Klebsiella oxytoca-producing IMP-1 Detected as the First Strain of Carbapenem-resistant Enterobacteriaceae in Our Hospital

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Abstract

We herein report a case of Klebsiella oxytoca-producing IMP-1 that was detected as a first isolate of carbapenem-resistant Enterobacteriaceae (CRE) at our facility. Since K. oxytoca is an uncommon strain for CRE, we speculated that the resistant organism had already spread out inside the hospital. Metallo-β-lactamases promotes antibiotic resistance in Enterobacteriaceae, which potentially yields problematic issues in clinical settings. Active surveillance of antibiotic resistant strains is important and should be repeatedly highlighted. Furthermore, appropriate methods should be established to detect highly resistant strains.

Key words: breakpoint, carbapenem-resistant Enterobacteriaceae (CRE), IMP-1, Klebsiella oxytoca


Introduction

The spread of carbapenem-resistant Enterobacteriaceae (CRE) is an emerging threat to public health (1). Widespread transmission of metallo-β-lactamase (MBL) genes among bacteria is mainly responsible for the present situation. In Japan, IMP-1 and IMP-6 have been reported to be spreading latently (2-5). Among the family of Enterobacteriaceae, Enterobacter spp, Escherichia coli, and Klebsiella pneumoniae are generally reported to be the main CRE pathogens (6). However, for Klebsiella oxytoca, CRE has only been detected sporadically (7), including in Japan (2, 8, 9). We herein present a case of IMP-1-producing K. oxytoca that was isolated as the first clinical strain of CRE at our hospital, in addition, we present the results of a genetic analysis and discuss the problems associated with this emerging pathogen.

Case Report

The patient was a woman in her sixties who had a previous history of hepatitis C virus infection (liver cirrhosis and hepatocellular carcinoma), chronic heart failure (post-aortic and mitral valve replacement, and pacemaker insertion more than 25 years previously) and Hashimoto thyroiditis. Two months prior, the patient received hepatectomy (posterior segmentectomy) for treatment of hepatocellular carcinoma at our hospital and had been admitted for 5 weeks postoperatively. Two weeks after the patient was discharged, she was readmitted to our hospital due to disturbed consciousness caused by hepatic encephalopathy (February 2014). She had been prescribed diuretics and a proton pump inhibitor (esomeprazole) at that time. The patient was first admitted to an intensive care unit for 2 days. A urine culture obtained on admission day was negative for any microorganisms. Next, the patient was moved to a general ward where she stayed for 5 days. Furthermore, she was transferred to another general ward, and one week after that, a multi-drug-resistant K. oxytoca was incidentally detected from her urine sample.

The minimum inhibitory concentration (MIC, μg/mL) of each antibiotic that was measured by the broth microdilution method using the dry plate Eiken test (Eiken Chemical, Tokyo, Japan) was as follows; piperacillin/tazobactum: >256, ceftazidime: >16, cefepime: 8, imipenem: 2, meropenem: 2, aztreonam: >16, gentamicin: 4, amikacin: < 1, ciprofloxacin: >8, levofloxacin: 8 and colistin: 0.5. Ac-
cording to the antimicrobial susceptible pattern, the pathogen was suspected to be a MBL producer, and therefore, a commercial test kit using the immune-chromatography method (Mizuho Medy, Tosu, Japan) for detecting the presence of the IMP-1 type gene was performed. The result was positive and the bacterial isolate was further analyzed.

A double-disc synergy test revealed a positive result for the sodium mercaptoacetate disk and a genetic investigation using polymerase chain reaction (primers, F1 5’-ACC GCA GCA GAG TCT TTG CC-3’ and R1 5’-ACA ACC AGT TTT GCC TTA CC-3’) verified the presence of the IMP-1 type MBL (10) and CTX-M-9 type β-lactamase (extended-spectrum β-lactamase) genes in the bacterial isolate. Next, sequencing analysis was performed to identify the type of MBL gene by using primers IMP-1F (5’-AAG GCG TTT ATG TTC ATA CTG CG-3’) and IMP-1R (5’-TTT AAC CGC CTG CTC TAA TGT AA-3’) (11). The sequence data were analyzed using the BLAST sequence homology search program at DNA Data Bank of Japan (DDBJ), and the strain was finally confirmed to possess the IMP-1 gene. Genetic investigations for aminoglycoside or quinolone resistance genes were not performed.

The patient was isolated to an individual room until discharged. The resistant organism did not cause any infections in the patient. A need of active surveillance for the MBL-producing strains across the hospital was discussed, but it was not enforced. Without screening, the true epidemiology of IMP-1-producing strains at our facility is unfortunately still uncertain. Currently, with the exception of the strain in this report, IMP-1-producing Enterobacteriaceae has not been isolated in our hospital laboratory.

Discussion

At our hospital, the IMP-1-producing K. oxytoca was isolated as the first recognized CRE strain. The patient was first admitted to an intensive care unit, and subsequently referred to two different general wards. A bacterial culture of a urine sample upon admission was negative for CRE; however, three weeks after admission, the IMP-1-producing K. oxytoca was isolated from her urine. It was highly suspected that the patient was infected with the resistant strain during hospitalization.

In Japan, MBL-producing K. oxytoca have only occasionally been reported. On September 2014, CRE was newly assigned as a notifiable infectious disease in Japan. According to the Infectious Diseases Weekly Report issued from the National Institute of Infectious Diseases (Japan) (8), more than 100 cases of CRE infections have been given notice within the first two months (through 38th week and 44th week in 2014). Among the Enterobacteriaceae, Enterobacter spp., followed by Escherichia coli, Klebsiella pneumoniae and Citrobacter spp., have been reported as frequent pathogens for CRE, while only one or two strains of K. oxytoca have been registered. We therefore speculate whether the strain in our case was really a first isolate of a IMP-1-producing Enterobacteriaceae at our facility.

Based on breakpoints set by the latest revision of Clinical and Laboratory Standards Institute (CLSI, M100-S23), susceptible categories of imipenem and meropenem for Enterobacteriaceae are defined as MICs of less than 1 μg/mL. As previously discussed (5), higher MIC breakpoints are still applied in many Japanese medical laboratories. The breakpoints data published by the authorized organizations, such as CLSI, are not updated appropriately, resulting in an underestimation of CRE and erroneous selection of treatment (1). Infections caused by CRE yield high mortality rates (12) and with limited treatment choices (13). To prevent misidentification of resistant strains, clinical breakpoints should be updated appropriately in medical laboratories. In the meantime, the MICs of Enterobacteriaceae need to be screened one by one at laboratories as a practical measure, especially in severe cases or for strains obtained from sterile samples.

Nevertheless, following MIC values would not be a sufficient screening method for detecting CRE. IMP-6-producing Enterobacteriaceae, which is prevailing in Japanese clinical settings, is known to be susceptible to imipenem and resistant to meropenem (3). Less frequently, other IMP-1-producing Enterobacteriaceae can also show this similar phenotypic pattern (14). Although the new Vitek 2 card, AST-N269, is reported to detect meropenem-resistant strains correctly (15), the MBL-producing Enterobacteriaceae can be categorized as susceptible to carbapenems by standard methods (16, 17). The dry plate Eiken method, which is used in our facility, may be reliable for detecting CRE (15); however, the accuracy of the test has not been fully evaluated. If CRE was suspected clinically or technically, there is no alternative but to examine strains by genetic analysis. The immune-chromatographic assay would be an easier method in medical laboratories (18). As with our patient, those with risk factors such as indwelling devices, prior use of cephalosporin, and a history of invasive procedures, should be actively screened for CRE (14).

In cases where CRE is isolated, the Center for Disease Control and Prevention recommends that active surveillance be conducted (19). However, an epidemiologic study was not performed at our hospital. Asymptomatically colonized patients are known to be a reservoir for CRE and active surveillance is recommended for high risk patients with a Charlson’s score greater than 3, immunosuppression, the presence of indwelling devices and prior antimicrobial exposures (20). Active surveillance, coupled with appropriate contact precautions and cohorting of carriers, have been an effective strategy to identify colonization and reduce the transmission of drug-resistant pathogens, and is also widely recognized (21-23).

The clinical threat of CRE is still oblivious to medical staff, including internists (24). The drug-resistant strains can be horizontally transmitted via plasmids harboring carbapenem resistance genes. To the best of our knowledge, there has been no Enterobacteriaceae isolates showing a similar
antimicrobial susceptibility pattern in our hospital. However, the isolate reported in this case could merely be the tip of an iceberg. A prospective investigation would be required for the comprehension of true epidemiology. By reporting our case, we would like to repeatedly highlight the importance of appropriate countermeasures for handling problems associated with CRE.

The authors state that they have no Conflict of Interest (COI).

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