IJID Regions 7 (2023) 77–83

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Epidemiology and prevention of hospital-acquired carbapenem-resistant Enterobacterales infection in hospitalized patients, Northeast Ethiopia

Agumas Shibabaw[a,*], Zenawork Sahle[b], Yeshi Metaferia[a], Asgdew Atlaw[b], Behailu Adenew[c], Alemu Gedefie[a], Mihret Tilahun[a], Endris Ebrahim[a], Yeshimebet Kassa[a], Habtu Debash[a], Shu-Hua Wang[d]

[a] Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wollo University, Dessie, Ethiopia
[b] Department of Medical Laboratory Sciences, Debre Berhan Health Science College, Debre Berhan, Ethiopia
[c] Department of Medical Laboratory Sciences, Debre Berhan Comprehensive Specialized Hospital, Debre Berhan, Ethiopia
[d] Department of Internal Medicine, Division of Infectious Diseases, College of Medicine, and Global One Health initiative, The Ohio State University, Columbus, OH, USA

ARTICLE INFO

Keywords:
Carbapenemase-producing Enterobacterales
Carbapenem-resistant Enterobacterales
Hospitalized patients
Hospital-acquired infection

ABSTRACT

Objective: Carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) are usually healthcare associated. The aim of this study was to investigate the epidemiology of hospital-acquired CRE and multi-drug-resistant infections, and identify associated risk factors in hospitalized patients in Northeast Ethiopia.

Methods: This cross-sectional study was conducted in patients admitted with sepsis between January and June 2021. Demographic and clinical data were collected using questionnaires. In total, 384 samples were collected and cultured based on source of infection. Bacterial species identification was performed using biochemical tests, and drug susceptibility testing was done using the Kirby–Bauer disk diffusion method. The modified carbapenem inactivation method was employed for carbapenemase detection. Data were analysed using Statistical Package for the Social Sciences.

Results: The overall rate of CP-CRE infection was 14.6%. Bloodstream infections and urinary tract infections were the predominant hospital-acquired infections (HAIs). The majority of CP-CRE were *Escherichia coli* and *Klebsiella pneumoniae*, and accounted for 4.9%. Chronic underlying disease (adjusted odds ratio (AOR): 7.9, 95% confidence interval (CI): 1.9–31.5), number of beds per room (AOR: 11, 95% CI: 1.7–75) and eating raw vegetables (AOR: 11, 95% CI: 3.4–40) were significantly associated with hospital-acquired CRE infection.

Conclusions: The rate of CP-CRE infection found in this study is concerning. There is a need for further evaluation of risk factors and measures to decrease HAI. Hand hygiene, increased laboratory capacity, improved infection prevention measures, and antimicrobial stewardship programmes are needed in healthcare settings to halt the transmission of CP-CRE.

Introduction

Enterobacterales are an order of Gram-negative, facultative anaerobes and non-spore-forming bacilli that inhabit the gastrointestinal tract of humans and animals [1–3]. Carbapenem-resistant Enterobacterales (CRE) are an important cause of multi-drug-resistant infections [4]. The prevalence of CRE is increasing globally and becoming a significant threat to public health [1,2]. Carbapenemase-producing CRE harbour an enzyme that inactivates most or all beta-lactam antibiotics, frequently encode on mobile genetic elements such as plasmids, and can be transmitted between organisms and spread rapidly in healthcare settings [5].

Due to extended drug resistance, therapeutic options are limited. The minimum inhibitory concentration (MIC) values of extended-spectrum beta-lactams such as cefepime and carbapenems vary depending on the isolate, and these antibiotics may be effective depending on the MIC. In addition, newer beta-lactam/beta-lactamase inhibitor and beta-lactam non-beta-lactam combination antimicrobials can also be active against CP-CRE [6].

The major risk factors of CRE acquisition include healthcare exposure and receiving broad-spectrum antibiotics, especially carbapenems [7]. The major risk factors for CRE colonization include underlying comorbid conditions, prior antibiotic exposure, prior hospitalization or

* Corresponding author at: Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wollo University, P. O. Box: 1145, Dessie, Ethiopia.
E-mail address: agumas2000@gmail.com (A. Shibabaw).

https://doi.org/10.1016/j.ijregi.2023.02.008
Received 16 January 2023; Received in revised form 23 February 2023; Accepted 24 February 2023

residence in a long-term care facility, invasive devices, and extended stay in an intensive care unit (ICU) [8].

Patients with CRE colonization or infection can serve as reservoirs. CRE can be transmitted between healthcare personnel, other patients, family members and the environment [9].

Infection prevention and control (IPC) measures are recommended to prevent the transmission of CRE by colonized and infected patients. Standard hand hygiene practices combined with contact precautions, such as isolation and use of personal protective equipment (PPE) by healthcare personnel, and environmental cleaning can decrease transmission in healthcare settings [10]. The burden of CP-CRE in Ethiopia is limited due to the lack of surveillance and limited availability of culture and drug susceptibility testing [11].

In 2017, the prevalence of CRE infection at Tikur Anbessa Specialize Hospital (TASH) in Addis Ababa was reported to be 12% [12]. A second study at TASH in 2018–2019 in adults and children reported that the prevalence of CP-CRE in urine specimens was 6.7% [13]. The management of patients with CRE infection and the implementation of IPC measures requires a large amount of resources. This study aimed to investigate the epidemiology of hospital-acquired CRE infection and its associated risk factors in Northeast Ethiopia in order to evaluate the need for strengthening laboratory capacity and IPC.

Methods

Study design and setting

A cross-sectional study was conducted from January to June 2021 at Debre Berhan Comprehensive Specialized Hospital (DBCSH), Amhara region, Northeast Ethiopia. Debre Berhan is located 130 km from Addis Ababa. DBCSH was established in 1937, has over 180 beds, and provides services such as paediatrics, emergency, surgical, medical, gynaecology/obstetrics, ICUs, psychiatry, ophthalmology, antiretroviral treatment, microbiological laboratories and other health care. It provides healthcare services for more than 2.8 million people in the catchment area. Although the hospital has an IPC and antibiotic stewardship programme (ASP), they are not yet fully functional.

All hospitalized patients with suspected bacterial infections who had been admitted for ≥48 h were included in the study. Physicians used clinical parameters such as signs and symptoms, site of primary complaint and haematological parameters to identify patients with possible bacterial infection. Patients who were monitored for <48 h, critically ill patients, and patients who were unable to provide a specimen were excluded from this study. Convenience sampling was used to recruit the study participants.

Demographic and clinical data collection

Sociodemographic and clinical data were collected from each study participant using a structured questionnaire. Checklists were employed to assess the IPC measures taken in the hospital. Written informed consent was obtained from each adult study participant and each child's guardians/parents. For participants who could not read and write, the required information was read to them and the consent form was signed before data collection.

Specimen collection, transportation and processing

Different types of clinical specimens were collected depending on the site of infection, such as blood, cerebrospinal fluid (CSF), urine, stool, rectal swab, sputum, wound swab and ear discharges. Blood samples were collected aseptically (70% alcohol):10 mL from adults, 5 mL from children and 2 mL from neonates [14].

Freshly voided midstream urine samples (approximately 10 mL) were collected using sterile, wide-mouthed, leakproof containers. Each participant was given proper instructions regarding how to collect a 3-g fresh stool sample in a sterile, clean cup container. Rectal swabs were collected from small children who could not provide a stool sample by an experienced nurse. A 2-mL sputum sample was collected from each study participant with bacterial respiratory infection symptoms using a clean, wide-necked, leakproof container. A 5–10-mL CSF sample was collected aseptically by a physician using a sterile tube with lumbar or ventricular puncture. Wound swab and ear discharges were collected using a sterile cotton tip applicator stick aseptically [14]. All specimens were transported to DBCSH Microbiology Laboratory immediately after collection for bacterial analysis.

Bacterial culture, isolation and identification

Blood samples were inoculated into tryptic soya broth (TSB), incubated immediately at 35–37°C, and inspected daily for signs of bacterial growth. Bacterial growth is evidenced by increased turbidity or colour changes. If no growth was detected after 7 days, the blood culture was reported as negative. If growth was detected, the isolate was subcultured on blood and MacConkey agar [12].

For urine specimens, a 10-μL well-mixed urine sample was inoculated using a calibrated wire loop on to cysteine lactose electrolyte-deficient agar, and subcultured on to MacConkey agar. Specimens with significant bacteriuria [$\geq 10^5$ colony-forming units (CFU)/mL] were further processed; specimens with $<10^5$ CFU/mL were considered insignificant or due to contamination.

Stool samples and rectal swabs were inoculated on to MacConkey agar. All clinical samples were incubated aerobically at 37°C for 18–24 h [15]. Colonies were sub-cultured on selective media such as eosin methylene blue (EMB) and xylose-lysine deoxycholate agar to detect significant bacteria. Pure colonies were collected for bacterial identification. All positive cultures were characterized to species level using colony morphology, Gram staining, and standard biochemical tests including indole, urease, triple sugar iron, citrate, motility, lysine decarboxylase and other tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with the guidelines of the Clinical Laboratory Standards Institute [16]. Bacterial inocula were prepared based on 0.5 McFarland standard. A bacterial suspension was spread over the entire surface of Mueller–Hinton agar, and antimicrobial disks were applied to the plate. Drug susceptibility testing of all Enterobacterales was performed using the disk diffusion method against ampicillin 10 μg, cefoxitin 30 μg, gentamicin 10 μg, ciprofloxacin 5 μg, trimethoprim-sulfamethoxazole 1.25/23.75 μg, imipenem 10 μg, meropenem 10 μg, amoxicillin-clavulanic acid 20/10 μg, cefotaxime 30 μg, ceftazidime 30 μg, ceftriaxone 30 μg, tetracycline 30 μg, cefepime 30 μg and chloramphenicol 30 μg. The inhibition zone was measured after incubation at 37°C for 16–18 h, and interpreted as susceptible, intermediate or resistant [16].

Screening for carbapenemase production

Enterobacterales were suspected to be carbapenemase producers if the isolate was resistant to imipenem or meropenem, or if the inhibition zone was ≤19 mm for imipenem or meropenem [16].

Phenotypic confirmation of carbapenemase production

The modified carbapenem inactivation method (mCIM) was used to detect CP-CRE. Isolates were emulsified into TSB, and a meropenem disk (10 μg) was added and incubated for 4 h. A McFarland standard equivalent suspension of a carbapenem-sensitive indicator organism (E. coli ATCC 25922) was inoculated on to Mueller–Hinton agar, and the meropenem disk was placed in TSB. After incubation for 24 h at 37°C,

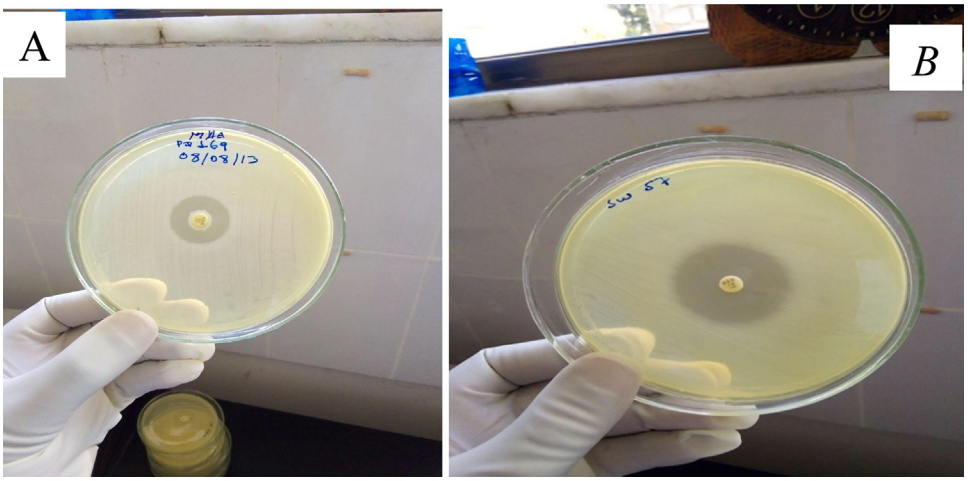

Figure 1. (A) Carbapenemase-positive (inhibition zone 8 mm) and (B) carbapenemase-negative (inhibition zone 20 mm) Enterobacterales from clinical samples using the modified carbapenem inactivation method at Debre Berhan Comprehensive Specialized Hospital during the study period.

the inhibition zone for meropenem was measured. Isolates were considered to be CP-CRE if the inhibition zone was between 6 and 15 mm, or if colonies were present within 16–18 mm [16].

Laboratory quality control

All quality control checks were made before, during and after data collection. Standard operating procedures were followed strictly for specimen collection, transportation, processing and laboratory analyses. Culture media was prepared according to the manufacturer's instructions, and sterility was checked by incubating 5% of the prepared media at 35–37°C overnight with observation for bacteria growth. Visual inspections of cracks in the media or plastic Petri dishes, unequal fills, haemolysis, evidence of freezing, bubbles and contamination were performed. Reference strains *Klebsiella pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706, were used as positive and negative controls for carbapenemase, respectively.

Data management and analysis

Data were entered using Epi-Data, and exported to Statistical Package for the Social Sciences Version 23 (IBM Corp., Armonk, NY, USA) for analysis. Descriptive statistics were carried out, and findings were displayed using tables and graphs. Binary logistic regression analysis was used to observe associations between dependent and independent variables. Variables with $P \leq 0.2$ on bivariate logistic regression analysis were further selected for multi-variate logistic regression analysis. $P \leq 0.05$ was considered to indicate statistical significance.

Results

Demographic and clinical characteristics of study participants

In total, 384 participants were enrolled in this study. The mean age of the participants was 22 (standard deviation 19) years, with a range of 0–75 years. More than half of the study participants were females (n=231, 60%) and lived in rural areas (n=240, 62.5%). One hundred and six participants (27.6%) reported a history of antibiotic use within the 2 weeks preceding admission. One hundred and sixty-four participants had Enterobacterales infections. Urinary tract infection (n=68, 41.5%) was the most common site of Enterobacterales infection, followed by bloodstream infection (n=44, 26.8%) (Table 1).

Epidemiology of CP-CRE infection

In total, 14.6% (24/164) of Enterobacterales infections were CP-CRE infections. Figure 1 shows carbapenemase producers and non-carbapenemase producers of Enterobacterales using the modified carbapenem inactivation method.

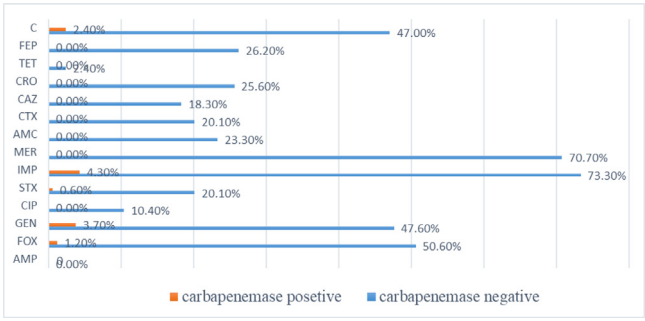

Figure 2. Drug susceptibility levels of carbapenemase-positive and carbapenemase-negative Enterobacterales at Debre Berhan Comprehensive Specialized Hospital from January to June 2021. IMP, imipenem; MER, meropenem; AMC, amoxicillin–clavulanic acid; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; TET, tetracycline; FEP, cefepime; C, chloramphenicol; AMP, ampicillin; FOX, cefoxitin; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; CIP, ciprofloxacin.

Bloodstream infection was the most common site of CP-CRE infection (n=11/24, 45.8%), followed by urinary tract infection (n=6/24, 25%), skin and soft tissue infection (n=5/24, 20.8%) and gastrointestinal infection (n=2/24, 8.3%). Most CP-CRE isolates were detected in blood (11/24) and urine (6/24) samples (Table 2).

The predominant CP-CRE isolates were *E. coli* (n=8, 4.9%) and *K. pneumoniae* (n=8, 4.9%). Other CP-CRE Enterobacterales were *Klebsiella oxytoca* (n=3, 1.8%), *Citrobacter koseri* (also called *C. divresus*) (n=2, 1.2%), *Providencia stuartii* (n=2, 1.2%) and *Klebsiella aerogenes* (previously called *Enterobacter aerogenes*) (n=1, 0.6%). Non-CP Enterobacterales included *Morganella morganii, Klebsiella ozaenae, Enterobacter cloacae, Citrobacter* spp. and *Citrobacter freundii*.

Drug resistance patterns of CP-CRE isolates

All CP-CRE isolates were multi-drug resistant, with at least one agent in three or more chemical classes of antibiotics. Among the CP-CRE isolates (carbapenemase-positive), seven, six and four isolates were susceptible to imipenem (4.3%), gentamicin (3.7%) and chloramphenicol (2.4%), respectively (Figure 2). Among the carbapenemase-negative isolates (non-CP-CRE), isolates showed high susceptibility to imipenem

Table 1
Demographic and clinical characteristics of patients infected with carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) among the 164 Enterobacterales infections detected at Debre Berhan Comprehensive Specialized Hospital during the study period.

Characteristics		Enterobacterales infection (*n*=164)		
		CRE infection		Total (*n*, %)
		Yes (*n*, %)	No (*n*, %)	
Site of CRE infection	Bloodstream infection	11 (6.7)	33 (20.1)	44 (26.8)
	Urinary tract infection	6 (3.6)	62 (37.8)	68 (41.4)
	Skin and soft tissue infection	5 (3.0)	20 (12.2)	25 (15.2)
	Gastrointestinal infection	2 (1.2)	23 (14)	25 (15.2)
	Pneumonia/respiratory tract infection	-	2 (1.2)	2 (1.2)
Type of invasive procedures	Urinary catheterization	5 (3)	5 (3)	10 (6)
	Surgery	1 (0.6)	4 (2.4)	5 (3)
	Others	1 (0.6)	-	1 (0.6)
	No invasive procedures	17 (10.4)	131 (79.9)	148 (90.2)
Patient's admission ward	Medical	3 (1.8)	35 (21.3)	38 (23.1)
	Surgical	6 (3.6)	18 (11)	24 (14.6)
	Pediatrics	9 (5.5)	36 (21.9)	45 (27.4)
	Neonatal ICU	4 (2.4)	23 (14)	27 (16.5)
	Adult ICU	-	5 (3)	5 (3)
	Obstetrics	2 (1.2)	23 (14)	25 (15.2)
Comorbid conditions	Diabetes mellitus	2 (1.2)	6 (3.7)	8 (4.9)
	HIV	2 (1.2)	10 (6.1)	12 (7.3)
	Hypertension	-	1 (0.6)	1 (0.6)
	No comorbidities	20 (12.2)	123 (75)	143 (87.2)
History of antibiotic use within 2 weeks preceding admission	Yes	12 (7.3)	57 (34.8)	69 (42.1)
	No	12 (7.3)	83 (50.6)	95 (57.9)
Sex	Male	14 (8.5)	62 (37.8)	76 (46.3)
	Female	10 (6.1)	78 (47.6)	88 (53.7)

ICU, intensive care unit; HIV, human immunodeficiency virus.

(73.3%, 120/164), meropenem (70.7%, 116/164), cefoxitin (50.6%, 83/164), and gentamicin (47.6%, 78/164) (Figure 2).

Risk factors for acquisition of hospital-acquired CP-CRE infection

In bivariate logistic regression, 22 independent variables were considered for analysis; only those variables with $P \leq 0.2$ underwent multivariate logistic analysis. Chronic underlying disease (AOR: 7.9, 95% CI: 1.9–31.5), number of beds per room (AOR: 11, 95% CI: 1.7–75) and eating raw vegetables (AOR: 11, 95% CI: 3.4–40) were significantly associated with acquisition of CP-CRE infection (Table 3).

Discussion

Identification of CP-CRE in the study hospital is alarming. Factors associated with transmission and methods to reduce transmission need to be explored further.

The finding that bloodstream CRE infection was the dominant HAI is of concern given the increased morbidity and mortality associated with bacteraemia. Urinary tract infection was the next most common site. A previous meta-analysis in Ethiopia also found that bloodstream infection and urinary tract infection were the predominant HAIs [17]. Possible reasons for the increase in HAIs may be due to poor hand hygiene practices, low adherence to IPC practices, resource constraints, low implementation of nursing processes and less attention given to HAIs.

The rate of CP-CRE infection in this study was 14.6% (95% CI: 9.8–20.7), which is similar to other studies in other parts of Ethiopia. For example, studies from Bahir Dar [14] and Addis Ababa [12] reported CPE infection rates of 16.5% and 12.2%, respectively. The rate found in the present study was higher than other Ethiopian cities, such as Addis Ababa (2.7%) [18], Arba Minchi (1.43%) [19] and Gondar (2.73%) [20]. Based on the present findings, the study area had poor IPC practices and a high rate of patient flow in the hospital which may favour the transmission of HAIs in the hospital environment and admitted patients. Moreover, a wide variation in prevalence was seen in low-to-middle-income countries (LMICs) such as Sudan (56%) [21], India (23.0%) [22], Uganda (22.4%) [23], India (12.4%) [24] and Morocco (2.8%) [25]. The discrepancy in these findings could be due to differences in methodology, local antibiotic prescribing habits, misuse or overuse of drugs, personal and environmental hygiene, and poor IPC strategies between healthcare settings.

The predominant CPE isolates were *E. coli* and *K. pneumoniae*. This finding was supported by a study in India [24]. The majority of CP-CRE identified in the hospital were from blood (41.7%) and urine (20.8%) samples. In addition, a significant number of CP-CRE isolates were detected from patients admitted to the paediatrics ward (37.5%, 9/24), surgical ward (25%, 6/24) and neonatal ICU (16.7%, 4/24). Another study supported the present finding by reporting that wards and ICU surfaces are frequently contaminated, and contribute to bacterial cross-transmission and patient colonization/infection [26]. The initial step in dealing with the problem of CP-CRE is identification of colonized and infected patients. Early CP-CRE detection is vital in patient care and management, and infection control in order to reduce the escalation of resistance [27]. Whole-genome sequencing (WGS) can be performed to identify phylogenetically linked strains to determine if an outbreak or ongoing transmission is occurring in the hospital environment. Unfortunately, resource limitations in LMICs may limit the availability of WGS.

The finding that all CP-CRE isolates were multi-drug resistant is of concern. Urgent implementation of IPC measures is important to prevent transmission. Although molecular techniques are regarded as the gold standard for detection of carbapenem resistance, this is cost-prohibitive and difficult to sustain in routine hospital laboratories in resource-limited countries. Thus, rapid and cost-effective phenotypic detection of CP-CRE is needed to initiate IPC, ASPs and other measures to decrease transmission. In Ethiopia, due to lack of equipment and limited supplies to perform culture, pathogen identification and drug susceptibility testing, empiric treatment is likely initiated prior to obtaining cultures. There are limited antimicrobial therapeutic options for treatment of CP-CRE, and this may be due to lack of implementation of ASPs in hospital settings and poor IPC measures such as hand hygiene, minimizing device use, environmental cleaning and isolation through contact precautions.

Table 2
Bacterial agents causing carbapenemase-producing carbapenem-resistant Enterobacterales infection found in clinical specimens at Debre Berhan Comprehensive Specialized Hospital from January to June 2021.

Bacterial isolate	Clinical sample	CPE infection (*n*=164)		Total
		Yes (n, %)	No (n, %)	
E. coli	Blood	3 (1.8)	14 (8.5)	17 (10.4)
	Urine	1 (0.6)	30 (18.3)	31 (18.9)
	Wound	4 (2.4)	10 (6.1)	14 (8.5)
	Sputum	-	2 (1.2)	2 (1.2)
	Stool	-	10 (6.1)	10 (6.1)
	Total	8 (4.9)	66 (40.2)	74 (45.1)
K. pneumoniae	Blood	3 (1.8)	10 (6.1)	13 (7.9)
	Stool	1 (0.6)	2 (1.2)	3 (1.8)
	Urine	4 (2.4)	19 (11.6)	23 (14.0)
	Wound	-	8 (4.9)	8 (4.9)
	Total	8 (4.9)	39 (23.8)	47 (28.7)
K. oxytoca	Blood	2 (1.2)	2 (1.2)	4 (2.4)
	Stool	1 (0.6)	2 (1.2)	3 (1.8)
	Total	3 (1.8)	4 (2.4)	7 (4.3)
C. koseri	Wound	-	1 (0.6)	1 (0.6)
	Stool	-	1 (0.6)	1 (0.6)
	Blood	2 (1.2)	4 (2.4)	6 (3.7)
	Urine	-	2 (1.2)	2 (1.2)
	Total	2 (1.2)	8 (4.9)	10 (6.1)
K. aerogenes	Wound	1 (0.6)	-	1 (0.6)
	Urine	-	3 (1.8)	3 (1.8)
	Stool	-	3 (1.8)	3 (1.8)
	Total	1 (0.6)	6 (3.7)	7 (4.3)
M. morganii	Urine	-	1 (0.6)	1 (0.6)
	Stool	-	1 (0.6)	1 (0.6)
	Total	-	2 (1.2)	2 (1.2)
K. ozaenae	Blood	-	3 (1.8)	3 (1.8)
	Urine	-	1 (0.6)	1 (0.6)
	Total	-	4 (2.4)	4 (2.4)
P. stuartii	Urine	1 (0.6)	2 (1.2)	3 (1.8)
	Blood	1 (0.6)	-	1 (0.6)
	Stool	-	1 (0.6)	1 (0.6)
	Total	2 (1.2)	3 (1.8)	5 (3.0)
E. cloacae	Urine	-	4 (2.4)	4 (2.4)
	Stool	-	2 (1.2)	2 (1.2)
	Total		6 (3.7)	6 (3.7)
Citrobacter spp.	Wound	-	1 (0.6)	1 (0.6)
C. freundii	Stool	-	1 (0.6)	1 (0.6)
Total	24 (14.6)	140 (85.4)	164(100)	

Table 3
Multi-variate analysis of risk factors for acquisition of hospital-acquired carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) infection at Debre Berhan Comprehensive Specialized Hospital from January to June 2021.

Variables	Categories	Hospital-acquired CP-CRE infection		COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
		Positive *n* (%)	Negative *n* (%)				
Handwashing before meals	Yes	23 (14.0)	112 (68.3)	Ref			
	No	1 (0.6)	28 (17.1)	5.7 (0.7–44)	0.094	7.9 (0.7–85)	0.088
Eating raw vegetables	Yes	16 (9.8)	17 (10.4)	14 (5.3–38)	0.07	11 (3.4–40)	0.000[a]
	No	8 (4.8)	123 (75.0)	Ref			
Previous history of invasive procedures	Yes	7 (4.3)	11 (6.7)	4.8 (1.6–14)	0.004	0.8 (0.1–4.5)	0.819
	No	17 (10.3)	129 (78.7)	Ref			
Chronic underlying disease	Yes	12 (7.3)	20 (12.2)	6 (2.3–15.2)	0.000	7.9 (1.9–31.5)	0.003[a]
	No	12 (7.3)	120 (73.2)	Ref			
Previous history of hospitalization	Yes	16 (9.8)	52 (31.7)	3.3 (1.3–8.4)	0.009	2.2 (0.6–8.1)	0.207
	No	8 (4.8)	88 (53.7)	Ref			
Number of beds per room	2–4	8 (4.8)	12 (7.3)	Ref			
	5–8	16 (9.8)	128 (78.0)	5.3 (1.8–15)	0.002	11 (1.7–75)	0.011[a]
Number of patients per room	2–4	13 (7.9)	48 (29.3)	Ref			
	5–8	11 (6.7)	92 (56.1)	2.2 (0.9–5.4)	0.067	0.4 (0.1–2.3)	0.376

COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval; Ref, reference.
[a] Statistically significant ($P<0.05$).

The association found between eating raw vegetables and CRE infection is interesting. A study in Ethiopia indicated that waste water environments (hospital and non-hospital) are a reservoir of antibiotic resistance and a potential source of novel resistance gene transfers to pathogens [28]. Drug-resistant pathogens including CP-CRE may be detected in hospital run-off waste waters. In rural communities, water sources from hospitals may be used for irrigation of fields, and if vegetables are not washed or cooked properly, the individual may be consuming drug-resistant bacteria on the raw vegetables. Another study conducted in Arba Minch, a rural community in Ethiopia, also

showed that eating raw food increased the risk of drug resistance [19].

The number of beds per room can be an indicator of overcrowding and a risk factor for transmission of drug-resistant bacteria. A study conducted at TASH, a large tertiary referral hospital, also showed that the number of beds per room was associated with CP-CRE infection [29]. The presence of chronic underlying disease was significantly associated with acquisition of CP-CRE infection, likely due to increased exposure to healthcare facilities, increased antibiotic prescriptions and increased HAIs. A study undertaken in China also showed that invasive procedures and bed transfers were associated with CRE colonization [30].

Conclusions

The identification of CP-CRE infection is of concern. Chronic underlying disease, number of beds per room and eating raw vegetables were significantly associated with acquisition of CP-CRE infection. There is a need to strengthen laboratory capacity to detect drug-resistant bacteria, improve IPC practices to increase hand hygiene to decrease the spread of drug-resistant bacteria, and implement contact precautions to respond and contain drug-resistant pathogens in order to block the transmission of CRE infection and drug-resistant bacteria in the hospital environment. In addition, ASPs should be implemented to monitor antimicrobial use in health facilities.

Conflict of interest statement

None declared.

Acknowledgements

The authors would like to acknowledge the staff of DBCSH for their efforts in the coordination of data collection, laboratory analysis and resource support. The authors also thank Wollo University, Amhara Regional Health Bureau and Debre Berhan Health Science College for funding and laboratory resources support. Finally, the authors would like to thank the study participants.

Ethical approval statement

Ethical approval was obtained from the College of Medicine and Health Sciences Ethical Review committee of Wollo University (Ref No: 213/2021). Data were collected from each study participant after obtaining informed consent, or assent from children's guardians/parents. The objectives and procedures of the study were explained to each study participant and parents/guardians during data collection. Data confidentiality was maintained using secret codes. All culture-positive cases were linked to the hospital for initiation of treatment and management.

Funding

This research work was supported by Wollo University and Amhara Regional Health Bureau. The funders had no role in data collection, study design, data analysis and interpretation.

Author contributions

AS, ZS and YM conceived and designed the study, performed laboratory investigations, acquired and curated the data, and undertook analysis and interpretation of data. AS and ZS wrote the original draft of the manuscript. AS, ZS, YM, MT, AG, AA, BA and SW reviewed and edited the final manuscript. All authors approved the final manuscript.

Availability of data and materials

All data that support the findings of the study are included.

References

[1] Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012;**18**:413–31.
[2] Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011;**53**:60–7.
[3] Adeolu M, Alnajar S, Naushad S, Gupta RS. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. *Int J Syst Evol Microbiol* 2016;**66**:5575–99.
[4] Bulens SN, Reses HE, Ansari UA, Grass JE, Carmon C, Albrecht V, et al. Carbapenem-resistant Enterobacterales in individuals with and without health care risk factors – emerging infections program, United States, 2012–2015. *Am J Infect Control* 2023;**51**:70–7.
[5] Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis* 2017;**215**:S28–36.
[6] Soontaros S, Leelakanok N. Association between carbapenem-resistant Enterobacteriaceae and death: a systematic review and meta-analysis. *Am J Infect Control* 2019;**47**:1200–12.
[7] Prabaker K, Lin MY, McNally M, Cherabuddi K, Ahmed S, Norris A. Transfer from high-acuity long-term care facilities is associated with carriage of Klebsiella pneumoniae carbapenemase-producing Enterobacteriaceae: a multihospital study. *Infect Control Hosp Epidemiol* 2012;**33**:1193–9.
[8] Yi J, Kim KH. Identification and infection control of carbapenem-resistant Enterobacterales in intensive care units. *Acute Crit Care* 2021;**36**:175–84.
[9] Gottesman T, Agmon O, Shwartz O, Dan M. Household transmission of carbapenemase-producing Klebsiella pneumoniae. *Emerg Infect Dis* 2008;**14**:859–60.
[10] World Health Organization *Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa in health care facilities*. Geneva: WHO; 2017.
[11] Sonda T, Kumburu H, van Zwetselaar M, Alifrangis M, Lund O, Kibiki G, et al. Meta-analysis of proportion estimates of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in East Africa hospitals. *Antimicrob Resist Infect Control* 2016;**5**:18.
[12] Legese MH, Weldearegay GM, Asrat D. Extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae among Ethiopian children. *Infect Drug Resist* 2017;**10**:27.
[13] Seman A, Sebre S, Awoke T, Yeshitela B, Asseffa A, Asrat D, et al. The magnitude of carbapenemase and ESBL producing Enterobacteriaceae isolates from patients with urinary tract infections at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia. *Adv Exp Med Biol* 2022;**1369**:117–28.
[14] Moges F, Eshetie S, Abebe W, Mekonnen F, Dagnew M, Endale A, et al. High prevalence of extended-spectrum beta-lactamase-producing Gram-negative pathogens from patients attending Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara region. *PLoS One* 2019;**14**:e0215177.
[15] Mulisa G, Selassie L, Jarso G, Shiferew T, Zewdu A, Abebe W, et al. Prevalence of extended spectrum beta-lactamase producing Enterobacteriaceae: a cross sectional study at Adama hospital, Adama, Ethiopia. *J Emerg Infect Dis* 2016;**1**:1–6.
[16] . Performance standards for antimicrobial susceptibility testing. Berwyn, PA: CLSI; 2020. p. 68–9.
[17] Alemu A, Endalamaw A, Bayih W. The burden of healthcare-associated infection in Ethiopia: a systematic review and meta-analysis. *Trop Med Health* 2020;**48**:77.
[18] Tekele SG, Teklu DS, Tullu KD, Birru SK, Legese MH. Extended-spectrum beta-lactamase and AmpC beta-lactamases producing Gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia. *PLoS One* 2020;**15**:e0241984.
[19] Aklilu A, Manilal A, Ameya G, Woldemariam M, Siraj M. Gastrointestinal tract colonization rate of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae and associated factors among hospitalized patients in Arba Minch General Hospital, Arba Minch, Ethiopia. *Infect Drug Resist* 2020;**13**:1517.
[20] Eshetie SUC, Gelaw A, Ayelign B, Endris M, Moges F. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. *Antimicrob Resist Infect Control* 2015;**4**:12.
[21] Dahab R, Ibrahim AM, Altayb HN. Phenotypic and genotypic detection of carbapenemase enzymes producing Gram-negative bacilli isolated from patients in Khartoum State. *F1000Res* 2017;**6**:1656.
[22] Singh S, Samant S, Bansal M, Talukdar A, Arif D. Phenotypic detection of carbapenemase producing Gram negative bacteria by modified Hodge test. *Int J Curr Microbiol App Sci* 2016;**5**:315–20.
[23] Andrew B, Kagirita A, Bazira J. Prevalence of extended-spectrum beta-lactamase-producing microorganisms in patients admitted at KRRH, Southwestern Uganda. *Int J Microbiol* 2017;**2017**:3183076.
[24] Nair PK, Vaz MS. Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. *J Microbiol Infect Dis* 2013;**3**:207–10.
[25] El Wartiti MA, Bahmani FZ, Elouennass M, Benouda A. Prevalence of carbapenemase-producing Enterobacteriaceae in a university hospital in Rabat, Morocco: a 19-month prospective study. *Int Arab J Antimicrob Agents* 2012;**2**.
[26] Blot S, Ruppé E, Harbarth S, Asehnoune K, Poulakou G, Luyt CE, et al. Healthcare-associated infections in adult intensive care unit patients: changes in epidemiology, diagnosis, prevention and contributions of new technologies. *Intensive Crit Care Nurs* 2022;**70**:103227.

[27] Magiorakos AP, Burns K, Rodríguez Baño J, Borg M, Daikos G, Dumpis U, et al. Infection prevention and control measures and tools for the prevention of entry of carbapenem-resistant Enterobacteriaceae into healthcare settings: guidance from the European Centre for Disease Prevention and Control. *Antimicrob Resist Infect Control* 2017;**6**:113.

[28] Moges F, Endris M, Belyhun Y, Walelign W. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. *BMC Res Notes* 2014;**7**:215.

[29] Desta K, Woldeamanuel Y, Azazh A, Mohammod H, Desalegn D, Shimelis D, et al. High gastrointestinal colonization rate with extended-spectrum β-lactamase-producing Enterobacteriaceae in hospitalized patients: emergence of carbapenemase-producing K. pneumoniae in Ethiopia. *PLoS One* 2016;**11**:e0161685.

[30] Zhao Z-c, Xu X-h, Liu M-b, Wu J, Lin J, Li B. Fecal carriage of carbapenem-resistant Enterobacteriaceae in a Chinese university hospital. *Am J Infect Control* 2014;**42**:e61–4.