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ANTIMICROBIAL RESISTANCE IN GRAM NEGATIVE BACILLI ISOLATED FROM BLOOD CULTURE

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Abstract

Healthcare-associated and community-acquired infections caused by Gram-negative bacteria are serious health concerns due to the rise of antibiotic resistance. Antimicrobial resistance (AMR) is a highly concerning issue in the field of medicine today, as it has significant impacts on morbidity, mortality, and socio-economic factors.

Blood samples from inpatients admitted at the Clinical Microbiology Laboratory during the study period from January 2022 until December 2023 were included in study analysis. Blood culture bottles for aerobic and anaerobic cultivation were incubated in automated BACT/ALERT® 3D.

For further analysis, isolated pure colonies were then identified to a bacterial species level using automated VITEK® 2 COMPACT system.

The most commonly isolated microorganism was *Escherichia coli* 22%. High proportion of *E. coli* isolates were resistant to ciprofloxacin (69%), ceftriaxone (60%), and trimethoprim sulfamethoxazole (58%). *Acinetobacter baumannii* was second most frequently isolated microorganism (11%) and showed highly resistance to almost all tested antibiotics.

All isolates were resistant to amikacin, fluoroquinolones and carbapenems. Highest sensitivity was found to tobramycin (35%). *Pseudomonas aeruginosa* showed highest resistance to gentamicin (71%), ciprofloxacin (71%), levofloxacin (75%), imipenem (76.5%) and meropenem (69%). *Klebsiella pneumoniae* was detected in 11% of bacterial isolates and showed highest resistance to third-generation cephalosporins (>70%), followed by ciprofloxacin (67%) and trimethoprim sulfamethoxazole (67%).

Our findings contribute valuable insights into local epidemiology and resistance trends, aiding clinicians in making informed treatment decisions. Continued surveillance and research in this area are essential for addressing the challenges posed by antimicrobial resistance and improving patient outcomes.

Keywords: blood culture, antimicrobial resistance, gram negative bacilli.

Introduction

Blood is a clean, sterile fluid. When microorganisms enter the bloodstream, it is a major factor in causing infectious diseases [1].

Recent studies have revisited the definition of bacteriemia and sepsis. Bacteremia refers to the presence of viable bacterial cells in the blood [2], while sepsis is a critical life threating condition characterized by bacteremia and systemic symptoms in due to a dysregulation of the immune system of the host. The pathogens release toxins into the bloodstream, triggering the release of cytokines. Patients with these infections typically experience high fever, chills, rapid heart rate, rapid breathing, and low blood pressure [3].

Healthcare-associated and community-acquired infections caused by Gram-negative bacteria are serious health concerns due to the rise of antibiotic resistance [4].

Antimicrobial resistance (AMR) is a highly concerning issue in the field of medicine today, as it has significant impacts on morbidity, mortality, and socio-economic factors. Within hospital settings, AMR leads to longer hospital stays, increased rates of complications, and higher healthcare costs [5,6].

The limitation in treatment options has detrimental effects on patient survival. Managing AMR effectively in both hospital and community settings is a complex task that requires a global strategy. It is important to note that no single intervention has proven completely effective in preventing and controlling AMR, thus the implementation of a comprehensive set of interventions has been recommended [7].

The frequent utilization of broad-spectrum antibiotics leads to the colonization of resistant Gram-negative bacteria, which in turn increases the risk of severe infections [8].

The Central Asian and Eastern European Surveillance of Antimicrobial Resistance-CAESAR as well as other important networks that are continuously monitoring antimicrobial resistance, such as, European Antimicrobial Resistance Surveillance Network -EARS-Net and The National Healthcare Safety Network-NHSN at the CDC have registered a significant increase in resistance levels over the last 10 years [9]. Also, it is of note that the prevalence of causative organisms and their resistance may vary from one region to another [5, 9].

The objective of this study was to identify the Gram-negative bacteria responsible for bloodstream infections and determine their susceptibility to antimicrobial drugs.

Material and methods

This cross-sectional study was conducted at the Institute of Microbiology and Parasitology, Faculty of Medicine, University Ss Cyril and Methodius in Skopje. Blood samples were obtained from outpatients and inpatients admitted at the Clinical Center ”Mother Teresa”, Skopje, Republic of North Macedonia and sent to our Clinical Microbiology Laboratory. Only samples from inpatients during the study period from January 2022 until December 2023 were included in study analysis.

Patients records were used for obtaining information on patient age, gender, date of admission and sampling date.

Blood samples (10 ml) were obtained aseptically using sterile techniques and transferred to each commercially available blood culture bottle (one for aerobic and one anaerobic cultivation) containing appropriate culture media specifically designed for the isolation and growth of bacteria from blood samples (bioMérieux), from patients suspected of having bloodstream infections caused by Gram-negative bacilli.

Blood culture bottles for aerobic and anaerobic cultivation were incubated in automated BACT/ALERT® 3D blood culture system (bioMérieux) as per manufacturer’s recommendations for a maximum of 5 days. Automatically detected positive hemoculture bottles were further assessed by Gram stain procedure as described previously [10].

Bottles with microscopically detected Gram-negative bacilli were subcultured on Columbia and chromogenic agar plates and incubated aerobically at an appropriate temperature of 37°C for 18-24 hours. For further analysis, isolated pure colonies were then identified to a bacterial species level using automated VITEK® 2 COMPACT system (bioMérieux) by using Gram-negative identification card (GN-ID) according to manufacturer instructions.

Isolated colonies were also used in antibiotic susceptibility testing (AST), performed and interpreted by Kirby-Bauer disk diffusion method following instructions of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [11].

Kirby Bauer disc diffusion AST was performed with commercially available paper antimicrobial susceptibility discs (Oxoid®) for following antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, gentamicin, tobramycin, amikacin, ciprofloxacin, levofloxacin, imipenem, meropenem, ampicillin, amoxicillin-clavulonic acid, ceftriaxone, ertapenem, trimethoprim-sulfamethoxazole, cefepime. Concurrently, AST was performed automatically by using the VITEK-2 System with the implementation specific antibiotic susceptibility testing card for AST for Gram-negative bacilli, GN-222 following manufacturers’ instructions.

Data obtained from clinical reports (age, gender, type of hospital) and laboratory analysis (isolated microorganims, antibiotic susceptibility testing results) was used to determine the prevalence

and patterns of antimicrobial resistance in Gram-negative bacilli isolated from hemoculture. Antimicrobial resistance profiles were compared among different isolated microorganisms.

Patient information data, bacterial isolate identification information and AST results were entered in the WHONET database. Also, an electronic Microsoft Excel Database was created and then further analysed using IBM SPSS 20.0 for Windows. Results in this study are presented as numbers and percentages.

Results

The main characteristics of patients are summarized in Table 1. It could be seen that highes proportion of samples were blood samples.

Out of these 160 isolates we selected data on antimicrobial resistance of Gram-negative bacilli that we used for study evaluation. The total number of isolated Gram-negative bacilli is 83 (50.3%).

The most commonly isolated microorganism was *Escherichia coli* 36 (22%), followed by *Acinetobacter baumannii* (11%), *Pseudomonas aeruginosa* (10%) and *Klebsiella pneumoniae* (7%).

Highest number of patients samples were noted to originate from the Internal Medicine Departments (40%), followed by Hematology and oncology (23%), Surgery (16%) and Intensive Care Unit (9%).

Escherichia coli was the most frequent isolate at the Hematology and/or Oncology Department *(42%)* followed by Internal Medicine Department (33%).

Lower percentage of isolation was seen in for Surgery Department (3%), Urology (8%) and Intensive Care Unit (6%).

Klebsiella pneumoniae was most frequently isolated from patients hospitalized at the Internal Medicine Department (33%), followed by Hematology and/or Oncology Department *(17%),* Surgery Department (17%) and Intensive Care Unit (17%).

Pseudomonas aeruginosa was found to be most frequently isolated from blood samples from Hematology and/or Oncology Department *(41%),* followed by Surgery Department (24%), Internal Medicine Department (18%), Urology (12%) and Intensive Care Unit (6%).

Acinetobacter baumannii was the most frequent isolate from patients from Surgery Department (50%), followed by the Intensive Care Unit (28%) and Internal Medicine Department (17%).

From patients of male gender there have been more bacterial isolates among all for Gram-negative bacilli. Of *Escherichia coli* and *Pseudomonas aeruginosa* isolates*,* were 72% were males, while 28% were of female gender for both bacterial isolates, respectively.

Table 1. Characteristics of patients represented by specific pathogen isolated from blood sample

	Escherichia coli	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Acinetobacter baumannii***
Number of isolates; n (%)	36 (22)	12 (7)	17 (10)	18 (11)
Sex n (%)				
Male	26 (72)	7 (58)	10 (59)	13 (72)
Female	10 (28)	5 (42)	7 (41)	5 (28)
Age in years n (%)				
0-4	2 (6)	0 (0)	1 (6)	0 (0)
5-19	1 (3)	0 (0)	0 (0)	0 (0)
20-64	19 (53)	7 (58)	7 (41)	10 (56)
65 and over	9 (25)	3 (25)	2 (12)	3 (17)
Unknown	5 (14)	2 (17)	7 (41)	5 (28)
Hospital department n (%)				
Haematology or oncology	15 (42)	2 (17)	7 (41)	0 (0)
Internal medicine	12 (33)	4 (33)	3 (18)	3 (17)
Surgery	14 (3)	2 (17)	4 (24)	9 (50)
Urology	3 (8)	0 (0)	2 (12)	0 (0)
Intensive care unit	2 (6)	2 (17)	1 (6)	5 (28)
Paediatrics or neonatal	2 (6)	1 (8)	0 (0)	0 (0)
Other	0 (0)	1 (8)	0 (0)	0 (0)
Unknown	1 (3)	0 (0)	0 (0)	1 (6)

From Table 2 it could be followed that *Escherichia coli*, showed highest percentage of antimicrobial resistance to ampicillin/amoxicillin (91%) and ciprofloxacin (69%), while the lowest resistance was observed in meropenem and imipenem, showing 0% resistance. Moderate resistance was seen to other antibiotics, trimethoprim-sulfamethoxazole (58%) and ceftriaxone (60%). Notable efficacy was presented by piperacillin-tazobactam, especially against *Escherichia coli*, with only 16% resistance. On the other hand, for *Klebsiella pneumoniae*, ampicillin/amoxicillin (100%) and ceftazidime (83%) both exhibit lowest efficacy, followed by ceftriaxone (77%), cefepime (75%) ciprofloxacin (67%). The lowest resistance was observed in amikacin (33%) and carbapenems (17%).

Table. 2. Resistance levels for *Escherichia coli* and *Klebsiella pneumoniae* among blood isolates in North Macedonia in 2022

Antibiotic	*E. coli*			*K. pneumoniae*		
	N	**n (%) R**	**n (%) S**	**N**	**n (%) R**	**n (%) S**
Ampicillin/amoxicillin	36	33 (91)	3 (9)	12	12 (100)	0 (0)
Amoxicillin-clavulanic acid	35	23 (66)	1 (34)	12	10 (83)	2 (17)
Piperacillin-tazobactam	36	6 (16)	30 (84)	12	8 (66)	4 (34)
Ceftriaxone	35	21 (60)	14 (40)	9	7 (77)	2 (23)
Ceftazidime	36	19 (53)	17 (47)	12	10 (83)	2 (17)
Gentamicin	35	11 (31)	24 (79)	12	5 (42)	7 (58)
Tobramycin	20	8 (40)	12 (60)	11	6 (54)	5 (46)
Amikacin	36	6 (17)	30 (83)	12	4 (33)	8 (67)
Ciprofloxacin	36	25 (69)	11 (31)	12	8 (67)	4 (33)
Imipenem	36	0 (0)	36 (100)	12	2 (17)	10 (83)
Meropenem	36	0 (0)	36 (100)	12	2 (17)	10 (83)
Ertapenem	34	2 (6)	32 (94)	12	2 (17)	10 (83)
Trimethoprim sulfamethoxazole	36	21 (58)	15 (42)	12	8 (67)	9 (33)
Cefepime	35	18 (51)	17 (49)	4	3 (75)	1 (25)

R – resistant; **S** – susceptible;

Table 3 represents data on antimicrobial resistance pattern of *Pseudomonas aeruginosa* and *Acientobacter baumannii* isolated from blood specimens. For *Pseudomonas aeruginosa*, the highest resistance is observed with imipenem (76.5%), levofloxacin (75%) and ciprofloxacin (71%), while amikacin and tobramycin showed relatively lower efficacy at 56% and 60% respectively.

In *Acinetobacter baumannii*, the highest resistance is uniformly seen with amikacin, ciprofloxacin, levofloxacin, imipenem, meropenem, all registering 100%, followed by gentamicin with 94% resistance. Tobramycin is relatively more efficient at 65%.

Table 3. Resistance levels for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* among blood and CSF isolates in North Macedonia in 2022

Antibiotic	*Pseudomonas aeruginosa*			*Acinetobacter baumannii*		
	N	**n (%) R**	**n (%) S**	**N**	**n (%) R**	**n (%) S**
Amikacin	17	10 (56)	7 (44)	18	18 (100)	0 (0)
Piperacillin-tazobactam	16	8 (50)	8 (50)	16	8 (50)	8 (50)
Cefepime	16	10 (62.5)	6 (37.5)	/	/	/
Ceftazidime	16	10 (62.5)	6 (37.5)	/	/	/
Gentamicin	17	12 (71)	12 (29)	18	17 (94)	1 (6)
Tobramycin	15	9 (60)	6 (40)	17	11 (65)	6 (35)
Amikacin	18	10 (59)	8 (41)	18	18 (100)	0 (0)
Ciprofloxacin	17	12 (71)	5 (29)	18	18 (100)	0 (0)
Levofloxacin	8	6 (75)	2 (25)	5	5 (100)	0 (0)
Imipenem	17	13 (76.5)	4 (23.5)	18	18 (100)	0 (0)
Meropenem	16	11 (69)	5 (31)	18	18 (100)	0 (0)

R – resistant; **S** – susceptible;

Discussion

The presented data reveals the intricate landscape of antimicrobial resistance among Gram-negative bacilli, specifically *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii*, isolated from hemocultures in the year 2022.

The key findings of our study show that both *E.coli* and *Klebsiella pneumoniae* exhibit high resistance to ampicillin and amoxicillin-clavulanic acid which is an indication that the beta-lactamase inhibitor combination is not universally effective against these strains. This underscores the limited efficacy of this drug against these pathogens and the need for alternative treatment options. Piperacillin-tazobactam is considerably higher in *K. pneumoniae* (66%) compared to *E.coli* (16%). This suggests differences in the susceptibility of these bacteria to beta-lactamase inhibitor combination.

Both bacteria exhibit substantial resistance to third-generations cephalosporins (in this case we tested on ceftriaxone and ceftazidime), indicating a potential challenge in treating infections caused by these drugs. Concerning the aminoglycoside resistance, there is variability in gentamicin resistance between the two bacteria, and tobramycin and amikacin resistance rates also differ. This emphasizes the importance of considering specific aminoglycosides based on susceptibility patterns.

Regarding antimicrobial resistance to fluoroquinolons, both bacteria show relatively high resistance to ciprofloxacin, indicating limited effectiveness of quinolones in treating infections caused by these strains.

While carbapenems stand out as a highly effective in treating infections caused by ESBL-producing bacteria, their frequent and improrer usage can lead to the emergence of resistance against these antibiotics [12].

The carbapenem resistance, specifically imipenem, meropenem and ertapenem shows divergence. While *E. coli* demonstrates low or no carbapenem resistance, *K. pneumoniae* shows some resistance, highlighting the importance of monitoring carbapenem use to prevent further emergence of resistance.

During a decade-long study encompassing 77.618 blood cultures in India, the study tracked the resistance of *E.coli* and *K. pneumoniae* strains to carbapenem and piperacillin-tazobactam. While the resistance rate for *E. coli* did not exhibit statistical significance over the years, there was a notable and statistically significant increase in resistance observed for *K. pneumoniae*.

The study interpreted this rise in resistance as a consequence of the escalating prevalence of ESBL-positive strains and a shift from third-generation cephalosporines to the use of carbapenems and piperacillin-tazobactam in the treatment of severe infections [13].
Last, but not least, both bacteria exhibit substantial resistance to trimethoprim-sulfametoxhazole, indicating imitations in using this combination for infections caused by these strains. Similar results were found in a study from 2019, where amikacin, meropenem, imipenem and ertapenem demonstrated the highest efficacy against against *E. coli* and *K. pneumoniae* isolates, each respectively. Conversely, the highest rates of resistance were observed in ceftriaxone, cefepime, trimethoprim-sulfamethoxazole and ciprofloxacin [12].

In question of the antimicrobial resistance to *Pseudomonas aeruginosa* and *Acinetobacter spp*., they both show broad-spectrum resistance, limiting the available therapeutic options.

The high resistance to aminoglycosides, fluoroquinolones and carbapenems underscores the limited efficacy of these drug classes. *Acinetobacter spp*. exhibits a 100% resistance to the tested carbapenems (imipenem, meropenem) and to the fluoroquinolones (ciprofloxacin, levofloxacin) which is highlighting the critical need for alternative treatment strategies and the challenges in managing infections caused by these bacteria, while with aminoglycosides, *P. aeruginosa* displays substantial resistance to fluoroquionolones and carbapenems, indicating limited effectiveness of these drug.

Currently, in various regions the resistance rates to imipenem among *Acinetobacter baumannii* isolates range from approximately 76% to 92% [14].

Research conducted in southern Iran revealed that oxacillinases, specifically OXA-type-B-lactamases, have emerged as a key factor contributing to the development of carbapenem resistance in clinical isolates of *Acinetobacter baumannii* [15].

A different investigation documented a notable decline in the resistance levels of *Pseudomonas aeruginosa* obtained from wound swabs, against ciprofloxacin, ceftazidime, meropenem and imipenem. The researches proposed that this decrease might be attributed to a reduction in the usage of ciprofloxacin [16].

A separate research study conducted in China observed a similar pattern regarding resistance of *Pseudomonas aeruginosa* to carbapenems. The resistance rate exhibited a decline between the years 2006-2014 [17].

Pseudomonas aeruginosa was also tested on piperacillin-tazobactam (50% resistance), which showed moderate resistance to this drug, while it showed higher resistance to third-generation cephalosporines (62.5%).

Conclusion

This study provides a comprehensive analysis of Gram-negative bacilli isolated from blood cultures, shedding light on their susceptibility patterns to commonly prescribed antibiotics. The findings contribute valuable insights into local epidemiology and resistance trends, aiding clinicians in making informed treatment decisions. Continued surveillance and research in this area are essential for addressing the challenges posed by antimicrobial resistance and improving patient outcomes.

References

1. Karuna T, Gupta A, Vyas A, et al. Changing Trends in Antimicrobial Susceptibility Patterns of Bloodstream Infection (BSI) in Secondary Care Hospitals of India. Cureus. 2023;15(4):e37800.
2. Martinez RM, Wolk DM. Bloodstream Infections. Microbiol Spectr. 2016;4(4):1-34.
3. Huerta LE, Rice TW. Pathologic Difference between Sepsis and Bloodstream Infections. J Appl Lab Med. 2019 Jan;3(4):654-663.
4. Akova M. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence. 2016 Apr 2;7(3):252-66.
5. Allel K, Stone J, Undurraga EA, Day L, Moore CE, Lin L, Furuya-Kanamori L, Yakob L. The impact of inpatient bloodstream infections caused by antibiotic-resistant bacteria in low- and middle-income countries: A systematic review and meta-analysis. PLoS Med. 2023 Jun 22;20(6):e1004199.
6. Stewardson AJ, Allignol A, Beyersmann J, Graves N, Schumacher M, Meyer R, Tacconelli E, De Angelis G, Farina C, Pezzoli F, Bertrand X, Gbaguidi-Haore H, Edgeworth J, Tosas O, Martinez JA, Ayala-Blanco MP, Pan A, Zoncada A, Marwick CA, Nathwani D, Seifert H, Hos N, Hagel S, Pletz M, Harbarth S; TIMBER Study Group. The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and Staphylococcus aureus in European hospitals, 2010 and 2011: a multicentre retrospective cohort study. Euro Surveill. 2016;21(33):30319.
7. Simonsen GS, Tapsall JW, Allegranzi B, Talbot EA, Lazzari S. The antimicrobial resistance containment and surveillance approach--a public health tool. Bull World Health Organ. 2004;82(12):928-34.
8. Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJ. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016 Jan 9;387(10014):176-87.
9. Saavedra JC, Fonseca D, Abrahamyan A, Thekkur P, Timire C, Reyes J, Zachariah R, Agudelo LG. Bloodstream infections and antibiotic resistance at a regional hospital, Colombia, 2019-2021. Rev Panam Salud Publica. 2023;47:e18.
10. Gram - Gerhardt P, Wood WA, Krieg NR, Murray R. Methods for general and molecular bacteriology. ASM 1994.
11. Matuschek E, Brown DF, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014;20(4):O255-66.
12. Bayraktar B, Pelit S, Bulut ME, Aktaş E. Trend in Antibiotic Resistance of Extended-Spectrum Beta-Lactamase-Producing Escherichia Coli and Klebsiella Pneumoniae Bloodstream Infections. Sisli Etfal Hastan Tip Bul. 2019;53(1):70-75.

13. Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ. A ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli & Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Res. 2012;135(6):907-12.
14. Asif M, Alvi IA, Rehman SU. Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist. 2018;11:1249-1260.
15. Pourabbas B, Firouzi R, Pouladfar G. Characterization of carbapenem-resistant Acinetobacter calcoaceticus-baumannii complex isolates from nosocomial bloodstream infections in southern Iran. J Med Microbiol. 2016;65(3):235-239.
16. Joseph NM, Devi S, Shashikala P, Kanungo R. Changing trend in the antibiotic resistance pattern of Pseudomonas aeruginosa isolated from wound swabs of out-patients and in-patients of a tertiary care hospital. J Clin Diagn Res. 2013;7(10):2170–2.
17. Karuna T, Gupta A, Vyas A, Kumar S, Sampath A, Goel P, Shukla P, Mishra V, Sharma S, Chakraborty S, Jaiswal SP, Mishra A, Gupta A, Sahu M, Tiwari S, Pal A, Nagendra M, Gautham H, Patel K, Asati S, Khadanga S. Changing Trends in Antimicrobial Susceptibility Patterns of Bloodstream Infection (BSI) in Secondary Care Hospitals of India. Cureus. 2023;15(4):e37800.