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RESEARCH

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Use of a national repository of Fourier-transform infrared spectroscopy spectra enables fast detection of silent outbreaks and prevention of spread of new antibiotic-resistant sequence types

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Abstract

Background The reference microbiology laboratory of Israel's National Institute for Antibiotic Resistance and Infection Control has established a national repository of isolates analyzed by Fourier-transform infrared (FTIR) spectroscopy and their spectra. Healthcare institutions send antibiotic-resistant isolates as part of outbreak investigation, periodic nation-wide collection of specific species, or point prevalence studies. Here, we describe the use of a national FTIR repository to detect the emergence and spread of new sequence types and resistance mechanisms.

Methods Using FTIR, we produced dendrograms of outbreaks and periodic country-level dendrograms of isolates from selected species. When FTIR identified new clusters that were distinct from previously characterized clusters, they were investigated further by whole genome sequencing.

Results FTIR analysis uncovered two clones new to Israel: NDM-5-producing *E. coli* ST650 harboring a novel plasmid, and NDM-producing *K. pneumoniae* ST307.

Conclusions Establishing regional or national FTIR repositories could serve as a simple and effective tool for early detection of new antibiotic-resistant clones.

Keywords Antibiotic resistance, Fourier-transform infrared spectroscopy, Surveillance

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Background

Bacterial typing is an important tool for outbreak investigations. Whole genome sequencing (WGS) is considered the gold standard; isolate analysis to determine relatedness is then based on either phylogeny, MLST or SNPs [1, 2]. The sequence data generated can be easily shared and is comparable between different laboratories. The main limitations of WGS are that it is both time- and resource-consuming, creating a significant lag between outbreak detection and analysis. Another tool for bacterial typing is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This tool maps whole-cell mass spectra of microbial proteins to two dimensional coordinates, based upon mass-to-charge ratio, creating a unique bacterial profile that can be compared to reference strains. Commonly used by clinical laboratories for species identification, MALDI-TOF MS has also been successfully used for outbreak investigation [3–5], and national epidemiological surveillance [6]. However, the discriminatory power of MALDI-TOF MS spectra is lower than that of WGS, and it displays low accuracy and reproducibility [7, 8].

Fourier-transform infrared spectroscopy (FTIR) is a spectrum-based technique that quantifies the absorption of infrared light by molecules present on the bacterial cell. The IR spectrum generated produces a specific fingerprint that reflects the composition of the cell surface, including nucleic acids, proteins, lipids, and carbohydrates [9]. This fingerprint can be used for phenotypic differentiation of microorganisms on the species and subspecies level [9]. FTIR has been demonstrated to be accurate and faster than conventional typing methods [10–15]. It can be used for fast and accurate detection of outbreaks, [16–21] and for detecting the introduction of new strains and sequence types (ST). When the dissemination of a new clone in a country or region is detected early, endemicity can be prevented.

The global spread of carbapenem-resistant Enterobacterales (CRE) and the rise of high-risk antibiotic-resistant strains threaten public health worldwide [22, 23]. One example is the rise of *Klebsiella pneumoniae* ST307, a high-risk clone that has been reported on several continents (North and South America, Europe, Asia and Africa) [24–27]. This ST was first described in Europe in 2008 of periodic collection of antibiotic-resistant isolates from acute care hospitals. All reported ST307 isolates shared a common capsular formation KL102 (associated with *wzi* allele 173), OCL O2V2, and contained a second capsule 2 cluster. Most isolates also carried a type IV secretion system and a π-fimbrial cluster [25].

In recent years the National Institute for Antibiotic Resistance and Infection Control (NIARIC) has been constructing a national repository of FTIR spectra to monitor Carbapenemase-producing Enterobacterales (CPE) in Israel. Here, we show how this approach allowed us to identify dissemination of NDM-producing *E. coli* ST650 and NDM-producing *K. pneumoniae* ST307, both previously undetected in Israel.

Methods

A national repository of antibiotic-resistant strains

An FTIR system (IR Biotyper, Bruker, Leipzig, Germany) was introduced into the reference laboratory of the NIARIC in 2018. Isolates came from three sources: First, as part of nation-wide surveillance, in 2020 we initiated a project of periodic collection of antibiotic-resistant isolates from acute care hospitals. We requested all CPE isolates (from both clinical and screening samples) isolated in specific months. Second, in 2022 the NIARIC performed point prevalence surveys for multidrug-resistant organisms in 18 post-acute care hospitals (PACHs). Third, since 2020, hospitals in Israel that experience an outbreak can send isolates to our laboratory for characterization to aid in outbreak analysis and infection control efforts. All of those isolates were analyzed by FTIR and the spectra were added to the national repository. As of August 2024, the repository contains spectra from 1394 *E. coli* and 723 *Klebsiella* spp. isolates. We have constructed a national FTIR dendrogram for the main clinically relevant species. The dendrogram was curated to include only one isolate per hospital per year in each cluster (to prevent over-representation of large local outbreaks). When new isolates are collected as part of an outbreak investigation or point-prevalence studies, they are added to the database of spectra, and a new FTIR dendrogram is created. If the FTIR indicates those isolates form new cluster that is distinct from previously characterized national clusters, it is investigated further by WGS.

Isolates

Here we describe two investigations that were triggered by FTIR analysis revealing isolates forming a previously unseen FTIR cluster. In the first investigation, periodic analysis of all *E. coli* isolates revealed a novel FTIR cluster. This cluster consisted of 12 carbapenem-resistant *E. coli* isolates that were collected between November 2021 and February 2022, during four epidemiologically unrelated CPE outbreaks in four different hospitals. The samples were obtained from rectal swabs ($n=9$), a wound ($n=1$), or unspecified body sites ($n=2$).

In the second investigation, periodic analysis of all *K. pneumoniae* isolates revealed a previously unseen cluster

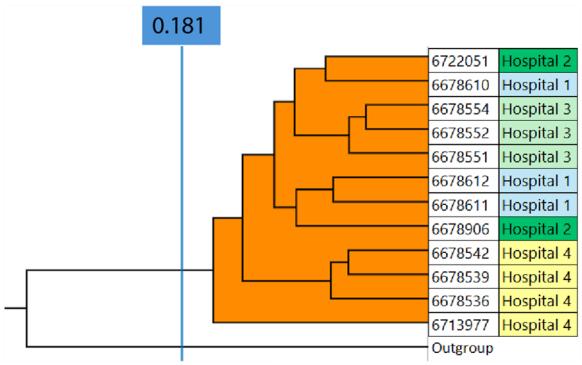

Fig. 1 FTIR biotyper dendrogram of the ST650 cluster. The blue line represents the cutoff value (0.181). Right column—color represents hospital. Orange—denotes a closely related cluster of isolates

of 12 carbapenem-resistant *K. pneumoniae* isolates. All were collected between September and December 2021, all in the context of four epidemiologically unrelated CPE outbreak investigations. Ten isolates were from 3 hospitals in the north of Israel and 2 from a single hospital in the east. All sample were sources from rectal swabs.

Strain identification and characterization

Isolates were sub-cultured on CPE selective media CHROMagar mSuperCARBA (HyLabs Laboratories Ltd, Israel). Species identification was performed by VITEK®2 (AST-GN) (bioMérieux SA, Marcy l'Etoile, France). Antibiotic susceptibility was determined by VITEK®2 (AST-N395) (bioMérieux SA, Marcy l'Etoile, France). Minimum inhibitory concentrations (MICs) were confirmed by broth microdilution (Sensititre™ Gram Negative DKMGN Kit, ThermoFisher Scientific, Oakwood Village, OH, USA) and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [23]. Isolates were tested for the presence of common carbapenemase genes using two multiplex PCR reactions: (1) bla_{OXA-48} and bla_{VIM} were detected as previously described [24, 25] and (2) bla_{KPC}, bla_{NDM} and bla_{IMI} were detected using the following primers: bla_{KPC}F/R: 5′-GACACACCCATCCGTTACG-3′/5′-GCATAGTCATTTGCCGTGC-3′; bla_{NDM} F/: 5′-CATTAGCCGCTGCATTGATGCT-3′/5′-TAGTGCTCAGTGTCGGCATCACC-3′and bla_{IMI} 5′-GCAAATGAACGATTTCCATTATGTA-3′/5′-GCACCAGGATAGAAGAGCTCTACTG-3′. Amplification was carried out for 2 min at 95 °C followed by 35 cycles of 10s at 95 °C, 15s at 62 °C, and 7s at 72 °C.

FTIR analysis

FTIR was performed separately for *E. coli* and *K. pneumoniae* according to the IR Biotyper manufacturer's instructions. Isolates were grown at 37 °C on TSBA-DEF sheep blood (Hylabs, Rehovot, Israel) for 24 h. Samples were prepared according to the manufacturer's instructions as previously described [26]. Spectra were recorded in transmission mode in a spectral range of 4000–400 cm^{-1} (mid-IR) using an IR spectrometer (Bruker Optics-Daltonik Gmbh&Co). Spectra were acquired, visualized, and processed by OPUS v8.2 software (Bruker Optics Gmbh&Co). IR Biotyper client software v4.0.3.7334 (Bruker Daltonik Gmbh&Co) built a dendrogram using the correlation average linkage clustering method. The cutoff value that defines a cluster was determined by visual inspection of the dendrogram and based on the species-specific cutoff range recommended by the IR Biotyper's manufacturer.

Whole genome sequencing

High molecular weight DNA was prepared from 12 *E. coli* and 12 *K. pneumoniae* isolates using the MagAttract HMW DNA Kit (Qiagen) according to the manufacturer's instructions. Whole genome sequencing (WGS) was performed at RUSH University Core Unit, Chicago IL using a Nextera XT library kit (Illumina Inc., CA, USA) on an Illumina Nextseq 500 device (2X150). Genome assembly was performed with CLC workbench [https://digitalinsights.qiagen.com/] and annotated with Prokka [27]. Presence of plasmids was detected using PlasmidFinder with default settings (https://cge.food.dtu.dk/services/PlasmidFinder/). One *E. coli* isolate (6,678,610) and one *K. pneumoniae* (6,678,339) were also sequenced by Oxford Nanopore WGS at RUSH University Core Facility in Chicago, IL. Plasmids were constructed by Unicycler version 0.4.8 using both long and short reads.

Genomic analysis

Genomes were classified into STs using pubMLST (https://pubmlst.org) with the Pasteur scheme for *K. pneumoniae* and the Achtman scheme for *E. coli*. Antibiotic resistance genes (ARGs) were detected using ResFinder (https://cge.food.dtu.dk/services/ResFinder/). Plasmids were detected using PlasmidFinder (https://cge.food.dtu.dk/services/PlasmidFinder/). Capsular polysaccharide (KL) and lipooligosaccharide outer core (OCL) synthesis groups were determined using Kaptive version 0.5. Serotyping was done with SerotypeFinder and the fimH allele was detected with FimType. Virulence factor content was detected using VirulenceFinder (https://cge.food.dtu.dk/services/VirulenceFinder/). Phylogenetic trees were constructed with a maximum

Table 1 Antibiotic resistance profile of *E. coli* ST650 isolates

Antibiotic agent		6,678,610	6,678,611	6,678,612	6,678,906	6,722,051	6,678,551	6,678,552	6,678,554	6,678,536	6,678,539	6,678,542	6,713,977
Trimethoprim/ sulfamethoxazole	SXT	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Piperacillin/ Tazobactam	P/T4	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Nitrofurantoin	NIT	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
Meropenem	MERO	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Gentamicin	GEN	*S*	*I*	*S*	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Fosfomycin	FOS	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
Ertapenem	EPT	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ciprofloxacin	CIP	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ceftriaxone	CTX	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ceftazidime	TAZ	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Amikacin	AMI	*S*	*S*	*S*	*S*	**R**	*S*	**R**	*S*	*S*	*S*	*S*	*S*
Aztreonam/ avibactam	ATM–AVI	*S*	*S*	*S*	*S*	**R**	*S*	**R**	*S*	*S*	*S*	*S*	*S*

Breakpoints determined by CLSI guidelines

Bold - Resistant, Italic - Intermediate/susceptible

R resistant, *S* sensitive, *I* intermediate

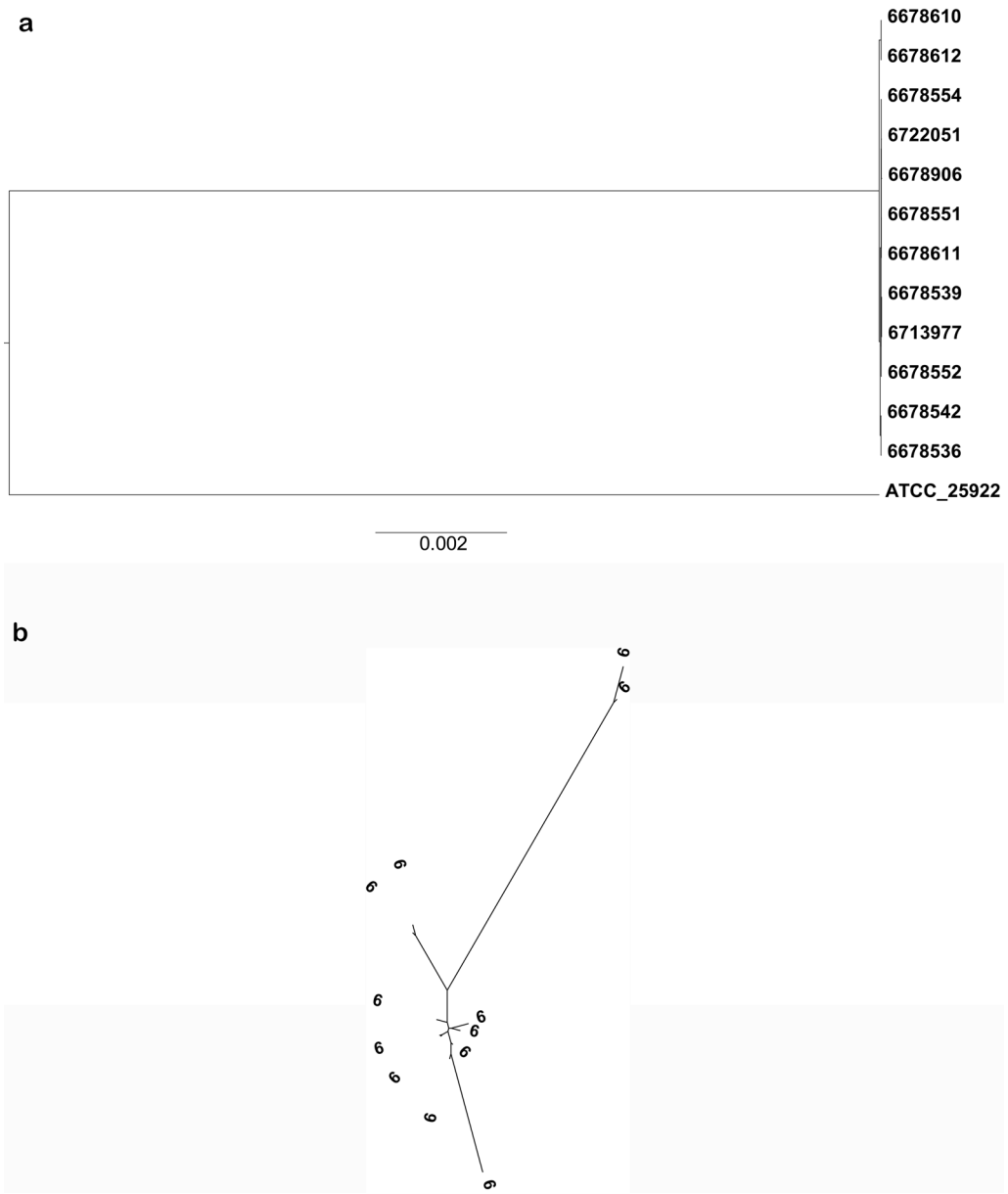

Fig. 2 A maximum likelihood whole genome phylogenetic tree of the outbreak isolates. **A** With ATCC25922 as outgroup. **B** An unrooted tree without an outgroup Scale bar—rate of substitutions per site

likelihood model using RaxML model version 8.2.12 with the GTR gamma model [28]. Plasmids were typed using the a pMLST website (https://pubmlst.org/organisms/plasmid-mlst). Single Nucleotide Polymorphism (SNPs) were calculated using Snippy.

Siderophore production analysis

Siderophore production was assessed using SideroTec AssayTM, according to manufacturer's instructions, and using media as described previously [28]. Each measurement was done in a triplicate, and production of siderophores was detected by color change. *E. coli* ATCC 25922 was used as a positive control.

Results

Identification of an *E. coli* ST650 cluster

The first novel cluster, identified by FTIR consisted of 12 carbapenem-resistant *E. coli* isolates (Fig. 1, Supplementary Fig. S1). All carried bla_{NDM}, had a meropenem MIC of > =8 mg/ml (Table 1). Three isolates were resistant to aztreonam/avibactam. Since there was no clear epidemiological connection between the 12

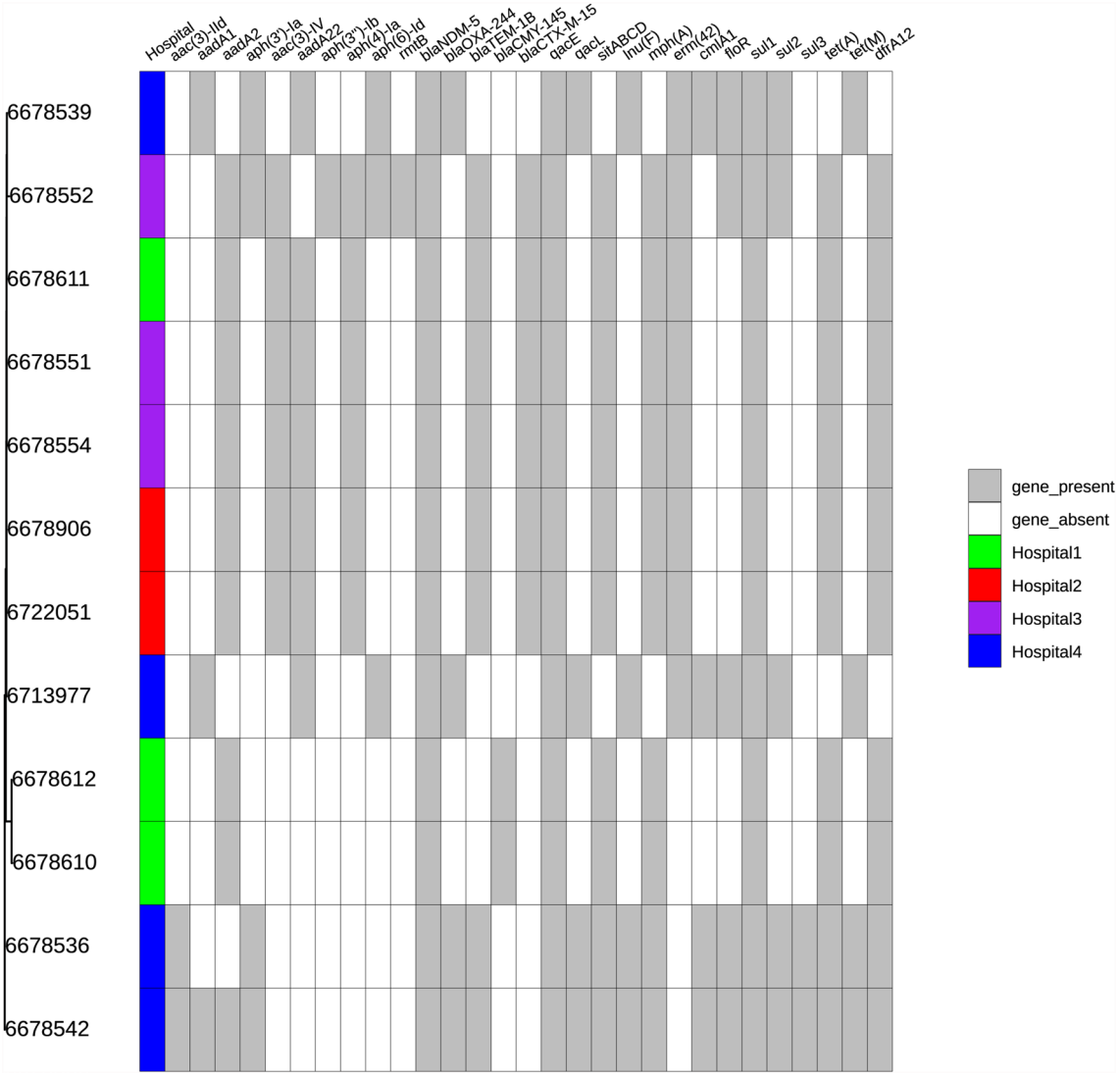

Fig. 3 Heatmap of antibiotic resistance genes in outbreak isolates

patients in the cluster, further analysis was performed. All 12 isolates underwent WGS. MLST analysis revealed that they all belonged to ST650, with serotypes O9/O9a and H30 and *fim* type H54. To test how closely the isolates were related, a phylogenetic maximum likelihood tree was constructed based on the whole genome, with ATCC25922 as an outgroup (Fig. 2A). This tree was concordant with the FTIR results and confirmed that all isolates belonged to a single clade. An unrooted tree confirmed that isolates from different hospitals were very closely related, with 2 isolates (610 and 612) slightly more distant from the other 10 isolates (Fig. 2B).

Genomic characterization of the *E. coli* ST650 cluster

All isolates carried *bla*NDM-5 and harbored D350N and S357N mutations in the PBP3 protein, known to confer antibiotic resistance to aztreonam/avibactam. Two of the three aztreonam/avibactam-resistant isolates also carried the *bla*CMY-145 allele.

As shown in Fig. 3, in addition to $bla_{\text{NDM-5}}$, the isolates carried an array of ARGs. All isolates contained *qacE* and *sul1* but differed in other ARGs.

As many ARGs are plasmid-borne, the presence of plasmids was assessed. All isolates carried IncFII, IncFIA and IncY *rep* genes (Supplementary Table 1). To determine the full plasmid content, isolate 6,678,610 was chosen for long-read Nanopore sequencing. Plasmid construction using Oxford Nanopore Technology revealed that the $bla_{\text{NDM-5}}$ allele was carried by the

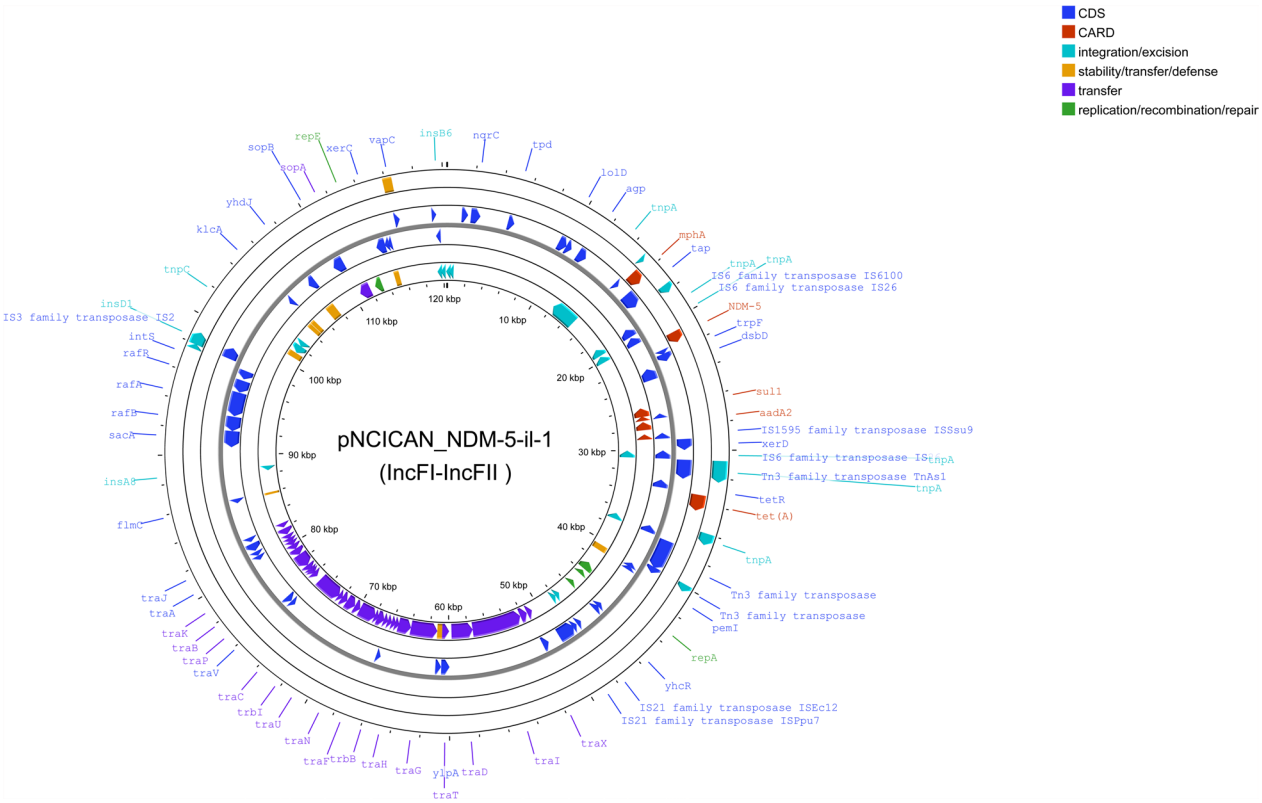

Fig. 4 The pNCICAN NDM-5-il-1 containing plasmid. Blue—CDS. RED—Resistance genes as per the CARD database. Cian—integration/excision genes. Orange—Stability related genes. Purple—transfer related genes. Green—Replication, recombination and repair genes. Arrows indicate ORF direction

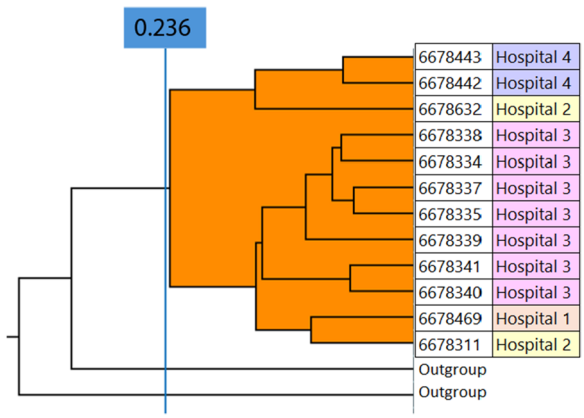

Fig. 5 FTIR image of the twelve study isolates with outgroups. Cutoff was determined at 0.236

IncFI-IncFII composite plasmid (Fig. 4). This plasmid was not previously reported in Israel, and we named it pNCICAN-NDM-5-il-1. It contained a large set of *tra* genes, various IS elements and the toxin-antitoxin system CcdAB. BlastN analysis of the plasmid revealed that it was 99.94% similar to pCMP42-1 (CP087579.1), previously detected in *E. coli* isolated from white-tailed deer in the US in 2021 (as per NCBI).

Identification of a *K. pneumoniae* ST307 cluster

The second novel cluster detected by routine review of national FTIR spectra consisted of 12 carbapenem-resistant *K. pneumoniae* isolates (Fig. 5, Supplementary Fig. S2). The isolates came from 10 patients in 4 hospitals. Although the isolates were obtained during periodic isolate collection and were not connected to a known outbreak, we investigated whether there was a previously overlooked epidemiological link between the cases. Seven *K. pneumoniae* isolates were collected in one hospital, Hospital 3, and 6 of them were from the same ward. We also found that one patient in Hospital 3 (isolate 339) was later admitted and re-screened in Hospital 1 (isolate 469). This patient apparently transmitted *K. pneumoniae* to another patient in Hospital 4 (isolate 443); although the two patients were in different wards, transmission could have occurred via shared consultants, equipment or contact in a common out of ward service. Another patient from Hospital 3 (isolate 337) was discharged and

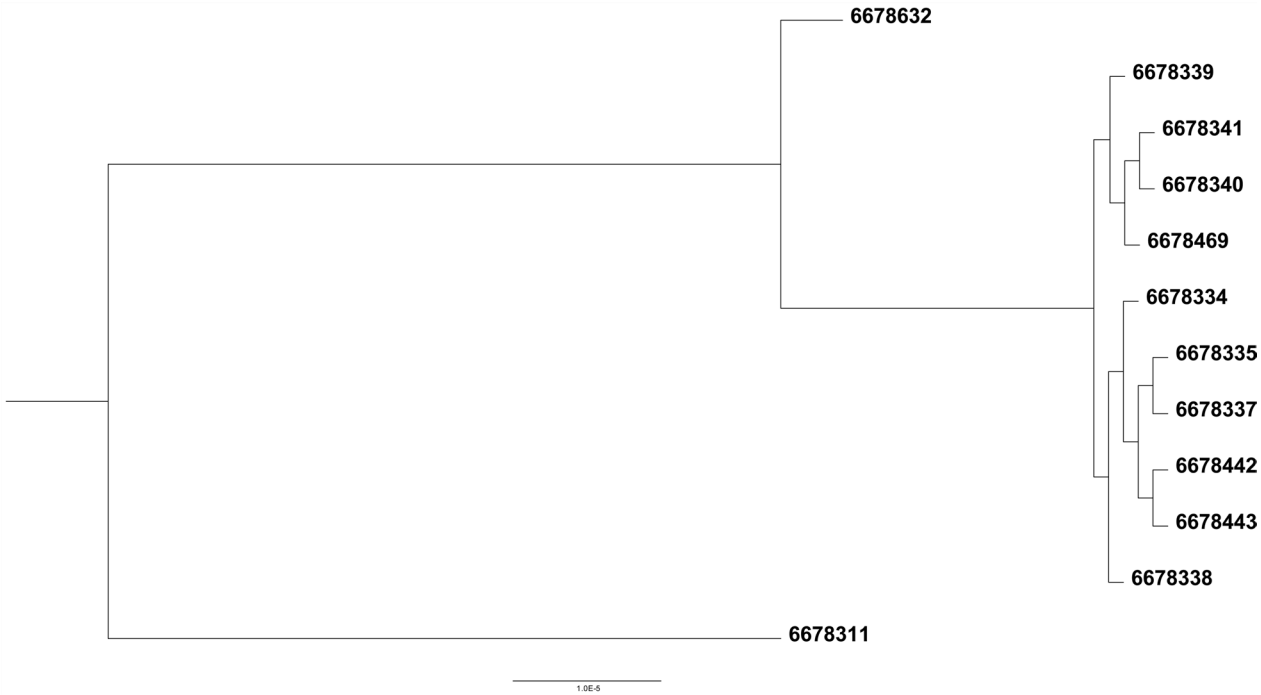

Fig. 6 A Phylogenetic tree of the 12 *K. pneumoniae* isolates. Branch lengths represent nucleotide substitutions per site

then admitted to Hospital 4 where he was screened again (isolate 442), with no further transmission detected. We could not find a link between any of these 3 hospitals and Hospital 2.

Genomic characterization of *K. pneumoniae* isolates

WGS of the 12 isolates revealed that they belonged to ST307 and carried KL102 and O2v2. Eleven isolates carried $bla_{\text{NDM-1}}$ and 1 (6,678,311) carried $bla_{\text{NDM-5}}$. Figure 6 shows the rooted phylogenetic tree of the 12 isolates: 10 were a single clade, while the 2 remaining isolates were not as closely related (Fig. 6). SNP analysis revealed that the 10 isolates in the clade differed by only 6–29 SNPs, while the other 2 isolates differed from those in the clade by hundreds of SNPs (Supplementary Fig. S3).

Characterization of *K. pneumoniae* isolates

All isolates were resistant to multiple antibiotics (Table 2). Ten isolates had an identical ARG content that included genes conferring resistance to β-lactams ($bla_{\text{NDM-1}}$, $bla_{\text{OXA-1}}$, bla_{TEM206}, $bla_{\text{CTX-M-15}}$ and bla_{SHV}), aminoglycosides (*aac(3)-IIa, aac(6')-Ib-cr, aph(3′)-VIb and aph(6)-Id*) as well as *tet(A), OqxA and OqxB, catB3, fosA, sul2, dfrA14* and *fosA*. The remaining isolates were diverse in gene content (Fig. 7).

Pan-genome analysis (Fig. 5) showed that most (4813/7632) genes were present in at least 11 of 12 strains, with only one strain (6,678,311, which is not one of the main group of ten isolates) having a different gene content, including $bla_{\text{NMD-5}}$ instead of $bla_{\text{NDM-1}}$. The ten closely-related strains also had a similar virulence factor content; all isolates carried 11/12 of the enterobactin genes, but not colibactin, allantion utilization or yersiniabactin. One gene, *iroE*, from salmochelin was found in all isolates. Only isolate 6,678,311 carried the aerobactin siderophore genes *iucA, iucB, iucC, iucD*. Production of siderophores by 6,678,311, but none of the other isolates, was confirmed in siderophore-indicative media (Supplementary Fig. S4).

The ten closely related isolates carried the same plasmid replicons: IncFII(K), IncFIB(K) and IncM1. The $bla_{\text{NDM-1}}$ gene was located on the same contig as the IncM1 rep. The two distinct isolates showed a different plasmid content: 6,678,311 has IncFIB(pNDM-MAR), IncH1B(pNDM-MAR), and ColRNAI, while 6,678,632 has IncFIB(pNDM-MAR), IncH1B(pNDM-MAR) replicon, Col440II, IncFIB(K), and IncFII(K). To determine the plasmid sequence, long read sequencing was performed for 6,678,339, and an assembly was constructed using long and short reads. Two different plasmids were constructed; one containing both IncFII(K) and IncFIB(K) rep types, and the second is an IncM1 type plasmid carrying NDM-1 (Fig. 8). We therefore named this plasmid pNCICAN-DM-ST307-il.

Table 2 Antibiotic resistance profile of *K. pneumoniae* ST307 isolates

Antibiotic agent		6,678,311	6,678,334	6,678,335	6,678,337	6,678,338	6,678,339	6,678,340	6,678,341	6,678,442	6,678,443	6,678,469	6,678,632
Meropenem	MERO	**R**	**R**	**R**	**R**	*I*	*I*	**R**	**R**	**R**	**R**	**R**	**R**
Amikacin	AMI	**R**	**R**	**R**	**R**	***S***	***S***	**R**	**R**	**R**	**R**	**R**	*S*
Gentamicin	GEN	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Aztreonam	AZT	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ciprofloxacin	CIP	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Piperacillin/Tazobactam	P/T4	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Amoxicillin/clavulanic acid	AUGC	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ceftolozane/Tazobactam	C/T	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Colistin	COL	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
Cefotaxime	FOT	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Tobramycin	TOB	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Tigecycline	TGC	***S***	*I*	*I*	*I*	*I*	*I*	***S***	***S***	**R**	**R**	*I*	***S***
Trimethoprim/sulfamethoxazole	SXT	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ceftazidime	TAZ	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ceftazidime/Avibactam	CZA	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Imipenem	IMI	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Ertapenem	EPT	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**

Breakpoints determined by CLSI guidelines

Bold - Resistant, Italic - Intermediate/susceptible

R resistant, *S* sensitive, *I* intermediate

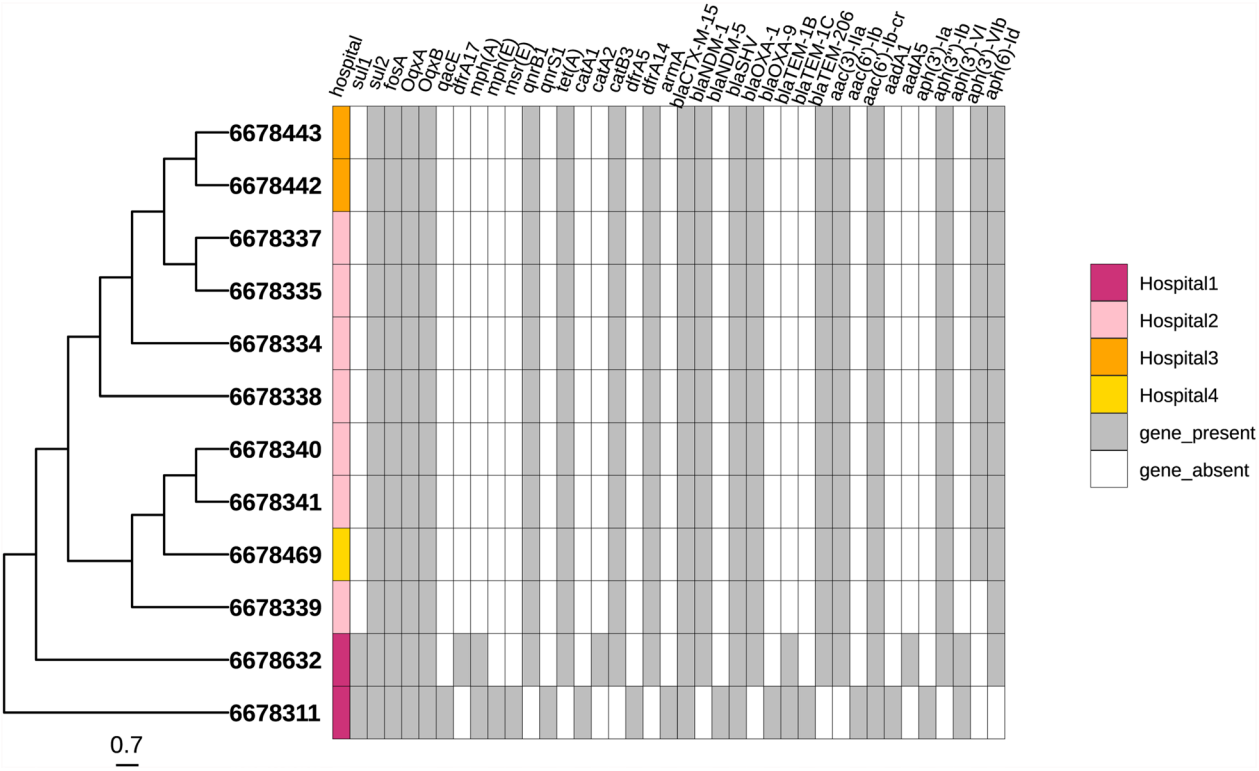

Fig. 7 Antibiotics resistance genes in *K. pneumoniae* isolates

Discussion

FTIR has recently emerged as a valuable tool for outbreak detection [10–15, 29] and retrospective epidemiological analysis [10]. It is easy to use, fast and cost-effective. Its accuracy compares well to other typing methods such as WGS and MALDI-TOF MS. Yet, FTIR's inter-laboratory reproducibility has been questioned [30]. Novais et al., have suggested that varying results are due to differences in culture conditions (culture media and incubation times) [30]. Consequently, a standardized FTIR protocol is needed to improve reproducibility. Until this aim is reached, a central national repository has an intrinsic advantage, as isolates from different hospitals are transferred to a single laboratory and analyzed using the same protocols.

Here we used a continually updated national repository of FTIR-typed CPE isolates to detect the emergence of carbapenem-resistant *E. coli* ST650 and *K. pneumoniae* ST307 clones. Without a central molecular typing repository, and in the absence of a clear epidemiological connection between hospitals and patients, these clones' emergence and spread would not have been suspected.

While ST650 is not considered a high-risk clone, it is a sequence type that has contributed to the spread and possible formation of carbapenemases. ST650 has been found to contain NDM-5 in several studies worldwide [19, 21] and in one case, even a novel single amino acid variant of NDM-5, NDM-33 [31, 32].

In recent years, there have been reports of NDM-5 carriage by the high-risk *E. coli* clones ST167 (Garcia-Fernandez et al. 2020; Alba et al. 2021) and ST648 [19]. NDM-5-producing, aztreonam/avibactam-resistant *E. coli* isolates were shown to carry both point mutations in the PBP3 protein and CMY-type alleles. Here, we showed that all study isolates carried D350N and S357N mutations in PBP3, but only the aztreonam/avibactam -resistant isolates 6,678,610 and 6,678,612 also carried the CMY-145 allele [33]. The reason for aztreonam/avibactam resistance in strain 6,722,051 remains unknown.

The novel pNCICAN-NDM-5-il-1 plasmid discovered in our study carried a large array of conjugation-facilitating *tra* genes, indicating that it is transferrable. The plasmid's closest available homolog in the NCBI database was isolated from white-tailed deer in 2021 (accession CP087579.1) [11], suggesting a potential cross between humans and animals. The role of wildlife from suburban and urban environments in the spread of antimicrobial resistance is not fully characterized [11]. In Israel, a study of eight petting zoos has indicated that they contribute to the spread of ESBLs between animal and human hosts [34, 35]. Taken

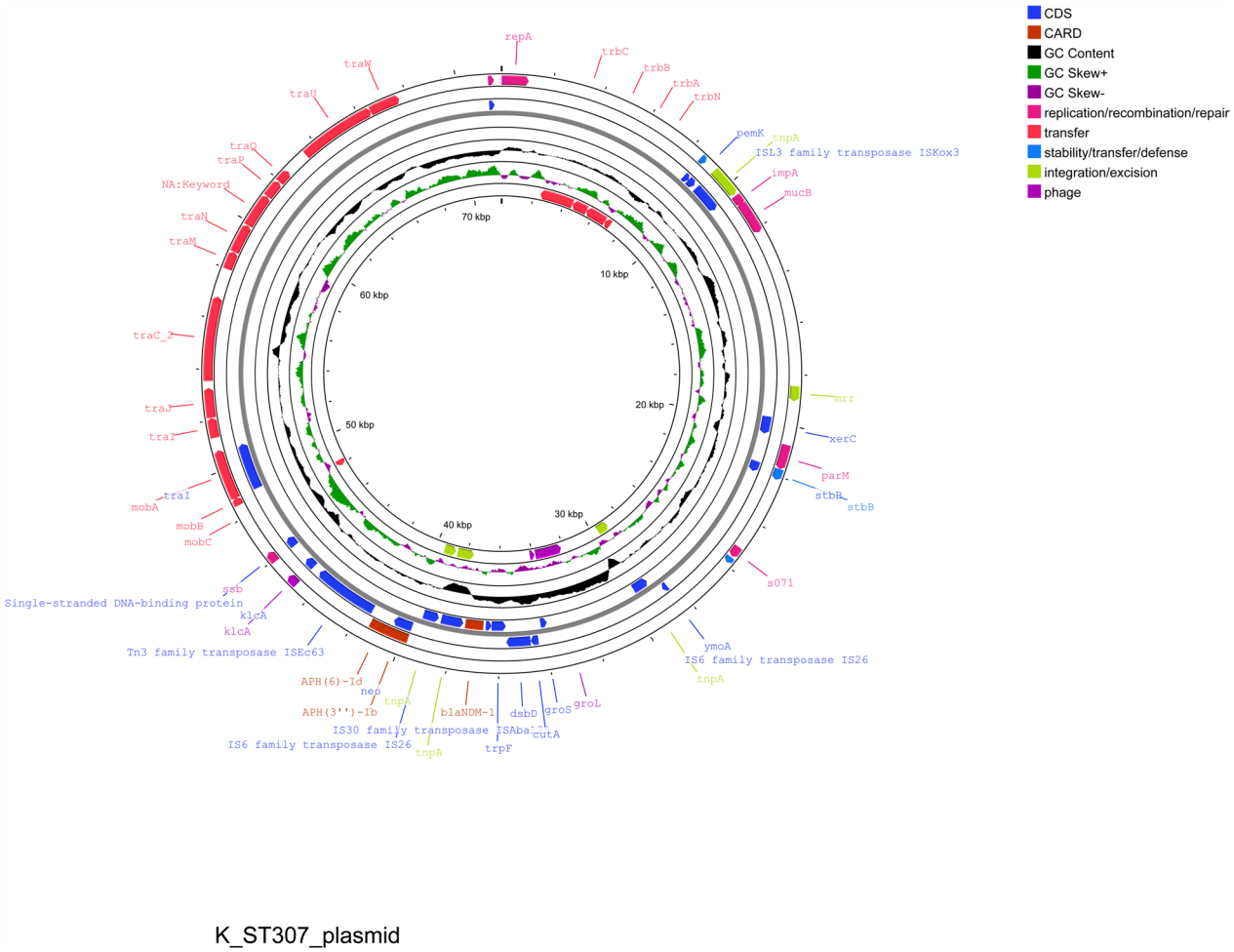

Fig. 8 pNCICAN-DM-ST307-il

together, these observations might indicate that the plasmid reservoir in animals can be introduced to and disseminate in human hosts, or vice versa.

K. pneumoniae clone ST307 has only recently came into focus as an emerging high-risk clone and genomic analysis shows that it encodes genetic features that might contribute to its adaptation to the hospital setting [24–27]. Several ST307 clones carrying $bla_{\text{NDM-1}}$ had been shown to cause outbreaks [26, 27]. Plasmids are a driving force of ST307 evolution [25]. It has long been known that IncM-type plasmids play a significant role in the spread of NDM-1 [36]. One report was made of an IncM1 that harbors an NDM-1 gene in Uruguay [37] but not in the Mediterranean. Thus, the detection of an IncM1 type plasmid harboring bla_{NDM-1} in Israel in novel and should be taken into consideration in regard to prevention measures to stop its potential spread.

Conclusions

Detecting the emergence and spread of new antibiotic-resistant clones and STs is challenging. If a single patient carrying a novel ST is admitted to a hospital and a second patient with the same ST is treated in another facility, it is unlikely that this ST will be detected before a wider outbreak occurs. Faster detection of novel bacterial STs improves the chances to prevent endemicity by implementing screening, isolation, and other infection control measures. Establishing regional or national FTIR repositories could serve as a simple and effective tool for early detection of emerging antibiotic-resistant clones.

Abbreviations

FTIR	Fourier-transform infrared
ST	Sequence type
CRE	Carbapenem-resistant enterobacterales
NIARIC	National institute for antibiotic resistance and infection control
CPE	Carbapenemase-producing enterobacterales
PACH	Post-acute care hospital
MIC	Minimum inhibitory concentration

CLSI	Clinical and laboratory standards institute
ARG	Antibiotic resistance gene
KL	Capsular polysaccharide
OCL	Lipooligosaccharide outer core
WGS	Whole genome sequencing
SNP	Single nucleotide polymorphism

Supplementary Information

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Supplementary file 1.

Supplementary file 2.

Supplementary file 3.

Supplementary file 4.

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Author contributions
YC and AKP conceived of the study and supervised the work. MNLW performed the analyses. MNLW, AKP and ET wrote the original manuscript. DBB, REE, OK, MB, GL and NR performed several experiments. All authors reviewed and approved the final manuscript.

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Declarations

Ethics approval
Institutional Review Board approval was not required for the study, which did not involve human subjects.

Competing interests
The authors declare no competing interests.

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References

1. Gilchrist CA, Turner SD, Riley MF, Petri WA Jr, Hewlett EL. Whole-genome sequencing in outbreak analysis. Clin Microbiol Rev. 2015;28(3):541–63.
2. Quainoo S, Coolen JP, van Hijum SA, Huynen MA, Melchers WJ, van Schaik W, Wertheim HF. Whole-genome sequencing of bacterial pathogens: The future of nosocomial outbreak analysis. Clin Microbiol Rev. 2017;30(4):1015–63.
3. Bar-Meir M, Berliner E, Kashat L, Zeevi DA, Assous MV. The utility of MALDI-TOF MS for outbreak investigation in the neonatal intensive care unit. Eur J Pediatr. 2020;179:1843–9.
4. Khennouchi NCEH, Loucif L, Boutefnouchet N, Allag H, Rolain JM. MALDI-TOF MS as a tool to detect a nosocomial outbreak of extended-spectrum-β-lactamase-and ArmA methyltransferase-producing Enterobacter cloacae clinical isolates in Algeria. Antimicrob Agents Chemother. 2015;59(10):6477–83.
5. Fiamanya S, Cipolla L, Prieto M, Stelling J. Exploring the value of MALDI-TOF MS for the detection of clonal outbreaks of *Burkholderia contaminans*. J Microbiol Methods. 2021;181:106130.
6. Giraud-Gatineau A, Texier G, Fournier PE, Raoult D, Chaudet H. Using MALDI-TOF spectra in epidemiological surveillance for the detection of bacterial subgroups with a possible epidemic potential. BMC Infect Dis. 2021;21:1–10.
7. Dauwalder O, Cecchini T, Rasigade JP, Vandenesch F. Matrix assisted laser desorption ionisation/time of flight (MALDI/TOF) mass spectrometry is not done revolutionizing clinical microbiology diagnostic. Clin Microbiol Infect. 2023;29(2):127–9.
8. Cuénod A, Aerni M, Bagutti C, Bayraktar B, Boz ES, Carneiro CB, et al. Quality of MALDI-TOF mass spectra in routine diagnostics: results from an international external quality assessment including 36 laboratories from 12 countries using 47 challenging bacterial strains. Clin Microbiol Infect. 2023;29(2):190–9.
9. Jun SY, Kim YA, Lee SJ, Jung WW, Kim HS, Kim SS, Lee K. Performance comparison between Fourier-transform infrared spectroscopy–based IR biotyper and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for strain diversity. Ann Lab Med. 2023;43(2):174–9.
10. Rakovitsky N, Frenk S, Kon H, Schwartz D, Temkin E, Solter E, Lellouche J. Fourier transform infrared spectroscopy is a new option for outbreak investigation: a retrospective analysis of an extended-spectrum-beta-lactamase-producing Klebsiella pneumoniae outbreak in a neonatal intensive care unit. Journal of Clin Microbiol. 2020;58(5):10–1128.
11. Silva L, Rodrigues C, Lira A, Leão M, Mota M, Lopes P, Peixe L. Fourier transform infrared (FT-IR) spectroscopy typing: a real-time analysis of an outbreak by carbapenem-resistant Klebsiella pneumoniae. European J Clin Microbiol Infect Dis. 2020;39:2471–5.
12. Sousa C, Silva L, Grosso F, Lopes J, Peixe L. Development of a FTIR-ATR based model for typing clinically relevant Acinetobacter baumannii clones belonging to ST98, ST103, ST208 and ST218. J Photochem Photobiol B. 2014;133:108–14.
13. Preisner O, Guiomar R, Machado J, Menezes JC, Lopes JA. Application of fourier transform infrared spectroscopy and chemometrics for differentiation of salmonella enterica serovar enteritidis phage types. Appl Environ Microbiol. 2010;76(11):3538–44.
14. Martak D, Valot B, Sauget M, Cholley P, Thouverez M, Bertrand X, et al. Fourier-transform infra red spectroscopy can quickly type gram-negative bacilli responsible for hospital outbreaks. Front Microbiol. 2019;10:1440.
15. Kon H, Lurie-Weinberger M, Cohen A, Metsamber L, Keren-Paz A, Schwartz D, et al. Occurrence, typing, and resistance genes of ESBL/AmpC-producing enterobacterales in fresh vegetables purchased in central Israel. Antibiotics. 2023;12(10):1528.
16. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, bla NDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046–54.
17. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis. 2011;11(5):355–62.
18. Khan AU, Maryam L, Zarrilli R. Structure, Genetics and Worldwide Spread of New Delhi Metallo-β-lactamase (NDM): a threat to public health. BMC Microbiol. 2017;17:1–12.

19. Hornsey M, Phee L, Wareham DW. A novel variant, NDM-5, of the New Delhi metallo-β-lactamase in a multidrug-resistant Escherichia coli ST648 isolate recovered from a patient in the United Kingdom. Antimicrob Agents Chemother. 2011;55(12):5952–4.
20. Liu Z, Xiao X, Li Y, Liu Y, Li R, Wang Z. Emergence of IncX3 plasmid-harboring blaNDM–5 dominated by *Escherichia coli* ST48 in a goose farm in Jiangsu. China Front Microbiol. 2019;10:2002.
21. Zou H, Jia X, Liu H, Li S, Wu X, Huang S. Emergence of NDM-5-producing escherichia coli in a teaching hospital in chongqing, china: IncF-type plasmids may contribute to the prevalence of blaNDM–5. Front Microbiol. 2020;11:334.
22. Ahmad N, Khalid S, Ali SM, Khan AU. Occurrence of blaNDM variants among enterobacteriaceae from a neonatal intensive care unit in a Northern India hospital. Front Microbiol. 2018;9:407.
23. Baloch Z, Lv L, Yi L, Wan M, Aslam B, Yang J, et al. Emergence of almost identical f36:A-:B32 plasmids carrying blandm-5 and QEPA in *Escherichia coli* from both Pakistan and Canada. Infect Drug Resist. 2019;12:3981–5.
24. Lowe M, Kock MM, Coetzee J, Hoosien E, Peirano G, Strydom KA, et al. Klebsiella pneumoniae ST307 with blaoxa-181, South Africa, 2014–2016. Emerg Infect Dis. 2019;25(4):739–47.
25. Villa L, Feudi C, Fortini D, Brisse S, Passet V, Bonura C, et al. Diversity, virulence, and antimicrobial resistance of the KPCproducing *Klebsiella pneumoniae* ST307 clone. Microb Genom. 2017;3(4):e000110.
26. Heiden SE, Hübner NO, Bohnert JA, Heidecke CD, Kramer A, Balau V, et al. A *Klebsiella pneumoniae* ST307 outbreak clone from Germany demonstrates features of extensive drug resistance, hypermucoviscosity, and enhanced iron acquisition. Genome Med. 2020;12(1):1–15.
27. Boonstra MB, Spijkerman DC, Voor AF, van der Laan RJ, Bode LG, van Vianen W, Severin JA. An outbreak of ST307 extended-spectrum beta-lactamase (ESBL)–producing Klebsiella pneumoniae in a rehabilitation center: an unusual source and route of transmission. Infect Control Hosp Epidemiol. 2020;41(1):31–6.
28. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller–Hinton broth. Diagn Microbiol Infect Dis. 2019;94(4):321–5.
29. Kon H, Lurie-Weinberger MN, Lugassy C, Chen D, Schechner V, Schwaber MJ, et al. Use of Fourier-transform infrared spectroscopy for real-time outbreak investigation of OXA-48-producing *Escherichia coli*. J Antimicrob Chemother. 2024;79(2):349–53.
30. Novais Â, Gonçalves AB, Ribeiro TG, Freitas AR, Méndez G, Mancera L, et al. Development and validation of a quick, automated, and reproducible ATR FT-IR spectroscopy machine-learning model for *Klebsiella pneumoniae* typing. J Clin Microbiol. 2024;62(2):e01211-e1223.
31. Wang T, Zhou Y, Zou C, Zhu Z, Zhu J, Lv J, et al. Identification of a novel bla NDM variant, bla NDM-33, in an *Escherichia coli* isolate from hospital wastewater in China. mSphere. 2021;6(5):10–1128.
32. Alba P, Taddei R, Cordaro G, Fontana MC, Toschi E, Gaibani P, et al. Carbapenemase IncF-borne blaNDM-5 gene in the *E. coli* ST167 high-risk clone from canine clinical infection Italy. Vet Microbiol. 2021;256:109045.
33. Sadek M, Juhas M, Poirel L, Nordmann P. Genetic features leading to reduced susceptibility to Aztreonam–Avibactam among Metallo-β-lactamase-producing *Escherichia coli* isolates. Antimicrob Agents Chemother. 2020;64(12):10–1128.
34. Ballash GA, Dennis PM, Mollenkopf DF, Albers AL, Robison TL, Adams RJ, et al. Colonization of white-tailed deer (*Odocoileus virginianus*) from urban and suburban environments with cephalosporinase- and carbapenemase-producing enterobacterales. Appl Environ Microbiol. 2022;88(13):e00465-e522.
35. Shnaiderman-Torban A, Steinman A, Meidan G, Paitan Y, Ahmad WA, Navon-Venezia S. Petting zoo animals as an emerging reservoir of extended-spectrum β-lactamase and AmpC-producing enterobacteriaceae. Front Microbiol. 2019;10:2488.
36. Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A. Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. PLoS ONE. 2015;10(5):e0123063.
37. Papa-Ezdra R, Caiata L, Palacio R, Outeda M, Cabezas L, Bálsamo A, et al. Prevalence and molecular characterisation of carbapenemase-producing enterobacterales in an outbreak-free setting in a single hospital in Uruguay. J Glob Antimicrob Resist. 2021;24:58–62.

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