# Supplementary information to "Virulence factor genes in invasive Escherichia coli are associated with clinical outcomes and disease severity in patients with sepsis: a prospective observational cohort study"

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**Supplementary Table S1.** Prevalence of virulence factors in ExPEC according to the source

| DESIGNATION | ADHESIN | FUNCTION AND ASSOCIATED INFECTIONS | Feces (%) | Cystitis (%) | APN (%) | Blood (%) | LCS (%) |
|---|---|---|---|---|---|---|---|
| Type 1 fimbriae | *fimH* | Colonization factor and biofilm formation in EIs, especially cystitis and meningitidis, by binding receptor D-Mannose | 68 to 99 | 62 to 100 | 88 to 99 | 90 to 98 | 92 |
| Curli fimbriae | *csgAB* | Attachment and invasion of host cells, interaction with host proteins, activation of the immune system, bacterial aggregation and biofilm formation | 34 to 36 | 44 to 100 | 24 | 53 to 92 | NF |
| P fimbriae | *papGII* | Proinflammatory activity and colonization factor in EIs especially pyelonephritis by binding GbO4 and GbO3 | 17 to 23 | 13 | 64 to 67 | 48 to 68 | 20 to 50 |
| | *papGIII* | Proinflammatory activity and colonization factor in EIs especially cystitis by binding receptors GbO5 and GloboA | 8 to 9 | 22 | 19 to 35 | 14 to 27 | 1 to 6 |
| S fimbriae | *sfaS* | Adhesion to intestinal and urinary tract cells and facilitate penetration into the tissues by binding receptors NeuNAc (α2-3)Gal in UTIs, meningitidis | 7 to 32 | 13 to 34 | 88 to 99 | 39 to 97 | 28 to 59 |
| F1C fimbriae | *focG* | Adhesion to epithelial and endothelial cells in the bladder and kidney | 3 to 6 | 14 to 39 | 35 | 21 | 1 to 4 |
| Auf fimbriae | *auf* | Auf fimbriae | 21 to 27 | 67 | NF | NF | 62 |
| RTX protein TosA | *tosA* | Adhesion to host cells derived from the upper urinary tract, enhances survival in disseminated infections and lethality during sepsis | 11 | 16 | 29 | | |
| Dr fimbriae | *dra* | Binding to the receptors DAF of epithelial cells, type IV collagen and CEACAMs. Promotes host cells invasion | 6 | 0 to 4 | 6 to 12 | 5 | NF |
| Afimbrial adhesin | *afa* | Binding to receptor DAF on the epithelial cells and hemagglutination capacity | 2 to 17 | 19 to 81 | 16 | 49 | 9 |
| IrgA homolog adhesin | *iha* | Iron-regulated-gene-homolog adhesin | 14 to 24 | 22 | 41 | 33 to 40 | 19 |
| **DESIGNATION** | **INVASIN** | **FUCNTION AND ASSOCIATED INFECTIONS** | **Feces (%)** | **Cystitis (%)** | **APN (%)** | **Blood (%)** | **LCS (%)** |
| hemagglutinin | *hek/hra* | Promote autoaggregation, hemagglutination, epithelial cell invasion and is associated with bacteremia in newborns | 14 to 28 | 28 | 33 to 66 | 45 | 11 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Endothelial brain invasion | *ibeA* | Cell invasion into the host tissues such as endothelial cell by binding Vimentine and Caspr1 to promote penetration of the blood–brain barrier | 1 to 15 | 13 to 23 | 29 | 4 to 11 | 32 to 38 |
| **DESIGNATION** | **TOXINS** | **FUCNTION AND ASSOCIATED INFECTIONS** | **Feces (%)** | **Cystitis (%)** | **APN (%)** | **Blood (%)** | **LCS (%)** |
| α-hemolysin | hly | Pore-forming RTX toxin responsible for kidney injury and inflammation | 4 to 15 | 15 to 43 | 38 to 47 | 34 to 77 | 9 to 30 |
| Cytotoxic necrotizing factor 1 | cnf1 | activation of Rho GTPases, engaging in cell necrosis and promoting resistance to phagocytosis, inflammation, recurrent UTIs and blood-brain barrier penetration | 3 to 13 | 13 to 39 | 28 to 47 | 17 to 49 | 6 to 27 |
| Cytolethal distending toxin | cdt | DNAse inducing apoptosis and cellular senescence resulting in cell distention | 0 to 1 | 9 to 17 | 12 | 0 to10 | 46 |
| Colibactin | clb | PK-NRP compounds inducing DNA damage resulting preferentially in apoptosis in immune cells and premature cellular senescence in epithelial cells. | 11 to 32 | 33 | 44 | 18 to 58 | 75 |
| **DESIGNATION** | **Autotransporters** | **FUCNTION AND ASSOCIATED INFECTIONS** | **Feces (%)** | **Cystitis (%)** | **APN (%)** | **Blood (%)** | **LCS (%)** |
| Antigen43 | *agn43* | Autotransporter involved in auto-aggregation, adhesion to host cells, biofilm development and persistence | 41 | 29 to 94 | 65 | 69 | NF |
| Secreted autotransporter toxin | Sat | Autotransporter having serine protease activity inducing cytotoxic effect and kidney injury | 14 to 24 | 23 to 56 | 55 to 68 | 26 to 39 | 49 |
| Vacuolating autotransporter toxin | Vat | Autotransporter having serine protease activity affecting mucins, facilitating epithelium colonization and inducing host cell vacuolization | 0 to 49 | 42 to 68 | 44 | 12 to 71 | 51 to 76 |
| Serin protease autotransporter | pic | Autotransporter exhibiting serine protease activity affecting mucins, facilitating epithelium colonization and damaging host cell membrane | 0 to 23 | 21 to 34 | 14 to 40 | 0 to 36 | NF |
| Contact-dependent growth inibition | cdiAB | Induce the growth inhibition of the bacterial target | NF | NF | NF | NF | NF |
| **DESIGNATION** | **IRON UPTAKE** | **FUNCTION AND ASSOCIATED INFECTIONS** | **Feces (%)** | **Cystitis (%)** | **APN (%)** | **Blood (%)** | **LCS (%)** |
| Enterobactin | ent, fepA | Acquisition of siderophore Enterobactin-Fe3 + | 100 | 100 | * | * | * |
| Aerobactin | aer, iuc, iut | Acquisition of siderophore Aerobactin-Fe ions in the host involved in UTI | 16 to 41 | 24 ot 52 | 38 to 77 | 54 to 80 | 61 to 88 |

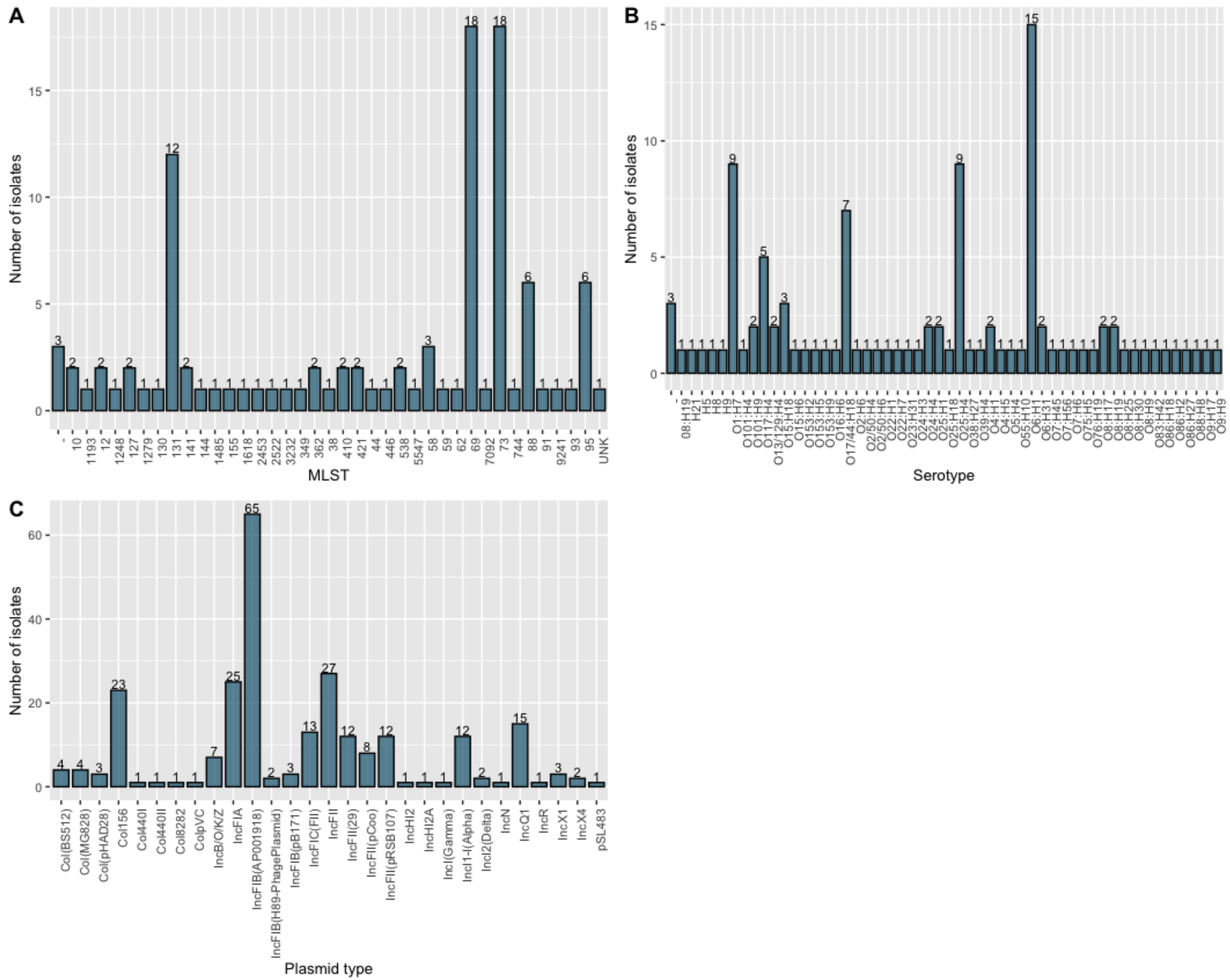
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Salmochelin | iroN | Acquisition of siderophore Salmochelin-Fe ions in the host involved in UTI and meningitidis | 5 to 26 | 33 to 74 | 76 to 78 | 44 to 63 | 38 to 75 |
| Yersiniabactin | irp, fuyA | Acquisition of siderophore Yersiniabactin-Fe ions in the host involved in UTI and meningitidis | 23 to 70 | 63 to 96 | 97 | 82 | 90 to 99 |
| ChuA, Hma | chu, hma | Acquisition of Fe from hemoglobin in the host system | 53 to 59 | 66 to 80 | 66 to 71 | 48 to 73 | 89 |
| Sit | sitDCBA | Transportation of Fe and Mn | 25 | 44 | 56 | 96 | 92 |
| IreA | ireA | Iron-regulated homolog of siderophore receptors involved in UTI | 7 to 19 | 13 | 47 | 32 | NF |
| K1 capsule | *kpsMI-neuA* | Enable resistance to complement activation and phagocytosis, immunological tolerance and intracellular survival promoting both bacteremia and meningitis | 0 to 27 | 35 | 13 to 47 | 8 to 82 | 75 to 90 |
| TcpC | tcpC | Impairs the innate immune response by inhibiting Toll-like receptor and MyD88-specific signaling | 8 | 22 | 42 | 32 to 39 | 0 |
| D-serine deaminase | dsdCXA | Promotes bacterial growth by using D-serine as carbon, nitrogen, and energy source, and prevent the bacteriostatic activity of D-serine in the urine and brain. | 0 to 4 | 20 to 22 | NF | 6 | 73 to 87 |
| TrapT | traT | Inhibition of the classical pathway of complement activity and serum survival | 43 to 88 | 36 to 68 | 49 to 81 | 50 to 81 | NF |
| Increased Serum Survival | Iss | Resistance to complement and serum survival | 4 to 20 | 13 | 35 | 10 to 34 | 25 |
| Outer membrane protein T | OmpT | Resistance to protamine and urine survival | 15 to 68 | 70 | 88 to 95 | 21 to 81 | 96 |

NF, data not found, *Present in almost all Escherichia coli genomes.

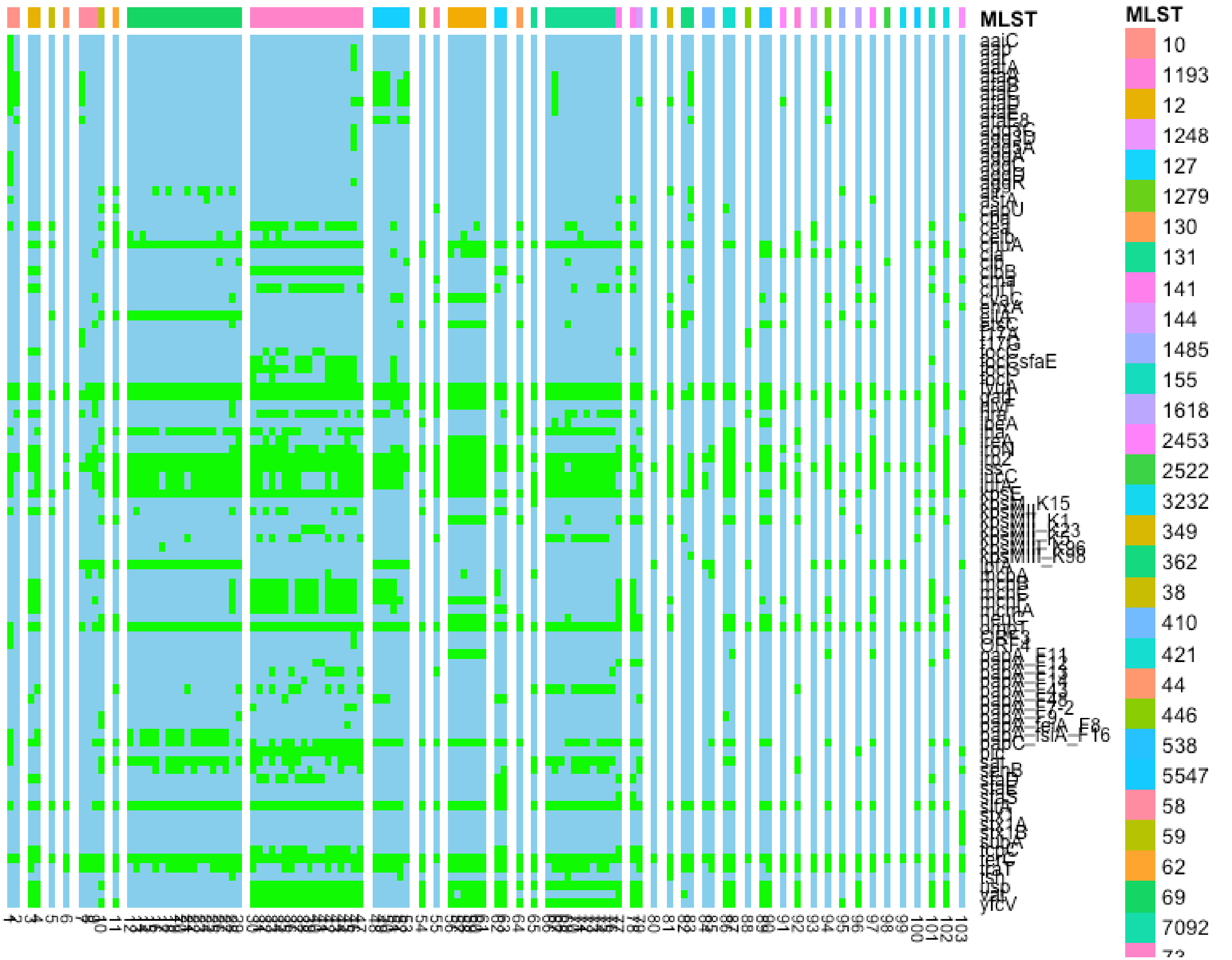
**Supplementary Table S2.** Literature search results

| PubmedID | Authors, year, journal | Country | Objectives |
|---|---|---|---|
| **Original research** | | | |
| 33235212 | Biggel M et al., 2020, Nat Commun | Belgium, Hungary Denmerk, Sweden UK, USA | a genome-wide association study to investigate the population structure, virulence determinants, and evolution of invasive UPEC isolates, as compared to non-invasive UPEC isolates |
| 32423949 | Fröding I. et al, 2020, Antimicrob Agents Chemother | Sweden | To study the molecular epidemiology of community-onset bloodstream infection caused by ESBL/pAmpC-EC |
| 22571989 | Skjøt-Rasmussen L et al., 2012, Int J Med Microbiol | Denmark | To study *E. coli* isolates from patients with bacteraemia of urinary tract origin according to virulence-associated genes, phylogroups, and antimicrobial resistance, and the relation of these factors to hospital- vs. community-acquired origin, sex, and mortality. |
| 31076319 | Hung WT et al. 2019, J Microbiol Immunol Infect | Taiwan | To study whether specific sequence type (ST) and VFs of extended-spectrum beta-lactamase-producing E. coli (ESBL-EC) are associated with different outcomes in patients with bloodstream infection. |
| 31245301 | Daga AP et al. 2019, Front Cell Infect Microbiol | Brazil | to characterize the VFs, antimicrobial sensitivity profile, and epidemiological data of E. coli isolates from the blood of patients |
| 23588104 | Rodríguez-Baño J et al., 2013, J Infect | Spain | To investigate the impact of VF and other microbiological determinants on the outcome of patients with invasive infections due to extended-spectrum β-lactamase-producing Escherichia coli |
| 26517481 | Cyoia PS et al., 2015, J Infect Dev Ctries | Brazil | To analyze the presence of VF encoding genes, PAI sequences and phylogenetic groups of ExPEC strains isolated from urine and blood of patients compare them with 50 faecal commensal strains from healthy individuals. |
| 33362261 | Paramita RI et al., 2020 Plos One | Indonesia | to characterize the serotypes, MLST, virulence genes, and antimicrobial resistance of E. coli isolated from bloodstream infection patients |
| 23801304 | Szemiako K et al., 2013, Eur J Clin Microbiol Infect Dis | Poland | To analyze the phylogenetic distribution and virulence genes of Escherichia coli isolates which predispose bacteria to translocate from the urinary tract to the bloodstream |

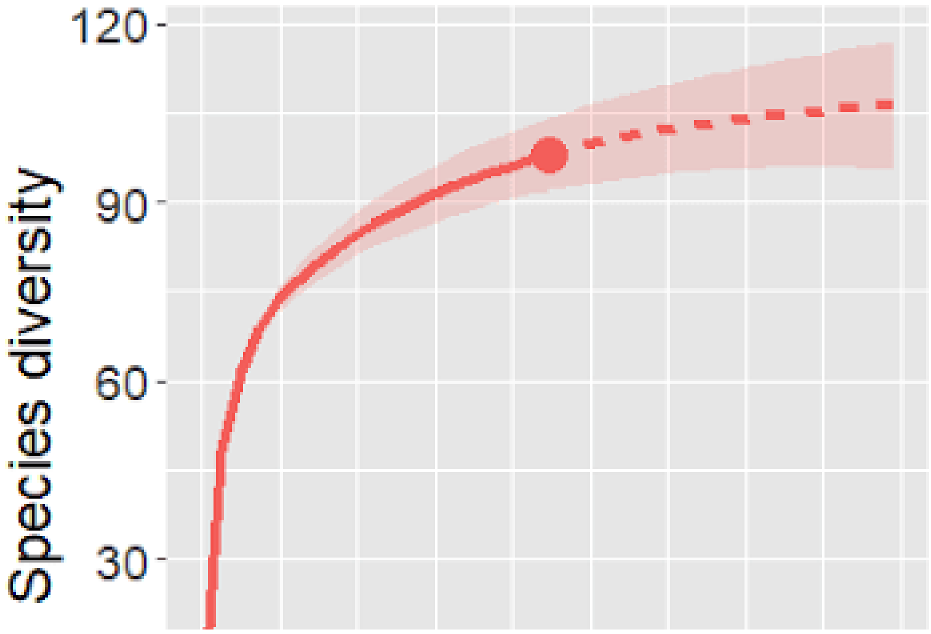
| | | | |
|---|---|---|---|
| 25654604 | Mora-Rillo M et al., 2015, Virulence | Spain | To study the characteristics of *E. coli* causing bacteremia and their relationship with sepsis severity and mortality. |
| **Reviews** | | | |
| 33101219 | Desvaux M et al., 2020, Front Microbiol | N.A. | To summarize the current knowledge on the structure and functional features of PAIs, on PAI-encoded E. coli pathogenicity factors and on the role of PAIs in host–pathogen interactions |



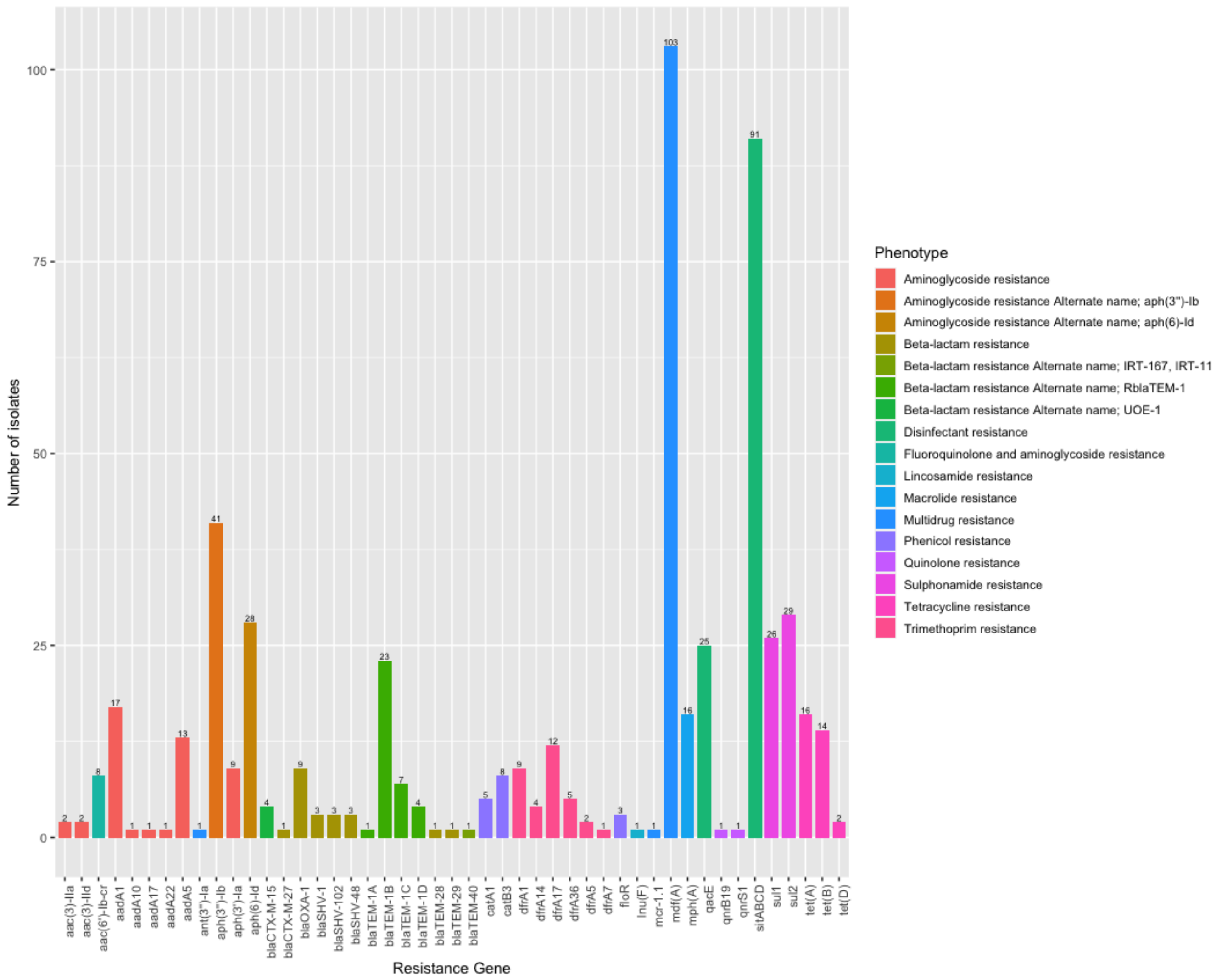

**Supplementary Figure S1. Isolate characteristics.** A: multilocus sequence type; B: serotype; C: plasmid types




**Supplementary Figure S2. Virulence factor genes per isolate per MLST.** The presence or absence of virulence factor genes is different between isolates within the same MLST. The isolates do not cluster per MLST.




**Supplementary Figure S3. Rarefaction curve.** The observed number of genes was plotted against the number of isolates. The solid line illustrates the observed species diversity, the dashed line the extrapolated diversity.




**Supplementary Figure S4. Antibiotic resistance genes** found in clinical isolates using the ResFinder database. Different colours represent resistance to different antibiotic classes.