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Characterization of the Multidrug-Resistant *Acinetobacter* species Causing a Nosocomial Outbreak at Intensive Care Units in a Korean Teaching Hospital: Suggesting the Correlations with the Clinical and Environmental Samples, Including Respiratory Tract-related Instruments

Hae-Sun Chung[1,2], Yangsoon Lee[2], Eun Suk Park[3], Dong Suk Lee[3], Eun Jin Ha[3], Myungsook Kim[2], Dongeun Yong[2], Seok Hoon Jeong[2], Kyungwon Lee[2,3], Yunsop Chong[2]

[1]Department of Laboratory Medicine, Ewha Womans University School of Medicine,
[2]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine,
[3]Department of Infection Control, Severance Hospital, Seoul, Korea

Background: *Acinetobacter* spp. is an important nosocomial pathogen for which increasing resistance to multiple antimicrobial agents has been observed. Prevalence of multidrug-resistant (MDR) *Acinetobacter* spp. in the intensive care unit (ICU) at a teaching hospital in Korea started to increase in 2008. The aim of this study was to determine the source of pathogen spread and to characterize the emerging strains at an early stage of outbreak.
Methods: Samples from respiratory instruments and fomites in the ICUs, as well as from the healthcare workers, were cultured to identify the sources of MDR *Acinetobacter* spp. Antimicrobial susceptibility was determined by the CLSI disk diffusion method. Pulsed field gel electrophoresis (PFGE) was performed for clinical and environmental isolates in order to determine clonality. Carbapenemase genes were detected by multiplex PCR. Infection control measures including peer-monitoring of hand washing, environmental cleaning and standard precautions were enforced.
Results: Among the samples from the ICU tools (105) and healthcare worker's hands (44), 31 (30%) and 2 (5%) respective samples yielded MDR *Acinetobacter* spp. Among the environmental samples, 90% were from respiratory-related equipment. The majority of clinical and environmental MDR *Acinetobacter* spp. (44/55) belonged to the pulsotype *A. baumannii* and carried both $bla_{\text{OXA-51}}$-like and $bla_{\text{OXA-23}}$-like genes. Even though infection-control measures were enforced, prevalence of MDR *Acinetobacter* spp. continues to increase.
Conclusion: An outbreak of MDR *Acinetobacter* spp. in a Korean hospital was caused by *A. baumannii* carrying the $bla_{\text{OXA-23}}$-gene and was correlated with contaminated respiratory-related instruments in the ICUs. More intensive measures for nosocomial infection control are needed for successful prevention of *Acinetobacter* spread in hospitals. **(Ann Clin Microbiol 2014;17:29-34)**

Key Words: *Acinetobacter,* Beta-lactamase OXA-23, Infection control, Disease outbreaks

INTRODUCTION

Acinetobacter spp. is an important nosocomial pathogen with an increase in resistance to multiple antimicrobial agents. Multidrug resistance (MDR) is defined as the resistance to representative antimicrobial agents of at least three different classes. The most commonly included antimicrobials are aminoglycosides, antipseudomonal penicillins, carbapenems, cephalosporins, and quinolones. The suggested definition of extreme drug resistance (XDR) in *Acinetobacter* spp. included resistance

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Correspondence: Dongeun Yong, Department of Laboratory Medicine, Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea. (Tel) 82-2-2228-2442, (Fax) 82-2-364-1583, (E-mail) deyong@yuhs.ac

to sulbactam, minocycline or doxycycline, and tigecycline in addition to the above mentioned antimicrobials. Furthermore, pandrug resistance (PDR) is defined as resistance to tigecycline and polymyxins along with the above mentioned antimicrobials [1].

Acinetobacter spp. is intrinsically less susceptible to antibiotics than *Enterobacteriaceae*, and has the propensity to acquire resistance [1,2]. Fournier, et al. [3] detected 45 acquired resistance genes in a MDR *Acinetobacter baumannii*, which were localized to the AbaR1 resistance island. The genetic surroundings of these resistance determinants provided more evidence for genetic promiscuity, with an array of broad-host-range mobile genetic elements identified, including three class 1 integrons, transposons, and insertion sequence (IS) elements.

A surveillance study in Korea in 2009 showed that resistance rates of *Acinetobacter* spp. were very high for fluoroquinolone (67%), amikacin (48%), ceftazidime (66%) and imipenem (51%) [4]. In The Surveillance Network study in the U.S, the resistance trend of *A. baumannii* was largely similar to that in Korea [5]. Antimicrobial susceptibilities patterns of *Acinetobacter* isolates from a Korean tertiary care hospital in 2009 showed that 14.9% and 41.8% of isolates were resistant to seven and to all eight antimicrobial agents, respectively [1].

In 2008, the number of MDR *Acinetobacter* spp. from clinical samples increased in a Korean university hospital, mainly due to the outbreak in the intensive care units (ICUs). The aims of this study were to characterize the molecular epidemiology of the MDR *Acinetobacter* spp. isolates in the early stage of their increase and to determine the source of their outbreak.

MATERIALS AND METHODS

1. Bacterial isolates from clinical and environmental samples of ICUs, including healthcare worker's hands

We analyzed clinical isolates recovered in the ICUs between April and December in 2008. The species were identified either by conventional biochemical tests or the VITEK 32GN system (bioMérieux, Marcy l'Etoile, France). The CLSI standardized disk diffusion methods [6] was used to determine the antimicrobial susceptibilities to ampicillin-sulbactam, piperacillin, aztreonam, ceftazidime, cefepime, cefotaxime, imipenem, amikacin, gentamicin, tobramycin, levofloxacin, trimethoprim-sulfamethoxazole and tetracycline. Modified-Hodge test and double disk synergy test using imipenem disk and EDTA+sodium mercaptoacetic acid (SMA) disk were used to characterize the carbapenem resistance mechanisms [7,8]. Twenty-eight MDR *Acinetobacter* isolates were randomly selected for further investigation.

The environmental samples from fomite and instruments in the ICUs were cultured to find the source of the MDR *Acinetobacter* strains. A detailed list of the samples includes ventilator, circuit, mask, O_2 meter, suction regulator, suction bottle, suction cup, suction cart, ambu bag, intra-venous pump machines, bed-side guard rails, and window sill around suction equipments. In addition, samples from the hands of 44 healthcare workers (HCWs; 3 physicians, 31 nurses, and 10 cleaners working in the ICUs) were also cultured. Among the environmental isolates, 27 strains were randomly selected for further investigation.

2. Typing using pulsed-field gel electrophoresis (PFGE)

To determine the relatedness of 55 MDR *Acinetobacer* isolates between the environment and clinical samples, comparision of their PFGE patterns of *Sma*I-restricted genome was carried out. *Sma*I-restricted genomic DNA from *Acinetobacter* isolates was separated by PFGE using a CHEF-DR II system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. The band patterns were analyzed according to the Tenover criteria [9].

3. Detection of OXA-type carbapenemase genes and IS*Aba1*

OXA carbapenemase genes were detected by multiplex PCR, as previously reported [10-12]. OXA carbapenemas genes were sequenced using amplicons generated with the following primers: OXA-51-like (5'-TAA TGC TTT GAT CGG CCT TG-3' and 5'-TGG ATT GCA CTT CAT CTT GG-3'), OXA-23-like (5'-GAT CGG ATT GGA GAA CCA GA-3' and 5'-ATT TCT GAC CGC ATT TCC AT-3'), OXA-24-like (5'-GGT TAG TTG GCC CCC TTA AA-3' and 5'-AGT TGA GCG AAA AGG GGA TT-3'), and OXA-58-like (5'-AAG TAT TGG GGC TTG TGC TG-3' and 5'-CCC CTC TGC GCT CTA CAT AC-3') [11]. To detect any upstream presence of IS*Aba1*, we used the primer IS*Aba*1F in combination with the reverse primers for the relevant OXA carbapenemase gene. The PCR amplification and sequencing of the $bla_{\text{OXA-182}}$ gene were carried out using primers OXA-143 F (5'-TTC TGT CAG TGC ATG CTC ATC-3'), OXA 143 R (5'-CAG GCA TTC CTT GCT TCA TT-3'), OXA-143 seq F (5'-GGC ACT CAA AAC TTT CCC TAA-3'), and OXA-143 seq R (5'-TTA TAT AAT CCC TAA ATT CTC TAA-3'). The nucleotide sequences were analyzed at a commercial laboratory (Macrogen, Seoul, Korea).

4. Infection control intervention

After recognition of the outbreak, infection control interventions were enforced more strictly since October 2008. The infection control efforts focused primarily on respiratory equipments because most of the *Acinetobacter* isolates were obtained from the respiratory specimens. Closed-suction system was introduced and the equipments used for suction, including cups and catheters, were prevented from contact with the contaminated environments prior to the usage. Hand hygiene was also emphasized. A task force team was organized to improve the hand-washing rates. Adherence to hand washing was monitored daily and provided on site. Furthermore, the team educated HCWs to encourage a behavior change through an improved understanding of the importance of the infection control measures.

RESULTS

1. Outbreak description

The laboratory data from 2008 show a noticeable increase in *Acinetobacter* spp. isolation in the hospital; the number of patients of *Acinetobacter* isolates significantly increased from 62 in January to 168 in August, 2008 (Fig. 1). The isolates from the ICU patients also increased from 26% to 40% (data not shown). More than 60% of the total isolates were from respiratory specimens. The number of *Acinetobacter* spp. isolated from clinical samples in the hospital was substantially reduced in October and November. However, the number of isolates increased again in December despite of the continuous effort to control the outbreak.

2. MDR *Acinetobacter* isolates from clinical and environmental samples and antimicrobial susceptibility testing

In September 2008, resistance rates of *Acinetobacter* isolates from clinical samples were 40% to imipenem, 47% to amikacin and 53% to levofloxacin. Among them, 21% were resistant to all 13 antimicrobial agents tested.

Of the 105 ICU environmental and 44 health-care worker's hand samples taken in October, 31 (30%) and 2 (5%) samples yielded MDR *Acinetobacter* spp., respectively (Table 1). The rate of MDR *Acinetobacter* spp. isolate from respiratory tract related instruments (38%) was much higher than those from formite (10%) and HCWs' hands (5%).

All of randomly selected 55 MDR *Acinetobacter* isolates (28 clinical isolates and 27 environmental isolates) were positive for Modified-Hodge test and negative for double disk synergy test using imipenem-EDTA plus sodium mercaptoacetic acid disks, which suggested presence of carbapenemase other than metalo-beta-lactamases (MBLs).

Intensive cleaning of the respiratory equipments and fomite in the ICUs with antiseptic solutions, and encouraging the hand washings resulted in a substantial decrease in MDR *Acineto-*

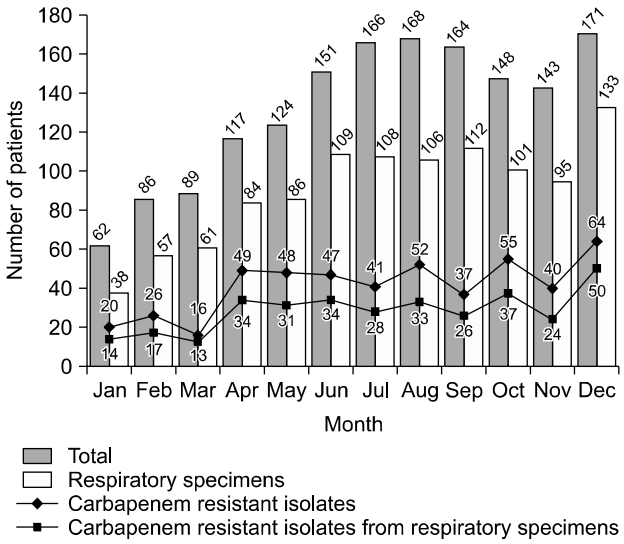

Fig. 1. Number of patients with *Acinetobacter* spp. isolates in a Korean hospital in 2008. There was noticeable increase in *Acinetobacter* spp. isolation in the hospital; the number of patients of *Acinetobacter* isolates significantly increased from 62 in January to 168 in August, 2008. The resistance rate to carbapenem was also increased to more than 50%. More than 60% of the total isolates were from respiratory specimens.

Table 1. MDR *Acinetobacter* spp. isolated from the ICUs environment and healthcare worker's hands

Specimen sources		No. of MDR *Acinetobacter* strains/total specimens (%)		
		October	November	December
Environment	Respiratory tract related instruments	28/74 (38)	25/90 (28)	9/42 (21)
	Fomites	3/31 (10)	6/36 (17)	3/35 (9)
Healthcare worker's hands		2/44 (5)	-	-

bacter isolation from respiratory tract samples, from 38% in October to 28% in November and 21% in December (Table 1).

3. Analysis of PFGE patterns and carbapenemase genes

Analysis of the PFGE results showed similar band patterns indicating the isolates are clonally related. The majority of the isolates from respiratory equipments and patients belonged to pulsotype A, indicating they are identical clones (Fig 2, Table 2). All of pulsotype A strains were positive for $bla_{\text{OXA-51}}$-like and $bla_{\text{OXA-23}}$-like genes, which suggested that they were *A. baumannii* and imipenem resistance were due to OXA-23 production. There were some sporadic clones harboring $bla_{\text{OXA-182}}$ and IS*Aba1*-associated $bla_{\text{OXA-51}}$ only (Table 2). Considering the PFGE pattern with OXA carbapenemase results indicated that the major imipenem resistance mechanism was OXA-23 production.

DISCUSSION

In this study, the majority of MDR *Acinetobacter* isolates from the patients and the environment, most importantly respiratory equipments, belonged to a single clone, pulsotype A. This finding suggests that the strains had been mostly transmitted via direct contact with respiratory droplets.

Majority of the MDR *Acinetobacter* isolates had $bla_{\text{OXA-23}}$-like and $bla_{\text{OXA-51}}$-like genes without IS*Aba1* insertion, which indicated that they were *A. baumannii* and the overproduction of the OXA-23 was the major mechanism for their carbapenem resistance. Outbreaks of OXA-type carbapenemase-producing *Acinetobacter* strains have been reported worldwide [1,2]. In re-

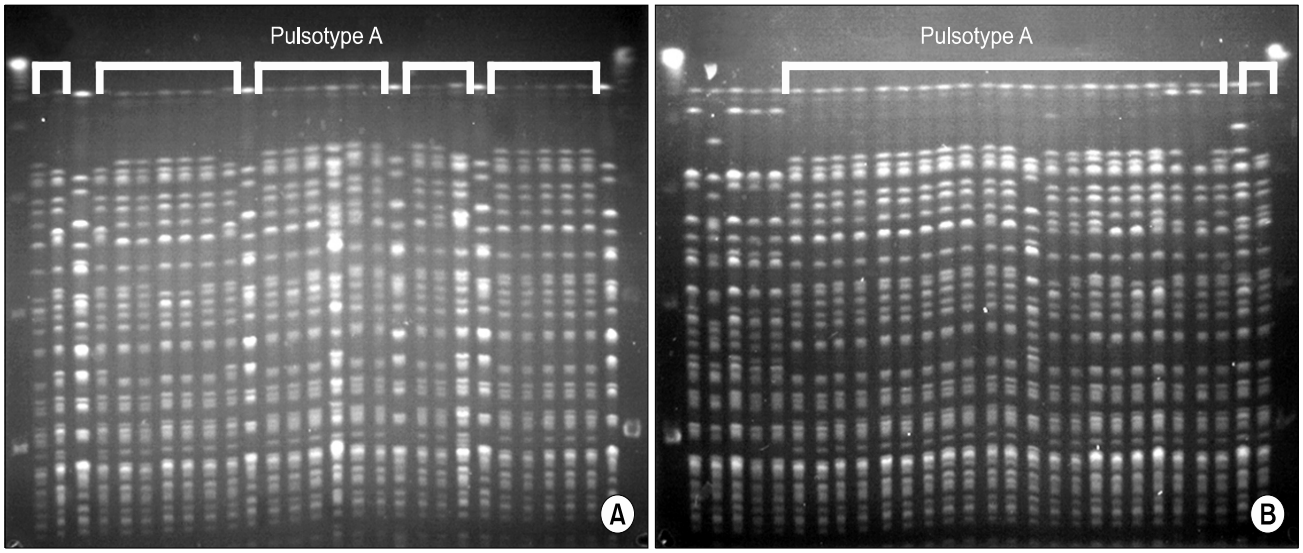

Fig. 2. PFGE patterns of *Acinetobacter* spp. isolates from ICU environment (A) and patients (B). The majority of the isolates from ICU environment and patients belonged to pulsotype A, indicating they are identical clones.

Table 2. Number of MDR *Acinetobacter* spp. isolates from clinical and environment samples according to the PFGE band patterns and OXA carbapenemase genes

PFGE pattern (No. of isolates)	No. of isolates		OXA carbapenemase genes detected		
	Clinical sample	Environmental sample	$bla_{\text{OXA-51}}$-like + $bla_{\text{OXA-23}}$-like	Upregulated $bla_{\text{OXA-51}}$-like only	$bla_{\text{OXA-51}}$-like + $bla_{\text{OXA-182}}$-like
A (44)	22	22	44		
B (4)		4	4		
C (1)		1		1	
D (4)	4		1		3
E (1)	1			1	
F (1)	1				1
Total (55)	28	27	49	2	4

cent studies, bla_{OXA-23}-like genes were the most prevalent among carbapenem-nonsusceptible isolates of *Acinetobacter* spp. from hospitals [1,12,13]. Another major mechanism for carbapenem resistance in *Acinetobacter* spp. in Korea is IS*Aba1*-activated bla_{OXA-51}-like genes [1,10,12,13]. However, in this study, only two isolates from the patients and the environment showed the upstream presence of IS*Aba1*.

Our findings further emphasize that PFGE and the detection of carbapenemase gene-associated genetic structures are warranted when investigating an outbreak caused by carbapenem-resistant *Acinetobacter* isolates. Intensive cleaning of respiratory equipments and fomite in the ICUs with antiseptic solutions, and hand washing were more emphasized in the ICUs since October 2008 as a measure of infection control. The isolation of MDR *Acinetobacter* spp. from respiratory equipments substantially decreased after infection control intervention. However, even though the infection control measures were continued in December, the number of isolates started to increase again. Patient isolation and ICU closure might be required in addition for a continuation of successful outbreak control.

In summary, the carbapenem-resistant *Acinetobacter* outbreak was correlated to contaminated respiratory-related instruments in the ICUs with single MDR *A. baumannii* clone carrying bla_{OXA-23}-like gene. A more intensive application of nosocomial infection control measures would be warranted for a successful prevention of an *Acinetobacter* spread in the hospital.

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=국문초록=

국내 한 대학병원의 중환자실에서 집단 발생한 다제내성 *Acinetobacter* species 성상 분석: 임상검체와 호흡기 관련 장비 등 환경 검체와의 연관

[1]이화여자대학교 의학전문대학원 진단검사의학교실, [2]연세대학교 의과대학 진단검사의학교실, 세균내성연구소, [3]세브란스병원 감염관리실

정혜선[1,2], 이양순[2], 박은숙[3], 이동숙[3], 하은진[3], 김명숙[2], 용동은[2], 정석훈[2], 이경원[2], 정윤섭[2]

배경: *Acinetobacter* spp.는 중요한 의료관련감염의 원인균으로 여러 항균제에 대해 내성이 증가하고 있다. 2008년 이후 국내 대학병원의 중환자실에서 다제내성 *Acinetobacter* spp.가 급증하였다. 본 연구에서는 집단감염발생 초기의 전파양상과 균주 특성에 대해 알아보고자 하였다.

방법: 다제내성 *Acinetobacter* spp.의 감염원을 찾기 위해 중환자실의 호흡기계관련기구와 주변환경 및 병원직원들에 대한 배양을 시행하였다. 항균제감수성검사는 CLSI 디스크확산법으로 수행하였다. 환경분리주와 임상분리주에 대해 PFGE를 시행하여 클론성을 규명하였다. 카바페넴분해효소 유전자는 다중 PCR을 이용하여 판정하였다. 손씻기 감시, 환경청소, 표준감염주의(standard precaution) 적용, 등을 포함한 감염관리를 강화하였다.

결과: 10월에 시행한 환경 검체 105개과 병원직원 손 검체 44개에 대한 배양에서 각각 31개(30%)와 2개(5%)의 검체에서 다제내성 *Acinetobacter* spp.가 분리되었다. 환경 검체 중에서는 90% (28/31)가 호흡기계기구 관련 검체였다. 임상과 환경 검체에서 분리된 다제내성 *Acinetobacter* spp.의 대부분(80%, 44/55)이 하나의 pulsotype에 속하였으며, 이들은 bla_{OXA-23}-like 유전자와 bla_{OXA-51}-like 유전자를 보유하고 있는 *A. baumannii*이었다. 감염관리를 강화하였음에도 불구하고, 임상검체에서 다제내성 *Acinetobacter* spp.의 분리는 다시 증가하였다.

결론: 집단검출된 다제내성 *Acinetobacter* spp.는 bla_{OXA-23} 유전자 양성 *A. baumannii*에 의한 것이었으며 중환자실의 오염된 호흡기계기구와 높은 관련이 있었다. 원내 *Acinetobacter* 전파를 막기 위하여는 더 높은 수준의 감염관리조치가 필요할 것이다. [Ann Clin Microbiol 2014;17:29-34]

교신저자 : 용동은, 120-752, 서울시 서대문구 연세로 50
연세대학교 의과대학 진단검사의학교실, 세균내성연구소
Tel: 02-2228-2442, Fax: 02-364-1583
E-mail: deyong@yuhs.ac