Section: Microbiology

Original Research Article

ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF CARBAPENEM-RESISTANT ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE ISOLATES AT A TERTIARY CARE HOSPITAL IN INDORE, INDIA

Manish Kumar Tiwari[1], Ramanath Karicheri[2], Himanshu Bhim Khatri[3]

[1]PhD scholar, Department of Microbiology, Index Medical Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[2]Professor, Department of Microbiology, Index Medical Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[3]Associate Professor, Department of Microbiology, Surabhi Institute of Medical Sciences, Siddipet, Telangana, India.

Received : 22/06/2023
Received in revised form : 04/08/2023
Accepted : 18/08/2023

Keywords:
Carbapenem-Resistant Enterobacteriaceae, Antibacterial Susceptibility, Antibiograms, Bacterial Sensitivity Tests, Escherichia coli, Klebsiella pneumoniae.

Corresponding Author:
Dr. Ramanath Karicheri,
Email: ramanath.karicheri@gmail.com

DOI: 10.47009/jamp.2023.5.4.348

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (4); 1744-1747

Abstract

Background: The injudicious use of carbapenems has led to the emergence of resistance in Enterobacteriaceae against these antibiotics. However, some isolates of carbapenem-resistant Enterobacteriaceae (CRE) are susceptible to older antibiotics and these antibiotics can be effectively used for the treatment of CREs. The aim of the study was to determine the antibiotic susceptibility profile of carbapenem-resistant Escherichia coli and Klebsiella pneumoniae isolates. **Materials and Methods:** Antibiotic susceptibility testing of carbapenem-resistant isolates of E.coli and K. pneumoniae was done according to CLSI guidelines. **Result:** A total of 156 carbapenem-resistant isolates were included in the study of which 96 were E.coli and 60 were K. pneumoniae. Of all the antibiotics used, only levofloxacin and amikacin had a susceptibility percentage of more than 50% for all the two isolates tested. Cephalosporins and β-lactam-β-lactamse inhibitor combinations had poor susceptibility for these isolates. **Conclusion:** Many isolates of E. coli and K. pneumoniae show good susceptibility to levofloxacin and amikacin, and the clinician should keep this in mind before starting empiric broad spectrum antibiotic therapy for these isolates. This could not only reduce the cost of treatment but also reduce the emergence of carbapenem-resistant strains.

INTRODUCTION

Carbapenems include beta-lactam antibiotics like imepenem, meropenem, ertapenem, and doripenem. They are usually considered as the last line of treatment for multidrug resistant (MDR) Gram negative bacteria.[1,2] However, lately, due to the injudicious use of carbapenems, the incidence of carbapenem resistant Gram negative bacteria is on the rise.[3] Moreover, antibiotic resistance can pass between bacteria through various genetic methods like horizontal gene transfer and has hence become a global threat.[4] Carbapenem-resistant Enterobacteriaceae (CRE) is defined as an isolate that has resistance to at least one of the carbapenems (ertapenem/meropenem/imipenem/doripenem) or there is documentation that the isolate possesses carbapenemase enzyme. For Enterobacteriaceae that have intrinsically elevated minimum inhibitory concentrations (MICs) to imipenem, resistance to carbapenems other than imipenem is required. This is a phenotypic definition given by the Centers of Disease Control (CDC), Atlanta in 2019.[5] CRE are broadly divided into two groups, namely CP-CRE and non-CP-CRE. CP-CRE stands for carbapenemase-producing CRE. These isolates produce the carbapenemase enzyme that is capable of hydrolyzing carbapenems. On the other hand, non-CP-CRE do not produce the carbapenemase enzyme but have other mechanisms of carbapenem resistance (like the possession of other β-lactamases with porin loss). CP-CRE has multiple carbapenemase genes that can be transferred horizontally to other bacteria. Thus, they have the highest potential to add to the global burden of antibiotic resistance.[6] These enzymes have emerged in practically all parts of the world. The commonly encountered organisms possessing carbapenemases include K. pneumoniae, E. coli, Pseudomonas spp., Acinetobacter sp., Citrobacter spp., and Enterobacter spp.[7] Carbapenemases are classified molecularly into three classes (A, B, and D). K. pneumonia carbapenemase (KPC) belonging to class A, New Delhi metallo β-lactamase (class B), and OXA48 (class D) are the most common carbapenemases produced by the Enterobacteriaceae family.[8,9] Many carbapenemase

ISSN (O): 2687-5365; ISSN (P): 2753-6556

enzymes confer resistance to almost all beta-lactam agents, including penicillins, cephalosporins, monobactams, and carbapenems.[7,8] Most are also resistant to beta-lactamase inhibitors. However, some carbapenemases are less active and require additional mechanisms to exhibit resistance.[7] A brief summary of the different carbapenemases is given in table 1.[10] Carbapenemase enzyme detection can be done phenotypically or genotypically using molecular techniques.[11,12] Phenotypic tests to identify CP CRE include the modified Hodge test (MHT), the Carba NP test and its variants, and the carbapenem inactivation method (CIM). All these phenotypic tests can detect carbapenemase production but the specific carbapenemase type produced cannot be identified.[13] Specific carbapenemase enzyme genes can be detected by molecular methods like the polymerase chain reaction (PCR).[14] Some strains of carbapenem resistant Gram negative bacteria may be susceptible to older non-carbapenem agents like gentamicin, amikacin, ciprofloxacin, cotrimoxazole, ampicillin-sulbactam, cefepime, minocycline, etc.[15-20] However, the susceptibility profiles are not predictable for most carbapenem-resistant Gram-negative bacteria, and therefore antibiotic susceptibility testing results should be used to guide the selection of any of these older agents. The aim of this study was to determine the antimicrobial susceptibility pattern of carbapenem resistant E.coli and Klebsiella pneumoniae. Isolates at our tertiary care hospital so as to guide the clinicians to make better treatment decisions for their patients.

MATERIALS AND METHODS

Study Design: Prospective laboratory-based observational study.
Study Setting: Department of Microbiology at a tertiary care hospital in Indore, India.
Study Period: December 2020 to December 2022
Ethical Consideration: Before the commencement of the study, clearance from the institutional ethics committee (IEC) was taken (IEC approval letter No: MU/Research/EC/Ph.D./2020/57). The study subjects were explained in detail the purpose of the study and were assured confidentiality of their identity. Written informed consent was taken from all the patients before collecting their samples.
Study population: All patients admitted in the hospital wards and ICUs or visiting the outpatient department of the hospital.
Sampling: All consecutive, non-duplicate samples were included till the sample size was met.
Inclusion criteria
Isolates of Escherichia coli, and Klebsiella pneumoniae isolates that were resistant to either ertapenem or meropenem. The breakpoint for determining resistance was equal to or less than 18 mm and 19 mm for ertapenem (10 µg) and meropenem (10 µg), respectively.
Exclusion Criteria
Isolates of Escherichia coli, Klebsiella pneumoniae isolates that were intermediate or susceptible to ertapenem and meropenem, and other Gram negative bacteria
Methodology
Clinical samples such as urine, pus, sputum, endotracheal aspirate (ETA), bronchoalveolar lavage (BAL), and blood were collected aseptically as per the standard operating procedure (SOP). Patients of all age groups were included in the study. Various non-selective and selective media were used for the isolation of organisms. Escherichia coli Klebsiella pneumoniae were identified based on conventional biochemical testing. The isolates that were resistant to either meropenem or ertapenem both were subjected to antibiotic susceptibility testing by the Kirby-Bauer disk diffusion method as per CLSI guidelines prevalent at the time. The antibiotics used for susceptibility testing were ampicillin (AMP):10µg, cefuroxime (CXM): 30µg, cefotaxime (CTX):30µg, ceftriaxone (CTR):30µg, cefepime (CPM): 30µg, piperacillin-tazobactam (PIT): 100/10µg, ciprofloxacin (CIP):5µg, levofloxacin (LE): 5µg, gentamicin (GE): 10µg, tobramycin (TOB): 10µg, amikacin (AK): 30µg, tetracycline (TE):30µg, doxycycline (DO): 30µg, nitrofurantoin (NIT):300µg, fosfomycin (FOS): 200µg, and cotrimoxazole (COT): 25µg.

RESULTS

Table 1: Classification of carbapenemases; KPC, K. pneumoniae carbapenemase; NDM: New Delhi Metallo‑β‑lactamase; OXA-48, Oxacillinase-48

Class	A common example in Enterobacteriaceae	Hydrolysis profile	Inhibition profile
Serine carbapeneases (Class A)	KPC	Carbapenems, oxyimino‑β‑lactams, Aztreonam, Cephamycins	Late cephalosporins, Clavulanate
Metallo-β-lactamases (Class B)	NDM	Carbapenems, Penicillins, Cephalosporins	Aztreonam, EDTA
Oxacillin-active carbapenemases (Class D)	OXA-48	Carbapenems, Penicillins, Oxacillin	Clavulanate, Aztreonam, EDTA

Table 2: Susceptibility percentage of E. coli, K. pneumonia isolates to antibiotics

Antibiotics	E. coli (n=96)	K. pneumoniae (n=60)
Ampicillin	54.39%	48.33%
Cefuroxime	23.46%	28.33%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Cefotaxime	8.52%	6.66%
Ceftriaxone	12.79%	30.00%
Cefepime	25.59%	38.33%
Piperacillin-tazobactum	20.26	6.66%
Ciprofloxacin	55.45%	26.66%
Levofloxacin	72.52%	68.33%
Gentamicin	40.53%	71.66%
Tobramycin	26.65%	53.33%
Amikacin	50.13%	73.33%
Tetracycline	25.59%	16.66%
Doxycycline	41.59%	41.66%
Nitrofurantoin	70.39%	21.66%
Fosfomycin	44.79%	25%
Cotrimoxazole	30.93%	46.66%

Table 3: Comparison of susceptibility percentages of isolates of E. coli.

Antibiotics	**E. coli**	
	Present study (n=96)	**Hamze et al. (n=104) [21]**
Ampicillin (10 µg)	54.39%	0%
Cefuroxime (30 µg)	23.46%	16.30%
Cefotaxime (30 µg)	8.52%	19.2%*
Ceftriaxone (30 µg)	12.79%	not tested
Cefepime (30 µg)	25.59%	30.80%
Piperacillin-tazobactum (100/10 µg)	20.26	3.8%†
Ciprofloxacin (5 µg)	55.45%	25%
Levofloxacin (5 µg)	72.52%	43.30%
Gentamicin (10 µg)	40.53%	62.50%
Tobramycin (10 µg)	26.65%	52.90%
Amikacin (30 µg)	50.13%	92.30%
Tetracycline (30 µg)	25.59%	10%‡
Doxycycline (30 µg)	41.59%	not tested
Nitrofurantoin (300 µg)	70.39%	67.8%§
Fosfomycin (200 µg)	44.79%	92.30%
Cotrimoxazole (25 µg)	30.93%	32.70%
* cefotaxime (5 µg), †piperacillin-tazobactum (30/4 µg) ‡ tetracycline (15 µg) §nitrofurantoin (100 µg)		

Table 4: Comparison of susceptibility percentages of isolates of Klebsiella pneumoniae.

Antibiotics	**Klebsiella pneumoniae**	
	Present study (n=60)	**Hamze et al. (n=25) [21]**
Ampicillin (10 µg)	48.33%	0%
Cefuroxime (30 µg)	28.33%	12%
Cefotaxime (30 µg)	6.66%	20%*
Ceftriaxone (30 µg)	30.00%	not tested
Cefepime (30 µg)	38.33%	20%
Piperacillin-tazobactum (100/10 µg)	6.66%	4%†
Ciprofloxacin (5 µg)	26.66%	40%
Levofloxacin (5 µg)	68.33%	44%
Gentamicin (10 µg)	71.66%	48%
Tobramycin (10 µg)	53.33%	56%
Amikacin (30 µg)	73.33%	72%
Tetracycline (30 µg)	16.66%	25%‡
Doxycycline (30 µg)	41.66%	not tested
Nitrofurantoin (300 µg)	21.66%	36%§
Fosfomycin (200 µg)	25%	52%
Cotrimoxazole (25 µg)	46.66%	40%
*cefotaxime (5 µg), †piperacillin-tazobactum (30/4 µg) ‡ tetracycline (15 µg) §nitrofurantoin (100 µg)		

A total of 156 isolates were included in the study of which 96 were E. coli and 60 were Klebsiella pneumoniae. The antibiotic susceptibility pattern of various isolates is shown in [Table 2]. Of all the antibiotics used, only levofloxacin and amikacin had a susceptibility percentage of more than 50% for all these two isolates. Furthermore; susceptibility of amikacin was higher for Klebsiella pneumoniae, 73.33% for K. pneumoniae and 52.22% for Escherichia. Coli. Gentamicin had a good susceptibility for carbapenem-resistant Klebsiella pneumoniae 71.66% but not for Escherichia coli (40.53%). On the other hand, ampicillin, ciprofloxacin and nitrofurantoin had a good

ISSN (O): 2687-5365; ISSN (P): 2753-6556

susceptibility for carbapenem-resistant Escherichia coli (54.39%, 55.45% and 70.39%, respectively) but not for Klebsiella pneumonia. Cephalosporins and β-lactam-β lactamase inhibitor (BLBLI) combinations had poor susceptibility against carbapenem-resistant isolates.

DISCUSSION

The present study details the susceptibility pattern of carbapenem-resistant isolates of Escherichia coli and Klebsiella pneumoniae at a tertiary care hospital in Indore, India. We could come across only one similar study by Hamze et al,[21] that had a sufficient sample size for comparison with our study. The comparison is detailed in [Table 3] and [Table 4] for E. coli and Klebsiella pneumoniae., respectively. For Escherichia coli, amikacin and nitrofurantoin were the only antibiotics that had a susceptibility percentage of more than 50% in both studies. In our study, susceptibility for fluoroquinolones was much higher (55.45% for ciprofloxacin and 72.52% for levofloxacin) than in the study by Hamze et al. (25% for ciprofloxacin and 43.3% for levofloxacin). In both studies, levofloxacin was more effective than ciprofloxacin. The study by Hamze et al. had a higher susceptibility for aminoglycosides (62.5%, 52.9%, and 92.3% for gentamicin, tobramycin and amikacin, respectively) as compared to our study (40.53%, 26.65% and 50.13% for gentamicin, tobramycin and amikacin, respectively). In both studies, amikacin was more effective than gentamicin and tobramycin. The susceptibility of cotrimoxazole was similar in both studies.

For Klebsiella pneumoniae amikacin was the only antibiotic that had a susceptibility percentage of more than 50% in both the studies. In both studies, almost two-thirds of isolates of Klebsiella pneumoniae were susceptible to amikacin. Here again, amikacin was more effective than gentamicin and tobramycin. The susceptibility for levofloxacin was higher in our study (68.33%) as compared to the study by Hamze et al. (44%). Nitrofurantoin was not as effective in both studies for Klebsiella pneumoniae as it was for Escherichia coli. The susceptibility of cotrimoxazole was also similar for Klebsiella pneumoniae in both studies.

Cephalosporins and beta-lactam-beta-lactamase inhibitor combinations had poor susceptibility for isolates of Escherichia coli and Klebsiella pneumoniae in both studies.

CONCLUSION

Many isolates of E. coli and Klebsiella pneumoniae. show good susceptibility to all older antibiotics, especially levofloxacin and amikacin, and the clinician should keep this in mind before starting empiric broad spectrum antibiotic therapy for these isolates. This could not only reduce the cost of treatment but reduce the emergence of carbapenem-resistant strains.

REFERENCES

1. Hu F, Chen S, Xu X, et al. Emergence of carbapenem resistant clinical Enterobacteriaceae isolates from a teaching hospital in Shanghai, China. J Med Microbiol.2012;61(Pt 1):132–136.
2. Sidjabat H, Nimmo GR, Walsh TR, et al. Carbapenem resistance in Klebsiella pneumoniae due to the New Delhi Metallo ß-lactamase. Clin Infect Dis.2011; 52(4):481–484.
3. Bakr AF. Intravenous lines related sepsis in newborn babies admitted to NICU in a developing country. J Trop Pediatr. 2003;49:295 7.
4. Read AF, Woods RJ. Antibiotic resistance management. Evol Med Public Health.2014;2014:147.
5. Center for Disease Control and Prevention. Carbapenem-resistant Enterobacterales. [Internet] [Updated 2019 November 22; Cited 2023 February 28]. Available from: https://www.cdc.gov/hai/organisms/cre/technical-info.html
6. NordmannP, NaasT, PoirelL. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis.2011;17:1791-8
7. Pitout JD. The latest threat in the war on antimicrobial resistance. Lancet Infect Dis.2010; 10:578-579.
8. Souli M, Galani I, Antoniadou A, et al. An outbreak of infection due to beta-lactamase Klebsiellapneumoniae carbapenemase-2 producing K. pneumoniae in a Greek University Hospital: molecular characterization, epidemiology, and outcomes. Clin Infect Dis.2010;50(3):364–373
9. Glasner C, Albiger B, Buist G, et al. European survey on carbapenemase-producing Enterobacteriaceae (EuSCAPE) working Group: carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill. 2013;18(28):20525
10. Queenan AM, Bush K. Carbapenemases: The Versatile β‐lactamases. Clin Microbiol Rev. 2007; 20:440‐58.
11. Van Dijk K, Voets GM, Scharringa J, et al. Disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae using phenyl boronic acid, dipicolinic acid and temocillin. ClinMicrobiol Infect.2014; 20(4):345–349.
12. Arend LN, Pilonetto M, Siebra CA, et al. Phenotypic and molecular characterization of 942 carbapenem-resistant Enterobacteriaceae (CRE) in southern Brazil. J Infect Chemother.2015; 21(4):316–318.
13. Lutgring JD, Limbago BM. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacteriaceae detection. J Clin Microbiol.2016; 54:529-34.
14. Bhatt P, Tandel K, Das NK, Grover N, Ranjan P, Rathi KR. Phenotypic detection and molecular characterization of carbapenem-resistant Enterobacteriaceae at a tertiary care center. J Mar Med Soc.2022; 24:S40-6.
15. Gonzalez-Padilla M, Torre-Cisneros J, Rivera-Espinar F, et al. Gentamicin therapy for sepsis due to carbapenem-resistant and colistin-resistant Klebsiella pneumoniae. J AntimicrobChemother.2015; 70:905–13.
16. Qureshi ZA, Hittle LE, O'Hara JA, et al. Colistin-resistant Acinetobacter baumannii: beyond carbapenem resistance. Clin Infect Dis.2015; 60:1295–303.
17. Falagas ME, Skalidis T, Vardakas KZ, Legakis NJ; Hellenic Cefiderocol Study Group. Activity of cefiderocol (S-649266) against carbapenem-resistant gram-negative bacteria collected from inpatients in Greek hospitals. J Antimicrob Chemother.2017; 72:1704–8.
18. Wei C, Ni W, Cai X, Zhao J, Cui J. Evaluation of trimethoprim/sulfamethoxazole (SXT), minocycline, tigecycline, moxifloxacin, and ceftazidime alone and in combinations for SXT-susceptible and SXT-resistant Stenotrophomonas maltophilia by in vitro time-kill experiments. PLoS One.2016; 11:e0152132.
19. Wang YL, Scipione MR, Dubrovskaya Y, Papadopoulos J. Monotherapy with fluoroquinolone or trimethoprim-sulfamethoxazole for treatment of Stenotrophomonas maltophilia infections. Antimicrob Agents Chemother. 2014; 58:176–82. 33.
20. Cho SY, Kang CI, Kim J, et al. Can levofloxacin be a useful alternative to trimethoprim-sulfamethoxazole for treating Stenotrophomonas maltophilia bacteremia? Antimicrob Agents Chemother.2014; 58:581–3.
21. Hamze N, Osman M, Mallat H, Nasr S, Raad EB, Achkar M. Epidemiology and antibiotic susceptibility patterns of carbapenem-resistant Gram negative bacteria isolated from two tertiary care hospitals in North Lebanon. The International Arabic Journal of Antimicrobial Agents.2018; 8(23):1-8.

ISSN (O): 2687-5365; ISSN (P): 2753-6556