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Prevalence, antimicrobial resistance patterns, and associated factors of extended-spectrum β-lactamase-producing *Enterobacteriaceae* at Livingstone University Teaching Hospital, Zambia

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Abstract

Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) are a major threat to antimicrobial therapy, particularly in low-resource settings with limited surveillance. This study investigated the prevalence of ESBL-PE and associated factors among clinical isolates at Livingstone University Teaching Hospital, Zambia. A total of 250 Enterobacteriaceae isolates recovered from routine clinical specimens were screened for reduced susceptibility to cefotaxime and/or ceftazidime. ESBL production was phenotypically confirmed using the double-disc synergy method, while antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. *Escherichia coli* (51.6%) and *Klebsiella pneumoniae* (24.4%) were the predominant isolates, with most recovered from urine specimens (86.0%). High resistance rates were observed for ampicillin (75.6%) and piperacillin (73.1%), whereas no resistance to imipenem was detected. Overall, 39 isolates were confirmed as ESBL producers, corresponding to a prevalence of 15.6%. ESBL production was significantly associated with department type and age group. In multivariable analysis, out-patient isolates had lower odds of ESBL production than in-patient isolates (aOR = 0.27, $p = 0.001$), while infant age group was independently associated with ESBL production (aOR = 7.72, $p = 0.022$). These findings highlight the need for strengthened antimicrobial stewardship and routine ESBL surveillance.

Introduction

Antimicrobial resistance (AMR) is a major global public health threat that compromises the effective treatment of bacterial infections and increases morbidity, mortality, and healthcare costs worldwide (1–3). Among resistant pathogens, extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) are of particular concern because they hydrolyse broad-spectrum β-lactam antibiotics and are frequently associated with multidrug resistance (MDR)(4, 5). ESBLs, commonly encoded by *blaCTX-M*, *blaSHV*, and *blaTEM* genes, inactivate penicillins, third- and fourth-generation cephalosporins, and monobactams, thereby limiting therapeutic options for severe bacterial infections (6, 7). As a result, carbapenems are often used as alternative treatment agents for infections caused by ESBL-producing organisms (8, 9). However, the emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) further threatens the effectiveness of last-line antibiotics because these organisms frequently harbour additional resistance mechanisms against fluoroquinolones, aminoglycosides, and other antimicrobial classes (9).

ESBL-PE have been increasingly reported in humans, animals, food products, and environmental reservoirs, highlighting their importance within the One Health framework. *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species are among the most frequently identified ESBL-producing pathogens globally (10, 11). In Zambia, Mumbula *et al*. reported ESBL production among invasive *K. pneumoniae* isolates recovered from blood culture specimens at the University Teaching Hospital (12), while Chishimba *et al*. demonstrated the presence of ESBL-producing *E. coli* in poultry with high resistance to β-lactam antibiotics and other antimicrobial agents (13). Similarly, ESBL-producing *E. coli* and *K. pneumoniae* strains with multidrug resistance phenotypes have been identified in environmental

samples and agricultural products from other settings (10, 11). Collectively, these findings demonstrate the widespread dissemination of ESBL-producing bacteria across multiple ecological niches and reinforce the importance of coordinated surveillance and antimicrobial stewardship strategies.

The growing burden of MDR bacterial infections in many healthcare settings has been partly attributed to the dissemination of ESBL-producing organisms, whose resistance genes are often plasmid-mediated and readily transferable between bacterial species (14). Previous studies conducted in Zambia have reported increasing prevalence of MDR bacterial isolates in both hospital clinical specimens and environmental samples (15–17). Despite these findings, routine laboratory detection of ESBL-PE remains limited in many healthcare facilities, particularly in low-resource settings where molecular diagnostic methods are often unavailable. Consequently, ESBL-producing isolates may remain underrecognized, potentially contributing to inappropriate antimicrobial therapy, treatment failure, and continued transmission of resistant organisms.

Despite increasing reports of ESBL-PE globally and regionally, data describing the burden and associated factors of ESBL production among clinical isolates in provincial referral hospitals in Zambia remain limited. Therefore, this study aimed to determine the prevalence of ESBL-PE among clinical isolates at Livingstone University Teaching Hospital in Zambia, and to identify demographic and clinical factors associated with ESBL production using a phenotypic double-disc synergy method.

Material and Methods

Materials

Biochemical identification media included sulphur indole motility (SIM) agar, triple sugar iron (TSI) agar, lysine iron agar, and urea medium from (Becton, Dickinson and company [BD], USA); citrate agar (Mast Group Ltd, UK), oxidase reagent (Himedia, India), API 20 for *Enterobacteriaceae* (bioMerieux® SA, France), and Gram staining reagents. Culture media included Columbia blood agar base (Himedia, India), MacConkey agar (Oxoid, UK) and Mueller-Hinton agar (BD, USA). Antibiotic susceptibility testing was performed using discs containing ampicillin (10 µg), piperacillin (100 µg), ciprofloxacin (5 µg), piperacillin/tazobactam (100/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), imipenem (10 µg), amoxicillin-clavulanate (20/10 µg), and cefotaxime-clavulanate (30/10 µg). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains and were obtained from the National Health Research Institute (NHRI), Ndola, Zambia.

Study design and site

A laboratory-based prospective cross-sectional study was conducted at the Microbiology Laboratory of Livingstone University Teaching Hospital (LUTH), Zambia, using bacterial isolates recovered from routine patient specimens. LUTH is a tertiary referral hospital serving Southern Province and contains several specialty departments, including internal medicine, surgery, paediatrics, and obstetrics and gynaecology.

The microbiology laboratory participates in an external quality assessment (EQA) programme for bacteriology and is accredited by the Southern African Development Community Accreditation Service (SADCAS).

Sample size. A previous study conducted at the University Teaching Hospital in Zambia reported carbapenem resistance rates of 6% in *Pseudomonas aeruginosa* and 18.2% in *Acinetobacter species* (18). In the absence of local prevalence data for ESBL-PE, the minimum sample size was estimated using OpenEpi software based on a prevalence of 18.2%, 95% confidence interval, and 5% margin of error, yielding a minimum sample size of 229 isolates.

Eligibility criteria. All consecutive non-duplicate Gram-negative *Enterobacteriaceae* isolates recovered during the study period were included. Isolates lacking patient demographic information, including age, gender, hospital location, or specimen type, were excluded from the analysis.

Bacterial culture and identification. Bacteriological analysis was performed according to national standard operating procedures and Clinical and Laboratory Standards Institute (CLSI) guidelines (19). Routine clinical specimens from in-patients and out-patients were cultured on MacConkey agar, blood agar, and chocolate agar, followed by aerobic incubation at 37°C for 18–24 h. Isolates were identified using Gram staining, colony morphology, motility testing, and standard biochemical tests including oxidase, triple sugar iron (TSI), indole production, urease production, and citrate utilization tests as previously described (18–20). API 20E strips were additionally used where necessary to confirm species identification.

Antimicrobial susceptibility profile. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar in accordance with CLSI guidelines (19). Bacterial suspensions were standardized to 0.5 McFarland turbidity prior to inoculation. Antibiotic discs tested included ampicillin, piperacillin, ciprofloxacin, piperacillin/tazobactam, cefotaxime, ceftazidime, imipenem, and amoxicillin-clavulanate. Following incubation, inhibition zone diameters were measured and interpreted according to CLSI breakpoints. Isolates demonstrating resistance to cefotaxime and/or ceftazidime were further evaluated phenotypically for ESBL production using the double-disc synergy method as previously described (19, 20).

Quality control. *Escherichia coli* ATCC 25922 was used for quality control of antimicrobial susceptibility testing, while *Pseudomonas aeruginosa* ATCC 27853 was used for quality control of culture media performance.

Determination of ESBL-PE with the ESBL-test.

Phenotypic detection of ESBL production was performed as previously described (19, 20). Briefly, isolates were initially screened for reduced susceptibility to third-generation cephalosporins using cefotaxime (30 µg) and ceftazidime (30 µg) discs. Isolates resistant to cefotaxime and/or ceftazidime

according to CLSI interpretive criteria were considered potential ESBL producers and subjected to confirmatory testing using the double-disc synergy method.

For confirmatory testing, standardized bacterial suspensions equivalent to 0.5 McFarland turbidity were inoculated onto Mueller-Hinton agar plates to produce a confluent lawn culture. Antibiotic discs containing cefotaxime alone and cefotaxime-clavulanate were placed 25–30 mm apart on the agar surface. Following incubation at 37°C for 24 h, inhibition zone diameters were measured. An increase of ≥ 5 mm in the inhibition zone diameter for cefotaxime-clavulanate compared with cefotaxime alone was interpreted as phenotypic confirmation of ESBL production according to CLSI guidelines (19).

Patient's demographics and clinical factors associated with ESBL-producing bacteria. Patient demographic and clinical information, including age, gender, hospital location (ward/clinic), and specimen type, were recorded for all non-duplicate *Enterobacteriaceae* isolates included in the study.

Data analysis.

Data analysis was performed using R version 4.5.2. Descriptive statistics, including frequencies, percentages, and mean, were used to summarize patient, specimen, and bacterial isolate characteristics. The prevalence of ESBL production was calculated as the proportion of phenotypically confirmed ESBL-producing isolates among all *Enterobacteriaceae* isolates analysed. Associations between ESBL production and categorical variables, including age group, department type, and gender, were assessed using Fisher's exact test. Variables associated with ESBL production were further evaluated using multivariable logistic regression analysis. A p-value < 0.05 was considered statistically significant.

Ethical considerations

This laboratory-based prospective study utilized bacterial isolates recovered from routine clinical specimens and did not involve direct patient contact or the use of patient identifiers. Ethical approval was obtained from the Mulungushi University School of Medicine and Health Sciences Research Ethics Committee (Ref: SMHS-MU4-2022-01). Authorization to conduct the study was granted by the National Health Research Authority, Zambia (Ref: NHRA0000020/31/10/2022), while permission to access and process clinical specimens was obtained from Livingstone University Teaching Hospital management.

Results

General patient, specimen, and bacterial isolate characteristics

A total of 250 Gram-negative bacterial isolates meeting the inclusion criteria were recovered from patient specimens. Most isolates were obtained from female patients (73.2%, n = 183), while 26.8% (n = 67) were from male patients. Patient age ranged from 5 days to 93 years. Based on age stratification, 12

(4.8%) patients were classified as infants (< 1 year), 36 (14.4%) as children (1–17 years), and 202 (80.8%) as adults (≥ 18 years). In addition, 98 (39.2%) isolates were obtained from in-patients, whereas 152 (60.8%) were from out-patients (Supplementary Table S1).

Urine specimens accounted for the majority of isolates (86.0%, *n* = 215). Other specimen types included pus (4.8%, *n* = 12), blood (3.2%, *n* = 8), and high vaginal swabs (2.4%, *n* = 6). Sputum, stool, urethral swabs, and body fluid samples each contributed less than 2% of total isolates (Table 1).

Table 1
Distribution of clinical specimens from which Gram-negative bacterial isolates were recovered

Specimen	Count	Percent (%)
Urine	215	86.0
Pus	12	4.8
Blood	8	3.2
HVS	6	2.4
Sputum	4	1.6
Stool	2	0.8
Urethral swab	2	0.8
Body fluid	1	0.4
Total	**250**	**100**

HVS: high vaginal swab

Escherichia coli was the predominant organism, accounting for 51.6% (*n* = 129) of isolates, followed by *K. pneumoniae* at 24.4% (*n* = 61) and *E. agglomerans* at 11.6% (*n* = 29). The remaining bacterial species each accounted for less than 3% of total isolates (Table 2).

Table 2
Distribution of Gram-negative bacterial isolates recovered from clinical specimens.

Organism	Count	Percentage (%)
Escherichia coli	129	51.6
Klebsiella pneumoniae	61	24.4
Enterobacter agglomerans	29	11.6
Enterobacter aerogenes	5	2
Enterobacter cloacae	5	2
Proteus mirabilis	5	2
Klebsiella oxytoca	4	1.6
Citrobacter diversus	3	1.2
Serratia marcescens	3	1.2
Morganella morganii	2	0.8
Salmonella typhi	2	0.8
Citrobacter freundii	1	0.4
Proteus vulgaris	1	0.4
Total	**250**	**100**

Most isolates were obtained from the Accident and Emergency Department (39.6%, *n* = 99), followed by the out-patient department (11.2%, *n* = 28), paediatric ward (10.4%, *n* = 26), gynaecological ward (6.8%, *n* = 17), surgical wards (5.6%, *n* = 14), and male admission ward (5.6%, *n* = 14). The remaining isolates originated from other hospital departments with comparatively lower frequencies (Supplementary Table S1).

Antibiotic susceptibility patterns among bacteria isolates

High resistance rates were observed for amoxicillin-clavulanate (75.9%, *n* = 186), ampicillin (75.5%, *n* = 188), and piperacillin (73.0%, *n* = 181). Moderate resistance was observed for third-generation cephalosporins, including cefotaxime (41.2%, *n* = 103) and ceftazidime (33.2%, *n* = 83), as well as ciprofloxacin (37.4%, *n* = 92). In contrast, resistance to piperacillin/tazobactam was low (2.0%). No resistance to imipenem was observed among the isolates tested (Table 3; Fig. 1A).

Table 3
Antibiotic resistance patterns among Gram-negative bacterial isolates

Ampicillin (10µg)	**Resistant**	**Intermediate**	**Sensitive**	**Total**
	n (%)	**n (%)**	**n (%)**	**n**
	188 (75.5)	**10 (4.0)**	**51 (20.5)**	**249**
Piperacillin (100µg)	181 (73.0)	16 (6.5)	51 (20.5)	248
Ciprofloxacin (5µg)	92 (37.6)	16 (6.5)	137 (55.9)	245
Piperacillin/Tazobactam(100/10µg)	5 (2.0)	6 (2.4)	238 (95.6)	249
Cefotaxime (30µg)	103 (41.4)	12 (4.8)	134 (53.8)	249
Ceftazidime (30µg)	82 (32.9)	17 (6.9)	150 (60.2)	249
Imipenem (10µg)	0	2 (0.8)	247 (99.2)	249
Amoxicillin-clavulanate (20/10µg)	186 (75.9)	24 (9.8)	35 (14.3)	245
Number(n), percent (%)				

Heatmap analysis demonstrated distinct antibiotic resistance patterns across *Enterobacteriaceae* isolates, with consistently high resistance to ampicillin and piperacillin observed across most bacterial species (Fig. 1A). *E. coli* and *K. pneumoniae* exhibited broader MDR profiles compared with less frequently isolated organisms. Age-stratified analysis showed broadly similar resistance patterns among infants, children, and adults, with high resistance to ampicillin and piperacillin observed across all age groups (Fig. 1B). Comparison by patient setting demonstrated generally higher resistance levels among in-patient isolates relative to out-patient isolates, particularly for third-generation cephalosporins and ciprofloxacin (Fig. 1C).

Among individual organisms, *E. cloacae* demonstrated resistance to the greatest number of antibiotics tested, followed by *E. coli* and *K. pneumoniae*. Moderate multidrug resistance patterns were also observed among *E. agglomerans*, *K. aerogenes*, and *K. oxytoca*, whereas *Serratia marcescens* demonstrated resistance to fewer antibiotics (Supplementary Table S2; Fig. 1A).

Evaluation of extended-spectrum beta-lactamase producing Enterobacteriaceae

Of the 250 *Enterobacteriaceae* isolates analysed, 108 (43.2%) screened positive for potential ESBL production based on resistance to cefotaxime and/or ceftazidime. Phenotypic confirmatory testing identified 39 isolates as ESBL producers, corresponding to a confirmation rate of 36.1% among screen-positive isolates and an overall ESBL prevalence of 15.6% (39/250) (Supplementary Table S1). Of these screen-positive isolates, the highest proportion of ESBL positivity was observed in *E. cloacae* (4/5), followed by *K. oxytoca* (2/4), *K. pneumoniae* (11/40), *E. agglomerans* (4/20), and *E. coli* (18/99) (Fig. 2).

Fisher's exact test was used to evaluate associations between phenotypically confirmed ESBL production and categorical variables due to the relatively small number of ESBL-positive isolates (n = 39). Significant associations were observed between ESBL production and gender (p = 0.039), department type (p < 0.001), and age group (p = 0.002). ESBL-producing isolates were more frequently identified among in-patient isolates and infants.

Multivariable logistic regression analysis demonstrated that out-patient isolates had significantly lower odds of ESBL production compared with in-patient isolates (adjusted odds ratio [aOR] = 0.27, 95% CI: 0.12–0.60; p = 0.001). Infant age group was independently associated with ESBL production (aOR = 7.72, 95% CI: 1.52–58.7; p = 0.022), whereas specimen type and the child age group were not significantly associated with ESBL positivity (Table 4).

Table 4
Multivariable logistic regression analysis of factors associated with phenotypically confirmed ESBL production

Variable	**aOR**	**95% CI**	***p*-value**
Department type			
Out-patient vs In-patient	0.27	0.12–0.60	**0.001**
Age group			
Children vs Adult	0.41	0.11–1.24	0.145
Infant vs Adult	7.72	1.52–58.7	**0.022**
Specimen type			
Urine vs others	1.46	0.44–6.17	0.566
aOR: adjusted odds ratio; CI: confidence interval			

Discussion

Extended-spectrum beta-lactamase (ESBL)-producing bacteria are an important public health concern because they limit treatment options and are frequently associated with multidrug resistance (5, 10, 11, 18, 21). Infections caused by ESBL-producing organisms may result in treatment failure, prolonged hospital stay, increased healthcare costs, and increased mortality risk. Furthermore, ESBL-producing bacteria can disseminate resistance genes to other bacterial species, thereby facilitating the spread of antimicrobial resistance within healthcare settings and the community (14). Therefore, effective infection prevention strategies, antimicrobial stewardship, and routine surveillance programs remain essential for the early detection and control of ESBL-producing organisms.

The present study demonstrated that urinary isolates from adult female out-patients constituted the majority of *Enterobacteriaceae* recovered, with *E. coli* and *K. pneumoniae* being the predominant

organisms. These findings are consistent with the well-established contribution of uropathogenic *Enterobacteriaceae*, particularly *E. coli*, to community- and hospital-associated urinary tract infections. Resistance profiling revealed high resistance rates to ampicillin and piperacillin, suggesting that these agents may no longer be reliable for empirical therapy in this study setting. Moderate resistance to third-generation cephalosporins and ciprofloxacin further highlights the growing burden of antimicrobial resistance among clinically important *Enterobacteriaceae* isolates. In contrast, the absence of resistance to imipenem suggests preserved carbapenem activity and supports its continued effectiveness against severe infections caused by MDR isolates.

The overall prevalence of phenotypically confirmed ESBL-PE in this study was 15.6%. The most common ESBL-producing organisms included *E. coli*, *K. pneumoniae*, *E. agglomerans*, *E. cloacae*, and *K. oxytoca*. Ibrahim *et al*. reported an ESBL prevalence of 58% among clinical isolates from tertiary hospitals in Kano, Nigeria, with *K. pneumoniae* being the predominant ESBL producer (20). In Pakistan, Yasmeen *et al*. reported an 8.4% prevalence of ESBL-producing *K. pneumoniae* among human samples (22). Similarly, Yu *et al*. reported increasing ESBL production among *K. pneumoniae* and *E. coli* isolates in Taiwan (23). The findings of the present study therefore support previous reports indicating that ESBL-producing *Enterobacteriaceae* remain an important challenge in hospital settings. However, unlike several previous studies in which *K. pneumoniae* predominated (20, 22, 23), *E. cloacae* isolates were the most common ESBL-producing bacteria in the present study. This difference may reflect variations in study design, sample distribution, healthcare practices, and local epidemiological patterns.

Analysis by Fisher's exact test demonstrated significant associations between ESBL production and gender, department type, and age group. However, these associations were further evaluated using multivariable logistic regression to account for potential confounding factors. In multivariable analysis, in-patient isolates were significantly associated with ESBL production compared with out-patient isolates. This finding is consistent with the recognized role of healthcare settings as important reservoirs for antimicrobial-resistant organisms due to increased antibiotic exposure, prolonged hospitalization, invasive procedures, and cross-transmission within hospital environments. Similar associations between hospitalization and ESBL carriage have been reported in previous studies conducted in sub-Saharan Africa and other low- and middle-income settings (10, 13, 24, 25).

Infant age group was independently associated with ESBL production. This observation may reflect increased healthcare contact, antibiotic exposure, or immature immune defences among infants. However, the relatively small number of infant isolates resulted in wide confidence intervals (aOR = 7.72, 95% CI: 1.52–58.7; $p = 0.022$), and this finding should therefore be interpreted cautiously. Specimen type was not independently associated with ESBL production after adjustment, suggesting that ESBL occurrence in this study was more strongly associated with healthcare-related factors than specimen source. Although gender showed an association with ESBL production in unadjusted analysis, this association was not retained after multivariable adjustment, indicating that gender was not an independent predictor of ESBL production in this population. Interpretation of subgroup analyses should consider the relatively small number of ESBL-positive isolates in certain categories, particularly among

infants, which may have affected estimate precision. In addition, ESBL characterization was based solely on phenotypic methods without molecular identification of ESBL-associated genes, which may have limited characterization of the underlying resistance mechanisms. Collectively, the findings suggest that ESBL production in this study setting is more strongly associated with healthcare exposure than demographic characteristics alone. The significantly higher burden among in-patient isolates underscores the importance of hospital-based antimicrobial stewardship, infection prevention, and continuous surveillance to limit the dissemination of resistant *Enterobacteriaceae*.

In conclusion, ESBL-PE constituted a substantial proportion of clinical isolates in this study and demonstrated considerable resistance to commonly used antibiotics, including third-generation cephalosporins and ciprofloxacin. These findings highlight the potential limitations of empirical therapy and reinforce the need for routine antimicrobial susceptibility testing to guide treatment decisions. The preserved activity of imipenem against all isolates suggests that carbapenems remain effective for severe infections caused by multidrug-resistant organisms. Furthermore, the study demonstrates that phenotypic detection using the double-disc synergy method remains a practical approach for ESBL surveillance in resource-limited settings lacking advanced molecular diagnostic capacity.

Declarations

We hereby confirm that the participants consented to participate in the study.

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References

1. Opintan JA, Newman MJ (2017) Prevalence of antimicrobial resistant pathogens from blood cultures: results from a laboratory based nationwide surveillance in Ghana. Antimicrob Resist Infect Control 6(1):64
2. Alali WQ, AlFouzan W, Dhar R (2021) Prevalence of antimicrobial resistance in Gram-negative clinical isolates from a major secondary hospital in Kuwait: a retrospective descriptive study. Germs 11(4):498–511
3. Kasanga M, Mukosha R, Kasanga M, Siyanga M, Mudenda S, Solochi BB et al (2021) Antimicrobial resistance patterns of bacterial pathogens their distribution in university teaching hospitals in

Zambia. Future Microbiol 16:811–824

4. Dantas Palmeira J, Ferreira HMN (2020) Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in cattle production - a threat around the world. Heliyon 6(1):e03206
5. Saravanan M, Ramachandran B, Barabadi H (2018) The prevalence and drug resistance pattern of extended spectrum β–lactamases (ESBLs) producing Enterobacteriaceae in Africa. Microb Pathog 114:180–192
6. Bradford PA, Sanders CC (1995) Development of test panel of beta-lactamases expressed in a common Escherichia coli host background for evaluation of new beta-lactam antibiotics. Antimicrob Agents Chemother 39(2):308–313
7. Bradford PA (2001) Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14(4):933–951
8. Mitgang EA, Hartley DM, Malchione MD, Koch M, Goodman JL (2018) Review and mapping of carbapenem-resistant Enterobacteriaceae in Africa: Using diverse data to inform surveillance gaps. Int J Antimicrob Agents 52(3):372–384
9. van Duin D, Doi Y (2017) The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 8(4):460–469
10. Kimera ZI, Mgaya FX, Mshana SE, Karimuribo ED, Matee MIN (2021) Occurrence of Extended Spectrum Beta Lactamase (ESBL) Producers, Quinolone and Carbapenem Resistant Enterobacteriaceae Isolated from Environmental Samples along Msimbazi River Basin Ecosystem in Tanzania. Int J Environ Res Public Health 18(16):8264
11. Song J, Oh S-S, Kim J, Shin J (2020) Extended-spectrum β-lactamase-producing Escherichia coli isolated from raw vegetables in South Korea. Sci Rep 10(1):19721
12. Mumbula EM, Kwenda G, Samutela MT, Kalonda A, Mwansa J, Mwenya D et al (2015) Extended spectrum β-lactamases producing Klebsiella pneumoniae from the neonatal intensive care unit at the university teaching hospital in Lusaka, Zambia. J Med Sci Technol 4:2
13. Chishimba K, Hang'ombe BM, Muzandu K, Mshana SE, Matee MI, Nakajima C et al (2016) Detection of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli in Market-Ready Chickens in Zambia. Int J Microbiol 2016:5275724
14. Castanheira M, Simner PJ, Bradford PA (2021) Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 3(3):dlab092
15. Chanda W, Manyepa M, Chikwanda E, Daka V, Chileshe J, Tembo M et al (2019) Evaluation of antibiotic susceptibility patterns of pathogens isolated from routine laboratory specimens at Ndola Teaching Hospital: A retrospective study. PLoS ONE 14(12):e0226676
16. Mulongo T, Kamvuma K, Phiri CN, Mulemena JA, Chanda W (2021) Elevators and staircase handrails as potential sources of nosocomial pathogens at Ndola Teaching Hospital, Zambia
17. Mwansa TN, Kamvuma K, Mulemena JA, Phiri CN, Chanda W (2022) Antibiotic susceptibility patterns of pathogens isolated from laboratory specimens at Livingstone Central Hospital in Zambia. PLOS Global Public Health 2(9):e0000623

18. Kaluba CK, Samutela MT, Kapesa C, Muma JB, Hang'ombe BM, Hachaambwa L et al (2021) Carbapenem resistance in Pseudomonas aeruginosa and Acinetobacter species at a large tertiary referral hospital in Lusaka, Zambia. Sci Afr 13:e00908
19. CLSI (2020) Performance Standards for antimicrobial Susceptibility Testing, 30 edn. Clinical and Laboratory Standards Institute, Wayne PA, p 296. January 2020
20. Ibrahim Y, Sani Y, Saleh Q, Saleh A, Hakeem G (2017) Phenotypic detection of extended spectrum beta lactamase and carbapenemase co-producing clinical isolates from two tertiary hospitals in Kano, North West Nigeria. Ethiop J health Sci 27(1):3–10
21. Islam MS, Sobur MA, Rahman S, Ballah FM, Ievy S, Siddique MP et al (2022) Detection of bla TEM, bla CTX-M, bla CMY, and bla SHV genes among extended-spectrum beta-lactamase-producing Escherichia coli isolated from migratory birds travelling to Bangladesh. Microb Ecol. :1–9
22. Yasmeen N, Aslam B, Fang LX, Baloch Z, Liu Y (2023) Occurrence of extended- spectrum β-lactamase harboring K. pneumoniae in various sources: a one health perspective. Front Cell Infect Microbiol 13:1103319
23. Yu WL, Chuang YC, Walther-Rasmussen J (2006) Extended-spectrum beta-lactamases in Taiwan: epidemiology, detection, treatment and infection control. J Microbiol Immunol Infect 39(4):264–277
24. Bush K, Bradford PA (2016) β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med. ;6(8)
25. Ndir A, Diop A, Faye PM, Cissé MF, Ndoye B, Astagneau P (2016) Epidemiology and Burden of Bloodstream Infections Caused by Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in a Pediatric Hospital in Senegal. PLoS ONE 11(2):e0143729

Figures

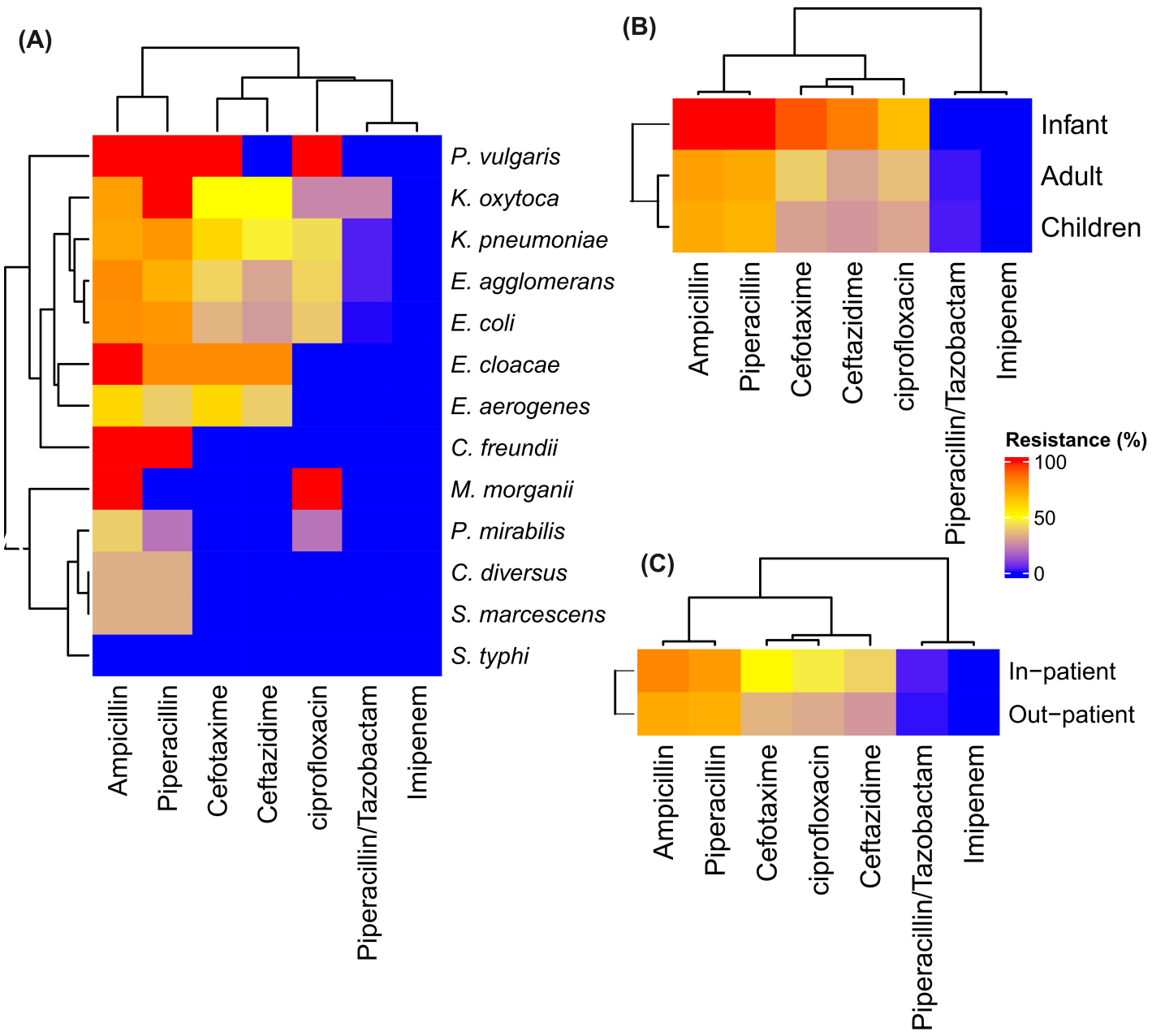


Figure 1

Antibiotic resistance patterns among *Enterobacteriaceae* isolates. (A) Hierarchically clustered heatmap showing the percentage resistance of *Enterobacteriaceae* species to the tested antibiotics. (B) Heatmap showing resistance patterns stratified by age group. (C) Heatmap showing resistance patterns stratified by patient setting (in-patient versus out-patient). Resistance percentages are represented by the color scale ranging from low resistance (0%, blue) to high resistance (100%, red).

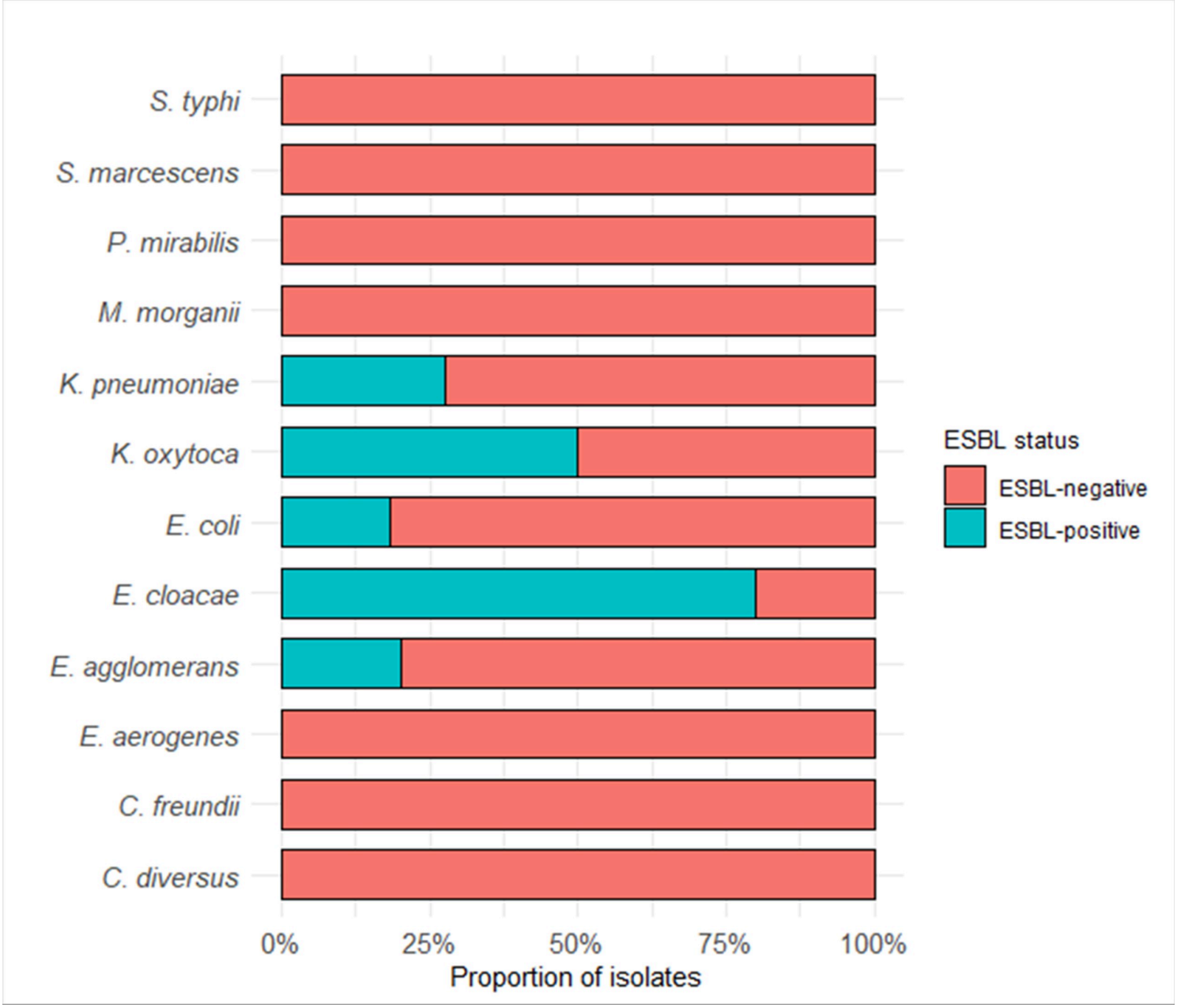


Figure 2

Distribution of ESBL-producing Enterobacteriaceae isolates by organism. Stacked bar plot showing the proportion of ESBL-positive and ESBL-negative isolates among *Enterobacteriaceae* species. ESBL status was determined phenotypically using cephalosporin-clavulanate combination testing.