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Whole Genome Sequencing of *Klebsiella pneumoniae* from Preterm Infant Meconium and Early Stool Reveals Multidrug Resistance and Virulence Gene Profiles

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Abstract: *Klebsiella pneumoniae* is a Gram-negative, non-motile, non-flagellated bacillus, and a facultative anaerobe belonging to the *Enterobacteriaceae* family. *Klebsiella pneumoniae* MPB 1 and MPB 17 were isolated from meconium/early stool samples of preterm infants in the Neonatal Intensive Care Unit (NICU) of Hospital Sultanah Aminah Johor Bahru, Malaysia. The draft genome of *K. pneumoniae* MPB 1 consists of 5,298,358 bp, with a G+C content of 57.44%, and a genome coverage of 87.7×. Genome of MPB 1 consists of 5,018 predicted genes, 4,936 protein-coding sequences, and 81 RNA-coding genes (tRNA: 77, rRNA: 4). *Klebsiella pneumoniae* MPB 17 consists of 5,477,999 bp, with a G+C content of 57.2%, and a genome coverage of 106.9×. Genome of MPB 17 consists of 5,176 predicted genes, 5,089 protein-coding sequences, and 86 RNA-coding genes (tRNA: 82, rRNA: 4). Subsequently, whole-genome sequences of MPB 1 and MPB 17 were analysed to identify the antibiotic resistance genes using ResFinder, and virulence genes were determined using the Virulence Factor Database (VFDB). Whole genome sequencing (WGS) analysis revealed that MPB 17 harbored a broader resistome (n= 16 resistance genes) compared to MPB 1 (n= 6 resistance genes), including β-lactamase genes (*bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{OXA\text{-}1}$, *bla*$_{TEM\text{-}1B}$), and resistance genes— aminoglycoside-modifying (aac(6′)-Ib-cr), phenicol (*catB3*), trimethoprim (*dfrA14*), sulfonamide (*sul2*), tetracycline (*tetA*), and others. Virulence gene profiling identified yersiniabactin-associated siderophore genes exclusively in MPB 17. KP1_RS17340 and KP1_RS17220 which are loci in the genes were also detected exclusively in MPB 17. One of the enterobactin-associated siderophore gene (*entD*) was detected exclusively in MPB 1. Type VI secretion system (T6SS) components were more abundant in MPB 17 (n= 11) than in MPB 1 (n= 7). These findings highlight the genomic diversity among *K. pneumoniae* colonizing preterm infants and underscore the importance of ongoing genomic surveillance in NICU settings to detect emerging multidrug-resistant (MDR) and potentially hypervirulent strains.

Keywords: *Klebsiella pneumoniae*; preterm infants; neonatal intensive care unit (NICU); preterm infant; antibiotic resistance; virulence; SDG 3 Good health and well-being

1. Introduction

Klebsiella pneumoniae is a Gram-negative, non-motile, non-flagellated bacillus, and a facultative anaerobe belonging to the *Enterobacteriaceae* family [1]. *Klebsiella pneumoniae* can be found across diverse environmental and host-associated settings. In the environment, *K. pneumoniae* can be found in soil, on plants, surface water, and sewage; in mammals, it can colonize mucosal surfaces, especially the gastrointestinal tract [1-3]. In addition, *K.*

pneumoniae has been detected on inanimate surfaces within hospital environments, such as floor and handwashing sink samples [4], chairs and sink's drain [5], and on medical devices including urinary catheters and endotracheal tubes [6].

Klebsiella pneumoniae is a major clinical and public health threat owing to increasing prevalence of nosocomial infections, healthcare-associated infections, and community-acquired infections caused by multidrug-resistant (MDR) strains producing extended-spectrum β-lactamases (ESBLs) and/or carbapenemases [7-13]. This species can be transmitted from person to person [14, 15]. It can cause a wide range of infections, including bacteremias, liver abscesses, meningitis, pneumonia, sepsis, urinary tract infections, and wound infections [16, 17]. Furthermore, the presence of numerous accessory gene-encoded virulence factors further enhances the severity of *K. pneumoniae* infections and/or the propensity to cause disease [7].

Importantly, the emergence of MDR *K. pneumoniae* strains has become a major public health concern, particularly in the Neonatal Intensive Care Units (NICUs) [18-20]. Preterm infants possess an immature gastrointestinal tract and immune system that impacts the developing preterm gut microbiome, making them highly susceptible to colonization by opportunistic pathogens [21-25]. More broadly, the gastrointestinal tract may serve as an important reservoir for *K. pneumoniae* infection, potentially contributing to subsequent infections and transmission within healthcare settings [17, 26, 27]. The rise in MDR *K. pneumoniae* among preterm infants in NICU settings is a growing concern [28-30]. Therefore, surveillance and genomic characterization of *K. pneumoniae* isolates recovered from the meconium/early stool of preterm infants are important for understanding bacterial adaptation, antibiotic resistance, and virulence potential.

Whole-genome sequencing (WGS) has provided extensive genomic information on various organisms, including clinical *Clostridioides difficile* isolates from Hong Kong [31], *Elizabethkingia anophelis* strain B2D isolated from dental plaque of a patient in Malaysia [32], *Vibrio parahaemolyticus* RP0132 isolated from shrimp in Malaysia [33], and various strains from the genus *Streptomyces*, including *Streptomyces griseiviridis* MUM 136J^{T} [34], *Streptomyces learnhanii* sp. nov. MUM 203J^{T} [35], *Streptomyces* sp. MUM 178J [36, 37], *Streptomyces pluripotens* MUM 16J [38], *Streptomyces albidoflavus* MUM256 [39], all of which were isolated from Malaysian mangrove soil. It is worth highlighting that in recent years, the concept of "Modern Actinobacteria" (MOD-ACTINO) had gained increasing attention in the exploration of Actinobacteria from unique and underexplored environements for the discovery of novel bioactive compounds and their potential applications [40, 41].

Notably, *Streptomyces*, a genus belonging to the phylum Actinobacteria, is a major producer of clinically important antibiotics and other bioactive secondary metabolites that have contributed significantly to human and animal health [34, 42-47].

Whole genome sequencing provides comprehensive insights into the genetic characterization of bacterial isolates, including species identification, sequence typing, detection of antimicrobial resistance and virulence genes, and phylogenetic analysis [48-53]. This is particularly relevant for *K. pneumoniae*, due to its large accessory genome comprising genes on plasmids and chromosomally encoded genes that encode specific virulence factors, various antibiotic-resistant enzymes and mechanisms [26]. While genomic surveillance of *K. pneumoniae* in adult populations is well-established, data from preterm infant populations in Malaysia remain limited. Given the clinical importance of MDR *K. pneumoniae* in NICUs, genomic characterization of antibiotic resistance genes and virulence genes has direct clinical implications. Such analyses improve understanding of the microorganism's pathogenic potential and resistance mechanisms, supporting antimicrobial stewardship, infection control, and genomic surveillance in the NICU [1, 7, 29, 54-56].

Therefore, the aims of this study were to: (i) perform WGS and comparative genomic analysis of *K. pneumoniae* MPB 1 and MPB 17 isolated from preterm infants in the NICU of Hospital Sultanah Aminah Johor Bahru, Malaysia as a follow-up to our previous studies whereby one of the study has been published [3] while the other is submitted for peer-review; (ii) characterize the antibiotic resistance gene profiles and predict phenotypic resistance; and (iii) identify virulence genes that may contribute to pathogenicity in this vulnerable population.

2. Materials and methods

Klebsiella pneumoniae MPB 1 and MPB 17 were isolated and identified from meconium/early stool samples of preterm infants in the NICU of Hospital Sultanah Aminah Johor Bahru, Malaysia [3]. Both isolates represent intestinal colonization strains rather than clinical infection isolates. In our previous study (submitted for peer-review), antimicrobial susceptibility testing (AST) was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines [57]. The inhibition zone was measured in triplicates, and the average diameter of zone of inhibition was obtained, recorded, and interpreted as susceptible, intermediate, or resistant based on CLSI breakpoints [57]. PCR-based virulence gene profiling of virulence genes *fimH*, *mrkD*, and *ybtS* was also performed. In this study, *K. pneumoniae* MPB 1 and MPB 17 were selected for WGS and subsequent antibiotic resistance and virulence gene analysis.

The genomic DNA sample was fragmented into short fragments. These DNA fragments were then end-repaired, A-tailed, and ligated with full-length adapters for Illumina sequencing before further size selection. PCR amplification was then conducted unless specified as PCR-free. Purification was then conducted through the AMPure XP system (Beverly). The resulting library was assessed on the Agilent Fragment Analyzer System (Agilent) and quantified to 1.5nM through Qubit (Thermo Fisher Scientific) and qPCR. The qualified libraries were pooled and sequenced on Illumina platforms, according to the effective library concentration and data amount required.

The raw sequence data were obtained in FASTQ format from the sequencer. The paired-end raw reads were assessed for quality using FastQC [58]. Based on the overall Phred score quality, the raw reads were trimmed for adapter sequences, low-quality bases that were below Phred score of 20 and ambiguous bases using Trimmomatic (v0.39) [59]. The quality-filtered sequences were subjected to de novo genome assembly using SPAdes (v4.2.0) with auto k-mer selection [60]. Subsequently, the contigs that were lower than 30× in coverage and shorter than 200 bp in length were removed. Quast (v5.2.0) was used to assess the quality of the assembled genomes [61]. The completeness of the assembled genomes was determined using BUSCO (Benchmarking Universal Single-Copy Orthologs) (v3.0.2) with reference to the *Enterobacteriaceae* family lineage in the BUSCO database [62]. The taxonomic similarity between strains was compared through calculation of average nucleotide identity (ANI) using the online ANI calculator [63]. The whole genome sequences of MPB 1 and MPB 17 were uploaded to the Pathogenwatch platform version 95 for comprehensive genotyping, including 7-gene multilocus sequence type (ST) calling (https://pathogen.watch/en).

The assembled genomes were subjected to gene annotation using Rapid Annotations using Subsystems Technology (RAST) [64] and Prokka pipeline [65]. Detection of antibiotic resistance genes and virulence genes were carried out using the ABRicate tool (v1. 0.1) (https://github. com/tseemann/abricate) with the Virulence Factor Database (VFDB) [66] and ResFinder [67] databases, as well as the Resistance Gene Identifier (RGI) in the Comprehensive Antibiotic Resistance Database (CARD) [68]. The plasmid sequences in the assembled genomes were determined using PlasmidFinder with the Enterobacteriales database [69].

3. Results

3.1. Genomic data for MPB 1 and MPB 17

The whole genome of MPB 1 comprised of 130 contigs, with G+C content of 57.44%, the genome size of 5,298,358 bp, and a genome coverage of 87.7-fold. The BUSCO score of

the whole genome of MPB 1 was 98.2%, indicating a reliable data output [70]. A total of 77 tRNA genes and 4 rRNA genes were predicted in the MPB 1 genome. On the other hand, the whole genome of MPB 17 comprised of 50 contigs, with G+C content of 57.2%, the genome size of 5,477,999 bp, and a genome coverage of 106.9-fold. The BUSCO score of the whole genome of MPB 17 was 98.2%, indicating a reliable data output [70]. A total of 82 tRNA genes and 4 rRNA genes were predicted in the MPB 17 genome (Table 1). The ANI analysis revealed that MPB 1 and MPB 17 genomes exhibited genomic similarity of 99.13%. Multilocus sequence typing analysis identified MPB 1 as *K. pneumoniae* ST152 and MPB 17 as *K. pneumoniae* ST985. The whole genome sequence of MPB 1 and MPB 17 have been deposited at DDBJ/EMBL/GenBank under accession number JBRSCA000000000 and JBRSBY000000000, respectively.

Table 1. Genome features of *Klebsiella pneumoniae* MPB 1 and MPB 17

Features	MPB 1	MPB 17
Genome size (bp)	5,298,358	5,477,999
Total number of contigs	130	50
GC content (%)	57.44	57.2
BUSCO completeness (%)	98.2	98.2
N50 (bp)	132,719	392,291
Genome coverage	87.7×	106.9×
Total number of predicted genes	5,018	5,176
Total number of protein coding sequences	4,936	5,089
Total number of rRNA-coding genes	4	4
Total number of tRNA-coding genes	77	82

3.2. Detection of resistance genes in MPB 1 and MPB 17 isolates and their phenotypic outcomes

AST findings from our previous study of MPB 1 and MPB 17 are summarized in Table S1 in the supplementary material. Among the 16 antibiotics tested, MPB 1 exhibited susceptibility only to 5 antibiotics, namely amoxicillin/clavulanate (20 µg/10 µg), ampicillin/sulbactam (10 µg/10 µg), cefoxitin (30 µg), tetracycline (30 µg), and chloramphenicol (30 µg). On the other hand, MPB 17 exhibited susceptibility to only 2 antibiotics, namely cefoxitin (30 µg) and chloramphenicol (30 µg). Both MPB 1 and MPB

17 were found to be MDR strains, with a multiple antibiotic resistance (MAR) index of 0.67 and 0.87, respectively (submitted for publication and under peer review). The high MAR index observed indicates that these isolates originated from a high-risk source of contamination with frequent antibiotic exposure and a potentially high level of resistance among the isolates [71, 72].

The complete list of antibiotic resistance genes in the whole genome sequences of MPB 1 and MPB 17 detected using ResFinder is provided in Supplementary Table S2. Genomic analysis demonstrated that MPB 1 and MPB 17 harbored multiple antibiotic resistance genes (Supplementary Table S2). A total of 17 antibiotic resistance genes were identified across *K. pneumoniae* MPB 1 and MPB 17. MPB 1 carried 6 antibiotic resistance genes, whereas MPB 17 harbored 16 antibiotic resistant genes, indicating a more extensive resistance gene repertoire. The detected genes included β-lactamase genes, as well as genes associated with resistance to aminoglycosides, fluoroquinolones, phenicol, efflux pumps (*oqxA* and *oqxB*), trimethoprim, fosfomycin, sulphonamides, and tetracyclines. These genes corresponded to the Center for Genomic Epidemiology (CGE)-predicted resistance phenotypes shown in Supplementary Table S2.

These findings were cross-referenced with the previously reported AST results to evaluate the concordance between WGS-detected resistance genes, CGE-predicted resistance phenotypes, and the observed phenotypic resistance patterns for each antibiotic (Table 2). Among the detected antibiotic resistance genes, those associated with CGE-predicted resistance phenotypes for antibiotics evaluated by AST in our previous study (submitted for peer-review) are presented in Table 2. For some antibiotics, multiple antibiotic resistance genes were associated with the same CGE-predicted resistance phenotype (Table 2). Overall, MPB 17 showed a broader resistance profile than MPB 1.

In Table 2, MPB 1 harbored less antibiotic resistance genes (n= 3), detecting only *aac(3)-IIa* and multidrug efflux pump genes *oqxA* and *oqxB*. Comparison of the CGE-predicted resistance phenotypes with the AST results of MPB 1 demonstrated concordance for 3 of the 11 antibiotics tested. Discordant results were observed for 8 antibiotics (Table 2**).** Of these, 5 (ampicillin, cefotaxime, ceftazidime, amikacin, and levofloxacin) were resistant by AST despite no corresponding antibiotic resistance genes being detected by WGS. However, resistance to ampicillin is expected, as *K. pneumoniae* has intrinsic resistance to this antibiotic, in accordance with CLSI guidelines [57]. In addition, 2 antibiotics (tobramycin and trimethoprim/sulfamethoxazole) were resistant by AST despite only partial detection of the antibiotic resistance genes associated with the corresponding CGE-predicted resistance

phenotype. Conversely, 1 antibiotic (chloramphenicol) was susceptible by AST despite partial detection of the corresponding antibiotic resistance genes.

In Table 2, MPB 17 harbored antibiotic resistance genes (n= 11) associated with a broader range of CGE-predicted resistance phenotypes, including ampicillin, amoxicillin/clavulanate, cefotaxime, ceftazidime, gentamicin, amikacin, tobramycin, tetracycline, levofloxacin, trimethoprim/sulfamethoxazole, and chloramphenicol**.** The broader predicted resistance profile of MPB 17 corresponded with its larger antibiotic resistance gene repertoire, which includes *oqxA*, *oqxB*, *aac(3)- IIa*, *aac(6')-Ib-cr*, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{TEM\text{-}1B}$, *catB3*, *dfrA14*, *sul2*, and *tet(A)*. Comparison of the CGE-predicted resistance phenotypes with the AST results of MPB 17 demonstrated concordance for 10 of the 11 antibiotics tested, with a discordant result observed for only 1 antibiotic (Table 2). The only discordant result was observed for chloramphenicol, where 3 chloramphenicol resistance-associated genes were detected, but the isolate was susceptible by AST.

Table 2. Comparison of antimicrobial susceptibility testing (AST) results with antibiotic resistance genes and Center for Genomic Epidemiology (CGE)-predicted antibiotic resistance phenotypes.

Antibiotic tested by antimicrobial susceptibility testing	**Antimicrobial susceptibility testing results**		**Resistance gene(s) associated with CGE-predicted antibiotic resistance phenotype**	**Presence of antibiotic resistance gene associated with CGE-predicted antibiotic resistance phenotype**	
	MPB 1	**MPB 17**		**MPB 1**	**MPB 17**
Ampicillin	Resistant	Resistant	$bla_{CTX\text{-}M\text{-}15}$	Not detected	Present
			$bla_{OXA\text{-}1}$	Not detected	Present
			$bla_{TEM\text{-}1B}$	Not detected	Present
Amoxicillin/Clavulanate	Susceptible	Resistant	$bla_{OXA\text{-}1}$	Not detected	Present
Cefotaxime	Resistant	Resistant	$bla_{CTX\text{-}M\text{-}15}$	Not detected	Present
Ceftazidime	Resistant	Resistant	$bla_{CTX\text{-}M\text{-}15}$	Not detected	Present
Gentamicin	Resistant	Resistant	*aac(3)-IIa*	Present	Present
Amikacin	Resistant	Resistant	*aac(6')-Ib-cr*	Not detected	Present
Tobramycin	Resistant	Resistant	*aac(3)-IIa*	Present	Present
			aac(6')-Ib-cr	Not detected	Present

Tetracycline	Susceptible	Resistant	*tet(A)*	Not detected	Present
Levofloxacin (Fluoroquinolone)	Resistant	Resistant	*aac(6')-Ib-cr*	Not detected	Present
Trimethoprim/ Sulfamethoxazole	Resistant*	Resistant	*oqxA*	Present	Present
			oqxB	Present	Present
			dfrA14	Not detected	Present
			sul2	Not detected	Present
Chloramphenicol	Susceptible	Susceptible	*oqxA*	Present	Present
			oqxB	Present	Present
			catB3	Not detected	Present

CGE: Center for Genomic Epidemiology

*For data analysis, results with intermediate susceptibility were considered resistant to provide a conservative estimate for antibiotic resistance.

3.3. Virulence gene profiling

The selected virulence genes (in percentage identity) detected in the whole genome sequences of strains MPB 1 and MPB 17 with reference to VFDB are presented in Table 3, while the complete list of detected genes is provided in Supplementary Table S3. MPB 17 exhibited a broader virulence gene repertoire than MPB 1. A total of 66 and 82 virulence genes were detected in MPB 1 and MPB 17, respectively (Supplementary Table S3). Both MPB 1 and MPB 17 shared key virulence genes, including type 1 and type 3 fimbrial adhesin genes and enterobactin-related genes (with *entD* detected exclusively in MPB 1). This was consistent with PCR detection results in our previous study (submitted for peer-review) whereby *fimH* (type 1 fimbrial adhesin) and *mrkD* (type 3 fimbrial adhesin) were detected in both MPB 1 and MPB 17. Eighteen virulence genes (marked with an asterisk) showed differences in detection between MPB 1 and MPB 17. Virulence gene profiling identified yersiniabactin-associated siderophore genes—*ybt* (AEPQSTUX), *fyuA/psn*, and *irp* (1 and 2)—exclusively in MPB 17. The detection of *ybtS* gene exclusively in MPB 17 was in agreement with the PCR detection findings in our previous study (submitted for peer-review). KP1_RS17340 and KP1_RS17220 which are loci in the genes were also detected exclusively in MPB 17. One of the enterobactin-associated siderophore gene (*entD*) was detected exclusively in MPB 1. Type VI secretion system components were more abundant

in MPB 17 (n= 11) than in MPB 1 (n= 7), with *icmF/tssM*, *tssF*, *tssG*, *sciN/tssJ* detected exclusively in MPB 17 (Table 3 and Supplementary Table S3).

Table 3. Selected virulence genes (in percentage identity) detected in the whole genome sequences of strains MPB 1 and MPB 17 with reference to Virulence Factor Database (VFDB).

VFDB accession number	**GenBank accession number**	**Length (bp)**	**Virulence gene**	**Gene product**	**MPB 1**	**MPB 17**
VFG000358	WP_002212885	2022	*fyuA/psn**	(fyuA/psn) pesticin/yersiniabactin receptor protein [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000359	WP_001088826	1578	*ybtE**	yersiniabactin siderophore biosynthetic protein [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000360	WP_002212799	804	*ybtT**	type II thioesterase YbtT [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000361	WP_000982866	1101	*ybtU**	yersiniabactin biosynthetic protein YbtU [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000362	WP_002212777	9492	*irp1**	yersiniabactin biosynthetic protein Irp1 [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000363	WP_002212775	6108	*irp2**	yersiniabactin biosynthetic protein Irp2 [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000364	WP_000140406	960	*ybtA**	transcriptional regulator YbtA [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000366	WP_002212761	1803	*ybtQ**	yersiniabactin ABC transporter ATP-binding/permease protein YbtQ [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG000367	WP_001286280	1281	*ybtX**	putative signal transducer [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100

VFDB accession number	GenBank accession number	Length (bp)	Virulence gene	Gene product	MPB 1	MPB 17
VFG000368	WP_000703040	1305	*ybtS**	salicylate synthase Irp9 [Yersiniabactin (VF0136) - Nutritional/Metabolic factor (VFC0272)] [Yersinia pestis CO92]	ND	100
VFG043623	WP_002916122	636	*mrkF*	type 3 fimbrial minor pilin subunit MrkF [Type 3 fimbriae (VF0567) - Biofilm (VFC0271)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG043624	WP_004149659	996	*mrkD*	fimbrial adhesin protein precursor MrkD [Type 3 fimbriae (VF0567) - Biofilm (VFC0271)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG043625	WP_000813718	2487	*mrkC*	fimbrial biogenesis outer membrane usher protein mrkC precursor [Type 3 fimbriae (VF0567) - Biofilm (VFC0271)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG043626	WP_000820818	702	*mrkB*	fimbrial chaperone protein mrkB precursor [Type 3 fimbriae (VF0567) - Biofilm (VFC0271)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG043627	WP_000756959	609	*mrkA*	type 3 fimbrial major pilin subunit MrkA [Type 3 fimbriae (VF0567) - Biofilm (VFC0271)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG044326	WP_001327262	1713	*ybtP**	yersiniabactin ABC transporter ATP-binding/permease protein YbtP [Ybt (VF0564) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	100
VFG048229	WP_015875142	606	*fimB*	tyrosine recombinase [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048237	WP_002916189	609	*fimE*	tyrosine recombinase [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048247	WP_002916190	549	*fimA*	type 1 major fimbrial subunit precursor [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100

VFDB accession number	**GenBank accession number**	**Length (bp)**	**Virulence gene**	**Gene product**	**MPB 1**	**MPB 17**
VFG048257	WP_004174507	537	*fimI*	type 1 pilus biosynthesis fimbrial protein [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048267	WP_015875145	726	*fimC*	periplasmic chaperone [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048277	WP_009308599	2613	*fimD*	outer membrane usher protein [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048287	WP_014906962	528	*fimF*	type 1 fimbrial minor component [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048297	WP_014906961	486	*fimG*	type 1 fimbrial minor component [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048307	WP_041937815	906	*fimH*	type 1 fimbrial adhesin precursor [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	99.67
VFG048317	WP_014906959	1413	*fimK*	transcriptional regulator [Type I fimbriae (VF0566) - Adherence (VFC0001)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048409	WP_086074289	747	*entA*	23-dihydroxybenzoate-23-dehydrogenase [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048419	WP_004210873	852	*entB*	23-dihydro-23-dihydroxybenzoate synthetase isochroismatase [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048429	WP_004183353	1608	*entE*	enterobactin synthase subunit E [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100

VFDB accession number	**GenBank accession number**	**Length (bp)**	**Virulence gene**	**Gene product**	**MPB 1**	**MPB 17**
VFG048439	WP_014907941	1176	*entC*	isochorismate synthase [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048449	WP_012737621	960	*fepB*	iron-enterobactin transporter periplasmic binding protein [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048468	WP_004176917	1008	*fepD*	iron-enterobactin transporter membrane protein [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048478	WP_004147521	993	*fepG*	iron-enterobactin transporter permease [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048488	WP_002893737	795	*fepC*	iron-enterobactin transporter ATP-binding protein [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048498	WP_012737619	3882	*entF*	enterobactin synthase subunit F [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048518	WP_012737617	2229	*fepA*	outer membrane receptor FepA [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048528	WP_004183335	630	*entD**	enterochelin synthetase component D [Ent (VF0562) - Nutritional/Metabolic factor (VFC0272)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	ND
VFG048632	WP_004176434	492	*vipA/tssB*	type VI secretion system contractile sheath small subunit VipA [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048643	WP_014907727	1545	*vipB/tssC*	type VI secretion system contractile sheath large subunit VipB [T6SS (VF0569) - Effector delivery system	100	100

VFDB accession number	**GenBank accession number**	**Length (bp)**	**Virulence gene**	**Gene product**	**MPB 1**	**MPB 17**
				(VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]		
VFG048654	WP_004224345	1344	*vasE/tssK*	type VI secretion system baseplate subunit TssK [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048665	WP_004176431	690	*dotU/tssL*	type VI secretion system protein DotU/TssL family [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048687	WP_002902160	492	*hcp/tssD*	type VI secretion system protein Hcp family [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048697	WP_015874721	2655	*clpV/tssH*	type VI secretion system ATPase TssH [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048755	WP_049127998	3080	*icmF/tssM**	type VI secretion protein TssM [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	89.12
VFG048771	WP_004190505	1755	*tssF**	type VI secretion system baseplate subunit TssF [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	100
VFG048779	WP_004190503	1086	*tssG**	type VI secretion system baseplate subunit TssG [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	100
VFG048787	WP_004176417	543	*sciN/tssJ**	type VI secretion system lipoprotein TssJ [T6SS (VF0569) - Effector delivery system (VFC0086)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	100

VFDB accession number	**GenBank accession number**	**Length (bp)**	**Virulence gene**	**Gene product**	**MPB 1**	**MPB 17**
VFG048808	WP_004899416	1371	*rfbK1*	O9 family phosphomannomutase RfbK1 [Capsule (VF0560) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG048972	WP_014907221	1127	KP1_RS17340*	polysaccharide export protein [Capsule (VF0560) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	99.29
VFG048980	WP_041937790	1440	KP1_RS17345	capsule assembly Wzi family protein [Capsule (VF0560) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG049029	WP_014907241	1209	KP1_RS17220*	glycosyltransferase [LPS (VF0561) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	ND	100
VFG049062	WP_015875020	1155	*rfbD*	UDP-galactopyranose mutase [LPS (VF0561) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG049084	WP_004175264	741	*rfbB*	O-antigen export ABC transporter ATP-binding protein RfbB [LPS (VF0561) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100
VFG049095	WP_002912373	768	*rfbA*	O-antigen export ABC transporter permease RfbA [LPS (VF0561) - Immune modulation (VFC0258)] [Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044]	100	100

VFDB: Virulence Factor Database; ND: not detected

* Genes marked with an asterisk indicate differences in detection between MPB 1 and MPB 17.

4. Discussion

4.1. Comparative genomic analysis of MPB 1 and MPB 17 K. pneumoniae isolates

Whole-genome analysis in this study showed that colonizing *K. pneumoniae* MPB 1 and MPB 17 isolated from meconium/early stool samples of preterm infants in the NICU of Hospital Sultanah Aminah Johor Bahru harbored both antibiotic resistance genes and virulence genes. MPB 1 and MPB 17 revealed comparable genomic features, including genome size, GC content, and predicted gene content. However, there were variations in their

predicted gene content, including differences in antibiotic resistance genes and virulence genes, which suggests genomic diversity among these colonizing strains that may influence their biological potential. These genetic differences may contribute to variation in their predicted resistance profiles and potential virulence among the NICU-associated *K. pneumoniae* isolates, providing insights into the genomic factors that influence clinically important traits. These findings highlight the importance of whole-genome characterization in identifying genetic factors associated with antibiotic resistance and virulence in *K. pneumoniae*. By providing genomic data from *K. pneumoniae* isolated from the meconium/early stool samples of preterm infants in Malaysia, this study contributes to a better understanding of *K. pneumoniae* isolates in this vulnerable population.

4.2. Resistance gene profiles and phenotypic correlates

With particular reference to the AST results and the WGS data in Table 2, the detected antibiotic resistance genes encompassed multiple antibiotic classes, including β-lactamase genes (*bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{OXA\text{-}1}$, and *bla*$_{TEM\text{-}1B}$), aminoglycoside-modifying resistance genes (*aac(3)-IIa* and *aac(6')-Ib-cr*), phenicol resistance genes (*catB3*), trimethoprim resistance gene (*dfrA14*), sulfonamide resistance gene (*sul2*), and tetracycline resistance genes (*tet(A)*). In addition, efflux pump-mediated resistance genes *oqxA* and *oqxB*, which encode the OqxAB efflux pump [73], were detected and were associated with the chloramphenicol- and trimethoprim-resistant phenotypes predicted by CGE. To facilitate discussion, the identified antibiotic resistance genes are presented according to their respective antibiotic classes and interpreted in relation to the phenotypic AST results.

4.2.1. β-lactam resistance genes (bla$_{TEM\text{-}1B}$*, bla*$_{CTX\text{-}M\text{-}15}$*, and bla*$_{OXA\text{-}1}$*)*

β-lactam antibiotics have traditionally been preferred for the treatment of *K. pneumoniae* infections due to the presence of efflux systems, aminoglycoside-modifying enzymes, and macrolide esterases that render the effectiveness of many other antibiotic classes [74]. However, treatment with β-lactams has become increasingly challenging in recent years due to the emergence of multiple classes of β-lactamases in clinical *Klebsiella* isolates [74].

β-lactamases belonging to the Temoneira (TEM), Cefotaximase-Munich (CTX-M), and Sulfhydryl Variable (SHV) families are classified as Ambler class A β-lactamases. The hydrolytic activity in class A varies from narrow-spectrum to carbapenem-hydrolysing enzymes and depends on a two-step process involving the catalytic Ser70 residue, a coordinated water molecule, and lysine and glutamic acid residues that are part of the active site [75]. On the other hand, Oxacillinase (OXA) or OXA-type β-lactamases (OXAs) is a large group of Ambler class D β-lactamases, that hydrolyze β-lactam antibiotics through an active-

site serine nucleophile that forms a covalent acyl-enzyme intermediate, followed by water-mediated deacylation of the β-lactam and product release [76-78]. The OXA-type β-lactamases exhibit a remarkably varied spectrum against β-lactam agents from narrow-spectrum to extended-spectrum and carbapenemases [78-80], whereas TEM- and SHV-type β-lactamases range from narrow-spectrum to ESBLs [75]. The CTX-M-type β-lactamases exhibit an extended-spectrum profile against β-lactam agents [75]. These β-lactamases are widely distributed among Gram-negative species, including members of the *Enterobacteriaceae* [78, 81]. Importantly, ESBL-encoding genes are commonly carried on conjugative plasmids or integron-like structures, enabling their effective dissemination to other bacterial strains and species [81].

The $bla_{TEM\text{-}1B}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{OXA\text{-}1}$ were detected only in MPB 17, while $bla_{SHV\text{-}187}$ was detected in both MPB 1 and MPB 17 (Supplementary Table S2). The resistance of MPB 17 to amoxicillin/clavulanate was consistent with the presence of $bla_{OXA\text{-}1}$, supporting the contribution of this resistance gene to the observed phenotype. $bla_{TEM\text{-}1B}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{OXA\text{-}1}$ corresponded to the CGE-predicted ampicillin-resistant phenotype. However, phenotypic resistance to ampicillin was observed in both isolates despite the absence of these genes in MPB 1. This finding is consistent with the intrinsic resistance of *K. pneumoniae* to ampicillin, as described in CLSI guidelines [57]. Although $bla_{CTX\text{-}M\text{-}15}$ was detected only in MPB 17, both MPB 1 and MPB 17 were resistant to cefotaxime and ceftazidime, indicating an inconsistency between the WGS-data, WGS-based predictions and the observed phenotypic resistance findings of MPB 1. It is likely that there is OFF-panel AMR mechanisms, for example, AmpC, other ESBLs, and porin mutations, may contribute to the resistance phenotype observed in MPB 1 [82].

The presence of bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} genes in the present study is in-line with previous studies, which have highlighted the widespread dissemination of these β-lactamase genes among *K. pneumoniae*. Msanga et al. [83] reported the prevalence genes bla_{TEM}, $bla_{CTX\text{–}M}$, and bla_{SHV} in 63.4%, 73.2%, and 75.6%, respectively in *K. pneumoniae* isolated from the blood and stool samples of neonates. Additionally, Geleta et al. [84] also reported high prevalence of resistance genes bla_{TEM}, $bla_{CTX\text{–}M}$, and bla_{SHV} in 94.1%, 96.1%, and 88.2% of ESBL-positive *K. pneumoniae* isolated from septic neonates. The authors found that most of the isolates (94.1%) carried at least two ESBL resistance genes [84]. Study by Chen et al. [85] also showed *K. pneumoniae* isolated from neonates carried various β-lactamase genes. Some isolates carried genes such as $bla_{TEM\text{-}1}$ variants, $bla_{CTX\text{-}M\text{-}15}$, bla_{SHV}, $bla_{OXA\text{-}1}$ among the various β-lactamase genes, which were also detected in MPB 17. Overall, these studies indicate that there is a high prevalence of bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} genes in *K. pneumoniae* isolated from infants.

The present study identified the *bla*$_{OXA-1}$ gene in *K. pneumoniae* MPB 17. In relation to *bla*$_{OXA}$ variants, particularly the *bla*$_{OXA-1}$ gene, Meng et al [80]. reported that among global *K. pneumoniae* isolates carrying *bla*$_{OXA}$ variants, *bla*$_{OXA-1}$ (51.5%) was the most prevalent variant, followed by *bla*$_{OXA-9}$ (17.3%), *bla*$_{OXA-48}$ (14.3%) and *bla*$_{OXA-232}$ (8.6%). A study in Bangladesh by Rahman et al [86]. reported that *K. pneumoniae* was the most common Gram-negative organism isolated from blood culture positive neonates suspected of sepsis. Among the 18 ESBL producers detected in their study, 9 belonged to *K.* pneumoniae and out of which 3 carried the *bla*$_{OXA-1}$ gene [86]. Another study in Russia reported that OXAs genes were detected in 10 *K. pneumoniae* strains (62.5%) isolated from newborns, with the *bla*$_{OXA-2}$ gene being the most prevalent (37.5%), while the *bla*$_{OXA-1}$ gene was detected in three strains (18.75%) [87]. A study in Malaysia by Lee et al. [29] also detected the ESBL-associated gene—*bla*$_{OXA-1}$ in 19 out of 39 isolates (48.7%) consisting of *K. pneumoniae* (36 isolates) and *Escherichia coli* (3 isolates) isolated from the stool and/or tracheal secretions of preterm infants. Another study reported of an alarming occurrence of extensively-drug resistant among *K. pneumoniae* isolates recovered from neonatal sepsis in Egypt, demonstrating that the *bla*$_{OXA–1}$ gene was present in 75% of the *K. pneumoniae* isolates [88]. Overall, these findings supported the occurrence of the *bla*$_{OXA-1}$ gene among *K. pneumoniae* isolated from infants.

4.2.2. Aminoglycoside-modifying resistance genes (aac(3)-IIa and aac(6')-Ib-cr)

One of the family of aminoglycoside-modifying enzymes is the aminoglycoside acetyltransferases (encoded by acetyltransferases (*aac*) genes), which confers resistance to aminoglycosides in members of the family *Enterobacteriaceae* [89]. *aac(3)-IIa* is widely observed among the members of *Enterobacteriaceae*, including *K. pneumoniae* [89]. While *aac(6')-Ib* gene encodes a common aminoglycoside acetyltransferase responsible for resistance to the aminoglycosides including kanamycin, amikacin and tobramycin [90], *aac(6')-Ib-cr* is a naturally occurring plasmid-associated gene that encodes a new variant of a common aminoglycoside acetyltransferase that is also capable of acetylating and reducing the activity of certain fluoroquinolones [90]. Notably, findings by Kim et al. [91] demonstrated that the *aac(6')-Ib-cr* variant was increasingly detected in bacterial genomes, *Klebsiella* and *Escherichia* being the major taxa harboring this gene. Relating these genes to *K. pneumoniae* isolates, Perez-Lopez et al. [92] reported that within the *K. pneumoniae* isolates (n= 19) in their NICU population, the prevalence of gene *aac(3)-II* (comprising of *aac(3)-IIa*, *aac(3)-IId*, *aac(3)-IVa*, *aac(3)-Via*) and *aac(6')-Ib-cr* were 42.1%, and 36.7%, respectively. Therefore, highlighting the occurrence of these genes in NICU-associated *K. pneumoniae*.

The aminoglycoside-modifying resistance gene *aac(3)-IIa* was detected in both MPB 1 and MPB 17. The detection of this gene was consistent with the CGE-predicted phenotypes and the AST results for gentamicin and tobramycin, Indicating genotype-phenotype concordance in both MPB 1 and MPB 17. However, with regards to one of the plasmid-mediated quinolone resistance (PMQR) gene *aac(6')-Ib-cr* [93], it was detected only in MPB 17 and was associated with CGE-predicted resistance to tobramycin, amikacin, and fluoroquinolones. The WGS-based prediction was concordant with the AST results of MPB 17; however, this genotype-phenotype correlation was not observed in MPB 1. With particular reference to the antibiotic amikacin, the absence of *aac(6')-Ib-cr* gene in MPB 1, was discordant with the AST result, which exhibited an amikacin-resistant phenotype. This genotype-phenotype discrepancy suggests that amikacin resistance in MPB 1 may not be attributed solely to *aac(6')-Ib-cr*. Regarding fluoroquinolone resistance, in particular levofloxacin, the presence of *aac(6')-Ib-cr* in MPB 17 and its absence in MPB 1, despite AST-confirmed resistance in both isolates, suggests that this gene alone may not have contributed greatly to the resistance observed. Similarly, Robicsek et al. [90] found that *aac(6')-Ib-cr* alone confers low-level ciprofloxacin resistance in *E. coli*, but act additively when added with another plasmid-encoded resistance mechanism mediated by *qnrA*. Hence, suggesting that additional determinants may be required for higher-level fluoroquinolone resistance.

4.2.3. Phenicol resistance gene (catB3) and Multidrug efflux pump genes (oqxA and oqxB)

The antibiotic resistance gene *catB3* encodes chloramphenicol acetyltransferases [94]. Sedrakyan et al. [95] reported a 52.4% prevalence of *catB3*, and included the presence of the gene in three chloramphenicol-susceptible clinical *K. pneumoniae* isolates. In our study, this gene was detected in MPB 17 but not in MPB 1, and was predicted by CGE to confer resistance to chloramphenicol. This gene has also been detected in a *K. pneumoniae* isolated from a premature infant in a study by Chen et al. [96]. The absence of *catB3* in MPB 1 was consistent with the chloramphenicol-susceptible AST result. However, the presence of *catB3* in MPB 17 despite chloramphenicol susceptibility indicates a discrepancy between the WGS-based resistance prediction and the phenotypic AST-based susceptibility result.

The *oqxA* and *oqxB* efflux pump genes are recognized as one of the PMQR determinants [93]. These genes encode the plasmid-encoded multidrug efflux pump OqxAB [73], which belongs to the resistance–nodulation–division (RND) superfamily [93, 97]. That being said, one study found *oqxA* and *oqxB* genes on the chromosome of clinical *K. pneumoniae* [73]. Another study reported that a chromosome-based *oqxAB* operon was detectable in all *K. pneumoniae* strains tested, including organisms isolated before the year

1984, and further suggested that the plasmid-borne *oqxAB* operon originated from the chromosome of *K. pneumoniae* and subsequently evolved to become more functionally active [98]. The OqxAB efflux pump is one of the resistant mechanisms that contribute to intrinsic, acquired, and phenotypic resistance by lowering intracellular antibiotic concentration and promoting site mutation accumulation [99]. This multidrug efflux pump could facilitate the development of resistance to multiple drugs (including chloramphenicol) and dissemination of antimicrobial resistance via horizontal transfer [100]. Nonetheless, it is worthy to note that *oqxAB* is subject to regulation by both RarA and RamA (AraC-type transcriptional activator) and OqxR (GntR-type transcriptional repressor) [100]. Notably, Kim et al. [73] reported that 74.1% of human clinical *K. pneumoniae* in their study were provisionally classified as positive for both *oqxA* and *oqxB* genes. In addition, Peltier et al. [101] found *K. pneumoniae* isolated from a NICU in their study encoded three PMQR, including *oqxA* and *oqxB*.

Both *oqxA* and *oqxB* genes were detected in MPB 1 and MPB 17. The CGE-predicted phenotypes associated with *oqxA* and *oqxB* included resistance to chloramphenicol and trimethoprim. For chloramphenicol, both MPB 1 and MPB 17 exhibited phenotypic susceptibility based on AST results, indicating a lack of complete concordance between WGS-based resistance predictions and the observed phenotype. In contrast, although *oqxA* and *oqxB* genes were associated with a CGE-predicted trimethoprim-resistant phenotype, direct comparison with AST results was not possible because the susceptibility testing was performed using the combination antibiotic trimethoprim/sulfamethoxazole rather than trimethoprim alone. Therefore, the CGE-predicted trimethoprim resistance could not be directly correlated with the observed AST result. Overall, the phenotypic susceptibility to chloramphenicol observed in MPB 1 and MPB 17 despite the presence of the resistance genes—*oqxA* and *oqxB* in MPB 1, and *catB3*, *oqxA*, and *oqxB* in MPB 17— may reflect an unexpressed/silent gene or reduced gene expression [67, 102].

4.2.4. Trimethoprim resistance gene (dfrA14), Sulfonamide resistance gene (sul2)

Resistance to trimethoprim is caused by modifications in the target enzyme dihydrofolate reductase (*dfr*), which is encoded by *dfr* genes that are usually associated with integrons [103]. Many *dfr* gene cassettes, including about half of the known *dfrA* alleles, lie within gene capture and expression systems known as integrons [104]. Notably, many *dfrA* alleles associated with integrons, transmissible plasmids, or both, are globally disseminated [104]. On the other hand, *sul2* is a plasmid-encoded sulfonamide resistance gene that produces dihydropteroate synthetase (DHPs) and induce resistance against sulfonamides [103, 105]. Sulfonamides are structural analogues of para-amino benzoic acid (PABA), which

competitively inhibit DHPs activity [105]. Therefore, the simultaneous prescription of dihydrofolate reductase (trimethoprim) with sulfonamides creates a synergistic antimicrobial activity in bacterial infections [105].

In this study, the concordance between the AST-confirmed resistance to trimethoprim/sulfamethoxazole and the presence of *dfrA14* and *sul2* in MPB 17 supports the contribution of these resistance genes to the observed phenotype, corresponding with the CGE-predicted resistance profiles for trimethoprim and sulfamethoxazole, respectively. Furthermore, the detection of *oqxA* and *oqxB* in both MPB 1 and MPB 17, and their association with CGE-predicted trimethoprim resistance, further support their potential contribution to the predicted resistance profile; however, their specific role in trimethoprim/sulfamethoxazole resistance remains uncertain. In contrast, the trimethoprim/sulfamethoxazole resistance observed in MPB 1 despite the absence of *dfrA14* and *sul2* suggests that additional mechanisms may contribute to this phenotype. Although *oqxA* and *oqxB* genes were present, trimethoprim/sulfamethoxazole resistance in *K. pneumoniae* is more commonly associated with *dfr* and *sul* resistance genes [94, 106], and the contribution of *oqxA* and *oqxB* to this phenotype requires further investigation.

4.2.5. Tetracycline resistance tet(A) gene

Tetracycline resistance *tet(A)* gene encodes a Class A tetracycline efflux pump that is highly prevalent in *Enterobacteriaceae*, including *K. pneumoniae* [107]. It is an efflux protein that is anchored in the inner membrane by 12 putative transmembrane α helices, which are connected by cytoplasmic and periplasmic loops [108]. In general, *tet* genes are commonly carried on mobile genetic elements (including plasmids, transposons, and integrons), and are frequently associated with additional resistance genes, enabling resistance to tetracycline and additional agents [109]. Nonetheless, chromosomal *tet* efflux determinants have also been reported [109]. The tetracycline resistance mechanism is mediated by the Major Facilitator Superfamily (MFS) of transporters [109, 110], and the gram-negative *tet* genes are controlled by the tetracycline-responsive *Tet*R repressor [109]. These *tet* efflux pumps extrude tetracycline out of a cell using proton exchange as the source of energy, resulting in antibiotic resistance [111].

The observed phenotypic resistance to tetracycline in MPB 17 was consistent with the detection of *tet(A)*, which corresponded with the CGE-predicted resistance phenotype of this antibiotic. In contrast, the absence of *tet(A)* gene in MPB 1 and the corresponding CGE prediction of susceptibility were in agreement with AST results. Overall, the concordance between *tet(A)* detection and tetracycline resistance, as well as the absence of this gene in the

susceptible isolate, suggests that *tet(A)* contributed to the observed tetracycline resistance phenotype in these isolates.

4.3. Antibiotic resistance genes in pediatric K. pneumoniae isolates

A study based on pediatric subjects by Flores-Valdez et al. [112] reported the prevalence of genes encoding resistance to β-lactams (β-lactamase genes: *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{TEM}$, and *bla*$_{CTX–M}$), aminoglycosides, phenicols, quinolones, trimethoprim, sulphonamides, and tetracycline, in *K. pneumoniae* isolated from the blood of paediatric patients were (53%, 53%, 5%, and 26%), 79%, 5%, 97%, 39%, 68%, and 45%, respectively. Additionally, Berglund et al. [113] reported a 21% prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) isolated from faecal sample of patients admitted to a Vietnamese NICU, which increased to 74% at discharge. They further showed that the resistance genes including *bla*$_{TEM}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA}$, *aac(6')-Ib-cr*, *oqxAB*, *dfrA*, *sul2*, and *tet(A)* genes were present in these isolates, with their frequency increasing from 12 to 27, 10 to 27, 25 to 81, 7 to 16, 29 to 85, 18 to 39, 8 to 13, and 11 to 14, respectively, between admission and discharge, suggesting clonal spread. The authors also observed that patients who were CRKP-positive at discharge had significantly longer NICU stays ($P < 0.001$), possibly due to the increased risk associated with prolonged exposure to the NICU environment [113].

Klebsiella pneumoniae isolated from NICU settings have been reported to harbour multiple antibiotic resistance genes spanning several antibiotic classes [96, 114]. This is supported by Chen et al. [96] who detected 12 antibiotic resistance genes in a *K. pneumoniae* isolate from a premature infant. Similar resistance determinants were detected in MPB 17, including β-lactamase genes, aminoglycoside-modifying resistance genes, phenicol resistance genes, sulfonamide resistance genes, and multidrug efflux pump-mediated genes. Another study which isolated carbapenemase-producing *K. pneumoniae* (from neonatal carriage, NICU environment, and neonatal bloodstream infections) from a NICU in Ghana, West Africa also detected resistance genes including *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *oqxA*, *oqxB*, *dfrA14*, *sul2*, and *tet(A)* [114], which were also reported in MPB 17.

4.4. Factors contributing to discrepancies between antibiotic resistance genes and AST results

Taken together, the detection of multiple antibiotic resistance genes suggests an increased resistance potential of these *K. pneumoniae* isolates. However, concordance between genotypic predictions and AST-based phenotypic results was not always observed. The discrepancy between detected WGS-based antibiotic resistance genes and the AST-based phenotypic result of the *K. pneumoniae* isolates in this study may be influenced by the

presence of resistance genes located on mobile genetic elements, such as plasmids [115]. Although WGS can detect many resistance-associated genes, the short-read sequencing approach used in this study has limited ability to accurately reconstruct plasmid structures and determine the genomic location of these genes [116-119]. Consequently, the observed genotype–phenotype discrepancies may be partly attributable to plasmid-borne resistance genes that could not be fully resolved using the current WGS approach. Furthermore, the current WGS-based resistance prediction approach may fail to detect resistance genes not represented in the ResFinder database, including resistance mechanisms arising from chromosomal mutations [67].

Beyond the current WGS-based resistance prediction approach, alternative or undetected resistance mechanisms, such as the presence of OFF-panel AMR mechanisms for example, AmpC, other ESBLs, and porin mutations that may have contributed to the observed discrepancies (particularly with the β-lactamase genes) [82]. Additionally, an unexpressed/silent gene or reduced gene expression may contribute to these discrepancies, as the presence of resistance genes does not necessarily result in the corresponding resistance phenotype [67, 102]. Thus, all these factors may contribute to the discrepancies between genotypic and phenotypic findings. Overall, highlighting the complexity of antibiotic resistance mechanisms and the need to interpret genotypic predictions alongside phenotypic results for an accurate interpretation of resistance profile as phenotypic testing remains essential for confirming the functional expression and clinical relevance of the detected genes.

Klebsiella pneumoniae MPB 1 and MPB 17 were recovered from the meconium/early stool samples of two preterm infants in the same NICU, representing intestinal colonization strains rather than clinical infection isolates. Both isolates showed different genomic antibiotic resistance profile (Table 2 and Supplementary Table S2). This may be influenced by differing antibiotic exposure between these two preterm infants. Antibiotic exposure likely constituted an important selective pressure, contributing to the detection of antibiotic resistance genes. Several studies have demonstrated an association between early-life antibiotic exposure and antibiotic resistance genes in infants [120-122]. Analyses of specific antibiotic resistance genes by Nogacka et al. [123] had also demonstrated a higher occurrence of some β-lactamase coding genes in infants whose mothers received intrapartum antimicrobial prophylaxis. Several other possible contributing factors that could influence the genomic antibiotic-resistance genes in infants include maternal transmission [124, 125], mode of delivery [126], gestational age [121], duration of lactation [121], type of feeding [120, 121, 127], and postnatal gut microbial ecology [128]. Additionally, horizontal gene transfer of AMR

genes within the NICU environment is probable [129]. Nonetheless, further investigation is warranted to better understand the underlying reasons for this finding.

4.5. Virulence determinants and pathogenic potential

In the genome of *K. pneumoniae*, mobile genetic elements including plasmids, prophages, transposons, and insertion sequences, improve bacterial viability and adaptability by mediating the horizontal transfer of virulence genes, antibiotic resistance genes, and other adaptive genes [130]. While antibiotic resistance genes enhance bacterial survival under antibiotic pressure, virulence factors contribute directly to pathogenicity. The pathogenicity of *K. pneumoniae* is attributed to multiple virulence factors that enable the bacterium to evade innate host defenses and persist within mammalian hosts [1]. The virulence factors of *K. pneumoniae* include capsule polysaccharide, lipopolysaccharide (LPS), fimbrial adhesins, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, which collectively contribute to survival and immune evasion during infection [131].

In this study, WGS was used to identify virulence genes as genomic indicators of virulence potential rather than direct evidence of expressed virulence. Based on PCR findings in our previous study (submitted for peer-review), virulence genes profiling revealed that *fim*H, *mrk*D, and *ybt*S were detected in 100%, 100%, and 82.14%, of *K. pneumoniae* isolates, respectively. The virulence genes *fimH* and *mrkD* were detected in both MPB 1 and MPB 17 whereas *ybtS* was detected only in MPB 17. These findings were consistent with the genomic results in this study (Table 3 and Supplementary Table S3). The presence of genes associated with major virulence factors, including capsular polysaccharide, LPS, fimbrial adhesins (Type 1 and Type 3), and siderophore-mediated iron acquisition systems, and Type VI secretion systems (T6SS), was discussed in relation to their potential contribution to *K. pneumoniae* pathogenicity.

4.5.1. Capsular polysaccharide and LPS

In general, members of the genus *Klebsiella* typically express 2 types of surface antigens—the K-antigen on the capsular polysaccharide and the O-antigen on the LPS—both of which contribute to their pathogenicity [132]. The capsule of *K. pneumoniae* is composed of an extracellular matrix that surrounds the bacterium [133]. Capsular polysaccharide facilitate host infection and enable pathogenic bacteria to evade the host immune system [134]. It contributes to the prevention of phagocytosis, hinder antibacterial activity of antimicrobial peptides, block complement components, preventing complement-mediated lysis and opsonization, and prevent fulminant activation of the immune response [133]. The capsule has an essential role in protecting *K. pneumoniae* from the hosts' immune responses but its

virulence may result from other factors [135]. It is worth noting that K2 strains represent the most commonly isolated clinical type of *K. pneumoniae*, followed by K1 strains [16]. The capsule-associated factors are *magA* for serotype K1, k_2A for serotype K2, and *rmpA*/*rmpA2* for the regulator of mucoid phenotype A [133]. With regard to resistance to antimicrobial peptides, the capsular polysaccharide located on the cell surface of *K. pneumoniae* acts as a protective shield against access from host-derived antimicrobial peptides, and the free capsule polysaccharides released from bacterial cells are able to trap antimicrobial polypeptides, reducing the number of antimicrobial polypeptides reaching the bacterial surface [131, 136, 137].

On the other hand, LPS, also known as endotoxin, is a major essential component of the outer leaflet of the cell membrane of all Gram-negative bacteria [16]. It protects *K. pneumoniae* against humoral defenses and activates immune system [16]. Lipopolysaccharide typically consists of an O-antigen, a core oligosaccharide, and lipid A [16, 131]. These components are encoded by genes in the *rfb*/*wb*, *waa*, and *lpx* gene clusters on the core genome, respectively [16, 138, 139]. The O-antigen prevents access of complement components to activators (for example, porins, and rough LPS), and therefore contributes to bacterial resistance against complement-mediated killing [131, 140]. The lipid A is a potent ligand of toll-like receptor 4 (TLR4), a pattern recognition receptor [16]. Activation of TLR4 triggers the release of chemokines and cytokines that recruit and activate cellular responses, including macrophages and neutrophils [16]. Hence, it may contribute to host inflammatory responses that may influence disease progression.

Based on Table 3 and Supplementary Table S3, KP1_RS17340, was annotated as a polysaccharide export protein associated with the capsule, while KP1_RS17220 was annotated as a glycosyltransferase associated with LPS. Both were not detected in MPB 1, but were present in MPB 17. Glycosyltransferases are involved in the biosynthesis of both capsular polysaccharide and LPS [141, 142]. In Gram-negative bacteria, there are 3 transporter-dependent systems (Wzx/Wzy, synthase, and ATP-binding cassette (ABC) transporter) capable of exporting capsular polysaccharides [134]. According to Rendueles et al. [141], *K. pneumoniae* capsule biosynthesis mainly relies on the Group I Wzx/Wzy-dependent pathway for capsular polysaccharide production and export. Capsule biosynthesis is initiated by a glycosyltransferase (either WcaJ or WbaP), which links the first moiety of glucose (WcaJ) or galactose (WbaP) to a lipid carrier—undecaprenyl phosphate, after which other oligosaccharides are added at the nonreducing end of the glycosyl-undecaprenyl phosphate [141].

These lipid-linked residues are flipped by Wzx across the inner membrane, from the cytoplasm to the periplasmic space and polymerized by virtue of the Wzy polymerase [141]. After it is regulated in length by Wzc, the capsule is exported out of the cell by an outer membrane exporter—Wza [141]. Lastly, the capsule is anchored to the cell surface, most likely by Wzi [141]. It is worth noting that glycosyltransferase WcaJ is commonly associated with capsule biosynthesis in *K. pneumoniae* [143, 144]. Furthermore, in antibiotic resistance, the negatively charged capsular polysaccharides contribute to bacterial protection by limiting the access of antimicrobial peptides to the bacterial envelope, hence, reducing their effectiveness [134]. The CPS and LPS protect *Klebsiella* spp. from the action of complement cascade and antimicrobial peptides, as well as from engulfment and phagocytosis by host immune cells [145, 146]. Therefore, the presence of loci encoding a polysaccharide export protein (KP1_RS17340) and glycosyltransferase (KP1_RS17220) in MPB 17 suggests the presence of genomic features associated with capsular polysaccharide and LPS biosynthesis, which may contribute to virulence potential. Nonetheless, their functional contribution to capsule or LPS production and any influence on antimicrobial susceptibility require further experimental validation.

4.5.2. Fimbrial adhesins (Type 1 and Type 3)

In terms of fimbrial adhesins, both classical and hypervirulent *K. pneumoniae* produces membrane-bound structures—type 1 and type 3 fimbriae [16]. Both types of fimbriae are important mediators of adhesion, and characterized as pathogenicity factors [16]. Type 1 fimbriae are members of the chaperone–usher class fimbriae family and are encoded by the *fimABCDEFGHIK* gene cluster [147]. Within this cluster, *fimA* encodes the primary structural subunit, whereas *fimH* encodes the adhesive subunit FimH, which has been demonstrated to facilitate adhesion to mannose-containing structures found on host tissue surfaces and within the extracellular matrix [147]. The other genes *fimF* and *fimG* encodes the minor structural subunits FimF and FimG; *fimC* encodes the fimbrial chaperone; *fimD* encodes an usher protein; *fimI* encodes an uncharacterized product necessary for type 1 fimbriae; and *fimK* has been theoretically proposed to play a role in the regulation of type 1 fimbriae [16]. Besides that, *fimB* and *fimE* encode two recombinases that mediate inversion of the switch, resulting in a fimbriated state when the switch is in the "on" position and a non-fimbriated state when it is in the "off" position [148, 149]. Interestingly, Struve et al. [148] found that type 1 fimbriae are a significant virulence factor of *K. pneumoniae* urinary tract infection, but type 1 fimbriae do not affect the ability of *K. pneumoniae* to infect the lungs or colonize the intestine.

Type 3 fimbriae also belong to the chaperone–usher class fimbriae and are encoded by the *mrkABCDF* gene cluster [147]. It is worth noting that *mrkE* has been reported in some

contexts and may have regulatory or accessory functions [150, 151]. The *mrkA* gene encodes the major structural subunit, while the *mrkD* gene encodes the fimbrial adhesin [147]. *mrkB*, *mrkC* and *mrkF* encode the chaperone, usher and scaffolding proteins, respectively, which are responsible for fimbrial assembly/stabilization [131, 152]. Similar to type 1 fimbriae, the type 3 fimbriae do not affect *K. pneumoniae* intestinal colonization, nor does it contribute significantly to the ability of *K. pneumoniae* to cause lung infection [150].

Type 3 fimbriae play an essential role in biofilm formation in *K. pneumoniae* [150, 152, 153]. Biofilm is a self-enclosed population of bacterial cells [154, 155]. Biofilm formation confers increased tolerance to antibiotics and the host immune system relative to growth as planktonic cells [153]. *Klebsiella pneumoniae* is a commonly encountered opportunistic pathogen associated with hospital-acquired infections that frequently affects patients with indwelling medical devices [152]. In these settings, type 3 fimbriae function as appendages mediating biofilm formation on both biotic and abiotic surfaces [152]. This would enable *K. pneumoniae* to seed vulnerable sites and persist in patients [16]. However, both type 1 and type 3 fimbriae are not expressed when *K. pneumoniae* is planktonic, and the expression of type 1 fimbriae is controversial [16, 147, 153]. Generally, biofilm is formed when planktonic bacteria encounter a surface, adhere to it, and subsequently form a complex three-dimensional (3D) structure [16]. Within this structure, bacteria often exhibit resistance to chemicals that planktonic cells may be susceptible to [16]. These biofilms can also release planktonically growing bacteria, which may serve as a source of infection [16].

Based on several studies, the prevalence of virulence genes associated with fimbrial adhesins (type 1 and type 3) in clinical *K. pneumoniae* seemed to be relatively high. A study based on *K. pneumoniae* isolated from the blood of paediatric patients reported that the prevalence of the fimbrial genes *fim* and *mrk* were 97% and 100%, respectively [112]. Another study based on *K. pneumoniae* isolated from different clinical specimens reported the prevalence of virulence genes *fimH-1* was 100%, while *mrkD* was 96.3% [1]. Consistent with our previous PCR-based finding (submitted for peer-review), virulence genes *fimH* and *mrkD* were detected in all *K. pneumoniae* isolates (including MPB 1 and MPB 17) recovered from the meconium/early stools of preterm infants. The detection of *fimH* and *mrkD* among NICU-associated *K. pneumoniae* isolates suggests the potential contribution of these adhesion-associated genes to bacterial adherence, which may enhance persistence and pathogenic potential of *K. pneumoniae* in this vulnerable population. The presence of these genes highlights the relevance of assessing virulence genes in understanding the pathogenic potential of *K. pneumoniae* isolates from NICU settings.

4.5.3. Siderophore-mediated iron acquisition systems

Iron is essential for bacterial growth and replication [156]. However, free iron is not readily accessible to pathogens, and the aim of this limitation is to restrict iron from invading pathogens [157]. Hence, in order to overcome this challenge, *K. pneumoniae* scavenges iron from the environment and the host during infection via multiple iron acquisition systems [157]. The ability to acquire iron is a key factor that enhances the invasive potential of *K. pneumoniae* during infection in immunocompetent individuals [156]. Iron acquisition in *K. pneumoniae* is primarily mediated by siderophores, which are low molecular-weight, high-affinity iron-chelating compounds crucial for bacterial survival and virulence [157]. Siderophores are secreted by the bacteria in response to iron deficiency to acquire iron from the environment [157]. It binds to Fe^{3+} ions in the environment, forming soluble complexes that can be recognized and internalized by specific receptors on the bacterial surface [157]. *Klebsiella pneumoniae* produces several siderophores, including enterobactin, yersiniabactin, aerobactin, and salmochelin, which contribute to iron acquisition and virulence [158]. A study by Flores-Valdez et al [112] reported that one of the *K. pneumoniae* isolates from the blood of a paediatric patient carried genes that code siderophores—enterobactin, yersiniabactin, aerobactin, and salmochelin. The production of multiple siderophores may enable *K. pneumoniae* to colonize and disseminate to various sites within the host, with each siderophore performing a niche-specific role [16].

Here, we focus only on enterobactin and yersiniabactin. Enterobactin is the primary siderophore of *K. pneumoniae* and is almost ubiquitous among both classical and hypervirulent [16]. The genes required for enterobactin biosynthesis are located on the chromosome within the *entABCDEF* gene clusters, whereas the *fepABCDG* gene cluster encodes the proteins involved in enterobactin transport, with fepA specifically encoding the uptake receptor [16].

The gene cluster *ybt* encoding yersiniabactin was initially identified on the high-pathogenicity island of *Yersinia* [157, 159]. fyuA (also named psn) encodes the outer membrane receptor for the siderophore yersiniabactin [160, 161]. The *fyuA* gene encodes the yersiniabactin receptor and is involved in the regulation and transport of yersiniabactin [162]. The proteins needed for yersiniabactin synthesis are encoded by *irp* genes, while the transporters needed for its secretion are predicted to be encoded by the *ybt* and *fyu* genes [16]. Yersiniabactin synthesis involves: *ybtD*, *ybtE*, *ybtS*, *ybtT*, *ybtU*, and two high-molecular weight proteins 1 and 2 [163]. *YbtA* is a transcriptional regulator in the AraC family [164]. *YbtA* is a negative regulator of its own expression and a transcriptional activator of the *irp2*, *psn*, and *ybtP* promoters [164]. *ybtQ* is believed to encode the uptake receptor, although this has not

yet been fully characterized in *K. pneumoniae* [16]. Additionally, *ybtX* is an inner membrane (IM) protein with 12 predicted transmembrane domains, is tentatively assigned as part of the *ybt* efflux system [164]. Notably, Paauw et al. [162] reported that yersiniabactin reduces the production of reactive oxygen species in activated innate immune cells, which decreases the bacterial killing capacity of innate immune cells, and thereby contributing to its virulence.

Since the virulence gene *ybtQ* was annotated as a yersiniabactin ABC transporter ATP-binding/permease protein in Table 3 and Supplementary Table S3, we provide a brief overview of ABC transporters, particularly in the context of siderophore and drug resistance. ABC transporters are located in the accessory genomes of bacterial pathogens and significantly contribute to both virulence and antimicrobial resistance [165]. In the context of siderophore-mediated iron uptake, iron-bound siderophores are translocated through a channel into the periplasmic space between inner and outer membranes [157]. Within the periplasm, they are recognized by specific periplasmic binding proteins and subsequently transferred to ABC transporters located in the inner membrane [157]. These ABC transporters then facilitate further movement into cytoplasm where specialized enzymes release ferrous iron from chelation for cellular use [157]. Beyond iron acquisition, ABC transporters also contribute to antimicrobial resistance through efflux mechanisms. In Gram-negative bacteria, specific ABC transporters are able to form part of tripartite efflux pumps that mediate transport across the outer membrane [166]. The tripartite efflux pumps span both inner and outer membranes to export and efflux noxious molecules, including antibiotics [166]. In particular, MacB, in concert with the adaptor MacA and the outer membrane exit duct, TolC, can underpin efflux of a variety of drugs and export of virulence factors from multiple Gram-negative bacterial species [166]. Hence, the presence of these transporters within accessory genomes may contribute to increased virulence and reduced treatment efficacy in clinical infections.

Our study detected iron acquisition-associated virulence genes in MPB 1 and MPB 17. Particularly genes related to the yersiniabactin siderophore system, including *ybt* (AEPQSTUX), *fyuA/psn*, and *irp* (1 and 2) were detected in MPB 17. Collectively, these genes indicate the presence of a yersiniabactin-associated siderophore system in MPB 17, which may contribute to iron-acquisition and virulence. Additionally, the presence of *ybtQ* in MPB 17 may contribute to its increased virulence and increased drug resistance in addition to its iron-acquisition capability. On the other hand, enterobactin siderophore system *entD* was detected in MPB 1. This suggests that MPB 1 harbours an enterobactin-associated siderophore system which may enhance iron acquisition and contribute to its virulence potential.

Studies have reported the presence of yersiniabactin and enterobactin virulence factor from the NICU-associated settings. Olwagen et al. [167] reported that yersiniabactin was the dominant virulence factor found in 64.7% (218/337 isolates) of the *K. pneumoniae* from South African infants ≤ 90 days, collected via observational hospital surveillance and postmortem sampling. Additionally, Goodman et al. [168] reported that with *Klebsiella* spp. obtained either from blood or faecal samples of neonates < 19 days old from Tanzania, 61% (11/18 isolates) have the same set of virulence genes, which are all involved in iron-uptake, including a set of *ybt* genes, *irp* and *fyuA*. Additionally, El Fertas-Aissani et al. [1] reported that the prevalence of siderophore genes *entB*, and *irp1-irp2-ybtS-fyuA* in *K. pneumoniae* isolated from different clinical specimens were 100%, and 46.3%, respectively. Similarly, Koczura et al. [160] also reported that all *K. pneumoniae* that were isolated from clinical specimens of different patients produced enterobactin (100%). In our previous study (submitted for peer-review), the prevalence of the virulence gene *ybtS* that was present in the meconium/early stool of preterm infants was 82.14%. In line with this, the presence of yersiniabactin-associated siderophores in NICU-associated *K. pneumoniae* isolates indicates the potential ability of these strains to utilise yersiniabactin-mediated iron acquisition, which may support bacterial survival under iron-restricted conditions encountered during infection.

4.5.4. Type VI Secretion System (T6SS)

Another important virulence factor of *K. pneumoniae* is the T6SS [169, 170]. It is a mechanism for protein transport across the cell envelope of Gram-negative bacteria [171]. Components that have been partially characterised include an intracellular multiplication protein F (IcmF) homologue, the ATPase ClpV, a regulatory forkhead-associated (FHA) domain protein and the secreted valine–glycine repeat protein G (VgrG) and hemolysin-coregulated protein (Hcp) [171]. The IcmF family protein—TssM is a conserved T6SS inner membrane protein [172]; while both VgrG and Hcp represents the hallmark of T6SS and are needed for the function of the T6SS machine [173]. The T6SS comprises multiple components that form a phage-like structure anchored to the bacterial membrane and typically consists of 13 core components designated TssA-M [174].

The core structure of T6SS consists of a membrane complex, baseplate, and tail tube/sheath complex [174]. The biological functions and regulatory mechanisms of the T6SS in *K. pneumoniae* have been reported by Liu et al. [175]. TssH (ClpV) is a AAA+-type ATPase [175, 176]. The baseplate, made up of TssE, TssF, TssG, and TssK, bridges the transmembrane complex to the tail [175]. It plays an important role in initiating the polymerization of the tube/sheath complex and anchoring it to the membrane complex, therefore initiating sheath

contraction while keeping the entire assembled system solid during sheath contraction [175]. The TssK interacts with TssG, while TssF helps maintain the stability of this interaction [175]. The membrane complex is composed of TssJ, TssL, and TssM, which anchor the system to the bacterial inner membrane and facilitate assembly of the rest of the structure [174]. Similar to the baseplates of contractile phages, T6SS baseplate comprises a central hub surrounded by at least six proteins, that are essential for assembly of the TssD tube and sheath and triggering sheath contraction [174]. The contractile sheath of T6SS is composed of TssB and TssC, which encase the inner TssD tube during assembly [174].

The T6SS contributes to bacterial competition, cell invasion and *in vivo* colonisation [169, 170]. It mediates contact-dependent injection of enzymes and toxins into competing bacteria or host cells, facilitating the colonization of a host organism [177]. Additionally, Barbosa et al. [169] reported that genes coding for iron uptake systems are encoded in adjacencies of T6SS, which suggest that *K. pneumoniae* T6SS might play a role in iron uptake. Notably, Merciecca et al. [178] reported that *K. pneumoniae* species had a high prevalence of T6SS clusters, with 98% of the strains harbouring at least one T6SS. Similarly, Liao et al [179] and Mohamed et al. [180] reported the frequency of T6SS genes among clinical *K. pneumoniae* isolates of 72.2% and 78.57%, respectively. Both studies reported that T6SS-positive strains (containing *icmF*, *vgrG*, and *hcp*) demonstrated higher biofilm-forming activity with high drug resistance [179, 180]. According to Liao et al. [179], T6SS-positive isolates had a higher frequency of virulence genes (*rmpA, fimH, entB, kfu, ybtS*), and the presence of these genes suggest higher virulence potential, and that T6SS may be located in the mobile genetic elements.

Based on our findings, *icmF*/*tssM*, *tssF*, *tssG*, *sciN*/*tssJ* in particular, were present only in MPB 17. Since T6SS has been reported to contribute to bacterial competition, cell invasion and *in vivo* colonisation, as well as been reported to be able to decrease antibiotic resistance and have biofilm forming potential, the presence of T6SS-associated virulence genes in MPB 17 may suggest enhanced virulence potential. The presence of T6SS-associated genes was also reported by Dentice Maidana et al. [181] who identified at least one cluster for T6SS in each clinical *K. pneumoniae* strain examined, with one of the strains composed of the genes *tssA, tssE, tssF, tssG, tssI* (*vgrG), tssJ, tssK, tssL, and tssM*. Nonetheless, the contribution of T6SS-associated genes to *K. pneumoniae* virulence cannot be inferred solely from their presence and requires further functional validation.

Overall, multiple virulence genes were detected in *K. pneumoniae* MPB 1 and MPB 17 recovered from stool samples of preterm infants without clinical evidence of infection. This suggests that the presence of virulence genes alone may not be sufficient to predict

pathogenic potential. In line with this, a study by Chen et al. [182] also reported no significant difference in siderophore production between healthy and NEC- and sepsis-associated *Klebsiella* isolates. This may be because the expression of virulence genes is regulated and influenced by bacterial and host-specific factors, including environmental cues, host immunity, and the availability of nutrients [183]. Therefore, MPB 1 and MPB 17 may have remained as asymptomatic intestinal colonizers while retaining the ability to cause opportunistic infections when host or environmental conditions become favourable.

To sum up, MPB 17 exhibited a broader virulence gene repertoire than MPB 1, characterized by the presence of yersiniabactin-associated iron acquisition genes exclusively in this isolate, additional capsule/LPS-associated genes (KP1_RS17340, KP1_RS17220), and a higher number of T6SS-associated genes (with *icmF/tssM*, *tssF*, *tssG*, *sciN/tssJ* detected exclusively in MPB 17). Both MPB 1 and MPB 17 shared key virulence-associated determinants, including type 1 and type 3 fimbrial adhesin genes and enterobactin-related genes (with *entD* detected exclusively in MPB 1), suggesting shared mechanisms involved in adhesion and iron acquisition. The wider range of virulence-associated genes identified in MPB 17 may suggest a greater potential for virulence-associated functions, particularly enhanced iron-acquisition through the exclusive presence of yersiniabactin-associated genes, alongside other traits related to colonization, persistence, survival, and pathogenicity [16]. Nonetheless, further functional studies are needed to determine whether these genomic differences correspond to enhanced virulence phenotypes.

4.6. Clinical implications and significance

Whole genome sequencing performed in this study provided a valuable approach for characterizing the genetic determinants associated with antibiotic resistance and virulence, enabling a more comprehensive understanding of *K. pneumoniae* populations circulating in a NICU setting in Malaysia. By characterizing these genomic findings, it may help inform infection prevention and control measures, antimicrobial stewardship, and genomic surveillance programmes aimed at limiting the dissemination of MDR *K. pneumoniae* within the neonatal settings in Malaysia. Furthermore, the genomic data generated from these NICU-associated *K. pneumoniae* isolates in Malaysia could contribute additional insights into the genomic characteristics of NICU-associated *K. pneumoniae* populations in Southeast Asian NICUs and may support future surveillance efforts, comparative studies, and the development of locally relevant infection control strategies.

In this study, the whole-genome analysis reported differences between *K. pneumoniae* MPB 1 and MPB 17, highlighting genomic diversity within the same NICU. Although *K. pneumoniae* MPB 1 and MPB 17 were obtained from intestinal colonization

samples of preterm infants rather than clinical infections, multiple antibiotic resistance genes and virulence-associated genes were detected. However, the detection of these determinants alone does not confirm active infection, as intestinal colonization by *K. pneumoniae* may occur in the absence of clinical disease [27]. Nevertheless, such colonization may serve as a reservoir for subsequent infection, especially in neonates [85].

Worryingly, the coexistence of antibiotic resistance genes and virulence genes in colonizing *K. pneumoniae* strains may pose a greater clinical concern, as these strains may limit treatment options and contribute difficult-to-treat infections should colonization progress to infection in preterm infants [7, 26]. Colonizing *K. pneumoniae* population may harbor, acquire and accumulate antibiotic resistance genes and virulence determinants through horizontal gene transfer, facilitating persistence under selective pressures and potentially increasing the risk of subsequent opportunistic infections [17, 26, 27, 130, 184]. These processes may contribute to the persistence of MDR *K. pneumoniae* within the host and may represent a concern particularly among vulnerable preterm infants. Furthermore, it has been reported that the most severe forms of neonatal sepsis with an non-favorable outcome were due to virulent strains of *K. pneumoniae* [156].

In this context, continued surveillance in NICU settings is essential due to the vulnerability of preterm infants to healthcare-associated infections caused by opportunistic pathogens such as *K. pneumoniae* [7, 21-25]. The emergence and dissemination of antibiotic-resistant *K. pneumoniae* carrying virulence-associated traits in the NICU settings represent a significant clinical concern, as these organisms may limit therapeutic options, and pose challenges for infection prevention and control in the vulnerable neonatal population [185, 186]. Therefore, integrating genomic approaches into surveillance efforts can enhance the understanding of *K. pneumoniae* populations by providing insights into the emergence and spread of strains carrying antibiotic resistance and virulence-associated traits in NICU settings [7].

4.7. Classical and hypervirulent K. pneumoniae: Convergence of resistance and virulence

K. pneumoniae are generally categorized into two major pathotypes: classical *K. pneumoniae* and hypervirulent *K. pneumoniae* [158]. The classical *K. pneumoniae* causes infections that occur more commonly in the health care setting, involving immunocompromised individuals or individuals with comorbidities [158]. Classical *K. pneumoniae* has become increasingly recognized due to its propensity to acquire antimicrobial resistance [156]. Among the most concerning are genes encoding ESBLs, which render bacteria resistant to cephalosporins and monobactams, as well as genes encoding carbapenemases, which renders bacteria resistant to almost all available β-lactams, including

the carbapenems [16, 158]. Notably, the carriage and expression of drug resistance make *K. pneumoniae* harder to treat without enhancing its virulence [16]. On the other hand, hypervirulent *K. pneumoniae* causes infections that occur mainly in the community setting and is described as a virulent pathogen that can infect healthy individuals of any age [158]. The high pathogenicity of hypervirulent *K. pneumoniae* is reflected in its invasiveness and its confrontation with the host immune system, which is mediated by various virulence factors carried by the strain [130].

Historically, hypervirulent *K. pneumoniae* have been largely susceptible to antibiotics [187, 188]. However, in *K. pneumoniae*, antimicrobial resistance and virulence determinants are increasingly interconnected [112, 185-187]. The convergence has led to the emergence of strains that are both MDR and hypervirulent [186]. Wyres et al. [7] reported that geographically, the focal point for convergence appears to be Asia, where both MDR and hypervirulence are common. This convergence has been observed in two ways: (i) classical *K. pneumoniae* isolates acquiring virulence genes or entire virulence plasmids, and (ii) hypervirulent *K. pneumoniae* isolates acquiring chromosomal or plasmid-encoded antibiotic resistance genes [187]. Together, these traits significantly enhance bacterial fitness and pose a huge challenge in clinical anti-infective treatment.

In the context of NICU settings, Shrestha et al. [185] reported an overlap between acquired antimicrobial resistance and accessory virulence genes in *K. pneumoniae* obtained from a NICU in Nepal. A study by Olwagen et al. [167] which included *K. pneumoniae* isolates obtained from South African infants ≥ 90 days (collected through observational hospital surveillance and postmortem sampling) also detected that 23.2% of the *K. pneumoniae* isolates harboured genes encoding *ybt* virulence and carbapenems resistance while 54.9% harboured genes encoding both *ybt* and ESBL production. Overall, this reflects the adaptability and complexity of *K. pneumoniae*.

4.8. Limitations and future directions

Several limitations should be considered when interpreting findings of this study. First, the number of *K. pneumoniae* isolates analysed were limited, which could restrict the generalisability of the findings to broader colonizing populations. Although WGS provides comprehensive detection of antibiotic resistance genes and virulence genes, the detection of these determinants represents the genetic potential of the isolate and does not necessarily confirm their expression, associated phenotypic trait, or contribution to antibiotic resistance and pathogenicity. Therefore, further phenotypic correlation and, where appropriate, functional studies would be required to establish the biological and clinical significance of these genomic findings [1, 16, 51, 66, 67, 189-192].

In this study, although ResFinder allows for a reliable detection of acquired antibiotic resistance genes, its predictions are dependent on the availability and completeness of the reference database, sequence quality, and read depth [67]. Furthermore, ResFinder primarily detects known acquired resistance genes and does not account for all mechanisms contributing to antimicrobial resistance, such as chromosomal mutations [67]. Therefore, resistance genes that are novel and/or absent from the database and/or mediated by mutations in chromosomal genes may not be identified. Similarly, the identification of virulence genes using the VFDB is dependent on the availability of the characterized virulence factor sequences within the database [66]. Consequently, novel or uncharacterized virulence determinants may not be detected, and the predicted virulence profile should be interpreted as representing the known virulence repertoire captured by VFDB rather than the complete virulence potential of the isolate.

The use of Illumina short-read sequencing in our study may also limit its ability to resolve genomic structure, which has major implications for tracking the spread of mobile genetic elements, including those which carry antimicrobial resistance determinants [117]. Furthermore, its use may result in fragmented genome assemblies, limiting the accurate resolution of plasmids and other mobile genetic elements involved in the dissemination of antimicrobial resistance determinants through horizontal gene transfer [118]. Therefore, long-read sequencing-based genome assembly may be required to determine whether these antibiotic resistance genes (including virulence genes) are associated with mobile genetic elements and infer their potential for horizontal transfer [116-119].

As WGS only identifies the presence of antibiotic resistance genes and virulence genes, future studies should integrate transcriptomic analyses such as RNA sequencing (RNA-seq), which simultaneously map transcribed regions and gene expression [193]. Transcriptomics reflects cellular functional activity by quantifying gene expression and revealing active resistance pathways, transcriptional response to antibiotic exposure, and stress-response mechanisms that contribute to bacterial survival without permanent genetic change [194]. Additionally, future studies should also incorporate functional assays to characterize the functional significance of antibiotic resistance genes and virulence genes identified by WGS. These may include virulence-associated assays, such as, capsule detection and hypermucoviscosity testing, hemagglutination assays, siderophore production assays, and biofilm formation assays to further characterize the contribution of these genes to the virulence in *K. pneumoniae* [1]. Lastly, future studies could employ comparative genomic and transcriptomic approaches to compare colonizing infant stool isolates with invasive clinical *K. pneumoniae* isolates, to identify genetic and expression profiles associated with the transition from gut colonization to infection. Together, the integration of

these approaches would improve understanding of the pathogenic potential of *K. pneumoniae* isolates and clarify the contribution of detected genes to antibiotic resistance and pathogenicity.

5. Conclusion

The acquisition of MDR along with virulence factors can worsen the course and prognosis of the diseases [156], complicates effective treatment, and may contribute to the dissemination of *K. pneumoniae* that are MDR as well as virulent. Genomic analysis revealed that *K. pneumoniae* colonizing preterm infants in Malaysian NICUs can harbor diverse antibiotic resistance gene profiles and virulence genes. While both MPB 1 and MPB 17 were MDR, MPB 17 exhibited a broader resistance gene repertoire and additional virulence genes, including yersiniabactin-associated siderophores which were exclusively detected in this isolate and a higher number of T6SS-associated genes. The discordance between WGS-predicted and observed phenotypic resistance highlights current limitations in genotype-based resistance prediction and underscores the need for continued integration of phenotypic and genomic surveillance. Future studies should integrate transcriptomic, functional, and comparative genomic approaches to further characterize the expression, biological significance, and pathogenic potential of antibiotic resistance genes and virulence genes identified by WGS among NICU-associated *K. pneumoniae* isolates. These findings support enhanced genomic surveillance and antimicrobial stewardship in neonatal intensive care settings.

Supplementary Materials: The materials are available online on the PMMB website along with the published article.

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References

1. El Fertas-Aissani R, Messai Y, Alouache S, *et al*. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Pathologie Biologie 2013; 61(5): 209-216. doi: https://doi.org/10.1016/j.patbio.2012.10.004.
2. Podschun R and Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews 1998; 11(4): 589-603. doi: https://doi.org/10.1128/cmr.11.4.589.

3. Thye AY-K, Kumari Y, Chan K-G, *et al*. Early colonization by *Enterobacteriaceae* in the developing gut microbiota of preterm infants: A culture-based insight. Progress In Microbes & Molecular Biology 2026; 9(1): a0000479. doi: https://doi.org/10.36877/pmmb.a0000479.
4. Rizki LP, Murni IK, Aman AT, *et al*. Environmental Metagenomic Analysis of" ESKAPE" Pathogens in the Pediatric Intensive Care Unit of General Hospital Yogyakarta Indonesia. Progress In Microbes & Molecular Biology 2024; 7(1): a0000398. doi: 10.36877/pmmb.a0000398.
5. Lordelo R, Branco R, Gama F, *et al*. Assessment of antimicrobial resistance, biofilm formation, and surface modification potential in hospital strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Heliyon 2024; 10(9). doi: 10.1016/j.heliyon.2024.e30464
6. Bellifa S, Hassaine H, Balestrino D, *et al*. Evaluation of biofilm formation of *Klebsiella pneumoniae* isolated from medical devices at the University Hospital of Tlemcen, Algeria. Afr J Microbiol Res 2013; 7(49): 5558-64. doi: 10.5897/AJMR12.2331.
7. Wyres KL, Lam MM, and Holt KE. Population genomics of *Klebsiella pneumoniae.* Nature Reviews Microbiology 2020; 18(6): 344-359. doi: https://doi.org/10.1038/s41579-019-0315-1.
8. Mohd Asri NA, Ahmad S, Mohamud R, *et al*. Global prevalence of nosocomial multidrug-resistant *Klebsiella pneumoniae*: a systematic review and meta-analysis. Antibiotics 2021; 10(12): 1508. doi: https://doi.org/10.3390/antibiotics10121508.
9. Juan C-H, Chuang C, Chen C-H, *et al*. Clinical characteristics, antimicrobial resistance and capsular types of community-acquired, healthcare-associated, and nosocomial *Klebsiella pneumoniae* bacteremia. Antimicrobial Resistance & Infection Control 2019; 8(1): 1. doi: https://doi.org/10.1186/s13756-018-0426-x.
10. Moradigaravand D, Martin V, Peacock SJ, *et al*. Evolution and epidemiology of multidrug-resistant *Klebsiella pneumoniae* in the United Kingdom and Ireland. MBio 2017; 8(1): 10.1128/mbio. 01976-16. doi: https://doi.org/10.1128/mbio.01976-16.
11. Monteiro AdSS, Silva MdO, Galvão VS, *et al*. High proportions of multidrug-resistant *Klebsiella pneumoniae* isolates in community-acquired infections, Brazil. Scientific Reports 2025; 15(1): 9698. doi: https://doi.org/10.1038/s41598-025-92549-w.
12. Zhang J, Zhou K, Zheng B, *et al*. High prevalence of ESBL-producing *Klebsiella pneumoniae* causing community-onset infections in China. Frontiers in microbiology 2016; 7: 1830. doi: https://doi.org/10.3389/fmicb.2016.01830.
13. Le T, Wang L, Zeng C, *et al*. Clinical and microbiological characteristics of nosocomial, healthcare-associated, and community-acquired *Klebsiella pneumoniae* infections in Guangzhou, China. Antimicrobial Resistance & Infection Control 2021; 10(1): 41. doi: https://doi.org/10.1186/s13756-021-00910-1.
14. Wang Y, Luo C, Du P, *et al*. Genomic epidemiology of an outbreak of *Klebsiella pneumoniae* ST471 producing extended-spectrum β-Lactamases in a neonatal intensive care unit. Infection and Drug Resistance 2020: 1081-1090. doi: https://doi.org/10.2147/IDR.S236212.
15. Sui W, Zhou H, Du P, *et al*. Whole genome sequence revealed the fine transmission map of carbapenem-resistant *Klebsiella pneumonia* isolates within a nosocomial outbreak. Antimicrobial Resistance & Infection Control 2018; 7(1): 70. doi: https://doi.org/10.1186/s13756-018-0363-8.

16. Paczosa MK and Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiology and molecular biology reviews 2016; 80(3): 629-661. doi: https://doi.org/10.1128/mmbr.00078-15.
17. Gorrie CL, Mirčeta M, Wick RR, *et al*. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. Clinical infectious diseases 2017; 65(2): 208-215. doi: 10.1093/cid/cix270.
18. da Silveira Ferreira IC, de Paula Menezes R, Lopes MSM, *et al*. Multidrug-resistant *Klebsiella pneumoniae* in critically ill neonates: evidence from a Brazilian cohort. Australian Critical Care 2025; 38(6): 101444. doi: https://doi.org/10.1016/j.aucc.2025.101444.
19. Zou H, Jia X, He X, *et al*. Emerging threat of multidrug resistant pathogens from neonatal sepsis. Frontiers in Cellular and Infection Microbiology 2021; 11: 694093. doi: https://doi.org/10.3389/fcimb.2021.694093.
20. Yap PSX, Ahmad Kamar A, Chong CW, *et al*. Intestinal carriage of multidrug-resistant gram-negative bacteria in preterm-infants during hospitalization in neonatal intensive care unit (NICU). Pathogens and global health 2016; 110(6): 238-246. doi: 10.1080/20477724.2016.1229884.
21. Collado MC, Cernada M, Neu J, *et al*. Factors influencing gastrointestinal tract and microbiota immune interaction in preterm infants. Pediatric research 2015; 77(6): 726-731. doi: https://doi.org/10.1038/pr.2015.54.
22. Sharma AA, Jen R, Butler A, *et al*. The developing human preterm neonatal immune system: a case for more research in this area. Clinical immunology 2012; 145(1): 61-68. doi: 10.1016/j.clim.2012.08.006.
23. Collins A, Weitkamp J-H, and Wynn JL. Why are preterm newborns at increased risk of infection? Archives of Disease in childhood-fetal and neonatal Edition 2018; 103(4): F391-F394. doi: https://doi.org/10.1136/archdischild-2017-313595.
24. Cuna A, Morowitz MJ, Ahmed I, *et al*. Dynamics of the preterm gut microbiome in health and disease. American Journal of Physiology-Gastrointestinal and Liver Physiology 2021; 320(4): G411-G419. doi: https://doi.org/10.1152/ajpgi.00399.2020.
25. Healy DB, Ryan CA, Ross RP, *et al*. Clinical implications of preterm infant gut microbiome development. Nature microbiology 2022; 7(1): 22-33. doi: https://doi.org/10.1038/s41564-021-01025-4.
26. Martin RM and Bachman MA. Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. Frontiers in Cellular and Infection Microbiology 2018; 8: 4. doi: https://doi.org/10.3389/fcimb.2018.00004.
27. Martin RM, Cao J, Brisse S, *et al*. Molecular epidemiology of colonizing and infecting isolates of *Klebsiella pneumoniae.* MSphere 2016; 1(5): 10.1128/msphere. 00261-16. doi: https://doi.org/10.1128/msphere.00261-16.
28. Milic M, Siljic M, Cirkovic V, *et al*. Colonization with multidrug-resistant bacteria in the first week of life among hospitalized preterm neonates in Serbia: Risk factors and outcomes. Microorganisms 2021; 9(12): 2613. doi: https://doi.org/10.3390/microorganisms9122613.
29. Lee YQ, Kamar AA, Velayuthan RD, *et al*. Clonal relatedness in the acquisition of intestinal carriage and transmission of multidrug resistant (MDR) *Klebsiella pneumoniae* and *Escherichia coli* and its

risk factors among preterm infants admitted to the neonatal intensive care unit (NICU). Pediatrics & Neonatology 2021; 62(2): 129-137.

30. El-Shanqetti EM, Albasri HM, Waznah MS, *et al*. Colonization of the gut by *Klebsiella pneumoniae* and its multidrug-resistant strains is well marked in preterm neonates. Frontiers in Cellular and Infection Microbiology 2026; 16: 1762624. doi: https://doi.org/10.3389/fcimb.2026.1762624.
31. Tan LT-H, Law JW-F, Chua K-O, *et al*. Phylogenomics, antimicrobial resistance, and mobile genetic elements in clinical *Clostridioides difficile* isolates from Hong Kong, China. Progress In Microbes & Molecular Biology 2026; 9(1): a0000651. doi: 10.36877/pmmb.a0000651.
32. Goh SY, Chua K-O, Khan SA, *et al*. Genomic and Resistome Analyses of *Elizabethkingia anophelis* Strain B2D isolated from Dental Plaque of Patient. Progress in Microbes and Molecular Biology 2024; 7(1): a0000440. doi: 10.36877/pmmb.a0000440.
33. Loo K-Y, Tan LT-H, Law JW-F, *et al*. Complete whole genome sequence of *Vibrio parahaemolyticus* RP0132 strain isolated from shrimp in Malaysia. Progress In Microbes & Molecular Biology 2023; 6(1): a0000388. doi: 10.36877/pmmb.a0000388.
34. Law JWF, Tan LT-H, Letchumanan V, *et al*. *Streptomyces griseiviridis* sp. nov., a novel "modern actinobacteria" isolated from Malaysia mangrove soil. Progress In Microbes & Molecular Biology 2023; 6(1): a0000270 doi: 10.36877/pmmb.a0000270.
35. Law JW-F, Letchumanan V, Hong K-W, *et al*. *Streptomyces learnhanii* sp. nov., unveiling a mangrove-derived novel "Modern Actinobacteria" in Malaysia. Progress In Microbes & Molecular Biology 2023; 6(1): a0000342. doi: 10.36877/pmmb.a0000342.
36. Loo K-Y, Tan LT-H, Chua K-O, *et al*. Complete whole-genome sequence of *Streptomyces* sp. MUM 178J, a potential anti-Vibrio agent. Progress In Microbes & Molecular Biology 2024; 7(1): a0000399. doi: 10.36877/pmmb.a0000399.
37. Loo K-Y, Tan LT-H, Law JW-F, *et al*. Detection of multidrug resistant *Vibrio parahaemolyticus* and anti-Vibrio *Streptomyces* sp. MUM 178J. Progress In Microbes & Molecular Biology 2023; 6(1): a0000347. doi: 10.36877/pmmb.a0000347.
38. Pusparajah P, Law JW-F, Chan K-G, *et al*. Whole-genome sequence of *Streptomyces pluripotens* strain MUM 16J, a potential resource of glycopeptide antibiotic and biocontrol agent against biofilm-forming bacteria. Progress In Microbes & Molecular Biology 2023; 6(1): a0000330. doi: 10.36877/pmmb.a0000330.
39. Tan LT-H, Law JW-F, Letchumanan V, *et al*. Pangenomics and Comparative Biosynthetic Diversity of Malaysian Mangrove-Derived *Streptomyces albidoflavus* MUM256 among *S. albidoflavus*-Assigned Genomes. Progress In Microbes & Molecular Biology 2026; 9(1): a0000657. doi: 10.36877/pmmb.a0000657.
40. Thye AY-K, Letchumanan V, Tan LT-H, *et al*. Malaysia's breakthrough in modern actinobacteria (MOD-ACTINO) drug discovery research. Progress in Microbes and Molecular Biology 2022; 5(1): a0000275. doi: 10.36877/pmmb.a0000275.
41. Law JW-F, Letchumanan V, Tan LT-H, *et al*. The rising of "modern actinobacteria" era. Progress In Microbes & Molecular Biology 2020; 3(1): a0000064. doi: https://doi.org/10.3687/pmmb.a0000064.
42. Procópio REdL, Silva IRd, Martins MK, *et al*. Antibiotics produced by *Streptomyces*. Brazilian Journal of Infectious Diseases 2012; 16(5): 466-471. doi: https://doi.org/10.1016/j.bjid.2012.08.014.

43. Watve MG, Tickoo R, Jog MM, *et al*. How many antibiotics are produced by the genus *Streptomyces*? Archives of microbiology 2001; 176(5): 386-390. doi: https://doi.org/10.1007/s002030100345.
44. Quinn GA, Banat AM, Abdelhameed AM, *et al*. *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. Journal of Medical Microbiology 2020; 69(8): 1040-1048. doi: https://doi.org/10.1099/jmm.0.001232.
45. Beroigui O and Errachidi F. *Streptomyces* at the heart of several sectors to support practical and sustainable applications: a review. Progress In Microbes & Molecular Biology 2023; 6(1): a0000345. doi: 10.36877/pmmb.a0000345.
46. Loo KY, Tan LTH, Law JWF, *et al*. *Vibrio parahaemolyticus*: Exploring its Incidence in Malaysia and the Potential of *Streptomyces* sp. as an Anti-Vibrio Agent. Progress in Microbes and Molecular Biology 2023; 6(1): a0000334. doi: 10.36877/pmmb.a0000334.
47. Goh JXH, Tan LT-H, Law JW-F, *et al*. *Streptomyces* sp. MUM 195J: A promising probiotic for controlling *Vibrio parahaemolyticus* infection in aquaculture. Progress In Microbes & Molecular Biology 2024; 7(1): a0000443. doi: 10.36877/pmmb.a0000443.
48. Kwong JC, McCallum N, Sintchenko V, *et al*. Whole genome sequencing in clinical and public health microbiology. Pathology 2015; 47(3): 199-210. doi: https://doi.org/10.1097/PAT.0000000000000235.
49. Yang J, Zhang K, Ding C, *et al*. Exploring multidrug-resistant *Klebsiella pneumoniae* antimicrobial resistance mechanisms through whole genome sequencing analysis. BMC microbiology 2023; 23(1): 245. doi: https://doi.org/10.1186/s12866-023-02974-y.
50. Marchionatti E, Kittl S, Sendi P, *et al*. Whole genome-based antimicrobial resistance, virulence, and phylogenetic characteristics of *Trueperella pyogenes* clinical isolates from humans and animals. Veterinary microbiology 2024; 294: 110102. doi: 10.1016/j.vetmic.2024.110102.
51. Tagini F and Greub G. Bacterial genome sequencing in clinical microbiology: a pathogen-oriented review. European journal of clinical microbiology & infectious diseases 2017; 36(11): 2007-2020. doi: https://doi.org/10.1007/s10096-017-3024-6.
52. Ferreira I, Beisken S, Lueftinger L, *et al*. Species identification and antibiotic resistance prediction by analysis of whole-genome sequence data by use of ARESdb: an analysis of isolates from the Unyvero lower respiratory tract infection trial. Journal of clinical microbiology 2020; 58(7): 10.1128/jcm. 00273-20. doi: https://doi.org/10.1128/jcm.00273-20.
53. Rasmussen LH, Dargis R, Højholt K, *et al*. Whole genome sequencing as a tool for phylogenetic analysis of clinical strains of Mitis group streptococci. European journal of clinical microbiology & infectious diseases 2016; 35(10): 1615-1625. doi: https://doi.org/10.1007/s10096-016-2700-2.
54. Arhoune B, El Fakir S, Himri S, *et al*. Intense intestinal carriage and subsequent acquisition of multidrug-resistant enterobacteria in neonatal intensive care unit in Morocco. PLoS One 2021; 16(6): e0251810.
55. Vasilyev I, Nikolaeva I, Siniagina M, *et al*. Multidrug-Resistant Hypervirulent *Klebsiella pneumoniae* Found Persisting Silently in Infant Gut Microbiota. International Journal of Microbiology 2020; 2020(1): 4054393.
56. Luo K, Tang J, Qu Y, *et al*. Nosocomial infection by *Klebsiella pneumoniae* among neonates: a molecular epidemiological study. Journal of Hospital Infection 2021; 108: 174-180.
57. Clinical and Laboratory Standards Institute *M100 Performace Standards for Antimicrobial Susceptibility Testing*. 2023, Clinical and Laboratory Standards Institute.

58. Andrews S *FastQC: A quality control tool for high throughput sequence data*. 2010, Babraham Bioinformatics, Babraham Institute: Cambridge, United Kingdom.
59. Bolger AM, Lohse M, and Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014; 30(15): 2114-2120. doi: https://doi.org/10.1093/bioinformatics/btu170.
60. Prjibelski A, Antipov D, Meleshko D, *et al*. Using SPAdes de novo assembler. Current Protocols in Bioinformatics 2020; 70(1): e102. doi: https://doi.org/10.1002/cpbi.102.
61. Gurevich A, Saveliev V, Vyahhi N, *et al*. QUAST: quality assessment tool for genome assemblies. Bioinformatics 2013; 29(8): 1072-1075. doi: https://doi.org/10.1093/bioinformatics/btt086.
62. Simão FA, Waterhouse RM, Ioannidis P, *et al*. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 2015; 31(19): 3210-3212. doi: https://doi.org/10.1093/bioinformatics/btv351.
63. Yoon S-H, Ha S-m, Lim J, *et al*. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 2017; 110(10): 1281-1286. doi: https://doi.org/10.1007/s10482-017-0844-4.
64. Aziz RK, Bartels D, Best AA, *et al*. The RAST Server: Rapid annotations using subsystems technology. BMC Genomics 2008; 9(1): Article#75. doi: https://doi.org/10.1186/1471-2164-9-75.
65. Seemann T. Prokka: Rapid prokaryotic genome annotation. Bioinformatics 2014; 30(14): 2068-2069. doi: https://doi.org/10.1093/bioinformatics/btu153.
66. Chen L, Yang J, Yu J, *et al*. VFDB: a reference database for bacterial virulence factors. Nucleic acids research 2005; 33(suppl_1): D325-D328. doi: https://doi.org/10.1093/nar/gki008.
67. Bortolaia V, Kaas RS, Ruppe E, *et al*. ResFinder 4.0 for predictions of phenotypes from genotypes. Journal of Antimicrobial Chemotherapy 2020; 75(12): 3491-3500. doi: https://doi.org/10.1093/jac/dkaa345.
68. Alcock BP, Raphenya AR, Lau TT, *et al*. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic acids research 2020; 48(D1): D517-D525. doi: https://doi.org/10.1093/nar/gkz935.
69. Carattoli A and Hasman H. *PlasmidFinder and in silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS)*, in *Horizontal gene transfer: methods and protocols*. 2019, Springer. p. 285-294. doi: https://doi.org/10.1007/978-1-4939-9877-7_20.
70. Feron R and Waterhouse RM. Assessing species coverage and assembly quality of rapidly accumulating sequenced genomes. GigaScience 2022; 11: giac006. doi: https://doi.org/10.1093/gigascience/giac006.
71. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Applied and environmental microbiology 1983; 46(1): 165-170. doi: https://doi.org/10.1128/aem.46.1.165-170.1983.
72. Woh PY, Yeung MPS, and Goggins III WB. Multiple antibiotic resistance index (MARI) of human-isolated *Salmonella* species: a practical bacterial antibiotic surveillance tool. Journal of Antimicrobial Chemotherapy 2023; 78(5): 1295-1299. doi: https://doi.org/10.1093/jac/dkad092.
73. Kim HB, Wang M, Park CH, *et al*. oqxAB encoding a multidrug efflux pump in human clinical isolates of *Enterobacteriaceae*. Antimicrobial agents and chemotherapy 2009; 53(8): 3582-3584. doi: https://doi.org/10.1128/aac.01574-08.

74. Broberg CA, Palacios M, and Miller VL. *Klebsiella*: a long way to go towards understanding this enigmatic jet-setter. F1000prime reports 2014; 6: 64. doi: 10.12703/P6-64.
75. Kaderabkova N, Bharathwaj M, Furniss RCD, *et al*. The biogenesis of β-lactamase enzymes. Microbiology 2022; 168(8): 001217. doi: 10.1099/mic.0.001217.
76. Hall BG and Barlow M. Revised Ambler classification of β-lactamases. Journal of Antimicrobial Chemotherapy 2005; 55(6): 1050-1051. doi: doi:10.1093/jac/dki130.
77. Schneider KD, Bethel CR, Distler AM, *et al*. Mutation of the active site carboxy-lysine (K70) of OXA-1 β-lactamase results in a deacylation-deficient enzyme. Biochemistry 2009; 48(26): 6136-6145. doi: 10.1021/bi900448u.
78. Poirel L, Naas T, and Nordmann P. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrobial agents and chemotherapy 2010; 54(1): 24-38. doi: https://doi.org/10.1128/aac.01512-08.
79. Liu L, Feng Y, Wei L, *et al*. KPC-2-producing carbapenem-resistant *Klebsiella pneumoniae* of the uncommon ST29 type carrying OXA-926, a novel narrow-spectrum OXA β-lactamase. Frontiers in microbiology 2021; 12: 701513. doi: https://doi.org/10.3389/fmicb.2021.701513.
80. Meng L, Liu Z, Liu C, *et al*. The distribution characteristics of global *blaOXA*-carrying *Klebsiella pneumoniae*. BMC Infectious Diseases 2023; 23(1): 182. doi: https://doi.org/10.1186/s12879-023-08156-5.
81. Wollheim C, Guerra IMF, Conte VD, *et al*. Nosocomial and community infections due to class A extended-spectrum β-lactamase (ESBLA)-producing *Escherichia coli* and *Klebsiella* spp. in southern Brazil. The Brazilian Journal of Infectious Diseases 2011; 15(2): 138-143.
82. Yee R, Dien Bard J, and Simner PJ. The genotype-to-phenotype dilemma: how should laboratories approach discordant susceptibility results? Journal of clinical microbiology 2021; 59(6): 10.1128/jcm. 00138-20. doi: https://doi.org/10.1128/jcm.00138-20.
83. Msanga DR, Minja CA, Härtel C, *et al*. Epidemiology, resistance and virulence of neonatal *Klebsiella pneumoniae* isolates from a tertiary hospital in Tanzania. The Journal of Infectious Diseases 2026: jiag253. doi: https://doi.org/10.1093/infdis/jiag253.
84. Geleta D, Abebe G, Tilahun T, *et al*. Molecular and clinical insights into extended-spectrum β-lactamase genes of *Klebsiella pneumoniae* isolated from neonatal sepsis in Ethiopia. BMC Infectious Diseases 2024; 24(1): 1442. doi: https://doi.org/10.1186/s12879-024-10344-w.
85. Chen C-m, Wang M, Li X-p, *et al*. Homology analysis between clinically isolated extraintestinal and enteral *Klebsiella pneumoniae* among neonates. BMC microbiology 2021; 21(1): 25. doi: https://doi.org/10.1186/s12866-020-02073-2.
86. Rahman MA, Uddin BMM, Ratan ZA, *et al*. Prevalence and Antimicrobial Resistance Pattern with Associated Gens of Extended Spectrum β-lactamase, Carbapenemases and Methicillin Resistant *Staphylococcus aureus* Producing Organisms isolated from Neonatal Sepsis in Dhaka City of Bangladesh. Bangladesh Journal of Medical Microbiology 2024; 18(2): 114-119. doi: https://doi.org/10.3329/bjmm.v18i2.78516.
87. Dubodelov D, Lubasovskaya L, Shubina E, *et al*. Genetic determinants of resistance of hospital-associated strains of *Klebsiella pneumoniae* to β-lactam antibiotics isolated in neonates. Russian Journal of Genetics 2016; 52(9): 993-998. doi: 10.1134/S1022795416090040.

88. Hassuna NA, AbdelAziz RA, Zakaria A, *et al*. Extensively-drug resistant *Klebsiella pneumoniae* recovered from neonatal sepsis cases from a major NICU in Egypt. Frontiers in microbiology 2020; 11: 1375. doi: https://doi.org/10.3389/fmicb.2020.01375.
89. Latifi B, Tajbakhsh S, Ahadi L, *et al*. Coexistence of aminoglycoside resistance genes in CTX-M-producing isolates of *Klebsiella pneumoniae* in Bushehr province, Iran. Iranian Journal of Microbiology 2021; 13(2): 161. doi: 10.18502/ijm.v13i2.5975.
90. Robicsek A, Strahilevitz J, Jacoby GA, *et al*. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nature medicine 2006; 12(1): 83-88. doi: https://doi.org/10.1038/nm1347.
91. Kim D-W, Thawng CN, Lee K, *et al*. Revisiting polymorphic diversity of aminoglycoside N-acetyltransferase AAC (6′)-Ib based on bacterial genomes of human, animal, and environmental origins. Frontiers in microbiology 2018; 9: 1831. doi: 10.3389/fmicb.2018.01831.
92. Perez-Lopez A SS, Al-Mana H, Tsui KM, Hasan MR, Suleiman M, Janahi M, Al Maslamani E, Tang P. Molecular Characterization of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* and *Klebsiella pneumoniae* Among the Pediatric Population in Qatar. Frontiers in microbiology 2020; 11: 581711. doi: 10.3389/fmicb.2020.581711.
93. Park KS, Kim MH, Park TS, *et al*. Prevalence of the plasmid-mediated quinolone resistance genes, *aac (6')-Ib-cr*, *qepA*, and *oqxAB* in clinical isolates of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* in Korea. Annals of Clinical & Laboratory Science 2012; 42(2): 191-197.
94. Muraya A, Kyany'a C, Kiyaga S, *et al*. Antimicrobial resistance and virulence characteristics of *Klebsiella pneumoniae* isolates in Kenya by whole-genome sequencing. Pathogens 2022; 11(5): 545. doi: https://doi.org/10.3390/pathogens11050545.
95. Sedrakyan A, Gevorgyan Z, Zakharyan M, *et al*. Molecular epidemiology and in-depth characterization of *Klebsiella pneumoniae* clinical isolates from Armenia. International journal of molecular sciences 2025; 26(2): 504. doi: https://doi.org/10.3390/ijms26020504.
96. Chen D, Hu X, Chen F, *et al*. Co-outbreak of multidrug resistance and a novel ST3006 *Klebsiella pneumoniae* in a neonatal intensive care unit: A retrospective study. Medicine 2019; 98(4): e14285. doi: http://dx.doi.org/10.1097/MD.0000000000014285.
97. Tian P, Guo M-J, Li Q-Q, *et al*. Discovery of clinical isolation of drug-resistant *Klebsiella pneumoniae* with overexpression of OqxB efflux pump as the decisive drug resistance factor. Microbiology Spectrum 2024; 12(10): e00122-24. doi: https://doi.org/10.1128/spectrum.00122-24.
98. Wong MHY, Chan EWC, and Chen S. Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of *Enterobacteriaceae.* Antimicrobial agents and chemotherapy 2015; 59(6): 3290-3297. doi: 10.1128/AAC.00310-15.
99. Gabr BM, Zamzam ASA, Eisa EA, *et al*. Detection of *oqxA* and *oqxB* efflux pump genes among nosocomial coliform bacilli: An observational cross-sectional study. Journal of Acute Disease 2021; 10(3): 117-121. doi: 10.4103/2221-6189.316676.
100. Li J, Zhang H, Ning J, *et al*. The nature and epidemiology of OqxAB, a multidrug efflux pump. Antimicrobial Resistance & Infection Control 2019; 8(1): 44. doi: https://doi.org/10.1186/s13756-019-0489-3.

101. Peltier F, Choquet M, Decroix V, *et al*. Characterization of a multidrug-resistant *Klebsiella pneumoniae* ST607-K25 clone responsible for a nosocomial outbreak in a neonatal intensive care unit. Journal of Medical Microbiology 2019; 68(1): 67-76. doi: https://doi.org/10.1099/jmm.0.000884.
102. Ing A and Humphries R. The Genotype—Phenotype Conundrum for Antimicrobial Susceptibility Testing. Clinical Chemistry 2023; 69(4): 316-318. doi: https://doi.org/10.1093/clinchem/hvac159.
103. Azizian R. Prevalence of *dfr*, *int* and *sul* Genes in Cotrimoxazole Resistance *Klebsiella pneumoniae* Isolated from Two Hospitals of Iran. 2014.
104. Labar AS, Millman JS, Ruebush E, *et al*. Regional dissemination of a trimethoprim-resistance gene cassette via a successful transposable element. PLoS One 2012; 7(5): e38142. doi: https://doi.org/10.1371/journal.pone.0038142.
105. Arabi H, Pakzad I, Nasrollahi A, *et al*. Sulfonamide resistance genes (*sul*) M in extended spectrum beta lactamase (ESBL) and non-ESBL producing *Escherichia coli* isolated from Iranian hospitals. Jundishapur Journal of Microbiology 2015; 8(7): e19961. doi: 10.5812/jjm.19961v2.
106. Manyahi J, Tellevik MG, Ndugulile F, *et al*. Molecular characterization of cotrimoxazole resistance genes and their associated integrons in clinical isolates of gram-negative bacteria from Tanzania. Microbial Drug Resistance 2017; 23(1): 37-43. doi: https://doi.org/10.1089/mdr.2016.0074.
107. Wang W, Guo Q, Xu X, *et al*. High-level tetracycline resistance mediated by efflux pumps Tet (A) and Tet (A)-1 with two start codons. Journal of Medical Microbiology 2014; 63(11): 1454-1459. doi: 10.1099/jmm.0.078063-0.
108. Linkevicius M, Sandegren L, and Andersson DI. Potential of tetracycline resistance proteins to evolve tigecycline resistance. Antimicrobial agents and chemotherapy 2016; 60(2): 789-796. doi: https://doi.org/10.1128/aac.02465-15.
109. Poole K. Efflux pumps as antimicrobial resistance mechanisms. Annals of medicine 2007; 39(3): 162-176. doi: https://doi.org/10.1080/07853890701195262.
110. Li X-Z and Nikaido H. Efflux-mediated drug resistance in bacteria. Drugs 2004; 64(2): 159-204. doi: https://doi.org/10.2165/00003495-200464020-00004.
111. Munita JM and Arias CA. Mechanisms of antibiotic resistance. Virulence mechanisms of bacterial pathogens 2016: 481-511. doi: https://doi.org/10.1128/microbiolspec.vmbf-0016-2015.
112. Flores-Valdez M, Ares MA, Rosales-Reyes R, *et al*. Whole genome sequencing of pediatric *Klebsiella pneumoniae* strains reveals important insights into their virulence-associated traits. Frontiers in microbiology 2021; 12: 711577. doi: 10.3389/fmicb.2021.711577.
113. Berglund B, Hoang NTB, Lundberg L, *et al*. Clonal spread of carbapenem-resistant *Klebsiella pneumoniae* among patients at admission and discharge at a Vietnamese neonatal intensive care unit. Antimicrobial Resistance & Infection Control 2021; 10(1): 162. doi: https://doi.org/10.1186/s13756-021-01033-3.
114. Labi A-K, Nielsen KL, Marvig RL, *et al*. Oxacillinase-181 carbapenemase-producing *Klebsiella pneumoniae* in neonatal intensive care unit, Ghana, 2017–2019. Emerging infectious diseases 2020; 26(9): 2235. doi: 10.3201/eid2609.200562.
115. Zhang J, Xu Y, Wang M, *et al*. Mobilizable plasmids drive the spread of antimicrobial resistance genes and virulence genes in *Klebsiella pneumoniae*. Genome medicine 2023; 15(1): 106. doi: 10.1186/s13073-023-01260-w.

116. Khedher MB, Ghedira K, Rolain J-M, *et al*. Application and challenge of 3rd generation sequencing for clinical bacterial studies. International journal of molecular sciences 2022; 23(3): 1395. doi: https://doi.org/10.3390/ijms23031395.
117. Wick RR, Judd LM, Gorrie CL, *et al*. Completing bacterial genome assemblies with multiplex MinION sequencing. Microbial genomics 2017; 3(10): e000132. doi: https://doi.org/10.1099/mgen.0.000132.
118. Foster-Nyarko E, Cottingham H, Wick RR, *et al*. Nanopore-only assemblies for genomic surveillance of the global priority drug-resistant pathogen, *Klebsiella pneumoniae*. Microbial genomics 2023; 9(2): mgen000936. doi: 10.1099/mgen.0.000936.
119. Akintayo I, Siroglavic M, Frolova D, *et al*. Tracking clonal and plasmid transmission in colistin-and carbapenem-resistant *Klebsiella pneumoniae*. MSystems 2025; 10(2): e01128-24. doi: https://doi.org/10.1128/msystems.01128-24.
120. Keshet A, Hochwald O, Lavon A, *et al*. Development of antibiotic resistome in premature infants. Cell Reports 2025; 44(4). doi: https://doi.org/10.1016/j.celrep.2025.115515.
121. Samarra A, Esteban-Torres M, Cabrera-Rubio R, *et al*. Maternal-infant antibiotic resistance genes transference: what do we know? Gut Microbes 2023; 15(1): 2194797. doi: https://doi.org/10.1080/19490976.2023.2194797.
122. Fadeyi TE, Oyedemi OT, Awe OO, *et al*. Antibiotic use in infants within the first year of life is associated with the appearance of antibiotic-resistant genes in their feces. PeerJ 2023; 11: e15015. doi: https://doi.org/10.7717/peerj.15015.
123. Nogacka A, Salazar N, Suárez M, *et al*. Impact of intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates. Microbiome 2017; 5(1): 93. doi: https://doi.org/10.1186/s40168-017-0313-3.
124. Gosalbes M, Vallès Y, Jiménez-Hernández N, *et al*. High frequencies of antibiotic resistance genes in infants' meconium and early fecal samples. Journal of developmental origins of health and disease 2016; 7(1): 35-44. doi: https://doi.org/10.1017/S2040174415001506.
125. de Vries LE, Vallès Y, Agersø Y, *et al*. The gut as reservoir of antibiotic resistance: microbial diversity of tetracycline resistance in mother and infant. PLoS One 2011; 6(6): e21644. doi: https://doi.org/10.1371/journal.pone.0021644.
126. Chatzigiannidou I, Johansen PL, Dehli RK, *et al*. Temporal dynamics and microbial interactions shaping the gut resistome in early infancy. Nature Communications 2025; 16(1): 8139. doi: https://doi.org/10.1038/s41467-025-63401-6.
127. Pärnänen KM, Hultman J, Markkanen M, *et al*. Early-life formula feeding is associated with infant gut microbiota alterations and an increased antibiotic resistance load. The American journal of clinical nutrition 2022; 115(2): 407-421. doi: https://doi.org/10.1093/ajcn/nqab353.
128. Taft DH, Liu J, Maldonado-Gomez MX, *et al*. Bifidobacterial dominance of the gut in early life and acquisition of antimicrobial resistance. MSphere 2018; 3(5): 10.1128/msphere. 00441-18. doi: 10.1128/mSphere.00441-18.
129. Price V, Dunn SJ, Moran RA, *et al*. Whole-genome sequencing enhances existing pathogen and antimicrobial-resistance surveillance schemes within a neonatal unit. Microbial genomics 2022; 8(6): 000841. doi: https://doi.org/10.1099/mgen.0.000841.
130. Pan T and Li Q. Mobile genetic elements in *Klebsiella pneumoniae*. Journal of Bacteriology 2025; 207(5): e00012-25. doi: https://doi.org/10.1128/jb.00012-25.

131. Li B, Zhao Y, Liu C, *et al*. Molecular pathogenesis of *Klebsiella pneumoniae*. Future microbiology 2014; 9(9): 1071-1081. doi: https://doi.org/10.2217/fmb.14.48.
132. Sikarwar A and Batra H. Identification of *Klebsiella pneumoniae* by capsular polysaccharide polyclonal antibodies. International journal of chemical Engineering and Applications 2011; 2(2): 130.
133. Monteiro AdSS, Cordeiro SM, and Reis JN. Virulence factors in *Klebsiella pneumoniae*: a literature review. Indian journal of microbiology 2024; 64(2): 389-401. doi: 10.1007/s12088-024-01247-0.
134. Masselot--Joubert L and Di Renzo MA. ATP-Binding Cassette (ABC) Transporters and Antibiotic Resistance: Specialized Systems for Capsular Polysaccharide Export in Gram-Negative Pathogens. Polysaccharides 2025; 6(2): 38. doi: https://doi.org/10.3390/polysaccharides6020038.
135. Wang G, Zhao G, Chao X, *et al*. The characteristic of virulence, biofilm and antibiotic resistance of *Klebsiella pneumoniae*. International journal of environmental research and public health 2020; 17(17): 6278. doi: https://doi.org/10.3390/ijerph17176278.
136. Llobet E, Tomas JM, and Bengoechea JA. Capsule polysaccharide is a bacterial decoy for antimicrobial peptides. Microbiology 2008; 154(12): 3877-3886. doi: https://doi.org/10.1099/mic.0.2008/022301-0.
137. Campos MA, Vargas MA, Regueiro V, *et al*. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infection and immunity 2004; 72(12): 7107-7114. doi: https://doi.org/10.1128/iai.72.12.7107-7114.2004.
138. Chen T, Ying L, Xiong L, *et al*. Understanding carbapenem-resistant hypervirulent *Klebsiella pneumoniae*: key virulence factors and evolutionary convergence. HLife 2024; 2(12): 611-624. doi: https://doi.org/10.1016/j.hlife.2024.06.005.
139. Follador R, Heinz E, Wyres KL, *et al*. The diversity of *Klebsiella pneumoniae* surface polysaccharides. Microbial genomics 2016; 2(8): e000073. doi: https://doi.org/10.1099/mgen.0.000073.
140. Merino S, Camprubí S, Albertí S, *et al*. Mechanisms of *Klebsiella pneumoniae* resistance to complement-mediated killing. Infection and immunity 1992; 60(6): 2529-2535. doi: https://doi.org/10.1128/iai.60.6.2529-2535.1992.
141. Rendueles O. Deciphering the role of the capsule of *Klebsiella pneumoniae* during pathogenesis: A cautionary tale. Molecular microbiology 2020; 113(5): 883-888. doi: 10.1111/mmi.14474.
142. Cote JM and Taylor EA. The glycosyltransferases of LPS core: a review of four heptosyltransferase enzymes in context. International journal of molecular sciences 2017; 18(11): 2256. doi: https://doi.org/10.3390/ijms18112256.
143. Pal S, Verma J, Mallick S, *et al*. Absence of the glycosyltransferase WcaJ in *Klebsiella pneumoniae* ATCC13883 affects biofilm formation, increases polymyxin resistance and reduces murine macrophage activation. Microbiology 2019; 165(8): 891-904. doi: https://doi.org/10.1099/mic.0.000827.
144. Cai R, Wang G, Le S, *et al*. Three capsular polysaccharide synthesis-related glucosyltransferases, GT-1, GT-2 and WcaJ, are associated with virulence and phage sensitivity of *Klebsiella pneumoniae*. Frontiers in microbiology 2019; 10: 1189. doi: https://doi.org/10.3389/fmicb.2019.01189.
145. Patro LPP and Rathinavelan T. Targeting the sugary armor of *Klebsiella* species. Frontiers in Cellular and Infection Microbiology 2019; 9: 367. doi: https://doi.org/10.3389/fcimb.2019.00367.

146. Pan Y-J, Lin T-L, Hsu C-R, *et al*. Use of a *Dictyostelium* model for isolation of genetic loci associated with phagocytosis and virulence in *Klebsiella pneumoniae.* Infection and immunity 2011; 79(3): 997-1006. doi: https://doi.org/10.1128/iai.00906-10.

147. Stahlhut SG, Struve C, Krogfelt KA, *et al*. Biofilm formation of *Klebsiella pneumoniae* on urethral catheters requires either type 1 or type 3 fimbriae. FEMS Immunology & Medical Microbiology 2012; 65(2): 350-359. doi: https://doi.org/10.1111/j.1574-695X.2012.00965.x.

148. Struve C, Bojer M, and Krogfelt KA. Characterization of *Klebsiella pneumoniae* type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. Infection and immunity 2008; 76(9): 4055-4065. doi: https://doi.org/10.1128/iai.00494-08.

149. Klemm P. Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli.* The EMBO journal 1986; 5(6): 1389-1393. doi: https://doi.org/10.1002/j.1460-2075.1986.tb04372.x.

150. Struve C, Bojer M, and Krogfelt KA. Identification of a conserved chromosomal region encoding *Klebsiella pneumoniae* type 1 and type 3 fimbriae and assessment of the role of fimbriae in pathogenicity. Infection and immunity 2009; 77(11): 5016-5024. doi: https://doi.org/10.1128/iai.00585-09.

151. Allen BL, Gerlach G-F, and Clegg S. Nucleotide sequence and functions of mrk determinants necessary for expression of type 3 fimbriae in *Klebsiella pneumoniae.* Journal of Bacteriology 1991; 173(2): 916-920.

152. Murphy CN and Clegg S. *Klebsiella pneumoniae* and type 3 fimbriae: nosocomial infection, regulation and biofilm formation. Future microbiology 2012; 7(8): 991-1002. doi: https://doi.org/10.2217/fmb.12.74.

153. Schroll C, Barken KB, Krogfelt KA, *et al*. Role of type 1 and type 3 fimbriae in *Klebsiella pneumoniae* biofilm formation. BMC microbiology 2010; 10(1): 179. doi: https://doi.org/10.1186/1471-2180-10-179.

154. Brożyna M, Paleczny J, Kozłowska W, *et al*. The antimicrobial and antibiofilm in vitro activity of liquid and vapour phases of selected essential oils against *Staphylococcus aureus.* Pathogens 2021; 10(9): 1207. doi: https://doi.org/10.3390/pathogens10091207.

155. Battah B, Rajab A, Shbibe L, *et al*. Evaluation of antibiofilm activity of *Thymus syriacus* essential oil against clinically isolated MDR bacteria. Progress in Microbes and Molecular Biology 2022: a0000284. doi: 10.36877/pmmb.a0000284.

156. Khaertynov KS, Anokhin VA, Rizvanov AA, *et al*. Virulence factors and antibiotic resistance of *Klebsiella pneumoniae* strains isolated from neonates with sepsis. Frontiers in medicine 2018; 5: 225. doi: https://doi.org/10.3389/fmed.2018.00225.

157. Lan P, Lu Y, Fu Y, *et al*. Siderophores and beyond: A comprehensive review of iron acquisition in *Klebsiella pneumoniae.* Virulence 2025; 16(1): 2550621. doi: https://doi.org/10.1080/21505594.2025.2550621.

158. Russo TA and Marr CM. Hypervirulent *klebsiella pneumoniae.* Clinical microbiology reviews 2019; 32(3): 10.1128/cmr. 00001-19. doi: https://doi.org/10.1128/cmr.00001-19.

159. Bach S, de Almeida A, and Carniel E. The Yersinia high-pathogenicity island is present in different members of the family *Enterobacteriaceae.* FEMS microbiology letters 2000; 183(2): 289-294. doi: https://doi.org/10.1111/j.1574-6968.2000.tb08973.x.

160. Koczura R and Kaznowski A. Occurrence of the Yersinia high-pathogenicity island and iron uptake systems in clinical isolates of *Klebsiella pneumoniae*. Microbial pathogenesis 2003; 35(5): 197-202. doi: https://doi.org/10.1016/S0882-4010(03)00125-6.

161. Jacobi CA, Gregor S, Rakin A, *et al*. Expression analysis of the yersiniabactin receptor gene *fyuA* and the heme receptor hemR of *Yersinia enterocolitica* in vitro and in vivo using the reporter genes for green fluorescent protein and luciferase. Infection and immunity 2001; 69(12): 7772-7782. doi: 10.1128/IAI.69.12.7772-7782.2001.

162. Paauw A, Leverstein-van Hall MA, van Kessel KP, *et al*. Yersiniabactin reduces the respiratory oxidative stress response of innate immune cells. PLoS One 2009; 4(12): e8240. doi: https://doi.org/10.1371/journal.pone.0008240.

163. Abbas R, Chakkour M, Zein El Dine H, *et al*. General overview of *Klebsiella pneumonia*: epidemiology and the role of siderophores in its pathogenicity. Biology 2024; 13(2): 78. doi: https://doi.org/10.3390/biology13020078.

164. Perry RD and Fetherston JD. Yersiniabactin iron uptake: mechanisms and role in *Yersinia pestis* pathogenesis. Microbes and infection 2011; 13(10): 808-817. doi: https://doi.org/10.1016/j.micinf.2011.04.008.

165. Farzand R, Rajakumar K, Barer MR, *et al*. A virulence associated siderophore importer reduces antimicrobial susceptibility of *Klebsiella pneumoniae*. Frontiers in microbiology 2021; 12: 607512. doi: https://doi.org/10.3389/fmicb.2021.607512.

166. Greene NP, Kaplan E, Crow A, *et al*. Antibiotic resistance mediated by the MacB ABC transporter family: a structural and functional perspective. Frontiers in microbiology 2018; 9: 950. doi: 10.3389/fmicb.2018.00950.

167. Olwagen CP, Izu A, Khan S, *et al*. *Genomic Characterization of Klebsiella pneumoniae Causing Invasive Disease in South African Infants: Observational Studies Between 2018 and 2023*. in *Open Forum Infectious Diseases*. 2026. Oxford University Press US.

168. Goodman RN, Moyo SJ, Memelis I, *et al*. Comparative genomics of blood and faecal *E. coli* and *K. pneumoniae* isolates from neonates with bloodstream infections in Tanzania. Communications Biology 2025; 8(1): 1603. doi: https://doi.org/10.1038/s42003-025-09008-5.

169. Barbosa VAA and Lery LMS. Insights into *Klebsiella pneumoniae* type VI secretion system transcriptional regulation. BMC Genomics 2019; 20(1): 506. doi: https://doi.org/10.1186/s12864-019-5885-9.

170. Hsieh P-F, Lu Y-R, Lin T-L, *et al*. *Klebsiella pneumoniae* type VI secretion system contributes to bacterial competition, cell invasion, type-1 fimbriae expression, and in vivo colonization. The Journal of Infectious Diseases 2019; 219(4): 637-647. doi: https://doi.org/10.1093/infdis/jiy534.

171. Bingle LE, Bailey CM, and Pallen MJ. Type VI secretion: a beginner's guide. Current opinion in microbiology 2008; 11(1): 3-8. doi: https://doi.org/10.1016/j.mib.2008.01.006.

172. Ma L-S, Narberhaus F, and Lai E-M. IcmF family protein TssM exhibits ATPase activity and energizes type VI secretion. Journal of Biological Chemistry 2012; 287(19): 15610-15621. doi: 10.1074/jbc.M111.301630.

173. Zhang J, Zhang H, Gao Z, *et al*. Structural basis for recognition of the type VI spike protein VgrG3 by a cognate immunity protein. FEBS letters 2014; 588(10): 1891-1898. doi: https://doi.org/10.1016/j.febslet.2014.04.016.

174. Zhu N, Zhao Y, Yi W, *et al*. Genomic profiling and experimental validation of type VI secretion system-associated proteins in *Klebsiella*. PLoS Genetics 2025; 21(9): e1011878. doi: https://doi.org/10.1371/journal.pgen.1011878.
175. Liu W, Li M, Cao S, *et al*. The biological and regulatory role of type VI secretion system of *Klebsiella pneumoniae*. Infection and Drug Resistance 2023: 6911-6922. doi: https://doi.org/10.2147/IDR.S426657.
176. Zhang Y, Xu Y, and Huang Y. Virulence genotype and correlation of clinical severeness with presence of the type VI secretion system in *Klebsiella pneumoniae* isolates causing bloodstream infections. Infection and Drug Resistance 2022: 1487-1497.
177. Agnetti J, Seth-Smith HM, Ursich S, *et al*. Clinical impact of the type VI secretion system on virulence of *Campylobacter* species during infection. BMC Infectious Diseases 2019; 19(1): 237. doi: https://doi.org/10.1186/s12879-019-3858-x.
178. Merciecca T, Bornes S, Nakusi L, *et al*. Role of *Klebsiella pneumoniae* Type VI secretion system (T6SS) in long-term gastrointestinal colonization. Scientific Reports 2022; 12(1): 16968. doi: https://doi.org/10.1038/s41598-022-21396-w.
179. Liao W, Huang H-h, Huang Q-s, *et al*. Distribution of type VI secretion system (T6SS) in clinical *Klebsiella pneumoniae* strains from a Chinese hospital and its potential relationship with virulence and drug resistance. Microbial pathogenesis 2022; 162: 105085. doi: https://doi.org/10.1016/j.micpath.2021.105085.
180. Mohamed NA, Alrawy MH, Makbol RM, *et al*. Type VI secretion system (T6SS) in *Klebsiella pneumoniae*, relation to antibiotic resistance and biofilm formation. Iranian Journal of Microbiology 2023; 15(5): 601. doi: 10.18502/ijm.v15i5.13865.
181. Dentice Maidana S, Elean M, Fukuyama K, *et al*. Hypermucoviscous carbapenem-resistant *Klebsiella pneumoniae* ST25 infect human intestinal epithelial cells and induce moderate inflammation. International journal of molecular sciences 2023; 24(10): 8804. doi: https://doi.org/10.3390/ijms24108804.
182. Chen Y, Brook TC, Soe CZ, *et al*. Preterm infants harbour diverse *Klebsiella* populations, including atypical species that encode and produce an array of antimicrobial resistance- and virulence-associated factors. Microbial genomics 2020; 6(6): e000377. doi: 10.1099/mgen.0.000377.
183. Nguyen TNT, Howells G, and Short FL. How *Klebsiella pneumoniae* controls its virulence. PLOS Pathogens 2025; 21(9): e1013499. doi: https://doi.org/10.1371/journal.ppat.1013499.
184. Han B, Feng C, Jiang Y, *et al*. Mobile genetic elements encoding antibiotic resistance genes and virulence genes in *Klebsiella pneumoniae*: Important pathways for the acquisition of virulence and resistance. Frontiers in microbiology 2025; 16: 1529157. doi: https://doi.org/10.3389/fmicb.2025.1529157.
185. Shrestha RK, Shrestha D, Kunwar AJ, *et al*. The overlap of accessory virulence factors and multidrug resistance among clinical and surveillance *Klebsiella pneumoniae* isolates from a neonatal intensive care unit in Nepal: a single-centre experience in a resource-limited setting. Tropical Medicine and Health 2024; 52(1): 30. doi: 10.1186/s41182-024-00595-3.
186. Wyres KL, Nguyen TN, Lam MM, *et al*. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. Genome medicine 2020; 12(1): 11. doi: https://doi.org/10.1186/s13073-019-0706-y.

187. Choby JE, Howard-Anderson J, and Weiss DS. Hypervirulent *Klebsiella pneumoniae*–clinical and molecular perspectives. Journal of internal medicine 2020; 287(3): 283-300. doi: 10.1111/joim.13007.
188. Marr CM and Russo TA. Hypervirulent *Klebsiella pneumoniae*: a new public health threat. Expert review of anti-infective therapy 2019; 17(2): 71-73. doi: https://doi.org/10.1080/14787210.2019.1555470.
189. Boolchandani M, D'Souza AW, and Dantas G. Sequencing-based methods and resources to study antimicrobial resistance. Nature Reviews Genetics 2019; 20(6): 356-370. doi: https://doi.org/10.1038/s41576-019-0108-4.
190. Casadevall A and Pirofski L-a. Virulence factors and their mechanisms of action: the view from a damage–response framework. Journal of water and health 2009; 7(S1): S2-S18. doi: 10.2166/wh.2009.036.
191. Martínez JL, Coque TM, and Baquero F. What is a resistance gene? Ranking risk in resistomes. Nature Reviews Microbiology 2015; 13(2): 116-123. doi: https://doi.org/10.1038/nrmicro3399.
192. Hughes D and Andersson DI. Environmental and genetic modulation of the phenotypic expression of antibiotic resistance. FEMS microbiology reviews 2017; 41(3): 374-391. doi: https://doi.org/10.1093/femsre/fux004.
193. Wang Z, Gerstein M, and Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 2009; 10(1): 57-63. doi: https://doi.org/10.1038/nrg2484.
194. Shahid U. From resistance genes to resistance states: A transcriptomics-driven precision medicine framework for antimicrobial therapy. The Journal of Precision Medicine: Health and Disease 2026; 7. doi: 10.1016/j.premed.2026.100030.



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