Laboratory and epidemiology communications

Isolation of OXA-181 Carbapenemase-Producing *Escherichia coli* and OXA-232 Carbapenemase-Producing *Klebsiella pneumoniae* from a Pregnant Woman Returning to Japan from India

Saeko Tamura¹, Kayoko Hayakawa^{1*}, Tohru Miyoshi-Akiyama², Misao Nakanishi¹, Tetsu Yano¹, Koichiro Shimoya³, Yuko Sugiki¹, Kazuhisa Mezaki¹, and Norio Ohmagari¹

¹National Center for Global Health and Medicine, and ²Research Institute, National Center for Global Health and Medicine, Tokyo 162-8655; and ³Kawasaki Medical School, Kurashiki 701-0192, Japan

Communicated by Motoyuki Sugai

OXA-48-carbapenemase producing *Enterobacteriaceae* (CPE) have rapidly emerged worldwide since it was first detected in 2001 (1). OXA-181 and OXA-232, the variants of OXA-48, have also been increasingly reported (1, 2). We report on a pregnant woman who resided in India and then returned to Japan, and was found to carry *Escherichia coli* positive for bla_{OXA-181} as well as *Klebsiella pneumoniae* positive for bla_{OXA-232}.

The patient is a 33-year-old Japanese woman who was 23 weeks pregnant upon presentation to our hospital and who had lived in India (Mumbai and New Delhi) for 3 years until 2 months ago. Three months ago, she had a febrile episode for 2 weeks and was diagnosed with typhoid based on a Widal test in India. She was treated with oral cefixime, azithromycin, and intravenous ceftriaxone at an outpatient clinic as well as 3 days of hospitalization. One-month ago, she went to another hospital in Japan where stool screening cultures (obtained on Day X to screen for *Salmonella* spp.) came back as positive for carbapenem-resistant (CR-) *Klebsiella pneumoniae* and *Escherichia coli* (the minimal inhibitory concentration [MIC] by Etest of meropenem was 16 mg/L, and > 32 mg/L, respectively), and her urine culture was also positive for CR-*K. pneumoniae* (MIC for meropenem >32 mg/L). The patient was referred to our hospital for prenatal care and delivery. Our hospital's infection control policy recommends screening for multidrug-resistant organisms (MDRO) among patients hospitalized overseas within the past year, before their admission to our facility (3). The results of screening stool culture (on day X + 7 weeks 3 days) were positive for *K. pneumoniae* and *E. coli* that were resistant to third and fourth generation cephalosporins (Table 1). Whole-genome sequences of isolates were obtained using a MiSeq system with Nextera XT library kits (Illumina, Tokyo, Japan) for the analysis of MLSTs and drug resistance genes as previously described (4). The resulting sequencing

data were registered with the DNA Data Bank of Japan (DDBJ) (DDBJ accession no. DRA005988).

Although the MICs for meropenem of *K. pneumoniae* and *E. coli* were lowered to less than 1 mg/L, both isolates were found to be positive for carbapenem-hydrolyzing class D β -lactamase (*E. coli*: OXA-181, *K. pneumoniae*: OXA-232) along with other resistance genes (Table 2).

During two additional prenatal visits, her stool cultures to screen for OXA-type CPE came back negative (Day X + 12 weeks 6 days, Day X + 19 weeks 5 days). Culture of vaginal secretions obtained at 39 week

Table 1. Minimal inhibitory concentrations of *Klebsiella pneumoniae* (KP) and *Escherichia coli* (EC)

Antibiotic ¹⁾	Minimal inhibitory concentration ²⁾ (μg/mL)		
	Mother		Child
	KP	EC	EC
ABPC	≥ 32	≥ 32	≥ 32
ABPC/SBT	≥ 32	≥ 32	≥ 32
PIPC/TAZ	≥ 256	32	16
CEZ	≥ 32	≥ 32	≥ 32
CMZ	1	8	128
CTRX	≥ 16	≥ 16	≥ 16
CEZ	16	≥ 32	≥ 32
CFPM	≥ 64	≥ 64	8
AZT	≥ 16	≥ 16	≥ 16
MEPM	0.125	0.125	≤ 0.03
IMP	0.5	1	0.25
AMK	2	2	2
GM	0.5	1	1
LVFX	0.06	≥ 32	32
FOM ³⁾	16	2	0.5

¹⁾ ABPC, ampicillin; ABPC/SBT, ampicillin/sulbactam; AMK, amikacin; AZT, aztreonam; CAZ, ceftazidime; CEZ, cefazolin; CFPM, cefepime; CMZ, cefmetazole; CTRX, ceftriaxone; GM, gentamicin; IMP, imipenem; LVFX, levofloxacin; MEPM, meropenem; MIC, minimum inhibitory concentrations; PIP/TAZ, piperacillin/tazobactam.

²⁾ MICs were determined by the broth microdilution method according to CLSI criteria (CLSI M100-S21).

³⁾ MICs were determined by the agar dilution method according to CLSI criteria (CLSI M100-S21).

Accepted April 16, 2019. J-STAGE Advance Publication May 31, 2019.

DOI: 10.7883/yoken.JJID.2018.432

*Corresponding author: Mailing address: Disease Control and Prevention Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan. Tel: +81-3-3202-7181, Fax: 81-3-3207-1038. E-mail: kayokohayakawa@gmail.com

Table 2. Resistant genes and MLST of *Klebsiella pneumoniae* (KP) and *Escherichia Coli* (EC)

Resistant gene	Mother		Child
	KP	EC	EC
Beta-lactams			
<i>bla</i> _{CMY-30}			positive
<i>bla</i> _{CMY-42}			positive
<i>bla</i> _{LAT-1}			positive
<i>bla</i> _{CTX-M-15}	positive	positive	
<i>bla</i> _{OXA-1}	positive	positive	
<i>bla</i> _{OXA-181}		positive	
<i>bla</i> _{OXA-232}	positive		
<i>bla</i> _{SHV-1}	positive		
<i>bla</i> _{TEM-1B}	positive		
<i>bla</i> _{TEM-30}			positive
Aminoglycoside			
<i>aac(6')-Ib-cr</i>	positive		
<i>aadA1</i>		positive	
<i>aacA4</i>	positive		
<i>aph(3')-Ia</i>	positive		
<i>aph(3')-Ic</i>	positive		
<i>strA/B</i>			positive
Fluoroquinolone			
<i>oqxA</i>	positive		
<i>qnrS1</i>		positive	
<i>qnrS4</i>		positive	
Macrolide			
<i>mphA</i>	positive	positive	
<i>ermB</i>	positive	positive	
Trimethoprim			
<i>dfrA1</i>		positive	
Tetracycline			
<i>tetB</i>		positive	positive
Chloramphenicol			
<i>catA1</i>		positive	
<i>catB3</i>	positive		
Fosfomycin			
<i>fosA</i>	positive		
MLST			
	ST 45 ¹⁾	ST 8654 ²⁾	ST 8655 ³⁾

¹⁾: gapA:2, infB:1, mdh:1, pgi:6, phoE:7, rpoB:1, tonB:12.

²⁾: adk:6, fumC:6, gyrB:22, icd:16, mdh:11, recA:7, and novel purA.

³⁾: adk:92, fumC:4, icd:96, mdh:70, purA:58, recA:2, and novel gyrB.

and 5 days was negative for drug-resistant bacteria or group B streptococcus. At 40 week and 3 days gestation, the patient delivered a boy by vacuum extraction. No perinatal antibiotics were given. The newborn baby was screened for possible stool carriage of CPE at 1 day-old; the result was negative although *E. coli* with AmpC-type beta-lactamase was detected (Table 2).

MLSTs of maternal and neonatal *E. coli* were distinct

(Table 2). As previously described (5), pulsed-field gel electrophoresis (PFGE) was performed, and the obtained fingerprinting patterns were analyzed by the unweighted-pair-group method with the Fingerprinting II Software (Bio-Rad Laboratories, Inc. CA, USA). Two *E. coli* isolates from the mother and the neonate were typed by PFGE (Fig. 1). The similarity was 84.21%, and both isolates were considered not to be genetically identical.

The analysis of genetic environments surrounding *bla*_{OXA-181} and *bla*_{OXA-232} showed that both β -lactamases genes were located near a replicase in plasmid ColKP3-1 [JN205800]), therefore, they would probably be located in plasmids although we cannot exclude possibilities that those are integrated into the chromosomes. Contig containing *bla*_{OXA-181} (12,624bp in size) was used to search using NCBI BLAST. Top 3 Blast hits were

Fig. 1. PFGE pattern of the *Escherichia coli* isolates from the mother and the neonate.

Escherichia coli M517 plasmid pM517-OXA181 DNA, *Enterobacter hormaechei* subsp. *xiangfangensis* M206 plasmid pM206-OXA181 DNA, and *Escherichia coli* strain EC21 plasmid pEC21-OXA-181. In the contig, transposase, detected genes encoded proteins included TnpA, OXA-181 β -lactamase, replication protein A, hypothetical protein, YdaA protein, Sea16 protein, Tn3 transposase, resolvase domain-containing protein, plasmid-mediated quinolone resistance protein and IS2 ORF1 protein based on the annotation of pM517-OXA181 (Genbank Accession number: AP018837.1).

A contig containing *bla*_{OXA-232} (6,141bp in size) was also searched. Top 3 Blast hits were *Klebsiella pneumoniae* strain DAARGOS_440 plasmid unnamed3, *Klebsiella pneumoniae* strain QS17-0029 plasmid pMR0617oxa, and *Klebsiella pneumoniae* strain kNICU5 plasmid pkNICU5. Of note, both of *Escherichia coli* M517 plasmid pM517-OXA181 DNA and *Klebsiella pneumoniae* strain DAARGOS_440 plasmid unnamed3 contained the a same replicase in plasmid ColKP3 [JN205800] as *bla*_{OXA-181} and *bla*_{OXA-232} isolated from the patient. In the contig, transposase, detected genes encoded proteins included MobB protein, protein MobD, hypothetical protein, OXA-48 family carbapenem-hydrolyzing class D β -lactamase OXA-232, hypothetical protein and plasmid mobilization relaxosome protein MobC based on the annotation of DAARGOS_440 plasmid unnamed3 (Genbank Accession number: CP023924.1).

Written informed consent was obtained from the patient for publication of this Case Report.

To the best of our knowledge, this is the first case demonstrating the isolation of OXA-232 carbapenemase-producing *K. pneumoniae* in Japan. The patient also carried OXA-181 carbapenemase-producing *E. coli*; only one report exists on OXA-181 carbapenemase-producing *K. pneumoniae* from Japan thus far in the literature (6). The first case in Japan was reported in a man who also had a history of hospitalization in India.

Stool of the newborn was positive for MDR (multidrug-resistant)-*E. coli* that possessed multiple resistance genes that had not been isolated in the mother. In our case, the horizontal acquisition carrying inside the hospital is unlikely because the culture was obtained only 1 day after delivery. PFGE and MLST analyses suggested that the maternal and neonatal *E. coli* isolates are genetically distinct. However, intrauterine infection of ascending bacteria from the lower genital tract, both clinical and subclinical, has been reported (7). The epidemiology and mechanism for vertical transmission of antibiotic-resistant bacteria from mother to child has remained unclear (8). Further study is warranted to reveal the exact processes of vertical transmission of resistant pathogens.

We recently reported a high rate (56.5%) of colonization of MDRO among patients with a history of overseas hospitalization (3). This case provided further evidence that colonization of MDRO could happen even

after admission for a very short period, in a healthy pregnant woman. In this case, mother's stool cultures reverted to negative for CPE after 3 months from the first positive culture which correlates with previous reports (9). As antimicrobial exposure has been reported to contribute to both increased and prolonged carriage of CPE (9), the avoidance of unnecessary antibiotics seems to be the key component in shortening the carriage of CPE.

As a limitation of this study, we were unable to determine the exact sequence of the plasmids containing *bla*_{OXA-181} and *bla*_{OXA-232} due to technical restrictions.

In the present times, the number of patients affected by the problem of drug-resistant pathogens not endemic to their home country would be even more diverse. Hospital infection control policies combined with microbiological technological platforms are key components for preventing the spread of MDR pathogens across borders.

Acknowledgments We wish to express our most sincere gratitude and appreciation to Kayo Shimada and Maki Nagashima for their help with microbiological analyses. This project was supported in part by Grants for International Health Research from the Ministry of Health, Labour and Welfare of Japan (28S1106) and AMED under Grant Number 18fm0108001 (Japan Initiative for Global Research Network on Infectious Diseases [J-GRID]).

Conflict of interest None to declare.

REFERENCES

1. Poirel L, Héritier C, Tolün V, et al. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*. 2004;48:15-22.
2. Yin D, Dong D, Li K, et al. Clonal dissemination of OXA-232 carbapenemase-producing *Klebsiella pneumoniae* in neonates. *Antimicrob Agents Chemother*. 2017;61: e00385-17.
3. Hayakawa K, Mezaki K, Sugiki Y, et al. High rate of multidrug-resistant organism colonization among patients hospitalized overseas highlights the need for preemptive infection control. *Am J Infect Control*. 2016;44:e257-e9.
4. Sherchan JB, Hayakawa K, Miyoshi-Akiyama T, et al. Clinical epidemiology and molecular analysis of extended-spectrum-beta-lactamase-producing *Escherichia coli* in Nepal: characteristics of sequence types 131 and 648. *Antimicrob Agents Chemother*. 2015;59:3424-32.
5. Shrestha B, Tada T, Shimada K, et al. Emergence of various NDM-type-metallo-beta-lactamase-producing *Escherichia coli* clinical isolates in Nepal. *Antimicrob Agents Chemother*. 2017;61:593-600.
6. Kayama S, Koba Y, Shigemoto N, et al. Imipenem-susceptible, meropenem-resistant *Klebsiella pneumoniae* producing OXA-181 in Japan. *Antimicrob Agents Chemother*. 2015;59:1379-80.
7. Fahey JO. Clinical management of intra-amniotic infection and chorioamnionitis: a review of the literature. *J Midwifery Womens Health*. 2008;53:227-35.
8. Seale J, Millar M. Perinatal vertical transmission of antibiotic-resistant bacteria: a systematic review and proposed research strategy. *BJOG*. 2014;121:923-8.
9. Ruppe E, Armand-Lefevre L, Estellat C, et al. High rate of acquisition but short duration of carriage of multidrug-resistant enterobacteriaceae after travel to the tropics. *Clin Infect Dis*. 2015;61:593-600.