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Short Communication

Repatriation of a patient with COVID-19 contributed to the importation of an emerging carbapenemase producer

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A B S T R A C T

Objectives: Patients hospitalised abroad can become colonised with multidrug-resistant bacteria and import them to their home countries. In this study, we characterised an OXA-484 carbapenemase-producing *Escherichia coli* strain from a Swiss patient infected by SARS-CoV-2 and repatriated from India.
Methods: At admission to Switzerland (April 2021), the patient undertook a nasopharyngeal swab to search for SARS-CoV-2 and a rectal swab to detect multidrug-resistant bacteria. Both SARS-CoV-2 and *E. coli* isolates were whole-genome sequenced and analysed for phylogenetic relatedness.
Results: The patient was infected with the SARS-CoV-2 B.1.617.2 lineage (VOC Delta), a lineage that began to be reported across Switzerland at that time. He was also colonised with a sequence type 410 (ST410) *E. coli* strain (L3452210II) producing OXA-484, a single amino acid variant of OXA-181. The $bla_{OXA-484}$ gene was carried by a 51.5 kb IncX3 plasmid identical to those described in $bla_{OXA-181}$-harbouring ST410 *E. coli* strains. Core genome analysis showed that L3452210II was identical ($\triangle$SNV $\leq$23) to two ST410 OXA-484 producers recently reported in Qatar and Germany, but differed from other ST410 OXA-181 producers reported worldwide.
Conclusion: The patient was infected by an emerging SARS-CoV-2 variant and also imported an *E. coli* producing OXA-484, an OXA-48-like carbapenemase not yet reported in Switzerland. The genetic background of L3452210II indicated that $bla_{OXA-484}$ shared the same plasmid as $bla_{OXA-181}$, but its bacterial host differed from most of the pandemic OXA-181-producing ST410 strains reported previously. This case description underlines that the COVID-19 crisis can contribute to the worldwide spread of emerging carbapenemase producers.

1. Introduction

The spread of carbapenemase-producing Enterobacterales (CPE) represents a worldwide public-health concern. In particular, the prevalence of OXA-48-type producers is increasing as their numerous OXA-48-like variants (e.g. OXA-181, OXA-232, OXA-244) emerge [1]. For instance, OXA-181 is the second most common OXA-48 derivative, which differs from OXA-48 by four amino acid substitutions. Its *bla* gene is commonly located on a 51.5-kb IncX3 plasmid that is frequently harboured by high-risk sequence type 410 (ST410) *Escherichia coli* strains. Such $bla_{OXA-181}$-possessing ST410 *E. coli* ($bla_{OXA-181}$-ST410-*Ec*) strains originated on the India subcontinent and are nowadays reported worldwide both in human and non-human settings [1,2].

One of the ways in which CPE spread throughout the world is by the transfer of patients between countries. In fact, it is well known that hospitalised subjects can become colonised at the intestinal level with multidrug-resistant Enterobacterales (MDR-E). Importation of these pathogens into the country of origin (i.e. after patient transfer) can lead to difficult-to-treat infections; importantly, their dissemination can contribute to the spread to other people and/or settings (e.g. animals, food chain and the environment) [3,4].

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In this context, the current COVID-19 (coronavirus disease 2019) pandemic crisis may amplify this phenomenon. Constant hygiene precautions of healthcare workers and pre-emptive isolation of patients repatriated from abroad are important elements to prevent the dissemination of MDR-E. However, the current COVID-19 pandemic has placed pressure on these precaution standards because healthcare workers are overworked and there is a lack of training of supplementary staff working in rooms with multiple beds and in intensive care units (ICUs) [5–7]. This may result in an uneven focus of hygiene precautions for COVID-19, while infection prevention and control measures linked to MDR-E are involuntarily (and unknowingly) relaxed.

In this study, we report a case of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection that was associated with intestinal colonisation of a rare OXA-48 variant in a Swiss patient transferred from India. State-of-the-art molecular and genomic techniques were implemented to characterise both the viral and bacterial pathogens.

2. Materials and methods

2.1. Routine laboratory tests

In April 2021, an adult patient hospitalised in India was transferred back to Switzerland. At admission, the patient underwent a nasopharyngeal swab to search for SARS-CoV-2 by implementing both the Cobas® SARS-CoV-2 and Influenza A/B assays on a Cobas® Liat® System (Roche), and the Xpert® Xpress SARS-CoV-2/Flu/RSV assay (Cepheid) was used as a confirmatory test to estimate the viral load. A rectal swab was also obtained to screen for MDR-E as part of the routine hygiene guidelines of the hospital. No further clinical data are available. The bacterial strain was named L3452210II to assure anonymity of the patient.

The rectal swab was plated on CHROMagar™ Orientation ESBL, mSuperCARBA and vancomycin-resistant enterococci (VRE) selective media and was incubated overnight at 36°C. Species identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker). Antimicrobial susceptibility testing was performed using broth microdilution ESB1F and GNX2F Sensititre panels (Thermo Fisher). Minimum inhibitory concentrations (MICs) for antibiotics were interpreted according to the 2021 European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (https://www.eucast.org/; version 11.0). eazyplex® SuperBug complete B assay was used to screen for the presence of the main *bla* carbapenemase genes (Amplex Diagnostics; https://www.eazyplex.com/).

2.2. Sequencing of the SARS-CoV-2 genome

The SARS-CoV-2 RNA genome was reverse transcribed and amplified following the ARTIC V3 protocol. Nanopore library preparation was performed with the sequencing kit SQK-LSK109, and whole-genome sequencing (WGS) was performed on a GridION X5 (Oxford Nanopore Technologies). Reagents, quality control and flow cell preparation were done as previously described [8,9]. Bioinformatic analyses followed the ARTIC workflow (v.1.1.3). Consensus sequences were generated using medaka and bcftools. All methods are described in detail in Supplementary File 1.

Overall, 59 291 Nanopore reads were generated (coverage 250 ×). The final assembly (29 775 bases, 98.67% covered bases) contained 8.92% unknown bases and a 7-nucleotide gap compared with the reference sequence of Wuhan-Hu-1 (GenBank **MN908947.3**). The resulting consensus sequence was deposited in GISAID (https://www.gisaid.org/) with accession no. EPI_ISL_1916510.

2.3. Characterisation of the Escherichia coli strain

Escherichia coli strain L3452210II underwent WGS implementing both NovaSeq 6000 (Illumina Inc.) and MinION (SQK-RBK004 library, FLO-MIN 106D R9 flow-cell; Oxford Nanopore Technologies) technologies as previously described [10–12]. The hybrid assembly was generated using Unicycler v.0.4.8, and annotation was performed with the NCBI pipeline. The final genome was analysed using tools from the Center for Genomic Epidemiology (www.genomicepidemiology.org/). Species identification was confirmed implementing the Type (Strain) Genome Server (https://tygs.dsmz.de/). Genome and plasmid assemblies were deposited in GenBank (**CP076527**– **CP076531**) under BioProject PRJNA735932.

2.4. Phylogenetic analyses for SARS-CoV-2 and Escherichia coli

All genomic sequences of SARS-CoV-2 B.1.617.2 lineages available for Switzerland ($n = 180$ as of 28 June 2021) were retrieved from GISAID. The multiple sequence alignment generated with MAFFT v.7.407 was further subjected to maximum likelihood phylogenetic reconstruction with IQ-TREE 2 using the TIM2+F+I model and 1000 ultrafast bootstrap replicates. All methods are described in detail in Supplementary File 1.

To study the link between *E. coli* L3452210II and other *bla*$_{OXA-181}$/*bla*$_{OXA-484}$-ST410-*Ec* strains, a core genome single nucleotide variant (SNV) analysis was performed as done previously [10,12]. Briefly, the genome assemblies of *bla*$_{OXA-181/OXA-484}$-ST410-*Ec* strains were retrieved from Enterobase and the NCBI genome database (access date 15 June 2021). Core genome alignment and calculation of the phylogenetic tree was performed with Parsnp v.1.2. Variants with no flags (PASS) were determined as reliable and were used for downstream SNV analysis with a custom R v.3.6.2 script.

3. Results and discussion

3.1. SARS-CoV-2

The routine nasopharyngeal swab resulted positive for the SARS-CoV-2 target (cycle threshold value of 25.9). According to WGS, the patient's case was classified as Pango lineage B.1.617.2 or VOC Delta (World Health Organization), which was first detected in India (https://covariants.org/variants/21A.Delta). The B.1.617.2 lineage harbours the L452R and P681R spike substitutions that impact antibody binding; moreover, it is associated with a more rapid transmission than other circulating SARS-CoV-2 variants [13].

The present COVID-19 case occurred in calendar Week 17, 3 weeks after the first Swiss case of VOC Delta was reported in GISAID from the Canton of Aargau (7 April 2021). Noticeably, during Weeks 14 to 17, a total of 3, 5, 12 and 21 Delta genome sequences were reported in Switzerland (GISAID), respectively. Phylogenetic comparison of the 180 VOC Delta genomes available in GISAID for Switzerland indicated that our case belonged to the initial wave of introduction of VOC Delta and suggests a multiple-origin introduction scenario (Supplementary Fig. S1).

3.2. Resistome and plasmidome of the OXA-484-producing Escherichia coli

An *E. coli* strain (L3452210II) was isolated from selective agar plates and was resistant to various antibiotics, including fluoroquinolones, cephalosporins, β-lactam/β-lactamase inhibitory combinations and ertapenem (Supplementary Table S1). The eazyplex® SuperBug assay indicated that the strain possessed *bla*$_{OXA-181}$, although this was not confirmed by WGS (see below).
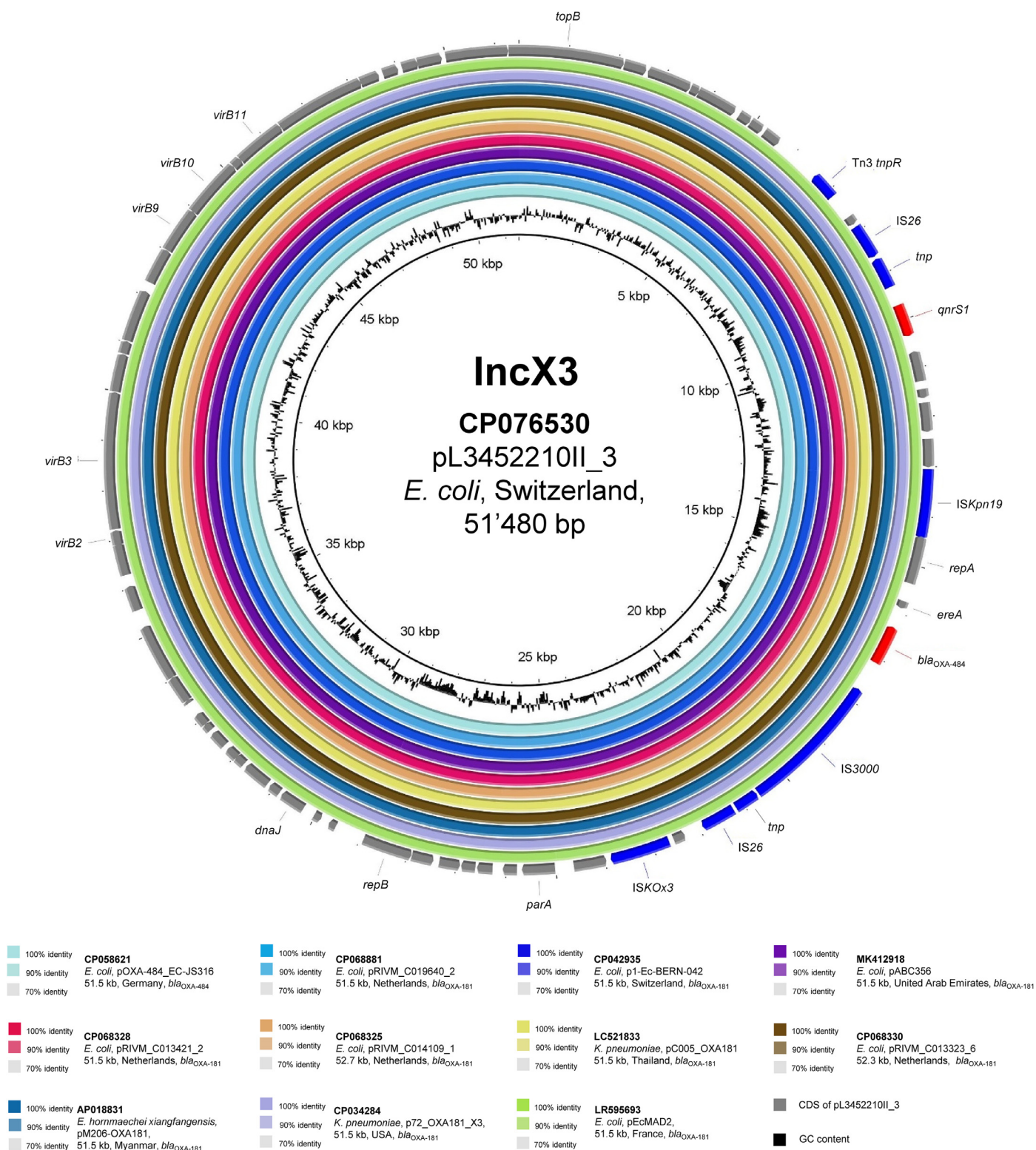
Fig. 1. BLASTn comparison of plasmid pL3452210II_3 (GenBank accession no. **CP076530**) against similar sequences. Plasmid sequences were selected based on high homology to pL3452210II_3 in a BLASTn search against the NCBI non-redundant nucleotide database (date of access 9 June 2021). Rings were constructed using BRIG (BLAST Ring Image Generator) v.0.95. Coloured rings represent similarities to the reference sequence. For each sequence we report GenBank accession, species of isolation, sequence name, plasmid size and carbapenemase gene. Coding sequences (CDS) are represented as arrows in the outermost circle. Mobile genetic elements are depicted in blue, antimicrobial resistance genes in red, and other CDS in grey.
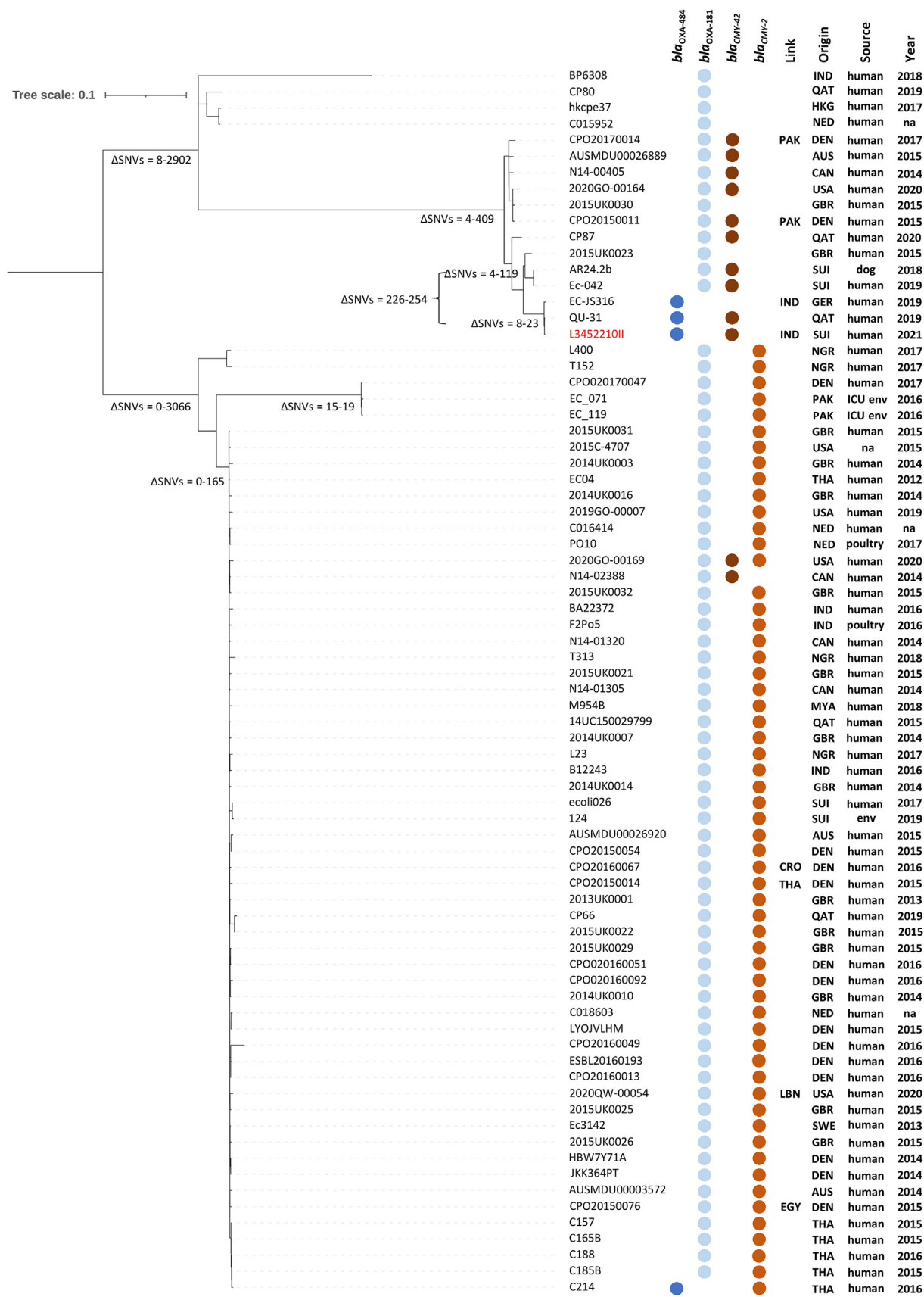
Fig. 2. Core genome analysis for *Escherichia coli* L3452210II (in red) and 75 *E. coli* ST410 possessing *bla*~OXA-484~ or *bla*~OXA-181~ obtained from the NCBI genome collection and Enterobase (date of access 15 June 2021). Core genome analysis of the whole-genome sequencing (WGS) assemblies is presented in a single nucleotide variant (SNV) hierarchical clustering dendrogram tree. The ΔSNV values correspond to the number of non-identical SNVs between two strains within a clade. We indicate association of the isolate with previous stay abroad (Link), country of origin, source of isolation and year of isolation. IND, India; QAT, Qatar; HKG, Hong Kong; NED, the Netherlands; PAK, Pakistan; DEN, Denmark; AUS, Australia; CAN, Canada; USA, United States of America; GBR, United Kingdom; SUI, Switzerland; GER, Germany; NGR, Nigeria; THA, Thailand; MYA, Myanmar; CRO, Croatia; LBN, Lebanon; SWE, Sweden; EGY, Egypt; ICU, intensive care unit; env., environment; na, not available.

According to the WGS analysis, strain L3452210II was of ST410 and its chromosome (4.7 Mb) possessed the *mdf(A)* antimicrobial resistance gene along with the GyrA Ser83Leu/Asp87Asn, ParC Ser80Ile and ParE Ser458Ala amino acid substitutions.

Strain L3452210II also possessed $bla_{OXA-484}$ and *qnrS1* within a composite transposon (IS*26*–*tnp*–IS*3000*–ΔIS*Ecp1*–$bla_{OXA-484}$–Δ*ereA*–Δ*repA1*–IS*Kpn19*–*tnp*–*qnrS1*–*tnp*–IS*26*) carried by a 51.5 kb IncX3 plasmid (pL3452210II_3). As shown in Fig. 1, this mobile genetic element (MGE) was indistinguishable (coverage 100%, identity 100%) from another $bla_{OXA-484}$-harbouring plasmid recently described in a ST410 *E. coli* (EC-JS316) isolated in Germany [14]. Moreover, with the exception of the expected single nucleotide substitution (responsible for Arg214Gly) in $bla_{OXA-484}$, this MGE was identical (coverage 100%, identity 99.99%) to the numerous $bla_{OXA-181}$-carrying plasmids reported worldwide, including those isolated in *E. coli* strains from human and non-human Swiss settings [2,11,15,16].

Strain L3452210II carried three additional plasmids: pL3452210II_1 (82.8 kb, IncY) without any antimicrobial resistance genes; pL3452210II_2 (67.3 kb, IncFIA/FII) carrying bla_{TEM-1B}, *aac(3)-IId, aph(3′′)-Ib, aph(6)-Id, tet*(B) and *sul2*; and pL3452210II_4 (34.2 kb; IncIγ) harbouring the bla_{CMY-42} plasmid-mediated AmpC β-lactamase [17]. We note that most of the $bla_{OXA-181/OXA-484}$-ST410-*Ec* strains previously reported carry bla_{CMY-42} in IncI1γ plasmids with a size of 34–51 kb. All of them shared (identity >99.62%) an ~30 kb region that included bla_{CMY-42} (Supplementary Fig. S2). The bla_{CMY-42} gene was located upstream of the outer membrane lipoprotein gene *blc* and downstream of an IS*1* family transposase. The same genetic context has been described in the IncIγ plasmid pISV_IncI_CMY-42 (GenBank **MN242251**) that was carried by an *E. coli* isolated from the stool of an Italian infant with recent travel history to India [18].

3.3. Global comparison of the ST410 $bla_{OXA-181/OXA-484}$-positive Escherichia coli strains

As shown in Fig. 2, core genome analysis indicated that L3452210II was identical (8–23 ΔSNVs) to the $bla_{OXA-484}$-ST410-*Ec* German isolate EC-JS316 [14], but also to one (QU-31) recently found in Qatar. In contrast, a third $bla_{OXA-484}$-ST410-*Ec* strain (C214) reported in Thailand was not clonally related. Nevertheless, analysis of the WGS assembly of C214 (GenBank **GCA_017902335**) with PlasmidFinder 2.0 indicated that the strain contained an IncX3 plasmid. Furthermore, the contigs containing the IncX3 replicon sequence (34 kb, GenBank **DADVDF010000036**) and the $bla_{OXA-484}$ gene (6.7 kb; GenBank **DADVDF010000052**) aligned with a coverage of 100% (identity 99.99%) to pL3452210II_4, indicating that C214 carried the same IncX3 plasmid (data not shown).

We also note that the majority of $bla_{OXA-181}$-ST410-*Ec* strains were not clonally related to the three identical $bla_{OXA-484}$-ST410-*Ec* isolates. Only several $bla_{OXA-181}$-ST410-*Ec* strains showed a certain homology (226 to 254 ΔSNVs) with those harbouring $bla_{OXA-484}$ (Fig. 2). Among them, we emphasise the presence of *E. coli* AR24.2b and *Ec*-042, both representative strains responsible for a large outbreak that occurred in the Swiss veterinary setting involving both pets and employees [2,11].

4. Conclusions

Numerous COVID-19 patients hospitalised in ICUs were found to have a further infection and/or colonisation due to CPE. However, all of these subjects acquired the CPE after their admission [5–7]. In contrast, our patient was already colonised with a rare OXA-484-producing *E. coli* originating from India and later imported to Switzerland where it had not yet been observed.

The $bla_{OXA-484}$ sequence was first reported (2017) in five *Klebsiella pneumoniae* isolates from the UK [19]. However, only recently the genetic background of this OXA-48-like carbapenemase has been described in EC-JS316, an *E. coli* strain obtained from a rectal swab of a patient hospitalised in Germany (December 2019) due to acute dengue fever following a stay in India [14]. The $bla_{OXA-484}$-ST410-*Ec* strain isolated in the present work also originated from India and possessed both an IncX3 $bla_{OXA-48-like}$-carrying plasmid and a core genome identical to EC-JS316 (23 ΔSNVs). Therefore, $bla_{OXA-484}$-ST410-*Ec* strains are probably endemic on the Indian subcontinent, as for the $bla_{OXA-181}$-ST410-*Ec* strains [1]. However, these two groups of bacteria (OXA-181 vs. OXA-484 producers) share only the same IncX3 plasmid (Fig. 1), whereas the *E. coli* host is different (Fig. 2). An exception could be noted for the $bla_{OXA-484}$-positive strain C214 (detected in Thailand in 2016) that harboured the IncX3 plasmid in a ST410 identical to those usually possessing $bla_{OXA-181}$. This unique finding suggests that both $bla_{OXA-484}$ and $bla_{OXA-181}$ may have evolved from a common ancestor and spread to different hosts. Importantly, the presence of these genes on a common IncX3 plasmid underlines the promiscuous ability of this MGE.

In conclusion, the COVID-19 crisis is contributing to the further expansion of life-threatening MDR bacteria endemic in some countries. This situation could also amplify the importation of these pathogens to low-prevalence nations [20], favouring their uncontrolled spread and the occurrence of outbreaks [5–7]. Therefore, in COVID-19 wards, infection prevention and control programmes must be rigorously maintained, or rapidly restored, to mitigate any future clinical impact due to CPE.

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.10.012.

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