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MECHANISMS OF RESISTANCE

Complete Nucleotide Sequences of *mcr-4.3*-Carrying Plasmids in *Acinetobacter baumannii* Sequence Type 345 of Human and Food Origin from the Czech Republic, the First Case in Europe

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ABSTRACT Here, we describe two plasmids carrying *mcr-4.3* in two *Acinetobacter baumannii* strains isolated from imported food and a clinical sample. The comparative analysis of these plasmids, with two other plasmids reported in the NCBI database, highlighted the common origin of the plasmidic structure carrying *mcr-4.3*. This is the first case of the *mcr-4.3* gene in a *A. baumannii* strain isolated from a clinical case in Europe. We hypothesize that food import is initiating the spread in Czech Republic.

KEYWORDS *Acinetobacter baumannii*, WGS, colistin resistance, *mcr-4.3*

The first report of the *mcr-4* gene was characterized on a ColE10 plasmid extracted from *Salmonella enterica* of pig origin in Italy (1). The *mcr-4.3* gene, which has two mutations leading to 2 codon changes of V179G and V236F compared with *mcr-4* (2), was first reported in 2014 on a ColE10-type plasmid carried by an *Enterobacter cloacae* isolate of human origin in Singapore (3). Here, we describe two plasmids carrying *mcr-4.3* in two *Acinetobacter baumannii* strains isolated from imported raw food for commercial use and from a clinical sample. Both isolates were selected from an ongoing screening survey for *mcr* genes in the Czech Republic. The first strain (LEV1449/17Ec) was obtained in 2017 from a frozen turkey liver imported from Brazil, while the second isolate (39741) was recovered in 2017 from a tracheal aspirate of a 94-year-old female patient in Karvina, Czech Republic. (These data have been presented as an oral communication at the ECCMID from 13 April to 16 April 2019.)

Antibiotic susceptibility profiling was performed using the Vitek2 system (bioMérieux, Marcy l'Etoile, France), while colistin susceptibility was determined by broth microdilution assay using EUCAST 2019 breakpoints (http://www.eucast.org/clinical_breakpoints/). A conjugation experiment was conducted using the strain *Escherichia coli* J62 as a recipient. *E. coli* DH10B and *A. baumannii* CIP7010 were transformed by plasmid DNA from the wild-type strains using electroporation (4). Genomic DNA extracted from the two strains was subjected to whole-genome sequencing using the MiSeq (Illumina Inc., San Diego, USA) and Sequel I (Pacific Biosciences, CA, USA) platforms. Reads from both Illumina MiSeq and Sequel sequencing were used to perform hybrid assembly. Sequence types and antibiotic resistance genes were detected upon uploading the assemblies to PubMLST (https://pubmlst.org/abaumannii/) and to ResFinder 3.2 (https://cge.cbs.dtu.dk/services/ResFinder/) (5), respectively. High-

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quality reads of LEV1449/17Ec were mapped to the 39741 chromosome using Bowtie v2.3.4 (6). Single nucleotide polymorphisms (SNPs) were called from mapped data using VarScan v2.4.3 (7). Detected SNPs were inspected manually by visually checking mapped data via Tablet v1.17 (8). The chromosome of 39741 was annotated using Prokka v1.13 (9) in order to examine the genetic context of the SNPs. Chromosomes of both samples were also compared and similarity statistics were obtained using QUAST tool v5.0.0 (10).

Both strains, namely, 39741 and LEV1449/17Ec, belonged to the sequence types 345 and 747 (ST345/ST747) according to the Pasteur and Oxford scheme, respectively. They exhibited colistin resistance (MIC, >16 mg/liter) while remaining susceptible to almost all other antibiotics tested (see Table S1 in the supplemental material). Moreover, both strains had 3 resistance genes in the chromosome, namely, intrinsic beta-lactamase-encoding genes bla_{OXA-67} and bla_{ADC-6} and *mcr-4.3* gene on a nonconjugative and nontransformable plasmid (see Table S2 in the supplemental material). Comparative analysis of chromosomal DNA showed that these isolates differ in almost 4% of their genetic content. Moreover, 18 regions of clustered SNPs and a total of 130 of nonclustered SNPs were found between their core genome sequences (see Table S3 in the supplemental material). Chromosomal mutations in the *pmrCAB* operon and in *lpxA*/*lpxC*/*lpxD* genes were screened by comparing the sequences with their respective ones corresponding to the reference strain ATCC 17978 (GenBank accession number CP000521) (11). Most of the amino acid substitutions in PmrCAB have not been previously reported (see Table S4 in the supplemental material). While certain mutations can be lineage specific and have no effect on colistin susceptibility/resistance, the impact of these amino acid substitutions in PmrCAB remains unknown, due to the absence of other genomes belonging to the same sequence type in the GenBank database.

Using long-read sequencing, four complete and circular contigs were obtained for the 39741 strain, namely, a 3,752,576-bp chromosome, a 135,229-bp group 13 plasmid (pEH_gr13), a 25,856-bp group 3 plasmid (pEH_gr3), and another 18,786-bp plasmid harboring the *mcr-4.3* gene that did not resemble any known plasmid Inc family (pEH_mcr4.3). The pEH_mcr4.3 plasmid had one copy of IS*Aba19*, two copies of two different type II toxin-antitoxin (TA) systems (PhD/YefM and RelE/ParE), and a *parA*-like gene (*copG*).

The LEV1449/17Ec strain had four complete and circular contigs, namely, a 3,708,164-bp chromosome, a 128,013-bp group 13 plasmid (pEC_gr13), a 76,956-bp group 6 plasmid (pEC_gr6), and a 43,093-bp plasmid harboring the *mcr-4.3* gene (pEC_mcr4.3). When comparing both *mcr-4.3* plasmids, pEH_mcr4.3 was fully represented in pEC_mcr4.3, with the IS*Aba19* being in the opposite direction. Moreover, pEC_mcr4.3 harbored an extra 24,307-bp segment that mostly contained a set of acyl-coenzyme A (CoA)-related genes which is expressed in low levels under normal conditions but strongly induced under metabolic stress and oxidizing agents (12).

Two isolates carrying plasmids with *mcr-4.3* have been recently reported in *A. baumannii* in the NCBI database; the first one carried a 35,502-bp plasmid (pAb-MCR4.3, GenBank accession number CP033872), which was isolated in 2008 in Brazil from a cerebrospinal fluid sample of a hospitalized patient (*A. baumannii* ST70/ST233). The second one carried a 25,602-bp plasmid isolated from a pig fecal sample (*A. baumannii* ST1303/ST1929) in 2018 in China (pAB18PR065, GenBank accession number CM013137) (13). The comparison of the four plasmids showed that pEH_mcr4.3 was the only common segment present within all the 4 plasmids (except for IS*Aba19*) (Fig. 1). The fact that IS*Aba19* seems to be involved in the addition or removal of the other plasmid segments infers that it plays an important role in the remodeling of the plasmid scaffold, as seen in the 4 discussed plasmids (13). Moreover, the presence of the pEH_mcr4.3 in all of the 4 plasmids suggests that this plasmidic structure is stable. Of note, even though pEH_mcr4.3 carries two copies of the TA systems and

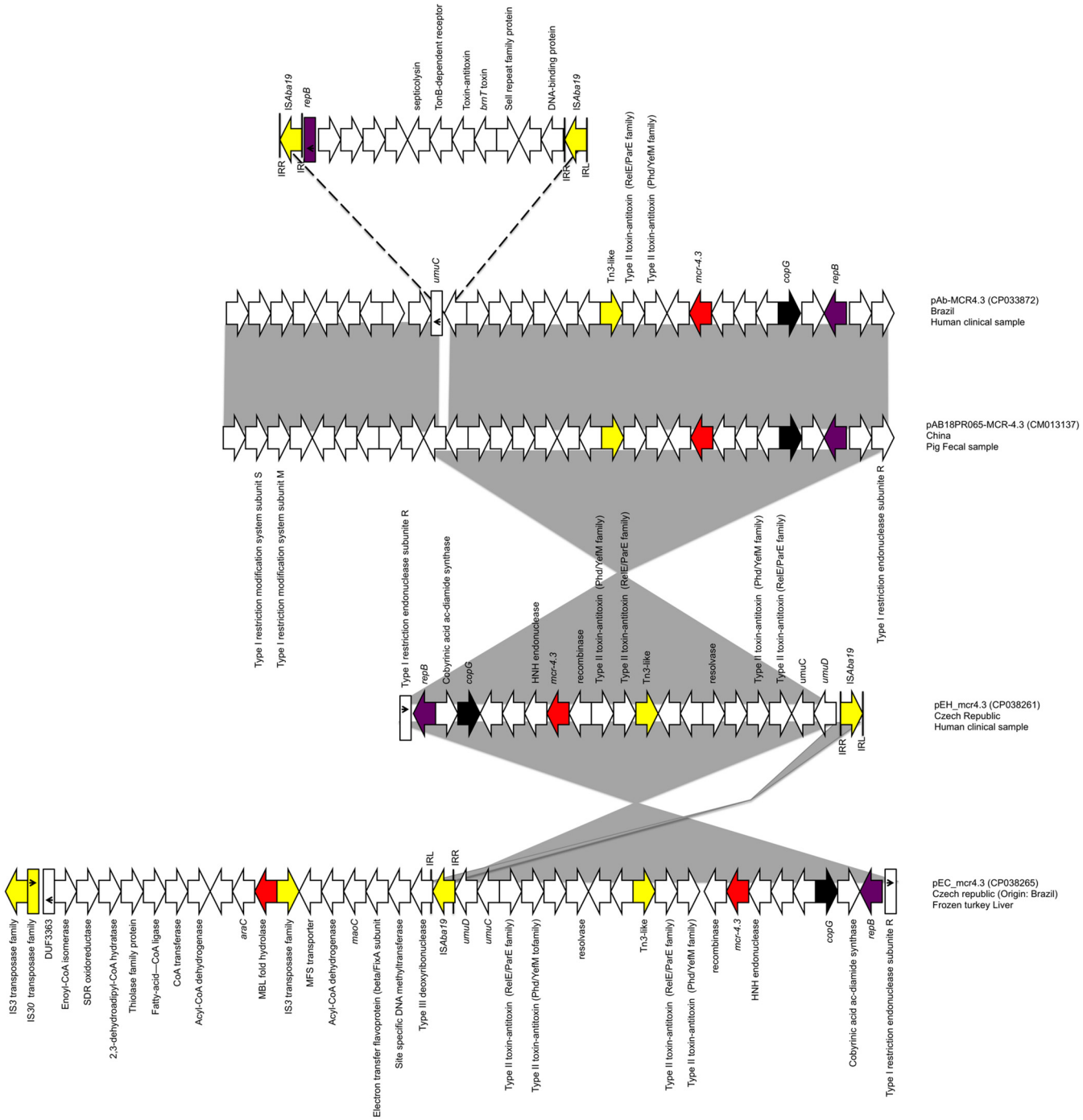

FIG 1 Linear map of the four *mcr-4.3*-carrying plasmids from *Acinetobacter baumannii*; arrows show the direction of transcription of open reading frames (ORFs), while rectangles represent truncated ORFs. Replication, partitioning genes, mobile elements, *mcr-4.3*, and other remaining genes are designated by violet, black, yellow, red, and white, respectively. Gray shaded areas represent similar sequences among the respective regions, while dashed lines represent the insertion point of the complex transposon.

was stable within the wild-type strains, it failed to be maintained upon transformation.

The results of this study highlight the possible role of global food trade in spreading of *mcr*-carrying plasmids into the human community. We hypothesize that the imported meat from Brazil could be an index case and a route of entry to the Czech Republic. The plasmid was probably not stable enough to keep its initial structure, probably due to the absence of selective pressure, fitness cost, and/or the ease of

site-specific recombination of IS*Aba19*, and lost a segment of 20 kb while retaining the rest as it reached the clinical settings. The comparative analysis of the plasmids highlighted the common origin of the plasmidic structure carrying *mcr-4.3* among all the reported plasmids. However, the possible risks of transfer of *A. baumannii* with plasmid-mediated colistin resistance from animals and food to human populations have to be elucidated further. To our knowledge, this is the first case of the *mcr-4.3* gene in *A. baumannii* isolated from a clinical case in Europe.

Data availability. The nucleotide sequences of the 39741 chromosome (EH), pEH_gr13, pEH_gr3, pEH_mcr4.3, LEV1449/17Ec chromosome (EC), pEC_gr13, pEC_gr6, and pEC_mcr4.3 have been deposited in GenBank under the accession numbers CP038258, CP038259, CP038260, CP038261, CP038262, CP038263, CP038264, and CP038265, respectively, and under BioProject accession number PRJNA529047.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01166-19.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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We have no conflicts to declare.

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