ORIGINAL ARTICLE

Assessment of carbapenem-resistant *Klebsiella pneumoniae* from clinical isolates of hospitalized patients

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ABSTRACT

Objective: *Klebsiella pneumoniae***,** a non-motile, Gram-negative rod of the *Enterobacteriaceae* family, is an opportunistic pathogen commonly associated with nosocomial infections and community-acquired diseases in both patients and healthy individuals. The aim of this study is to assess the carbapenem resistance and alternative antibiotic options in clinical isolates of *Klebsiella pneumoniae*.

Materials and Methods: The present study was conducted in CMH, Multan, for 3-5 months. One hundred various clinical samples (blood, BAL, urine, pus, sputum, tissue, and wound fluids) that were received in microbiology laboratory of the hospital were processed for bacterial growth on blood and MacConkey agar plates. The clinical isolates of *Klebsiella pneumoniae* were identified, and their antibiogram profiles were studied. Isolates resistant to Imipenem, meropenem alone or both were considered carbapenem resistant.

Results: Of 110 clinical samples, 50% yielded *Klebsiella pneumoniae*, of which 67.3% were carbapenem resistant. Most patients were aged 50–70 years (mean 58.04 ± 17.31); 65.5% isolates were from males. Strains were mainly recovered from blood (38.2%), pus (27.3%), and sputum (12.7%). High resistance was observed to imipenem (69.1%) and meropenem (47.3%), while sensitivity to colistin and tigecycline was 100% and 72.7%, respectively.

Conclusion: In conclusion, carbapenem resistance was frequent among clinical isolates of *Klebsiella pneumoniae*, and there should be combination therapy of colistin, tigecycline, and meropenem against infections of carbapenem-resistant *Klebsiella pneumoniae*.

Keywords: Carbapenem-resistant, Colistin, *Klebsiella pneumoniae*, Meropenem, Tigecycline

BACKGROUND

Carl Friedlander discovered *Klebsiella pneumoniae* (*K. pneumoniae*) in the lungs of a person suffering from pneumonia in 1882.[1] *K. pneumoniae* is a Gram-negative rod and non-motile bacterium that belongs to the *Enterobacteriaceae* family.[2] It is an opportunistic bacterium that causes frequent nosocomial infections (NIs) and community-related diseases (CRDs) in patients and immunocompetent individuals, respectively.[3] 83% of hospital-acquired pneumonia is usually caused by *K. pneumoniae* in patients.[4] The poor treatment of infections by this bacteria can elevate the proportion of deaths among patients.[5]

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The increasing antibiotic resistance among infectious bacteria is one of the major health concerns worldwide.[6] These bacteria have evolved with time and adapted resistance to many crucial antibiotic classes.[7] Hospital-acquired infections (HAIs) are frequently caused by *A. baumannii*, *E. coli*, *E. faecium*, *Enterobacter spp.*, *K. pneumoniae*, *P. aeruginosa*, *and S.* aureus. All these infectious bacteria have adapted multi-drug resistance (MDR).[8] Carbapenems are the most potent antibiotics against ESBL- or AmpC-producing *Enterobacterales*. Unfortunately, misuse of carbapenems leads to carbapenem resistance (CR) in Gram-negative bacteria of the *Enterobacterales*.[9] CR is defined as the ability of microorganism to resist the effect of carbapenems.[10] The carbapenem resistance in bacteria is mainly due to the production of carbapenemases.[11]

CR bacteria (CRB) show resistance to β-lactam antibiotics as well as possess pathways giving resistance to other antibiotic classes like monobactams.[12] Nowadays only few antibiotics such as fosfomycin, polymyxins, aminoglycosides, tigecycline, and combination of different drugs are effective against these bacteria.[13] Three important resistant pathways have been identified in *Enterobacterales*.[10] (1) active

expression of efflux pumps to remove drugs from cells, (2) active production of carbapenemases to hydrolyze drugs, (3) alternation in permeability of cell wall or cell membrane through the production of AmpC beta-lactamases and point mutations.[10,12,14]

Due to a lack of new treatment options and the misuse of antibiotics, cases of CRB are drastically increasing in the southern Punjab region of Pakistan. There is also a lack of local literature regarding serious CR in *K. pneumoniae* and alternative treatments for patients suffering from its chronic infections. Hence, local studies are crucial and help physicians select the correct antibiotic therapy to treat infections of CR *K. pneumoniae* (CR-KP). The objective of the present study was to identify the frequency of CR-KP among clinical samples and analyze the sensitivity of CR-KP strains to other antibiotics.

MATERIAL AND METHODS

The present cross-sectional study was conducted in the microbiology section of Combined Military Hospital (CMH) Multan from January 1st to May 31st, 2024. A sample size of 110 was calculated by employing WHO sample size calculator: $n = z^2 (p)(q)/d^2$ (anticipated prevalence (p) = 7.7%[15], z = 1.96, q = 100-p, d = 5%). Those patients who were showing relevant signs of infection were included in this study, while patients who were already taking antibiotics were not. An ethical consent was taken from patients to use their samples for this study.

A total of 110 different clinical samples received in laboratory were processed as per standard microbiology techniques. All samples were inoculated on freshly prepared blood and MacConkey agar plates, followed by incubation at 37 °C for 24 hours. The samples showing the growth of *K. pneumoniae* were included in the present study. The recognition of *K. pneumoniae* was performed through growth colonies, Gram staining, and standard biochemical tests like Catalase, Indole ,TSI and Citrate. The antibiogram profile of these selected isolates was studied through an antibiotic susceptibility test. The freshly prepared Muller-Hinton agar (MHA) plates were utilized for this experiment. A 0.5 MacFarland suspension of each strain was separately prepared in 5 mL of saline test tubes. Every suspension was aseptically swabbed on MHA plates, followed by the careful placement of specific antibiotic discs at approximate distances with the help of sterile syringes. The plates were incubated overnight at 37 °C, and zones of inhibition (ZOIs) were measured and recorded. ZOIs were interpreted as per Clinical and. Laboratory Standards Institute (CLSI) guidelines for 2024.

SPSS software version 25 was used to analyze recorded data. The quantitative data like age was calculated as mean and standard deviation. Qualitative data like gender and isolated bacteria was presented as frequency and percentages. Confounding variables like age, gender was collected through stratification of data and chi- square was applied. P value was considered significant as <0.05.

RESULTS

110 different clinical samples were examined for *K. pneumoniae* growth in the laboratory. According to the results, 55 (50%) samples showed the growth of *K. pneumoniae*. Out of 55 strains, 19 (34.5%) were isolated from females and 36 (65.5%) from males. The mean age of the patients was calculated as 58.04 ± 17.314 years. The majority of strains were isolated from blood samples (38.2%) followed by pus (27.3%). Table-I displays the age, gender, and sample-wise distribution of strains.

Significant carbapenem resistance was detected in *K. pneumoniae* isolates. Out of 55 strains, 26 (47.3%) and 38 (69.1%) strains were resistant to meropenem and imipenem, respectively. 37 (67.2) were resistant to both imepem and meropenem and cosidered as carbapenem resistant. Out of these CR-KP strains, 24 were isolated from blood samples, and 19 were isolated from pus samples. However, 55 (100%) and 40 (72.7%) CR-KP strains were susceptible to colistin and tigecycline, respectively (Table-II & III). This is the significant result of the present study.

Table-I: Relation of *K. pneumoniae* with age, gender and nature of specimen.

Characteristics	KP (%) (n = 55)	p-value[a]
Age		
< 10 days	9 (16.3)	
01 to 44 years	12 (21.8)	0.64
50 to 70 years	23 (41.8)	

> 70 years	11 (20)	
Gender		
Male	36 (65.5)	0.637
Female	19 (34.5)	
Nature of specimen		
Blood	21 (38.2)	
Pus	15 (27.3)	
Sputum	4 (7.3)	
Tissue	4 (7.3)	
Urine	7 (12.7)	0.754
Canulla tip	1 (1.8)	
Wound fluid	1 (1.8)	
Ascitic fluid	1 (1.8)	
BAL	1 (1.8)	

a. As in above table p-values are >0.05, so the relationship between variables is not significant.

Table-II: Antibiogram profile of *K. pneumoniae* isolates (n = 55).

Antibiotic (n = 12)	S[a] (%)	I[b] (%)	R[c] (%)
Amikacin	18 (32.7)	8 (14.5)	29 (52.7)
Augmentin	13 (23.6)	5 (9.1)	37 (67.3)
Ceftazidime	10 (18.2)	0	45 (81.8)
Ceftriaxone	9 (16.4)	0	46 (83.6)
Ciprofloxacin	11 (20.0)	0	44 (80.0)
Colistin	55 (100)	0	0
Cotrimoxazole	12 (21.8)	0	43 (78.2)
Imipenem	15 (27.3)	2 (3.6)	38 (69.1)
Levofloxacin	16 (29.1)	4 (7.3)	35 (63.6)
Meropenem	27 (49.1)	2 (3.6)	26 (47.3)
Tazocin	9 (16.4)	10 (18.2)	36 (65.5)
Tigecycline	40 (72.7)	5 (9.1)	10 (18.2)

a. S = sensitive
b. I = intermediate resistance
c. R = resistant

Table-III: Antibiogram profile of CR-KP isolates (n = 37).

Antibiotic (n = 10)	CR-KP (%)	p-value[a]
Amikacin	26 (70.2)	$<0.001^{S}$
Augmentin	31 (83.7)	$<0.001^{S}$
Ceftazidime	34 (91.8)	0.005^{S}
Ceftriaxone	34 (91.8)	0.018^{S}
Ciprofloxacin	32 (86.4)	0.085^{NS}
Colistin	-	-
Cotrimoxazole	32 (86.4)	0.033^{S}
Levofloxacin	29 (78.3)	0.04^{S}
Tazocin	33 (89.1)	$<0.001^{S}$
Tigecycline	9 (24.3)	0.159^{NS}

a. All p-values are significant (<0.05) except two values, representing a significant relationship between variables (S = significant, NS = not significant).

DISCUSSION

The present study reported 55.5% of the samples had the growth of *K. pneumoniae*. The antibiotic susceptibility order of strains was colistin (100%) > tigecycline (72.7%) > meropenem (49.1%) > amikacin (32.7%) > levofloxacin (29.1%) > imipenem (27.3%) > augmentin (23.6%) > cotrimoxazole (21.8%) > ciprofloxacin (20.0%) > ceftazidime (18.2%) > ceftriaxone (16.4%). However, the antibiotic resistance order was ceftriaxone (83.6%) > ceftazidime (81.8%).

Hussain *et al*. 2019 reported the isolation of 130 *K. pneumoniae* strains from respiratory (34%), pus (27%), blood (18.5%), urine (11.5%), and other clinical samples. This study reported 82 out of 130 *K.*

pneumoniae isolates as CR-KP. This study also reported similar antibiogram results to the present study. According to the results, colsitin and tigecycline were the only antibiotics to which CR-KP strains were sensitive, whereas alarming resistance was reported against carbapenems, cephalosporins, and aminoglycosides. However, this study reported the resistance of CR-KP strains to colistin, while the present study reported 100% sensitivity to colistin. [16] Ugrakli *et al*. 2020 reported the significant distribution of a total of 2452 strains of *K. pneumoniae* among various samples: blood (32.2%), bronchio alveolar lavage (36.5%), catheters (2.6%), pleural draines (6%), miscellaneous samples (1.6%), peritoneal fluid (6%), tracheal aspirates (3.9%), and urine (2.5%). This study showed that K. pneumoniae had the highest level of resistance to carbapenem, but the strains were mostly sensitive to tigecycline.[17]

Negm *et al*. 2021 reported the frequent isolation of *K. pneumoniae* (33.5%) from clinical samples of ICU patients. This study reported a very low sensitivity of carbapenems (19%) against *K. pneumoniae* strains, while significant colistin sensitivity (96%) was reported.[18] Al-Baz *et al*. 2022 reported the isolation of 142 (21.8%) strains of *K. pneumoniae* from clinical samples. Out of these isolated strains, 60% and 30% were identified as XDR and MDR, respectively. According to the antibiotic susceptibility test, 36 (25.4%) strains were identified as CR-KP, and all CR-KP strains showed complete resistance to amoxicillin/clavulanic acid, amikacin, aztreonam, ampicillin/sulbactam, ciprofloxacin, cefepime, ceftazidime, ceftriaxone, cefoxitin, cefotaxime, gentamycin, levofloxacin, nitrofurantoin, norfloxacin, and pipracillin/ tazobactam.[19] Ali *et al*. 2024 reported the isolation of 57 CR-KP strains (66.3%) from clinical cases. The isolated strains showed resistance levels of 98% to ceftolozane/ tazobactam, 82.2% to ceftazidime/ avibactam, 65% to cefiderocol, and 52% to eravacycline.[20]

CONCLUSION

This study reported the high prevalence of carbapenem resistance in *Klebsiella pneumoniae* isolated from different clinical samples. All CR-KP strains showed a increased pattern of resistance to other antibiotics, except colistin and tigecycline. As per previous research studies, it was concluded that colistin, tigecycline, and meropenem can be employed as an effective treatment options of CR-KP infections.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Mehreen Afzal: Study design, data acquisition, manuscript drafting, final approval, accountable for all aspects of publication.

Inam UIlah Khan: Data acquisition, critical review, final approval, final approval, accountable for all aspects of publication.

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