

Molecular Characterization and Antimicrobial Resistance of Uropathogenic Bacteria Among Elderly Residents of Nursing Care Homes in Accra, Ghana

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Abstract

Urinary tract infections (UTIs) pose a major health concern among the elderly in institutional care settings. We sampled urine samples from 118 elderly residents in 5 nursing homes in Accra and diagnosed UTI in 34 of them via dipstick and microbial culture. The pathogens causing the UTI were identified via MALDI-TOF identification and 16S rRNA sequencing. The Kirby-Bauer disc diffusion method was used to determine the antibiogram of these pathogens, and the carriage of ESBL genes and virulence genes was detected via PCR. Four gram-negative pathogens: *E. coli* (38.2%), *K. pneumoniae* (20.6%), *P. mirabilis* (20.6%), and *E. cloacae* (11.8%); and two gram-positive bacteria: *A. junii* (5.9%) and *B. cereus* (2.9%) were identified. Virulence genes of adhesion were also detected amongst the *E. coli* isolates, which include Pap E (18.5%), Sfas (40.8%), Sfa (25.9%), Afa (11.1%), and allele II (3.7%). 38.4% and 30.8% of the *E. coli* isolates belong to phylogenetic groups B2 and D, respectively, while 15.4% of them were found to belong to each of phylogenetic groups A and B1. High resistances were exhibited by *E. coli* to cefepime (84.6%) and azithromycin (76.9%), while *K. pneumoniae* showed complete resistance to imipenem. A total of 189 ESBL genes were detected, most predominant being *bla*TEM and *bla*CTX-M-825.

Introduction

Urinary tract infection is one of the commonest diseases diagnosed amongst the elderly and affects approximately 150 million people annually, leading to an associated loss of \$659 million and \$936 million in direct and indirect costs, respectively (1). The most significant etiologic factor in both community-acquired and nosocomial UTIs is *Escherichia coli*, which is responsible for 50–80 percent of cases. Apart from *E. coli*, other uropathogenic bacterial samples amongst humans include *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis*, and *Acinetobacter junii*, amongst others (2). Amongst the elderly dwelling in long-term care facilities, UTIs are the leading cause of infections and the second most common infection amongst those in community settings (1, 3). There has been a rampant increment in the emergence of resistant uropathogens to antimicrobial agents among the elderly, in both hospitals and community settings. This is attributable to the application of antimicrobial therapy in the management of UTI (4). The burden of antimicrobial resistance is more significant in the developing world due to limited treatment options. Microbiological conventional methods, such as biochemical and serological testing, culture media, antibiotic sensitivity, and microscopy, have been applied to classify pathogens over the years. In recent times, however, bacterial identification has been revolutionized by the application of molecular methods; notable amongst these methods is the 16S (rRNA) gene sequencing. In addition to accuracy and a faster approach to bacterial identification, this method also allows for discrimination between bacteria that are phenotypically identical (5). The ease of amplification and high conserved nature of the 16S rRNA gene make it possible to be routinely employed in the study of phylogenetic diversity of different bacterial genomes and comparative analysis (6). It has thus become the gold standard molecular tool for identification, differentiation, and comparison of bacterial genomes and establishing the genetic relationship between them (7). In both hospital and community settings, there has been an increment in extended-spectrum beta-lactamase (ESBL)-

producing bacterial infections (8). These strains extend their resistance beyond most beta-lactams, and they result in an increase in morbidity and mortality. Their occurrence is fueled by the indiscriminate use of antimicrobial agents, which enhances the selection of multidrug-resistant bacteria (9, 10). The impact of ESBL in bloodstream infections, including delays in antibiotic therapy, length of stay, and cost, is well characterized, but that of UTI remains unclear. To enhance the best procedure for UTI diagnosis, therefore, further studies are required (11). The ability of Uropathogenic *E. coli* (UPEC) to colonize the urinary tract and cause diseases is mainly determined by a range of virulence determinants (12). Chief amongst these determinants are adhesin-encoding genes, which include *papA*, *papC*, *Sfas*, *Sfa*, *Afa*, and their allelic variants of Allele II and Allele III. They can colonize host defense systems and lead to multi-drug-resistant strains (13, 14). The study was carried out to characterize bacterial pathogens causing urinary tract infections amongst the aged population of care homes in Accra.

Materials and Methods

Study design and Sample Collection

The study was conducted between December 2022 and December 2024 amongst 118 elderly persons who are residents of five nursing care homes in Accra, Ghana. Prior to sample collection, all recruited participants were anonymized with random code identifiers. Individuals were excluded from the study if they did not fall within the age group of 55 to 100 years. Early morning mid-stream urine samples were collected aseptically from the participants into sterile calibrated urine collection tubes. Participant demographics and other clinical data were also collected. The urine specimen was preserved at low temperatures (2–8°C) in an ice chest with ice packs and transported immediately to the microbiology laboratory of the Biomedical and Public Health Research Unit.

Diagnosis of Urinary Tract Infections

The collected urine samples were analyzed for the diagnosis of urinary tract infection based on instructions for processing URIT 10V strips. The examination was done within 2 hours of collection of the samples. For diagnosing infections, a positive dipstick was a test result with a $\geq 70/\mu\text{L}$ leukocyte esterase and/or nitrite positivity and/or a reflex microscopy requiring ≥ 10 WBC/HPF for the diagnosis of a UTI (15, 16).

Isolation of UTI-positive Bacteria

The urine samples that tested positive for UTI were cultured on cysteine–lactose–electrolyte-deficient (CLED) agar for primary bacteria isolation. For secondary isolation, the isolates were inoculated on MacConkey and Blood Agar plates (Oxoid Limited, Hampshire, United Kingdom) and incubated for bacterial growth. Bacterial colonies that recorded a colony-forming unit of less than 10^5 were classified as “insignificant growth,” while those that recorded a CFU of equal to or greater than 10^5 were classified

as “significant growth” and were a confirmation of urinary tract infection (17). When there was no growth at all, the isolate was described as “no growth.” Any observed mixed growth that contained more than one colony type was excluded from the study. Biochemical tests were performed to confirm the identity of these bacterial pathogens; these included the indole, methyl red, Voges-Proskauer, and citrate tests. The identity of the pathogens was also identified using the MALDI-TOF equipment (18).

DNA extraction

4 to 5 cultured pure colonies were picked using a sterile inoculation loop and mixed in 200 µL of genomic lysis buffer. The DNAs were then extracted using the Zymogen extraction kit, as per the protocols of the manufacturer, with some modifications

16SrRNA gene amplification of bacterial isolates

The DNAs of all the isolated bacterial isolates were screened for the presence of the 16S rRNA gene for molecular confirmation. The PCR products were sequenced. The protocol for the PCR was as follows: PCR amplification was carried out in a 25 µL reaction. Each reaction included 5x Q5 reaction buffer of 5 µl, 2x high-fidelity Taq polymerase master mix of 0.25 µl (New England Biolabs, USA), 1.25 µl of 10 µM of each primer, 0.5 µl of 10 mM dNTP mix, 12.75 µl of nuclease-free water, and 4.0 µl of the template DNA. Conditions of the reaction mixtures include 3 minutes at 94°C initial denaturation followed by 30 cycles of 94°C denaturation for 30s, annealing at the various annealing temperatures for 30s, and elongation at 68°C, then a final elongation at 68°C for 5 minutes. The primer sequences and their corresponding gene sizes, as well as annealing temperature, are stated in Table 1. The PCR products were then sequenced by the Sanger method of sequencing with the BigDye Terminator v3.1 sequencing kit.

Table 1
16S rRNA Primers for confirmation and sequencing of bacterial samples

Pathogen	5'-3' sequence	Size (bp)	Tm(°C)	Reference
Gram-negative	CCCCCTGGACGAAGACTGAC ACCGCTGGCAACAAAGGATA.	401	56.7	Awad et al., 2020
Gram-positive	CAGGCCTAACACATGCAAGTA GGGCGGWGTGTACAAGGC	1300	50	Suwanto et al., 2000

Antimicrobial Susceptibility Testing

All identified bacterial isolates were subjected to the Kirby-Bauer disc-diffusion method of antimicrobial susceptibility testing (AST) based on the 2023 CLSI guidelines (19). The results were interpreted using the same guidelines (20).Briefly, a bacterial suspension was prepared, and its turbidity was adjusted to the 0.5 McFarland standard for comparison. The following antibiotics (Oxoid Ltd., Basingstoke, Hants,

UK) were then tested: imipenem (IPM, 10 µg), ertapenem (ERT, 10 µg), aztreonam (AZM, 15 µg), cefepime (FEP, 30 µg), nitrofurantoin (F, 50 µg), cefuroxime (CXM, 10 µg), gentamicin (CN, 10 µg), amikacin (AK, 30 µg), ciprofloxacin (CIP, 5 µg), and levofloxacin (LEV, 10 µg). Isolates that are resistant to at least three categories of drugs were considered multidrug resistant (MDR) (21). As a quality control, all antimicrobial agents were tested against *E. coli* ATCC 25,922 for Gram-negative bacteria and *Staphylococcus aureus* ATCC 29,123 for Gram-positive bacteria.

Multiplex PCR for Molecular characterization of *E. coli* isolates

We developed a multiplex PCR to screen for the presence of five virulence genes amongst our *E. coli* isolates. These are PapE, Afa, Sfas, Sfa, and Allele II. We also determined the phylogenetic groups amongst the same isolates based on the triplex PCR method as described by Clemont et al., 2000(22). The method grouped the isolates into four phylogenetic groups (A, B1, B2, and D), based on the presence and absence of genes (*chuA*, *yjaA*, and *TspE4.C2*). Isolates assigned to phylogenetic group A harbor none of the markers, *while* those classified under group B1, harbor only the *TspE4.C2* marker. Group B2 isolates harbor *the chuA* and *yjaA* markers, whereas phylogenetic Group D isolates would harbor only the *chuA* marker as illustrated by (Armah et al., 2024,(23) (Fig. 1). For each of the multiplex PCRs above, the protocol was carried out in a 25 µL reaction. Each reaction included 5x Q5 reaction buffer of 5 µL, 2x high-fidelity Taq polymerase master mix of 0.25 µL (New England Biolabs, USA), 1.25 µL of 10 µM of each primer, 0.5 µL of 10 mM dNTP mix, 12.75 µL of nuclease-free water, and 4.0 µL of the template DNA. The PCR cycling conditions included an initial denaturation of 94°C for 3 minutes, 30 cycles of denaturation for 45s at 94°C, 45s at the various annealing temperatures, 1 min of extension at 68°C, and a final extension step of 5 min at 68°C. The primer sequences, amplicon sizes, and their respective annealing temperatures are listed in Table 2

Table 2

Primer sequence, amplicon sizes, annealing temperatures of Virulence profiles, and phylogenetic grouping of *E. coli* isolates

Virulence genes	Primers 5'-3' sequence	Size (bp)	Tm(°C)
	PapE F-GCAACAGCAACGCTGGTTGCATCAT	326	63.0
	R-AGAGAGAGCCACTCTTATACGGACA	559	
	Afa F-GGCAGAGGGCCGGCAACAGGC	240	
	R-CCCGTAACGCGCCAGCATCTC	407	
	Sfas F- GTGGATACGACGATTACTGTG	190	
	R-CCGCCAGCATTCCCTGTATTC		
	Sfa F-CTCCGGAGAACTGGGTGCATCTTAC		
	R-CGGAGGAGTAATTACAAACCTGGCA		
	Allele II F-GGGATGAGCGGGCCTTTGAT		
	R-CGGGCCCCCAAGTAACTCG		
Phylogenetic grouping	TspE4.C2 F-GAGTAATGTCGGGGCATTCA	840	52.9
	R-CGCGCCAACAAAGTATTACG	854	
	chuA F-GACGAACCAACGGTCAGGAT	593	
	R-TGCCGCCAGTACCAAAGACA		
	yjaA F-TGAAGTGTGAGGAGACGCTG		
	R-ATGGAGAATGCGTTCCTCAAC		

Detection of ESBL genes amongst bacterial isolates

We screened all bacterial samples for the presence of 10 ESBL genes as described by Kiiru et al., 2012 (24). PCR amplification was carried out in a 25 µL reaction protocol. Each reaction included 5x Q5 reaction buffer of 5 µL, 2x high-fidelity Taq polymerase master mix of 0.25 µL (New England Biolabs, USA), 1.25 µL of 10 µM of each primer, 0.5 µL of 10 mM dNTP mix, 12.75 µL of nuclease-free water, and 4.0 µL of the template DNA. The PCR cycling conditions included an initial denaturation of 94°C for 3 minutes, 30 cycles of denaturation for 45 s at 94°C, 45 s at the various annealing temperatures, 1 min of extension at 68°C, and a final extension step of 5 min at 68°C. The primer sequences, amplicon sizes, and their respective annealing temperatures are listed in Table 3.

Table 3
Primer sequence, amplicon sizes, and annealing temperatures of ESBL genes

Target Gene	5'–3' Sequence	Size (bp)	Ann. Temp (°C)
<i>bla</i> _{TEM}	ATGAGTATTCAACAT TTC CG CCAATGCTTAATCAGTGAGG	840	55.1
<i>bla</i> _{SHV}	TTCGCCTGTGTATTATCTCCCTG TTAGCGTTGCCAGTGYTCG	854	51.2
<i>bla</i> _{MA}	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAAYCAGCGG	593	51.2
<i>bla</i> _{CTX-M 825}	CGC TTT GCC ATG TGC AGC ACC GCTCAGTACGATCGAGCC	307	57.7
<i>bla</i> _{CTX-M 914}	GCT CAG TAC GAT CGA GCC GTAAGCTGACGCAACGTCTG	474	56.3
<i>bla</i> _{CMY-1}	GTGGTGGATGCCAGCATCC GGTCGAGCCGGTCTTGTTGAA	915	56.1
<i>bla</i> _{CMY-2}	GCACTTAGCCACCTATACGGCAG GCTTTTCAAGAATGCGCCAGG	758	52.5
<i>bla</i> _{OXA-1}	ATGAAAAACACAATACATATCAACTTCGC GTGTGTTTAGAATGGTGATCGCATT	820	51.2
<i>bla</i> _{OXA-2}	ACGATAGTTGTGGCAGACGAAC ATYCTGTTTGGCGTATCRATATTC	602	56.1
<i>CF</i>	ATGATGAAAAAATCGTTATGC TTGCAGCTTTTCAAGAATGCGC	1200	56.1

Molecular analysis

Alignment of the nucleotide sequences was performed using ClustalW in MEGA XI software (25). The aligned sequences were subsequently used to construct the phylogenetic tree. Phylogeny was inferred using the maximum likelihood method and the Tamura-Nei model of nucleotide substitutions (26). The

trees with the highest log likelihood (-4,673.35) are shown. The percentage of replicate trees in which the associated taxa clustered together (based on 500 replicates) is indicated next to the branches.

Statistical Analysis

All collected data were cleaned and stored in Microsoft Excel and imported into STATA 14 (Strata Corp., College Station, TX, USA) and SPSS for analysis. The data included ESBL and virulence gene profiles and phylogenetic and antibiogram status, as well as demographics. Carriage and distribution of the various genes were checked for significance (P-value) and calculated with the chi-square test of independence.

Ethical Considerations

Before the commencement of the study, approval of ethics was obtained from the Council for Scientific and Industrial Research—Institutional Review Board (CSIR-IRB), Ghana (Protocol ID: RPN 011/CSIR-IRB/2022). Oral and written informed consent were obtained from the study participants, their family members, and managers of the various homes

Results

Prevalence of Urinary Tract infection

Out of a total of 118 urine samples, 28.8%(n = 34) tested positive for urinary tract infection. Amongst these, 58.5% (20) were women, while 41.2% (14) were men. Amongst the positive UTI urine samples, six different pathogens were identified; 4 are gram-positive, and the remaining 2 are gram-negative. 38.2% (13) of the urinary tract-infected samples were *Escherichia coli*; 20.6% (7) were recorded for each of *Klebsiella pneumoniae* and *Proteus mirabilis*; 11.8% (4) were positive for *Enterobacter cloacae*; and the remaining three were Gram-positive bacteria: 5.9% (2) were *Acinetobacter junii*, and the remaining 2.9% (1) was *Bacillus cereus*. Table 4

Table 4
Prevalence of UTI-positive bacteria

Gram-negative	Bacterial pathogen	Frequency(n = 34)	Percentage (%)
	<i>Escherichia coli</i>	13	38.2
	<i>Klebsiella pneumoniae</i>	7	20.6
	<i>Proteus mirabilis</i>	7	20.6
	<i>Enterobacter cloacae</i>	4	11.8
Gram-positive	<i>Acinetobacter Junii</i>	2	5.9
	<i>Bacillus cereus</i>	1	2.9

16Sr RNA sequence analysis of bacterial uropathogens

The 34 different bacterial species were further evaluated for 16S rRNA sequencing via BigDye sequencing. The sequences were then submitted to NCBI, and accession numbers were assigned to each sequence. The sequences were aligned, and maximum likelihood phylogenetic trees were constructed with each set of sequences. For each bacterial pathogen, similar sequences (red) that showed sequence identities between 90 and 99% were downloaded and used to construct the phylogenetic tree with our sequences (black). For each of the Gram-negative bacteria, *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*, the outgroup used was *Bacillus subtilis* (LT4437.1). For *B. cereus* bacterial sequences, the outgroup was *S. aureus* (D12572.1), while *N. subflava* (NZ_MAYX02000004) was used as an outgroup for *A. junii* sequences.

In the *E. coli* phylogeny tree, eleven of our sequences clustered close with five other *E. coli* sequences from the NCBI database, with *Bacillus subtilis* (LT4437.1) as an outgroup. This indicates close genetic relatedness. The remaining two sequences, on the other hand, formed distant clades, suggesting unique evolutionary divergence from these strains. The majority of the clusters were assigned high bootstrap values, ranging from 80 to 100, an indication of strong clustering confidence, while a few ranged between 39 and 50. (Fig. 2)

The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). In the figure, colour red = sequences downloaded from the NCBI database, colour black = our sequences, and colour blue = outgroup (*Bacillus subtilis* L14437.1). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018). In the *K. pneumoniae* phylogeny, the tree produced two distinct major clades with five other *K. pneumoniae* sequences from the NCBI database, with *Bacillus subtilis* (LT4437.1) as an outgroup. The reference sequences formed a close upper cluster with moderate to strong bootstrap values, between 53 and 99, and relatively shorter branch lengths. This is an indication that they are very genetically identical. On the other hand, our sequence showed distinct sub-clades and several multiple internal branching points with longer branch lengths and more branching nodes. They also showed low to high bootstrap values (36–88) (Fig. 3). Thus, although our sequences are genetically related to the reference strains, they show local diversification.

The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). In the figure, colour red = sequences downloaded from the NCBI database, colour black = our sequences, and colour blue = outgroup (*Bacillus subtilis* L14437.1). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

In the *E. cloacae* phylogeny, we aligned our sequences with five other *E. cloacae* sequences from the NCBI database. The tree was rooted using *Bacillus subtilis* (LT4427.1) as the outgroup. Our sequences clustered together in several sub-branches with relatively average bootstrap values, ranging from 16 to 74. This indicates the existence of internal diversity. Although the reference sequences were found on separate clades, they were closely related to our sequences. Their clustering gave high bootstrap values (45–92) (Fig. 4)

The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). In the figure, colour red = sequences downloaded from the NCBI database, colour black = our sequences, and colour blue = outgroup (*Bacillus subtilis* L14437.1). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

With the *P. mirabilis* sequence analysis, we aligned our sequences with four other sequences from the NCBI database, with *Bacillus subtilis* (LT4437.1) serving as an outgroup. There were overall very high bootstrap values (90–100), indicating high clustering confidence. Our sequences clustered together, forming a distinct clade from the reference sequences. On the other hand, the reference strains clustered more tightly (Fig. 5). This separation indicates that our sequences are genetically distinct from the reference strains

The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). In the figure, colour red = sequences downloaded from the NCBI database, colour black = our sequences, and colour blue = outgroup (*Bacillus subtilis* L14437.1). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018). Our only *B. cereus* sequence was aligned with three sequences of *B. cereus* from the NCBI database. Our sequence clustered closely and formed a clade with one of the reference sequences. The other two reference sequences formed a clade on their own and separated from the other clades. The bootstrap values are very high (99 and 100%), and this indicates the highest level of confidence in the constructed tree rooted firmly by the outgroup *S. aureus* (D12572.1), which served as the outgroup (Fig. 6).

The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). In the figure, colour red = sequences downloaded from the NCBI database, colour black = our sequences, and colour blue = outgroup (*Staphylococcus aureus* D12572.1). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

In the *A. junii* phylogenetic tree, there was a clear evolutionary distinction between our sequences and four sequences from the NCBI database. Our sequences clustered together on a single clade, while the reference sequences also clustered on a clade with *Neisseria subflava* as an outgroup (MAYX02000004). The observed bootstrap values were fairly high (65–91%), rendering high confidence in the phylogeny. (Fig. 7)

The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). In the figure, colour red = sequences downloaded from the NCBI database, colour black = our sequences, and colour blue = outgroup (*N. subflava* NZ_MAYX02000004). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018)

5.5.3 Virulence genes distribution amongst bacterial

A total of 27 virulence genes were detected amongst 11 out of the 13 *E. coli* isolates. The highest count was the Sfas gene, which was 11 (40.8%), and the lowest was Allele II with 1 count (3.7%). Sfa, PapE, and

Afa were 7 (25.9%), 5 (18.5%), and 3 (11.1%), respectively. (Fig. 8). One sample contains 5 virulence genes, while 3 samples harbor 3 virulence genes each. Six samples harbor two genes each, while one sample contains a single gene. Two samples harbor no virulence gene at all.

Sfas (240 bp), Sfas (407 bp), PapE (306 bp), Afa (559 bp), and AlleleII (190 bp). M is a 100 bp marker, and NC is a negative control. The Sfas gene, which was 11 (40.8%), and the least was Allele II with 1 count (3.7%). Sfa, PapE, and Afa were 7 (25.9%), 5 (18.5%), and 3 (11.1%), respectively (Table 5)

Table 5
Prevalence of virulence genes amongst *E. coli*

genes	Prevalence n = 27(%)
Virulence Pap E	5(18.5)
genes Sfas	11(40.8)
Sfa	7(25.9)
Afa	3(11.1)
Allele II	1(3.7)
Phylogenetic A	2(15.4)
grouping B1	2(15.4)
B2	5(38.4)
D	4(30.8)

Antibiogram of bacterial pathogens

The antimicrobial resistance profile in bacterial isolates showed variability across different species and antibiotics. Among the 13 *Escherichia coli* isolates, the highest resistance was against cefepime (84.6%), followed by azithromycin (76.9%) and both cefuroxime and nitrofurantoin (69.2%). Moderate resistance was seen with ciprofloxacin (61.5%), imipenem, and gentamicin (53.8%). Lower resistance was observed for levofloxacin (38.5%) and amikacin (46.2%). *Klebsiella pneumoniae* isolates (n = 7) were fully resistant to imipenem (100%) and also demonstrated high resistance to ertapenem and cefepime (85.7%). Resistance to aminoglycosides was high, which is 85.7% for amikacin and 71.4% for gentamicin. Ciprofloxacin, cefuroxime, and nitrofurantoin resistance were recorded to be 42.9%. *Proteus mirabilis* (n = 7) showed consistent high resistance to antibiotics like cefepime, levofloxacin, and cefuroxime (each 71.4%), with high resistance to imipenem and ertapenem (71.4%) and lower resistance to ciprofloxacin (42.9%). *Enterobacter cloacae* (n = 4) exhibited complete resistance to azithromycin (100%) and high resistance to imipenem, cefuroxime, and ciprofloxacin (75%). Resistance to cefepime and amikacin was 25%, with no resistance to levofloxacin. *Acinetobacter junii* (n = 2) showed total resistance to several antibiotics, including ertapenem, azithromycin, cefepime, and nitrofurantoin (100%), while resistance to imipenem, cefuroxime, levofloxacin, and gentamicin was 50%. No resistance was found against

ciprofloxacin or amikacin. The single *Bacillus cereus* isolate (n = 1) was resistant to most tested antibiotics, such as ertapenem, azithromycin, cefepime, nitrofurantoin, levofloxacin, cefuroxime, and gentamicin, but remained susceptible to imipenem, ciprofloxacin, and amikacin (Table 6)

Table 6
Antibiogram of bacterial pathogens against 10 antibiotics

ERT (10 ug)	<i>E. coli</i> (n = 13)	<i>K. pneum.</i> (n = 7)	<i>P.mirabilis</i> (n = 7)	<i>E. cloacae</i> (n = 4)	<i>A. junii</i> (n = 2)	<i>B. cereus</i> (n = 1)
	6(46.2%)	6(85.7%)	5(71.4%)	2(50%)	2(100%)	1(100%)
IPM (10 µg)	7(53.8%)	7(100%)	5(71.4%)	3(75%)	1(50%)	0(0%)
AZM (15 µg)	10(76.9%)	4(57.1%)	4(57.1%)	4(100%)	2(100%)	1(100%)
FEP (30ug)	11(84.6%)	6(85.7%)	5(71.4%)	1(25%)	2(100%)	1(100%)
F (50ug)	9(69.2%)	3(42.9%)	4(57.1%)	2(50%)	2(100%)	1(100%)
LEV (10 µg)	5(38.5%)	5(71.4%)	5(71.4%)	0(0%)	1(50%)	1(100%)
CXM (10 µg)	9(69.2%)	3(42.9%)	5(71.4%)	3(75%)	1(50%)	1(100%)
CN (10 µg)	7(53.8%)	5(71.4%)	4(57.1%)	2(50%)	1(50%)	1(100%)
CIP (5 ug)	8(61.5%)	3(42.9%)	3(42.9%)	3(75%)	0(0%)	0(0%)
AK (30ug)	6(46.2%)	6(85.7%)	6(85.7%)	1(25%)	0(0%)	0(0%)

Carriage of ESBL amongst bacterial pathogens

The distribution of the 10 different ESBL genes across the six bacterial pathogens was observed. A total of 189 ESBL genes were detected. High ESBL prevalence was detected amongst *E. coli* (29), *K. pneumoniae* (24), *E. cloacae* (16), and *P. mirabilis* (14). The remaining two bacterial pathogens recorded low ESBL prevalence: *A. junii* (4) and *B. cereus* (2). A similar trend was observed for the divergence of ESBL genes amongst these pathogens. The most diverse pathogen was *E. coli*; it contained all the 10 different ESBL genes. Both *K. pneumoniae* and *P. mirabilis* contain all ESBL genes, except bla_{MA}. *cloacae* also contain all ESBL genes, except CF. *A. junii* contains bla_{TEM}, bla_{SHV}, bla_{CMY-2}, and OXA-2, while *B. cereus* contains only bla_{TEM} and bla_{SHV}. With regard to the specific ESBL genotype, the most prevalent genes were bla_{TEM} and bla_{CTXM-825}; 16 of each of them were detected. This was followed closely by bla_{SHV} and bla_{CMY-1}, recording a total of 15 and 13, respectively. 6 and 7 bla_{CMY-1} and bla_{CMY-2} were detected, respectively, while 5 each of bla_{OXA-1} and bla_{OXA-2} were observed, and the least represented was CF (3) (Fig. 9).

Discussion

One of the significant healthcare challenges amongst elderly residents of institutional settings, particularly in home care, is urinary tract infections. Age-related immunological and physiological changes are largely associated with this occurrence. As one ages, factors such as reduced immune competence, frequent comorbidities, and particularly incomplete bladder emptying become apparent and increase the chances of acquiring a UTI (27, 28). The present study recorded a UTI prevalence of 28.8% amongst elderly populations in five home cares in the Greater Accra region of Ghana. This falls within the average and is consistent with similar studies, in which prevalence ranged from 20% to 50% (29, 30). The most predominant pathogen, which accounted for 38.2% of the isolates, was *Escherichia coli*. While this is in line with several previous research studies (31), the observed prevalence is lower than the commonly observed 50–80%. This may be attributable to several occurrences that influence pathogen distribution, including differences in antimicrobial usage, geographical variation, and institutional practices (32). Our results also indicated that non-*E. coli* Gram-negative organisms also contribute significantly to the occurrence of UTIs in the elderly. Both *Klebsiella pneumoniae* and *Proteus mirabilis* each accounted for **20.6%** of infections, while *Enterobacter cloacae* accounted for 11.8%. We also observed that gram-positive organisms also contributed to the occurrence of UTIs, although mildly. *Acinetobacter junii* and *Bacillus cereus* were responsible for 5.9% and 2.9% of the infections, respectively. This broadens the scope of profiles of pathogen-causing UTIs and may as well indicate an ascendancy in antimicrobial pressure in the study settings. (33). The study used 16S rRNA gene sequencing as a tool to identify the bacterial pathogens and also to access the genetic variability that exists amongst the bacterial isolates, as compared to similar sequences from the NCBI database. The variability was assessed through genetic alignment and subsequent construction of phylogenetic trees to reveal the genetic relatedness. Clustering of our *E. coli* isolates with the reference strains revealed close genetic relatedness, with the majority of the clusters attaining bootstrap values ranging from 80 to 100. The few divergent clusters observed amongst the *E. coli* phylogeny may represent the presence of local evolution or strains that are not well associated with global sequence databases. Isolates of *Proteus mirabilis* and *B. cereus* demonstrated even a closer clustering, showing very high bootstrap values: 90–100% for *Proteus mirabilis* and 99–100% for *B. cereus*. This is indicative of the existence of relatively highly conserved and unique lineages within the population. It also reveals the expected high degree of genetic conservation of these pathogens as reported previously (34). On the other hand, isolates of *K. pneumoniae* demonstrated a more complex phylogeny, presenting heterogenous clustering patterns and longer branch lengths. This affirms the existence of intra-species diversity as well as adaptation to environmental settings (35). Isolates of *A. juni* and *E. cloacae* also demonstrated fair genetic divergence, although small numbers of isolates were used. The *E. coli* isolates were screened for the presence of various virulence-associated genes as well as phylogenetic genes to determine the various phylogenetic groups. The most predominant virulence factors are S fimbrial adhesins, *sfa* and *sfas*, which recorded 40.8% and 25.9% detection rates, respectively. These factors are key to enhancing the attachment of the bacteria to uroepithelial cells and thereby establishing the pathogenesis of UTI (36). Other adhesin-related genes observed included PapE (p fimbrial adhesins, 18.5%), Afa (A fimbrial adhesins, 11.1%), and Allele II (fimbrial adhesins, 3.7%). The variation on virulence gene profiles is indicative of varying pathogenic capacity amongst these strains. While the majority of the *E. coli* samples harbored various

virulence genes at different rates, 2 of them harbored no gene at all, although they are pathogenic. This implies that host susceptibility, as well as microbial factors, also contributes to disease conditions. In an immunocompromised population like the elderly, isolates who lack virulence genes may still cause disease (30). The majority of the *E. coli* isolates belong to phylogroups B2 (38.4%) and D (30.8%). These groups are commonly associated with extraintestinal pathogenic *E. coli* (ExPEC) strains and are famous for harboring virulence genes that enhance their role in the pathogenesis of UTIs (22). A few isolates were observed to belong to A and B1, 15.4% each. These two groups are normally associated with commensal *E. coli* strains; their occurrence in pathogenic *E. coli*, therefore, suggests that comorbidities and other host-related factors play key roles in the development of diseases, especially among vulnerable populations. This study observed a worrying pattern of resistance to selected antimicrobials, especially for an immunocompromised population like the elderly. There were general high resistances of bacterial pathogens across several antibiotic categories, including carbapenems, fluoroquinolones, and beta-lactams. Of particular concern is the observed resistance to imipenem, a last-resort carbapenem for treating severe infections. The high resistance of this antibiotic, particularly amongst *K. pneumoniae*, *E. coli*, and *P. mirabilis*, is alarming and indicative of the emergence of highly resistant strains among this population, as reported by (37). *E. coli* and *K. pneumoniae* also exhibited widespread resistance to cefepime and other cephalosporins. This observed pattern suggests that the effectiveness of commonly used empirical therapies is decreasing and also lends credence to the current global trend of the rise of resistance to beta-lactam antibiotics (38). The efficacy of oral treatment is further limited by the observed resistance to fluoroquinolones, including ciprofloxacin. In addition to age-related physiological changes, the observed resistance pattern could also be driven by inappropriate antibiotic use and horizontal transfer of resistance genes within the environment. This observed pattern was further elucidated by the occurrence of ESBL genes detected in the bacterial samples. *E. coli* and *K. pneumoniae* isolates harbored high rates of blaCTX-M-825 and blaCTX-M-914, which are variants of blaCTX-M, a well-known beta-lactamase mostly associated with plasmid-mediated dissemination (39). The spread and persistence of beta-lactam enzymes amongst study participants was also further affirmed by the co-detection of *bla TEM* and *bla SHV*. The study also detected several AmpC-type genes amongst bacterial isolates: blaCMY-1 and blaCMY-2. The observation complicates treatment strategies, as these enzymes are known to resist cephalosporins but not inhibited by conventional beta-lactamase inhibitors (40). *Acinetobacter junii* and *Bacillus cereus*, which is gram-positive, also harbored several resistance genes, although the numbers are insignificant.

Conclusion and recommendation

Our results highlight the critical significance of routine molecular surveillance of uropathogens within home care settings in Accra, Ghana. The observed high prevalence of multidrug-resistant organisms and diverse resistance mechanisms represents an alarming incidence, especially for gram-negative uropathogens. It also provides critical insights into the molecular epidemiology of uropathogens and the genetical variation that exists within microbial populations amongst the study population. This observation calls for an urgent need for more population-specific surveillance data to further inform

evidence-based treatment guidelines and interventions. Antimicrobial stewardship programs and infection prevention and control measures should be implemented to enhance the health outcomes of the elderly populations.

Declarations

Author Contribution

Author contributions Conceptualisation, E.A., L.O.-N., M.Y.O.-A. and N.T.K.D.D.; methodology, E.A., L.O.-N., M.Y.O.-A., Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, F.N.A and N.T.K.D.D.; validation, L.O.-N., M.Y.O.-A., F.C.N.K., Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, F.N.A and N.T.K.D.D.; formal analysis, E.A., L.O.-N., Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, F.N.A and F.C.N.K.; investigation, E.A., B.I., M.K.A., I.A., F.B.K., Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, and F.N.A; resources, E.A., L.O.-N., F.C.N.K. and M.Y.O.-A.; data curation, E.A., B.I., M.K.A., I.A., F.B.K., M.Y.O.-A. and N.T.K.D.D.; writing—original draft preparation, E.A., F.C.N.K., Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, F.N.A and N.T.K.D.D.; writing—review and editing, E.A., L.O.-N., B.I., M.K.A., I.A., F.B.K., F.C.N.K., M.Y.O.-A. Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, F.N.A. and N.T.K.D.D.; visualisation, E.A., L.O.-N., F.C.N.K., M.Y.O.-A., Q.D.Q, N.A.K, J.W, G.A.K, A.K.O.S.E, J.A, O.N.A,R.E, F.N.A and N.T.K.D.D.; supervision, L.O.-N., M.Y.O.-A. and N.T.K.D.D.; project administration, E.A., L.O.-N., M.Y.O.-A. and N.T.K.D.D.;

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Ethics approval and declaration

Ethical approval

for this study was obtained from the Council for Scientific and Industrial Research—Institutional Review Board (CSIR-IRB), Ghana (Protocol ID: RPN 011/CSIR-IRB/2022). Participants provided oral and written informed consent prior to the commencement of the study. Permissions of the study were also sought from the management of the various care homes as well as the family members of the study participants. The research was conducted in compliance to the Helsinki Declaration

Data Availability

The datasets generated and/or analysed during the current study are available in the NCBI repository

Organism	Accession no	Web link
<i>E. coli</i>	PX501814.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501814.1?report=fasta
<i>E. coli</i>	PX501815.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501815.1?report=fasta
<i>E. coli</i>	PX501816.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501816.1?report=fasta
<i>E. coli</i>	PX501817.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501817.1?report=fasta
<i>E. coli</i>	PX501818.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501818.1?report=fasta
<i>E. coli</i>	PX501819.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501819.1?report=fasta
<i>E. coli</i>	PX501820.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501820.1?report=fasta
<i>E. coli</i>	PX501821.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501821.1?report=fasta
<i>E. coli</i>	PX501822.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501822.1?report=fasta
<i>E. coli</i>	PX501823.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501823.1?report=fasta
<i>E. coli</i>	PX501824.1	https://www.ncbi.nlm.nih.gov/nuccore/PX501824.1?report=fasta
<i>E. coli</i>	PX517365.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517365.1?report=fasta
<i>E. coli</i>	PX517366.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517366.1?report=fasta
<i>K. pneumoniae</i>	PX517347.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517347.1?report=fasta
<i>K. pneumoniae</i>	PX517349.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517349.1?report=fasta
<i>K. pneumoniae</i>	PX517350.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517350.1?report=fasta
<i>K. pneumoniae</i>	PX517351.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517351.1?report=fasta
<i>K. pneumoniae</i>	PX517357.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517357.1?report=fasta
<i>K. pneumoniae</i>	PX517362.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517362.1?report=fasta
<i>K. pneumoniae</i>	PX517363.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517363.1?report=fasta
<i>E. cloacae</i>	PX517348.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517348.1?report=fasta
<i>E. cloacae</i>	PX517352.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517352.1?report=fasta
<i>E. cloacae</i>	PX517353.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517353.1?report=fasta
<i>E. cloacae</i>	PX517354.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517354.1?report=fasta
<i>E. cloacae</i>	PX517356.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517356.1?report=fasta
<i>E. cloacae</i>	PX517358.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517358.1?report=fasta
<i>E. cloacae</i>	PX517360.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517360.1?report=fasta
<i>P. mirabilis</i>	PX517355.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517355.1?report=fasta

Organism	Accession no	Web link
<i>P. mirabilis</i>	PX517359.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517359.1?report=fasta
<i>P. mirabilis</i>	PX517361.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517361.1?report=fasta
<i>P. mirabilis</i>	PX517364.1	https://www.ncbi.nlm.nih.gov/nuccore/PX517364.1?report=fasta
<i>A. junii</i>	PX421550.1	https://www.ncbi.nlm.nih.gov/nuccore/PX421550.1?report=fasta
<i>A. junii</i>	PX421553.1	https://www.ncbi.nlm.nih.gov/nuccore/PX421553.1?report=fasta
<i>B. cerus</i>	PX421552.1	https://www.ncbi.nlm.nih.gov/nuccore/PX421552.1?report=fasta

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Figures

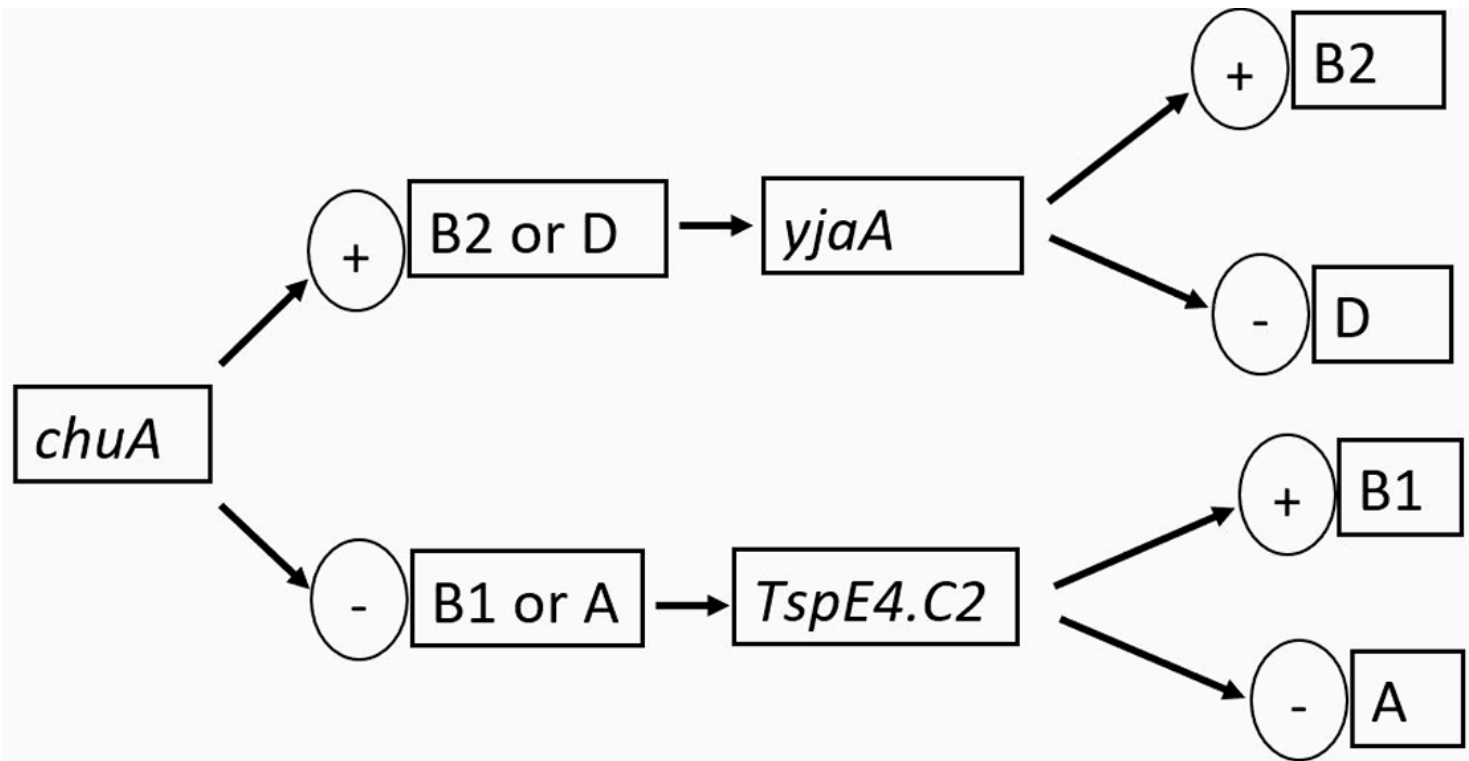


Figure 1

Dichotomous tree of triplex phylogenetic grouping. (Armah et al., 2024)

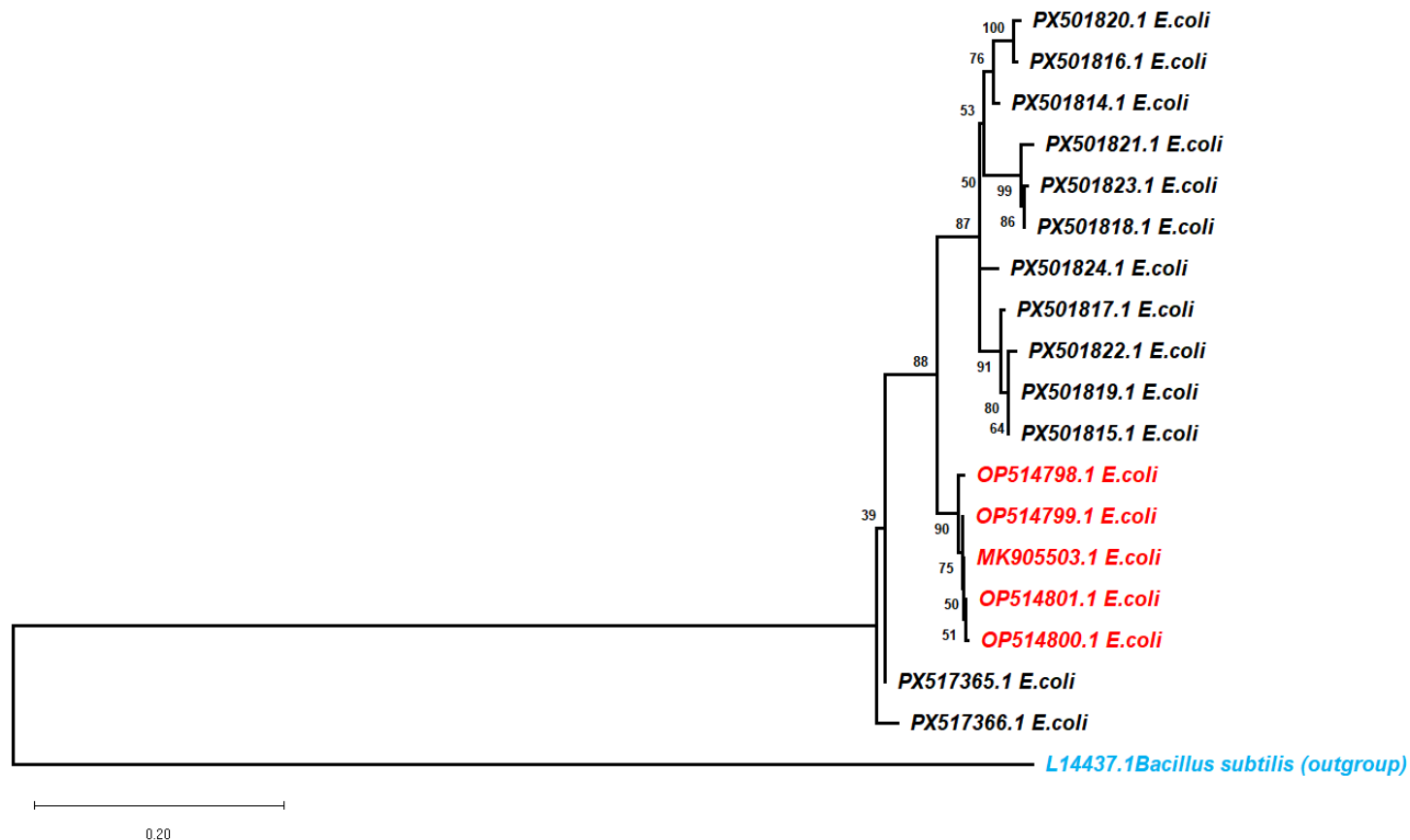


Figure 2

Phylogenetic tree from 16S rRNA gene sequences of *E. coli*.

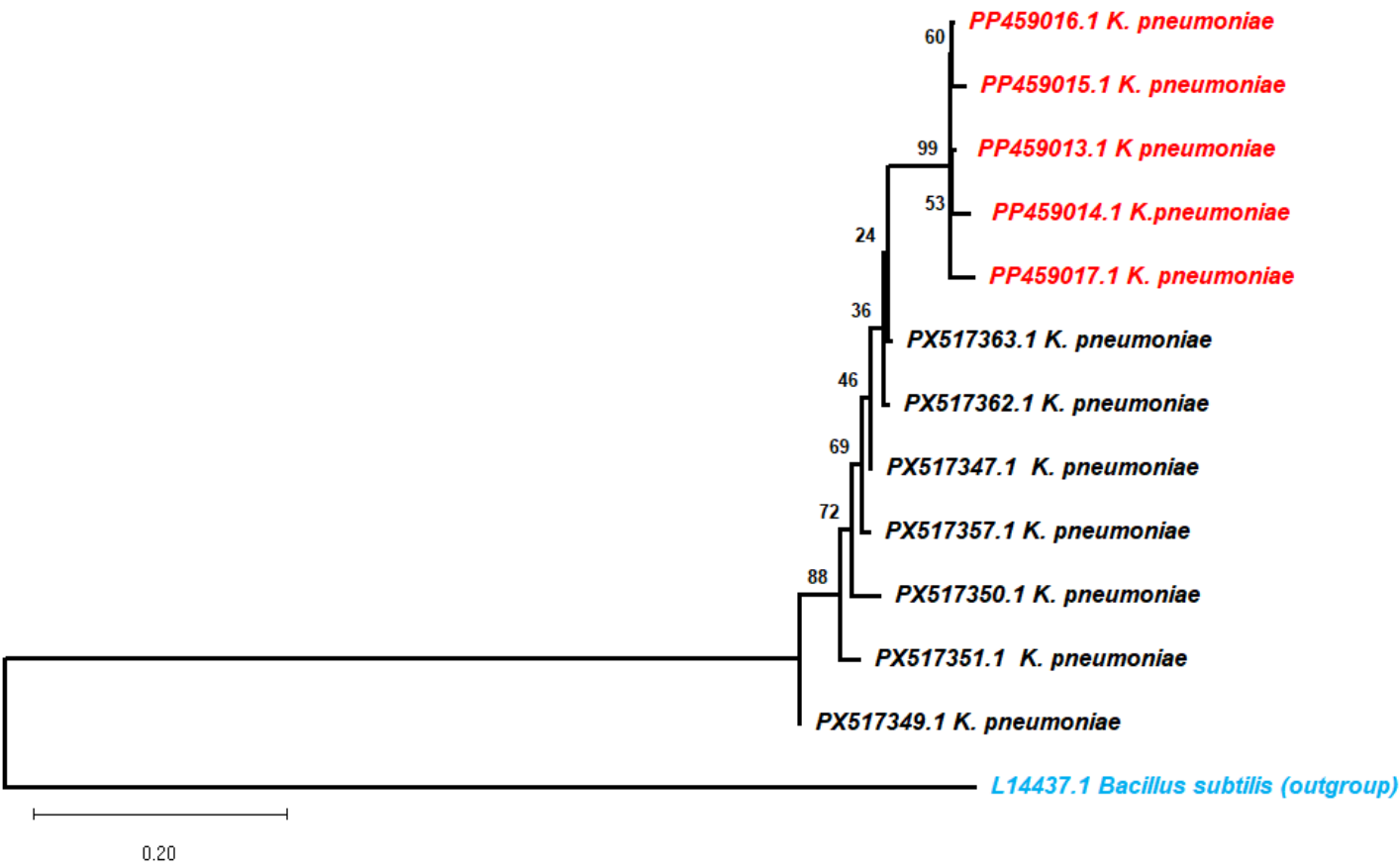


Figure 3

Phylogenetic tree from 16S rRNA gene sequences of *K. pneumoniae*.

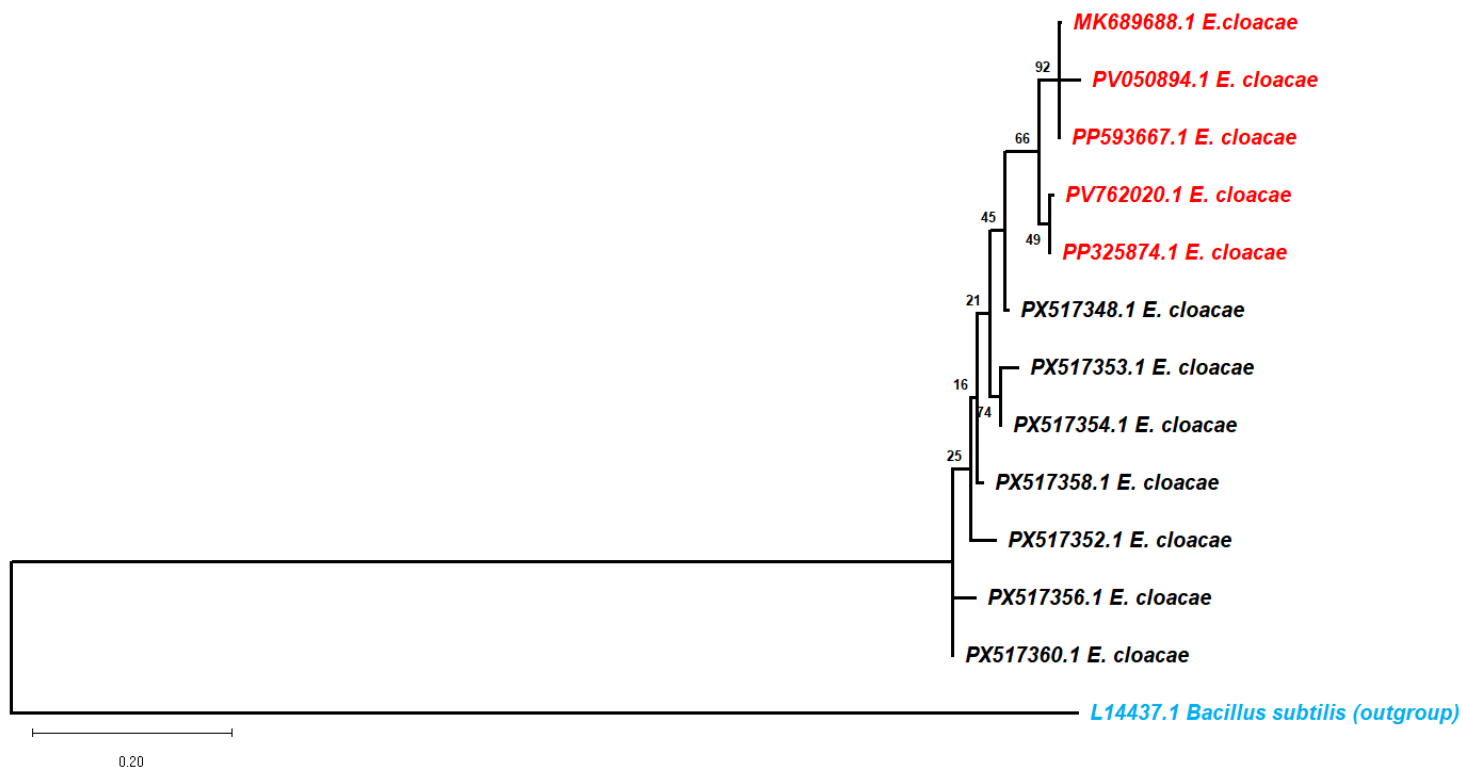


Figure 4

Phylogenetic tree from 16S rRNA gene sequences of *E. cloacae*.

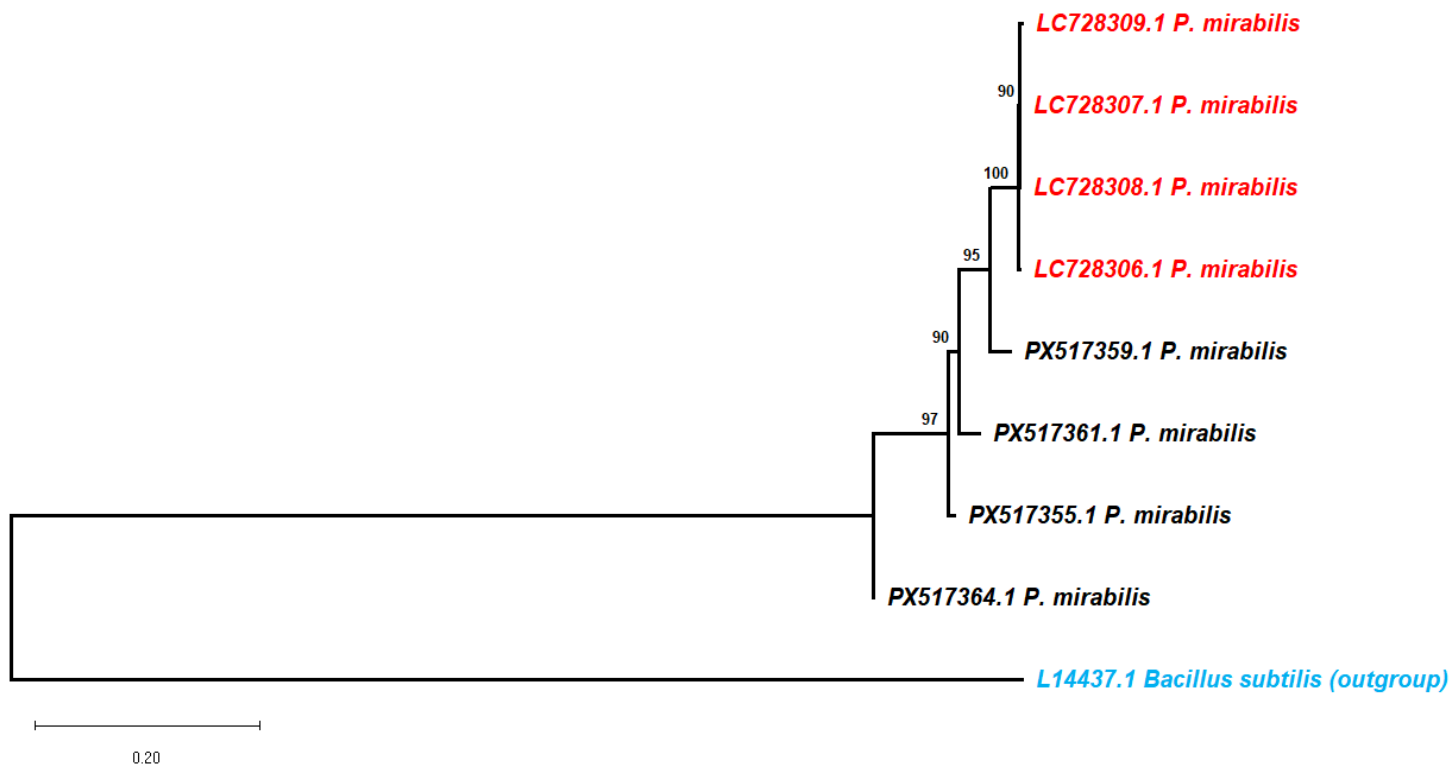


Figure 5

Phylogenetic tree from 16S rRNA gene sequences of *P. mirabilis*.

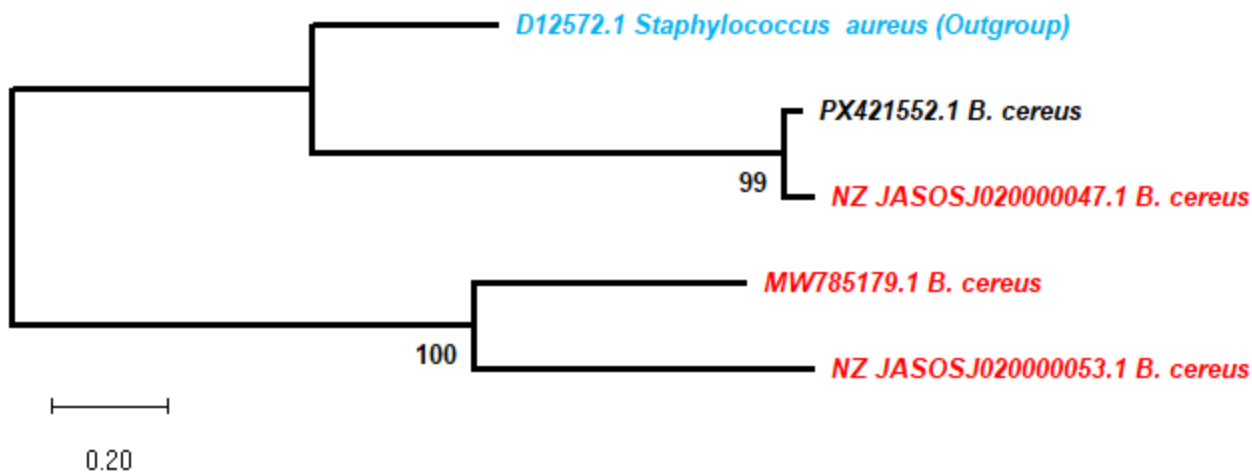


Figure 6

Phylogenetic tree from 16S rRNA gene sequences of *B. cereus*.

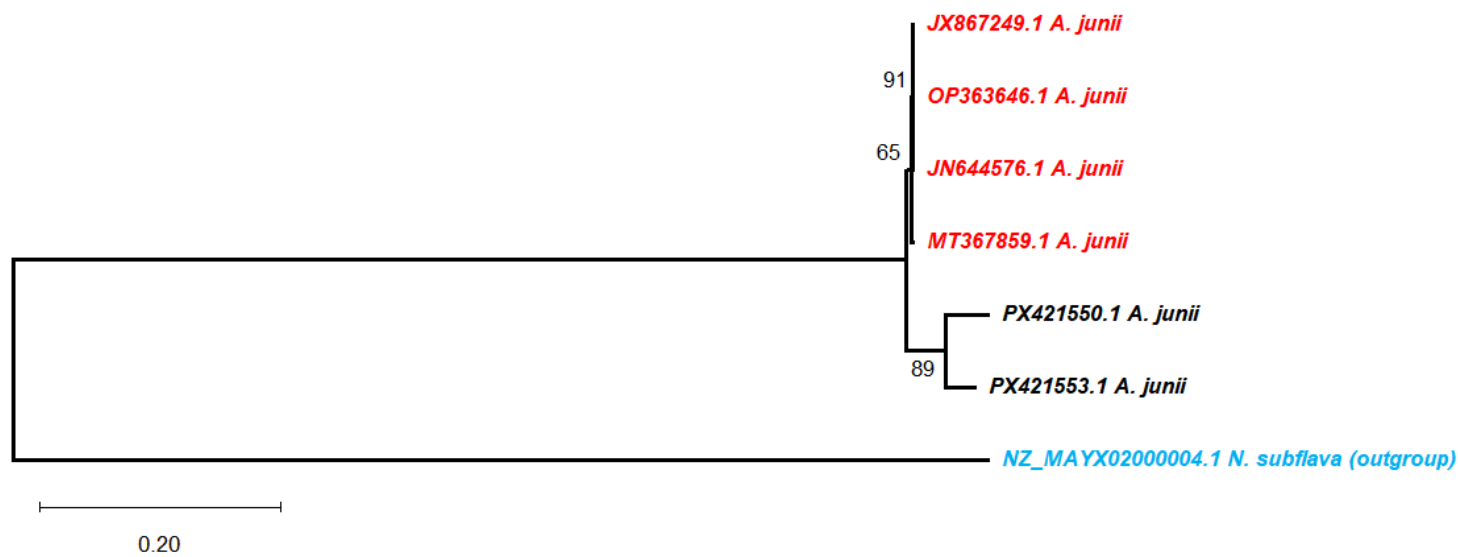


Figure 7

Phylogenetic tree from 16S rRNA gene sequences of *A. junii*.

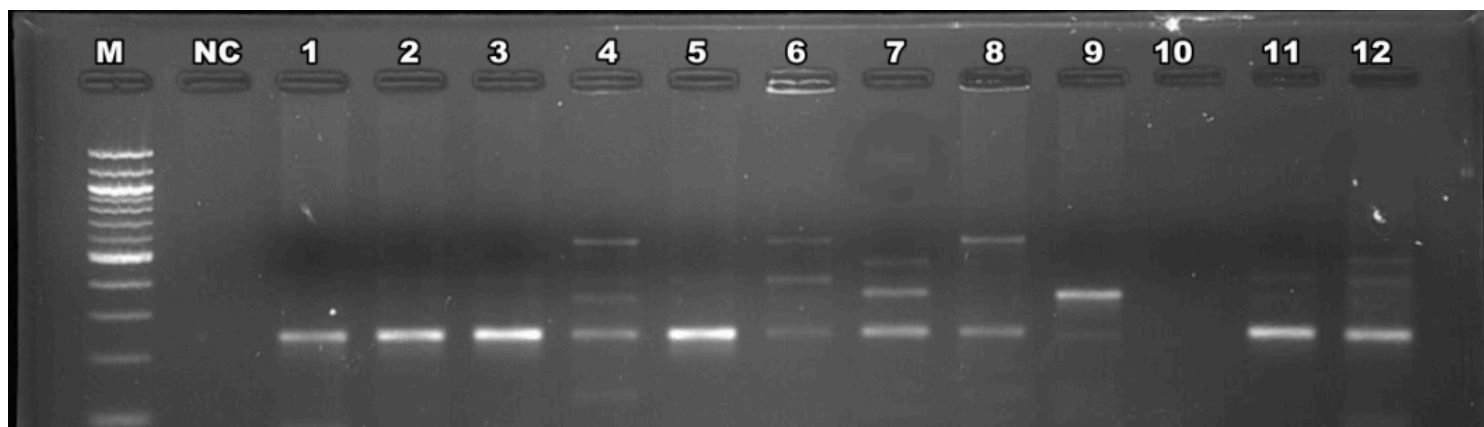


Figure 8

PCR amplification of the virulence genes.

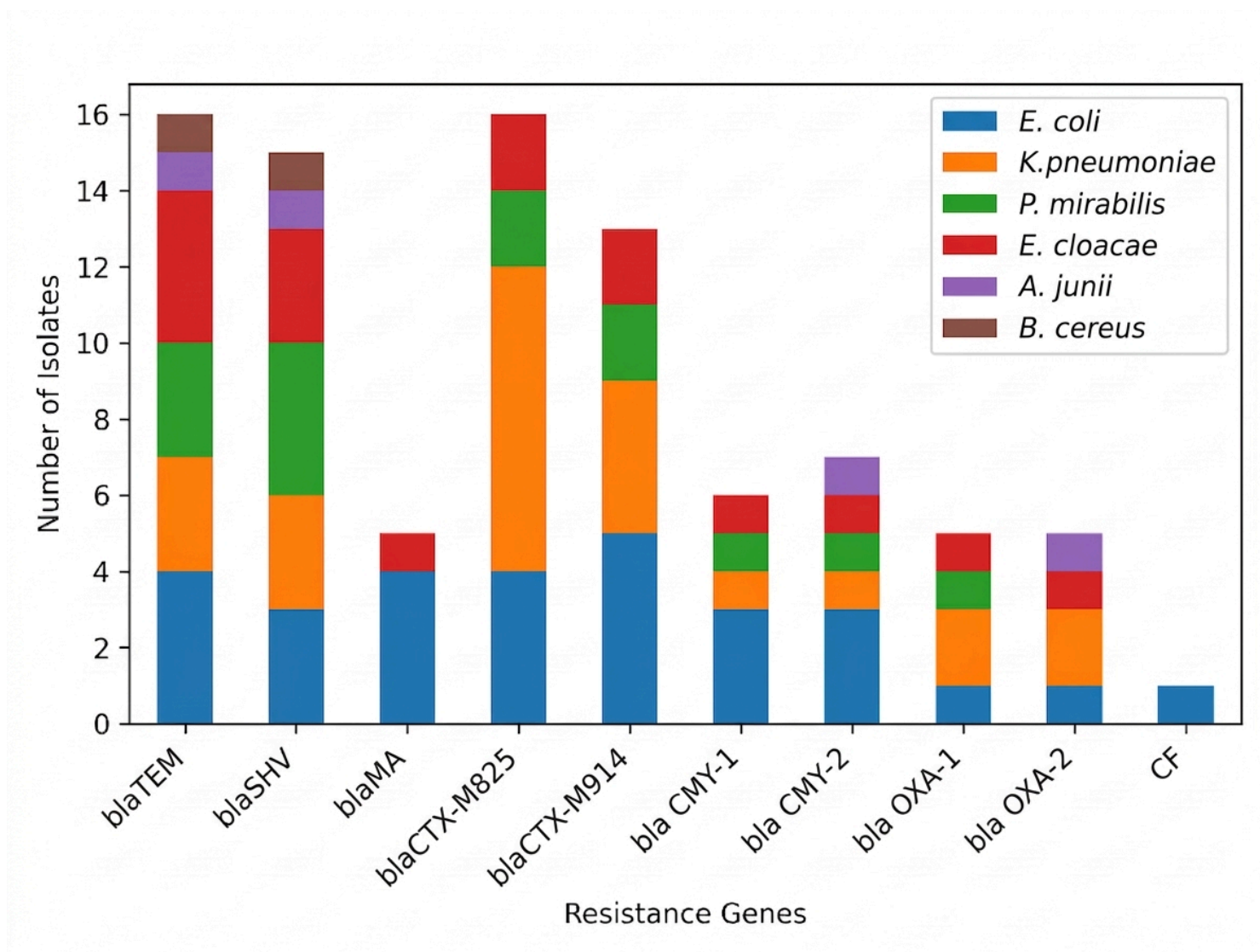


Figure 9

Distribution of ESBL genes amongst bacterial isolates

Supplementary Files

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