



# Phenotypic Detection of Carbapenem-Resistant Gram-Negative Bacilli from a Clinical Specimen in Sidama, Ethiopia: A Cross-Sectional Study

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**Background:** Carbapenem-resistant gram-negative bacteria are an emergent source of both community-acquired and healthcare-associated infection that poses a substantial hazard to public health. This study aimed to conclude the magnitude of carbapenem resistance gram-negative bacteria from a clinical specimen at Hawassa University Comprehensive Specialized Hospital.

**Methods:** A hospital-based cross-sectional study was accompanied from February 13 to June 7, 2020, in which consecutive patients with 103 gram-negative bacteria were encompassed. The isolates included were 54 urine, 17 blood, 17 pusses, 4 cerebrospinal fluid (CSF), 3 aspirates, 3 effusions, 2 stools, 2 ear discharges, and 1 nasal swab. A semi-structured questionnaire was used to gather socio-demographic data from the attendant and clinical data from the patient's chart. Patients admitted in any wards and visited outpatients department were included for the study if gram-negative bacteria was identified for those who accepted the consent. A routine manual culture, Gram's staining and biochemical tests used to identify the bacteria. Antibiotic susceptibility was determined for twelve antibiotics including cotrimoxazole, ceftazidime, meropenem, gentamycin, chloramphenicol, ampicillin, ciprofloxacin, cefotaxime, cefuroxime, nitrofurantoin, piperacillin-tazobactam, and amikacin using the Kirby-Bauer disc diffusion method. Modified carbapenem inactivation (mCIM) method was used to determine carbapenem resistance using meropenem disk as per the recommendation of Clinical and Laboratory Standards Institute guideline. Statistical package for social science software version 21 was used for data entry and analysis. The odds ratio at 95% confidence interval (CI) and p-value <0.05 were taken as a statistically significant association.

**Results:** Generally, 111 gram-negative bacteria were identified from 103 patients. Of 111 isolates, thirteen isolates (nine resistance and four intermediates) were identified in disk diffusion testing for meropenem. Of this, 10 isolates were carbapenemases producer with the overall rates of 9% in the Modified carbapenem inactivation method (mCIM). *Pseudomonas* spp. 3 (30.0%), *E. coli*, *K. pneumonia*, *Acinetobacter* spp. each two (20.0%), and *K. oxytoca* 1 (10.0%) were identified as carbapenemases positive. The rates of the multidrug, extensive, pan drug were 86.5, 43.3, and 1.8, respectively. Ampicillin 94 (97.9%), followed by cefuroxime 52 (91.2%), cefotaxime 94 (88.7%), cotrimoxazole 58 (88.1%), ceftazidime 40 (83.3%), ciprofloxacin 47 (77.1%), nitrofurantoin 35 (70.0%), gentamycin 71 (65.7%), with high level of resistance. However, piperacillin-tazobactam 41 (48.8%), chloramphenicol 25 (47.2%), meropenem 13 (11.7%), and amikacin 9 (8.5%) were with low rates of resistance. In this study, there were no variables statically associated with carbapenem resistance that is  $p > 0.05$ .

**Conclusion:** Our study showed that carbapenem-resistant gram-negative bacilli are 9% in the study area. Our finding signposts that ampicillin, cefuroxime, cefotaxime, cotrimoxazole, ceftazidime, ciprofloxacin, nitrofurantoin, and gentamycin with a high rate of resistance >50%. However,

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piperacillin-tazobactam, chloramphenicol, meropenem, and amikacin were at low rates of resistance. Therefore, a measure should be taken to contain carbapenem resistance gram-negative bacteria in the study area. Further, study with better method needs to be conducted to conclude the real scenario of carbapenem resistance.

**Keywords:** phenotypic, carbapenem resistance, gram-negative bacilli, clinical specimen, Hawassa, Ethiopia

## Background

Carbapenem-resistant gram-negative bacteria (CR-GNB) are an evolving cause of both community-acquired and healthcare-associated infection (HCAI) that pose a significant threat to public health.<sup>1</sup> Carbapenem is bactericidal  $\beta$ -lactam antimicrobials with demonstrated efficacy in severe infections caused by extended-spectrum  $\beta$ -lactamase (ESBL) producing bacteria.<sup>2</sup> Drugs like meropenem, ertapenem, and imipenem are used to treat most of the infections affected by multidrug-resistant bacteria and can be castoff for the screening of carbapenem resistance in the bacteriology laboratory.<sup>3</sup> Carbapenem resistance can be intrinsic or acquired by mutation or gene acquisition via horizontal gene transfer.<sup>4</sup>

Both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) annually define the susceptibility breakpoints to commercially available carbapenem's, including doripenem, ertapenem, imipenem, and meropenem for gram-negative species, although EUCAST no longer provides doripenem breakpoints.<sup>5,6</sup> When a strain is found to be non-susceptible to carbapenem's (ie, intermediate or resistant), the mechanism of resistance is still unknown.<sup>7-9</sup> Thus, to confirm the production of carbapenemases and/or presence of other mechanisms, further biochemical assays and/or gene-based tests must be performed.<sup>6,7,10</sup> The modified Carbapenem Inactivation Method (mCIM) is a simple phenotypic test that detects carbapenemases-producing gram-negative bacteria that have only been evaluated for use on bacterial colonies.<sup>11</sup>

Gram-negative bacteria, especially Enterobacteriaceae, are communal sources of both community-acquired and hospital-acquired infections, comprising urinary tract, bloodstream, and lower respiratory tract infections. These bacteria can gain genes encoding numerous antibiotic resistance mechanisms, containing ESBLs, AmpCs, and carbapenemases.  $\beta$ -Lactam drugs are regularly the key therapeutic choice for serious infections, and carbapenem, in particular, are frequently considered agents of last remedy.<sup>12</sup> However, the rise of unusual  $\beta$ -lactamases with straight carbapenem-hydrolyzing action has contributed to an augmented prevalence of carbapenem-resistant

Enterobacteriaceae (CRE). CRE is principally challenging assumed the occurrence with which Enterobacteriaceae causes infections.<sup>13</sup>

*Klebsiella species* and *Escherichia coli* (*E. coli*) are models of Enterobacteriaceae, a regular part of the human belly bacteria that can be converted carbapenem-resistant. Patients whose care involves strategies like ventilators (breathing machines), urinary (bladder) catheters, or intravenous (vein) catheters and patients who are taking extended courses of certain antibiotics are most at menace for CRE infections.<sup>14,15</sup> Also, patients with comorbidities, ICU stay immunosuppression, the two extremities of age, and exposure to multiple antibiotics before the initial culture considered as risk factors for acquiring CR.<sup>16,17</sup> Now a day, the medical effect of carbapenem resistance has become a public health problem around the world in terms of increased mortality, longer hospital stays, and costs of treatment.<sup>18</sup>

The frequency of CRE differs extensively between diverse class and different topographical areas. In the USA, carbapenem-resistance rates are revealed as 0.1% and 5.3% for *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*), correspondingly, whereas in Europe, most nations report resistance rates below 1% for both pathogens.<sup>19</sup> Even though studies from Africa stated a carbapenem resistance of (3.8–52.6%),<sup>20-23</sup> still there are a few data on CR-GNB in Africa.<sup>21</sup> While there are a few studies in Ethiopia conveyed on CR with a range of 2–27.1%,<sup>24-27</sup> however, there is limited data in the study area. Therefore, this study intended to determine the magnitude of CR-GNB identified from clinical samples at Hawassa University Comprehensive Specialized Hospital (HUCSH).

## Methods

### Study Design, Area, and Period

A hospital-based cross-sectional study was accompanied at HUCSH since February 13 to June 7, 2020. The hospital is found in the capital city of Sidama at Hawassa, 275 kilometers (KMs) far from Addis Ababa, the capital city of Ethiopia. The altitude of the town is 1697m beyond sea level with a mean annual temperature and rainfall of 20.9 o C and 997.6 mL correspondingly. HUCSH was established

in November 2005 and it serves about 12 million peoples. Patients looking for medical care obtain services at different outpatient and inpatient units (surgery, gynecology, and obstetrics, internal medicine, paediatrics, ophthalmology, psychiatry, radiology, pathology). The laboratory in the hospital analyzes arrays of tests, including parasitological, microbiological, immunological, hematological, and biochemical analysis. In the microbiology section, all aerobic culture and sensitivity testing done regularly from Monday to Sunday for 24 hours. The microbiology laboratory at HUCSH was established in 2010. It is the only laboratory in the region that participates for obtaining accreditation.

## Population

All patients that visited the microbiology laboratory for routine culture and susceptibility testing through the study period were the source population. All patients confirmed with gram-negative isolates were the study population.

## Eligibility

All patients that requested for culture and those agreed to join in the study included and patients refuse to participate in the study and patients with gram-positive isolates omitted from the study.

## Variables of the Study

Carbapenem resistance is the dependent variable, whereas, age, sex, residence, wards, another hospital stays, length of hospital stay, sites of infection, an external device used, length of device indwelled, previous antibiotics usage, class of antibiotics, and underlined disease.

## Sampling Technique

Convenient sampling technique employed to collect 103 consecutive patients with gram-negative isolates. The patient's result and data were included if they are volunteering to contribute to the study.

## Data Collection

### Socio-Demographic and Clinical Data Collection

Trained data collectors used an organized questionnaire to gather socio-demographic as well as clinical data from patients and patient's charts. If there is a positive growth for gram-negative bacteria in the laboratory the microbiologist inform the person in the reception and attach the questionnaires with laboratory report form. For those in

cable patients and children, the attendant or caregivers were requested for participation, otherwise for the ambulatory patients directly interviewed for the socio-demographic data. The clinical data were collected from the patients' chart and attending physician.

## Sample Collection

All samples were collected based on the standard operating procedure (SOP) of HUCSH microbiology laboratory. Urine, puss, blood, ear discharge, eye swab, stool, cerebrospinal fluid (CSF), sputum and nasal swab sample suspected for any bacterial infection. The samples were sent from different wards. All samples were cultured on appropriate culture media. Samples collected for routine culture purposes were included if gram-negative bacteria were isolated.

## Laboratory Diagnosis

### Culturing

The samples were inoculated based on the essentiality of samples. Blood culture was collected with the sterile procedure and immediately inoculated to tryptone soy broth (TSB) at the site of collection. Then the bottle was transported for incubation in the microbiology laboratory. It is incubated at 37 C<sup>0</sup> for five days. The bottle checked daily for the presence of growth indicators, ie, gas, pellicles, clot, and hemolysis. The sample subcultured blood agar plate (BAP), chocolate agar plate (CAP), and MacConkey agar (MAC) and gram stain were conducted at 24 hours incubation even if there were no growth indicators. If there were grown in solid media, identification performed based on their gram staining characteristics. Gram-negative organisms were included in this study. Finally, on day five, the bottle subcultured solid media if there were no growth reported as negative. Urine and puss were inoculated with BAP and MAC. Chocolate agar was included for ear discharge and nasal swab. Colony characteristics and gram staining are used as preliminary identification of isolates.

### Biochemical Testing

Once the organism was identified as gram-negative in gram staining, serial biochemical testing that was prepared routinely performed to identify the isolates. Triple sugar iron agar, urea, citrate, mannitol fermentation, lysine iron agar, sulfur indole motility testing, and oxides used to distinguish the isolates to the species level.

## Antibiotic's Susceptibility Testing

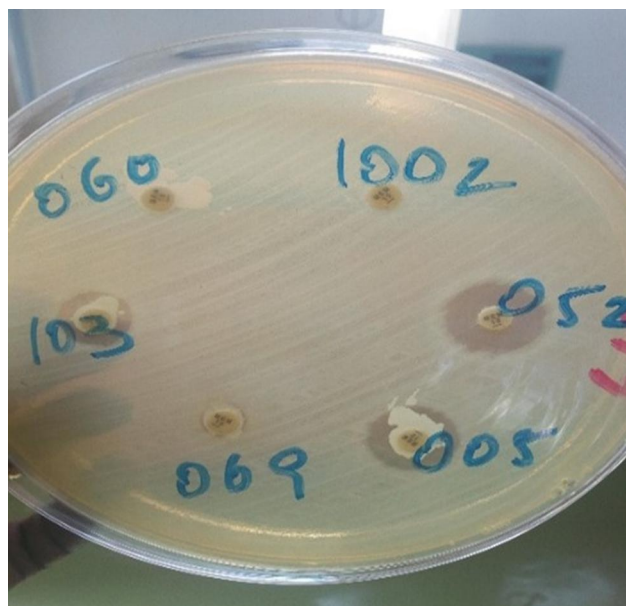
The Kirby disk diffusion technique was used to perform the susceptibility testing on Muller Hinton agar (MHA).<sup>5</sup> Twelve different antibiotics performed including cotrimoxazole - COT (1.25/23.75µg), ceftazidime - CAZ (30µg), meropenem - MER (10µg), gentamycin - GEN (10µg), chloramphenicol - CAF (30µg), ampicillin - AMP (10µg), ciprofloxacin - CIP (5µg), cefotaxime - CTX (30µg) cefuroxime - CRX (30µg), nitrofurantoin - NIT (5 µg), piperacillin-tazobactam - PIT (100/10 µg) and amikacin - AMK (30 µg). The recent clinical laboratory standard institute guideline as sensitive, intermediate, and resistant will interpret the result of measuring the zone of inhibition. Carbapenem resistance is determined by modifying the inactivation of carbapenem in which meropenem disk is used.

## Modified Carbapenem Inactivation Method (mCIM)

Sterile inoculating loop, 1 µL for *Enterobacteriaceae*, and 10 µL for *Pseudomonas* and *Acinetobacter* spp. of test organisms were added into a tube having 2 mL of tryptic soy broth (TSB). The bacterial suspension was mixed for 10–15 seconds. Subsequently, a 10-µg MER disk was carefully added to the already prepared suspension. The suspension with disk was then incubated for 4 hours ± 15 minutes at 35°C ± 2°C in an ambient atmosphere. Just before the end of the 4-hour carbapenem inactivation step, a suspension of the mCIM quality control strain (*E. coli* ATCC 25,922) with turbidity equivalent to a 0.5 McFarland standard was arranged and the surface of an MHA dish inoculated using the technique for standard disk diffusion susceptibility testing. The meropenem disk then detached from the TSB suspension by a 10-µL inoculating loop disk employed onto the inoculated MHA plate, which then incubated in an upturned situation for 18–24 hours at 35°C ± 2°C in ambient air.<sup>5</sup>

### mCIM Result Interpretation

The width of the zone of inhibition around each MEM disk was measured a zone diameter of 6–10 mm was taken to be a positive result (ie, carbapenemases production detected), 11–19 mm an indeterminate result and ≥20 mm a negative result (ie, No carbapenemases production detected). A thin ring of growth adjoining the MEM disk, representative of the leftover of the test organism from the TSB, was snubbed<sup>5</sup> (Figure 1).



**Figure 1** Modified carbapenem inactivation method (mCIM) to determine carbapenem resistance of gram-negative bacteria from clinical specimens at tertiary care hospital, southern Ethiopia.

### Quality Control

Data quality warranted using standardized data gathering tools, pretesting of the questionnaires, appropriate training earlier the start of data assortment and rigorous supervision throughout data assemblage by the authors. For laboratory investigation, pre-analytical, analytical, and post-analytical phases of quality assurance amalgamated into Standard operating procedures (SOPs) of the microbiology laboratory stringently tracked. Besides, well-trained and skilled laboratory professionals participated in the laboratory investigation system. Media sterility checked after preparation by incubating for 24hrs. Quality control bacteria such as *E. coli* (ATCC-25,922), and *P. aeruginosa* (ATCC-27,853) found from the Ethiopian public health institute (EPHI) to check the characteristic of the colony while growing of respective media and biochemical tests.

### Data Processing and Analysis

Data were managed using SPSS statistical software version 21 and presented in table and graph. The bivariate and multivariate logistic regression model used to check the real forecasters of a dependent variable. The odds ratio at 95% confidence level and p-value <0.05 was used for statistical significance.

### Ethical Consideration

Hawassa University College of Medicine and Health Sciences institutional review board (IRB) has approved

the proposal with a Ref. No: IRB/268/12. At that moment support letter was gained from the hospital management. Socio-demographic data and specimen were collected after written and agreement gained from each child's parents and patients. All information kept secret using codes and locking on the board. The result of the patient-reported to the clinician within three or four days and those who are culturally positive treated accordingly.

## Operational Definitions

Multidrug-resistant bacteria (MDR): Bacteria that are resistant to at least one agent in three or more antibiotic categories.

Extensively drug-resistant (XDR) is non-susceptibility to at least one agent in all but two or fewer antibiotic categories (ie, bacterial isolates remain susceptible to only one or two categories).

Pan drug-resistant (PDR) is non-susceptibility to all agents in all antibiotic categories.<sup>28</sup>

## Result

### Socio-Demographic Characteristics

One-hundred three (103) patients with gram-negative isolates were involved in the study, of these 54 (52.4%) of them were male. Regarding age 62 (60.2%) was <5 years and the rest 40 (38.8%) was >6 years age group. The mean and standard deviation (SD) of the age for the study subject was  $11.52 \pm 16.2$  years that range from 1 day to 70 years. Fifty-five (53.4%) of them were from urban and the rest 48 (46.6%) was a rural resident (Table 1).

### Clinical Features and Associated Risk Factors

Patients enrolled in this study were from eight different wards that include paediatrics 38 (36.9%), surgical 16 (15.5%) neonatal intensive care units (NICU) 27 (26.2%), and others (medical, intensive care unit (ICU), ENT) 22 (21.4%). Thirty-two (31.1%) patients had another hospital stay and the rest 711 (68.9%) have no history of hospital stay. The length of hospital stay for <10 days was 64 (62.1%) and the rest 39 (37.9%) stay for >11 days that ranged (0–40 days). The mean and SD of hospital stay was  $11.6 \pm 9.12$  days. The sites of infection identified from this study were 54 (52.4%) was a urinary tract infection and the rest 49 (47.6%) was from other sites of infection (bloodstream, surgical site, intestinal, respiratory, nervous system, soft tissue, otitis). Most of the study

subjects 85 (82.5%) used one or more external devices during a hospital stay in which the mean and standard deviation of length of device usage was  $14.7 \pm 10.8$  in the days that ranged from 1 to 60 days. Antibiotics were used by 92 (89.3%) of patients as treatment or prophylaxis before sample collection and the rest 11 (10.7%) of them were not taking on antibiotics. Of this was 70 (76.1%) used a single class of antibiotics and the rest 22 (23.9%) taken a combined class of antibiotics. Our study tried to perform a bivariate analysis for socio-demographic as well as for clinical data. There were no candidate variables for multi-variable analysis in bivariate analysis  $p < 0.25$ . Therefore, there are no statically significant associated variables for carbapenem resistance  $p > 0.05$  (Table 1).

### Specimen Type

Of total 103 specimen, 54 (48.6%) was urine, 17 (15.3%) blood, 17 (15.3%) puss, 4 (3.6%) cerebrospinal fluid (CSF), 3 (2.7%) aspirates, 3 (2.7%) effusion, 2 (1.8%) stool, 2 (1.8%) ear discharge, and 1 (0.9%) nasal swab (Figure 2).

### Frequency of Isolated Bacteria

Of 103 patients enrolled in the study, overall 111-gram negative bacteria were isolated. The utmost isolate was *E. coli* 34 (30.6%). Followed by *K. pneumoniae* 31 (27.9%), *Acinetobacter* spp. 11 (9.9%), *Klebsiella oxytoca* (*K. oxytoca*) 9(8.1%), *P. aeruginosa* 8 (7.2%), *Klebsiella ozaenae* (*K. ozaenae*) 6 (5.4%), *Morganella morganii* (*M. morganii*) 5 (4.5%), *Klebsiella rhinosclolaris* (*K. rhinosclolaris*), *Citrobacter diversus* (*C. diversus*) and *Proteus mirabilis* (*P. mirabilis*) 2 (1.8%) each, and *Enterobacter agglomerus* (*E. agglomerus*) 1 (0.9%) (Figure 3).

### Antibiotic Resistance Patterns

The resistance patterns of antibiotics for each bacteria were determined accordingly, the most resisted antibiotic was ampicillin 94 (97.9%), followed by cefuroxime 52 (91.2%), cefotaxime 94 (88.7%), cotrimoxazole 58 (88.1%), ceftazidime 40 (83.3%), ciprofloxacin 47 (77.1%), nitrofurantoin 35 (70.0%), gentamycin 71 (65.7%), piperacillin-tazobactam 41 (48.8%), chloramphenicol 25 (47.2%), meropenem 13 (11.7%) and amikacin 9 (8.5%) (Table 2).

### The Magnitude of Multidrug Resistance

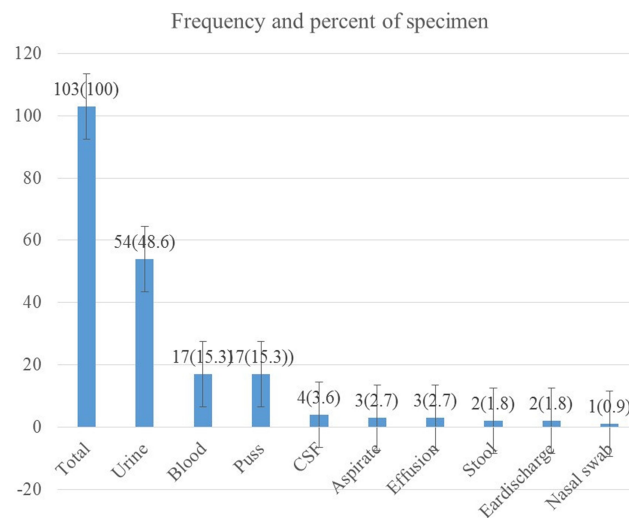
The level of multidrug resistance (MDR) checked for each isolate considering that bacteria that were

**Table 1** Socio-Demographic and Clinical Characteristics of Patients for the Study of Carbapenem Resistance of Gram-Negative Bacteria from Clinical Specimens at Tertiary Care Hospital, Southern Ethiopia, February 13–June 7, 2020

Variables	Frequency (%)	Carbapenemases (%)		COR (95% CI)	p-value
		Yes	No		
Age group (in years)					
<5	62 (60.2)	6 (9.7)	56 (90.3)	0.500 (0.096–2.609)	0.411
>6	40 (38.8)	2 (5.0)	38 (95.0)	1	
Sex					
Male	54 (52.4)	3 (5.6)	51 (94.4)	1.93 (0.437–8.547)	0.385
Female	49 (47.6)	5 (10.2)	44 (89.8)	1	
Residence					
Urban	55 (53.4)	5 (9.1)	50 (90.9)	0.667 (0.151–2.949)	0.593
Rural	48 (46.6)	3 (6.3)	45 (93.8)	1	
Wards					
Paediatrics	38 (36.9)	3 (7.9)	35 (92.1)	1.167 (0.180–7.582)	0.872
Surgical	16 (15.5)	2 (12.5)	14 (87.5)	0.700 (0.088–5.578)	0.736
NICU	27 (26.2)	1 (3.7)	26 (96.3)	2.600 (0.220–30.745)	0.448
Others	22 (21.4)	2 (9.1)	20 (90.9)	1	
Another Hospital stays					
Yes	32 (31.1)	3 (9.4)	29 (90.6)	1	0.683
No	71 (68.9)	5 (7.0)	66 (93.0)	0.732 (0.164–3.271)	
Length of hospital stay (days)					
< 10	64 (62.1)	5 (7.8)	59 (92.2)	0.983 (0.222–4.364)	0.982
>11	39 (37.9)	3 (7.7)	36 (92.3)	1	
Sites of infection					
UTI	54 (52.4)	5 (9.5)	49 (90.7)	0.639 (0.144–2.827)	0.555
Others	49 (47.6)	3 (6.1)	46 (93.9)	1	
External device used					
Yes	85 (82.5)	7 (8.2)	78 (91.8)	0.655 (0.076–5.683)	0.701
No	18 (17.5)	1 (5.6)	17 (94.4)	1	
Length of device indwelled (days)					
<5	22 (21.4)	2 (9.1)	20 (90.9)	1	0.897
>6	61 (59.2)	5 (8.2)	56 (91.8)	0.893 (0.160–4.974)	
Previous antibiotics usage					
Yes	92 (89.3)	7 (7.6)	85 (92.4)	1.21 (0.135–10.908)	0.862
No	11 (10.7)	1 (9.1)	10 (90.9)	1	
Class of antibiotics					
Single	70 (76.1)	6 (8.6)	64 (91.4)	0.508 (0.058–4.465)	0.541
Combined	22 (23.9)	1 (4.5)	21 (95.5)	1	
Underlined disease					
Yes	36 (35.0)	4 (11.1)	32 (88.9)	0.508 (0.119–2.165)	0.360
No	67 (65.0)	4 (6.0)	63 (94.0)	1	

resistant for three or more groups of antibiotics as MDR. Based on this definition, the level of MDR ranges from (0–100%). *Pseudomonas* spp., *K. ozaenae*, *K. oxytoca* was 100% MDR followed by

*K. pneumoniae* 96.8%, *E. coli* 82.4%, *Acinetobacter* spp. 81.8%, *M. morgani* 80%, *K. rhinoscolaris* and *C. diversus* 50%, and the rest *P. mirabilis* and *E. agglomerus* with 0% (Table 3).



**Figure 2** Frequency of specimens included for the study of carbapenem resistance of gram-negative bacteria from clinical specimens at tertiary care hospital, southern Ethiopia February 13–June 7, 2020.

The overall MDR, XDR and PDR were 86.5%, 43.2%, 1.8%, respectively (Figure 4).

## The Magnitude of Carbapenem Resistance

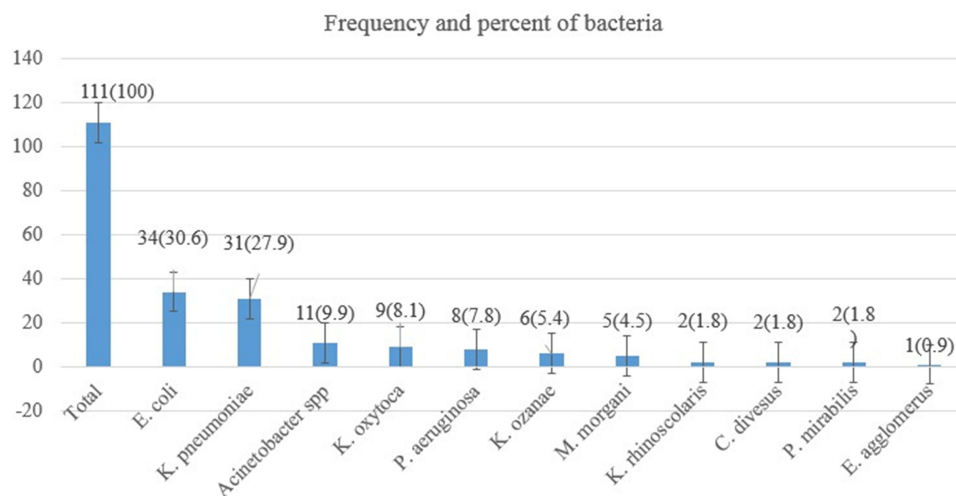
In Kirby-disc diffusion techniques, 111 isolates were tested against meropenem disk, of these 13 isolates (nine bacteria were resistant and four isolates were intermediate). In modified carbapenem inactivation techniques 10 out of the 13 confirmed as carbapenem resistance with overall carbapenem resistance was 9.0% (3.6–14.6%) (Figure 5), of these isolates the predominant was *Pseudomonas* spp. 3(30.0%), followed by *E. coli*,

*K. pneumoniae*, *Acinetobacter* spp. each two (20.0%) and *K. oxytoca* 1(10.0%). All CR bacteria showed MDR, XDR, and one of the isolates was PDR.

## Discussion

The arrival of resistance to carbapenem's in gram-negative bacteria is a significant and increasing threat to public health in Africa.<sup>2</sup> A load of infectious diseases is high in Africa,<sup>29</sup> and gram-negative bacteria cause most of the common clinical infections such as UTI, septicemia, pneumonia, meningitis, and peritonitis. Years of deprived therapeutic antibiotic prescribing and lack of infection, prevention strategies in African hospitals have put patients in danger of acquiring these difficult to treat bacterial infections.<sup>30</sup> The scourge of forged and substandard drug has only made the problem worse. Medical tourism has now resulted in patients acquiring these resistant bacteria in countries outside the continent and bringing them back to African hospitals.<sup>31</sup> Our study conducted on all clinical specimen from different outpatient and inpatients sent for routine culture at HUCSH and only included for the study if gram negative bacteria isolated.

Our study shows that the overall CR-GNB was 9.0% (3.6%–14.6%). This is comparable to a study reported in India (14.6%).<sup>32</sup> In contrast to our study, a higher rate of studies was reported from India (65%),<sup>33</sup> (30%),<sup>34</sup> Ethiopia (27.1%),<sup>35</sup> and (25%),<sup>36</sup> and lower prevalence studies reported from Ghana (2.9%),<sup>37</sup> Addis Ababa, Ethiopia (2%),<sup>25</sup> Germany (0.22%).<sup>38</sup> This difference might be due to the study period, area, and laboratory method employed. Carbapenem is the last chance of



**Figure 3** Frequency of bacteria isolated for the study of carbapenem resistance of gram-negative bacteria from clinical specimens at tertiary care hospital, southern Ethiopia February 13–June 7, 2020.

**Table 2** Antibiotics Resistance Patterns of Isolates for the Study of Carbapenem Resistance of Gram-Negative Bacteria from Clinical Specimens at Tertiary Care Hospital, Southern Ethiopia, February 13–June 7, 2020

Isolates Tested	Antibiotics Tested											
	Cot (67)	CAZ (48)	MER (111)	GEN (108)	CAF (53)	AMP (96)	CIP (61)	CTX (106)	CXR (57)	AMK (106)	NIT (50)	PIT (84)
	R	R	R	R	R	R	R	R	R	R	R	R
<i>K. pneumoniae</i>	14 (100)	11 (91.7)	3 (9.7)	26 (86.7)	11 (64.7)	28 (100)	10 (66.7)	29 (96.7)	21 (100)	4 (12.9)	11 (100)	11 (52.4)
<i>E. coli</i>	23 (88.5)	16 (84.2)	3 (8.8)	18 (52.9)	0 (0.0)	25 (100)	22 (84.6)	26 (83.9)	16 (84.2)	2 (6.2)	11 (47.8)	15 (50)
<i>P. aeruginosa</i>	4 (100)	1 (100)	3 (37.5)	2 (28.6)	4 (100)	7 (100)	2 (100)	7 (87.5)	2 (100)	2 (25.0)	4 (100)	2 (33.3)
<i>Acinetobacter</i> spp.	4 (66.7)	1 (33.3)	3 (27.3)	7 (63.6)	6 (85.7)	10 (90.9)	2 (50.0)	9 (81.8)	2 (50)	1 (9.1)	3 (100)	5 (62.5)
<i>K. ozaenae</i>	3 (100)	3 (100)	0 (0.0)	5 (83.3)	3 (75.0)	6 (100)	3 (100)	6 (100)	3 (100)	0 (0.0)	1 (50)	2 (40)
<i>K. oxytoca</i>	5 (71.4)	4 (100)	1 (11.1)	7 (77.8)	0 (0.0)	7 (100)	5 (100)	9 (100)	4 (100)	0 (0.0)	2 (66.7)	3 (42.9)
<i>K. rhinoscolaris</i>	2 (100)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	2 (100)	0 (0.0)	1 (100)	NT	0 (0.0)	1 (100)	1 (100)
<i>C. diversus</i>	1 (100)	1 (100)	0 (0.0)	1 (50.0)	0 (0.0)	2 (100)	1 (100)	2 (100)	2 (100)	0 (0.0)	1 (100)	NT
<i>M. morgani</i>	3 (100)	2 (100)	0 (0.0)	4 (80.0)	0 (0.0)	5 (100)	2 (100)	5 (100)	2 (100)	0 (0.0)	1 (50)	2 (66.7)
<i>P. mirabilis</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50)	0 (0.0)	0 (0.0)	NT	0 (0.0)	NT	0 (0.0)
<i>E. agglomerus</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	NT	0 (0.0)	NT	0 (0.0)
Total	58 (88.1)	40 (83.3)	13 (11.7)	71 (65.7)	25 (47.2)	94 (97.9)	47 (77.1)	94 (88.7)	52 (91.2)	9 (8.5)	35 (70.0)	41 (48.8)

**Abbreviations:** R, resistance; NT, not tested; COT, cotrimoxazole; CAZ, ceftazidime; MER, meropenem; GEN, gentamycin; CAF, chloramphenicol; AMP, ampicillin; CIP, ciprofloxacin; CTX, cefotaxime; CXR, cefuroxime; AMK, amikacin; NIT, nitrofurantoin; PIT, piperacillin-tazobactam.

treatment used in serious infections; however, due to the increment of carbapenem resistance bacteria, it is challenging for treating MDR, XDR, and PDR bacteria.<sup>25</sup> In our study, all the isolates that are carbapenem resistant showed (100%) MDR, XDR, and (10%) PDR in which the other one isolate is indeterminate for carbapenemases.

The risk factors for the gaining of antibiotic-resistant bacterium demarcated as the antibiotic consumption and acquaintance, the use of the external device, healthcare exposure.<sup>39–41</sup> Our study displays that CR-GNB is high among <5 years;

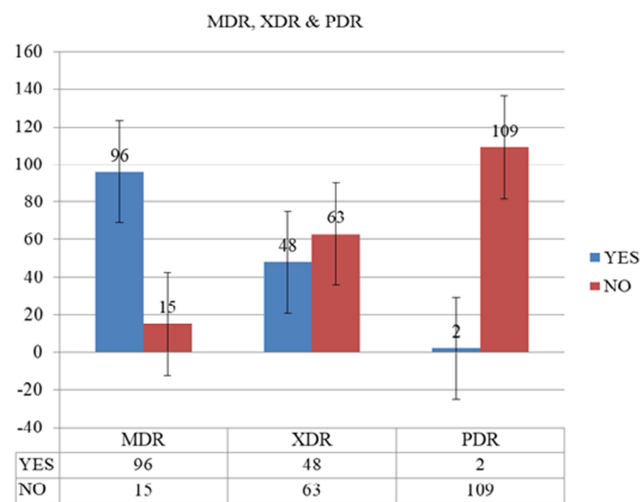
however, it is not statistically significant (COR: 0.500, 95% CI: 0.096–2.609, p = 0.411). Similarly, a study from Nigeria reported as there is a difference in the proportion of age groups but not statistically associated.<sup>42</sup> In study conducted in Northwest Ethiopia showed that being female (OR 4.46; P = 0.018), age (OR 1.08; P = 0.001), hospitalization (OR 5.23; P = 0.006), and prior antibiotic use (OR 3.98; P = 0.04) were associated risk factors for MDRE.<sup>42</sup> Even though our study agrees as the rates of carbapenem resistance are high among age <5 years, with female sex and for those who had antibiotic

**Table 3** Multidrug Resistance Isolates from the Study of Carbapenem Resistance of Gram-Negative Bacteria from Clinical Specimens at Tertiary Care Hospital, Southern Ethiopia from February 13 to June 7, 2020

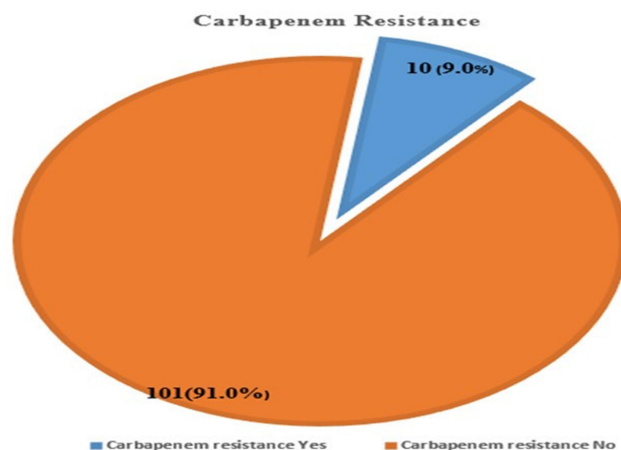
Bacteria	Class of Antibiotics									MDR ≥ R3
	R1(6)	R2	R3	R4	R5	R6	R7	R8	R9	
<i>K. pneumoniae</i> (31)	0 (0.0)	1 (3.2)	7 (22.6)	9 (29.0)	1 (3.2)	8 (25.8)	4 (12.9)	1 (3.2)	0 (0.0)	30 (96.8)
<i>E. coli</i> (34)	1 (2.9)	5 (14.7)	5 (14.7)	9 (26.5)	3 (8.8)	5 (14.7)	5 (14.7)	1 (2.9)	0 (0.0)	28 (82.4)
<i>P. aeruginosa</i> (8)	0 (0.0)	0 (0.0)	3 (37.5)	2 (25.0)	0 (0.0)	2 (25.0)	0 (0.0)	1 (12.5)	0 (0.0)	8 (100)
<i>Acinetobacter</i> spp. (11)	2 (18.2)	0 (0.0)	2 (18.2)	1 (9.1)	1 (9.1)	4 (36.4)	0 (0.0)	0 (0.0)	1 (9.1)	9 (81.8)
<i>K. ozaenae</i> (6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (50.0)	1 (16.7)	1 (16.7)	1 (16.7)	0 (0.0)	0 (0.0)	6 (100)
<i>K. oxytoca</i> (9)	0 (0.0)	0 (0.0)	3 (33.3)	1 (11.1)	3 (33.3)	1 (11.1)	0 (0.0)	1 (11.1)	0 (0.0)	9 (100)
<i>K. rhinoscolaris</i> (2)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50)
<i>C. diversus</i> (2)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50)
<i>M. morgani</i> (5)	0 (0.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (80)
<i>P. mirabilis</i> (2)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>E. agglomerus</i> (1)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	6 (5.4)	9 (8.1)	21 (18.9)	26 (23.4)	10 (9.0)	24 (21.6)	10 (9.0)	4 (3.6)	1 (0.9)	96 (86.5)

**Notes:** R1–9 – resistance to 1-classes, 2-classes, 3- classes ... 9-classes.  
**Abbreviation:** MDR, multidrug resistances.





**Figure 4** Rates of MDR, XDR and PDR bacteria from clinical specimens at tertiary care hospital, southern Ethiopia from February 13 to June 7, 2020.



**Figure 5** Magnitude of carbapenem resistance of gram-negative bacteria from clinical specimens at tertiary care hospital, southern Ethiopia from February 13 to June 7, 2020.

usage before culture in general, but it is not statistically associated as  $p > 0.05$ . This difference might be explained with female patients are more susceptible for UTI as a natural phenomenon more than half of CR-GNB was isolated from UTI patients, ie, 5 out of 8 patients. The high rates of CR-GNB for those who had previous exposure of antibiotics may be explained that our hospital already started prescribing the carbapenem class of antibiotics and the bacteria may acquire these resistance genes. In studies elsewhere, patients with comorbidities, ICU stay, immunosuppression, the two extremities of age, and exposure to multiple antibiotics before the initial culture considered as risk factors for acquiring CR.<sup>17,18</sup>

In this finding, bacteria, which are CR, were *Pseudomonas* spp., *Klebsiella* spp., *E. coli*, and *Acinetobacter* spp. This is in agreement with studies

testified in a systematic review from East Africa,<sup>43</sup> India.<sup>34,44</sup> This study identified as CR, three of four gram-negative organisms considered as ESKAPE, which are recognized as the most important emerging threats of this century that is *Pseudomonas*, *Klebsiella*, *Acinetobacter*, and *Enterobacter*.<sup>45,46</sup>

In this finding the overall MDR was 86.5% (79.1--91.0%), this value is comparable to a study reported from Ethiopia, in Bahir Dar (80.0%),<sup>47</sup> Addis Ababa (81.5%),<sup>48</sup> and Jimma (85%).<sup>49</sup> In contrast to our study, a lower rate of MDR was reported from Debre Markos (72.2%),<sup>50</sup> Bahir Dar (65.2%),<sup>51</sup> Addis Ababa (68.3%),<sup>52</sup> Northwest Ethiopia (54.3%),<sup>53</sup> India (37.1%),<sup>54</sup> (30%).<sup>34</sup> However, a higher rate of MDR was reported from Gonder (92%),<sup>55</sup> Addis Ababa (94.5%).<sup>56</sup>

Our study determined antibiotic susceptibility patterns of each isolate, based on this ampicillin are the most resisted antibiotics followed by cefuroxime, cefotaxime, cotrimoxazole, ceftazidime, ciprofloxacin, nitrofurantoin, and gentamycin, in descending order with greater than 50% of the resistance. In line with our study similarly a high resistance level of ampicillin was reported from Addis Ababa,<sup>35,56</sup> Bahir Dar,<sup>53</sup> Hawassa.<sup>57</sup> In contrast to our study, a lower rate of resistance to ciprofloxacin and gentamycin was described,<sup>51,53,56</sup> this designates that the resistance rates of commonly prescribed antibiotics getting a higher chance of resistance. In this study, lower rates of antibiotic resistance were observed for piperacillin-tazobactam, chloramphenicol, meropenem, and amikacin for all isolates in descending order, which is in line with reports from various parts of Ethiopia.<sup>53,58</sup> This can be justified that the least prescribed antibiotics are still good but needs attention when introducing these drugs in the market as we are running out of effective antibiotics to cure the most vulnerable patients.

## Conclusion

Our study showed that carbapenem-resistant gram-negative bacilli are 9% in the study area. The level of MDR is still alarmingly higher. Our finding signposts that ampicillin, cefuroxime, cefotaxime, cotrimoxazole, ceftazidime, ciprofloxacin, nitrofurantoin, and gentamycin with a high level of resistance >50%. However, piperacillin-tazobactam, chloramphenicol, meropenem, and amikacin were at low rates of resistance. In this study, there were no variables statically associated with carbapenem resistance. Therefore, a measure should be taken to decrease the increment of carbapenem resistance. The clinician

should follow strictly the guideline for treating patients as we are running out of effective antibiotics. A study with a large sample of a gram-negative organism should be conducted for a better understanding of carbapenem resistance in the region. Finally, we recommend if empirical treatment is mandatory piperacillin-tazobactam, chloramphenicol, meropenem, and amikacin are more effective.

## Limitation of the Study

- The drawback of this study is it only based on the phenotypic characteristic of carbapenem resistance that means not genotypic confirmed due to the budgetary issue and unavailability of setup.
- With this result, we could not generalize the carbapenem resistance in the region, as most of the patients come to this hospital after taking numerous treatments.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics Approval and Consent to Participate

Hawassa University College of Medicine and Health Sciences institutional review board (IRB) has approved the proposal (Ref. No. IRB/268/12). At that moment support letter was gained from the hospital management. Socio-demographic, clinical and laboratory data were collected after written informed consent and agreement gained from each child's parents and patients. All information kept secret using codes and locking on the board. The result of the patient-reported to the clinician within three or four days and those who are culturally positive treated accordingly. This study was conducted following the declaration of Helsinki.

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## Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be

published, and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest for this work.

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