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CASE REPORT

Chronological Emergence of a Class A Carbapenemase-producing *Enterobacter Aerogenes* in Taiwan

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This study reports the case of a 77-year-old, long-term, bedridden patient, with a nosocomial wound infection caused by a multidrug-resistant strain of *Enterobacter aerogenes* (*E. aerogense*). The isolate produced an Ambler-class A carbapenemase, which was demonstrated by the Modified Hodge test (MHT) and a confirmatory inhibition test. However, no known carbapenemase genes were discovered in this isolate by polymerase chain reactions (PCRs) with specific primers. New carbapenemase or other resistant mechanisms could be explored from the isolate of carbapenem-resistant *E. aerogense,* according to the revised criteria (CLSI, 2012).

Key words: Carbapenemase, *Enterobacter aerogenes, Taiwan*

INTRODUCTION

Enterobacter aerogenes was not an important pathogen until it was reported as one of the common pathogens causing nosocomial infections in recent decades.[1-3] Strains of this species are naturally resistant to ampicillin, amoxicillin-clavulanate, cefazolin, and cefuroxime, due to the constitutive production of chromosomal AmpC β-lactamases.[2,3] In recent studies, the clinical isolates of this species has not only shown the extended-spectrum β-lactamase TEM-24, which results in resistance to β-lactam antibiotics,[4-6] but also resistance to quinolones, tetracycline, and chloramphenicol, due to reduced drug uptake, by losing porins on the outer membrane.[7,8] In the past 10 years, the carbapenem-resistant *E. aerogenes* has been found in many countries.[3-5,9,10] With the emergence of multidrug-resistant *E. aerogenes* strains, the mortality rate of their infected patients has also increased.[8]

This study reports the case of a 77-year-old, long-term, bedridden patient having a nosocomial wound infection due to a multidrug-resistant (MDR) strain of *E. aerogenes*, which was also resistant to carbapenems. The isolate developed a phenotype of the class A carbapenemase after imipenem treatment for three weeks.

This is the first report of carbapenem-resistant *E. aerogenes* that demonstrates a phenotype of class A carbapenemase, in Taiwan.

CASE REPORT

A 77-year-old male has had a history of old cerebral vascular accident, with right hemiparesis, stupor consciousness, hypertension, and type-2 diabetes mellitus with nephropathy, for years. He had no recent traveling history. Regular hemodialysis was initiated a year ago. He was admitted to create an arteriovenous shunt over his left arm. The operation was performed on hospitalization day 2. Subsequently, the patient presented with shortness of breath, fever, and dyspnea with leukocytosis. Aspiration pneumonia was diagnosed on hospitalization day 4. Arterial blood gas analysis revealed a pH of 7.71, $PaCO_2$ of 15.9 mmHg, and PaO_2 of 63.1 mmHg. After an emergent endotracheal tube insertion, he was transferred to the Intensive Care Unit (ICU) on hospitalization day 9. Isolates of *E. aerogenes* were obtained from two sets of sputum culture, and they were found to be sensitive to amikacin, piperacillin, third-generation cephalosporin, imipenem, ertapenem, and intermittent to ciprofloxacin on hospitalization day 5. Imipenem-sensitive *Pseudomonas aeruginosa* (*P. aeruginosa*) was isolated from his sputum on hospitalization day 17. The patient received tracheostomy on hospitalization day 28 because of ventilator dependence. In his infection course [Table 1], despite administering the serial anti-microbial agent, prescriptions

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Table 1. Chronological emergence of carbapenem-resistant phenotypes for *Pseudomonas aeruginosa* and *Enterobacter aerogenes* during hospitalization

Hospital weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Carbapenem sensitivity of *E. aerogenes*	S	S	S		S											R				
Carbapenem sensitivity of *P. aeruginosa*			S		S		S		S	S		R		R		R	R	R		
Specimen type	*S*	*S*	*S*		*S*		*CVP*		*S*	*S*		*S*	*B*	*S*		*W*	*S*	*S*	*S*	*S*
Antibiotic usage																				
Augcin: amoxicillin + clavulanate	D4			D19-D25																
Augmentin: amoxicillin + clavulanate										D62										
Tazocin: Piperacillin +tazobacta		D4-D17								D64-D71										
Veterin: Cefazolin	D1-D3																			
Ceflexin: Cephalexin first generation Cephalosporins	D3-D4																			
Cefin (Cefepime) fourth generation Cephalosporins						D31-D59							D83-D97							
Maxipime(Cefepime) : fourth generation Cephalosporins																	D111-D118			
Vanco: Vancomycin		D6-D17					D45-D59													
Mepem: Meropenem															D104-D111					
Tienam: IMIPENEM											D71-D83									
Ciproxin: Ciprofloxacin										D62-D83										
BAKTAR: Trimethoprim + Sulfamethoxazole													D80-D104							
Acemycin: Aminoglycosides																	D111-D118			
Zyvox: Linezolid														D90-D104						

including amoxicillin, piperacillin, first- and fourth-generation cephalosporin, vancomycin, and ciprofloxacin, the *P. aeruginosa*-related pneumonia persisted. The antibiograms of the *P. aeruginosa* isolate remained unchanged until an imipenem-resistant *P. aeruginosa* was noted on hospitalization day 81. A few weeks later, a 5 × 8 cm^2 pressure sore was noted over his sacral region, owing to his being bedridden for a long period of time. Mixed infections of *P. aeruginosa* and *E. aerogenes* were found in the wound culture on hospitalization day 107. Not only did the *E. aerogenes* isolate remain resistant to carbapenems, it also became resistant to carbapenems. According to the antibiograms of the two imipenem-resistant strains, the patient was then treated with amikacin (1000 mg, q.d.) and cefepime (1000 mg, q.d.). Although his wound infection was controlled, the nosocomial pneumonia caused by the imipenem-resistant *P. aeruginosa* persisted. At present, he is still hospitalized in a Respiratory Care Center for controlling the nosocomial pneumonia.

LABORATORY RESULT

Both imipenem-resistant *P. aeruginosa* and *E. aerogenes* were isolated from the wound swab on hospitalization day 107. The Vitek-2 ID 32 GN and AST-N044 systems (bioMerieux Vitek, Marcy-l'Etoile, France) were used for species identification and the antimicrobial susceptibility test. All antibiotics, except amikacin, showed high-level minimum inhibitory concentration (MIC) values. Both disk diffusion and microdilution methods were also performed and revealed compatible results with the MIC values of Vitek-2 (MIC: amikacin <4 μg/ml, cefepime: 8 μg/ml, imipenem >8 μg/ml, ertapenem >4 μg/ml, meropenem: 8 μg/ml, tigecycline: 2 μg/ml, colistin: 0.25 μg/ml, polymyxin B: 0.5 μg/ml). Carbapenem susceptibilities for Enterobacteriaceae are interpreted according to the new Clinical and Laboratory Standards Institute (CLSI) criteria, 2012. The Modified Hodge test was performed to detect whether carbapenemase was produced by the two imipenem-resistant isolates.[11] A strong positive result was revealed for the isolate of *E. aerogenes*, but not for the isolate of *P. aeruginosa* [Figure 1a]. We used a carbapenemase-inhibitor-impregnated agar to test and classify the carbapenem-resistant strains.[12,13] Aminophenylboronic acid (APBA) was employed to inhibit class A carbapenemase. Class B metallo-carbapanemase was suppressed by ethylenediaminetetraacetic acid (EDTA), and 6-pyridinedicarboxylic acid (DPA) and cloxacillin were utilized to inhibit β-lactamase. The size differences of the inhibition

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zone on the meropenem disks between the control and inhibitor of the APBA-impregnated Mueller-Hinton-agar were measured with a cut-off value of 4 mm, while those between the control and inhibitors of EDTA, DPA, and cloxacillin were measured with a cut-off value of 5 mm. The positive result was noted only on the meropenem disk with APBA [Figure 1b]. The *E. aerogenes* isolate was therefore phenotypically typed as a strain-producing Ambler class A carbapenemase. However, the isolate of imipenem-resistant *P. aeruginosa* showed a negative result in the Modified Hodge test.

To verify the class A carbapenemase genes, PCRs were performed with primers specific for the Ambler class A ($bla_{KPC,}$ $bla_{GES,}$ $bla_{SME,}$ bla_{NMC-A}), B ($bla_{IMP,}$ $bla_{VIM,}$ $bla_{GIM,}$ $bla_{SIM,}$ bla_{SPM}), or D (bla_{OXA}) genes, as in the previous studies.[14-18] However, both isolates revealed no meaningful carbapenemase gene bands on the electrophoresis gel.

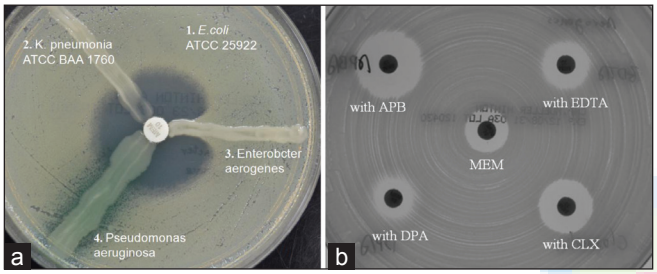

Figure 1. Modified Hodge Test and (a) confirmatory inhibition test (b) for the *Enterobacter aerogenes*. 1: *E. coli* ATCC 25922 as a reporter strain; 2: *K. pneumonia* ATCC BAA 1760 as the negative control; 3: *Enterobacter aerogenes* isolate showed a positive result; 4: *Pseudomonas aeruginosa* isolate showed a negative result; APB: aminophenylboronic acid, DPA: 6-pyridinedicarboxylic acid, CLX: cloxacillin sodium salt monohydrate, EDTA: ethylenediaminetetraacetic acid

DISCUSSION

The imipenem-resistant mechanism of *E. aerogenes* has been well investigated in Western countries.[10,19] However, there is still no report about the emergence of imipenem-resistant *E. aerogenes* in East Asia. Transport of the carbapenemase gene via plasmids has been reported among different species.[20] From our antibiotic-sensitive vigilance system, it is noted that the imipenem-resistant rate of *P. aeruginosa* has increased from 9% (2009) to 24% (2011). There has been no *E. aerogenes* resistant to imipenem in our record, before 2011. So far, the imipenem-resistant rate of *E. aerogenes* has been about 1.2% (2/169). Although imipenem-resistant isolates of *P. aeruginosa* and *E. aerogenes* had been obtained from the same wound culture concomitantly on hospital day 107, the chronological transfer of plasmids between the two isolates has been ruled out because of their different results in the modified Hodge tests. The carbapenem resistance of these two isolates may be due to the different mechanisms involved.

According to publications in the past two decades, *E. aerogenes* develops imipenem-resistance in three main mechanisms [Table 2]. They are, (I) reduction in drug uptake due to the loss of porin in the outer membrane,[7,21,22] (II) increase in production of carbapenemase,[10,19,23] and (III) combination with loss of porin in the outer membrane and production of extended spectrum beta-lactamase (ESBL).[8,24] The mechanism of porin loss on the outer membrane has been investigated

Table 2. Literature review for carbapenem-resistant *Enterobacter aerogenes*

Year	Country	Clinical isolates	Carbapenemase	Other mechanisms (Cephalosporinase or porin loss)	MIC of imipenem (mg/L)	References
1999	France	2	ND	Porin loss	ND	Claude Bosi
2000	France	4	ND	TEM-24 + porin loss	≥16 mg/L	Charléric Bornet
2002	America	1	ND	Porin loss	16 mg/L	Hesna Yigit
2005	France	1	ND	Porin loss	4-8mg/L	Aure´lie Thiolas
2008	France	10	ND	Porin loss	8-32 mg/L	M. Biendo
		3	ND	Porin loss	>32 mg/L	
		20	IMP-1	TEM-24, SHV-12	>8 mg/L	
		2	IMP-1	TEM-20		
		1	VIM-2	TEM-24, SHV-5		
		1	VIM-2	SHV-5		
2008	China	1	ND	(DHA-1, TEM-1, SHV-5, CTX-M-3, CTX-M-14) + porin loss	32 mg/L	Ya-Gang Chen
2010	China	1	ND	CTX-M-14, DHA-1, qnrS1	32 mg/L	Qiwen Yang
		1	ND	(TEM-1, CTX-M-3)+ porin loss	8 mg/L	
2012	Greece	7	KPC	ND	>32 mg/L	Georgia Vrioni

ND = not detectable.

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clearly.[7,8,21,22,25] *E. aerogenes* is shown to be capable of adapting rapidly to its permeability by regulating the expression of porin, in the previous studies. Long-term use of imipenem has been shown to be associated with *in vivo* development of porin-deficient mutants.[21] Moreover, the carbapenem resistance of *E. aerogense* has been found to be reversible after discontinuing the prescription of carbapenems.[7,8] Moreover, Mallea *et al.* have also described clinical *E. aerogenes* strains presenting complex resistant strategies associated with β-lactamase production, impermeability, and active efflux pumps.[25]

Long-term use of carbapenems against the ESBL- and AmpC-producing *E. aerogenes* could lead to the emergence of carbapenem resistance. Several β-lactamases, including, Ambler class A (CTX-M-3, CTX-M-14, TEM-1, and SHV-12) and Ambler class D (DHA-1) β-lactamases were reported to result in carbapenem resistance.[9,24] Moreover, the specific carbapenemases (Ambler classes A and B) encoded by plasmids were also identified from the strains of *E. aerogenes* in some Western countries.[10,19] The Ambler class A ($bla_{KPC,}$ bla_{GES}), B ($bla_{IMP,}$ $bla_{VIM,}$ $bla_{GIm,}$ $bla_{SIM,}$ $bla_{SPM,}$), and D (bla_{OXA}) genes were not found in this isolate. Hence, further investigation to detect whether the *E. aerogenes* isolate contains an unknown carbapenemase is needed.

According to CLSI 2012, the carbapenem-resistant MIC (μg/mL) breakpoints for Enterobacteriaceae shifted from ≥16 μg/ml to ≥4 μg/ml. After applying the revised carbapenem interpretation criteria for Enterobacteriaceae, more strains of imipenem-resistant *E. aerogenes* were isolated, but none of them produced carbapenemase. This is the first isolate of imipenem-resistant *E. aerogenes* producing the Ambler class A carbapenemase in Asia. In order to sensitively isolate carbapenem-resistant *E. aerogenes*, the revised interpretation criteria should be implemented. Moreover, further investigation on the carbapenemase genes from those isolates or other resistant mechanisms should also be conducted.

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DISCLOSURE

All authors declare that they have no competing financial interests.

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