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Identification of Carbapenem Resistant Klebsiella Species obtained from Clinical Isolates

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INTRODUCTION

Nowadays the major global problem found is antibiotic resistance or antimicrobial resistance (AMR). The extrajudicial use of antibacterial agents has led to antimicrobial resistance among both pathogenic and commensal organisms. There are two possible ways through which drug resistance can be developed, one is horizontal gene transfer and the other is dissimilar chromosomal loci through mutations. The dissemination of carbapenem resistance among the family of *Enterobacteriaceae* is one of the most potent intimidations to an individual's health these days. The genre *Klebsiella* associates with the family of *Enterobacteriaceae*. The genus *Klebsiella* consists of gram -ve rods, capsulated, 1-2 micrometer long and non-motile organisms. Their favorable environment for growth is moist environment. They are facultative anaerobes. Klebsiella species are disease-causing organisms and are also reported to cause community-acquired and healthcare-associated infections. The genus *Klebsiella* consists of *Klebsiella pneumoniae*, *Klebsiella terrigena*, *Klebsiella* ozaenae, Klebsiella *planticola*, *Klebsiella rhinoscleromatis*, *Klebsiella oxytoca* and *Klebsiella ornithinolytica*. K. Pneumoniae has two major antibiotic resistance mechanisms in them. One pathway involves the expression of extended-spectrum β-lactamases (ESBLs) that contributes to resistance of K. Pneumoniae against cephalosporin and monobactam. Another extremely worse resistance mechanism is that the expression of Carbapenemases by *K. Pneumoniae*, which contributes to the resistance of *K. Pneumoniae* against most offered β-lactams as well as the carbapenems. In this research, the foremost focus is on carbapenemase-producing *Klebsiella species*. Researchers have given the terminology "carbapenem-hydrolyzing enzymes" to the term "carbapenemases". Carbapenemases are particular beta-lactamases with the flexibility to degrade Carbapenems. Carbapenems are a genre of beta-lactam ring containing antibiotics and include meropenem imipenem, ertapenem, biapenem, doripenem & panipenem. Carbapenems are highly effective antibiotics that attach to penicillin-binding proteins thus constraining the synthesis of the outer cellular wall that leads to bacterial killing so if the resistance against carbapenem emerges than it will subsequently lead to narrow therapeutic options. The foremost purpose of this research is the determination of carbapenem resistance among *Klebsiella species*.

Aim: The aim of this study was to determine the carbapenem resistance among Klebsiella spp.

METHODOLOGY

Fifty (n=50) isolates of *Klebsiella species* were collected from the Centralized Diagnostic Laboratory of Karachi, Pakistan. All the isolates were aseptically streaked on EMB (eosin methylene blue) agar to differentiate them from other Enterobacteriaceae and MacConkey agar to check lactose fermentation. The morphological identification of the isolates was done microscopically by gram staining and capsule staining. Capsule staining was performed by Manvel's method. Biochemical tests such as Indole, Methyl red, Voges Proskauer, citrate (IMViC), and Triple Sugar Iron (TSI) were carried out to differentiate *Klebsiella species*. Nitrate reduction test was also performed for the differentiation of *Klebsiella species*. Carbapenem resistance against Imipenem and Meropenem was identified by the disc diffusion method based as per the CLSI guidelines. This technique is one of the most used and efficient in microbiology for antibiotic susceptibility checking.

RESULTS

Out of 50 Gram-negative isolates, 26 isolates were identified as *Klebsiella species*. In these 26 isolates, 10 isolates were identified as *Klebsiella* pneumoniae by using standard microbiological techniques. All ten isolates gave lactose fermenting pink colonies on MacConkey agar and pink to purple colonies on EMB agar. They appeared as gram -ve large rods in gram staining Biochemical tests were positive for Voges Proskauer, citrate, nitrate, gas production, lactose, and sucrose and glucose fermentation. Indole, methyl red test, and H2S production were negative confirming *K. Pneumoniae*. Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method which showed that *K. pneumoniae* was highly resistant to Meropenem as compared to Imipenem. All ten isolates of *K. Pneumoniae* were resistant to Meropenem, zones for Meropenem resistance were ranging between 13mm to 19mm and while only two isolates of *K. Pneumoniae* were resistant to Imipenem and the rest were sensitive to Imipenem, zones for Imipenem resistance were 17mm and 18 mm and Imipenem sensitive were ranging between 25mm to 31mm.

DISCUSSION

Nowadays one of the major issues is antibiotic resistance amongst microorganisms. Resistance against carbapenems mainly amongst Gram -ve microorganism is an ongoing public challenge for the globe. Carbapenems are frequently given as a final therapeutic choice when sufferers with infections grow to be severely unwell or they're suspected of harboring resistant pathogens. Unfortunately, nowadays the emergence of multi-drug resistant (MDR) pathogens seriously threatens this class of life-saving antibiotics. Several latest studies have shown that resistance to carbapenems is increasing at some stage in the world. Resistance mechanism against carbapenems in *K. Pneumoniae* occurs due to Beta-lactamases production, alteration in the expression or function of Penicillin Binding Proteins, or porins due to mutations or efflux pump system. Combinations of these mechanisms can lead to a high level of resistance against Carbapenems among *K. Pneumoniae*. Notwithstanding the type of carbapenemase that is carried, carbapenem-resistant *K. Pneumoniae* segregation is called CRE for carbapenem-resistant *Enterobacteriaceae*. In this research, the primary attention turned into carbapenem-resistant *K. Pneumoniae*, for this motive Imipenem and Meropenem (both belong to the carbapenem class) were used. Results showed that *K. Pneumoniae* is relatively more resistant against Meropenem as compared to Imipenem. Similar to our study, it was found in research that all 38 experimental isolates have been resistant to beta-lactam antibiotics in which 37 strains out of 38 experimental isolates were resistant to imipenem which was also confirmed by genotypic characterization of carbapenemase gene.

CONCLUSION

In this research, it was found that *K. Pneumoniae* was highly resistant to Meropenem and less to Imipenem. Thus, it is concluded that the emergence of resistance against carbapenems due to the production of carbapenemases is increasing day by day and it is now a major concern for public health. The provided data can be used to develop rapid and appropriate treatment of patients so that in the future we can use alternative drugs or cocktail of drugs to treat infections caused by *Klebsiella*. This eventually will lead to lower medical costs, shorten hospital stays, and decreased mortality rates.