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Phenotypic detection of carbapenem-resistant Gram-negative bacteria from different clinical samples in a tertiary care hospital

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Abstract

Carbapenem-resistant gram-negative bacteria have emerged as a significant threat, contributing to both community-acquired and healthcare-associated infections that pose a serious risk to public health. This study was conducted to assess the prevalence and impact of carbapenem-resistant gram-negative bacteria isolated from clinical specimens at Hawassa University Comprehensive Specialised Hospital. By examining these pathogens, the research aims to shed light on the extent of this resistance within the hospital environment, highlighting the challenges posed by these formidable microorganisms in the fight against infectious diseases.

Methods: A total of 2,890 clinical samples were processed, yielding 1,293 instances of Gram-negative bacilli growth. From these, 220 carbapenem-resistant strains were

isolated and identified across diverse clinical specimens, including blood, urine, pus, endotracheal secretions, cerebrospinal fluid, body fluids, stool, and sputum. Of the 1293 GNB, 220 organisms exhibited resistance to carbapenem antibiotics, including imipenem and meropenem, as determined by the disc diffusion.

Results: Among 220 CRE, the most predominant organism was *Klebsiella spp.* with 118 isolates (53.7%), followed by *Escherichia coli*, with 35(15.9%), *Acinetobacter spp.*, with 28(12.7%), *Pseudomonas aeruginosa* with 26 isolates (11.8%) and *Enterobacter spp.*, 13(5.9%)

Conclusion: carbapenemase-producing Carbapenem-resistant Enterobacteriaceae (CP-CRE) spread easily in communities and cause significant morbidity and mortality. Early detection is vital in low-resource countries like India, where these carbapenemase producers are prevalent. Timely identification can improve treatment plans and infection control policies, preventing further community spread and shifting the paradigm of carbapenem resistance.

Introduction

Carbapenem-resistant gram-negative bacteria (CR-GNB) represent a growing challenge in both community-acquired infections and healthcare-associated infections (HCAs). These pathogens have developed resistance to carbapenems, a class of antibiotics commonly used as a last resort for treating severe bacterial infections. The emergence and spread of CR-GNB are concerning not only due to their ability to resist treatment but also because they can cause serious infections, including pneumonia, bloodstream infections, and urinary tract infections, which can lead to increased morbidity and mortality. The rise of these resistant organisms poses a significant threat to public health, as they are increasingly implicated in outbreaks within healthcare settings and can contribute to prolonged hospital stays, higher healthcare costs, and a greater risk of death. Preventing the spread and impact of CR-GNB necessitates urgent attention to antibiotic stewardship, infection control measures, and ongoing surveillance efforts.[1]

Carbapenems are a powerful class of bactericidal β -lactam antimicrobials known for their effectiveness against severe infections. These agents are particularly valuable in treating infections caused by bacteria that produce extended-spectrum β -lactamases (ESBLs), enzymes that confer resistance to many commonly used antibiotics. Due to their broad-spectrum activity and ability to effectively penetrate bacterial cell walls, carbapenems

play a critical role in managing life-threatening infections, particularly in cases where other treatments may fail. [2]

Drugs such as meropenem, ertapenem, and imipenem are commonly used to treat infections caused by multidrug-resistant bacteria and can be utilized for screening carbapenem resistance in bacteriology laboratories. Carbapenem resistance can be intrinsic or acquired through mutation or gene acquisition via horizontal gene transfer.[3]

Gram-negative bacteria, particularly those belonging to the Enterobacteriaceae family, represent a significant source of both community-acquired and hospital-acquired infections. These pathogens are commonly implicated in various serious conditions, including urinary tract infections, bloodstream infections, and lower respiratory tract infections. One of the alarming characteristics of these bacteria is their ability to acquire genes that confer a range of antibiotic resistance mechanisms, which include extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, and carbapenemases. [4]

β -Lactam antibiotics are often the cornerstone of treatment for these severe infections due to their effectiveness; however, carbapenems are frequently viewed as the last line of defence when other treatments fail. The emergence of resistance in these bacteria poses a daunting challenge for healthcare providers, as it limits the options for effective treatment and raises the stakes for patient outcomes. [5]

Carbapenem-resistant Enterobacteriaceae (CRE) pose a significant challenge in healthcare settings due to the increasing prevalence of infections caused by these bacteria that are resistant to carbapenems. This resistance complicates treatment options, making infections more difficult to manage and increasing the risk of severe patient outcomes. Understanding the mechanisms behind the emergence of CRE and its transmission is crucial for developing effective prevention and control strategies. [5]

Klebsiella species and Escherichia coli (E. coli) serve as key representatives of the Enterobacteriaceae family, a group of bacteria commonly found in the human gut microbiota. These bacteria play a crucial role in digestion and overall gut health. However, they have the potential to develop resistance to carbapenems, a class of potent antibiotics used to treat serious infections. This resistance can occur through various mechanisms, making these organisms a growing concern in the field of infectious diseases.

The goal of the research is to determine how often carbapenem-resistant gram-negative bacteria are among hospitalised patients and to characterise the resistance mechanism involved by phenotypic methods.

Materials and Methods

A total of 220 carbapenem-resistant *Enterobacteriaceae* were isolated from different clinical samples collected from outpatients and inpatients of Index Medical College, Hospital & Research Centre, Indore, M.P.

This observational study was conducted from October 2018 to October 2021, following approval from the ethics committee.

Study samples: Various clinical samples, including pus, urine, blood, body fluids, and endotracheal tubes, were processed using standard laboratory methods to isolate carbapenem-resistant *Enterobacteriaceae*. All isolates were subjected to antibiotic susceptibility testing per Clinical and Laboratory Standards Institute guidelines. [7]

Modified carbapenem inactivation methods(mCIM) were performed for Carbapenemase production in all isolated *Enterobacteriaceae*. [8]

Phenotypic methods used for confirmation of MBL production

1. **Double Disk Synergy Test (DDST):** This test was performed with an overnight broth culture of the test strain inoculated on the MHA plate and allowed to dry. 10 µl of the 0.5 M EDTA solution was added to a 6-mm blank filter paper disk (Whatman filter paper, no. 1), which contained approximately 750 µg of EDTA. A 10 µg imipenem disk was placed in the centre of the plate, flanked by an EDTA disk at a distance of 20 mm centre-to-centre from a blank disc containing 10 µl of 0.5 M EDTA (750 µg). After overnight incubation, the presence of an enlarged zone of inhibition towards the EDTA disk was interpreted as positive for an MBL producer [9]

2. **E-Test:** E-test Metallo-beta-lactamase strips consisted of a double-sided seven dilution range of imipenem IP (4 to 256µg/ml) and IP (1 to 64µg/ml) overlaid with a constant gradient of EDTA. Individual colonies were picked from overnight agar plates and suspended in 0.85% saline to a turbidity of 0.5 McFarland's standard. A sterile cotton swab was dipped into the suspension of the inoculum, and a lawn culture of the inoculum was performed on an MHA plate. The excess moisture was allowed to be absorbed for about 15 min before the E-test MBL (AB bioMerieux) strip was applied. Plates were incubated for 16 to 18 h at 37°C. The MIC endpoints were read where the inhibition ellipses intersected the strip. A reduction of imipenem MIC=3 by two-fold in the presence of EDTA will be interpreted as suggesting MBL production. [10]

Results

Table 1: Age-wise distribution of total carbapenem-resistant organisms

Age	Number	Percentage
0-12Months	49	22.2%
1-10Y	57	25.9%
11-20Y	34	15.45%
21-30Y	12	5.4%
31-40Y	19	8.6%
41-50Y	7	3.1%
51-60Y	29	13.18%
61-70Y	8	3.6%
71-80Y	3	1.3%
81Y-Above	2	0.9%
Total	220	100%

The prevalence of CRGNB is seen more in 1-10Y with 25.9%, followed by 0-12 M with 22.2%, and fewer isolates are from 71-80Y with 1.3%. [Table 1]

Table 2: Prevalence of Carbapenem resistant Gram-negative bacilli.

Organisms	Number	Percentage
<i>Klebsiella spp</i>	118	53.7%
<i>Escherichia coli</i>	35	15.9%
<i>Acinetobacter pp</i>	28	12.7%
<i>Pseudomonas aeruginosa</i>	26	11.8%
<i>Enterobacter spp.,</i>	13	5.9%

Out of 220 isolates, the most predominant organism was *Klebsiella spp.* with 118 isolates (53.7%), followed by *Escherichia coli*, with 35(15.9%), *Acinetobacter spp.*, with 28(12.7%), *Pseudomonas aeruginosa* with 26 isolates (11.8%) and *Enterobacter spp.*, 13(5.9%) [table 2]

Table 3: Isolation of CRGNB from different clinical samples

Organisms	Samples							
	Blood	CS F	Endotracheal Secretions	Pus & Wound	Sputum & Bal	Stool	Urine	Grand Total
<i>Acinetobacter spp.,</i>	13	1	8	4	1	1		28
<i>Ecoli</i>	6		6	2	4	11	6	35
<i>Enterobacter spp.,</i>	5		2	3	2		1	13
<i>Klebsiella spp.,</i>	27	1	27	26	23	4	10	118
<i>Pseudomonas aeruginosa</i>	3	1	6	12	2		2	26
GrandTotal	54	3	49	47	32	16	19	220

Table 4: Antibiotic susceptibility and resistance pattern of all 220 isolates of Enterobacteriaceae

Antibiotics	Resistant	Sensitive

Amikacin	73%	27%
Ceftazidime	73.5%	26.5%
Ceftriaxone	76%	24%
Cefepime	68.5%	31.5%
ciprofloxacin	66.5%	33.5%
Aztreonam	67.5%	23.5%
Meropenem	17%	83%
Imipenem	17%	83%
Pipracillin/Tazobactam	63.5%	36.5%
Colistin	0	100
Polymyxin B	0	100

The antibiotic sensitivity pattern amongst isolates. 76% were resistant to ceftriaxone, followed by 73.5% to ceftazidime, 73% to Amikacin, 68.5% to cefepime, 63.5% to Pipracillin/tazobactam, 66.5% to ciprofloxacin, and 17% to meropenem & imipenem. Colistin and Polymyxin B were found to be 100% sensitive, followed by meropenem & Imipenem 83%, Pipracillin/tazobactam 73(36.5%), cefepime 63(31.5%), ciprofloxacin 33.5%, amikacin 27%, ceftazidime 26.5%, and aztreonam 23.5%.

Discussion

Members of the Enterobacteriaceae family, including genera like *Escherichia*, *Klebsiella*, and *Enterobacter*, are commonly found in the gastrointestinal tracts of humans and animals. They play a vital role in gut health but can also harbour pathogenic strains. When they enter the environment through faecal contamination, they risk contaminating water and food, leading to the spread of infections. This is particularly concerning in healthcare settings where vulnerable populations may be exposed to antibiotic-resistant strains. Understanding how Enterobacteriaceae colonise and spread is essential for developing effective control strategies. [11]

Carbapenem-resistant Enterobacteriaceae (CRE) represent a major public health challenge globally. These multidrug-resistant organisms are increasingly recognized as a leading cause of healthcare-associated infections, especially in patients with weakened immune systems or those undergoing invasive procedures. The emergence of CRE is alarming due to the limited treatment options, which often result in high rates of morbidity and mortality among those infected.

Our study aimed to detect metallo-beta-lactamases in carbapenem-resistant clinical isolates of Enterobacteriaceae from clinical samples received at the hospital laboratory.

We are thrilled to announce the successful isolation of strains of carbapenem-resistant bacteria from patients across diverse hospital units, including the critical intensive care unit, the high-stakes surgical department, and general medicine wards. This significant discovery underscores the pressing need for rigorous surveillance and enhanced infection control measures as we confront this rising threat to patient health. Our findings hold the potential to inform future treatment strategies and significantly improve recovery outcomes for those affected. Together, we can meet this challenge head-on and foster a safer environment for every patient in our care.

A total of 220 isolates from the family Enterobacteriaceae were included in our study. Out of 220 Enterobacteriaceae isolates, the Majority were collected from male patients, with 131 isolates and 89 isolates from females, respectively.

This was in accordance with an observation by Offner et al. (1999, whose study proved that having a masculine gender increases the risk of serious surgical site infections. Another study by Esther and Edwin 2017) claimed males are 66.8% more likely to harbour carbapenem resistant bacteria. Gaynes and Edwards, in a 2005 study, highlighted the role of *Pseudomonas spp.*, *Klebsiella spp.*, and *Enterobacteriaceae* members in HAI. [12,13]

1-10years showed maximum prevalence (25.9%) of carbapenem resistance in our study, followed by 0-12Months with 22.2%. These studies present an upsetting drift of drug resistance in the pediatric age group, according to Averbuch et al. (2017). 22% of CRGNB was observed in critically ill children, whereas a high incidence in children was observed in Italy and Egypt, with 50-66% Caselli, 2016. [14]

24.5% of the CRGNB was isolated from blood samples, followed by endotracheal (ET) secretions (22.3%). Our study is similar to Loqman et al. (2021), in which the prevalence of CRGNB was isolated from blood samples at 56.4%. [15]

Members of the *Klebsiella spp.* were predominant in most of the samples considered, followed by *Escherichia coli*. This was in accordance with the observations made by Kang et al. (2005) and Loqman et al. (2021), with a predominance of 59% *Klebsiella*. [15,16]

A study conducted by the group showed *Escherichia coli* and *Klebsiella spp.* to be predominantly present in bloodstream infections. The predominant organisms isolated from sputum samples are *Klebsiella spp.*, with 71.8% which is comparable with the research of Sriram et al. (2018) (77.8%). The second predominant *Escherichia coli* strain was isolated more frequently from stool samples, at 68.8%, which is comparable to Ravikant et al. (2019), who reported 71.4%. [17] The overall CRE prevalence isolated from stool samples in our study is 6.8% which is comparable with the research of Ola et al., 2021, with 7.7%. Whereas according to Mohan et al. (2020), the prevalence rate of CRE in stool samples is reported to be high, with 18.1% which evidently specifies the need for bracing the antimicrobial stewardship in healthcare settings, and proper hand hygiene techniques should be promptly implemented, as these organisms are a budding cause of endogenous diseases. [18] Therefore, screening of CRE must be done to avoid this illness. Carbapenemase resistance was primarily investigated by a rapid and effective in-house Carba NP test (Rudresh et al., 2017). 207 of the 220 CRGNB showed the

production of carbapenemase within 2 hours with 94%. However, this value varies according to the geographical location. [19]

For example, the prevalence was found to be 36, 56, 19, 2.9 and 5.99 in Egypt (Makharita et al, 2020), Pakistan (Ain et al, 2018), Algeria (Bourafa et al.,2018), Ghana(Codjoe,2016) and Morocco (Mahrach et al., 2019). [20,21,22,23,24]

Conclusion

Carbapenemase-producing Carbapenem-resistant Enterobacteriaceae (CP-CRE) have a high tendency to spread within communities and are associated with significant morbidity and mortality. Early detection of CP-CRE is especially important in low-resource countries like India, which serves as a substantial reservoir for carbapenemase producers. Timely identification can help formulate better treatment plans to avoid therapeutic failures and design effective infection control policies, thereby preventing the further spread of carbapenemase producers in the community. This proactive approach can ultimately shift the paradigm of carbapenem resistance.

References:

1. WHO. Guidelines for the prevention and control of carbapenem- resistant Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. 2017
2. Codjoe FS, Eric S. Carbapenem resistance: a review. *Med Sci*. 2018;6 (1):1. doi:10.3390/medsci6010001
3. Hu FCS, Xu X, Guo Y, Liu Y, Zhu D, Zhang Y. Emergence of carbapenem-resistant clinical Enterobacteriaceae isolates from a teaching hospital in Shanghai, China. *J Med Microbiol*. 2012;61 (1):132–136. doi:10.1099/jmm.0.036483-0
4. . Gupta NLB, Patel J, Kallen A, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis*. 2011;53(1):60–67. doi:10.1093/cid/cir202.
5. LJal B. The problem of carbapenemase-producing carbapenem-resistant Enterobacteriaceae detection. *J Clin Microbiol*. 2016;JCM. 02771–02715.
6. Alemayehu, Asnake S, Azerefehn E, et al Phenotypic Detection of Carbapenem-Resistant Gram-Negative Bacilli from a Clinical Specimen in Sidama, Ethiopia: A Cross-Sectional Study. *Infection and Drug Resistance* 2021;14 369–380.

7. CLSI performance standard for antimicrobial susceptibility testing, 35th edition. CLSI document M100-S24. 2025
8. Pica˜o, R. C., S. S. Andrade, A. G. Nicoletti, E. H. Campana, G. C. Moraes, R. E. Mendes, and A. C. Gales. 2008. Metallo- β -lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. *J. Clin. Microbiol.* 46:2028–2037.
9. Bae IK, Suh B, Jeong SH, et al. Molecular epidemiology of *Pseudomonas aeruginosa* clinical isolates from Korea producing β -lactamases with extended-spectrum activity. *Diagn Microbiol Infect Dis.* 2014;79(3):373–377.
10. Srinivasan VB, Rajamohan G, Pancholi P, Stevenson K, Tadesse D, Patchanee P, Marcon M, Gebreyes WA. Genetic relatedness and molecular characterisation multidrug-resistant *Acinetobacter baumannii* isolated in central Ohio, USA. *Annals clinical microbiology and antimicrobials.* 2009;8(1):21.
11. Nordmann P, Poirel L, et al. The emerging NDM carbapenemases. *Trends Microbiol.* 2011;19(12):588-95. *Journal of Antimicrobial Chemotherapy.* 2003; 52:1.
12. Offner PJ, Moore EE, Biffl WL. Male gender is a risk factor for major infections after surgery. *The Archives of Surgery.* 1999; 134(9): 935-940
13. Esther J, Edwin D, Uma. Prevalence of carbapenem-resistant non-fermenting Gram-negative bacterial infection and identification of carbapenemase-producing NFGNG isolates by simple phenotypic tests. *Journal of clinical and diagnostic research.* 2017; 11: DC10-DC13
14. D.Averbuch, C. Avaky, M. Harit, P. Stephensky, I. Fried, T. Ben-Ami, et al. Non-fermentative Gram-negative rods bacteremia in children with cancer: a 14-year single-centre experience. *Infection*, 45(2017), 327-334
15. Loqman S, Soraa N, Diene SM, Rolain JM. Dissemination of Carbapenemases (OXA-48, NDM and VIM) Producing Enterobacteriaceae isolated from Mohamed VI University Hospital in Marrakech, Morocco. *Antibiotics (Basel)*. 2021;10(5):492. Published 2021 Apr 26
16. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, Oh M, Choe KW. Bloodstream infections caused by antibiotic-resistant Gram-negative bacilli: Risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrobial Agents and Chemotherapy.* 2005; 49(2): 760-766.
17. Ravi Kant, M. Jeya and A. John William FELIX, Faecal Carriage of Carbapenem-Resistant Enterobacteriaceae among Hospitalised Children in Chidambaram, India: Prevalence and Risk Factors. (2020). *Int J Pharm Sci.* 11(3), b119128 <http://dx.doi.org/10.22376>
18. Mohan, B., Prasad, A., Kaur, H., Hallur, V., Gautam, N., Taneja, N. Fecal carriage of carbapenem-resistant enterobacteriaceae and risk factor analysis in hospitalised patient: A single centre study from India. *Indian J. Med Microbiol.* 2017; 35, 555-562
19. Rudresh SM, Ravi GS, Sunitha L, Hajira SN, Kalaiarasan E, Harish BN. Simple, rapid and cost-effective modified Carba NP test for carbapenemase detection among Gram-negative bacteria. *Journal of Laboratory Physicians.* 2017; 9(4): 303-307
20. Makharia RR et al. Antibiogram and genetic characterization of carbapenem-resistant Gram-negative pathogens implicated in healthcare-associated infections. *Infection and drug resistance.* 2020; 13: 3991.

21. Ain NU, Iftikhar A, BukhariSS, et al. High frequencyand molecular epidemiology of metallo betalactamase producing Gram-negative bacilli in a tertiary care hospital in Lahore, Pakistan. Antimicrobial Infection and Resistance control. 2018; 7, 128.
22. Bourafa et al. Molecular characterization of carbapenem-resistant Gram-negative bacilli clinical isolates in Algeria. Infection and Drug resistance. 2018; 11: 735.
23. Bouza E, Munoz P. Monotherapy versus combination therapy for bacterial infections.
24. Codjoe FS. Detection and characterization of carbapenem-resistant Gram-negative bacilli infections in Ghana. <http://shura.shu.ac.uk/15577/>
25. Mahrach Y., et al. Phenotypic and molecular study of carbapenemase-producing Enterobacteriaceae in a regional hospital in northern Morocco. Journal of Clinical and Medical Science. 2019; 3: 113.