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Emergence of *Salmonella* Strain That Produces IMP-1-Type Metallo- β -Lactamase in a Japanese Patient

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Drug resistance has been reported in many bacterial species isolated from clinical specimens in Japan. Multidrug resistance in gram-negative bacilli is particularly important clinically. Recently, metallo- β -lactamase (MBL)-producing *Enterobacteriaceae* have been reported (1).

MBLs belong to class B in the Ambler classification of β -lactamases (2). MBLs can be classified into the types, such as IMP, NDM, VIM, and SPM and can break down almost all β -lactam antibiotics, including carbapenems. Furthermore, β -lactamase inhibitors are ineffective against MBLs.

Here, we report the isolation of an IMP-1-type MBL-producing strain of the *Salmonella enterica* subspecies *enterica* serovar Agona from the feces of a male in-patient admitted to our hospital.

In 1999, the patient underwent bone marrow transplantation for combined immunodeficiency syndrome at the age of 13 years. In 2012, he was hospitalized at the Department of Pediatrics, Fujita Health University Hospital, to undergo treatment for iatrogenic adrenocortical insufficiency. On day 12 of hospitalization, he developed diarrhea, and a stool culture was performed.

Bacteria in the stool culture were identified, and an antimicrobial susceptibility test was performed using MicroScan WalkAway 96 SI and Neg Combo 6.11J panels (Siemens Healthcare Diagnostics K.K., Tokyo, Japan). The results were assessed according to the Clinical and Laboratory Standards Institute M100-S17 (2007) criteria (3).

O-antigen and H-antigen were identified using *Salmonella* antisera (Denka Seiken Co., Ltd., Tokyo, Japan) and *Salmonella* phase induction antisera (Denka Seiken), respectively. Sodium mercaptoacetic acid (SMA) disks (Eiken Chemical Co., Ltd., Tokyo, Japan) were used for MBL detection. The disks contained imipenem (IPM) or ceftazidime (CAZ) (Japan Becton

Dickinson Inc., Tokyo, Japan), and the double-disk method was used to assess the size of the growth inhibitory zone (1,4).

The stool sample was cultured at 35°C under aerobic conditions for 18 h. Many colonies with black centers were observed on a *Salmonella*-*Shigella* (SS) agar plate. Gram staining of the grown colonies confirmed the presence of gram-negative bacilli. Identification using the MicroScan WalkAway 96 system revealed the presence of *Salmonella* spp. with a 99% probability. The serotype was identified as O4:G:–; therefore, the strain was typed as *Salmonella* Agona.

Table 1 shows the results of the antimicrobial susceptibility test. The strain was susceptible to IPM but resistant to many β -lactam antibiotics, including CAZ. In addition, the results of the MBL detection test were positive, as indicated by the increased size of the inhibitory zone.

Next, polymerase chain reaction was performed to determine the MBL type. In the test, the IMP-1 gene was amplified. Gene sequencing results showed that the IMP-1 gene (accession no. S71932) was matched with a 100% probability. The VIM-2, NDM-1, OXA-48, and KPC genes were not amplified (1,5).

Table 1. Antimicrobial susceptibility of *Salmonella* Agona

Antimicrobial agent	MIC (mg/L)	MIC interpretation
Ampicillin	> 16	R
Piperacillin	16	S
Clavulanic acid/amoxicillin	> 16	R
Cefazolin	> 16	—
Cefotiam	> 16	—
Cefpodoxime	> 4	R
Cefcapene	> 1	R
Cefotaxime	> 32	R
Ceftazidime	> 16	R
Cefpirome	≤ 8	S
Cefmetazole	> 32	—
Imipenem	2	S
Aztreonam	≤ 8	S
Amikacin	≤ 4	—
Gentamicin	2	—
Levofloxacin	≤ 1	S
Sulbactam/cefoperazone	> 32	R

S, susceptible; I, intermediate; R, resistant.

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The IMP-type MBL gene was first reported in Japan in 1994, when a carbapenem-resistant *Serratia marcescens* strain was isolated. The gene and amino acid sequences were determined and reported in 1994 (6). Subsequently, the sequences were reported in bacteria belonging to the family *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella*, and glucose nonfermenters, such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. (1).

Salmonella Agona was detected in a dry cereal outbreak in USA in 2008 (7). The current patient and his family had no history of overseas travel. From the time of hospitalization, piperacillin/tazobactam and clindamycin were administered. From day 7 of hospitalization, antimicrobial treatment was switched to ceftriaxone because of respiratory tract infection. *Salmonella* Agona was detected after these antimicrobial treatments. The patient's diarrhea resolved without specific treatment, and he was discharged from the hospital on day 26 of hospitalization. To the best of our knowledge, this is the first report of the clinical isolation of an MBL-producing *Salmonella* spp.

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Conflict of interest None to declare.

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