Genetic Plurality of OXA/NDM-Encoding Features Characterized From Enterobacterales Recovered From Czech Hospitals

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The aim of this study was to characterize four Enterobacterales co-producing NDM- and OXA-48-like carbapenemases from Czech patients with travel history or/and previous hospitalization abroad. Klebsiella pneumoniae isolates belonged to “high risk” clones ST147, ST11, and ST15, while the Escherichia coli isolate was assigned to ST167. All isolates expressed resistance against most β-lactams, including carbapenems, while retaining susceptibility to colistin. Furthermore, analysis of WGS data showed that all four isolates co-produced OXA-48- and NDM-type carbapenemases, in different combinations (Kpn47733: blaNDM−5 + blaOXA−181; Kpn50595: blaNDM−1 + blaOXA−181; Kpn51015: blaNDM−1 + blaOXA−244; Eco52418: blaNDM−5 + blaOXA−244). In Kpn51015, the blaOXA−244 was found on plasmid p51015_OXA-244, while the respective gene was localized in the chromosomal contig of E. coli Eco52418. On the other hand, blaOXA−181 was identified on a ColKP3 plasmid in isolate Kpn47733, while a blaOXA−181-carrying plasmid being an IncX3-ColKP3 fusion was identified in Kpn50595. The blaNDM−1 gene was found on two different plasmids, p51015_NDM-1 belonging to a novel IncH plasmid group and p51015_NDM-1 being an IncF1K1-FIB replicon. Furthermore, the blaNDM−5 was found in two IncFII plasmids exhibiting limited nucleotide similarity to each other. In both plasmids, the genetic environment of blaNDM−5 was identical. Finally, in all four carbapenemase-producing isolates, a diverse number of additional replicons, some of these associated with important resistance determinants, like blaCTX−M−15, arr-2 and ermB, were identified. In conclusion, this study reports the first description of OXA-244-producing Enterobacterales isolated from Czech hospitals. Additionally, our findings indicated the genetic plurality involved in the acquisition and dissemination of determinants encoding OXA/NDM carbapenemases.

Keywords: OXA-244, OXA-181, NDM-1, NDM-5, mobile genetic elements
INTRODUCTION

The increased incidence of multidrug-resistant (MDR) Gram-negative bacteria worldwide over the last decade has been worrisome (Bassetti et al., 2019). Carbapenems are considered the drug of choice in treating such infections. However, the increased usage of these antibiotics has led to the emergence of carbapenem-resistant strains (Roberts et al., 2020). Center for Disease Control (CDC) considers carbapenem-resistant Enterobacterales (CRE) as a serious global threat to patient health that limits treatment options, especially in chronically ill patients in intensive care units (ICU) and long-term care facilities (McConville et al., 2017; Gupta et al., 2019). Resistance to carbapenems is caused by various mechanisms, such as porin loss, increased efflux pump activity and most importantly the production of carbapenemases (Ye et al., 2018).

Acquired carbapenem-hydrolyzing β-lactamases are enzymes of the Ambler class A KPC type, class B type, including IMP-, VIM-, and NDM-like metallo-β-lactamas (MβLs), or the class D OXA-48 type. However, in Enterobacterales, the most clinically significant metallo-β-lactamas are the NDM-like enzymes (Nordmann et al., 2011). NDM-like enzymes efficiently hydrolyze broad range of β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, with the exception of monobactams such as aztreonam (Nordmann and Poirel, 2014). In 2008, NDM-1 was reported for the first time (Yong et al., 2009) and, shortly after, 24 distinct NDM enzymes have been described worldwide, most of them originating from India, China, Nepal, or Near East (Horseby et al., 2011; Kaase et al., 2011; Nordmann et al., 2012; Williamson et al., 2012; Rogers et al., 2013; Tada et al., 2013; Wang et al., 2014; Wu et al., 2019). Compared to NDM-1, NDM-5 MβL has two amino acid substitutions (Val88Leu and Met154Leu) (Horseby et al., 2011). NDM-5 was reported, for the first time, from a clinical Escherichia coli strain in the United Kingdom (Hornsey et al., 2011). In the Czech Republic, NDM-1 and NDM-5 enzymes were reported for the first time in 2011 and in 2016, respectively (Hrabak et al., 2012; Paskova et al., 2018).

Also, OXA-48-producing Enterobacterales pose an important public threat, mainly due to their challenging detection and the rapid horizontal transfer of OXA-48-like plasmids (Skalova et al., 2017). OXA-48-like enzymes hydrolyze penicillins at a high level and carbapenems at a low level, sparing broad-spectrum cephalosporins, and are not susceptible to β-lactamase inhibitors (Poirel et al., 2004). Since its first report in Turkey in 2004, 11 variants with few amino acid substitutions or deletions emerged globally (Bakthavatchalam et al., 2016; Mairi et al., 2018). In 2006–2007, OXA-181 was reported for the first time in India and since then it is considered one of the most disseminated OXA-48-like enzymes worldwide especially in patients with travel history to the Indian continent (Castanheira et al., 2011; Rojas et al., 2017). In 2011, OXA-244 was reported for the first time in Spain, since then there has been only limited number of reports, indicating limited dissemination (Oteo et al., 2013; Fursova et al., 2015; Potron et al., 2016; van Hattem et al., 2016). Recently, co-production of NDM- and OXA-48-like carbapenemase has been increasingly described, especially in patients with travel history to Italy (Marchetti et al., 2019), South Korea (Baek et al., 2019), Turkey (Otu et al., 2018), Singapore (Balm et al., 2013) and the United States (Doi et al., 2014; Contreras et al., 2020).

Thus, the aim of this study was to genomically characterize four isolates (three Klebsiella pneumoniae and one E. coli) co-producing NDM- and OXA-48-like carbapenemases from Czech patients with travel history or/and previous hospitalization abroad.

MATERIALS AND METHODS

Case Presentations

The first case was reported, in December 2018, from a Czech patient admitted to the Neuro Intensive Unit Care (ICU) of the Military University Hospital in Prague for head injury and concussion. The patient had traveled shortly before admission to India, where he/she was hospitalized due to a motorcycle accident, and then transferred back to Prague. A rectal swab was collected, highlighting a K. pneumoniae isolate (Kpn47733) co-producing NDM- and OXA-48-like carbapenemases.

The second case referred to an inpatient of the Rehabilitation Unit of the Malvazinky Clinic in Prague, who underwent an orthopedic surgery for hip replacement in May 2019. During rehabilitation, the patient developed a urinary tract infection. Urine culture confirmed the presence of a K. pneumoniae isolate (Kpn50595), coproducing NDM- and OXA-48-like carbapenemases. The patient had a travel history 2 weeks before admission (April 2019) to Mauritius, but didn’t have any history of hospitalization there.

The third case was a patient admitted to Hepatogastroenterology Unit of the Institute of Clinical and Experimental Medicine in Prague for bile duct obstruction, in June 2019. As a part of the screening process, a rectal swab was performed, and culture highlighted the presence of a K. pneumoniae isolate (Kpn51015), co-producing NDM- and OXA-48-like enzymes. The patient had a travel history to Egypt in December 2018, without hospitalizations.

The fourth case was reported in August 2019, when a female patient was admitted to the Nephrology Ambulatory of the University Hospital of Ostrava, showing urinary tract infection symptoms. Urine sample culture identified the presence of an E. coli isolate (Eco52148), co-producing NDM- and OXA-48-like enzymes. The patient’s hospitalization history showed that she had kidney transplantation shortly before being admitted. Additionally, the patient was recently repatriated from northern part of Africa.

Carbapenem Production and Susceptibility Testing

These four isolates, mentioned above, were selected to be further characterized, since they were the only Enterobacterales, co-producing NDM- and OXA-48-like enzymes, referred from local microbiological laboratories to our lab, during 2018–2019. Species identification of the four strains was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) through MALDI Biotyper.
software (Bruker Daltonics, Bremen, Germany). MALDITOF MS meropenem hydrolysis assay was used to confirm carbapenem production (Rotova et al., 2017). Production of carbapenemases (metallo-β-lactamase, OXA-48 and KPC) was assessed using the double-disc synergy test with EDTA, temocillin disc test and phenylboronic acid test (Lee et al., 2003; Doi et al., 2008; Glupczynski et al., 2012). The isolates were screened by PCR for the presence of bla<sub>NDM</sub>-like bla<sub>AMR</sub>-like, bla<sub>KPC</sub>-like and bla<sub>OXA−48</sub>-like genes (Papagiannitsis et al., 2015). Antimicrobial susceptibility was performed using broth microdilution according to European Committee on AntimicrobialSusceptibility Testing (EUCAST) guidelines. Susceptibility to fosfomycin was performed using agar dilution based on EUCAST guidelines. Susceptibility data were interpreted according to the criteria (version 10.0) of the EUCAST<sup>1</sup>.

**Transfer of Carbapenemase-Encoding Genes**

The conjugal transfer of carbapenemase-encoding genes was tested in liquid medium using the *E. coli* A15 strain (Azd<sup>6</sup>) as recipient. Transconjugants were selected on MacConkey agar (Scharlab, SL, Barcelona, Spain) plates containing sodium azide (100 mg/L) (Sigma-Aldrich, St. Louis, MO, United States) and ampicillin (100 mg/L) (Sigma-Aldrich). The presence of bla<sub>NDM</sub>-like and bla<sub>OXA−48</sub>-like was confirmed by PCR.

**Whole-Genome Sequencing and Analysis**

Genomic DNA was extracted from the four clinical isolates using NucleoSpin Microbial DNA kit (Macherey-Nagel, Germany). Whole genome sequencing (WGS) was performed on the Sequel I platform (Pacific biosciences, Menlo Park, CA, United States). Microbial multiplexing protocol was used for the library preparation according to the manufacturer instructions for Sheared DNA. DNA shearing was performed using the Megaruptor 2 (Diagenode, Liege, Belgium) using long hydro pores producing 15kb long inserts. No size selection was performed during the library preparation. Microbial Assembly pipeline offered by the SMRT Link v8.0 software was used to perform the assembly and circularization with minimum seed coverage of 30×. Assembled sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Antibiotic resistant genes, plasmid replicons, mobile elements and multilocus sequence types (MLST) were determined through uploading the assembled sequences to ResFinder 4.1 and CARD (Zankari et al., 2017; Alcock et al., 2020), PlasmidFinder (Carattoli et al., 2014), ISfinder (Siguier et al., 2006), and MLST 2.0 (Larsen et al., 2012), respectively. Comparative genome alignment was done using Mauve v.2.3.1<sup>3</sup> and BLAST Ring Image Generator (BRIG) (Alikhan et al., 2011). Diagrams and gene organization were sketched using Inkscape 0.92.4<sup>1</sup>.

**Nucleotide Sequence Accession Numbers**

The nucleotide sequences of the genomes and plasmids of Kpn47733, Kpn50595, Kpn51015, and Eco52148 has been uploaded to GenBank under the accession numbers CP050360-CP050370, CP050371-CP050375, CP050376-CP050381, and CP050382-CP050384 respectively.

**RESULTS**

All isolates expressed resistance to ampicillin, ciprofloxacin, piperacillin, piperacillin-tazobactam, cefotaxime, meropenem, and ertapenem, while retaining susceptibility to colistin. Moreover, all isolates, except for Eco52148, showed resistance against gentamicin, amikacin, netilmicin and tobramycin. On the other hand, isolates Eco52148 and Kpn47733 showed resistance to tetracycline (Table 1).

WGS performed on the Sequel I platform and assembly performed on Microbial Assembly pipeline resulted in complete, closed chromosomes and plasmids shown in Table 2. WGS revealed that *K. pneumoniae* isolates Kpn47733, Kpn50595, and Kpn51015 belonged to sequence types (STs) 147, 11 and 15, respectively. All these three STs have been considered as "high risk" clones (Woodford et al., 2011). The *E. coli* isolate Eco52148 was assigned to ST167. Several studies have reported the association of ST167 *E. coli* with the dissemination of resistance genes, especially of the carbapenemase-encoding gene bla<sub>NDM</sub>−5 (Mani et al., 2017; Sánchez-Benito et al., 2017; Sun et al., 2018; Xu et al., 2019).

Furthermore, analysis of WGS data showed that all four isolates carried different combinations of carbapenemase-encoding genes (Kpn47733: bla<sub>NDM</sub>−5 + bla<sub>OXA−48</sub>; Kpn50595: bla<sub>NDM</sub>−1 + bla<sub>OXA−181</sub>; Kpn51015: bla<sub>NDM</sub>−1 + bla<sub>OXA−244</sub>; Eco52148: bla<sub>NDM</sub>−5 + bla<sub>OXA−244</sub>) (Table 2). Noteworthy, based on our knowledge, this is the first report of *Enterobacteriales*, carrying bla<sub>OXA−244</sub> gene, isolated from the Czech Republic. Additionally, all isolates exhibited a wide variety of resistance genes conferring resistance to β-lactams, aminoglycosides, sulfonamides, macrolides, lincosamides, streptogramin b, fosfomycin (low-level resistance), fluoroquinolones, chloramphenicol, tetracyclines, and/or rifampicin (Table 2).

The carbapenem resistance phenotypes of all clinical strains were transferred to azide-resistant *E. coli* A15 by conjugation (Supplementary Table 1). For isolates Kpn47733, Kpn50595 and Kpn51015, all transconjugants carried both carbapenemase-encoding genes, while only the bla<sub>NDM</sub>−5 gene was identified in the transconjugants of the *E. coli* isolate Eco52148.

Analysis of contigs carrying carbapenemase-encoding genes showed that, in isolate Kpn51015, the bla<sub>OXA−244</sub> gene was found on a plasmid (p51015_OXA−244) of 71402 bp in size, while the respective gene was localized in the chromosomal contig of *E. coli* isolate Eco52148. In both isolates, the bla<sub>OXA−244</sub> gene was bounded by two copies of IS1 insertion sequence in parallel orientation (Supplementary Figure 1), forming a composite transposon, named Tn51098. In the *E. coli* isolate Eco52148, the
TABLE 1 | Susceptibility profiles of Enterobacteria, co-producing NDM- and OXA-48-like carbapenemases, isolates collected in Czech hospitals, during the study.

| Isolate    | ST    | Amp     | Pip     | Tzp     | Ctx     | Caz     | Mem     | Etp     | Gm      | Amk     | Tm      | Net     | Tet     | Tgc     | Col     | Fos     |
|------------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| K. pneumonia Kpn47733 | 147 | >128   | >128   | >128   | >8      | >16     | >16     | >2      | >32     | >64     | >8      | >16     | 1       | 0.25    | 128     |
| K. pneumonia Kpn50596 | 11  | >128   | >128   | >128   | >8      | >16     | >16     | >2      | >32     | >64     | >8      | >16     | 2       | 0.5     | 32      |
| K. pneumonia Kpn51015 | 15  | >128   | >128   | >128   | >8      | >16     | 8       | >2      | >32     | >64     | >8      | >16     | 1       | 0.25    | 64      |
| E. coli Eco52148 | 167 | >128   | >128   | >128   | >8      | >16     | 4       | >2      | 4       | 64      | 0.5     | 1       | >32     | 0.25    | 0.5     |

MIC, minimum inhibitory concentration; Amp, ampicillin; Pip, piperacillin; Tzp, tazobactam; Ctx, cefotaxime; Caz, ceftazidime; Mem, meropenem; Etp, ertapenem; Gm, gentamicin; Amk, amikacin; Tm, tobramycin; Net, netilmicin; Tet, tetracycline; Tgc, tigecycline; Col, colistin; Fos, fosfomycin.

TABLE 2 | WGS data of Enterobacteria, co-producing NDM- and OXA-48-like carbapenemases, isolates recovered from Czech hospitals.

| Isolate    | ST    | Replicons | Size   | Plasmid name | Inc group | Carbapenemase encoding-genes | Other resistance genes | GenBank accession no. |
|------------|-------|-----------|--------|--------------|-----------|-------------------------------|------------------------|----------------------|
| Kpn47733   | 147   | Chromosome| 5401559 bp |             |           |                                |                        | CP050360             |
| Plasmid    | 103085 bp | p47733_NDM-5 | IncFII | blaNDM-5    |           | aadA2, mrtB, blatem, mepB, mepH, sul1, dfrA12 |                        | CP050367             |
| Plasmid    | 6812 bp | p47733_OXA-181 | ColKp3 | blaOXA-181  |           | rmF, blatem, mepH, catA2, aac(6')-Ib-cr, qnrB1, dfrA12, dfrA14 |                        | CP050368             |
| Plasmid    | 119961 bp | p47733_CTX-M-15 | R      | –           |           | –                             |                        | CP050364             |
| Plasmid    | 107451 bp | p47733_ARR-2 | IncFII | –           |           | –                             |                        | CP050361             |
| Plasmid    | 115360 bp | p47733_IndFIB | IncFIB | –           |           | –                             |                        | CP050365             |
| Plasmid    | 2101 bp | p47733_Col_BSS12 | Col | –           |           | –                             |                        | CP050362             |
| Plasmid    | 1546 bp | p47733_Col_MG828 | Col | –           |           | –                             |                        | CP050363             |
| Plasmid    | 2056 bp | p47733_Col_PVC | Col | –           |           | –                             |                        | CP050370             |
| Plasmid    | 4715 bp | p47733_S | NT    | –           |           | –                             |                        | CP050369             |
| Plasmid    | 55119 bp | p47733_L | NT    | –           |           | –                             |                        | CP050366             |
| Kpn50596   | 11    | Chromosome| 5339674 bp |             |           |                                |                        | CP050371             |
| Plasmid    | 193462 bp | p50595_NDM-1 | IncFIB-IndFIB | blaNDM-1 |           | aac(3)-Ia, aac(6')-Ib-cr, mrtF, blatem, catB, arn-2 |                        | CP050374             |
| Plasmid    | 51140 bp | p50595_OXA-181 | IncX3-ColKp3 | blaOXA-181 |           | qnrS1 |                        |                        | CP050375             |
| Plasmid    | 76387 bp | p50595_ERM | IncFII | –           |           | emr(B), mepH(A) |                        | CP050372             |
| Plasmid    | 127925 bp | p50595 | IncFII-IndFIB | –           |           | –                             |                        | CP050373             |
| Kpn51015   | 15    | Chromosome| 5306656 bp |             |           |                                |                        | CP050376             |
| Plasmid    | 353810 bp | p51015_NDM-1 | IncFIB-IndFIB | blaNDM-1 |           | aaph(3)-la, aaph(3')-VIII, armA, mepH(A), mepH(E), mar(E), qnrS1, sul1, sul2, dfrA5 |                        | CP050380             |
| Plasmid    | 71402 bp | p51015_OXA-244 | IncFII | blaOXA-244 |           | –                             |                        | CP050381             |
| Plasmid    | 225540 bp | p51015_CTX-M-15 | IncFII-IndFIB | –           |           | aac(3)-IId, aac(6')-Ib-cr, aaph(3)-Ib, aaph(3')-Ia, aaph(3)-Id, blatem, blatem, mepH(A), catB3, sul2 |                        | CP050379             |
| Plasmid    | 18651 bp | p51015_ColRNAI | ColRNAI | –           |           | –                             |                        | CP050378             |
| Plasmid    | 1565 bp | p51015 | NT    | –           |           | –                             |                        | CP050377             |
| Eco52148   | 167   | Chromosome| 4859628 bp |             |           |                                |                        | CP050382             |
| Plasmid    | 121872 bp | p52148_NDM-5 | IncFII-IndFIA | bblaOXA-244 |           | mdr(A) |                        |                        | CP050384             |
| Plasmid    | 4081 bp | p52148 | NT    | –           |           | –                             |                        | CP050383             |

NT, non-typed.
Tn51098 transposon was integrated into an open reading frame (ORF) encoding an HNH endonuclease (nts 576701 to 580010 in GenBank accession no. CP050382), as described previously (Potron et al., 2016; Hoyos-Mallecot et al., 2017). Direct repeats of 9 bp (TGAATTGCT) were found at the boundaries of the bla_{OXA-244}-carrying composite transposon, suggesting its transposition into the *E. coli* chromosome. However, unlike the isolate Eco52418, an ORF encoding a LysR transcriptional regulator wasn’t found between bla_{OXA-244} (downstream) and IS1, in plasmid p51015_OXA-244. Plasmid p51015_OXA-244, which belonged to the incompatibility group FII (IncFII), exhibited extensive similarity with IncFII plasmids from *E. coli* strains D181 and F5176C6 (GenBank accession nos. CP024250 and CP024669, respectively) (Supplementary Figure 2A). Unlike p51015_OXA-244, those plasmids were negative for the presence of *bla*_{OXA-244} gene. Among p51015_OXA-244, no resistance genes other than *bla*_{OXA-244} were identified.

On the other hand, the *bla*_{OXA-181} carbapenemase-encoding gene was identified on a ColKP3 plasmid (p47733_OXA-181) of 6812 bp in size, in isolate Kpn47733, while a *bla*_{OXA-181}-carrying plasmid (p50595_OXA-181; 51140 bp), being an IncX3-ColKP3 fusion, was identified in isolate Kpn50595. In both plasmids, the *bla*_{OXA-181} genes were surrounded by identical sequences (Supplementary Figure 3). In comparison with the archetypal ColE2-type plasmid pKP3-A carrying *bla*_{OXA-181} (Potron et al., 2011), p47733_OXA-181 was composed only of repA and *bla*_{OXA-181} genes (Supplementary Figure 2A). The *mob* genes, encoding proteins that form a plasmid mobilization system, were not found in plasmid p47733_OXA-181. One additional difference between the two plasmids was the presence of Tn5403 transposon in p47733_OXA-181. The Tn5403 transposon has previously been found in *bla*_{NDM-1}-positive IncN2 plasmids, like plasmid pN24NDM1 characterized from a ST405 *E. coli* from China (Hao et al., 2019). On the other hand, plasmid p50595_OXA-181 was almost identical to plasmids p1-EcBERN-042 (100% coverage, 99.99% identity; GenBank accession no. CP042935) and pOXA181_29144 (100% coverage, 99.99% identity) (Supplementary Figure 2A). Plasmid pOXA181_29144 was previously characterized from a ST18 *K. pneumoniae* strain (Kpn-29144) isolated, in 2015, in the Czech Republic (Skalova et al., 2017). Similar to pOXA181_29144, which was transferable by conjugation (Skalova et al., 2017), a complete *tra* locus was found in the sequence of p50595_OXA-181. Also, the *qnrS1* gene, conferring low-level resistance to fluoroquinolones, was identified in the sequence of p50595_OXA-181.

The *bla*_{NDM-1} carbapenemase-encoding gene was found on two different plasmid types (Supplementary Figure 4). In isolate Kpn51015, a *bla*_{NDM-1}-positive plasmid (p51015_NDM-1) of 353810 bp in size was identified, while a 193462-bp plasmid (p50595_NDM-1) carrying *bla*_{NDM-1} was found in isolate Kpn50595. Plasmid p51015_NDM-1 exhibited extensive similarity to *bla*_{NDM-5}-carrying plasmid pKPvST383I (99% coverage, 99% identity; GenBank accession no. CP034201) (Supplementary Figure 2B), characterized from a ST383 *K. pneumoniae* recovered in London. p51015_NDM-1 belonged to a novel IncH plasmid group, harboring FIB and HIB replicons previously observed in *bla*_{NDM-1}-carrying plasmid pNDM-MAR characterized from a ST15 *K. pneumoniae* isolated in Morocco (Villa et al., 2012). In the MDR of p51015_NDM-1, beside *bla*_{NDM-1}, *aph(3′)-la, aph(3′)-VI, armA, mph(A), mph(E), msr(E), qnrS1, sul1, sul2, and dfrA5 genes, conferring resistance to aminoglycosides, macrolides, streptogramin b, fluoroquinolones, sulfonamides and trimethoprim, were found (Figure 1). Additionally, plasmid p51015_NDM-1 carried tellurium resistance genes (*terZABCDEF*), commonly associated with this plasmid family (Zingali et al., 2020). Unlike p51015_NDM-1, p50595_NDM-1 was an IncF1-K-FIB plasmid being a fusion derivative of previously characterized plasmids, like pUCLAOX232-3, p51015_CTX_M_15, pKPX-1, and pGR-1870 (GenBank accession no. CP012564, CP050379, AP012055 and KF874498) (Supplementary Figure 2B). In p50595_NDM-1, the *bla*_{NDM-1} gene was found in a genetically distant MDR region than observed in plasmid p51015_NDM-1. The MDR region of p50595_NDM-1 also contained *bla*_{CTX-M-15}, *aac(3)-Ⅱa, aacA4* (2 copies), *rmtF* (2 copies), *catB* (2 copies) and *arr-2* (2 copies) genes conferring resistance to β-lactams,
aaminoglycosides, chloramphenicol and rifampicin (Figure 1). Additionally, plasmid backbone of p50595_NDM-1 included an arsenate resistance region. In both blaNDM-1-carrying plasmids, several insertion sequences (ISs) that could be involved in the organization of their MDR regions were found.

Furthermore, the blaNDM-5 gene was found in two IncFII plasmids, p47733_NDM-5 and p52418_NDM-5, which contained complete tra operons. The FII (allele 2) plasmid p47733_NDM-5 (103085 bp), found in K. pneumoniae Kpn47733, showed extensive similarity to the blaNDM-5-carrying plasmid pCRKP-2297_2 (99% coverage, 99.96% identity; GenBank CP050384) (Supplementary Figure 2B) characterized from K. pneumoniae strain CRKP-2297 recovered in South Korea. In p47733_NDM-5, the blaNDM-5 gene was found in a MDR region of 31159 bp (nts 38315-69473 in GenBank accession no. CP050367) (Supplementary Figure 2B). The blaNDM-5 gene was bounded by two copies of IS26 element, also included blrTEM-1, aadA2 (2 copies), sul1, rmtB, mphA and ermB genes conferring resistance to β-lactams, aminoglycosides, sulfonamides, macrolides, lincosamides, and streptogramin B. An additional resistance gene, dfrA12, being the unique gene cassette of a class 1 integron was identified ~26 Kb upstream of the p47733_NDM-5 MDR region (Figure 2). While both blaNDM-5-carrying plasmids belonged to IncFII group, the FII (allele 36) plasmid p52148_NDM-5 (121872 bp), found in E. coli isolate Eco52148, harbored a second replicon, FIA (allele 4) (nts 85531-86286 in GenBank accession no. CP050384). Plasmid p52148_NDM-5 exhibited limited nucleotide similarity (53% coverage, 100% identity) against p47733_NDM-5. However, it was highly similar to blaNDM-5-carrying plasmid p1ESCUMpO83_CORR (100% coverage, 99.98 identity; GenBank accession no. CP033159) (Supplementary Figure 2B) characterized from a pathogenic E. coli strain in India. In p52148_NDM-5, the blaNDM-5 gene was found in a MDR region of 28400 bp in size (nts 105368-121872 and 1-11895 in GenBank accession no. CP050384). In both plasmids, p47733_NDM-5 and p52148_NDM-5, the genetic environment of blaNDM-5 was identical (Figure 2). The aadA2, dfrA12, sul1, mphA and tetA resistance genes were also identified in the MDR region of p52148_NDM-5. The MDR region of p52418_NDM-5 was inserted downstream the pemIK operon, as it was also observed in p47733_NDM-5.

Finally, in all four carbapenemase-producing isolates, a diverse number of additional replicons were identified (Table 2). Some of these replicons were associated with important resistance determinants, like blaCTX-M-15, arr-2 and ermA.

DISCUSSION

Previous studies have reported the spread of blaOXA-48-like and blaNDM-like genes in Enterobacteriales recovered from Czech hospitals (Skalova et al., 2017; Paskova et al., 2018). However, based on our knowledge, this study reports the first description of OXA-244-producing Enterobacteriales isolated from Czech hospitals. Both OXA-244-producing isolates, also expressed NDM-1 or NDM-5 β-lactamases, were identified. Overall, horizontal gene transfer is the main mechanism involved in the dissemination of blaNDM-like and blaOXA-48-like genes, but certain clones have been associated with the spread of these resistance genes (Pitout et al., 2019). Carbapenemase-producing K. pneumoniae and E. coli isolates, characterized during this study, belonged to clones (ST11, 15, and 147 in K. pneumoniae, and ST167 in E. coli; Table 2), which have been previously characterized as “high risk clones,” and have been reported in association with these carbapenem-resistance mechanisms (Woodford et al., 2011; Pitout et al., 2019). In all cases, carbapenemase-producers were recovered from patients with travel history or previous hospitalization abroad. The endemicity of OXA-181 and NDM-5 carbapenemases among Enterobacteriales isolated in the Indian subcontinent has been reported in several studies (Lascols et al., 2013; Krishnaraju et al., 2015; Ahmad et al., 2019;
Interestingly, a recent report from the United States described the characterization of a ST147 *K. pneumoniae* harboring *bla*$_{NDM-5}$ and *bla*$_{OXA-181}$ from a patient, who was previously hospitalized in India (Rojas et al., 2017). Also, North African countries, and especially Egypt, represent a geographical region, where *bla*$_{OXA-48}$-like and/or *bla*$_{OXA-NDM}$-like genes are highly disseminated among *Enterobacterales* (Tafoukt et al., 2017; Soliman et al., 2020a,b). Studies from Italy have described the import of NDM-1-producing *K. pneumoniae* isolates from Egypt (Principe et al., 2016; Nucleo et al., 2020). Additionally, the import of OXA-244-producing *E. coli* isolates from countries in Northern Africa was observed in a surveillance from Denmark (Hammerum et al., 2020). Finally, two studies have documented the spread of NDM-1-producing *K. pneumoniae* isolates in Mauritius (Poirel et al., 2012; Holman et al., 2017), speculating a link with India, due to the geographical and cultural links between the two countries. In 2018, another study described the characterization of a *K. pneumoniae* isolate co-producing NDM-1 and OXA-181 carbapenemases, recovered from a patient, who had previously went to Mauritius (Allyn et al., 2018). These findings highly underline that import of carbapenemase-producing isolates via travel or/and hospitalization abroad could represent a risk for a further dissemination of these isolates in Czech hospitals. However, epidemiological data don’t confirm the scenario regarding the spread of *Enterobacterales* co-producing NDM- and OXA-48-like carbapenemases in Czech hospitals (Hrabak, unpublished results).

Although the main limitation of this study was the small number of *Enterobacterales* isolates co-producing OXA- and NDM-type carbapenemases, which were collected in clinical microbiology laboratories, analysis of WGS data revealed that all four isolates harbored a huge variety of genes conferring resistance to several categories of antibiotics. Additionally, inspection of contigs carrying carbapenemase-encoding genes showed that different genetic structures and replicon types were involved in the dissemination of these resistance determinants. These contigs also included a huge variety of insertion sequences that might be involved in the organization of MDR regions, conferring resistance to several antibiotic categories thus, limiting therapeutic options. Thus, in addition to different combinations of carbapenemase-encoding genes, the variability of replicon types, genetic structures, resistance genes and mobile elements observed among the studied isolates indicated the genetic plurality involved in the acquisition and dissemination of determinants encoding OXA/NDM carbapenemases.

### DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, CP050360–CP050384.

### AUTHOR CONTRIBUTIONS

CP and IB played an important role in interpreting the results and in writing the manuscript. KC, LK, and JH helped to acquire data. KC, LK, VM, and IB carried out experimental work. CP supervised the experiments and revised the final manuscript, which was approved by all authors.

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### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.641415/full#supplementary-material

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