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RESEARCH ARTICLE

Detection of KPC-3 producing *Escherichia coli* ST410 in Volos, Greece

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ABSTRACT

Escherichia coli A382 was isolated in July 2024 from a positive blood culture obtained from the central venous catheter of a male patient undergoing chemotherapy at the Hospital of Volos, Thessaly, Greece. Whole-genome sequencing analysis revealed that the isolate A382 is *E. coli* belonging to the ST410 high-risk clone, which co-harbors the bla_{KPC-3} and $bla_{SHV-182}$ genes on an IncX3 plasmid. It also harbors bla_{TEM-1} and has five replicons, as follows: IncX3, IncQ1, CoIRNAI, IncF1A, and IncFIB. Complete genome analysis revealed that *E. coli* A382 isolate carries a range of virulence factors (*iutA*, *iucC*, *fimH*, *fdeC*, *yehA*, *yehD*, *yehC*, *yehB*, *cgs*, *ahha*, *ccI*, *hlyE*, *papC*, *irp2*, *fyuA*, *lpfA*, and *nlpl*) and many other non-beta-lactam resistance determinants, including *dfrA14* and *sul2*, but it is susceptible to aminoglycosides, nitrofurantoin, tigecycline, colistin and ceftazidime-avibactam. In conclusion in this study, we describe the phenotypic and genome characteristics of an extensively drug-resistant *E. coli* ST410.

KEYWORDS

Escherichia coli, KPC-3, bloodstream infection

INTRODUCTION

Escherichia coli is considered to be one of the most important pathogens for humans, as it causes a range of infections in humans, including urinary tract infections and other community-acquired infections [1]. The ability of *E. coli* to accumulate resistance genes, particularly those conferring resistance to beta-lactams, has led to increasing challenges in treatment. Among these resistance mechanisms, extended-spectrum beta-lactamases (ESBLs) such as CTX-M-15 are particularly prevalent [2]. However, the emergence of carbapenemase-producing *E. coli* has become a serious issue in the field of healthcare [3].

In addition to ESBLs, *E. coli* has also been increasingly associated with carbapenem resistance, primarily due to the acquisition of carbapenemase genes. Recent reports have shown the spread of bla_{OXA}, bla_{NDM} and the bla_{KPC} genes among *E. coli* isolated from patients in different geographical regions [4]. Initially identified in *Klebsiella pneumoniae*, KPC-type enzymes have now spread to *E. coli* and to other *Enterobacteriaceae*, mainly through plasmids and transposons [5–7]. In recent years, the presence of KPC in the *E. coli* sequence type 131 strain has raised concerns [8]. In Greece, the emergence of *E. coli* type 410 with KPC-2 lactamase was reported by Efthymia Petinaki et al. in 2010 [9]. However, *E. coli* ST410 strain is known to be associated with OXA, NDM and CTX-M-15 beta-lactamase production [10–12].

Beyond beta-lactamases, *E. coli* can harbor other resistance mechanisms, including aminoglycoside-modifying enzymes, which inactivate aminoglycosides, efflux pumps and mutations in target sites such as *gyrA* and *parC,* leading to fluoroquinolone resistance.

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Additionally, resistance to colistin a last-resort antibiotic, has emerged through modifications in the *pmrAB* and *phoPQ* regulatory systems, and by acquisition of the *mcr*-1 gene [10–13]. The accumulation of multiple resistance determinants within a single strain can lead to extensively drug-resistant (XDR) phenotypes, significantly narrowing the options for effective treatment [12, 13].

This study presents the phenotypic and genomic characterization of A382 an XDR *E. coli* ST410 strain isolated from a patient in Volos, Greece. Whole genome analysis of the A382 strain revealed multiple resistance genes to beta-lactams like $bla_{\text{KPC-3}}$, $bla_{\text{CTX-M-15}}$, and to other classes of antibiotics, like *dfr*A14 and *sul2*. It also revealed the presence of multiple virulence genes like *iutA* and *iucC* for the synthesis and transport of iron transferring molecule aerobactin.

MATERIALS AND METHODS

Collection of strain

The strain was recovered from a blood culture of a male patient who was receiving chemotherapy in the Hospital of Volos, Greece, representing an *E. coli* isolate fulfilling the phenotypic criteria for carbapenemase production.

Susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using an automated method with a Vitek-2 system (Biomerieux). The MIC of ceftazidime-avibactam was determined with E-test (Biomerieux). The MIC of colistin was determined using the broth microdilution method. The interpretive criteria of the European Committee on Antimicrobial Susceptibility testing (EUCAST) were used (https://www.eucast.org/clinical_breakpoints (accessed on 14-07-2024)).

Whole-genome sequencing

For genome sequencing, total DNA was extracted using a PureLinkTM Quick Gel Extraction Kit (Life Technologies, CA, USA). Whole-genome sequencing was performed in a private laboratory in Greece (Cemia). Libraries were prepared using Ion Torrent technology and Ion Chef workflows (Thermo Scientific). Sequencing was performed using the S5XLS system and the analysis of primary data was conducted with the Ion Torrent Suite software (v.5.10.0). Resistance genes were identified using Resfinder-4.6.0. Mobile genetic elements were identified using MobileElementFinder-1.0.3. The core genome ST was identified using the cgMLSTFinder-1.2 Server. The replicons were identified using the PlasmidFinder-2.0 Server. The CH type was identified using the CHTyper-1.0 Server. CHTyper is a web tool for the subtyping of extra-intestinal pathogenic *E. coli* based on the *fum*C and *fim*H alleles [14]. The pathogenicity was predicted using PathogenFinder. Finally, the phylogroup was predicted using Clermon typing [15].

RESULTS AND DISCUSSION

The isolate exhibited resistance to cefotaxime, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ertapenem, trimethoprim-sulfamethoxazole and ciprofloxacin; it exhibited sensitivity to aminoglycosides, nitrofurantoin, tigecycline, colistin and ceftazidime-avibactam (Table 1).

Genotyping of *E. coli* A382 indicated that it has a genome size of 4,872,137 bp and G+C content of 57.16% (Table 2). The isolate belonged to the sequence type (ST) ST410, according to the MLST allelic profile of Achtman's scheme, which uses the sequences of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*). The *E. coli* of ST410 lineage has presented increasing worldwide spread and, due to its association with multiple antibiotic determinants and its efficient escalation in healthcare settings, it is now considered a highly successful pandemic clone compared to ST131. This international clone has shown a global context in 14 countries encompassing Europe, North and South America, Asia, and Africa [16]. In Greece the *E. coli* of ST410 lineage appeared in 2010 [9]. The persistence of this lineage in 2024 with the acquisition of $bla_{\text{KPC-3}}$ gene is of great concern.

The detection of *fimH* demonstrated that the isolate carries the *fimH24* allele, resulting in the clonotyping CH4-24 type. The *fimH24* subtype has been described by Roer et al. as a successful sub-clonal lineage among ST410 *E. coli* strains [14].

Table 1. Susceptibility of *E. coli* ST410 strain A382 to antibiotics

Antimicrobial	MIC (mg L^{-1})
Ampicillin	≥32
Ampicillin/Sulbactam	≥32
Amoxycillin/Clavulanic Acid	≥32
Piperacillin	≥128
Piperacillin/Tazobactam	≥128
Cefuroxime	≥64
Cefuroxime Axetil	≥64
Ceftriaxone	8
Ceftazidime	16
Aztreonam	≥64
Ceftazidime/Avibactam	1
Cefotaxime	4
Ertapenem	2
Imipenem	≥16
Meropenem	≥16
Amikacin	≤2
Gentamycin	≤1
Tobramycin	≤1
Ciprofloxacin	≥4
Levofloxacin	≥8
Moxifloxacin	≥8
Tigecycline	≤0.5
Nitrofurantoin	≤16
Colistin	0.5
Trimethoprim	≥16
Trimethoprim/Sulfomethoxazole	≥320

Table 2. Genome of *E. coli* ST410 strain A382

Parameter	Values
# contigs	98
# contigs (≥0 bp)	124
# contigs (≥1,000 bp)	82
Largest contig	357,858
Total length	4,872,137
Total length (≥0 bp)	4,879,386
Total length (≥1,000 bp)	4,860,675
N50	147,993
N90	33,229
auN	148,317
L50	12
L90	39
GC (%)	50.62
Mismatches	
#N's per 100 kbp	0
#N's	0

According to Clermon typing, the strain belongs to phylogroup C. This phylogroup is correlated with hemolytic uremic syndrome in humans [15].

The genome of *E. coli* A382 demonstrated an MDR genotype, carrying genes conferring resistance to aminoglycosides (*aph(6)-Id*, *aph(3″)*), sulfonamides (*sul2*), trimethoprim (*dfrA14*), macrolides (*mph(A)*), tetracyclines (*tet(B)*), and chloramphenicol (*catA1*). In addition to $bla_{\text{KPC-3}}$, Resfinder identified $bla_{\text{SHV-182}}$ and $bla_{\text{TEM-1B}}$ (Table 3). Also, it carried point mutation in genes *par*C, *gyr*A conferring resistance to fluoroquinolones, *glpT* conferring resistance to fosfomycin.

Table 3. Resistance genes, point mutations, virulence genes and replicons in *E.coli* ST410 strain A382

1. Antibiotic resistance genes
1a. β-lactams
$bla_{\text{KPC-3}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{SHV-182}}$, $bla_{\text{SHV-159}}$, $bla_{\text{SHV-158}}$
1b. other antibiotics
*dfr*A14 trimethoprim
*sul*2 sulfamethoxazole
mph(A) erythromycin
*cat*A1 chloramphenicol
tet(B) tetracycline
2. Point mutations
parC, parE, gyrA, gyrB, acrR, acrB quinolone
*rpo*B rifamycin
glpT_E448K fosfomycin
pmrB_Y358N colistin
3. Tolerance to antiseptics:
*sit*ABCD hydrogen peroxide
4. Virulence-associated genes:
*iut*A aerobactin receptor synthesis; *iucC* aerobactin synthesis
*sit*A Iron transport protein, *irp2* high-molecular-weight protein 2 non-ribosomal peptide synthetase, *fyuA* siderophore receptor
*fim*H type 1 fimbriae, *papC* outer membrane usher P fimbriae, *lpfA* long polar fimbriae
*fde*C intimin like adhesin
yehA, *yehC*, *yehD, yehB* outer membrane lipoprotein, YHD fimbrial cluster
cgsA curlin major subunit
hha hemolysin expression regulator, *hly*E Avian *E. coli* hemolysin, *ccI* cloacin
nlpl lipoprotein precursor
5. Resistance to heavy metals:
*ter*C tellurium iron resistance protein
6. Replicons:
IncX3, IncQ1, CoIRNAI, IncF1A, and IncFIB

The presence of many virulence genes in the genome of *E. coli* A 382 indicates that it is an extraintestinal pathogen.

Five replicons were detected as follows: IncX3, incQ1, CoIRNAI, InCF1A, and IncFIB. The $bla_{\text{KPC-3}}$-carrying plasmid IncX3 has been acquired by *E. coli* ST410, which is known to be associated with CTX-M, KPC-2, and NDM production [16–20]. The fusion of $bla_{\text{KPC-3}}$ in a common pathogen, such as *E. coli,* has been earlier reported [13]. The core genome of *E. coli* A382 belongs to ST114429. The input organism was predicted as a human pathogen, with a probability of being a human pathogen of 0.874.

CONCLUSIONS

This study documented the acquisition of $bla_{\text{KPC-3}}$ by *E. coli* belonging to ST410. It is the first time that a $bla_{\text{KPC-3}}$ gene in an *E. coli* strain carried by transposon Tn4401 on an IncX3 plasmid has been reported in Greece. The persistence of this lineage in Greece since its first appearance in 2010 [9] indicates insufficient infection control in healthcare settings.

These findings, which suggest that the KPC-3-encoding transposon Tn4401 was acquired by an IncX3 replicon, highlight the continued need for molecular surveillance of multidrug-resistant pathogens. They also emphasize the growing clinical significance of the IncX3 plasmid family.

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Informed consent statement: Not applicable due to the retrospective nature of this study.

Data availability statement: The whole-genome shotgun sequence for the *E. coli* strain A382 was deposited in Genbank under the BioProject PRJNA1146892 accession number.

Conflicts of interest: The authors declare no conflicts of interest.

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