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Original research article

Screening carbapenem resistance in *Klebsiella pneumoniae*: A laboratory based study

Dr. Satyendu Saha*

Professor and Head, Department of Microbiology, Shri Shankaracharya Institute Medical Science, Chattisgarhm;
Email id: sstayin@gmail.com

Abstract

Introduction: In recent years, *K. pneumoniae* has developed resistance to multiple antibacterial agents including carbapenem. Carbapenems are one among the most effective antibacterial drugs prescribed for treatment of life threatening infections. The present study was conducted with an aim to study the prevalence of carbapenem resistance in *K. pneumoniae* isolated from clinical specimen.

Methods: The carbapenemase production in *K. pneumoniae* was screened and interpreted as per the Clinical and Laboratory Standard Institute (CLSI) guidelines. Disc diffusion tests were performed on Muller Hinton agar plates by Kirby-Baurer method.

Results: *K. pneumoniae* (80.6%) was the predominant spp. from the genus Klebsiella. A total of 62 (53.4%) isolates were resistant to meropenem whereas imipenem resistance was seen in 53 (45.7) isolates.

Conclusion: As the present study shows high prevalence of carbapenem resistance in *Klebsiella pneumoniae* more emphasis should be given to strict compliance with infection prevention and control practices and judicious use of antibiotics.

Keywords: Carbapenem resistance, imipenem, *Klebsiella pneumoniae,* meropenem.

Introduction.

Klebsiella pneumoniae is one of the most commonly isolated bacteria from the Enterobacteriaceae family.[1] The family Enterobacteriaceae consists of aerobic or facultative anaerobic Gram negative bacilli. They naturally inhabit intestinal tract human and animals. Enterobacteriaceae consists of species that are both commensals and pathogens.[2] Pathogenic and opportunistic pathogenic bacteria from the Enterobacteriaceae family are common causes of both hospital and community acquired infections.[3]

K. pneumoniae causes a wide spectrum of clinical manifestations ranging from pneumonia to urinary tract infections as well as wound and surgical site infections, bloodstream infection, and meningitis. It is also an important cause of health-care associated infections. In recent years, *K. pneumoniae* has developed resistance to multiple antibacterial agents including carbapenem.[1, 4]

Carbapenems are structurally similar to penicillin but possess additional sulphur group at C1 position.[5] This class of antibiotics has the most potent wide spectrum and is utilized for used for treating life threatening and serious infections.[6] The prevalence of superbugs like carbapenem resistant *K. pneumoniae* highly depends on compliance with an antibiotic policy of hospital, carriage rate among health care workers, and adherence to infection control practices. [7] The present study was conducted with an aim to study the prevalence of carbapenem resistance in *K. pneumoniae* isolated from clinical specimen.

Material and methods.

Study design: The present descriptive cross sectional, laboratory based study was undertaken at Department of Microbiology of a tertiary care teaching hospital for a period of 1 year (January to December 2019).

Bacterial isolates and specimens: *K. pneumoniae* isolated from various clinical specimens received in Department of Microbiology for culture and sensitivity were included in the study. The processing of the clinical specimens was done by following routine standard operative procedures (SOPs).

All clinical specimens were inoculated onto MacConkey's and Blood agar and incubated at 37°C for overnight. After incubation the culture plates were examined for presence of growth. If the growth was seen, the colonies were processed for identification.

In case of blood culture, a drop of Brain Heart Infusion broth after overnight incubation at 37°C was inoculated on MacConkey agar and Blood agar. The inoculated culture plates were incubated at 37°C for overnight.

Identification of *K. pneumoniae* isolates was done on the basis of colony morphology, Gram staining, motility and biochemical reactions. *K. pneumoniae* isolated in a pure and predominant growth were only considered in the present study. These isolated were maintained in in 0. 2% semisolid agar for further tests.

Screening of carbapenem resistance: The carbapenemase production in *K. pneumoniae* was screened and interpreted as per the Clinical and Laboratory Standard Institute (CLSI) guidelines. Disc diffusion tests were performed on Muller Hinton agar plates by Kirby-Baurer method. The carbapenem drug tested were Imipenem (10ug) and Meropenem (10ug). Both antibiotic discs were procured from HiMedia Pvt. Ltd, Mumbai. The diameter of the zones of inhibition was recorded and interpreted as sensitive (S), intermediate (I) and resistant (R).

Results

Clinical specimen	**Number (%)**
Urine	763 (32.4)
Pus	417 (17.7)
Blood	348 (14.8)
Sputum	254 (10.8)
Feces	243 (10.3)
Cerebrospinal fluid	237 (10.1)
Pleural fluid	58 (2.5)
Ascitic fluid	36 (1.5)
Total	2356

During the study period, a total of 2356 clinical specimens were received in the laboratory for culture and sensitivity. As shown in table 1, urine samples followed by pus and blood were most common clinical specimens.

Out of these, 876 (37.2%) showed growth. Among these 876 isolates, 781 (89.2%) were bacteria and 95 (10.8%) were fungi. The bacterial isolates from various clinical specimens is showed in table 2. The rate of isolation of Klebsiella spp. was 18.4%.

Bacterial isolates	Number (%)
Escherichia coli	176 (22.5)
Klebsiella spp.	144 (18.4)
Staphylococcus aureus	122 (15.6)
Coagulase negative Staphylococci	119 (15.2)
Pseudomonas spp.	111 (14.2)
Proteus spp.	61 (7.8)
Citrobacter spp.	24 (3.1)
Enterobacter spp.	18 (2.3)
Salmonella spp.	04 (0.5)
Vibrio cholerae	02 (0.3)
Total	781

As shown in figure 1, *K. pneumoniae* (80.6%) was the predominant spp. from the genus Klebsiella.

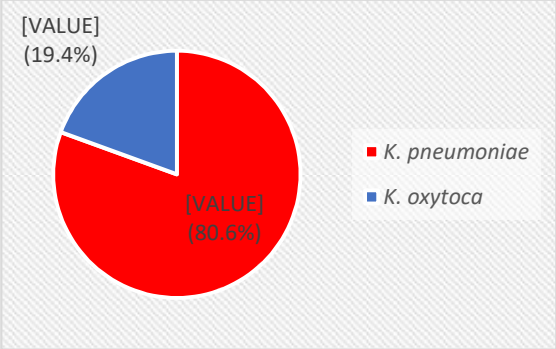

Figure 1: Klebsiella spp. isolated from various clinical specimens.

Carbapenem resistance in *K. pneumoniae* isolates is shown in table 3. A total of 62 (53.4%) isolates were resistant to meropenem whereas imipenem resistance was seen in 53 (45.7) isolates.

Table 3: Carbapenem resistance in *K. pneumoniae* isolates

Carbapenem drug	Resistance (%)	Sensitive (%)	Total
Imipenem	53 (45.7)	63 (54.3)	116
Meropenem	62 (53.4)	54 (46.6)	116

Discussion.

Health and diseases have always been matter of concern to the mankind. Infectious diseases are a major cause of patient illness and death. They are attributed to bacteria, fungi, parasites and viruses. In the present study, 89.2% of organisms isolated in culture were bacteria. As compared to other counterparts, bacteria are major causes of infectious diseases.

Urine (32.4%) followed by pus (17.7%), blood (14.8%) and sputum (10.8) was the major clinical specimen received in laboratory for culture and sensitivity testing. Similar to our observation Parimala *et al* (2017), reported urine and pus as major clinical specimens in their study. [3]Nair *et al* (2013), reported blood, sputum and urine as major clinical specimens received in their study.[8] In the study of Sailaja *et al*. (2019) respiratory specimens followed by pus, urine and blood were the major clinical specimens.[9] Urinary tract infections, blood stream infections, wound infections and respiratory tract infections are common infections encountered in clinical practice.

In the present study, a total of 429 (54.9%) bacterial isolates out of 781 belonged to *Enterobacteriaceae* family. Bacteria belonging to *Enterobacteriaceae* family cause a wide range of community acquired and health-care associated infections.[5] Additionally, they can easily acquire and transfer genes responsible for resistance to various classes of antibiotics through plasmids and transposons. *Enterobacteriaceae* are capable to colonize the gut of patients and spread through the community via the faeco-oral route. [5]

Klebsiella spp. is one of the most common isolate from *Enterobacteriaceae*. In the present study, a total of 144 (18.4%) isolates were Klebsiella spp. *K. pneumoniae* (80.6%) was the predominant spp. from the genus Klebsiella. Recent studies have reported emergence of multi drug resistance strains of *K. pneumoniae*.[3, 10] These strains demonstrate resistance to multiple antibiotics including extended spectrum β-lactam and carbapenem agents. [3, 10]Clinically, resistance to carbapemens is of great concern because these drugs are often considered as the last resort for bacteria producing extended spectrum beta-lactamases (ESBL) in the *Enterobacteriaceae* in general and *E. coli* and *K. pneumoniae* in particular.[11] This class of antibiotics is also prescribed for infections caused by *Pseudomonas aeruginosa* and *Acinetobacter* spp. Imipenem, meropenem, ertapenem, doripenam, panipenem and biapenem are examples of carbapenems drugs recommended for treating critical and life threatening infections.[12]

The most common and elucidated mechanism carbapenem resistance is the production of carbapenemases.[3] As these carbapenemases are β lactamases enzymes having ability of hydrolyzing both carbapenem and all other β lactam antibiotics, all carbapenemases are β lactamases but not all β lactamases are carbapenemases. [3] These enzymes also led to overexpression of multidrug efflux pumps by the bacterial cell membrane. [2] Members of *Enterobacteriaceae* be intrinsically resistant or may acquire resistance during the course of therapy.[8] Few strains may demonstrate both intrinsic and acquired resistance. As *K. pneumoniae* is intrinsically susceptible to carbapenems, it usually acquires secondary resistance due to production of carbapenem-hydrolysing β-lactamases.[8, 13] In *K. pneumonia*e, carbapenemases (KPC) were first identified in North Carolina in 2001.[14]

Although molecular methods like polymerase chain reaction (PCR) are considerd as the 'gold standard' techniques for detection of carbapenem resistance, they are not cost effective for routine diagnostic laboratory setup.[8] In the present study, a total of 53 (45.7%) isolates were resistant to imipenem whereas 62 (53.4) demonstrated resistance to meropenem by disc diffusion method.

Using the same technique, Parimal *et al* (2017) reported high resistance to meropenem (31.1%) compared to to imipenem (13.2%).[3] Similarly, Temple *et al*. (2015) also reported high meropenem resistance (57%) compared imipenem resistance (49. 5%).[15] Increased usage of meropenem compared to imipenem may be one of the reasons for high meropenem resistance. [2, 15]Strict compliance with infection prevention and control practices,

judicious use of antibiotics and adherence to antibiotic policy is important to prevent emergence of drug resistant strains.[16]

Conclusion.

As the present study shows high prevalence of carbapenem resistance in *Klebsiella pneumoniae* more emphasis should be given to strict compliance with infection prevention and control practices and judicious use of antibiotics.

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