ORIGINAL ARTICLE

Antibiotic Resistance Pattern of Multi-drug Resistant *Klebsiella pneumoniae* and Detection of Carbapenem-resistance Genes

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Abstract:

Background: Resistance to carbapenem is increasing over the years, which comes as one of the therapy of last resort therapy for critically ill patients. The current scenario is such that the antibiotics in hand are few for carbapenem-resistant *Klebsiella pneumoniae* and has created a major problem in our setup. *Aim and Objectives:* Our study highlights the detection of antibiotic resistance pattern of Multidrug Resistant (MDR) *Klebsiella pneumoniae,* carbapenemase genes involved and Minimum Inhibitory Concentration (MIC) of Meropenem and Colistin in MDR *Klebsiella pneumoniae*. *Material and Methods:* A total of 51 MDR *Klebsiella pneumoniae* were obtained from various clinical samples over 6 months from patients admitted to two tertiary care hospitals. MIC for Meropenem and Colistin by microbroth dilution and Xpert Carba R assay for carbapenemase genes detection was done for MDR *Klebsiella pneumoniae*. *Results:* In our setup,45 (88.2%) MDR *Klebsiella pneumoniae* were resistant to three or more classes of antimicrobial agents. Among the carbapenem-resistant strains, New Delhi metallobetalactamases-1 (NDM-1), Oxacillinase-48 (OXA-48) and both were detected in 17, 7 and 5 patients respectively. *Conclusion*: NDM-1 was the most frequently associated gene with carbapenem resistance. Infections with MDR *Klebsiella* are a therapeutic challenge for clinicians. Combination therapy, double carbapenem therapy, beta-lactam inhibitor combination like ceftazidime avibactum, are the only few options that become available for clinicians, especially when OXA-48 genes are detected.

Keywords: Carbapenem-resistant *Klebsiella*, Xpert Carba R, Carbapenemase

Introduction:

The emergence of antimicrobial resistance among microorganisms has been a great concern for the medical fraternity in the present era [1]. *Klebsiella pneumoniae* belonging to the family Entero-bacteriaceae has been known both as a community and hospital-acquired pathogen for over a hundred years[2]. *Klebsiella pneumoniae* identified to produce plasmid-mediated enzymes that hydrolyze beta-lactams [3]. Multidrug resistance accounts for the development of resistance to three or more classes of antimicrobial agents. *K. pneumoniae* has developed resistance to carba-penems, aminoglycosides, beta-lactams, and fluoroquinolones as a result of plasmids and hence termed Multi-Drug Resistant (MDR) [4-7].

Over the years, *Klebsiella pneumoniae* has been evolving, developing resistance to the common antibiotics used in the treatment like cephalosporin, beta-lactams, quinolones, etc. As a result of various mechanisms involved, like the production of enzymes like Extended Spectrum Beta Lactamases (ESBL), lack of permeability to these drugs, and efflux pumps. They are ideal for adaptation as they evolve rapidly, thereby providing the bacteria enough scope for the spread of resistance, ensuring their survival. The antibiotic resistance in these bacteria may be attributed to mutations in gene

encoding the enzymes produced by the organisms, leading to carbapenem resistance [8].

According to the Centre for Disease Dynamics, Economics and Policy (CDDEP), resistance to cephalosporins and carbapenems is upto 80% and 60% in Indian *K. pneumoniae* isolates. In India, the prevalence of hospital acquired infections among Carbapenem resistant *Klebsiella pneumonia* is around 95% [9]. The increase in hospital-acquired infections and the emergence of resistant *Klebsiella pneumoniae* has led to a significant decline in the antibiotic options available. Therefore, synergistic combination therapies have been tried with meropenem, tobramycin, ceftazidime, ciproflo-xacin, imipenem with varying results, which depends on local resistance pattern and the Minimum Inhibitory Concentrations (MIC) of the antibiotic. Infections caused by these beta-lactamase producing *Klebsiella pneumoniae* have a high mortality rate and is the upcoming challenge as a nosocomial infection, mainly due to lack of valid antimicrobial combinations [9-10].

This study was taken up to determine the resistance pattern of MDR *Klebsiella pneumoniae*, detect the molecular basis for Carbapenem resistance from clinical specimens, and detect the MIC for meropenem and colistin, in this particular geographical area, to support the clinicians in the treatment of MDR *Klebsiella pneumoniae*.

Material and Methods:

The study was a prospective, laboratory-based study. MDR *Klebsiella pneumoniae* isolates obtained from various clinical specimens collected over 6 months were studied. MDR *Klebsiella pneumoniae* isolates, resistant to 3 or more classes of antibiotics were obtained from the hospital clinical samples. The identification and the antibiotic resistant profile was determined by the Vitek 2 compact system (Biomeurieux, France) and the isolates were stored at -70°C for micro broth dilution and Xpert Carba R test. The patient details and possible risk factors data were compiled for correlation. The MIC for meropenem and colistin was determined by the micro broth dilution method as described by CLSI 2012 [11]. MIC_{50} is the representation of the MIC value at which greater than or equal to 50% of the samples are inhibited in the population under study. The MIC_{90} is the representation of the greater than or equal to 90% inhibition. These values are always represented as concentrations. Breakpoint resistance is a chosen concentration of an antibiotic above which a species of bacteria is resistant to the antibiotic [11]. The breakpoint values for colistin and Meropenem are indicated in Table 1. The genes NDM-1, OXA-48, IMP, Verona Imipenemase (VIM), KPC responsible for Carbapenem resistance were detected by Xpert Carba-R assay (Cepheid, Sunnyvale, US), a real-time PCR assay. Only MDR culture isolates were alliqoted into the cartridge along with the positive and negative controls provided in the kit and processed as per manufacturer's instructions [12]. Quality control: Positive control: *K. pneumonia* ATCC BAA 1705 and *K. pneumonia* BAA 1706 (negative control) was used.

Statistical Analysis:

Data was entered and analyzed in SPSS (Statistical Package for Social Sciences) version 11.5. The categorical data were analyzed in the form of frequency and proportion. The results were expressed in means ± SD, medians (IQR), and percentages and MIC values. P-value of less than 0.05 was considered statistically significant.

Table 1: MIC for Colistin and Meropenem in MDR *Klebsiella pneumonia*

Antibiotic	MIC_{50} µg/ml	MIC_{90} µg/ml	MIC Range	Susceptibility to antibiotics µg/ml			Breakpoint value µg/ml		
				Sensitive	Inter-mediate	Resistant	Sensitive	Inter-mediate	Resistant
Colistin	<0.25	0.25	0.25-4	51	-	0	⩽2	-	>2
Meropenem	8	32	0.125-32	19	5	27	⩽2	2	⩾4

MIC: Minimum Inhibitory Concentration

Results:

During the study period, among 101 Gram negative bacilli, *Klebsiella* was the major pathogen isolated constituting 53 (50.4%), 18 (17.8%) *Acinetobacter* species, 15 (14.8%) *Escherichia coli* and 16 (15.8%) *Pseudomonas* species. Fifty one of *Klebsiella pneumoniae* isolates were multi-drug resistant as per the definition criteria. Of these 39 were from males, 12 were from females. The age of the patients ranged from 42 to 81, and the median age is 65. Out of all the samples collected, only 6 were not fitting into the definition of MDR. Therefore, the total number of MDR accounted for 45 (88.2%). The isolates were from respiratory samples (sputum, broncho-alveolar lavage, endo-tracheal tube) 33 (65%), blood 6 (12%), urine 8 (16%), skin and soft tissue 4 (8%). Risk factors in the population included 29.4% diabetes mellitus (P=0.003), 27.5% hypertension (P=0.001), 12% were admitted in the Intensive Care Unit (ICU) (P=0.001). About 10% had CKD (P<0.001), CLD 6% (P=0.001), COPD 4% (P<0.001), and 94.1 % had previous exposure to antibiotics (P=0.000). Chi square tests showed that all the risk factors were statistically significant (P <0.05). Antibiotics used in the treatment of patients, 33.3% were exposed to one antibiotic, 45.1% to 2 antibiotics, and 15.7 % to 3 or more antibiotics. The table shows the antibiotic resistance pattern of 51 isolates of *Klebsiella pneumoniae* (Table 2). Increasing resistance pattern were observed among fluoroquinolones (41%), third generation cephalosporins (45%) with susceptibility to tigecyclin being 80%.

Out of 51 isolates, 27 (52.9%) were Meropenem and Imipenem resistant and hence were Carbapenem-resistant strains. Among these carbapenem-resistant strains, NDM-1 and OXA-48 were detected in 17 and 7 isolates, respectively. Both NDM-1 and OXA 48 genes were identified in 5 isolates. And surprisingly, one sensitive strain had NDM-1 and the other had both NDM-1 and OXA-48.

All 51 isolates were colistin sensitive while 19 isolates were sensitive to meropenem, 5 were inter-mediate and 27 were resistant. MIC range for colistin was 0.25 ug/ml to 4 ug/ml and for mero-penem was 0.125 ug/ml to 8 ug/ml. The phenotypi-cally sensitive strains which carried carbapenem genes had MIC of 2ug/ml and 0.5ug/ml (Table 1). Meropenem MIC was 2ug/ml in the isolate possessing both NDM and OXA-48 genes and 0.25ug/ml in the isolate NDM-1 gene.

Table 2: Antibiotic Susceptibility Pattern among the MDR *Klebsiella* (n= 51)

Antibiotic	Sensitive (%)	Resistant (%)
Amikacin	20 (39.2%)	31(60.8%)
Amoxiclav	5 (9.8 %)	46(90.2%)
Ampicillin	1(2%)	50(98%)
Aztreonam	6 (11.8%)	45(88.2%)
Cefoperazone	7(13.7%)	43(84.3%)
Cefotaxim	6(11.8%)	45(88.2%)
Ceftazidine	6(11.8%)	45(88.2%)
Cefoperazone Sulbactum	16(31.4%)	35(68.6%)
Ciprofloxacin	10(19.6%)	41(80.4%)
Cotrimoxazole	9(17.6%)	42(82.4%)
Gentamycin	12(23.5%)	39(76.5%)
Ertapenem	21(41.2%)	30(58.8%)
Imipenem	24(47.1%)	27(52.9%)
Meropenem	24(47.1%)	27(52.9%)
Netillin	10(19.6%)	41(80.4%)
Ofloxacin	10(19.6%)	41(80.4%)
Piperacillin Tazobactam	13(25.5%)	38(74.5%)
Tigecycline	41(80.4%)	10(19.6%)

MDR: Multidrug resistant

Discussion:

The pace of development of antibiotics has not matched the development of resistance to them by the bacteria [13-14]. In this era, the medical fraternity faces a significant challenge in finding therapeutic regimens for bacterial diseases. Multidrug resistance, as defined earlier, is the tool that helps to compare the extent of the spread of resistance [15-16]. Out of the 51 isolates we studied, 88.2% were MDR. Most of the isolates were resistant to the first-line drugs used for the

treatment of *Klebsiella pneumonia*. Multiple studies conducted in India showed that almost 80% of all *Klebsiella pneumoniae* are ESBL producers, 30% are carbapenemase producers and pan drug resistant [9,19]. Being a ubiquitous pathogen, *Klebsiella pneumoniae* causes a multitude of infections. We obtained majority of CRE *Klebsiella* spp from respiratory tract (65%) followed by blood (12%), urine (16%) and tissue (6%).

Our study was conducted in Mangalore and samples were also obtained from the nearby areas of Kannur, Shimoga, Kodagu, Kasargod, Chikmagalur, and Hassan. Being a coastal area and a travel hub where several travelers arrive for health check-ups, thus the MDR *K. pneumonia* appears to be widespread geographically in and around Mangalore. Table 2 shows the presence of multidrug resistance in the region.

In our study, we came across patients with diabetes mellitus, hypertension, chronic liver disease, chronic kidney disease, prolonged ICU stay, and prior exposure to antibiotics as some of the significant predisposing factors. Other studies also identified prolonged ICU stay as one of the most significant factor in the development of resistance, mostly because of the transmission of bacteria via air and contact [17-18]. Prolonged use of broad-spectrum antibiotics, a necessary evil in the ICU leads to the emergence of resistance [18]. The development of infections in the ICU like Ventilator-associated Pneumonia (VAP) and catheter infections comes with the inherent risk of resistant microbes due to nosocomial pathogens [19]. Not only does MDR affect the therapeutics, but it also causes prolonged illness, higher deaths, adds on to the medical cost, decreases the effectiveness of other drugs [20].

The resistance pattern is unique to each geographical area attributed to the varied bacterial flora and is identified by determining the minimum inhibitory concentration [21]. Our study shows that most of the samples are resistant to meropenem while all were sensitive to colistin (51). All the study isolates did not show any resistance to the colistin antibiotic as supported by the MIC_{50} and MIC_{90} values for colistin.

Many studies have shown the drug resistance pattern for the carbapenems and the extensive spread of the same [22]. Carbapenem resistance initially came as a huge drawback as it was the first-line drug forthe treatment of *Klebsiella* resistant to beta-lactams due to the production of beta-lactamase enzyme. Similarly, the carbapenems are inactivated by the production of carbapenemase by these *Klebsiella* [23].The line of treatment in these patients included were colistin, tigecycline or fosfomycin or a combination therapy [24].The carbapenem resistant bacteria are showing increasing resistance to colistin, which is not the case in our setup [24]. The increase in the use of these has resulted in resistance to the last-resort drugs too, which entails a threat in treating critically ill patients [7-8].

Our study showed that in all isolates, which were carbapenem resistant, one or both i.e. NDM-1 and OXA-48 genes were detected and two Carbapenem sensitive strains also harboured the NDM-1 and OXA-48 genes, but these genes were not expressed by the organism. This signifies that both phenotypic and genotypic methods have to be incorporated for interpretation of results. IMP, VIM and KPC genes were not detected in our study as reported in some studies where KPC was predominant [25]. In India, resistance rates in

Klebsiella spp is around 54% with predominantly being NDM-1, OXA-48 like and KPC being found rarely. Highest carbapenem resistance rates of 68% was noted in Greece, followed by India and eastern Mediterranean [26].

Ceftazidime-avibactum is best used when KPC or OXA-48 genes are expressed and not useful for NDM-1, IMP, and VIM. Hence it becomes important to perform carbapenemase gene detection for choosing appropriate therapy.

Conclusion:

In this study, NDM-1 and OXA-48 strains were prevalent. Increase in resistance to meropenem was noted while colistin was susceptible in all patients. To improve the current situation of the shortage of new drugs, and to address the issue of drug resistance, testing for the prevalence of resistance in Carbapenems becomes important to guide the clinicians in the treatment of critically ill patients and in hospital infection control measures.

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