**Original Research Article**

**Genotypic Analysis of Klebsiella Pneumoniae among patients admitted in critical care settings in a tertiary care center of Bangladesh**

**Abstract**

Objective: Klebsiella pneumoniae, a multidrug-resistant pathogen, is a leading cause of respiratory infections in critically ill patients, particularly those in intensive care units (ICUs). The increasing prevalence of carbapenemase-producing strains, including KPC, NDM, and OXA-48, has significantly limited treatment options and contributed to high mortality rates. This study aims to investigate the antibiotic resistance patterns of K. pneumoniae and identify the responsible resistance genes.

Methods: A single-center cross-sectional study was conducted at Chittagong Ma-O-Shihsu Medical College, Bangladesh, from January to March 2024. Endotracheal aspirates were collected from ICU patients undergoing mechanical ventilation. Biochemical assays and phenotypic tests were used for bacterial identification, and antimicrobial susceptibility was assessed using the modified Kirby-Bauer disc diffusion method. Conventional polymerase chain reaction (PCR) was employed to detect resistance genes (KPC, OXA-48, NDM, QnrB, AacB, and Sul-2). Data were analyzed using SPSS version 25.

Results: A high prevalence of antibiotic resistance was observed, particularly against ampicillin, cefuroxime, and cefotaxime. Ceftazidime-avibactam exhibited a lower resistance rate, while colistin resistance was minimal. Carbapenemase gene production was detected in 86% (KPC), 96% (OXA-48), and 74% (NDM) of isolates. Additional resistance genes, including qnrb (80%) and sul-2 (88%), were also prevalent. Mortality among infected patients was approximately 50%.

Conclusion: The study highlights the severe antibiotic resistance pattern and high mortality rate associated with K. pneumoniae infections in ICU patients in Bangladesh. The findings underscore the urgent need for stringent antibiotic stewardship and enhanced surveillance to curb the further spread of resistance.

Keywords: Klebsiella pneumoniae, antibiotic resistance, resistance genes, ICU, Bangladesh

**Introduction**

Worldwide, infections continue to be one of the leading causes of mortality associated with intensive care units [1]. In critically ill patients, respiratory infections, particularly ventilation-associated pneumonia (VAP) and community-acquired pneumonia (CAP), are prevalent and can be life-threatening. which is largely associated with Klebsiella pneumoniae, a multidrug-resistant organism. The species typically infects humans by integrating into the human gastrointestinal microbiota, although it also colonizes the respiratory tract. The high rate of acute infection is a direct result of the organism's extensive virulence spectrum, which is explicitly attributed to the plasmid-associated gene [2]. Additionally, the bacteria's polysaccharide vesicles enable it to significantly evade the immune system. Furthermore, the organism swiftly acquires the extended-spectrum beta lactamase or carbapenemase gene, which results in resistance to third-generation cephalosporin or carbapenem. This significantly reduces the treatment options [3]. In addition, there is a growing number of strains that are reported to produce carbapenemases of functional class A (KPC), class B (NDM), and class D (OXA-48), as well as co-producing more than one type of carbapenemas[4]. Wyres et al. [5] have demonstrated that antibiotic resistance is linked to specific genetic determinants for distinct genetic lineages of the organism. By comprehending the resistance pattern, it is possible to prevent the development of additional resistance.   
There has been a twofold increase in the reporting of ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*) pathogens between 2015 and 2018 in Bangladesh, indicating an increase in the prevalence of multi-drug-resistant K. pneumonia [6]. Their dissemination has been further facilitated by the absence of awareness, monitoring, and antibiotic stewardship. Additionally, the prevalence of pandrug-resistant carbapenemase-resistant K pneumonia in Bangladesh has increased by nearly 14% [7]. Their increasing gene resistance is further exacerbated by factors such as horizontal gene transfers and transposition of genes. Delhi Metallo-beta-lactamase (NDM), oxacillinase (OXA), and sulfhydryl variables are the most prevalent [6]. It is imperative to comprehend the resistant pattern of this organism due to the ever-increasing hazard of resistance, the vulnerability of critical care patients, and the limited treatment options. The objective of this investigation was to examine the resistant pattern of K. pneumonia in an intensive care unit and identify the gene that is responsible for the resistance.

**Materials & Methods**

This single-center cross-sectional study was conducted at Chittagong Ma-O-Shihsu Medical College in Chattogram, Bangladesh, from January 2024 to March 2024. Endotracheal aspirates were obtained from ICU patients who were undergoing mechanical ventilation. The organism was identified through biochemical assays, colony morphology, and other phenotypic characteristics following inoculation in Triple Sugar Iron, Motility Indole Urea, and Citrate agar media. The final selection consisted of fifty samples. Areas of inhibition were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations, and all isolates were tested for antimicrobial susceptibility using modified Kirby-Bauer disc diffusion on Mueller–Hinton agar plates. The virulence primers for thermal cycler amplification were identified using conventional polymerase chain reaction (PCR) for the following genes: Kpc, Oxa 48, Ndm, Qnrb, Aadb, and Sul-2. The PCR products were identified by ultraviolet transillumination after electrophoresis on a 1.5% agarose gel with 1X TAE buffer and ethidium bromide. SPSS version 25 was employed to aggregate and analyze the data.

**Results and discussion**

Result of this study is presented in table 1, and Figure 1,2,3.

This is one of the first studies to investigate the antibiotic resistant pattern and potential resistant gene of K. pneumonia in Bangladesh, where the information is still limited.   
Our study demonstrates that critical care patients are particularly susceptible to K. pneumonia infection. In our investigation, infection resulted in the deaths of nearly half of the patients. Our discovery is comparable to a meta-analysis that identified a 47.66% mortality rate for carbapenem-resistant K. pneumonia [8] The treatment option is severely restricted and the mortality rate is further exacerbated by the propensity for the rapid development of resistance to the most commonly used antibiotics [9].   
  
All samples in our study exhibit a high rate of resistance to ampicillin, cefuroxime, and cefotaxime, as well as multidrug resistance. The combination of ceftazidime and avibactam exhibited a lower resistant rate, while colistin only exhibited one instance of complete resistance. Our results are comparable to those of Aminul et al. [10] who identified a resistant pattern that is nearly identical to ours. The resistance to ceftriaxone, ceftazidime, gentamicin, and colistin is particularly high, while the resistance to colistin is the lowest. Colistin's restricted use in the ICU is the reason for its minimal resistance [10]  
The production of carbapenemase genes was observed in 86% (KPC), 96% (Oxa 48), and 74% (NDM) of the samples. Aminul et al. [10] discovered NDM in 23.34% of samples, OXA-48 in 8%, and KPC in 11% of samples. The significantly increased gene production in comparison to other studies may be attributed to the specific selection of ICU admitted patients. NDM is a gene that is relatively noble and has been endemic to India, Pakistan, and Bangladesh, which has allowed it to develop a unique resistant pattern (Lee et al., 2016). Ballén et al. [11] have ascribed the production of the aadb gene to extensive drug resistance and gentamicin resistance in all of our samples. The qnrb gene, which is responsible for fluoroquinolone resistance, was produced in 80% of the samples [12]. Sul-2 accounted for 88% of our samples. Kashefieh et al. [13] identified Sul-2 in 43% of co-trimoxazole-resistant Klebsiella samples.

This investigation was undertaken on a highly specific population and was conducted at a single center. Therefore, the outcome may not be applicable in other contexts and may not be generalizable. In order to generate more generalizable findings, it is necessary to conduct future research at multiple centers with a diverse array of patients.

The study emphasizes the high mortality rate and significant antibiotic resistance pattern in critically ill patients in Bangladesh. It is imperative to exercise antibiotic stewardship in order to prevent the emergence of additional resistance.

**References**

[1] Mayr VD, Dünser MW, Greil V, Jochberger S, Luckner G, Ulmer H, et al. Causes of death and determinants of outcome in critically ill patients. Crit Care 2006;10:R154. https://doi.org/10.1186/cc5086.

[2] Calvo M, Stefani S, Migliorisi G. Bacterial Infections in Intensive Care Units: Epidemiological and Microbiological Aspects. Antibiotics 2024;13:238. https://doi.org/10.3390/antibiotics13030238.

[3] Petrosillo N, Giannella M, Lewis R, Viale P. Treatment of carbapenem-resistant *Klebsiella pneumoniae* : the state of the art. Expert Rev Anti Infect Ther 2013;11:159–77. https://doi.org/10.1586/eri.12.162.

[4] Lan P, Jiang Y, Zhou J, Yu Y. A global perspective on the convergence of hypervirulence and carbapenem resistance in Klebsiella pneumoniae. J Glob Antimicrob Resist 2021;25:26–34. https://doi.org/10.1016/j.jgar.2021.02.020.

[5] Wyres KL, Wick RR, Judd LM, Froumine R, Tokolyi A, Gorrie CL, et al. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of Klebsiella pneumoniae. PLoS Genet 2019;15:e1008114. https://doi.org/10.1371/journal.pgen.1008114.

[6] Tanni AA, Sultana N, Ahmed W, Hasan MdM, Hossain MdS, Noyon SH, et al. Investigating Antimicrobial Resistance and ESBL Producing Gene in Klebsiella Isolates among Neonates and Adolescents in Southern Bangladesh. Canadian Journal of Infectious Diseases and Medical Microbiology 2022;2022:1–10. https://doi.org/10.1155/2022/7071009.

[7] Okanda T, Haque A, Koshikawa T, Islam A, Huda Q, Takemura H, et al. Characteristics of Carbapenemase-Producing Klebsiella pneumoniae Isolated in the Intensive Care Unit of the Largest Tertiary Hospital in Bangladesh. Front Microbiol 2021;11. https://doi.org/10.3389/fmicb.2020.612020.

[8] Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. Ann Clin MicrobiolAntimicrob 2017;16:18. https://doi.org/10.1186/s12941-017-0191-3.

[9] Büyüktuna SA, Hasbek M, Çelik C, Ünlüsavuran M, Avcı O, Baltacı S, et al. YoğunBakımÜnitesindeGelişen Klebsiella pneumoniae Enfeksiyonları: KarbapenemDirencive Hasta Mortalitesiileİlgili Risk Faktörler. Mikrobiyol Bul 2020;54:378–91. https://doi.org/10.5578/mb.69679.

[10] Aminul P, Anwar S, Molla MdMA, Miah MdRA. Evaluation of antibiotic resistance patterns in clinical isolates of Klebsiella pneumoniae in Bangladesh. Biosaf Health 2021;3:301–6. https://doi.org/10.1016/j.bsheal.2021.11.001.

[11] Ballén V, Gabasa Y, Ratia C, Ortega R, Tejero M, Soto S. Antibiotic Resistance and Virulence Profiles of Klebsiella pneumoniae Strains Isolated From Different Clinical Sources. Front Cell Infect Microbiol 2021;11. https://doi.org/10.3389/fcimb.2021.738223.

[12] Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A, et al. *qnrB* , Another Plasmid-Mediated Gene for Quinolone Resistance. Antimicrob Agents Chemother 2006;50:1178–82. https://doi.org/10.1128/AAC.50.4.1178-1182.2006.

[13] Kashefieh M, Hosainzadegan H, Baghbanijavid S, Ghotaslou R. The Molecular Epidemiology of Resistance to Antibiotics among Klebsiella pneumoniae Isolates in Azerbaijan, Iran. J Trop Med 2021;2021:1–9. https://doi.org/10.1155/2021/9195184.

Table 1: Antibiotics and gene production frequency of the Klebsiella

Figure 1: Resistance pattern of *Klebsiella* to different antibiotics

Figure 2: Frequency of gene production of *Klebsiella* samples

Figure 3: Outcome of the infected patients