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Original Research Article

Prevalence of Carbapenemase producing *Klebsiella pneumoniae* in Hospital acquired infections and Community acquired infections: A comparative study from a tertiary care hospital

Meera Rajeev Nair[1], Madhumati Jagadish Patil[2*]

[1]Dept. of Microbiology, Jawaharlal Nehru Medical College, KAHER University, Belagavi. Karnataka, India

Abstract

Background: The incidence of carbapenem-resistant bacteria causing community-acquired and healthcare-associated infections has significantly increased globally due to the irrational use of antibiotics. Among the Enterobacteriaceae family, primarily *Klebsiella pneumoniae*, are most commonly responsible for deadly infections. Carbapenems are the last-resort medications for treating drug-resistant Gram-negative bacterial infections. But recently, it has been observed that there has been an increase in the prevalence of carbapenem-resistant Klebsiella species resulting in treatment failure. Hence, it is imperative that a suitable and economical phenotypic method to be used to identify *Klebsiella pneumoniae* that produce carbapenemase in order to prevent infections from spreading further.

Materials and Methods: A hospital-based cross-sectional study was carried out in Microbiology laboratory. *Klebsiella pneumoniae* was isolated from various clinical sample and were classified using a thorough clinical history into hospital-acquired and community-acquired infections. Antibiotic susceptibility testing (AST) was performed by the Kirby–Bauer disc diffusion method. The Modified Carbapenemase Inactivation Test (m-CIM) was performed to detect carbapenemase producers.

Results: A total of 123 *Klebsiella pneumonia* were isolated from various clinical sample. Out of which, 71(58%) were associated with Hospital acquired infection, 52(42%) were causing Community acquired infections and 33(27%) were positive for m -CIM test. Tigecycline showed the highest sensitivity 46(89%), followed by meropenem and imipenem 45(87%) in Community acquired infections. Carbapenemase producing *K. pneumonia* showed higher resistance to Ampicillin (100%) and Piperacillin –Tazobactam (100%).

Conclusion: The rate of carbapenemase production was high in the *K. pneumoniae* isolates. Tigecycline could be the drug of choice for the empirical treatments of carbapenemase-producing *K. pneumoniae*. Our study provides a better understanding of antibiotic resistance threat and enables physicians to select the most appropriate antibiotics.

Keywords: Hospital infection, Community acquired infection, Carbapenemase, Klebsiella pneumonia, Carbapenems, Bacterial antibiotic resistance.

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1. Introduction

Globally, the number of multi-drug-resistant organisms that cause healthcare-associated infections (HAI) and community-acquired infections (CAI) are increasing rapidly, posing a serious threat to society and public health.[1] The main cause of the situation is the increased abuse of antibiotics in veterinary care, agriculture, and human medicine.[1] Bacteria that cause hospital-acquired illnesses or diseases in the community are becoming alarmingly more resistant to antibiotics. Among them, Enterobacteriaceae, especially *Klebsiella pneumoniae*, are most commonly responsible for deadly diseases.[2]

Organisms that produce Ampicillin cephalosporinase (AmpC) and Extended-spectrum β-Lactamase (ESBL) are resistant to all β-Lactams except carbapenems. Therefore, carbapenems are frequently the preferred treatment for infections caused by these organisms. But recently, it has been found that there is an increase in carbapenem-resistant Klebsiella species resulting in treatment failure *Klebsiella pneumoniae* is an essential source of transferable antibiotic resistance.[3] Clinical manifestation of this organism can go

*Corresponding author: Madhumati Jagadish Patil
Email: drmadhumatipatil@gmail.com

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from colonization of the skin and mucous membrane to severe life-threatening infections, leading to substantial mortality and morbidity.[4]

Identifying the risk factors for colonization by Carbapenem-Resistant Enterobacterales (CRE) is crucial for both clinical and epidemiological understanding. Key risk factors include extended hospital stays, immunosuppression, prior surgeries, invasive procedures, and long-term use of broad-spectrum antibiotics.[5] Although the transmission of CRE within healthcare settings is well established, the occurrence and clinical significance of CRE infections that originate in the community remain poorly understood.[6]

Carbapenem resistance pose a treatment challenge to physicians. Therefore, a major clinical and public health concern is the emergence and dissemination of *Klebsiella pneumoniae* that is resistant to carbapenem. The increase in antibiotic resistance has multiple underlying causes. Lack of proper antibiotic stewardship contributes significantly to the emergence of resistance by causing misuse of antibiotics, improper empirical coverage, delays in accurate diagnosis, and de-escalation therapy. As time goes on, there are fewer and fewer antimicrobials available that are effective in treating these infections, and so, the problem further escalates.[7]

Thus, to reduce the increase in hospital and community-acquired infections, screening and identification of carbapenemase producers, as well as the selection and application of suitable treatment plans, are crucial. Though molecular techniques are considered as gold standard they may not be able to identify unknown carbapenemase genes that are not included in the gene panel. Biochemical assays based on a technique to detect the hydrolysis of a carbapenem's β lactam rings have been developed in order to overcome all of these limitations.

Resistant strains are isolated from hospitals and communities worldwide, along with the increase in the incidence of organisms producing Carbapenemase enzymes; this leads to limited treatment options. Hence it is of the highest priority to know the prevalence of these pathogens to formulate treatment policies.

The current study was conducted to determine the prevalence of Carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) among infections acquired in hospitals and the community.

2. Materials and Methods

In the present study 123 distinct *K. pneumoniae* clinical isolates from a range of clinical samples were examined. Only one isolate per patient was considered. The samples were collected between January and December 2020. Infections were categorised as Hospital Acquired (HAI) or Community Acquired (CAI) depending on the following criteria

2.1. Criteria for HAIs[8]

Patients who satisfy the following criteria were included in this category

1. Infection acquired in a facility by a patient admitted for reasons other than the infection in the context.
2. The infection should not be in the incubation period, or no symptoms should be present when getting admitted.
3. Symptoms appearing after 48hrs of admission or within 30 days of leaving the facility.

2.2. Criteria for CAI

They are contracted outside of a health care facility or diagnosed within 48 hours of admission without any previous encounter.

Classification of isolates into a community-acquired infection (CAI), Healthcare-associated infections (HAI) was done based on taking a detailed history of the patient regarding previous hospital stay, any antibiotic regimen taken during the stay, and any recurrent hospital admissions.

Antibiotic sensitivity testing was done on Mueller Hinton Agar by Kirby Bauer disc diffusion method according to CLSI guideline.[9] All *Klebsiella pneumoniae* isolates were screened for carbapenemase production using Ertapenem (10µg) on Mueller Hinton agar. Then it was confirmed for Carbapenemase production using a Modified Carbapenemase Inactivation Test (mCIM).

2.3. Modified carbapenemase inactivation test (mCIM) [10]

For the mCIM test, each isolate which was resistant to Ertapenem was emulsified (1µl) in two ml of Tryptic Soy Broth (TSB) and Meropenem disc (10µg) was placed in the broth and incubated at 35°C, 4 hours ± 15 minutes. After the required time, the meropenem disc was taken out from TSB and placed on a Mueller Hinton agar, freshly inoculated with 0.5 McFarland suspension of Carbapenemase sensitive E.coli ATCC 25922 strain. The inoculated plates were incubated at 35°C for 18- 24hrs, interpretation of the result was made according to CLSI guidelines 2019.[9]

1. Carbapenemase positive: zone size 6 – 15mm
2. Carbapenemase negative: zone size >19mm
3. Carbapenemase intermediate: 16 – 18mm.

2.4. Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 16. Chi-square test at 95% confidence interval (CI) was performed to evaluate the association between antibiotic sensitivity of CAI and HAI.

3. Results

Among the sixty-four female (n=64, 52%) and fifty-nine male (n=59, 48%) patients who were included in the study,

42(59%) female and 34(48%) male patients had a HAI. Most of the participants were 44(35%) between 1-30 years, followed by 37(30%) between 31-60 years. Among 18(14%) neonates below 23 days, 16(22%) had a hospital-acquired neonatal infection. *Klebsiella pneumoniae* was commonly isolated from wound swabs 43(35%) with 27(38%) samples from HAI mainly linked to surgical site infection (SSI) followed by 34(27%) urine samples and 16(23%) were linked to catheter-associated urinary tract infection (CAUTI). The majority of the blood samples 12(17%) were also linked to HAIs, mainly causing neonatal bloodstream infections.

The **Table 2** shows the pattern of antibiotic sensitivity to infections acquired in hospitals and the community. The sensitivity to ampicillin (p=0.019), cefotaxime (p=0.002), levofloxacin (p<0.001), nitrofurantoin, and fosfomycin was clearly lower in HAIs than that of community-acquired infections when the two groups were compared. In contrast, HAIs had higher sensitivity to tobramycin, gentamicin, imipenem, meropenem and tigecycline.

Table 3 indicates that most CP-KP 30(42.2%) cases are linked to healthcare settings, where as smaller proportion of CP-KP 3(5.7%) infections occur in community.

Higher Resistance was noted in CP-KP, Ampicillin (100%), Cefotaxime (100%), Piperacillin-Tazobactam (100%). Tobramycin and Gentamicin show high resistance (above 60%), highlighting a pattern of resistance against common aminoglycosides. (**Figure 1**) Non CP-KP shows lower resistance to many of the antibiotics compared to CP-KP. Imipenem (16%) and Meropenem (14%) resistance suggests that these carbapenems are still effective against Non CP-KP, though some resistance is present.

Table 1: Comparison of Hospital-acquired infection (HAI) and Community-acquired infection (CAI) based on demographic features and samples

Variables		**Hospital Acquired Infection n= 71**	**Community Acquired Infection n= 52**
Gender	Male n=59	34 (48%)	25(48%)
	Female n=64	42(59%)	24(46%)
Age	1-23 days n=18	16(22%)	2(4%)
	1-30 yrs n=44	24(34%)	20(38%)
	31-60yrs n=37	19(27%)	18(35%)
	61-90yrs n=24	12(17%)	12(23%)
Sample	Wound Swab n=43	27(38%)	16(31%)
	Urine n=34	16(23%)	18(35%)
	Sputum n=21	11(15%)	10(19%)
	Blood n=15	12(17%)	3(6%)
	Ear Swab n=3	--	3(6%)
	Tracheal Aspirate n=5	5(7%)	--
	Pleural Fluid n=1	--	1(2%)
	Mothers milk n=1	--	1(2%)

Table 2: Comparison of antibiotic sensitivity pattern of *Klebsiella pneumoniae* causing HAI & CAI

Antibiotics	**Sensitivity HAI (n-71)**	**Sensitivity CAI (n-52)**	**Chi-Sq(X2)**	**p value**
Ampicillin	4(6%)	10(19%)	5.505	0.019
Tobramycin	29(41%)	43(83%)	21.659	<0.001
Gentamycin	31(44%)	44(85%)	21.157	<0.001
Amoxiclav	27(38%)	24(46%)	0.817	0.366
Cefotaxime	20(28%)	29(46%)	9.54	0.002
Imipenem	34(48%)	45(87%)	19.517	<0.001
Meropenem	32(45%)	45(87%)	22.047	<0.001
Ertapenem	32(45%)	47(90.3%)	22.36	<0.001
Piperacillin-Tazobactam	22(31%)	38(73%)	21.284	<0.001
Tigecycline	36(54%)	46(89%)	20.17	<0.001
Levofloxacin	26(36%)	30(48%)	9.56	<0.001
Nitrofurantoin	4(44%)	17(71%)	18.715	<0.001

Table 3: Detection of Carbapenemase producing *Klebsiella pneumoniae* by m -CIM

Type of Infection	Ertapenem Sensitive	Ertapenem Resistant	m-CIM positive
HAI	32 (45%)	39(54%)	30(42.2%)
CAI	47 (90.3%)	5(9.6%)	3(5.7%)
Total	**123**	**44(35.7%)**	**33(27%)**

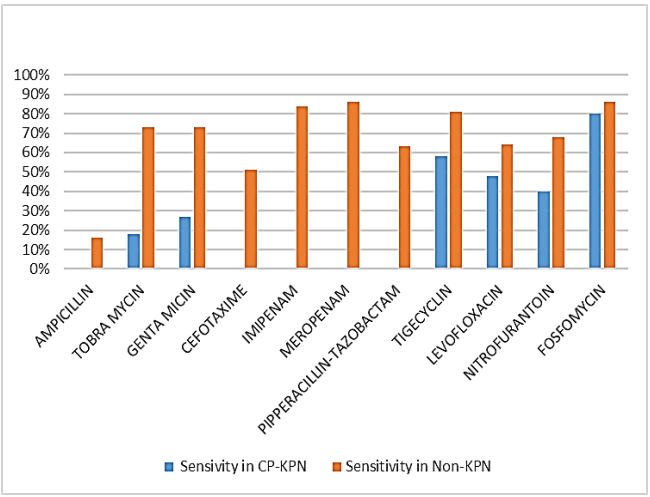

Figure 1: Antibiotic sensitivity in carbapenemase and non carbapenemase producing Klebsiella pneumonia

4. Discussion

The prevalence of Carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) can vary significantly across geographical regions and healthcare settings, highlighting the need for localized surveillance. In our study, 27% of the 123 *K. pneumoniae* isolates were confirmed as CP-KP, which is consistent with the 33% prevalence reported by Nair *et al.* in Mumbai. This finding emphasizes a concerning but relatively stable trend in CP-KP prevalence in urban Indian settings.[11]

Contrasting our results, the study conducted in Tumkur, Karnataka, reported a higher prevalence of 42.5%. However, it is important to note that their methodology included all Enterobacteriaceae organisms and relied solely on the Modified Hodge Test for detecting Carbapenemase production.[12] The recent CLSI 2019 guidelines recommend the mCIM test, which has improved sensitivity and specificity, thus potentially explaining the discrepancies in findings between studies.[9]

Age distribution analysis revealed that the most affected demographic was individuals aged 1-30 years 44(35%) followed by 37(30%) between 31-60 years. This aligns with findings from Thomas *et al.*, suggesting that younger adults are particularly vulnerable to CP-KP infections. Furthermore, our results indicate a substantial burden of healthcare-associated infections (HAI), with 58% of isolates categorized as HAI, further reinforcing the notion that nosocomial settings are hotspots for multidrug-resistant organisms. This finding is consistent with studies by Koteb *et al.*[13] However, the 95% prevalence of CP-KP in nosocomial infections reported by Veeraraghavan *et al.* underscores the variable nature of these infections and highlights the need for continued monitoring.[1]

The distribution of CP-KP among various clinical samples indicated that wound swabs (35%) were the most common source, particularly from surgical site infections (38%), highlights the role of this pathogen in postoperative complications, followed by urine samples (23%) with 23% linked to catheter-associated urinary tract infections (CAUTI), underlines the importance of catheter care. These results corroborate findings from studies conducted by Pawar *et al.*[15,16] which emphasize the critical role of invasive procedures and wounds in the dissemination of resistant pathogens. Additionally, our study identified a notable incidence of Klebsiella infections among neonates (17%), with 78% of these cases being carbapenem-resistant. This is in agreement with observations made by Mukherjee *et al.*[17] drawing attention to the vulnerability of neonatal populations to antibiotic-resistant infections.

When comparing antibiotic sensitivity patterns between healthcare-associated infections and community-acquired infections (CAI), we observed a marked difference. Imipenem and meropenem showed the highest sensitivity in CAI (87%) compared to HAI (48%), illustrating that community-acquired strains may retain more susceptibility to carbapenems. This significant disparity highlights the impact of healthcare environments on resistance patterns, as noted in the studies by Trojan *et al.*[18]

In our study, Ertapenem disc (10µg) was used to screen the isolates, and modified Carbapenemase inactivation (mCIM) test was done to confirm the production of a carbapenemase enzyme. Out of 44 isolates resistant to ertapenem, only 33 were positive by mCIM confirming the carbapenemase enzyme production. There are different mechanisms responsible for carbapenem resistance in *K. pneumoniae* other than production of carbapenemases, hyperproduction of ESBL or AmpC enzymes combined with the major outer membrane proteins (OMPs) porin loss or upregulated efflux pump.[19] CLSI approves both the phenotypic identification of Carbapenemase resistant *Klebsiella pneumoniae*.[9] mCIM test is used in this study as it is simple, cost-effective, easy to interpret; the sensitivity and specificity are also above 97% compared to molecular methods, according to a study done by Pierce *et al.*[7] Another vital point is that this test can be done in any microbiology laboratory with basic facilities, where molecular confirmation cannot be done.

Most of CP-KP cases (42.2%) are linked to healthcare settings, where the risk of acquiring multidrug-resistant pathogens is higher due to invasive procedures, immuno compromised patients, and antibiotic use. A smaller proportion of CP-KP infections (5.2%) occur outside

healthcare settings, possibly indicating the pathogen's limited spread in the community. CP-KP is predominantly associated with HAIs, underscoring the need for stringent infection prevention and control measures in healthcare facilities. The relatively low percentage of CAI suggests community transmission is less common but may still pose a potential threat, requiring public health monitoring.[20] Overall, CP-KP infections constitute a significant proportion (27%) of the infections analysed, highlighting their clinical and epidemiological importance.

Susceptibility patterns in our study revealed alarmingly high resistance rates among CP-KP when compared to Non CP-KP, CP-KP showed higher sensitivity to tigecycline(58%), suggesting this drug as potential choice for treating infections caused by carbapenem-resistant *K. pneumoniae* strains. In a study conducted by Kumar Kaushal showed a higher sensitivity (80%) to tigecycline compared to our study.[21]

As CP-KP producing bacteria continue to spread, guidelines regarding the best use of these antibiotics is urgently needed, either alone or in combination with other partially effective antibiotics, for treating infections caused by KPC-producing bacteria.[22]

Newly approved and upcoming antimicrobial agents show great promise in treating infections caused by CP-KP. Ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam are promising treatments that have been associated with improved outcomes and reduced mortality in carbapenemase producing *K. pneumoniae* infections. However, they are ineffective against metallo-β-lactamases (MBL). New β-lactamase inhibitors (BLIs) in advanced stages of development, such as aztreonam-avibactam, cefepime-zidebactam, cefepime-taniborbactam, and meropenem-nacubactam, along with cefiderocol, have shown in vitro activity against both KPC and MBL.[23]

Intensive infection control measures have successfully reduced the incidence of CP-KP producing bacterial infections in intensive care units and long-term acute care hospitals. In order to control the spread of these bacteria, a combined strategies are essential, including enhanced environmental cleaning, active surveillance culturing, contact precaution, and antibiotic stewardship.[24]

5. Conclusion

Our study highlights the urgent need for robust antimicrobial stewardship programs and regular surveillance of CP-KP in both community and healthcare settings. Understanding the local prevalence and resistance patterns is essential to combat the rising threat of antibiotic-resistant organisms and to implement effective infection control measures. Addressing the spread of multidrug-resistant organisms will ultimately require concerted efforts at multiple levels, including healthcare policy reform, public health education, and enhanced laboratory capabilities.

6. Ethical Approval

This study was approved by the Institutional Ethics Committee of our institution and was carried out according to the guidelines of the Committee vide letter No. MDC/DOME/270 dated 24-12-2019.

7. Source of Funding

None.

8. Conflict of Interest

None.

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