Phenotype–genotype discordance in antimicrobial resistance profiles of Gram-negative uropathogens recovered from catheter-associated urinary tract infections in Egypt

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Abbreviations: AMR, antimicrobial resistance; CAUTI, catheter-associated urinary tract infection; DDT, disc diffusion test; ESBL, extended spectrum β-lactamase; HAI, healthcare-associated infection; ICU, intensive care unit; MDR, multidrug-resistant; ME, major error; VME, very major error; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection; XDR, extensively drug-resistant.

Data availability: The sequence data included in the study are available under BioProject PRJNA1208035.

ABSTRACT

Catheter-associated urinary tract infections (CAUTIs) are among the most common healthcare-associated infections in low- and middle-income countries (LMICs), contributing to the clinical burden of antimicrobial resistance (AMR). In this study, phenotypic (EUCAST AMR profiles) and genomic data were generated for 132 isolates (67 *Escherichia coli*, 14 *Pseudomonas aeruginosa*, 11 *Klebsiella pneumoniae*, 9 *Proteus mirabilis*, 8 *Providencia* spp., 5 *Enterobacter hormaechei*, and 18 rare uropathogens) recovered from CAUTIs in an Egyptian hospital. Several (22/132, 16.6 %) isolates were multidrug-resistant, while almost half (62/132, 46.9 %) were extensively drug-resistant. Comparison of phenotypic data with genotypic data from three different AMR-profiling tools (ResFinder, CARD, AMRFinder) highlighted phenotype–genotype discordance as an important consideration in resistome studies in Egypt, with a total concordance of 88.4 %, 85.7 %, and 80.3 % ResFinder, CARD and AMRFinder, respectively. *Pseudomonas*, at the species level, exhibited the greatest discordance. At the antimicrobial level, meropenem was subject to greatest discordance. In addition to the findings from our comparative analyses, new AMR variants are reported for Egypt for *Pseudomonas* (*OXA-486*, *OXA-488*, *OXA-905*, *IMP-43*, *PDC-35*, *PDC-45*, *PDC-201*) and *Escherichia coli* (*TEM-176*, *TEM-190*), along with several new multilocus sequence types (for *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) for the country. Two *Pseudomonas aeruginosa* isolates harbouring pBT2436-like megaplasmids were identified, encoding resistance genes (*aph(3'')-Ib*, *aph(6)-Id*). In summary, we show that there is significant phenotype–genotype discordance in AMR profiling among CAUTI isolates, highlighting the need for comprehensive approaches in resistome studies. We also show the genomic diversity of Gram-negative uropathogens contributing to disease burden in a little-studied LMIC setting.

Introduction

The healthy bladder is not sterile as urine harbours a complex microbial community even in healthy, asymptomatic individuals. This microbial community performs critical functions in bladder homeostasis and promotion of normal immune function (Ackerman & Chai, 2019). However, uropathogens can cause complicated or uncomplicated, symptomatic or asymptomatic urinary tract infections (UTIs) via different mechanisms for survival in response to stresses (e.g. starvation) and immune responses in the bladder.

UTIs are an increasing public health problem caused by a range of uropathogens. The frequency of healthcare-associated UTIs is 12.9 %, 19.6 % and 24 %, respectively, in the United States, Europe and developing countries (Tandogdu & Wagenlehner, 2016). In 2019, bacterial antimicrobial resistance (AMR) contributed to 48,700 deaths associated with UTIs out of 541,000 cases of infectious syndromes (Mestrovic et al., 2022). Resistance to almost all antibiotics in healthcare-associated UTIs is above 20 % and there is a significant geographical variation (Tandogdu & Wagenlehner, 2016). However, meta-analysis and surveillance data are scarce from low- and middle-income countries (LMICs) (Allegranzi et al., 2011; Öztürk & Murt, 2020). Egypt initiated a national surveillance program for healthcare-associated infections (HAIs) with international collaborators and national entities to establish benchmarks to outline the microbiological profiles and reduce AMR in clinical settings. Phase one of the National Action Plan showed nearly 50 % and 75 % of isolates [*Escherichia* (*Esc.*) *coli* and *Klebsiella pneumoniae*] isolated from urine were resistant to quinolones and cephalosporins, respectively (WHO, 2018a). Across 27 countries, Egypt came in third place for total consumption of antibacterials (1355.4 tonnes) in 2020 (WHO, 2022).

The most common bacteria causing UTIs are uropathogenic *Esc. coli* (UPEC), *K. pneumoniae*, *Pseudomonas* (*Pse.*) *aeruginosa* and *Proteus* (*Pro.*) *mirabilis*. High recurrence rates and increasing AMR threaten to greatly increase the economic burden of infections caused by these microbes (Flores-Mireles et al., 2019; Flores-Mireles et al., 2015). Generally, UPEC is the main cause of community-acquired or healthcare-associated UTIs. UPEC is followed in prevalence by *K. pneumoniae, Pro. mirabilis, Pse. aeruginosa,* enterococci, *Staphylococcus saprophyticus* and *Candida albicans* (Mancuso et al., 2023). In a recent review, the prevalence of

Gram-negative uropathogens across Egyptian centers was, in descending order, *Esc. coli*, *K. pneumoniae*, *Pse. aeruginosa*, and other *Enterobacterales* (El-Kholy et al., 2021).

K. pneumoniae and *Pse. aeruginosa* are common multidrug-resistant (MDR) pathogens in nosocomial infections. These bacteria are now becoming resistant to many frontline antibiotics for both nosocomial and community-acquired infections (Cottalorda et al., 2021; Hola et al., 2012; Krawczyk et al., 2022). AMR is particularly important due to the acquisition of extended spectrum β-lactamase- (ESBL) and carbapenemase-encoding genes by uropathogens (Bush & Bradford, 2020). Uropathogens have a high incidence of mortality due to limited treatment options especially if ESBL and carbapenemases are coharboured with genes encoding for aminoglycoside-modifying enzymes, quinolone resistance determinants, altered permeability and efflux pumps (Hooper & Jacoby, 2015; Ramirez & Tolmasky, 2010).

In Egypt, catheter-associated UTIs (CAUTIs) are among the most common types of HAIs affecting patients in intensive care units (ICUs) (El-Kholy et al., 2021; Kotb et al., 2020; See et al., 2013; Talaat et al., 2016). In Egypt between 2011 and 2012, 15 % (69/472) of HAIs were UTIs and the rate of CAUTIs was 1.2 per 1,000 device-days (See et al., 2013). Another surveillance showed that UTIs represented 15 % (406/2688) of HAIs, the rate of CAUTI development increased up to 1.9/1,000 device-days and CAUTIs represented 98.3 % of UTIs (Talaat et al., 2016). An epidemiological study (between 2011 and 2017) of carbapenem-resistant *Enterobacteriaceae* in Egyptian ICUs showed that UTIs accounted for 14.8 % (236/1598) of HAIs, with 63.8 % of UTIs catheter-related (Kotb et al., 2020). The second most common HAIs were UTIs (12.2 %) in Egyptian clinical settings (El-Kholy et al., 2021).

There are few reports on the genomic diversity of uropathogens found in Egyptian clinical settings (Baiomy et al., 2023; Edward et al., 2022; Eladawy et al., 2023; Sherif et al., 2021; Zakaria et al., 2021, 2022). However, high rates of AMR are observed in Egypt beside documented transmission of carbapenem-resistant strains from Egypt to other countries (Hassuna et al., 2020; Kohler et al., 2020; van Duin et al., 2014). We collected a range of Gram-negative uropathogens (n = 132 isolates) from an Egyptian hospital over a 9-month period, and generated genotypic and phenotypic data for them. Our aim was to investigate the AMR patterns associated with UTIs in an Egyptian clinical setting and provide evidence that could inform the development of effective antimicrobial surveillance plans and targeted infection control policies.

In addition, we assessed the agreement/concordance between phenotypic and genotypic data to evaluate the usefulness of such data from a diagnostic perspective.

Methods

Recovery of isolates

Isolates (n = 132) were recovered from urinary catheters between December 2020 and August 2021 by staff at the Urology and Nephrology Center, Mansoura, Egypt during routine diagnostic procedures. Anonymized isolates with no patient data were recovered onto blood agar, and Cystine-Lactose-Electrolyte-Deficient (CLED) agar after incubation aerobically at 37 °C. Purification of Gram-negative isolates was further confirmed by inoculating colonies onto MacConkey's agar in the microbiology laboratory at the Faculty of Pharmacy, Mansoura University. The study of anonymized clinical isolates beyond the diagnostic requirement was approved by the Urology and Nephrology Center, Mansoura, Egypt. No other ethical approval was required for the use of the clinical isolates.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion test (DDT) on Mueller-Hinton agar (Oxoid Ltd, UK), with overnight cultures diluted to 0.5 McFarland standard and swabbed onto the agar plates, followed by aerobic incubation at 37 °C for 24 h. Twelve different antimicrobial discs (Oxoid Ltd, UK) from seven different antimicrobial classes were used: penicillins – piperacillin/tazobactam (30/6 µg); cephalosporins – ceftazidime (10 µg), cefepime (30 µg); monobactams – aztreonam (30 µg); carbapenems – doripenem (10 µg), meropenem (10 µg); aminoglycosides – tobramycin (10 µg), amikacin (30 µg); quinolones – ciprofloxacin (5 µg), levofloxacin (5 µg); miscellaneous – nitrofurantoin (100 µg), sulfamethoxazole/trimethoprim (1.25-23.75 µg).

Inhibition zone diameters were determined and recorded according to breakpoints of EUCAST guidelines version 12.0, 2022 (http://www.eucast.org/clinical_breakpoints/). The recommended EUCAST reference strains - *Esc. coli* ATCC 25922 and *Pse. aeruginosa* ATCC 27853 - were used for quality control purposes in this study. Classification system proposed by Magiorakos et al. (Bitew & Tsige, 2020; Magiorakos et al., 2012) was applied to categorize bacterial isolates into R1 (resistant to one antimicrobial category), R2 (resistant to two

antimicrobial categories), MDR (non-susceptible to at least one agent in three or more antimicrobial categories), and extensively drug-resistant (XDR) (non-susceptible to at least one agent in all antimicrobial categories except two or fewer).

DNA extraction and whole-genome sequencing

DNA was extracted from isolates as described previously (Eladawy et al., 2023). Illumina sequencing (Nextera XT Library Prep Kit; HiSeq/NovaSeq; 2 ×250 bp paired-end reads; min. ~30× coverage) was performed by MicrobesNG (Birmingham, United Kingdom). Reads were adapter-trimmed to a minimum length of 36 nt using Trimmomatic v0.30 (Bolger et al., 2014) with a sliding window quality cut-off of Q15. *De novo*-assembled genomes (SPAdes v3.7; (Bankevich et al., 2012)) were returned to us by MicrobesNG. In-house-generated MinION sequence data were generated as per (Eladawy et al., 2023) and base called using Guppy v6.5.7. Adapters were trimmed, and hybrid MinION/Illumina assemblies performed as per our previous report (Eladawy et al., 2023).

Bioinformatic analyses

Contigs with fewer than 500 bp were filtered from draft genomes using reformat.sh of BBmap 39.01 (Bushnell, 2014). CheckM2 v0.1.3 was used to assess genome quality (Parks et al., 2015). Bakta v1.5.1 (database v4.0) was used for annotating genes within genomes (Schwengers et al., 2021).

sourmash v4.8.4 was used to generate k-mer (k31) signatures for all genomic data. These were then compared (sourmash compare) with one another to generate a similarity matrix and associated dendrogram (sourmash plot). The dendrogram was retained to visualize relationships among isolates (Pierce et al., 2019). Species identities of genomes (**Supplementary Table 1**) were determined using sourmash lca with the Genome Taxonomy Database (release 214). Those genomes that could not be assigned to a species by sourmash were initially identified using ribosomal MLST (https://pubmlst.org/species-id; (Jolley et al., 2012)), then compared against relevant reference sequences (**Supplementary Table 1**) using FastANI v1.33 (Jain et al., 2018). Species identities were confirmed by NCBI upon submission of sequence data to GenBank.

Serotypes were determined via ECTyper v1.0.0 (database v1.0) (Bessonov et al., 2021). Phylogrouping of *Esc. coli* was achieved using Ezclermont v0.7.0 (Beghain et al., 2018). Multilocus sequence type (MLST) of each isolate was determined using *mlst* v2.23.0 (developed by Torsten Seemann, available at Github https://github.com/tseemann/mlst) and relevant MLST schemes from http://pubmlst.org (Bai et al., 2012; Curran et al., 2004; Diancourt et al., 2010; Diancourt et al., 2005; Jolley et al., 2018; Miyoshi-Akiyama et al., 2013; Wirth et al., 2006).

AMR gene markers were identified using three different AMR databases (accessed February 2025); the Resistance Gene Identifier (RGI; v6.0.3) tool of the Comprehensive Antibiotic Resistance Database (CARD) v4.0.0 (McArthur et al., 2013), ResFinder software v4.6.0, ResFinder database v2.4.0, PointFinder database v4.1.1 (Florensa et al., 2022), and AMRFinder v4.0.3, database version 2024-12-18.1 (Feldgarden et al., 2019). Genes responsible for efflux pumps were excluded from this study, as suggested by (Mahfouz et al., 2020). Only AMR genes that showed a perfect or strict match (identity ≥ 90 %) were reported in this study.

For the *Esc. coli* genomic data, a phylogenetic tree was constructed using core genes that were identified using Panaroo v1.3.4 (Tonkin-Hill et al., 2020) running in strict mode with the sequence identity default threshold set to 98 % and the alignment option set to “core”. Genes were required to be present in all 67 *Esc. coli* strains to be considered core. All core genes were aligned with prank (v170427) (Löytynoja, 2014). The gene alignments were concatenated with FASconCAT-G (v1.02) into a single alignment (Kück & Longo, 2014). A phylogenetic analysis was performed using the concatenated sequence and RaxML v1.2.0 with model GTR+G8+IO (Stamatakis, 2014). Node support was determined using 1000 non-parametric bootstrap replicates. The phylogenetic tree was visualized using iTOL v6.9 (Letunic & Bork, 2019).Virulence traits encoded in genomes were detected via BLASTP (≥90 % coverage, ≥70 % identity) with the protein sequences of the core dataset from the Virulence Factors Database (VFDB) (accessed August 2023) (Chen et al., 2005).

Kleborate v3.1.2 was used to characterize genotypes of *K. pneumoniae* isolates and MLSTs of *Esc. coli* genomes using the Pasteur MLST scheme (Lam et al., 2021; Wyres et al., 2016).

Identification of pBT2436-like megaplasmids in *Pse. aeruginosa* genomes

A BLASTN search (--outfmt 6) was made using the megaplasmid pBT2436-like core gene sequences (*repA*, *parA*, *virB4*) described by (Cazares et al., 2020) against the contigs of our newly generated short-read genome sequence data. Hybrid (Illumina/MinION) genome sequences were generated for those genomes found to encode the three core gene sequences, as described previously (Eladawy et al., 2023). We compared the newly identified pBT2436-like megaplasmids against *Pse. aeruginosa* plasmid pP9Me1, identified in our previous study, our curated megaplasmid database (Eladawy et al., 2023; Hoyles, 2023a) and additional *Pseudomonas* megaplasmid sequences identified by (Wang et al., 2024). The megaplasmid sequences were annotated using Bakta v1.5.1 (database v4.0). FastANI v1.33 (Jain et al., 2018) was used to determine how similar the sequences of the newly identified megaplasmids were to those of pBT2436 and our database of megaplasmid sequences. Predicted protein sequences of all megaplasmids were concatenated, sorted by length (longest to shortest) and clustered as described previously (Eladawy et al., 2023). Core sequences found in single copies in all 54 plasmids were concatenated and used to generate a sequence alignment (MAFFT v7.490, BLOSUM 62; Geneious Prime v2023.0.1) from which a GAMMA BLOSUM62 substitution model maximum likelihood tree (RAxML 8.2.11; parameters selected to generate best-scoring maximum likelihood tree, 100 bootstraps; Geneious Prime v2023.0.1) was generated. The R script (Eladawy et al., 2023) associated with processing of the sequence data is available for download (Hoyles, 2023b).

Comparison of AMR genotypes and phenotypes

According to EUCAST guidelines, “susceptible, standard dosing regimen” (S) and “susceptible, increased exposure” (I) categories are grouped under the term “susceptible”. Using three different databases – CARD, ResFinder, and AMRFinder – genomic data of isolates were compared with DDT results for 132 isolates against 12 antimicrobials [except when certain species lacked breakpoints; i.e. nitrofurantoin was used only for *Esc. coli* (n = 67), sulfamethoxazole/trimethoprim was used only for *Enterobacterales* (n = 114)]. For each combination, concordance was considered positive if a) WGS data were predicted to encode AMR genes and the isolate had a phenotypic resistant profile “true resistant” (WGS-R/DDT-R) or b) WGS data were not predicted to encode AMR genes and the isolate had a phenotypic

susceptible profile “true susceptible” (WGS-S/DDT-S) (Rebelo et al., 2022; Vanstokstraeten et al., 2023). Discordance was considered positive where major errors (ME) or very major errors (VME) were detected. MEs or false positives (WGS-R/DDT-S) are defined as a resistant genotype and susceptible phenotype. VMEs or false negatives (WGS-S/DDT-R) are defined as a susceptible genotype and resistant phenotype. To detect AMR gene mutations, PointFinder tool for ResFinder and Point mutations tool for AMRFinder results were also included in concordance analyses.

Results

Genome characterization

The draft genomes assembled from short-read and, in some instances, long-read data consisted of between 2 and 677 contigs. CheckM2 analysis confirmed the genomes were of high quality (>90 % complete, <5 % contamination; **Supplementary Table 1**). The number of coding sequences predicted to be encoded within the genomes was between 3267 and 6636. Sourmash was used to identify and compare the isolates to each other **Figure (1A**). Sourmash analysis using the Genome Taxonomy Database (release 214) assigned all genomes to the phylum *Pseudomonadota*; and, except for Me47, all genomes were assigned to the class *Gammaproteobacteria* (**Supplementary Table 1**). The majority (124/132) of genomes could be identified to the species level using sourmash (**Supplementary Table 1**), with taxonomic affiliations of the eight remaining genomes determined using ribosomal MLST, ANI and NCBI quality control checks. Among our isolates, UPEC dominated (50.7 %, 67/132), followed by *Pse. aeruginosa* (10.6 %, 14/132), *K. pneumoniae* (8.3 %, 11/132), other uropathogens (6.8 %, 9/132), *Pro. mirabilis* (6.8 %, n = 9/132), *Providencia* spp. (6 %, n = 8/132), *Enterobacter* (*Ent.*) *hormaechei* (3.7 %, n = 5/132), *Alcaligenes* spp. (3 %, n = 4/132), *Serratia* spp. (2.2 %, n = 3/132) and *Pse. vlassakiae* (1.51 %, n = 2/132).

Antimicrobial susceptibility of isolates

The isolates were highly resistant to ceftazidime (68.9 %, 91/132) followed by ciprofloxacin (62.1 %, 82/132). The lowest resistance was recorded for nitrofurantoin (5.9 %, 4/67) and meropenem (18.2 %, 24/132) (**Figure (1B)**, **Supplementary Table 2**). *Ent.*

hormaechei (60 %, 3/5) and *K. pneumoniae* (100 %, n = 11) were associated with the highest prevalence of MDR and XDR, respectively. The prevalence of susceptible, R1, and R2 categories are 19.6%, 7.6%, 9.1% respectively. Moreover, 16.6 % (n = 22/132) of isolates were MDR, while 46.9 % of isolates (n=62/132) were XDR (**Figure (1C)**).

AMR determinants

The main AMR genes responsible for resistance to antimicrobials tested in this study were identified using different databases: namely, ResFinder, CARD and AMRFinder (**Supplementary Table 3**). New AMR variants reported for uropathogens in Egypt are *OXA-486*, *OXA-488*, *OXA-905*, *IMP-43*, *PDC-35*, *PDC-45*, and *PDC-201* for *Pse. aeruginosa*, and *TEM-176* and *TEM-190* for *Esc. coli*.

Comparison of WGS data and DDT results (with respect to predicted AMR genes and actual resistance phenotypes) yielded a total concordance of 88.4 %, 85.7 %, and 80.3 % for ResFinder, CARD and AMRFinder, respectively. Variations in discordance were detected for different databases, where the MEs (WGS-R/DDT-S) were 7.8 %, 11.9 %, 15.7 % while VMEs (WGS-S/DDT-R) were 3.7 %, 2.2 %, 3.8% for ResFinder, CARD, and AMRFinder, respectively (**Figure (2)**).

The highest discordance was associated with *Pseudomonas* (n = 16) in three databases [42.1% (ResFinder), 31.2% (AMRFinder), and 21.2% (CARD)], while the highest concordance was recorded for *Alcaligenes* (n = 4) species in three databases; AMRFinder (97.7%), ResFinder (97.2%) and CARD (100 %) (**Supplementary Table 7**).

Concordance for aminoglycosides, quinolones, and miscellaneous antibiotics was higher than 87.8 % in all databases. Meropenem was associated with highest discordance in AMRFinder (48.4%) and CARD (47.7%); however, it showed 14.3% discordance in ResFinder.

Moreover, fluctuations of concordance were observed for β-lactam antibiotics in the three databases. The range of total discordance of β-lactam antibiotics for three databases was as follows: ResFinder 11.3–18.9%, CARD 9–47.7%, AMRFinder 15.1–48.4% (**Supplementary Table 6**)

Clones, sequence types, and capsule types

The clonal diversity among isolates showed 24, 10, 9 and 3 different sequence types (STs) for *Esc. coli*, *Pse. aeruginosa*, *K. pneumoniae* and *Ent. hormaechei*, respectively. New STs of *Esc. coli* (14, 24, 44, 73, 99, 127, 141, 156, 405, 641, 648, 847, 1193, 1431, 1487, 2279, 2659, 3090, 12869), *Enterobacter* (114, 152, 923), *K. pneumoniae* (152, 3328, ST147-1LV, ST1787-1LV) and *Pse. aeruginosa* (12, 292, 555, 1145, 2021, 2618, 4529) for Egypt were submitted to the respective PubMLST isolate databases. Eight different capsule loci (n = 8/11, 72.7 %) were identified for *K. pneumoniae* (KL17, KL24, KL51, KL62, KL64, KL108, KL148, KL149; **Supplementary Table 1**). Three different O locus types (O4, O1/O2v, O1/O2v2) were identified for *K. pneumoniae* isolates.

Detailed analyses of UPEC isolates

The pangenome of UPEC strains consisted of 10453 genes, of which 3136 (30 %) were core genes, while 6303 (60.3 %) were accessory genes and 1014 (9.7 %) were unique. A phylogenetic tree generated based on pangenomic analysis of core genes is shown in **Figure (3).** Using phylogroups as a broad typing method, UPEC from phylogroup B2 were responsible for most of the reported UTIs (n = 36/67, 53.7 %) (**Figure (3)**). Other phylogroups were B1 (19.4 %), A (11.9 %), D (7.4 %), F (5.9 %) and C (1.4 %). We identified 29 different serotypes (-:H16, -:H4, -:H5, -:H6, O1:H6, O102:H6, O102:H9, O108:H2, O153:H18, O153:H4, O18:H5, O2/O50:H18, O2:H1, O25:H4, O54:H28, O6:H31, O64:H21, O75:H5, O8:H25, O89:H17, O89:H21, O89:H4, O89:H5, O89:H9, O9:H10, O9:H4, O9:H9, O91:H21, O96:H19) for UPEC. The most common serotypes were O25:H4 (n = 12/67, 17.9 %) and O75:H5 (n = 11/67, 16.4 %), with both serotypes belonging only to phylogroup B2.

Comparative analysis with VFDB demonstrated that the principal virulence genes associated with UPEC strains (n = 67) were type 1 fimbriae (*fimH* 100 %), outer membrane protein (*ompA* 100 %), enterobactin (*entS* 100 %), curli fibers (*csgA* 98 %, n = 66), common pilus (*ecp* 95 %, n = 64), nutritional and metabolic factors (*fyuA* 79 %, n = 53, and *chuA* 67 %, n = 45), K1 capsule type (*kpsM* 66 %, n = 44), aerobactin (*iutA* 60 %, n = 40), P fimbriae (*papC* 52 %, n = 35, *papG* 33 %, n = 22), α-hemolysin (*hlyA* 24 %, n = 16), iron siderophores (*iroN* 24 %,

n = 16), exotoxins (*cnf1* 13 %, n = 9), and S fimbriae and F1C fimbriae (*sfa* and *foc* 6 %, n = 4) (**Figure (3)**).

In terms of virulence, *fimH* was present in all phylogroups, while all *sfa* genes were only associated with phylogroup B2 except *sfaX*, which was present in all phylogroups. *entD* was only in phylogroup B1, while all *dra* genes were present only in one strain (Me12A) from phylogroup B2. *cnf-1* was present in all phylogroup B2 and only one isolate (Me87) from B1.

In terms of β-lactam resistance, *blaOXA−1* and *blaTEM-1* were present in all phylogroups, *TEM-176* was present only in phylogroup B1, while other *blaTEM* variants were present in only phylogroup A. *dha* gene variants were only in phylogroup B2, while *blaNDM−5* were absent in the same group. *blaCTX−M−15* was present in all phylogroups, while *blaCTX−M−14* and *blaCTX−M−16* were present in phylogroup F only. In terms of quinolone resistance, *qnrB4* was present in phylogroup B2 only while *qnrS1* was present in all phylogroups. Mutations of *gyrA* and *parC* genes were present in all phylogroups. Genes of aminoglycoside-modifying enzymes were present in all phylogroups.

Kleborate analysis of UPEC isolates showed that there were 24 different STs based on the Achtman MLST scheme, 23 different STs based on the Pasteur MLST scheme and 12 different clonal complexes (**Supplementary Table 8**). ST1193 Achtman (ST53 Pasteur) was the most common ST (12 isolates), while CC ST14 was the most common clonal complex (19 isolates) for UPEC.

Virulence genes of non-UPEC isolates

According to analyses using the VFDB, *Pse. aeruginosa* was associated with the highest number of virulence genes (range: 280-321), followed by *Acinetobacter baumannii* (124 genes), *Esc. coli* strains (range: 109-201), and *K. pneumoniae* (range: 82-106). All other remaining isolates and rare uropathogens encoded fewer than 92 virulence genes. The highest total count of virulence genes was recorded for two *Pse. aeruginosa* isolates: Me23 (321 genes) and Me31B (310 genes). Total count of different virulence genes is listed for each isolate according to virulence categories in **Supplementary Table 4**.

The most abundant virulence genes for *Pse. aeruginosa* (n = 14) were *algD*, *pvdA*, *exoT*, *flgD*, *phzM*, *plcH*, *lasA* and *lasB*, all of which were present in all 14 *Pse. aeruginosa* strains. *toxA*

(93 %, n = 13), *exoY* (93 %, n = 13) and *exoS* (78.5 %, n = 11) were also present in most strains. The highest abundant virulence genes for *K. pneumoniae* are *fimH* (100 %), *fepA/iutA* (100 %), *mrkA/mrkD* (100 %), acrA (100 %), *rcsA/rcsB* (100 %), *ompA* (100 %), *iroE* (90.9 %), *ybt* (63.6 %), *rmpA* (27.2 %), *iucA* (27.2 %).

Kleborate analysis showed three (Me106, Me107, Me96) of the *K. pneumoniae* isolates to encode the hypermucoidy locus *rmpADC* (KpVP-1) and aerobactin (*iuc1*). Two of these isolates (Me106 and Me96) harboured the plasmid-encoded gene *rmpA2*, which is also associated with the hypermucoid virulent phenotype (**Supplementary Table 9**).

Detection of pBT2436-like megaplasmids in *Pse. aeruginosa*

We previously characterized the first pBT2436-like *Pse. aeruginosa* megaplasmid found in an Egyptian clinical setting (Eladawy et al., 2023). Using the core genes (*repA, virB4, parA*) used in our previous study in BLASTN searches with the contigs for each of our genome sequences, we identified two *Pse. aeruginosa* isolates (Me23 and Me31B) encoding pBT2436-like megaplasmids. Similarity was 97.1 %, 98.9 % and 99.6 % for *repA, virB4* and *parA*, respectively, for both sets of sequences. After generating MinION/Illumina hybrid assemblies for the two isolates, comparative analyses of their megaplasmid sequences showed they shared 96.80–97.15 % ANI with megaplasmid pP9Me1 (Eladawy et al., 2023), and were most closely related to *Pse. aeruginosa* AR439 plasmid unnamed2 (98.72–99.19 % ANI; **Supplementary Table 5**), one of the pBT2436-like reference sequences of (Cazares et al., 2020). Phylogenetic analysis of pBT2436-like core sequences confirmed the affiliation of the newly sequenced megaplasmids with AR439 plasmid unnamed2 (**Figure 4**). The two plasmids are predicted to encode resistance genes (*aph(3'')-Ib* and *aph(6)-Id*).

Discussion

Characterization of uropathogens, with respect to their AMR genotypes and phenotypes, recovered from patients with CAUTIs has received little attention in LMICs, particularly Egypt. WGS is the most efficient way to detect novel resistance determinants - especially MDR genotypes - in pathogens, but has had limited application in LMICs in the past because of expense. As access to WGS technologies and sequence data improves in LMICs, understanding

how AMR genotypic and phenotypic profiles correlate is of the utmost importance because – as shown here and in our previous work (Eladawy et al., 2023) – uropathogens identified in countries such as Egypt encode a range of novel AMR genes not currently screened for using PCR-based assays in LMIC clinical laboratories. As such, understanding AMR genotype–phenotype discordance will improve proper management of UTIs and, eventually, the prevention of AMR. Our main aim in this study was to conduct a detailed study on a large collection of Gram-negative CAUTI-associated bacteria (n = 132 isolates) isolated in Egypt to investigate AMR genotype–phenotype concordance in relation to gene data available in widely used, public databases. We also sought to understand the diversity of virulence factors encoded by the main species contributing to CAUTIs in an Egyptian clinical setting. In the current study, we found UPEC isolates to be the main causative agents of CAUTIs (n = 67, 50.7 %) followed by *P. aeruginosa* (n = 14, 10.6 %), *K. pneumoniae* (n = 11, 8.3 %) and *P. mirabilis* (n = 9, 6.8 %), along with other, rare uropathogens (*Alcaligenes* spp., *Citrobacter portucalensis*, *Leclercia adecarboxylata*, *Morganella morganii*, *Paenalcaligenes*, *Providencia* spp., *Stenotrophomonas maltophilia*) (**Figure (1)**). In Egyptian clinical settings, UPEC was previously reported as the main cause of UTIs for adults and neonates followed by other *Enterobacterales* or Gram-positive bacteria (Baz et al., 2021; Hassan et al., 2020; Mohamed et al., 2020; Shrief et al., 2022). *Providencia* was considered a rare pathogen, but is now increasingly recognized as an opportunistic pathogen capable of causing serious UTIs (Rajni et al., 2022). Notably, *Providencia* spp. emerged as a causative agent of UTIs in this study, representing 6 % (n = 6) of all isolates. A small outbreak of *blaNDM-1*-producing *Providencia* isolated from burns has previously been detected in an Egyptian hospital (Soliman et al., 2020).

Empirical therapy plays an important role in treating UTIs until culturing results are available. Therefore, it is important to have an idea about AMR rates for different antibiotics to be able to choose the best option, save time or even alter treatment guidelines in relation to AMR rates. As shown in **Figure (1)**, the isolates were highly resistant (> 50 % resistance) to quinolones and carbapenems. Unsurprisingly, symptomatic CAUTIs are treated like complicated UTIs with 2nd or 3rd generation cephalosporins while uncomplicated pyelonephritis is treated with a short course of fluoroquinolones as the first line therapy, according to Egyptian Urological guidelines (Nouh et al., 2021). AMR for quinolones (ciprofloxacin and levofloxacin) and cephalosporins (cefepime and ceftazidime) was higher than 64 % among MDR uropathogens in

Egyptian clinical settings (Elshamy et al., 2020). A previous report showed that more than half of UPEC isolates (57.8 %) showed high-level ciprofloxacin resistance and *gyrA* mutations were detected in 76.7 % of isolates, so the authors recommended revision of the antibiotics used for empirical treatment of UTIs (Esmaeel et al., 2020).

Esc. coli seemed to be most susceptible to nitrofurantoin (3 % resistance). Nitrofurantoin was reported as a drug of choice for UTI treatment by previous Egyptian studies (Abdel-Aziz Elzayat et al., 2017; Shash et al., 2019), but it still has some limitations, resulting in dark brownish coloured urine and is contraindicated in patients with glucose-6-phosphate dehydrogenase deficiency and during the 3rd trimester of pregnancy (Recht et al., 2022). However, it seemed to be promising for *in silico* AMR prediction using CARD as it showed 95.5 % concordance with phenotypic results (**2)**). In a recent Egyptian study, the resistance level to nitrofurantoin was 51 % in *Klebsiella* spp., but it remains effective for *Esc. coli* UTIs in males and females (92 % sensitivity). In comparison, *Esc. coli* and *Klebsiella* spp. showed elevated resistance rates to cephalosporins: 75 % and 81 %, respectively (Tarek et al., 2024).

This study showed that uropathogens are predicted to encode a wide range of determinants responsible for resistance to β-lactams (*NDM*, *OXA*, *PDC*, *SHV*, *VEB*, *VIM*, *SHV*), quinolones (*QnrA1*, *QnrB17*, *QnrB4*, *QnrB76*, *QnrD1*, *QnrS1*, *QnrS2*, *QnrVC1*) and aminoglycosides [*AAC(2')-Ia*, *AAC(3)-IVa*, *AAC(6')-Ib-cr5*, *ANT(2'')-Ia*, *ANT(3'')-IIa*, *APH(3')-Ia*, *APH(4)-Ia*, *APH(6)-Id*] (**Supplementary Table (3)**). The AMR variants *OXA-486*, *OXA-488*, *OXA-905*, *TEM-176*, *TEM-190*, *IMP-43*, *PDC-35*, *PDC-45* and *PDC-201* are reported for uropathogens in Egypt for the first time.

Pangenomic approaches highlight genetic diversity, revealing adaptations, virulence factors and resistance traits among collections of genomes. In our study, we used panaroo to investigate draft genomes of 67 *Esc. coli* (**Figure (3)**), revealing a total of 10,453 genes, of which 3136 (30 %) were core genes and 6303 (60.3 %) were accessory genes. Multiple pangenome studies of UPEC strains have been performed for both global collections (Fatima et al., 2023) and individual countries, including Ireland and Bangladesh (Hossain et al., 2020; Whelan et al., 2024), with the smallest pangenome consisting of at least 16,797 genes in total, the number of core genes ranging from 2,926 to 2,945, and the number of unique genes ranging from 2,900 to 15,266.

CTX-M-15 variant was detected in 42 % (n = 28/67) of UPEC strains, while the main phylogroup was B2 (n = 36/67, 53.7 %) in this study. In Egypt, CTX-M-15 is the most predominant extended spectrum β-lactamase-producing type among uropathogenic and diarrheagenic *Esc. coli* strains (Abdelrahim et al., 2021). In previous reports from Egypt and Iran, B2 was the most predominant phylogenetic group and strains belonging to this phylogroup were most resistant to commonly used antibiotics among patients with UTIs (Farahat et al., 2021; Halaji et al., 2022; Iranpour et al., 2015). However, another Egyptian study showed that the most prevalent phylogroup was A followed by B2 (El-Mahdy et al., 2021). Although group B2 strains are uncommon among the commensal microbiota, they are highly virulent when present; phylogroup B2 strains have been shown to persist in the intestinal microbiota of infants (Nowrouzian et al., 2006) while also being associated with wildlife and livestock (Aguirre-Sánchez et al., 2022), and herbivorous and omnivorous mammals (Gordon & Cowling, 2003).

Screening the UPEC strains for *Esc. coli* virulence factors from VFDB revealed that those with highest prevalence include *fimH* (adhesin), *ompA* (outer membrane protein), *csgA* (curli fibers), *ecp* (common pilus), and *entS* (enterobactin). In contrast, genes associated with iron acquisition, capsule formation and K1 capsule type showed more variability. Aerobactin and P fimbriae genes were detected at moderate levels, while α-hemolysin and iron siderophores were less common. Exotoxins and S-fimbriae were rare [**Figure (3)**]. Similar to (Hossain et al., 2020), who found *fim*, *ecp*, and *csg* gene families as the main part of the core genetic pool, we find adhesion factors are prevalent in the UPEC pangenome. In contrast, a PCR-based study from Mansoura reported a much higher prevalence of afimbrial adhesins such as *afa/dra* (14 %) and S-fimbriae (*sfaS*, 60.6 %) (El-Baz et al., 2022), which were largely absent in our dataset. Additionally, our data shows a higher prevalence of P fimbriae genes (*papG*, 33 %; *papC*, 52 %) compared to El-Baz et al. (2022), who found these genes in only 21.3 % and 49.3 % of isolates, respectively. Similarly, our detection of iron acquisition genes such as *iutA* (60 %) and *chuA* (67 %) exceeds their reported values of 34.6 % and 54 %. Notably, *cnf1*, which encodes cytotoxic necrotizing factor 1, was much rarer in our dataset (13 %) compared to 42 % in El-Baz et al. (2022), suggesting potential differences in strain pathogenicity.

Overall, our results highlight a strong presence of core adhesion and iron acquisition systems in UPEC strains, with notable variations in toxin-associated genes compared to previous

studies. These findings emphasize the genetic diversity of UPEC virulence factors and the importance of comparing detection methods when assessing pathogenic potential.

The prevalence of pBT2436-like megaplasmids in different countries was reported by us previously (Eladawy et al., 2023). These mobile genetic elements represent an emerging family of clinically important mobile genetic elements (Cazares et al., 2020). While megaplasmid pP9Mel was predicted to encode virulence genes but not AMR genes (Eladawy et al. 2023), the two megaplasmids identified in this study (pMe23_1 and pMe31B_1; **Figure (4)**) do not encode virulence genes but are predicted to encode the AMR genes *aph(3'')-Ib* and *aph(6)-Id*, aminoglycoside phosphotransferases encoded by plasmids, transposons, integrative conjugative elements and chromosomes found in a range of Gram-negative bacteria. The identification of two more pBT2436-like megaplasmids in *Pse. aeruginosa* in Egypt in this study further emphasizes the potential for horizontal gene transfer in shaping resistance patterns.

Increasing the knowledge base of *in silico* AMR prediction via WGS as a competitor to phenotypic susceptibility testing will provide a more precise definition of AMR determinants across various organisms. There is a strong possibility that WGS might soon take over phenotypic testing for surveillance purposes, particularly in situations where the low error rate has minimal consequences (Ellington et al., 2017). In the current study, ResFinder/PointFinder (overall concordance, 88.4%) showed better *in silico* AMR prediction than CARD/RGI (85.7 %) and AMRFinder with Point mutations (80.3 %) (**Figure (2)**). Notably, of the species described in the current study, PointFinder only tackles chromosome mutation-mediated resistance in *Esc. coli* and *Klebsiella.* A combined *in silico* AMR prediction from ResFinder and PointFinder for ciprofloxacin has previously been shown to result in improved prediction performance and a reduced VME rate (Mahfouz et al., 2020). Point mutations are important to tackle and to be considered especially when analysing quinolone-resistant members of *Enterobacteriaceae,* where high frequency of *gyrA* mutations is associated with quinolone resistance in urine (Shenagari et al., 2018; Thompson et al., 2024).

In the current study, variations in discordance were detected for different databases, where the MEs were 7.8 %, 11.9 %, 15.7 % while VMEs were 3.7 %, 2.2 %, 3.8% for ResFinder, CARD, and AMRFinder, respectively. The use of an ineffective therapeutic agent in treatment due to a VME could result in treatment failure, while an ME may restrict therapeutic choices and

create complications in the treatment process. The more severe impact of VMEs is evident in FDA regulations for the approval of diagnostic tests, where the FDA mandates that VMEs be kept below 1.5 % and MEs below 3 % for the approval of a new AMR diagnostic test or device (Jorgensen & Ferraro, 2009).

High rates for MEs or/and VMEs have been reported previously. Mahfouz et al. (2020) compared CARD and ResFinder for two global collections and a total of 2,587 isolates comprising five pathogens of medical importance; ME rates were higher in CARD (42 %) than ResFinder (25 %). However, CARD showed almost no VMEs (1.1 %) compared with ResFinder (4.4 %) (Mahfouz et al., 2020). In comparison, another report showed an overall concordance of 91 % for ResFinder while ME and VME rates were 6.2 % and 2.1 %, respectively, for 488 different isolates, where *Pse. aeruginosa* had the highest percentage of discordant results (44.4 %) (Rebelo et al., 2022). In our study, *Pseudomonas* also showed the highest discordance for the three databases used in this study; 42.1 % for ResFinder, 31.2 % for AMRFinder and 21.2 % for CARD. In this study, 33 % of MEs tested phenotypically "susceptible, increased exposure" and were therefore considered as not resistant due to the EUCAST update of susceptibility definitions 2019 "*by the pooling of S and I isolates together into one category*" (Nabal Díaz et al., 2022). Notably in another study, 22.73 % of ME tested phenotypically as 'susceptible at higher exposure', in 234 non-duplicate *Esc. coli* strains (Vanstokstraeten et al., 2023). Discordant bioinformatic predictions of AMR from WGS have previously been shown through analysis of seven clinical isolates with three different databases (ResFinder, CARD and ARG-ANNOT) and where the overall ME "false positives" and VME "false negatives" were 20 % and 14 %, respectively (Doyle et al., 2020). In the current study, the overall MEs and VME in three databases were 12.1 % and 3.2 %, respectively. The high MEs rate may be also due to low expression levels of enzymes, insufficient understanding of gene regulation, mutations in intergenic regions and de novo rRNA mutations.

For the best outcomes for *in silico* AMR prediction, the understanding of phenotypic–genotypic correlations must be improved to achieve FDA requirements for clinical microbiology diagnostic testing below 3% MEs and 1.5% VMEs. Recommendations have been made previously with respect to excluding genes associated with efflux pump mechanisms from detected AMR genes (Mahfouz et al., 2020). Percentage identity thresholds for AMR gene

detection should be ≥ 90 % for different databases (Doyle et al., 2020). Point mutations leading to AMR should be considered alongside acquired resistance determinants, especially if they are reported from specific clinical sources like urine, as the most common site for discordance was urine for both *Enterobacterales* and *Pseudomonas aeruginosa* while high frequency of mutations in the *gyrA* gene was associated with quinolone resistance (Ku et al., 2021; Shenagari et al., 2018). Databases should replace general terminology (β-lactams, quinolones, etc.) of antimicrobial class as a prediction to confer resistance to all members of the same antimicrobial class, so *in silico* AMR prediction should be tailored to antibiotics themselves rather than antibiotic classes to avoid MEs. For example the aminoglycoside gene *aac(6')-1* leads to amikacin and tobramycin resistance, while *aac(3)-IIa* leads to gentamicin and tobramycin resistance (Krause et al., 2016). This was reported recently as poor correlation (85.9 %) for aminoglycosides between EUCAST and WGS data using ARG-ANNOT and CARD databases, and the authors developed a phenotype-based algorithm to reflect mechanisms responsible for the corresponding aminoglycoside resistance phenotypes (Mancini et al., 2019).

In addition to the above consideration, in-depth curation of *in silico* databases is required. Some AMR genes listed in databases are not truly responsible for AMR. For example, the *crpP* gene identified by ResFinder and AMRFinder was previously known as a novel ciprofloxacin-modifying enzyme (Chávez-Jacobo et al., 2018). However, recent studies showed that *crpP* presence is not always associated with ciprofloxacin resistance (Hernández-García et al., 2021; López et al., 2022; Zubyk & Wright, 2021); in this study *crpP* is responsible for seven of the eight MEs generated by AMRFinder and all seven MEs generated by ResFinder when analysing ciprofloxacin resistance. *crpP* was detected in only one strain that was resistant to ciprofloxacin, Me73B, and was the only quinolone resistance determinant detected by ResFinder for that strain. However, AMRFinder also detected a point mutation in *gyrA*, T83I, which most likely is the true explanation of ciprofloxacin resistance in this strain. Variations between databases can be found. For example, the AMR phenotype associated with the gene *armA* by ResFinder was amikacin and tobramycin, while AMRFinder predicted resistance to gentamicin. Moreover, the phenotypic prediction for *aac(6')-Ic* in ResFinder is resistance to amikacin and tobramycin, while AMRFinder only provides aminoglycoside as a subclass. Another example, the *smeF* gene in *Stenotrophomonas maltophilia*, responsible for quinolones resistance (Jia et al., 2015) was detected only by AMRFinder. *smeF* was detected in only one strain in this study, and that

resulted in a major error, thus explaining the difference in MEs between ResFinder and AMRFinder for ciprofloxacin for our dataset (7 MEs in ResFinder and 8 MEs for AMRFinder) [**Figure (2)**].

Pseudomonas and *Acinetobacter* spp. had poor concordance in this study and previous studies, so we need to track causes of discordance in such species (Eladawy et al., 2023; Ellington et al., 2017; Rebelo et al., 2022). In contrast, *Enterobacterales* seemed to be more promising for AMR prediction. Previously, urine was the most common site of discordance for both *Enterobacterales* and *Pseudomonas aeruginosa*. However, while *Pseudomonas* isolates exhibited a significant rate of discordance between sites, this was not observed for *Enterobacterales* (Ku et al., 2021).

Creating a suitable framework to integrate *in silico* AMR findings into clinical settings is a must. Despite its evident potential, the existing public methods, especially databases, are not ready-made tools for predicting AMR from WGS data universally in a clinical context.

Conclusion

Our study provides crucial insights into the genomic and AMR landscape of CAUTI-associated pathogens in Egypt, highlighting significant phenotype–genotype discordance in AMR profiling. The discovery of novel AMR variants and sequence types underscores the genomic diversity of Gram-negative uropathogens and the evolving resistance mechanisms in this LMIC. These findings reinforce the need for integrative phenotypic and genomic surveillance strategies to improve AMR diagnostics, guide treatment decisions, and inform infection control policies in resource-limited settings. Promoting Egypt's national action plan for AMR, in partnership with the WHO, among healthcare professionals in hospitals and communities is essential to reduce the risk of bacterial resistance, especially among infants and elderly patients in Egypt (WHO, 2018b).

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ME – did all phenotypic work; extracted DNA for sequencing; all bioinformatics associated with clinical isolates; characterized the AMR and virulence genes encoded by the isolates and their plasmids; MLST analysis and summary; interpreted virulence and AMR data; pangenome analysis. JCT – MinION sequencing and hybrid genome assembly; pangenome analysis and associated supervision. NH, DN – library preparation and genome sequencing of some clinical isolates; LH – sourmash analyses; analyses of megaplasmid sequences; supervised the study. ME, JCT and LH wrote the original version of the manuscript, and all authors approved the final version.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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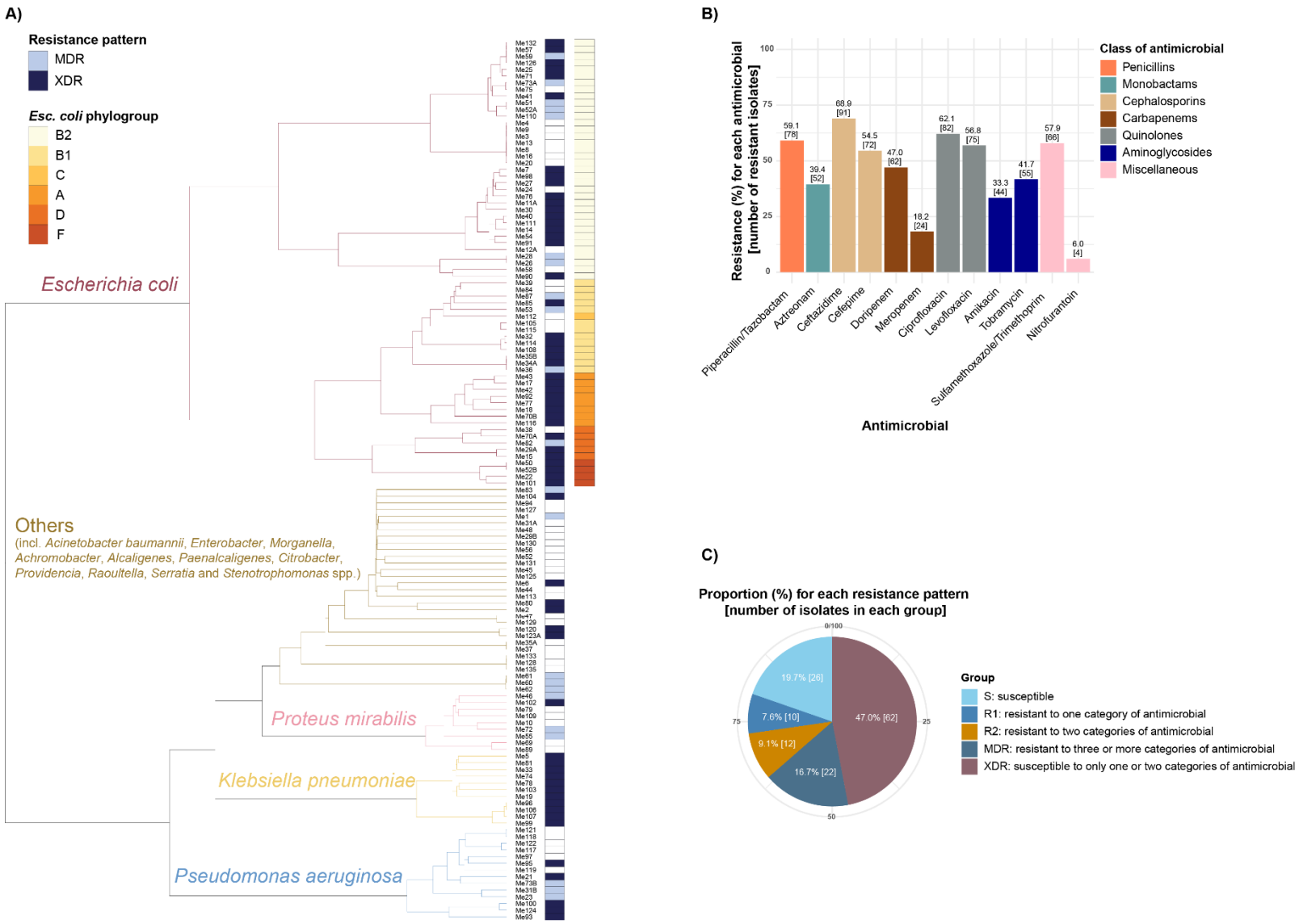
A)
Resistance pattern
MDR
XDR
Esc. coli phylogroup
B2
B1
C
A
D
F
Escherichia coli
Others
(incl. Acinetobacter baumannii, Enterobacter, Morganella, Achromobacter, Alcaligenes, Paenalcaligenes, Citrobacter, Providencia, Raoultella, Serratia and Stenotrophomonas spp.)
Proteus mirabilis
Klebsiella pneumoniae
Pseudomonas aeruginosa
B)
Resistance (%) for each antimicrobial [number of resistant isolates]
100
75
50
25
0
59.1 [78]
39.4 [52]
68.9 [91]
54.5 [72]
47.0 [62]
18.2 [24]
62.1 [82]
56.8 [75]
33.3 [44]
41.7 [55]
57.9 [66]
6.0 [4]
Piperacillin/Tazobactam
Aztreonam
Ceftazidime
Cefepime
Doripenem
Meropenem
Ciprofloxacin
Levofloxacin
Amikacin
Tobramycin
Sulfamethoxazole/Trimethoprim
Nitrofurantoin
Antimicrobial
Class of antimicrobial
Penicillins
Monobactams
Cephalosporins
Carbapenems
Quinolones
Aminoglycosides
Miscellaneous
C)
Proportion (%) for each resistance pattern [number of isolates in each group]
0/100
25
50
75
19.7% [26]
47.0% [62]
16.7% [22]
9.1% [12]
7.6% [10]
Group
S: susceptible
R1: resistant to one category of antimicrobial
R2: resistant to two categories of antimicrobial
MDR: resistant to three or more categories of antimicrobial
XDR: susceptible to only one or two categories of antimicrobial

967

968

Figure (1): AMR profiles of uropathogens included in this study. A) Dendrogram generated from comparison of sourmash signatures for uropathogens showing their species affiliations and whether they are MDR or XDR. Phylogroup affiliations are given for *Esc. coli* isolates. B) Resistance summary for the 132 uropathogens using seven different antimicrobial classes according to EUCAST guidelines. (C) Data presented using the category designations suggested by (Bitew & Tsige, 2020; Magiorakos et al., 2012). Penicillins: piperacillin/tazobactam. Monobactams: aztreonam. Cephalosporins: ceftazidime and cefepime. Carbapenems: doripenem and meropenem. Quinolones: ciprofloxacin and levofloxacin. Aminoglycosides: amikacin and tobramycin. Miscellaneous: sulfamethoxazole/trimethoprim and nitrofurantoin.

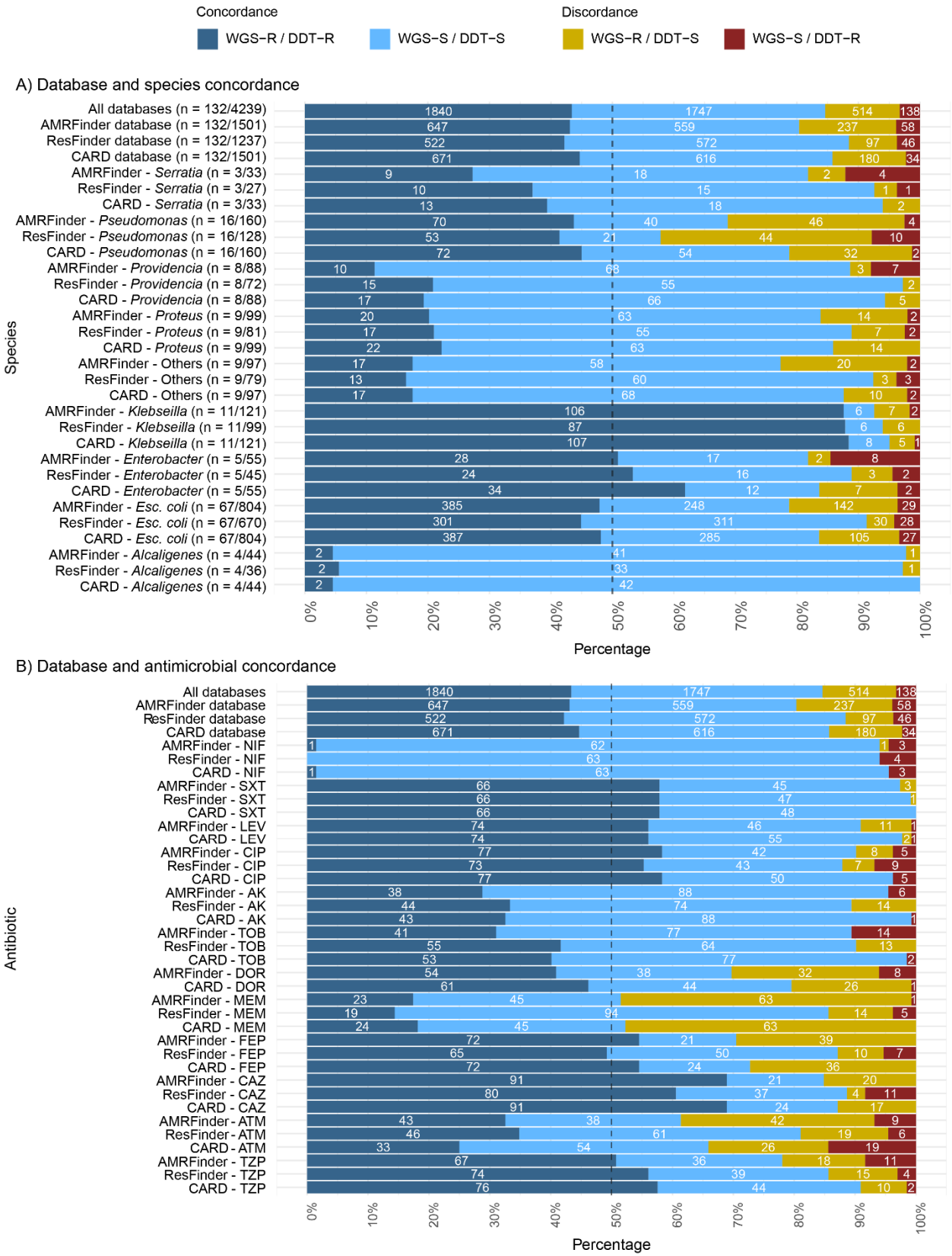
Concordance
WGS-R / DDT-R
WGS-S / DDT-S
Discordance
WGS-R / DDT-S
WGS-S / DDT-R
A) Database and species concordance
Species
All databases (n = 132/4239)
AMRFinder database (n = 132/1501)
ResFinder database (n = 132/1237)
CARD database (n = 132/1501)
AMRFinder - Serratia (n = 3/33)
ResFinder - Serratia (n = 3/27)
CARD - Serratia (n = 3/33)
AMRFinder - Pseudomonas (n = 16/160)
ResFinder - Pseudomonas (n = 16/128)
CARD - Pseudomonas (n = 16/160)
AMRFinder - Providencia (n = 8/88)
ResFinder - Providencia (n = 8/72)
CARD - Providencia (n = 8/88)
AMRFinder - Proteus (n = 9/99)
ResFinder - Proteus (n = 9/81)
CARD - Proteus (n = 9/99)
AMRFinder - Others (n = 9/97)
ResFinder - Others (n = 9/79)
CARD - Others (n = 9/97)
AMRFinder - Klebseilla (n = 11/121)
ResFinder - Klebseilla (n = 11/99)
CARD - Klebseilla (n = 11/121)
AMRFinder - Enterobacter (n = 5/55)
ResFinder - Enterobacter (n = 5/45)
CARD - Enterobacter (n = 5/55)
AMRFinder - Esc. coli (n = 67/804)
ResFinder - Esc. coli (n = 67/670)
CARD - Esc. coli (n = 67/804)
AMRFinder - Alcaligenes (n = 4/44)
ResFinder - Alcaligenes (n = 4/36)
CARD - Alcaligenes (n = 4/44)
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Percentage
B) Database and antimicrobial concordance
Antibiotic
All databases
AMRFinder database
ResFinder database
CARD database
AMRFinder - NIF
ResFinder - NIF
CARD - NIF
AMRFinder - SXT
ResFinder - SXT
CARD - SXT
AMRFinder - LEV
CARD - LEV
AMRFinder - CIP
ResFinder - CIP
CARD - CIP
AMRFinder - AK
ResFinder - AK
CARD - AK
AMRFinder - TOB
ResFinder - TOB
CARD - TOB
AMRFinder - DOR
CARD - DOR
AMRFinder - MEM
ResFinder - MEM
CARD - MEM
AMRFinder - FEP
ResFinder - FEP
CARD - FEP
AMRFinder - CAZ
ResFinder - CAZ
CARD - CAZ
AMRFinder - ATM
ResFinder - ATM
CARD - ATM
AMRFinder - TZP
ResFinder - TZP
CARD - TZP
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Percentage

Figure (2): Genotype–phenotype discordance among the 132 uropathogens. (A, B) Percentage and number of genotype–phenotype concordances and discordances are shown, when comparing WGS data and DDT results using ResFinder, CARD, and AMRFinder. The bars are coloured according to Concordance or Discordance (refer to colour legend at the top of the figure). White numbers on top of the bars correspond to the respective number of results. (A) Species-level data, (B) antimicrobial-level data. Numbers in parentheses in (A) correspond to the number of isolates per species / the number of isolate-antimicrobial combinations. (A) Others: *Paenalcaligenes suwonensis*, *Raoultella ornithinolytica*, *Citrobacter portucalensis*, *Leclercia adecarboxylata*, *Morganella morganii*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, and *Achromobacter xylosoxidans*. (B) Nitrofurantoin concordance was analyzed only via CARD/RGI. Levofloxacin and doripenem were not analyzed with ResFinder/PointFinder. Piperacillin/tazobactam (TZP), aztreonam (ATM), ceftazidime (CAZ), cefepime (FEP), doripenem (DOR), meropenem (MEM), ciprofloxacin (CIP), levofloxacin (LEV), amikacin (AK), tobramycin (TOB), sulfamethoxazole/trimethoprim (SXT), and nitrofurantoin (NIF). Major errors (ME) are WGS-R / DDT-S, while very major error (VME) are WGS-S / DDT-R.

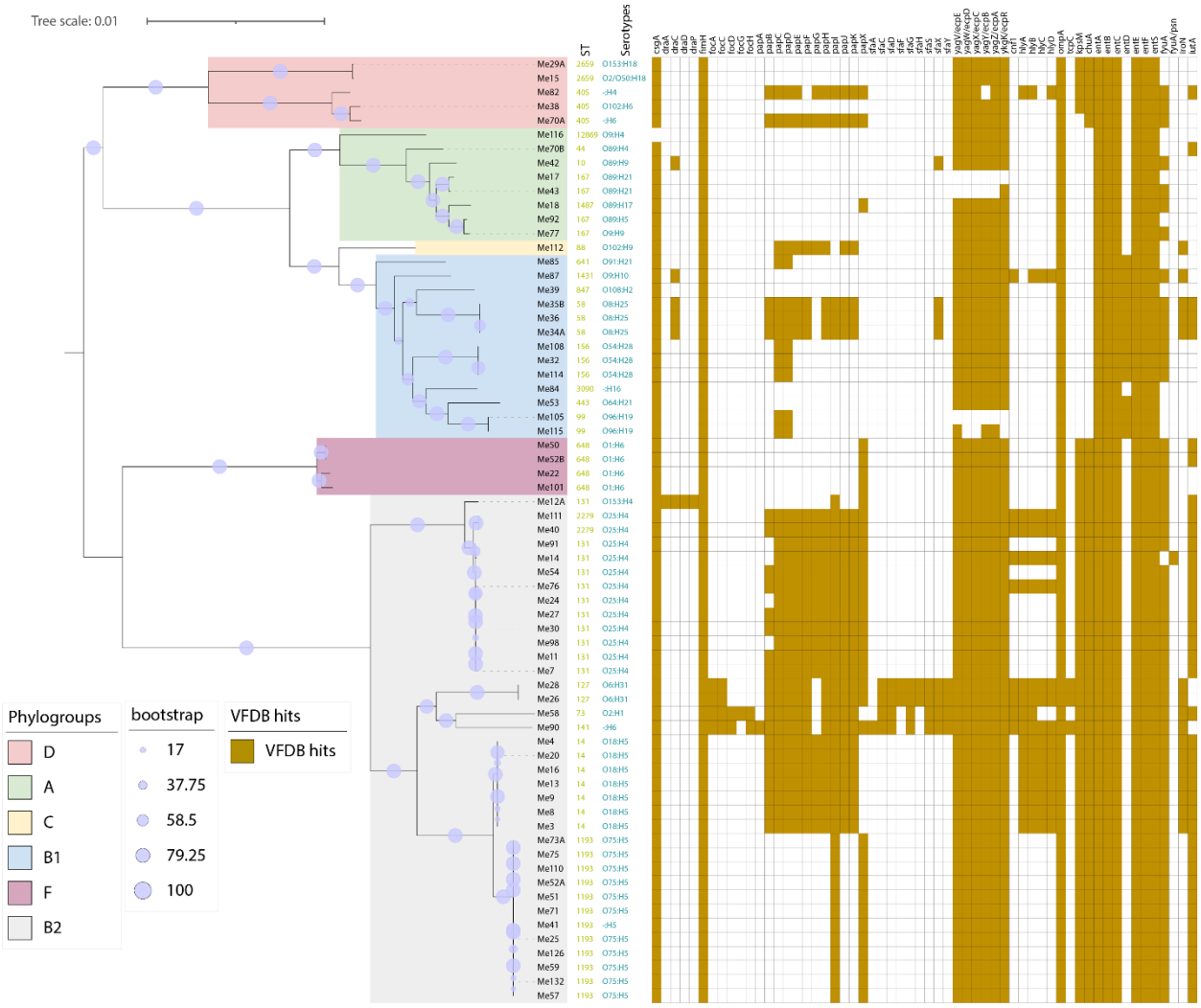

Figure (3): Phylogenetic tree for 67 *Esc. coli* isolates constructed using pangenome analysis of core genes, via panaroo and RaxML. UPEC strains are grouped into clusters (A, B1, B2, C, D, F) depending on EzClermont phylogroups. The maximum-likelihood tree was annotated using iTOL, with bootstrap values expressed as a percentage of 1000 replicates. Scale bar, nucleotide substitutions per site. Virulence genes were identified through BLASTp with VFDB based on coverage >= 90 % and identity >=70 %.

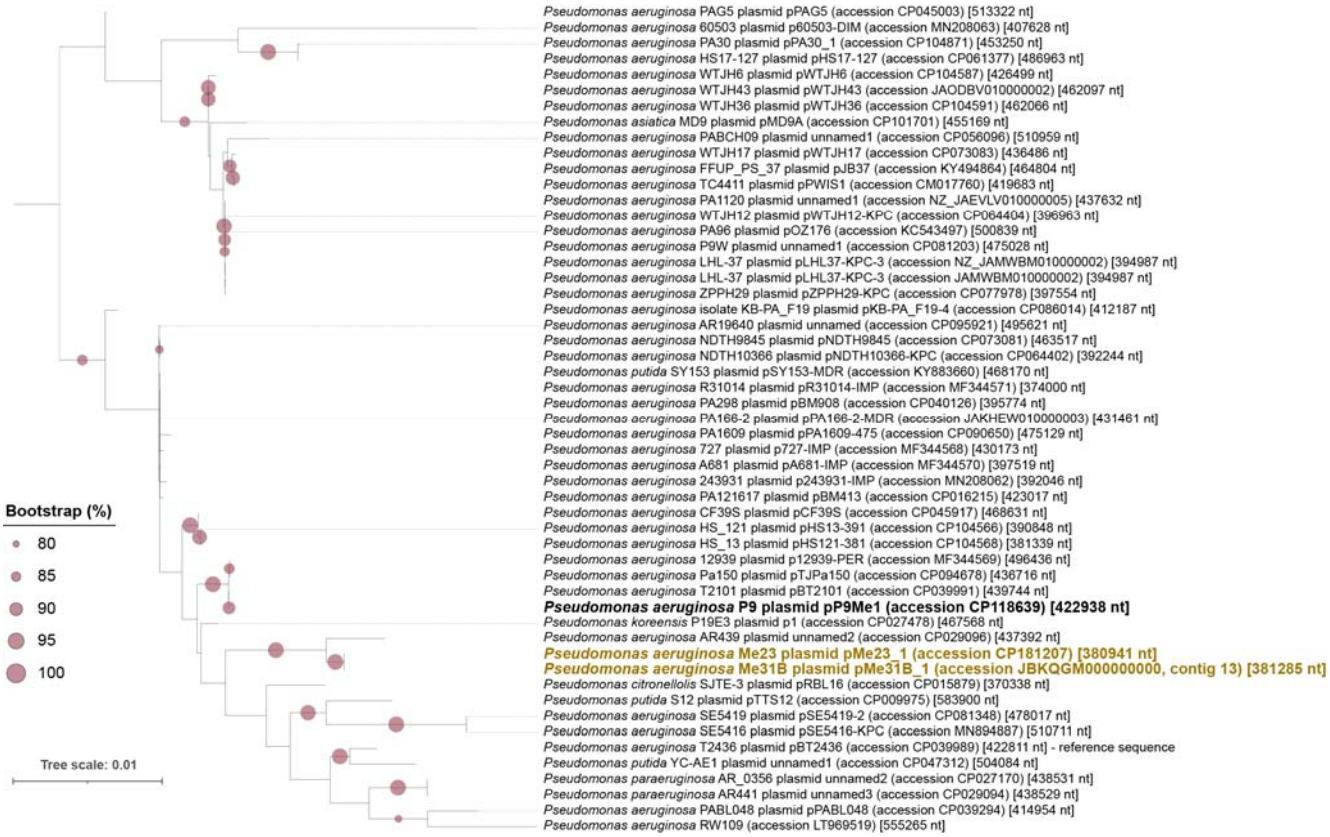

Figure (4): Phylogenetic (maximum likelihood) tree showing relationships of megaplasmids pMe23_1 and pMe31B_1 with other pBT2436-like megaplasmids. The tree, rooted at the midpoint, was built from a multiple-sequence alignment of 30,845 aa, comprising the sequences of 111/261 core proteins described by (Cazares et al., 2020). Megaplasmids shown in gold were found in Egyptian CAUTI isolates and newly sequenced in this study, while that in black bold type was recovered from an Egyptian CAUTI isolate we described previously (Eladawy et al., 2023). Scale bar, average number of amino acid substitutions per position. The tree shown represents the best-scoring maximum likelihood tree as determined using RAxML (parameters -f a -x 1). Bootstrap values determined based on 100 replications.