

## ORIGINAL ARTICLE

# Antimicrobial Activity of Gram Negative Bacteria in Blood Cultures: A Study with Blood Agar in ICU of Lahore General Hospital, Lahore

HIRA GHAFFAR<sup>1</sup>, SAIRA ZAFAR<sup>2</sup>, ZAFAR IQBAL<sup>3</sup>, GHAZIA FATIMA<sup>4</sup>, SIBGHA NAZ<sup>5</sup>, FAIZ AHMAD FAIZ<sup>6</sup>, GHAFFAR ALI<sup>7</sup>, AQEELA ASHRAF<sup>8</sup>, DIL NAWAZ KHALIL<sup>9</sup>

<sup>1</sup>Assistant Professor Microbiology, Continental Medical College, Lahore

<sup>2</sup>Associate Professor Microbiology, Continental Medical College, Lahore

<sup>3</sup>Associate Professor Pathology, Continental Medical College, Lahore

<sup>4</sup>Assistant Professor Histopathology, Continental Medical College, Lahore

<sup>5</sup>Demonstrator Microbiology, Continental Medical College, Lahore

<sup>6</sup>Assistant professor Hematology, Islam Medical College, Sialkot

<sup>7</sup>Lab. Technologist, Mafazatul Hayat Hospital, Lahore

<sup>8</sup>Assistant Prof. Biology Department, Lahore Garrison University, DHA, Lahore

<sup>9</sup>MS Bioinformatics Scholar, Virtual University of Pakistan, Lahore

Correspondence to Dr. Hira Ghaffar, Assistant Professor Microbiology

## ABSTRACT

**Aim:** To evaluate gram-negative rods' prevalence and antibiotic resistance, which are responsible for bloodstream infections in ICU patients.

**Study design:** Cross-sectional study

**Place and duration of study:** Lahore General Hospital, Lahore from 1<sup>st</sup> July 2022 to 31<sup>st</sup> December 2022.

**Methodology:** Total 200 patients in ICU suspected to have bloodstream infection were enrolled in this study. Blood samples were cultured on Blood agar and then subjected to antibiotic susceptibility testing by the disk diffusion method. Statistical analysis was performed by using SPSS Software.

**Results:** 135 were positive for Gram-negative bacterial strains and 13 show gram positive strain with 25 showing mixed growth and 27 showing no growth. In all positive cases for different bacterial isolates resistance for amikacin was 60.8%, cefepime 66.8%, ceftriaxone 79%, ceftazidime 75.6%, cotrimoxazole 57.4%, ciprofloxacin 61.4%, gentamycin 60.8%, levofloxacin 59.4%, imipenem 58.7%, meropenem 65.5%, doxycycline 51.3%, tazobactam 71.6%, and piperacillin + tazobactam 74.3%. Overall gram-negative bacterial isolates exhibited a high resistance rate for cefotaxime at 79% and the lowest resistance rate for doxycycline at 51.3%. Out of 148 positive cases, 70(35%) were male and 78(39%) were females. Most positive cases were reported in the age group of 16-25 years (33.3%).

**Conclusion:** All the positive samples showed gram-negative rods and gram positive on gram staining. Klebsiella 37(18.5%) was the most prevalent pathogen found in blood samples of ICU patients.

**Keywords:** Prevalence, antibiotic resistance, bloodstream infections, gram-negative rods.

## INTRODUCTION

Gram-negative bacteria are a broad term and are divided into two groups: The Enterobacteriaceae family is a heterogeneous group of bacteria that can be found all over the world. They account for over 80% of gram-neg bacterial isolates in people, producing a variety of diseases such as Meningitis, Pneumonia, Diarrhea, Urinary tract infections, Endotoxic shock, Sepsis, and many more. Among the bacteria that commonly infect people are: Escherichia, Enterobacter, Citrobacter, Yersinia, Klebsiella, Proteus, Salmonella, and Shigella<sup>1</sup>.

In comparison to Enterobacteriaceae, non-fermenter gram-negative bacilli have a lower frequency of isolation; yet, they are a significant group because they potentially cause, lethal infections, particularly in hospitals. In the Intensive care unit patients undergoing invasive operations, also trigger opportunistic illnesses. The most common gram-negative bacilli that cause illness in humans are: Alcaligenes, Moraxella, Stenotrophomonas, Pseudomonas aeruginosa, Burkholderiaceae, and Acinetobacter baumannii<sup>2</sup>.

ICUs (Intensive care units) were developed in sixties to care for physiologically unwell and critically sick individuals. These are built with high-technology devices and effective medications e.g., antimicrobials, which help stabilize the patient's clinical condition and treat the sickness, exposing the patients under their care to danger of acquiring hospital's pathogenic infections. The ICU setting is a center for the growth and propagation of antimicrobial-resistant bacteria due to its specific features. In hospitalized patients who acquired hospital infections, the death rate was 33.7%, compared to 14.5% in those who didn't get infections<sup>3</sup>.

The objective of the study was to evaluate gram-negative rods' prevalence and antibiotic resistance, which are responsible for bloodstream infections in ICU patients.

## METHODOLOGY

This cross sectional study was conducted at Lahore General Hospital, Lahore during from the period 1<sup>st</sup> July 2022 to 31<sup>st</sup> December 2022 Permission was granted by Institutional Ethical Review Board to start this research. A total of 200 patients in ICU suspected to have bloodstream infection. Patient in ICU and diagnosed with any other bacterial infection were excluded. Samples from all the patients admitted to the ICU were collected. Using a sterilized syringe take 05 ml of blood sample in blood culture bottles in a strict sterilized condition. Every sample was assigned a distinct identification number. Translucent protective cap off the bottle's lid took off with care to reveal the rubber seal. With alcohol or an iodized solution, disinfect the stopper. Take five milliliters of blood by using a syringe. With the needle, inoculate the blood through the rubber seal on the top of each bottle (approximately 5ml in bottle). Remove the transparent protective cap and replace it. All of the samples were tested for septicemia bloodstream infection by performing blood culture and antibiotic susceptibility testing. All the candidates were informed about their test results.

Place the heat-fixed smear on the staining tray. Flood smear carefully with crystal violet and set aside for 1 minute. To carefully clean the slide, tilt it slightly and fill a wash bottle with distilled water. Give the Gram's iodine one minute to absorb into the smear. To carefully clean the slide, tilt it slightly and fill a wash bottle with distilled water. A purple circle will show on the slide as a smear. Decolorize using 95% ethyl alcohol or acetone. Pour the alcohol drop by drop for five to ten sec, tilting the slide gently, until

Received on 05-01-2023

Accepted on 10-03-2023

the alcohol flows virtually clear. Take care not to decolorize too much. Rinse immediately with water. To counter-stain, slightly flood with safranin and then let stay for 45 sec. Gently tilt the slide and use a wash bottle to slightly clean it with distilled water. Let the slide dry. Examine the smear using an oil-immersion light microscope. Statistical analysis was performed by using SPSS Software.

## RESULTS

A total of 200 ICU patients were included. Out of 200 subjects, 104 were male and 96 were female. The detail of results is given in tables.

Table 1: Gram-negative Bacteria on Blood Agar

Frequency	Growth on Blood Agar	Suspected organisms
30(14.9%)	Mucoid, nonpigmented, and domed	Acinetobacter
17(8.5%)	Opaque shiny grey	Citrobacter
2(1%)	Large dull-grey	Enterobacter
7(3.5%)	Medium greyish smooth colonies	Escherichia coli
4(2%)	Beta-hemolytic grey colonies	Salmonella
13(6.5%)	Gamma-hemolytic grey colonies	Salmonella
25(12.5%)	Greyish Green colonies	Pseudomonas
37(18.5%)	Heavily mucoid Colonies	Klebsiella
13(6.5%)	Small raised, hemolytic colonies	Gram-Positive Bacteria
25(12.5%)	Mixed growth spots	Mixed Growth
27(13.4%)	-	No Growth

Table 2: Biochemical Tests for Different Bacterial Isolates

Bacterial Isolates	n	Biochemical Tests				
		Indole	Citrate	Urease	TSI	Motility
Acinetobacterspp	30	Negative	Positive	Negative	Positive	Negative
Citrobacterspp	17	Negative	Positive	Negative	Positive	Positive
Pseudomonas	37	Negative	Positive	Negative	Positive	Positive
Klebsiella	25	Negative	Positive	Positive	Negative	Negative
Salmonella	17	Negative	Positive	Negative	Negative	Positive
Escherichia coli	07	Positive	Negative	Negative	Positive	Positive
Enterobacter	02	Negative	Positive	Positive	Negative	Positive

Table 3: Percentage of Susceptible Gram-negative Bacteria from ICU

Bacterial Isolates (no.)	AK	FEP	CEF	CTX	CAZ	CIP	COR	GN	IMP	LEV	MEM	COL	DOX	TAZ	PIP+TAZ
Acinetobacter(30)	7	4	4	4	9	7	14	20	10	8	8	5	19	9	5
Citrobacter(17)	4	1	2	1	1	6	6	6	1	6	1	1	11	1	1
Pseudomonas (37)	14	17	11	9	11	14	11	11	20	16	18	9	10	14	15
Klebsiella (25)	7	7	8	2	1	6	6	6	10	6	7	9	4	1	2
Salmonella (17)	9	4	4	3	3	7	8	9	3	10	4	3	11	3	4
Escherichia coli(7)	4	4	3	1	1	1	3	3	5	1	2	1	2	4	1
Gram+ve Bacteria (13)	7	6	4	5	4	9	9	7	6	7	5	4	8	4	4
Enterobacter (2)	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0
All Isolates (148)	52	43	36	25	30	51	57	52	55	84	45	33	66	36	32

Table 4: Percentage of Resistant Gram-negative Bacteria from ICU

Bacterial Isolates(no.)	AK	FEP	CEF	CTX	CAZ	CIP	COR	GN	IMP	LEV	MEM	COL	DOX	TAZ	PIP+TAZ
Acinetobacter(30)	23	26	25	16	21	23	16	20	20	22	22	25	11	21	25
Citrobacter (17)	13	16	15	16	16	11	11	11	6	11	16	16	6	16	16
Pseudomonas(37)	11	8	14	16	14	11	14	14	5	9	7	16	15	11	10
Klebsiella(25)	24	24	23	29	30	25	25	25	21	25	24	22	27	30	29
Salmonella (17)	8	13	13	14	14	10	9	8	14	7	13	14	6	14	13
Escherichia coli(7)	3	3	4	6	6	6	4	4	2	6	5	6	5	3	6
Gram+veBacteria(13)	7	7	9	8	9	4	4	6	7	6	8	9	5	9	9
Enterobacter (2)	2	2	2	2	2	1	2	2	2	2	2	1	1	2	2
All Isolates	90	99	105	117	112	91	85	90	87	88	97	109	76	106	110

## DISCUSSION

Our study shows that among our participants we find an infectivity rate of 148(74%) that is positive cases out of 200 participants were identified for the presence of bloodstream infections caused by different gram-negative rods positive using blood culture and antibiotic susceptibility testing. In this study, all samples are taken from hospitalized infection patients in a public health care facility in Lahore.

It has been shown that using some antimicrobial drugs contributes to rising antibiotic resistance. According to a study conducted in Southwest China, *Escherichia coli* was the most commonly isolated bacteria responsible for bloodstream infections, responsible for 32.03%<sup>4</sup>. Consistent with previous studies, from 2011 to 2020, percentages of *E. coli* resistance to various antibiotics in China continued to remain stable<sup>5</sup>. According to this study, *Klebsiella pneumoniae* (18.5%) is the most prevalent pathogen responsible for bloodstream infections in critically ill patients.

Among all antibiotics, doxycycline had the highest rate (79%) and cefotaxime had the lowest rate (51.4%) of antibiotic resistance for all species of gram-negative rods. The highest rate of resistance for *Acinetobacter* was 17.5% of cefepime, for *Citrobacter* was 10.8% of cefepime, cefotaxime, ceftazidime, meropenem, colistin, tazobactam, and piperacillin + tazobactam, for *Pseudomonas* was 10.8% of cefepime and colistin, for *Klebsiella* was 20% of ceftazidime and tazobactam, for *Salmonella* was 20.7% of cefepime, cefotaxime, imipenem, colistin and tazobactam, for *E. coli* was 4.05% of cefotaxime, ceftazidime, imipenem, colistin, and piperacillin + tazobactam. According to a previous study, five hundred and seventeen different gram-negative bacterial strains in total were reported. Bronchial secretions were the most prevalent positive culture. In the PICU and NICU, *Pseudomonas aeruginosa* predominated, but *Escherichia coli* was frequent in the AICU. Generally, gram-negative bacteria showed high levels of resistance to the following drugs: Ceftazidime (73.19%), Cefazoline (75%), Aztreonam (75.23%), Ceftriaxone (77.41%), Cefotaxime (78.07%), Piperacillin (82.93%), Cefuroxime (84.17%) and Ampicillin (95.85%). The rates

of cephalosporin, fluoroquinolone, carbapenem, and penicillin resistance in gram-negative bacteria from various ICUs vary significantly<sup>6</sup>(Uc-Cachón et al., 2019).

In this study, colonial appearance on Blood agar shows *Acinetobacter* 30(14.9%), *Citrobacter* 17(8.5%), *Enterobacter* 2(1.0%), *Escherichia coli* 7(3.5%), *Salmonella* 4(2%), *Salmonella* 13(6.5%), *Pseudomonas* 25(12.4%), *Klebsiella* 37(18.5%) and gram-positive bacteria 13(6.5%). While (13.4%) show no growth and the remaining 25(12.5%) show mixed growth when cultured on the MacConkey and Blood agar.

In research conducted by Chaudhari et al<sup>7</sup> in 2022, out of 941 ICU samples, 322 were positive. The most isolated pathogen was *Klebsiella* (37.26%); results consistent with this study, followed by *E.coli* (16.45%), *Pseudomonas* (12.42%) and *Staphylococcus aureus* (7.45%). Antibiotics notably colistin (96.26%) and tigecycline (83.40%) were the most effective against Gram-negative bacteria, followed by carbapenems (71.79%), aminoglycosides (71.36%), and fluoroquinolones (67.2%).

On Blood agar, *Acinetobacter* showed mucoid, nonpigmented, and domed colonies, *Citrobacter* showed colorless but after 24 hours became pink, *Enterobacter* showed large, mucoid, and pink colonies, *Escherichia coli* showed pink, flat, dry, and non-mucoid colonies, *Salmonella* showed some convex, colorless, and serrated marginated colonies, and some convex, colorless, and serrated marginated colonies, *Pseudomonas* showed colorless, smooth, and flat colonies, *Klebsiella* showed heavily mucoid pinkish colonies and gram-positive bacteria showed some pinkish / others colorless colonies.

## CONCLUSION

The highest sensitivity was to doxycycline 66/148 isolates (44.5%) and the lowest sensitivity was to cefotaxime 36/148 isolates (24.2%). Out of 148 positive cases patients 70(35%) were male and 78 (39%) were females. Most positive cases were reported in age group of 16-25 years (33.3%). All the positive samples showed gram-negative rods on gram staining (100%). *Klebsiella* 37(18.5%) was the most prevalent pathogen found in blood samples of ICU patients.

**Conflict of interest:** Nil

## REFERENCES

- Oliveira, J., & Reygaert, W. C. (2019). Gram negative bacteria.
- Perry, J. D. (2017). A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. *Clinical Microbiology Reviews*, 30(2), 449–479.
- Costantini, M., Donisi, P. M., et al. (1987). Hospital acquired infections surveillance and control in intensive care services. Results of an incidence study. *European Journal of Epidemiology*, 3(4), 347–355.
- Yang, S., Xu, H., Sun, J. et al. (2019). Shifting trends and age distribution of ESKAPEc resistance in bloodstream infection, Southwest China, 2012–2017. *Antimicrobial Resistance & Infection Control*, 8(1), 1–10.
- Hu, F., Zhu, D., Wang, F., et al. (2018). Current status and trends of antibacterial resistance in China. *Clinical Infectious Diseases*, 67(suppl\_2), S128–S134.
- Uc-Cachón, A. H., Gracida-Osorno, C., Luna-Chi, I. G. et al. (2019). High prevalence of antimicrobial resistance among gram-negative isolated bacilli in intensive care units at a tertiary-care hospital in Yucatán Mexico. *Medicina*, 55(9), 588.
- Chaudhari, D. M., Bhavsar, H. K., Thummar, S. G. et al. (2022). Study of prevalence and antimicrobial susceptibility pattern of organisms isolated from various clinical specimen of the patients admitted in intensive care unit of tertiary care hospital. *National Journal of Physiology, Pharmacy and Pharmacology*, 13(3).