stain.

2.3 Antimicrobial Susceptibility Testing

Isolated microorganism was emulsified into the normal saline; turbidity was compared with 0.5 MacFarland turbidity standards. Antimicrobial susceptibility testing (AST) was done by Kirby-Bauer disc diffusion method using Muller Hinton agar (MHA) which was then incubated for 16 hours at 37°C. Antimicrobial susceptibility testing was performed on selected antibiotics recommended by Tanzania standard Treatment Guidelines (Ministry of Health, 2017). Other antimicrobials are available in the pharmacies around Moshi Municipality, were also included in the study. The antimicrobial drugs tested were: ampicillin (10 µg), chloramphenicol (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), trimethoprim (5 µg), gentamicin (10 µg), piperacillin-tazobactam (100/10 µg), amoxicillin-clavunic (20/10 µg), ceftazidime (30 µg), meropenem (10 µg), ceftriaxone (30 µg) and cefuroxime (30 µg).

The zone of bacterial growth inhibition by the antibiotics was measured in millimeter (mm) according to Clinical Laboratory Standard Institute (CLSI) guideline of 2013. *Escherichia coli* ATCC 25922 was used as quality control strain. The following zone of inhibition was considered as resistant; ≤13 mm for ampicillin and amoxicillin/clavunic acid, ≤12 mm for gentamicin and chloramphenicol, ≤17 mm for piperacillin-tazobactam and ceftazidime, ≤19 mm for ceftriaxone and meropenem, ≤20 mm for

ciprofloxacin, ≤14 mm for amikacin, ≤10 mm for trimethoprim and ≤14 mm for cefuroxime.

2.4 Detection of ESBL

All isolates resistant to cefuroxime were tested for ESBL production whereby ceftazidime was set 2 cm away from amoxicillin/clavunic acid and synergism effect was determined. A test was considered as positive when a decreased susceptibility to ceftazidime was combined with a clear-cut enhancement of the inhibition zone of ceftazidime in front of amoxicillin/clavunic disc, often resulting in a characteristic shape-zone referred to as champagne-cork or keyhole [16].

2.5 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 20 was used for data analysis. Descriptive statistics were summarized by frequency and proportions. Continuous data were summarized as median along with interquartile range (IQR) values.

3. RESULTS AND DISCUSSION

3.1 Results

A total of 282 archived rectal swabs collected from hospitalized children, 139 (49%) from KCMC, 74 (26%) from Mawenzi and 69 (25%) from Pasua were included in the study. The majority of the children were males (56.7%). Most of participants (39%) were aged between 3 and 11 months with the median age of 18 months (IQR 8.0-44.25). The carriage rate of *Escherichia coli* to the sample collected was 100%, as *Escherichia coli* were isolated from all study children. All isolates were susceptible to amikacin and meropenem. However, AMR was observed to ampicillin and Trimethoprim with 83.3% and 75.9% of the isolates been resistant. Resistance in the isolates was observed against ceftazidime 29.4%; ciprofloxacin 27.7%; gentamicin 21.3%; piperacillin-tazobactam 8.2%; amoxicillin/clavunic 24.5%; ceftriaxone 30.1% and cefuroxime 32.6% (Table 1).

Out of 282 *E. coli* isolates in this study 37 (13.1%) were found to be ESBL producers. All 37 (100%) ESBL producers were resistant to ampicillin and cefuroxime while varying degree of AMR was shown by 28 (75%) to ciprofloxacin, 33 (89.2%) to trimethoprim 20 (54.1%) to gentamicin 31 (83.8%) to ceftazidime and 36 (97.3%) to

ceftriaxone. All ESBL producers were 100% sensitive to amikacin and meropenem (Table 2).

Among 245 non ESBL producers, high resistance was observed to ampicillin 198 (80.8%) and trimethoprim 181 (73.9%). All non ESBL producers were 100% sensitive to amikacin and meropenem (Table 3).

3.2 Discussion

All *E. coli* isolates that were analyzed demonstrated high rates of resistance to commonly used classes of antimicrobial. Similar

observations have been reported by previous studies in Mwanza [9,15].

Escherichia coli showed a high rate of resistance to ampicillin. This high rate of resistance of *E. coli* to ampicillin could be contributed by its easy availability in the pharmacy at inexpensive prices affordable by everyone and is highly prescribed by clinicians since is a first line antibiotic as per National standard antibiotic guidelines [17] sometimes without doing culture and antimicrobial susceptibility testing leading to its overuse and misuse [18,19].

Table 1. Number (%) of *E. coli* isolates resistant to different antibiotics

Antibiotics	Pasua (n=69)	Mawenzi (n=74)	KCMC (n=139)	Overall (N=282)
AMP(10 µg)	48 (69.6)	66 (89.2)	121 (87)	235 (83.3)
C(30 µg)	10 (14.5)	9 (12.2)	27 (19.4)	46 (16.3)
AK(30 µg)	0 (0)	0 (0)	0 (0)	0 (0)
CIP(5 µg)	9 (13)	19 (25.7)	50 (36)	78 (27.7)
W(5 µg)	51 (73.9)	48 (64.9)	115 (82.7)	214 (75.9)
CN(10 µg)	3 (4.3)	12 (16.2)	45 (32.4)	60 (21.3)
TZP(100/10 µg)	5 (7.2)	3 (4.1)	15 (10.8)	23 (8.2)
AMC (20/10 µg)	1 (1.4)	21 (28.4)	25 (18)	69 (24.5)
CAZ(30 µg)	8 (11.6)	43 (58.1)	54 (38.8)	83 (29.4)
CRO(30 µg)	9 (13)	23 (31.1)	52 (38.1)	85 (30.1)
MEM(10 µg)	0 (0)	0 (0)	0 (0)	0 (0)
CXM(30 µg)	10 (14.5)	24 (32.4)	58 (41.7)	92 (32.6)

Key: AMP, Ampicillin; C, Chloramphenicol; AK, Amikacin; CIP, Ciprofloxacin; W, Trimethoprim; CN, Gentamicin; TZP, Piperacillin-Tazobactam; AMC, Amoxicillin/Clavunic; CAZ, Ceftazidime; MEM, Meropenem; CRO, Ceftriaxone; CXM, Cefuroxime; µg, Microgram

Table 2. Antimicrobial susceptibility to commonly used antimicrobial classes among ESBL producing *E. coli* isolates (N=37) at Pasua, Mawenzi and KCMC

Antimicrobial agent	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Penicillin			
AMP (10 µg)	37(100)	0(0)	0(0)
AMC (20/10 µg)	18(48.6)	10(27.0)	9(24.3)
TZP (100/10 µg)	8(21.6)	11(29.7)	18(48.6)
Cephalosporin			
CRO (30 µg)	36(97.3)	0(0)	1(2.7)
CAZ (30 µg)	31(83.8)	3(8.1)	3(8.1)
CXM (30 µg)	37(100)	0(0)	0(0)
Aminoglycoside			
CN (10 µg)	20(54.1)	1(2.7)	16(43.2)
AK (30 µg)	0(0)	0(0)	37(100)
C (30 µg)	8(21.6)	0(0)	29(78.4)
Sulphonamide			
W (5 µg)	33(89.2)	0(0)	4(10.8)
Fluoroquinolones			
CIP (5 µg)	28(75.7)	4(10.8)	5(37)
Carbapenem			
MEM (10 µg)	0(0)	0(0)	37(100)

Key: AMP, Ampicillin; C, Chloramphenicol; AK, Amikacin; CIP, Ciprofloxacin; W, Trimethoprim; CN, Gentamicin; TZP, Piperacillin-Tazobactam; AMC, Amoxicillin/Clavunic; CAZ, Ceftazidime; MEM, Meropenem; CRO, Ceftriaxone; CXM, Cefuroxime; µg, Microgram

Table 3. Antimicrobial susceptibility to commonly used antimicrobial classes among non-extended spectrum beta lactamase producing *Escherichia coli* isolates (N=245) at Pasua, Mawenzi and KCMC

Antimicrobial agent	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Penicillin			
AMP (10 µg)	198(80.8)	5(2.0)	42(17.1)
AMC (20/10 µg)	51(20.8)	65(26.5)	129(52.7)
TZP (100/10 µg)	15(6.1)	51(20.8)	179(73.1)
Cephalosporin			
CRO (30 µg)	49(20.0)	2(0.8)	194(79.2)
CAZ (30 µg)	52(21.2)	84(34.3)	109(44.5)
CXM (30 µg)	55(22.4)	8(3.3)	182(74.3)
Aminoglycoside			
CN (10 µg)	40(16.3)	0(0)	205(83.7)
AK (30 µg)	0(0)	0(0)	245(100)
C (30 µg)	38(15.5)	1(0.4)	206(84.1)
Sulphonamide			
W (5 µg)	181(73.9)	1(0.4)	63(25.7)
Fluoroquinolones			
CIP (5 µg)	50(20.4)	13(5.3)	182(74.3)
Carbapenem			
MEM (10 µg)	0(0)	0(0)	245(100)

Key: AMP, Ampicillin; C, Chloramphenicol; AK, Amikacin; CIP, Ciprofloxacin; W, Trimethoprim; CN, Gentamicin; TZP, Piperacillin-Tazobactam; AMC, Amoxicillin/Clavunic; CAZ, Ceftazidime; MEM, Meropenem; CRO, Ceftriaxone; CXM, Cefuroxime; µg, Microgram

All *Escherichia coli* isolates were susceptible to amikacin and meropenem similar to previous reports [20,7,21]. No resistance to amikacin and meropenem could be explained by the rare use of these antimicrobial since are second line according to National standard antibiotic guidelines [17].

The prevalence of ESBL producing *E. coli* in this study was higher as compared to reports on globally isolated *E. coli* [21]. However, it was lower as compared to the findings in hospitalized children in Dar es Salaam [22] and in Bahran [20] and Rwanda [23]. The easily accessible and the habit of using third generation cephalosporin which is a broad spectrum without culture and antibiotic susceptibility testing may influence on the overuse of the drugs. AMR among ESBL producing bacteria to cephalosporin has been reported to be associated with over exposure to this class of drugs [24,25].

The high rate of antimicrobial resistance was observed to cephalosporins followed by penicillin, sulphonamide and fluoroquinolones among ESBL producing *E. coli*. These are easily available and commonly prescribed by clinicians, hence creating the pressure of excessive antimicrobial use in Moshi. Similarly, a study in Mwanza, Tanzania reported an increase in the

rate of AMR to third generation cephalosporins overtime among commonly prescribed antibiotics [9]. The increases of pressure in antimicrobial use have been reported to promote development of AMR amongst different bacterial species and strains through horizontal gene transfer and thus contribute to the rapid dispersal in the community [10].

4. CONCLUSION AND RECOMMENDATION

Amikacin and meropenem is still the antibiotic of choice as a second line for the infections caused by resistant *Escherichia coli*. Both ESBL and non ESBL producing *Escherichia coli* showed resistance to commonly used antibiotics, hence further studies are warranted to understand mechanisms used by non ESBL producing *Escherichia coli* to become resistant. Use of Ampicillin and trimethoprim in treatment or control of *Escherichia coli* infection should be reviewed. Culture and antibiotic susceptibility testing should be emphasized prior antibiotic prescription in patient management. Epidemiological surveys on antibiotic resistance among isolated bacteria should be conducted regularly to prevent and control its spread. The use of archived sample is considered to be the limitation of this study.

ETHICAL APPROVAL

The study was carried out in accordance with existing ethical guidelines. Ethical clearance was obtained from the Kilimanjaro Christian Medical University College Research Ethics Committee with certificate number 2030. The permission to use the sample was granted by the head of Laboratory department Kilimanjaro Christian Medical University College.

ACKNOWLEDGEMENT

We are grateful to the staff of the teaching laboratory of KCMU Co for their participation in this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
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