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Emergence of *Klebsiella pneumoniae* Carbapenemase (KPC)-Producing *Klebsiella pneumoniae* from clinical isolates in tertiary care center in BMCH, Chitradurga.

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Abstract:
Background and Objectives: *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality. KPCs are an important mechanism of resistance for an increasingly wide range of Gram-negative bacteria and are no longer limited to *Klebsiella pneumoniae*. KPC-producing bacteria are often misidentified by routine microbiological susceptibility testing and incorrectly reported as sensitive to carbapenems; The present study was undertaken to know the prevalence of KPC producing *Klebsiella pneumoniae* from clinical isolates and their antibiotic resistance pattern.
Methods: A total of 76 *Klebsiella pneumonia* were recovered from various clinical specimens. All the samples were processed for routine bacterial culture and antimicrobial susceptibility test as per standard protocol. They were further subjected to KPC detection by Imipenem+ EDTA combined disc test and Modified Hodge test.
Results: By Imipenem-EDTA combined disk test 26(34.21%) isolates were found to be KPC positive. By Modified Hodge test 23(30.26%) isolates were found to be KPC positive. Majority of KPC producers were resistant to Gentamicin (65.38%), Levofloxacin (84.61%), Piperacillin+Tazobactum(69.23%) and Norfloxacin (84.61%) respectively. All isolates were sensitive to Polymyxin B.
Conclusion: The prevalence of *Klebsiella pneumoniae Carbapenemases* was 34.21% among *Klebsiella pneumoniae*. Significantly higher resistance rate was observed by these isolates to almost all the drugs routinely used

Keywords: *Klebsiella pneumoniae*., KPC.,multi-drug resistant, carbapenem-resistant, Imipenem-EDTA combined disk test., Modified Hodge test.

Introduction

A Swedish patient of Indian origin traveled to New Delhi, India, and acquired a urinary tract infection caused by a carbapenem-resistant *Klebsiella pneumonia* strain that typed to the sequence type 14 complex. The isolate, *Klebsiella pneumoniae* 05-506, was shown to possess a metallo-β-lactamase (Klebsiella

Pneumoniae Carbapenemases) but was negative for previously known Klebsiella Pneumoniae Carbapenemases genes[1].

Infections caused by bacteria-producing *Klebsiella pneumoniae* carbapenemases (KPCs) are becoming an increasingly significant problem worldwide since the first detection of these enzymes greater than a decade ago.[1] Although KPCs do not represent the first or the sole mechanism of carbapenem resistance, they are remarkable because they are often not detected by routine susceptibility screening and possess an exceptional potential for dissemination. In addition to the infection control challenges that have arisen, infections caused by these organisms present clinicians with serious treatment challenges, due to limited antibiotic options

Efforts are underway to address these varied clinical challenges and have concentrated on enhanced infection control practices, better screening methods, determination of optimal usage of existing antibiotics, and development of novel antimicrobials.

AIMS AND OBJECTIVES:

- Detection of Klebsiella pneumoniae Carbapenemase producing Klebsiella pneumoniae .
- Study of antibiotic resistance pattern of Klebsiella pneumoniae Carbapenemase producing Klebsiella pneumoniae .
- Risk factors associated with the Klebsiella pneumoniae Carbapenemase producing Klebsiella pneumoniae infection.

MATERIALS AND METHODS:

The present study was undertaken at the Department of Microbiology, Basaveshwara medical collage Chitradurga from Dec 2013 to march 2014.

Source of data:

Clinical samples such as pus, urine, blood, body fluids etc. obtained from patients admitted in Basaveshwara medical collage hospital Chitradurga and received at the department of Microbiology.

Inclusion criteria:

Non repetitive, consecutive Klebsiella pneumoniae isolated from clinical samples obtained from hospitalised patients (IPD) received during study period.

Sample processing:

All the samples were processed for routine bacterial culture as per standard protocol.[4]

- **Antimicrobial susceptibility test:[6,7]**

Antimicrobial susceptibility test was carried out with modified Kirby-Bauer disk diffusion method using current CLSI[9] recommendations. Commercially available antibiotic disks (Himedia, Mumbai) were used. The antibiotic susceptibility profile against Gentamicin, Amikacin, Norfloxacin, Levofloxacin, Cephalosporins (Cefoxitin, Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime), Piperacillin-Tazobactam, Imipenem and Polymyxin B were studied. *Pseudomonas aeruginosa* ATCC 27853 was used as control strain.[6]

The isolates were further subjected to following tests:

a. Klebsiella pneumoniae Carbapenemase (KPC) production detected by Combined disk diffusion method using Imipenem+EDTA combined disk.[8.9.10.11]

b. Modified Hodge test[9,10]:

- **Detection of KPC production:[6]**

Imipenem resistant isolates were selected for detection of KLEBSIELLA PNEUMONIAE CARBAPENEMASES production[8].

- **Imipenem-EDTA combined disc test:**

A suspension of the test isolate equivalent to 0.5 McFarland turbidity was swabbed on Muller Hinton agar plate and two 10µg imipenem disks were placed on the plate. 10µl of EDTA solution was added to one of them to obtain the desired concentration (750µg). After overnight incubation at 37^0 C, the inhibition zones of imipenem and imipenem+EDTA disks were compared.

Interpretation:

- If the increase in inhibition zone with imipenem-EDTA disc is ≥7mm than the imipenem disc alone, it is considered as KLEBSIELLA PNEUMONIAE CARBAPENEMASES positive.

- **Modified Hodge test:**

A lawn culture of *Escherichia coli* ATCC 25299 prepared on MHA plate. 10µg imipenem disks were placed on the center of plate.A suspension of the test isolate equivalent to 0.5 McFarland turbidity was streaked on Muller Hinton agar plate in perpendicular direction 3mm close to center disc.

Interpretation:

- A positive test appears as a flattening or indentation of the imipenem inhibition zone in the vicinity of the test disk.
- A negative test will have undistorted zone.[6,7]

Statistical analysis:

Chi square test was used with appropriate correction to see the significance of difference between the sensitivity of various drugs in KLEBSIELLA PNEUMONIAE CARBAPENEMASES producing strains using SPSS software. $p \leq 0.05$ was considered significant.

Ethical consideration:

The protocol for this study was approved by the institutional Ethical Committee. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice, quality control ensured and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for Human Experimentation in Biomedical Research[45]. Approval was obtained from the subjects by taking informed consent.

Figure 1: KPC -Combined disc diffussion test: a) Imipenem (b) Imipenem+EDTA. Test negative for KLEBSIELLA PNEUMONIAE CARBAPENEMASES production

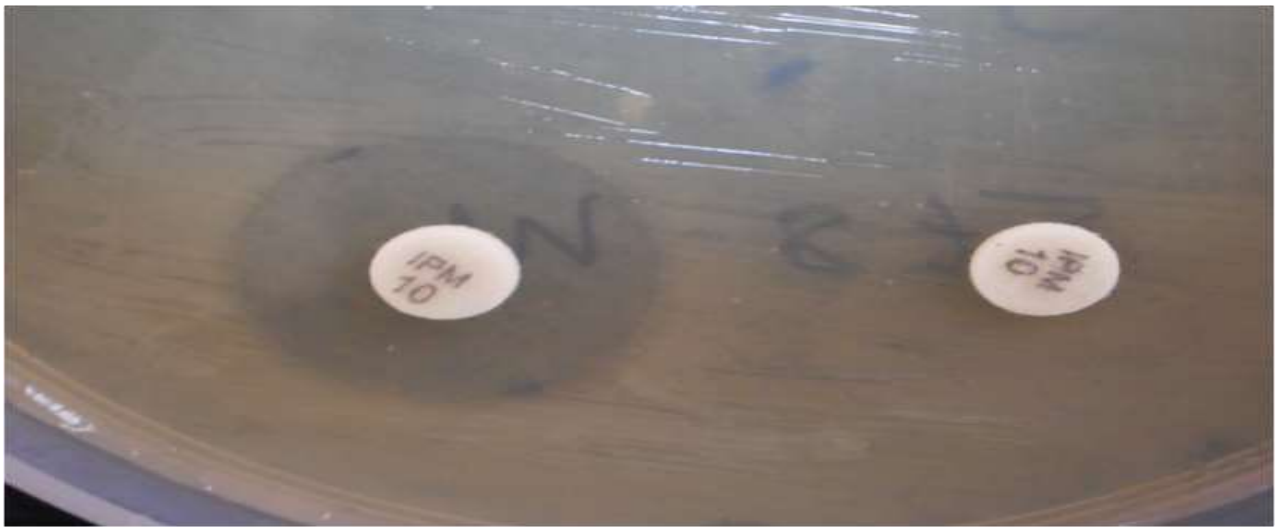

Figure 2: KPC- Combined disc diffussion test: a) Imipenem (b) Imipenem+EDTA. Test positive for KLEBSIELLA PNEUMONIAE CARBAPENEMASES production.

RESULTS AND OBSERVATIONS:

A prospective study was conducted to know the prevalence of different β-lactamases among Klebsiella pneumoniae isolated from various clinical specimens received at the Department of Microbiology, Basaveshwara medical collage Chitradurga from Dec 2013 to March 2014.

Of 572 bacterial isolates 76 (13.28%) were Klebsiella pneumoniae recovered from various clinical specimens like pus (19), sputum(18), urine(15), ear discharge (8), blood (4), cerebrospinal fluid (3), pleural fluid (3), ascitic fluid (2), post operative drain (1), aspiration from liver abcess (1), corneal scraping (1) and tracheal secretion (1).

Table 1: Detection of Klebsiella pneumonia carbapenemasesby Imipenem-EDTA combined disc test positive:

Organism	No. of isolates resistant to Imipenem	Imipenem-EDTA combined disc test positive isolates No (%)

Klebsiella pneumonia (76)	26	26 (34.21)
Others (496)	21	21 (4.23)
TOTAL (572)	47	47 (8.21)

Among the 76 total isolates Klebsiella pneumonia 26 were resistant to imipenem and 26 (34.21%) were detected as Klebsiella pneumonia carbapenemases producers by Imipenem-EDTA combined disc test and 23 (30.26) by modified Hodge test.

- Maximum number of Klebsiella pneumonia carbapenemases producing organisms were isolated from pus (30.76%) followed by urine (23.07%) and sputum (19.23%).

Table 2: Prevalence of Klebsiella pneumonia carbapenemases among different Klebsiella pneumonia organisms by Modified Hodge test.

Organism	Klebsiella pneumonia carbapenemases positive No (%) by Modified Hodge test.
Klebsiella pneumonia (76)	23 (30.26)
Others (496)	18 (3.62)
TOTAL (572)	41 (7.16)

Table 3: Co-existence of AmpC β-lactamases and Klebsiella pneumonia carbapenemases among different organisms.

Organism (no)	AmpC and Klebsiella pneumonia carbapenemases positive

	no (%)
Klebsiella pneumonia (76)	5 (6.57)
Others (496)	3 (0.6)
TOTAL (572)	8 (1.39)

5 (6.57) isolates demonstrated the coexistence phenotype of both Klebsiella pneumonia carbapenemases and AmpC β-lactamases.

Table 4: Distribution of Klebsiella pneumonia carbapenemases positive Klebsiella pneumonia isolate in the hospital ward.

Wards	Klebsiella pneumonia carbapenemases positive no (%)
Surgery(24)	6 (23.07)
Medicine (20)	5 (19.23)
Orthopedics (8)	2(7..69)
Burns (8)	2(7..69)
ENT(4)	2(7..69)
OBG (3)	1(3..84)
Pediatric (3)	1(3..84)
Ophthalmology (3)	1(3..84)
NICU (3)	1(3..84)
Total(76)	26(34.21)

Maximum number of the Klebsiella pneumonia carbapenemases harbouring Klebsiella pneumonia isolates were obtained from the Surgery, Medicine and Orthopedics wards

Table 5: Comparison of Antibiotic resistance pattern of Klebsiella pneumonia carbapenemases positive and Klebsiella pneumonia carbapenemases negative Klebsiella pneumonia:

Antibiotics	Klebsiella pneumonia carbapenemases negative KLEBSIELLA PNEUMONIAE n=50		Klebsiella pneumonia carbapenemases positive KLEBSIELLA PNEUMONIAE n=26		p value
	Resistant	%	Resistant	%	
Gentamicin	8	16	17	65.38	0.01
Amikacin	4	8	15	57.69	0.01
Norfloxacin	5	10	21	80.76	0.01
Levofloxacin	7	14	22	84.61	0.01
Cefipime	8	16	24	92.30	0.01
Ceftazidime	12	24	26	100	0.01
Piperacillin+ Tazobactum	1	2	18	69.23	0.01
Imipinem	0	0	26	100	0.0001
Polymyxin B	0	0	0	0	

KLEBSIELLA PNEUMONIAE CARBAPENEMASES producing organisms were more drug resistant, difference was statistically significant towards all the antibiotics used in the study.

All the KPC were sensitive to Polymyxin B.

Table 6: Analysis of the risk factors for Klebsiella pneumonia infection by Klebsiella pneumonia carbapenemases positive isolates.

Risk factors	KLEBSIELLA PNEUMONIAE CARBAPENEMASES positive No (n=26) (%)
Burns (8)	2 (7.69)
Carcinomas (14)	3(11.53)
Catheterization(29)	16(61.53)
Chronic ailment(24)	11(42.3)
Diabetis mellitus. (21)	8(30.76)
HIV Positive(8)	0
Hospitalization of 5 days or more (71)	22(84.61)
ICUs (Intensive care units) (27)	9(34.61)
Neurological Disorders(12)	4(15.58)
Sepsis (23)	8(30.76)
Surgical Intervention(31)	16 (61.53)

The major risk factors for infection with Klebsiella pneumonia carbapenemases producing Klebsiella pneumonia were hospitalization of 5 days or more, surgical intervention and catheterization.

Discussion:

A prospective study was conducted to know the prevalence of different β-lactamases among Klebsiella pneumoniae isolated from various clinical specimens received at the Department of Microbiology, Basaveshwara medical collage Chitradurga from Dec 2013 to March 2014.

Of 572 bacterial isolates 76 (13.28%) were Klebsiella pneumoniae recovered from various clinical specimens like pus (19), sputum(18), urine(15), ear discharge (8), blood (4), cerebrospinal fluid (3), pleural fluid (3), ascitic fluid (2), post operative drain (1), aspiration from liver abcess (1), corneal scraping (1) and tracheal secretion (1).

The growing increase in the rates of antibiotic resistance is a major cause for concern in both nonfermenting bacilli and isolates of the *Enterobacteriaceae* family. β-Lactams have been the mainstay of treatment for serious infections, and the most active of these are the carbapenems, which are advocated for use for the treatment of infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumonia*. However, carbapenemases are increasingly being reported; and the most prevalent of these would appear to be KPC, which has recently been characterized in the United States, Israel, Turkey, China, India, the United Kingdom, and Nordic countries. KPC has invariably been found in *K. pneumoniae*, although recent reports indicate that it can cross species boundaries[1].

Significance of *Klebsiella pneumoniae* carbapenemases:

In 1983, the first report of plasmid-mediated beta-lactamases capable of hydrolyzing extended-spectrum cephalosporins was made. They were named extended-spectrum beta-lactamases (ESBLs) and they have since been described worldwide.[1] The fact that carbapenems are the treatment of choice for serious infections caused by ESBLs, along with an increasing incidence of fluoroquinolone resistance among *Enterobacteriaceae*, has led to an increased reliance on carbapenems in clinical practice.[2]

In 2001, the first KPC-producing *K pneumoniae* isolate was reported in North Carolina.[3] The enzyme (KPC-1), an Ambler class A beta-lactamase, was not the first carbapenemase to be detected in *K pneumoniae*, as isolates harboring Ambler class B metallo-beta-lactamases capable of hydrolyzing carbapenems had previously been reported in Japan as early as 1994.[1] However, metallo-beta-lactamases are uncommon in the US and the production of KPC enzymes has become the most prevalent mechanism of carbapenem resistance in the US today.[4] KPCs are encoded by the gene bla_{KPC}, whose potential for inter-species and geographic dissemination is largely explained by its location within a Tn3-type transposon, Tn4401. This transposon is a genetic element which is capable of inserting into diverse plasmids of Gram-negative bacteria. Plasmids

carrying *bla*$_{KPC}$ are often also associated with resistance determinants for other antibiotics. Although *K pneumoniae* remains the most prevalent bacterial species carrying KPCs.

In 2009, the Centers for Disease Control and Prevention (CDC) released a report on KPC-producing bacteria in which the term Carbapenem-Resistant Enterobacteriaceae (CRE) was proposed as more accurate, given the understanding that multiple species of Gram-negative bacteria can harbor the KPC-resistant element.[6] We have used the terminology 'KPC,' given the preponderance of the literature using this term to date.

Microbiological Testing for KPC-producing Organisms

Misidentification of KPC-producing bacteria is common with standard susceptibility testing. It has been reported that automated systems will identify seven to eighty-seven percent of KPC-producing *K pneumoniae* as susceptible to imipenem or meropenem.[14] The great variability that has been observed in carbapenem minimal inhibitory concentrations (MICs) by routine testing is likely related to the phenotypic heterogeneity among isolates, giving the appearance of susceptibility *in vitro*. It is thought that additional factors such as reduced outer membrane permeability may be needed for the KPC-producing organism to achieve full resistance to carbapenems, which would make them easier to detect by clinical laboratories. Several groups have made the observation that an ertapenem MIC in the resistant range by standard susceptibility testing may be the most sensitive indicator for presence of a KPC. In two different studies, one involving 33 cases of KPC-producing *Enterobacter* spp and another involving 28 cases of KPC-producing *K pneumoniae*, determination of ertapenem MICs by automated testing identified all cases as ertapenem-resistant.[15,16] Ertapenem resistance has been found to be the most sensitive clinical test of KPC production regardless of the method used and is recommended by the CDC.[17]

Confirmatory testing for KPC-producing bacteria is recommended in geographical locations where *Enterobacteriaceae* are noted to have decreased susceptibility to carbapenems or resistance to most

non-carbapenem beta-lactams by routine testing. The most easily performed confirmatory test for KPCs is the modified Hodge test, which has been found to be 100% sensitive for the detection of a carbapenemase, although not specific for KPC production.[17] This test is performed first by culturing a susceptible *Escherichia coli (E coli)* isolate on a Mueller-Hinton plate, after which a carbapenem disk is placed in the center. Isolates suspected of carbapenemase production then are streaked from the disk to the outer margin of the plate. Growth of *E coli* near the disk or along the isolate streak indicates that a carbapenemase is present. In January 2009, the Clinical and Laboratory Standards Institute (CLSI) recommended all *Enterobacteriaceae* with elevated but susceptible carbapenem MICs be tested with a modified Hodge test.[6] Another emerging test is a chromogenic medium CHROMagar KPC, which has been shown to have a sensitivity of 100% and specificity of 98.4% relative to polymerase chain reaction (PCR).[18]Definite confirmation of KPC production requires molecular methods such as PCR, but these are costly and rarely available outside of reference laboratories.[14]

Yigit H, Queenan AM, et al[3],. reported that imipenem+EDTA combined disc test is more than 90% sensitive and modified Hodge test is more than 95% sensitive for KPC detecton.

Bratu S, Landman D, et al[8],. reported that imipenem+EDTA combined disc test and modified Hodge test is equally sensitive for KPC detecton.

Clinical Features of KPCs:

Infections caused by KPC-producing *K pneumoniae* have been associated with increased cost and length of stay as well as frequent treatment failures and death.[6]Risk factors for infection include advanced age,[19] being severely ill,[20] previous treatment with antibiotics,[7] organ or stem-cell transplantation, mechanical ventilation, and long hospital stays.[21] Reports are mixed as to whether previous carbapenem use is associated with the development of infections caused by KPC-producing bacteria.[7,19,16,22] In at least one study, prior fluoroquinolone and extended-spectrum cephalosporin use were both independently associated with infection or colonization with KPCs.[20]

Poor outcomes from infections with KPC-producing bacteria have been reported since the first reports of KPC outbreaks in New York City hospitals. A small series of patients with bloodstream infections caused by KPC-producing bacteria from New York City hospitals in 2005 revealed mortality rates of 47% to 66%.[7,19] The experience outside the US has been similar, as shown by a matched retrospective historical cohort study of 32 Israeli patients with bacteremia caused by carbapenem-resistant *K pneumoniae* compared to patients with infections caused by susceptible *K pneumoniae* that showed a crude mortality of 72%, and an attributable mortality of 50%.[23] None of the patients in this study received appropriate empiric antibiotics. In a cohort of 99 cases with KPC-producing *K pneumoniae* and 99 controls with susceptible *K pneumoniae*, KPC-production was associated with greater than two-fold increased risk of death.[21]

The difficulty of detecting KPC production with routine testing appears to have contributed to the poor outcomes observed with infections caused by KPC-producing bacteria by causing a critical delay in treatment. In the aforementioned study of 33 KPC-producing *Enterobacter* spp compared to imipenem-sensitive controls, significantly higher mortality was noted in conjunction with less frequent appropriate empiric antibiotics.[15] Weisenberg et al looked at 28 cases of confirmed KPC-producing *K pneumoniae* and found 46% of clinical isolates to have been reported inappropriately as imipenem-susceptible, which in turn led to the majority of these cases being treated with imipenem or meropenem.[16] KPC-producing bacteria present a significant problem in clinical situations where administration of effective empiric antibiotics is essential to preventing mortality. This applies to serious infections such as bacteremia, but also extends to other infections in patients undergoing organ transplants and cancer treatment, where the immunocompromised status of patients requires effective empiric antibiotics. Mathers et al reported two cases of orthotopic liver transplant recipients that died as a result of infections caused by KPC-producing *K pneumoniae*. Both patients were initially treated with meropenem based on the results of routine susceptibility testing.[24]

Enterobacteriaceae are among the leading causes of nosocomial infections.[25] Early identification of KPC-producing bacteria with *in vitro* testing is of paramount importance to the success of infection control

efforts.[6] In the appropriate setting, active surveillance can improve infection control by detecting colonization and preventing horizontal spread.[26] A study of 36 patients in an intensive care unit (ICU) in New York City during a KPC outbreak revealed 39% of all patients had gastrointestinal colonization while only 14% were identified by previous clinical culture.[7] The CDC released guidelines for surveillance in March 2009 recommending the use of active surveillance in outbreaks, and that even non-endemic acute care facilities review all clinical cultures within the last 6-12 months for previously unrecognized KPCs.[6]

Aggressive infection control efforts have been effective at decreasing rates of infections with KPC-producing bacteria in intensive care units and long-term acute care hospitals.[26,27] Bundled interventions including enhanced environmental cleaning, active surveillance culturing and contact precautions, as well as antimicrobial stewardship are important in controlling KPC-producing bacteria.[19,28]

Yigit H, Queenan AM, et al[3],, reported that clinically KPC producing organisms are hospital acquired and resistant to routinely used antibiotics

Bratu S, Landman D, et al[8]., reported that clinically KPC producing organisms are hospital acquired and multidrug resistant.

Phillips M, Sharma S et al[9]., reported that clinically KPC producing organisms are hospital acquired and resistant to almost all the antibiotics tested except polymyxin B

Naas T, Nordmann P, et al[10]., reported that clinically KPC producing organisms are hospital acquired and sensitive to higher quinolones.

Detection of Klebsiella pneumonia carbapenemases by Imipenem-EDTA combined disc test

In our present study among the 76 total isolates Klebsiella pneumonia 26 were resistant to imipenem and 26 (34.21%) were detected as Klebsiella pneumonia carbapenemases producers by Imipenem-EDTA combined disc test.

Yigit H, Queenan AM, et al[3]., reported that 15% Klebsiella pneumonia were KPC positive by Imipenem-EDTA combined disc test

Bratu S, Landman D, et al[8]., reported that 25% Klebsiella pneumonia were KPC positive by Imipenem-EDTA combined disc test

Phillips M, Sharma S et al[9]., reported that 18% Klebsiella pneumonia were KPC positive by Imipenem-EDTA combined disc test

Naas T, Nordmann P et al[10]., reported that 34% Klebsiella pneumonia were KPC positive by Imipenem-EDTA combined disc test

Prevalence of Klebsiella pneumonia carbapenemases among different Klebsiella pneumonia organisms by Modified Hodge test.

Of the 76 Klebsiella pneumonia isolates 23 (30.26%) were Klebsiella pneumonia carbapenemases positive by Modified Hodge test.

Yigit H, Queenan AM, et al[3]., reported that 11% Klebsiella pneumonia were KPC positive by Modified Hodge test.

Bratu S, Landman D, et al[8]., reported that 25% Klebsiella pneumonia were KPC positive by Modified Hodge test.

Phillips M, Sharma S et al[9]., reported that 18% Klebsiella pneumonia were KPC positive by Modified Hodge test.

Naas T, Nordmann P et al[10]., reported that 38% Klebsiella pneumonia were KPC positive by Modified Hodge test.

Distribution of Klebsiella pneumonia carbapenemases positive Klebsiella pneumonia isolate in the clinical samples:.

Maximum number of Klebsiella pneumonia carbapenemases producing organisms were isolated from pus (30.76%) followed by urine (23.07%) and sputum (19.23%).

Yigit H, Queenan AM, et al[3]., reported that Klebsiella pneumonia carbapenemases producing organisms were isolated from pus (53%) followed by urine (21%) and sputum (12%).

Bratu S, Landman D, et al[8]., reported that Klebsiella pneumonia carbapenemases producing organisms were isolated from pus (36%) followed by blood (26%) and urine (15%).

Phillips M, Sharma S et al[9]., reported that Klebsiella pneumonia carbapenemases producing organisms were isolated from pus (33%) followed by urine (29%) and blood (10%).

Naas T, Nordmann P et al[0]., reported that Klebsiella pneumonia carbapenemases producing organisms were isolated from pus (31%) followed by ear discharge (28%) and urine (15%).

Co-existence of AmpC β-lactamases and Klebsiella pneumonia carbapenemases among different organisms.

Total of 5 (6.57%) isolates demonstrated the coexistence phenotype of both Klebsiella pneumonia carbapenemases and AmpC β-lactamases.

Naas T, Nordmann P et al[10]., reported the coexistence phenotype of both Klebsiella pneumonia carbapenemases and AmpC β-lactamases.

Distribution of Klebsiella pneumonia carbapenemases positive Klebsiella pneumonia isolate in the hospital ward.

Maximum number of the Klebsiella pneumonia carbapenemases harbouring Klebsiella pneumonia isolates were obtained from the Surgery(53%), Medicine(31%) and Orthopedics wards (11%).

Yigit H, Queenan AM et al[3]., reported that the Klebsiella pneumonia carbapenemases harbouring Klebsiella pneumonia isolates were obtained from the Surgery(39%), Orthopedics (21%) and ENT wards (9%).

Bratu S, Landman D,, et al[8]., reported that the Klebsiella pneumonia carbapenemases harbouring Klebsiella pneumonia isolates were obtained from the Surgery(33%), Medicine(25%) and Orthopedics wards (12%).

Phillips M, Sharma S et al[9]., reported that the Klebsiella pneumonia carbapenemases harbouring Klebsiella pneumonia isolates were obtained from the Surgery(41%), Medicine(34%) and ENT wards (19%).

Naas T, Nordmann P et al[10]., the Klebsiella pneumonia carbapenemases harbouring Klebsiella pneumonia isolates were obtained from the Surgery(41%), OBG (32%) and Orthopedics wards (18%).

Comparison of Antibiotic resistance pattern of Klebsiella pneumonia carbapenemases positive and Klebsiella pneumonia carbapenemases negative Klebsiella pneumonia

Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant, difference was statistically significant towards all the antibiotics used in the study. All were sensitive to Polymyxin B

Yigit H, Queenan AM, et al[3]., reported that Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant and sensitive to Polymyxin B.

Rhomberg PR et al[2]., reported that Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant and sensitive to quinolones.

Lee J, Patel G et al[4]., reported that Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant and sensitive to quinolones and piperacillin+tazobactum.

Bratu S, Landman D, et al[8]., reported that Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant and sensitive to quinolones and aminoglycosides.

Phillips M, Sharma S et al[9]., reported that Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant and sensitive to quinolones.

Naas T, Nordmann P et al[10]., reported that Klebsiella Pneumoniae Carbapenemases producing organisms were more drug resistant and sensitive to aminoglycosides.

Analysis of the risk factors for Klebsiella pneumonia infection by Klebsiella pneumonia carbapenemases positive isolates.

The major risk factors for infection with Klebsiella pneumonia carbapenemases producing Klebsiella pneumonia were hospitalization of 5 days or more(84.61%), surgical intervention(61.53%)and catheterization(61.53%).

Studies have reported that chronic debilitated (22% to 32%) and diabetes (18% to 21%) and prolonged antibiotics use (24% to 36%) are major risk factors for infection with Klebsiella pneumonia carbapenemases producing Klebsiella pneumonia [3,7,9,10].

Current therapeutic options

With the spread of KPC-producing bacteria, clinicians are becoming increasingly dependent on polymyxins and tigecycline for treatment of these infections.[4] Some experts suggest that high-dose continuous infusion of a carbapenem may be helpful, though clear evidence of efficacy is lacking.[29] There is currently a need for information regarding the optimal use of these antibiotics with or without other partially active antibiotics in the treatment of infections caused by KPC-producing bacteria.

Polymyxins are a class of cyclic polypeptide antibiotics consisting of groups A-E, of which Polymyxin B and E (colistin) are currently available.[30] *In vitro* susceptibility to polymyxins among clinical KPC-producing isolates ranges from 90-100%.[13,8]Polymyxins achieve concentration-dependent bactericidal killing and are often the only agents active against KPC-producing bacteria that achieve adequate levels in the serum to treat serious bloodstream infections. However, in the past polymyxins were used infrequently, largely due to their associated nephrotoxicity and neurotoxicity. There is a small amount of retrospective data describing use of polymyxins as monotherapy for the treatment of KPCs. Three bloodstream infections during a Manhattan outbreak caused by KPC-producing *K pneumoniae* isolates susceptible to polymyxins were treated with polymyxin B, and one survived.[19]

Polymyxins are commonly used in combination with other antimicrobials, although there are no prospective data to evaluate the efficacy of this approach. Combination therapy may improve outcomes and be helpful in preventing bacterial resistance. Lee et al looked at 16 patients with persistent infections caused by KPC-producing *K pneumoniae* and found that three of twelve treated with polymyxin monotherapy developed polymyxin resistance during treatment. None of four cases treated with polymyxin combined with tigecycline developed resistance to either antibiotic.[4] In terms of outcomes, there are limited data examining combination therapy in humans, but what is present suggests non-inferiority of monotherapy compared with combination therapy.[31] During an outbreak of KPC-2 infections in Greece, 88% of the patients were treated with combination therapy and 22% of the cases resulted in clinical failure.[22]

Tigecycline is a novel glycylcycline that is often used in treatment of infections caused by KPC-producing bacteria and other multidrug-resistant (MDR) Gram-negative bacteria. Using the Federal Drug Administration (FDA) susceptibility breakpoint of a MIC <2 mg/L, tigecycline has excellent *in vitro* activity against KPC-producing bacteria. All 95 KPC isolates from Brooklyn hospitals in 2005[8] and 73 KPC isolates from 2000-2005 in seven different states[32] were susceptible to tigecycline by *in vitro* testing. However, in the United Kingdom an increase in tigecycline resistance has been noted in *Klebsiella* spp from 2001-2006.[33]

Despite use in clinical practice for off-label indications, few studies have evaluated outcomes of serious MDR-*Enterobacteriaceae* infections treated with tigecycline, and even fewer have been dedicated to infections caused by KPC-producing bacteria. In a review of 10 studies including only 33 patients with infections caused by MDR-*Enterobacteriaceae*, Kelesidis et al reported favorable outcomes with tigecycline treatment in 70% of cases.[34] However, 49% of these were cases of intra-abdominal infections, for which tigecycline has been approved. In contrast, several studies reported delayed clearance of the organism, recurrence of pathogens, and the need for prolonged administration of the antibiotic to achieve favorable outcomes.[34]Tigecycline is not recommended for treatment of infections of the blood or urine, where it has low concentrations. Development of resistance during therapy has been described in several reports and remains a concern.[35,36] Between January and July of 2009 in one New York City hospital, 14 *K pneumoniae* isolates were identified as intermediate or resistant to tigecycline and six were intermediate or resistant to polymyxin; two of these *K pneumoniae* isolates were resistant to tigecycline, polymyxin and other antibiotics.[9]

Aminoglycoside resistance is increasing among KPC-producing bacteria. In susceptible strains, *in vitro* data have shown rapid bactericidal activity of gentamicin against gentamicin-susceptible strains.[8] Members of the successful ST258 clone usually remain sensitive to gentamicin.[37] However, other lineages may carry gentamicin-modifying enzymes and other aminoglycosides, namely amikacin and tobramycin, which have been shown to be less effective against KPC-producing *K pneumoniae*than other forms of MDR-*K pneumoniae*.[38] When aminoglycoside susceptibility is identified, aminoglycosides are an important therapeutic option for the treatment of KPC-producing bacteria. However, pan-resistant bacteria have been reported that are resistant to tigecycline, polymixin, and aminoglycosides.[9,39]

Other older antimicrobials including fosfomycin or nitrofurantoin have been discussed for use in noninvasive infections such as urinary tract infections, but data relating to clinical efficacy is absent.[14,40]

Therapeutic options in development

Available beta-lactamase inhibitors such as clavulanic acid may restore activity of beta-lactams *in vitro* against KPC-producing bacteria, but such additions do not lower the MIC values of beta-lactam antibiotics to within the susceptible range and should not be used.[41] A novel beta-lactamase inhibitor, NXL104 has activity against the KPC enzyme and is currently in development.[41,42] Several novel synthetic polymyxin derivatives, including NAB739 and NAB740, have been developed that may be less nephrotoxic yet retain equal antibacterial activity.[43] Two other novel members of existing antibacterial classes are in development including a novel tetracycline PTK-0796,[37] and a novel aminoglycoside, ACHN-490.[38]

Although there are new agents within existing classes of antimicrobials, currently there are no new classes of antimicrobials in the later phases of development with activity against MDR-Gram-negative bacteria.

Conclusion:

A total of 76 *Klebsiella pneumoniae*.were recovered from various clinical specimens.The prevalence of *Klebsiella pneumoniae* Carbapenemases was 30.21% among *Klebsiella pneumoniae*. By Imipenem-EDTA combined disk test 26 isolates were found to be KPC positive. By Modified Hodge test 23 isolates were found to be KPC positive. Majority of KPC producers were resistant to Gentamicin (65.38%), Levofloxacin (84.61%), Piperacillin+Tazobactum(69.23%) and Norfloxacin (80.76%) respectively. All isolates were sensitive to Polymyxin B.

After initial outbreaks in the northeastern United States, KPC-producing bacteria have emerged in multiple species of Gram-negative bacteria across the world. They have created significant clinical challenges for clinicians as they are not consistently identified by routine screening methods and are highly drug-resistant, resulting in delays in effective treatment and a high rate of clinical failures. Effective antibiotics are limited to polymyxins, tigecycline and occasionally aminoglycosides. Hospitals must prepare so that they can identify these organisms early and institute enhanced infection control efforts when necessary. Clinical microbiology

laboratories need to recognize the signature of ertapenem resistance as a marker for KPC-producing bacteria, and should alert physicians to assume cross resistance to all carbapenems when it is present. Furthermore, clinicians need to appreciate that KPC-production can occur in many Gram-negative bacilli and become familiar with the limited effective antibiotics against KPC-producing bacteria as the frequency of KPC-producing bacteria is expected to continue to increase.

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