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Original Article

Prevalence of Antimicrobial Resistance and Virulent Genes in Hypervirulent Klebsiella Pneumonia Isolates

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Abstract

Objective: To determine the antimicrobial resistance and virulence of hypervirulent Klebsiella pneumoniae by phenotypic and genotypic methods.

Methods: This was a cross-sectional study done for 6 months at Microbiology department, King Edward Medical University, Lahore. Antimicrobial susceptibility of 76 isolates was assessed,phenotypic string test was performed to check the hypermucoviscosity, and the presence of carbapenem resistance. Presence of genes i.e. New Delhi metallo-β-lactamase-1 (NDM1), extended-spectrum β-lactamase (blaCTX-M-15) and iron uptake chelate A (iucA) encoding aerobactin responsible for virulence was established by PCR.

Results: Out of 76 patients, 54(71%) were males and 22(29%) were females. Among these 76 isolates 99.5% were Polymyxin B, Colistin (PB, CT) susceptible, 2.6% were gentamycin (CN) and Cefoperazone/sulbactam (SCF) susceptible, 46.1% had Imipenem (IPM) susceptibility and 18.4% had overall susceptibility of PB, CT, IPM, SCF & CN. Whereas, 78.9% isolates showed positive string test. The percentages of genes present were 68.4% iucA, 40.8% blaCTX-M-15 and 19.7% blaNDM. The association of positive string test with the presence of virulent genes was statistically borderline significant for blaCTX-M-15 and significant for blaNDM.

Conclusion: All the genes analyzed in hvKp isolates including NDM1, blaCTX-M-15 and iucA were prevailing with iucA(78.9%) being most prevalent. In addition to it carbapenems resistance was found to be increasing in hvKp in our study.

Keywords: Hypervirulent Klebsiella pneumoniae, antimicrobial resistance, prevalence

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Introduction

Klebsiella pneumoniae (K. pneumoniae) is a widely recognized in hospital-acquired infections (HAIs) in immunocompromised individuals and can colonize the skin, nasopharynx, and gastrointestinal tract. It is from Enterobacteriaceae family, a gram negative, encapsulated, non-motile, lactose fermenting bacterium capable of causing a variety of diseases, including wound infections, bacteremia, pneumonia, and urinary tract infections.[1] There are two distinct pathotypes of Klebsiella pneumoniae: the most prevalent subtype, known as classical (cKp), which has gained more attention because of its propensity to develop multidrug resistance, and a recently discovered new variety known as hypervirulent Klebsiella (hvKp). Although the prevalence of the HvKp varies greatly, with China having 12% to 45%[2] and India 87.5%.[3]

The hvKp isolates have recently documented the rise of multidrug-resistant (MDR) strains. In Taiwan, K. pneumoniae infection was noted in 1986 patients.[4] With no underlying biliary tract disease, seven healthy community members developed pyogenic liver abscesses. They also experienced purulent meningitis, pulmonary embolization, septic endophthalmitis, and prostatic abscess.[5]

World Health Organization (WHO) considers carbapenems resistant K.pneumoniae isolates to be of "critical concern". A "hypermucoid appearance" on agar plate known as hypermucoviscous (HMV) Kp is a distinguishing feature of hvKp which is due to overproduction of its polysaccharide capsule.[6] There are small molecules known as siderophores that have the ability to steal iron from host iron-chelating proteins. Enterobactin (entB), yersiniabactin (YbtS), and aerobactin are among the siderophores that are expressed by K. pneumoniae. Aerobactin, which is more frequently seen in hvKP, increases the pathogenicity of the bacteria. The hvKp strains have 6-10 times greater siderophore activity in comparison to cKp. Aerobactin is a crucial virulence factor for hvKp, which frequently coexists with the mucoid phenotype.[7]

A multi-center study in China first described the clinical and molecular traits of aerobactin-positive hvKp.

This characteristic of siderophore production, in turn, has increased the resistance of hvKP against complement mediated antimicrobial activity.[1] Although there were a number of cases of hvKp documented in Europe and the America, the pandemic spread of hvKp largely in Asian nations, including Taiwan, China, South Korea, and Iran. For instance, 84/369 clinical isolates of K. pneumoniae linked to different invasive diseases in China were found to be hvKP in 22.8% of the cases. The hypermucoviscosity trait was present in 42.2% of K. pneumoniae strains recovered from bacteremia patients in South Korea.[2,3] Extended-spectrum -lactamases (ESBLs) of the CTX-M type have become widespread over the past ten years. In remote| geographic areas (Germany, France, and Argentina) in the early 1990s, the first microbes expressing this kind of -lactamase were discovered as lone and epidemic clinical isolates. In numerous hospitals in Spain, the United Kingdom, China, Canada and Korea it has recently been noted that the proportion of various CTX-M variants to Temoniera (TEM) and Sulf-hydryl variable (SHV) derived extended-spectrum-lactamase (ESBLs) is rapidly increasing.[3] Carbapenem-hydrolyzing beta lactamases are carried either on DNA or through plasmid. According to the ambler classification beta lactamases have four major classes (A,B,C,D) among which Metallo-beta-lactamases (MBLs) are more common and fall in class B and include enzymes such as IMP, VIM, GIM, SIM and NDM1. NDM has the ability to hydrolyze all beta lactamases with the exception of aztreonam just like other acquired MBLs, aztreonam is often inactivated by co-produced ESBLs or Amp C beta lactamases. Because of its origin from Indian subcontinent, many studies have included samples from Pakistan and India to observe the prevalence and dissemination of this enzyme.[4]

In many parts of the world, comprehensive characterization of ESBL-producing K. pneumoniae has been carried out, but limited information is available from Pakistan. Keeping in view the aforementioned emerging risk of highly resistant klebsiella pneumoniae, our study was aimed to see the prevalence of virulent and resistant genes in isolates of klebsiella pneumoniae in this local Pakistani community.

Methods

It was a Cross-sectional study conducted at Microbiology Lab, Department of pathology, King Edward Medical University, Mayo Hospital, Lahore for 6 months 1st January 2024 to 30th June 2024 after ethical approval of synopsis vide letter No.158/RC/KEMU dated 21-12-2023. Sample size of 76 was estimated by using 95% confidence level, 10% absolute precision. Presumptive Klebsiella Pneumoniae containing samples collected from the microbiology lab underwent antibiotic susceptibility test by using the most common method the Kirby-Bauer disk diffusion method. The strains then categorized into susceptible, intermediate, or resistant in accordance with the Clinical Laboratory Standards Institute (CLSI) 2023 guidelines.[6] All isolates were subjected to string test as an HvKp screen test. An inoculation loop was used to stretch a colony established on the agar plate and the hyper viscosity was identified by evaluating the formation of mucoviscous string >5mm in length. CLSI 2023 guidelines were used to interpret the results[7]. The ESBL phenotypes were confirmed by disc diffusion and double disc synergy tests while inoculating the Muller Hinton Agar with the standard inoculums of test isolates.[8] Boiling method was used to extract DNA. Several PCR amplifications were carried out on thermal cycler for the genes encoding carbapenems, blaNDM, blaCTX-M and iucA9. The primers, sizes of the expected amplification product, and annealing temperatures used for PCR amplification were noted.

After this, isolates were subjected to agarose gel electrophoresis using TAE buffer.[10-13] Association of phenotypic and genotypic method was seen using chi square test.

Results

Out of 76 patients, 54(71%) were males and 22(29%) were females. Among these 76 isolates 99.5% were Polymyxin B, Colistin (PB, CT) sensitive, 2.6% were gentamycin (CN) and Cefoperazone/sulbactam (SCF) sensitive, 46.1% had Imipenem (IPM) susceptibility and 18.4% had overall sensitivity of PB, CT, IPM, SCF & CN. 78.9% isolates showed positive string test. The percentages of genes present were 68.4% iucA,40.8% blaCTX-M-15 and 19.7% BlaNDM. The association of positive string test with the gene's presence was statistically insignificant for iucA (p=1.00), boarderline significant for blaCTX-M-15(p=0.05) and highly significant for blaNDM (p=0.001).

Figure 1: *Positive String Test*

Table 1: *Demographics of Patients*

n	76
Resistance among females	22(29%)
Resistance among males	54(71%)
String test positive	60(79%)
String test negative	16(21%)
iucA	52(68.4%)
BlaCTXM-15	31(40.8%)
BlaNDM	15(19.7%)

Table 2: *Association of String Test and Genes Using Chi Square Test (n=76)*

String Test	iuc-A		P-value	Interpretation
	Present	Absent		
Positive	41	19	1.00	Not signifycant
Negative	11	05		
Total	52(68.4%)	24(31.6%)		
	BlaCTXM-15		0.05	Significant (Boarder line)
	Present	Absent		
Positive	08	25		
Negative	23	20		
Total	31(40.8%)	45(59.2%)		
	BlaNDM		0.001	Significant
	Present	Absent		
Positive	5	50		
Negative	10	11		
Total	15(19.7%)	61(80.3%)		

Discussion

In our study we found increasing prevalence of carbapenem resistance in hvKp isolates and among the prevalent genes iucA (68.4%) was most prevalent. Hypervirulent K. pneumoniae (hvKp) was initially reported in 1986. In contrast to cKp, the hvKp strains had special characteristics. In addition to sick patients, immunocompetent and young healthy persons can also contract serious infections from hvKp strains due to their hypermucoviscosity.

A 2-year survey in Ireland revealed twenty-eight carbapenem resistant hypervirulent K. pneumoniae ST23 strains. All these K. pneumoniae strains were recovered from various clinical samples, including rectal swab, feces (as colonizer) as well as from blood culture specimens (from persistent infections). All hypervirulent K. pneumoniae strains displayed multiple virulence markers suggesting the hypervirulent pathotype namely, rmpA2, iuc, iro, ybt.[14]

Five important acquired virulence loci linked to invasive infections were investigated in a study on the genetic and phenotypic characterization of Klebsiella pneumoniae. These loci consist of the siderophores yersiniabactin (ybt), aerobactin (iuc), and salmochelin (iro), the genotoxin colibactin (clb), and regulator of mucoid phenotype A (rmpACD and/or rmpA2).99 out of 112 LAKp were discovered to have at least one of the pathogenicity loci indicated above.[15]

Rao shamitha and collegues studied that carbapenem resistance and multidrug resistance were observed in 35.29% of the hvKp strains. The mortality rate in patients infected with hvKp was 23.52%.The combination of genetic markers such as rmpA with iucA has high reported diagnostic accuracy.[16]

In another study at Dutch patients, it was concluded that hvKp strains do occur but are relatively uncommon in Dutch patients and differ from international strains. No carbapenems-producers were found among study isolates.[17]

In a recent study it was concluded that HvKp are increasingly being reported in hospital-acquired settings, complicating treatment strategies. In particular, while hvKp have historically been antibiotic-susceptible, multidrug-resistant (MDR) strains have emerged and this posed a significant public health threat.[18]

In this research the hvKP strains were collected from mayo hospital Lahore and the prevalence of antimicrobial resistance and virulent genes were studied. All the results of this study represented an alarming situation that the hvKP is an emerging high risk pathogen with the increasing resistance to carbapenems and aminoglycosides.

Conclusion

All the virulent genes analyzed in hvKp isolates including iucA (68.4%), blaMTX-M-15(40.8%) and blaNDM (19.7%) were prevailing but iucA was the most prevalent gene found among these isolates. In addition to it carbapenems resistance was found to be increasing in hvKP in our study. Thus, iucA high prevalence can be used as a marker of hypervirulence. The high multidrug resistance is concerning leading to difficult to treat infections.

Ethical Approval: The IRB/EC approved this study via letter no. 158/RC/KEMU dated December 21, 2023.

Conflict of Interest: None

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Authors' Contribution

HB: Conception.

TK: Design of the work.

RM, KA: Data acquisition, analysis, or interpretation.

RM, KA, TK: Draft the work.

HB: Review critically for important intellectual content.

All authors approve the version to be published.

All authors agree to be accountable for all aspects of the work.

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