Integrative Genomic and Phenotypic Characterization of Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* (ESBL-E) and Related Bacterial Species Isolated from Pediatric Diarrheal Cases in Bangladesh.

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Dedicated To

My Respected Parents and Teachers

Abstract

Extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) pose a major global threat to antimicrobial therapy, particularly in pediatric populations where diarrheal diseases remain a leading cause of morbidity and mortality. This study provides an integrative genomic–phenotypic assessment of ESBL-producing pathogens isolated from diarrheal stool samples of hospitalized children (<8 years) in Bangladesh. A total of 38 bacterial isolates obtained from 32 samples were identified to the species level using MALDI-TOF MS, with *Escherichia coli* (47.37%) and *Klebsiella pneumoniae* (10.53%) being predominant. Whole genome sequencing (WGS) revealed genome sizes ranging from 3.36–6.63 Mb and 3,095–6,056 annotated coding sequences. Genomic screening using ABRicate across five AMR databases identified ESBL-associated genes in 55.3% (21/38) of isolates. The $bla_{CTX\text{-}M\text{-}15}$ gene was the most prevalent variant (76.19%), primarily among *E. coli*, followed by bla_{TEM} and bla_{SHV} alleles. Phenotypic ESBL detection via CLSI-recommended double-disk synergy testing confirmed ESBL activity in 39.5% of isolates. Genotype–phenotype concordance was 78.95%, with genotype-positive/phenotype-negative mismatches likely linked to silent gene expression, masking by co-produced β-lactamases, or complex resistance mechanisms. Plasmid replicon analysis identified diverse incompatibility groups, including IncF, IncI, IncHI, IncX, IncR, and Col plasmids, supporting the mobility and dissemination of ESBL genes. Virulence profiling showed substantial heterogeneity, with ESBL-positive isolates carrying multiple adhesion, iron acquisition, and stress-response determinants that may enhance pathogenic potential.

This study represents one of the first comprehensive genomic–phenotypic investigations of ESBL-producing *Enterobacteriaceae* in pediatric diarrheal cases in Bangladesh. The predominance of $bla_{CTX\text{-}M\text{-}15}$, the complexity of plasmid-mediated gene dissemination, and notable genotype–phenotype discrepancies underscore the need for integrated WGS-based surveillance alongside refined phenotypic diagnostics. These findings offer critical insights for early detection, infection control, and therapeutic decision-making in resource-limited healthcare settings.

Keywords: *ESBL, Whole genome sequencing, Pediatric diarrhea, Antimicrobial resistance, Virulence gene, Plasmid, Mobilome Analysis*

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List of Abbreviations

AMR	Antimicrobial Resistance
ESBL-E	Extended-spectrum β-lactamase-producing *Enterobacteriaceae*
MGEs	Mobile Genetic Elements
BLAST	Basic Local Alignment Search Tool

WGS	Whole Genome Sequencing
CDC	Centers for Disease Control and Prevention
CDS	Coding Sequence
CNS	Central Nervous System
CI	Confidence Interval
DDT	Disc Diffusion Test
DNA	Deoxyribonucleic Acid
ETEC	Enterotoxigenic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EDTA	Ethylenediaminetetraacetic Acid
HP	Hypothetical Protein
HGT	Horizontal Gene Transfer
HCCA	Hydroxycinnamic acid
MALDI-TOF MS	Matrix-assisted Laser Desorption Ionization-time of Flight Mass Spectrometry
NGS	Next Generation Sequencing
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
PPI	Protein-protein interaction
rRNA	Ribosomal Ribonucleic Acid
rMLST	Ribosomal Multilocus Sequence Typing
SRA	Sequence Read Archive
SMART	Study for Monitoring Antimicrobial Resistance Trends
VFDB	Virulence Factor Database
WHO	World Health Organization

Chapter 1

Introduction

1.1. Background

Extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) pose a prominent global health risk because they can hydrolyze broad-spectrum β-lactam antibiotics, causing many first-line treatments ineffective. In turn, the right treatment is often delayed, which increases the risk of death [1], [2]. Infections resulting from these resistant organisms leads to significant amounts of morbidity and mortality around the world [3]. ESBL-E has a significant impact on public health, infecting approximately 290,220 hospitalized patients annually in the U.S. [4]. In 2015, ESBL-producing *Escherichia coli* (*E. coli*) caused about 300,000 infections and 9,000 deaths in Europe, while *Klebsiella pneumonia*e (*K. pneumoniae*) led to 70,000 infections and over 3,500 deaths [5]. In 2019, AMR resulted in 1.27 million direct deaths and 4.95 million associated deaths worldwide [6]. By 2050, it is expected that there could be up to 10 million fatalities per year globally, resulting in an economic burden of $100 trillion. The extensive presence of ESBLs significantly contributes to the current antibiotic resistance crisis [7]. According to global projections, over 50% of *K. pneumoniae* and *E. coli* bacteria will be resistant to third-generation cephalosporins by 2030 [8]. WHO classified third-generation cephalosporin-resistant Enterobacterales as critical group pathogens in the bacterial priority pathogens list, 2024 [9]. According to a landmark study presented at the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Global 2025 annual congress found that over 3 million children around the world died from AMR-related infections in 2022. This shows how important it is to have targeted surveillance and control strategies, especially for ESBLs-producing pathogens, which are an essential factor of pediatric antimicrobial resistance [10]. Notably, Colonization with ESBL-E is a big risk factor for subsequent infections that are caused by antibiotic-resistant bacteria [11]. ESBL-E have emerged as a global concern, becoming endemic in numerous countries since their initial identification in 1983 [12], [13], mainly because of the dissemination of ESBL genes associated with mobile genetic elements (MGEs) like plasmids [14]. These plasmid-mediated ESBL genes are a significant public health threat because they transmit easily between microbes through MGEs [15]. In the past, the TEM and SHV types of ESBLs were the most common families of ESBLs [16]. Now, particularly CTX-M type ESBLs, demands a multi-stake holder attention [17]. ESBL-E inactivate beta-lactam antibiotics containing an oxyimino group, particularly third- and fourth-generation cephalosporins and monobactams, but not cephamycins or carbapenems. Unlike AmpC enzymes, ESBLs are

inhibited by beta-lactamase inhibitors, including tazobactam and clavulanic acid [18]. Some ESBL-producing *E. coli* strains also carry virulence genes, which enhance their pathogenic potential. Genes such as *eilA*, *air*, and *lphA* aid in bacterial adherence, *senB* and *astA* encode enterotoxins, while *gad* supports survival in acidic environments. The co-occurrence of ESBL and virulence genes is linked to increased disease severity, higher mortality, and greater healthcare costs [19]. Identifying these resistance (ESBLs) genes is critical for a quick diagnosis, effective treatment, and developing strategies to prevent further spread.

Over the recent decades, many past studies have focused on the distribution and genomic characterization of ESBL-E for their contribution in the worldwide crisis of antimicrobial resistance. Studies in Bangladesh found that high-risk, multidrug-resistant lineages, such ST131, which are mostly linked to cephalosporin resistance and virulence genes, were highly prevalent and in circulation, warranting close monitoring [20]. Hypervirulent *K. pneumoniae* strains, which harbored the most common ESBL gene, $bla_{\text{CTX-M-15}}$ were also found in circulation [21]. In addition, a study by International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), found a high prevalence of multidrug resistant and carbapenem-resistant *Klebsiella pneumoniae* which revealed the dominance of resistance genes like $bla_{\text{CTX-M-1}}$ (91%), bla_{TEM} (76.1%), and bla_{SHV} (68.7%), in addition to carbapenemase genes such as bla_{KPC} (55.2%) and $bla_{\text{NDM-1}}$ (13.4%). Virulence factors like fimH (71.6%) and ugeF (58.2%) were also occasionally detected, suggesting severe pathogenic potential [22]. However, Despite these findings, most studies lacked comprehensive data on community level colonization or regional diversity [21]. Furthermore, the survivability of the ESBL-E plasmids and resistance gene transmission across cells in hospitalized infections remain unclear [23]. Using whole genome sequencing (WGS) [24], this study fills in critical gaps by inscribing a more detailed characterization and transmission dynamics of ESBL-E isolates by exploring their prevalence, genome diversification, resistance profiles and scrutinizing different antibiotics against isolates obtained from hospital patients in Bangladesh.

The introduction of WGS has revolutionized the detection and characterization of antimicrobial resistance (AMR), offering a high-resolution tool for identifying resistance genes, tracking transmission dynamics, and understanding bacterial evolution. When combined, WGS, antibiogram data, and systems biology provide a potent platform to get overcome the drawbacks

of traditional phenotypic techniques and narrow-scope molecular techniques. Together, these strategies significantly improve early identification of new resistance threats, genotype-phenotype correlation, and comprehensive surveillance [25], [26]. While several studies in Bangladesh have reported ESBL-producing organisms from various sources, including poultry, meat, wastewater, and clinical specimens [27], [28], [29], [30], with most relying solely on phenotypic or limited molecular techniques. So far, no published work has used a combined WGS-based ESBL gene detection and phenotypic validation method on diarrhea stool samples from kids under five, who are very likely to get infections caused by AMR. Previous research has mostly looked at either genotypic detection or phenotypic characterisation of ESBL-producing organisms. They hadn't combined both methods to integrate both approaches for comprehensive ESBL gene identification and validation [31], [32]. This study aligns with the WHO Global Action Plan on Antimicrobial Resistance (2015) by contributing to improved surveillance and understanding of ESBL-producing pathogens, thereby supporting evidence-based strategies for antimicrobial stewardship and infection prevention [33].

Hence, aiming to achieve a better and more comprehensive understanding of ESBL-E in pediatric diarrheal cases, we conducted WGS of isolates from children under eight in Bangladesh. ESBL genes were identified from AMR profiles using ABRicate with multiple databases (CARD, NCBI, MEGARes, ResFinder, and ARG-ANNOT), and phenotypically validated via double-disk synergy test (DDST). Finally, we present an integrative analysis correlating genotypic predictions with phenotypic expression, highlighting the diagnostic value of combining in silico and laboratory-based surveillance to strengthen AMR monitoring strategies in resource-limited settings.

1.2. Objectives:

- To isolate and identify bacterial pathogens associated with pediatric diarrhea using culture and MALDI-TOF MS.
- To determine the genomic characteristics and ESBL gene profiles of diarrheal isolates using whole genome sequencing (WGS).
- To phenotypically validate ESBL production through the double-disk diffusion test (DDT) following CLSI M100-ED35 guidelines.
- To compare and correlate genotypic (WGS-based) and phenotypic (DDT-based) ESBL detection methods for diagnostic accuracy.
- To highlight the epidemiological significance of ESBL-producing *Enterobacteriaceae* in pediatric diarrheal infections in Bangladesh.
- To analyze mobile genetic elements and their role in horizontal gene transfer using WGS data.

Chapter 2

Literature Review

2.1. Introduction to the Problem

Antimicrobial resistance (AMR) is now an emerging global health problem and many patients succumb to death due to drug-resistant organisms and the unavailability of appropriate antibiotics [34]. In developing countries, antibiotic resistance is rising due to unregulated sales, overuse, and easy access to low-cost generic drugs. According to data from **Market Data Forecast** (accessed June 2025), the global antibiotics market was estimated at USD 53.10 billion in 2025 and is projected to grow to USD 72.67 billion by 2033. In the Asia-Pacific region, the market accounted for the largest share and is expected to expand significantly over the forecast period [35]. This growing market demand, along with rising resistance, makes it even more important for continuous monitoring and molecular characterization of resistant pathogens [36]. The World Health Organization (WHO) has indeed referred to antimicrobial resistance (AMR) as a "silent pandemic" due to its severe and growing threat to global health, development, and security [37]. This term highlights how AMR is a slow-moving but devastating crisis that often goes unnoticed by the public, despite causing millions of deaths and posing a major risk to the effectiveness of modern medicine. Alarming projections suggest that, without effective interventions, drug-resistant infections could claim up to 10 million lives annually by 2050 [7], [38]. Among the numerous resistant pathogens, members of the family *Enterobacteriaceae* occupy a particularly critical position [39]. This family encompasses several clinically important genera, including *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Morganella*, and *Proteus*, which are ubiquitous in the environment, human gut microbiota, and hospital settings [40], [41]. The widespread distribution and genetic adaptability of *Enterobacteriaceae* facilitate the acquisition and dissemination of resistance determinants, especially those encoding extended-spectrum β-lactamases (ESBLs) [42]. These enzymes confer resistance to a wide array of β-lactam antibiotics, including third-generation cephalosporins and monobactams, which are cornerstone agents for the management of severe bacterial infections [18], [23].

ESBLs are plasmid-mediated enzymes capable of hydrolyzing β-lactam antibiotics such as penicillins, cephalosporins (including cefotaxime, ceftazidime, and ceftriaxone), and aztreonam, but they are inhibited by β-lactamase inhibitors like clavulanic acid, tazobactam, and sulbactam [18]. The most prevalent ESBL families are blaTEM**,** blaSHV, and blaCTX-M, with various CTX-M variants dominating globally [1], [43]. Several investigators highlighted the widespread

presence of CTX-M-producing *E. coli*, reporting CTX-M-14 as the most common ESBL type. However, subsequent studies by the same group observed a shift toward CTX-M-15–producing isolates, which gradually replaced the earlier CTX-M-14–producing population [44]. The global dissemination of *blaCTX-M* genes has changed the epidemiology of β-lactam resistance and is now considered a hallmark of community- and hospital-acquired infections [45]. ESBL-producing *Enterobacteriaceae* (ESBL-E) are frequently associated with multidrug resistance (MDR) phenotypes due to co-localization of resistance genes on the same plasmid, thereby limiting therapeutic options and complicating infection management [46]. The clinical relevance of ESBLs extends beyond treatment failure; their presence often correlates with increased morbidity, prolonged hospital stays, higher healthcare costs, and elevated mortality rates [19].

The burden of ESBL-producing pathogens is particularly alarming in developing countries, where antibiotic misuse, limited diagnostic infrastructure, and poor infection control practices contribute to rapid dissemination [47]. South Asian countries, including Bangladesh, have reported a high prevalence of ESBL-E in both community and hospital settings. The over-the-counter availability of antibiotics, inadequate wastewater treatment, and poor sanitation further promote the horizontal transfer of resistance genes among bacterial populations [48]. In Bangladesh, diarrheal diseases remain one of the most common causes of morbidity and mortality, especially among children under five years of age [49]. According to WHO and UNICEF reports, diarrheal infections account for a substantial proportion of pediatric hospital admissions and deaths each year [50]. These infections are frequently caused by enteric bacteria such as *E. coli*, *K. pneumoniae*, *Enterobacter spp.*, and *Citrobacter spp.*, many of which have developed resistance to multiple antibiotics, including β-lactams [51]. The co-existence of ESBL-mediated resistance in diarrheagenic *Enterobacteriaceae* not only complicates clinical treatment but also poses a significant risk of resistance gene dissemination to other commensal and pathogenic bacteria within the gut [52].

Children are particularly vulnerable to infections caused by multidrug-resistant bacteria because of their underdeveloped immune systems, frequent exposure to contaminated food and water, and limited access to appropriate healthcare facilities in resource-constrained settings [53], [54]. Furthermore, the use of broad-spectrum antibiotics in pediatric populations often prescribed empirically creates selective pressure that facilitates the emergence and spread of ESBL-

producing pathogens. The gastrointestinal tract of children serves as a reservoir where resistant bacteria can thrive, multiply, and exchange genetic material via horizontal gene transfer (HGT) [55]. This scenario underscores the necessity of systematic surveillance and detailed molecular characterization of ESBL-producing bacteria from pediatric diarrheal cases to inform treatment strategies and containment measures.

Understanding the molecular basis of ESBL-mediated resistance requires a multifaceted approach that integrates both phenotypic and genotypic analyses. Phenotypic methods such as the double-disk diffusion test (DDT) and combination disk assays remain standard for detecting ESBL activity in clinical microbiology laboratories[56]. However, these techniques have limitations, including false negatives arising from the co-production of other β-lactamases(like AmpC β-lactamases) or changes in membrane permeability and false positivity due to hyperproduction of narrower-spectrum β-lactamases combined with altered permeability [44]. In contrast, genotypic approaches, particularly those based on whole genome sequencing (WGS), offer comprehensive insights into the resistome, mobilome, and virulome of bacterial isolates. WGS enables the identification of resistance genes, plasmid replicon types, mobile genetic elements (MGEs), and virulence-associated factors, thereby providing a deeper understanding of how ESBLs evolve, spread, and persist in bacterial populations. Integrating phenotypic and genomic data thus enhances diagnostic accuracy and supports evidence-based antimicrobial stewardship [57], [58].

Recent advances in bioinformatics have further strengthened genomic investigations. Databases such as the Comprehensive Antibiotic Resistance Database (CARD), ResFinder, ARG-ANNOT, and the NCBI AMR database allow for systematic screening of resistance determinants [59], [60], [61], [62], while plasmid detection tools like PlasmidFinder and MOB-suite facilitate mobilome analysis [63]. Similarly, the use of virulence factor databases (VFDB) and phylogenetic tools such as rMLST and core genome alignment enable species confirmation and evolutionary relationship mapping. Such integrative approaches not only delineate the genetic architecture of resistance but also reveal possible evolutionary pathways and epidemiological linkages among isolates [64], [65]. By comparing phenotypic resistance profiles with genotypic predictions, researchers can identify discrepancies that may indicate emerging mechanisms or

silent resistance genes, contributing to a more comprehensive understanding of the AMR landscape.

Within this framework, the study of ESBL-producing *Enterobacteriaceae* from pediatric diarrheal samples in Bangladesh holds significant clinical and epidemiological relevance. It bridges the gap between routine phenotypic surveillance and advanced genomic characterization, offering insights into both resistance determinants and their associated virulence factors. Moreover, understanding the role of MGEs in the dissemination of ESBL genes provides essential information for developing targeted interventions aimed at limiting the spread of resistant pathogens. The incorporation of phylogenetic analyses adds an evolutionary dimension, allowing researchers to trace genetic relationships and potential transmission patterns within and across bacterial species [66].

The purpose of this literature review is to provide a comprehensive overview of the existing knowledge surrounding ESBL-producing *Enterobacteriaceae*, with a particular focus on their genomic and phenotypic characteristics, resistance mechanisms, virulence factors, and mobile genetic elements. The review also explores how integrative genomic and phenotypic approaches enhance our understanding of resistance evolution and inform clinical decision-making. By synthesizing evidence from global and regional studies, this chapter aims to contextualize the current research on pediatric diarrheal pathogens in Bangladesh and highlight the critical need for genomic-informed surveillance to combat the growing threat of antimicrobial resistance.

2.2. Global epidemiology of ESBL-producing *Enterobacteriaceae*

ESBL-E have become a major global public health concern, with rapidly increasing prevalence in both community and hospital settings. Over the past two decades, ESBL-producing pathogens particularly *E. coli* and *K. pneumoniae* have spread across continents at an unprecedented rate, driven largely by horizontal gene transfer, antibiotic misuse, international travel, and globalization of the food supply chain [15], [67]. Unlike earlier decades, when ESBLs were predominantly reported in hospital-acquired infections, current evidence shows that community-

associated ESBLs now represent a substantial portion of global infections, affecting people across all age groups, including neonates and young children. This global expansion has been accompanied by rising multidrug resistance, limiting the effectiveness of commonly used antibiotics and complicating treatment protocols in both high- and low-income countries [68], [69].

2.3. Global Prevalence and Trends

The prevalence of ESBL-producing *Enterobacteriaceae* varies widely across regions, but increasing trends have been consistently documented worldwide. The highest reported burdens exist in Asia, Sub-Saharan Africa, Latin America, and the Middle East, where inappropriate antibiotic use and limited diagnostic capacity contribute to rapid dissemination [70], [71], [72].

Some clinical studies in India have reported extremely high ESBL rates among *Enterobacteriaceae*, sometimes in the range of 60–80%, though this level of prevalence is not uniformly reported across all countries in South Asia [73], [74], [75]. In Pakistan, high levels of ESBL-producing ***E. coli*** have also been documented about 48% [76], [77]. In Bangladesh, meta-analyses suggest more modest pooled prevalence (~17%) in *E. coli* isolates, indicating considerable regional variation in ESBL burden [78]. A significantly high proportion of AMR, MDR, and ESBL producers were detected in the period from 2015 to 2020 (**Table 1**)[48]. Studies from Asian countries report ESBL rates in clinical *Enterobacteriaceae* isolates often exceeding 60–70%, significantly higher than those observed in Europe or North America [48], [79], [80]. For example, A landmark study analyzed 178,825 *Enterobacteriaceae* isolates collected from 1997 to 2016 across 199 hospitals in 42 countries under the SENTRY Antimicrobial Surveillance Program. The proportion of isolates with an ESBL phenotype rose from 10.3% in 1997–2000 to 24.0% in 2013–2016. Among ESBL producers, the dominant species were *E. coli* (~47.5%) and *K. pneumoniae* (~43.7%). Regionally, the increase was significant in all world regions, such as Latin America saw a ~22.4% rise; Asia-Pacific ~17.6%; Europe ~16.2%; U.S. ~10.9% [81].

Table 1. Current status of ESBL as reported in Bangladesh, India, and Pakistan public health sectors from 2015 to 2023.

	Country	ESBL	*Enterobacteriaceae*	Source	Prevalence	Reference
1	Bangladesh	*blaCTX-M-15*	*E. coli*	Urine	80%	[79]
2	India	*blaCTX-M-15*	*E. coli*	Skin and soft tissue	70%	[82]
3	India	*blaCTX-M-15*	*E. coli*	Urine, pus, extra intestinal clinical samples	25%	[83]
4	Bangladesh	*blaTEM*	*E. coli*	Urine	50%	[84]
5	Bangladesh	*blaCTX-M-15*	*E. coli*	Rectal swabs	48.20%	[85]
		blaCTX-M-1			11.10%	
		blaSHV-12			11.10%	
		blaCTX-M-14			7.40%	
		blaCTX-M-27			7.40%	
		blaCTX-M-9			3.70%	
		blaCTX-M-14b			3.70%	
		blaSHV-28			3.70%	
		blaTEM-12			3.70%	
6	Bangladesh	*blaCTX-M-1*	*E. coli*	Clinical specimens	33.90%	[30]
		blaCTX-M-1	*K. pneumoniae*		51.40%	
7	Bangladesh	Non-specific	*E. coli*	Urine	25.84%	[86]
		Non-specific	*Klebsiella pneumoniae*		6.60%	
8	Bangladesh	*blaTEM*	*E. coli*	Urine	22.70%	[87]
		blaCTX-M			24.20%	
		blaSHV			4.30%	
9	Bangladesh	Non-specific	*K. pneumoniae*	Tracheal swabs, sputum, wound swabs, pus, blood, urine	50%	[88]
		Non-specific	*K. oxytoca*		25%	
10	Bangladesh	*blaCTX-M-3*	*Pseudomonas spp.*	Urine, swab, pus	78.00%	[89]
		blaCTX-M- 14			80.00%	
11	Bangladesh	*blaTEM*	*E. coli*	Stool	41%	[90]
		blaCTX–M–group–1			96%	
12	India	*blaCTX-M-15*	*E. coli*	Urine	52%	[91]
		blaOXA-2			8%	
13	India	Non-specific	*E. coli*	Pus	9.80%	[92]
				Urine	82.60%	
14	North-East India	*blaCTX-M*	*E. coli*	Urine, sputum, vaginal discharge	54.34%	[93]
		blaTEM			60.86	
		blaSHV			63.04%	
15	South India	*blaCTX-M-15*	*E. coli*	Urine, wound swab, sputum, pus, endotracheal secretions, bronchoalveolar lavage, bile fluid	90%	[94]

16	Bihar, India	*blaTEM*	*E. coli*	Stool	51.80%	[95]
		blaSHV			68%	
		blaCTX-M			86.10%	
17	India	*blaSHV*	*Pseudomonas aeruginosa*	Urine, blood, sputum, endotracheal aspirate	15.10%	[74]
		blaTEM			57.10%	
18	Pakistan	*blaCTX-M-15*	*E. coli*	Fecal samples	86.20%	[96]
19	North-West Pakistan	Non-specific	*P. aeruginosa*	Burn patients	35.85%	[97]
20	Lahore, Pakistan	*blaCTX - M*	*E. coli, Klebsiella spp., Pseudomonas aeruginosa, Enterobacter spp., Acinetobacter spp.*	Urine, pus, wound swabs	76%	[98]
		blaTEM			28%	
		blaSHV			21%	
21	Faisalabad, Pakistan	*blaCTX-M-1*	*E. coli*	Dog owners	59%	[99]
				Cat owners	73.90%	
				Veterinary professionals	80%	
22	Pakistan	*blaCTX-M1*	*K. pneumoniae*	Hospital waste	71%	[100]
		blaTEM			53%	
		blaSHV			6%	
23	Lahore, Pakistan	*blaCTX-M-I*	*E. coli*	Clinical specimens	72.10%	[98]
		blaCTX-M-II			8.50%	
24	Peshawar, Pakistan	*blaCTX-M-15*	*Pseudomonas aeruginosa*	Clinical specimens	19.71%	[101]
25	Lahore, Pakistan	Non-specific	*E. coli*	Healthy individuals	57.00%	[102]
				Patients	53.00%	
26	Faisalabad, Pakistan	*blaCTXM-1*	*E. coli*	Urine	70%,	[103]
		blaTEM-1			74.40%	
		blaCTXM-15			49%	

According to the SMART Antimicrobial Surveillance Program, ESBL-producing *Enterobacteriaceae* have shown a clear and consistent upward trend globally. A 10-year review (2002–2011) demonstrated increasing ESBL prevalence in *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*, with the steepest rises observed in Asia, Latin America, and the Middle East, and smaller but notable increases in Europe and North America [104][105]. ESBL rates in intra-abdominal infections increased significantly across Asia, Europe, Latin America, and the Middle East, while urinary tract infections showed marked increases mainly in Asia and the Middle East. Despite the rise in ESBL producers, most *E. coli* and *K. pneumoniae* isolates remained susceptible to carbapenems during this period. ESBL production was also strongly associated

with fluoroquinolone resistance, especially among *E. coli*. Regional analyses, such as a 2008 Latin American SMART report, revealed particularly high ESBL rates (~26.8% in *E. coli* and ~37.7% in *K. pneumoniae*). More recent global SMART data (2015–2019) further confirmed increasing ESBL non–carbapenem-resistant phenotypes, with over 50% ESBL positivity in *E. coli* in several countries including India, Thailand, Vietnam, China, Russia, Mexico, Kenya, and Kuwait, alongside rising ESBL *K. pneumoniae* rates in Latin America and North America. Together, SMART findings highlight a sustained global rise in ESBL-producing *Enterobacteriaceae* with strong geographic variation [105], [106].

In Europe, although prevalence is generally lower, marked geographic heterogeneity exists. Southern and Eastern European countries, including Italy, Greece, Romania, and Bulgaria, report significantly higher ESBL rates compared to Northern Europe. In contrast, Scandinavian countries and the Netherlands maintain relatively low prevalence due to stringent antibiotic stewardship programs and robust surveillance frameworks [107], [108]. In North America, ESBL rates range from 10–15% in most reports, though certain states have reported higher community-associated ESBL infections, particularly among urinary tract isolates. Africa and Latin America remain underrepresented in global surveillance data; however, available studies show high ESBL prevalence, often associated with limited diagnostic access, informal antibiotic markets, and inadequate sanitation infrastructure [109], [110], [111].

International travel also contributes substantially to the spread of ESBL-producing strains. Several studies demonstrate that travelers returning from South Asia, Southeast Asia, or Africa frequently acquire ESBL-producing *E. coli*, even without developing symptoms, and may transmit them to household contacts. Foodborne transmission is another emerging concern, with ESBL-producing *Enterobacteriaceae* detected in poultry, livestock, vegetables, and retail meat products, highlighting the importance of a One Health perspective [15], [112], [113], [114].

2.4. Pediatric Burden: Incidence, Morbidity, and Mortality

Children represent one of the most vulnerable groups in the global ESBL epidemic. Pediatric patients, especially those under five years of age, are more susceptible to gastrointestinal infections due to immature immune systems, frequent environmental exposure, and malnutrition

an important risk factor in low-income settings. ESBL-producing *Enterobacteriaceae* are increasingly associated with pediatric diarrheal diseases, urinary tract infections, bloodstream infections, neonatal sepsis, and hospital-acquired infections[115], [116].

Globally, diarrheal diseases remain a leading cause of morbidity and mortality among children [117]. IHME data (published in Lancet Infectious Diseases) estimate 1.2 million diarrheal deaths in 2021, with high burden in sub-Saharan Africa and South Asia [118] There is good evidence that *E. coli* (various pathotypes) contributes to diarrheal disease and that ESBL-producing *E. coli* (and other Enterobacterales) cause antimicrobial challenges [119]. Although not all diarrheal cases are bacterial, enteric bacteria such as *E. coli*, *Klebsiella spp.*, *Enterobacter spp.*, and *Citrobacter spp.* are significant contributors, and the rise of ESBL-producing strains exacerbates clinical outcomes [51], [120], [121]. Studies from India, Nepal, Pakistan, and Bangladesh reveal that ESBL-producing *E. coli* and *K. pneumoniae* are frequently isolated from pediatric diarrheal stool samples, often exhibiting multidrug resistance patterns that severely limit therapeutic choices [48], [122], [123], [124], [125].

Neonatal infections caused by ESBL-producing *Enterobacteriaceae* (ESBL-E), such as *K. pneumoniae, Enterobacter spp.,* and *E. coli*, pose a serious challenge in South Asia and Africa. Outbreaks in neonatal units are linked to high mortality rates ranging from 20% to 60%. These infections are often acquired through maternal transmission, contaminated birth environments, or prolonged hospitalization. Treatment is difficult due to resistance to first-line antibiotics like ampicillin and cephalosporins. Mortality among neonates with ESBL bloodstream infections has been reported at around 36%, significantly higher than in non-ESBL infections, underscoring the urgent need for improved infection control and antibiotic stewardship in these regions [126], [127], [128].

In Bangladesh, the pediatric burden is particularly significant. Studies conducted at icddr,b, Dhaka Medical College, and other tertiary centers consistently report high ESBL prevalence in enteric bacterial isolates from children. Factors contributing to this include widespread antibiotic misuse, poor sanitation, over-the-counter antibiotic sales, and limited infection control practices. The gastrointestinal tract of Bangladeshi children often acts as a reservoir for ESBL-producing

bacteria, increasing the likelihood of transmission within households and communities [78], [124], [129], [130], [131].

2.5. Temporal Trends: Evolution of *CTX-M, SHV*, and *TEM* Families

Extended-spectrum β-lactamases (ESBLs) are enzymes—mainly Ambler class A and some class D β-lactamases—that can break down many broad-spectrum penicillins and cephalosporins, including oxyimino-β-lactams. They are inhibited by β-lactamase inhibitors and are grouped under Bush–Jacoby–Medeiros functional classification subgroups 2be and 2d [132], [133], [134]. ESBLs are typically defined as acquired β-lactamases, most often carried on plasmids [135], [136]. They represent a relatively recent evolutionary adaptation, with the first clinical isolates harboring ESBLs reported in Germany in 1983 [137], [138]. By 1985, the first hospital outbreaks involving ESBL-producing bacteria emerged in France, followed by similar occurrences in the United States toward the late 1980s and early 1990s [139], [140], [141]. Since their emergence in 1983, the number of identified ESBL variants has steadily increased, highlighting their rapid evolutionary diversification. Based on amino acid sequence analysis, ESBLs are grouped into nine structural or evolutionary families, including *TEM, SHV, CTX-M, PER, VEB, GES, TLA, BES*, and *OXA*. By the early 2000s, the *TEM* and *SHV* families remained the predominant groups, representing the earliest ESBL types discovered. The majority of what we know regarding the evolution of ESBL is derived from research on *TEM* and *SHV* enzymes, which are primarily structural mutants of *TEM-1, TEM-2*, and *SHV-1* penicillinases, respectively [142].

The molecular epidemiology of ESBLs has shifted dramatically over time. Initially, ESBLs were predominantly derived from mutations in *blaTEM* and *blaSHV* genes, which were common in hospital settings during the 1980s and 1990s. These enzymes were primarily associated with nosocomial outbreaks, particularly among *K. pneumoniae* isolates. However, the emergence of the *CTX-M* family in the early 2000s marked a turning point in global ESBL epidemiology [104], [143], [144].

The *blaCTX-M* group, particularly *CTX-M-14* and *CTX-M-15*, has become the most prevalent ESBL worldwide. *CTX-M-15* is strongly linked with the globally disseminated *E. coli* clone ST131, driving widespread community and hospital infections. The genes encoding *CTX-M* enzymes are often on mobile IncF plasmids, facilitating their rapid international spread. Currently, *CTX-M* ESBLs represent over 70% of ESBL phenotypes in many regions, surpassing *TEM* and *SHV* types as the dominant resistance mechanism [72], [94], [145], [146].

Despite the dominance of *CTX-M* enzymes, the *TEM* and *SHV* families remain epidemiologically important. Mutated variants such as *TEM-135* and *SHV-12* continue to circulate, especially in Asia, Africa, and Latin America [147], [148], [149]. Co-existence of multiple ESBL genes within single isolates is increasingly reported, contributing to complex resistance phenotypes. These molecular trends reflect ongoing evolutionary pressure driven by heavy antibiotic use in clinical medicine, agriculture, and the environment [52].

2.6. Positioning ESBLs Within Modern β-Lactamase Classification Frameworks

The first β-lactamase classification that included ESBLs was proposed in 1989 by Karen Bush. She defined group 2b' enzymes as those that can break down oxyimino-β-lactams (like cefotaxime, ceftazidime, and aztreonam) at ≥10% of the rate for benzylpenicillin and are strongly inhibited by clavulanate (**Figure 1**). Later, this group was renamed as group 2be in the functional classification developed by Bush, Jacoby, and Medeiros [150], [151]. In this classification, ESBLs are defined as class A β-lactamases that can hydrolyze extended-spectrum β-lactam antibiotics and are inhibited by common β-lactamase inhibitors like clavulanate, sulbactam, and tazobactam. Initially, only plasmid-mediated ESBLs were included, but the updated system now recognizes that ESBL genes can move between plasmids and chromosomes. Traditional ESBLs are blocked by both older inhibitors (clavulanate, sulbactam, tazobactam) and newer ones such as avibactam, relebactam, and vaborbactam. Although some suggest classifying any enzyme that breaks down oxyimino-β-lactams as an ESBL, the strict definition still requires that the enzyme must be inhibited by clavulanate [152], [153], [154], [155].

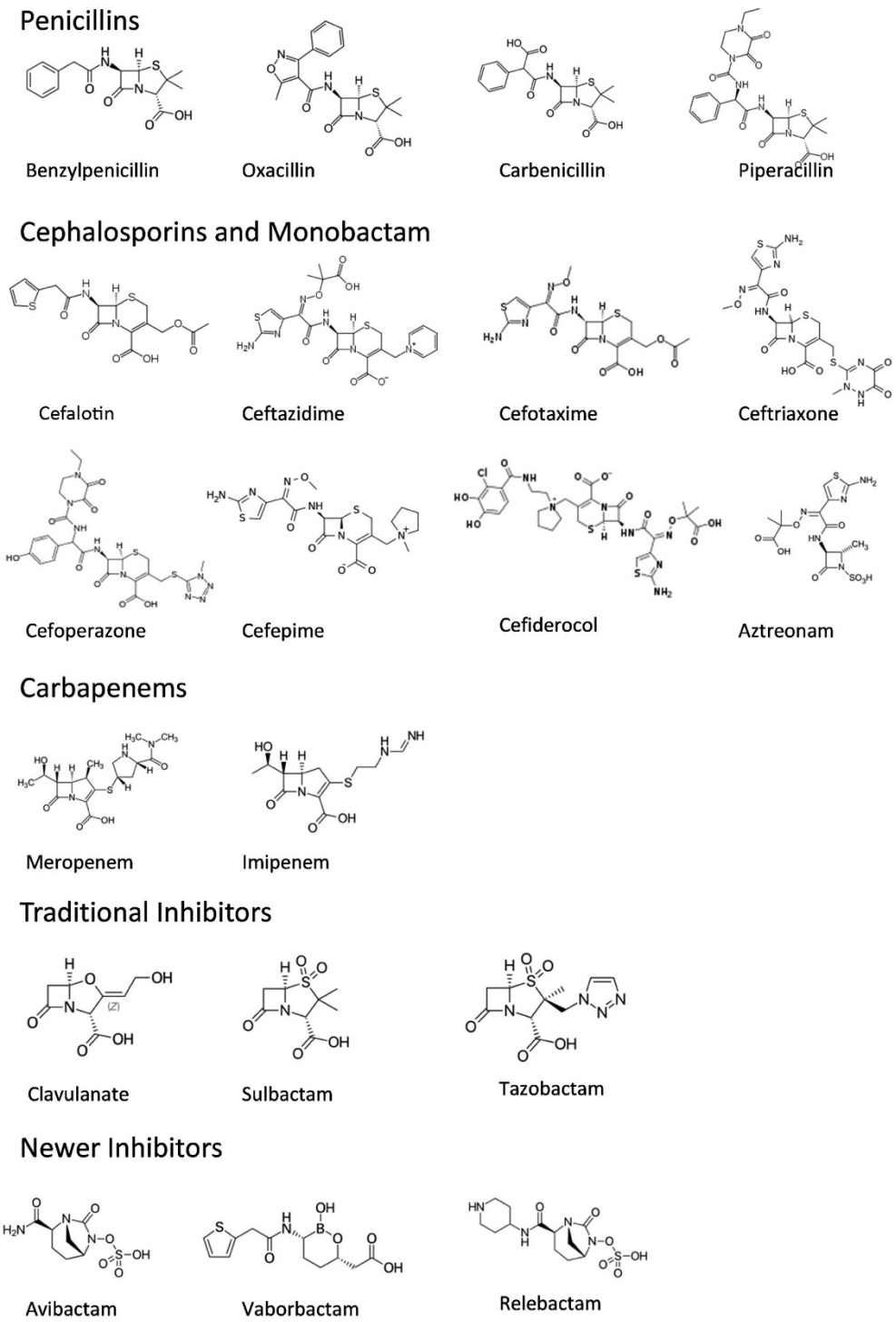


Figure 1. Chemical structures of β-lactam antibiotics and the inhibitors designed to block β-lactamase activity [44].

2.7. Overview of Predominant ESBL Families and Their Global Significance

ESBLs share similar functions, like breaking down extended-spectrum β-lactam antibiotics and being blocked by clavulanate, but their genes are different. They are grouped into several families [143]. TEM and SHV types are very similar, with only small differences in their amino acids, while CTX-M types are more diverse. Each family also has its own unique features.

Table 2. Classification of ESBL Families [156].

Family	Nomenclature	Characteristics
TEM	Temoneira, the patient infected with the first isolate expressing *TEM-1*	Point mutation variants of *TEM-1* or *TEM-2*
SHV	Sulfhydryl reagent variable	Point mutation variants of *SHV-1*
IRT	Inhibitor-resistant *TEM*	TEM variants that are resistant to inhibition by clavulanate and sulbactam, but do not have ESBL phenotype
CMT	Complex mutant derived from *TEM-1*	TEM variants that are resistant to inhibition by clavulanate and sulbactam and also have ESBL phenotype
CTX-M	Cefotaxime-hydrolysing β-lactamase isolated in Munich	Derived from the chromosomal β-lactamase from *Kluyvera* spp.
		Preferentially hydrolyses cefotaxime
GES	Guiana-extended spectrum	More prevalent in *P. aeruginosa* than Enterobacterales
		Some variants also hydrolyse carbapenems
PER	*Pseudomonas*extended resistant	More prevalent in *P. aeruginosa* and *A. baumannii* than Enterobacterales
		Inhibition by newer β-lactamase inhibitors is variable
VEB	Vietnam extended-spectrum β-	Preferentially hydrolyses ceftazidime and

	lactamase	aztreonam compared with cefotaxime
		Inhibition by newer β-lactamase inhibitors is variable
BEL	Belgium extended β-lactamase	Preferentially hydrolyses ceftazidime and aztreonam compared with cefotaxime
TLA	Named after the Tlahuica Indians (Mexico), from whom the first isolate was obtained	Preferentially hydrolyses ceftazidime and aztreonam compared with cefotaxime
SFO	From *Serratia fonticola*	Inducible
OXY	From *Klebsiella oxytoca*	Chromosomally encoded

2.7.1. *TEM*

TEM-type ESBLs are derived from the original plasmid-mediated β-lactamase, *TEM-1*, first identified in the 1960s from an *E. coli* isolate of a Greek patient named Temoneira [157], [158]. *TEM-2*, the first derivative, has a single amino acid change (Gln39Lys) but the same substrate profile as *TEM-1* and became the ancestor of many TEM-type ESBLs. The first *TEM* variant showing the ESBL phenotype was *TEM-3*, reported in 1989. To date, 243 *TEM* variants have been described, though not all are ESBLs [143], [159], [160]. Amino acid changes in *TEM* enzymes occur at specific positions that affect the ESBL phenotype [143]. Key residues include Gly238 and Glu240 on the β3 sheet, Arg164 on the Ω loop, and Glu104 near the active site. Changes like Gly238Ser and Glu240Lys have the greatest effect (**Figure 3**). Some newer variants, such as *TEM-184*, show altered substrate preferences, hydrolyzing aztreonam better than other drugs. While many new variants are found through WGS, few are tested phenotypically, though computer models can predict whether a variant is broad-spectrum, ESBL, or inhibitor-resistant [161], [162], [163], [164]. *TEM-3* was common in France but rare in the USA [165], where *TEM-10* was the most prevalent TEM-type ESBL [166]. TEM-26 has been found worldwide. However, with the global rise of CTX-M-type β-lactamases, TEM-type ESBLs have become rare, appearing in less than 1% of ESBL-producing *E. coli* and Klebsiella pneumoniae in recent European surveys [165], [167], [168], [169], [170].

Figure 2. Amino acid alignments of *TEM-1*, *SHV-1* and *CTX-M-1*. The amino acid sequences WP_000027057.1 (*TEM-1*), WP_001620095.1 (SHV-1) and WP_013188473.1 (*CTX-M-1*) were obtained from NCBI and aligned using Clustal Omega. Numbering according to Ambler. Asterisk (*) indicates positions that have a single, fully conserved residue. Colon (:) indicates conservation between groups of strongly similar properties. Period (.) indicates conservation between groups of weakly similar properties. The yellow highlights show the active site Ser70-X-X-Lys active site common to all serine β-lactamases. Red amino acids denote residues where substitutions provide ESBL phenotype (*TEM* and *SHV*). Blue amino acids denote where substitutions provide inhibitor resistance phenotype. Green indicates position 240 in *CTX-M-1*, which has been identified as being associated with increased hydrolysis of cefotaxime [44].

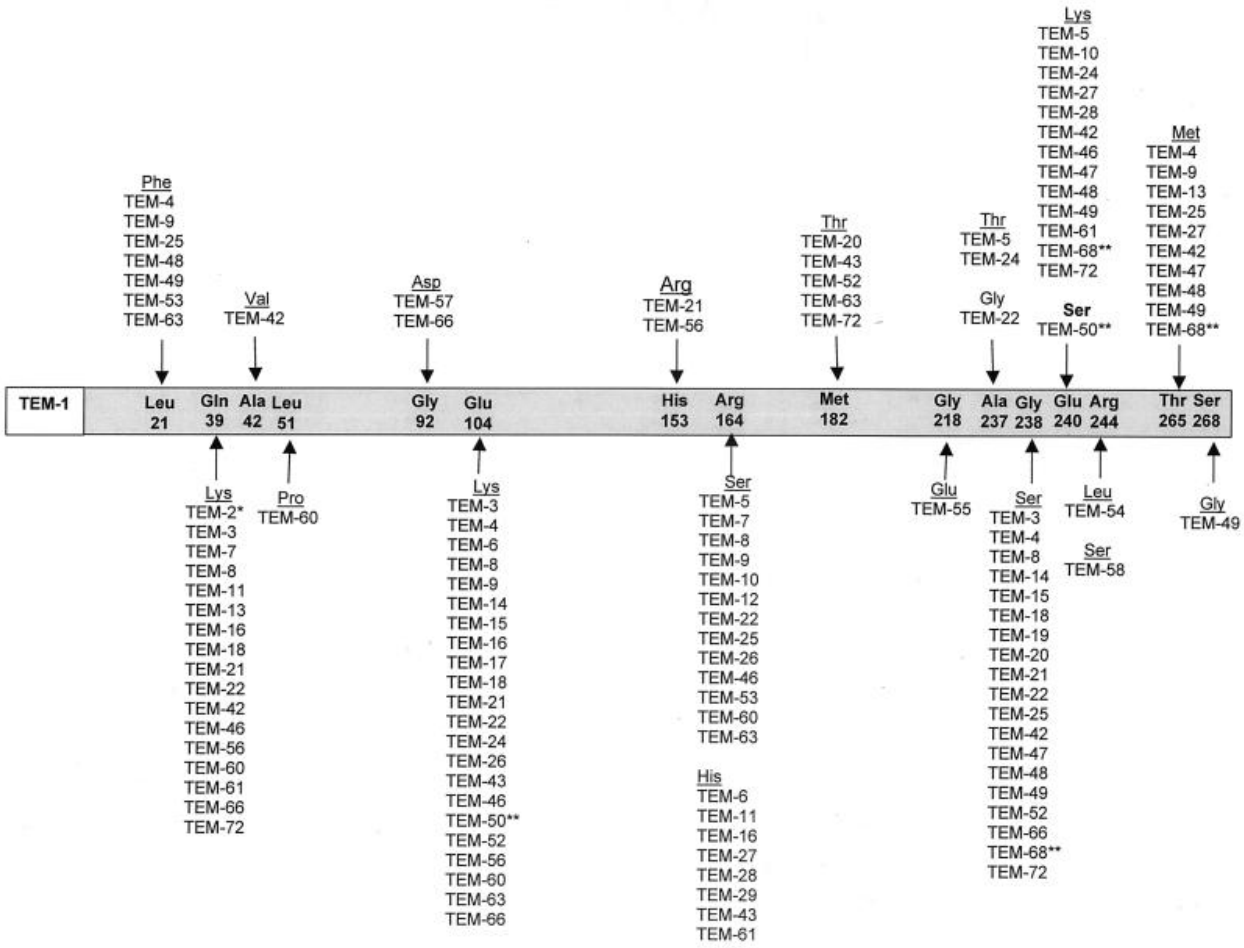


Figure 3. Amino acid changes in *TEM* ESBL derivatives are shown relative to *TEM-1* (grey bar) using Ambler numbering. Variants may have multiple substitutions. TEM-2, although not an ESBL, is included as a *TEM-1* derivative; its Gln39Lys change does not cause the ESBL phenotype, but many ESBLs originate from *TEM-2. TEM-50* and *TEM-68* have substitutions shared by both ESBL and IRT types. Only amino acid changes common to TEM-type ESBLs are shown [143].

2.7.2. *SHV*

SHV-type β-lactamases (named for "sulfhydryl reagent variable") originated as chromosomal enzymes in *K. pneumoniae*. The first ESBL, *SHV-2*, was reported in 1985 from a Klebsiella ozaenae strain in Germany and differed from *SHV-1* by a single amino acid change, Gly238Ser [135], [171]. Like TEM-type ESBLs, most SHV-type ESBLs have mutations at *Ambler* positions 238 (Gly→Ser) and 240 (Lys→Glu). The Gly238Ser change is key for hydrolyzing ceftazidime, while Lys240 is important for cefotaxime hydrolysis. Mathematical models have explored how these substitutions affect substrate profiles [171], [172]. So far, 228 SHV variants have been identified, though not all have been tested for

ESBL activity. Globally, *SHV-5 and SHV-12* are the most common ESBL variants in Enterobacterales [173], [174]. Recent European surveillance found SHV-type ESBLs in 3.1%–17.0% of K. pneumoniae isolates, depending on the region. In a clinical trial on ceftazidime-resistant pathogens from complicated intra-abdominal and urinary tract infections, SHV-type ESBLs were rare and only appeared in strains also producing AmpC or carbapenemases [170], [175]. While TEM- and SHV-type ESBLs are still detected, their impact on clinical isolates is now minimal.

2.7.3. *CTX-M*

CTX-M-type β-lactamases were first reported in the late 1980s in multiple locations. The name *CTX-M* (cefotaximase from Munich) came from Germany [176], while the same enzymes were called FEC-1 and Toho-1 in Japan, and *MEN-1* in France [177]. These enzymes quickly spread, leading to what is now called the '*CTX-M* pandemic.' Since the early 2000s, *CTX-M* enzymes have become the most common ESBLs, overtaking *TEM* and *SHV* types. *CTX-M* variants are found in *Enterobacterales, P. aeruginosa*, and *Acinetobacter spp.*, in hospitals, communities, animals, food, and the environment [178], [179], [180].

Most *CTX-M* enzymes are grouped into five clusters based on sequence similarity: *CTX-M-1, -2, -8, -9, and -25.* The *CTX-M-1* group is the most common, with *CTX-M-15, CTX-M-3, and CTX-M-1* being frequent [177], while *CTX-M-9, -14*, and recently -27 dominate the *CTX-M-9* group [181], [182], [183], [184], [185]. CTX-M-2, -8, and -25 are the main variants in their groups. Studies show *CTX-M-2* genes originated from Kluyvera ascorbata (*KLUA-1*), *CTX-M-134* from *KLUC-1* of *Kluyvera cryocrescens*, and *CTX-M-9* from *KLUG-1* of *Kluyvera georgiana*. *CTX-M* enzymes also share structural and hydrolytic features with other environmental class A β-lactamases, like those from Erwinia persicina and Rahnella aquatilis [186], [187], [187]. Early *CTX-M* variants were efficient at hydrolyzing cefotaxime and ceftriaxone, which gave them the name "cefotaximase." Unlike *TEM*- and *SHV*-type ESBLs, they initially had low activity against ceftazidime. Later, variants such as *CTX-M-15* (CTX-M-1 group) and *CTX-M-27* (*CTX-M-9* group) were found to hydrolyze ceftazidime effectively [177].

CTX-M-15 evolved from *CTX-M-3* through a single amino acid change at position 240 (Asp→Gly) in the B3 β-strand, which helps accommodate the bulkier ceftazidime molecule. This change modestly increases hydrolytic activity but significantly raises ceftazidime MIC values [188], [189], [190]. *CTX-M-27* has the same residue at position 240, resulting in elevated ceftazidime MICs despite lower activity against other

substrates compared to *CTX-M-14*. Clinical data show that ceftazidime MICs for CTX-M-producing isolates range widely, from 0.25 to >32 mg/L [191].

In 2019, Poirel et al. [192] described *CTX-M-33*, a *CTX-M* variant with a single amino acid change at position 109 (Asp→Ser) from *CTX-M-15*. This change reduced ceftazidime hydrolysis but increased meropenem hydrolysis, causing only a modest MIC rise in lab strains. In a clinical *K. pneumoniae* isolate, reduced permeability led to a meropenem MIC of 8 mg/L. *CTX-M* enzymes are widespread, and when combined with other resistance mechanisms or single mutations that expand hydrolysis, they may limit the effectiveness of meropenem and newer antibiotics.

2.7.4. Inhibitor-resistant β-lactamases

Inhibitor-resistant β-lactamases are TEM- and SHV-derived enzymes with amino acid changes that make them resistant to clavulanate and sulbactam, classified as functional group 2br. Most are still inhibited by tazobactam and avibactam. Most originate from *TEM-1* and were formerly called IRT (inhibitor-resistant *TEM*), but now follow sequential *TEM* numbering [155], [193], [194], [195]. Common amino acid changes in *TEM* variants occur at Met69, Ser130, Arg244, Arg275, and Asn276. These mutations confer resistance to clavulanate and sulbactam but reduce hydrolysis of some penicillins and cephalosporins, like cefalotin [196], [197]. Although rare, inhibitor-resistant *TEM-30* was found in KPC-producing *K. pneumoniae* during a CRE outbreak in New York City. SHV-type inhibitor-resistant β-lactamases, such as *SHV-49*, *-56*, and *-107*, have also been reported in clinical *K. pneumoniae* isolates from Europe [194], [198], [199], [200].

2.7.5. ESBL phenotype *OXA*-type β-lactamases

OXA-type β-lactamases, which hydrolyze oxacillin, belong to *Ambler* class D and Bush functional group 2d. They are diverse in sequence and substrate profile, and some variants can hydrolyze cephalosporins, cephems, or monobactams, showing an ESBL-like phenotype (Bush subgroup 2de). However, whether these oxacillinases are true ESBLs is debated, as they are not in the 2be group and are poorly inhibited by clavulanate and similar inhibitors [132], [154], [155]. According to a recent review, there are 27 oxacillinase enzymes described as extended spectrum. Most extended-spectrum oxacillinases derive from *OXA-10* (also named PSE-2) and *OXA-2*. The *OXA-10* derivatives include *OXA-11, OXA-13,*

OXA-14, OXA-16, OXA-17, OXA-19 and *OXA-28*. In addition, *OXA-16* has only a partial sequence submitted as its first description (GenBank #AF043100). Among the *OXA-2* derivatives, *OXA-15, OXA-32, OXA-34, OXA-36* (partial sequence), *OXA-53, OXA-141, OXA-161, OXA-210* and *OXA-226* have been described. Many *OXA-2* and *OXA-10* derivatives are detected in isolates of *P. aeruginosa* [201], [202].

2.8. Public Health and Economic Impact

The rise of ESBL-producing *Enterobacteriaceae* has profound public health and economic consequences. Infections caused by ESBL-E are associated with delayed effective therapy, prolonged hospitalization, increased need for second- or third-line antibiotics (including carbapenems), and higher treatment costs. Studies estimate that ESBL infections increase healthcare expenses by 1.5- to 3-fold compared to susceptible infections, largely due to extended hospital stays and more expensive medications [203], [204].

From a public health standpoint, ESBL-E pose a serious challenge to infection control efforts, particularly in resource-limited settings where diagnostic tests are often unavailable. Without routine screening and surveillance, outbreaks can spread unchecked within hospitals. Community spread is equally concerning, especially in areas with poor sanitation and high population density, such as slums and urban settlements in Bangladesh [205], [206], [207].

The environmental dimension of ESBL dissemination further amplifies the crisis. ESBL-producing bacteria have been detected in wastewater, rivers, household water, livestock, and vegetables, demonstrating that AMR is no longer restricted to clinical environments. These reservoirs facilitate continuous circulation of resistance genes, increasing the risk of transmission to vulnerable populations, including children [22], [108], [208].

2.9. Molecular Biology of ESBLs: Mechanisms and Classification

2.9.1. Enzymatic Mechanism of Beta-Lactam Hydrolysis

ESBLs are enzymes produced by certain bacteria that confer resistance by hydrolyzing beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams. The core catalytic process involves the acylation and deacylation of the enzyme's active site, primarily utilizing a serine residue to hydrolyze the beta-lactam ring (**Figure 4b**), a crucial structure in these antibiotics [209]. The hydrolysis mechanism resembles that of other serine beta-lactamases, binding the antibiotic substrate in the active site, where the serine hydroxyl group attacks the electrophilic carbon of the beta-lactam ring, forming a covalent acyl-enzyme intermediate. Subsequent deacylation releases the inactivated antibiotic, regenerating the free enzyme for another catalytic cycle [209], [210].

In oxyimino cephalosporins such as cefotaxime and ceftazidime, the bulky R1 and R2 side chains sterically hinder entry into the catalytic pocket of class A β-lactamases. However, ESBLs have acquired structural adaptations, particularly enlargements and increased flexibility of the active-site and Ω-loop regions, that enable efficient accommodation and hydrolysis of these expanded-spectrum substrates. The Ω-loop is a flexible, conserved loop located at the entrance to the active site of class A β-lactamases. It contains the essential Glu166 residue, which is crucial for the deacylation step in the hydrolysis of the β-lactam ring. Without this loop, the deacylation apparatus is eliminated. [16], [211], [212]. The conserved catalytic residues **Ser70, Lys73, Ser130, Glu166, and Asn170** (**Figure 4a**), which are characteristic of class A β-lactamases, coordinate nucleophilic attack, proton transfers, and deacylation, forming a catalytic machinery analogous to that of other serine hydrolases [189], [213].

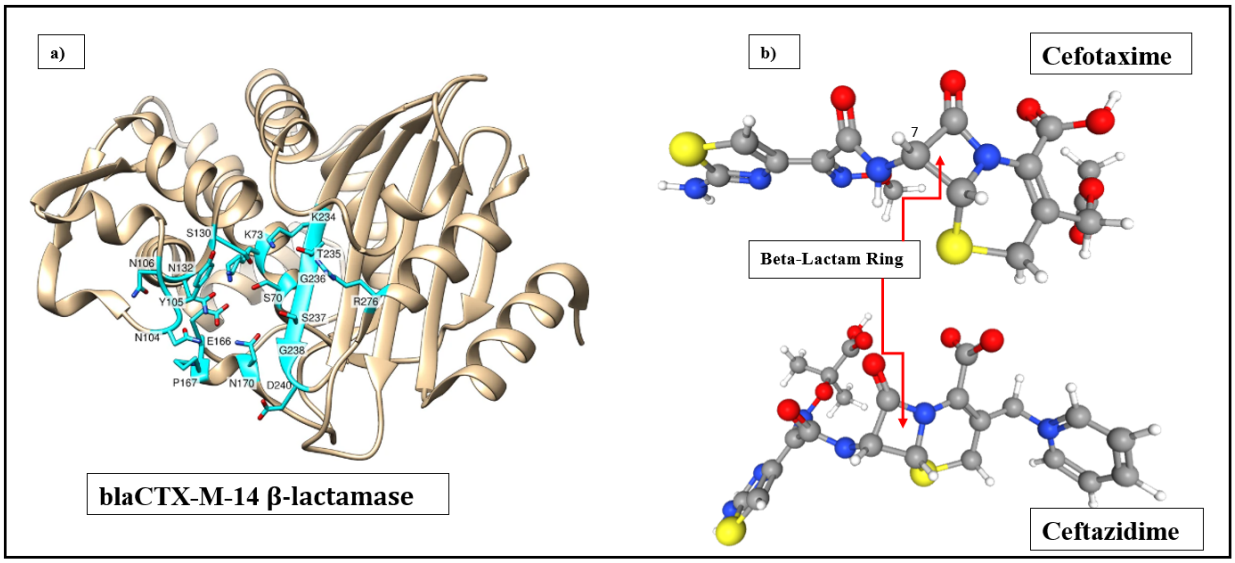


Figure 4. (a) X-ray crystal structure (PDB: 1YLT) of CTX-M-14 β-lactamase. 17 active site residues are highlighted in cyan. Heteroatoms of amino acid side chains are represented in red (oxygen) and blue (nitrogen) [212]. **(b)** Chemical structures of β-lactam antibiotics. The oxyimino-cephalosporins cefotaxime and ceftazidime are shown.

2.9.2. Phenotypic Implications

The hydrolytic activity of ESBLs results in resistance primarily to third-generation cephalosporins, aztreonam, and other beta-lactams. In clinical laboratories, phenotypic detection often involves susceptibility testing with antibiotics such as cefotaxime or ceftazidime, coupled with inhibitor-based tests using clavulanic acid or tazobactam. A characteristic feature is the "keyhole" or synergy zone in disc diffusion assays, indicating inhibition of ESBL activity by beta-lactamase inhibitors [16], [214].

However, phenotypic tests can sometimes be misleading due to co-existing resistance mechanisms such as AmpC beta-lactamases or carbapenemases that can mask ESBL activity [135], [215]. The limitations affect both sensitivity (e.g. false negatives due to the co-production of an AmpC β-lactamase) and specificity (e.g. false positivity due to hyperproduction of narrower-spectrum β-lactamases combined with altered permeability) [44]. Therefore, molecular detection of ESBL genes complements phenotypic assays, guiding effective treatment strategies.

2.9.3. Evolution: Mutation, Selection, and Spread

2.9.3.1. Mutation and Adaptation

ESBLs have evolved primarily through point mutations in bla genes, which encode beta-lactamase enzymes. These mutations often alter amino acids within or near the enzyme's active site, expanding the substrate profile to hydrolyze a broader range of beta-lactam antibiotics, especially third-generation cephalosporins and oxyimino-cephalosporins. A key region for such mutations is the Omega loop (residues 164–179), particularly in CTX-M-type ESBLs, where alterations modify the enzyme's conformation and enhance its ability to bind and hydrolyze extended-spectrum antibiotics efficiently [48], [216], [217].

Evolutionary studies of ESBLs, including TEM and SHV families, reveal a stepwise accumulation of point mutations and sometimes recombination events leading to diverse enzyme variants with extended activity. For example, TEM-derived ESBLs emerged from the narrow-spectrum TEM-1 and TEM-2 penicillinases through multiple independent mutational events, creating variants such as TEM-3, TEM-4, and others, each with altered catalytic efficiency and inhibitor susceptibility. Similarly, SHV enzymes, ancestral to chromosomal penicillinases in Klebsiella pneumoniae, evolved into ESBLs through single or multiple amino acid substitutions resulting in functional diversification [216], [218], [219].

These mutations primarily affect critical residues in the active site surroundings, such as those influencing substrate binding and hydrolysis rates. In CTX-M enzymes, mutations within the Omega loop can increase substrate affinity for ceftazidime and cefotaxime, driving the extended-spectrum activity characteristic of these enzymes. This molecular adaptation has facilitated the global spread of CTX-M-type ESBLs, now the most prevalent ESBL family worldwide [48], [217].

The evolutionary pressure from widespread use of beta-lactam antibiotics selects for these mutations, enabling bacteria harboring ESBLs to survive antimicrobial treatment and

disseminate resistance. The genetic mobility of bla genes, often carried on conjugative plasmids, further accelerates their horizontal transfer among bacteria, exacerbating the public health threat posed by multidrug-resistant pathogens [216], [219].

2.9.3.2. Horizontal Gene Transfer

The rapid dissemination of ESBL genes predominantly occurs via plasmids, especially conjugative ones belonging to IncF, IncI, IncHI, and other incompatibility groups. The high mobility of these plasmids facilitates horizontal gene transfer between bacterial species and strains, promoting outbreaks of multidrug-resistant infections [220], [221].

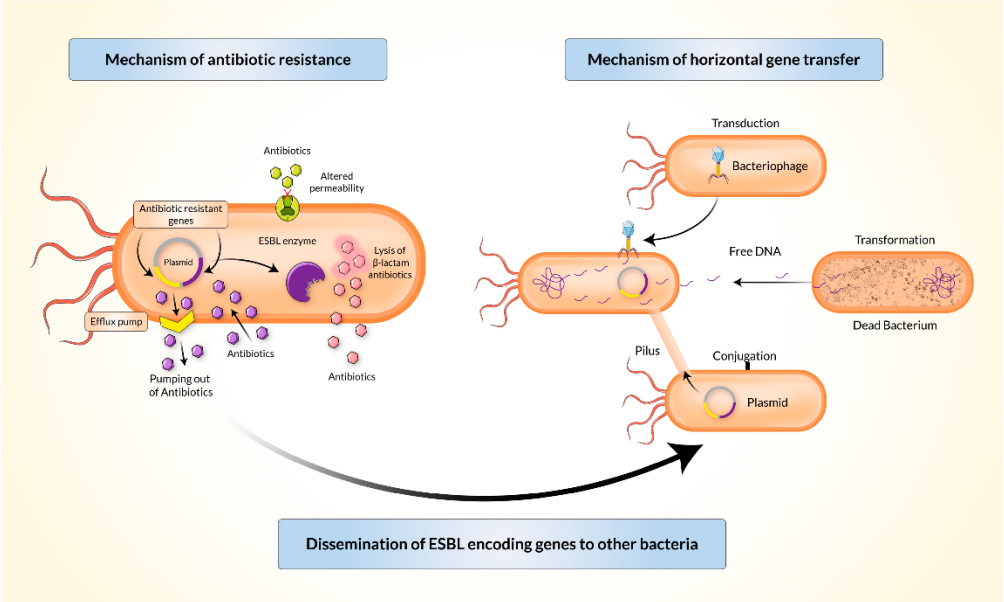


Figure 5. Mechanisms of antibiotic resistance and horizontal gene transfer by extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) [48].

2.9.3.3. Selection Pressure

Intense antibiotic use in both human medicine and agriculture has exerted selective pressure favoring the spread of ESBL producers. The widespread use of third-generation cephalosporins, aztreonam, and related compounds has maintained and expanded the reservoir of ESBL genes in pathogenic and commensal bacteria [16], [222], [223], [224].

2.9.3.4. Global Spread

Global dissemination is driven by travel, trade, and inadequate infection control, with *bla*$_{\text{CTX-M-15}}$ dating back to origins in South Asia but now prevalent across continents. The ability of ESBL genes to reside on transferable plasmids and integrate into various bacterial genomes has facilitated their persistence and expansion worldwide [14], [23], [48].

Chapter 3

Methods and Materials

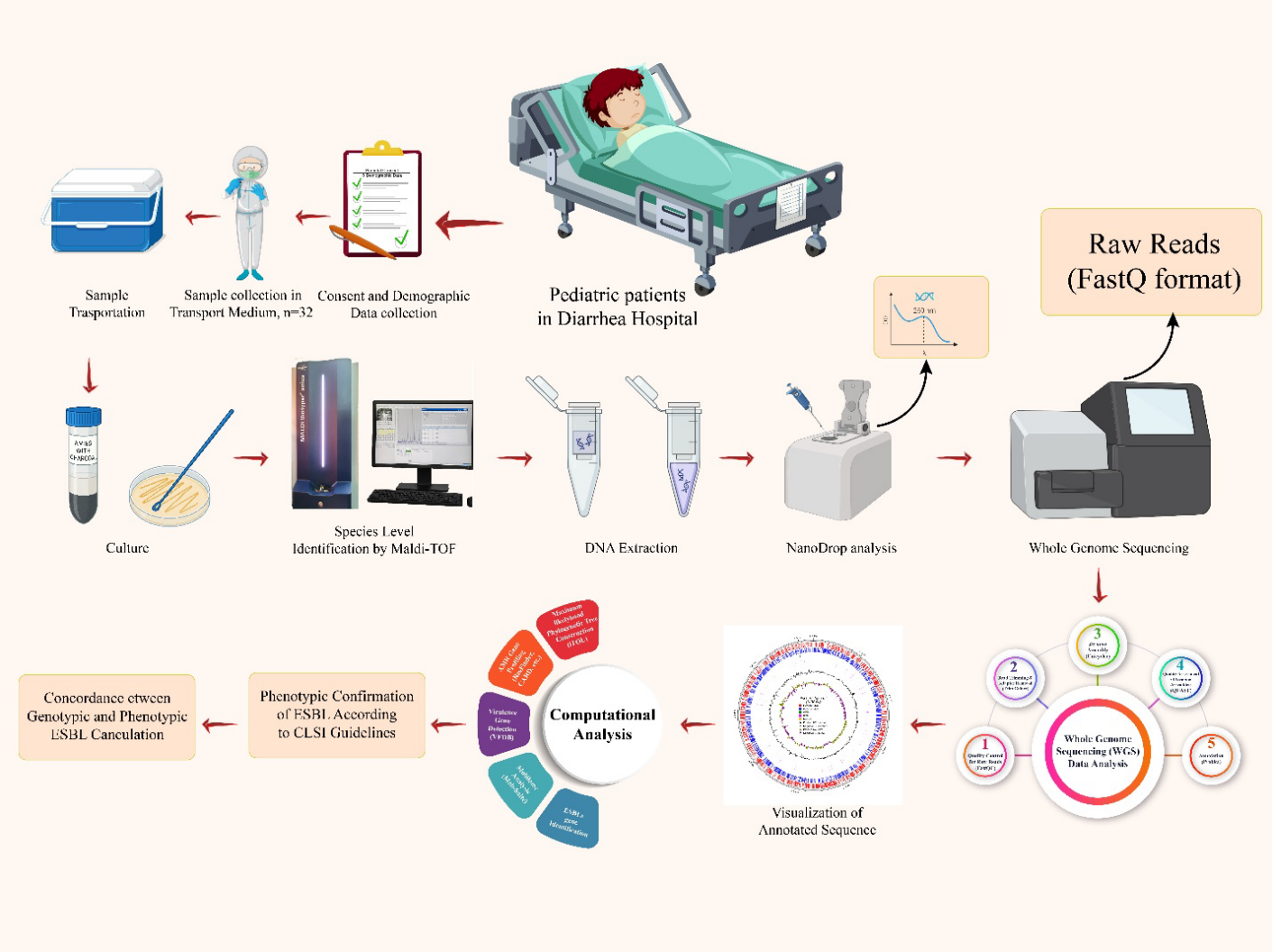


Figure 6. Overview of Sample Processing, Whole-Genome Sequencing, and Computational Analysis Workflow for Characterizing ESBL-Producing *Enterobacteriaceae* in Pediatric Diarrheal Patients.

A. Clinical Bacterial Isolates Associated with Diarrhea

3.1. Collection of Clinical Specimens

A total of 32 stool samples were obtained from children under 8 years old undergoing diarrhea at Dhaka Shishu Hospital & Institute in Dhaka (23.7732° N, 90.3628° E), and Lakshmipur Sadar Hospital in Laxmipur (22.9347°N, 90.8329°E). The sample demographic metadata were collected, which includes demographic details about the hospitalized participants age, origin, gender, and residence (**Supplementary Table S1)**. Clinical samples were collected under strict aseptic conditions using sterile containers with Amies transport medium **(Figure 7)** and were transported promptly within two hours to the respective laboratories maintaining 4 degree Celsius (Microbial Biotechnology Laboratory, National Institute of Biotechnology for Dhaka Shishu Hospital samples, and the Microbiology Department, NSTU for Lakshmipur Sadar Hospital samples) for comprehensive microbial examination, including isolation and identification.

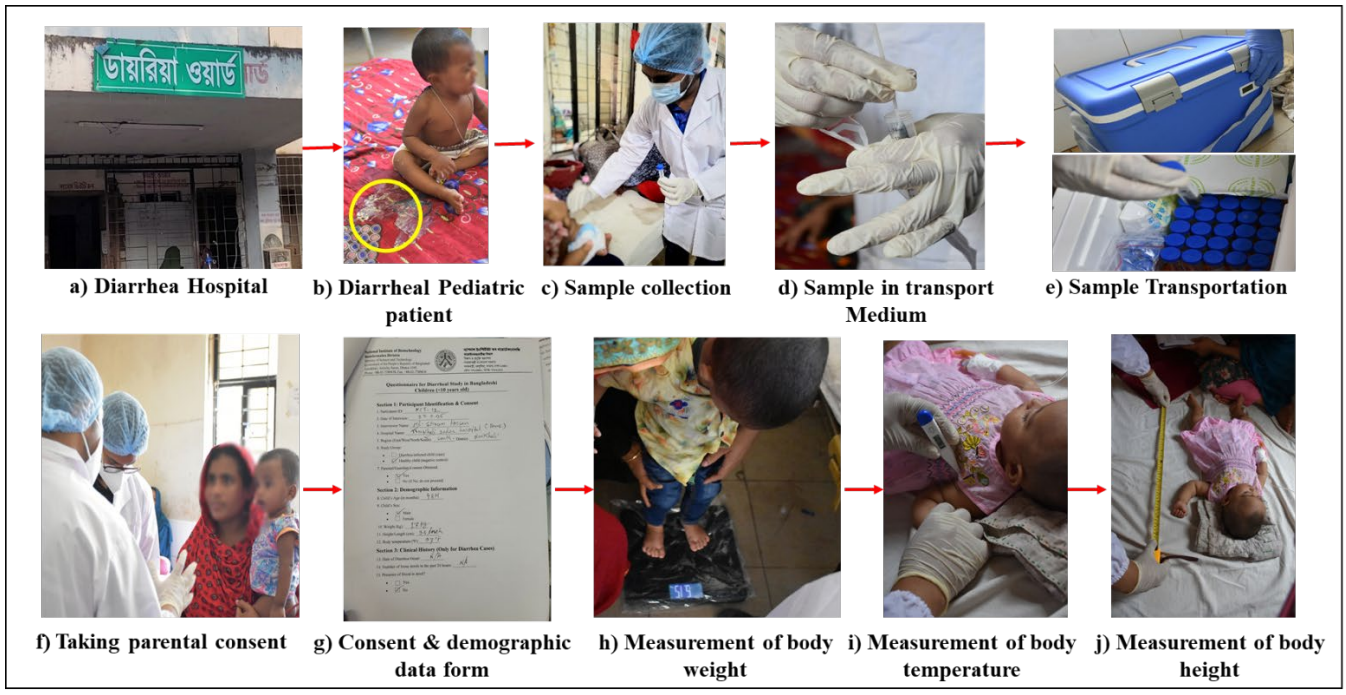


Figure 7. Sample collection and data acquisition process. **(a–j)** Collection of diarrheal stool samples from children and obtaining informed consent, along with demographic information from their parents or guardians.

3.2. Isolation of Bacteria

The sample was vortexed thoroughly to ensure homogeneity, and a loopful was then inoculated on MacConkey agar (Oxoid, UK) plate and incubated at 37°C for 24 hours. Bacteria that fermented lactose made pink or red colonies, while bacteria that didn't ferment lactose made pale or colorless colonies (**Figure 8**). Representative colonies were sub-cultured on fresh MacConkey agar to acquire pure isolates. Suspected colonies of both lactose fermenters and non-lactose fermenters from the selective media were subsequently streaked onto nutrient agar plates and incubated at 37°C for 18 to 24 hours in order to perform biochemical analysis.

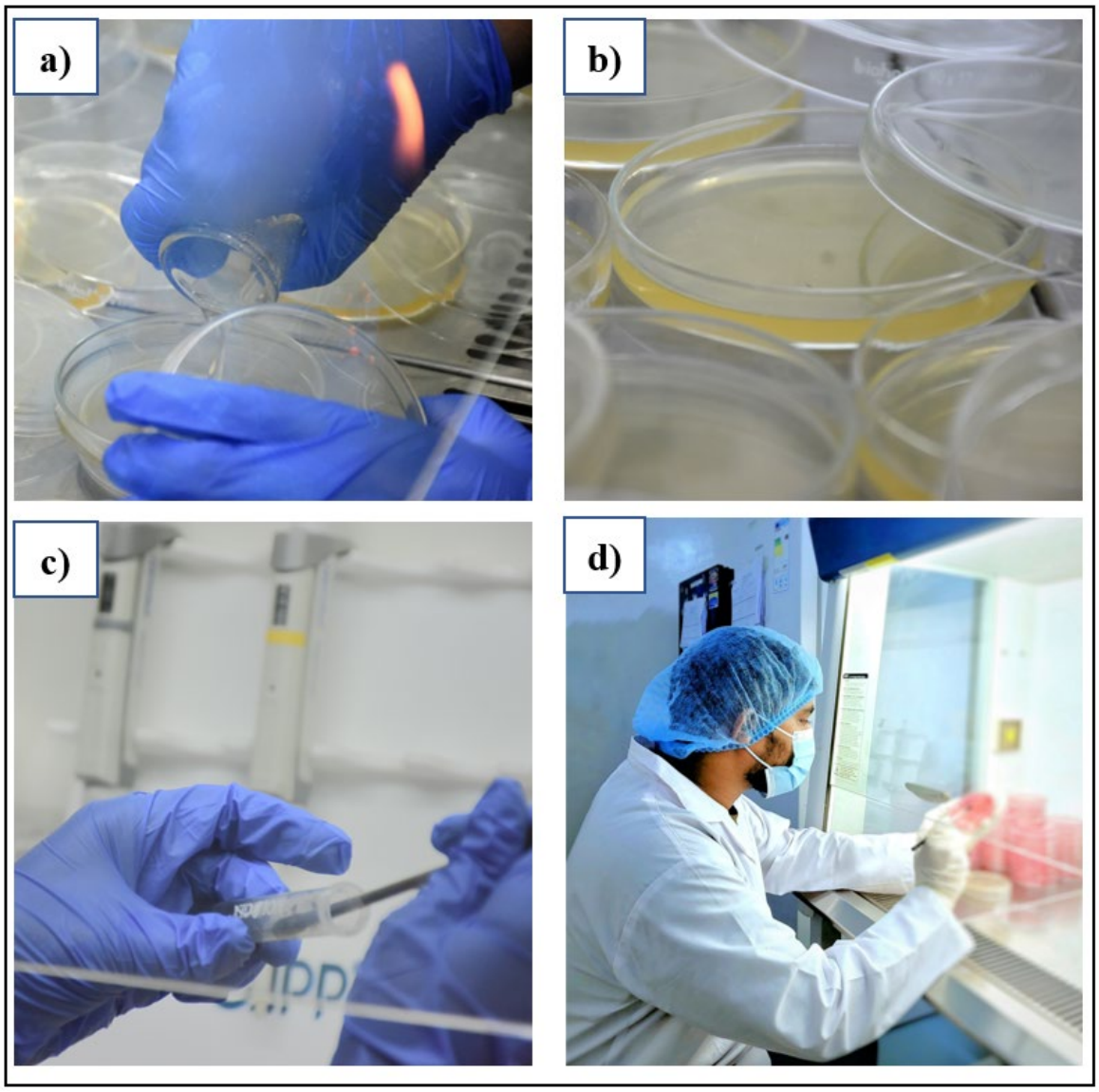


Figure 8. (a-b) Media preparation and **(c-d)** sample processing for bacterial culture.

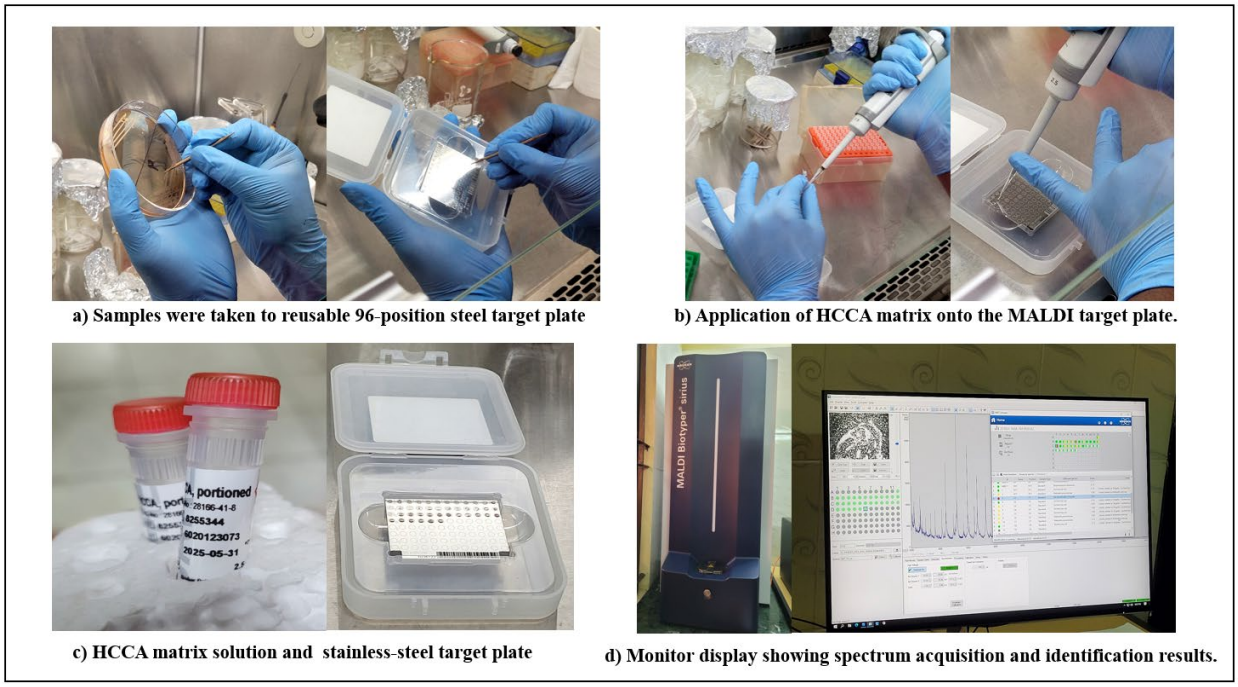
3.3. Species identification of clinical isolates

Colonies grown on nutrient agar were examined using MALDI-TOF mass spectrometry for species-level identification [225].

3.3.1. Identification of Bacteria through MALDI-TOF MS (Matrix-assisted laser desorption ionization-time of flight mass spectrometry)

1 µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was applied on top of the bacterial colonies that had been moved to a designated spot on the target plate (**Figure 9**). The plate was placed into the Bruker MALDI Biotyper® Sirius system after being allowed to air-dry at room temperature for five to ten minutes in order to allow crystallization. The device measured the time-of-flight (TOF) of ionized proteins to generate mass spectra. Based on distinctive mass-to-charge (m/z) ratios, the resulting spectral profiles were compared to the Bruker reference database in order to identify the bacterial species.

MALDI-TOF MS identifies bacteria by detecting the mass-to-charge (m/z) ratios of ribosomal proteins, generating a unique spectral fingerprint for each species. Instead of targeting specific biomarker peaks, it compares the overall mass profile to a reference database to determine the closest match [226].



a) Samples were taken to reusable 96-position steel target plate

b) Application of HCCA matrix onto the MALDI target plate.

c) HCCA matrix solution and stainless-steel target plate

d) Monitor display showing spectrum acquisition and identification results.

Figure 9. MALDI-TOF MS Biotyper and sample identification workflow**. (a–d)** Stepwise process of bacterial identification using the MALDI-TOF MS Biotyper, including sample spotting, matrix application, plate loading, spectrum acquisition, and result interpretation.

B. Genomic insights of diarrheal pathogen

3.4. Bacterial Extraction Genomic DNA and Purification

Genomic DNA from bacterial isolates was extracted using the Thermo Scientific GeneJET Genomic DNA Purification Kit, following the manufacturer's instructions. The kit's spin column system enabled fast and efficient high-quality genomic DNA isolation. DNA concentration and purity were checked using a NanoDrop™ 2000/2000c Spectrophotometer.

3.5. NanoDrop analysis

The NanoDrop system was utilized to quantify double-stranded DNA (dsDNA) using microvolume analysis, with a detection range of 2–3700 ng/µL and a 10 mm pathlength to measure absorbance. Before sample analysis, the NanoDrop spectrophotometer was calibrated by first measuring 1 µL of ultrapure water as a blank, followed by a second blank measurement using 1 µL of elution buffer from the Thermo Scientific GeneJET Genomic DNA Purification Kit. Bacterial DNA extracts were then quantified in duplicate (two technical replicates), with 1 µL of each sample used for measurement. The NanoDrop ND-1000 3.3 software was used to record the data (**Figure 10**). DNA concentrations and absorbance ratios, were exported as .txt files for further processing.

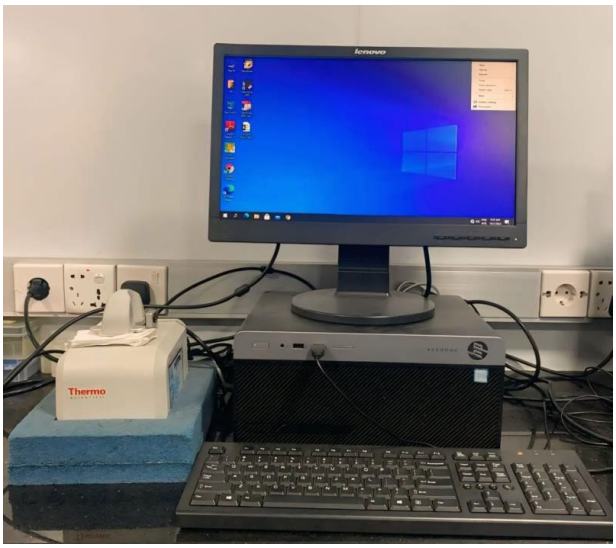


Figure 10. NanoDrop spectrophotometer for the measurement of DNA concentration and purity ratios.

3.6. Whole Genome Sequencing (WGS)

The MALDI-TOF validated bacterial 38 isolates underwent WGS on the Illumina MiSeq platform at the Center for Next-Generation Sequencing (NGS) facility at the National Institute of Biotechnology (NIB) (**Figure 11**). Genomic DNA was fragmented into 400–550 bp segments utilizing the M220 Focused-ultrasonicator (Covaris Ltd., Brighton, UK). Sequencing libraries were constructed employing the Nextera XT DNA Library LT kit (Illumina, San Diego, CA, USA), in accordance with the manufacturer's guidelines. The MiSeq Reagent Kit v3, Illumina, was used for WGS to sequence 300 bp paired-end reads.

Figure 11: Next Generation Sequencing (NGS) of the clinical isolates at NIB.

3.7. NGS Data Pre-processing and Quality Control

Raw FASTQ files generated by the MiSeq instrument were processed using a suite of bioinformatics tools within a Linux environment. Quality assessment was performed using FastQC (v0.12.1), followed by adapter and low-quality bases trimming with Trimmomatic (v0.6.10) [227], utilizing the NextTera adapter sequence. This procedure obtained high-quality paired-end reads in FASTQ format.

3.8. WGS data Assembly and Annotation

De novo genome assembly was performed using Unicycler (v0.5.1) [228], which utilized SPAdes (v4.0.0) genome assembler to construct a De Bruijn graph-based assembly with multiple k-mer sizes for error correction to minimize mismatching (**Figure 12**). The quality of the assembled genomes was subsequently assessed using QUAST (v5.3.0) [229]. This tool provides detailed insights into the assembly, including key metrics such as the N50 value, which reflects the minimum contig length needed to cover 50% of the genome. Once high-quality assemblies were confirmed, Prokka (v1.14.6) [230] was used to annotate the contigs, identifying protein-coding genes, tRNAs, and small RNAs. The resulting annotations were then explored and reviewed using a Python tool [231], allowing for visualization and better understanding of the genomic features.

Figure 12: Genome assembly using the Unicycler (v0.5.1) utilizing SPAdes (v4.0.0) genome assembler in the Linux operating system

3.9. Genomic exploration using computational analysis

3.9.1. Phylogenetic Tree Construction from Pangenome-Derived Core Gene Alignment

A pangenome analysis was performed using Roary v3.13.0 [232], which processed the Prokka-annotated GFF files and produced a core gene alignment (core_gene_alignment.aln) (**Figure 13**). This alignment was then used as input for FastTree to construct a maximum-likelihood phylogenetic tree, generating the final output in Newick format. The resulting tree was uploaded to iTOL (Interactive Tree of Life) v7 for visualization and annotation, where a circular layout was selected to make the evolutionary clustering among the isolates easier to compare and interpret.

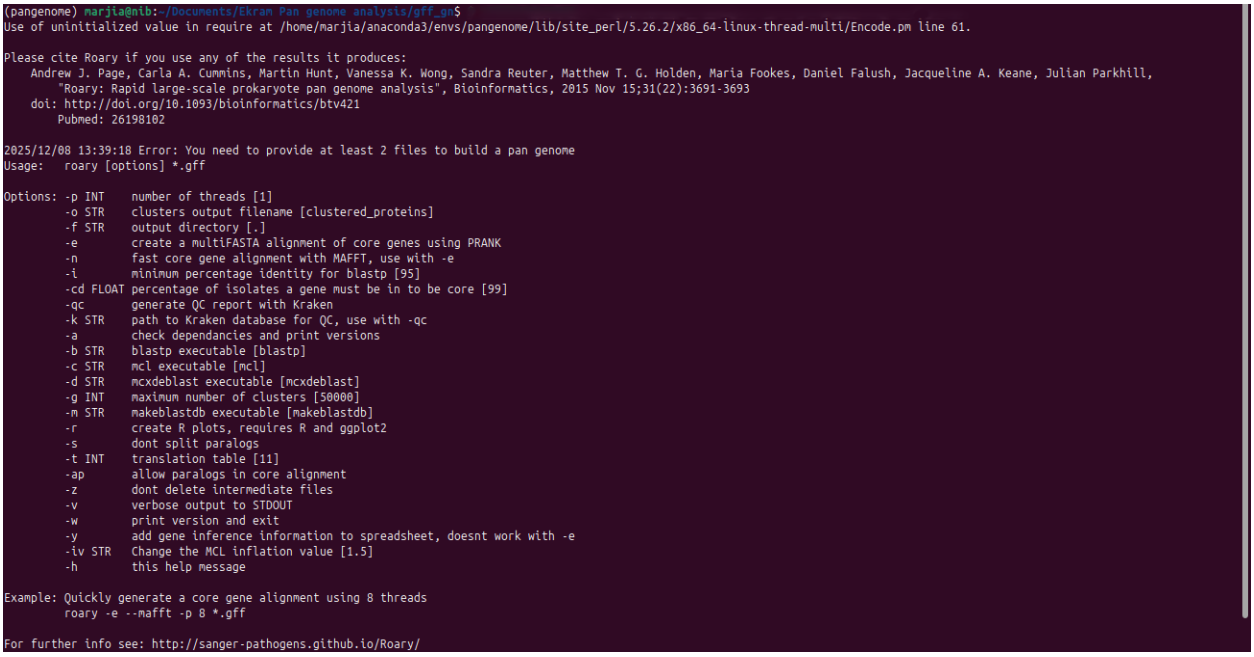


Figure 13. Core genome alignment produced by Roary v3.13.0 for downstream phylogenetic analysis.

3.9.2. Antimicrobial Resistance Gene Profiling Using ABRicate

Antimicrobial resistance (AMR) gene screening was conducted using ABRicate v1.0.1 [233] across multiple resistance gene databases (**Figure 14**), including CARD [234], ResFinder [31],

NCBI [62], MEGARes [235], and ARG-ANNOT [61]. A minimum DNA identity (minid) of 95% and a minimum DNA coverage (mincov) of 90% were applied as thresholds. The complete antibiotic resistance databases were utilized to identify potential AMR gene candidates. Output data were compiled into summary tables for comparative analysis, with a focus on detecting genes associated with ESBL production.

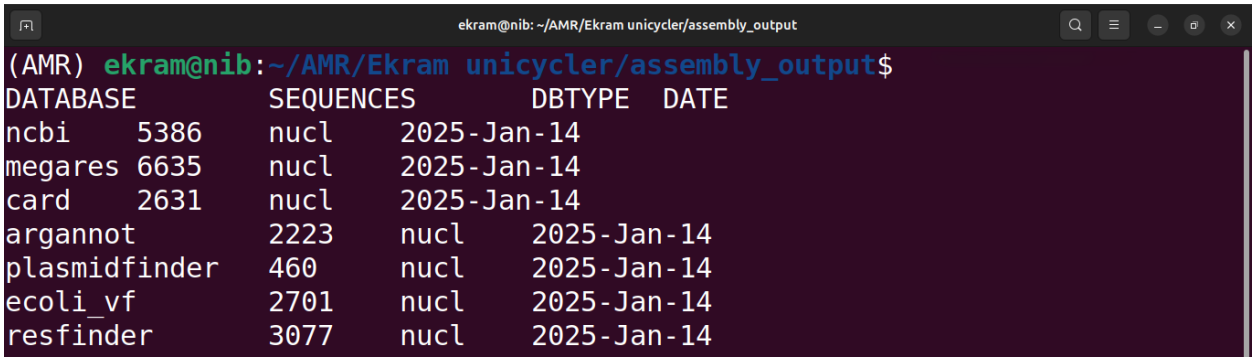


Figure 14. Databases utilized for curation of AMR genes in Linux

3.9.3. Virulence Gene Detection

Virulence-associated genes were identified using ABRicate with the VFDB database. Assembled genome sequences of all isolates were screened with a minimum coverage of 90% and minimum identity of 95% **(Figure 15)**. Only high-confidence hits were retained for further analysis to determine the virulence gene profiles of the isolates.

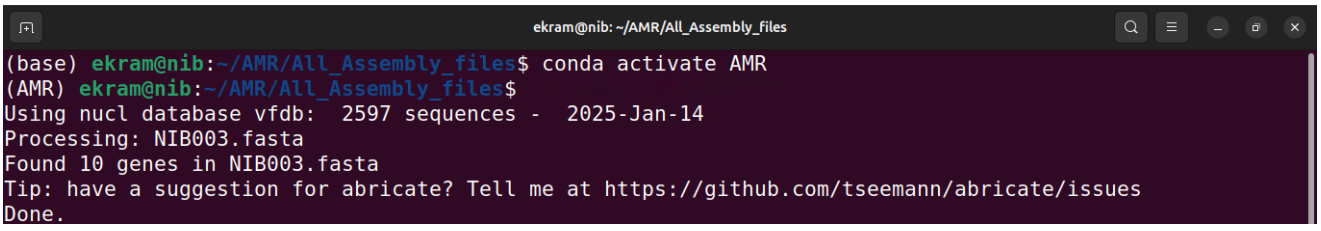


Figure 15. Identification of virulence genes in isolates using ABRicate against the VFDB database (mincov 90%, minid 95%).

3.10. Mobilome Analysis Revealing the Presence of Mobile Genetic Elements

Mobilome analysis was performed to identify mobile genetic elements (MGEs) associated with horizontal gene transfer and antimicrobial resistance dissemination [236]. Mobile genetic elements, including plasmids and insertion sequence (IS) elements, were identified using the MOB-suite toolkit (version 3.1.9) [237], which offers an integrated approach for plasmid reconstruction and typing (**Figure 16**). To begin, mob_recon v3.1.9 was used to rebuild plasmid sequences from the assembled genomes and then IS elements were analysed by mob_typer module of MOB-suite. PlasmidFinder [233] was used to detect plasmid replicons within the genomic assemblies.

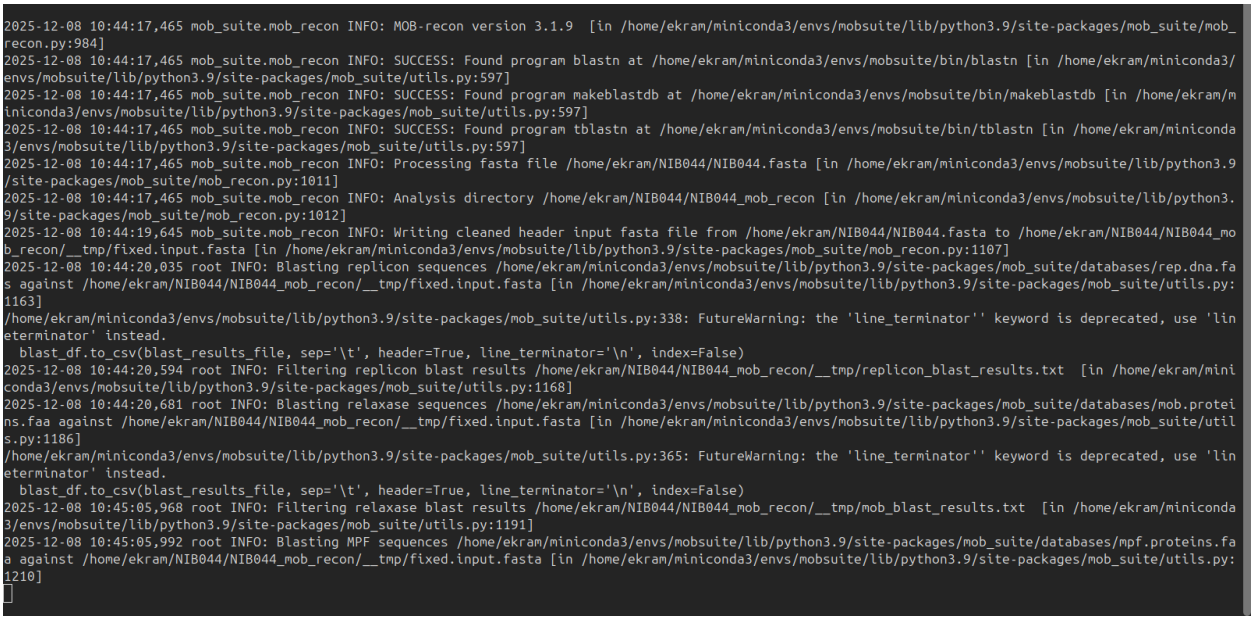


Figure 16. Executing the MOB-suite Plasmid Reconstruction Pipeline via Terminal.

C. Wet Lab validation for ESBL

3.11. The Disk Diffusion phenotypic testing of ESBL

The phenotypic identification of ESBL production was conducted by the Disk Diffusion Test (DDT) in compliance with CLSI guidelines (CLSI M100-Ed35). A standardized bacterial suspension, equivalent the 0.5 McFarland standard, was inoculated onto Mueller-Hinton agar (MHA; Oxoid, UK) plates with a sterile cotton swab to ensure uniform distribution (**Figure 17**). cefotaxim (**CTX**, 30µg), cefotaxime-clavulanic acid (**CTC**, 30/10 µg), ceftazidime (**CAZ**, 30 µg) and ceftazidime-clavulanic acid (**CZC**, 30/10 µg) discs (Biomaxima, Poland) were placed on the agar surface with a center-to-center distance of 25–30 mm between the β-lactam and β-lactam/β-lactamase inhibitor combination discs. The plates were incubated at **35 ± 2 °C for 16–18 hours**. According to CLSI M100-ED35:2025 guidelines, an increase of ≥5 mm in zone diameter for CTC or CZC compared to CTX or CAZ alone indicated ESBL production.

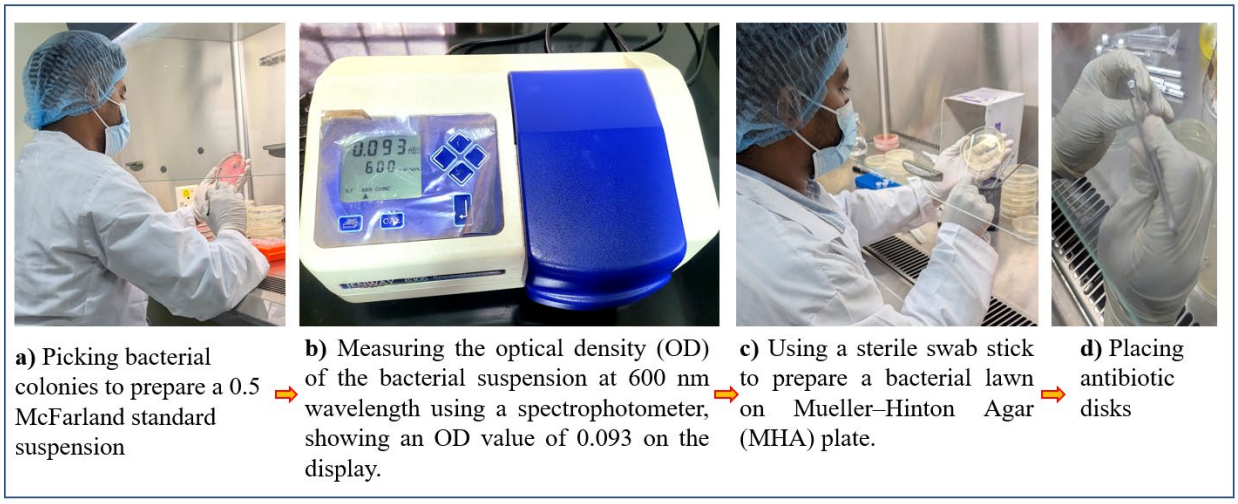


Figure 17. Preparation and standardization of bacterial inoculum for double-disc diffusion testing, maintaining a 0.5 McFarland standard ($OD_{600} \approx 0.08–0.1$), equivalent to approximately 1.5×10^8 CFU/mL cells.

Chapter 4

Results

4.1. Clinical Sample Overview

A total of 32 pediatric patients were included in the study. Among them, 56.25% (n=18) were male, and 43.75% (n=14) were female **(Table 3)**. The majority of the patients (53.12%, n=17) were within the age group of 0–12 months, followed by 34.38% (n=11) aged 13–24 months, and 12.50% (n=4) aged 25–96 months. In terms of residence, 56.25% (n=18) of the participants were from urban areas, while 43.75% (n=14) were from rural regions.

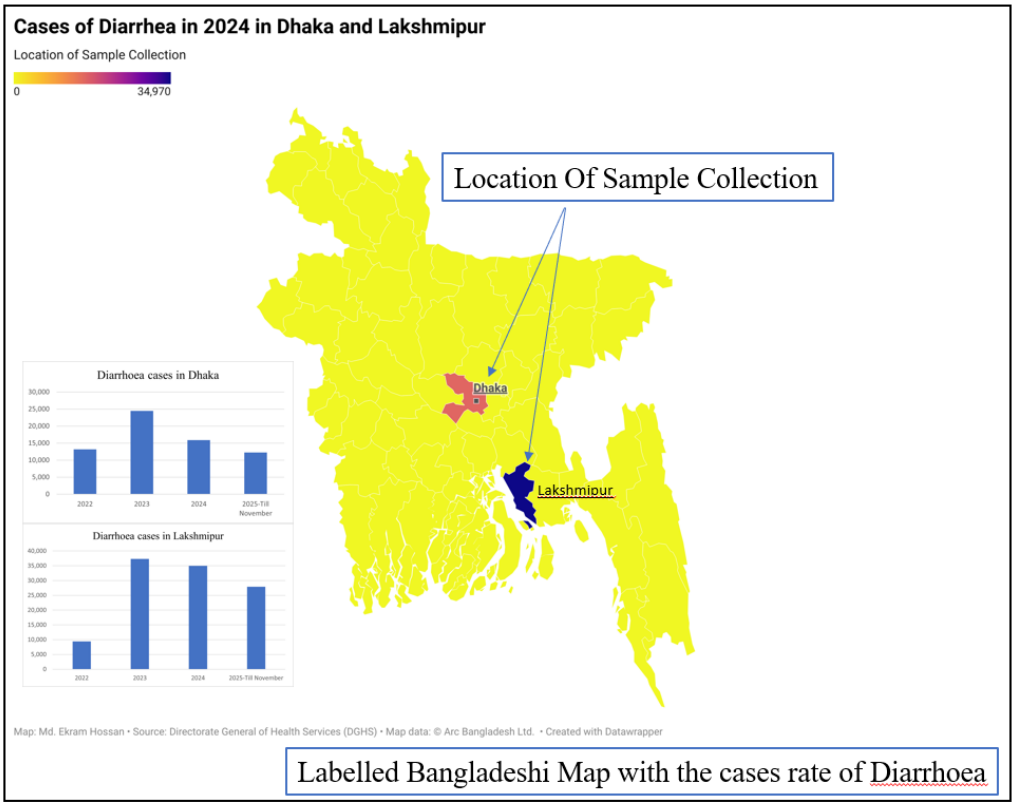


Figure 18. Geographical distribution of diarrheal cases in 2024 across Dhaka and Lakshmipur, Bangladesh, indicating sample collection sites.

The patients were enrolled from two hospitals: 42.63% (n=13) from Lakshmipur Sadar Hospital **(Figure 18)** and 59.37% (n=19) from Dhaka Shishu Hospital & Institute. Regarding clinical status, all participants (100%, n=32) were hospitalized patients, with no outpatients included in the study. Most of the patients had been suffering for 6–7 days (46.88%, n=15), followed by ≤5 days (37.50%, n=12) and ≥8 days (15.63%, n=5)**.**

Table 3: Demographic and Clinical Characteristics of the Pediatric Patients Enrolled in the Study. (N=32)

Category	Subcategory	Count	Percentage
Total Patients	-	32	100.00
Sex	Male	18	56.25
	Female	14	43.75
Age (Months)	0-12	17	53.12
	13-24	11	34.38
	25-96	4	12.50
Residence	Urban	18	56.25
	Rural	14	43.75
Location	Lakshmipur Sadar Hospital	13	42.63
	Dhaka Shishu Hospital & Institute	19	59.37
Clinical Status	Outpatient	0	0
	Inpatient	32	100
Days of Suffering	≤5	12	37.50
	6-7	15	46.88
	≥8	5	15.63

4.2. Bacterial Isolation and Preliminary Identification

From 32 pediatric stool samples, 38 bacterial isolates were obtained using MacConkey Agar medium. Distinct colonies based on color, morphology and lactose fermentation **(Figure 19)** were sub-cultured and preserved. Subculture on MacConkey agar yielded two distinct colony types: pink, lactose-fermenting colonies and pale, non-lactose-fermenting colonies, enabling preliminary differentiation of *Enterobacteriaceae* and non-fermenting pathogens **(Figure 20a & 20b)**.

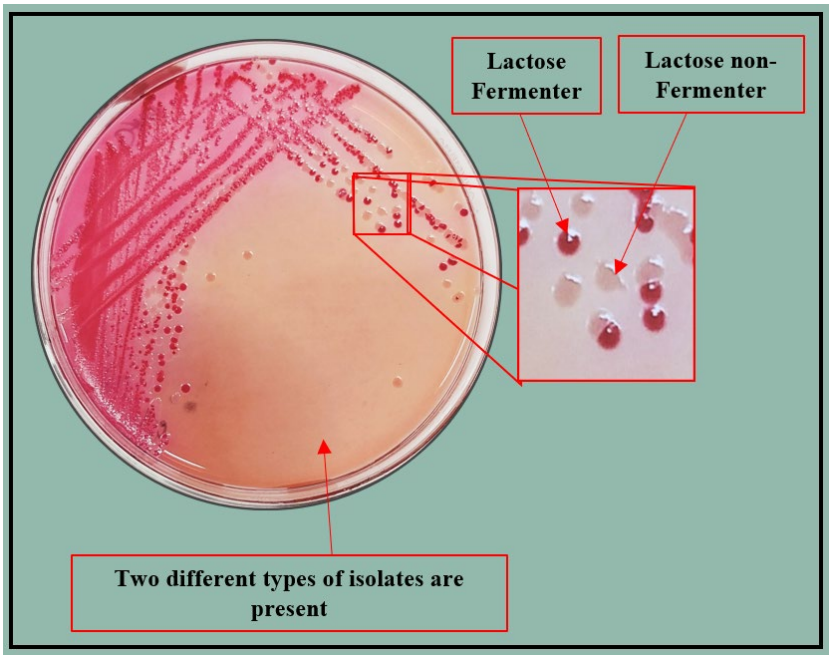


Figure 19. Preliminary screening of Gram-negative bacteria on MacConkey agar medium. Representative MacConkey medium culture plates showing two types of distinct colony morphology Gram-negative bacteria isolated from diarrheal stool samples as part of the preliminary ESBL screening.

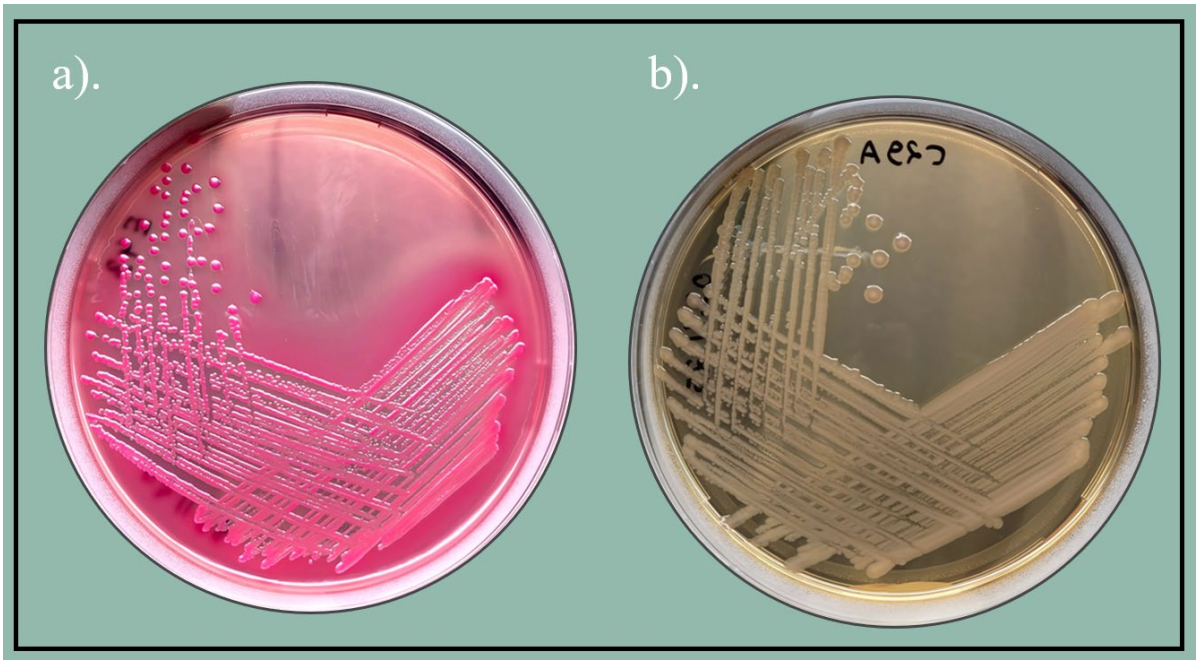


Figure 20. Subculture on MacConkey agar showing **(a)** pink lactose-fermenting and **(b)** pale non-lactose-fermenting colonies.

4.3. Species-Level Identification of Clinical Isolates Using MALDI-TOF

MALDI-TOF MS successfully identified all 38 bacterial isolates recovered from pediatric diarrheal stool samples. Identification was based on spectral matching against the Bruker Biotyper® reference database, with score values indicating high-confidence results (≥2.0) in most cases.

Table 4. Distribution of bacterial species identified by MALDI-TOF MS and associated confidence scores.

Strain Number	Rank (Quality)	Top Match Organism	Score Value
NIB001	(+++)	*Plesiomonas shigelloids*	2.13
NIB002	(+)	*Providencia stuartii*	1.91
NIB003	(+++)	*Citrobacter werkmanii*	2.13
NIB006	(+)	*Morganella morganii*	1.99
NIB007	(+)	*Enterobacter hormaechei*	1.9
NIB008	(+++)	*Escherichia coli*	2.03
NIB009	(+++)	*Escherichia coli*	2.01
NIB010	(+++)	*Escherichia coli*	2.11
NIB011	(+)	*Escherichia coli*	1.83
NIB012	(+++)	*Klebsiella pneumoniae*	2.33
NIB013	(+++)	*Enterobacter hormaechei*	2.33
NIB014	(+++)	*Klebsiella pneumoniae*	2.44
NIB015	(+++)	*Escherichia coli*	2.22
NIB016	(+++)	*Stutzerimonas stutzeri*	2.01
NIB017	(+++)	*Acinetobacter variabilis*	2.3
NIB018	(+++)	*Escherichia fergusonii*	2.21
NIB019	(+++)	*Pseudomonas aeruginosa*	2.44
NIB020	(+++)	*Escherichia coli*	2.12
NIB021	(+)	*Escherichia coli*	1.97
NIB022	(+++)	*Escherichia coli*	2.18
NIB023	(+++)	*Escherichia coli*	2.32

NIB024	(+++)	*Escherichia coli*	2.27
NIB025	(+++)	*Proteus mirabilis*	2.05
NIB026	(+++)	*Escherichia coli*	2.16
NIB027	(+++)	*Escherichia coli*	2.13
NIB028	(+++)	*Pseudomonas aeruginosa*	2.28
NIB029	(+++)	*Enterobacter chuandaensis*	2.2
NIB030	(+++)	*Pseudomonas aeruginosa*	2.18
NIB031	(+++)	*Escherichia coli*	2.23
NIB032	(+)	*Klebsiella pneumoniae*	1.76
NIB033	(+++)	*Escherichia coli*	2.01
NIB034	(+++)	*Escherichia coli*	2.23
NIB035	(+++)	*Escherichia coli*	2.11
NIB036	(+++)	*Escherichia coli*	2.3
NIB037	(+++)	*Klebsiella pneumoniae*	2.26
NIB039	(+++)	*Pseudomonas mendocina*	2.1
NIB041	(+++)	*Proteus faecis*	2.2
NIB044	(+++)	*Escherichia coli*	2.21

A diverse distribution of Gram-negative organisms was observed. The most frequently identified species was *E. coli* (n = 18, 44.7%), followed by *K. pneumoniae* (n = 4, 10.53%). Other notable species included *P. aeruginosa* (n=3) and *E. hormaechei* (n = 2) **(Figure 21)**.

Each isolate was assigned a rank score reflecting the quality of identification: high confidence (+++), low confidence (+), or no organism detected (-), with the majority falling in the high range. **(Table 4).** provides the full distribution of identified species, associated strain numbers, and MALDI-TOF score values.

This initial species-level identification laid the foundation for subsequent whole genome sequencing (WGS), resistance gene profiling, and phenotypic validation of antimicrobial resistance traits.

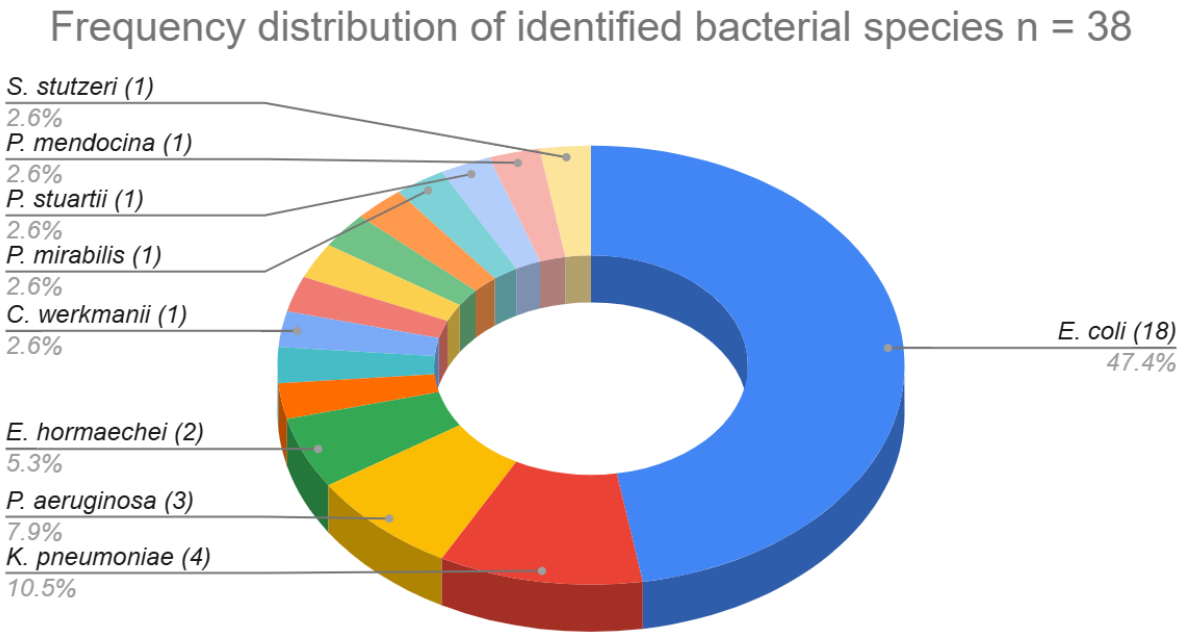


Figure 21. Distribution of identified bacterial species using MALDI-TOF MS *(n = 38)*.

4.4. Whole Genome Sequencing (WGS) and Assembly Metrics

Genome assembly and annotation of 38 bacterial isolates were performed using the Unicycler assembler, which generated high-quality draft genomes ranging from 3.36 Mb to 6.63 Mb in size **(Table 6)**. Assembly quality metrics were evaluated using the QUAST tool, which revealed considerable variation in the number of contigs, with *P. aeruginosa* having the highest (517 contigs) and *M. morganii* the lowest (9 contigs). The contig N50 values, indicating the length of the shortest contig to cover 50% of the genome, ranged from 15,970 bp in *P. mirabilis* to 2,569,229 bp in *M. morganii*. GC content remained relatively consistent across isolates, ranging from 38% to 66.25%. Genome annotation was carried out using the Prokka tool, identifying coding DNA sequences (CDS) ranging from 3,095 in *P. shigelloides* to 6,056 in *P. aeruginosa*, with rRNA counts between 0 and 6, tRNAs ranging from 45 to 98, and one tmRNA detected in all isolates except *P. mendocina*.

Table 6. Genome Assembly and Annotation Statistics of Bacterial Isolates Based on WGS Analysis.

Bacteria name	Strain	Genome size (mb)	Number of contig	Contig N50	GC%	CDS	rRNA	tRNA	tmRNA	Host	Isolation source
A. variabilis	NIB017	3.36	122	66949	42.01	3181	2	74	1	Homo sapiens	stool
C. werkmanii	NIB003	4.77	61	204028	52.07	4464	4	77	1	Homo sapiens	stool
E. chuandaensis	NIB029	4.73	18	672565	55.62	4376	3	74	1	Homo sapiens	Stool
E. hormaechei	NIB013	4.87	58	210580	54.99	4598	4	75	1	Homo sapiens	stool
	NIB007	4.63	77	114007	55.24	4390	5	76	1	Homo sapiens	stool
E. coli	NIB008	4.54	112	103035	50.84	4216	5	78	1	Homo sapiens	stool
	NIB009	5.44	113	196560	50.35	5098	3	86	1	Homo sapiens	stool
	NIB010	4.81	147	62674	50.9	4490	5	83	1	Homo sapiens	stool
	NIB011	5.07	85	169647	50.65	4733	3	78	1	Homo sapiens	stool
	NIB015	4.93	121	142889	50.71	4564	4	81	1	Homo sapiens	stool
	NIB020	4.54	215	69252	50.75	4216	3	45	1	Homo sapiens	stool
	NIB021	5.18	265	140555	50.48	4742	2	74	1	Homo sapiens	stool
	NIB022	5.11	93	169601	50.68	4779	3	77	1	Homo sapiens	stool
	NIB023	5.06	98	126956	50.5	4688	5	86	1	Homo sapiens	stool
	NIB024	4.83	289	62616	50.83	4486	3	60	1	Homo sapiens	stool
	NIB026	4.9	222	165393	50.74	4587	3	78	1	Homo sapiens	stool
	NIB027	5.09	98	216715	50.62	4756	4	80	1	Homo sapiens	stool
	NIB031	5.21	187	159959	50.51	4869	4	80	1	Homo sapiens	stool
	NIB033	5.04	266	163213	50.7	4679	3	79	1	Homo sapiens	stool
	NIB034	4.71	172	148118	50.68	4397	4	71	1	Homo sapiens	stool
	NIB035	5.3	339	196966	50.48	4902	4	83	1	Homo sapiens	stool
	NIB036	5.16	256	172592	50.62	4812	3	76	1	Homo sapiens	stool
	NIB044	4.65	173	81140	50.75	4400	4	74	1	Homo sapiens	stool
E. fergusonii	NIB018	4.54	44	516532	49.93	4220	4	80	1	Homo sapiens	Stool
K. pneumoniae	NIB012	5.34	143	242100	57.36	4945	6	64	1	Homo sapiens	Stool

	NIB014	5.42	63	212697	57.39	5057	4	78	1	Homo sapiens	Stool
	NIB032	5.36	94	142775	57.42	4973	3	68	1	Homo sapiens	Stool
	NIB037	5.66	194	183445	57.06	5303	6	65	1	Homo sapiens	Stool
M. morganii	NIB006	3.7	9	2569229	51.16	3446	3	67	1	Homo sapiens	Stool
P. shigelloides	NIB001	3.62	64	291173	51.93	3095	4	98	1	Homo sapiens	stool
Proteus faecis	NIB041	3.93	45	184401	38	3450	5	75	1	Homo sapiens	stool
P. mirabilis	NIB025	3.89	389	15970	38.73	3394	4	54	1	Homo sapiens	stool
P. stuartii	NIB002	4.34	51	301266	41.31	4006	3	69	1	Homo sapiens	stool
	NIB019	6.63	73	203123	65.97	6056	3	67	1	Homo sapiens	stool
P. aeruginosa	NIB028	6.35	213	56815	66.25	5856	3	70	1	Homo sapiens	stool
	NIB030	6.55	517	22629	65.75	6018	3	62	1	Homo sapiens	stool
P. mendocina	NIB039	5.2	25	333874	62.7	4792	0	59	0	Homo sapiens	stool
S. stutzeri	NIB016	4.84	279	61330	50.84	4495	3	55	1	Homo sapiens	stool

Circular genome maps were generated for all 38 isolates. A representative genome map is shown in **Figure 22**, highlighting key genomic features including coding sequences, resistance genes, and GC content. All other genome maps are provided in **Supplementary Figures F13–F50**.

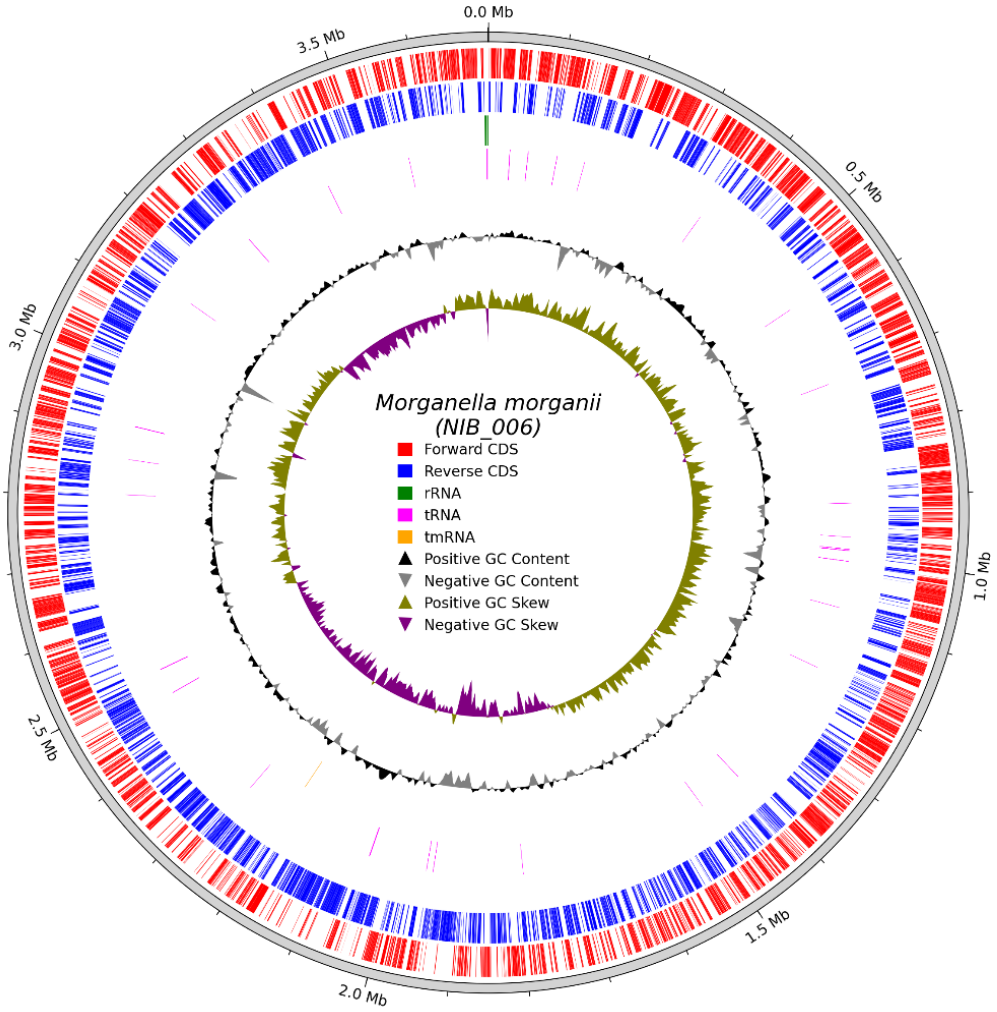
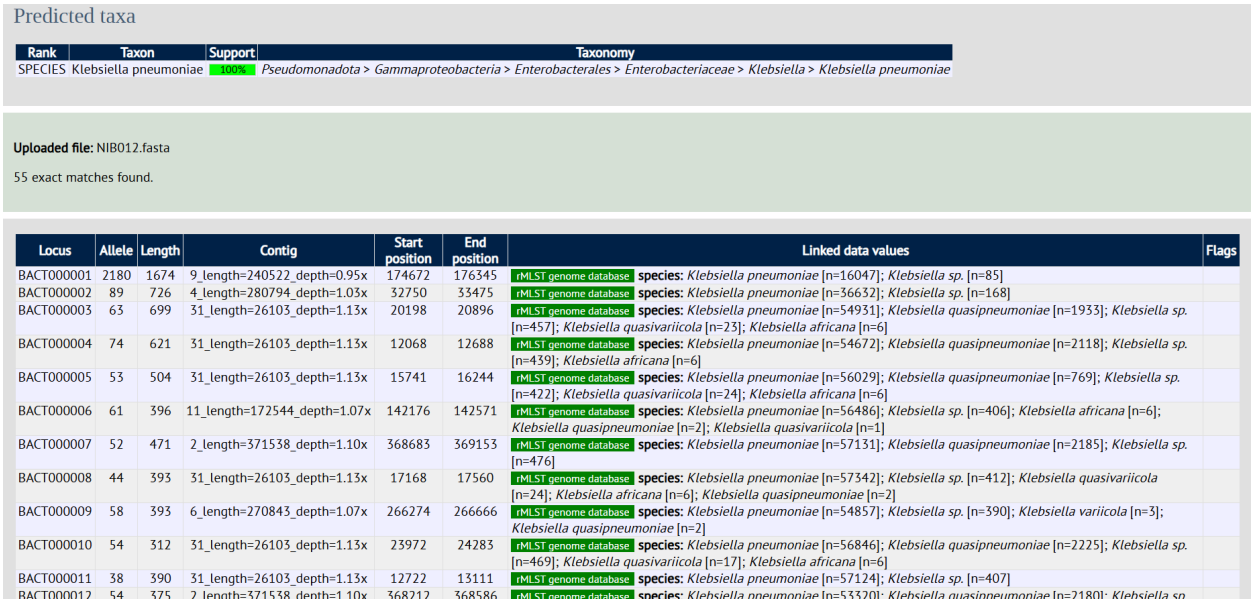


Figure 22. Circular representation of the whole genome of *Morganella morganii* (NIB006) isolated from a pediatric diarrheal sample.

The circular genome map illustrates key genomic features including forward coding sequences (CDS, red), reverse CDS (blue), rRNA (green), tRNA (purple), and tmRNA (orange). The inner rings represent GC content (black) and GC skew (green/purple), which indicate nucleotide composition bias across the genome. The genome size of *M. morganii* NIB006 was approximately 3.7 Mb with a GC content of 51.16%. This graphical representation provides an overview of the genomic architecture, distribution of functional genes, and compositional asymmetry, serving as a representative genome map for *Enterobacteriaceae* isolates characterized in this study.

4.5. Species Identification Based on Whole Genome Analysis Using rMLST (PubMLST Platform)

Whole genome sequences of the isolated strains were analyzed using the rMLST (ribosomal Multilocus Sequence Typing) database available at PubMLST (**Figure 23**). This tool identifies bacterial species by comparing the 53 conserved ribosomal protein subunit genes across genomes. The analysis confirmed accurate species-level identification of the isolates, supporting the initial MALDI-TOF results. The rMLST-based prediction showed high sequence similarity, validating the taxonomic classification of each isolate.



Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Klebsiella pneumoniae	100%	*Pseudomonadota* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Klebsiella* > *Klebsiella pneumoniae*

Uploaded file: NIB012.fasta

55 exact matches found.

Locus	Allele	Length	Contig	Start position	End position	Linked data values	Flags
BACT000001	2180	1674	9_length=240522_depth=0.95x	174672	176345	rMLST genome database **species:** *Klebsiella pneumoniae* [n=16047]; *Klebsiella sp.* [n=85]	
BACT000002	89	726	4_length=280794_depth=1.03x	32750	33475	rMLST genome database **species:** *Klebsiella pneumoniae* [n=36632]; *Klebsiella sp.* [n=168]	
BACT000003	63	699	31_length=26103_depth=1.13x	20198	20896	rMLST genome database **species:** *Klebsiella pneumoniae* [n=54931]; *Klebsiella quasipneumoniae* [n=1933]; *Klebsiella sp.* [n=457]; *Klebsiella quasivariicola* [n=23]; *Klebsiella africana* [n=6]	
BACT000004	74	621	31_length=26103_depth=1.13x	12068	12688	rMLST genome database **species:** *Klebsiella pneumoniae* [n=54672]; *Klebsiella quasipneumoniae* [n=2118]; *Klebsiella sp.* [n=439]; *Klebsiella africana* [n=6]	
BACT000005	53	504	31_length=26103_depth=1.13x	15741	16244	rMLST genome database **species:** *Klebsiella pneumoniae* [n=56029]; *Klebsiella quasipneumoniae* [n=769]; *Klebsiella sp.* [n=422]; *Klebsiella quasivariicola* [n=24]; *Klebsiella africana* [n=6]	
BACT000006	61	396	11_length=172544_depth=1.07x	142176	142571	rMLST genome database **species:** *Klebsiella pneumoniae* [n=56486]; *Klebsiella sp.* [n=406]; *Klebsiella africana* [n=6]; *Klebsiella quasipneumoniae* [n=2]; *Klebsiella quasivariicola* [n=1]	
BACT000007	52	471	2_length=371538_depth=1.10x	368683	369153	rMLST genome database **species:** *Klebsiella pneumoniae* [n=57131]; *Klebsiella quasipneumoniae* [n=2185]; *Klebsiella sp.* [n=476]	
BACT000008	44	393	31_length=26103_depth=1.13x	17168	17560	rMLST genome database **species:** *Klebsiella pneumoniae* [n=57342]; *Klebsiella sp.* [n=412]; *Klebsiella quasivariicola* [n=24]; *Klebsiella africana* [n=6]; *Klebsiella quasipneumoniae* [n=2]	
BACT000009	58	393	6_length=270843_depth=1.07x	266274	266666	rMLST genome database **species:** *Klebsiella pneumoniae* [n=54857]; *Klebsiella sp.* [n=390]; *Klebsiella variicola* [n=3]; *Klebsiella quasipneumoniae* [n=2]	
BACT000010	54	312	31_length=26103_depth=1.13x	23972	24283	rMLST genome database **species:** *Klebsiella pneumoniae* [n=56846]; *Klebsiella quasipneumoniae* [n=2225]; *Klebsiella sp.* [n=469]; *Klebsiella quasivariicola* [n=17]; *Klebsiella africana* [n=6]	
BACT000011	38	390	31_length=26103_depth=1.13x	12722	13111	rMLST genome database **species:** *Klebsiella pneumoniae* [n=57124]; *Klebsiella sp.* [n=407]	
BACT000012	54	375	2_length=371538_depth=1.10x	368212	368586	rMLST genome database **species:** *Klebsiella pneumoniae* [n=53320]; *Klebsiella quasipneumoniae* [n=2180]; *Klebsiella sp.*	

Figure 23. rMLST Analysis of Bacterial Isolates on PubMLST for species identification using WGS.

4.6. Phylogenetic Analysis of Bacterial Isolates Based on Whole-Genome Sequences

The radial phylogenetic tree shows a clear separation of the bacterial isolates into distinct, species-specific clades **(Figure 24)**. A large cluster of *E. coli* isolates forms a tight, cohesive group with short branch lengths, indicating high genetic similarity. Nearby but separate, *Enterobacter* species form their own clade, showing moderate divergence from *E. coli* while

remaining within the *Enterobacteriaceae*. *K. pneumoniae* isolates also cluster closely together and are clearly separated from both *Escherichia* and *Enterobacter*. In contrast, *Pseudomonas aeruginosa* forms an independent and genetically distant lineage, consistent with its divergence from *Enterobacteriaceae* within the *Gammaproteobacteria*. Additional species, including *Pseudomonas mendocina*, *Acinetobacter variabilis*, *Morganella morganii*, *Proteus mirabilis*, and *Plesiomonas shigelloides*, appear as isolated branches with longer branch lengths, indicating greater evolutionary distance. Overall, the tree demonstrates strong clustering within species and clear separation between genera, confirming robust phylogenetic relationships among the isolates.

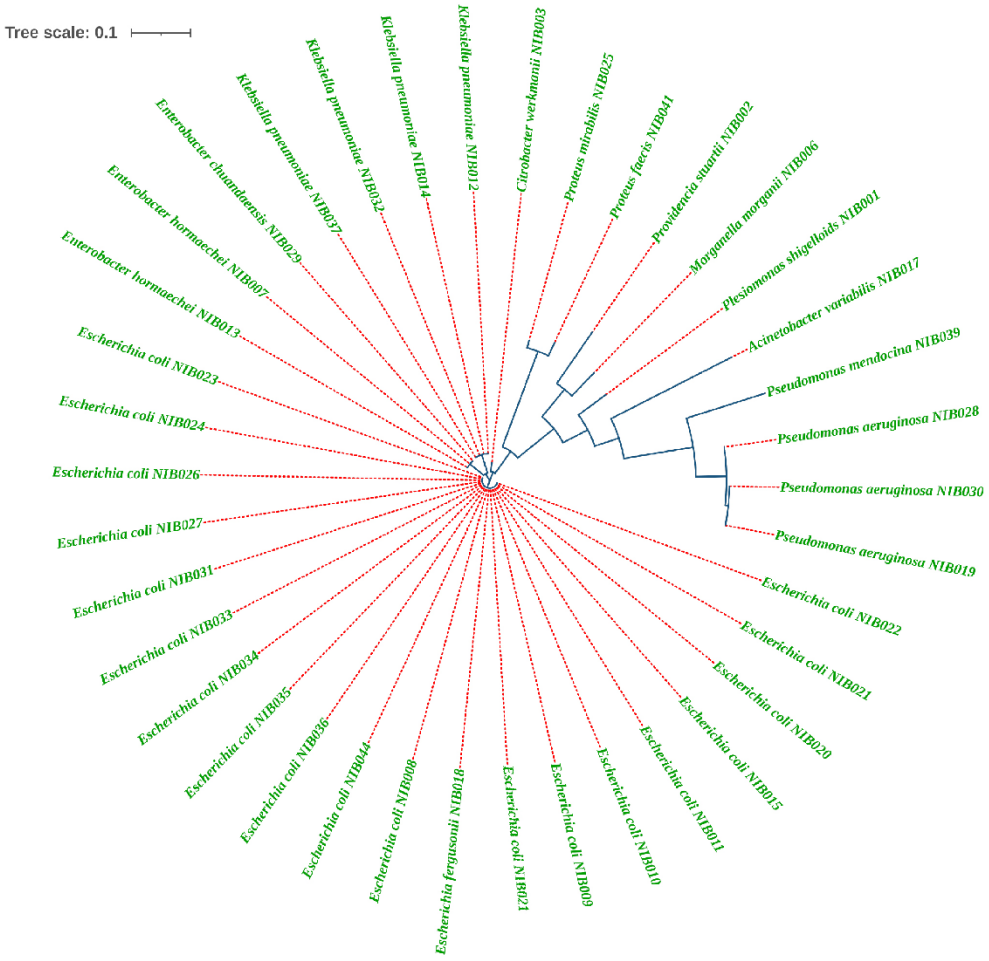


Figure 24. Circular phylogenetic tree illustrating the evolutionary relationship among 38 bacterial isolates recovered from pediatric diarrheal samples based on whole-genome sequence analysis.

4.7. Antimicrobial Resistance (AMR) Gene Profiling

Whole-genome-based antimicrobial resistance (AMR) gene screening of 38 diarrheal bacterial isolates revealed a diverse array of resistance determinants across multiple antibiotic classes (**Figure 25**). Using ABRicate v1.0.1 with the ResFinder database (minid ≥95%, mincov ≥90%), a total of 65 distinct AMR genes were identified, indicating a high prevalence of multidrug resistance among the isolates. AMR genes were also detected from other databases (CARD, NCBI, ARGANNOT and Megares), which are given in **Supplementary Table S2**. The detected genes were distributed among resistance categories for β-lactams, aminoglycosides, fluoroquinolones, sulfonamides, tetracyclines, macrolides, fosfomycin, phenicols, trimethoprim, and miscellaneous.

Among these, the most clinically significant findings were the β-lactamase–encoding genes associated with extended-spectrum β-lactamase (ESBL) activity. The *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{SHV-11}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{OXA-10}}$, and *bla*$_{\text{DHA-1}}$ genes were frequently identified, particularly in *E. coli* and *K. pneumoniae* isolates. Notably, *K. pneumoniae* isolates (NIB012, NIB014, NIB032, NIB037) exhibited multiple ESBL determinants such as blaSHV, blaTEM, and blaOXA families, which are well known for conferring resistance to third-generation cephalosporins. Similarly, *E. coli* isolates harbored *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{TEM-1B}}$, consistent with global patterns of ESBL-mediated resistance. The presence of *bla*$_{\text{NDM-5}}$, a carbapenemase gene, was also observed in certain isolates, indicating the possible emergence of carbapenem resistance within the sample population.

Beyond β-lactam resistance, several aminoglycoside-modifying enzymes (AMEs) such as aac(3)-IIa, aac(6′)-Ib, aadA2, and aph(3′)-IIb were detected, suggesting a broad resistance profile to aminoglycosides. Fluoroquinolone resistance genes, including qnrA1, qnrB, qnrS1, and oqxA/B were also widely distributed, indicating potential plasmid-mediated quinolone resistance. Sulfonamide (sul1, sul2, sul3) and tetracycline [tet(A), tet(B)] resistance determinants were commonly identified across both *Enterobacteriaceae* and non-fermenter groups, emphasizing multidrug resistance dissemination.

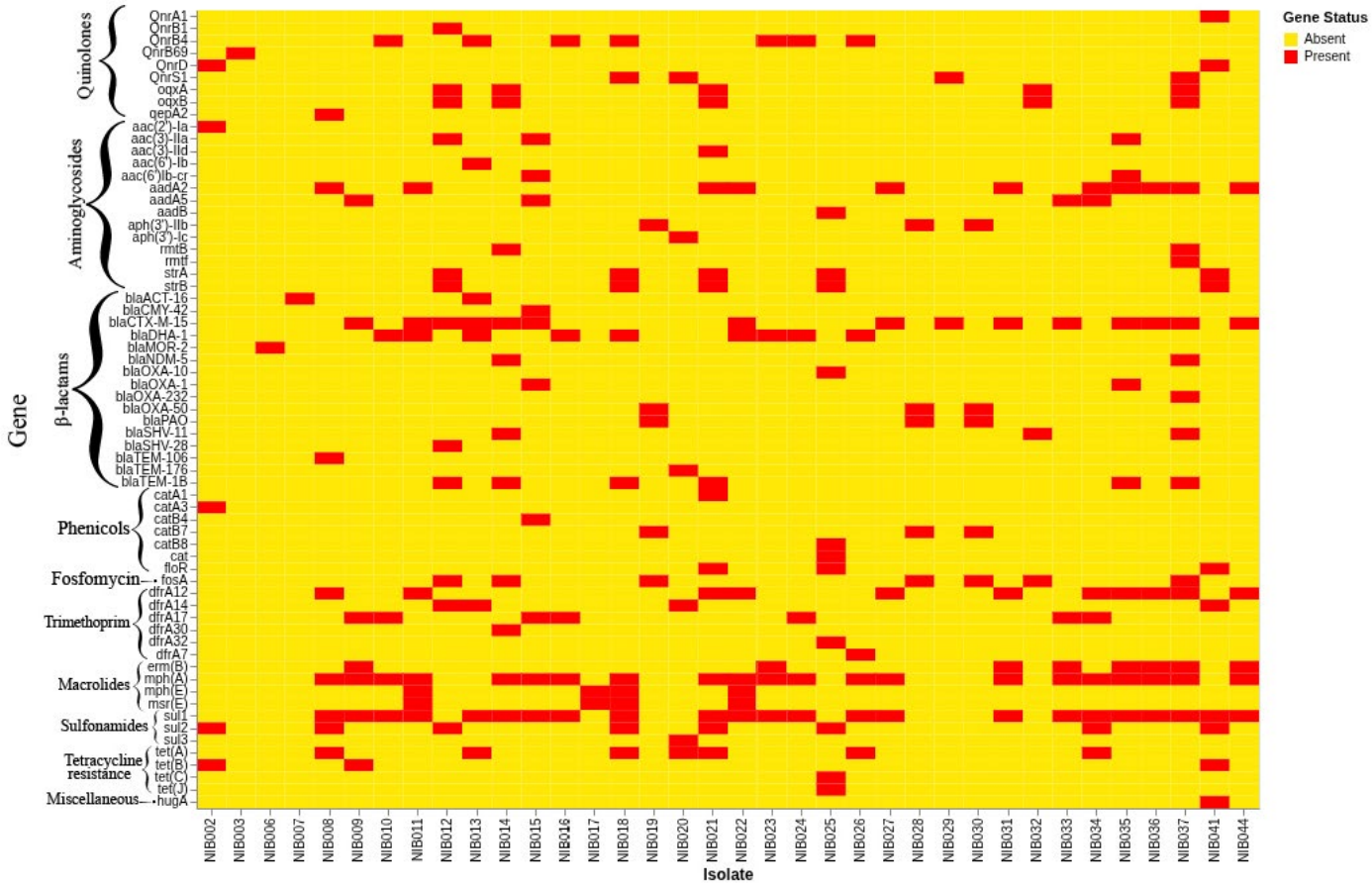


Figure 25. Heatmap showing the distribution of antimicrobial resistance (AMR) genes among 38 diarrheal bacterial isolates identified by ABRicate using the ResFinder database.

The heatmap representation visualized the distribution and co-occurrence of these resistance genes among the 38 isolates, showing clustering of *E. coli* and *K. pneumoniae* based on shared ESBL and efflux pump–associated genes. In contrast, non-fermenters such as *P. aeruginosa* (NIB019, NIB028, NIB030) and *A. variabilis* (NIB017) displayed species-specific resistance determinants including $bla_{\text{OXA-50}}$ and *catB* variants. Collectively, these findings highlight the widespread presence of ESBL-producing isolates in diarrheal stool samples and underline the genomic evidence of horizontally transferable resistance mechanisms in both enteric and opportunistic pathogens.

4.8. Extended-Spectrum β-Lactamase (ESBL) Genes

Detection of ESBL genes was performed utilizing multiple resistance gene databases, including CARD, ResFinder, NCBI AMR, and ARG-ANNOT. Among the 38 isolates, 21 were found to harbor ESBL-associated genes, with ***bla*$_{CTX-M-15}$** being the most prevalent. Other detected ESBL genes included *bla*$_{SHV}$ variants (e.g., *bla*$_{SHV-11}$, *bla*$_{SHV-106}$, *bla*$_{SHV-110}$, *bla*$_{SHV-158}$, *bla*$_{SHV-182}$) and *blaTEM* variants (e.g., *bla*$_{TEM-105}$, *bla*$_{TEM-176}$, *bla*$_{TEM-135}$) **(Table 5)**. Several isolates harbored multiple ESBL genes concurrently. It is noteworthy that while some isolates carried ***bla*$_{TEM-1}$**, this gene alone is not classified as an ESBL-producing gene and was therefore not considered in the ESBL-positive count **Supplementary Table S2** [238].

Table 5: Summary of detected ESBL genes in each isolate based on ABRicate results across multiple databases.

Strain Number	Organism	ESBLs Gene
NIB008	*E. coli*	***bla*$_{TEM-135}$, *bla*$_{TEM-106}$**
NIB009	*E. coli*	***bla*$_{CTX-M-15}$**
NIB011	*E. coli*	***bla*$_{CTX-M-15}$**
NIB012	*K. pneumoniae*	***bla*$_{SHV-106}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-105}$**
NIB013	*E. hormaechei*	***bla*$_{CTX-M-15}$**
NIB014	*K. pneumoniae*	***bla*$_{CTX-M-15}$, *bla*$_{SHV-158}$, *bla*$_{TEM-105}$, *bla*$_{SHV-182}$**
NIB015	*E. coli*	***bla*$_{CTX-M-15}$**
NIB018	*E. fergusonii*	***bla*$_{TEM-105}$**
NIB020	*E. coli*	***bla*$_{TEM-176}$**
NIB021	*E. coli*	***bla*$_{TEM-105}$**
NIB022	*E. coli*	***bla*$_{CTX-M-15}$**
NIB026	*E. coli*	***bla*$_{CTX-M-15}$**
NIB027	*E. coli*	***bla*$_{CTX-M-15}$**
NIB029	*E. chuandaensis*	***bla*$_{CTX-M-15}$**
NIB031	*E. coli*	***bla*$_{CTX-M-15}$**
NIB032	*K. pneumoniae*	***bla*$_{SHV-110}$**

NIB033	*E. coli*	***bla*$_{CTX-M-15}$**
NIB035	*E. coli*	***bla*$_{CTX-M-15}$, *bla*$_{TEM-105}$**
NIB036	*E. coli*	***bla*$_{CTX-M-15}$**
NIB037	*K. pneumoniae*	***bla*$_{CTX-M-15}$, *bla*$_{SHV-11}$, *bla*$_{SHV-158}$, *bla*$_{SHV-182}$, *bla*$_{TEM-105}$**
NIB044	*E. coli*	***bla*$_{CTX-M-15}$**

The distribution of ESBL type gene variants for *E. coli, K. pneumoniae, E. hormaechei, E. fergusonii,* and *E. chuandaensis* was analyzed **(Table 6)**. These bacteria were found from a total of 21 genotypically positive ESBL-E isolates. 16 of these isolates tested positive for the gene variant, *bla*$_{CTX-M-15}$ (16/21, 76.19%); most of them belonged to Escherichia coli (11/14, 78.57%). The *bla*$_{CTX-M-15}$ gene variant distribution for the remaining 3 isolates are as follows, 3 (75%) in *K. pneumoniae*, 1 (100%) in *E. hormaechei*, and 1 (100%) in *E. chuandaensis*. Variants of the *bla*$_{TEM}$ gene were also prevalent (6/21, 28.57%); as *bla*$_{TEM-105}$ was detected in the *E. coli* isolates (2/14, 14.28%), *K. pneumoniae* (3/4, 75%) isolates and *E. fergusonii* (1/1, 100%) isolates. Other variants of *bla*$_{TEM}$ such as, *bla*$_{TEM-135}$ and *bla*$_{TEM-176}$, were also found in *E. coli,* but their distribution rates were lower (7.14% in each). It's worth mentioning that *bla*$_{TEM-105}$ was the most widespread variant of the *bla*$_{TEM}$ family in *E. coli* and *K. pneumoniae*. All the gene variants of *bla*$_{SHV}$ such as, *bla*$_{SHV-11}$, *bla*$_{SHV-106}$, *bla*$_{SHV-110}$, *bla*$_{SHV-158}$ and *bla*$_{SHV-182}$, were exclusively detected in *K. pneumoniae*. Notably, *bla*$_{SHV-158}$ and *bla*$_{SHV-182}$ were the most prevalent, each present in 50% of the *K. pneumoniae* isolates, while *bla*$_{SHV-11}$, *bla*$_{SHV-106}$, and *bla*$_{SHV-110}$ were found at a lower frequency of 25% each.

Table 6. Distribution of ESBLs genes among 21 ESBL-E isolates from diarrheal stool sample.

ESBL type	Gene variant	*E. coli* (n = 14)[a,b]	*K. pneumoniae* (n =4)[a,b]	*E. hormaechei* (n = 1)[a,b]	*E. fergusonii* (n =1)[a,b]	*E. chuandaensis* (n =1)[a,b]	Total (n = 21)
***bla*$_{CTX-M}$**	***bla*$_{CTX-M-15}$**	11 (78.57)	3 (75)	1 (100)	0	1 (100)	16 (76.19)
***bla*$_{TEM}$**	***bla*$_{TEM-105}$**	2 (14.28)	3 (75)	0	1 (100)	0	6 (28.57)
	***bla*$_{TEM-135}$**	1(7.14)	0	0	0	0	1 (4.76)
	***bla*$_{TEM-176}$**	1 (7.14)	0	0	0	0	1 (4.76)
***bla*$_{SHV}$**	***bla*$_{SHV-158}$**	0	2 (50)	0	0	0	2 (9.52)
	***bla*$_{SHV-182}$**	0	2 (50)	0	0	0	2 (9.52)
	***bla*$_{SHV-11}$**	0	1 (25)	0	0	0	1 (4.76)
	***bla*$_{SHV-106}$**	0	1 (25)	0	0	0	1 (4.76)
	***bla*$_{SHV-110}$**	0	1 (25)	0	0	0	1 (4.76)

[a]The number of isolates (Percentages) is represented

[b]Some isolates have multiple resistance genes, so the total number of isolates is not simply the sum of the values in each column.

Among the 32 pediatric patients included in this study, ESBL genes were detected in 16 patients (50%), while the remaining 16 patients (50%) carried isolates without any identifiable ESBL genes (**Figure 26**). This equal distribution highlights the heterogeneous nature of ESBL-mediated resistance within the study population and indicates that both ESBL-producing and non-ESBL-producing *Enterobacteriaceae* were associated with pediatric diarrheal cases.

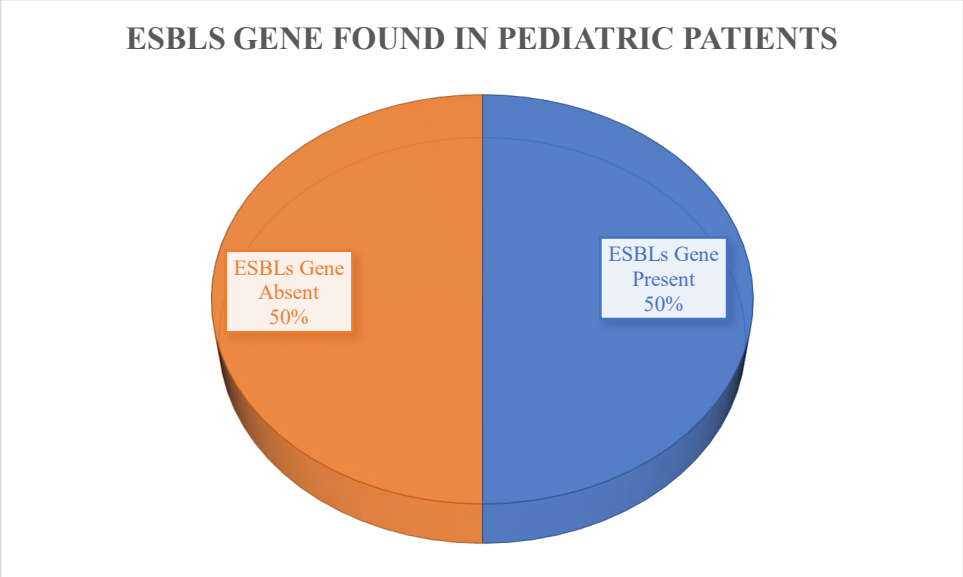


Figure 26. The rate of ESBLs gene absent and present from total 32 pediatric diarrheal patients.

4.9. Virulence Gene Profile Heatmap

The distribution of virulence genes varied considerably among the isolates analyzed. A collective total of 338 virulence genes were detected across in 25 isolates (**Supplementary Table S3**). Key virulence factors identified included genes associated with adhesion (e.g., fimbriae), iron acquisition (siderophores), toxin production, and biofilm formation. Some isolates possessed a diverse complement of virulence genes, indicating a high pathogenic potential, while others harbored fewer virulence determinants. This heterogeneity reflects varied infection strategies and potential clinical outcomes. A heatmap was generated to visualize the distribution of virulence-associated genes of those bacterial isolates (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. stutzeri*, and *E. fergusonii*), categorized into 10 functional categories (**Figure 27**). The color intensity in the heatmap corresponds to the number of genes identified in each functional category per isolate.

The analysis revealed distinct virulence gene profiles among the isolates. *P. aeruginosa* strains (NIB019, NIB028, NIB030) exhibited the most diverse and abundant virulence gene repertoire, with high counts across nearly all categories, particularly in Motility and Chemotaxis**,** Capsule, LPS, and Biofilm Formation**,** Type III and Type VI Secretion Systems**,** and Type IV Pili. In contrast, most *E. coli* and other isolates showed a more restricted profile, with dominant representation in Iron Uptake & Metabolism, Adhesion, and Colonization Factors.

Notably, isolates such as NIB014 and NIB037 (*K. pneumoniae*) and NIB018 (*E. fergusonii*) displayed minimal virulence gene counts, with genes almost exclusively limited to iron acquisition systems. Several *E. coli* isolates (e.g., NIB011, NIB022, NIB027, NIB033, NIB036) also carried genes associated with adhesion and iron uptake, but lacked most secretion system components and toxin genes.

These findings highlight significant interspecies and intraspecies variation in virulence gene content, suggesting adaptations to specific pathogenic niches and mechanisms of host interaction.

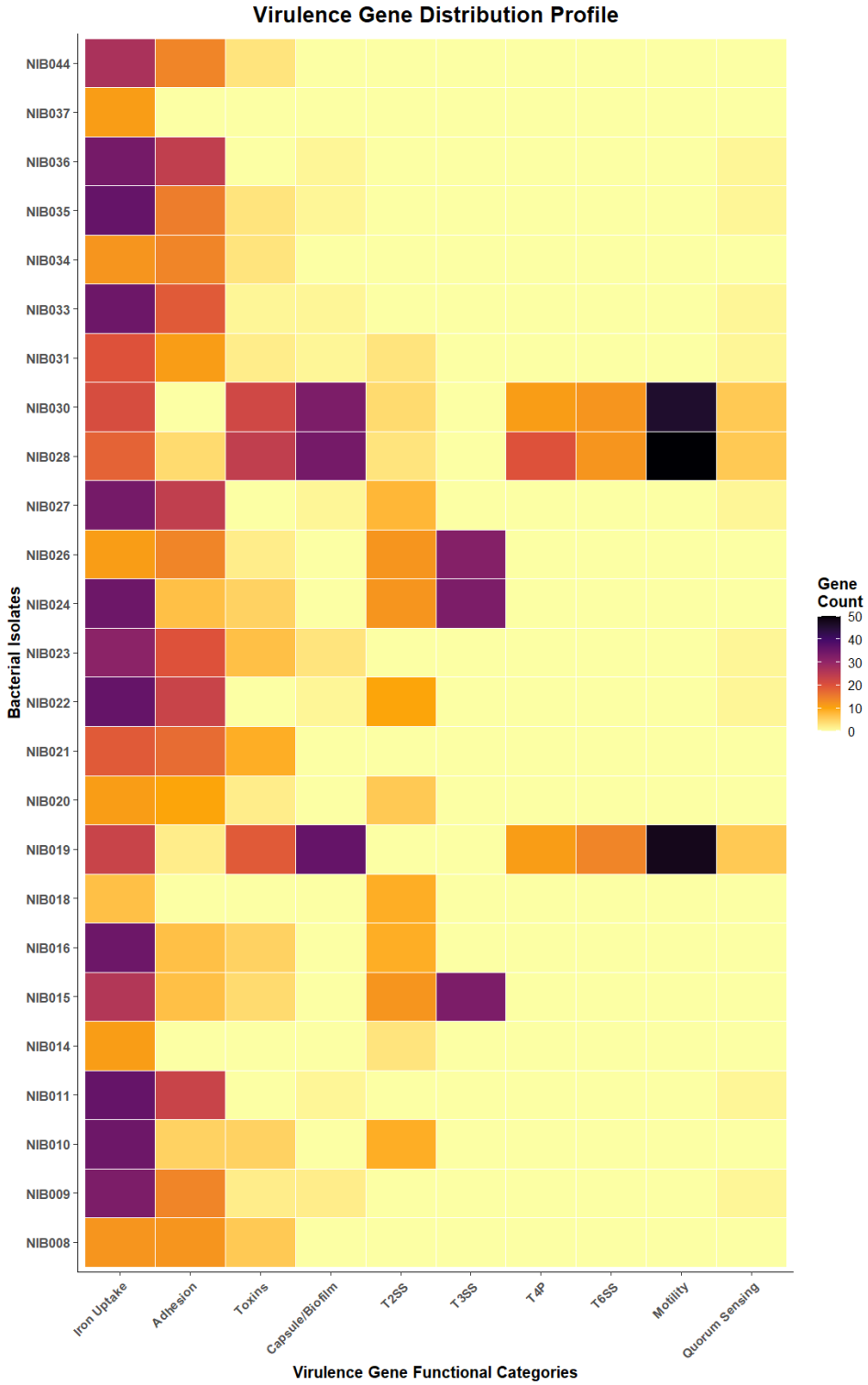


Figure 27. Heatmap of Virulence-Associated Gene Distribution across Bacterial Isolates.

4.10. Plasmid Replicon Diversity and ESBL Gene Dissemination

A total of 38 *Enterobacteriaceae* and related isolates (species: *E. coli*, *K. pneumoniae*, *E. hormaechei*, *E. fergusonii*, *S. stutzeri*, and *P. faecis*) were screened for plasmid replicon types using the PlasmidFinder database and found plasmids in 25 isolates. The plasmid analysis revealed a diverse range of incompatibility (Inc) groups, predominantly belonging to IncF**,** IncI**,** IncHI**,** IncX**,** IncB/O/K/Z**,** IncR, and multiple Col-type plasmids (Table 4). These plasmid families are well known for their ability to acquire and disseminate antimicrobial resistance genes, particularly ESBLs, across *Enterobacteriaceae* populations [23], [239].

Among *E. coli* isolates, the IncF family [IncFIA, IncFIB(AP001918), IncFII, and IncFII(pRSB107)] was the most frequently detected replicon type. These plasmids are well known for carrying *blaCTX-M-15*, which aligns with global dissemination patterns. Several isolates also harbored the p0111-type plasmid, while IncX1 and IncI1_Alpha were additionally detected in a few *E. coli* strains.

In *K. pneumoniae*, plasmids belonging to IncFIB(K) and IncR, along with accessory Col plasmids such as ColKP3 and Col(BS512), were identified. *E. hormaechei* carried a combination of IncHI1A**,** IncHI1B(R27), and IncR, which are large, conjugative plasmids associated with MDR spread.

Overall, blaCTX-M-15 was the most prevalent ESBL gene, frequently co-occurring with IncF plasmids. Additionally, several isolates carried blaTEM variants (e.g., TEM-105, TEM-106, TEM-135, TEM-176) and blaSHV variants (e.g., SHV-106, SHV-110, SHV-158, SHV-182). Notably, five isolates lacked ESBL genes despite harboring plasmid replicons, indicating the presence of non-ESBL plasmids or plasmids associated with other functional traits.

Table 12. Plasmid Replicon Types and ESBL Genes detected among clinical isolates.

Strain ID	Species	Plasmid Replicon(s)	ESBLs genes Detected in same Isolate
NIB008	*E. coli*	p0111_1	*blaTEM-135, blaTEM-106*
NIB009	*E. coli*	IncY_1, IncFIB(AP001918)_1, IncFIA_1, IncFII(pRSB107)_1_pRSB107	*blaCTX-M-15*
NIB010	*E. coli*	IncFII(pRSB107)_1_pRSB107, IncFII_1	*No ESBLs gene*
NIB011	*E. coli*	IncFIB(AP001918)_1, IncFIA_1	*blaCTX-M-15*
NIB012	*K. pneumoniae*	IncFIB(K)_1_Kpn3	*blaSHV-106, blaCTX-M-15, blaTEM-105*
NIB013	*E. hormaechei*	IncHI1A_1, IncHI1B(R27)_1_R27, IncR_1, ColpVC_1	*blaCTX-M-15*
NIB014	*K. pneumoniae*	IncFIB(pQil)_1_pQil, IncFII_1, ColpVC_1	*blaCTX-M-15, blaSHV-158, blaTEM-105, blaSHV-182*
NIB015	*E. coli*	IncFIB(AP001918)_1, IncI1_1_Alpha, IncFIA_1, Col156_1, Col(BS512)_1, Col(BS512)_1__NC_010656_dupe	*blaCTX-M-15*
NIB016	*S. stutzeri*	IncFII(pRSB107)_1_pRSB107, IncFII_1	*No ESBLs gene*
NIB018	*E. fergusonii*	p0111_1	*blaTEM-105*
NIB020	*E. coli*	IncX1_1	*blaTEM-176*
NIB021	*E. coli*	IncI1_1_Alpha	*blaTEM-105*
NIB022	*E. coli*	IncFIB(AP001918)_1, IncFIA_1, IncFII_1	*blaCTX-M-15*
NIB023	*E. coli*	IncFII(pRSB107)_1_pRSB107, IncFIB(AP001918)_1	*No ESBLs gene*
NIB024	*E. coli*	IncFII(pRSB107)_1_pRSB107, IncFII_1	*No ESBLs gene*
NIB027	*E. coli*	IncI2_1, IncFIA_1, IncFIB(AP001918)_1, IncFII_1	*blaCTX-M-15*
NIB031	*E. coli*	p0111_1, IncB/O/K/Z_1, IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	*blaCTX-M-15*
NIB032	*K. pneumoniae*	IncFIB(K)_1_Kpn3	*blaSHV-110*
NIB033	*E. coli*	IncFIB(AP001918)_1, Col156_1, IncFIA_1, IncFII(pRSB107)_1_pRSB107	*blaCTX-M-15*
NIB034	*E. coli*	p0111_1	*No ESBLs gene*
NIB035	*E. coli*	IncFIA_1, IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	*blaCTX-M-15, blaTEM-105*
NIB036	*E. coli*	IncFIA_1, IncFIB(AP001918)_1, IncFII_1	*blaCTX-M-15*
NIB037	*K. pneumoniae*	IncR_1, IncFIB(K)_1_Kpn3, IncFII_1, ColKP3_1, Col(BS512)_1, Col(BS512)_1__NC_010656_dupe	*blaCTX-M-15, blaSHV-11, blaSHV-158, blaSHV-182, blaTEM-105*
NIB041	*P. faecis*	Col3M_1	*No ESBLs gene*
NIB044	*E. coli*	IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107, IncFII_1	*blaCTX-M-15*

4.11. Mobile Genetic Elements Profiling and Clustering Analysis

A clustered heatmap was generated to examine the distribution of mobile genetic elements across the 36 isolates, using MOB-suite (MobTyper) IS family counts with hierarchical clustering (Ward's linkage, Euclidean distance). The heatmap displays absolute counts of 24 IS families and reveals pronounced heterogeneity in MGE content (**Figure 28**).

Overall, ISEc, IS, and ISKpn were the most common IS families, though their abundance varied widely. Isolates NIB031, NIB011, NIB015, NIB021, NIB022, and NIB027 exhibited high ISEc copy numbers (7 copies), suggesting elevated genomic plasticity and potential roles in AMR gene mobilization. In contrast, a cluster of isolates (NIB003, NIB006, NIB007, NIB025, NIB028, NIB029) showed very low IS content, indicating comparatively stable genomes.

Several isolates carried lineage-specific IS families. NIB017 uniquely harbored ISAba (12 copies), ISAha (4), ISAjo (1), and ISAlw (1), representing a distinct MGE signature. NIB002 exclusively contained ISYal, suggesting unique evolutionary origins. NIB037 displayed a high ISKpn burden (6 copies).

Across the dataset, ISEc was the most widespread family (21/36 isolates, 58.33%), followed by ISKpn (15 isolates), ISCro (11), ISSen (11), and ISEcp. Their co-occurrence in multiple isolates suggests shared reservoirs or coordinated mobilization pathways.

Clustering identified a high-MGE-content group (e.g., NIB037, NIB012, NIB014, NIB013) enriched for ISEc, IS, ISKpn, and occasional rare IS families, indicating elevated genomic rearrangement potential. In contrast, a low-MGE-content group (NIB002, NIB007, NIB024, NIB025, NIB029, NIB006, NIB003, NIB028) exhibited minimal insertion sequences.

Overall, the clustering highlights both common and isolate-specific IS profiles, reflecting ongoing evolutionary diversification and suggesting that high-copy IS elements in selected isolates may be driven by selective pressures such as antibiotic exposure.

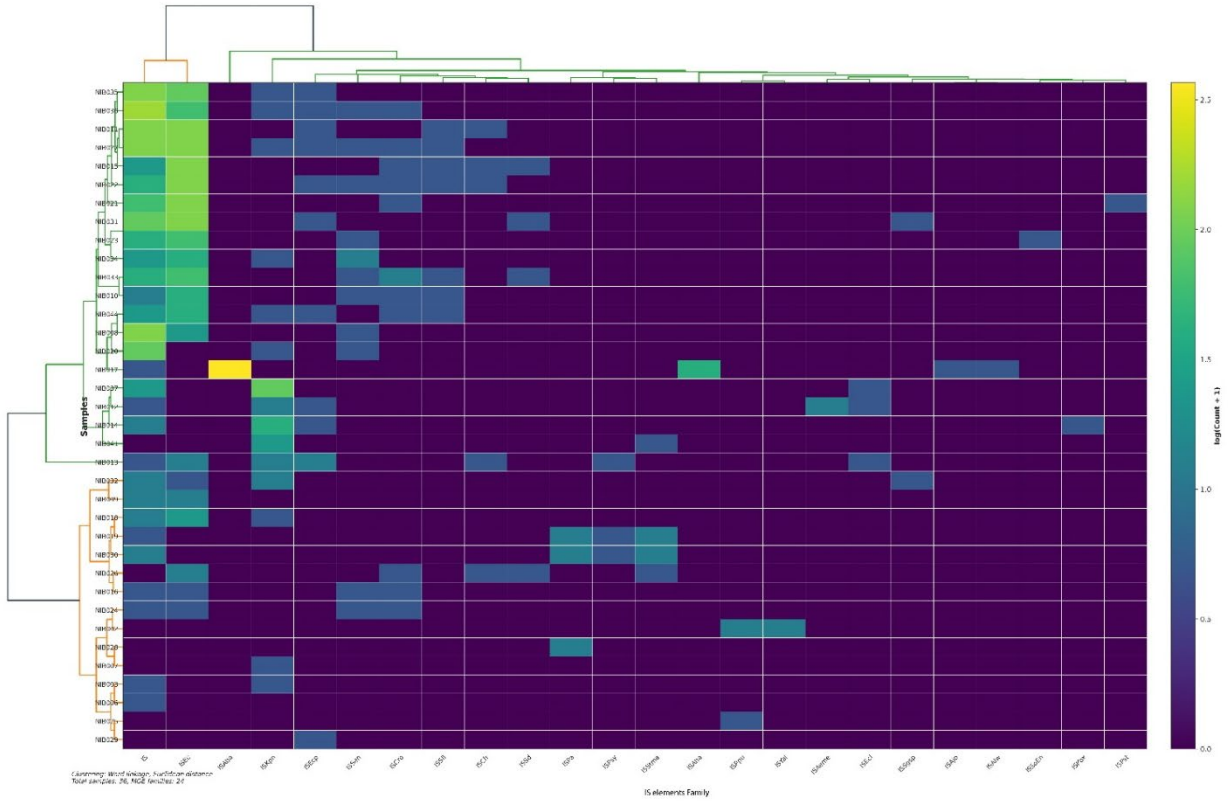


Figure 28. Hierarchical Clustering of Clinical Isolates Based on the Abundance and Diversity of Mobile Genetic Elements (Insertion Sequence Families)

4.12. Distinct IS Family Co-occurrence Modules Reveal Heterogeneous MGE Patterns Across Clinical Isolates

The correlation analysis of IS families showed that most insertion sequences were distributed independently across isolates, with only weak or negligible associations. Even so, a few small clusters of strongly correlated IS families stood out, hinting at specific genetic linkages. The tightest cluster, ISAha, ISAjo, and ISAlw, displayed almost perfect positive correlations, mainly because they appeared together in high copy numbers within a single isolate (NIB014) (**Figure 29**). Moderate correlations among the ISEc, ISEcp, and ISEcl families suggested they frequently occur together across multiple isolates, while ISKpn also showed positive associations with several IS families, consistent with its typical presence on plasmids. A smaller grouping among

ISP family elements (ISPpu, ISPst, ISPsy) was also noticeable. Since many IS families were rare, some strong correlations likely reflect the influence of only a few isolates. Notably, the clusters involving ISEcp and ISKpn, both well-known for mobilizing antimicrobial resistance genes, highlight their potential role in spreading MGEs that may carry resistance determinants.

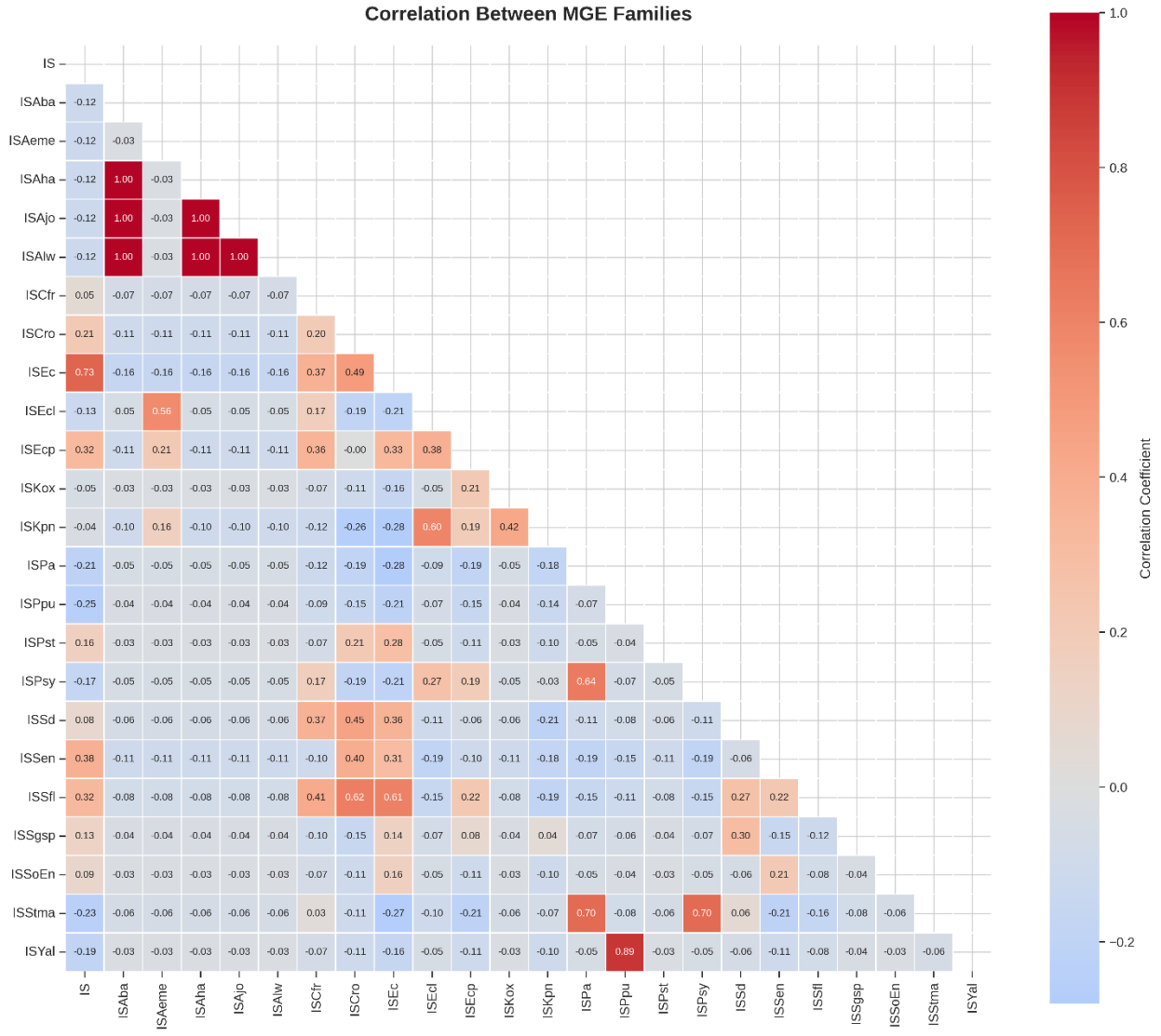


Figure 29. Correlation Heatmap Showing Co-occurrence Patterns Among 24 IS Families Across 36 Isolates.

4.13. Phenotypic Confirmation of ESBL Production Following CLSI Guidelines

Phenotypic detection of ESBL production was performed using the Disc Diffusion Test (DDT) (**Figure 30**). This method evaluates the enhancement of inhibition zones for cephalosporin-clavulanic acid combinations such as cefotaxime-clavulanic acid (CTC, 30/10 µg) and ceftazidime-clavulanic acid (CZC, 30/10 µg), compared to cephalosporin alone (cefotaxime [CTX, 30 µg] and ceftazidime [CAZ, 30 µg]). Based on CLSI M100-ED35:2025 guidelines, an increase of ≥5 mm in zone diameter for CTC or CZC compared to CTX or CAZ alone was interpreted as phenotypic ESBL production.

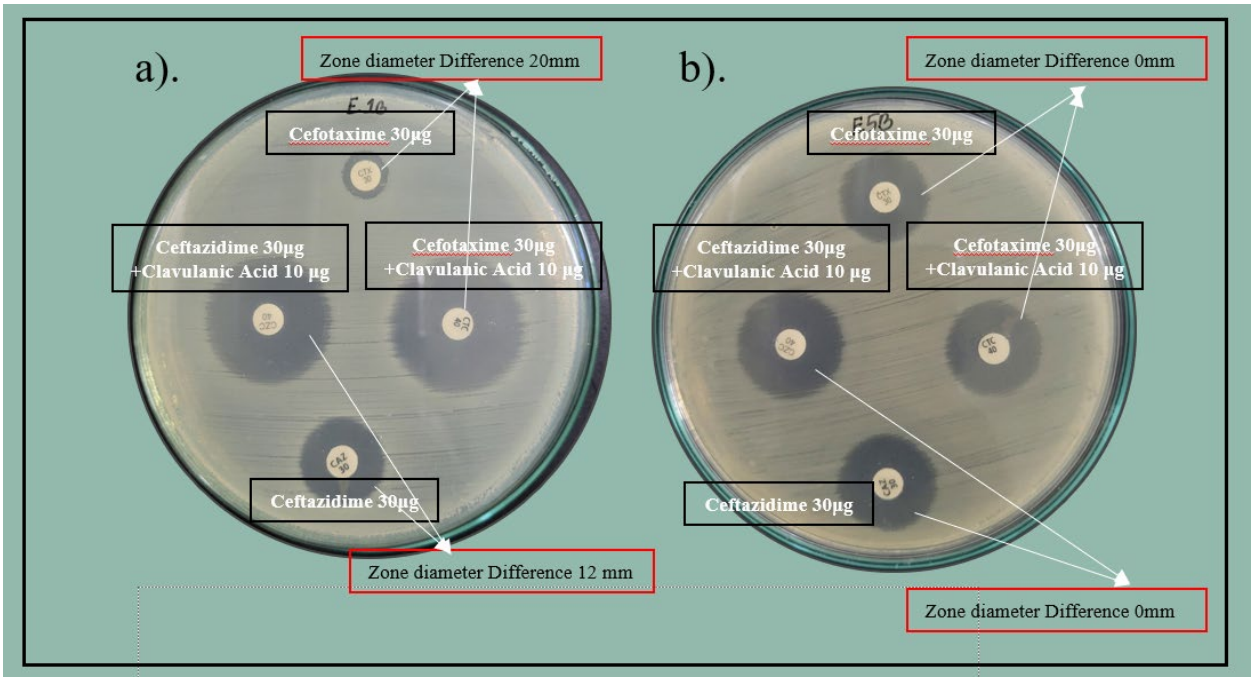


Figure 30. Image of representative DDST plate confirming ESBL (a) positive *E. coli* (NIB036) and (b) negative *Enterobacter hormaechei* (NIB007) distribution

Table 7. Phenotypic Detection of ESBL Production via Disc Diffusion Test (DDT) Based on Zone Diameter Differences Between Cephalosporin and Cephalosporin-Clavulanic Acid Combinations of Genotypically positive isolates.

Strain Number	Organism	Cefotaxim (CTX, 30µg) (mm)	Cefotaxime-Clavulanic acid (CTC, 30/10 µg) (mm)	Ceftazidime (CAZ, 30µg) (mm)	Ceftazidime-Clavulanic acid (CTC, 30/10 µg) (mm)	(CTC – CTX) (mm)	(CZC-CAZ) (mm)
NIB008	*E. coli*	30	30	30	30	0	0
NIB009	*E. coli*	0	28	14	27	28	13
NIB011	*E. coli*	0	25	15	23	25	8
NIB012	*K. pneumoniae*	9	28	16	25	19	9

NIB013	*E. hormaechei*	7	22	13	19	15	6
NIB014	*K. pneumoniae*	0	7	0	7	7	7
NIB015	*E. coli*	0	7	0	8	7	8
NIB018	*E. fergusonii*	24	27	24	26	3	2
NIB020	*E. coli*	30	31	27	27	1	0
NIB021	*E. coli*	28	28	25	26	0	1
NIB022	*E. coli*	0	30	17	23	30	6
NIB026	*E. coli*	25	26	21	23	1	2
NIB027	*E. coli*	0	23	16	27	23	11
NIB029	*E. chuandaensis*	10	27	17	27	17	10
NIB031	*E. coli*	0	25	16	25	25	9
NIB032	*K. pneumoniae*	25	25	26	26	0	0
NIB033	*E. coli*	0	23	19	23	23	4
NIB035	*E. coli*	0	26	15	24	26	9
NIB036	*E. coli*	7	30	15	27	23	12
NIB037	*K. pneumoniae*	0	0	0	0	0	0
NIB044	*E. coli*	9	26	14	23	17	9

The zone diameter measurements of genotypically positive 21 isolates tested revealed notable differences in inhibition zones. Among them, several isolates showed significant increases in inhibition zones for CTC and CZC compared to CTX and CAZ, respectively, with differences reaching up to 30 mm **(Table 7)**. This suggests phenotypic ESBL activity. For instance, isolate NIB022 (*E. coli*) showed a 30 mm increase in the CTC-CTX comparison, while isolate NIB009 (*E. coli*) showed a 28 mm increase. Conversely, a few isolates (e.g., NIB008, NIB032, and NIB037) showed no significant change (0 mm difference), indicating a negative ESBL phenotype.

Table 8: Phenotypic Detection of ESBL Production via Disc Diffusion Test (DDT) Based on Zone Diameter Differences Between Cephalosporin and Cephalosporin-Clavulanic Acid Combinations of Control (Genotypically negative isolates).

Strain Number	Organism	Cefotaxim (CTX, 30µg) (mm)	Cefotaxime-Clavulanic acid (CTC, 30/10 µg) (mm)	Ceftazidime (CAZ, 30µg) (mm)	Ceftazidime-Clavulanic acid (CTC, 30/10 µg) (mm)	(CTC – CTX) (mm)	(CZC – CAZ) (mm)
NIB001	*P. shigelloids*	26	27	24	24	1	0
NIB002	*P. stuartii*	24	28	22	23	4	1
NIB003	*C. werkmanii*	29	30	27	27	1	0
NIB006	*M. morganii*	35	35	29	30	0	1
NIB007	*E. hormaechei*	23	23	21	21	0	0
NIB010	*E. coli*	26	27	22	22	1	0
NIB016	*S. stutzeri*	32	36	41	42	4	1
NIB017	*A. variabilis*	26	29	26	30	3	4
NIB019	*P. aeruginosa*	17	19	25	27	2	2
NIB023	*E. coli*	24	25	21	21	1	0
NIB024	*E. coli*	25	28	21	23	3	2
NIB025	*P. mirabilis*	34	34	30	30	0	0
NIB028	*P. aeruginosa*	16	20	23	24	4	1
NIB030	*P. aeruginosa*	18	21	27	27	3	0
NIB034	*E. coli*	10	26	19	24	16	5
NIB039	*P. mendocina*	20	23	23	23	3	0
NIB041	*P. faecis*	34	34	30	30	0	0

Results for 17 control isolates showed that most exhibited minimal differences in zone diameters between cephalosporins and their respective clavulanic acid combinations, typically less than 5 mm **(Table 8)**. This pattern suggests an absence of ESBL activity. However, a few exceptions were observed, for instance, isolate NIB034 (*E. coli*) showed a 16 mm increase between CTC and CTX and 5 mm between CZC and CAZ, indicating a positive ESBL-like response. Such results may arise due to factors like overexpression of other β-lactamases or cell wall permeability alterations.

Together, these results highlight the utility of DDT in identifying potential ESBL producers based on measurable differences in zone diameters on disc diffusion assays.

4.14. Genotype-Phenotype Correlation of ESBL detection

Table 9. Comparison of Genotypic and Phenotypic ESBL Detection in Pediatric Diarrheal Bacterial Isolates

Serial No.	Sample ID	Strain Number	Organism	ESBLs Gene	ESBL Genotype	ESBL Phenotype
1	**H-11**	NIB001	*P. shigelloids*	No ESBL gene detected	Negative	Negative
2	**I2**	NIB002	*P. stuartii*	No ESBL gene detected	Negative	Negative
3	**J4**	NIB003	*C. werkmanii*	No ESBL gene detected	Negative	Negative
4	**Z3**	NIB006	*M. morganii*	No ESBL gene detected	Negative	Negative
5	**Z6**	NIB007	*E. hormaechei*	No ESBL gene detected	Negative	Negative
6	**G4**	NIB008	*E. coli*	***bla*$_{TEM-135}$, *bla*$_{TEM-106}$**	Positive	Negative
7	**H2**	NIB009	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
8	**C7**	NIB010	*E. coli*	No ESBL gene detected	Negative	Negative
9	**1**	NIB011	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
10	**J8**	NIB012	*K. pneumoniae*	***bla*$_{SHV-106}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-105}$**	Positive	Positive
11	**17**	NIB013	*E. hormaechei*	***bla*$_{CTX-M-15}$**	Positive	Positive
12	**16**	NIB014	*K. pneumoniae*	***bla*$_{CTX-M-15}$, *bla*$_{SHV-158}$, *bla*$_{TEM-105}$, *bla*$_{SHV-182}$**	Positive	Positive
13	**22**	NIB015	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
14	**R6**	NIB016	*S. stutzeri*	No ESBL gene detected	Negative	Negative
15	**E2**	NIB017	*A. variabilis*	No ESBL gene detected	Negative	Negative
16	**P6**	NIB018	*E. fergusonii*	***bla*$_{TEM-105}$**	Positive	Negative
17	**24**	NIB019	*P. aeruginosa*	No ESBL gene detected	Negative	Negative
18	**D1**	NIB020	*E. coli*	***bla*$_{TEM-176}$**	Positive	Negative

19	**12**	NIB021	*E. coli*	***bla*$_{TEM-105}$**	Positive	Negative
20	**4**	NIB022	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
21	**25**	NIB023	*E. coli*	No ESBL gene detected	Negative	Negative
22	**C2**	NIB024	*E. coli*	No ESBL gene detected	Negative	Negative
23	**Z5**	NIB025	*P. mirabilis*	No ESBL gene detected	Negative	Negative
24	**J3**	NIB026	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Negative
25	**7**	NIB027	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
26	**8**	NIB028	*P. aeruginosa*	No ESBL gene detected	Negative	Negative
27	**J2**	NIB029	*E. chuandaensis*	***bla*$_{CTX-M-15}$**	Positive	Positive
28	**23**	NIB030	*P. aeruginosa*	No ESBL gene detected	Negative	Negative
29	**A1**	NIB031	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
30	**3**	NIB032	*K. pneumoniae*	***bla*$_{SHV-110}$**	Positive	Negative
31	**Q5**	NIB033	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
32	**9**	NIB034	*E. coli*	No ESBL gene detected	Negative	Positive
33	**M2**	NIB035	*E. coli*	***bla*$_{CTX-M-15}$, *bla*$_{TEM-105}$**	Positive	Positive
34	**5**	NIB036	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive
35	**21**	NIB037	*K. pneumoniae*	***bla*$_{CTX-M-15}$, *bla*$_{SHV-11}$, *bla*$_{SHV-158}$, *bla*$_{SHV-182}$, *bla*$_{TEM-105}$**	Positive	Negative
36	**shig**	NIB039	*P. mendocina*	No ESBL gene detected	Negative	Negative
37	**S5**	NIB041	*P. faecis*	No ESBL gene detected	Negative	Negative
38	**R4**	NIB044	*E. coli*	***bla*$_{CTX-M-15}$**	Positive	Positive

A total of 38 bacterial isolates were subjected to genotypic and phenotypic analysis for extended-spectrum β-lactamase (ESBL) production. Genotypic screening using *Abricate* and curated resistance gene databases revealed that 21 isolates (55.3%) **(Table 10)** harbored ESBL-associated genes such as *bla*$_{CTX-M-15}$, *bla*$_{SHV-158}$, *bla*$_{TEM-105}$, *and bla*$_{SHV-182}$ **(Table 9)**. The remaining 17 isolates (44.7%) did not carry any detectable ESBL genes (genotypically negative). In contrast, Fifteen isolates (39.5%) were phenotypically positive, while 23 (60.5%) were negative.

Comparison of genotypic and phenotypic findings showed that 14 isolates were both genotypically and phenotypically (wet lab validation) positive, confirming ESBL production. However, 7 isolates were genotypically positive but phenotypically negative (false negative) **(Table 10)**. Among them, one notable case was a *K. pneumoniae* (Strain Number: NIB037) isolate carrying multiple ESBL genes (*bla*$_{CTX-M-15}$, *bla*$_{TEM-105}$, and *bla*$_{SHV-158}$), yet it exhibited

complete resistance with no inhibition zone (0 mm) for CTX, CTC, CAZ, or CZC **(Table 7)**. This failed to meet the ≥5 mm increase threshold required for phenotypic confirmation, leading to a false-negative result in the phenotypic test. It suggests high-level resistance possibly due to co-existing carbapenemases (*bla*$_{NDM-5}$, *bla*$_{OXA-232}$, *bla*$_{OXA-181}$) and other resistance determinants (e.g., *rmtF*, *oqxAB*, *fosA6*), which can mask ESBL activity in phenotypic tests.

In contrast, one *E. coli* isolate (strain NIB034) demonstrated an ESBL-positive phenotype (false positive), although no known ESBL genes were detected through WGS. This discrepancy may be attributed to the hyperproduction of non-ESBL β-lactamases, alterations in outer membrane permeability (CLSI M100 ED35:2025), the presence of novel or uncharacterized ESBL variants, or limitations within current resistance gene databases.

Overall, the concordance between genotypic and phenotypic methods was moderate, underscoring the limitations of phenotypic detection particularly in isolates with complex resistance mechanisms such as AmpC β-lactamase coproduction, carbapenemase presence, or porin loss.

Table 10. Summary of genotypic and phenotypic ESBL detection among 38 isolates.

Result Type	Number of Isolates
Genotypically Positive	21
Genotypically Negative	17
Phenotypically Positive	15
Phenotypically Negative	23
Genotype & Phenotype Positive	14
Genotype Positive, Phenotype Negative	7
Genotype Negative, Phenotype Positive	1

4.15. Concordance Between Genotypic and Phenotypic ESBL Detection

The comparison of ESBL detection methods across 38 bacterial isolates demonstrated 78.95% overall concordance (30/38), with 36.84% (14/38) positive agreement (both genotype and phenotype positive) and 42.10% (16/38) negative agreement (both methods negative) (**Table 11**). Discordant results were observed in 21.05% (8/38) of isolates, predominantly as genotype-positive/phenotype-negative cases (18.42%, 7/38), possibly due to underexpressed genes or phenotypic assay limitations. A single isolate (2.63%, 1/38) displayed phenotypically ESBL positive without the presence of ESBL genes in WGS data. The frequent detection of *bla*$_{CTX\text{-}M\text{-}15}$ in concordant positives underscores its clinical relevance. These findings underscore the importance of integrating whole genome sequencing (WGS) with phenotypic methods for accurate ESBL detection, while emphasizing the urgent need to curb the spread of ESBL genes.

Table 11. Analytical Summary of ESBL Detection Methods in Pediatric Diarrheal Isolates (n=38)

Category	Number of Isolates	Percentage (%)
Agreement (Total Concordance)	**30**	**78.95**
True Positive (TP) = Genotype +, Phenotype +	14	36.84
True Negative (TN) = Genotype –, Phenotype –	16	42.10
Disagreement (Total Discordance)	**8**	**21.05**
Genotype+/Phenotype- (FN)	7	18.42
Genotype-/Phenotype+ (FP)	1	2.63
Concordance Formula: Concordance = $\frac{TP+TN}{Total\ isolates(n)}$		
Sensitivity (Genotype predicting Phenotype): Sensitivity = $\frac{TP}{TP+FN} = \frac{14}{14+7} \times 100\% = 66.67\%$ Specificity = $\frac{TN}{TN+FP} = \frac{16}{16+1} \times 100\% = 94.12\%$ Positive Predictive Value (PPV) = $\frac{TP}{TP+FP} = \frac{14}{14+1} \times 100\% = 93.3\%$ Negative Predictive Value (NPV) = $\frac{TN}{TN+FN} = \frac{16}{16+6} \times 100\% = 72.73\%$		

Chapter 5

Discussion

Diarrhoea is defined as the passage of three or more loose or liquid stools per day, excluding frequent formed stools or the naturally loose stools of breastfed infants. It remains a major global health concern, ranking as the third leading cause of death among children aged 1–59 months. The World Health Organization (WHO) estimates that every year, diarrhoea causes approximately 443,832 deaths in children under five, along with 50,851 deaths among children aged 5–9 years, and accounts for nearly 1.7 billion childhood cases worldwide [240]. Although preventable and treatable, diarrhoeal disease continues to contribute significantly to childhood malnutrition [241]. While dehydration was once the primary cause of diarrhoea-related mortality, septic bacterial infections are now increasingly responsible for severe outcomes [242].

Antimicrobial resistance (AMR) has emerged as a major global concern in the 21st century due to the rapid rise in AMR infection rates and the limited development of new antimicrobial agents [243]. ESBL-E represent a significant part of this AMR threat and have become a global issue, increasing in prevalence across many countries since their first detection in 1983 [12], [13]. ESBLs represent the most significant resistance mechanism against β-lactam antibiotics in *Enterobacteriales*. According to the CLSI, ESBL testing is primarily useful for epidemiological studies and infection prevention involving *E. coli, K. pneumoniae*, and *P. mirabilis*. ESBLs are diverse, plasmid-mediated enzymes capable of hydrolyzing nearly all β-lactam antibiotics, with the exception of cephamycins and carbapenems [238]. According to the World Health Organization's priority list for antibiotic research and development, ESBL-E is ranked as the most critical pathogen [244].

Previous studies have highlighted the rapid global expansion of ESBL-producing *Enterobacteriaceae*, particularly the dominance of CTX-M-type enzymes [245], [246], [247], [248]. **Cantón et al. (2008b)** [249] reported that CTX-M ESBLs have replaced the earlier TEM and SHV families and are now the most prevalent worldwide, largely due to their association with mobile genetic elements and epidemic plasmids [250], [251], [252]. Similarly, **Bevan et al. (2017)** suggested that plasmid-mediated *blaCTX-M-15* has become the most widely disseminated ESBL variant, contributing substantially to community and hospital infections in South Asia [14]. In another genomic investigation, **Stoesser et al. (2016)** demonstrated that ESBL genes, particularly *blaCTX-M*, frequently co-occur with other AMR determinants and virulence factors on transferable plasmids, accelerating both pathogenicity and resistance spread [253].

Comparable findings have been reported in Bangladesh. Souverein et al. (2017) documented widespread circulation of *blaCTX-M-15*, *blaTEM*, and *blaSHV* among clinical *E. coli* and *K. pneumoniae* [85]. A recent study by Rahman et al. (2025) [254] reported high levels of multidrug-resistant ESBL-producing *E. coli* in animals, while M. S. Hossain et al. (2021) [255] identified ESBL genes in *E. coli* from faecal sludge treatment plants in Rohingya camps. However, ESBL-producing *Enterobacteriaceae* in pediatric diarrheal patients, together with virulence gene profiling and mobilome characterization, remain largely unexplored in Bangladesh.

To address this issue, the present study combined whole genome sequencing, ESBL gene identification, virulence profiling, plasmid identification, and phenotypic ESBL testing to provide a more comprehensive picture of ESBL-producing *Enterobacteriaceae* in pediatric diarrheal cases. This study systematically assessed ESBL production among clinical isolates through complementary genotypic and phenotypic approaches, revealing several critical insights into resistance mechanisms and their implications.

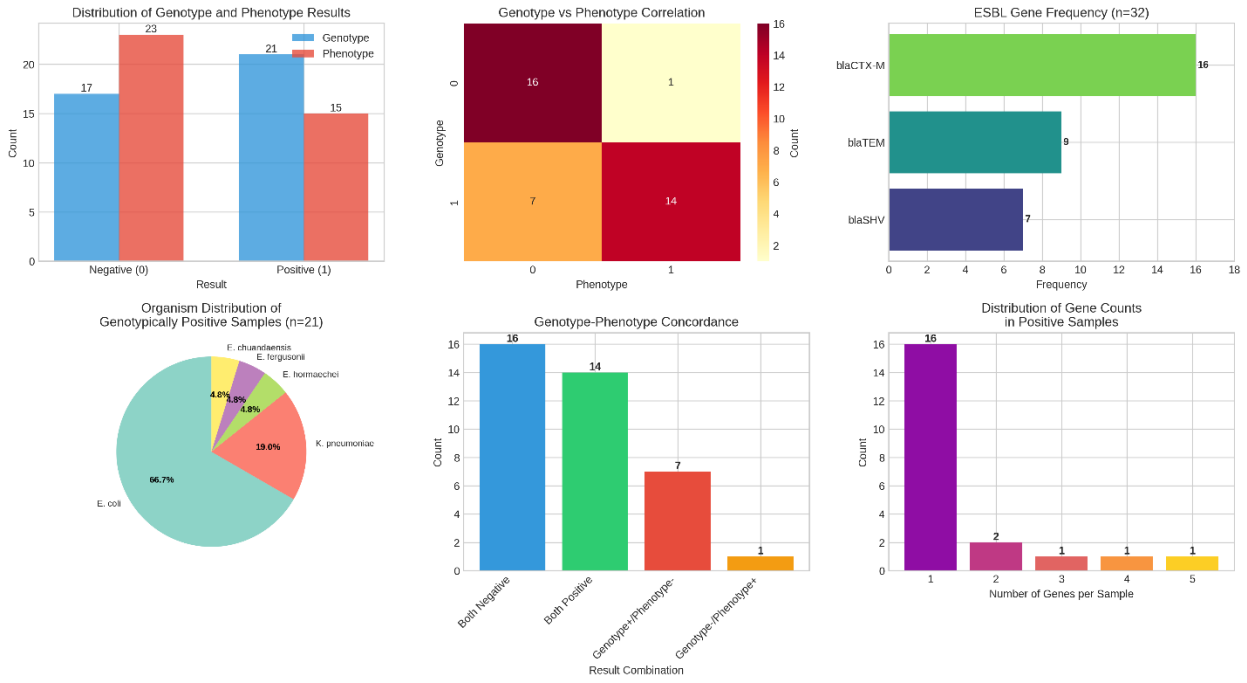


Figure 31. ESBL Phenotypic and Genotypic analysis.

Genotypic screening using WGS identified ESBL-related genes in 55.3% (21/38) of isolates, with *blaCTX-M-15* emerging as the predominant ESBL variant detected in 76.19% (16/21) of positive isolates, primarily in *E. coli* (78.57%) (**Figure 31**). This finding support prior Bangladeshi and international studies and aligns with global trends recognizing *blaCTX-M-15* as the most widespread ESBL gene variant, reflecting its broad host range and transmissibility across species, including *K. pneumoniae, E. hormaechei, and E. chuandaensis* [250], [256], [257]. *blaTEM* variants appeared in 28.57% of isolates, with *blaTEM-105* being the most common across *E. coli* and *K. pneumoniae*. blaSHV variants were exclusive to *K. pneumoniae*, notably *blaSHV-158* and *blaSHV-182*, highlighting strain-specific resistance gene distribution [258], [259]. The detection of multiple ESBL gene variants (*blaTEM*, *blaSHV*) within single isolates indicates potential co-selection and horizontal gene transfer, intensifying resistance profiles [52], [77].

Phenotypic analysis using disk diffusion tests, based on CLSI guidelines, identified ESBL production in 39.5% of isolates. Concordance between genotypic and phenotypic methods was moderate at 78.95%, with 36.84% positive agreement and 42.1% negative agreement. Discordant findings included genotypic-positive but phenotypic-negative isolates (false negatives), possibly due to masking effects from co-produced AmpC β-lactamase, carbapenemases and other resistance mechanisms, as exemplified by a *K. Pneumoniae* isolate (NIB037) showing high-level resistance without typical phenotypic confirmation [44]. Conversely, one *E. coli* isolate (NIB034) was phenotypically positive yet lacked detected ESBL genes (false positive), potentially explained by hyperproduction of non-ESBL β-lactamases, porin modifications, or novel gene variants not captured in databases. These discrepancies underscore limitations of phenotypic testing alone, especially when resistance profiles are complex [260], [261], [262].

Plasmid replicon analysis revealed a diverse assemblage of incompatibility groups, predominantly IncF, IncI, IncHI, IncX, IncB/O/K/Z, IncR, and Col-type plasmids, well-known vectors facilitating the spread of ESBL genes. The IncF family was dominant among *E. coli* isolates, consistent with their global role in disseminating blaCTX-M-15, while *K. pneumoniae* harbored IncFIB(K), IncR, and multiple Col plasmids. The presence of plasmids in isolates without detectable ESBL genes suggests other accessory functions and stresses the importance of integrated plasmid and resistance gene surveillance to fully capture resistance

dynamics [263], [264], [265]. The high-MGE-content group (e.g., NIB037, NIB012, NIB014, NIB013) showed an abundance of ISEc, IS, ISKpn, and several uncommon IS families, suggesting an increased capacity for genomic rearrangements.

Virulence profiling displayed marked heterogeneity. *P. aeruginosa* isolates demonstrated extensive virulence gene repertoires, reflecting their adaptability and pathogenic versatility. In contrast, *E. coli* isolates housed consistent core virulence determinants tied to adhesion and iron acquisition, supporting persistence with less overt aggressiveness. The interplay between virulence factors and resistance genes, particularly in ESBL carriers, poses clinical challenges by potentially enhancing colonization, evasion, and infection severity.

Collectively, the observed high prevalence of blaCTX-M-15, the complexity revealed by plasmid replicon diversity, and the phenotypic-genotypic discordances highlight the need for integrated diagnostic strategies combining WGS and phenotypic assays. Enhanced molecular surveillance coupled with refined phenotypic methodologies is critical to accurately identify ESBL producers, guide therapy, and curb the spread of multidrug resistant pathogens in clinical settings.

Notably, the integrative analysis of ESBL genes, virulence determinants, and mobilome components using WGS has not been extensively conducted in Bangladesh, and even globally, only a limited number of studies have focused specifically on pediatric diarrheal isolates with such a comprehensive genomic–phenotypic approach. Therefore, the present work fills a critical gap by offering detailed insights into resistance gene transmission, plasmid dynamics, and virulence potential in a vulnerable pediatric population.

Chapter 6

Conclusion

This study provides one of the most detailed looks to date at both the genomic and phenotypic features of ESBL-producing *Enterobacteriaceae* found in children with diarrheal illness in Bangladesh. We observed that blaCTX-M-15 remains the dominant ESBL gene, accompanied by several *blaTEM* and *blaSHV* variants, an indication that highly transmissible resistance determinants continue to spread among vulnerable pediatric populations.

The moderate agreement between WGS-based genotyping and phenotypic ESBL results also serves as an important reminder: no single diagnostic method tells the full story. Silent genes, masked resistance mechanisms, and plasmid-driven gene exchange can all complicate interpretation when relying on phenotypic testing alone. Likewise, the wide range of plasmid replicon types detected highlights how effectively mobile genetic elements can move resistance and virulence traits across bacterial species.

Together, these findings underscore the urgent need to pair routine whole-genome sequencing with traditional phenotypic assays to achieve more accurate detection, surveillance, and understanding of ESBL-associated resistance. Strengthening genomic surveillance systems, especially in pediatric healthcare settings, will be essential for guiding antimicrobial stewardship, informing infection control policies, and slowing the spread of multidrug-resistant pathogens in resource-limited environments.

Ultimately, this work provides a crucial genomic foundation that can support national AMR monitoring efforts and help shape future interventions aimed at protecting children from severe and resistant infections.

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Appendix A

Supplementary Tables

S1: Demographic Data

Serial No.	Patient ID	Location	Sex	Age (Months)	Days of suffering	Residence	Clinical Status	Strain No.	Occurrence (n)
1	A	Lakshmipur Sadar Hospital	Female	8	6	Rural	Inpatient	NIB031	1
2	B	Lakshmipur Sadar Hospital	Female	18	7	Rural	Inpatient	NIB033	1
3	C	Lakshmipur Sadar Hospital	Female	24	5	Urban	Inpatient	NIB010, NIB024	2
4	D	Lakshmipur Sadar Hospital	Female	18	6	Urban	Inpatient	NIB020	1
5	E	Lakshmipur Sadar Hospital	Male	13	7	Rural	Inpatient	NIB016	1
6	F	Lakshmipur Sadar Hospital	Male	11	8	Rural	Inpatient	NIB017	1
7	G	Lakshmipur Sadar Hospital	Female	18	5	Urban	Inpatient	NIB008	1
8	H	Lakshmipur Sadar Hospital	Male	13	7	Urban	Inpatient	NIB009	1
9	I	Lakshmipur Sadar Hospital	Male	24	5	Rural	Inpatient	NIB029	1
10	J	Lakshmipur Sadar Hospital	Male	24	6	Rural	Inpatient	NIB012, NIB026	2
11	K	Lakshmipur Sadar Hospital	Female	70	7	Rural	Inpatient	NIB018	1
12	L	Lakshmipur Sadar Hospital	Female	6	6	Urban	Inpatient	NIB035	1
13	M	Lakshmipur Sadar Hospital	Male	14	7	Rural	Inpatient	NIB044	1
14	N	Dhaka Shishu Hospital & Institute	Male	8	8	Urban	Inpatient	NIB014	1
15	O	Dhaka Shishu Hospital & Institute	Male	22	5	Urban	Inpatient	NIB022, NIB032	2
16	P	Dhaka Shishu Hospital & Institute	Female	12	4	Urban	Inpatient	NIB027, NIB036	2
17	Q	Dhaka Shishu Hospital & Institute	Male	7	6	Rural	Inpatient	NIB034	1
18	R	Dhaka Shishu Hospital & Institute	Female	8	5	Urban	Inpatient	NIB021	1
19	S	Dhaka Shishu Hospital & Institute	Male	8	6	Urban	Inpatient	NIB011, NIB013	2
20	T	Dhaka Shishu Hospital & Institute	Male	9	25	Rural	Inpatient	NIB037	1
21	U	Dhaka Shishu Hospital & Institute	Male	12	11	Urban	Inpatient	NIB019	1
22	V	Dhaka Shishu Hospital & Institute	Male	4	7	Rural	Inpatient	NIB015	1
23	W	Dhaka Shishu Hospital & Institute	Male	8	3	Urban	Inpatient	NIB023	1
24	X	Dhaka Shishu Hospital & Institute	Male	15	6	Rural	Inpatient	NIB028	1
25	Y	Dhaka Shishu Hospital & Institute	Female	6	4	Urban	Inpatient	NIB003	1
26	Z	Dhaka Shishu Hospital & Institute	Male	4	3	Rural	Inpatient	NIB039	1
27	A1	Dhaka Shishu Hospital & Institute	Female	1	5	Urban	Inpatient	NIB041	1
28	A2	Dhaka Shishu Hospital & Institute	Male	1	4	Rural	Inpatient	NIB030	1
29	A3	Dhaka Shishu Hospital & Institute	Female	10	6	Urban	Inpatient	NIB025	1
30	A4	Dhaka Shishu Hospital & Institute	Male	54	10	Urban	Inpatient	NIB006	1
31	A5	Dhaka Shishu Hospital & Institute	Female	30	4	Urban	Inpatient	NIB002	1
32	A6	Dhaka Shishu Hospital & Institute	Female	96	7	Urban	Inpatient	NIB001, NIB007	2
									Total=38

S2: List of AMR genes Identified from 38 Isolates using 5 different databses.

Strain Number	Organism	CARD Database	Resfinder Database	NCBI Database	ARGANNOT database	Megares Database	ESBLs Gene	Carbapenemase producing gene
NIB001	***Plesiomonas shigelloids***	CRP	Not Detected	Not Detected	Not Detected	CRP	Not Detected	Not Detected
NIB002	***Providencia stuartii***	tet(B), sul2, catIII, QnrD1, AAC(2')-Ia, CRP	tet(B)_1, sul2_2, catA3_1, qnrD1_1, aac(2')-Ia_1	tet(B), sul2, catA3, qnrD1, aac(2')-Ia	(Tet)tetB, (Sul)sul2, (Phe)catA3, (Flq)qnrD, (AGly)aac2-Ia	TETB, TETD, SULII, CATA, QNRD, AAC2-PRIME, CRP	Not Detected	Not Detected
NIB003	***Citrobacter werkmanii***	kdpE, baeR, mdtC, mdtB, CRP, QnrB69, cpxA, tolC, bacA, H-NS, CMY-98, marA, msbA, acrD, yojI, pmrF, emrB, emrA, emrR, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH	qnrB69_1, blaCMY-98_1	qnrB69, blaCMY-159	(Bla)Penicillin_Binding_Protein_Ecoli, (Flq)qnrB37, (Bla)blaCMY-83, (Bla)ampH_Ecoli	PBP2, KDPE, KDEA, BAER, BAES, MDTC, MDTB, MDTI, CRP, QNRB, SOXS, CPXAR, CPXAR, BACA, EMRD, HNS, ROBA, CMY, CMY, MARA, MARR, MSBA, ACRD, BCR, YOGI, KPNO, PMRF, EMRB, EMRA, EMRR, ACRA, ACRB, AMPH	Not Detected	Not Detected
NIB006	***Morganella morganii***	DHA-20, CRP	blaMOR-2	blaMOR-2	(Bla)blaMOR-2	DHA, CRP	Not Detected	Not Detected
NIB007	***Enterobacter hormaechei***	ACT-25, FosA2, CRP, marA, Klebsiella_pneumoniae_KpnF, bacA, baeR, mdtC, mdtB, mdtA, cpxA, ramA, msbA, acrD, oqxB, oqxA, Enterobacter_cloacae_acrA, acrB, emrB, Klebsiella_pneumoniae_KpnG, emrR, H-NS	blaACT-16, fosA, oqxB, oqxA	blaACT-83 fosA oqxB9 oqxA9	(Bla)blaACT-16, (Fcyn)FosA2, (Bla)Penicillin_Binding_Protein_Ecoli, (Flq)OqxBgb, (Flq)OqxA	FOSA, ACT, HNS, CRP, CPXAR, MARR, MARA, MDTA, MDTB, MDTC, BAER, KPNO, SOXS, MSBA, ACRD, OQXB, OQXA, RAMA, PBP2, KDEA, BACA, KPNF, ACRB, EMRB, KPN, EMRR	Not Detected	Not Detected
NIB008	***Escherichia coli***	msbA, Escherichia_coli_mdfA, mdtP, mdtO, mdtN, eptA, Escherichia_coli_ampC, cpxA, kdpE, emrR, emrA, emrB, mphB, acrD, Escherichia_coli_ampC1_beta-lactamase, evgS, evgA, emrK, emrY, mdtG, mdtH, H-NS, acrS, acrE, acrF, marA, tolC, bacA, gadX, gadW, mdtF, mdtE, CRP, pmrF, yojI, mdtM, QepA4, dfrA12, aadA2, sul1, mphA, baeR, baeS, mdtC, mdtB, mdtA, ugd, sul2, tet(A), TEM-135, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH	mdf(A), qepA4, dfrA12, aadA2, sul1, mph(A), sul2, tet(A), blaTEM-106	blaEC-15, qepA4, dfrA12, aadA2, sul1, mph(A), sul2, tet(A), blaTEM-135	(Bla)AmpC2_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)AmpC1_Ecoli, (Flq)qepA, (Tmt)dfrA12, (AGly)aadA2, (Sul)sul1, (MLS)mph(A), (Sul)sul2, (Tet)tetR, (Tet)tetA, (Bla)blaTEM-106, (Bla)ampH_Ecoli	MSBA, MDFA, MDTP, MDTO, MDTN, EPTA, BLAEC, CPXAR, CPXAR, PBP2, KDPE, EMRR, EMRA, EMRB, MPHB, ACRD, PBP4B, EVGS, EMRK, EMRY, MDTG, MDTH, HNS, ROBA, ACRS, ACRE, ACRF, MARR, MARA, BACA, GADX, GADW, MDTF, MDTE, CRP, PMRF, YOGI, BCR, MDTM, EMRD, SOXS, QEPA, DFRA, ANT3-DPRIME, SULI, MPHA, BAER, BAES, MDTC, MDTB, MDTA, ASMA, UGD, SULII, MDTI, MDTJ, MDTK, TETA, TEM, ACRA, ACRB, AMPH	blaTEM-135, blaTEM-106	Not Detected
NIB009	***Escherichia coli***	mphB, acrD, Escherichia_coli_ampC1_beta-lactamase, evgS, evgA, emrK, emrY, pmrF, yojI, baeR, baeS, mdtC, mdtB, mdtA, ugd, Escherichia_coli_ampC, bacA, tolC, mdtP, mdtO, mdtN, eptA, cpxA, kdpE, Escherichia_coli_mdfA, H-NS, marA, emrR, emrA, emrB, mdtM, msbA, mdtG, mdtH, dfrA17, aadA5, sul1, mphA, Escherichia_coli_ampH, acrB, Escherichia_coli_acrA, Escherichia_coli_emrE, CTX-M-15, ErmB, CRP, mdtE, mdtF, gadW, gadX, tet(B), acrS, acrE, acrF	mdf(A), dfrA17, aadA5, sul1, mph(A), blaCTX-M-15, erm(B), tet(B)	blaEC-19, dfrA17, aadA5, sul1, mph(A), blaCTX-M-15, erm(B), tet(B)	(Bla)AmpC1_Ecoli, (Bla)AmpC2_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Tmt)dfrA17, (AGly)aadA5, (Sul)sul1, (MLS)mph(A), (Bla)ampH_Ecoli, (Bla)blaCTX-M-15, (MLS)erm(B), (Tet)tetB	MPHB, ACRD, PBP4B, EVGS, EMRK, EMRY, PMRF, KPNO, YOGI, BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, UGD, BLAEC, BACA, SOXS, MDTP, MDTO, MDTN, EPTA, CPXAR, PBP2, KDPE, MDFA, EMRD, HNS, MARA, MARR, MDTK, MDTJ, MDTI, EMRR, EMRA, EMRB, MDTM, ROBA, MSBA, MDTG, MDTH, DFRA, ANT3-DPRIME, SULI, MPHA, AMPH, ACRB, ACRA, MVRC, CTX, ERMB, CRP, MDTE, MDTF, GADW, GADX, TETB, ACRS, ACRE, ACRF	blaCTX-M-15	Not Detected
NIB010	***Escherichia coli***	mdtP, mdtO, mdtN, eptA, Escherichia_coli_ampC, emrR, emrA, emrB, tolC, bacA, msbA, cpxA, acrS, acrE, acrF, Escherichia_coli_ampH, yojI, H-NS, pmrF, mdtA, mdtB, mdtC, baeS, baeR, mdtM, mphB, acrD, Escherichia_coli_mdfA, Escherichia_coli_ampC1_beta-lactamase, kdpE, marA, mdtH, acrB, Escherichia_coli_acrA, emrY, emrK, evgA, evgS, mdtE, mdtF, gadW, gadX, QnrB4, DHA-1, sul1, mphA, dfrA17, CRP, mdtG	mdf(A), qnrB4, blaDHA-1, sul1, mph(A), dfrA17	blaEC, qnrB4, blaDHA-1, sul1, mph(A), dfrA17	(Bla)AmpC2_Ecoli, (Bla)ampH_Ecoli, (Bla)AmpC1_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Flq)qnrB4, (Bla)blaDHA-1, (Sul)sul1, (MLS)mph(A), (Tmt)dfrA17	MDTP, MDTO, MDTN, EPTA, BLAEC, EMRR, EMRA, EMRB, BACA, MSBA, MDTK, CPXAR, ACRS, ACRE, ACRF, AMPH, YOGI, BCR, HNS, PMRF, MDTA, MDTB, MDTC, BAES, BAER, MDTM, MPHB, ACRD, MDFA, PBP4B, EMRD, KDPE, PBP2, MARR, MARA, ROBA, MDTH, SOXS, ACRB, ACRA, EMRY, EMRK, EVGS, MDTE, MDTF, GADW, GADX, MDTI, MDTJ, QNRB, DHA, SULI, MPHA, DFRA, CRP, MDTG	Not Detected	Not Detected
NIB011	***Escherichia***	emrR, emrA, emrB, ugd, mdtA, mdtB,	mdf(A),	blaEC-5,	(Bla)AmpC2_Ecoli	EMRR, EMRA, EMRB,	blaCTX-M-15	Not Detected

	coli	mdtC, baeS, baeR, yojI, pmrF, Escherichia_coli_ampC, acrD, cpxA, mdtH, mdtG, Escherichia_coli_mdfA, emrY, emrK, evgA, evgS, mdtE, mdtF, gadW, gadX, marA, Escherichia_coli_emrE, CRP, CTX-M-15, acrF, acrE, acrS, bacA, tolC, mdtM, kdpE, H-NS, sul1, aadA2, dfrA12, mphA, msrE, mphE, DHA-1, Escherichia_coli_ampH, acrB, Escherichia_coli_acrA, mdtP, mdtO, mdtN, eptA, msbA	blaCTX-M-15, sul1, aadA2, dfrA12, mph(A), msr(E), mph(E), blaDHA-1	blaCTX-M-15, sul1, aadA2, dfrA12, mph(A), msr(E), mph(E), blaDHA-1	(Bla)blaCTX-M-15 (Bla)Penicillin_Binding_Protein_Ecoli (Sul)sul1 (AGly)aadA2 (Tmt)dfrA12 (MLS)mph(A) (MLS)msr(E) (MLS)mph(E) (Bla)blaDHA-1 (Bla)ampH_Ecoli	UGD, ASMA, MDTA, MDTB, MDTC, BAES, BAER, BCR, YOGI, KPNO, PMRF, BLAEC, ACRD, CPXAR, CPXAR, MDTH, MDTG, MDFA, EMRD, EMRY, EMRK, EVGS, MDTE, MDTF, GADW, GADX, MARR, MARA, MDTI, MDTJ, MDTK, MVRC, CRP, CTX, ACRF, ACRE, ACRS, BACA, ROBA, MDTM, MVRC, PBP2, KDPE, HNS, SULI, ANT3-DPRIME, DFRA, MPHA, MSRE, MPHE, DHA, AMPH, ACRB, ACRA, SOXS, MDTP, MDTO, MDTN, EPTA, MSBA		
NIB012	***Klebsiella pneumoniae***	Klebsiella_pneumoniae_OmpK37, Klebsiella_pneumoniae_KpnE, Klebsiella_pneumoniae_KpnF, SHV-106, marA, H-NS, baeR, mdtC, mdtB, ramA, cpxA, FosA6, CRP, Klebsiella_pneumoniae_KpnH, Klebsiella_pneumoniae_KpnG, emrR, oqxB, oqxA, CTX-M-15, TEM-1, APH(6)-Id, APH(3")-Ib, sul2, QnrB17, dfrA14, AAC(3)-IIe, acrD, acrB, Klebsiella_pneumoniae_acrA, msbA	blaSHV-106, fosA6, oqxB, oqxA, blaCTX-M-15, blaTEM-1B, aph(6)-Id, aph(3")-Ib, sul2, qnrB1, dfrA14, aac(3)-IIa	blaSHV-106, fosA6, oqxB19, oqxA5, blaCTX-M-15, blaTEM-1, aph(6)-Id, aph(3")-Ib, sul2, qnrB1, dfrA14, aac(3)-Iie	(Bla)blaSHV-106, (Bla)Penicillin_Binding_Protein_Ecoli, (Fcyn)FosA6, (Flq)OqxBgb, (Flq)OqxA, (Bla)blaCTX-M-15, (Bla)blaTEM-105, (AGly)strB, (AGly)strA, (Sul)sul2, (Flq)qnrB1, (Tmt)dfrA14, (AGly)aac3-IIa, (Bla)ampH	OMP37, KPNE, KPNF, SHV, MARA, KMRA, HNS, KPNO, BAER, MDTC, MDTB, ASMA, SOXS, PBP2, RAMA, EMRD, CPXAR, FOSA, CRP, EMRB, KPN, EMRR, OQXB, OQXA, PHOR, CTX, TEM, APH6, APH3-DPRIME, SULII, QNRB, DFRA, AAC3, ACRD, AMPH, ACRB, ACRA, KDEA, MSBA	blaSHV-106, blaCTX-M-15, blaTEM-105	Not Detected
NIB013	***Enterobacter hormaechei***	bacA, marA, cpxA, mdtA, mdtB, mdtC, baeR, emrB, Klebsiella_pneumoniae_KpnG, emrR, ACT-16, FosA2, tet(A), CTX-M-15, AAC(6')-Ib9, sul1, acrB, Enterobacter_cloacae_acrA, ramA, DHA-1, QnrB4, msbA, dfrA14, CRP, H-NS, oqxA, oqxB, acrD	blaACT-16, fosA, tet(A), blaCTX-M-15, aac(6')-Ib, sul1, blaDHA-1, qnrB4, dfrA14, oqxA, oqxB	blaACT-46, fosA, tet(A), blaCTX-M-15, aac(6')-Ib-D181Y, sul1, blaDHA-1, qnrB4, dfrA14, oqxA9, oqxB9	(Bla)blaACT-16, (Fcyn)FosA2, (Tet)tetA, (Tet)tetR, (Bla)blaCTX-M-15, (AGly)aac6-Ib, (Sul)sul1, (Bla)blaDHA-1, (Flq)qnrB4, (Tmt)dfrA14, (Bla)Penicillin_Binding_Protein_Ecoli, (Flq)OqxA, (Flq)OqxBgb	BACA, KPNO, MARR, MARA, CPXAR, CPXAR, SOXS, MDTA, MDTB, MDTC, BAER, EMRB, KPN, EMRR, ACT, ACT, FOSA, CTX, TETA, CTX, SULI, ACRB, ACRB, RAMA, DHA, QNRB, MSBA, DFRA, CRP, KDEA, PBP2, HNS, OQXA, OQXB, ACRD	blaCTX-M-15	Not Detected
NIB014	***Klebsiella pneumoniae***	cpxA, CTX-M-15, Klebsiella_pneumoniae_OmpK37, Klebsiella_pneumoniae_KpnE, Klebsiella_pneumoniae_KpnF, SHV-182, marA, mdtB, mdtC, baeR, ramA, H-NS, msbA, NDM-5, determinant_of_bleomycin_resistance, sul1, dfrA30, mphA, TEM-1, rmtB, acrB, Klebsiella_pneumoniae_acrA, FosA6, acrD, Klebsiella_pneumoniae_KpnH, Klebsiella_pneumoniae_KpnG, emrR, oqxB, oqxA, CRP	blaACT-16, fosA, tet(A), blaCTX-M-15, aac(6')-Ib, sul1, blaDHA-1, qnrB4, dfrA14, oqxA, oqxB	blaCTX-M-15, blaSHV-158, blaNDM-5, ble-MBL, sul1, dfrA30, mph(A), blaTEM-1, rmtB1, fosA6, oqxB, oqxA	(Bla)blaCTX-M-15, (Bla)blaSHV-11, (Bla)blaNDM-5, (Sul)sul1, (Tmt)dfrA30, (Bla)Penicillin_Binding_Protein_Ecoli, (MLS)mph(A), (Bla)blaTEM-105, (AGly)rmtB, (Bla)ampH, (Fcyn)FosA6, (Flq)OqxBgb, (Flq)OqxA	SOXS, PHOR, CPXAR, CPXAR, CTX, OMP37, KPNE, KPNF, SHV, MARA, KMRA, KPNO, MDTB, MDTC, BAER, ASMA, RAMA, CTX, HNS, MSBA, NDM, BRP, SULI, DFRA, PBP2, KDEA, MPHA, TEM, RMTB, AMPH, ACRB, ACRA, FOSA, ACRD, EMRB, KPN, EMRR, OQXB, OQXA, CRP	blaCTX-M-15, blaTEM-105, blaSHV-158, blaSHV-182	blaNDM-5
NIB015	***Escherichia coli***	tolC, marA, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH, kdpE, CRP, bacA, msbA, Escherichia_coli_mdfA, H-NS, mdtH, mdtG, pmrF, yojI, baeR, baeS, mdtC, mdtB, mdtA, emrY, emrK, evgA, evgS, mdtE, mdtF, gadW, gadX, emrB, emrA, emrR, cpxA, CMY-59, mdtM, Escherichia_coli_ampC, eptA, mdtN, mdtO, mdtP, acrE, acrF, dfrA17, aadA5, sul1, Escherichia_coli_ampC1_beta-lactamase, acrD, mphB, mphA, CTX-M-15, AAC(3)-IIe, AAC(6')-Ib-cr, OXA-1	mdf(A), blaCMY-148, dfrA17, aadA5, sul1, mph(A), blaCTX-M-15, aac(3)-IIa, aac(6')-Ib-cr, blaOXA-1	blaCMY-148, blaEC-15, dfrA17, aadA5, sul1, mph(A), blaCTX-M-15, aac(3)-IIe, aac(6')-Ib-D181Y, blaOXA-1	(Bla)ampH_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)blaCMY-42, (Bla)AmpC2_Ecoli, (Tmt)dfrA17, (AGly)aadA5, (Sul)sul1, (Bla)AmpC1_Ecoli, (MLS)mph(A), (Bla)blaCTX-M-15, (AGly)aac3-IIa, (Bla)blaOXA-1, (Phe)catB4	CTX, MARR, MARA, MDTI, MDTJ, MDTK, ACRA, ACRB, AMPH, PBP2, KDPE, CRP, BACA, MSBA, MDFA, ROBA, HNS, MDTH, MDTG, PMRF, KPNO, YOGI, BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, EMRY, EMRK, EVGS, MDTE, MDTF, GADW, GADX, EMRB, EMRA, EMRR, CPXAR, CPXAR, EMRD, CMY, MDTM, BLAEC, EPTA, MDTN, MDTO, MDTP, SOXS, ACRE, ACRF, DFRA, ANT3-DPRIME, SULI, PBP4B, ACRD, MPHB, MPHA, CTX, AAC3, OXA	blaCTX-M-15	blaOXA-1
NIB016	***Stutzerimonas stutzeri***	acrF, acrE, acrS, bacA, tolC, H-NS, msbA, dfrA17, cpxA, mphB, acrD, Escherichia_coli_ampH, Escherichia_coli_ampC, eptA, baeR, baeS, mdtC, mdtB, mdtA, mdtH, Escherichia_coli_mdfA, kdpE, mdtP, mdtO, mdtN, Escherichia_coli_acrA, acrB, marA, Escherichia_coli_ampC1_beta-lactamase, mdtM, pmrF, yojI, gadX, gadW, mdtF, mdtE, emrR, emrA, emrB, CRP, emrY, emrK, evgA, evgS, mdtG, QnrB4, DHA-1, sul1, mphA	dfrA17, mdf(A), qnrB4, blaDHA-1, sul1, mphA	dfrA17, blaEC, qnrB4, blaDHA-1, sul1, mphA	(Tmt)dfrA17, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)ampH_Ecoli, (Bla)AmpC2_Ecoli, (Bla)AmpC1_Ecoli, (Flq)qnrB4, (Bla)blaDHA-1, (Sul)sul1, (MLS)mph(A)	ACRF, ACRE, ACRS, BACA, HNS, MSBA, DFRA, MDTK, CTX, PBP2, CPXAR, MPHB, ACRD, AMPH, BLAEC, EPTA, BAER, BAES, MDTC, MDTB, MDTA, MDTH, MDFA, KDPE, MDTP, MDTO, MDTN, EMRD, ACRA, ACRB, MARR, MARA, PBP4B, MDTM, PMRF, YOGI, BCR, GADX, GADW, MDTF, MDTE, ROBA, EMRR, EMRA, EMRB, SOXS, MDTJ, MDTI, CRP, EMRY, EMRK, EVGS, MDTG, QNRB, DHA, SULI, MPHA	Not Detected	Not Detected

NIB017	***Acinetobacter variabilis***	mphE, msrE	mph(E)_1, msr(E)_1	mph(E), msr(E)	(MLS)mph(E), (MLS)msr(E)	MPHE, MSRE	Not Detected	Not Detected
NIB018	***Escherichia fergusonii***	Escherichia_coli_acrA, acrB, Escherichia_coli_ampH, tolC, bacA, mdtF, mdtM, acrF, emrR, emrA, emrB, tet(A), QnrB4, DHA-1, sul1, TEM-1, APH(6)-Id, APH(3")-Ib, sul2, acrD, Escherichia_coli_ampC1_beta-lactamase, pmrF, yojI, msbA, QnrS1, mphE, msrE, mphA, Escherichia_coli_ampC, kdpE, baeR, baeS, mdtC, mdtB, mdtA, marA, mdtH, H-NS, cpxA, CRP	tet(A), qnrB4, blaDHA-1, sul1, blaTEM-1B, aph(6)-Id, aph(3")-Ib, sul2, qnrS1, mph(E), msr(E), mph(A)	tet(A), qnrB4, blaDHA-1, sul1, blaTEM-1, aph(6)-Id, aph(3")-Ib, sul2, qnrS13, mph(E), msr(E), mph(A), blaEC-8	(Bla)ampH_Ecoli, (Tet)tetA, (Tet)tetR, (Flq)qnrB4, (Bla)blaDHA-1, (Sul)sul1, (Bla)blaTEM-105, (AGly)strB, (AGly)strA, (Sul)sul2, (Bla)AmpC1_Ecoli, (Flq)qnr-S1, (MLS)mph(E), (MLS)msr(E), (MLS)mph(A), (Bla)AmpC2_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli	ACRA, ACRB, AMPH, BACA, MDTF, MDTM, ACRF, EMRR, EMRA, EMRB, TETA, QNRB, DHA, SULI, TEM, APH6, APH3-DPRIME, SULII, ACRD, PBP4B, PMRF, YOGI, MSBA, QNRS, MPHE, MSRE, MPHA, ROBA, BLAEC, SOXS, PBP2, KDPE, BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, MARR, MARA, MDTK, MDTH, HNS, EMRD, CPXAR, CPXAR, CRP	blaTEM-105	Not Detected
NIB019	***Pseudomonas aeruginosa***	OprM, MexB, MexA, PmpM, OXA-50, fosA, Pseudomonas_aeruginosa_CpxR, mexV, mexW, MexC, MexD, OprJ, MexE, MexF, OprN, OpmB, MuxC, MuxB, MuxA, TriA, TriB, TriC, ArmR, mexL, mexJ, mexK, arnA, mexP, mexQ, opmE, mexX, mexY, Pseudomonas_aeruginosa_soxR, Pseudomonas_aeruginosa_emrE, OpmH, basS, mexM, mexN, Pseudomonas_aeruginosa_catB7, opmD, mexI, mexH, mexG, bcr-1, APH(3')-IIb, PDC-3	blaOXA-50, fosA, catB7, aph(3')-IIb, blaPAO	blaOXA-50, fosA-354827590, catB7, aph(3')-IIb, blaPDC-374	(Bla)blaOXA-50, (Phe)catB7, (Phe)bcr1, (AGly)aph(3")-IIb, (Bla)blaPDC-119	MEXB, MEXA, MEXR, PMPM, OXA, FOSA, CPXAR, MEXC, MEXD, MEXE, MEXF, OPRN, OPMB, MUXC, MUXB, MUXA, ARMR, MEXL, MEXJ, MEXK, ARNA, MEXQ, MEXQ, MEXQ, MEXX, MEXY, SOXR, EMRE, OPMH, BASRS, MEXN, MEXN, CAT, OPMD, MEXI, MEXH, MEXG, BCR1, APH3-DPRIME, PDC	Not Detected	blaOXA-50
NIB020	***Escherichia coli***	yojI, pmrF, emrY, emrK, evgA, evgS, Escherichia_coli_ampC1_beta-lactamase, acrD, mphB, mdtN, mdtO, mdtP, TEM-176, sul3, mdtH, mdtG, QnrS1, dfrA14, marA, cpxA, Escherichia_coli_mdfA, tolC, bacA, acrB, Escherichia_coli_acrA, eptA, acrS, acrE, acrF, baeR, baeS, mdtC, mdtB, mdtA, tet(A), APH(3')-Ia, CRP, mdtE, mdtF, gadW, gadX, msbA, mdtM, Escherichia_coli_ampH, Escherichia_coli_ampC, emrR, emrA, emrB, H-NS, Escherichia_coli_emrE, kdpE	blaTEM-176, sul3, qnrS1, dfrA14, mdf(A), tet(A), aph(3')-Ia	blaTEM-176, sul3, qnrS1, dfrA14, tet(A), aph(3')-Ia, blaEC-15	(Bla)AmpC1_Ecoli, (Bla)blaTEM-176, (Sul)sul3, (Flq)qnr-S1, (Tmt)dfrA14, (Bla)Penicillin_Binding_Protein_Ecoli, (Tet)tetR, (Tet)tetA, (AGly)aph(3")-Ia, (Bla)ampH_Ecoli, (Bla)AmpC2_Ecoli	BCR, YOGI, PMRF, EMRY, EMRK, EVGS, PBP4B, ACRD, MPHB, MDTN, MDTO, MDTP, TEM, SULIII, MDTH, MDTG, QNRS, DFRA, MARA, MARR, PBP2, CPXAR, CPXAR, MDFA, BACA, ACRB, ACRA, EPTA, ACRS, ACRE, ACRF, SOXS, BAER, BAES, MDTC, MDTB, MDTA, ROBA, TETA, CTX, APH3-DPRIME, CRP, MDTE, MDTF, GADW, GADX, MSBA, MDTI, MDTJ, MDTK, MDTM, ASMA, AMPH, BLAEC, EMRD, EMRR, EMRA, EMRB, CTX, HNS, MVRC, KDPE	blaTEM-176	Not Detected
NIB021	***Escherichia coli***	Escherichia_coli_mdfA, msbA, mdtG, mdtH, H-NS, mdtM, acrF, acrE, acrS, mdtN, mdtO, mdtP, emrB, emrA, emrR, pmrF, yojI, baeR, baeS, mdtC, mdtB, mdtA, ugd, cpxA, Escherichia_coli_ampC, mdtE, mdtF, gadW, gadX, kdpE, mphB, acrD, Escherichia_coli_ampC1_beta-lactamase, evgS, evgA, emrK, emrY, eptA, Escherichia_coli_emrE, floR, tet(A), APH(6)-Id, APH(3")-Ib, sul2, catI, dfrA12, aadA2, sul1, mphA, CRP, oqxA, oqxB, marA, TEM-1, AAC(3)-IId, tolC, bacA, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH	mdf(A), floR, tet(A), aph(6)-Id, aph(3")-Ib, sul2, catA1, dfrA12, aadA2, sul1, mph(A), oqxA, oqxB, blaTEM-1B, aac(3)-IId	blaEC-15, floR, tet(A), aph(6)-Id, aph(3")-Ib, sul2, catA1, dfrA12, aadA2, sul1, mph(A), oqxA2, oqxB, bleO, blaTEM-1, aac(3)-Iid	(Bla)AmpC2_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)AmpC1_Ecoli, (Phe)floR, (Tet)tetA, (Tet)tetR, (AGly)strB, (AGly)strA, (Sul)sul2, (Phe)catA1, (Tmt)dfrA12, (AGly)aadA2, (Sul)sul1, (MLS)mph(A), (Flq)OqxA, (Flq)OqxBgb, (Bla)blaTEM-105, (AGly)aac3-IId, (Bla)ampH_Ecoli	MDFA, MSBA, MDTG, MDTH, HNS, MDTM, ROBA, ACRF, ACRE, ACRS, MDTN, MDTO, MDTP, SOXS, EMRB, EMRA, EMRR, EMRD, PMRF, YOGI, BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, UGD, CPXAR, CPXAR, BLAEC, MDTE, MDTF, GADW, GADX, PBP2, KDPE, MPHB, ACRD, PBP4B, EVGS, EMRK, EMRY, EPTA, MVRC, FLOR, TETA, APH6, APH3-DPRIME, SULII, CATA, DFRA, ANT3-DPRIME, SULI, MPHA, CRP, OQXA, OQXB, MDTK, MDTJ, MDTI, MARA, MARR, BLE, TEM, AAC3, BACA, ACRA, ACRB, AMPH	blaTEM-105	Not Detected
NIB022	***Escherichia coli***	Escherichia_coli_emrE, marA, mdtP, mdtO, mdtN, eptA, acrD, Escherichia_coli_mdfA, acrS, acrE, acrF, cpxA, H-NS, mdtH, mdtG, ugd, mdtA, mdtB, mdtC, baeS, baeR, yojI, pmrF, emrY, emrK, evgA, evgS, CTX-M-15, msbA, bacA, tolC, mdtM, gadX, gadW, mdtF, mdtE, CRP, sul1, aadA2, dfrA12, kdpE, msrE, mphE, Escherichia_coli_ampH, acrB, Escherichia_coli_acrA, mphA, DHA-1, emrR, emrA, emrB, Escherichia_coli_ampC	mdf(A), blaCTX-M-15, sul1, aadA2, dfrA12, msr(E), mph(E), mph(A), blaDHA-1	blaCTX-M-15, sul1, aadA2, dfrA12, msr(E), mph(E), mph(A), blaDHA-1, blaEC-5	(Bla)blaCTX-M-15, (Sul)sul1, (AGly)aadA2, (Tmt)dfrA12, (Bla)Penicillin_Binding_Protein_Ecoli, (MLS)msr(E), (MLS)mph(E), (Bla)ampH_Ecoli, (MLS)mph(A), (Bla)blaDHA-1, (Bla)AmpC2_Ecoli	MVRC, MDTK, MDTJ, MDTI, MARA, MARR, SOXS, MDTP, MDTO, MDTN, EPTA, ACRD, MDFA, ACRS, ACRE, ACRF, CPXAR, CPXAR, HNS, MDTH, MDTG, UGD, ASMA, MDTA, MDTB, MDTC, BAES, BAER, BCR, YOGI, KPNO, PMRF, EMRD, EMRY, EMRK, EVGS, CTX, MSBA, BACA, MVRC, ROBA, MDTM, GADX, GADW, MDTF, MDTE, CRP, SULI, ANT3-DPRIME, DFRA, PBP2, KDPE, MSRE, MPHE, AMPH, ACRB, ACRA, MPHA, DHA, EMRR, EMRA, EMRB, BLAEC	blaCTX-M-15	Not Detected
NIB023	***Escherichia coli***	tolC, bacA, acrS, acrE, acrF, mdtE, mdtF, gadW, gadX, cpxA, CRP, eptA,	mdf(A), mph(A),	blaEC-8, mph(A),	(Bla)AmpC2_Ecoli, (MLS)mph(A), (Bla)Penicillin_Binding_Protein_Ecoli,	BACA, ACRS, ACRE, ACRF, MDTE, MDTF,	Not Detected	Not Detected

		mdtN, mdtO, mdtP, baeR, baeS, mdtC, mdtB, mdtA, Escherichia_coli_mdfA, H-NS, mdtM, pmrF, marA, yojI, Escherichia_coli_ampC, ugd, mphA, mphB, acrD, evgS, evgA, emrK, emrY, Escherichia_coli_emrE, kdpE, sul1, DHA-1, QnrB4, emrB, emrA, emrR, msbA, mdtG, ErmB, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH, mdtH	sul1, blaDHA-1, qnrB4, erm(B)	sul1, blaDHA-1, qnrB4, erm(B)	(Sul)sul1, (Bla)blaDHA-1, (Flq)qnrB4, (MLS)erm(B), (Bla)ampH_Ecoli	GADW, GADX, EMRD, CPXAR, CPXAR, CRP, EPTA, MDTN, MDTO, MDTP, SOXS, BAER, BAES, MDTC, MDTB, MDTA, ASMA, MDFA, HNS, MDTM, ROBA, PMRF, MDTJ, MDTI, MARA, MARR, BCR, YOGI, KPNO, BLAEC, MDTK, UGD, MPHA, MPHB, ACRD, EVGS, EMRK, EMRY, MVRC, KDPE, PBP2, SULI, DHA, QNRB, EMRB, EMRA, EMRR, MSBA, MDTG, ERMB, ACRA, ACRB, AMPH, MDTH		
NIB024	***Escherichia coli***	acrS, acrE, acrF, tolC, bacA, H-NS, dfrA17, msbA, kdpE, cpxA, Escherichia_coli_ampH, baeR, baeS, mdtC, mdtB, mdtA, Escherichia_coli_mdfA, CRP, marA, Escherichia_coli_ampC1_beta-lactamase, acrD, mphB, mdtM, gadX, gadW, mdtF, mdtE, mdtH, emrB, emrA, emrR, acrB, Escherichia_coli_acrA, pmrF, yojI, emrY, emrK, evgA, evgS, mdtG, QnrB4, DHA-1, sul1, mphA, Escherichia_coli_ampC, eptA, mdtN, mdtO, mdtP	dfrA17, mdf(A), qnrB4, blaDHA-1, sul1, mph(A)	dfrA17, qnrB4, blaDHA-1, sul1, mph(A), blaEC	(Tmt)dfrA17, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)ampH_Ecoli, (Bla)AmpC1_Ecoli, (Flq)qnrB4, (Bla)blaDHA-1, (Sul)sul1, (MLS)mph(A), (Bla)AmpC2_Ecoli	ACRS, ACRE, ACRF, BACA, HNS, DFRA, MSBA, CTX, MDTK, PBP2, KDPE, CPXAR, CPXAR, AMPH, BAER, BAES, MDTC, MDTB, MDTA, MDFA, CRP, EMRD, MARA, MARR, PBP4B, ACRD, MPHB, MDTM, GADX, GADW, MDTF, MDTE, ROBA, MDTH, EMRB, EMRA, EMRR, SOXS, ACRB, ACRA, MDTJ, MDTI, PMRF, YOGI, BCR, EMRY, EMRK, EVGS, MDTG, QNRB, DHA, SULI, MPHA, BLAEC, EPTA, MDTN, MDTO, MDTP	Not Detected	Not Detected
NIB025	***Proteus mirabilis***	catA4, floR, tet(C), APH(6)-Id, APH(3")-Ib, sul2, OXA-10, catB8, ANT(2")-Ia, dfrA32, CRP, tet(J)	cat, floR, tet(C), aph(6)-Id, aph(3")-Ib, sul2, blaOXA-10, catB8, ant(2")-Ia, dfrA32, tet(J)	catA4, floR, tet(C), aph(6)-Id, aph(3")-Ib, sul2, blaOXA-10, catB8, ant(2")-Ia, dfrA32, tet(J)	(Phe)catA4, (Phe)floR, (Tet)tetC, (AGly)strB, (AGly)strA, (Sul)sul2, (Bla)blaOXA-10, (Phe)catB8, (AGly)aadB, (Tmt)dfr32, (Tet)tetJ	OMPD, CATA, FLOR, TETC, APH6, APH3-DPRIME, SULII, ANT2-DPRIME, DFRA, CRP, TETJ	Not Detected	blaOXA-10
NIB026	***Escherichia coli***	pmrF, yojI, baeR, baeS, mdtC, mdtB, mdtA, ugd, Escherichia_coli_ampH, acrB, Escherichia_coli_acrA, eptA, mdtN, mdtO, mdtP, acrF, acrE, acrS, kdpE, msbA, Escherichia_coli_mdfA, bacA, tolC, mphB, acrD, Escherichia_coli_ampC1_beta-lactamase, evgS, evgA, emrK, emrY, cpxA, marA, emrR, emrA, emrB, Escherichia_coli_ampC, mphA, sul1, DHA-1, QnrB4, tet(A), dfrA7, CRP, mdtE, mdtF, gadW, gadX, mdtM, mdtH, mdtG, H-NS	erm(B), qepA4, mdf(A), blaCTX-M-15, dfrA12, aadA2, sul1, mph(A)	blaEC-13, mph(A), sul1, blaDHA-1, qnrB4, tet(A), dfrA7	(Bla)ampH_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)AmpC1_Ecoli, (Bla)AmpC2_Ecoli, (MLS)mph(A), (Sul)sul1, (Bla)blaDHA-1, (Flq)qnrB4, (Tet)tetA, (Tet)tetR, (Tmt)dhfr7	PMRF, KPNO, YOGI, BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, UGD, AMPH, ACRB, ACRA, EPTA, MDTN, MDTO, MDTP, SOXS, ACRF, ACRE, ACRS, PBP2, KDPE, MSBA, MDFA, BACA, EMRD, MPHB, ACRD, PBP4B, EVGS, EMRK, EMRY, CPXAR, CPXAR, MARA, MARR, EMRR, EMRA, EMRB, MDTK, MDTJ, MDTI, BLAEC, MPHA, SULI, DHA, QNRB, TETA, DFRA, CRP, MDTE, MDTF, GADW, GADX, ROBA, MDTM, MDTH, MDTG, HNS	blaCTX-M-15	Not Detected
NIB027	***Escherichia coli***	Escherichia_coli_mdfA, kdpE, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH, mdtP, mdtO, mdtN, eptA, Escherichia_coli_ampC, H-NS, CTX-M-15, acrD, Escherichia_coli_emrE, marA, emrY, emrK, evgA, evgS, cpxA, CRP, mdtE, mdtF, gadW, gadX, Escherichia_coli_emrE, pmrF, yojI, baeR, baeS, mdtC, mdtB, mdtA, ugd, tolC, bacA, acrS, acrE, acrF, mdtM, dfrA12, aadA2, sul1, mphA, mdtH, mdtG, msbA, emrR, emrA, emrB	mdf(A), blaCTX-M-15, dfrA12, aadA2, sul1, mph(A)	blaEC-5, blaCTX-M-15, dfrA12, aadA2, sul1, mph(A)	(Bla)Penicillin_Binding_Protein_Ecoli, (Bla)ampH_Ecoli, (Bla)AmpC2_Ecoli, (Bla)blaCTX-M-15, (Tmt)dfrA12, (AGly)aadA2, (Sul)sul1, (MLS)mph(A)	MDFA, KDPE, PBP2, ACRA, ACRB, AMPH, SOXS, MDTP, MDTO, MDTN, EPTA, BLAEC, HNS, EMRD, CTX, ACRD, MVRC, MARR, MARA, MDTI, MDTJ, EMRY, EMRK, EVGS, CPXAR, CPXAR, CRP, MDTE, MDTF, GADW, GADX, MVRC, PMRF, KPNO, YOGI, BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, UGD, BACA, ACRS, ACRE, ACRF, MDTM, DFRA, ANT3-DPRIME, SULI, MPHA, ROBA, MDTH, MDTG, MSBA, MDTK, EMRR, EMRA, EMRB	blaCTX-M-15	Not Detected
NIB028	***Pseudomonas aeruginosa***	mexK, mexJ, mexL, ArmR, Pseudomonas_aeruginosa_CpxR, opmD, mexI, mexH, mexG, fosA, MexE, MexF, OprN, OpmB, MuxC, MuxB, MuxA, Pseudomonas_aeruginosa_emrE, mexV, mexW, mexM, mexN, TriC, TriB, TriA, arnA, mexP, mexQ, opmE, MexC, MexD, OprJ, Pseudomonas_aeruginosa_catB7, mexX, mexY, MexA, MexB, OprM, OXA-50, PmpM, basS, APH(3')-IIb,	fosA, catB7, blaOXA-50, aph(3')-IIb, blaPA	fosA-354827590, catB7, blaOXA-50, aph(3')-IIb, blaPDC-374	(Phe)catB7, (Bla)blaOXA-50, (AGly)aph(3")-IIb, (Bla)blaPDC-119, (Phe)bcr1	MEXK, MEXJ, MEXL, ARMR, CPXAR, OPMD, MEXI, MEXH, MEXG, FOSA, MEXE, MEXF, OPRN, OPMB, MUXC, MUXB, MUXA, EMRE, MEXN, MEXN, ARNA, MEXQ, MEXQ, MEXQ, MEXC, MEXD, CAT, MEXX, MEXY, MEXR, MEXA, MEXB, OXA, PMPM, BASRS, APH3-	Not Detected	blaOXA-50

		PDC-8, OpmH, Pseudomonas_aeruginosa_soxR, bcr-1				DPRIME, PDC, OPMH, SOXR, BCR1		
NIB029	***Enterobacter chuandaensis***	acrB, Enterobacter_cloacae_acrA, QnrS1, CTX-M-15, ramA, msbA, oqxA, oqxB, marA, H-NS, cpxA, emrR, Klebsiella_pneumoniae_KpnG, emrB, bacA, baeR, mdtC, mdtB, FosA2, ACT-29, CRP, acrD	qnrS1, blaCTX-M-15, mdf(A), oqxA, oqxB, fosA, blaACT-6	qnrS1, blaCTX-M-15, oqxA10, oqxB9, fosA, blaACT-80	(Flq)qnr-S1, (Bla)blaCTX-M-15, (Bla)Penicillin_Binding_Protein_Ecoli, (Flq)OqxA, (Flq)OqxBgb, (Fcyn)FosA2, (Bla)blaACT-6	ACRB, ACRB, QNRS, CTX, RAMA, PBP2, KDEA, MSBA, OQXA, OQXB, MARA, HNS, CPXAR, CPXAR, EMRR, KPN, EMRB, BACA, BAER, MDTC, MDTB, FOSA, ACT, ACT, CRP, ACRD	blaCTX-M-15	Not Detected
NIB030	***Pseudomonas aeruginosa***	OXA-50, mexM, mexN, Pseudomonas_aeruginosa_CpxR, ArmR, PDC-3, MexE, MexF, TriC, TriB, TriA, mexY, bcr-1, basS, mexI, mexH, mexG, OprJ, MexD, MexC, mexV, mexW, fosA, arnA, Pseudomonas_aeruginosa_emrE, opmD, mexP, mexQ, OprM, MexB, MexA, OpmH, Pseudomonas_aeruginosa_soxR, APH(3')-IIb, mexL, mexJ, mexK, OpmB, MuxC, MuxB, MuxA, Pseudomonas_aeruginosa_catB7	blaOXA-50, blaPAO, fosA, aph(3')-IIb, catB7	blaOXA-50, blaPDC-374, fosA-354827590, aph(3')-IIb, catB7	(Bla)blaOXA-50, (Bla)blaPDC-119, (Phe)bcr1, (AGly)aph(3")-IIb, (Phe)catB7	OXA, MEXN, MEXN, CPXAR, ARMR, PDC, MEXE, MEXF, MEXY, BCR1, BASRS, MEXI, MEXH, MEXG, MEXD, MEXC, FOSA, ARNA, EMRE, OPMD, MEXQ, MEXQ, MEXB, MEXA, MEXR, OPMH, SOXR, APH3-DPRIME, MEXL, MEXJ, MEXK, OPMB, MUXC, MUXB, MUXA, CAT	Not Detected	blaOXA-50
NIB031	***Escherichia coli***	marA, emrR, emrA, emrB, cpxA, msbA, acrB, Escherichia_coli_acrA, Escherichia_coli_mdfA, kdpE, ugd, mdtA, mdtB, mdtC, baeS, baeR, CTX-M-15, mdtM, Escherichia_coli_ampH, evgS, evgA, emrK, emrY, pmrF, yojI, mphA, sul1, aadA2, dfrA12, tolC, bacA, acrS, acrE, acrF, gadX, gadW, mdtF, mdtE, CRP, ErmB, mdtP, mdtO, mdtN, eptA, Escherichia_coli_ampC, mphB, acrD, Escherichia_coli_ampC1_beta-lactamase, mdtG, mdtH, H-NS	mdf(A), blaCTX-M-15, mph(A), sul1, aadA2, dfrA12, erm(B)	blaCTX-M-15, mph(A), sul1, aadA2, dfrA12, erm(B), blaEC-19	(Bla)Penicillin_Binding_Protein_Ecoli, (Bla)blaCTX-M-15, (Bla)ampH_Ecoli, (MLS)mph(A), (Sul)sul1, (AGly)aadA2, (Tmt)dfrA12, (MLS)erm(B), (Bla)AmpC2_Ecoli, (Bla)AmpC1_Ecoli	MARR, MARA, MDTI, MDTJ, MDTK, EMRR, EMRA, EMRB, CPXAR, CPXAR, MSBA, EMRD, ACRB, ACRA, MDFA, KDPE, PBP2, UGD, ASMA, MDTA, MDTB, MDTC, BAES, BAER, CTX, MDTM, ROBA, AMPH, EVGS, EMRK, EMRY, PMRF, KPNO, YOGI, BCR, MPHA, SULI, ANT3-DPRIME, DFRA, BACA, ACRS, ACRE, ACRF, GADX, GADW, MDTF, MDTE, CRP, ERMB, SOXS, MDTP, MDTO, MDTN, EPTA, BLAEC, MPHB, ACRD, PBP4B, MDTG, MDTH, HNS	blaCTX-M-15	Not Detected
NIB032	***Klebsiella pneumoniae***	oqxA, oqxB, emrR, Klebsiella_pneumoniae_KpnG, Klebsiella_pneumoniae_KpnH, FosA6, H-NS, marA, SHV-110, ramA, msbA, CRP, cpxA, Klebsiella_pneumoniae_OmpK37, Klebsiella_pneumoniae_KpnE, Klebsiella_pneumoniae_KpnF, acrD, mdtB, mdtC, baeR, acrB, Klebsiella_pneumoniae_acrA	oqxA, oqxB, fosA6, blaSHV-110	oqxA, oqxB, fosA6, blaSHV-110	(Flq)OqxA, (Flq)OqxBgb, (Fcyn)FosA6, (Bla)blaSHV-110, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)ampH	OQXA, OQXB, EMRR, KPN, EMRB, FOSA, HNS, MARA, SHV, SOXS, RAMA, MSBA, KPNO, KDEA, CRP, CPXAR, PHOR, PBP2, OMP37, KPNE, KPNF, ACRD, KMRA, ASMA, MDTB, MDTC, BAER, AMPH, ACRB, ACRA	blaSHV-110	Not Detected
NIB033	***Escherichia coli***	Escherichia_coli_emrE, marA, eptA, mdtN, mdtO, mdtP, Escherichia_coli_ampH, acrB, Escherichia_coli_acrA, acrD, evgS, evgA, emrK, emrY, cpxA, H-NS, baeR, baeS, mdtC, mdtB, mdtA, ugd, acrF, acrE, acrS, bacA, tolC, emrB, emrA, emrR, Escherichia_coli_mdfA, kdpE, yojI, pmrF, CTX-M-15, msbA, mdtG, mdtH, dfrA17, aadA5, sul1, mphA, ErmB, mdtM, CRP, mdtE, mdtF, gadW, gadX, Escherichia_coli_ampC	mdf(A), blaCTX-M-15, dfrA17, aadA5, sul1, mph(A), erm(B)	blaCTX-M-15, dfrA17, aadA5, sul1, mph(A), erm(B), blaEC-5	(Bla)ampH_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)blaCTX-M-15, (Tmt)dfrA17, (AGly)aadA5, (Sul)sul1, (MLS)mph(A), (MLS)erm(B), (Bla)AmpC2_Ecoli	MVRC, MDTK, MDTJ, MDTI, MARA, MARR, EPTA, MDTN, MDTO, MDTP, SOXS, AMPH, ACRB, ACRA, ACRD, EVGS, EMRK, EMRY, CPXAR, HNS, BAER, BAES, MDTC, MDTB, MDTA, ASMA, UGD, ACRF, ACRE, ACRS, BACA, EMRB, EMRA, EMRR, MDFA, KDPE, PBP2, BCR, YOGI, KPNO, PMRF, CTX, MSBA, MDTG, MDTH, DFRA, ANT3-DPRIME, SULI, MPHA, EMRD, ERMB, MDTM, ROBA, CRP, MDTE, MDTF, GADW, GADX, BLAEC	blaCTX-M-15	Not Detected
NIB034	***Escherichia coli***	pmrF, emrY, emrK, evgA, evgS, Escherichia_coli_ampC1_beta-lactamase, acrD, mphB, bacA, tolC, CRP, msbA, acrF, acrE, acrS, mdtP, mdtO, mdtN, kdpE, marA, mdtM, cpxA, ugd, mdtA, mdtB, mdtC, baeS, baeR, mdtE, mdtF, gadW, gadX, emrB, emrA, emrR, yojI, H-NS, sul2, mphA, dfrA12, aadA2, sul1, tet(A), eptA, Escherichia_coli_ampC, QepA4, dfrA17, aadA5, Escherichia_coli_mdfA, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH, mdtG, mdtH	sul2, mph(A), dfrA12, aadA2, sul1, tet(A), qepA4, dfrA17, aadA5, mdf(A)	sul2, mph(A), dfrA12, aadA2, sul1, tet(A), blaEC-15, qepA4, dfrA17, aadA5	(Bla)AmpC1_Ecoli, (Bla)Penicillin_Binding_Protein_Ecoli, (Sul)sul2, (MLS)mph(A), (Tmt)dfrA12, (AGly)aadA2, (Sul)sul1, (Tet)tetA, (Tet)tetR, (Bla)AmpC2_Ecoli, (Flq)qepA, (Tmt)dfrA17, (AGly)aadA5, (Bla)ampH_Ecoli	PMRF, EMRY, EMRK, EVGS, PBP4B, ACRD, MPHB, BACA, CRP, MSBA, ACRF, ACRE, ACRS, SOXS, MDTP, MDTO, MDTN, PBP2, KDPE, MARR, MARA, MDTM, MDTI, MDTJ, MDTK, CPXAR, CPXAR, UGD, ASMA, MDTA, MDTB, MDTC, BAES, BAER, BCR, EMRD, MDTE, MDTF, GADW, GADX, ROBA, EMRB, EMRA, EMRR, YOGI, KPNO, HNS, SULII, MPHA, DFRA, ANT3-DPRIME, SULI, TETA, EPTA, BLAEC, QEPA, DFRA, ANT3-DPRIME, MDFA, ACRA, ACRB, AMPH, MDTG, MDTH	Not Detected	Not Detected
NIB035	***Escherichia coli***	mphB, acrD, Escherichia_coli_ampC1_beta-lactamase, evgS, evgA, emrK, emrY,	blaCTX-M-15, blaTEM-	blaCTX-M-15, blaTEM-1,	(Bla)AmpC1_Ecoli, (Bla)blaCTX-M-15, (Bla)Penicillin_Binding_Protein_Ecoli,	MPHB, ACRD, PBP4B, EVGS, EMRK, EMRY, PMRF, KPNO, YOGI,	blaCTX-M-15, blaTEM-105	Not Detected

		pmrF, yojI, baeR, baeS, mdtC, mdtB, mdtA, acrS, acrE, acrF, CTX-M-15, CRP, gadX, gadW, mdtF, mdtE, TEM-1, cpxA, msbA, Escherichia_coli_mdfA, kdpE, H-NS, marA, emrB, emrA, emrR, ugd, mdtM, Escherichia_coli_ampC, eptA, mdtN, mdtO, mdtP, dfrA12, aadA2, sul1, mphA, Escherichia_coli_acrA, acrB, Escherichia_coli_ampH, tolC, bacA, ErmB, AAC(3)-IIe, AAC(6')-Ib-cr, OXA-1, mdtG, mdtH	1B, mdf(A), dfrA12, aadA2, sul1, mph(A), erm(B), aac(3)-IIa, aac(6')-Ib-cr, blaOXA-1	blaEC-18, dfrA12, aadA2, sul1, mph(A), erm(B), aac(3)-IIe, aac(6')-Ib-D181Y, blaOXA-1	(Bla)blaTEM-105, (Bla)AmpC2_Ecoli, (Tmt)dfrA12, (AGly)aadA2, (Sul)sul1, (MLS)mph(A), (Bla)ampH_Ecoli, (MLS)erm(B), (AGly)aac3-IIa, (Bla)blaOXA-1	BCR, BAER, BAES, MDTC, MDTB, MDTA, ASMA, ACRS, ACRE, ACRF, CTX, CRP, PBP2, GADX, GADW, MDTF, MDTE, CPXAR, CPXAR, MSBA, MDFA, KDPE, ROBA, HNS, MARA, MARR, MDTK, MDTJ, MDTI, EMRB, EMRA, EMRR, UGD, MDTM, BLAEC, EPTA, MDTN, MDTO, MDTP, SOXS, DFRA, ANT3-DPRIME, SULI, MPHA, ACRA, ACRB, AMPH, BACA, ERMB, AAC3, EMRD, OXA, MDTG, MDTH		
NIB036	***Escherichia coli***	ugd, mdtA, mdtB, mdtC, baeS, baeR, yojI, pmrF, H-NS, Escherichia_coli_emrE, acrD, cpxA, marA, Escherichia_coli_mdfA, kdpE, emrY, emrK, evgA, evgS, emrB, emrA, emrR, acrF, acrE, acrS, bacA, tolC, mdtH, mdtG, msbA, mdtM, mphA, sul1, aadA2, dfrA12, Escherichia_coli_ampC, CTX-M-15, Escherichia_coli_ampH, acrB, Escherichia_coli_acrA, ErmB, gadX, gadW, mdtF, mdtE, CRP, mdtP, mdtO, mdtN, eptA	mdf(A), mph(A), sul1, aadA2, dfrA12, blaCTX-M-15, erm(B)	mph(A), sul1, aadA2, dfrA12, blaEC-5, blaCTX-M-15, erm(B)	(Bla)Penicillin_Binding_Protein_Ecoli, (MLS)mph(A), (Sul)sul1, (AGly)aadA2, (Tmt)dfrA12, (Bla)AmpC2_Ecoli, (Bla)blaCTX-M-15, (Bla)ampH_Ecoli, (MLS)erm(B)	UGD, ASMA, MDTA, MDTB, MDTC, BAES, BAER, BCR, YOGI, KPNO, PMRF, HNS, ROBA, MVRC, ACRD, CPXAR, MDTJ, MDTI, MARA, MARR, MDFA, KDPE, PBP2, EMRD, EMRY, EMRK, EVGS, EMRB, EMRA, EMRR, ACRF, ACRE, ACRS, BACA, MDTH, MDTG, MSBA, MDTM, MPHA, SULI, ANT3-DPRIME, DFRA, BLAEC, CTX, AMPH, ACRB, ACRA, MDTK, ERMB, GADX, GADW, MDTF, MDTE, CRP, SOXS, MDTP, MDTO, MDTN, EPTA	blaCTX-M-15	Not Detected
NIB037	***Klebsiella pneumoniae***	rmtF, acrB, Klebsiella_pneumoniae_acrA, msbA, oqxA, oqxB, emrR, Klebsiella_pneumoniae_KpnG, Klebsiella_pneumoniae_KpnH, cpxA, acrD, baeR, mdtC, mdtB, FosA6, ramA, Klebsiella_pneumoniae_KpnF, Klebsiella_pneumoniae_KpnE, Klebsiella_pneumoniae_OmpK37, NDM-5, determinant_of_bleomycin_resistance, sul1, aadA2, dfrA12, OXA-232, CTX-M-15, SHV-182, marA, TEM-1, rmtB, mphA, arr-2, AAC(6')-Ib9, ErmB, H-NS, QnrS1, CRP	rmtF, oqxA, oqxB, fosA6, blaNDM-5, sul1, aadA2, dfrA12, blaOXA-232, blaCTX-M-15, blaSHV-182, blaTEM-1B, rmtB, mph(A), ARR-3, erm(B), qnrS1	rmtF1, oqxA6, oqxB25, fosA6, blaNDM-5, ble-MBL, sul1, aadA2, dfrA12, blaOXA-232, blaCTX-M-15, blaSHV-158, blaTEM-1, rmtB1, mph(A), arr-2, aac(6')-Ib-G, erm(B), qnrS1, catB	(AGly)rmtF, (Bla)ampH, (Flq)OqxA, (Flq)OqxBgb, (Fcyn)FosA6, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)blaNDM-5, (Sul)sul1, (AGly)aadA2, (Tmt)dfrA12, (Bla)blaOXA-181, (Bla)blaCTX-M-15, (Bla)blaSHV-11, (Bla)blaTEM-105, (AGly)rmtB, (MLS)mph(A), (Rif)arr2, (MLS)erm(B), (Flq)qnrS1, (Phe)catB_variant1	RMTF, AMPH, ACRB, ACRA, KMRA, MSBA, SOXS, OQXA, OQXB, EMRR, KPN, EMRB, PHOR, CPXAR, ACRD, KPNO, BAER, MDTC, MDTB, ASMA, FOSA, KDEA, PBP2, RAMA, KPNF, KPNE, OMP37, CTX, NDM, BRP, SULI, ANT3-DPRIME, DFRA, OXA, SHV, MARA, TEM, RMTB, MPHA, ARR, AAC6-PRIME, ERMB, HNS, QNRS, CRP	blaCTX-M-15, blaSHV-11, blaSHV-158, blaSHV-182, blaTEM-105	blaNDM-5, blaOXA-232, blaOXA-181
NIB039	***Pseudomonas mendocina***	Pseudomonas_aeruginosa_CpxR, mexW, MexF, MexB, mexK	Not Detected	Not Detected	Not Detected	CPXAR, MEXF, MEXB, MEXK	Not Detected	
NIB041	***Proteus faecis***	tet(B), sul2, APH(3")-Ib, APH(6)-Id, sul1, QnrA1, dfrA14, floR, QnrD1, CRP	mecA, msr(A), mph(C), dfrG, fosD, cat, erm(C), aac(6')-aph(2"), cfr	tet(B), sul2, aph(3")-Ib, aph(6)-Id, sul1, qnrA1, dfrA14, floR, hugA, qnrD1	(Bla)mecA, (Bla)mecI, (Bla)blaARL-2, (MLS)msr(A), (MLS)mph(C), (Tmt)dfrG, (Fcyn)FosD, (Phe)catA8, (MLS)erm(C), (AGly)aadC, (AGly)aac6-Aph2, (MLS)cfr(A), (AGly)apH-Stph	MECA, MECA, MECI, ARL, MSRA, MPHC, DFRG, FOSD, CATA, QACJ, ERMC, QACJ, AAC6-PRIME, CFRA, APH3-PRIME	Not Detected	Not Detected
NIB044	***Escherichia coli***	Escherichia_coli_ampC1_beta-lactamase, acrD, mphB, msbA, evgS, evgA, emrK, emrY, ErmB, bacA, tolC, CRP, QepA4, emrR, emrA, emrB, mdtH, mdtG, cpxA, mdtP, mdtO, mdtN, eptA, marA, acrS, acrE, acrF, baeR, baeS, mdtC, mdtB, mdtA, acrB, Escherichia_coli_acrA, H-NS, Escherichia_coli_mdfA, Escherichia_coli_emrE, CTX-M-15, kdpE, Escherichia_coli_ampC, mdtM, dfrA12, aadA2, sul1, mphA, Escherichia_coli_ampH, pmrF, yojI	erm(B), qepA4, mdf(A), blaCTX-M-15, dfrA12, aadA2, sul1, mph(A)	erm(B), qepA4, blaCTX-M-15, blaEC-18, dfrA12, aadA2, sul1, mph(A)	(Bla)AmpC1_Ecoli, (MLS)erm(B), (Flq)qepA, (Bla)Penicillin_Binding_Protein_Ecoli, (Bla)blaCTX-M-15, (Bla)AmpC2_Ecoli, (Tmt)dfrA12, (AGly)aadA2, (Sul)sul1, (MLS)mph(A), (Bla)ampH_Ecoli	PBP4B, CTX, ACRD, MPHB, MSBA, EVGS, EMRK, EMRY, ERMB, BACA, CRP, QEPA, EMRR, EMRA, EMRB, MDTH, MDTG, MDTK, MDTJ, MDTI, CPXAR, CPXAR, MDTP, MDTO, MDTN, EPTA, PBP2, MARA, MARR, SOXS, ACRS, ACRE, ACRF, BAER, BAES, MDTC, MDTB, MDTA, ASMA, ACRB, ACRA, ROBA, HNS, MDFA, MVRC, CTX, KDPE, BLAEC, MDTM, DFRA, ANT3-DPRIME, SULI, MPHA, AMPH, PMRF, KPNO, YOGI, BCR	blaCTX-M-15	Not Detected
							Total Carbapenemase Positive Isolates= 21	Total Carbapenemase Positive Isolates= 7

S3: List of Virulence genes detected in 25 isolates.

Isolates	Name of Isolates	Functional Category	Genes	Count of Genes
NIB008	*E. coli*	Adhesion and Colonization Factors	ompA, fimB, fimC, fimF, fimG, fimH, ykgK/ecpR, yagZ/ecpA, yagY/ecpB, yagX/ecpC, yagW/ecpD, yagV/ecpE	40
		Iron Uptake and Metabolism	entA, entB, entC, entD, entE, entF, entS, fepA, fepB, fepC, fepD, fes	
		Toxins and Effectors	espX1, espX4, espX5, espY2, espL1, espL4	
		Type II Secretion (T2SS)	gspC, gspD, gspE, gspF, gspH, gspI, gspJ, gspK, gspL, gspM	
NIB009	*E. coli*	Adhesion and Colonization Factors	ykgK/ecpR, yagZ/ecpA, yagY/ecpB, yagX/ecpC, yagW/ecpD, yagV/ecpE, draP, draD, afaC-I, afaB-I, afaA, daaF, ompA, papX	57
		Iron Uptake and Metabolism	ybtS, ybtX, ybtQ, ybtP, ybtA, irp2, irp1, ybtU, ybtT, ybtE, fyuA, fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA, iucB, iucC, iucD, chuS, shuA, chuT, chuW, shuX, chuY, chuU, chuV	
		Toxins and Effectors	espX4, espL1	
		Capsule, LPS, and Biofilm Formation	kpsM, kpsD	
		Type II Secretion (T2SS)	gspD, gspG, gspH, gspK, gspL	
		Quorum Sensing, Regulation, and Miscellaneous	aslA	
NIB010	*E. coli*	Adhesion and Colonization Factors	fimC, fimI, fimE, fimB, ompA	45
		Iron Uptake and Metabolism	iucA, iucB, iucC, iucD, iutA, entA, entB, entE, entC, fepB, entS, fepD, fepC, entF, fes, fepA, ybtS, ybtX, ybtQ, ybtP, ybtA, irp2, irp1, ybtU, ybtT, ybtE, fyuA, shuS, shuA, shuT, chuW, shuX, chuY, chuU, chuV	
		Toxins and Effectors	espL1, espL4, espX1, espY2, espY3	
NIB011	*E. coli*	Adhesion and Colonization Factors	fimH, fimG, fimF, fimD, fimC, fimI, fimE, fimB, afaA, afaB-I, afaC-I, draP, daaF, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, fdeC, papX, ompA	61
		Iron Uptake and Metabolism	aslA, chuS, chuA, chuT, chuW, chuX, chuY, chuU, chuV, ybtS, ybtX, ybtQ, ybtP, ybtA, fyuA, ybtE, ybtT, ybtU, irp1, iroE, iroD, iucD, iucC, iucB, iucA, fepA, fes, entF, fepC, fepG, fepD, entS, fepB, entC, entE, entB, entA	
		Capsule, LPS, and Biofilm Formation	kpsD	
		Toxins and Effectors	sat	
NIB014	*K. pneumoniae*	Iron Uptake and Metabolism	fyuA, ybtE, ybtT, ybtU, irp1, irp2, ybtA, ybtP, ybtQ, ybtX, ybtS	11
NIB015	*E. coli*	Adhesion and Colonization Factors	yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, ompA	46
		Iron Uptake and Metabolism	entD, fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA, iucB, iucC, iucD, ybtS, ybtX, ybtQ, ybtP, ybtA, irp2, irp1, ybtU, ybtT, ybtE, fyuA	
		Toxins and Effectors	espL1, espX1, espX5, espL4	
		Type II Secretion (T2SS)	gspM, gspL, gspK, gspJ, gspI, gspH, gspF, gspE, gspD	
NIB016	*S. stutzeri*	Adhesion and Colonization Factors	fimG, fimF, fimB, fimE, fimI, fimC, ompA	47
		Iron Uptake and Metabolism	iucA, iucB, iucC, iucD, iutA, fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA, chuV, chuU, chuY, shuX, chuW, shuT, shuA, shuS, fyuA, ybtE, ybtT, ybtU, irp1, irp2, ybtA, ybtP, ybtQ, ybtX, ybtS	
		Toxins and Effectors	espL1, espX1, espY3, espL4, espY2	
NIB018	*E. fergusonii*	Iron Uptake and Metabolism	fepA, fes, fepC, fepD, fepB, entC, entB	10
		Type II Secretion (T2SS)	gspD, gspH, gspL	
NIB019	*P. aeruginosa*	Adhesion and Colonization Factors	fha1, fimV	205
		Iron Uptake and Metabolism	fpvA, pvdA, pvdE, pvdF, pvdG, pvdH, pvdL, pvdM, pvdN, pvdO, pvdP, pvdQ, pvdS, pchA, pchB, pchC, pchD, pchR, pvcA, pvcB, pvcC, pvcD, mbtH-like	
		Toxins and Effectors	aprA, toxA, exoS, exoT, exoY, lip1, plcH, lasA, lasB, tse1, tse2, tse3, vgrG1a, vgrG1b, phzH, phzM	
		Capsule, LPS, and Biofilm Formation	alg44, alg8, algA, algB, algC, algD, algE, algF, algG, algI, algJ, algK, algL, algP/algR3, algQ, algR, algU, algW, algX, algZ, mucA, mucB, mucC, mucD, mucE, mucP, waaA, waaC, waaF, waaG, waaP, wzy, wzz	
		Type II Secretion (T2SS)	xcpA/pilD, xcpP, xcpQ, xcpR, xcpS, xcpT, xcpU, xcpV, xcpW, xcpX, xcpY, xcpZ	

		Type III Secretion (T3SS)	pscB, pscC, pscD, pscE, pscF, pscG, pscH, pscI, pscJ, pscK, pscL, pscN, pscO, pscP, pscQ, pscR, pscS, pscT, pscU, exsA, exsB, exsC, exsD, exsE, popB, popD, popN, pcr1, pcr2, pcr3, pcr4, pcrD, pcrG, pcrH, pcrR, pcrV	
		Type VI Secretion (T6SS)	hcp1, hsiA1, hsiB1/vipA, hsiC1/vipB, hsiE1, hsiF1, hsiG1, hsiH1, hsiJ1, icmF1/tssM1, dotU1, clpV1, ppkA, pppA	
		Motility and Chemotaxis	flgA, flgB, flgC, flgD, flgE, flgF, flgG, flgH, flgI, flgJ, flgK, flgL, flgM, flgN, flhA, flhF, fliA, fliC, fliD, fliE, fliF, fliG, fliH, fliI, fliJ, fliK, fliL, fliM, fliN, fliO, fliP, fliQ, fliR, fliS, fleI/flag, fleN, fleP, fleQ, fleR, fleS, motA, motB, motC, motD, motY, chpA, chpB, chpC, chpD, chpE	
		Quorum Sensing, Regulation, and Miscellaneous	lasI, rhlI, rhlA, rhlB, rhlC, ptxR, pppA, tagF/pppB, tagQ, tagR, tagS, tagT, pvdS	
		Type IV pili (T4P) biogenesis and regulatory	pilE, pilF, pilG, pilH, pilI, pilJ, pilK, pilM, pilN, pilO, pilP, pilQ, pilR, pilS, pilT, pilU, pilV, pilW, pilX, pilY2	
NIB020	*E. coli*	Adhesion and Colonization Factors	fimH, fimG, fimF, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, ompA	32
		Iron Uptake and Metabolism	entA, entB, entC, entE, entF, entS, fepA, fepB, fepC, fepD, fes	
		Toxins and Effectors	espX5, espL1	
		Type II Secretion (T2SS)	gspC, gspD, gspE, gspF, gspG, gspH, gspJ, gspK, gspL	
NIB021	*E. coli*	Adhesion and Colonization Factors	fimB, fimC, fimD, fimE, fimF, fimG, fimH, papB, papI, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, ompA	54
		Iron Uptake and Metabolism	entA, entB, entC, entE, entF, entS, fepA, fepB, fepC, fepD, fes, shuS, shuA, shuT, chuW, shuX, chuY, chuU, chuV	
		Toxins and Effectors	astA, espL1, espX1, espX2, espX4, espX5, espY1, espY2, espY3	
		Type II Secretion (T2SS)	gspC, gspD, gspE, gspF, gspH, gspI, gspJ, gspK, gspL, gspM	
NIB022	*E. coli*	Adhesion and Colonization Factors	fimB, fimC, fimD, fimE, fimF, fimG, fimH, afaA, afaB-I, afaC-I, draP, daaF, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, fdeC, papX, ompA, sat	61
		Iron Uptake and Metabolism	aslA, fyuA, ybtE, ybtT, ybtU, irp1, ybtA, ybtP, ybtQ, ybtX, ybtS, iucA, iucB, iucC, iucD, chuV, chuU, chuY, chuX, chuW, chuT, chuA, chuS, fepA, fes, entF, fepC, fepG, fepD, entS, fepB, entC, entE, entB, entA, iroD, iroE	
NIB023	*E. coli*	Adhesion and Colonization Factors	fimB, fimC, fimD, fimE, fimF, fimG, fimH, aap/aspU, agg3A, agg3B, agg3C, agg3D, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, ompA	68
		Iron Uptake and Metabolism	aslA, fyuA, ybtE, ybtT, ybtU, irp1, irp2, ybtA, ybtP, ybtQ, ybtX, ybtS, chuV, chuU, chuY, chuX, chuW, chuT, chuA, shuS, shuA, shuT, shuX, fepA, fes, entF, fepC, fepG, fepD, entS, fepB, entC, entE, entB, entA, kpsD, kpsT, kpsM	
		Toxins and Effectors Genes	astA	
		Type II Secretion (T2SS)	gspD, gspG, gspH, gspI, gspJ, gspK	
		Type III Secretion (T3SS)	espL1, espL4, espX4, espX5, espY3, espY4	
NIB024	*E. coli*	Adhesion and Colonization Factors	fimB, fimC, fimE, fimF, fimG, fimI, ompA	47
		Iron Uptake and Metabolism	iucA, iucB, iucC, iucD, iutA, fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA, chuV, chuU, chuY, chuW, shuX, shuT, shuA, shuS, ybtS, ybtX, ybtQ, ybtP, ybtA, irp2, irp1, ybtU, ybtT, ybtE, fyuA	
		Toxins and Effectors Genes	espL1, espL4, espX1, espY2, espY3	
NIB026	*E. coli*	Adhesion and Colonization Factors	fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR	37
		Iron Uptake and Metabolism	fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA	
		Toxins and Effectors Genes	espL1, espX5	
		Type II Secretion (T2SS)	gspC, gspD, gspE, gspF, gspG, gspH, gspI, gspJ, gspK, gspL	
NIB027	*E. coli*	Adhesion and Colonization Factors	fimB, fimC, fimD, fimE, fimF, fimG, fimH, draP, afaC-I, afaB-I, afaA, daaF, ompA, papB, papX, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR	60
		Iron Uptake and Metabolism	entA, entB, entE, entC, fepB, entS, fepD, fepG, fepC, entF, fes, fepA, ybtA, ybtP, ybtQ, ybtX, ybtS, aslA, chuS, chuA, chuT, chuW, chuX, chuY, chuU, chuV, fyuA, ybtE, ybtT, ybtU, irp1, iucD, iucC, iucB, iucA	
		Capsule, LPS, and Biofilm Formation Genes	kpsD	

		Quorum Sensing, Regulation, and Miscellaneous Genes	sat	
NIB028	*P. aeruginosa*	Adhesion and Colonization Factors	fimU, fimT, fimV, pilE, pilF, pilG, pilH, pilI, pilJ, pilK, pilM, pilN, pilO, pilP, pilQ, pilR, pilS, pilT, pilV, pilW, pilX, pilY2	215
		Iron Uptake and Metabolism	fptA, mbtH-like, pvdA, pvdG, pvdH, pvdL, pvdO, pvdQ, pvdS, pchA, pchB, pchC, pchD, pchR, pvcA, pvcB, pvcC, pvcD	
		Toxins and Effectors Genes	aprA, exoS, exoT, exoY, lasA, lasB, plcH, phzC1, phzD1, phzE1, phzF1, phzG1, phzH, phzM, phzS, tse1, tse2, tse3, lip1	
		Capsule, LPS, and Biofilm Formation Genes	algA, algB, algC, algD, algE, algF, algG, algI, algJ, algK, algL, algP/algR3, algQ, algR, algU, algW, algX, algZ, alg44, alg8, mucA, mucB, mucC, mucD, mucE, wzz, wzy, waaA, waaC, waaF, waaG, waaP, tagF/pppB, tagQ, tagR, tagS, tagT	
		Type II Secretion (T2SS)	gspC, gspD, gspE, gspF, gspG, gspH, gspI, gspJ, gspK, gspL, xcpA/pilD, xcpP, xcpQ, xcpR, xcpS, xcpT, xcpU, xcpV, xcpW, xcpX, xcpY, xcpZ	
		Type III Secretion (T3SS)	pscB, pscC, pscD, pscE, pscF, pscG, pscH, pscI, pscJ, pscK, pscL, pscN, pscO, pscP, pscQ, pscR, pscS, pscT, pscU, pcr1, pcr2, pcr3, pcr4, pcrD, pcrG, pcrH, pcrR, pcrV, exsA, exsB, exsC, exsD, exsE, popB, popD, popN	
		Type VI Secretion (T6SS)	hcp1, hsiA1, hsiB1/vipA, hsiC1/vipB, hsiE1, hsiF1, hsiG1, hsiH1, hsiJ1, icmF1/tssM1, clpV1, vgrG1a, ppkA, pppA, tagF/pppB	
		Motility and Chemotaxis Genes	flgA, flgB, flgC, flgD, flgE, flgF, flgG, flgH, flgI, flgJ, flgK, flgL, flgM, flgN, fliA, fliC, fliD, fliE, fliF, fliG, fliH, fliI, fliJ, fliK, fliL, fliM, fliN, fliO, fliP, fliQ, fliR, fliS, fleI/flag, fleN, fleP, fleQ, fleR, fleS, motA, motB, motC, motD, motY, chpA, chpB, chpC, chpD, chpE	
		Quorum Sensing, Regulation, and Miscellaneous Genes	lasI, rhlA, rhlB, rhlC, ptxR, pppA, tagQ, tagR, tagS, tagT	
NIB030	*P. aeruginosa*	Adhesion and Colonization Factors	pilG, pilH, pilI, pilJ, pilK, pilM, pilR, pilS, pilT, pilU	195
		Iron Uptake and Metabolism	fpvA, mbtH-like, pvdA, pvdE, pvdF, pvdG, pvdH, pvdM, pvdN, pvdP, pvdQ, pvdS, pchA, pchB, pchD, pchR, pvcA, pvcB, pvcC, fptA	
		Toxins and Effectors Genes	aprA, exoS, exoT, exoY, lasA, lasB, plcH, lip1, tse1, tse3, phzA1, phzD1, phzE1, phzF1, phzG1, phzH, phzM, phzS, lasA, toxA	
		Capsule, LPS, and Biofilm Formation Genes	algA, algB, algC, algD, algE, algF, algG, algI, algJ, algK, algL, algQ, algR, algU, algW, algZ, alg44, alg8, mucA, mucB, mucC, mucD, mucE, wzz, wzy, waaA, waaC, waaF, waaG, waaP, tagF/pppB, tagQ, tagR	
		Type II Secretion (T2SS)	xcpA/pilD, xcpP, xcpQ, xcpR, xcpS, xcpT, xcpU, xcpV, xcpW, xcpX, xcpY, xcpZ, gspC, gspD, gspE, gspF, gspG, gspH, gspI, gspJ, gspK, gspL	
		Type III Secretion (T3SS)	pscB, pscC, pscD, pscE, pscF, pscG, pscH, pscI, pscJ, pscK, pscL, pscN, pscO, pscP, pscQ, pscR, pscS, pscT, pscU, pcr1, pcr2, pcr4, pcrD, pcrG, pcrH, pcrR, pcrV, exsA, exsB, exsC, exsD, exsE, popB, popN	
		Type VI Secretion (T6SS)	hcp1, hsiB1/vipA, hsiC1/vipB, hsiE1, hsiF1, hsiG1, hsiH1, hsiJ1, icmF1/tssM1, clpV1, vgrG1a, dotU1, tagF/pppB, pppA	
		Motility and Chemotaxis Genes	flgA, flgB, flgC, flgD, flgE, flgF, flgG, flgH, flgI, flgJ, flgK, flgL, flgM, flgN, fliA, fliC, fliD, fliE, fliF, fliG, fliH, fliI, fliJ, fliK, fliL, fliM, fliN, fliO, fliP, fliQ, fliR, fliS, fleI/flag, fleN, fleP, fleQ, fleR, fleS, motA, motB, motC, motD, motY, chpB, chpC, chpD, chpE	
		Quorum Sensing, Regulation, and Miscellaneous Genes	lasI, rhlA, rhlB, rhlC, ptxR	
NIB031	*E. coli*	Adhesion and Colonization Factors	yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, ompA, aggD, aggC, aggB, aap/aspU	43
		Iron Uptake and Metabolism	aslA, entA, entB, entE, entC, fepB, entS, fepD, fepG, fepC, entF, fes, fepA, chuV, chuU, chuY, shuX, chuW, chuT, shuA, chuS	
		Type II Secretion (T2SS)	gspD, gspF, gspG, gspH, gspI, gspJ, gspK, gspL	
		Capsule, LPS, and Biofilm Formation Genes	kpsD	
		Toxins and Effectors Genes	espL1, espR3	
		Adhesion and Colonization Factors	aggD, aggC, aggB, aap/aspU	
NIB033	*E. coli*	Adhesion and Colonization Factors	fdeC, ykgK/ecpR, yagZ/ecpA, yagY/ecpB, yagX/ecpC, yagW/ecpD, yagV/ecpE, fimH, fimG, fimF, fimD, fimC, fimI, fimE, fimB, ompA, papB, papX	60
		Iron Uptake and Metabolism	aslA, fyuA, ybtE, ybtT, ybtU, irp1, irp2, ybtA, ybtP, ybtQ, ybtX, ybtS, entA, entB, entE, entC, fepB, entS, fepD, fepG, fepC, entF, fes, fepA, iucA, iucB, iucC, iucD, chuS, chuA, chuT, chuW, chuX, chuY, chuU, chuV, sat	
		Type II Secretion (T2SS)	gspD, gspG, gspK	
		Capsule, LPS, and Biofilm Formation Genes	kpsD	

		Toxins and Effectors Genes	senB	
NIB034	*E. coli*	Adhesion and Colonization Factors	fimB, fimE, fimI, fimC, fimD, fimF, fimG, fimH, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR	33
		Iron Uptake and Metabolism	entD, fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA	
		Type II Secretion (T2SS)	gspM, gspL, gspD, gspC	
		Toxins and Effectors Genes	espX5, espX1, espL1	
NIB035	*E. coli*	Adhesion and Colonization Factors	afaF-III, draA, draB, afaC-I, draP, papB, papX, ompA, ykgK/ecpR, yagZ/ecpA, yagY/ecpB, yagX/ecpC, yagW/ecpD, yagV/ecpE	59
		Iron Uptake and Metabolism	aslA, fyuA, ybtE, ybtT, ybtU, irp1, irp2, ybtA, ybtP, ybtQ, ybtX, ybtS, fepA, fes, entF, fepC, fepD, entS, fepB, entC, entE, entB, entA, chuV, chuU, chuY, shuX, chuW, chuT, shuA, chuS, iroD, iroE, iucA, iucB, iucC, iucD, sat	
		Type II Secretion (T2SS)	gspK, gspG, gspD	
		Toxins and Effectors Genes	espX1, espX4, espL1	
NIB036	*E. coli*	Adhesion and Colonization Factors	fimH, fimG, fimF, fimD, fimC, fimI, fimE, fimB, afaC-I, afaB-I, afaA, daaF, draP, papB, papX, ompA, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, fdeC, chuV, chuU, chuY, chuX, chuW, chuT, chuA, chuS	60
		Iron Uptake and Metabolism	ybtS, ybtX, ybtQ, ybtP, ybtA, aslA, entA, entB, entE, entC, fepB, entS, fepD, fepG, fepC, entF, fes, fepA, kpsD, irp1, ybtU, ybtT, ybtE, fyuA, iucA, iucB, iucC, iucD, sat	
NIB037	*K. pneumoniae*	Iron Uptake and Metabolism	fyuA, ybtE, ybtT, ybtU, irp1, irp2, ybtA, ybtP, ybtQ, ybtX, ybtS	11
NIB044	*E. coli*	Adhesion and Colonization Factors	ompA, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR, afaF-III, draA, draB, draC, draD, draP, draE	56
		Iron Uptake and Metabolism	entA, entB, entE, entC, fepB, entS, fepD, fepC, entF, fes, fepA, ybtS, ybtX, ybtQ, ybtP, ybtA, irp2, irp1, ybtU, ybtT, ybtE, fyuA, iutA, iucA, iucB, iucC, iucD	
		Toxins and Effectors	espL1, espX5, espX1	

S4: List of IS elements and their presence in genetic material genes detected in 25 isolates.

Sample	MGE_Type	MGE_Subtype	Position	GC_Content	MGE Length (bp)
NIB002	ISPpu12	ISL3	chromosome	41.17	3372
NIB002	ISPpu12	ISL3	chromosome	41.17	3372
NIB002	ISYal1	IS3	chromosome	41.58	1187
NIB002	ISYal1	IS3	chromosome	36.78	1187
NIB003	IS911	IS3	chromosome	51.64	1251
NIB003	ISKpn25	ISL3	chromosome	51.62	2239
NIB006	IS1400	IS3	chromosome	51.01	1207
NIB007	ISKpn8	IS3	chromosome	56.51	1443
NIB008	IS100X	IS21	chromosome	50.28	2247
NIB008	IS103	IS3	chromosome	51.19	1446
NIB008	IS186A	IS4	plasmid	51.34	1345
NIB008	IS30D	IS30	chromosome	50.28	1221
NIB008	IS6100	IS6	plasmid	61.28	5466
NIB008	IS6100	IS6	plasmid	61.28	5466
NIB008	IS6100	IS6	plasmid	61.28	5466
NIB008	ISEc17	IS3	chromosome	46.89	1263
NIB008	ISEc26	ISAs1	chromosome	50.04	1257
NIB008	ISEc38	ISL3	chromosome	50.28	1695
NIB008	ISSen6	IS200/IS605	chromosome	50.79	1746

NIB009	IS1397	IS3	chromosome	48.46	1432
NIB009	IS4	IS4	chromosome	49.6	1427
NIB009	ISEc38	ISL3	chromosome	47.81	1695
NIB009	ISEc5	ISAs1	chromosome	38.26	1291
NIB010	IS186A	IS4	chromosome	51.34	1345
NIB010	IS630	IS630	chromosome	48.39	1160
NIB010	ISCro1	IS66	chromosome	51.75	2699
NIB010	ISEc22	IS66	chromosome	47.17	2454
NIB010	ISEc36	IS3	chromosome	51.06	1320
NIB010	ISEc43	IS66	chromosome	54.17	2532
NIB010	ISEc5	ISAs1	chromosome	48.66	1291
NIB010	ISSen6	IS200/IS605	chromosome	51.49	1746
NIB010	ISSfl3	IS66	chromosome	51.58	2735
NIB011	IS100X	IS21	chromosome	54.06	2247
NIB011	IS103	IS3	chromosome	44.08	1446
NIB011	IS1397	IS3	chromosome	50.12	1432
NIB011	IS186A	IS4	plasmid	56.88	1345
NIB011	IS4	IS4	chromosome	43.1	1427
NIB011	IS629	IS3	chromosome	54.06	1314
NIB011	IS640	IS21	chromosome	52.38	2131
NIB011	ISCfr12	ISL3	plasmid	51.83	1316
NIB011	ISEc12	IS21	chromosome	51.27	2582
NIB011	ISEc17	IS3	chromosome	42.17	1263
NIB011	ISEc23	IS66	chromosome	53.19	2534
NIB011	ISEc27	IS3	chromosome	48.51	1329
NIB011	ISEc38	ISL3	chromosome	45.75	1695
NIB011	ISEc49	IS66	plasmid	54.99	1103
NIB011	ISEc5	ISAs1	chromosome	50.43	1291
NIB011	ISEcp1	IS1380	chromosome	47.22	2656
NIB011	ISSfl4	IS110	chromosome	54.06	1468
NIB012	IS903	IS5	plasmid	46.4	1057
NIB012	ISAeme2	ISAs1	plasmid	51.08	6172
NIB012	ISAeme2	ISAs1	plasmid	51.08	6172
NIB012	ISEcl1	IS3	plasmid	53.38	1336
NIB012	ISEcp1	IS1380	plasmid	51.08	2656
NIB012	ISKpn1	IS3	chromosome	50.38	1445
NIB012	ISKpn34	IS3	plasmid	46.4	1221
NIB013	IS640	IS21	plasmid	43.34	2131
NIB013	ISCfr12	ISL3	chromosome	54.23	1316
NIB013	ISEc17	IS3	plasmid	42.46	1263
NIB013	ISEc21	IS110	plasmid	46.47	1374
NIB013	ISEcl1	IS3	plasmid	49.11	1336

NIB013	ISEcp1	IS1380	plasmid	49.11	2656
NIB013	ISEcp1	IS1380	plasmid	49.11	2656
NIB013	ISKpn21	ISNCY	plasmid	52.13	2278
NIB013	ISKpn8	IS3	chromosome	56.25	1443
NIB013	ISPsy24	IS3	chromosome	54.9	1245
NIB014	IS1400	IS3	chromosome	49.77	1207
NIB014	IS903B	IS5	chromosome	57.94	1057
NIB014	ISEcp1	IS1380	chromosome	56.96	2656
NIB014	ISKox1	IS66	chromosome	49.19	2526
NIB014	ISKpn1	IS3	chromosome	50.26	1445
NIB014	ISKpn25	ISL3	plasmid	53.89	2239
NIB014	ISKpn34	IS3	chromosome	56.79	1221
NIB014	ISKpn38	IS3	chromosome	58.6	1598
NIB015	IS103	IS3	plasmid	44.39	1446
NIB015	IS186A	IS4	chromosome	52.23	1345
NIB015	IS903	IS5	chromosome	50.19	1057
NIB015	ISCfr12	ISL3	chromosome	48.69	1316
NIB015	ISCro1	IS66	plasmid	54.08	2699
NIB015	ISEc1	ISAs1	chromosome	37.89	1291
NIB015	ISEc17	IS3	chromosome	51.21	1263
NIB015	ISEc22	IS66	plasmid	55.31	2454
NIB015	ISEc26	ISAs1	chromosome	51.79	1257
NIB015	ISEc38	ISL3	chromosome	50.78	1695
NIB015	ISEc5	ISAs1	chromosome	51.53	1291
NIB015	ISEc5	ISAs1	chromosome	38.54	1291
NIB015	ISSd1	IS3	chromosome	45.85	1339
NIB015	ISSfl4	IS110	chromosome	57.47	1468
NIB016	IS630	IS630	chromosome	48.15	1160
NIB016	ISCro1	IS66	plasmid	51.81	2699
NIB016	ISEc5	ISAs1	chromosome	48.66	1291
NIB016	ISSen6	IS200/IS605	chromosome	49.61	1746
NIB017	IS18	IS30	chromosome	43.35	1074
NIB017	ISAba12	IS5	chromosome	41.36	1039
NIB017	ISAba12	IS5	chromosome	42.03	1039
NIB017	ISAba12	IS5	plasmid	38.23	1039
NIB017	ISAba125	IS30	plasmid	37.26	2175
NIB017	ISAba14	IS3	plasmid	38.71	1283
NIB017	ISAba18	IS3	chromosome	39.35	1321
NIB017	ISAba18	IS3	plasmid	34.97	1321
NIB017	ISAba21	IS3	chromosome	41.72	1274
NIB017	ISAba26	IS256	plasmid	39.18	1318
NIB017	ISAba32	ISNCY	chromosome	41.35	1482

NIB017	ISAba32	ISNCY	plasmid	38.23	1482
NIB017	ISAba8	IS21	plasmid	43.84	1920
NIB017	ISAha2	IS5	chromosome	42.26	1040
NIB017	ISAha2	IS5	chromosome	42.36	1040
NIB017	ISAha3	IS5	plasmid	34.12	1040
NIB017	ISAha3	IS5	plasmid	42.43	1040
NIB017	ISAjo2	ISNCY	plasmid	39.48	1482
NIB017	ISAlw1	IS5	chromosome	41.25	1039
NIB018	IS4	IS4	plasmid	53.23	1427
NIB018	IS911	IS3	chromosome	49.7	1251
NIB018	ISEc17	IS3	chromosome	48.57	1263
NIB018	ISEc5	ISAs1	chromosome	48.57	1291
NIB018	ISEc5	ISAs1	chromosome	48.57	1291
NIB018	ISKpn26	IS5	plasmid	50.71	1197
NIB019	IS222	IS3	chromosome	61.69	1236
NIB019	ISPa32	IS3	chromosome	64.69	1237
NIB019	ISPa32	IS3	plasmid	64.11	1237
NIB019	ISPsy11	IS3	chromosome	66.53	1323
NIB019	ISStma11	ISL3	chromosome	65.55	4426
NIB019	ISStma11	ISL3	chromosome	65.55	4426
NIB020	IS100X	IS21	chromosome	50.6	2247
NIB020	IS103	IS3	chromosome	50.79	1446
NIB020	IS186A	IS4	chromosome	51.22	1345
NIB020	IS30D	IS30	chromosome	52.45	1221
NIB020	IS903	IS5	chromosome	51.62	1057
NIB020	IS903	IS5	plasmid	46.53	1057
NIB020	ISKpn26	IS5	chromosome	51.62	1197
NIB020	ISSen6	IS200/IS605	chromosome	50.75	1746
NIB021	IS100X	IS21	chromosome	50.55	2247
NIB021	IS6100	IS6	plasmid	58.24	5466
NIB021	IS6100	IS6	plasmid	58.24	5466
NIB021	IS6100	IS6	plasmid	58.24	5466
NIB021	IS629	IS3	chromosome	47.69	1314
NIB021	ISCro1	IS66	plasmid	50.53	2699
NIB021	ISEc16	IS3	chromosome	47.06	1233
NIB021	ISEc17	IS3	chromosome	48.21	1263
NIB021	ISEc23	IS66	chromosome	47.69	2534
NIB021	ISEc31	IS3	chromosome	47.42	1294
NIB021	ISEc36	IS3	plasmid	53.01	1320
NIB021	ISEc38	ISL3	chromosome	50.55	1695
NIB021	ISEc38	ISL3	chromosome	46.57	1695
NIB021	ISPst2	ISL3	chromosome	47.69	2985

NIB022	IS1397	IS3	chromosome	49.66	1432
NIB022	IS186A	IS4	plasmid	56.68	1345
NIB022	IS4	IS4	chromosome	50.23	1427
NIB022	IS640	IS21	chromosome	52.82	2131
NIB022	ISCfr12	ISL3	plasmid	51.84	1316
NIB022	ISCro1	IS66	chromosome	55.12	2699
NIB022	ISEc12	IS21	plasmid	47.37	2582
NIB022	ISEc17	IS3	chromosome	42.16	1263
NIB022	ISEc36	IS3	chromosome	45.92	1320
NIB022	ISEc38	ISL3	chromosome	45.92	1695
NIB022	ISEc38	ISL3	plasmid	46.21	1695
NIB022	ISEc49	IS66	chromosome	52.19	1103
NIB022	ISEc5	ISAs1	chromosome	50.4	1291
NIB022	ISEcp1	IS1380	chromosome	47.12	2656
NIB022	ISSen6	IS200/IS605	chromosome	51.31	1746
NIB022	ISSfl4	IS110	chromosome	52.63	1468
NIB023	IS100X	IS21	chromosome	48.73	2247
NIB023	IS1203	IS3	plasmid	47.34	1310
NIB023	IS1397	IS3	chromosome	51.3	1432
NIB023	IS4	IS4	plasmid	49.31	1427
NIB023	ISEc17	IS3	chromosome	49.09	1263
NIB023	ISEc31	IS3	chromosome	52.74	1294
NIB023	ISEc38	ISL3	chromosome	49.09	1695
NIB023	ISEc5	ISAs1	chromosome	50.14	1291
NIB023	ISEc5	ISAs1	chromosome	51.45	1291
NIB023	ISSen6	IS200/IS605	chromosome	48.73	1746
NIB023	ISSoEn2	IS256	plasmid	49.31	1314
NIB024	IS630	IS630	chromosome	47.63	1160
NIB024	ISCro1	IS66	plasmid	51.81	2699
NIB024	ISEc5	ISAs1	chromosome	48.66	1291
NIB024	ISSen6	IS200/IS605	chromosome	49.61	1746
NIB025	ISPpu12	ISL3	plasmid	48.88	3372
NIB026	ISCfr12	ISL3	chromosome	51.02	1316
NIB026	ISCro1	IS66	chromosome	51.02	2699
NIB026	ISEc26	ISAs1	chromosome	38.54	1257
NIB026	ISEc38	ISL3	chromosome	50.39	1695
NIB026	ISSd1	IS3	chromosome	50.95	1339
NIB026	ISStma11	ISL3	plasmid	63.32	4426
NIB027	IS103	IS3	chromosome	50.36	1446
NIB027	IS1397	IS3	chromosome	49.78	1432
NIB027	IS186A	IS4	plasmid	50.65	1345
NIB027	IS6100	IS6	plasmid	58.5	5466

NIB027	IS6100	IS6	plasmid	58.5	5466
NIB027	IS6100	IS6	plasmid	58.5	5466
NIB027	IS640	IS21	chromosome	52.38	2131
NIB027	ISCro1	IS66	plasmid	55.38	2699
NIB027	ISEc12	IS21	chromosome	49.9	2582
NIB027	ISEc23	IS66	chromosome	53.08	2534
NIB027	ISEc27	IS3	chromosome	47.32	1329
NIB027	ISEc38	ISL3	chromosome	45.27	1695
NIB027	ISEc38	ISL3	chromosome	47.95	1695
NIB027	ISEc49	IS66	chromosome	52.21	1103
NIB027	ISEc5	ISAs1	chromosome	50.36	1291
NIB027	ISEcp1	IS1380	chromosome	49.62	2656
NIB027	ISKpn34	IS3	chromosome	51.07	1221
NIB027	ISSen6	IS200/IS605	chromosome	51.21	1746
NIB027	ISSfl4	IS110	chromosome	56.3	1468
NIB028	ISPa32	IS3	chromosome	61.05	1237
NIB028	ISPa37	IS30	chromosome	64.86	1453
NIB029	ISEcp1	IS1380	chromosome	55.76	2656
NIB030	IS222	IS3	chromosome	63.37	1236
NIB030	IS222	IS3	chromosome	58.83	1236
NIB030	ISPa32	IS3	chromosome	54.28	1237
NIB030	ISPa32	IS3	plasmid	59.92	1237
NIB030	ISPsy11	IS3	chromosome	65.39	1323
NIB030	ISStma11	ISL3	chromosome	64.64	4426
NIB030	ISStma11	ISL3	chromosome	64.64	4426
NIB031	IS100X	IS21	plasmid	40.23	2247
NIB031	IS103	IS3	chromosome	49.09	1446
NIB031	IS30D	IS30	plasmid	44.11	1221
NIB031	IS6100	IS6	plasmid	58.56	5466
NIB031	IS6100	IS6	plasmid	58.56	5466
NIB031	IS6100	IS6	plasmid	58.56	5466
NIB031	ISEc12	IS21	plasmid	47.35	2582
NIB031	ISEc17	IS3	plasmid	53.83	1263
NIB031	ISEc22	IS66	chromosome	49.26	2454
NIB031	ISEc22	IS66	chromosome	52.26	2454
NIB031	ISEc31	IS3	chromosome	47	1294
NIB031	ISEc38	ISL3	chromosome	47.82	1695
NIB031	ISEc38	ISL3	plasmid	46.04	1695
NIB031	ISEcp1	IS1380	chromosome	49.23	2656
NIB031	ISSd1	IS3	chromosome	49.26	1339
NIB031	ISSgsp1	IS66	chromosome	49.26	3044
NIB032	IS1400	IS3	chromosome	56.3	1207

NIB032	IS1400	IS3	chromosome	56.3	1207
NIB032	ISEc63	Tn3	chromosome	47.98	5981
NIB032	ISKpn1	IS3	plasmid	50.21	1445
NIB032	ISKpn34	IS3	plasmid	46.83	1221
NIB032	ISSgsp1	IS66	plasmid	55.78	3044
NIB033	IS103	IS3	chromosome	44.07	1446
NIB033	IS1397	IS3	chromosome	50.1	1432
NIB033	IS186A	IS4	chromosome	51.34	1345
NIB033	IS30D	IS30	chromosome	49.1	1221
NIB033	ISCro1	IS66	chromosome	52.48	2699
NIB033	ISCro1	IS66	chromosome	52.48	2699
NIB033	ISEc12	IS21	plasmid	47.35	2582
NIB033	ISEc16	IS3	chromosome	46.69	1233
NIB033	ISEc23	IS66	plasmid	52.96	2534
NIB033	ISEc38	ISL3	chromosome	46.06	1695
NIB033	ISEc5	ISAs1	chromosome	50.37	1291
NIB033	ISSd1	IS3	chromosome	52.48	1339
NIB033	ISSen6	IS200/IS605	chromosome	51.67	1746
NIB033	ISSfl4	IS110	chromosome	53.45	1468
NIB034	IS103	IS3	chromosome	44.07	1446
NIB034	IS186A	IS4	plasmid	51.38	1345
NIB034	IS629	IS3	plasmid	54.39	1314
NIB034	ISEc17	IS3	plasmid	53.5	1263
NIB034	ISEc26	ISAs1	chromosome	48.12	1257
NIB034	ISEc38	ISL3	chromosome	50.04	1695
NIB034	ISEc5	ISAs1	chromosome	50.16	1291
NIB034	ISKpn26	IS5	chromosome	50.02	1197
NIB034	ISSen6	IS200/IS605	chromosome	49.13	1746
NIB034	ISSen6	IS200/IS605	chromosome	52.15	1746
NIB035	IS100X	IS21	chromosome	52.87	2247
NIB035	IS103	IS3	chromosome	43.59	1446
NIB035	IS1397	IS3	chromosome	50.68	1432
NIB035	IS186A	IS4	chromosome	51.3	1345
NIB035	IS30D	IS30	chromosome	49.01	1221
NIB035	IS609	IS200/IS605	chromosome	50.4	1753
NIB035	IS640	IS21	chromosome	49.85	2131
NIB035	ISEc17	IS3	chromosome	43.59	1263
NIB035	ISEc27	IS3	chromosome	49.14	1329
NIB035	ISEc43	IS66	chromosome	43.59	2532
NIB035	ISEc5	ISAs1	chromosome	51.58	1291
NIB035	ISEc5	ISAs1	chromosome	48.91	1291
NIB035	ISEc5	ISAs1	chromosome	44.45	1291

NIB035	ISEcp1	IS1380	chromosome	51.93	2656
NIB035	ISKpn26	IS5	plasmid	54.47	1197
NIB036	IS103	IS3	chromosome	49.37	1446
NIB036	IS1397	IS3	chromosome	49.93	1432
NIB036	IS186A	IS4	plasmid	51.12	1345
NIB036	IS4	IS4	chromosome	50.59	1427
NIB036	IS6100	IS6	plasmid	58.59	5466
NIB036	IS6100	IS6	plasmid	58.59	5466
NIB036	IS6100	IS6	plasmid	58.59	5466
NIB036	IS640	IS21	chromosome	52.38	2131
NIB036	ISCro1	IS66	plasmid	55.46	2699
NIB036	ISEc12	IS21	plasmid	47.37	2582
NIB036	ISEc38	ISL3	chromosome	45.58	1695
NIB036	ISEc38	ISL3	plasmid	47.45	1695
NIB036	ISEc49	IS66	chromosome	52.25	1103
NIB036	ISEc5	ISAs1	chromosome	50.34	1291
NIB036	ISEcp1	IS1380	chromosome	49.36	2656
NIB036	ISKpn34	IS3	chromosome	51.07	1221
NIB036	ISSen6	IS200/IS605	chromosome	51.67	1746
NIB037	IS102	IS5	plasmid	50.29	1055
NIB037	IS1400	IS3	chromosome	52.94	1207
NIB037	IS630	IS630	chromosome	55.94	1160
NIB037	ISEcl1	IS3	plasmid	53.44	1336
NIB037	ISKpn21	ISNCY	plasmid	52.03	2278
NIB037	ISKpn24	IS66	plasmid	56.85	2454
NIB037	ISKpn26	IS5	chromosome	54.71	1197
NIB037	ISKpn28	IS481	plasmid	47.81	1105
NIB037	ISKpn34	IS3	chromosome	55.85	1221
NIB037	ISKpn38	IS3	chromosome	57.78	1598
NIB041	ISKpn20	IS3	chromosome	47.85	1196
NIB041	ISKpn26	IS5	chromosome	47.85	1197
NIB041	ISKpn26	IS5	chromosome	47.85	1197
NIB041	ISStma11	ISL3	plasmid	47.86	4426
NIB044	IS1203E	IS3	chromosome	47.96	1311
NIB044	IS186A	IS4	chromosome	51.38	1345
NIB044	IS903B	IS5	chromosome	51.87	1057
NIB044	ISCro1	IS66	plasmid	50.99	2699
NIB044	ISEc1	ISAs1	chromosome	49.07	1291
NIB044	ISEc17	IS3	chromosome	49.13	1263
NIB044	ISEc23	IS66	chromosome	50.71	2534
NIB044	ISEc38	ISL3	chromosome	50.71	1695
NIB044	ISEcp1	IS1380	chromosome	45.25	2656

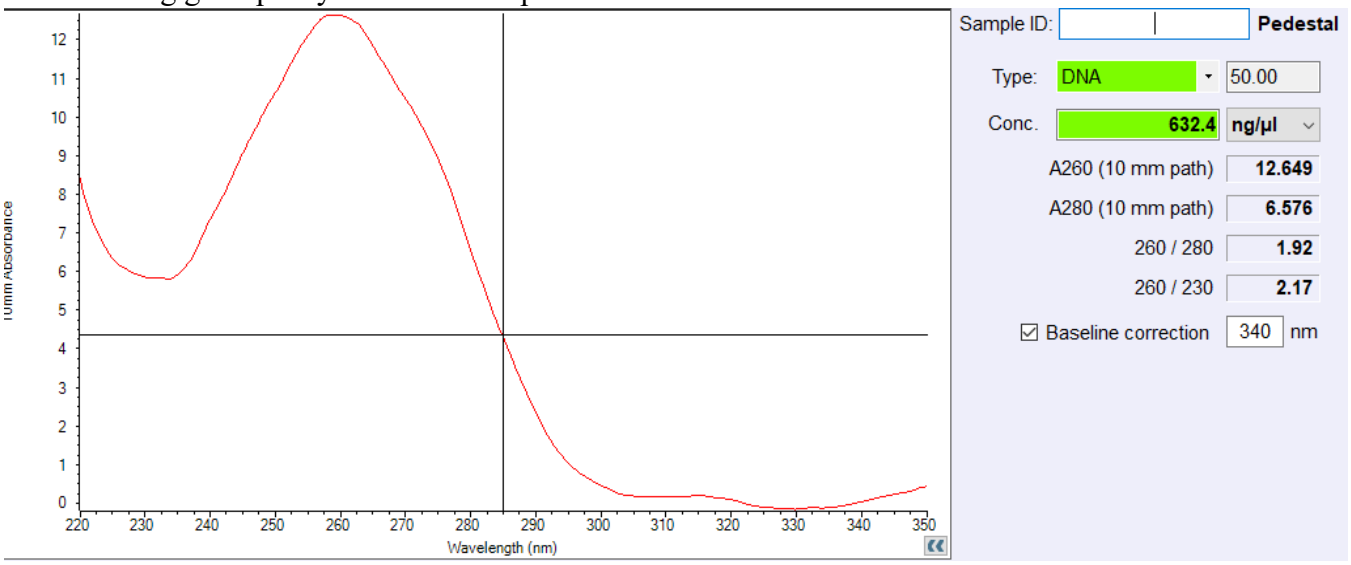
NIB044	ISKpn26	IS5	chromosome	46.06	1197
NIB044	ISSfl4	IS110	chromosome	47.96	1468

Appendix B

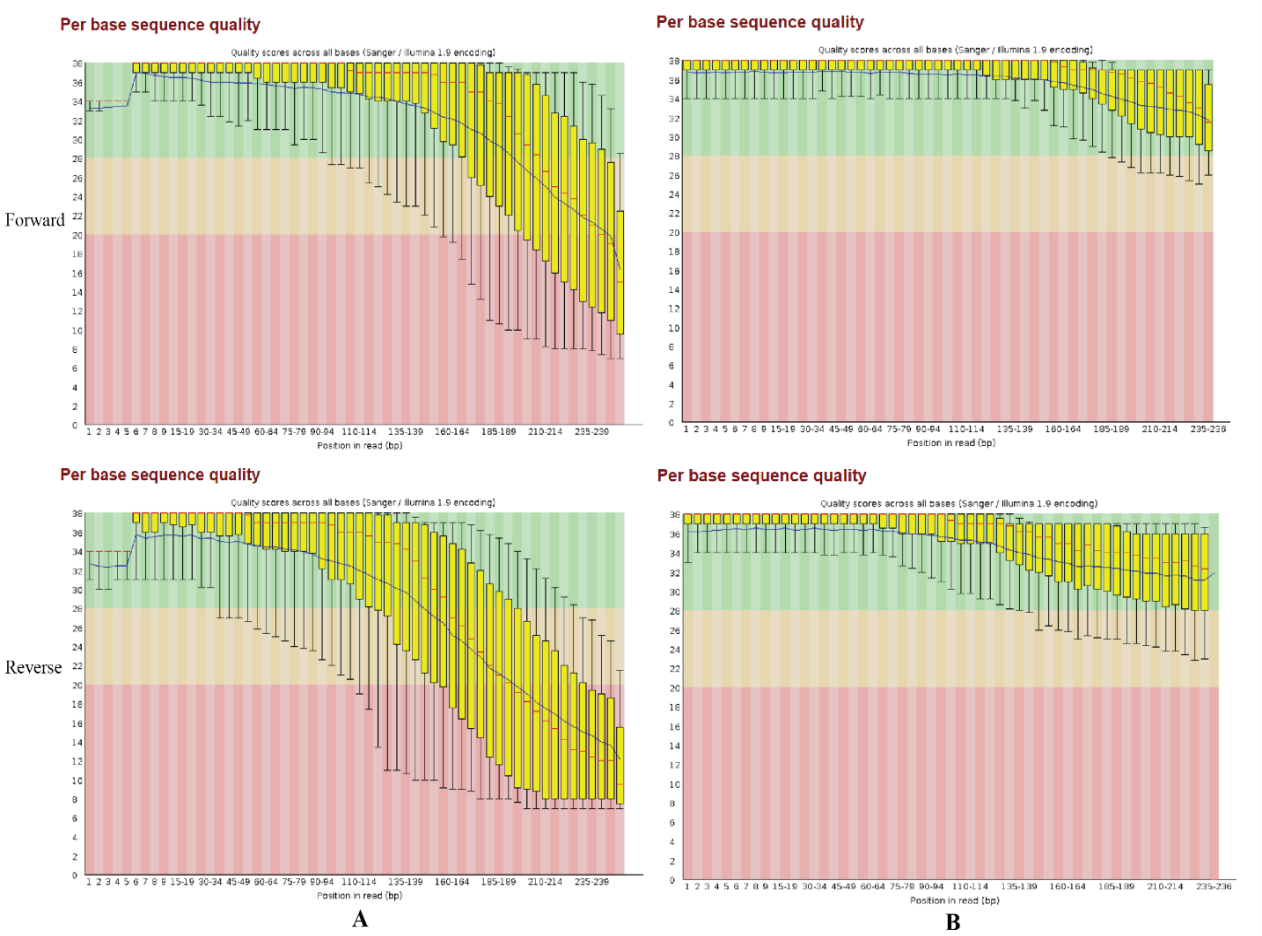
Supplementary Figures

F1: **Nanodrop spectrophotometer profile showing the absorbance spectrum and purity ratios of the extracted DNA.**

The DNA sample exhibited a concentration of 632.4 ng/µL, indicating a high yield suitable for downstream applications. The A260/A280 ratio was 1.92, and the A260/A230 ratio was 2.17, showing good purity with minimal protein or solvent contamination.



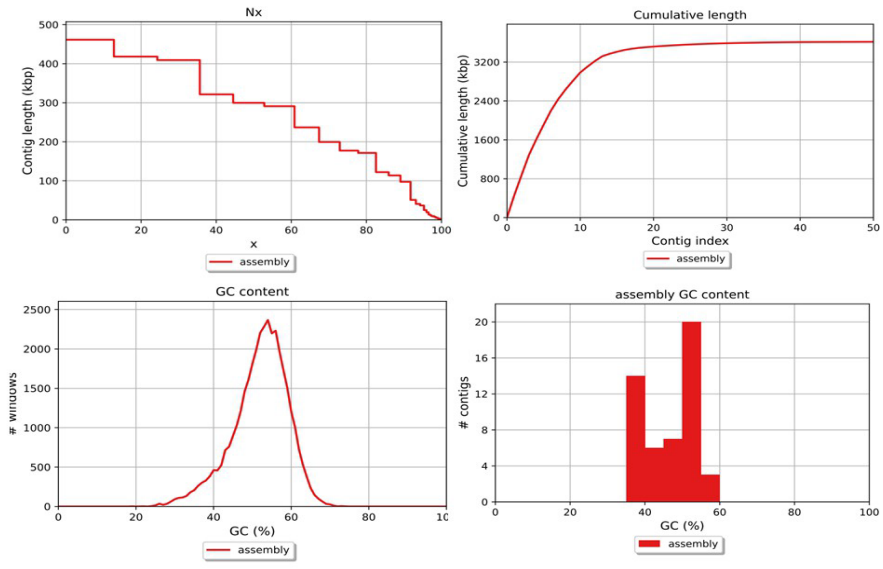
F2: Per base sequence quality for forward and reverse sequence. Here, (**A**) are the raw reads and (**B**) are the trimming reads.



F3: Assembly quality of the *Plesiomonas shigelloides*

Report

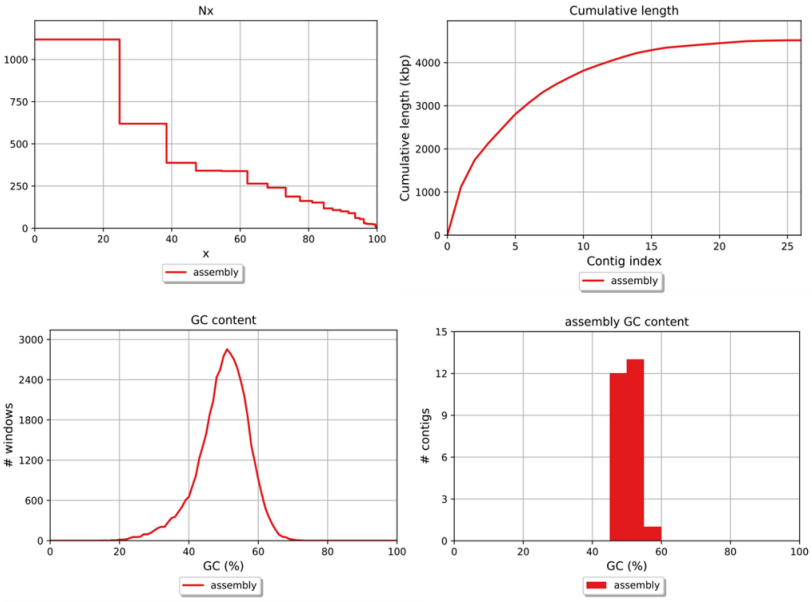
	assembly
# contigs (>= 0 bp)	66
# contigs (>= 1000 bp)	41
# contigs (>= 5000 bp)	28
# contigs (>= 10000 bp)	20
# contigs (>= 25000 bp)	16
# contigs (>= 50000 bp)	14
Total length (>= 0 bp)	3621225
Total length (>= 1000 bp)	3612030
Total length (>= 5000 bp)	3579420
Total length (>= 10000 bp)	3519141
Total length (>= 25000 bp)	3448725
Total length (>= 50000 bp)	3371035
# contigs	50
Largest contig	461416
Total length	3617790
GC (%)	51.92
N50	299831
N90	97454
auN	286092.1
L50	5
L90	13
# N's per 100 kbp	0.00



F4: Assembly quality of the *Escherichia fergusonii*

Report

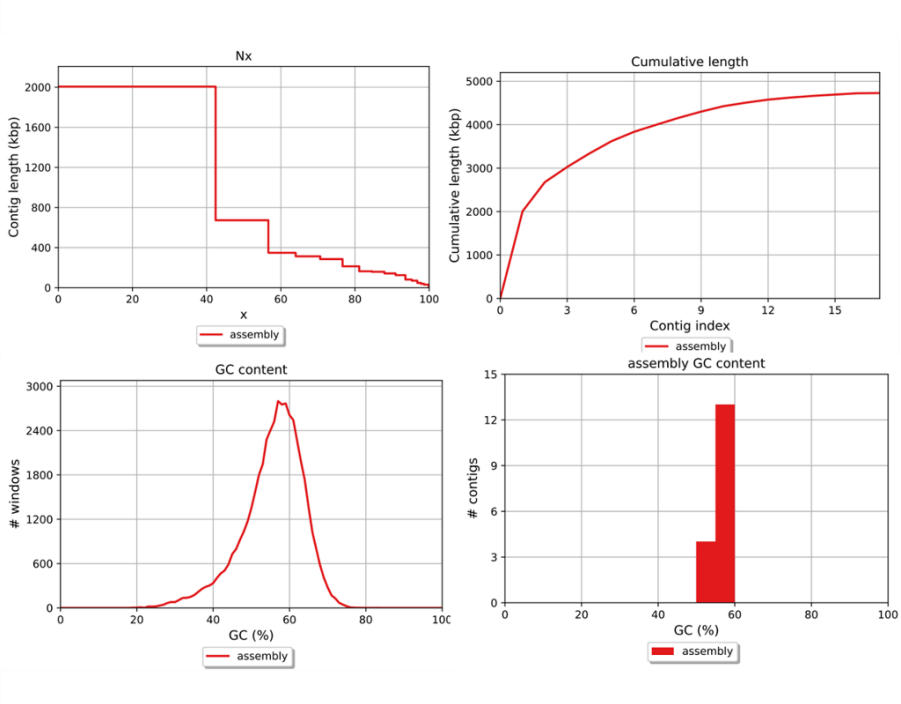
	assembly
# contigs (>= 0 bp)	26
# contigs (>= 1000 bp)	25
# contigs (>= 5000 bp)	25
# contigs (>= 10000 bp)	22
# contigs (>= 25000 bp)	20
# contigs (>= 50000 bp)	16
Total length (>= 0 bp)	4517282
Total length (>= 1000 bp)	4516677
Total length (>= 5000 bp)	4516677
Total length (>= 10000 bp)	4495540
Total length (>= 25000 bp)	4449070
Total length (>= 50000 bp)	4342427
# contigs	26
Largest contig	1118853
Total length	4517282
GC (%)	49.92
N50	341080
N90	100020
auN	505589.8
L50	4
L90	13
# N's per 100 kbp	0.00



F5: Assembly quality of the *Enterobacter chuandaensis*

Report

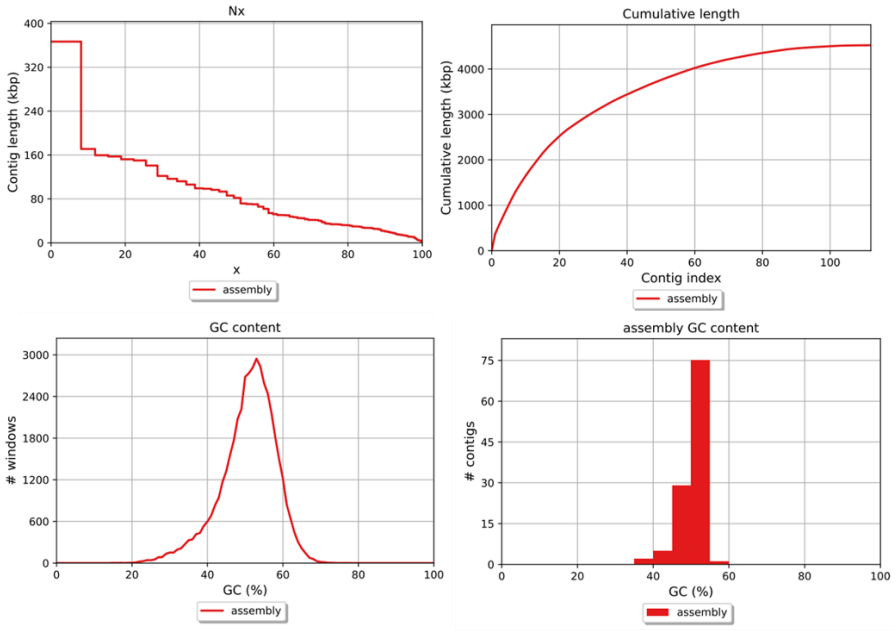
	assembly
# contigs (>= 0 bp)	18
# contigs (>= 1000 bp)	17
# contigs (>= 5000 bp)	16
# contigs (>= 10000 bp)	16
# contigs (>= 25000 bp)	16
# contigs (>= 50000 bp)	12
Total length (>= 0 bp)	4728407
Total length (>= 1000 bp)	4728294
Total length (>= 5000 bp)	4723487
Total length (>= 10000 bp)	4723487
Total length (>= 25000 bp)	4723487
Total length (>= 50000 bp)	4576875
# contigs	17
Largest contig	2005841
Total length	4728294
GC (%)	55.62
N50	672565
N90	142106
auN	1041754.4
L50	2
L90	9
# N's per 100 kbp	0.00



F6: Assembly quality of the *Escherichia coli*

Report

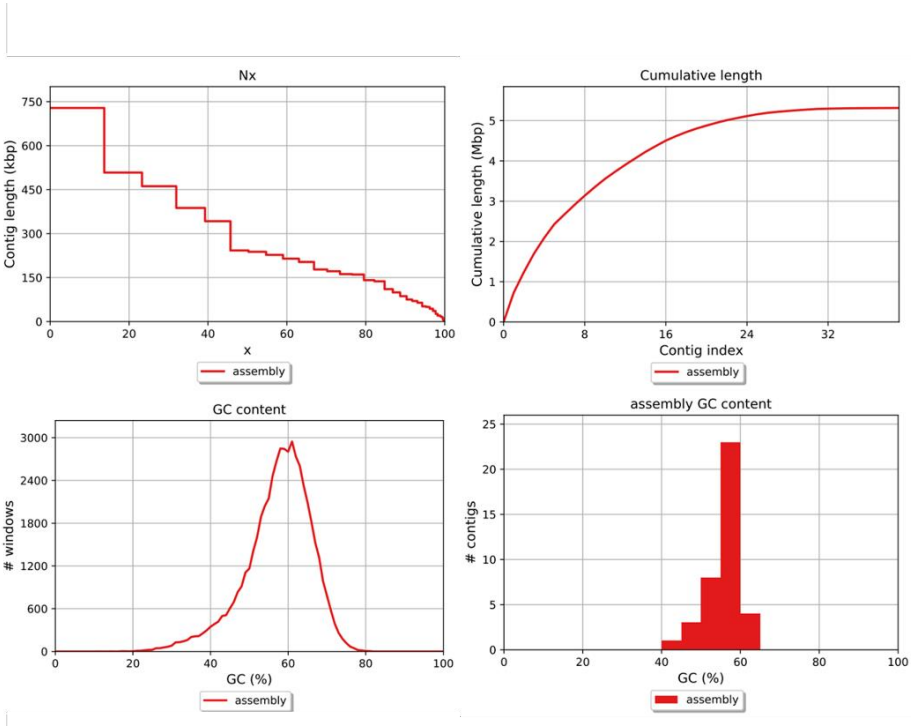
	assembly
# contigs (>= 0 bp)	113
# contigs (>= 1000 bp)	107
# contigs (>= 5000 bp)	92
# contigs (>= 10000 bp)	87
# contigs (>= 25000 bp)	59
# contigs (>= 50000 bp)	27
Total length (>= 0 bp)	4524061
Total length (>= 1000 bp)	4520167
Total length (>= 5000 bp)	4466654
Total length (>= 10000 bp)	4430707
Total length (>= 25000 bp)	3999686
Total length (>= 50000 bp)	2907245
# contigs	112
Largest contig	366709
Total length	4523630
GC (%)	50.77
N50	81866
N90	20717
auN	103047.8
L50	17
L90	63
# N's per 100 kbp	0.00



F7: Assembly quality of the *Klebsiella pneumoniae*

Report

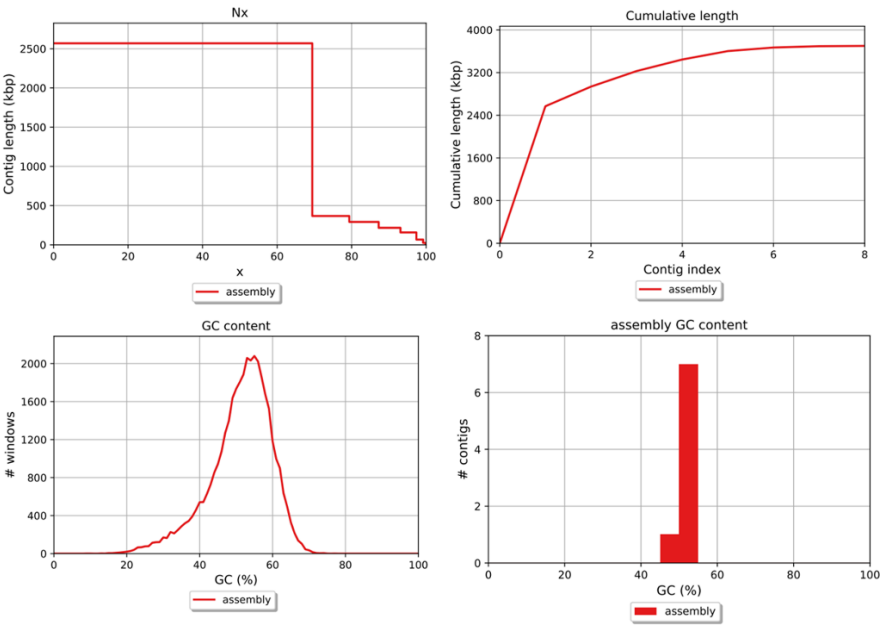
	assembly
# contigs (>= 0 bp)	58
# contigs (>= 1000 bp)	52
# contigs (>= 5000 bp)	45
# contigs (>= 10000 bp)	38
# contigs (>= 25000 bp)	33
# contigs (>= 50000 bp)	26
Total length (>= 0 bp)	4874853
Total length (>= 1000 bp)	4872922
Total length (>= 5000 bp)	4855539
Total length (>= 10000 bp)	4806955
Total length (>= 25000 bp)	4725826
Total length (>= 50000 bp)	4455155
# contigs	53
Largest contig	491659
Total length	4873742
GC (%)	54.99
N50	210580
N90	59655
auN	213807.2
L50	9
L90	25
# N's per 100 kbp	0.00



F8: Assembly quality of the *Morganella morganii*

Report

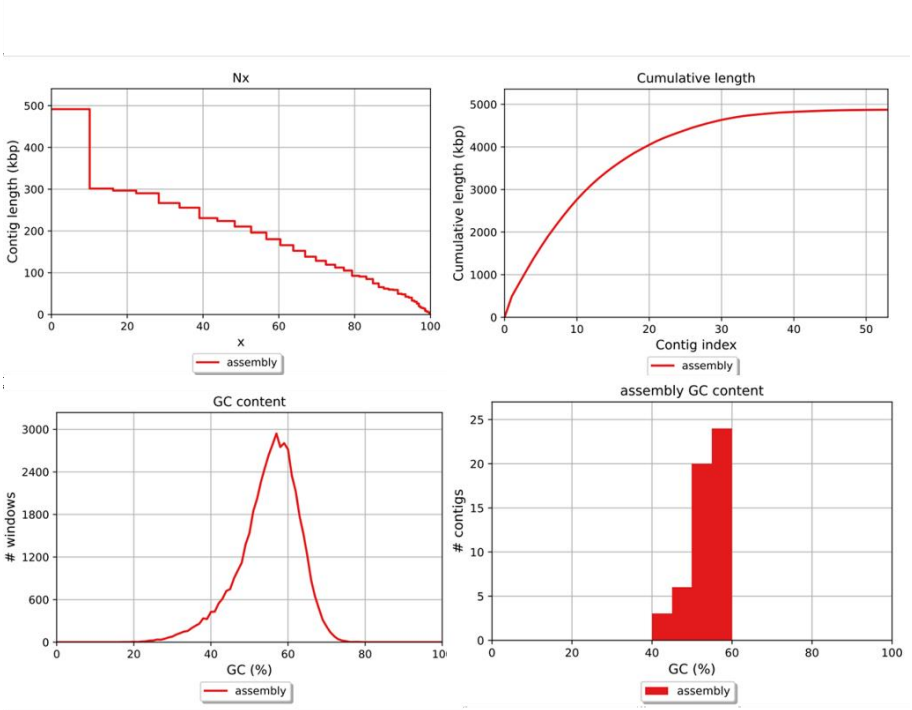
	assembly
# contigs (>= 0 bp)	9
# contigs (>= 1000 bp)	8
# contigs (>= 5000 bp)	8
# contigs (>= 10000 bp)	7
# contigs (>= 25000 bp)	6
# contigs (>= 50000 bp)	6
Total length (>= 0 bp)	3700411
Total length (>= 1000 bp)	3700258
Total length (>= 5000 bp)	3700258
Total length (>= 10000 bp)	3694842
Total length (>= 25000 bp)	3670044
Total length (>= 50000 bp)	3670044
# contigs	8
Largest contig	2569229
Total length	3700258
GC (%)	51.16
N50	2569229
N90	216692
auN	1864249.2
L50	1
L90	4
# N's per 100 kbp	0.00



F9: Assembly quality of the *Enterobacter hormaechei*

Report

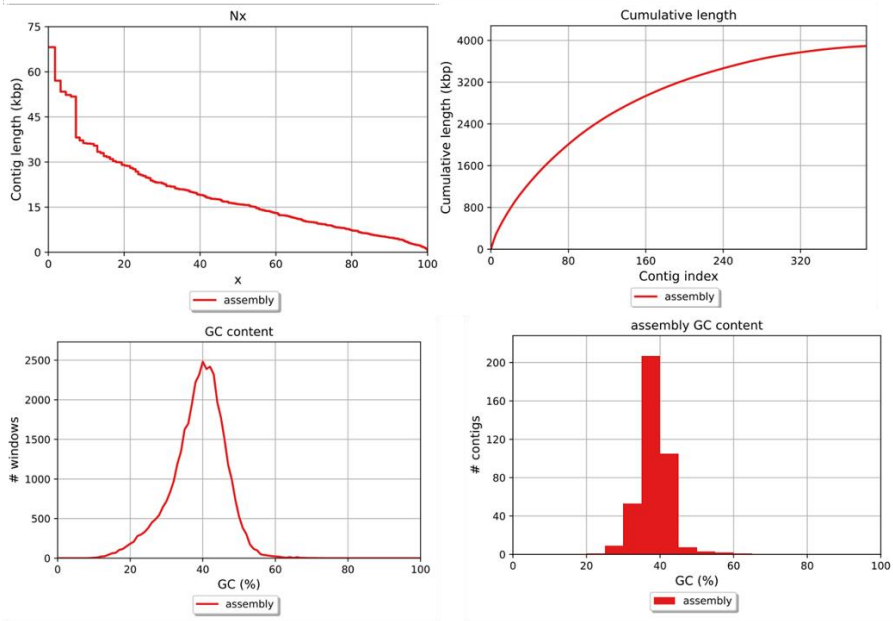
	assembly
# contigs (>= 0 bp)	58
# contigs (>= 1000 bp)	52
# contigs (>= 5000 bp)	45
# contigs (>= 10000 bp)	38
# contigs (>= 25000 bp)	33
# contigs (>= 50000 bp)	26
Total length (>= 0 bp)	4874853
Total length (>= 1000 bp)	4872922
Total length (>= 5000 bp)	4855539
Total length (>= 10000 bp)	4806955
Total length (>= 25000 bp)	4725826
Total length (>= 50000 bp)	4455155
# contigs	53
Largest contig	491659
Total length	4873742
GC (%)	54.99
N50	210580
N90	59655
auN	213807.2
L50	9
L90	25
# N's per 100 kbp	0.00



F10: Assembly quality of the *Proteus mirabilis*

Report

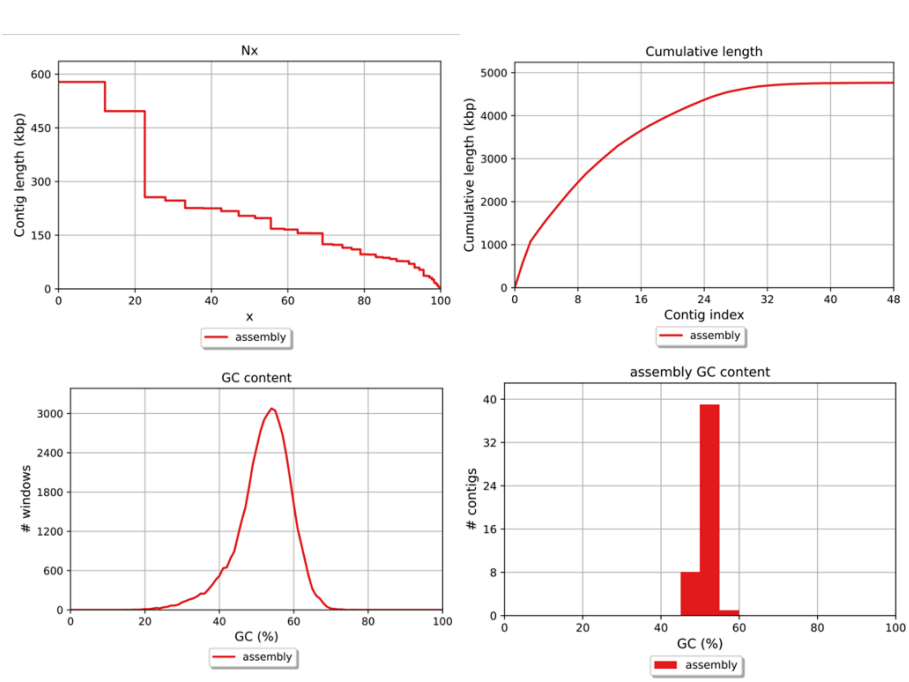
	assembly
# contigs (>= 0 bp)	389
# contigs (>= 1000 bp)	388
# contigs (>= 5000 bp)	245
# contigs (>= 10000 bp)	136
# contigs (>= 25000 bp)	28
# contigs (>= 50000 bp)	5
Total length (>= 0 bp)	3891737
Total length (>= 1000 bp)	3891584
Total length (>= 5000 bp)	3489694
Total length (>= 10000 bp)	2712965
Total length (>= 25000 bp)	999068
Total length (>= 50000 bp)	282704
# contigs	388
Largest contig	68187
Total length	3891584
GC (%)	38.73
N50	15970
N90	4893
auN	19253.1
L50	77
L90	248
# N's per 100 kbp	0.00



F11: Assembly quality of the *Citrobacter werkmanii*

Report

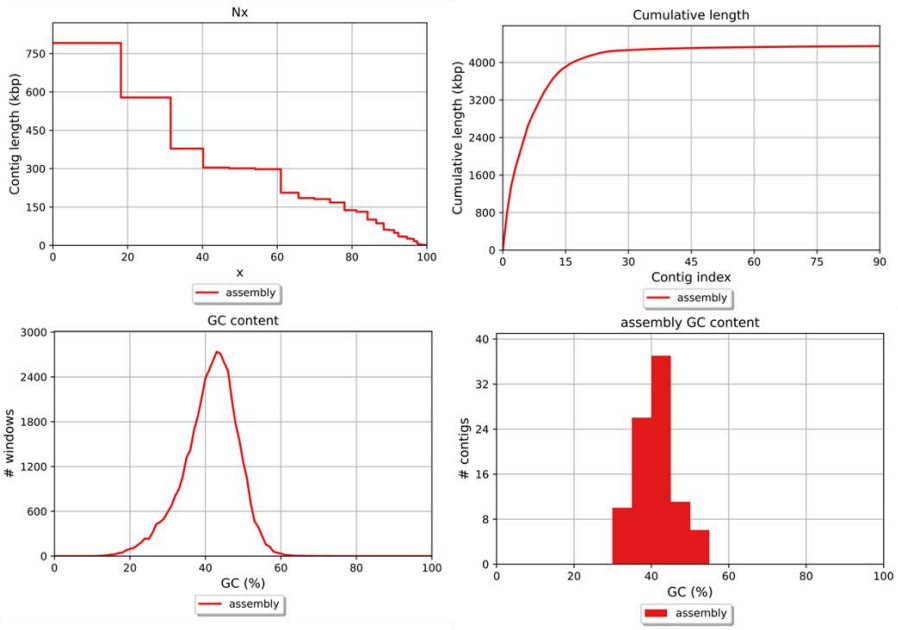
	assembly
# contigs (>= 0 bp)	61
# contigs (>= 1000 bp)	43
# contigs (>= 5000 bp)	37
# contigs (>= 10000 bp)	34
# contigs (>= 25000 bp)	31
# contigs (>= 50000 bp)	27
Total length (>= 0 bp)	4768703
Total length (>= 1000 bp)	4762017
Total length (>= 5000 bp)	4747868
Total length (>= 10000 bp)	4727228
Total length (>= 25000 bp)	4682378
Total length (>= 50000 bp)	4552294
# contigs	48
Largest contig	578310
Total length	4765791
GC (%)	52.07
N50	204028
N90	77554
auN	244706.7
L50	8
L90	23
# N's per 100 kbp	0.00



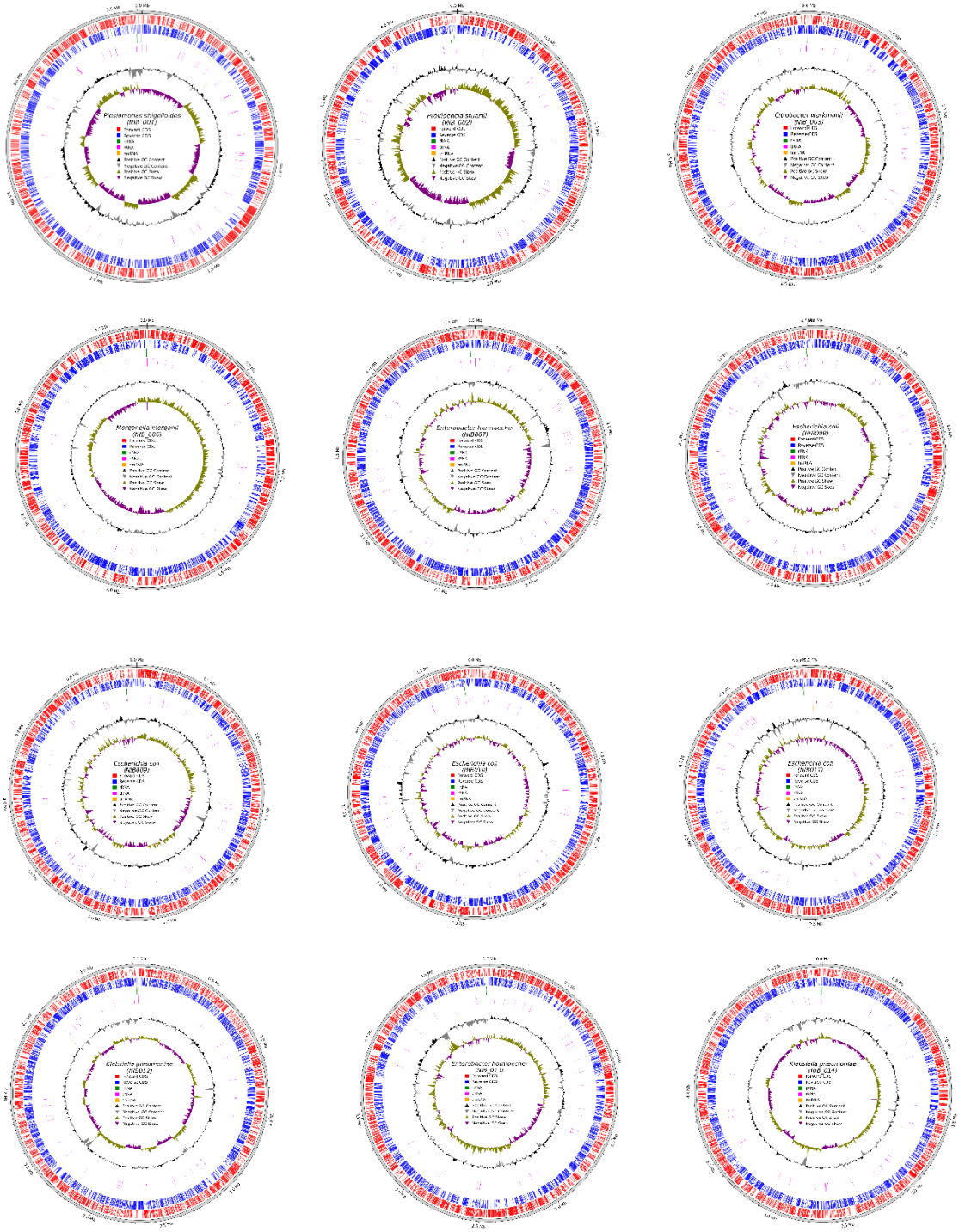
F12: Assembly quality of the *Providencia stuartii*

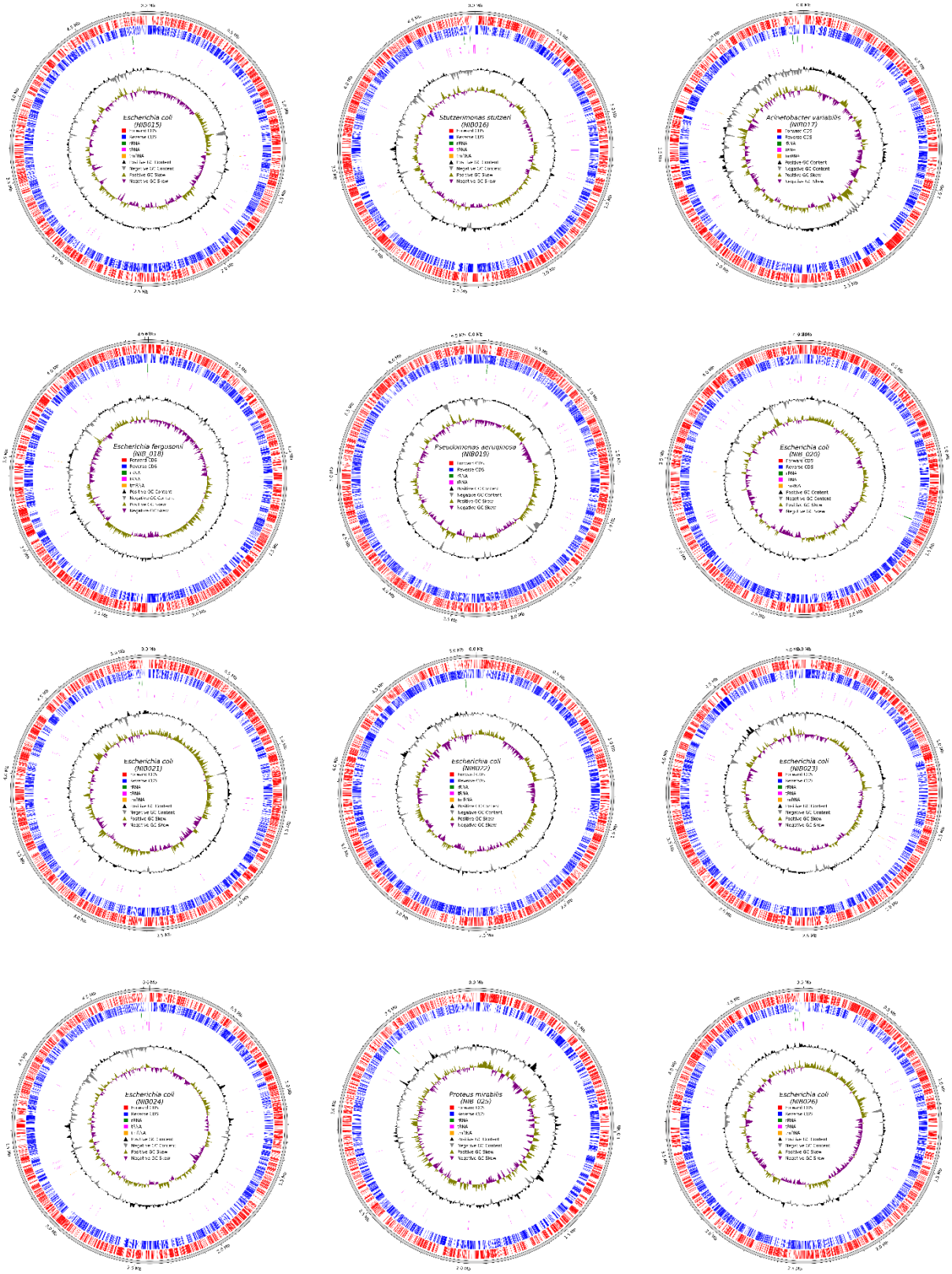
Report

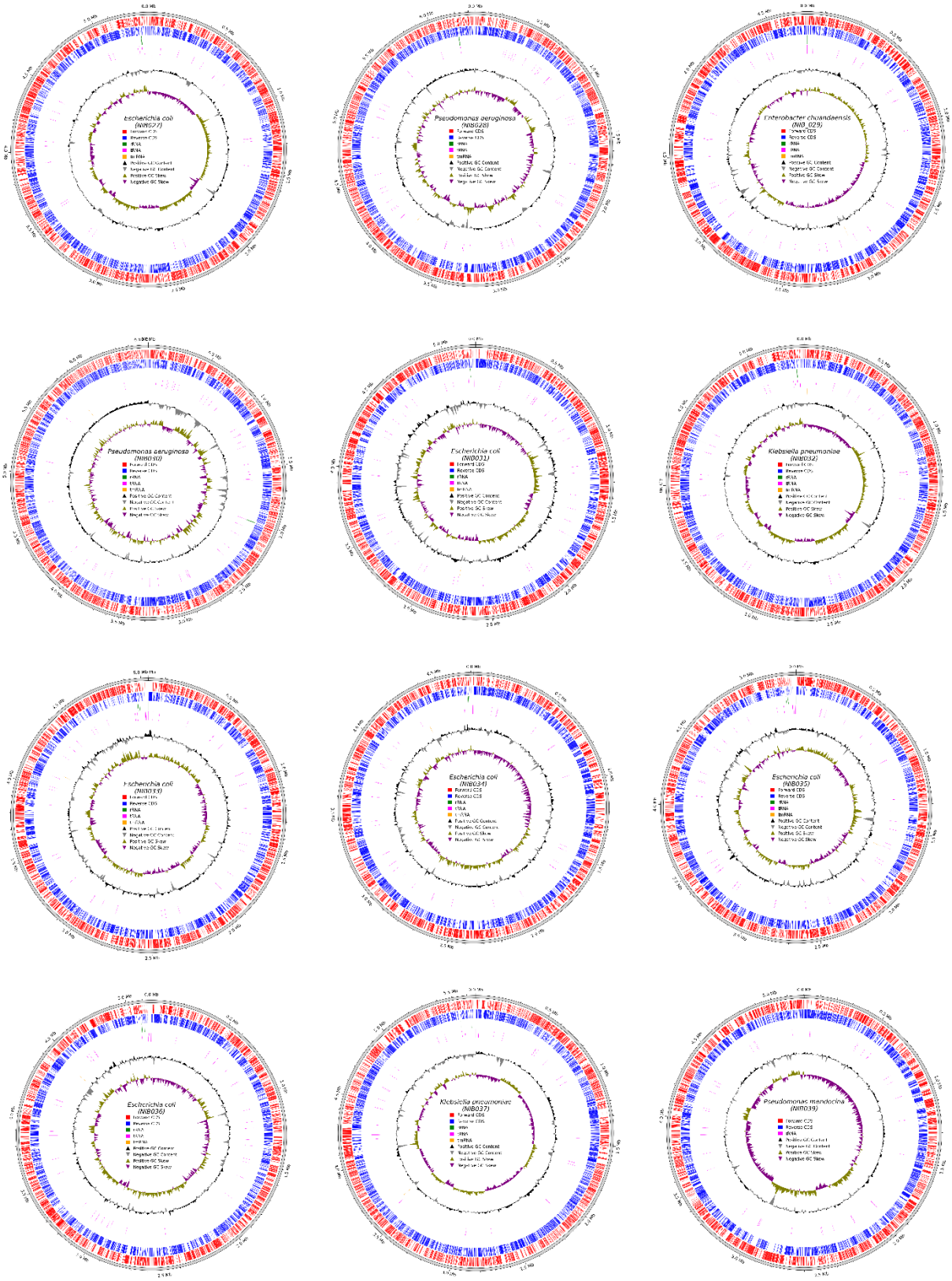
	assembly
# contigs (>= 0 bp)	147
# contigs (>= 1000 bp)	63
# contigs (>= 5000 bp)	28
# contigs (>= 10000 bp)	26
# contigs (>= 25000 bp)	23
# contigs (>= 50000 bp)	16
Total length (>= 0 bp)	4362054
Total length (>= 1000 bp)	4331035
Total length (>= 5000 bp)	4254275
Total length (>= 10000 bp)	4242708
Total length (>= 25000 bp)	4198215
Total length (>= 50000 bp)	3969023
# contigs	90
Largest contig	791279
Total length	4348919
GC (%)	41.31
N50	301266
N90	59891
auN	364108.9
L50	5
L90	16
# N's per 100 kbp	0.00



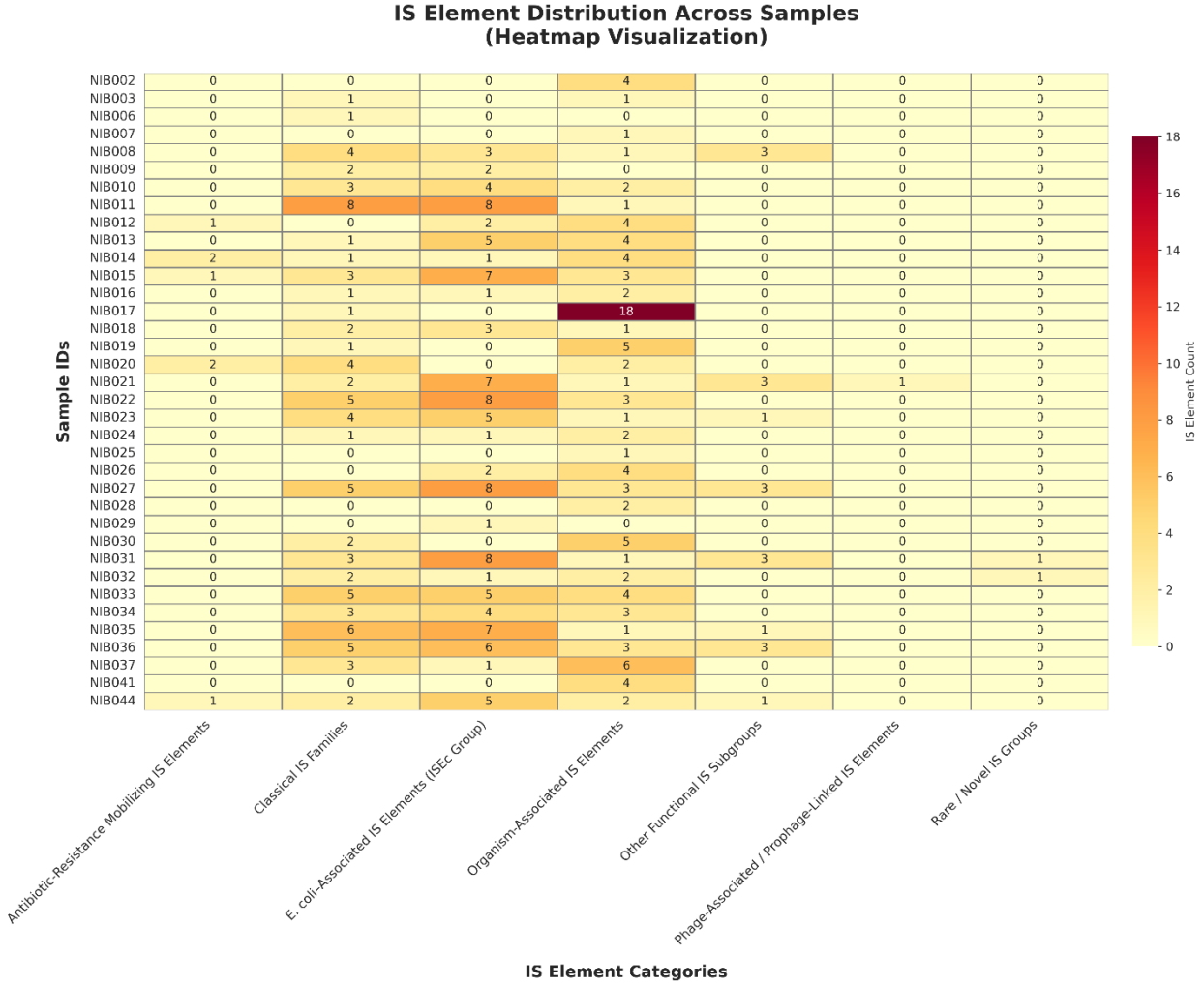
F13-F50: Circular genome maps were generated for all 38.




Escherichia coli (NIB015)
Stutzerimonas stutzeri (NIB016)
Achromobacter xylosoxidans (NIB017)
Escherichia fergusonii (NIB_018)
Pseudomonas aeruginosa (NIB019)
Escherichia coli (NIB_020)
Escherichia coli (NIB021)
Escherichia coli (NIB022)
Escherichia coli (NIB023)
Escherichia coli (NIB024)
Proteus mirabilis (NIB_025)
Escherichia coli (NIB026)



F51: Distribution of Insertion Sequence (IS) Element Categories Across Bacterial Samples.



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