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Case Report

Isolation of three distinct carbapenemase-producing Gram-negative bacteria from a Vietnamese medical tourist

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ABSTRACT

Carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli*, multidrug-resistant *Pseudomonas aeruginosa* and vancomycin-resistant *Enterococcus faecium* were isolated from a single patient. The patient came to Japan for advanced medical treatment after having undergone laparoscopic cholecystectomy and hospitalization in Vietnam. Whole-genome sequence analysis revealed that *K. pneumoniae* harbored bla_{OXA-48} that was found on a Col156 -type small plasmid, *E. coli* harbored bla_{NDM-5} and *P. aeruginosa* harbored both bla_{NDM-1} and 16S rRNA methyltransferase (*rmtB*). To the best of our knowledge, this is the first report of detection of *K. pneumoniae* harboring bla_{OXA-48} on a Col156-type small plasmid in the world and *P. aeruginosa* coharboring genes encoding NDM-1 and RmtB in Japan.

1. Introduction

Reports of carbapenemase-producing *Enterobacteriaceae* are increasing worldwide [1,2]. Moreover, high-level resistance to aminoglycosides mediated by production of 16S rRNA methyltransferases is increasingly reported among various Gram-negative bacteria [3]. Thus far, sporadic clinical isolates of OXA-48 Carbapenemase- and New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriaceae* have been reported [4,5], and the prevalence of multidrug-resistant (MDR) bacteria in Japan is lower than in other Asian countries [1]. However, tourism has increased and Asian visitors accounted for about 80% of all foreign visitors to Japan [6]. Medical tourism, tourism for the purpose of advanced medical treatment and rigorous medical examination, has become more commonplace, though the number of such visitors remains low [7]. Future mass gatherings include the 2020 Summer Olympic and Paralympic Games, which are estimated to attract 36 million visitors to Japan [8]. With the growing number of potential tourists, especially those who are hospitalized in areas that have high prevalence rates of MDR organisms (MDROs) before visiting, an influx of MDROs may occur.

Here, we describe the case of a Vietnamese patient who was admitted to our hospital after spending a few weeks hospitalized in Ho Chi Minh City, Vietnam. Carbapenem-resistant *Enterobacteriaceae*, multidrug-resistant *P. aeruginosa* (MDRP) and vancomycin-resistant *E. faecium* (VRE) were isolated from the patient. Although treatment for the infection caused by MDR bacteria was successful, colonization of carbapenem-resistant *K. pneumoniae*

Abbreviations: NDM, New Delhi metallo-beta-lactamase; MDRO, multidrug-resistant organism; MDRP, multidrug-resistant *Pseudomonas aeruginosa*; VRE, vancomycin-resistant *Enterococcus faecium*; ST, sequence type; MLST, multilocus sequence typing; SNP, single nucleotide polymorphism; MIC, minimum inhibitory concentration.

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and VRE in the gut persisted and a subsequent VRE outbreak occurred in our hospital.

2. Case report

A Vietnamese man in his 60s came to our emergency department complaining of severe dyspnea in the late 2010s. Before coming to Japan, he underwent laparoscopic cholecystectomy and suffered from fatigue and loss of appetite for a month after the surgery. He had been admitted to a general internal medicine ward in Ho Chi Minh City for 2 weeks and received medical therapy with antimicrobial agents, intravenous infusion and blood transfusion. Then, he came to Japan, where his relatives reside, to seek treatment after obtaining the "Visa for Medical Stay." On admission to our hospital, chest X-ray and echocardiography revealed a massive pericardial effusion and emergent pericardiocentesis was carried out, however, its etiology was unknown.

On day 10 after admission, his body temperature significantly decreased to 34.8 °C and heart rate increased to 120 beats/min. His respiratory condition worsened, and he was intubated and required mechanical ventilation. A computed tomographic (CT) scan showed free air in his abdomen. Exploratory laparotomy was performed, which revealed duodenal perforation, and surgical repair followed.

Carbapenem-resistant *K. pneumoniae* and *E. coli*, MDRP and VRE were isolated from stool, ascites and sputum. In the perioperative period, meropenem (1 g every 8 h) and amikacin (400 mg/day) were administered and then switched to combination therapy comprising meropenem, tigecycline (50 mg every 12 h), colistin (150 mg/day) and linezolid (600 mg every 12h) for 10 days with negative pressure wound therapy for the treatment of intra-abdominal infection and the prevention of surgical site infection.

Carbapenem-resistant *K. pneumoniae* and VRE continued to be isolated from the surgical wound and stool after antimicrobial drug therapy (Fig. 1). The patient's general condition gradually improved and his wound eventually healed, and he was discharged from our hospital on day 56 after admission.

Draft whole-genome sequence analysis was performed using the Nextera XT DNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA) and the MiSeq system (Illumina). Paired-end reads of every 300 cycles on MiSeq using the 600-cycle Reagent Kit v3 (Illumina) were assembled by SPAdes version 3.12.0 *de novo* [9]. Identification of acquired antimicrobial resistance genes, plasmids and sequence type (ST) by multilocus sequence typing (MLST) were performed using ResFinder version 3.1, PlasmidFinder version 2.0 and MLST version 2.0 in the Center for Genomic Epidemiology server (http://www.genomicepidemiology.org/), respectively. Core-genome single nucleotide polymorphism (SNP)-based phylogenetic analysis of *K. pneumoniae* ST15 was performed according to a previous report [10], and *K. pneumoniae* PMK1 ST15 genome (DNA Data Bank of Japan [DDBJ] accession no. CP008929.1) was used as the reference sequence for SNP detection. *K. pneumoniae* (TUM17816, -17817, −17822 and −17823) belonged to ST15 and harbored $bla_{\text{OXA-48}}$ and other β-lactamases ($bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-28}}$ and $bla_{\text{TEM-1B}}$). Only *K. pneumoniae* TUM17816 of the four $bla_{\text{OXA-48}}$-positive *K. pneumoniae* was susceptible to meropenem [minimum inhibitory concentration (MIC)≤1 mg/L] and non-susceptible to imipenem (MIC: 2 mg/L). While no or one SNP was found among *K. pneumoniae* TUM17817, -17822 and −17823, 22–23 SNPs were found between these three strains and TUM17816 in core-genome SNP-based phylogenetic analysis (99.1% of the reference genome, 4,846,391/5,317,001 bp). $bla_{\text{OXA-48}}$ genes of *K. pneumoniae* TUM17816, -17817, −17822 and −17823 were found on Col156-type small plasmids (Fig. 2) [pMTY17816_OXA48 (DDBJ accession no. AP019554), pMTY17817_OXA48 (AP019555), pMTY17822_OXA48 (AP019556), and pMTY17823_OXA48 (AP019557), respectively] Will be obtained soon. Nucleotide sequence similarity of these four $bla_{\text{OXA-48}}$ genes harbored on Col156-type small plasmids was ≥99.8%. In *E. coli* (TUM17824), which belonged to ST617, genes encoding all classes of β-lactamases in the Ambler classification ($bla_{\text{NDM-5}}$, $bla_{\text{DHA-1}}$, $bla_{\text{CTM-M-15}}$ and $bla_{\text{OXA-1}}$) were detected. *P. aeruginosa* (TUM17825 and -17826) belonged to ST233 and harbored $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-50}}$ and 16S rRNA methyltransferase (*rmtB*) (Table 1).

3. Discussion

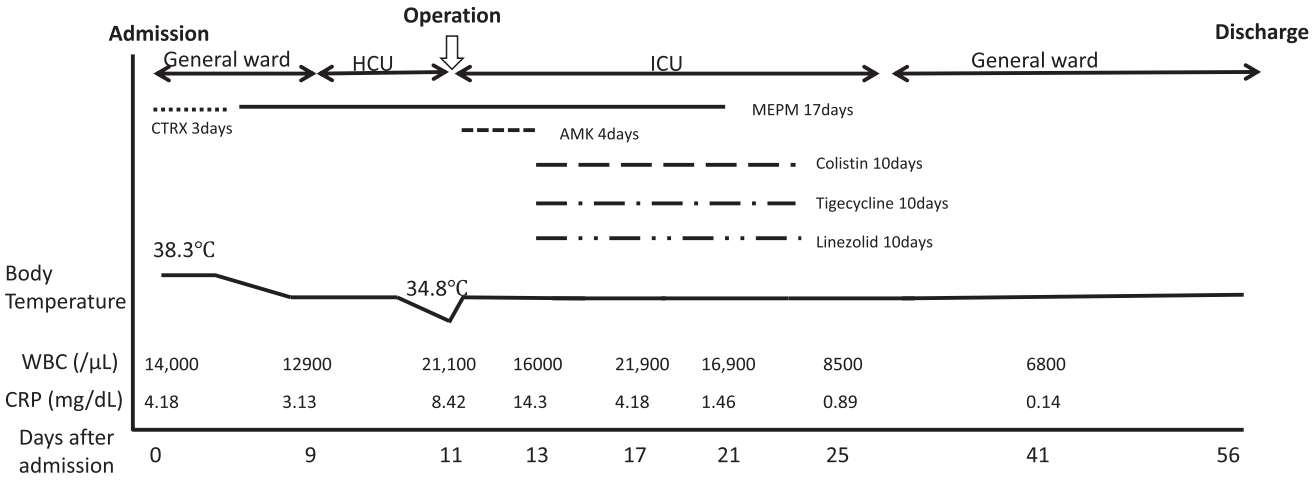
NDM -producing *Enterobacteriaceae* was reported to spread from the Indian subcontinent throughout the world [1,2]. In addition, emergence of MDR bacteria through further acquisition of 16S rRNA methyltransferase genes, which are responsible for extremely high levels of resistance against various aminoglycosides, threatens to become a serious clinical problem [3]. MDRP clinical isolates coharboring genes encoding both carbapenemase and 16S rRNA methyltransferase were reported for the first time in 2007 in Brazil [11], and subsequently reported in India [12] and Nepal [13] in 2015–2017. The present case might suggest the rapid spread of *P. aeruginosa* coproducing carbapenemase and 16S rRNA methyltransferase in Asian medical settings.

In Vietnam, dissemination of $bla_{\text{KPC-2}}$-, $bla_{\text{NDM-1}}$-, $bla_{\text{NDM-4}}$- and $bla_{\text{OXA-48}}$- positive carbapenem-resistant *K. pneumoniae* ST15 was reported at a hospital [14]. While most of the previously reported $bla_{\text{OXA-48}}$ were harbored on IncL/M plasmids, $bla_{\text{OXA-48}}$ in this case was located on Col156-type small plasmids (pMTY17816_OXA48, pMTY17817_OXA48, pMTY17822_OXA48, and pMTY17823_OXA48). Therefore, there was no direct genetic relationship between $bla_{\text{OXA-48}}$-positive *K. pneumoniae* ST15 in this study and the strain previously reported from Vietnam. The establishment of pMTY17816_OXA48, pMTY17817_OXA48, pMTY17822_OXA48 and pMTY17823_OXA48 was estimated to occur by the insertion of two copies of inverted IS*1R* into the non-coding region of a small plasmid pIGJC156 (Fig. 2). The difference in carbapenem susceptibility between *K. pneumoniae* TUM17816 and the other three *K. pneumoniae* strains was explained by differences in their clones.

As for the incidence of VRE in Asia, the prevalence rate in acute care hospitals in Singapore was about 14%, which was higher than that in intermediate-term care facilities (7.6%) and long-term care facilities (0.8%) [15]. Taiwan Nosocomial Infections Surveillance showed that the prevalence of nosocomial infections caused by VRE in intensive care units (ICUs) increased from 3% in 2003 to 24% in 2011 [16]. According to Japan Nosocomial Infections Surveillance, the rate of VRE was 0.8% of 52,127 *E. faecium* clinical isolates in Japanese hospitals in 2017 [17]. However, VRE outbreaks have occurred in tertiary hospitals in Japan [18]. Thus, it is necessary to pay careful attention to the prevalence of VRE in health care settings.

In the present case, although the laboratory should have been more suspicious of potential infection by MDROs due to the patient's recent hospitalization in a foreign country, no extensive screening was conducted until consultation with the infectious disease specialist. After the described MDROs were detected, strategies for preventing the spread of MDROs including isolation, hand hygiene, contact precautions and environmental cleaning were strictly adopted. Nonetheless, a VRE outbreak occurred mainly in the ICU.

Therapy for invasive infections caused by MDROs is thought to be challenging. Combination therapies are reported to have better outcomes for high-risk patients [19]. In this case, though combination therapy with carbapenem, amikacin, colistin, tigecycline and linezolid was used, colonization of OXA-48-producing



Culture results

Days after ad.	0	1	2	3	4	9	10	13	15	18	24	31	39
sputum	K.P* (CPE), normal oral flora					VRE, C.tropicalis		VRE, K.p(CRE), P.a, C.tropicalis		P. a, K.p(CRE), VRE			
stool		Normal flora						K.p(CRE), MDRP*, VRE, E.coli*(CRE)		P.a, K.p*(CRE), VRE, normal flora	VRE, K.p(CRE), P.a	VRE, K.p(CRE), P.a, normal flora	VRE, K.p(CRE), P.a
blood				(-)	(-)		(-)		(-)				
others		Pericardial fluid(-)	Pleural effusion (-)				Intraoperative ascites: K.p*(CRE), VRE, C.tropicalis	Catheter: MDRP*, VRE	Drainge catheter: K.p(CRE), VRE	Wound: K.p*(CRE),VRE, ascites(-), urine(-), CD Ag(+), CD toxin(-)	Catheter: (-), CD Ag(-), CD toxin(-)	Wound: K.p(CRE), VRE	

Fig. 1. Clinical course of the patient. *Strains included in whole-genome sequence analysis (shown in Table 1). HCU, high care unit; ICU, intensive care unit; CTRX, ceftriaxone; MEPM, meropenem; AMK, amikacin; WBC, white blood cell; CRP, C-reactive protein; K.p, *Klebsiella pneumoniae*; *C. tropicalis, Candida tropicalis*; P.a, *Pseudomonas aeruginosa*; CD, *Clostridioides difficile*; CPE, carbapenemase-producing *Enterobacteriaceae*; CRE, carbapenem-resistant *Enterobacteriaceae*.

K. pneumoniae and VRE continued in the patient's gastrointestinal tract until hospital discharge. Persistence of MDROs in the gut might contribute not only to the patient's more severe infection caused by these bacteria but also to further human-to-human transfer.

In conclusion, the isolation of three distinct carbapenemase-producing Gram-negative bacteria and VRE from a single Vietnamese patient suggests a heavy burden of various MDROs in Vietnamese hospitals. To reduce further spread of MDROs worldwide, susceptibility testing, hand hygiene, environmental cleaning and

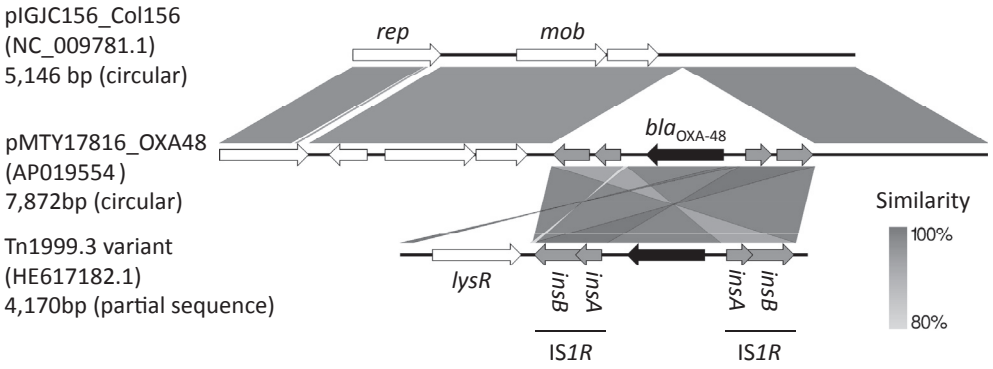


Fig. 2. Comparison of pMTY17816_OXA48 (GenBank accession number AP019554) carrying blaOXA-48 with pIGJ156_Col156 (NC_009781.1) and Tn1999.3 variant (HE617182.1) drawn with Easyfig version 2.1. Arrows indicate confirmed or putative open reading frames (ORFs) and their orientations. Arrow size is proportional to the predicted ORF length. Black, antibiotic resistance genes; gray, transposase gene; white, other genes.

Table 1
Results of antimicrobial sensitivity testing, gene analysis and multilocus sequence typing.

Species	date	MIC(mg/L)									Carbapenemase	Other β-lactamases	Aminoglycoside/ Fluoroquinolone	Aminoglycoside	Fluoroquinolone	ST
(strain ID)	site	GM	AMK	ABPC	AZT	CAZ	LVFX	IPM	MEPM	FOM	CL	TGC					
K.pneumoniae (TUM17816)	day 0 sputum	>16	≦8	>32	>16	>16	>8	2	≦1	<4	–	–	bla_{OXA-48}	bla_{SHV-28}	*aac (6')Ib-cr*	*strA, strB*	*oqxA, oqxB, qnrB66*	15
K.pneumoniae (TUM17817)	day 10 ascites	>16	≦8	>32	>16	>16	>8	>8	>8	16	<2	–	bla_{OXA-48}	$bla_{CTX-M-15}$, bla_{TEM-1B}		*strA, strB*	*oqxA, oqxB, qnrB66*	15
K.pneumoniae (TUM17822)	day18 wound	>16	≦8	>32	>16	>16	>8	>8	>8	–	–	8	bla_{OXA-48}	$bla_{CTX-M-15}$, bla_{SHV-28}, bla_{TEM-1B}	*aac (6')Ib-cr*	*strA, strB*	*oqxA, oqxB*	15
K.pneumoniae (TUM17823)	day18 stool	>16	≦8	>32	>16	>16	>8	>8	>8	16	–	16	bla_{OXA-48}	$bla_{CTX-M-15}$, bla_{SHV-28}, bla_{TEM-1B}		*strA, strB*	*oqxA, oqxB*	15
E.coli (TUM17824)	day13 stool	<2	≦8	>32	>16	>16	>8	>8	>8	<4	<2	0.25	bla_{NDM-5}	bla_{DHA-1}, $bla_{CTX-M-15}$, bla_{OXA-1}	*aac (6')Ib-cr*	*strA, strB, aadA5, aadA2*	*qnrB4*	617
P.aeruginosa (TUM17825)	day13 stool	>16	>64	>32	>16	>16	>8	>8	>8	16	<2	–	bla_{NDM-1}	bla_{OXA-50}		*rmtB, aph (3')-Ib, aac (3)-Id, aac (6')-Ia, aph (3')-VIa, aph (3')-VIb*	*qnrVC1*	233
P.aeruginosa (TUM17826)	day13 catheter	>16	>64	>32	>16	>16	>8	>8	>8	16	<2	–	bla_{NDM-1}	bla_{OXA-50}		*rmtB, aph (3')-Ib, aac (3)-Id, aac (6')-Ia, aph (3')-VIb*	*qnrVC1*	233

MICs of aminoglycoside, β-lactams and levofloxacin were determined by BD Phoenix 100 (Becton Dickinson); fosfomycin and colistin by MicroScan WalkAway 96 Plus (Beckman Coulter) and tigecycline by Etest (bioMérieux).
GM, gentamicin; AMK, amikacin; ABPC, ampicillin; AZT, aztreonam; CAZ, ceftazidime; LVFX, levofloxacin; IPM, imipenem; MEPM, meropenem; FOM, fosfomycin; CL, colistin; TGC, tigecycline.

antimicrobial stewardship are always necessary [20]. Especially in endemic areas, a simple screening test to detect MDROs and early containment are needed. In low-prevalence countries, it is imperative that all patients who have been recently hospitalized in high-prevalence counties are screened for MDROs on admission and that these patients are quarantined until screening results become available.

Accession numbers

Draft whole-genome sequences data were deposited in DDBJ under accession numbers BIFI01000001–BIFI01000311 (TUM17816), BIFJ01000001–BIFJ01000261 (TUM17817), BIFK01000001–BIFK01000360 (TUM17822), BIFL01000001–BIFL01000370 (TUM17823), BIFM01000001–BIFM01000320 (TUM17824), BIFN01000001–BIFN01000219 (TUM17825) and BIFO01000001–BIFO01000193 (TUM17826).

Authorship statement

TK and HM treated the patient. NHH, NM and KH critically advised treatment regimens. KA and YI performed whole genome sequence analysis. NHH, KA, MT and YI participated in manuscript preparation. All authors meet the ICMJE authorship criteria.

Conflicts of interest

The authors declare no conflict of interest.

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