BIOSCIENCE JOURNAL

INCREASED RESISTANCE RATES OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING *Enterobacterales* ISOLATED FROM BLOOD CULTURES IN BRAZIL

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How to cite: DA SILVA, R.E., et al. Increased resistance rates of extended-spectrum β-lactamase-producing *Enterobacterales* isolated from blood cultures in Brazil. *Bioscience Journal*. 2024, **40**, e40040. https://doi.org/10.14393/BJ-v40n0a2024-69945

Abstract

This study aimed to identify and evaluate the antimicrobial susceptibility profile of extended-spectrum β-lactamase (ESBL)-producing *Enterobacterales* isolated from blood cultures of patients with suspected sepsis. This retrospective cross-sectional epidemiological study was performed for four years (2015 to 2018) by accessing the medical records of patients diagnosed with bloodstream infections in two tertiary public hospitals in Brazil. Culture results and susceptibility tests of bacterial strains isolated from blood samples were analyzed. During four years, 2,263 blood culture exams were performed, and *Enterobacterales* were isolated in 694 (30.7%) samples. The disk approximation test detected ESBL production in 171 (24.6%) of the 694 isolated *Enterobacterales* ($p<0.05$), with 125 (73.1%) corresponding to *Klebsiella* spp., 28 (16.2%) to *Escherichia coli*, eight (4.6%) to *Enterobacter* spp., five (2.9%) to *Serratia* spp., three (1.7%) to *Proteus* spp., and two (1.2%) to *Citrobacter* spp. All tested antibiotics demonstrated low susceptibility (2.9% to 26.9%). The classes of monobactams, cephalosporins, and penicillin showed high resistance indices. The evaluated *Enterobacterales* isolates exhibited expressive antimicrobial resistance to drugs commonly used as empirical treatment in bloodstream infections. The findings reinforce the relevance of assessing the bacterial antimicrobial susceptibility profile, as it drives antibiotic therapy, potentially detecting multiresistance cases.

Keywords: Beta-lactamases. Blood culture. Drug resistance.

1. Introduction

Over the last decades, the increase in resistant bacteria that cause infections in hospitalized patients has become a relevant global public health concern. Extended-spectrum beta-lactamase (ESBL) production is one of the main mechanisms of antimicrobial resistance found especially among Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae* (Lepe and Martínez-Martínez 2022).

The treatment options for infections caused by ESBL-producing bacteria are limited and often fail due to the resistance to carbapenems and other antibiotic classes, such as aminoglycosides and fluoroquinolones (Rawat and Nair 2010; Mishra et al. 2012).

ESBL-producing *E. coli* and *K. pneumoniae* have often been involved in outbreaks and isolated from hospitalized patients with sepsis, a disease with high mortality rates (Scheuerman et al. 2018; de Lastours et al. 2020; Carvalho et al. 2021). Usually, bloodstream infections caused by ESBL-producing *Enterobacterales* (EPE) are associated with worse clinical outcomes and longer hospitalization times (Adeolu et al. 2016; Ling et al. 2021). Delays in receiving appropriate antibacterial therapy and longer hospitalization times also contribute as independent risk factors for mortality (Shamsrizi et al. 2020). The primary risk factors for acquired sepsis include ages of 65 or older, long-term hospitalization (13.6 days or more), and invasive procedures (including central vascular catheter, emergency abdominal surgery, or ventilatory assistance) (Nivesvivat et al. 2018).

The prevalence of EPE isolated from blood cultures and the rates of multidrug resistance have increased. According to geographic region and other risk factors, the mean mortality rate among bloodstream infection patients is around 31% and may range up to 83% (Rottier et al. 2012). In this context, the present study evaluated the resistance rate of EPE isolated from blood culture samples of patients with suspected sepsis.

2. Material and Methods

The Research Ethics Committee of the Federal University of Piauí (PI, Brazil) approved this cross-sectional and retrospective study (protocol number 66139617.4.0000.5669). The research accessed the medical records of patients with suspected sepsis from 2015 to 2018 in two Brazilian tertiary public hospitals in Uberaba (MG, Brazil) and Parnaíba (PI, Brazil). The blood culture and blood sample antibiogram results were analyzed. Additional information, such as the patient's age and sex, isolated microorganisms, antimicrobial susceptibility, and disk approximation tests for ESBL phenotypic detection, were also evaluated. The study excluded medical exams outside the evaluated period and blood cultures without an antibiogram or the concomitant disk approximation test.

Patient identity was preserved throughout the study. Chi-square or Fisher's exact test analyzed the data in SPSS 23.0 software. The p-value ≤ 0.05 was significant.

3. Results

There were 2,263 positive blood culture exams among all patients admitted to the hospitals during the study period, with 2,013 (89%) from the hospital in Uberaba (Hospital 1) and 250 (11%) from the hospital in Parnaíba (Hospital 2). Patient ages ranged from <1 to 108 (average of 43.3 ± 29.1 years). Regarding sex, male patients represented 1,287 (56.9%), and 976 (43.1%) were female.

As for the isolated microorganisms on blood cultures, *Enterobacterales* were responsible for 694 (30.7%) cases. Biochemical methods and automation (Vitek®) identified the bacteria. *Klebsiella* spp. was the most prevalent microorganism, detected in 315 (45.4%) samples, followed by *Escherichia coli* and *Enterobacter* spp., detected in 157 (22.6%) and 130 (18.7%) blood cultures, respectively.

The disk approximation test detected ESBL production in 171 (24.6%) of the 694 isolated enterobacteria ($p<0.05$), with 125 (73.1%) corresponding to *Klebsiella* spp., 28 (16.2%) to *E. coli*, eight (4.6%) to *Enterobacter* spp., five (2.9%) to *Serratia* spp., three (1.7%) to *Proteus* spp., and two (1.2%) to *Citrobacter* spp. The age of patients with positive blood cultures caused by EPE ranged from <1 to 94 (average of 39.2 ± 31.9 years).

Table 1 exhibits the antimicrobial susceptibility profile of EPE. Overall, all tested antibiotics showed low susceptibility. Resistance to all cephalosporins classes, from the first to the fourth generation, was demonstrated. Most isolates (72.5%, n=124) were resistant to cephalothin, cefoxitin, cefotaxime, ceftazidime, and cefepime. Cefadroxil (94.1%) and ceftriaxone (92.4%) resistances were even more widespread among EPE. Likewise, more than 70% of isolates were resistant to β-lactams amoxicillin

(72.5%), ampicillin (73.7%), and piperacillin (72.5%). The highest resistance rates occurred for carbapenem ertapenem (96.5%) and aztreonam, belonging to the monobactam group (94.7% of EPE).

The non-EPE isolates presented low resistance to the evaluated antimicrobials, and ceftriaxone and aztreonam resistances were the most prevalent (29.8% and 14.3%, respectively) (Table 1).

Table 1. Profile of antimicrobial susceptibility of ESBL-positive *Enterobacterales* (n=171) and non-ESBL-producing *Enterobacterales* (n=523).

Antibiotic	Antimicrobial susceptibility							
	Susceptible		Resistant		Intermediate		Not applicable	
	N (%)		N (%)		N (%)		N (%)	
	ESBL+	Non-ESBL	ESBL+	Non-ESBL	ESBL+	Non-ESBL	ESBL+	Non-ESBL
Amoxicillin	-	-	124 (72.5)	-	-	-	47 (27.5)	523 (100)
Ampicillin	5 (2.9)	24 (4.6)	126 (73.7)	8 (1.5)	1 (0.6)	-	39 (22.8)	491 (93.9)
Aztreonam	5 (2.9)	215 (41.1)	162 (94.7)	75 (14.3)	-	3 (0.6)	4 (2.3)	230 (43.9)
Cefadroxil	10 (5.8)	52 (9.9)	161 (94.1)	44 (8.4)	-	3 (0.6)	-	424 (81.1)
Cefepime	-	334 (63.9)	124 (72.5)	51 (9.7)	-	5 (0.9)	47 (27.5)	133 (25.4)
Cefotaxime	43 (25.1)	88 (16.8)	124 (72.5)	7 (1.3)	2 (1.2)	4 (0.7)	2 (1.2)	424 (81.1)
Cefoxitin	46 (26.9)	89 (17)	124 (72.5)	6 (1.1)	-	-	1 (0.6)	428 (81.8)
Ceftazidime	-	320 (61.2)	124 (72.5)	64 (12.2)			47 (27.5)	139 (26.6)
Ceftriaxone	12 (7)	343 (65.6)	158 (92.4)	156 (29.8)	-	1 (0.2)	1 (0.6)	23 (4.4)
Cephalothin	-	-	124 (72.5)	-	-	-	47 (27.5)	523 (100)
Ertapenem	5 (2.9)	325 (62.1)	165 (96.5)	44 (8.4)	-	1 (0.2)	1 (0.6)	153 (29.2)
Imipenem	-	395 (75.5)	124 (72.5)	3 (0.6)	-	-	47 (27.5)	125 (23.9)
Meropenem	-	401 (76.7)	124 (72.5)	3 (0.6)	-	-	47 (27.5)	119 (22.7)
Piperacillin	-	-	124 (72.5)	-	-	-	41 (24)	507 (96.9)

ESBL: Extended-spectrum beta-lactamases.

4. Discussion

The prevalence of ESBL-producing *Enterobacterales* (EPE) in nosocomial infections has been widely reported. Antibiotic resistance is increasing worldwide; consequently, bloodstream infections from multidrug-resistant microorganisms are causing substantial morbidity and mortality, mainly due to limited available therapies and worse clinical outcomes (Rugini et al. 2015; Lepe and Martínez-Martínez 2022). Considering the prevalence differences of ESBL-producing strains among regions, epidemiological studies are relevant to establish an appropriate therapeutic intervention that might limit the spread of these microorganisms (Dirar et al. 2020).

Gram-negative bacteria have been significantly associated with sepsis (Kabi et al. 2020). *Klebsiella* spp., *E. coli*, and *Enterobacter* spp. were the primary beta-lactamase-producing species in the present study, corresponding to 73.1% (n=125), 16.2% (n=28), and 4.6% (n=8), respectively. *Klebsiella pneumoniae* is frequently associated with resistance to the highest-priority critically important antimicrobial agent (Carvalho et al. 2021). These findings agree with other studies identifying these species as the most prevalent cause of bloodstream infection in hospitalized patients (Sangare et al. 2016; Gaibani et al. 2020; Carvalho et al. 2021).

In Brazil, studies have demonstrated the occurrence of infections caused by ESBL-positive *Enterobacterales*. A total of 2,197 positive cultures were evaluated, with 30.3% (n=666) of ESBL-producing isolates, and the most common species were *K. pneumoniae, E. coli*, and *Enterobacter* spp. Also, high antimicrobial resistance rates were detected in ESBL-producing *E. coli* and *Klebsiella* spp. that caused urinary tract infection, and the resistance to cephalosporins ranged from 93.1% to 96.6% in the 3,418 evaluated samples over four years (Rios-Neto et al. 2017).

The antimicrobial susceptibility test in this study showed decreased sensitivity to different antibiotic classes in EPE, especially to ampicillin, aztreonam, and ertapenem. Conversely, all tested antibiotics presented high resistance (72.5% to 96.5%).

Multidrug-resistant microorganisms have been a global concern. A report by the New York Presbyterian Hospital/Weill Cornell Medical Center on bacteremia demonstrated that 78 (4.2%) of 1,857 cases due to *E. coli* and *K. pneumoniae* were caused by strains resistant to piperacillin and tazobactam but

susceptible to ceftriaxone. These isolates were not susceptible to ampicillin and sulbactam. The 32 (1.7%) isolates susceptible to piperacillin, tazobactam, and ceftriaxone were sequenced but did not exhibit ESBL production (Baker et al. 2018).

A single EPE was sensitive to cefoxitin in this study, representing the lowest sensitivity rate (0.6%; n=1). Susceptibility to antibiotics, such as imipenem and meropenem, was higher than 90%, and there was resistance to ceftriaxone (26.7%; n=140) and gentamicin (24%; n=126) compared to non-EPE.

Another Brazilian study evaluated the antimicrobial sensitivity profile of ESBL-producing strains in nosocomial infections isolated from inpatients at a teaching hospital. As in the present study, those isolates showed resistance rates from 66% to 100% for ceftriaxone and ceftazidime antibiotics. However, *K. pneumonia, E. coli*, and *Enterobacter cloacae* were the most prevalent isolated species (Rocha et al. 2019).

Studies have demonstrated that ESBL-producing *E. coli* caused bacteremia in 326 (58.9%) cases of 544 hospitalized patients in Detroit, United States. The urinary tract infection was the primary site of ESBL-positive *E. coli* that caused the bloodstream infection (Chopra et al. 2015). Usually, infections from EPE delay the onset of effective therapy. Consequently, patient hospitalization extends, leading to higher hospital costs. The lack of effective treatment also increases mortality rates (Peirano and Pitout 2019).

The resistance profile of the isolates in the present study agrees with previous data. The high rates of antibiotic resistance by EPE is a significant public health concern. Thus, it is worth noting the relevance of consciously administering antimicrobial drugs and the need for improved epidemiological surveillance of these resistant microorganisms to reduce the dissemination of bacterial resistance determinants.

5. Conclusions

ESBL production occurred in 171 (24.6%) of 694 *Enterobacterales* isolates, with 125 (73.1%) being *Klebsiella* spp. and 28 (16.2%) *Escherichia coli*. There was a high susceptibility to monobactams, cephalosporins (including the first, second, third, and fourth generation), and penicillin.

Additionally, bacterial multidrug resistance is a relevant public health problem, requiring continuous monitoring of the spread of these microorganisms to understand the epidemiological reality and determine prevention and control actions.

Authors' Contributions: DA SILVA, R. E.: Conception and design, analysis and interpretation of data, and drafting the manuscript; ALVES, N. S.: Drafting the manuscript, design and critical review; DO NASCIMENTO, C. K. R.: Acquisition of data, analysis and interpretation of data; DOS SANTOS, R. M. M.: Acquisition of data, analysis and interpretation of data; BARRETO, W. L. P.: Acquisition of data, analysis and interpretation of data; REIS FILHO, J. S.: Acquisition of data, analysis and interpretation of data; MARTINS, V. B. D.: Acquisition of data, analysis and interpretation of data; SEVERINO, E. C. V.: Acquisition and data analysis; ARAÚJO, M. C.: Acquisition and data analysis; RIOS-NETO, M. A.: Acquisition and data analysis; PAIVA, A. D.: Interpretation of data and critical review; FERREIRA-PAIM, K.: Data analysis; drafting the manuscript and critical review; FONSECA, F. M.: Conception and design, analysis of data, and drafting the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Approved by Research Ethics Committee of Federal University of Piauí (CAAE: 66139617.4.0000.5669)

Funding: Not applicable.

References

ADEOLU, M., et al. 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. *International Journal of Systematic and Evolutionary Microbiology*. 2016, **66**(12), 5575-5599. https://doi.org/10.1099/ijsem.0.001485

BAKER, T.M., et al. Epidemiology of Bloodstream Infections Caused by *Escherichia coli* and *Klebsiella pneumoniae* That Are Piperacillin-Tazobactam-Nonsusceptible but Ceftriaxone-Susceptible. *Open Forum Infectious Diseases*. 2018, **5**(12), 1-7. https://doi.org/10.1093/ofid/ofy300

CARVALHO, I., et al. Multidrug-resistant Klebsiella pneumoniae harboring extended spectrum β-lactamase encoding genes isolated from human septicemias. *PLoS One*. 2021, **16**(5), e0250525. https://doi.org/10.1371/journal.pone.0250525

CHOPRA, T., et al. Risk factors for bloodstream infection caused by extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: a focus on antimicrobials including cefepime. *American Journal of Infection Control*. 2015, **43**(7), 719-723. https://doi.org/10.1016/j.ajic.2015.02.030

DE LASTOURS, V., et al. Mortality in *Escherichia coli* bloodstream infections: antibiotic resistance still does not make it. *Journal of Antimicrobial Chemotherapy.* 2020, **75**(8), 2334–2343. https://doi.org/10.1093/jac/dkaa161

DIRAR, M.H., et al. Prevalence of extended-spectrum β-lactamase (ESBL) and molecular detection of *bla*TEM, *bla*SHV and *bla*CTX-M genotypes among *Enterobacteriaceae* isolates from patients in Khartoum, Sudan. *Pan African Medical Journal*. 2020, **37**(213), 1-11. https://doi.org/10.11604/pamj.2020.37.213.24988

GAIBANI, P., et al. Bloodstream infection caused by KPC-producing *Klebsiella pneumoniae* resistant to ceftazidime/avibactam: epidemiology and genomic characterization. *Clinical Microbiology and Infection*. 2020, **26**(4): 516.e1-516.e4. https://doi.org/10.1016/j.cmi.2019.11.011

KABI, A., et al. Clinical spectrum and risk factors for hospital-acquired septicemia in a tertiary care centre of North-East India. *Journal of Family Medicine and Primary Care*. 2020, **9**(8): 3949-3954. https://doi.org/10.4103/jfmpc.jfmpc_469_20

LEPE, J.A. and MARTÍNEZ-MARTÍNEZ, L. Resistance mechanisms in Gram-negative bacteria. *Medicina Intensiva (English Edition).* 2022, **46**(7): 392-402. http://doi.org/10.1016/j.medine.2022.05.004

LING, W., et al. Adverse clinical outcomes associated with infections by *Enterobacterales* producing ESBL (ESBL-E): a systematic review and meta-analysis. *JAC-Antimicrobial Resistance*. 2021, **3**(2), 1-11. https://doi.org/10.1093/jacamr/dlab068

MISHRA, S.K., et al. Extended-spectrum beta-lactamase and metallo-beta-lactamase-producing bacterial strains among the patients attending a tertiary care center in Nepal. *International Journal of Infectious Diseases*. 2012, **16**S, e317–e473. https://doi.org/10.1016/j.ijid.2012.05.592

NIVESVIVAT, T., et al. Clinical epidemiology, risk factors and treatment outcomes of extended-spectrum beta-lactamase producing *Enterobacteriaceae* bacteremia among children in a Tertiary Care Hospital, Bangkok, Thailand. *BMC Research Notes*. 2018, **11**(1), 1-5. https://doi.org/10.1186/s13104-018-3729-3

PEIRANO, G. and PITOUT, J.D.D. Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae*: Update on Molecular Epidemiology and Treatment Options. *Drugs*. 2019, **79**, 1529–1541. https://doi.org/10.1007/s40265-019-01180-3

RAWAT, D. and NAIR, D. Extended-spectrum ß-lactamases in gram negative bacteria. *Journal of Global Infectious Diseases*. 2010, **2**(3), 263–274. https://doi.org/10.4103/0974-777X.68531

RIOS-NETO, M.A., et al. High rates of antimicrobial resistance of ESBL-producing *Enterobacteriaceae* isolated from clinical samples in Northeast of Brazil. *Infectious Diseases*. 2017, **50**(3), 229-231. https://doi.org/10.1080/23744235.2017.1381987

ROCHA, F.R., et al. High Frequency of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* Nosocomial Strains Isolated from a Teaching Hospital in Brazil. *Microbial Drug Resistance*. 2019, **25**(6), 909-914. https://doi.org/10.1089/mdr.2018.0142

ROTTIER, W.C., AMMERLAAN, H.S.M. and BONTEN, M.J.M. Effects of confounders and inter- mediates on the association of bacteraemia caused by extended-spectrum b-lactamase-producing *Enterobacteriaceae* and patient outcome: a meta- analysis. *Journal of Antimicrobial Chemotherapy*. 2012, **67**(6), 1311–1320. https://doi.org/10.1093/jac/dks065

RUGINI, C.L., SOBOTTKA, A.M. and FUENTEFRIA, D.B., Occurrence and sensitivity profile of extended spectrum beta-lactamase-producing *Enterobacteriaceae* at a tertiary hospital in Southern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 2015, **48**(6), 692-698. https://doi.org/10.1590/0037-8682-0211-2015

SANGARE, A.S., et al. Prevalence of ESBL-producing *Enterobacteriaceae* isolated from blood cultures in Mali. *The Journal of Infection in Developing Countries.* 2016, **10**(10), 1059-1064. https://doi.org/10.3855/jidc.7536

SCHEUERMAN, O., et al. Comparison of Predictors and Mortality Between Bloodstream Infections Caused by ESBL-Producing *Escherichia coli* and ESBL-Producing *Klebsiella pneumoniae*. *Infection Control & Hospital Epidemiology*. 2018, **39**(6), 660–667. https://doi.org/10.1017/ice.2018.63

SHAMSRIZI, P., et al. Variation of effect estimates in the analysis of mortality and length of hospital stay in patients with infections caused by bacteria-producing extended-spectrum beta-lactamases: a systematic review and meta-analysis. *BMJ Open*. 2020, **10**(1), 1-10. http://dx.doi.org/10.1136/bmjopen-2019-030266

Received: 26 May 2023 | **Accepted:** 27 July 2024 | **Published:** 19 September 2024