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Research article

Chromosomal and plasmid-encoded virulence and multidrug resistance of *Escherichia coli* ST58/24 infecting a 2-year-old sickle cell patient with sepsis in Kampala Uganda, East Africa

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ABSTRACT

Sepsis and drug resistance represent a complex of the most common global causes of mortality in intensive care units (ICUs) especially among patients with comorbidities. Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains are highly implicated in systemic infections, with multidrug resistance exacerbating the risk of chronic conditions and patient mortality. The diversity of virulence and evolution of multidrug resistance are yet to be fully deciphered. In this work, we aimed at unveiling the pathogens and their genomic determinants of virulence and drug resistance relevant to increased sepsis in a sickle cell child admitted to ICU. From a rectal swab, we isolated a strain of *E. coli* from the patient and phenotypically tested it against a panel of selected beta lactams, fluoroquinolones, macrolides, aminoglycosides and colistin. We then sequenced the entire genome and integrated multiple bioinformatic pipelines to divulge the virulence and multidrug resistance profiles of the isolate. Our results revealed that the isolate belongs to the sequence type (ST) 58/24, which (ST58), is a known ExPEC. With the use of PathogenFinder, we were able to confirm that this isolate is a human pathogen ($p = 0.936$). The assembled chromosome and two plasmids encode virulence factors related to capsule (antiphagocytosis), serum survival and resistance, type 6 secretion system (T6SS), multiple siderophores (iron acquisition), and biosynthetic gene clusters for polyketides and nonribosomal peptides exhibiting host cell damaging activity *in silico*. The genome also harbors multidrug resistance genotypes including extended spectrum beta lactamase (ESBL) genes such as *blaTEM*-1A/B, sulfonamide resistance genes *sul1/2*, fluoroquinolone resistance genes *dfrA5* and nonsynonymous mutations of the gene *pmrB*, conferring intrinsic colistin resistance. Conclusively, this pathogen holds the potential to cause systemic infection and might exacerbate sickle cell anemia in the patient. The virulence and multidrug resistance profiles are encoded by both the chromosome and plasmids. Genomic

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surveillance of pathogens with multidrug resistance among patients with commodities is crucial for effective disease management.

1. Introduction

Sepsis and multidrug resistance are among the major causes of patient mortality in intensive care units globally. Among other Enterobacteriaceae species, *E. coli* has been implicated in gastrointestinal tract, urinary tract, respiratory tract, blood stream infections and sepsis, with high mortality of patients in tertiary care hospitals worldwide [1,2]. Recently, multidrug-resistant *E. coli* has been implicated in sepsis, colonization of hospital devices and patients with comorbidities in the Mulago National Referral Hospital of Uganda [3,4]. This is strongly suggestive of possible occurrence of unnoticed *E. coli* pathogens in the clinics, which calls for attention especially to critical ICU-admitted patients for better intervention. Cumulative evidence has genetically and experimentally distinguished a high-risk group of *E. coli* categorized as extraintestinal pathogenic *E. coli* (ExPEC) strains, which portray the capacity to transition from gastrointestinal tract to multiple systems including the urinary tract, respiratory tract, the nervous system as well as the blood stream [5]. Very common virulence factors carried by most *E. coli* strains include fimbriae, capsular polysaccharides, lipopolysaccharide O antigens, invasion of brain endothelial cells (Ibes), toxins such as shiga-like toxin, secretion systems as well as siderophores such as enterobactin, yersiniabactin, aerobactin and salmochelin, among others [6]. Evidence shows that most blood stream infectious *E. coli* strains also possess multidrug resistance to common antibiotic regimes, posing threat to the management of nosocomial infections [7]. Recently from Uganda, *E. coli* isolates carrying extended spectrum beta-lactamases (ESBLs) [8,9] have been reported along with colistin resistant strains from Mulago hospital [10].

Despite increased evidence for prevalence of multidrug resistant blood stream pathogens, genomic information on virulence, serotypes and multidrug resistance profiles of pathogens afflicting patients with comorbidities is scarce. In this work, we aimed at unveiling the pathogens and their genomic determinants of virulence and drug resistance relevant to increased sepsis in a sickle cell child admitted to ICU. Here, we report the chromosomal and plasmid-encoded virulence and multidrug resistance profiles of *Escherichia coli* ST58/24, strain RSM044, associated with sepsis under sickle commodity from ICU in Uganda, East Africa.

2. Materials and methods

2.1. Patients' brief information

A 2-year-old female patient was admitted to the ICU in the Mulago National Referral Hospital for advanced respiratory support and other device supports such as urinary catheter, nasogastric tube, central line, peripheral line and tracheal tubes. The patient had sickle cell anemia comorbidity, without previous indwelling devices, ICU history and had not been under any antibiotic regimen before admission. Diagnosed with sepsis, the patient received an intravenous (IV) injection of levofloxacin.

Table 1
Antibiotic susceptibility test (AST), colistin MIC and mcr phenotypic test results.: Cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), imipenem (IMP), amoxicillin–clavulanate (AMC), piperacillin–tazobactam (TPZ), chloramphenicol (C), gentamycin (GEN), amikacin (AMK), trimethoprim-sulfamethoxazole, Ciprofloxacin (CIP), levofloxacin (LVX), fosfomycin (FF), Tigecycline (TIG), Colistin (COL).

AST				ESBL	Colistin resistance tests		
Antibiotic	Wt (μg)	ZI (mm)	Results	ESBL	MIC phenotype	MCR (CPD-E)	MCR (BMD)
AMC	20/10	18	S	Positive	R	Negative	Positive
CAZ	30	25	S				
CRO	30	20	I				
FEP	30	27	S				
CXM	30	8	R				
TPZ	100/10	22	S				
C	30	24	S				
GEN	10	18	S				
SXT	1.25/23.75	6	R				
LVX	5	21	I				
CIP	5	21	I				
TIG	5	18	S				
SXT	1.25/23.75	6	R				
AMK	10	19	S				
FF	200	20	S				
IMP	10	27	S				
COL	10	13	S				

Table interpretation keyStructured• ESBL: Testing results for extended spectrum beta lactamasesStructured• COL MIC: Phenotypic results from colistin MIC testing• MCR CPD-E: MCR Colistin Pre-Diffusion and inhibition with EDTA test• MCR BMD: MCR broth microdilution method, E-testStructured• S: SusceptibleStructured• R: ResistantStructured• I: Indeterminate phenotype.

2.2. Specimen collection and bacterial strain isolation

A sterile swab was carefully inserted into the anal canal, approximately one inch (2.5 cm) beyond the anal sphincter, rotated for approximately 10 s to sample the anal crypts, and then carefully removed to recover fecal specimens. Under ice, the specimens were moved from ICU to the Medical Microbiology Laboratory and kept in Brain Heart Infusion (BHI) containing 30 % glycerol until required for bacterial isolation. The swabs were suspended in 5 ml Buffered Peptone Water (BPW), vortexed for complete mixing, and incubated overnight for 24 h at 37 °C in aerobic conditions. Thereafter, an aliquot of 10 μl from each sample was inoculated onto an in-house selective MacConkey agar containing 5 μg/ml of colistin sulfate and 305 μg/ml of ampicillin, followed by aerobic incubation at 35–37 °C for 24 h. Then *E. coli* colonies were identified from colony appearance, colony characteristics, and biochemical tests including urease citrate, hydrogen sulfide gas and indole, motility in semi-solid agar, along with utilization fermentation patterns of sugars such as lactose, sucrose, and glucose.

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed on a panel of 16 antibiotics (Table 1) using the Kirby-Bauer disc diffusion method or the broth microdilution method (for colistin). The interpretation of susceptibility and resistance was based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). We also checked for the presence of plasmid mcr-mediated colistin resistance by using the Colistin Pre-Diffusion and inhibition with EDTA test (CPD-E), as described by Condor and colleagues [11]. *E. coli* strain ATCC 25922, known for intrinsic colistin resistance, was used to quality-control of the susceptibility testing, MIC colistin, and CPD-E test procedures according to CLSI and EUCAST guidelines. Additionally, broth microdilution method (BMD) was used as the reference susceptibility test method as recommended by CLSI and EUCAST for polymyxins. BMD was performed with a volume of 0.05–0.1 ml in plain microtitration trays, cation-adjusted Mueller-Hinton broth (CA-MHB), a range of 2-fold dilutions of polymyxins (ranging from 0.12 to 512 μg/ml), and a final bacterial inoculum of 5 × 105 CFU/ml in each well.

2.4. DNA extraction and whole genome sequencing

DNA extraction was performed using a ZymoBIOMICS DNA Miniprep Kit (ZR D4300), according to the manufacturer's instructions. Then a DNA library was constructed using a TruSeq DNA PCR-Free kit, and sequenced with the Illumina NovaSeq 6000 platform, producing paired-end 21,743,824 with maximum sequence length of 151 bp with an average length of 149 bp.

2.5. Read quality control, genome assembly and annotation

Quality control of the raw reads was performed using FASTQC (v0.115) based on quality Phred score cutoff of 20 and maximum trimming error rate of 0.1. High quality reads were then *de novo*-assembled into contigs using Unicycler v0.48 [12], minimum contig length was set to be 300 bp. The contigs were further assembled into chromosomes with default parameters of MeDuSa v1.3 [13]. Plasmids were predicted using the Inc. typing method to search for corresponding plasmids from the PlasmidFinder database https://cge.cbs.dtu.dk/services/PlasmidFinder/. Individual chromosomes and plasmids were annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) available as a free service from the National Center for Biotechnology Information (NCBI) available at https://www.ncbi.nlm.nih.gov/genome/annotation_prok/.

2.6. Genome-based phylogrouping and serotyping

The Bacterial and Viral Bioinformatics Resource Center (BV-BRC, v3.32.13) (https://www.bv-brc.org/app/ComprehensiveGenomeAnalysis) was used for preliminary identification of relative strains and genomic virulence and antimicrobial resistance features. Then genome sequences of the relative strains were retrieved from NCBI and phylogenetically compared with our genome using the TYGS server (https://tygs.dsmz.de/). To predict relevant serotypes, assembled contigs were analyzed using SerotypeFinder-2.0 (https://cge.food.dtu.dk/services/SerotypeFinder/) in which the selected percentage identity threshold was set to 85 %, with minimum length of 60 %. We further used Orthovenn 3 (https://orthovenn3.bioinfotoolkits.net/document) to predict shared orthologous clusters between our isolate and other strains recovered from blood stream infections. To predict sequence types (STs), the genome was analyzed with multilocus sequence typing (MLST) pipelines available from http://mlst.warwick.ac.uk/mlst/dbs/Ecoli and http://bigsdb.web.pasteur.fr/ecoli/.

2.7. Genomic analysis of virulence factors

Virulence factors were first predicted from the virulence factor database (VFDB) using the VFanalyzer pipeline (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFanalyzer). Virulence factors from secondary metabolite gene clusters were further analyzed by subjecting the contigs to version 7 of the antibiotics and secondary metabolite analysis shell' (antiSMASH) for biosynthetic gene clusters (BGCs) through the polyketide synthase (PKS) and nonribosomal peptide synthetase (NRP) pathways (https://antismash.secondarymetabolites.org/#!/start). Molecular assembly and structural prediction of the encoded putative toxins were performed using the PRediction Informatics for Secondary Metabolomes (PRISM, v4.0) toolkit (https://prism.adapsyn.com/). The compounds structurally elucidated from PRISM were converted into the structured data file (SDF) format using PyMol (v2.4) available at PyMOL |

pymol. org. To find if these compounds can damage red blood cells, we retrieved spectrin, a component of RBC cytoskeletal system from the Protein Data Bank (PDB, https://www.rcsb.org/), removed water molecules and docked the structures using Autodock Vina under Seamdock [14] using the parameters: mode = 2, energy range = 5 and exhaustiveness = 10.

2.8. Genomic analysis of multidrug resistance analysis

Both chromosomes and plasmids were analyzed for resistance genotypes with respect to specific drugs using ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/) with default parameters as follows; selected percentage identity threshold for ResFinder was set to 90 %, minimum length of 60 %, selected percentage identity threshold for PointFinder was 90 % and minimum length for PointFinder was 60 %. The genome was further analyzed using the Resistance Gene Identifier (RGI) to predict resistome(s) based on homology and SNP models of the comprehensive antimicrobial resistance database (CARD) (https://card.mcmaster.ca/analyze/rgi). Selected resistance genotypes encoded by plasmids revealed by ResFinder analysis were annotated and mapped with PlasMapper (v3.0) available at https://plasmapper.ca/.

3. Results

3.1. Antimicrobial resistance phenotypes

While indeterminate phenotypes were observed for ceftriaxone, levofloxacin, and ciprofloxacin, the isolate demonstrated susceptibility to 11 of the tested drugs but resistance to cefuroxime and cotrimoxazole and exhibited positive ESBL. The MIC results are shown in Table 1. However, conflicting results were observed with colistin, i.e. while colistin-susceptible under AST, it was resistant under MIC. While the isolate tested mcr-negative from the CPD-E method, it exhibited mcr-positive results the from BMD method.

3.2. Assembly and annotation results

Upon quality control, the reads were reduced to 14,033,900. Unicycler *de novo* assembly generated 471 contigs, with annotation features summarized in Supplementary Table 1. From search for incompatibility group I1 (IncI1) plasmids, the assembly matched with

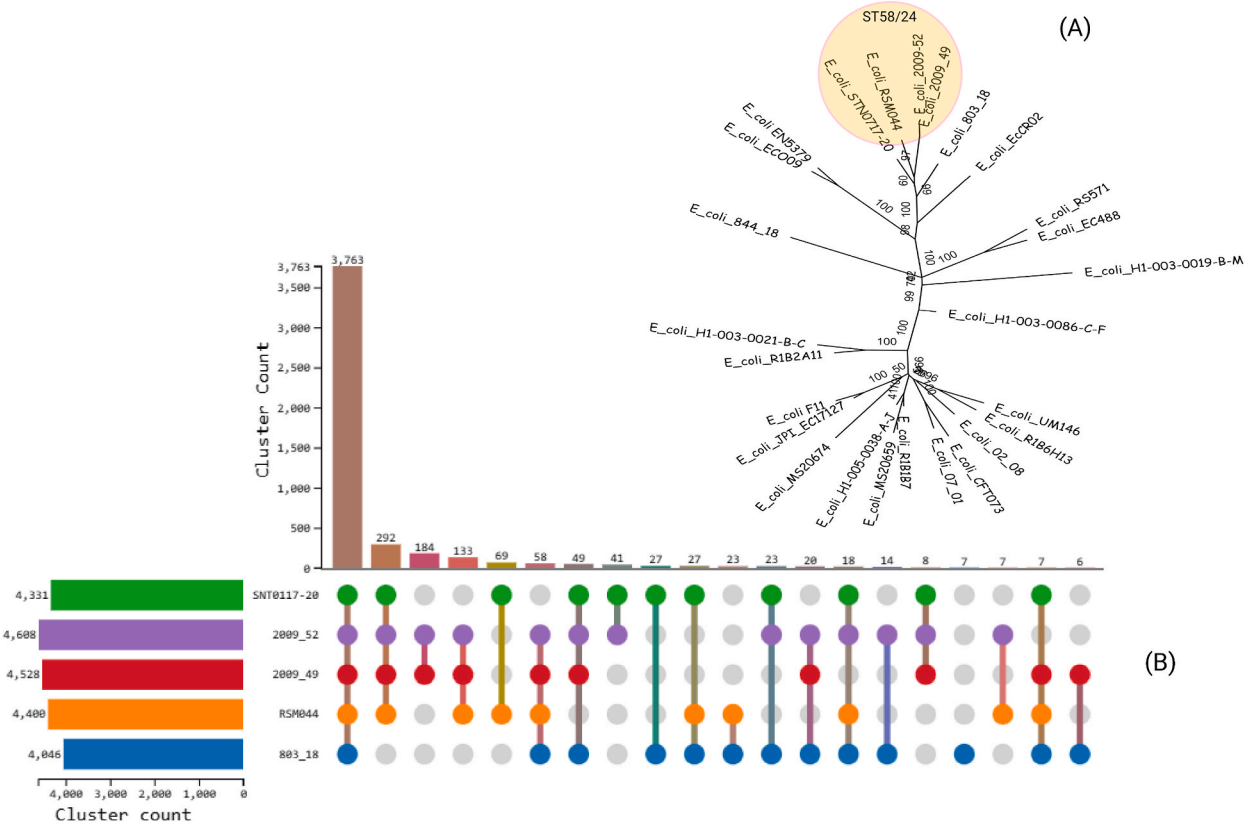
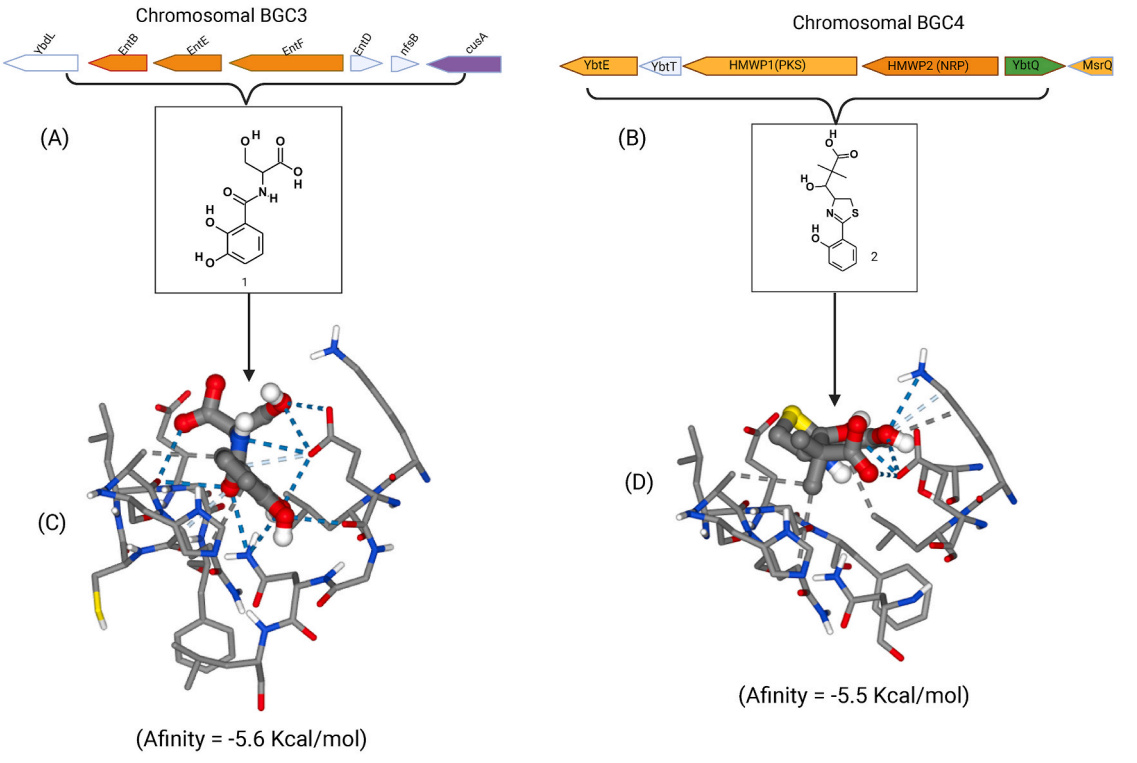
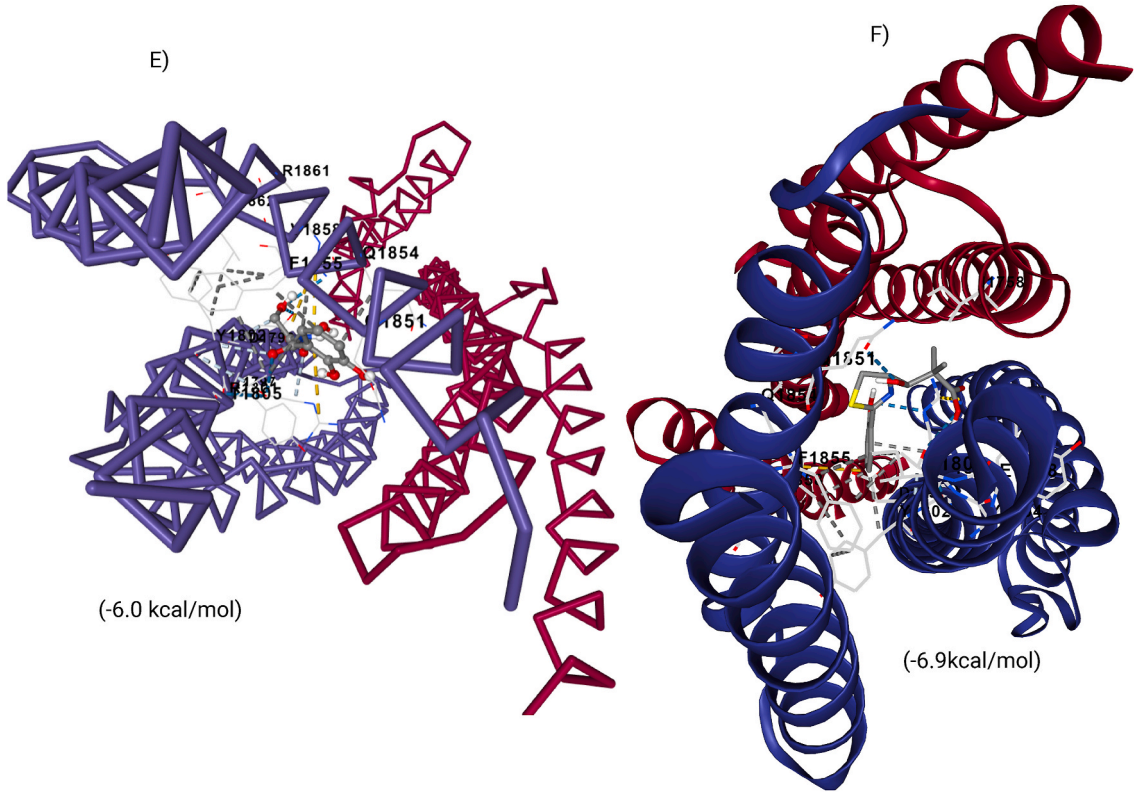


Fig. 1. Phylogrouping of *E. coli* strain RSM044 from proteome-based phylogeny generated from TYGS (A). The bar plot represents Orthovenn 3-uncovered shared orthologous clusters between *E. coli* strain RSM044 and the close relatives with which it clusters together (B).





(caption on next page)

Fig. 2. Biosynthetic gene clusters of compound 1 ((2-[(2,3-dihydroxybenzoyl)amino]-3-hydroxypropanoic acid), and compound 2 (3-(4,5-dihydro-2-(2-hydroxyphenyl)thiazol-4-yl)-3-hydroxy-2,2-dimethylpropanoic acid). The structure of each is shown below the cluster. Putative mechanism of RBC damage of each compound is shown by docking to spectrin alpha and beta domains with corresponding affinities (C–D). The interaction to ankyrin binding domain for each putative toxin is also indicated, with corresponding Vinna energy scores in kcal/mol (E–F).

three plasmid types, i.e., IncFII_1 (GenBank accession no. AY458016), IncFII_1 (GenBank accession no. AY458016), IncFIA/B _1 (GenBank accession no. AP001918), Col (pHAD28)_1 and (KU674895) also reported from other blood stream *E. coli* pathogens [15]. The two plasmids were successfully assembled and confirmed i.e., pRSM044_p1 (GenBank accession no. CP133102.1) and pRSM044_p2 (GenBank accession no. CP133103.1).

3.3. Phylogrouping and sequence typing

Combining TYGS proteome phylogeny, ANI, and MLST, the closest relatives include *E. coli* STN0717-20 (GenBank accession no. AP022482.1, ANI = 99.93), *E. coli* 2009–49 (GenBank accession no. NXEP00000000.1, ANI 99.90 %) and *E. coli* 2009–52 (GenBank accession no. NXEO00000000.1, ANI 99.90 %) (Fig. 1A). Initial MLST analysis utilized seven housekeeping genes namely *adk, fumC, gyrB, icd, mdh, purA* and *recA* to assign the organism to ST58. Alternatively, using eight genes *dinB*, *icdA*, *pabB*, *polB*, *putP*, *trpA*, *trpB* and *uidAI,* the organism was assigned to ST24, hence *E. coli* ST58/24. From SerotypeFinder analysis, the strain RSM044 is serotype H10/O8/O9/9a. i.e. H10 and O8 (% 100 % GenBank accession no. AB010150.1), O9 (99.7 % D43637.1) and O9a (99.92 % GenBank accession no. AB010293.1). These were inferred from correspondence to the genes required for lipopolysaccharide O antigen assembly namely ATP binding component of ABC-transporter (*wzt*), and integral membrane component of ABC-transporter (*wzm*). The relative strains STN0717-20, 2009–49 and 2009–52 are serotypes H10/O8 and H25/O8 respectively, associated with sepsis [16]. Analysis of orthologous clusters showed a close similarity with clinical strains associated with blood stream infection and extraintestinal invasion [15,16], sharing 3763 clusters (Fig. 1B). Existing datasets place ST58 together with extraintestinal pathogenic *E. coli* (ExPEC) lineages [5]. Five unique clusters were found, one with the role as an efflux pump (Supplementary Table 2) Combining our findings from SerotypeFinder with Orthovenn cluster of ortholog, we identify this pathogen as an ExPEC strain, similar to those characterized from other clinical reports [15,17].

From PathogenFinder analysis, the isolate was confirmed to be a human pathogen (p = 0.936). Relevant virulence factors include the colonization factor antigen I (*CFA/I*) fimbriae genes (*cfaA cfaB*, *cfaC*), well described in enterotoxigenic *E. coli* [18], also important in adherence. Others include *E. coli* laminin-binding fimbriae (ELF; *elfA*, *elfC*, *elfD*, *elfG*), adhesin (*upaG/ehaG*), *E. coli* hemorrhagic pili

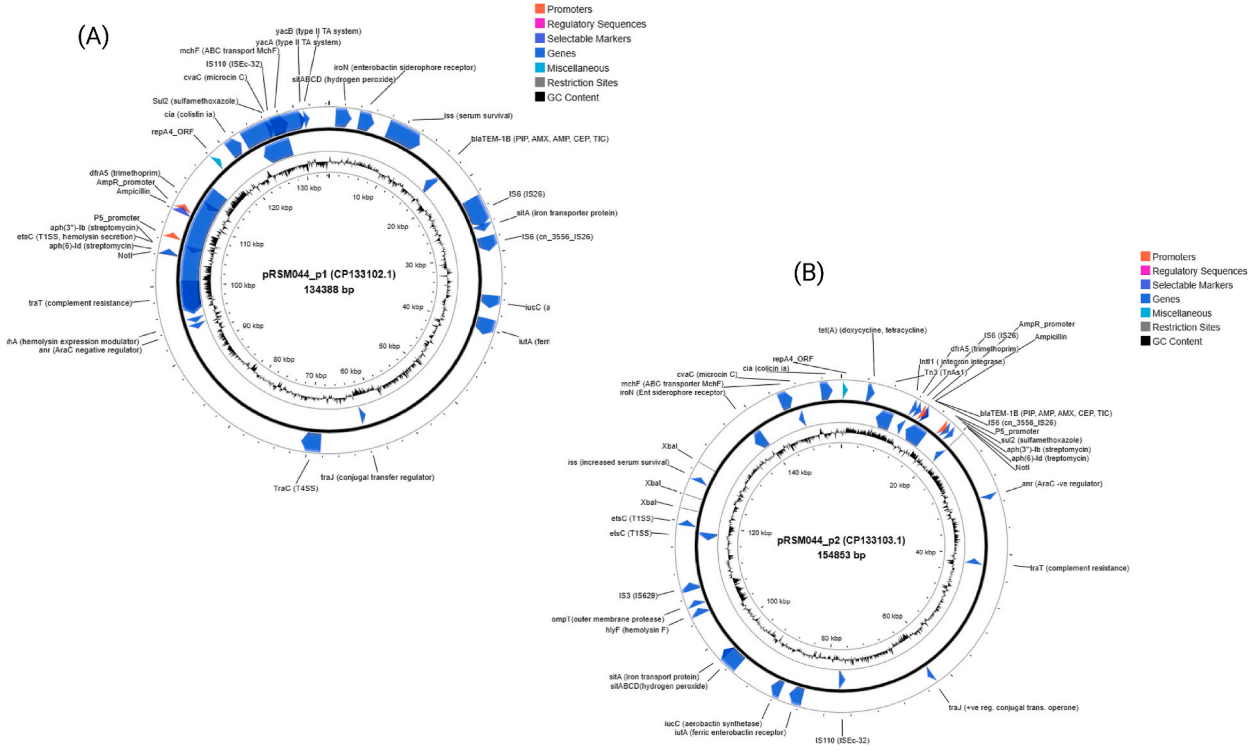


Fig. 3. Plasmid-encoded virulence and multidrug resistance (A–B) profiles. The position of each gene is indicated in blue. The profile of multidrug resistance is summarized in the comparative heatmap genotypes from CARD RIG analysis indicating the relatedness of *E. coli* strain RSM044 with other strains from other studies (C).

(*hcpA*, *hcpB*, *hcpC*) and the genes for type I fimbriae (*fimA*, *fimB*, *fimC*, *fimD*, *fimE*, *fimF*, *fimG*, *fimH*, *fimI*), which are implicated in hemorrhagic and disseminated infection [17]. The strain also carries capsular genes *wcaI*, *wzc*, *wzi*, and the serum resistance LPS rfb locus, all of which play role in polysaccharide assembly, promoting antiphagocytosis and survival in the blood stream [19]. Furthermore, The chromosome encodes the invasion of brain endothelial cells (*Ibes*) (*ibeB* and *ibeC*), causing systemic infection and meningitis [20]. Furthermore, type 6 secretion system (T6SS) was identified along with the toxin gene hemolysin/cytolysin A (*hlyE/clyA*), which could account for hemolysis and sepsis [21]. We observed that the most predominant genomic virulence factors of the stain RSM044 are iron uptake genes encoding iron/manganese transport, aerobactin, yersiniabactin and salmochelin, which are the most common siderophores enabling *E. coli* to acquire iron and survive in the blood stream, exacerbating sepsis [6,22].

Secondary metabolite BGCs for virulence factors detected from antiSMASH were enterobactin, yersiniabactin and aerobactin, as well as NRPS-independent siderophore. Chromosome analysis with PRISM predicted five clusters: cluster 1 (PKS), cluster 2 (Class II/III Confident Bacteriocin), cluster 3 NRP compound 1 (2-[(2,3-dihydroxybenzoyl)amino]-3-hydroxypropanoic acid), cluster 4 PK/NRP compound 2 (3-(4,5-dihydro-2-(2-hydroxyphenyl)thiazol-4-yl)-3-hydroxy-2,2-dimethylpropanoic acid), and cluster 5 NRPS-independent siderophore synthase (NIS). From all the five BGCs, BGC3 and BGC4, whose structures were successfully resolved are presented in Fig. 2 (A, B), while their corresponding putative modes of action are shown in Fig. 2 (C, D). We found that while compound 1 comes from the enterobactin BGC, the NR-PK compound 2, is closely related to metabolites in the yersiniabactin BGC characterized by Pfeifer and colleagues [23]. From our *in silico* molecular docking shown Fig. 2C-D, both compounds exhibit affinity (Vinna score: 5.6 and 5.5 kcal/mol) for alpha chain of human erythroid spectrin repeats 8 and 9 (PDB accession https://www.rcsb.org/structure/1S35), a protein essential in the stability of RBC cytoskeletal system [24]. Interestingly, the two metabolites exhibited higher affinity (Vinna score −6.0 to −6.9 kcal/mol) for the ankyrin binding domain (beta chain repeats 14 and 15) of spectrin (https://www.rcsb.org/structure/3F57) (Fig. 2E and F), suggesting inhibition of spectrin-ankyrin interaction necessary for RBC membrane integrity [24]. Details of forces of interaction and participating amino acids are presented in the Supplementary Table 3.

Notably, the plasmids harbor both antimicrobial resistance and virulence factors that enhance the pathogen's fitness to invade and survive in the gut, urinary tract and the blood stream (Fig. 3). These include the anticomplement TraT, ABC transporter protein MchF, putative type I secretion outer membrane protein etsC, and the increased serum survival protein iss, among others. Siderophore encoding virulence factor genes include *fyuA, irp1, irp2, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU,* and *ybtX,* which are known from blood steam infectious *E. coli* [5,25].

3.4. Antimicrobial resistance genotypes

From BV-BRC comprehensive genome analysis, we found that efflux pumps contribute to the most abundant mechanism, with up to 30 pumps (Fig. 3, Table 2). We also noticed that the genome of strain *E. coli* RSM044 carries multiple antibiotic inactivating genes, including those conferring aminoglycoside resistance [*H(3″)-I, APH(6)-Ic/APH(6)-Id*, *Mph(A)*] and beta lactamases of the *BlaEC*, *CTX-M* and *TEM*-families.

Chromosomal ResFinder revealed the AMR operon *sitABCD* (sitABCD_AY598030), conferring hydrogen peroxide resistance. Our CARD analysis revealed that the chromosome carries multiple peptide antibiotic resistance genotypes, conferring a target modification mechanism of polymyxin resistance [15,26]. Established mechanisms of resistance the polymyxin, colistin involve cell wall lipopolysaccharide (LPS) lipid A modification through addition of phosphoethanolamine (PEtN) by phosphoethanolamine transferase and 4-amino-4-deoxy-L-arabinose (L-Ara4N) by the genes *EptA*, *EptB*, *pmrCABCD* and *ArnT*, among others [26,27]. Here we detected nonsynonymous mutations of the gene *pmrB* (D283G and Y358 N), known to confer colistin resistance in mcr-negative *E. coli* [28]. Other mutations involved the beta-lactamase *ampC* promoter (g.-18G > A), *parC*:p.E62K, *ampC*-promoter (g.-1C > T), commonly associated with multidrug resistance among *E. coli* strains [29].

Our findings show that while the chromosome is devoted to multidrug efflux pumps and peptide antibiotic resistance, the largest proportion of RSM044 antimicrobial resistome is plasmid-encoded, involving resistance to beta lactam antibiotics, fluoroquinolones, macrolides, aminoglycosides and sulfonamides. The first plasmid, pRSM044_p1 harbors multiple AMR genotypes including the operon

Table 2
Genotypes underlying multidrug resistance recovered from BV-BRC comprehensive genome annotation. Only genotypes related to mechanism of drug resistance were selected for presentation.

AMR mechanism	Gene
Antibiotic activation enzyme	KatG
Antibiotic inactivation enzyme	APH(3″)-I, APH(6)-Ic/APH(6)-Id, BlaEC family, CTX-M family, Mph(A) family, TEM family
Antibiotic resistance gene cluster, cassette, or operon	MarA, MarB, MarR
Antibiotic target modifying enzyme	Erm(B)
Antibiotic target protection protein	BcrC
Efflux pump conferring antibiotic resistance	AcrAB-TolC, AcrAD-TolC, AcrEF-TolC, AcrZ, EmrAB-TolC, EmrD, EmrE, EmrKY-TolC, MacA, MacB, MdfA/Cmr, MdtABC-TolC, MdtEF-TolC, MdtL, MdtM, QacE, SugE, Tet(A), TolC/OpmH
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, PgsA
Regulator modulating expression of antibiotic resistance genes	AcrAB-TolC, EmrAB-TolC, GadE, H-NS, OxyR

sitABCD (hydrogen peroxide), aminoglycoside O-phosphotransferases *APH(3″)-Ib* and *APH(6)-Id (*streptomycin*)*, sulfonamide-resistant dihydropteroate synthase *sul2* (sulfomethoxazole), trimethoprim-resistant dihydrofolate reductase *dfrA5* (trimethoprim) and the broad-spectrum class A beta-lactamase *TEM-1* (*blaTEM-1A*), which confers resistance to amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin (Fig. 3A). The second plasmid pRSM044_p2 carries six AMR genes namely *sitABCD*, *Tet(A)* for doxycycline resistance, *sul2* for sulfamethoxazole resistance, *dfrA5* trimethoprim resistance, the tetracycline efflux MFS transporter *Tet(A)*, the broad-spectrum class B beta-lactamase *TEM-1 (blaTEM-1B*) conferring resistance to amoxicillin, ampicillin, cephalothin, piperacillin, and ticarcillin resistance, as well as the genes *aph(6)-Id* and *aph(3″)-Ib* for streptomycin resistance (Fig. 3B). In both cases, the *dfrA5* gene is flanked within a cassette of mobile genetic elements, i.e. insertion sequences IS6, IS26 and the class 1 integron (*intI1*), which strongly associated with increased carriage and expression of multidrug resistance genes including *dfrA* and some efflux pumps [16,17].

From orthologous cluster analysis five unique clusters were annotated as functionally related to periplasmic space, carbohydrate transport, two sequence-specific DNA binding proteins and a transmembrane transporter activity protein. The latter was identified as the multidrug resistance protein *MdtB*, comprising of the efflux RND transporter permease subunit subunits (GenBank accession nos. WP_307897709.1 and WP_307897711.1). This accounts for an intrinsic mechanism of multidrug resistance, also characterized from other bacterial strains [9].

4. Discussion

Sepsis is one of the most notorious causes of mortality among immunocompromised individuals as well as people with comorbidities [17,25]. Recently, *E. coli* ST58 was reported among the high risk extraintestinal pathogenic strains and has been strongly associated with sepsis [17]. This could explain why our ST58/24 isolate was associated with sepsis in this work. In addition, phylogenetic and MLST analyses have demonstrated clearly that this strain clusters with strains isolated and reported from blood stream infections [15,16]. Our findings, therefore, underscore the placement of the strain RSM044 within the ExPEC group, holding the potential to migrate from the gastrointestinal tract and cause blood stream infection in the sickle cell patient. The virulence factors T1SS, T6SS and hemolysin/cytolysin *hlyE/clyA* suggest that the pathogen is highly capable of disseminating across a range of systems and could be the cause of the septic diagnosis in this case [16]. Recent evidence from immuno-biochemical experiments verified the involvement of the protein TraT in infection complement resistance and serum survival [30]. This protein could also play a significant role as a virulence factor required for RSM044 blood stream survival, exacerbating the risk of anemia in the child. From molecular docking, the spectrin-binding of compounds 1 and 2 suggests that these metabolites could deployed to demolish RBCs, and thus increase the availability of iron to siderophores, aggravating the risk of anemia in the sickle cell patient. In addition, evidence shows that the virulence factors *iro*N and iss pose one of the highest mortality risks in patients with sepsis [19]. Thus, the possession of these virulence factors by this pathogen accounts for its mortality risk to this patient with sickle cell comorbidity. This calls for more attention to sickle cell patients, through improving our screening of bacterial infections for better management of the disease condition.

The chromosomal intrinsic mechanisms of multidrug resistance could be attributed to increased use of antibiotics in the clinical setting or transmission of the pathogens from the environment as recently reported from the same context [8], strongly suggesting existence of unnoticed antimicrobial resistant pathogens circulating in the clinic. This highlights the importance of genomic surveillance of multidrug resistant pathogens in leveraging therapeutics and management of patients with sepsis and commodities. Although indeterminate phenotypes were observed for the fluroquinolone antibiotics levofloxacin and ciprofloxacin, the multitude chromosomal resistance genotypes including *marA, emrA/B/R*, *mdtF*, *mdtM*, *AcrA/B* and *TolC*, should suffice to consider the strain RSM044 as a fluoroquinolone resistant pathogen.

The role of the phosphoethanolamine transferases *eptA*, *ArnT* and *pmrB* in colistin resistance has been clearly substantiated in *E. coli* [15,28]. Together' the *pmrB* mutations D283G and Y358 N, along with *eptA*, *ArnT* and *pmrF* could account for the colistin resistance phenotypically observed from MIC in this study. The *mcr*-positive results observed from MCR Phenotype (BMD) could be attributed to sequence homology between the *eptA, eptB* and/or *ArnT* and the *mcr* genes, suggesting their functional relatedness, including their role in colistin resistance [15,31]. Since most colistin resistance phenotypes have been attributed to plasmid-encoded mcr genes, this observation essentially underscores the role of chromosomal *mcr*-like genes in the colistin resistance repertoire. Therefore, our findings suggest that the isolation of *E. coli* ST58/24 from this patient is an important alarm for existence of virulent multidrug resistant ExPEC strains invading sickle cell patients and increasing the risk of patient mortality in the ICUs.

In conclusion**,** the strain isolated in this work is part of groups characterized by McKinnon et al. [16] and Reid et al. [32] from blood stream infections. These features are equivalently contributed by chromosomal and plasmid genes. Taken together, our findings strongly attribute *E. coli* strain RSM044 to the diagnosed septic condition of this patient, and that surveillance would enhance the efficacy of our management of sickle cell disease among patients.

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Ethical approval

Our project was approved by the Higher Degree and Graduate Research Ethics Committee (HDREC) of Makerere University College of Health Sciences: Approval number SBS-2021-47. The permission of study was obtained from Mulago National Referral Hospital Management, Mulago ICU leadership and Department of Medical Microbiology. Laboratory numbers but not names were used for identification of samples and consent from the by proxy were obtained before sample collection. Collected samples were coded and secured with restricted access.

Data availability statement

All nucleotide sequences used in this study were deposited into the National Center for Bioinformatics Information (NCBI), where the chromosome and the two plasmids are accessible at https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_030908565.1/.

CRediT authorship contribution statement

Reuben S. Maghembe: Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Conceptualization. **Maximilian A.K. Magulye:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Emmanuel Eilu:** Writing – review & editing, Validation, Resources, Methodology. **Simon Sekyanzi:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Funding acquisition. **Savannah Mwesigwa:** Writing – review & editing, Visualization, Supervision, Software, Data curation. **Eric Katagirya:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30187.

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