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Identification of extended-spectrum β-lactamase (ESBL)-producing hypervirulent *Klebsiella pneumoniae* KL1-ST23 bloodstream isolates in South Korea

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ABSTRACT

Background: Hypervirulent *Klebsiella pneumoniae* (hvKp) KL1-ST23 is a predominant lineage associated with invasive infections in East Asia and has traditionally been susceptible to most antimicrobial agents. However, hvKp carrying acquired resistance determinants, including extended-spectrum β-lactamase (ESBL)-encoding and carbapenemase genes, has increasingly been reported worldwide. This study aimed to identify and characterize KL1-ST23 hvKp bloodstream isolates carrying acquired antimicrobial resistance determinants in South Korea.

Methods: Between 2014 and 2024, 2,835 bloodstream *K. pneumoniae* isolates from a tertiary hospital in South Korea were screened by capsular serotyping, identifying 339 KL1-ST23 isolates. Among these, two ESBL-producing strains were identified in 2022 (SMC2209-053) and 2023 (SMC2308-038). Antimicrobial susceptibility was determined using VITEK2 and broth microdilution according to CLSI guidelines. Capsular type KL1 was confirmed by *magA*-targeted PCR, and sequence type was determined by multilocus sequence typing (MLST). Hypermucoviscosity was assessed by the string test, and conjugation assays were performed using azide-resistant *Escherichia coli* J53. Complete genomes were generated by PacBio long-read sequencing and analyzed using Kleborate (v3.1.2).

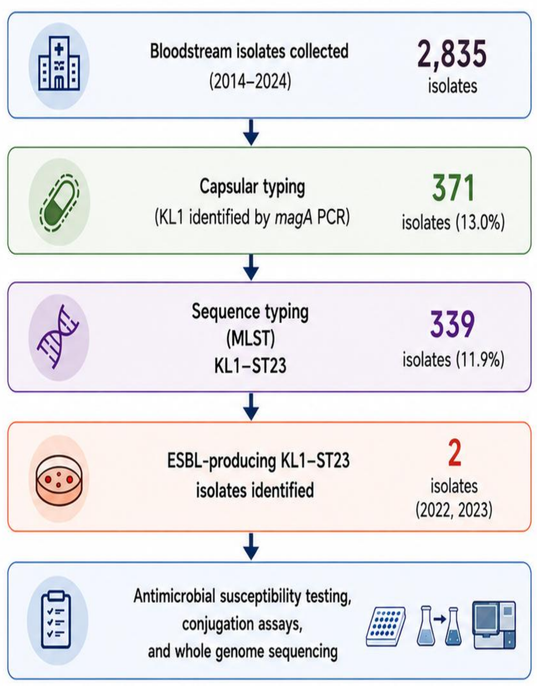
Results: Both isolates belonged to KL1-ST23 and carried key virulence loci with identical virulence scores, although structural variation was observed in the *rmp* operon. SMC2308-038 was positive by the string test, whereas SMC2209-053 was negative. Long-read genome analysis revealed distinct ESBL plasmid architectures: SMC2209-053 carried a Tn3-like plasmid harboring *bla*CTX-M-15, *bla*TEM-1b, and *qnrS1*, while SMC2308-038 carried ESBL plasmids encoding *bla*SHV-12 and *bla*TEM-1b. Both isolates also possessed hybrid IncHI1B–IncFIB plasmids. Phenotypically, both isolates showed ESBL activity with resistance to third- and fourth-generation cephalosporins; SMC2209-053 additionally exhibited resistance to ciprofloxacin and trimethoprim–sulfamethoxazole. Conjugation experiments demonstrated limited transferability, yielding no transconjugants from SMC2209-053 and only three colonies from SMC2308-038 across triplicate assays. Whole-genome sequencing–based phylogenetic analysis showed that the two isolates belonged to different CG23 sublineages.

Conclusion: Two ESBL-producing hvKp KL1-ST23 bloodstream isolates were identified in South Korea through decade-long surveillance. Distinct ESBL plasmid structures, limited transferability, and genomic separation within CG23 suggest sporadic and independent acquisition of resistance determinants within this hypervirulent lineage. Continued genomic surveillance is warranted to monitor early resistance–virulence convergence in KL1-ST23.

BACKGROUND

- Hypervirulent *Klebsiella pneumoniae* (hvKP) KL1-ST23 is a predominant lineage associated with community-acquired liver abscesses in Asian countries, including Taiwan and South Korea, over the past tree decades (Choby JE et al., 2020)
- Recently, antimicrobial-resistant KL1-ST23 hvKP carrying acquired resistance determinants, including ESBL-encoding genes (Chung et al., 2017) and carbapenemase genes (ECDC, 2024), has been increasingly reported.
- This study investigated the prevalence and molecular characteristics of acquired antimicrobial resistance genes among **KL1-ST23 *K. pneumoniae*** bloodstream isolates in South Korea.

MATERIALS AND METHODS



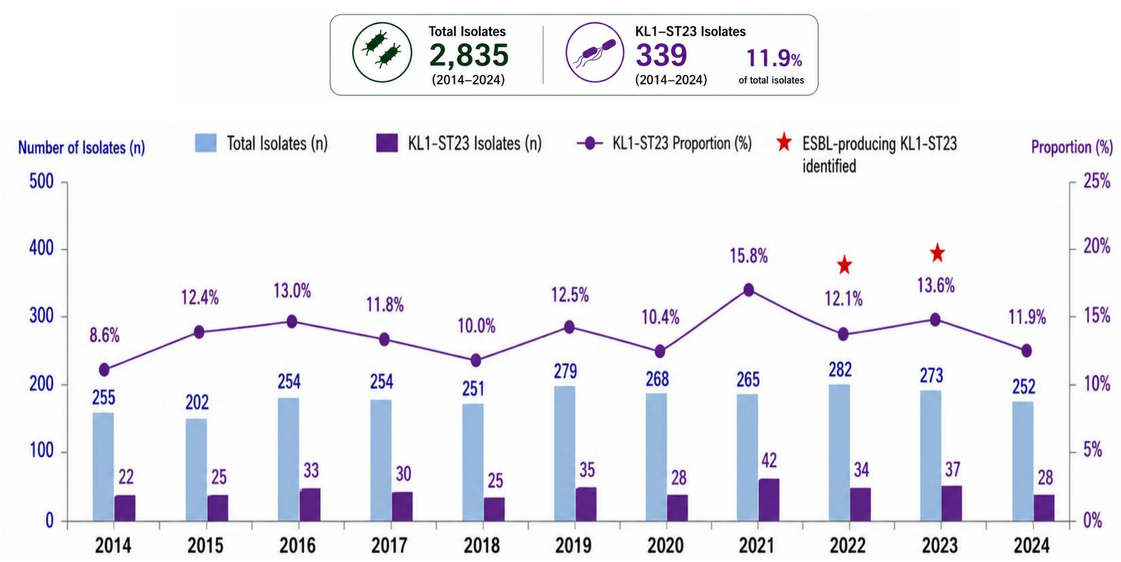
Molecular and phenotypic characterization
- KL1 confirmed by *magA* PCR
- Sequence type determined by MLST
- Antimicrobial susceptibility testing by VITEK2 and broth microdilution according to CLSI guidelines (2025)

Genomic analysis
- PacBio whole-genome sequencing
- Genomic analysis using Kleborate v3.1.2
- Core genome phylogeny was inferred using Roary and RAxML (v8.2.9) and visualization with iTOL.

Transferability assay
- Conjugation assay using azide-resistant *E. coli* J53

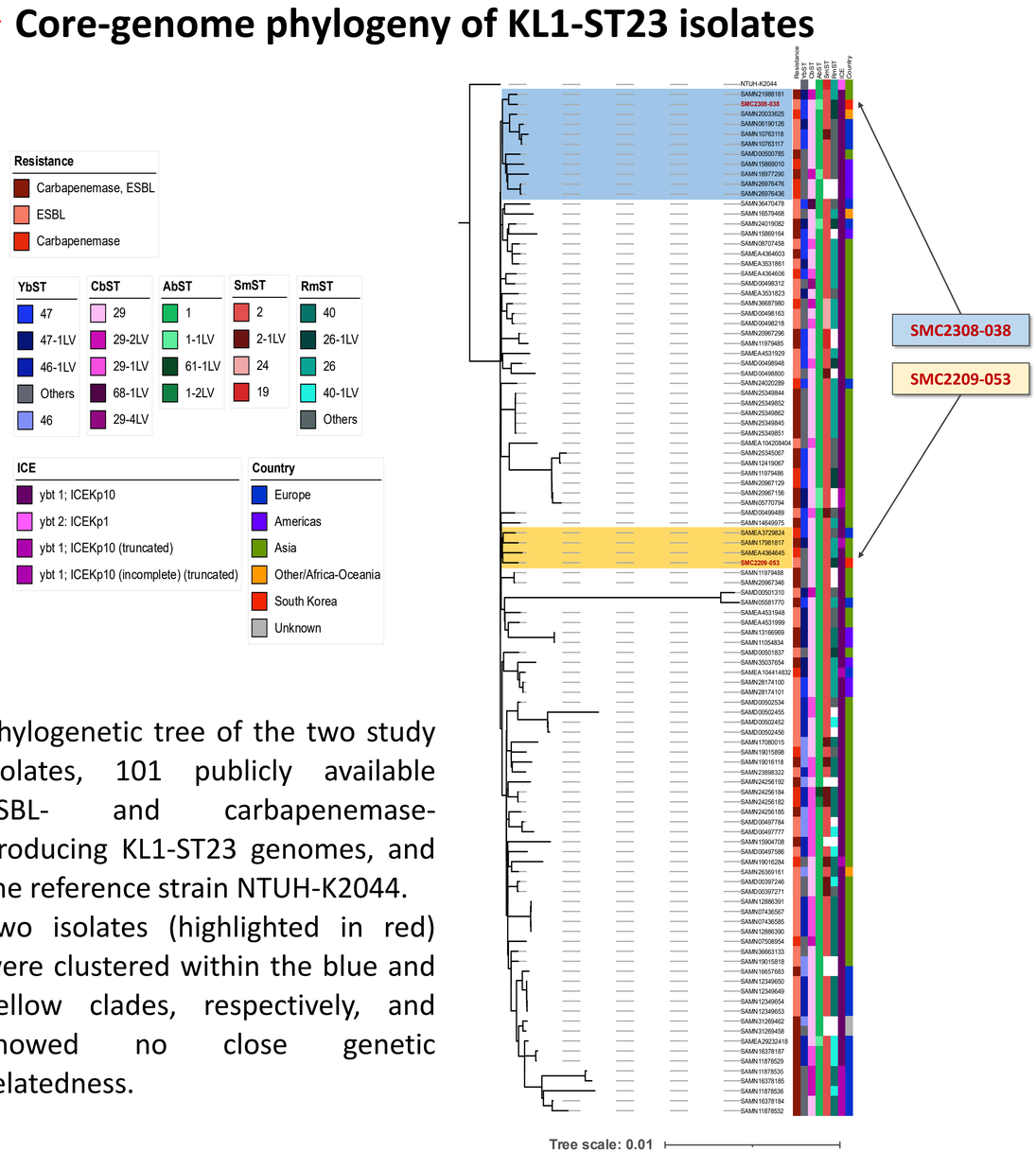
RESULTS

❖ Prevalence of KL1-ST23 isolates (2014-2024)



- 339/2,835 (11.9%) isolates belonged to KL1-ST23.
- Two ESBL –producing KL1-ST23 isolates were identified in 2022 and 2023.

❖ Core-genome phylogeny of KL1-ST23 isolates



- Phylogenetic tree of the two study isolates, 101 publicly available ESBL- and carbapenemase-producing KL1-ST23 genomes, and the reference strain NTUH-K2044.
- Two isolates (highlighted in red) were clustered within the blue and yellow clades, respectively, and showed no close genetic relatedness.

❖ Characteristics of ESBL-producing KL1-ST23 isolates

Characteristics	SMC2209-053	SMC2308-038
Source (year)	Bloodstream (2022)	Bloodstream (2023)
Genome size (bp)	5,751,212	5,869,655
K locus-sequence type	KL1-ST23	KL1-ST23
O-antigen serotype	O1/O2v2	O1/O2v2
Number of plasmids	3	5
Resistance gene profile	***bla*CTX-M-15**, ***bla*TEM-1B**, *qnrS1*	***bla*SHV-12**, ***bla*TEM-1A**, ***bla*OXA-9**, ***bla*OXA-18**, *aadA1*, *aac(6')-Ib*, ***bla*TEM-1B**
Antibiotic resistance profile	AMP, AZT, CAZ, FEP, CTX, CIP, SXT, CZO	AMP, AZT, CAZ, FEP, CTX, CZO
Conjugation result (to *E. coli* J53)	No transconjugants	3 transconjugant colonies
String test	Negative	Positive

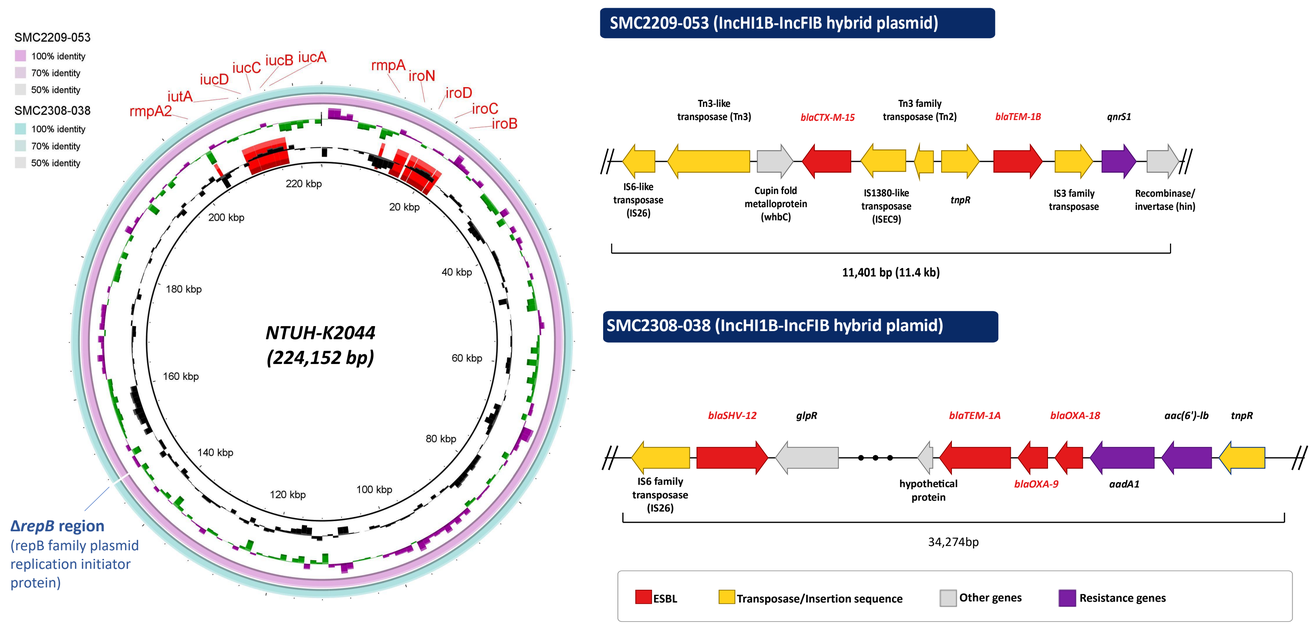
AMP, ampicillin; AZT, aztreonam; CAZ, ceftazidime; FEP, cefepime; CTX, cefotaxime; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CZO, cefazolin

- The two ESBL-producing KL1-ST23 isolates differed in resistance gene profiles, plasmid content, and transferability.

❖ Comparative analysis of hypervirulence and ESBL plasmids

A Hypervirulent plasmid comparison

B Structural analysis of ESBL gene contexts



- Hypervirulent plasmids from both isolates showed high structural similarity to the reference strain NTUH-K2044.
- Both ESBL-carrying plasmids belonged to the IncHI1B-IncFIB hybrid replicon type and carried distinct ESBL genes along with antimicrobial resistance determinants.

CONCLUSIONS

- ✓ Two ESBL-producing hypervirulent KL1-ST23 isolates were identified in South Korea.
- ✓ Genomic and plasmid analyses indicated independent acquisition of ESBL determinants without evidence of clonal spread, underscoring the importance of ongoing surveillance for resistance-virulence convergence.