

Research Article

Detection of carbapenem resistant *Klebsiella* species isolated from clinical samples

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Abstract

The dissemination of carbapenem resistance among the family of *Enterobacteriaceae* is one of the most potent intimidation to an individual's health nowadays. In this research, the foremost focus is on carbapenemase-producing *Klebsiella* species. Carbapenems are a genre of beta-lactam ring containing antibiotics which includes meropenem imipenem, ertapenem, biapenem, doripenem & panipenem. Carbapenems are highly effective antibiotics as they arrest the cell wall of bacteria 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. Fifty (n=50) gram-negative samples were collected from the Centralized Diagnostic Laboratory of Karachi, Pakistan. All the samples were sub-cultured on EMB (eosin methylene blue) agar to differentiate them from other *Enterobacteriaceae*s. MacConkey agar was used to check lactose fermentation. Gram staining and capsule staining for morphological identification, biochemical tests (Indole, Methyl red, Voges Proskauer, Citrate, (IMViC), Triple Sugar Iron (TSI)) were carried out to differentiate among *Klebsiella* species. Carbapenem resistance against Imipenem and Meropenem was identified by the disc diffusion method based on the Clinical Laboratory Standard Institute (CLSI) guidelines. Out of which 26 isolates were identified as *Klebsiella* spp. In these 26 isolates, 10 isolates were identified as *Klebsiella pneumoniae*. Further results showed that *K. pneumoniae* isolates were more resistant to Meropenem as compared to the Imipenem. All ten isolates of *K. pneumoniae* were found resistant to Meropenem while only two isolates of *K. pneumoniae* were resistant to Imipenem. This study was done to determine the carbapenem resistance among *Klebsiella* so that data can be provided which will help in 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*.

Keywords: Beta-lactamases; Carbapenems; Emergence; *Klebsiella pneumoniae*; Resistance

Introduction

The Genus *Klebsiella* consists of gram -ve rods, capsulated, 1-2 micrometer long and non-motile organisms, favorably grew in a moist environment. They are facultative anaerobes. The Genus *Klebsiella* associated with the family of *Enterobacteriaceae* [1,

2]. *Klebsiella* is amidst the foremost probable elements of a spread of society and healthcare-associated infections. Healthcare-associated diseases of *Klebsiella* cannot be easily discriminated medically from other microorganism involved in different diseases. However,

society-associated diseases of *Klebsiella* may have some indicative options [1]. Historically, Genus *Klebsiella* has been thought to be a very important reason for community-acquired respiratory disorders. *Klebsiella pneumoniae* followed by *Klebsiella oxytoca* is the most prominent species of the genus, found to be associated with human clinical specimens [1].

Nowadays the major global problem found is antibiotic resistance or antimicrobial resistance (AMR). The extrajudicial use of antibacterial agents has led to antimicrobial resistance both in pathogenic and commensal organisms [3]. There are two possible ways through which drug resistance can develop, one is horizontal gene transfer and the other is dissimilar chromosomal loci through mutations [4].

K. pneumoniae have two major antibiotic resistance mechanism in them. One pathway involves the expression of extended-spectrum β -lactamases (ESBLs) that contributes to produce resistance in *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 [5].

The foremost report of *K. pneumoniae* presenting a carbapenemase was known in 1996 in the North geographical area & therefore, this sort of carbapenemase is termed as *K. pneumoniae* carbapenemase (KPC) [6]. Carbapenemases present the adjustable clan of β -lactamases with a wider spectrum unequaled by different beta-lactam-degrading enzymes. Researchers have given the terminology “carbapenem-hydrolyzing enzymes” to the term “carbapenemases” [7]. Carbapenemases are particular beta-lactamases with the flexibility to degrade carbapenems [8].

Carbapenems come under the class of Beta-lactam antibiotics that attach to penicillin-binding proteins thus constraining the synthesis of the outer cellular wall that

leads to bacterial killing [9]. Carbapenems have a fully wider spectrum. For Gram-positive micro-organisms, effective antibiotics are doripenem, imipenem and panipenem. For Gram-negative micro-organisms, potent antibiotics are meropenem, ertapenem, doripenem and biapenem [10]. Resistance mechanism against carbapenems in *K. pneumoniae* occurs due to beta-lactamases/carbapenemase production, alteration in the expression or function of Penicillin Binding Proteins (PBPs) or porins due to mutations and efflux pump system. Together these mechanisms can lead to a high level of resistance against Carbapenems among *K. pneumoniae* [11]. In this research, the main focus was to detect Carbapenem resistance among *K. pneumoniae* which is due to the expression of Carbapenemases. So, that spread of resistant strains can be restricted with proper therapy and effective drugs.

Material and methods

Specimen collection

Fifty (n=50) gram-negative samples were collected from the Centralized Diagnostic Laboratory of Karachi, Pakistan. All the isolates were aseptically streaked on MacConkey agar and EMB agar. Identification and characterization of bacterial isolates were performed by using standard microbiological techniques.

Antibiotic susceptibility checking (By disc diffusion-kirby-bauer technique)

This technique is one of the most used and efficient in microbiology for antibiotic susceptibility checking. In this test, a suspension of inoculum is prepared and then compared to the McFarland standard. Inoculum is then spread evenly on the Mueller Hinton Agar (MHA). Small filter paper discs impregnated with specific antibiotics are then placed onto an agar plate to which bacteria have been spread. Plates are incubated overnight at 37°C. Inhibition zone following the impregnated discs is measured on the next day according to CLSI guidelines. Zone of inhibition can state either the microorganism is sensitive

(S), intermediate or resistant (R) to the given antibiotics. The commercially prepared antibiotic disc used for the samples of *Klebsiella species* were: Imipenem (10ug) and Meropenem (10ug) (both belonging to Carbapenem family).

Results

Out of 50 Gram-negative isolates, 26 isolates were identified as *Klebsiella* spp. In these 26 isolates, 10 isolates were identified as *Klebsiella pneumoniae* by using

standard microbiological techniques. According to zone of inhibitions showed in (Fig. 1), *K. pneumoniae* were found highly resistant to Meropenem as compared to Imipenem. All ten isolates of *K. pneumoniae* were resistant to Meropenem whereas two isolates of *K. pneumoniae* showed resistance against Imipenem and the rest were sensitive to Imipenem as mentioned in the (Table 1).

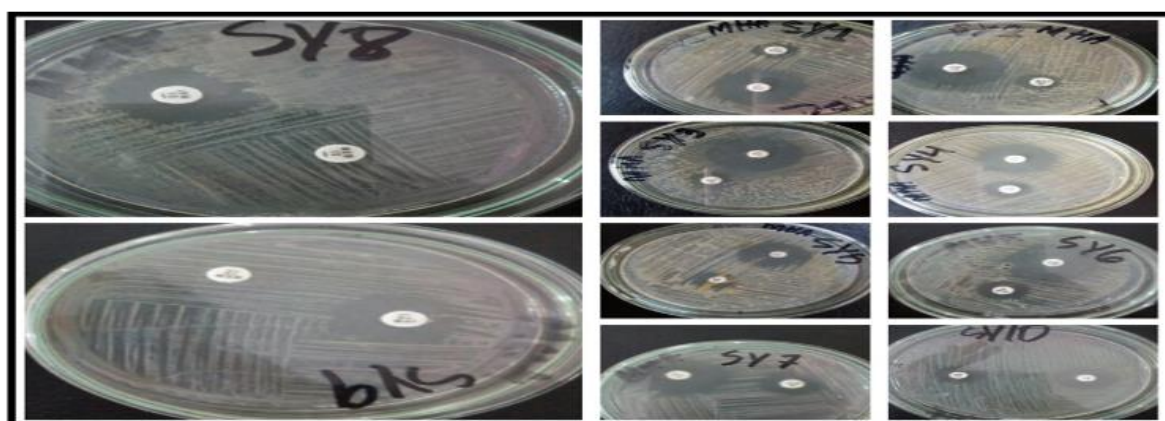


Figure 1. Antibiotic susceptibility testing performed by disc diffusion-Kirby Bauer technique shows different zones of inhibition of Imipenem and Meropenem against *Klebsiella pneumoniae*

Table 1. Antibiotic susceptibility test performed by disc diffusion-Kirby Bauer technique as shown in figures 1(a-c)

Organisms	Antibiotic disc (10UG)	Zone of inhibitions (MM)	Interpretation a/c to clsi data
Sy1	Imipenem	25 mm	Sensitive
Sy2	Imipenem	30 mm	Sensitive
Sy3	Imipenem	31 mm	Sensitive
Sy4	Imipenem	25 mm	Sensitive
Sy5	Imipenem	27 mm	Sensitive
Sy6	Imipenem	31 mm	Sensitive
Sy7	Imipenem	30 mm	Sensitive
Sy8	Imipenem	18 mm	Resistant
Sy9	Imipenem	17 mm	Resistant
Sy10	Imipenem	27 mm	Sensitive
Sy1	Meropenem	13 mm	Resistant
Sy2	Meropenem	13 mm	Resistant
Sy3	Meropenem	12 mm	Resistant
Sy4	Meropenem	15 mm	Resistant
Sy5	Meropenem	19 mm	Resistant
Sy6	Meropenem	16 mm	Resistant
Sy7	Meropenem	15 mm	Resistant
Sy8	Meropenem	No zone	Resistant
Sy9	Meropenem	No zone	Resistant
Sy10	Meropenem	19 mm	Resistant

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. Beta lactams are the maximum used antibiotics internationally, consist of Penicillin, Cephalosporin, Monobactams, and Carbapenems. They all have a common beta-lactam ring and for this reason, they are capable of inactivating penicillin-binding proteins that are an important function for the bacterial wall [12]. Carbapenems are the beta-lactams that can be used for to Gram -ve and Gram +ve bacteria presenting a vast spectrum of antibacterial activity. 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 [6]. Carbapenem-resistant in *K. Pneumoniae* is in particular due to the expression of carbapenemases. Other mechanisms of resistance to carbapenems are the manufacturing of beta-lactamases, efflux pumps, and mutations that modify the expression or function of porins and Penicillin-binding proteins. Combinations of those mechanisms can cause excessive ranges of resistance to carbapenems in certain bacterial species which include *K. Pneumoniae* [13]. Notwithstanding the type of carbapenemase that is carried, carbapenem-resistant *K. Pneumoniae* segregation is called CRE for carbapenem-resistant Enterobacteriaceae [5]. 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 a 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. [14].

Conclusion

In this research, it was found that *K. pneumoniae* were highly resistant to Meropenem and less to Imipenem. Thus, it is concluded that the emergence of resistance against carbapenems due to production of carbapenemases is increasing day by day and it is now a major concern for public health.

Authors' contributions

Conceived and designed the experiments: T Malik, Performed the experiments: SY Ali, Analyzed the data: T Malik & SY Ali, Contributed reagents/ materials/ analysis tools: T Malik & SY Ali, Wrote the paper: SY Ali.

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