

ESBL-producing and colistin-resistant Enterobacterales among Czech international travellers: genomic relatedness to community and environmental isolates[☆]

Suhanya Prasad^a, Barbora Dratvova^a, Milena Antuskova^a, Marie Brajerova^a, Elka Nycova^b, Eva Adamova^b, Milan Trojaneck^{c,d}, Jan Tkadlec^a, Pavel Drevinek^a, Marcela Krutova^{a,*}

^a Department of Medical Microbiology, Second Faculty of Medicine, Charles University and Motol University Hospital, Czech Republic

^b Department of Clinical Microbiology, University Hospital Bulovka, Czech Republic

^c Department of Infectious Diseases, University Hospital Bulovka, Czech Republic

^d Department of Infectious Diseases and Travel Medicine, Second Faculty of Medicine, Charles University and Motol University Hospital, Czech Republic

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ABSTRACT

Background: While antimicrobial resistance in Enterobacterales is closely monitored in healthcare settings, much less is known about the circulation of these bacteria in the community, particularly in high-risk groups such as travellers. This study aimed to determine the prevalence of intestinal carriage of extended-spectrum β -lactamase (ESBL)-producing, carbapenem- and colistin-resistant Enterobacterales in returning symptomatic Czech travellers, and to investigate whether travel contributes to colonisation with resistant strains distinct from those circulating in the Czech Republic.

Methods: Stool samples were cultured using a selective chromogenic medium following enrichment. Isolates with phenotypic resistance were characterised by short and long-read whole-genome sequencing. Genomes of ESBL and *mcr-1*-carrying *E. coli* isolates from travellers were compared with available genomes from various Czech sources, including wastewater treatment plants, hospital sewage, river water, patients with urinary tract infections, rectal swabs from medical students, and meat samples.

Results: Between September 2019 and October 2021, 165 stool samples (male = 91, 55.2 %, average age 41 years) were analysed. Thirty-two travellers (19.4 %) were culture-positive on the ESBL screening media, with ESBL genes identified in 17.6 % (29/165). The *bla*_{CTX-M-15} gene was dominant (59 %, 17/29), located either on the plasmid or the chromosome. Three non-ESBL *E. coli* isolates carried the *mcr-1* gene (1.8 %, 3/165). No carbapenem-resistant isolates were recovered. No clonal relatedness was observed between ESBL-carrying *E. coli* isolates or their plasmids from travellers and other sources.

Conclusion: Czech travellers frequently carried diverse ESBL-producing Enterobacterales, suggesting that travel contributes to the dissemination of bacterial strains and mobile genetic elements harbouring antimicrobial resistance genes.

1. Introduction

Intestinal colonisation by multidrug-resistant organisms (MDRO) increases the risk of infection and the dissemination of antimicrobial resistance in both hospitalised patients and the community [1,2]. Antimicrobial resistance-encoding genes, often carried on plasmids such

as extended-spectrum β -lactamases (ESBLs), *mcr*-genes-mediated colistin resistance, and carbapenemases, have spread via horizontal gene transfer among bacterial populations across diverse ecosystems, including humans, companion animals, livestock, and the environment [3].

Extended-spectrum β -lactamases (ESBLs) are rapidly evolving

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* Corresponding author. Department of Medical Microbiology, Second Faculty of Medicine, Charles University and Motol University Hospital, V Uvalu 84, Prague 5, 150 06, Czech Republic.

E-mail address: marcela.krutova@lfmotol.cuni.cz (M. Krutova).

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enzymes that confer resistance to a broad spectrum of β -lactam antibiotics, including penicillins, cephalosporins, and monobactams. Over the past two decades, ESBL producers have emerged as significant opportunistic pathogens. In Europe, a high prevalence of 13.8 % and 6 % ESBL-producing *Escherichia coli* was reported in hospital and community settings, respectively [4]. Currently, more than 350 ESBL variants have been identified. ESBL genes are commonly carried on transferable plasmids, often located near insertion sequences and transposons, but can also be integrated into bacterial chromosomes [5].

Carbapenems are highly effective antibiotics due to their broad-spectrum activity and resilience against most beta-lactam resistance mechanisms. However, the prevalence of carbapenem-resistant Enterobacterales is increasing and shows considerable regional variability [6]. Resistance mechanisms primarily involve acquired genes encoding carbapenemases (e.g., *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48}), which have diverse hydrolytic properties. Additionally, carbapenem resistance can be mediated by mutations that lead to the loss or inactivation of outer membrane porins, reducing membrane permeability to carbapenems [7].

Colistin has regained importance in healthcare as a last-line treatment for infections caused by multidrug-resistant gram-negative bacteria. For many years, colistin resistance remained low and was primarily associated with chromosomal mutations. However, its widespread use, particularly in veterinary medicine and livestock production, has contributed to a variable increase in resistance, further limiting treatment options [8,9].

Multiple studies have documented that foreign travel significantly increases the risk of acquiring multidrug-resistant (MDR) pathogens [10, 11]. The reported prevalence rates vary between studies, depending on travel destination and screening strategy employed [12,13]. Colonisation rates also differ during travel and after return [14]. Most studies focus on a single type of resistance or the combined detection of ESBL and carbapenemase-mediated resistance [15,16].

This study aimed to assess the intestinal carriage of ESBL-producing, carbapenem-, and colistin-resistant Enterobacterales in returning travellers and to investigate whether international travel contributes to colonisation with resistant strains distinct from those already circulating in the Czech Republic.

2. Methods

2.1. Sample collection and culture

Stool samples from Czech travellers with a history of international travel submitted for parasitological examination at the Department of Medical Microbiology, Bulovka Hospital, Prague, between September 2019 and October 2021 were included in the study. The primary indication for testing was gastrointestinal symptoms, predominantly diarrhoea with a suspected parasitic origin (118/165; 71.5 %). Seven samples (4.2 %) were associated with malaria, and four (2.4 %) with suspected viral infections without gastrointestinal symptoms. Five travellers required hospitalisation abroad, and seven received outpatient care. The remaining 36 samples (21.8 %) were submitted for other systemic complaints, including fever, or without a specific indication. The time between return from travel and sample submission was <6 months for 77 individuals (46.7 %), 6–12 months for 28 (17.0 %), 1–2 years for 20 (12.1 %), and >2 years for 11 (6.7 %; 11/165); data were missing for 29 (17.6 %) travellers.

Samples were first enriched overnight using (*Enterobacteriaceae* Enrichment Broth-Mossel, ThermoFisher) and subsequently cultured on a selective chromogenic media for the detection of ESBL-producing (CHROMagar™ Orientation + ESBL supplement ES372, CHROMagar, France), carbapenem (CHROMagar™ Orientation KPC, CHROMagar, France) and/or colistin-resistant Enterobacterales (Brilliance™ UTI Clarity™ Agar, ThermoFisher, US) supplemented with colistin 3.5 mg/L (Sigma-Aldrich, US).

The bacterial species were identified using matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry (MALDI-TOF MS, Biotyper v3.1, Bruker, US). Antimicrobial susceptibility testing for 24 antibiotics was performed using the broth microdilution (BMD) (Erba Mannheim, Germany) and was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [17]. Except for susceptibility to cefoperazone, netilmicin, and tetracycline, which were interpreted based on the Clinical Laboratory Standards Institute (CLSI, M100, 33rd Edition, 2023) breakpoints [18]. *E. coli* ATCC 25922 and the *mcr-1*-positive *E. coli* NCTC 13846 were included as quality control strains. In colistin-resistant isolates, the presence of *mcr* genes (1–8) was determined by PCR as previously described [19].

2.2. Whole genome sequencing and bioinformatic analysis

Whole-genome sequencing was performed on isolates with confirmed cefotaxime resistance and those carrying the *mcr* gene. DNA was extracted using the MasterPure Complete DNA & RNA Purification kit (BioSearch Technologies, UK), and libraries for short-read sequencing were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, US). The libraries were sequenced on an Illumina NovaSeq 6000 sequencer (Illumina, US). Whole-genome assemblies were performed using Spades v3.15.5 [20]. Genome annotation was performed using the RASTtk server (<https://rast.nmpdr.org/rast.cgi>).

Antimicrobial resistance (AMR) determinants, including acquired resistance genes and chromosomal point mutations, were identified using ResFinder v4.4.1 [21] and PointFinder [22]. *In silico* multilocus sequence typing (MLST) was performed using MLST v2.0 [23]. These tools are available at the Centre for Genomic Epidemiology website (<https://cge.cbs.dtu.dk>).

The sequence type in *Enterobacter cloacae* and *Citrobacter freundii* isolates was assigned using the PubMLST database (<http://www.pubmlst.org>) [24].

Identification of chromosomal mutations associated with colistin and carbapenem resistance in *E. cloacae* strain (S566) was performed by Snippy v4.6.0 [25] using the reference genome *Enterobacter cloacae* subsp. *dissolvens* SDM (GenBank NC_018079).

A core-SNP maximum-likelihood phylogenetic tree was constructed using kSNP4 [26] with the assembled genomes, and the Kchooser4 function was used to determine the optimal k-mer size for SNP detection. The resulting tree was then visualised and edited using iTOL v6.9.1 [27] and Inkscape v1.3.2. Phylogroup classification of the *E. coli* isolates was performed using *in silico* Clermont Typing (<http://clermonttyping.iame-research.center/>) [28].

Long-read sequencing was performed to determine the localisation of ESBL and *mcr-1* genes and to identify the types of corresponding plasmids. DNA libraries were prepared using the Ligation Sequencing gDNA Kit (SQK-LSK109), the Native Barcoding Kit (SQK-NBD114.24), and the Rapid Barcoding Kit (SQK-RBK114.24). Sequencing was performed on R9.4.1 or R10.4.1 MinION flow cells (both Oxford Nanopore Technologies, UK). Hybrid assembly of long and short reads was performed using Flye assembler v2.9.2 [29], Medaka v1.9.1 (<https://github.com/nanoporetech/medaka>), and Polypolish v0.5.0 [30]. PlasmidFinder v2.1 was used to identify plasmid replicons in the hybrid genome assemblies, applying a minimum threshold of 80 % identity and coverage [31]. MobileElementFinder (MGE v1.0.3) detected mobile elements associated with resistance and virulence genes on plasmids [32].

2.3. Comparative dataset

To assess whether resistant strains acquired abroad are distinct from those already circulating in different reservoirs within the country, we performed comparative analysis with available genomes of the same bacterial species and resistance profiles from other Czech studies, including municipal wastewater treatment plants (WWTPs), hospital

sewage, river water, patients with urinary tract infections (UTI) [33], rectal swabs of medical students [34] and meat samples imported to or originated in the Czech Republic [35].

2.3.1. Plasmid and associated bacterial strain comparative analysis

Complete plasmid sequences from this study were reoriented to begin at the *repA* gene using Circlator v1.5.5 [36]. Hypothetical proteins annotated by RASTtk were searched via BLASTN/BLASTP against the NCBI and UniProt databases to infer potential functions based on their homology.

Comparative analysis was performed for ESBL genes-carrying plasmids, such as IncB/O/K/Z, IncI1-I(Alpha), IncN, and a multi-replicon plasmid IncFIB (AP001918)/IncFIA/IncFIC (FII), due to their detection in this study and in a previous study by Gerzova et al. [33]. First, short reads were mapped to complete plasmids from this study using BWA-MEM [37] and SAMtools v1.18 [38], and sequence similarity and coverage were calculated. Second, plasmid contigs were assembled using plasmidSPAdes [39] with default parameters. For circular comparative analysis, the longest sequence of the complete plasmid from this study was used as a reference for each plasmid. Additionally, plasmid similarity was searched using BLASTN against publicly available sequences in the NCBI database.

The genetic organisation of IncX4 (n = 2) plasmids carrying the *mcr-1* gene was compared with plasmids from rectal swabs of Czech medical students [34], meat samples imported to or originating from the Czech Republic [35], and top hits retrieved from the NCBI nucleotide database using the BLASTN search. IncX4 plasmid contigs were obtained from genome assemblies of isolates derived from meat sources using MOB-recon, part of the MOB-suite v3.0.3 package (<https://github.com/phac-nml/mob-suite>).

The IncI2 (Delta) plasmid carrying the *mcr-1* gene was compared with top hits retrieved from the NCBI nucleotide database using the BLASTN search. Visualization of circular comparative analysis was performed with BRIG v0.95 [40].

The genetic relatedness of isolates selected based on common sequence types (STs), ESBL/*mcr* variants, and corresponding plasmids was determined using whole-genome multilocus sequence typing (wgMLST) (Bionumerics v8.1, bioMérieux, France). These included ESBL-producing *E. coli* isolates (n = 24) from the Gerzova et al. study [33], and *mcr-1* carrying *E. coli* isolates from rectal swabs of Czech medical students (n = 3) [34], and meat sources (n = 5) [35].

2.4. Statistical analysis

Associations between ESBL colonisation and categorical variables, including continent, age group, and gender, were assessed using Pearson's chi-squared test or Fisher's exact test, as appropriate. Odds ratios (ORs) with 95 % confidence intervals were calculated in R v4.4.2. For the analysis, countries were grouped into four regions: Asia, Africa, Europe, and "Others", with the latter category including North America, South America, Central America, and Oceania.

3. Results

3.1. Study cohort characteristics

Between September 2019 and October 2021, 165 stool samples were collected from Czech travellers, including 91 males (55.2 %) and 74 females (44.8 %). The average age was 41 years (median 39 years; range 3–85 years), Table 1. The most frequently visited continents were Asia (61/165, 36.9 %) and Africa (37, 22.4 %). Within Asia, India was the most frequently visited country (21/61; 34.4 %). A list of individual countries visited is provided in Supplementary Table S1.

Table 1
Study cohort.

Characteristics	Total (%)
Number of travellers	165
Average age in years (SD)	41 ± 14.7
Sex	
Female	74 (44.8 %)
Male	91 (55.2 %)
Destination of travel (by continent) ^a	
Asia	61 (36.9 %)
Africa	37 (22.4 %)
Europe (including Russia)	32 (19.4 %)
North and Central America	21 (12.7 %)
South America	9 (5.5 %)
Oceania	3 (1.8 %)
Multi-continent	2 (1.2 %)

^a Continents reflect the most recent trip. For two travellers, return dates were not reported; these multi-continent trips are shown separately (Asia and Africa, n = 1; Asia and North America, n = 1) and are not included in the single-continent totals.

3.2. Bacterial culture and antimicrobial resistance detection

3.2.1. ESBL screening media

From 165 stool samples, 19.4 % (n = 32) Enterobacterales isolates grew on ESBL chromogenic agar. These included *E. coli* (n = 28), *K. pneumoniae* (n = 2), *E. cloacae* (n = 1), and *C. freundii* (n = 1), Table 2. All isolates were resistant to ampicillin, cefotaxime, cefazolin, and cefuroxime. Antimicrobial susceptibility testing (AST) results for all tested antimicrobials and breakpoints used are summarised in Supplementary Table S2.

The ESBL genes were identified in 27 *E. coli* and 2 *K. pneumoniae* isolates. Among travellers colonised with ESBL-producing Enterobacterales (ESBL-PE), 58.6 % (17/29) presented within one year of returning from travel; one had been hospitalised abroad for a viral infection, and two received outpatient treatment. Two travellers presented 1–2 years post-return, two after more than two years, and eight had no recorded return date. Gastrointestinal symptoms were reported in 23 of the 29 ESBL-PE carriers (79.3 %), while the remaining six were tested for non-gastrointestinal indications. Most ESBL-PE colonised travellers had recently visited Asia (48.3 %, 14/29), and among them, 6 (42.8 %) had travelled to India. However, the association between travel to Asia and ESBL carriage was not statistically significant (OR 1.7, 95 % CI 0.76–3.88; p = 0.18), Table 3.

A total of 86 % (25/29) isolates encoded *bla*_{CTX-M}-type ESBLs, among which the *bla*_{CTX-M-15} gene was predominant (68 %, 17/25). In 27.5 % (n = 8) of *bla*_{CTX-M}-type ESBL producers, the *bla*_{OXA-1} gene was also carried. The presence of other β-lactamase genes is shown in Fig. 1 and listed in the Supplementary Table S2.

The ESBL-producing isolates were not genetically related (SNP range 52–22,037, Supplementary Table S3). The isolates clustered according to their ST and phylogroup (Fig. 1). *E. coli* isolates carrying ESBL or AmpC

Table 2
Bacterial culture results on selective agar media.

Bacterial strains (total n)	CHROMagar™ Orientation + ESBL	Brilliance™ UTI Clarity™ Agar + colistin
<i>Escherichia coli</i> (47)	28 (59.5 %)	19 (40.4 %)
<i>Klebsiella pneumoniae</i> (6)	2 (33.3 %)	4 (66.6 %)
<i>Citrobacter freundii</i> (2)	1 (50 %)	1 (50 %)
<i>Enterobacter cloacae</i> complex (3)	1 (33.3 %)	2 (66.6 %)
<i>Escherichia hermannii</i> (1)	–	1 (100 %)
<i>Klebsiella oxytoca</i> (1)	–	1 (100 %)

*No carbapenem-resistant isolate was cultured on CHROMagar™ Orientation KPC agar.

Table 3

Summary of traveller characteristics by ESBL colonisation status.

Characteristic	Total N	ESBL-PE positive	ESBL-PE negative	OR (95 % CI)	p-value
Age group					0.94
1–17	8	2 (25.0 %)	6 (75.0 %)	1.59 (0.15–9.58)	0.63
18–30	28	5 (17.8 %)	23 (82.1 %)	1.02 (0.27–3.14)	1
31–50	92	17 (18.5 %)	75 (81.5 %)	1.15 (0.51–2.59)	0.73
51–60	21	3 (15.0 %)	17 (85.0 %)	0.75 (0.13–2.88)	1
>60	16	2 (12.5 %)	14 (87.5 %)	0.64 (0.06–3.08)	0.74
Gender					0.21
Male	91	19 (20.8 %)	72 (79.1 %)	1.69 (0.73–3.90)	–
Female	74	10 (13.5 %)	64 (86.5 %)	–	–
Continent^b					0.03 ^a
Asia	61	14 (22.9 %)	47 (77 %)	1.72 (0.76–3.88)	0.18
Africa	37	8 (21.6 %)	29 (78.3 %)	1.37 (0.55–3.43)	0.48
Europe	32	7 (21.8 %)	25 (78.1 %)	1.38 (0.53–3.60)	0.5
Others	33	0 (0 %)	33 (100.0 %)	0.05 (0–0.86)	0.002

^a p-value calculated using Pearson’s chi-squared; OR= Odds ratio; CI= Confidence interval. Each category was tested for significance.

^b Two travellers who went to more than one continent (Asia and Africa, Asia and North America), whose travel return was not specified, are not included in this analysis.

genes belonged to 14 STs, using the Achtman MLST scheme [41]. The most prevalent was ST131 (8/28, 28.6 %), followed by ST38 and ST69 (3/28, 10.71 %). These *E. coli* isolates were assigned to five phylogroups, with A, B2, and D each representing 28.6 % (8/28), followed by B1 with 14.3 % (3/28), and one isolate classified as phylogroup F (3.5 %). Individual STs and phylogroups are provided in the [Supplementary Table S2](#).

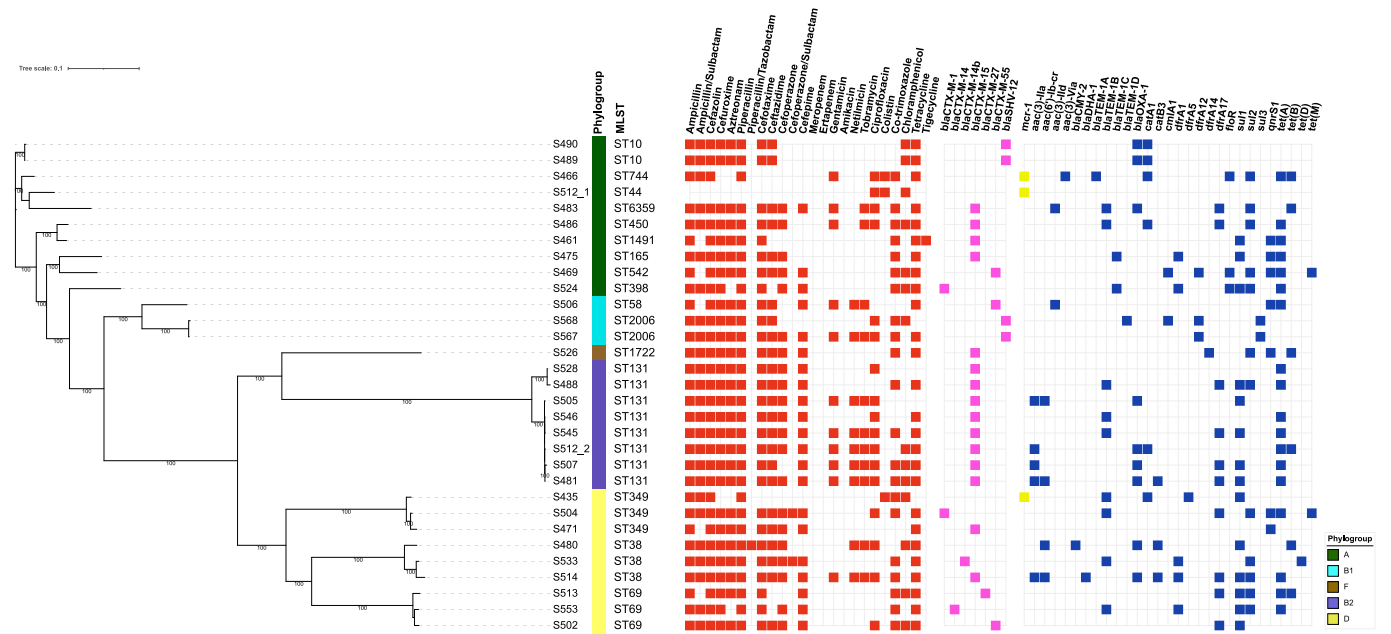


Fig. 1. Core-SNP phylogenetic tree of ESBL (n = 27) and non-ESBL *E. coli* isolates (*mcr-1* n = 3 and *ampC* n = 1) A Maximum likelihood core-SNP phylogenetic tree was constructed using the kSNP4 tool [26]. Red-filled squares represent phenotypic resistance to tested antibiotics. Pink and yellow-filled squares denote the presence of ESBLs and *mcr-1* genes, respectively. Blue squares denote the presence of other β -lactamases and non- β -lactam AMR resistance genes. The numbers on the branches denote bootstrap support values.

3.2.2. Colistin resistance screening media

Overall, 17 % (28/165) Enterobacterales isolates were cultured on the colistin chromogenic agar. Colistin resistance was confirmed in 46.4 % (13/28) of isolates, *E. coli* (n = 8), *K. pneumoniae* (n = 3), *E. hermannii* (n = 1), and *K. oxytoca* (n = 1). The presence of the *mcr-1* gene was confirmed in three *E. coli* isolates. No *mcr* genes were detected in the remaining 10 isolates. The *mcr-1*-positive isolates belonged to STs 349, 44, and 744; phylogroup A (n = 2), and phylogroup D (n = 1). Travellers colonised by *E. coli* carrying the *mcr-1* gene had travelled to India, Indonesia, and East Africa.

In *mcr-1*-positive isolates, two were multidrug resistant, and the remaining isolate exhibited resistance to colistin and ciprofloxacin only. Two *mcr-1*-positive isolates carried *bla*_{TEM}, the β -lactamase gene. One *mcr-1*-positive *E. coli* (S435) isolate has an amino acid substitution in the GyrA (S83L) but was susceptible to ciprofloxacin.

The mechanism of colistin resistance was not analysed in non-*mcr* colistin-resistant isolates except for the *E. cloacae* isolate cultured on ESBL agar, where the missense variants in the *phoPQ*, *pmrAB*, *eptA*, *arnT*, and *arnC* genes were identified ([Supplementary Table S4](#)).

3.2.3. Carbapenem resistance

No isolates grew on the KPC screening agar media. However, the *E. cloacae* isolate cultured on ESBL agar exhibited resistance to ertapenem (MIC >2 mg/L). Missense variants in genes for outer membrane porins and penicillin-binding proteins (*ompC*, *ompF*, *mrdA*, and *ftsI*) were identified ([Supplementary Table S5](#)).

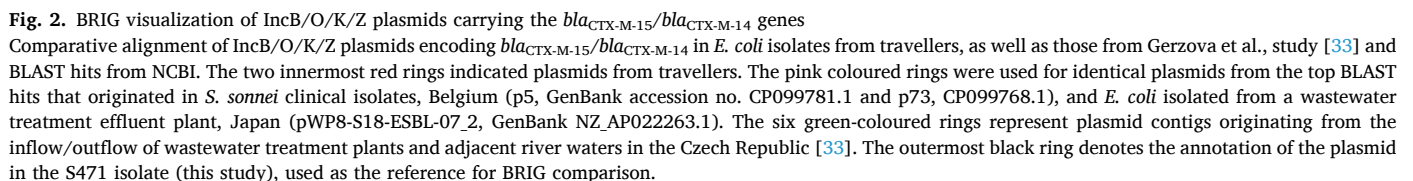
3.2.4. Discrepancies between phenotype and genotype

The antimicrobial resistance predicted by ResFinder was compared to antimicrobial susceptibility data. Ampicillin/sulbactam, cefazolin, cefuroxime, cefoperazone, and cefoperazone/sulbactam were excluded from the comparison because the ResFinder v.4.4.1 database does not provide resistance predictions for these antibiotics.

The 100 % agreement was found for ampicillin and piperacillin. Cefepime resistance was predicted in 29 isolates based on the presence of the *bla*_{CTX-M-15}, *bla*_{SHV-12} genes, or the co-presence of the *bla*_{OXA-1} gene; however, eight isolates (8/29, 28 %) were phenotypically susceptible.

Ciprofloxacin resistance was predicted in 11 ESBL- and one *mcr-1*-positive isolate based on the presence of *qnrS1*, *qnrB1*, *qnrB10*, *oxqAB* genes, and/or amino acid substitution in the GyrA (S83L). Two cotrimoxazole-sensitive isolates were predicted to be resistant based on the presence of the *sul1* gene. Chloramphenicol resistance was predicted

On the other hand, no resistance mechanism was identified in eleven phenotypically resistant isolates. These included isolates resistant to chloramphenicol (n = 2), tetracycline (n = 3), tigecycline (n = 4), ciprofloxacin (n = 1), and one strain resistant to both gentamicin and tobramycin, [Supplementary Table S6](#).



%). The NCBI search identified the closest matches (95–100 % query coverage and 99–100 % identity) to plasmids p5 (GenBank CP099781.1) and p73 (GenBank CP099768.1) found in clinical *S. sonnei* isolates from Belgium, as well as an *E. coli* plasmid pWP8-S18-ESBL-07.2 (GenBank AP022263.1) obtained from a wastewater effluent sample in Japan, Fig. 2 and Supplementary Table S8.

The IncI1-I (Alpha) plasmids carried by three *E. coli* isolates (S502, S553, S524) of STs 69 and 398 varied in size (87, 92, and 106 kb) and encoded different ESBLs *bla*_{CTX-M-55}, *bla*_{CTX-M-14}, and *bla*_{CTX-M-1} genes, respectively. The mapping of short-sequence reads from two *E. coli* isolates (Czech UTI patients) [33] to each of the three IncI1-I (Alpha) plasmids showed coverage ranging from 89.4 % to 93.5 % (average 91.5 %). The NCBI search of IncI1-I (Alpha) plasmids retrieved similar plasmids (exhibited 99.9 % similarity at 91–97 % query coverage) in isolates from pig faeces (Netherlands, pESBL-283, GenBank CP008736.1) [42], pig caecum (Denmark, p15078279, Genbank MK181566.1) [43], and one human inpatient isolate (China, p30, GenBank LT968716.1), Fig. 3 and Supplementary Table S8.

The IncN plasmid was carried in one *E. coli* isolate S469 of ST542, which had a sequence length of 51,855 bp. The mapping of short-reads from two isolates from WWTP outflow and upstream river water from the Gerzova et al. study [33] to the IncN plasmid from this study showed 76–85 % coverage. The NCBI search retrieved similar plasmids (exhibited 99 % similarity at 87 % query coverage in *E. coli* isolates from hospital wastewater (Sweden, pWE_H_2, GenBank MW574948.1), sheep faeces (UK, pRHB24-C01_2, GenBank CP057511.1), and in *Escherichia marmotae* isolate from wild game meat (Switzerland, pW49-2-b, GenBank CP093241.1), Supplementary Fig. S1 and Supplementary Table S8.

The multi-replicon plasmids IncFIB (AP001918)/IncFIA/IncFIC (FII) were carried by *E. coli* isolates (S506 and S512.2) of STs 58 and 131, with sizes of 139 and 142 kb, respectively. Due to the limited resolution of short-read sequencing for resolving multi-replicon ESBL-carrying plasmids, raw read mapping to isolates from other sources [33] was not undertaken. The NCBI search retrieved similar plasmids (99.9 % similarity, at 76–95 % coverage) in isolates from a community-associated UTI patient by *E. coli* in the USA (pCA14, GenBank CP009231) and another from dairy cattle in the UK (pI1-34TF, GenBank LN850163.1), as shown in Supplementary Fig. S2 and Supplementary Table S8.

3.3.2. *mcr-1*-encoding plasmids

The *mcr-1* gene was located on the IncX4 plasmid (33.3 kb) in two *E. coli* isolates (S435 and S466) of STs 349 and 744, and the IncI2 (Delta) plasmid (60.8 kb) in an *E. coli* isolate (S512.1) of ST44. No other antimicrobial-resistant determinants were present in these plasmids. The IncX4 plasmids from this study exhibited high homology (99.8–100 % identity, 97–100 % coverage) with plasmids from rectal swabs of Czech medical students [34] and meat samples from cattle, chicken, and pig [35], as shown in Supplementary Fig. S3 and Supplementary Table S8. The NCBI search of IncX4 plasmids retrieved identical plasmids (99.9 % identity at 100 % coverage) found in *E. coli* isolates from a German turkey farm (pVet5, GenBank OR724983.1; pVet2, GenBank OR724980.1), and found in *Salmonella enterica* from meat-derived products in Spain (pIncX4_LSP295/15, GenBank OK642378). The NCBI search of the IncI2 (Delta) plasmids retrieved identical plasmids (99.9 % identity, 100 % coverage) in *E. coli* isolates from sheep (China, pSX23-3-*mcr-1*, GenBank CP142791.1), Chicken (China, pAH62-1, Genbank CP055260.1) and one human faecal sample (Ecuador, pPSS-07_2, GenBank AP027675.1), as shown in Supplementary Fig. S4 and Supplementary Table S8.

3.4. ESBL-producing and *mcr-1*-positive *E. coli* isolates analysis

To investigate the genetic relatedness of isolates, the ESBL-producing (n = 27) and *mcr-1*-positive (n = 3) *E. coli* genomes from this study were compared with 32 isolates from various sources [33–35] (their list and reason for inclusion are in Supplementary Fig. S5 and Supplementary

Table S9). The wgMLST analysis, including 8172 loci, showed 98–938 allele differences between isolates from travellers and other sources, suggesting non-genetic relatedness. The clonal isolates (1–9 alleles) were identified between isolates from the study of Gerzova et al., namely, ST38 from hospital sewage (n = 3), ST69 from river upstream (n = 2) and downstream (n = 1), ST131 from hospital sewage (n = 1) and WWTP inflow (n = 1) and ST117 from two clinical UTI patients [33].

4. Discussion

Data on the antimicrobial resistance in the community remain limited in the Czech Republic. Existing data come primarily from recent studies investigating the nasal and intestinal carriage of multidrug-resistant pathogens in medical students, as well as from analyses of meat products and environmental samples, including municipal wastewater and river water [33–35]. International travel has been identified as a significant risk factor for acquiring MDR pathogens [44].

In our previous work, we focused on the intestinal carriage of colistin-resistant Enterobacterales in Czech travellers and expatriates [45]. In the present study, we expanded the design to include Enterobacterales that produce ESBLs, are resistant to carbapenems, and/or to colistin. We also incorporated comparative genomic analysis to assess the genetic relatedness of our isolates and plasmids against those from the aforementioned Czech environmental and carriage studies [33–35]. This enables us to assess the impact of international travel on the diversity of bacterial strains, plasmids, and antimicrobial resistance determinants.

In our study, the prevalence of ESBL-producing Enterobacterales (29/165) among returning travellers was 17.6 %. The most common travel destination among colonised individuals was Asia (14/29, 48.3 %); however, this association was not statistically significant. Compared with previous European cohorts, our observed prevalence was lower, which may reflect that 79.3 % (23/29) of ESBL-PE carriers in our study reported diarrhoea and that only 46.7 % of travellers were sampled within six months of their return, a factor likely to influence the detection rate given the transient nature of colonisation [14,46].

Higher colonisation rates have been reported in travellers from the Netherlands (34.3 %, 633/1847; 2012–2013) [47], Germany (23 %, 53/230; 2016–2017) [48], and Switzerland (69.4 %, 118/170; 2012–2013) [49]. Across these studies, travel destination, traveller's diarrhoea (TD), and antibiotic use were consistently identified as key risk factors for ESBL acquisition. The Dutch COMBAT study found particularly high acquisition rates among travellers to South Asia (75.1 %, 136/181; 95 % CI 68.4–80.9) with persistent diarrhoea after return (50.6 %, 44/87; adjusted odds ratio [aOR] 2.31, 95 % CI 1.42–3.76; p = 0.001) and antibiotic use during travel (aOR 2.69, 95 % CI 1.79–4.05; p < 0.001) as independent predictors [47]. Similarly, the German travel-clinic study identified travel to Asia to be an independent risk factor (47 %, 16/34; aOR 4.6, 95 % CI 1.9–11), whereas TD and antibiotic use were not [48]. In contrast, the Swiss cohort of travellers to South Asia, mainly India, reported the highest colonisation rate (86.8 %, 95 % CI 78.5–95.0), again without significant effects of TD or antibiotic use [49].

The prevalence of *mcr-1*-positive Enterobacterales in returning travellers was 1.8 %, closely aligning with our earlier findings of 1.1 % (2/146.7 %) reported between 2018 and 2019 [45]. These rates are also in line with the *mcr-1* carriage rate in Czech medical students (1.7 %, 3/178) [34] and are higher than the prevalence observed in hospitalised patients (0.21 %, 4/1922) [19]. By comparison with other traveller cohorts, other studies have documented considerably higher rates of *mcr-1* gene carriage, such as 10.5 % (4/38) among Swiss travellers returning from India [50] and 15.8 % (3/19) of Japanese travellers returning from Vietnam [51]. In our cohort, the three colistin-resistant *mcr-1*-positive *E. coli* isolates originated from travellers who visited India, Indonesia (Bali), and East Africa (Madagascar). In our previous

cohort, *mcr-1*-positive isolates were derived from outpatients residing in the Czech Republic who had recently returned from New Zealand and Italy [45]. These findings suggest that the acquisition of the *mcr-1* gene-carrying strain could be linked to diverse travel destinations, including both high- and low-endemicity regions.

No carbapenem-resistant Enterobacterales were detected in our cohort. Earlier prospective studies of travellers conducted between 2009 and 2011 also reported no CRE colonisation [52,53], whereas later studies from 2012 to 2018 documented only sporadic cases, primarily among travellers returning from Asia and North Africa [14,54,55]. These findings highlight the importance of continued surveillance to detect and monitor the emerging carbapenem-resistant Enterobacterales in returning travellers.

WGS enabled high-resolution typing, essential for outbreak investigation and surveillance [56]. Although WGS-based resistance prediction shows promising potential, challenges remain in achieving full genotype-phenotype concordance, especially for MIC values near clinical breakpoints [57–59]. For instance, discrepancies in cephalosporin resistance were observed in our isolates. Four isolates carried *bla*_{CTX-M} genes but were phenotypically susceptible. Two had MICs of 4 mg/L (borderline resistance), and two had MICs of 1 mg/L. Similarly, ciprofloxacin-susceptible isolates (MICs of 0.5 mg/L) often harboured the *qnr* gene or a single *gyrA* mutation. In contrast, ciprofloxacin-resistant isolates exhibited a combination of resistance mechanisms, *oqxAB*, *qnr* variants, and multiple chromosomal mutations in *gyrA*, *parC*, and *parE*, reflecting the known multifactorial nature of fluoroquinolone resistance [60].

In this study, the *E. coli* isolates harbouring ESBL or *mcr* genes represented a diverse group of sequence types, with no evidence of clonality, as indicated by the core-genome SNP analysis. This heterogeneity is consistent with other studies, which show that returning travellers commonly acquire a wide variety of *E. coli* strains resistant to multiple antibiotics [61], a pattern likely amplified by the wide range of travel destinations. Phylogroup A (32 %, 10/31) predominated among the ESBL and *mcr-1*-positive *E. coli* isolates, reflecting the fact that commensal strains are often acquired via environmental or foodborne routes [15]. However, a notable proportion belonged to phylogroups B2 and D, associated with extraintestinal pathogenic *E. coli*. All B2 isolates (ST131) and most D isolates (ST38, ST69, ST349) were linked to travel in Asian and Africa, consistent with previous findings of ST131 carriage in expatriates from these regions [62].

Among ESBL-encoding plasmids, *bla*_{CTX-M} genes were detected in 76.5 % (13/17) of isolates and were distributed across diverse incompatibility groups. IncF-family plasmids were the most prevalent, typically appearing as multi-replicon plasmids unique to each isolate, followed by IncB/O/K/Z and Inc11-I(Alpha) plasmids. Comparison with data from Gerzova et al. [33] revealed low to moderate similarity between plasmids. In contrast, a search against publicly available international sequences showed a close match with plasmids reported abroad, suggesting that the strains carried by our travellers were likely acquired during travel. This is also supported by the non-relatedness of isolates by wgMLST.

For *mcr-1*, our isolates carried IncX4 and IncI2 (delta) replicons, aligning with global trends where over 90 % of *mcr-1*-positive plasmids belong to IncX4, IncI2, or IncHI2 types [63]. The plasmids detected in isolates from travellers showed close similarity to those from Czech meat samples, students, and isolates from food-producing animals in Germany, Spain, China, and one healthy resident in Ecuador. This highlights the global dissemination of *mcr-1*-harbouring plasmids across human, animal, and environmental reservoirs [64]. However, the wgMLST analysis revealed no clonal relatedness between the ESBL/*mcr-1* producing *E. coli* from travellers and those from environmental samples and animal sources, suggesting that while the plasmids carrying the *mcr-1* gene are widespread, the traveller-derived strains themselves were unique, possibly reflecting acquisition abroad.

4.1. Study limitations

Our study has several limitations. First, travellers were sampled only after their return, and pre-travel carriage status was not assessed, preventing us from distinguishing between pre-existing and newly acquired colonisation. However, the genetic unrelatedness of ESBL-producing Enterobacterales and/or their plasmids circulating in the Czech Republic, combined with their close relatedness to plasmids from other countries, supports the hypothesis that travellers most likely acquired these strains abroad. In addition, most participants presented with gastrointestinal symptoms and the interval between return and sample collection varied considerably, from less than 6 months to more than 2 years, which may therefore have influenced the prevalence rates, though colonisation with ESBL-producing Enterobacterales is known to be transient [14,46]. Future research, including pre-travel sampling, defined post-travel sampling and more detailed data on travel-related exposures and behaviours, is needed to better define risk factors and outcomes associated with the acquisition of multidrug-resistant bacteria during travel.

5. Conclusion

Our findings suggest that international travel may play a role in the acquisition of multidrug-resistant Enterobacterales, particularly ESBL- and *mcr-1*-positive *E. coli*, among Czech travellers. Although no carbapenemase-producing isolates were detected, continued monitoring remains important given global trends. Whole-genome sequencing revealed considerable genetic diversity in both bacterial strains and resistance plasmids, with limited overlap between travel-associated isolates and those previously identified in the Czech environment or community. These findings suggest that resistant strains were likely acquired abroad, highlighting the role of travel in introducing novel resistance determinants into local settings. The detection of globally disseminated plasmid types further underscores the importance of international collaboration and One Health approaches in combating antimicrobial resistance. Routine genomic surveillance, especially among returning travellers, could enhance early detection and containment of emerging antimicrobial resistance threats.

CRedit authorship contribution statement

Suhanya Prasad: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Barbora Dratvova:** Writing – review & editing, Investigation, Formal analysis. **Milena Antuskova:** Writing – review & editing, Investigation, Formal analysis. **Marie Brajerova:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Elka Nycova:** Writing – review & editing, Formal analysis, Data curation. **Eva Adamova:** Writing – review & editing, Investigation, Formal analysis. **Milan Trojanek:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Jan Tkadlec:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Pavel Drevinek:** Writing – review & editing, Funding acquisition, Conceptualization. **Marcela Krutova:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethical statement

The study was approved by the Ethics Committee of the Motol University Hospital and Second Faculty of Medicine, Charles University, Prague, Czech Republic. As this was a surveillance-based study, informed consent from individual patients was not required.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tmaid.2025.102930>.

Data availability

Illumina and Nanopore raw reads for all isolates have been submitted under the BioProject accession ID PRJNA1268026. GenBank accession numbers were assigned for the following plasmid sequences: IncB/O/K/Z (PV764921, PV764922, PV764923), IncI-I (Alpha) (PV764924, PV764925, PV764926), IncFIB (AP001918)/IncFIA/IncFIC (FII) (PV764927, PV764928), IncI2 (Delta) (PV764929), IncX4 (PV764930, PV764931), and IncN (PV764932).

References

- Wielders CCH, Van Duijken E, Van Den Bunt G, Meijis AP, Dierikx CM, Bonten MJM, et al. Seasonality in carriage of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in the general population: a pooled analysis of nationwide cross-sectional studies. *Epidemiol Infect* 2020;148:e68. <https://doi.org/10.1017/S0950268820000539>.
- Zurfluh K, Stephan R, Widmer A, Poirel L, Nordmann P, Nüesch HJ, et al. Screening for fecal carriage of MCR-producing *Enterobacteriaceae* in healthy humans and primary care patients. *Antimicrob Resist Infect Control* 2017;6:28. <https://doi.org/10.1186/s13756-017-0186-z>.
- Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. *J Antimicrob Chemother* 2018;73(5):1121–37. <https://doi.org/10.1093/jac/dkx488>.
- Bezabih YM, Bezabih A, Dion M, Batard E, Tekla S, Obolo A, et al. Comparison of the global prevalence and trend of human intestinal carriage of ESBL-producing *Escherichia coli* between healthcare and community settings: a systematic review and meta-analysis. *JAC Antimicrob Resist* 2022;4(3):dlac048. <https://doi.org/10.1093/jacamr/dlac048>.
- Castanheira M, Simmer PJ, Bradford PA. Extended-spectrum β -lactamases: an update on their characteristics, epidemiology, and detection. *JAC Antimicrob Resist* 2021;3(3):dlab092. <https://doi.org/10.1093/jacamr/dlab092>.
- Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis* 2017; 215(suppl_1):S28–36. <https://doi.org/10.1093/infdis/jiw282>.
- Bulman ZP, Krapp F, Pincus NB, Wenzler E, Murphy KR, Qi C, et al. Genomic features associated with the degree of phenotypic resistance to carbapenems in carbapenem-resistant *Klebsiella pneumoniae*. *mSystems* 2021;6(5):e0019421. <https://doi.org/10.1128/mSystems.00194-21>.
- Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016;16(2):161–8. [https://doi.org/10.1016/S1473-3099\(15\)00424-7](https://doi.org/10.1016/S1473-3099(15)00424-7).
- Nang SC, Li J, Velkov T. The rise and spread of mcr plasmid-mediated polymyxin resistance. *Crit Rev Microbiol* 2019;45(2):131–61. <https://doi.org/10.1080/1040841X.2018.1492902>.
- Raffelsberger N, Bucek DJ, Svendsen K, Småbrekke L, Pöntinen AK, Löhr IH, et al. Community carriage of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: a cross-sectional study of risk factors and comparative genomics of carriage and clinical isolates. *mSphere* 2023;8(4):e0002523. <https://doi.org/10.1128/msphere.00025-23>.
- von Wintersdorff CJ, Penders J, Stobbering EE, Oude Lashof AM, Hoebe CJ, Savelkoul PH, et al. High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. *Emerg Infect Dis* 2014 Apr;20(4): 649–57. <https://doi.org/10.3201/eid2004.131718>.
- Frost I, Van Boeckel TP, Pires J, Craig J, Laxminarayan R. Global geographic trends in antimicrobial resistance: the role of international travel. *J Travel Med* 2019;26(8):taz036. <https://doi.org/10.1093/jtm/taz036>.
- Wendt S, Böhm P, Daniel J, Lippmann N, Lübbert C. How are travellers colonized with antimicrobial-resistant bacteria? *J Travel Med* 2020;27(1):taz068. <https://doi.org/10.1093/jtm/taz068>.
- Schaumburg F, Sertic SM, Correa-Martinez C, Mellmann A, Köck R, Becker K. Acquisition and colonization dynamics of antimicrobial-resistant bacteria during international travel: a prospective cohort study. *Clin Microbiol Infect* 2019;25(10): 1287.e1–7. <https://doi.org/10.1016/j.cmi.2019.03.002>.
- Worby CJ, Sridhar S, Turbett SE, Becker MV, Kogut L, Sanchez V, et al. Gut microbiome perturbation, antibiotic resistance, and *Escherichia coli* strain dynamics associated with international travel: a metagenomic analysis. *Lancet Microbe* 2023;4(10):e790–9. [https://doi.org/10.1016/S2666-5247\(23\)00147-7](https://doi.org/10.1016/S2666-5247(23)00147-7).
- van Hattem JM, Arcilla MS, Bootsma MC, van Genderen PJ, Goorhuis A, Grobusch MP, et al. Prolonged carriage and potential onward transmission of carbapenemase-producing *Enterobacteriaceae* in Dutch travelers. *Future Microbiol* 2016;11:857–64. <https://doi.org/10.2217/fmb.16.18>.
- The European Committee on antimicrobial susceptibility testing. Breakpoint tables for interpretation of MICs and zone diameters, Version 13.0; 2023. <http://www.eucast.org>.
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 33rd. CLSI informational supplement M100. Wayne, PA: CLSI; 2023.
- Tkadlek J, Kalova A, Brajerova M, Gelbicova T, Karpiskova R, Smelikova E, et al. The intestinal carriage of plasmid-mediated colistin-resistant *Enterobacteriaceae* in tertiary care settings. *Antibiotics (Basel)* 2021;10(3):258. <https://doi.org/10.3390/antibiotics10030258>.
- Prijbelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes De Novo Assembler. *Curr Protoc Bioinform* 2020;70(1):e102. <https://doi.org/10.1002/cpbi.102>.
- Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattorri V, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; 75(12):3491–500. <https://doi.org/10.1093/jac/dkaa345>.
- Zankari E, Allesøe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J Antimicrob Chemother* 2017;72(10):2764–8. <https://doi.org/10.1093/jac/dkx217>.
- Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 2012;50(4):1355–61. <https://doi.org/10.1128/JCM.06094-11>.
- Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018;3:124. <https://doi.org/10.12688/wellcomeopenres.14826.1>.
- Seeman Torsten. Snippy: fast bacterial variant calling from NGS reads. Available from, <https://github.com/tseemann/snippy>; 2015.
- Hall BG, Nisbet J. Building phylogenetic trees from genome sequences with kSNP4. *Mol Biol Evol* 2023;40(11):msad235. <https://doi.org/10.1093/molbev/msad235>.
- Letunic I, Bork P. Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 2021;49(W1):W293–6. <https://doi.org/10.1093/nar/gkab301>.
- Beghain J, Bridier-Nahmias A, Le Nagard H, Denamur E, Clermont O. ClermontTyping: an easy-to-use and accurate in silico method for *Escherichia coli* strain phylogeny. *Microb Genom* 2018;4(7):e000192. <https://doi.org/10.1099/mgen.0.000192>.
- Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 2019;37(5):540–6. <https://doi.org/10.1038/s41587-019-0072-8>.
- Wick RR, Holt KE, Polypolish. Short-read polishing of long-read bacterial genome assemblies. *PLoS Comput Biol* 2022;18(1):e1009802. <https://doi.org/10.1371/journal.pcbi.1009802>.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014;58(7):3895–903. <https://doi.org/10.1128/AAC.02412-14>.
- Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, Petersen TN. Detection of mobile genetic elements associated with antibiotic resistance in *Salmonella enterica* using a newly developed web tool: mobileelementfinder. *J Antimicrob Chemother* 2021;76(1):101–9. <https://doi.org/10.1093/jac/dkaa390>.
- Davidova-Gerzova L, Lausova J, Sukkar I, Nesporova K, Nechutna L, Vlkova K, et al. Hospital and community wastewater as a source of multidrug-resistant ESBL-producing *Escherichia coli*. *Front Cell Infect Microbiol* 2023;13:1184081. <https://doi.org/10.3389/fcimb.2023.1184081>.
- Smelikova E, Drevenek P, Nyc O, Brajerova M, Tkadlek J, Krutova M. To screen or not to screen medical students for carriage of multidrug-resistant pathogens? *J Hosp Infect* 2023;140:15–23. <https://doi.org/10.1016/j.jhin.2023.06.028>.
- Sismova P, Sukkar I, Kolidentsev N, Palkovicova J, Chytilova I, Bardon J, et al. Plasmid-mediated colistin resistance from fresh meat and slaughtered animals in the Czech Republic: nation-wide surveillance 2020–2021. *Microbiol Spectr* 2023;11(5):e0060923. <https://doi.org/10.1128/spectrum.00609-23>.
- Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, Harris SR. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biol* 2015;16:294. <https://doi.org/10.1186/s13059-015-0849-0>.
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25(14):1754–60. <https://doi.org/10.1093/bioinformatics/btp324>.

- [38] Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009;25(16):2078–9. <https://doi.org/10.1093/bioinformatics/btp352>.
- [39] Antipov D, Hartwick N, Shen M, Raiko M, Lapidus A, Pevzner PA. plasmidSPAdes: assembling plasmids from whole genome sequencing data. *Bioinformatics* 2016;32(22):3380–7. <https://doi.org/10.1093/bioinformatics/btw493>.
- [40] Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genom* 2011;12:402. <https://doi.org/10.1186/1471-2164-12-402>.
- [41] Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 2006;60(5):1136–51. <https://doi.org/10.1111/j.1365-2958.2006.05172.x>.
- [42] Brouwer MS, Bossers A, Harders F, van Essen-Zandbergen A, Mevius DJ, Smith HE. Complete genome sequences of IncI1 plasmids carrying extended-spectrum β -Lactamase genes. *Genome Announc* 2014;2(4):e00859. <https://doi.org/10.1128/genomeA.00859-14>.
- [43] Valcek A, Roer L, Overballe-Petersen S, Hansen F, Bortolaia V, Leekitcharoenphon P, et al. IncI1 ST3 and IncI1 ST7 plasmids from CTX-M-1-producing *Escherichia coli* obtained from patients with bloodstream infections are closely related to plasmids from *E. coli* of animal origin. *J Antimicrob Chemother* 2019;74(8):2171–5. <https://doi.org/10.1093/jac/dkz199>.
- [44] Brouwer MS, Bossers A, Harders F, van Essen-Zandbergen A, Mevius DJ, Smith HE. Unwanted souvenirs: travel and multi-resistant bacteria. *J Travel Med* 2011;18(5):297–8. <https://doi.org/10.1111/j.1708-8305.2011.00541.x>.
- [45] Krutova M, Kalova A, Nycova E, Gelbicova T, Karpiskova R, Smelikova E, et al. The colonisation of Czech travellers and expatriates living in the Czech Republic by colistin-resistant *Enterobacteriaceae* and whole genome characterisation of *E. coli* isolates harbouring the *mcr-1* genes on a plasmid or chromosome: a cross-sectional study. *Travel Med Infect Dis* 2021;39:101914. <https://doi.org/10.1016/j.tmaid.2020.101914>.
- [46] ÖstholmBalkhed Å, Tärnberg M, Nilsson M, Nilsson LE, Hanberger H, Hällgren A, Southeast Sweden Travel Study Group. Duration of travel-associated faecal colonisation with ESBL-producing *Enterobacteriaceae* - a one year follow-up study. *PLoS One* 2018;13(10):e0205504. <https://doi.org/10.1371/journal.pone.0205504>.
- [47] Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MCJ, van Genderen PJJ, Goorhuis A, et al. Import and spread of extended-spectrum β -lactamase-producing *Enterobacteriaceae* by international travellers (COMBAT study): a prospective, multicentre cohort study. *Lancet Infect Dis* 2017;17(1):78–85. [https://doi.org/10.1016/S1473-3099\(16\)30319-X](https://doi.org/10.1016/S1473-3099(16)30319-X).
- [48] Meurs L, Lempp FS, Lippmann N, Trawinski H, Rodloff AC, Eckardt M, et al. Intestinal colonization with extended-spectrum beta-lactamase producing *Enterobacteriales* (ESBL-PE) during long distance travel: a cohort study in a German travel clinic (2016–2017). *Travel Med Infect Dis* 2020;33:101521. <https://doi.org/10.1016/j.tmaid.2019.101521>.
- [49] Kuenzli E, Jaeger VK, Frei R, Neumayr A, DeCrom S, Haller S, et al. High colonization rates of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* in Swiss travellers to South Asia- a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. *BMC Infect Dis* 2014;14:528. <https://doi.org/10.1186/1471-2334-14-528>.
- [50] Bernasconi OJ, Kuenzli E, Pires J, Tinguely R, Carattoli A, Hatz C, et al. Travelers can import colistin-resistant *Enterobacteriaceae*, including those possessing the plasmid-mediated *mcr-1* gene. *Antimicrob Agents Chemother* 2016;60(8):5080–4. <https://doi.org/10.1128/AAC.00731-16>.
- [51] Nakayama T, Kumeda Y, Kawahara R, Yamaguchi T, Yamamoto Y. Carriage of colistin-resistant, extended-spectrum β -lactamase-producing *Escherichia coli* harboring the *mcr-1* resistance gene after short-term international travel to Vietnam. *Infect Drug Resist* 2018;11:391–5. <https://doi.org/10.2147/IDR.S153178>.
- [52] Paltansing S, Vlot JA, Kraakman ME, Mesman R, Bruijning ML, Bernards AT, et al. Extended-spectrum β -lactamase-producing *enterobacteriaceae* among travelers from the Netherlands. *Emerg Infect Dis* 2013;19(8):1206–13. <https://doi.org/10.3201/eid1908.130257>.
- [53] Kantele A, Lääveri T, Mero S, Vilkinen K, Pakkanen SH, Ollgren J, et al. Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing *Enterobacteriaceae*. *Clin Infect Dis* 2015;60(6):837–46. <https://doi.org/10.1093/cid/ciu957>.
- [54] Ruppé E, Armand-Lefevre L, Estellat C, Consigny PH, El Mniai A, Boussadia Y, et al. High rate of acquisition but short duration of carriage of multidrug-resistant *Enterobacteriaceae* after travel to the tropics. *Clin Infect Dis* 2015;61(4):593–600. <https://doi.org/10.1093/cid/civ333>.
- [55] Reuland EA, Sonder GJ, Stolte I, Al Naiemi N, Koek A, Linde GB, et al. Travel to Asia and traveller's diarrhoea with antibiotic treatment are independent risk factors for acquiring ciprofloxacin-resistant and extended spectrum β -lactamase-producing *Enterobacteriaceae*-a prospective cohort study. *Clin Microbiol Infect* 2016;22(8):731.e1–7. <https://doi.org/10.1016/j.cmi.2016.05.003>.
- [56] Snitkin ES, Zelazny AM, Thomas PJ, Stock F, NISC Comparative Sequencing Program Group, Henderson DK, Palmore TN, Segre JA. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci Transl Med* 2012;4(148):148ra116. <https://doi.org/10.1126/scitranslmed.3004129>.
- [57] Cipriani G, Helmersen K, Mazzon RR, Wagner G, Aamot HV, Ferreira FA. Evaluation of whole-genome sequencing protocols for detection of antimicrobial resistance, virulence factors and mobile genetic elements in antimicrobial-resistant bacteria. *J Med Microbiol* 2025;74(3):001990. <https://doi.org/10.1099/jmm.0.001990>.
- [58] Rebelo AR, Bortolaia V, Leekitcharoenphon P, Hansen DS, Nielsen HL, Ellermann-Eriksen S, et al. One day in Denmark: comparison of phenotypic and genotypic antimicrobial susceptibility testing in bacterial isolates from clinical settings. *Front Microbiol* 2022;13:804627. <https://doi.org/10.3389/fmicb.2022.804627>.
- [59] Vanstokstraeten R, Piérard D, Crombé F, De Geyter D, Wybo I, Muyldermans A, et al. Genotypic resistance determined by whole genome sequencing versus phenotypic resistance in 234 *Escherichia coli* isolates. *Sci Rep* 2023;13(1):449. <https://doi.org/10.1038/s41598-023-27723-z>.
- [60] Morgan-Linnell SK, Becnel Boyd L, Steffen D, Zechiedrich L. Mechanisms accounting for fluoroquinolone resistance in *Escherichia coli* clinical isolates. *Antimicrob Agents Chemother* 2009;53(1):235–41. <https://doi.org/10.1128/AAC.00665-08>.
- [61] Vading M, Kabir MH, Kalin M, Iversen A, Wiklund S, Naucré P, et al. Frequent acquisition of low-virulence strains of ESBL-producing *Escherichia coli* in travellers. *J Antimicrob Chemother* 2016;71(12):3548–55. <https://doi.org/10.1093/jac/dkw335>.
- [62] Campos-Madueno EI, Aldeia C, Roumet MC, Limacher A, Sendi P, Endimiani A. Epidemiology and risk factors of expatriates returning to Switzerland colonized at the intestinal level with multidrug-resistant *Enterobacteriales*. *Eur J Clin Microbiol Infect Dis* 2025;44(4):1007–14. <https://doi.org/10.1007/s10096-025-05069-w>.
- [63] Bastidas-Caldes C, de Waard JH, Salgado MS, Villacís MJ, Coral-Almeida M, Yamamoto Y, et al. Worldwide prevalence of *mcr*-mediated colistin-resistance *Escherichia coli* in isolates of clinical samples, healthy humans, and Livestock-A systematic review and meta-analysis. *Pathogens* 2022;11(6):659. <https://doi.org/10.3390/pathogens11060659>.
- [64] Jeannot K, Bolard A, Plésiat P. Resistance to polymyxins in Gram-negative organisms. *Int J Antimicrob Agents* 2017;49(5):526–35. <https://doi.org/10.1016/j.ijantimicag.2016.11.029>.