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Japhet Anania Peter

japhetpeter190@gmail.com

Manyara Regional Referral Hospital

Yesige Mutajwaa

Manyara Regional Referral Hospital

Fedrick Malakatete

Manyara Regional Referral Hospital

Aisha Elias

Manyara Regional Referral Hospital

Erick Monzo

Manyara Regional Referral Hospital

Anania Tippe

Haydom Lutheran Referral Hospital

Vickfarajaeli Daudi

Haydom Lutheran Referral Hospital

Ramadhan Rashid Rasul

Haydom Lutheran Referral Hospital

Peter Ponsian Kunambi

Muhimbili University of Health and Allied Science

Mecky Isaac Matee

Muhimbili University of Health and Allied Science

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High Burden of Multidrug Resistance and β -Lactamase-Associated Resistance Phenotypes among Gram-Negative Bacilli in Regional Healthcare Facilities in Northern Tanzania

Authors;

Japhet Anania Peter^{1*}, Yesige Mutajwaa¹, Fedrick Malakatete¹, Aisha Elias¹, Erick Monzo¹, Loveness Paulo¹, Charity Gasper¹, Anania Tippe², Vicky Daudi², Ramadhan Rashidi², Peter Kunambi³, Mecky Isaac Matee³

Affiliations

¹Department of Microbiology and Immunology, Manyara Regional Referral Hospital, Manyara, Tanzania

²Department of Microbiology and Immunology, Haydom Lutheran Referral Hospital, Manyara, Tanzania

³Department of Microbiology and Immunology, Muhimbili University of Health and Allied Science, College of Medicine, Dar es Salaam, Tanzania

Author emails

JAP japhetpeter190@gmail.com- **ORCID iD is 0009-0009-3501-4641**

YM yesige2006@yahoo.com **ORCID iD is 0009-0004-1217-198X**

FM fmalakatete@gmail.com **ORCID iD is 0009-0000-9414-0941**

AE aishaelias40@gmail.com **ORCID iD is 0009-0004-4739-4737**

EBM ercbrian@gmail.com **ORCID iD is 0009-0003-4563-391X**

LPM paullovenes5@gmail.com **ORCID iD is 0009-0005-2904-0326**

CGN charitygasper16@gmail.com **ORCID iD is 0009-0002-7742-0786**

AT danielanania@yahoo.com **ORCID iD is 0009-0003-9962-519X**

VZD farajaali@yahoo.com **ORCID iD is 0009-0007-4298-7973**

RRR ramarasul199@gmail.com ORCID iD is 0009-0003-1907-9580

PPK ponsianpeter@gmail.com ORCID iD is 0009-0002-0702-5625

MIM mateemecky@yahoo.com ORCID iD is 0000-0002-6900-1533.

*Correspondence author: JAP japhetpeter190@gmail.com

Abstract

Background:

Antimicrobial resistance (AMR) among Gram-negative bacteria poses a growing threat to effective treatment of bacterial infections, yet data from regional healthcare facilities in Tanzania remain limited. Most AMR studies in Tanzania are performed in tertiary hospitals.

Objective: To determine antimicrobial resistance patterns and the prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), ESBL, and AmpC resistance phenotypes among clinical Gram-negative bacterial isolates from two healthcare facilities in Manyara Region, Tanzania.

Methods: A laboratory-based cross-sectional study was conducted from October 2025 to June 2026 at Manyara Regional Referral Hospital and Haydom Lutheran Hospital. A total of 370 non-duplicate Gram-negative bacterial isolates were included. Bacterial identification was performed using conventional biochemical methods and API identification systems. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. ESBL- and AmpC-associated resistance were assessed phenotypically. MDR and XDR were classified according to internationally accepted criteria.

Results: *Escherichia coli* (37.3%) and *Klebsiella pneumoniae* (23.8%) were the most common isolates. Resistance was particularly high against ampicillin (82.7%), cefoxitin (72.7%), and trimethoprim–sulfamethoxazole (72.2%). Lower levels of resistance were against imipenem (10.5%) and meropenem (6.2%). Overall, 292 (79.0%) isolates were MDR and 178 (48.1%) were XDR. MDR was highest among *Acinetobacter baumannii* (93.8%) and *K. pneumoniae* (89.8%), while XDR was highest among *A. baumannii* (75.0%) and *K. pneumoniae* (72.7%). ESBL-associated resistance was observed in 64/315 (20.3%) Enterobacterales isolates, while phenotypic AmpC-associated resistance occurred in 148/370 (40.0%) isolates and inducible AmpC-associated resistance in 34/370 (9.2%). Among Enterobacterales, ESBL-associated resistance and

constitutive AmpC-associated resistance were significantly associated with MDR ($p = 0.027$ and $p = 0.046$, respectively), whereas inducible AmpC-associated resistance was not ($p = 0.727$).

Conclusion: Regional healthcare facilities in Manyara showed a substantial burden of MDR, XDR, and β -lactamase-associated resistance phenotypes. Strengthening laboratory-based AMR surveillance, facility-specific antibiograms, antimicrobial stewardship, and infection prevention and control is warranted.

Keywords: Antimicrobial resistance; Gram-negative bacteria; multidrug resistance; extensively drug-resistant; ESBL; AmpC; Manyara; Tanzania.

Background

Antimicrobial resistance (AMR) among Gram-negative bacteria, including multidrug-resistant (MDR) and β -lactamase-producing organisms, is a major global health threat (Macesic et al. 2025; WHO 2024, 2025). *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are important causes of bloodstream, urinary tract, respiratory, and healthcare-associated infections and are increasingly associated with limited treatment options, prolonged hospitalization, increased healthcare costs, and excess mortality (Miller and Arias 2024; Macesic et al. 2025; Murray et al. 2022; WHO 2024, 2025).

The emergence and spread of Gram-negative AMR are driven by interacting factors, including antimicrobial selection pressure and inappropriate antimicrobial use, inadequate infection prevention and control (IPC), limited diagnostic capacity, and weaknesses in surveillance systems (Nguyen et al. 2024). β -lactamase-mediated resistance is particularly concerning because it compromises widely used β -lactam antibiotics and can substantially reduce therapeutic options (Koenig and Kuti 2024). Extended-spectrum β -lactamases (ESBLs) hydrolyse penicillins and many cephalosporins, whereas AmpC β -lactamases hydrolyse penicillins, most cephalosporins, and cephamycins and are generally not inhibited by β -lactamase inhibitors such as clavulanate (Bush and Bradford 2020; Tamma et al. 2024). Surveillance of these resistance phenotypes is therefore important for empirical treatment, antimicrobial stewardship (AMS), IPC, and AMR surveillance.

The burden of AMR is particularly substantial in low- and middle-income countries, where capacity to prevent, diagnose, monitor, and respond to resistant infections remains constrained (Lewnard et al. 2024). Across sub-Saharan Africa, high levels of resistance to commonly used antimicrobial agents have been reported among Gram-negative pathogens, with resistance to third-generation cephalosporins representing a major concern (Kimera & Matee 2026; Guido et al. 2026). Although molecular and genomic approaches can identify specific resistance determinants and, when integrated with epidemiological data, provide insights into the emergence and transmission of resistant organisms, their routine implementation remains limited in many resource-constrained settings (Kajumbula et al. 2024; Derar et al. 2026). Consequently, routine phenotypic antimicrobial susceptibility testing remains essential for informing clinical management, AMS, IPC, and AMR surveillance, particularly where molecular diagnostic capacity is limited (Essack et al. 2017; Africa CDC 2018).

In Tanzania, studies from tertiary referral hospitals have documented a substantial burden of AMR among Gram-negative pathogens, including MDR and β -lactamase-associated resistance, particularly ESBL-associated resistance (Seni et al. 2021; Mshana et al. 2022; Peter et al. 2025). However, evidence from regional healthcare facilities, particularly in northern Tanzania and Manyara Region, remains limited. Resistance patterns observed in tertiary referral hospitals may not be directly generalizable to regional healthcare facilities because of differences in patient populations, antimicrobial-use patterns, referral pathways, laboratory capacity, and other healthcare-system factors (Knettel et al. 2020). Characterizing resistance at the regional level is therefore important for developing locally relevant antibiograms, informing empirical antimicrobial therapy, strengthening AMS, and contributing data to regional and national AMR surveillance systems (Tamma et al. 2024; WHO 2025).

Manyara Region is one such under-characterized setting. Manyara Regional Referral Hospital and Haydom Lutheran Hospital provide important hospital-based healthcare services to populations in northern Tanzania, yet published data on AMR and phenotypic β -lactamase-associated resistance among clinical Gram-negative isolates from these facilities remain limited. This evidence gap may constrain development of facility-specific antibiograms, optimization of empirical antimicrobial treatment, and

contribution of regional laboratory data to national AMR surveillance. Therefore, this study aimed to **characterize antimicrobial resistance patterns and determine the prevalence of MDR, XDR, ESBL-associated resistance, AmpC-associated resistance, and carbapenem non-susceptibility among Gram-negative bacterial isolates recovered from clinical specimens at two hospitals in Manyara Region, northern Tanzania.**

Methods

Study design and setting

This laboratory-based cross-sectional study was conducted at Manyara Regional Referral Hospital and Haydom Lutheran Hospital in Manyara Region, northern Tanzania, from October 2025 to June 2026. Both facilities provide inpatient and outpatient healthcare services and routinely perform bacterial culture, organism identification, and antimicrobial susceptibility testing (AST). The study included non-duplicate Gram-negative bacterial isolates recovered from routine clinical specimens submitted for microbiological investigation during the study period.

Study population, sampling, and eligibility criteria

The study population comprised Gram-negative bacterial isolates recovered from clinical specimens from patients of all ages and both sexes. Consecutive sampling was used, whereby all eligible Gram-negative isolates recovered during the study period were considered for inclusion. The unit of analysis was the non-duplicate bacterial isolate. Only isolates with complete organism identification and antimicrobial susceptibility results required for analysis were included. Duplicate isolates of the same bacterial species recovered from the same patient during the same infection episode were excluded to avoid over-representation of individual patients. Gram-positive bacterial isolates, fungal isolates, and isolates lacking essential microbiological information were also excluded.

Sample size

The minimum sample size was calculated using the Kish–Leslie formula for estimating a single population proportion (Kish 1965):

$$n = Z^2 \times p \times (1 - p) / d^2$$

where **n** is the required sample size, **Z** is the standard normal deviate corresponding to a 95% confidence level (1.96), **p** is the estimated prevalence of antimicrobial resistance (60%) based on previously reported findings among Gram-negative bacterial isolates in Tanzania (Peter et al. 2025), and **d** is the desired margin of error (5%).

Thus:

$$n = (1.96)^2 \times 0.60 \times (1 - 0.60) / (0.05)^2$$

$$n = 3.8416 \times 0.60 \times 0.40 / 0.0025$$

$$n = 368.8 \approx 369$$

The minimum calculated sample size was therefore 369 isolates, which was rounded up to **370 isolates** for the study.

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Data collection and bacterial identification

Demographic and microbiological data, including age, sex, healthcare facility, patient care setting, specimen type, bacterial species, and AST results, were extracted from laboratory records using a structured data-collection tool.

Clinical specimens were processed according to standard laboratory procedures. Gram-negative bacterial isolates were identified using colony morphology, Gram staining, and appropriate biochemical tests. Where required, species-level identification was confirmed using API identification systems (bioMérieux, France) according to the manufacturer's instructions.

Antimicrobial susceptibility testing

AST was performed using the Kirby–Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) *Performance Standards for Antimicrobial Susceptibility Testing*, M100, 35th edition (2025) (CLSI 2025). Bacterial suspensions equivalent to a 0.5 McFarland standard were prepared and inoculated onto Mueller–Hinton agar. Plates were incubated at $35 \pm 2^\circ\text{C}$ for 16–18 hours, after which inhibition-zone diameters were measured and interpreted using applicable CLSI 2025 breakpoints.

The antimicrobial agents tested included ampicillin (10 µg), amoxicillin–clavulanate 30mcg (20/10mcg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg),

trimethoprim-sulfamethoxazole (25 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin–tazobactam (100/10 µg), and tetracycline (30 µg). The agents tested varied according to bacterial species and applicable as per CLSI 2025 recommendations.

Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality-control strains in accordance with CLSI recommendations (CLSI 2025).

Phenotypic detection of ESBL- and AmpC-associated resistance

Phenotypic ESBL production was assessed among Enterobacterales using the combined-disk method with cefotaxime and ceftazidime tested alone and in combination with clavulanic acid. An increase of ≥ 5 mm in the inhibition-zone diameter for either antimicrobial agent when combined with clavulanic acid, compared with the corresponding agent alone, was interpreted as a positive ESBL phenotype (CLSI 2025).

AmpC-associated resistance was initially screened using cefoxitin disk diffusion. Isolates with a cefoxitin inhibition zone <18 mm were considered screen-positive and further evaluated using the laboratory's phenotypic disk-approximation method. Because molecular confirmation of AmpC genes was not performed, the findings were reported as phenotypic AmpC-associated resistance and were not interpreted as definitive evidence of AmpC gene carriage or enzyme production.

Carbapenem non-susceptibility and MDR/XDR classification

Carbapenem non-susceptibility was assessed using imipenem and meropenem susceptibility results interpreted according to CLSI M100, 35th edition (2025). An isolate was considered carbapenem non-susceptible when it showed non-susceptibility to either imipenem or meropenem. Multidrug resistance (MDR) and extensively drug-resistant (XDR) phenotypes were defined according to the international expert criteria proposed by Magiorakos et al. (2012). MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories, while XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. Intrinsic or known inherent resistance was excluded from MDR and XDR classification. Antimicrobial agents were grouped according to their respective antimicrobial categories rather than counted as separate categories when multiple agents from the same category were tested.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 27. Continuous variables are presented as medians with interquartile ranges (IQRs), and categorical variables as frequencies and percentages. Resistance to individual antimicrobials and the proportions of isolates classified as MDR, XDR, ESBL-associated, AmpC-associated, or

carbapenem non-susceptible were calculated overall and by bacterial species, healthcare facility, and specimen type.

Categorical variables were compared using Pearson's chi-square test or Fisher's exact test where appropriate. All tests were two-sided, with $p < 0.05$ considered statistically significant. Analyses used the observations available for each variable.

Ethical considerations

Ethical approval was obtained from the National Institute for Medical Research National Health Research Ethics Committee (NIMR-NHREC), Tanzania (Reference No. NIMR/HQ/R.8a/Vol.IX/5088). Permission was obtained from the participating healthcare facilities. A waiver of informed consent was granted because the study involved routinely collected, de-identified laboratory data and archived bacterial isolates. Data were anonymized and securely stored, with access restricted to the research team.

Results

Demographic and clinical characteristics

A total of 370 non-duplicate Gram-negative bacterial isolates were recovered from the two study facilities. The median age of patients was 40 years (IQR: 21–61), with the largest proportion aged 25–44 years (26.2%), followed by 45–64 years (24.3%) and ≥65 years (20.3%). Females accounted for 54.3% (201/370) of isolates and males for 45.6% (168/370); sex was missing for one isolate. Most isolates were recovered from the outpatient department (37.3%). Pus was the most frequently submitted specimen (38.4%), followed by urine (32.7%) and blood (17.0%) (Table 1).

Table 1. Demographic and clinical characteristics of patients with Gram-negative bacterial isolates (n = 370)

Variable	Frequency, n	Percent, %
Age group (years)		
<5	56	15.1
5–14	21	5.7
15–24	31	8.4
25–44	97	26.2
45–64	90	24.3
≥65	75	20.3

Median age, years (IQR) 40 (21–61)

Sex

Male	168	45.6
Female	201	54.3
Missing	1	0.3

Department/ward

OPD	138	37.3
Medical ward	53	14.3
NICU	42	11.4
Surgical ward	39	10.5
Female ward	35	9.5
Pediatrics	25	6.8
Male ward	29	7.8
ICU	2	0.5
Bima	5	1.4
OBGY	1	0.3
Postnatal	1	0.3

Specimen type

Pus	142	38.4
Urine	121	32.7
Blood	63	17.0
HVS	22	5.9
Sputum	15	4.1
Stool	4	1.1

CSF	2	0.5
Pleural fluid	1	0.3

Abbreviations: CSF, cerebrospinal fluid; HVS, high vaginal swab; ICU, intensive care unit; IQR, interquartile range; NICU, neonatal intensive care unit; OBGY, obstetrics and gynecology; OPD, outpatient department.

Distribution of bacterial species

Escherichia coli was the most predominant isolate 138/370 (37.3%) followed by *Klebsiella pneumoniae* (88/370, 23.8%) and *Pseudomonas aeruginosa* (35/370, 9.5%). Together, *E. coli* and *K. pneumoniae* accounted for 226/370 (61.1%) of all isolates (Figure 1).

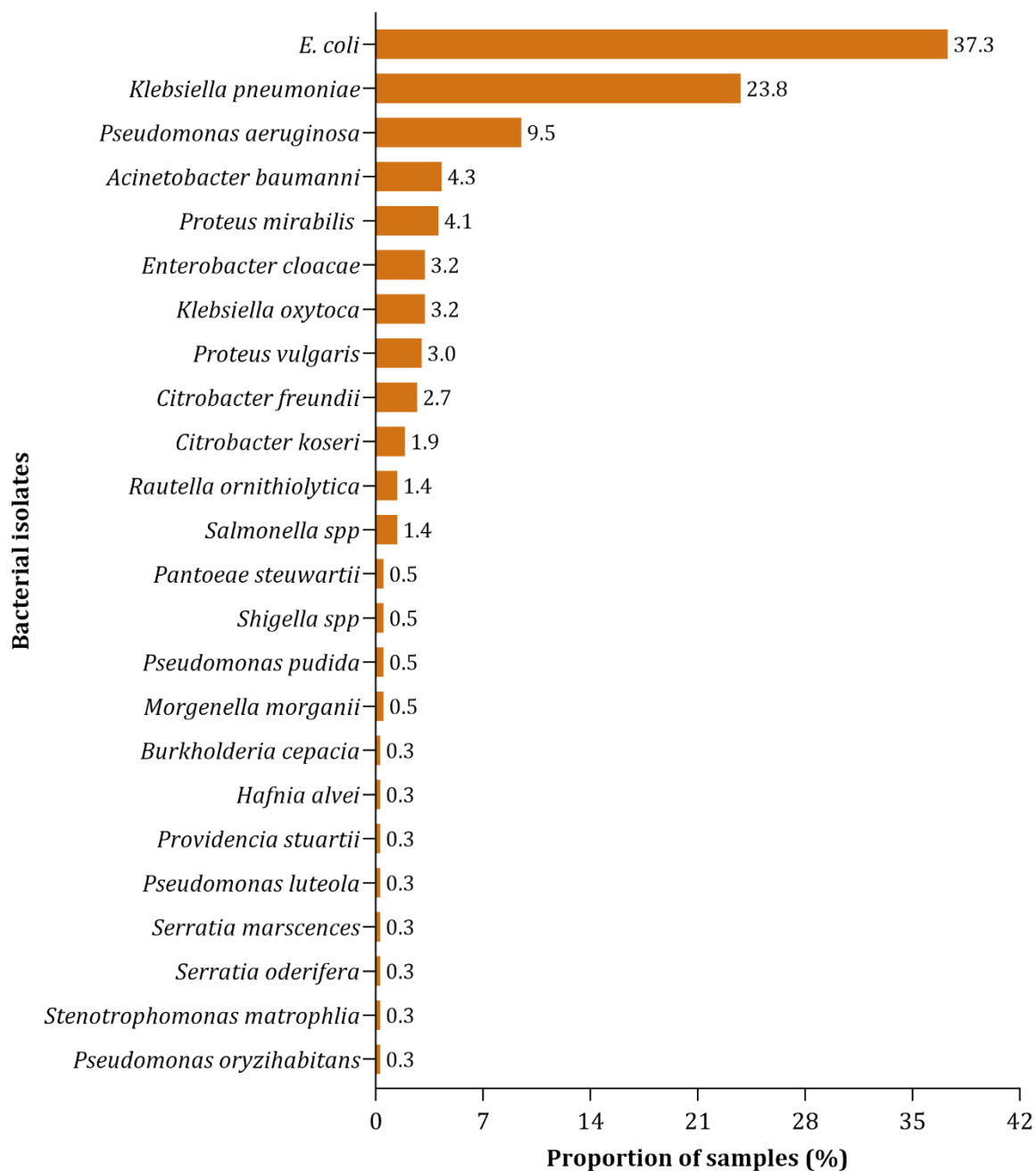


Figure 1. Distribution of Gram-negative bacterial isolates recovered from clinical specimens

Antimicrobial resistance patterns

Resistance varied across the antimicrobial agents tested (Figure 2). The highest overall resistance was observed to ampicillin (82.7%), followed by cefoxitin (72.7%), trimethoprim–sulfamethoxazole (72.2%), and tetracycline (61.0%). Resistance to meropenem and imipenem was 6.2% and 10.5%, respectively.

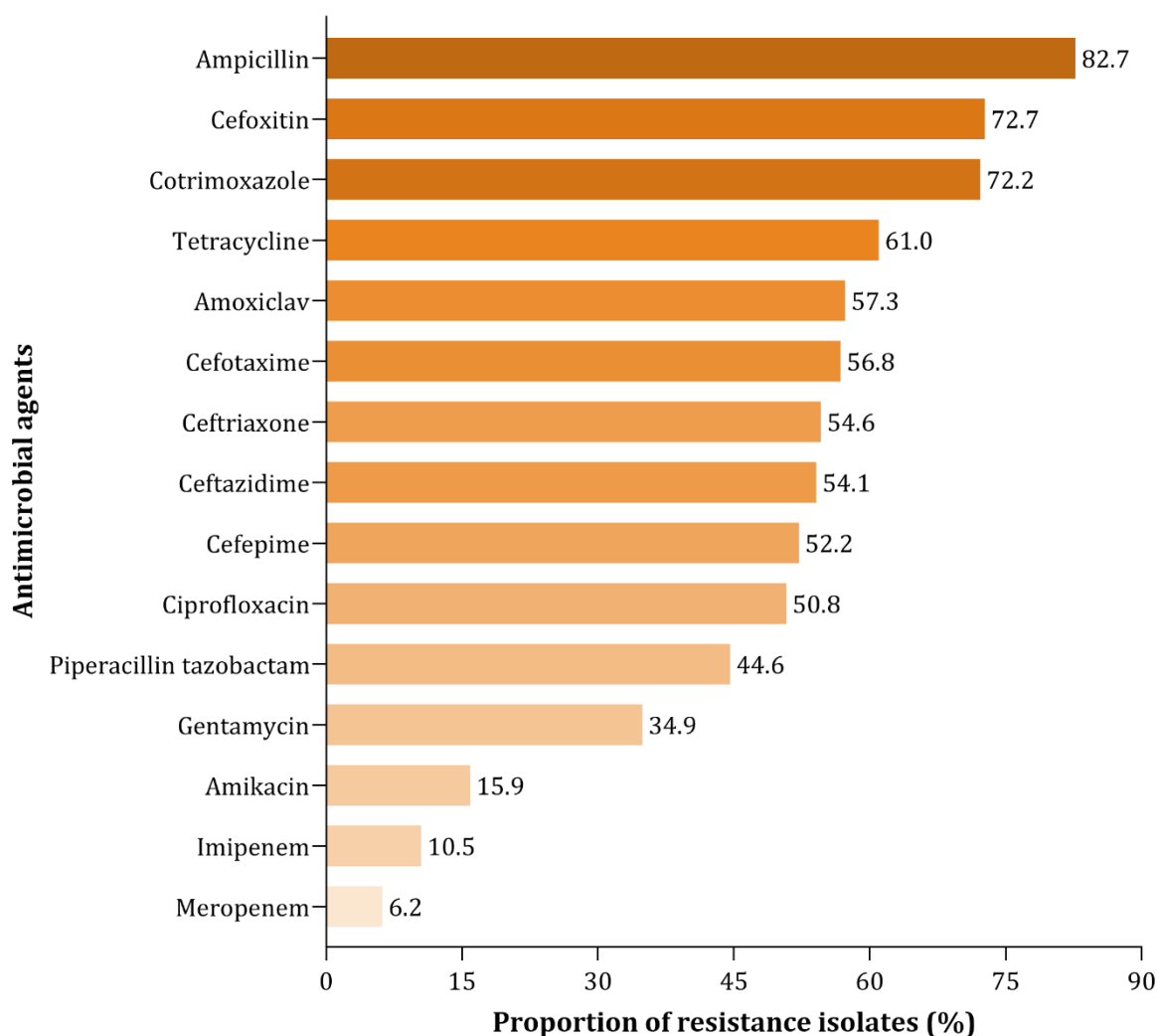


Figure 2. Proportion of Gram-negative bacterial isolates resistant to selected antimicrobial agents

Resistance patterns differed by bacterial species (Table 2). Among the predominant species, *E. coli* showed high resistance to ampicillin (84.1%), cefoxitin (67.4%), and trimethoprim–sulfamethoxazole (69.9%), while corresponding resistance among *K. pneumoniae* was 80.7%, 83.0%, and 71.3%, respectively. *A. baumannii* showed high resistance to ampicillin (93.8%), cefoxitin (87.5%), and ceftazidime (81.3%). Carbapenem resistance was 7.2% to imipenem and 2.9% to meropenem among *E. coli*, compared with 10.2% and 2.3%, respectively, among *K. pneumoniae*.

Table 2. Antimicrobial resistance profiles of clinical Gram-negative bacterial isolates by species

Isolate	N	FOX	AMC	CAZ	CTX	CTR	FEP	MEM	IP	GEN	AK	CIP	TZP	AMP	COT	T
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<i>E. coli</i>	138	67.4	45.7	51.4	55.8	52.9	52.9	2.9	7.2	29.0	13.0	52.9	47.8	90.6	84.1	69.9
<i>K. pneumoniae</i>	88	83.0	60.2	71.6	69.3	68.2	70.5	2.3	10.2	50.0	22.7	72.7	56.8	89.8	80.7	71.3
<i>P. aeruginosa</i>	35	0.0	0.0	45.7	62.9	57.1	31.4	8.6	11.4	17.1	17.1	28.6	31.4	0.0	0.0	0.0
<i>P. mirabilis</i>	15	46.7	53.3	20.0	26.7	20.0	26.7	13.3	20.0	40.0	20.0	26.7	26.7	66.7	46.7	40.0
<i>A. baumannii</i>	16	87.5	62.5	81.3	68.8	75.0	75.0	50.0	43.8	56.3	31.3	43.8	68.8	93.8	75.0	68.8
<i>E. cloacae</i>	12	91.7	75.0	66.7	66.7	66.7	58.3	0.0	16.7	58.3	8.3	58.3	25.0	100.0	66.7	41.7
<i>K. oxytoca</i>	12	75.0	58.3	50.0	58.3	58.3	50.0	8.3	8.3	41.7	25.0	58.3	50.0	100.0	66.7	75.0
<i>C. freundii</i>	10	60.0	50.0	30.0	20.0	20.0	20.0	0.0	0.0	30.0	0.0	10.0	20.0	70.0	60.0	50.0
<i>P. vulgaris</i>	11	45.5	54.5	27.3	27.3	27.3	27.3	0.0	9.1	9.1	9.1	27.3	27.3	90.9	54.5	54.5
<i>C. koseri</i>	7	71.4	14.3	42.9	42.9	42.9	42.9	14.3	0.0	28.6	0.0	28.6	0.0	85.7	71.4	85.7
Other species†	26	69.2	65.4	42.3	46.2	42.3	38.5	7.7	7.7	23.1	7.7	38.5	26.9	73.1	73.1	53.8
Total	370	72.7	57.3	54.1	56.8	54.6	52.2	6.2	10.5	34.9	15.9	50.8	44.6	82.7	72.2	61.0

Values are percentages of isolates resistant to each antimicrobial agent.

Multidrug-resistant and extensively drug-resistant phenotypes

Overall, 292/370 (79.0%) isolates were classified as MDR and 178/370 (48.1%) as XDR (Table 3). MDR prevalence was highest among *A. baumannii* (93.8%), *K. pneumoniae* (89.8%), and *K. oxytoca* (83.3%). XDR prevalence was highest among *A. baumannii* (75.0%) and *K. pneumoniae* (72.7%). MDR and XDR were observed in 80.4% and 47.8% of *E. coli* isolates, respectively, compared with 54.3% and 11.4% among *P. aeruginosa*.

Table 3. Distribution of multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes among clinical Gram-negative bacterial isolates (n = 370)

Bacterial species	N	MDR, n (%)	XDR, n (%)
<i>Escherichia coli</i>	138	111 (80.4)	66 (47.8)
<i>Klebsiella pneumoniae</i>	88	79 (89.8)	64 (72.7)
<i>Pseudomonas aeruginosa</i>	35	19 (54.3)	4 (11.4)
<i>Acinetobacter baumannii</i>	16	15 (93.8)	12 (75.0)

<i>Proteus mirabilis</i>	15	10 (66.7)	4 (26.7)
<i>Enterobacter cloacae</i>	12	9 (75.0)	7 (58.3)
<i>Klebsiella oxytoca</i>	12	10 (83.3)	7 (58.3)
<i>Citrobacter freundii</i>	10	8 (80.0)	0 (0.0)
<i>Proteus vulgaris</i>	11	6 (54.5)	3 (27.3)
Other Gram-negative bacilli*	33	25 (75.7)	11 (33.3)
Total	370	292 (79.0)	178 (48.1)

*Other Gram-negative bacilli comprised species represented by fewer than 10 isolates in Table 3.

ESBL and AmpC β -lactamase phenotypes

Among the 315 Enterobacterales isolates, 64 (20.3%) were ESBL-positive, while constitutive AmpC production was detected in 125 (39.7%). Inducible AmpC was detected in 30 (9.5%) Enterobacterales isolates. ESBL and constitutive AmpC production were highest among *Enterobacter cloacae* (50.0% and 75.0%, respectively), followed by *K. pneumoniae* (30.7% and 42.0%, respectively). Among the 55 non-fermentative Gram-negative rods, constitutive AmpC production was detected in 23 (41.8%) isolates and was highest among *A. baumannii* (50.0%). Inducible AmpC was detected in 4 (7.3%) non-fermentative isolates (Table 4).

Table 4. Distribution of ESBL, AmpC β -lactamase, and inducible AmpC phenotypes among Gram-negative bacterial isolates

Group	n	ESBL n (%)	AmpC n (%)	Inducible AmpC n (%)
Enterobacterales	315	64 (20.3)	125 (39.7)	30 (9.5)
<i>E. coli</i>	138	19 (13.8)	54 (39.1)	20 (14.5)
<i>K. pneumoniae</i>	88	27 (30.7)	37 (42.0)	3 (3.4)
<i>P. mirabilis</i>	15	1 (6.7)	4 (26.7)	1 (6.7)
<i>E. cloacae</i>	12	6 (50.0)	9 (75.0)	1 (8.3)
...	...	...	...	...

Group	n	ESBL n (%)	AmpC n (%)	Inducible AmpC n (%)
Non-fermentative GNR	55	—	23 (41.8)	4 (7.3)
<i>P. aeruginosa</i>	35	—	15 (42.9)	1 (2.9)
<i>A. baumannii</i>	16	—	8 (50.0)	2 (12.5)
...	...	...	...	...

Multidrug resistance by study site

MDR was detected in 164/200 (82.0%) isolates from Haydom Lutheran Hospital and 128/170 (75.3%) from Manyara Regional Referral Hospital (Table 5). Among *K. pneumoniae*, MDR was detected in 43/47 (91.5%) isolates from Haydom and 36/41 (87.8%) from Manyara. Among *A. baumannii*, MDR was detected in 8/9 (88.9%) isolates from Haydom and 7/7 (100.0%) from Manyara. For *P. aeruginosa*, MDR was detected in 8/8 (100.0%) isolates from Haydom and 11/27 (40.7%) from Manyara.

Table 5. Comparison of multidrug resistance among Gram-negative bacterial isolates by study site

Bacterial species	Haydom Hospital, n/N (%)	Manyara Hospital, n/N (%)
<i>E. coli</i>	63/78 (80.8)	48/60 (80.0)
<i>K. pneumoniae</i>	43/47 (91.5)	36/41 (87.8)
<i>P. aeruginosa</i>	8/8 (100.0)	11/27 (40.7)
<i>P. mirabilis</i>	7/11 (63.6)	3/4 (75.0)
<i>A. baumannii</i>	8/9 (88.9)	7/7 (100.0)
<i>E. cloacae</i>	6/8 (75.0)	3/4 (75.0)
<i>K. oxytoca</i>	5/6 (83.3)	5/6 (83.3)
<i>C. freundii</i>	6/8 (75.0)	2/2 (100.0)
<i>P. vulgaris</i>	4/6 (66.7)	2/5 (40.0)
<i>C. koseri</i>	3/4 (75.0)	2/3 (66.7)
Other Gram-negative bacilli	11/15 (73.3)	9/10 (90.0)

Total	164/200 (82.0)	128/170 (75.3)
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Association between β -lactamase phenotypes and MDR

Among Enterobacterales, MDR was more frequent among ESBL-positive isolates than ESBL-negative isolates (90.6% vs. 78.5%; $p = 0.027$). Similarly, MDR was more frequent among isolates with constitutive AmpC production than among those without constitutive AmpC (86.4% vs. 77.4%; $p = 0.046$). MDR prevalence did not differ significantly between isolates with and without inducible AmpC production (83.3% vs. 80.7%; $p = 0.727$) (Table 6).

Table 6. Association between ESBL, constitutive AmpC β -lactamase, inducible AmpC, and multidrug resistance among Enterobacterales (n = 315)

Variable	MDR, n (%)		Non-MDR, n (%)	p-value
ESBL				
Positive	58 (90.6)	6 (9.4)		0.027
Negative	197 (78.5)	54 (21.5)		
Constitutive AmpC β -lactamase				
Positive	108 (86.4)	17 (13.6)		0.046
Negative	147 (77.4)	43 (22.6)		
Inducible AmpC				
Positive	25 (83.3)	5 (16.7)		0.727
Negative	230 (80.7)	55 (19.3)		

Note: Percentages are calculated within each β -lactamase phenotype category.

Discussion

Principal Findings

This study demonstrates a substantial burden of antimicrobial resistance (AMR) among clinical Gram-negative bacterial isolates from two regional healthcare facilities in Manyara Region, Tanzania. *Escherichia coli* and *Klebsiella pneumoniae* predominated, with high resistance to several commonly used antimicrobials, substantial MDR and XDR phenotypes, and a considerable burden of phenotypic AmpC and ESBL

production. These findings extend the growing Tanzanian AMR evidence base to Manyara Region, which remains relatively underrepresented in published surveillance.

Distribution and Resistance Patterns

The predominance of *E. coli* and *K. pneumoniae* is consistent with recent Tanzanian studies (Msemo et al. 2019; Kibwana et al. 2026). The high resistance observed to commonly used antimicrobials, including third-generation cephalosporins, is also consistent with recent national evidence (Kafaiya et al. 2026) and raises concerns about the reliability of conventional empirical treatment in these settings. The substantial β -lactam resistance is further consistent with Tanzanian reports of ESBL- and AmpC-associated resistance among clinical Gram-negative pathogens (Mshana et al. 2025).

Although less frequently isolated, *Acinetobacter baumannii* had the highest MDR and XDR proportions and extensive resistance across antimicrobial classes, consistent with recent Tanzanian reports (Mshana et al. 2025). This finding highlights the importance of including non-fermenting Gram-negative pathogens in routine AMR surveillance and infection prevention and control activities.

MDR, XDR and β -Lactamase Phenotypes

The substantial MDR and XDR burden, particularly among *K. pneumoniae* and *A. baumannii*, is consistent with previous Tanzanian findings (Kamori et al. 2024; Augustine et al. 2023). Phenotypic AmpC production exceeded ESBL production overall and was particularly common among *E. cloacae* and *K. pneumoniae*. Among Enterobacterales, ESBL and constitutive AmpC phenotypes were significantly associated with MDR, whereas inducible AmpC was not. These associations indicate a relationship between selected β -lactamase phenotypes and broader resistance but do not establish causality or specific genetic mechanisms. Molecular characterization would be required to define the underlying resistance determinants.

Recent Tanzanian genomic studies have identified multiple acquired resistance determinants among highly resistant *E. coli* and *K. pneumoniae*, highlighting the genetic complexity of AMR in these organisms (Bahati & Maghembe 2025; Silago et al. 2026). Molecular surveillance could therefore complement phenotypic monitoring in Manyara by clarifying resistance mechanisms and detecting emerging determinants.

Carbapenem Resistance and AMR Control

Carbapenem resistance was comparatively low, particularly among *E. coli* and *K. pneumoniae*, consistent with recent Tanzanian evidence (Kafaiya et al. 2026). Nevertheless, its occurrence among highly resistant pathogens warrants continued surveillance. Recent Tanzanian genomic surveillance has identified clinically important carbapenemase determinants, including *bla*NDM-5 and *bla*OXA-181, among carbapenemase-producing Enterobacterales (Mapunda et al. 2025). Although the

present study did not characterize resistance genes, these findings reinforce the importance of early detection of emerging carbapenem resistance.

The relatively low carbapenem resistance observed should not encourage broader empirical carbapenem use but rather supports preserving these agents through susceptibility-guided prescribing and antimicrobial stewardship. The high MDR/XDR burden supports regular facility-specific antibiograms, timely culture and susceptibility testing, and integration of AMR surveillance with antimicrobial stewardship and infection prevention and control. These approaches are consistent with WHO recommendations emphasizing microbiological surveillance and laboratory-informed antimicrobial use (World Health Organization, 2025a). Incorporating regional facilities into national AMR surveillance would further improve geographic representation and provide locally relevant evidence for treatment and stewardship policies.

Strengths and Limitations

This study included 370 non-duplicate clinical Gram-negative isolates and provides AMR data from regional facilities in Manyara, an underrepresented setting in the Tanzanian literature. Characterization of pathogen distribution, antimicrobial susceptibility, MDR/XDR phenotypes, and β -lactamase-associated resistance provides a useful baseline for regional surveillance.

However, the cross-sectional design and inclusion of only two facilities limit assessment of temporal trends and generalizability. Small numbers for some species reduce the precision of species-specific estimates. Phenotypic β -lactamase testing did not establish specific resistance genes or transmission pathways, while the absence of antimicrobial-use, patient-level, IPC, and clinical-outcome data limited assessment of the drivers and consequences of resistance. Future studies should incorporate longitudinal surveillance, antimicrobial-use and clinical data, and molecular characterization where feasible.

Conclusion

The substantial AMR burden identified among clinical Gram-negative bacteria in regional healthcare facilities in Manyara highlights the need to strengthen facility-level surveillance, laboratory-informed antimicrobial stewardship, timely susceptibility testing, and infection prevention and control. Continued surveillance, including molecular characterization where feasible, will be important for detecting emerging resistance, particularly carbapenem resistance, and guiding locally responsive AMR interventions.

Abbreviations

Abbreviation Full term

AK	Amikacin
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Abbreviation Full term

AMR	Antimicrobial resistance
AMC	Amoxicillin–clavulanic acid
AmpC	AmpC β -lactamase
AMP	Ampicillin
AMS	Antimicrobial stewardship
API	Analytical Profile Index
ATCC	American Type Culture Collection
CAZ	Ceftazidime
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
COT	Trimethoprim–sulfamethoxazole
CSF	Cerebrospinal fluid
CTR	Ceftriaxone
CTX	Cefotaxime
ESBL	Extended-spectrum β -lactamase
FEP	Cefepime
FOX	Cefoxitin
GEN	Gentamicin
HVS	High vaginal swab
ICU	Intensive care unit
IP	Imipenem
IPC	Infection prevention and control
IQR	Interquartile range

Abbreviation Full term

MEM	Meropenem
MDR	Multidrug-resistant
MHA	Mueller–Hinton agar
NICU	Neonatal intensive care unit
OBGY	Obstetrics and gynecology
OPD	Outpatient department
TZP	Piperacillin–tazobactam
T	Tetracycline
WHO	World Health Organization
XDR	Extensively drug-resistant

Declarations**Ethical consideration**

Ethical approval for the study was obtained from the National Institute for Medical Research (NIMR) National Health Research Ethics Committee (Reference No. NIMR/HQ/R.8a/Vol.IX/5088). Administrative permission to access routinely collected laboratory data and archived, de-identified bacterial isolates was obtained from the participating healthcare facilities.

Consent to Participate

A waiver of informed consent was granted because the study involved secondary analysis of routinely collected laboratory data and archived, de-identified bacterial isolates without direct patient contact. All data were anonymized and handled confidentially throughout the study.

Consent for publication

Permission for publication was granted by NIMR **Ref No. BD.242/437/01D/193**

Availability of data and material

All data generated during this study were included in this manuscript

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

JAP, YM, and FM conceived and designed the study. JAP, RRR, EBM, and LPM conducted data collection and laboratory investigations. JAP and PKP performed the statistical analyses and interpreted the findings. JAP and MIM drafted the manuscript. MIM, YM, FM, VZD, ADT, RR, and AE critically reviewed and revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript.

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AI disclosure: Generative artificial intelligence tools were not used to generate, analyze, or interpret the study data. AI-assisted tools were used solely for language editing and improving manuscript clarity. All scientific content, interpretations, and final decisions were reviewed and approved by the authors.

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