Genomic Landscape of Hypervirulent *Klebsiella pneumoniae* (hvKp) in Bangladesh: Comparative Analysis with Global hvKp Lineages

By

Arnob Sharma
Student ID: 23176019

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Masters of Science in Biotechnology

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Examining Committee:

Supervisor:
(Member)

Fahim Kabir Monjurul Haque, PhD
Associate Professor
Department of Microbiology,
School Of Life Sciences
BRAC University, Bangladesh

External Expert Examiner:
(Member)

Mohd. Raeed Jamiruddin, PhD
Associate Professor;School of Pharmacy,
BRAC University, Bangladesh

Departmental Head:
(Chair)

Iftekhar Bin Naser, PhD
Associate Professor and Chairperson
Department of Biotechnology
School of Life Sciences
BRAC University, Bangladesh

Ethics Statement

All data used in this study were obtained from publicly accessible genomic repositories and contained no patient-identifiable information. Consequently, no ethical approval or informed consent was required.

Abstract

Hypervirulent *Klebsiella pneumonia* (hvKp) has emerged as a major global public health concern due to its ability to cause severe, invasive infections and its increasing convergence with antimicrobial resistance (AMR). While hvKp has been extensively studied in East and Southeast Asia, genomic data from South Asia, particularly Bangladesh remain limited. This thesis presents a comprehensive comparative genomic analysis of hvKp isolates from Bangladesh in a global context, focusing on population structure, virulence determinants, antimicrobial resistance profiles, and capsular diversity. Using publicly available whole-genome sequencing data from the Pathogen Watch platform, a total of 20,430 *K. pneumoniae* genomes were analyzed, including 601 isolates from Bangladesh. Hypervirulent strains were defined using established genomic markers, primarily the presence of the aerobactin (iuc) locus, ensuring high specificity. Comparative analyses were conducted between Bangladeshi and global isolates using sequence typing, virulence scoring, resistance burden metrics, and multivariate statistical approaches. The results reveal that the *K. pneumoniae* population in Bangladesh represents a limited subset of global diversity, dominated by a small number of enriched sequence types, notably ST11, ST16, ST48, and ST147. Hypervirulent strains accounted for only 4.5% of Bangladeshi isolates, substantially lower than the global average. Bangladeshi hvKp exhibited strong enrichment of key virulence loci, including aerobactin, yersiniabactin, and rmpA/rmpA2, but showed lower prevalence of colibactin compared to global hvKp populations. Although Bangladeshi hvKp isolates demonstrated high levels of multidrug resistance—particularly to aminoglycosides, fluoroquinolones, and sulfonamides—the prevalence of carbapenem-resistant hvKp remained low. Capsular and O-antigen analyses identified a distinctive regional serotype profile dominated by K20, K2, and O1 variants, differing markedly from global patterns. Multivariate analyses confirmed that Bangladeshi hvKp form a genetically constrained

cluster with limited virulence–AMR convergence. Overall, this study highlights Bangladesh as being in an early epidemiological phase of hvKp evolution, presenting a critical opportunity for targeted surveillance, antimicrobial stewardship, and region-specific intervention strategies before the widespread emergence of highly resistant hypervirulent clones.

Keywords: Hypervirulent *Klebsiella pneumonia*; Bangladesh; Global; Sequence type; Antimicrobial Resistance; Virulence determinants; Genomic epidemiology.

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List of Acronyms

Acronym	Full Form
AMR	Antimicrobial Resistance
ARGs	Antimicrobial Resistance Genes
BD	Bangladesh
CAUTIs	Catheter-Associated Urinary Tract Infections
clb	Colibactin locus
CR-hvKp	Carbapenem-Resistant Hypervirulent *Klebsiella pneumoniae*
cKp	Classical *Klebsiella pneumoniae*
CTX-M	Cefotaximase-Munich β-lactamase
ESBL	Extended-Spectrum Beta-Lactamase
hvKp	Hypervirulent *Klebsiella pneumoniae*
ICEKp	Integrative and Conjugative Element of *Klebsiella pneumoniae*
ICU	Intensive Care Unit
iuc	Aerobactin synthesis locus
iutA	Aerobactin receptor gene
iro	Salmochelin synthesis locus
KPC	*Klebsiella pneumoniae* Carbapenemase
Kp	*Klebsiella pneumoniae*
LMICs	Low- and Middle-Income Countries
MDR	Multidrug Resistant
MGEs	Mobile Genetic Elements
MLST	Multilocus Sequence Typing
NDM	New Delhi Metallo-β-lactamase
OXA	Oxacillinase

PCA	Principal Component Analysis
PDR	Pandrug Resistant
rmpA / rmpA2	Regulator of Mucoid Phenotype
SD	Standard Deviation
SHV	Sulfhydryl Variable β-lactamase
ST	Sequence Type
WHO	World Health Organization
XDR	Extensively Drug Resistant
ybt	Yersiniabactin locus

Chapter 1

Introduction

1.1 *Klebsiella pneumonia* and Its Clinical Significance.

Klebsiella pneumonia is part of a group of pathogens known as ESKAPE, which includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species (1). *Klebsiella pneumonia* are opportunistic gram-negative bacteria that are encapsulated, non-motile, and rod-shaped. They are widespread, living primarily in soil, water, and sewage ecosystems and colonizing the gastrointestinal and nasopharynx tracts (2,3). Infections caused by *Klebsiella pneumonia* include pneumonia, meningitis, septicemia, and lung abscesses (4). These infections pose a serious risk to newborns, the elderly, and people with compromised immune systems (5).

Extensive drug resistance (XDR) is common in bacteria due to horizontally acquired antimicrobial resistance (AMR) genes (5,6). The presence of virulence factors in *Klebsiella pneumonia*, such as surface antigens, fimbriae, capsules, outer membrane proteins, and siderophores, facilitates invasion and proliferation (2,7). Rapid changes in virulence factors and AMR genes are possible due to its adaptability, which includes chromosomal recombination and plasmid exchange (8). The genomic epidemiology of *Klebsiella pneumonia* in resource-constrained environments, such as Bangladesh, is poorly understood despite its prominence.

1.2 Clinical Impact of *Klebsiella pneumonia* Infections

Klebsiella pneumonia's linked to a number of nosocomial infections, including bloodstream infections, septicemia, pneumonia, urinary tract infections, surgical site infections, and soft tissue infections (9). Some of the community acquired infections by *Klebsiella pneumonia* may cause cough, fever, pleuritic chest pain, and shortness of breath (9,10). It is also associated with lung abscesses, meningitis, endophthalmitis, pyelonephritis, wound infections, pyogenic liver abscesses, and burns (2,11). Survivors frequently have impaired lung function, and recovery can take a long time (12).

1.3 Risks Factors Influencing Susceptibility to *Klebsiella pneumonia* Infection

Due to their immature gastrointestinal mucosal barriers and underdeveloped immune systems, newborns are at a higher risk. Prior antibiotic use significantly increases the risk of

patients acquiring Klebsiella, particularly when broad-spectrum or multiple antibiotics are used (13,14). Numerous factors can affect one's susceptibility to *Klebsiella pneumonia* infection (Table 1).

Table 1. Factors Influencing Susceptibility to *Klebsiella pneumonia* Infection.

Type	Factors	References
Pathogen-related	Antibiotic resistance and virulence factors	(15).
Host intrinsic	Age, diabetes, cancer, liver and gallbladder diseases, chronic obstructive pulmonary disease, renal failure, and nutritional status are all factors	(15)(16).
Extrinsic	Using antibiotics, eating poorly, alcoholism, chemotherapy, transplantation, hospital and intensive care unit stays, and dialysis	(17).

1.4 *Klebsiella pneumonia* Colonization, Infection, and Transmission in Healthcare Settings

Highly resistant *Klebsiella pneumonia* strains benefit from multi-drug resistance in hospitals where antibiotic use is prevalent (18). The main reservoirs for *Klebsiella* transmission in hospitals, besides medical devices and blood products, are patients' gastrointestinal tracts and hospital staff members' hands (17). In a recent study, the prevalence of *Klebsiella pneumonia* colonic colonization ranged from 5.8 to 35% in Western countries and 18.8 to 87.7% in Asia (17,19). In Western countries, 1-5% of healthy individuals were nasopharyngeally colonized with *Klebsiella pneumonia*, while children under 10 in Brazil and Vietnam showed rates of 1.4% and 1.6% respectively (19).

Klebsiella primarily inhabits both environmental sources (surface water, sewage, soil, and plants) and healthcare facilities, and antibiotic use influences its colonization frequency (2,20). In healthcare settings, distinguishing between colonization and infection is critical because different rates of colonization by *Klebsiella pneumonia* occur in different people depending on their environment and exposures (2,3).

1.5 Pathogenesis of *Klebsiella pneumonia*

A variety of virulence factors are used by *Klebsiella pneumonia* to establish infections, suppress host immune responses, and ensure its survival (21). AMR genes are often found on mobile genetic elements (MGEs) that help bacteria cause infections. These MGEs also contain virulence factors like capsules, adhesins, and siderophores (2,22). Additionally,

Klebsiella pneumonia can create biofilms on catheters, which can lead to Catheter-Associated Urinary Tract Infections (CAUTIs), and thus make infection control more difficult (23).

Klebsiella pneumonia's polysaccharide capsule is an important virulence factor that protects it from host defenses. Lipopolysaccharides on the bacterium's surface cause inflammation, and contribute to sepsis (24). The bacterium attaches to host cells via adhesins like fimbriae (types 1 and 3) and pili, enabling adhesion to diverse surfaces. Additionally, type 3 fimbriae mediate the formation of biofilms on both biotic and abiotic surfaces (25). Moreover, capsule modification and the presence of iron-absorbing molecules such as enterobactin, yersiniabactin, salmochelin, and aerobactin contribute to virulence, immune evasion, and pathogenicity in various strains (26). Effector delivery systems and genes like AcrAB play a role in mediating antibacterial activity and resistance, respectively (27,28).

Hypervirulent *Klebsiella pneumonia* (hvKP), first discovered in Taiwan in 1986, is a virulent bacterium that causes serious infections like pneumonia and liver abscesses. The hvKP strains depend on virulence factors for survival and infection, with siderophores, adhesion, and increased capsule production being the most important ones (29–31). Over the last few decades, however, a more aggressive and clinically significant pathotype has emerged hvKp. Since its first recognition in the 1980s, hvKp has rapidly gained global attention due to its enhanced virulence and distinct capacity to cause severe, invasive infections—even in otherwise healthy, immunocompetent individuals (32). Unlike cKp, which is largely restricted to hospital settings, hvKp frequently causes severe community-acquired infections, including pyogenic liver abscesses, pneumonia, meningitis, endophthalmitis, and necrotizing fasciitis (29). This fundamental shift in pathogenicity underscores hvKp as a major and escalating threat to global public health (32).

1.6 Genomic Biomarkers vs. Phenotypic Identification

Historically, hvKp was identified by the "string test," where a positive result is defined by a viscous string >5 mm. However, recent genomic evidence suggests that hypermucoviscous phenotypes do not always correlate with high virulence (31). The "gold standard" has shifted toward molecular biomarkers located on the pLVPK-like virulence plasmid. Key markers include the siderophores aerobactin (*iuc*A) and salmochelin (iroB), and the mucoid phenotype regulators (rmpA/rmpA2) (33). Aerobactin is currently regarded as the most accurate predictor of the hypervirulent phenotype, as it allows the bacteria to thrive in iron-poor host

environments (34). Utilizing these genomic markers provides a more definitive classification of "true" hvKp compared to the often-unreliable phenotypic string test.

1.7 Antimicrobial Resistance (AMR) in *Klebsiella pneumonia*: A Growing Concern

Klebsiella pneumonia can acquire antimicrobial resistance genes (ARGs), which leads to the emergence of XDR and Multi drug resistance (MDR) strains (26). Its acquired resistance to a variety of antibiotic classes is caused by a combination of chromosomal and plasmid-encoded genes (35).

Klebsiella pneumonia was identified as the main causative organism in a recent meta-analysis that linked infections with carbapenem-resistant strains with mortality rates ranging from 26% to 44% (36). New Delhi metallo-beta-lactamase (NDM) is clinically significant among newly emerging carbapenemases because of its increased resistance, evolution, and widespread distribution (37). Since 2008, 16 new blaNDM alleles have developed from NDM, with Asia serving as a significant source of plasmid-mediated transfer (38). The issue arises because beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, constitute up to 60% of the available treatment choices for antibiotic-resistant gram-negative bacteria (37).

1.8 Virulence Mechanisms and Distinctive Features of hvKp

The hypervirulent phenotype of hvKp is underpinned by a specific suite of genetic and phenotypic traits that clearly distinguish it from cKp. Key among these traits is the production of a thick capsular polysaccharide and the display of a hypermucoviscous phenotype, both of which significantly contribute to resistance against host immune clearance, enhanced survival in host tissues, and increased invasiveness (33). Furthermore, hvKp frequently harbors genes encoding high-affinity siderophore-mediated iron uptake systems (e.g., aerobactin, yersiniabactin, salmochelin), enabling efficient iron acquisition in the iron-limiting host environment, which facilitates bacterial growth and virulence (29). Other virulence factors, such as fimbriae (for adhesion), outer membrane proteins, lipopolysaccharide, and various secretion systems, also contribute to effective colonization, immune evasion, tissue invasion, and the successful establishment of infection (29). Given this formidable combination of virulence determinants, hvKp is capable of causing severe, invasive disease often characterized by rapid onset and systemic dissemination, even in community settings and in individuals lacking classic infection risk factors (39). Because of

the inherent genetic and phenotypic diversity among hvKp isolates, definitive identification remains challenging, necessitating the proposed use of specific molecular biomarkers—such as the simultaneous detection of genes like *iucA, iroB, peg-344, and rmpA/rmpA2*—as the most reliable approach for hvKp surveillance and diagnosis (39).

1.9 The Dual Threat: Convergence of Hypervirulence and Antimicrobial Resistance

The Emergence of Resistance in hvKp

Initially, hvKp strains were generally susceptible to most antibiotics (apart from intrinsic resistances), allowing for relatively effective clinical management despite their heightened virulence (19,39). However, a troubling global trend has emerged: hvKp strains are increasingly acquiring AMR determinants. This acquisition has led to the dangerous convergence of hypervirulence with MDR, which critically includes resistance to last-line antibiotics such as carbapenems (19,39,40). This resulting "dual-threat" combination—high virulence coupled with high resistance—significantly complicates established treatment strategies, severely reduces therapeutic options, and inherently raises the risk of severe clinical outcomes, particularly in environments with limited antimicrobial resources (41).

The Global Crisis of Carbapenem-Resistant hvKp (CR-hvKp)

The most alarming development is the emergence and spread of carbapenem-resistant hvKp (CR-hvKp) strains, which carry carbapenemase genes. Recent global surveillance networks, including a 2024 report from the World Health Organization (WHO), confirm that isolates of the high-risk hvKp sequence type (ST) 23, now harboring carbapenem-resistance genes, have been detected across all WHO global regions (42). The proliferation of CR-hvKp threatens to undermine last-line antibiotic therapies worldwide and represents a looming public health crisis, especially in low- and middle-income countries (LMICs) where access to advanced antibiotics or robust infection control practices is often limited (43).

1.10 Genomic Diversity and Geographic Distribution of *Klebsiella pneumonia* Sequence Types (ST)

In a study led by Christian G. et al, multilocus sequence typing (MLST) was performed on *Klebsiella pneumonia* strains associated with the spread of blaNDM-1 in India, Sweden, and the United Kingdom. They revealed various sequence types with genetic diversity among ST11, ST14, ST15, ST26, ST101, ST147, ST149, ST231, ST258, ST627, and ST977 (44).

Some sequence types exhibit clear geographic associations, while others develop into epidemic or endemic diseases. For instance, ST258 has become well-known in North America, Latin America, and some of Europe, demonstrating its impact on the world (9,45). Other than that, ST11 is widely distributed throughout South America and Asia, and ST627 has only recently appeared in Egypt (9,45). According to a two-year epidemiological study conducted in Korea, different *Klebsiella pneumonia* sequence types are endemic and exhibit varying degrees of AMR (46). In Greece, it was reported that ST258 coexisted with other *Klebsiella* sequence types, creating difficulties for treatment and infection control in healthcare facilities (47).

1.11 One Health Perspective: Environmental and Transmission Dynamics

In countries like Bangladesh, the transmission of *K. pneumoniae* is a "One Health" issue involving humans, animals, and the environment. High population density and inadequate wastewater treatment in cities like Dhaka facilitate the movement of AMR genes between hospital effluents and municipal water sources (48). *K. pneumoniae* serves as a "hub" for horizontal gene transfer, where environmental strains in soil or sewage can acquire virulence and resistance plasmids through contact with clinical waste (49). This environmental persistence creates a continuous cycle of re-infection and dissemination, making the genomic monitoring of environmental reservoirs as critical as clinical surveillance.

1.12 *Klebsiella pneumonia* Infections: A Global Concern

Klebsiella pneumonia causes roughly one-third of all gram-negative bacterial infections (9). It was accountable for around 15% of gram-negative infections in hospital intensive care units (ICUs) in the United States between 1993 and 2004 (50). Furthermore, worldwide, *Klebsiella pneumonia*'s the causative agent in 11.8% of cases of hospital-acquired pneumonia (10). In special care newborn units (SCANU), *Klebsiella pneumonia*'s a significant concern, particularly in low-resource settings. These infections can account for up to 50% of neonatal deaths in hospitals (2,23). It is the third most common bloodstream pathogen in children, according to global surveillance data(51). An estimated 2.5 million newborns worldwide died within the first 28 days of birth in 2018, with nearly 80% of these deaths taking place in South Asia and sub-Saharan Africa (52). The worldwide burden of infant mortality, as of 2019, stands at a rate of 28 deaths per 1,000 live births (53). This emphasizes the imperative

need for comprehensive strategies to address *Klebsiella pneumonia* infections as a pressing global health concern.

The emergence of CR-hvKp is a particularly significant growing hazard in Bangladesh. Previous local studies discovered the prevalence of high-risk multidrug-resistant hvKp clones—including ST11, ST14, and ST307—in Dhaka (54). These clones demonstrate a troubling synergy, being linked to both high antimicrobial resistance rates and the presence of critical virulence genes. This complex interplay between virulence and resistance is compounded in Bangladesh by factors such as inadequate antimicrobial stewardship programs and elevated rates of antibiotic misuse. Despite increased awareness of hvKp, the genomic and phenotypic diversity of the pathogen within Bangladesh remains poorly understood, with limited information available on its precise genetic landscape, ST, and local antimicrobial resistance patterns. This critical knowledge gap makes it exceptionally difficult to establish effective, targeted surveillance and appropriate intervention techniques.

1.13 *Klebsiella pneumonia* and Antibiotic Resistance Challenges in Bangladesh

In South Asian regions, the indiscriminate and irrational use of antibiotics frequently leads to prolonged, unsuccessful treatments, and an escalating disease burden (55). Poor outcomes are particularly likely to affect newborns, the elderly, and immunocompromised people (56). *Klebsiella* in Bangladesh exhibits resistance to various antibiotic classes, including aminoglycosides, macrolides, and beta-lactams (57). In a notable 2021 study, 30% of CPKP isolates (43 out of 145) were observed, with 14% of them classified as pandrug-resistant (PDR) strains (58). NDM-1 has been discovered to be highly prevalent in various environmental samples of ESBL-producing *Enterobacteriaceae* in Bangladesh, including clinical samples and samples taken from sewage systems and natural water sources (58).

Third-generation cephalosporin resistance has been detected in many *Klebsiella pneumonia* isolates from clinical samples (60.6% of cefpodoxime, 59.9% of ceftriaxone, and 52.1% of cefotiam). Recent studies on *Klebsiella* revealed resistance to nitrofurantoin (91%) and amoxicillin (91%) (59,60). Treatment effectiveness has been compromised due to NDM, oxacillinase (OXA), and sulfhydryl variables (SHV-201, SHV-202). Effective policies to combat antibiotic resistance are hindered due to the lack of knowledge, resources, and widespread monitoring (61).

Klebsiella pneumonia ranked third among the organisms recovered from clinical specimens in Bangladesh's national AMR surveillance study, behind *Pseudomonas* species and *Escherichia coli*. The mortality of young children with pneumonia has been directly impacted by the rise in *Klebsiella pneumonia* resistance rates, according to a study between 2014 and 2017 (62). According to an in-country report, the percentage of MDR *Klebsiella pneumonia* has risen over time, reaching 80%. As a result, carbapenem has become the preferred drug for treating infections caused by MDR *Klebsiella pneumonia (63,64)*.

1.14 Specific Carbapenemase Alleles in the Bangladesh Context

In Bangladesh, the genomic landscape of resistance is dominated by specific carbapenemase alleles that complicate treatment. While bla_{NDM-1} is the most widespread, there is an increasing prevalence of bla_{OXA-48-like} (notably bla_{OXA-181}) and bla_{KPC-2} (58). These genes are frequently located on highly mobile IncFII or IncL/M plasmids, which are often found in high-risk clones like ST11 and ST147 (65,66). The co-existence of these carbapenemases with ESBL genes, such as bla_{CTX-M-15}, provides a genetic foundation for the emergence of pandrug-resistant (PDR) strains in local clinical settings.

This thesis, therefore, seeks to close this substantial gap by performing a comprehensive genomic analysis of the genetic diversity, virulence profiles, and antibiotic resistance trends of hvKp strains circulating in Bangladesh. This study will examine an extensive collection of *K. pneumoniae* isolates to compare local hvKp strains with established global lineages in terms of ST, detailed virulence factors, AMR, and capsular diversity. The findings will critically expand our knowledge about the prevalence and specific genetic characteristics of hvKp in Bangladesh, which will, in turn, guide public health interventions and bolster global initiatives aimed at addressing this complex, emerging pathogen.

1.15 Objectives of the Study

The primary objective of this study was to characterize and compare hypervirulent *Klebsiella pneumonia* (hvKp) isolates from Bangladesh with global hvKp lineages in terms of sequence type (ST), virulence determinants, antimicrobial resistance (AMR), and capsular diversity.

The secondary objectives are structured to provide a detailed genomic profile:

1. Population Structure: To identify dominant STs in Bangladeshi hvKp isolates and identify any STs present globally but absent in Bangladesh.
2. Virulence Determinants: To characterize major virulence loci (iuc, iro, rmpA/rmpA2, ybt, clb) in Bangladeshi hvKp, compare their prevalence with global isolates, and highlight any unique or enriched virulence gene combinations.
3. Antimicrobial Resistance (AMR): To identify specific AMR genes and resistance classes in Bangladeshi hvKp, compare their resistance profiles globally, and assess the convergence of virulence and AMR (hvKp carrying high-risk resistance genes).
4. Capsular Diversity: To determine K- and O-locus types in Bangladeshi hvKp and compare them with classical hypervirulent types globally (e.g., K1, K2, KL64, O1, O2).
5. hvKp vs Non-hvKp Comparisons: To compare STs, virulence, and AMR profiles between hvKp and non-hvKp isolates in Bangladesh and determine whether these patterns align with global observations.
6. Unique Features of Bangladeshi hvKp: To identify virulence and AMR gene combinations unique to Bangladesh and highlight STs and capsular types enriched in Bangladesh compared to global datasets.

By achieving these explicit objectives, this study will provide crucial genomic data on hvKp strains in Bangladesh, helping to fill the knowledge gap and offering valuable insights for improving local and regional surveillance, diagnostics, and treatment strategies for hvKp infections.

1.16 Rationale

HvKp poses a growing global health threat due to its enhanced virulence and increasing convergence with multidrug resistance. While hvKp has been well characterized in East and Southeast Asia, South Asia — and Bangladesh in particular — remains understudied despite being a hotspot for antimicrobial resistance. Current genomic data on Bangladeshi hvKp are limited, with few studies addressing ST diversity, virulence profiles, and AMR patterns in a representative set of isolates. Understanding the genomic landscape of hvKp in Bangladesh is essential to:

- Identify high-risk clones circulating locally.
- Assess the prevalence and distribution of virulence and AMR determinants.
- Compare Bangladeshi hvKp strains with global lineages to detect unique features.

- Inform public health strategies by highlighting strains that may pose higher clinical risk due to combined virulence and AMR.

This study addresses these gaps by analyzing a large dataset of *K. pneumoniae* isolates from Bangladesh and placing them in a global context using ST, virulence, and AMR profiles.

Chapter 2

Methodology

1. Study Design and Analytical Overview

This study employed a comparative genomic epidemiology approach to characterize the population structure, virulence architecture, and AMR profiles of *Klebsiella pneumonia* isolates from Bangladesh, with a specific focus on hvKp. Bangladeshi isolates were analysed in parallel with a large, curated global dataset to contextualize national trends within worldwide hvKp lineages.

All analyses were conducted using publicly available whole-genome sequencing metadata and implemented through reproducible bioinformatic and statistical workflows in Linux, Mac and R Studio.

2. Data Source and Genome Collection

2.1 Source Database

Genomic metadata for *Klebsiella pneumonia* were obtained from Pathogen Watch, a publicly accessible genomic surveillance platform developed by the Centre for Genomic Pathogen Surveillance. Pathogen Watch integrates the Kleborate framework to infer sequence types (STs), virulence loci, capsular (K) and O-antigen loci, and acquired antimicrobial resistance determinants from genome assemblies.

2.2 Dataset Composition

A total of 20,430 *K. pneumoniae* genomes were retrieved, representing all isolates available in Pathogen Watch as of 27 October 2025. The dataset spanned 41 countries and multiple years of global sampling, enabling broad geographic and evolutionary comparisons.

- Bangladesh: 601 isolates
- Non-Bangladesh (Global): 19,829 isolates

2.3 Exclusion of United States Isolates

Isolates originating from the United States were deliberately excluded from the analysis. The U.S. dataset alone comprised more than 12,000 genomes, which would have dominated the

global sample and exceeded the intended analytical scope and available computational resources.

This exclusion was implemented to maintain balance across regions and to ensure that global comparisons reflected diverse international lineages rather than being disproportionately influenced by a single country. Consequently, results referring to “global” isolates should be interpreted as representative rather than exhaustive, particularly for lineages heavily sampled in the United States.

3. Metadata Extraction and Data Integration

3.1 Retrieved Variables

The following variables were downloaded for each isolate:

- Sequence type (ST) based on MLST
- Virulence loci, including:
    - Aerobactin (*iucA–D, iutA*)
    - Salmochelin (iroB–D, iroN)
    - Yersiniabactin/irp locus (ybt genes, irp1)
    - Colibactin (clbA–X)
    - Capsule regulators (rmpA, rmpA2, rmpC, rmpD)
- Antimicrobial resistance genes, grouped by antibiotic class
- Capsular (K) locus types
- O-antigen (O) locus types
- Kleborate virulence score

3.2 Data Merging and Cleaning

Individual metadata files were concatenated using standard Unix shell commands and imported into R Studio for further processing. Data cleaning involved:

- Removal or flagging of incomplete entries
- Identification of assemblies with reported quality issues
- Standardization of variable names and categorical labels
- Conversion of gene presence/absence calls into binary indicators

The final curated dataset was stored in a structured tabular format suitable for downstream statistical and graphical analyses.

4. Definition of Hypervirulent *K. pneumoniae* (hvKp)

To ensure biological relevance and analytical robustness, hvKp was defined using two complementary genomic criteria.

4.1 Primary Definition (High Specificity)

An isolate was classified as hvKp if it met either of the following conditions:

- Presence of the aerobactin locus (iuc) alone, or
- Presence of iuc together with rmpA and/or rmpA2(67)

Rationale: Aerobactin is widely recognized as the most reliable single genetic marker of hypervirulence due to its essential role in iron acquisition and invasive disease. This conservative definition emphasizes specificity and reproducibility.

All principal analyses in this study were based on this primary definition.

4.2 Sensitivity Analysis (Broader Coverage)

As an independent validation, hvKp was also identified using a Kleborate virulence score ≥3, which integrates multiple virulence loci (iuc, iro, ybt, clb).(31)

This secondary approach was used to confirm trends observed under the primary definition and to ensure that conclusions were not sensitive to classification thresholds.

5 Quantification of Virulence Burden

5.1 Individual Virulence Loci

Presence or absence of each virulence gene or operon was analysed individually to identify loci enriched in hvKp relative to non-hvKp populations.(68)

5.2 Composite Virulence Score

To summarise the cumulative virulence potential of each isolate, a composite virulence score was calculated based on the number of key virulence determinants present:

Virulence Factor	Gene / Locus	Primary Function
Aerobactin	*iuc*	High-affinity iron acquisition system; key marker of hypervirulence
Salmochelin	*iro*	Siderophore-mediated iron uptake and immune evasion
Capsule regulators	*rmpA / rmpA2*	Regulation of capsule production and hypermucoviscosity
Yersiniabactin / irp locus	*ybt / irp*	Iron acquisition and enhanced survival in host tissues
Colibactin	*clb*	Genotoxin associated with DNA damage and increased virulence

This score enabled quantitative comparison of virulence burden between hvKp and non-hvKp isolates and across geographic regions.

6. Quantification of Antimicrobial Resistance (AMR)

AMR burden was assessed using three complementary metrics, capturing both breadth and intensity of resistance:

1. Resistance Score (0–3)
   Reflects the number of major antibiotic classes affected by acquired resistance determinants.
2. Number of Resistance Classes
   Counts the distinct antibiotic classes for which resistance genes or mutations were detected.
3. Total Number of AMR Genes
   Measures the absolute number of acquired resistance genes per isolate.(69)

Using multiple metrics ensured that conclusions were not dependent on a single operational definition of resistance burden.

7. Comparative Analyses

7.1 Bangladesh vs Global Comparisons

Analyses were conducted at multiple levels, including:

- Sequence-type (ST) distributions
- hvKp prevalence
- Virulence gene frequencies
- AMR profiles
- K-type and O-type diversity

Bangladeshi isolates were compared with non-Bangladeshi global isolates to identify locally enriched clones, absent global lineages, and unique regional patterns.

7.2 ST-Stratified Analyses

To control for population structure, AMR and virulence metrics were also compared within major STs shared between Bangladesh and the global dataset.

8. Multivariate and Clustering Analyses

8.1 Principal Component Analysis (PCA)

PCA was performed on hvKp isolates using integrated virulence and AMR variables to explore multidimensional relationships and to visualize functional diversity between Bangladeshi and global hvKp populations.

8.2 Hierarchical Clustering

A balanced subset of hvKp isolates (50 Bangladeshi and 50 global) was analysed using hierarchical clustering with Ward's D2 method based on Euclidean distances. This approach assessed whether Bangladeshi hvKp formed distinct functional groupings or represented a constrained subset of global diversity.

9. Software and Computational Environment

- Operating system: Linux and Mac
- Statistical analysis and visualization: R Studio
- Data manipulation: Base R and tidyverse packages

Linux was selected for its stability and compatibility with large-scale genomic datasets, while R Studio provided flexibility for statistical modelling and figure generation. Packages Used:

- Data Manipulation: tidyverse, data.table
- Statistical Analysis: stats, vegan
- Genomic Analysis: biomaRt, GenomicRanges, seqinr
- Clustering & PCA: cluster, factoextra
- Visualization: ggplot2, gplots, heatmap3.

10. Ethical Considerations

All data used in this study were obtained from publicly accessible genomic repositories and contained no patient-identifiable information. Consequently, no ethical approval or informed consent was required.

11. Study Limitations

This study is subject to several limitations:

- Reliance on publicly available genomes may introduce sampling bias, as sequencing intensity varies between countries and STs.
- Exclusion of U.S. isolates may result in underrepresentation of certain globally dominant AMR lineages, particularly carbapenem-resistant clones.
- The number of Bangladeshi hvKp isolates was relatively small, which limits statistical power for some correlation analyses.

Despite these limitations, the dataset remains one of the most comprehensive comparative genomic assessments of hvKp in Bangladesh to date.

Chapter 3

Results

1. Population Structure of *Klebsiella pneumonia* in Bangladesh in a Global Context

1.1 Sequence Type (ST) Diversity and Distribution

Within the compiled dataset, 1,465 unique STs were identified. Among the Bangladeshi isolates, only 115 STs were identified, suggesting that the *K. pneumoniae* population in Bangladesh constitutes a limited component of the global diversity. Still, these 115 STs include several high-risk lineages that are well-known around the world.

A modest number of STs made the most of the Bangladeshi collection. Together, the ten most common STs (ST11, ST16, ST48, ST147, ST14, ST340, ST502, ST15, ST307, and ST17) were responsible for 57.7% of all isolates from Bangladesh.

ST11 was the most prevalent lineage in Bangladesh (15.5%), followed by ST16 (9.5%), ST48 (8.5%), ST147 (8.2%), ST14 (3.2%), and ST340 (3.0%). Several of these lineages showed marked enrichment in Bangladesh relative to their global frequencies, including:

Sequence Type (ST)	Bangladesh (%)	Global (%)	Fold Enrichment (BD vs Global)
ST48	8.5	1.0	~8.7-fold
ST16	9.5	3.5	~2.7-fold
ST340	3.0	1.0	~3.1-fold
ST147	8.2	4.8	~1.7-fold

These results imply that certain high-risk clones may be spreading locally in Bangladesh.

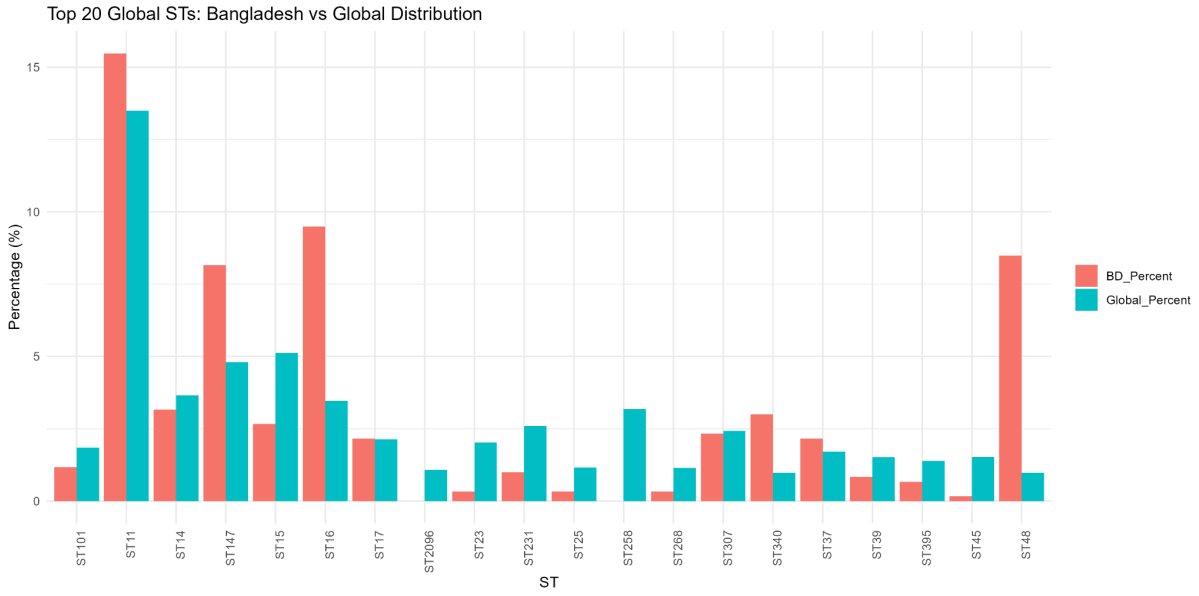

Figure 1:Top 20 STs distribution of BD vs Global

1.2 Comparison of Dominant Bangladeshi STs with Global Populations

The top 20 STs that are most common worldwide were compared between Bangladesh and the global dataset (*Figure: 1*), revealing both similarities and differences in the population structure of Bangladesh. Bangladesh participates in important globally dispersed *K. pneumoniae* lineages, as evidenced by the fact that ST11, the most prevalent ST globally (13.5%), was also the dominating lineage in Bangladesh (15.5%).

On the other hand, a number of STs that are widespread throughout the world were not as prevalent in Bangladeshi isolates. For instance, ST15 accounted for 5.1% of isolates worldwide but only 2.7% in Bangladesh; ST23 for 2.0% worldwide against 0.3% in Bangladesh; and ST231, ST25, ST268, ST39, ST395, and ST45 each contributed more than 1% of the global collection but less than 1% of isolates from Bangladesh. Despite accounting for 3.2% and 1.1% of all isolates worldwide, respectively, two of the most prevalent lineages, ST258 and ST2096, were completely absent from the Bangladeshi dataset.

Overall, these data indicate that the Bangladeshi *K. pneumoniae* population is characterized by a combination of regionally enriched clones (ST16, ST48, ST147, and ST340) and globally disseminated high-risk lineages (e.g., ST11), while several canonical global epidemic STs (especially ST258 and ST2096) seem rare or absent.

Together, these data indicate that Bangladesh shares some globally successful lineages but exhibits a distinct ST composition, characterised by regional enrichment and absence of several globally dominant clones. The entire data analysis for this is in supplementary table 1.

2. Global context of the dominant Bangladeshi sequence types:

In the current dataset, 20 STs were unique to Bangladesh (BD_Proportion_Global = 100%). Additionally, 10 STs had ≥50% of their worldwide isolates coming from Bangladesh. Importantly, several of these were not rare isolated individuals but rather lineages with multiple Bangladeshi isolates, such as ST244 and ST626, which had 56.3% and 52.9% of all documented isolates, respectively, from Bangladesh. These data suggest that, in addition to sharing globally distributed clones, Bangladesh contains a group of STs that are either exclusive to or highly concentrated in this region.

The ten most prevalent STs in Bangladesh were ST11, ST16, ST48, ST147, ST14, ST340, ST502, ST15, ST307, and ST17, accounting for 57.7% of Bangladeshi isolates but only 37.1% of the global collection *(Figure 2)*. ST11 was the most common lineage in both contexts (15.5% in Bangladesh and 13.5% internationally). Several STs were enriched in Bangladesh relative to their global frequency: ST16 (9.5% vs 3.5%), ST48 (8.5% vs 1.0%), ST147 (8.2% vs 4.8%), ST340 (3.0% vs 1.0%), and, most notably, ST502 (2.8% vs 0.09%). On the other hand, ST15 was less common in Bangladesh (2.7%) than internationally (5.1%), but ST14, ST307, and ST17 were equally prevalent in both datasets.

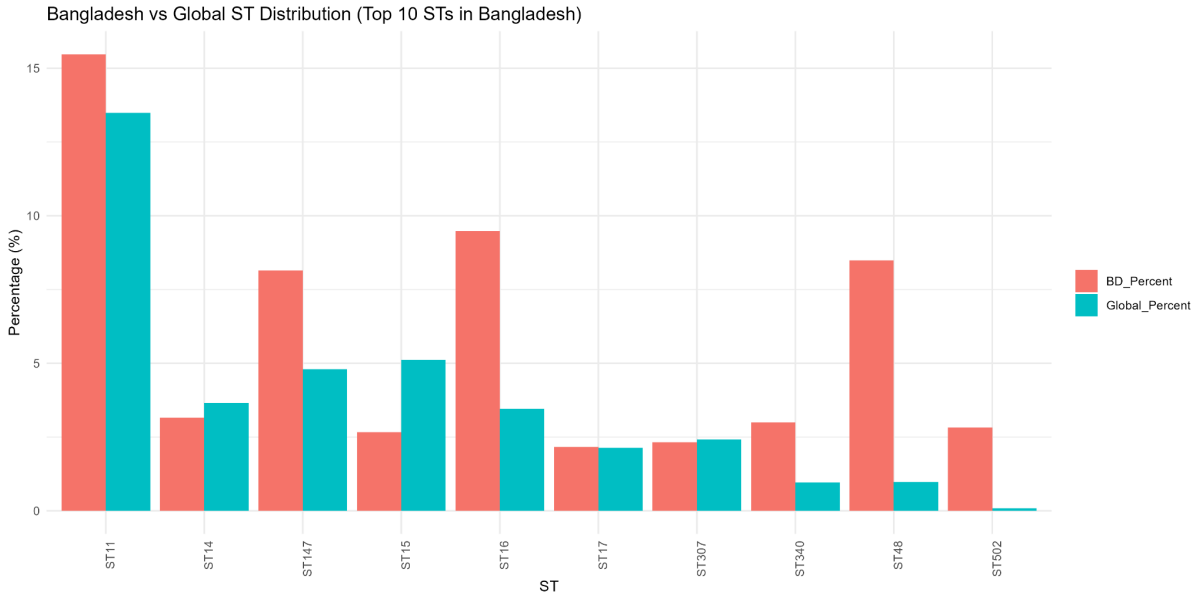

Figure 2:Top 10 dominant BD STs

When it comes to the global presence of these major Bangladeshi lineages, Bangladesh accounts for nearly half of all ST502 isolates (48.6%), as well as significant numbers of ST48 (20.9%), ST340 (8.6%), and ST16 (7.7%). For the globally common clones ST11 and ST147, isolates from Bangladesh made up 3.4% and 4.9% of the total world burden, respectively. The whole analysis is in supplementary table 2

4. Globally Prevalent STs Absent from Bangladesh

We examined all STs in the combined dataset that included no Bangladeshi isolates to identify *K. pneumoniae* lineages that are common globally but have not yet been observed in Bangladesh. The global collection contained 1,350 STs but did not include any from Bangladesh. These "non-Bangladeshi" STs accounted for 7,090 of 19,829 worldwide isolates, or 35.8% of the total dataset. Thus, more than one-third of all *K. pneumoniae* isolates collected worldwide belonged to STs that were not found in Bangladesh in this investigation.

The 20 most common STs that were prevalent globally but not in Bangladesh generated 2,370 isolates (12.0% of total global isolates) (*Figure 3*). ST258 was the most common lineage, accounting for 632 isolates (3.2% of the global collection), yet it was completely absent from the Bangladesh dataset. Other globally important but locally absent STs were ST2096 (214 isolates, 1.1%), ST2621 (164 isolates, 0.8%), ST86 (161 isolates, 0.8%), ST323 (131 isolates, 0.7%), ST661 (110 isolates, 0.6%), ST512 (93 isolates, 0.5%), and ST36 (88 isolates, 0.4%). Additional STs, such as ST12, ST13, ST1828, ST219, ST111, ST348, ST412, ST133, ST280, ST461, ST2938, and ST405 each contributed 50–85 isolates globally but were absent from Bangladeshi isolates.

Taken together, these findings reveal that, whereas Bangladesh shares several major STs with the global *K. pneumoniae* population, a significant fraction of globally ubiquitous lineages—including well-represented clones like ST258 and ST2096—were not found in this collection. The entire analyzed data is in supplementary table 3.

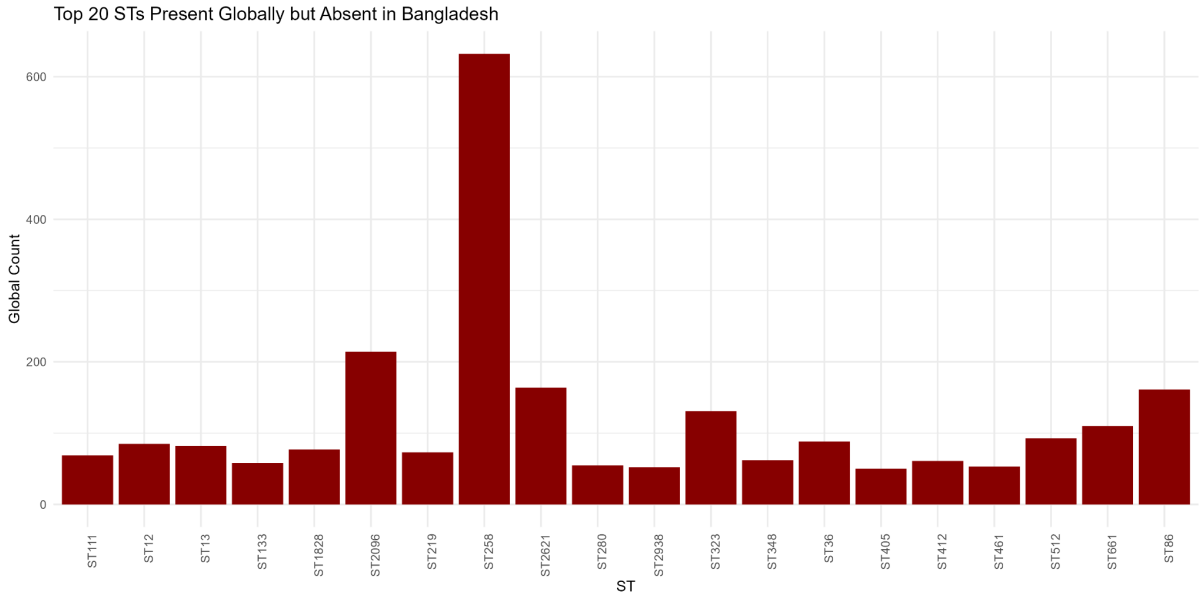

Figure 3:Top20 STs present globally but absent in Bangladesh

5. Sequence Types Unique to Bangladesh

To examine the existence of regionally confined *K. pneumoniae* lineages in Bangladesh, we identified STs exclusive to Bangladeshi isolates and absent in isolates from any other country within the aggregated dataset. A total of 20 STs met the criteria for being classified as Bangladesh-unique (Global_Count = BD_Count). These lineages represented 30 of 601 isolates from Bangladesh (4.99%), indicating that the bulk of isolates were associated with internationally shared STs, while a minor subset consisted of clones that were either geographically confined or rarely collected. The whole analyzed data is in Table.3 supplementary table 4.

The main STs in Bangladesh were singletons (BD_Count = 1), which means they had an uneven or limited spread. However, several sequence categories included multiple isolates (Figure 4). The primary unique lineage identified was ST24821LV, comprising five isolates.Three isolates each represented two supplementary sequence types, ST19911LV and ST6366, while two isolates each represented ST31751LV and ST4411LV. The other 15 STs, which were only found in one Bangladeshi isolate, were ST1111LV, ST1685, ST1746, ST1804, ST2136, ST2341LV, ST4085, ST421LV, ST4321, ST4494, ST5043LV, ST5468, ST564, ST6758, and ST861LV.

Table 2: Unique STs present in Bangladesh.

ST	BD_Count	ST	BD_Count
ST24821LV	5	ST2341LV	1
ST19911LV	3	ST4085	1
ST6366	3	ST421LV	1
ST31751LV	2	ST4321	1
ST4411LV	2	ST4494	1
ST1111LV	1	ST5043LV	1
ST1685	1	ST5468	1
ST1746	1	ST564	1
ST1804	1	ST6758	1
ST2136	1	ST861LV	1

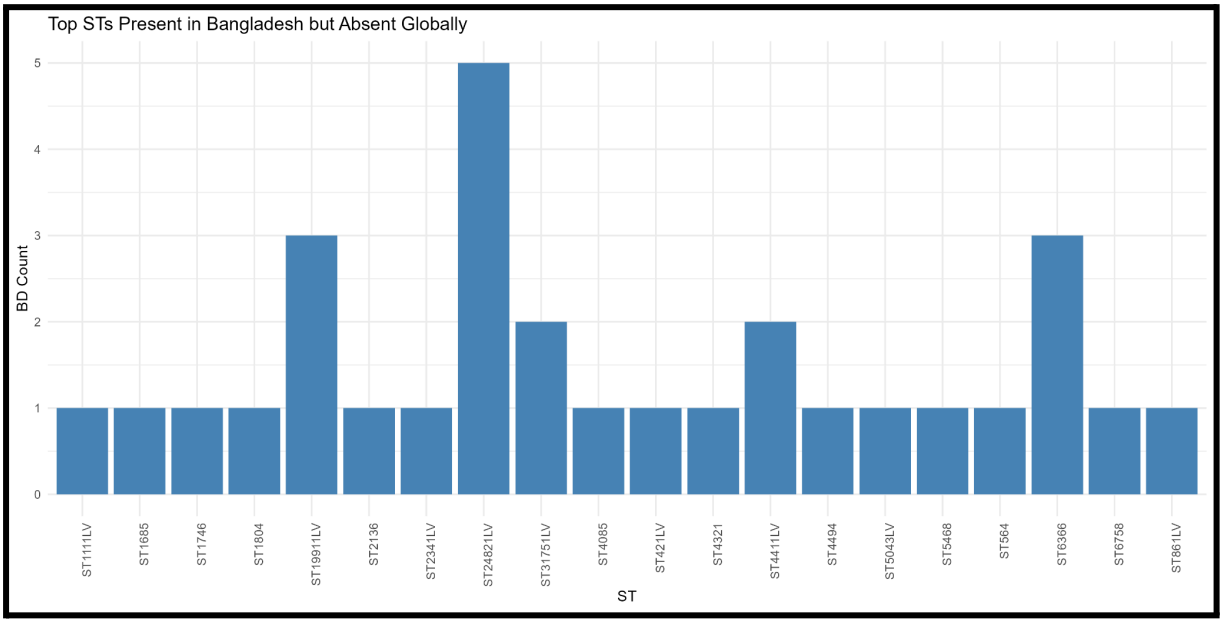

Figure 4: STs present in Bangladesh but absent in Global

These findings indicate that while Bangladesh shares the majority of significant *K. pneumoniae* lineages with the global population, it also harbors a collection of seemingly country-specific STs, some of which (e.g., ST24821LV, ST19911LV, and ST6366) encompass multiple isolates and may signify the emergence of local clones. Additional genomic and

epidemiological analysis of these Bangladesh-specific STs may elucidate whether they are genuinely region-specific or merely underrepresented in other global locales.

6. Prevalence of Hypervirulent *K. pneumoniae* (hvKp)

We employed genomic criteria for hvKp, characterized by specific hypervirulence loci, to assess the prevalence of hvKp and non-hvKp isolates in Bangladesh in comparison to other global regions. A total of 19,829 samples were collected from various countries ("Global"), whereas 601 samples were obtained from Bangladesh. This process resulted in a total of 20,430 isolates. The entire analyzed data is in Table 4. supplementary table 5.

Table 3: Comparison of of BD and Global hvKp and non-hvKp

Label	Count (n)	Percent	Group
non-hvKP	574	95.50749	Bangladesh
hvKP	27	4.492512	Bangladesh
non-hvKP	16772	84.58319	Global
hvKP	3057	15.41681	Global
non-hvKP	17346	84.90455	Combined
hvKP	3084	15.09545	Combined

Only 27 of 601 isolates (4.5%) in Bangladesh were categorized as hvKp, while 574 of 601 (95.5%) were categorized as non-hvKP. Of the 19,829 isolates in the non-Bangladeshi global dataset, 3,057 (15.4%) were found to be hvKP, while 16,772 (84.6%) were found to be non-hvKP. In the non-Bangladeshi global dataset, 3,057 out of 19,829 isolates (15.4%) were identified as hvKP, while 16,772 (84.6%) were classified as non-hvKP. When all isolates were aggregated, hvKP included 3,084 out of 20,430 isolates (15.1%), whereas the remaining 84.9% comprised non-hvKP (*Figure 5*). The relative frequency of hvKP in Bangladesh was around one-third that of the global average (4.5% compared to 15.4%).

Thus, hvKp prevalence in Bangladesh was approximately one-third of the global average, indicating that non-hypervirulent lineages dominate the Bangladeshi *K. pneumoniae* population.

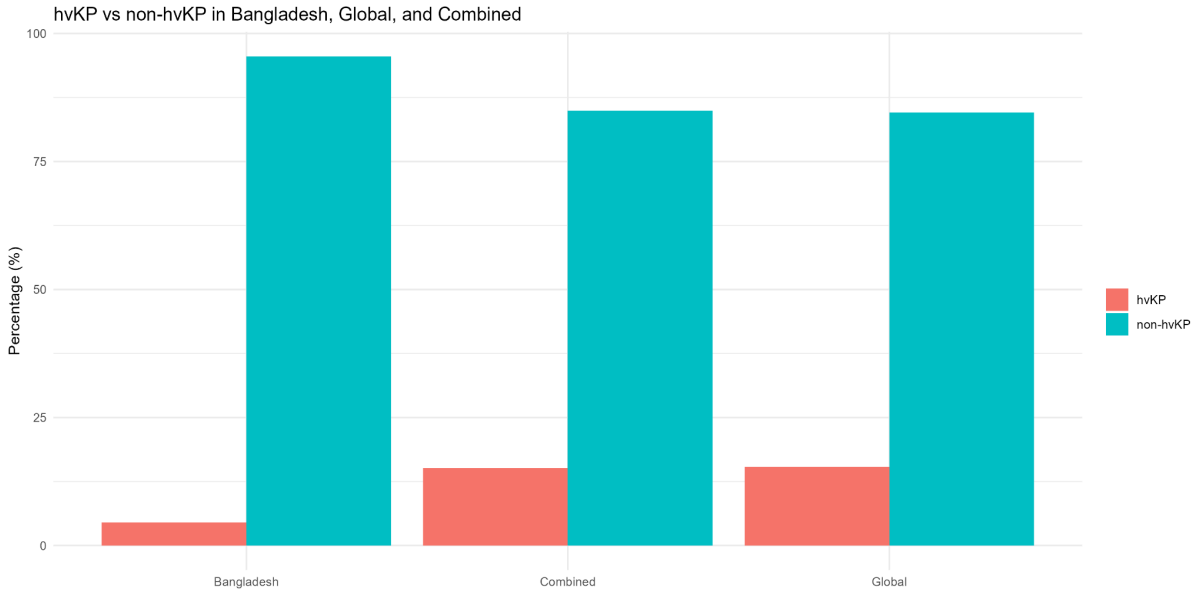

Figure 5: hvKP and hvKP comparing BD vs Global

7. Sequence-Type Distribution among hvKp and Non-hvKp Isolates

Across the combined dataset, hvKp displayed a highly clonal population structure. We looked at ST distributions across the combined dataset (20,430 isolates) to compare the population structure of hvKp with non-hypervirulent isolates. In all, 17,346 isolates (84.9%) were categorized as non-hvKP and 3,084 isolates (15.1%) as hvKP. The hvKp population was highly clonal: the top 15 hvKP STs accounted for 86.3% of all hvKP isolates, whereas the same STs represented only 25.9% of non-hvKP isolates (*Figure 6*). The whole analyzed part on this supplementary table 6.

The most common lineage among hvKP was ST11, which made up 27.6% of hvKP isolates. This was followed by ST23 (12.5%), ST231 (12.1%), ST2096 (5.9%), ST15 (5.3%), ST86 (4.6%), ST65 (3.7%), and ST395 (3.5%). In contrast, these STs were much less frequent among non-hvKP: ST23, ST65, and ST86 each accounted for ≤0.2% of non-hvKP isolates, ST2096 for 0.2%, and ST231 and ST395 for <1.0%. Several additional STs—ST218, ST268, ST383, ST412, and ST420—each contributed 1.1–1.6% of hvKP but ≤0.1% of non-hvKP. These patterns indicate a strong association of ST23, ST231, ST2096, ST65, ST86, and related lineages with the hypervirulent phenotype.

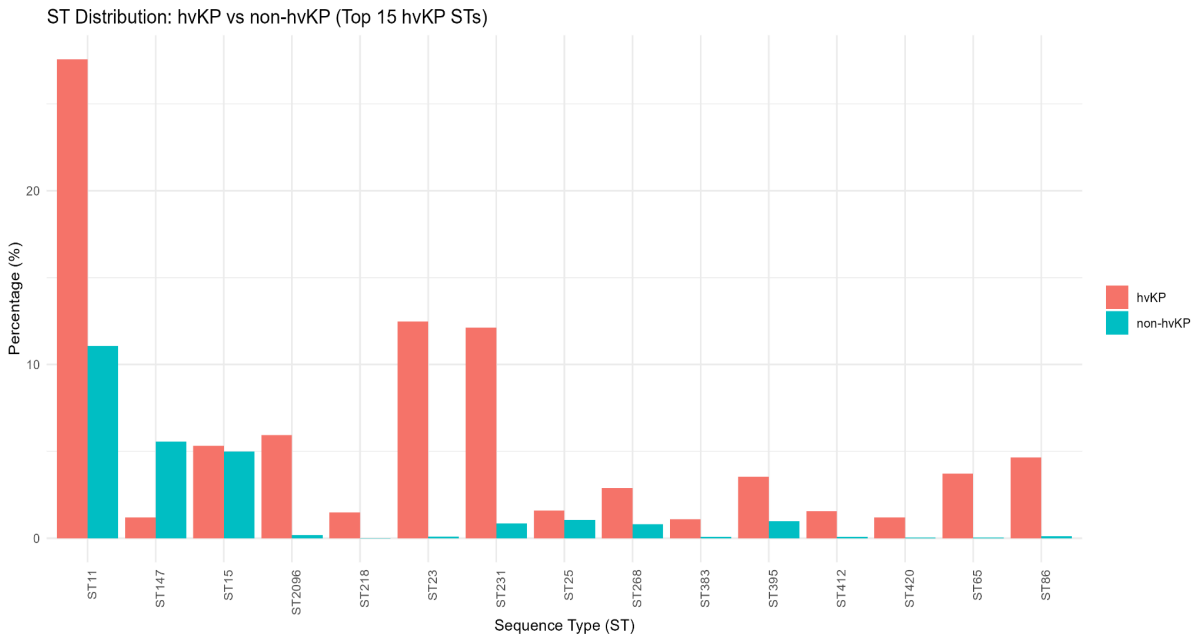

Figure 6: Top 15 hvKp and non-hvKp STs distribution

By comparison, some STs were more evenly distributed between hvKp and non-hvKp or were predominantly non-hvKp. ST11 remained frequent in both groups (27.6% of hvKp vs. 11.1% of non-hvKp), indicating that it is a widely distributed clone. ST15 had equal representation in hvKp and non-hvKp (5.3% vs. 5.0%); however, ST147 was primarily found in non-hvKp (1.2% of hvKp vs. 5.6%). Overall, these findings indicate that hvKP is concentrated in a small number of STs that are relatively uncommon among non-hvKP, whereas some high-risk lineages, such as ST11 and ST15, occur in both hypervirulent and non-hypervirulent populations.

8. Virulence Architecture of hvKp versus Non-hvKp

Distribution of loci for hypervirulence:

Hypervirulence loci exhibited significant clustering within the hvKP group when analyzed among 3,084 hvKP isolates and 17,346 non-hvKP isolates (Figure 7). By definition, hvkp_presence was absent from all isolates that were not hvKp and present in 100% of hvKp. In hvKp, the aerobactin locus (iucA–D and iutA) was nearly universal (99.8–99.9%), whereas in non-hvKp, it was virtually absent (≤0.04% for each gene).

Although the overall incidence was lower, the salmochelin locus (iroB, iroC, iroD, and iroN) displayed a similar pattern. IroB, iroC, and iroD were found in approximately 36% of hvKp

isolates and iroN in 47.9%, while each of these genes was found in less than 0.7% of non-hvKP isolates. The yersiniabactin/irp1 locus (ybt/irp) was, on the other hand, common in both groups but enriched in hvKp: irp1_presence and its related ybt genes were detected in approximately 83.3% of hvKp as opposed to approximately 48.8% of non-hvKp. This suggests that yersiniabactin is more common among hypervirulent lineages rather than being unique to them.

Additionally, there was a substantial correlation between hvKp and classical hypermucoidy regulators. While rmpA2 was found in 67.3% of hvKp isolates and essentially missing from non-hvKp isolates (0.02%), rmpA was found in 78.2% of hvKp isolates but only 0.6% of non-hvKp isolates. The downstream genes rmpC and rmpD were found in around 49.2% and 47.9% of hvKp, respectively, while they were found in approximately 0.6% of non-hvKp. Together, these findings demonstrate that aerobactin, salmochelin, yersiniabactin/irp1, and rmpA/rmpA2-associated loci are tightly combined in hvKp isolates, while these determinants are uncommon or nonexistent in non-hvKp isolates. The whole analyzed data is in supplementary data 7.

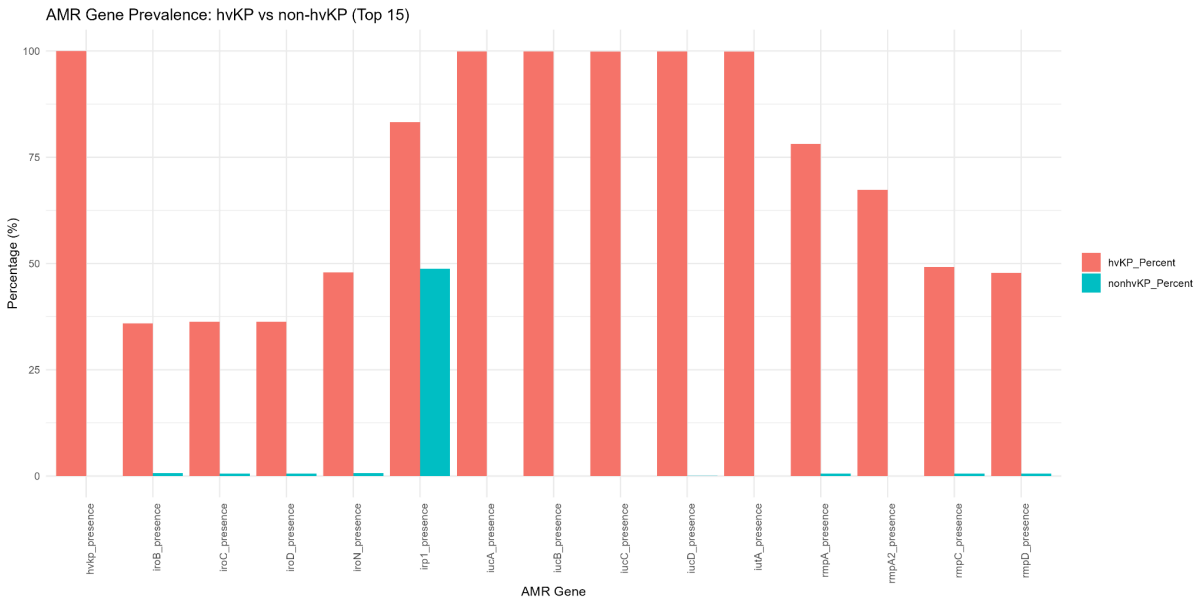

Figure 7: Top 15 AMR genes that cause of hvKP and non-hvKP

Total burden of antimicrobial resistance:

We evaluated a composite resistance score between hvKP and non-hvKP isolates to ascertain if hypervirulence was associated with an increased burden of resistance (Table 5). The

average resistance scores for hvKP and non-hvKP isolates were 1.44 (SD 0.94) and 1.44 (SD 0.82) to two decimal places, respectively (3.084 vs. 17,346 isolates). The average resistance burden in this dataset did not significantly differ between hvKP and non-hvKP, as the mean differences were small (~0.003 units) and well within the overlap of the two distributions.

While the overall level of acquired resistance is similar to that of non-hvKP isolates, these findings indicate that hvKP isolates are significantly enriched in conventional hypervirulence loci, including aerobactin, salmochelin, yersiniabactin/irp1, and rmpA/rmpA2. The presence of resistance determinants in hvKP lineages, comparable to those found in the broader *K. pneumoniae* population, suggests a convergence between hypervirulence and antibiotic resistance. The entire analyzed data is on table 5 supplementary table 8.

Table 4: Resistance score of hvKp and non-hvKp

hvKP	mean_res_score	sd_res_score	n
FALSE	1.43572	0.820705	17346
TRUE	1.438716	0.944008	3084

9. Virulence determinants in hvKP versus non-hvKP

Distribution of virulence genes

Genes such as rmpA, rmpA2, and hvKp presence are almost exclusive to hvKp isolates. These are critical virulence factors that promote capsule production, which is a hallmark of hypervirulence in Klebsiella. The presence of genes like aerobactin and iron uptake genes (iroB, iroC, iroD) highlights the ability of hvKp to acquire iron efficiently, further supporting its ability to evade host defences and establish infections.

The top 15 virulence genes were almost exclusively confined to the hvKP population (*Figure 8*). By construction, hvkp_presence and Aerobactin_presence were detected in essentially all hvKP isolates and were absent from non-hvKP. The aerobactin operon (iucA, iucB, iucC, iucD and iutA) showed the same pattern: each gene was present in nearly 100% of hvKP isolates, whereas only a tiny fraction of non-hvKP carried any of these determinants (bars are close to zero on the plot). The whole analyzed data is in supplementary table 9.

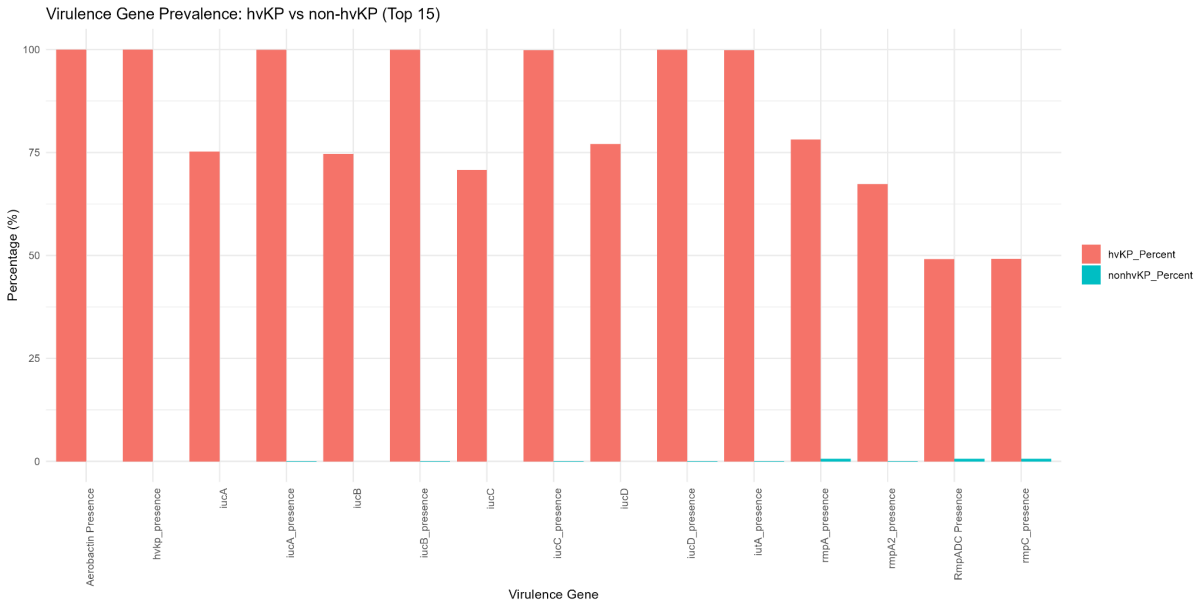

Figure 8: Top 15 virulence genes of hvKP and non-hvKP.

Similarly, genes associated with hypermucoidy, including rmpA, rmpA2 and the combined rmpADC locus, were highly enriched in hvKP. Around two-thirds to three-quarters of hvKP isolates carried rmpA and rmpA2, and about half carried the broader rmpADC cluster, while these genes were detected only sporadically among non-hvKP isolates. Overall, the bar chart shows a striking dichotomy: for each of the 15 virulence markers displayed, hvKP isolates have very high frequencies (typically ≥70–100%), whereas non-hvKP isolates show an almost complete absence of these loci.

These data confirm that hvKP, as defined in this study, is characterised by a tightly linked virulence cassette comprising aerobactin (iucA–D, iutA) and hypermucoidy regulators (rmpA/rmpA2/rmpADC), which is largely missing from the broader non-hvKP population.

Composite virulence score

To summarise the overall virulence gene burden per isolate, we calculated a virulence score based on the number of key virulence determinants present. As shown in (*Table 6*), hvKP and non-hvKP are separated by this metric. Non-hvKP isolates had a low mean virulence score of ~0.5, indicating that most carried none or only a single virulence gene from the panel. In contrast, hvKP isolates had a mean virulence score of ~4.0, consistent with the carriage of multiple virulence loci in combination. The whole data analysis is on table 6. supplementary table 10.

Table 5: Virulence score of hvKp and non-hvKp

hvKP	mean_virulence_score	sd_virulence_score	n
FALSE	0.507956	0.538375	17346
TRUE	4.005512	0.581615	3084

Taken together, these findings show that hvKP lineages are distinguished from non-hvKP not only by the presence of individual virulence genes but also by a highly conserved, multi-gene virulence module, reflected in a markedly elevated virulence score. This strong genetic separation backs up the hvKP definition that was used and shows how dangerous these lineages could be for causing serious invasive disease.

10. Antimicrobial resistance profiles of Bangladeshi hvKP compared with global hvKP

We next compared the distribution of acquired antimicrobial resistance (AMR) determinants between Bangladeshi hvKP (n = 27) and hvKP from the rest of the world (n = 3,057) across major resistance classes (*Figure 9*). Overall, Bangladeshi hvKP showed a broad MDR profile but had a distinct pattern compared with global hvKP, particularly for carbapenem, colistin and trimethoprim resistance.

Both groups had high prevalence of aminoglycoside resistance genes (AGly_presence), with Bangladesh having a slightly higher prevalence (74.1% vs. 70.8%). Likewise, chromosomal β-lactam resistance markers (Bla_chr_presence) were very common in hvKP globally and in Bangladesh (77.8% vs 76.1%). ESBL genes (Bla_ESBL_presence) were more frequent in Bangladeshi hvKP than in global hvKP (74.1% vs 57.9%), and the category “any β-lactam resistance gene” (Bla_presence) also showed higher prevalence in Bangladesh (66.7% vs 40.0%). For several non-β-lactam classes, Bangladeshi hvKP again exhibited higher rates of resistance: fluoroquinolone resistance genes (Flq_presence) were present in 66.7% of Bangladeshi hvKP compared with 57.0% of global hvKP, macrolide–lincosamide–streptogramin (MLS_presence) in 63.0% vs 41.1%, sulfonamide resistance (Sul_presence) in 70.4% vs 56.8%, and tetracycline resistance (Tet_presence) in 59.3% vs 39.2%.

In contrast, several high-priority resistance mechanisms were less frequent or absent among Bangladeshi hvKP. Carbapenemase genes (Bla_Carb_presence) occurred in only 3.7% of

Bangladeshi hvKP, compared with 63.5% of global hvKP, indicating that carbapenem-resistant hvKP are still relatively uncommon in the Bangladeshi collection. Similarly, colistin resistance mutations (Col_mutations_presence), fosfomycin resistance (Fcyn_presence) and rifampicin resistance (Rif_presence) were not detected in Bangladeshi hvKP (0% for each), whereas these determinants were present in 7.3%, 12.5%, and 20.0% of global hvKP, respectively. Resistance to trimethoprim (Tmt_presence) and phenicol antibiotics (Phe_presence) was also lower in Bangladesh (22.2% and 14.8%) than in global hvKP (56.8% and 39.8%).

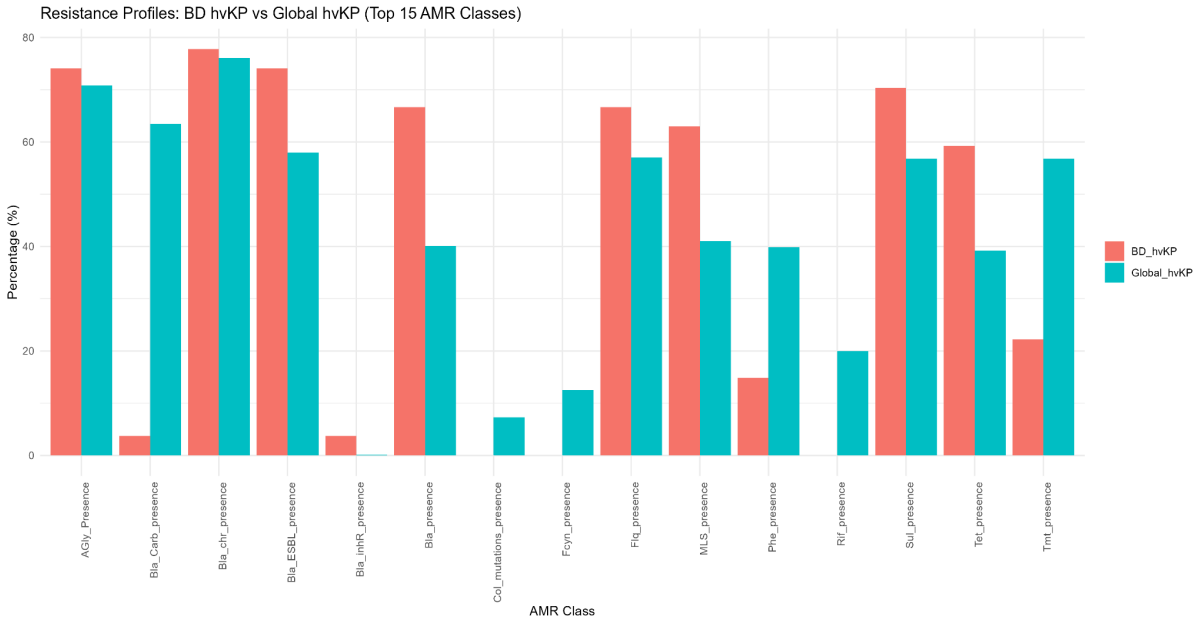

Figure 9: AMR class comparison between BD hvKP and Global hvKP

The whole analyzed data is in supplementary table 11.

Taken together, these data indicate that Bangladeshi hvKP isolates are highly multi-drug resistant, with particularly high levels of ESBL, fluoroquinolone, sulfonamide, tetracycline, and MLS resistance, but they currently show much lower rates of carbapenemase, colistin, fosfomycin, rifampicin, trimethoprim, and phenicol resistance than hvKPs circulating globally. This pattern suggests substantial MDR pressure in Bangladesh, but with a resistance profile that is, for now, less dominated by carbapenemase- and colistin-resistant hvKP than in many other regions.

11. Antimicrobial Resistance Burden in hvKp versus Non-hvKp

AMR Burden (Resistance Classes) hvKp vs. non-hvKp

The graphic shows how the AMR load is different between hvKp and non-hvKp strains based on the number of resistance classes. The hvKp strains (teal box plot) have a greater range of resistance classes than the non-hvKp strains (red box plot). The mean (the line in the middle of the box) for hvKp is much higher, which means that hvKp strains tend to have more resistance classes (*Figure 10*). This means that hvKp strains have more ways to become resistant to antibiotics. Non-hvKp Shows Less AMR Burden: The red box plot for non-hvKp reveals a lower range of resistance classes, which means that non-hvKp strains usually have fewer ways to resist.The line that crosses the box is the lower median, which shows a reduced overall AMR burden in non-hvKp strains and a higher chance of antibiotic response.

Outliers in HvKp: HvKp strains have a number of outliers (dots outside the box), which suggests that some of them are particularly resistant to antibiotics. This could be due to some changes or resistance genes that were picked up. If this happens, it would mean that some hvKp isolates are very resistant to many drugs. This makes it much harder to find treatment options.

From the summary statistics, non-hvKP isolates harboured on average **6.58 resistance classes** (SD 3.24), while hvKP carried **6.34 classes** on average (SD 3.98). Thus, hvKP did **not** show a higher class-level resistance burden; if anything, the mean number of resistant classes was slightly lower in hvKP, although the difference was small relative to the wide spread in both groups. The boxplots illustrate this overlap, with comparable medians and ranges for hvKP and non-hvKP. Supplementary table 12

Table 6: AMR burden of hvKp and non-hvKp

hvKP	mean_classes	sd_classes	mean_genes
FALSE	6.579384	3.241302	9.626196
TRUE	6.340791	3.983131	8.549611

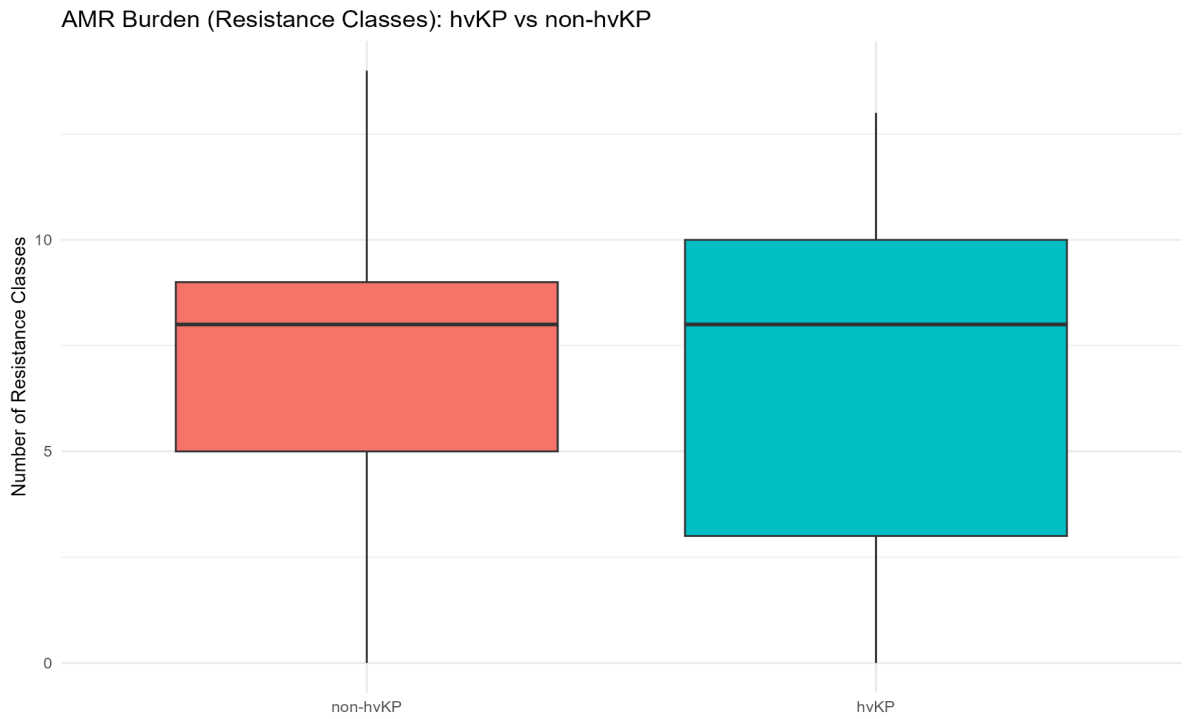

Figure 10: Resistance classes AMR burden of hvKP and non-hvKP

Number of resistance genes per isolate:

The second boxplot (*Figure 11*) illustrates the total number of resistance genes present in each genome. Both groups displayed broad distributions, with a notable proportion of isolates exhibiting multiple AMR genes. The average number of resistance genes in non-hvKP isolates was 9.63 (SD 5.90), whereas the average number of genes in hvKP isolates was 8.55 (SD 6.14). Notable outliers (>30 genes) absent from hvKP isolates are apparent in non-hvKP boxplots, suggesting significant overlap between hvKP and non-hvKP groups.

The findings indicate that hvKP isolates do not exhibit a significantly greater antimicrobial resistance burden compared to non-hvKP isolates, based on the total count of resistance genes or resistance classes. Conversely, hvKP seem to exhibit a similar, if somewhat reduced, average resistance burden, corroborating prior findings that indicate a convergence of hypervirulence and elevated antimicrobial resistance, although this phenomenon is not yet universally observed throughout hvKP lineages.

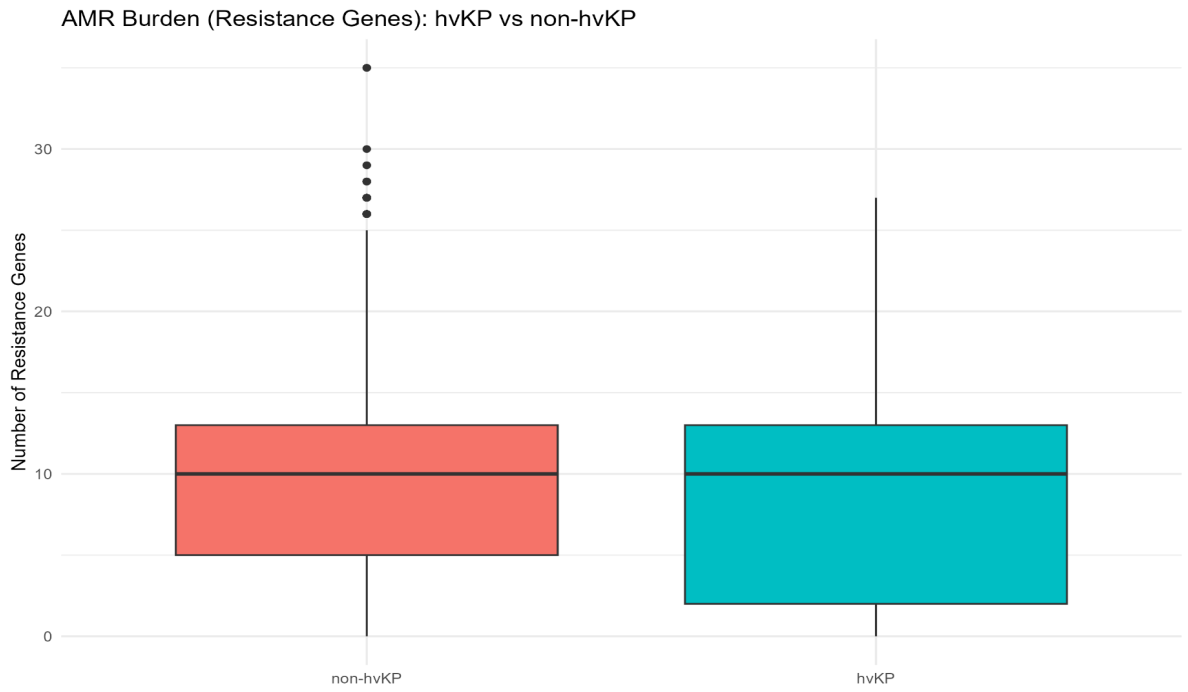

Figure 11: AMR burden resistance gene of hvKP and non-hvKP

12. Virulence Loci

Analysis of major top 25 virulence loci revealed distinct differences between hypervirulent *Klebsiella pneumonia* (hvKp) strains from Bangladesh and those globally (*Figure 12)*. The colibactin (clb) operon, comprising clbA to clbX, exhibited a minimal prevalence of around 3.7% among Bangladeshi hvKp isolates. This was much lower than global estimates for hvKp, which were typically around 9%. The lower presence of colibactin in Bangladesh suggests that typical hvKp lineages such as ST23–K1 are relatively rare in that region. On the other hand, siderophore-associated pathogenicity genes, especially those from the iuc locus, were found much more often. The aerobactin-related genes iucB and iucC were very common in Bangladeshi hvKp (about 90–100%), sometimes even higher than global levels. On the other hand, iucA and iucD were fairly common but not as common as they are in global hvKp datasets. In the same way, the iutA receptor gene was more common in isolates from Bangladesh than in the world comparator group. The salmochelin genes (iroB, iroC, iroD, and iroN) were moderately common in Bangladeshi hvKp isolates. This was a little lower than world trends but still about the same overall. The rmpA and rmpA2 regulatory genes linked to the hypermucoviscous phenotype were found more often in Bangladesh (about 45–50%) compared to the world hvKp (about 38–40%). This suggests that the hypermucoid

phenotype may be more common among strains that circulate locally. Furthermore, yersiniabactin genes (ybtA, ybtE, ybtS, ybtQ, ybtT, ybtU, and ybtX) were consistently more common in Bangladeshi hvKp, with prevalence rates of 45–50%. This suggests that ICEKp-associated chromosomal virulence modules are present in large numbers. Collectively, these results show that Bangladeshi hvKp has a unique virulence architecture, with fewer classical colibactin-positive lineages and more modern plasmid-associated hypervirulence factors like iuc, rmpA, and the ICEKp-linked yersiniabactin system. New worldwide hvKp clones, such as ST11, ST15, and ST231, which exhibit varying degrees of virulence and antibiotic resistance, in this instance, better fit the pattern. This implies that the hvKp populations in Bangladesh might have developed concurrently. The entire analyzed data is in supplementary table 13.

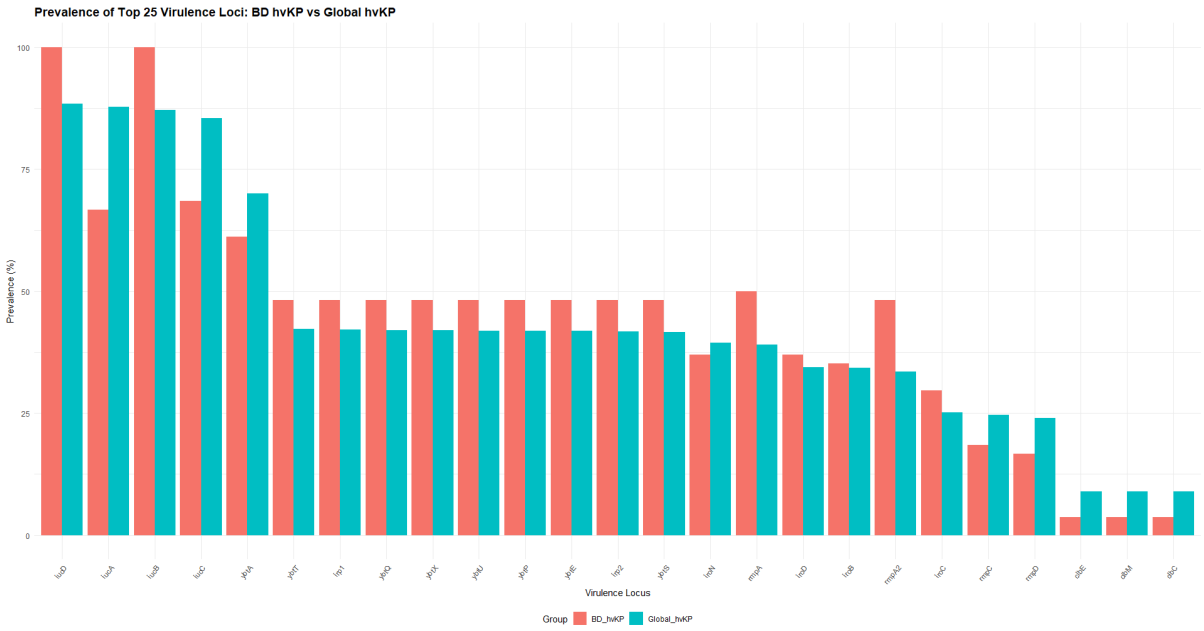

Figure 12: Most virulence loci between BD hvKP and Global hvKP

13. Capsular (K-type) and O-antigen (O-type) Diversity.

Diversity of Bangladeshi hvKp in Capsular (K-type) and O-antigen (O-type) Compared to Global hvKp

The capsular polysaccharide (K antigen) and lipopolysaccharide (O antigen) are important surface features that have a big effect on how *Klebsiella pneumonia* causes disease, avoids the immune system, and spreads in the population. We analysed and compared the K- and O-locus distributions of circulating hypervirulent *K. pneumoniae* (hvKp) in Bangladesh with

those of hvKp populations from around the world, using a dataset of hvKp isolates from other continents. The data shows that Bangladeshi hvKp and their worldwide counterparts have completely unique types of capsules and antigens, which suggests that they have different clonal expansions and virulence-associated features in different regions of the world.

Distribution of K-type in Bangladeshi hvKp

The distribution of capsule types in Bangladeshi hvKp isolates did not align with global patterns (*Figure 13)*. K20 emerged as the most prevalent capsule type in Bangladesh, accounting for 14.8% of all hvKp isolates, in contrast to only 4.8% globally. This uneven distribution indicates that K20 may constitute a capsular lineage that is more prevalent in specific regions and has the potential to expand within local hospital or community environments. K2 was the second most prevalent capsule type in Bangladesh, accounting for 11.1% of cases. This is comparable to the global prevalence of 13.9% and illustrates the importance of K2 as a classic hypervirulent serotype associated with severe invasive infections.

K1 was historically among the most prevalent forms of hvKp capsules worldwide, particularly in relation to ST23 strains and liver abscesses. However, it was less prevalent in Bangladesh—only 7.4% of isolates in comparison to 12.7% overall. This suggests that the dominant ST23-K1 lines may not be spreading as much in Bangladesh. This indicates that hvKp isolates within the country may originate from various sequence types or possess diverse virulence profiles.

Table 7: Key K types of BD and Global hvKp

K_type	BD_Count	Percent_BD	Global_Count	Percent_Global
K1	2	7.407407	388	12.69218
K2	3	11.11111	424	13.86981

K30 and K57 are observed at modest prevalence rates globally, at 1.4% and 3.5%, respectively. They were identified in Bangladeshi hvKp but remain relatively uncommon. K64 was exceptionally uncommon in Bangladesh (3.7%) in contrast to its markedly higher prevalence (32.5%) globally, particularly in Asia, where it is predominantly associated with high-risk carbapenem-resistant hvKp clones, notably ST11. The infrequent occurrence of

K64 in Bangladesh indicates that these high-risk hvKp-AMR hybrid lineages have not yet become predominant in the region. The entire data is in supplementary table 14.

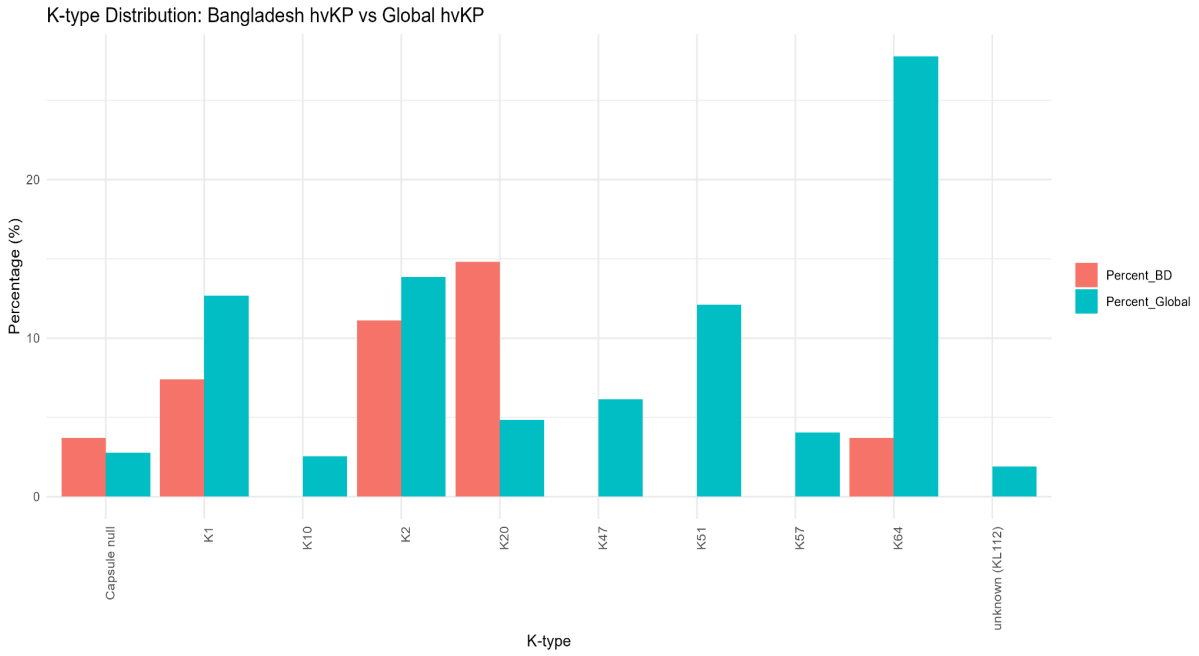

Figure 13: K-type distribution of Bangladesh and Global hvKP

Globally prevalent capsules such as K47, K51, and K57—each associated with significant hvKp or multidrug-resistant lineages—were either entirely absent or only minimally detectable in Bangladeshi samples. This overall trend indicates that the capsular diversity of Bangladeshi hvKp is limited, primarily concentrated around K20 and K2, and less influenced by AMR-associated hvKp serotypes that are disseminating globally.

Comparison with Global K-type Diversity

The distribution of K-types among hvKp isolates from Bangladesh reveals significant geographic differentiation when considered in a global context. K64, K2, K1, K20, and K51 are the most common capsule types worldwide, representing both traditional hypervirulent lineages (K1, K2) and newly emerging AMR-hypervirulent hybrids (K64, K51). Bangladeshi hvKp isolates exhibit a disproportionate dependence on a limited subset, specifically K20, K2, and K1. This limited capsular diversity indicates localized clonal expansion instead of widespread introduction of the various hvKp capsular types currently present globally.

The limited availability of K64 in Bangladesh is important. K64 is associated with the epidemic ST11 and ST147 carbapenem-resistant hypervirulent *Klebsiella pneumonia* lineages

in China, India, and Southeast Asia. The low prevalence in Bangladesh suggests that these high-risk clones may be emerging at a slower rate in the region or confined to isolated outbreaks rather than widespread dissemination.

O-type distribution in Bangladeshi hvKp

The O-type distribution in Bangladeshi hvKp exhibited more significant variations when compared to global hvKp populations, as indicated by the O-antigen study (*Figure 14*). The O1αβ,2α serotype predominated among Bangladeshi hvKp isolates, accounting for 74.1% of all cases—nearly three times the global hvKp percentage of 25.4%. The pronounced skew indicates a significant regional fitness advantage or clonal proliferation of hvKp bacteria with O1αβ,2α, perhaps influenced by circulating lineages harboring virulence plasmids or other ICEKp-related virulence factors.

Table 8: O types of BD and Global

O_type	BD_Count	Percent_BD	Global_Count	Percent_Global
O1αβ,2α	20	74.07407	776	25.38436
O1αβ,2β	5	18.51852	982	32.123
O2α	2	7.407407	840	27.47792
O11α,2α	NA	NA	1	0.032712
O11αβ,2α	NA	NA	1	0.032712
O13	NA	NA	204	6.673209
O1α,2α	NA	NA	6	0.196271
O1α,2β	NA	NA	6	0.196271
O2β	NA	NA	83	2.71508
O3αβ	NA	NA	30	0.981354
O3γ	NA	NA	103	3.369316
O4	NA	NA	7	0.228983
O5	NA	NA	18	0.588813

In Bangladesh, the second most prevalent O-type was O1αβ,2β (18.5%), which is reasonably common worldwide (32.1%); however, it remains less dominant than in several global hvKp datasets. Conversely, O2α, which constitutes a significant 27.5% of hvKp strains globally, was infrequently observed in Bangladesh (7.4%). This study suggests that O2-associated lineages, particularly the numerous ST23 and ST65 classical hvKp, exert a relatively minor influence on local hvKp epidemiology.

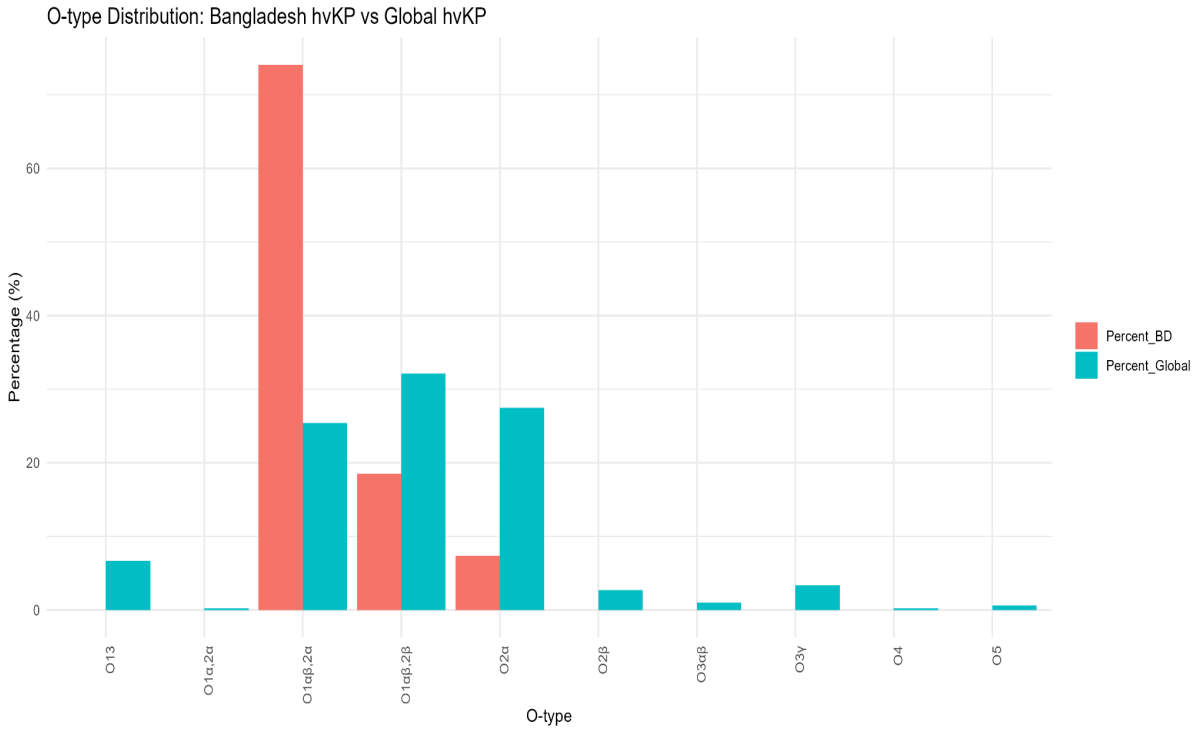

Figure 14: O-type distribution of BD and Global hvKP

A diverse array of minor O-types—namely O3, O3b, O5, O4, and others—was identified internationally, although entirely lacking in Bangladeshi hvKp isolates. The lack of O-antigen diversity suggests a homogeneous local hvKp population predominantly consisting of O1 variants, particularly O1αβ and 2α, with negligible integration of alternative O-antigen lineages. The entire analyzed data is in supplementary table 15.

Global O-type Trends and Comparison With Bangladesh

Globally, hvKp strains demonstrate increased diversity in O-types. The most common serotypes are O1 and O2, while there are a few less common O-types as well. The global prevalence of O2α and O1αβ,2β suggests their correlation with classical hvKp and emerging

hybrid AMR-hvKp lineages. However, Bangladesh does not exhibit this wider variety, as the O1 lineage accounts for the majority of hvKp isolates there.

The predominance of O1αβ,2α in Bangladesh suggests that one or a few predominant clones are spreading extensively within the region. As a result, certain K-types, like K20 and K2, are becoming more common. This tendency indicates that the hvKp population in Bangladesh may have undergone restricted clonal selection or founder effects, leading to reduced antigenic diversity relative to the global hvKp landscape.

The combined K-type and O-type tests reveal a unique regional hvKp serotype signature in Bangladesh. The frequency of O1αβ, 2α, and K20 underscores the dissemination of particular local hypervirulent clones that are inadequately documented in global datasets. The diminished prevalence of conventional K1-ST23 lineages and the limited presence of globally relevant AMR-associated serotypes such as K64 further distinguish Bangladesh's hvKp population from current international patterns. These data indicate that the hvKp in Bangladesh is influenced by many evolutionary and epidemiological factors, elucidating the limited yet systematic distribution of capsular and O-antigen types. The local serotype pattern significantly influences the creation of vaccines targeting capsular antigens, the transmission of the disease, its diagnosis, and its severity.

14. Resistance Score vs Virulence Score

Relationship Between Antimicrobial Resistance and Virulence in Bangladeshi and Global hvKp Populations:

It is essential to investigate the correlation between antibiotic resistance and virulence characteristics in hvKp, as the interactions of these elements results in the development of high-risk strains with major health consequences. Isolates received a Resistance Score (0–3) based on the number of AMR gene classes found, and a Virulence Score (0–5) determined according to the presence of key virulence loci (iuc, iro, rmpA/rmpA2, ybt, clb). Scores were analyzed between Bangladeshi isolates and global hvKp to assess the direction and intensity of correlation using regression analysis.

Visual Patterns of the Resistance and Virulence Relationship:

In the scatterplot we see that the Bangladeshi sample isolates had higher resistance scores with lower virulence ratings and created a negative resistance virulence trendline (*Figure 15*).

On the other hand, global hvKp visualized mini positive correlation between resistance and virulence. This divergence reflects different evolutionary pressures on Bangladeshi and global hvKp populations.

Bangladeshi isolates clustered around lower virulence and resistance values, indicating a narrower hvKp phenotypic range. Global hvKp isolates were more common across both axes, indicating increased diversity and more resistance–virulence hybrid strains.

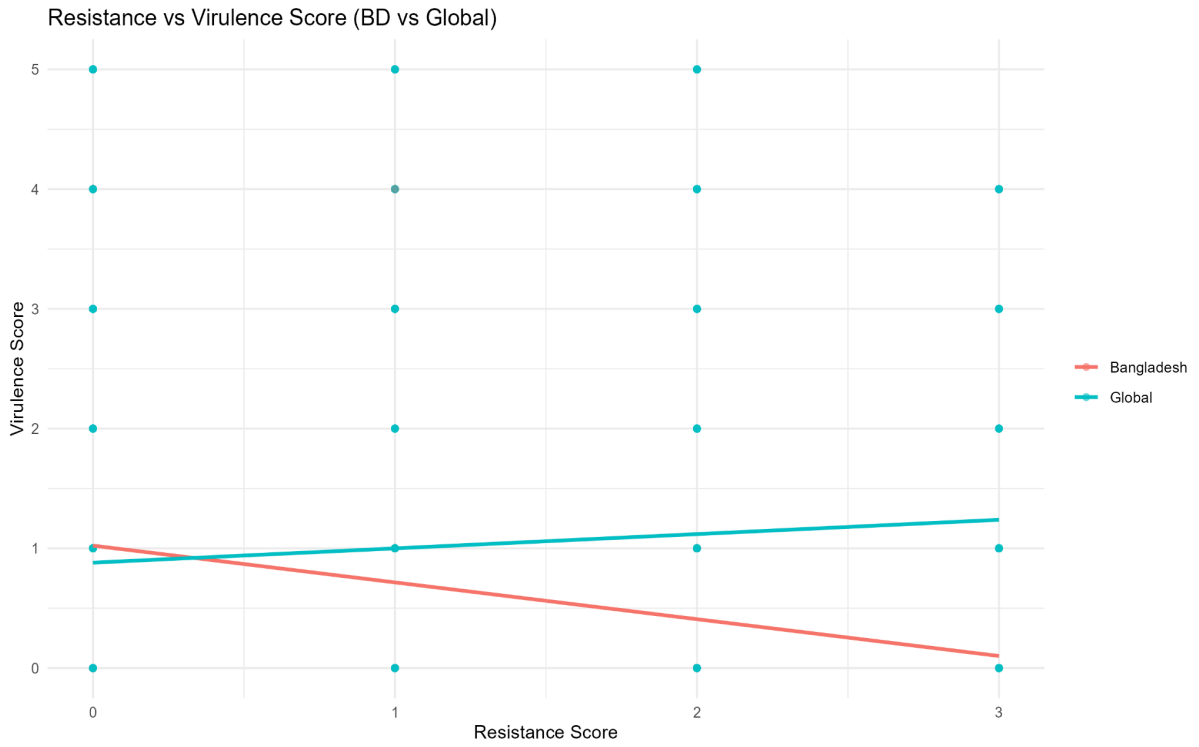

Figure 15: Antibiotic Resistance and Virulence score of BD and Global isolates

Bangladesh hvkp: Strong negative resistance–virulence relationship and hvKp are highly virulent but largely antibiotic-susceptible. Suggests dominance of classical hvKp lineages with minimal AMR acquisition. Indicates that hvKp–AMR convergence has not yet occurred at scale in Bangladesh.

Global hvKp: Significant positive resistance–virulence relationship. Reflects rising prevalence of AMR-hypervirulent hybrid strains. Suggests global propagation of hvKp strains carrying both virulence plasmids and MDR/carbapenemase genes.

Bangladesh in a Global context

The observed correlations suggest that Bangladesh is in an earlier stage of hvKp epidemiology, characterised by virulent yet non-antimicrobial-resistant clones, while global hvKp populations seem to have advanced towards a convergence of resistance and virulence. This situation has significant clinical implications: Bangladesh may currently be experiencing severe hvKp infections; however, treatment options are still available. On the other hand, Global hvKp populations pose two risks since they are more virulent and resistant, which makes it harder to treat and control infections. If no one does anything, Bangladesh may follow the global trend as antimicrobial resistance plasmids spread through hypervirulent *Klebsiella pneumonia* lines.

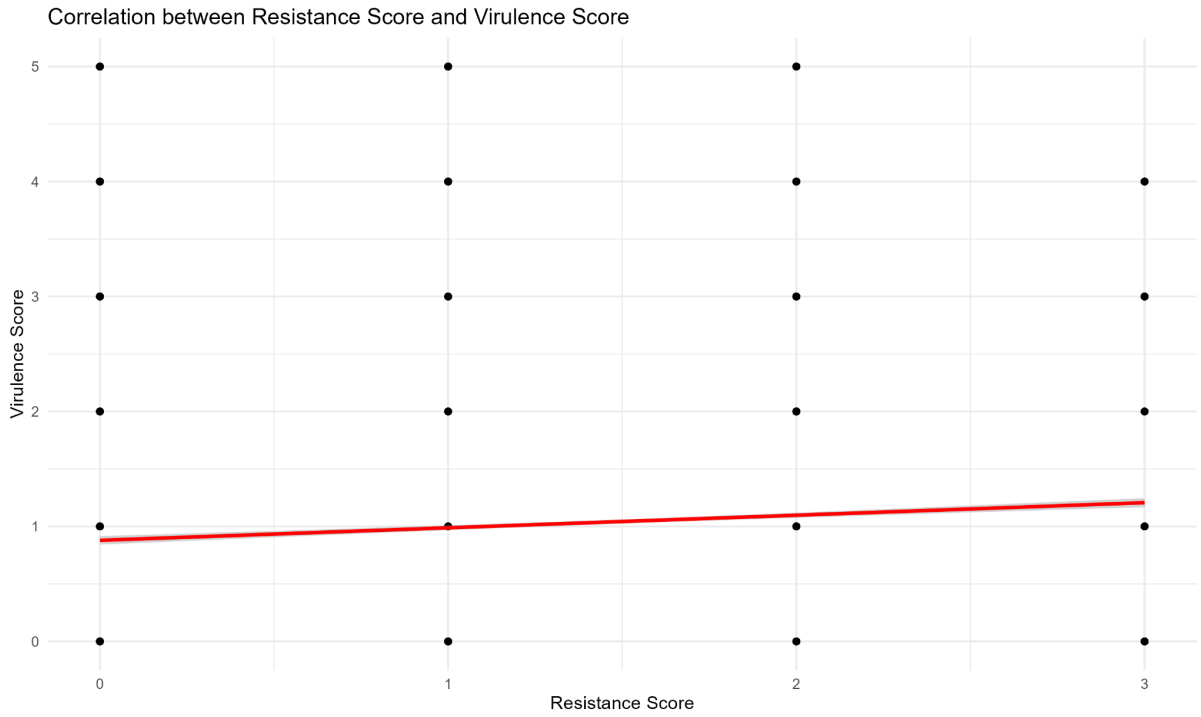

Figure 16: Correlation between Antibiotic Resistance and Virulence score.

15. AMR Burden Across STs: Bangladesh Shows Higher AMR Load in Most Lineages

Across almost all sequence types tested, Bangladeshi hvKp had a higher mean AMR load (number of resistance classes) than their global counterparts (*Figure 17)*. This trend was consistent across ST11, ST147, ST15, ST16, ST307, ST340, and ST48. For example:

ST11: BD ≈ 8.5 classes vs. Global ≈ 7.5

ST147: BD ≈ 9.0 vs. Global ≈ 8.3

ST16: BD ≈ 9.1 vs. Global ≈ 8.2

ST307: BD ≈ 8.3 vs. Global ≈ 7.8

ST340: BD ≈ 8.5 vs. Global ≈ 8.2

This pattern indicates that Bangladeshi hvKp lineages carry a heavier AMR load than equivalent global lineages, despite the country's hvKp generally showing weaker virulence signatures (as reported earlier). The magnitude of difference is most pronounced for:

ST16 (one of the highest AMR burdens in Bangladesh)

ST147, a global high-risk clone strongly linked to MDR phenotypes

ST307, also notorious for ESBL and carbapenemase dissemination

ST14 was one of the few exceptions where global isolates showed a slightly higher AMR burden than Bangladesh. This suggests that the AMR expansion in Bangladesh is not uniform across STs but is concentrated in several high-risk clones. The whole analyzed data is in supplementary table 16.

Table 9: STs AMR burden classes

ST	Group	mean_classes	mean_genes
ST11	Bangladesh	8.978495	13.6129
ST11	Global	7.566355	9.415327
ST14	Bangladesh	8.210526	14.84211
ST14	Global	8.627586	15.54069
ST147	Bangladesh	9.306122	15.95918
ST147	Global	8.969538	14.00945
ST15	Bangladesh	8.1875	14.8125
ST15	Global	7.857143	11.81478
ST16	Bangladesh	9.473684	16.01754

ST16	Global	8.431487	11.71866
ST17	Bangladesh	7.153846	10
ST17	Global	5.938534	8.56974
ST307	Bangladesh	8.785714	13.14286
ST307	Global	7.877083	11.99167
ST340	Bangladesh	9.055556	16.16667
ST340	Global	8.989583	13.42188
ST48	Bangladesh	8.254902	12.98039
ST48	Global	7.34715	12.36269
ST502	Bangladesh	6.764706	11.47059
ST502	Global	6.5	9.777778

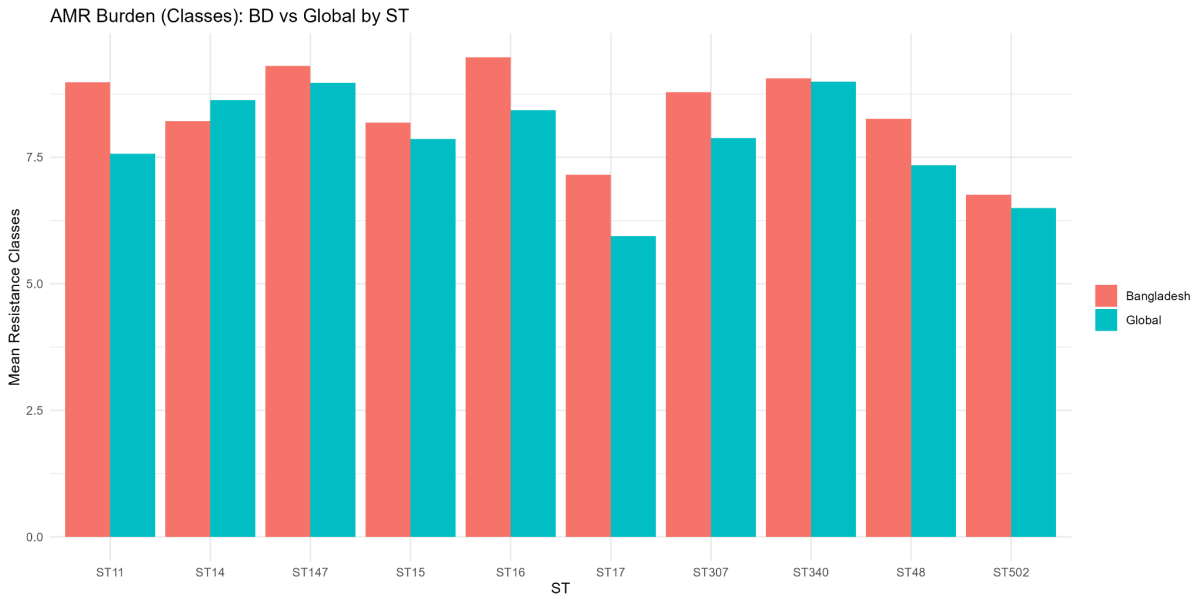

Figure 17: ST BD vs Global AMR Burden Classes

Resistance Score by ST: Bangladesh Again Shows Higher AMR but Not Uniformly

The average resistance score (0–3) followed the same trends as the AMR burden. In most STs, Bangladeshi isolates had resistance levels that were the same as or higher than global isolates (*Figure 18*). Notable high scores for Bangladesh were:

ST11 (BD = 2.0, Global = 1.95)

ST16 (BD = 2.0, Global = 1.93)

ST48 (≈ 1.5 for BD and ≈ 1.5 for Global)

ST502 (BB = 2.0 vs. G = 1.3)

A very high resistance score of 2.0 was seen for ST502 in Bangladesh compared to a score of 1.3 for the whole world. This means that Bangladesh may have a forceful local ST502 lineage, even if it's not very common.

Frequently, ST17 was the major exception that isolated from around the world it had slightly higher resistance scores than Bangladesh (1.1 vs. 1.15). Overall, the resistance-score trends support the idea that Bangladeshi hvKp lineages carry higher AMR loads across different STs. This indicates that there is a lot of selection pressure for resistance in the area. The analyzed data is in supplementary table 17.

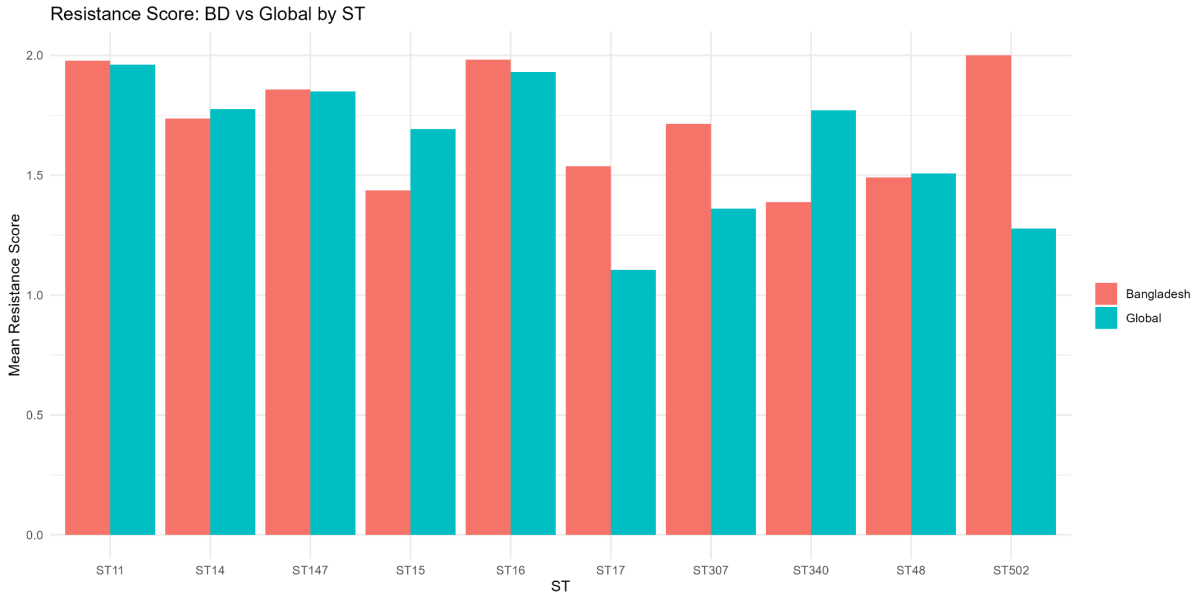

Figure 18: ST BD vs Global Resistance Score

16. Virulence Score by ST: Global Lineages Exhibit Stronger Virulence Than Bangladesh

The global dominance of ST11–K64 hypervirulent-AMR hybrids is seen in the large disparity between BD ≈ 0.1 and Global = 1.8 (*Figure 19)*.

ST15: Global ≈ 1.05 vs. BD ≈ 0.2 shows that, in contrast to Bangladesh, virulence plasmids are frequently carried by global ST15 hvKp.

ST16: BD ≈ 0.18 vs Global ≈ 0.8

ST17: BD ≈ 0.62 vs. Global ≈ 0.45

Bangladesh exhibits increased pathogenicity in ST17, the sole lineage.

ST307: The BD and global levels are still very low (≈ 0.3), which indicates that ST307 is mostly an AMR lineage around the world.

ST48: Global ≈ 1.1 versus BD ≈ 1.8

ST48 is usually a weak germ globally, but in Bangladesh, it has evolved into a much more dangerous and aggressive "superbug".

Because iuc-/iro-positive virulence plasmids and ICEKp-associated yersiniabactin are common in global hvKp clones, these data show that global hvKp lineages generally exhibit greater virulence markers.

However, in a few STs (ST17 and ST48), Bangladesh exhibits selectively enhanced virulence, indicating pockets of hvKp proliferation rather than general virulence-plasmid diffusion. The analyzed data is in supplementary table 18.

Table 10: STs based virulence score BD and Global

ST	Group	mean_vir_score	sd_vir_score	n
ST11	Bangladesh	0.11828	0.324689	93
ST11	Global	1.826916	1.530403	2675
ST14	Bangladesh	0.894737	0.315302	19
ST14	Global	0.816552	0.482571	725
ST147	Bangladesh	0.612245	0.606092	49
ST147	Global	0.798319	0.688861	952
ST15	Bangladesh	0.1875	0.403113	16

ST15	Global	1.054187	1.11473	1015
ST16	Bangladesh	0.157895	0.367884	57
ST16	Global	0.85277	0.480465	686
ST17	Bangladesh	0.615385	0.50637	13
ST17	Global	0.453901	0.613534	423
ST307	Bangladesh	0.214286	0.425815	14
ST307	Global	0.225	0.499896	480
ST340	Bangladesh	0.055556	0.235702	18
ST340	Global	0.473958	0.500627	192
ST48	Bangladesh	1.803922	1.456291	51
ST48	Global	1.088083	0.864528	193
ST502	Bangladesh	0	0	17
ST502	Global	0	0	18

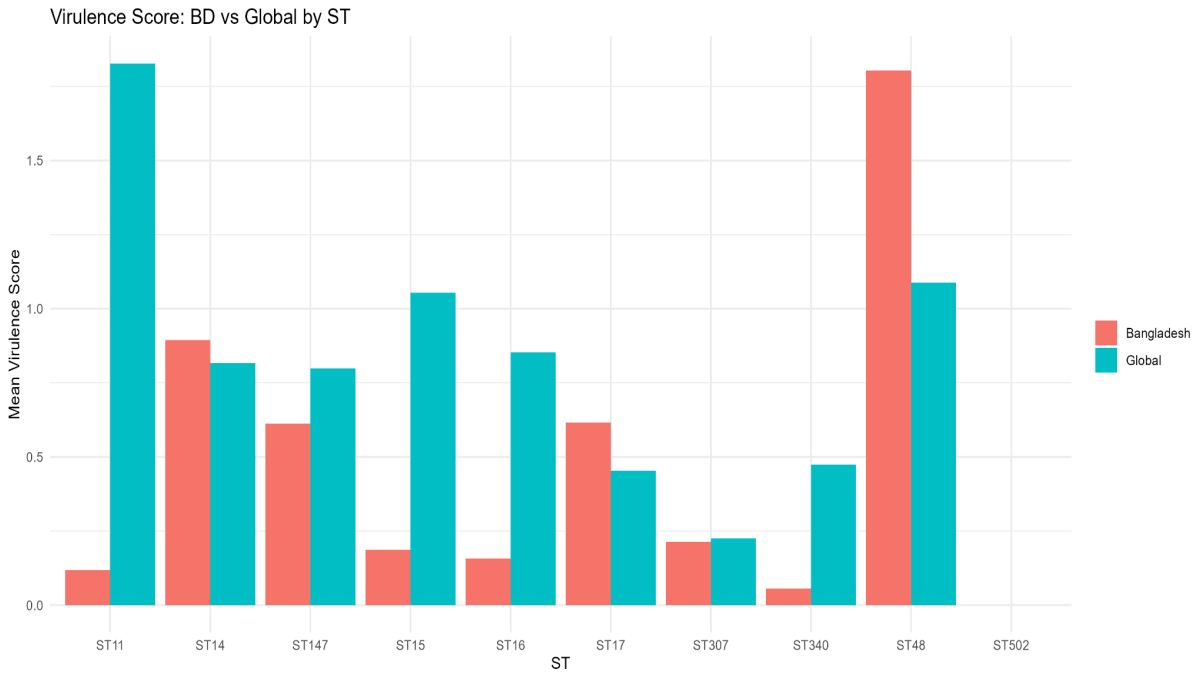

Figure 19: ST_BD_vs_Global_VirulenceScore

17. Principal Component Analysis of Combined Virulence and AMR Profiles among hvKp Isolates

To examine the relationship between Bangladeshi hvKp isolates and those circulating globally, a Principal Component Analysis (PCA) was conducted using combined virulence and AMR profiles. PCA was employed to reduce the dimensionality of the dataset and to visualize patterns of similarity and divergence between Bangladeshi and global isolates based on their composite genomic characteristics.

The PCA plot revealed that the first two principal components (PC1 and PC2) captured a substantial proportion of the overall variation in virulence and AMR profiles. The distribution of isolates along these axes demonstrated the presence of three major clusters, indicating heterogeneity among hvKP strains at the global level.

Importantly, Bangladeshi hvKP isolates did not form a distinct or separate cluster. Instead, they were largely embedded within the broader global hvKP population. Most Bangladeshi isolates were concentrated within the central cluster, where they overlapped extensively with isolates from several other Asian countries. This overlap suggests a high degree of similarity between Bangladeshi hvKP strains and globally circulating hvKP, particularly those originating from the Asian region.

The first cluster, located primarily in the upper-left region of the PCA plot, was dominated by global isolates and appeared to represent hvKP strains with strong virulence signatures and relatively lower AMR complexity. Bangladeshi isolates were sparse or absent in this cluster, indicating limited representation of this classical hypervirulent profile among the Bangladeshi samples analyzed.

The central cluster contained the majority of Bangladeshi isolates along with a substantial number of global isolates. This cluster likely reflects hvKP strains with mixed virulence and resistance characteristics, suggesting a transitional or intermediate profile. The co-clustering of Bangladeshi isolates with global isolates in this region indicates that Bangladeshi hvKP strains share common virulence–AMR patterns with globally prevalent lineages rather than exhibiting unique local adaptations.

The third cluster, positioned at higher values of PC1, was dominated by global isolates and appeared to represent hvKP strains with a higher burden of antimicrobial resistance while retaining virulence traits. Bangladeshi isolates were rare or absent from this cluster,

suggesting that, in comparison to some global counterparts, Bangladeshi hvKP strains have not yet extensively converged toward highly resistant hypervirulent lineages.

These observations were further supported by hierarchical clustering analysis using Ward's method, which showed that Bangladeshi isolates were interspersed among global isolates rather than forming a separate branch. Together, the PCA and dendrogram analyses consistently indicate that Bangladeshi hvKP isolates are part of the broader global hvKP population, with no evidence of country-specific clustering.

Overall, the PCA-based comparison demonstrates that Bangladeshi hvKP isolates are genomically and phenotypically similar to global hvKP strains, particularly those from Asia. While global hvKP populations exhibit considerable diversity and form multiple distinct clusters based on virulence and AMR profiles, Bangladeshi isolates predominantly align with shared global patterns rather than representing a distinct evolutionary lineage.

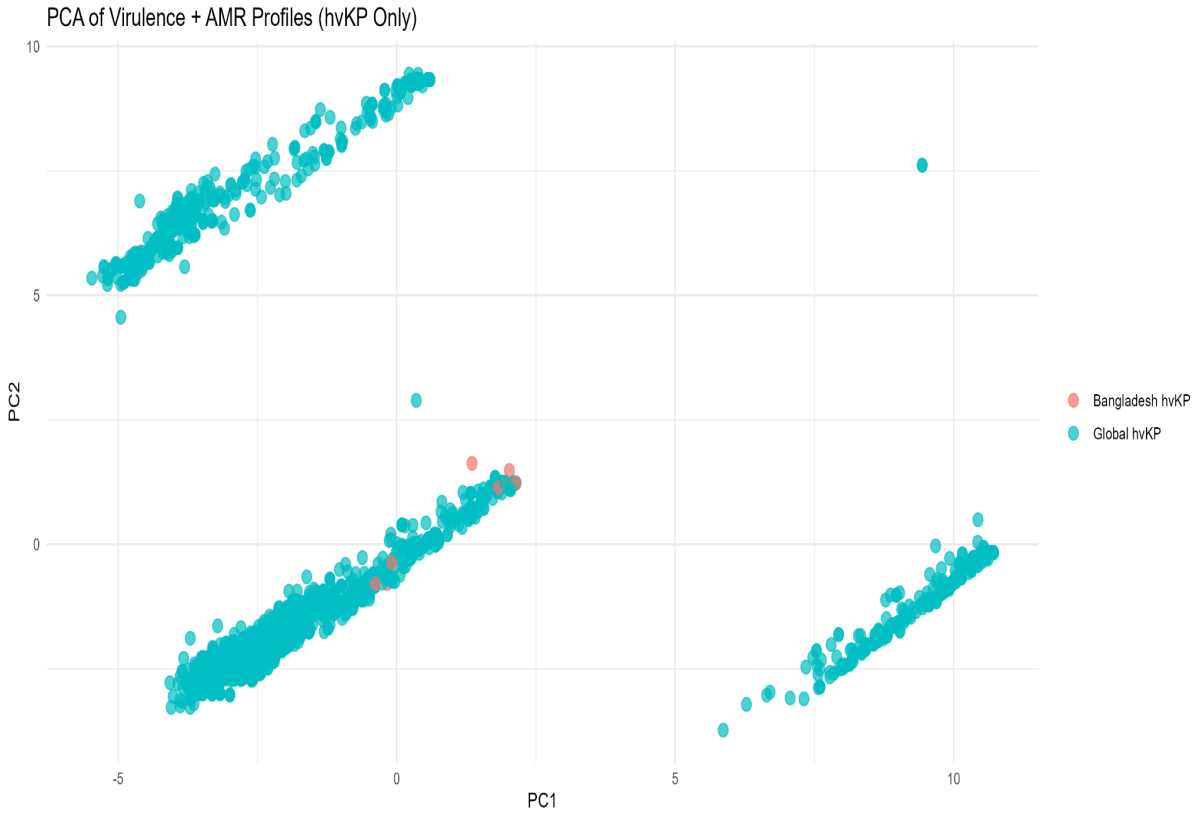

Figure20: PCA hvKP Virulence AMR BD vs Global

18. Hierarchical Clustering of hvKp Virulence–AMR Profiles (Balanced Bangladesh vs Global Subset)

We did a hierarchical clustering analysis on a balanced dataset of 50 Bangladeshi hvKp isolates and 50 randomly chosen worldwide hvKp isolates to see how closely related they are to each other (*Figure 21*). The grouping was predicated on Euclidean distances derived from integrated virulence-gene presence/absence profiles and AMR gene-class data. To find groups that have the least amount of variance within them, we used Ward's D2 approach. The dendrogram shows if Bangladeshi hvKp have their own unique lineage patterns or if they work the same way as other hvKp populations throughout the world.

The dendrogram clearly showed that the isolates were split into two main groups that split apart at a fairly high point on the tree. There is a lot of functional and genomic diversity in the world's hvKp population, as shown by this separation. Several smaller groups were seen within these two main ones. Each one was made up of a mix of isolates from different countries. The known variety of hvKp is shown by this pattern. Virulence plasmids, ICEKp elements, and AMR determinants mix and match across sequence types and geographical regions. The worldwide hvKp isolates were spread out a lot across several subclusters, which fits with the PCA results that showed a lot of variation in the virulence and AMR gene combos.

But Bangladeshi hvKp isolates were grouped in a completely unique way. Most of the Bangladeshi isolates were closely grouped into a few subclusters that were also closely linked to each other. The virulence and AMR profiles of these subclusters were very similar, and the branch lengths were very long. Additional evidence corroborates previous findings that Bangladeshi hvKp isolates exhibit minimal variation in capsule types, O-antigen structures, virulence gene architectures, and antimicrobial resistance scores. Several Bangladeshi isolates clustered with global hvKp strains, indicating occasional crossover with certain international lineages. However, the majority of them stayed in a small cluster that was separate from many of the global hvKp sublineages that were studied.

It's also important to note that the dendrogram shows that Bangladeshi hvKp isolates are not part of a completely separate lineage but are instead part of the global hvKp diversity spectrum. This overlapping and constrained pattern suggests that local hvKp strains are not evolutionarily distinct but rather originate from a restricted set of globally circulating

hypervirulent backgrounds. Their tighter clustering relative to global isolates indicates reduced variation in virulence plasmids, lower diversity of ICEKp-associated yersiniabactin loci, and a more uniform antimicrobial resistance burden among Bangladeshi hvKp.

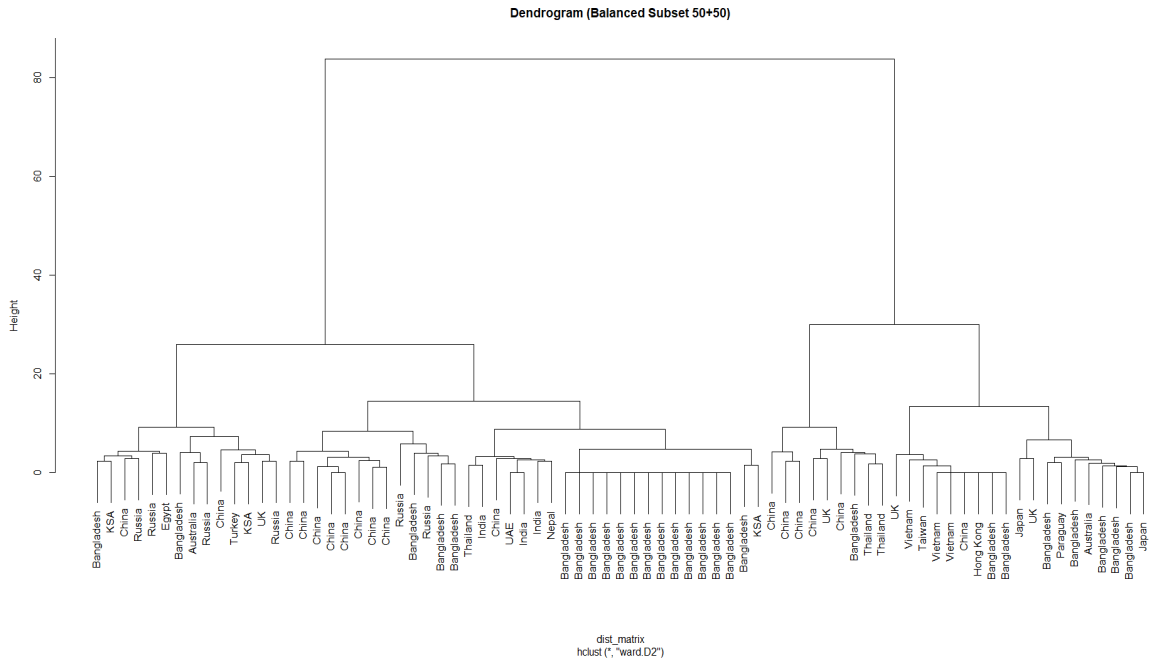

Figure21: Dendrogram BalancedSubset hvKP

Overall, the hierarchical clustering analysis reinforces the key conclusion from PCA and ST-based comparisons:

Bangladeshi hvKp isolates represent a smaller and less diverse segment of the global hvKp population. They have similar virulence–AMR profiles and not much lineage heterogeneity.

At the same time, global hvKp isolates show a lot of functional and genomic variety, which suggests that virulence and AMR have become more similar through a number of separate evolutionary events. The findings underscore the stability of hvKp populations in Bangladesh and emphasise the necessity of monitoring the introduction or emergence of high-risk, globally disseminated hvKp clones.

Chapter 4

Discussion

This study's findings give us new information about the genetic makeup and spread trends of highly contagious *Klebsiella pneumonia* (hvKp) strains from Bangladesh. The primary objectives of this study were to examine the STs, virulence profiles, AMR trends, and capsular diversity of hvKp strains present in Bangladesh, and to compare these findings with strains from various global regions. The main outcomes of the study are outlined below:

Types of Sequences: Among Bangladeshi hvKp isolates, the study found 115 different STs, the most prevalent of which were ST11, ST16, ST48, and ST147. In comparison to the global population of *K. pneumoniae*, the ten most common sequence types (STs) accounted for 57.7% of the isolates, indicating a relatively low diversity.

Virulence Determinants: The majority of Bangladeshi hvKp isolates exhibited critical virulence loci, such as aerobactin (iuc), yersiniabactin (ybt), and rmpA/rmpA2, which correlate with enhanced pathogenic capability. It was found that colibactin (clb), which is often thought of as a key indicator of hypervirulence in different world strains, was not always present in Bangladeshi isolates.

Antimicrobial Resistance (AMR): The hvKp isolates from Bangladesh exhibited significant resistance to commonly used antibiotics, including aminoglycosides, fluoroquinolones, and sulfonamides. Nevertheless, the incidence of carbapenem resistance was quite low relative to global patterns. Carbapenem-resistant hvKp (CR-hvKp) is in the nascent phase of prevalence in Bangladesh.

In Bangladeshi hvKp, K20 was found to be the most common capsule type, followed by K2. This finding is different from the fact that K1 is common around the world and is often tied to serious infections like liver abscesses. The study also revealed that the K64 capsular type, associated with multidrug-resistant high-risk clones, was infrequently observed in Bangladesh.

The findings of this study both confirm and contrast with prior research on hvKp in different locales. Similarly recorded the prevalence of ST11 in Bangladesh, underscoring the importance of this global high-risk clone(54). ST16 and ST48 are more common in Bangladesh than elsewhere. The proliferation of these STs indicates that Bangladesh may be undergoing local clonal expansions of specific high-risk strains, potentially affecting public health surveillance and response measures.

The results we obtained regarding virulence determinants align with studies carried out in East Asia and Southeast Asia, where hvKp strains frequently exhibit the presence of aerobactin and yersiniabactin. Colibactin is infrequently detected in Bangladeshi isolates, yet it is commonly present in South Korean and Chinese ST23 strains, where this locus is often linked to severe conditions like liver abscesses. Based on the changes seen between regions, it's possible that hvKp strains in Bangladesh have their own unique pathogenicity pattern. The difference could be because of how they evolved locally or how the host and pathogen interact with each other.

This study found that carbapenems were unable to kill 3.7% of the types of hvKp found in Bangladesh. This figure is significantly lower than those reported globally, particularly in China and India(70). The observed difference may be attributed to the dissemination of carbapenemase genes in various regions, coupled with the lower usage of antibiotics in Bangladesh compared to Europe or Southeast Asia, where carbapenem-resistant hvKp is increasingly prevalent.

This study's results present several important public health implications for Bangladesh:

Emergence of multidrug-resistant hypervirulent *Klebsiella pneumonia*. The significant multidrug resistance identified in Bangladeshi hvKp strains presents a considerable risk to public health, particularly due to the restricted access to effective antimicrobial agents. The emergence of resistance to aminoglycosides, fluoroquinolones, and sulfonamides is a significant concern, given their frequent application in treating hospital-acquired infections. The low prevalence of carbapenem resistance indicates that carbapenemase-producing hvKp has not yet become widespread in Bangladesh. This presents an opportunity to implement preemptive surveillance and antibiotic stewardship programs to avert its emergence.

Virulence Factors' Regional Specificity: The identification of unique virulence profiles in Bangladeshi hvKp isolates, such as the prevalence of iuc and rmpA/rmpA2, suggests that there may be region-specific virulence mechanisms that affect the severity and outcomes of infections. Global hvKp studies may therefore need to be adjusted to account for regional variations in virulence loci. This could help develop regionally appropriate diagnostic and treatment approaches.

The restricted capsular diversity observed in Bangladeshi hvKp strains, predominantly K20 and K2, carries significant implications for vaccine development. Current global vaccination

initiatives primarily concentrate on K1 and K2, which are linked to classical hvKp lineages such as ST23. The prevalence of K20 in Bangladesh indicates that a targeted vaccine strategy for this region should include this capsule type. The lack of K64 indicates that Bangladesh might not encounter the same high-risk AMR-associated hvKp clones found in other regions, presenting a unique opportunity for the development of region-specific strategies.

Strengths and Limitations of the Study

This research is valuable because it examines the genetic setting of hvKp in Bangladesh across a broad range of areas. This research employs genetic and epidemiological data to compare Bangladeshi strains with global hvKp isolates.

Yet, there are several limitations:

Sample Representation: This study utilized publicly accessible sources, which may not comprehensively reflect the geographic diversity of hvKp in Bangladesh. Future research should employ geographically diverse isolates from urban and rural regions to enhance comprehension of the hvKp population.

Lack of Clinical Data: This study examined the genomics of hvKp; however, the lack of clinical outcome data prevents direct correlation between virulence factors and AMR profiles and infection severity. The clinical implications of these findings require further research, including clinical surveillance.

The dataset provides a snapshot of hvKp in Bangladesh and does not account for strain prevalence, virulence, or resistance changes. We need longitudinal investigations to follow hvKp strain evolution and antimicrobial adaptation.

Future research should focus on the following to address the limitations of this study:
Longitudinal surveillance involves monitoring hvKp strains in Bangladesh over an extended period to track changes in virulence and antibiotic resistance. This will aid in assessing the broader dissemination of multidrug-resistant hvKp and carbapenem resistance.

Identifying the Role of Specific Virulence Factors in Pathogenicity: More study with animal models and in vitro experiments is needed to determine how these genes affect host infection. Adding isolates from rural and remote areas to the dataset would help us get a better picture

of the hvKp population in Bangladesh. This would help us learn more about how strain frequency and virulence change from region to region.
A global comparison of the genetic diversity and virulence patterns of hvKp strains from Bangladesh with those from other South Asian nations could reveal the existence of analogous patterns in the region and elucidate Bangladesh's role within the broader context of hvKp epidemiology.

In conclusion, this article provides a thorough examination of Bangladeshi hypervirulent *Klebsiella pneumonia* (hvKp) isolates. This study aims to shed light on the genetic diversity of these isolates, along with their virulence factors and profiles of antimicrobial resistance. The findings indicate that while carbapenem-resistant hvKp is not currently widespread in Bangladesh, the increasing prevalence of multidrug-resistant strains presents a significant threat to public health. The specificity of virulence loci and capsular types in certain regions underscores the necessity of creating diagnostic, therapeutic, and preventative strategies that are customised for the particular context. The results of this study provide valuable insights that can guide public health policy, enhance surveillance programmes, and aid in the creation of vaccines tailored to the local hvKp strains currently present in Bangladesh.

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