Original Article

http://dx.doi.org/10.3349/ymj.2013.54.1.177
pISSN: 0513-5796, eISSN: 1976-2437

Yonsei Med J 54(1):177-182, 2013

YMJ

Molecular and Epidemiological Characterization of Carbapenem-Resistant *Acinetobacter baumannii* in Non-Tertiary Korean Hospitals

Sunok Park, Hwa-Su Kim, Kyeong Min Lee, Jung Sik Yoo, Jae Il Yoo, Yeong Seon Lee, and Gyung Tae Chung

Division of Antimicrobial Resistance, Center for Infectious Diseases, Korea National Institute of Health, Cheongwon, Korea.

Received: September 29, 2011
Revised: Febarary 27, 2012
Accepted: March 12, 2012
Corresponding author: Dr. Gyung Tae Chung, Division of Antimicrobial Resistance, Center for Infectious Diseases, Korea National Institute of Health, 187 Osongsaengmyeong 2-ro, Osong-eup, Cheongwon 363-700, Korea.
Tel: 82-43-719-8241, Fax: 82-43-719-8269
E-mail: gtchung@nih.go.kr

· The authors have no financial conflicts of interest.

Purpose: The increasing prevalence and global spread of carbapenem-resistant *Acinetobacter baumannii* (*A. baumannii*) has become a serious problem. The aim of this study was to investigate molecular and epidemiological characteristics of carbapenem-resistant *A. baumannii* isolates collected from Korean non-tertiary hospitals. **Materials and Methods:** Thirty six non-duplicated carbapenem-resistant *A. baumannii* isolates were collected from 17 non-tertiary hospitals in Korea between 2004 and 2006. Isolates were typed by multilocus sequence typing and repetitive-sequence-based PCR (rep-PCR). Detection of genes encoding OXA carbapenemase and their relationship with ISA*ba1* was performed by PCR. **Results:** Two clones were prevalent among 36 isolates: ST69 (17 isolates, 47.2%) and ST92 (19 isolates, 52.8%). Rep-PCR patterns were diverse and revealed that all isolates were clustered into eight band patterns. The ISA*ba1*-activated *bla*OXA-23-like and ISA*ba1*-activated *bla*OXA-51-like genes were prevalent among the carbapenem-resistant *A. baumannii* isolates. **Conclusion:** The class D β-lactamase genes of *A. baumannii* were distributed nationwide in non-tertiary Korean hospitals.

Key Words: *Acinetobacter baumannii*, Carbapenem-resistant, OXA carbapenemase

INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) has emerged worldwide as an important opportunistic nosocomial pathogen, especially in intensive care unit (ICU) patients.[1] Carbapenems have been widely used to treat serious infections associated with multidrug-resistant (MDR) *A. baumannii*, but carbapenem resistance has been increasingly reported worldwide.[2,3] Moreover, most carbapenem-resistant *A. baumannii* strains are multidrug or even pandrug resistant.[4] In Korea, imipenem-resistant *A. baumannii* isolates from a university hospital reached 32.5%.[5] Among the various mechanisms of carbapenem resistance in *A. baumannii*, carbapenemase of the molecular class D OXA enzymes has emerged as the main source of carbapenem resistance.[6] A previous study reported that among ten university hospitals in Korea,

78.3% of carbapenem-resistant *A. baumannii* isolates were caused by OXA-23-like enzymes, among which ST22 was the most prevalent type.[7] Park, et al.[8] reported that two major clones of carbapenem-resistant *A. baumannii*, ST22 and ST28, were found in five tertiary care hospitals.[8] However, these two clones have so far been studied only in tertiary hospital isolates. This study was performed to analyze the genetic diversity and clonal distribution of carbapenem-resistant *A. baumannii* isolates among non-tertiary hospitals in Korea.

MATERIALS AND METHODS

Bacterial isolates and reference strains

A total of 315 consecutive *A. baumannii* clinical isolates were collected from 24 non-tertiary South Korea hospitals between 2004 and 2006. Among these isolates, 50 were carbapenem-resistant, of which 36 nonrepetitive isolates from 17 hospitals in 8 provinces were selected for this study. The strains were propagated at 37°C in Luria-Bertani broth or agar. The clinical isolates were identified by using the Vitek II system (bioMerieux, Marcy-I'Etoile, France). Species identification was performed by partial *rpoB* gene sequencing.[9]

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were performed using the minimal inhibitory concentrations of Etest (AB BIODISK, Piscataway, NJ, USA), based on the results reported by the Clinical and Laboratory Standards Institute.[10] Eleven antimicrobial agents were tested: amikacin, gentamicin, ticarcillin-clavulanic acid, piperacillin, imipenem, meropenem, ciprofloxacin, ceftazidime, cefepime and polymyxin B, according to the manufacturer's instructions. *Escherichia coli* ATCC 25922 was used for quality control in antimicrobial susceptibility testing.

Multilocus sequencing typing (MLST)

MLST was performed for all isolates according to the method described previously.[11] Fragments of seven housekeeping genes (*gltA, gyrB, gdhB, recA, cpn60, gpi, rpoD*) were amplified by PCR and sequenced. Allele numbers were assigned as sequence types (STs) after distinct allele sequences were submitted to a dedicated data base (http://pubmlst.org). A clonal complex was used to assess the genetic relatedness of the STs; the most stringent definition-sharing alleles at 6 of 7 loci-was used.[12]

Repetitive-sequence-based PCR (Rep-PCR)

Rep-PCR was performed by using the DiversiLab system (bioMerieux SA, Grenoble, France) to investigate the clonal relatedness of *A. baumannii* isolates. Results were analyzed with DiversiLab software using the Kullback-Leibler method to determine distance matrices, and the unweighted pair group method with arithmetic averages to create a dendrogram. Sample relationships were designated as follows: pattern, no band differences (indistinguishable); group, 1 to 2 band differences (similar); and different group, 3 or more band differences. Each group corresponded to an outbreak.

Identification of carbapenemase genes

PCR was performed to detect the genes encoding MBLs (IMP-1 variants and VIM-2 variants) and OXA carbapenemase (OXA-23-like, OXA-24-like, OXA-51-like and OXA-58-like) according to the method described previously.[13] The sequence analysis of PCR products was carried out by request at Macrogen (http://macrogen.co.kr).

RESULTS

Antimicrobial susceptibilities

The isolated carbapenem-resistant *A. baumannii* isolates showed 100% resistance to gentamicin, piperacillin, ciprofloxacin, ceftazidime and cefepime. In addition, they showed a high resistance against amikacin (34/36, 94.4%), but a complete susceptibility to polymyxin B (Table 1).

Distribution and molecular epidemiology of ß-lactamase genes

Two unique STs among the 36 carbapenem-resistant *A. baumannii* isolates were revealed by MLST analysis (Table 2). Two clones were prevalent among 36 isolates: ST69 (17 isolates, 47.2%) and ST92 (19 isolates, 52.8%). All 36 *A. baumannii* isolates carried the *bla*OXA-51-like gene. Fourteen isolates (38.9%) had the ISA*ba1*-activated *bla*OXA-23-like gene, and 25 isolates (69.4%) had the ISA*ba1*-activated *bla*OXA-51-like gene. Coexistence of *bla*OXA-23 and ISA*ba1*-activated *bla*OXA-51-like genes was detected in three isolates (8.3%). No *bla*OXA-24-like, *bla*OXA-58 like, IMP-1 or VIM-2 genes were found.

Clonal relationship

The results of rep-PCR showed that carbapenem-resistant *A. baumannii* isolates were clustered into eight distinctive band

Table 1. The Results of Antimicrobial Susceptibility in 36 Carbapenem-Resistant *A. baumannii* Isolates

No.	Strains	Antibiotics									
		AMK	GEN	TIM	PIP	IMP	MER	CIP	CAZ	CPM	PMB
1	5303	R	R	R	R	R	R	R	R	R	S
2	5A037	I	R	R	R	R	R	R	R	R	S
3	5A035	R	R	R	R	I	R	R	R	R	S
4	5A067	R	R	R	R	S	R	R	R	R	S
5	6A115	R	R	R	R	S	R	R	R	R	S
6	6A030	R	R	R	R	R	R	R	R	R	S
7	6A032	R	R	R	R	R	R	R	R	R	S
8	6A109	R	R	R	R	R	R	R	R	R	S
9	5A114	R	R	R	R	S	R	R	R	R	S
10	5A127	R	R	R	R	S	R	R	R	R	S
11	5A042	R	R	R	R	R	R	R	R	R	S
12	6A018	R	R	R	R	R	R	R	R	R	S
13	6A076	R	R	R	R	R	R	R	R	R	S
14	6A111	R	R	R	R	R	R	R	R	R	S
15	6A107	R	R	R	R	R	R	R	R	R	S
16	5620	R	R	R	R	I	R	R	R	R	S
17	5A023	R	R	R	R	R	R	R	R	R	S
18	5345	R	R	R	R	R	R	R	R	R	S
19	5821	R	R	R	R	I	R	R	R	R	S
20	5A010	R	R	R	R	R	R	R	R	R	S
21	6A061	R	R	R	R	R	R	R	R	R	S
22	5A031	R	R	R	R	R	R	R	R	R	S
23	5A018	R	R	R	R	R	R	R	R	R	S
24	5621	R	R	R	R	R	R	R	R	R	S
25	5A022	R	R	R	R	S	R	R	R	R	S
26	5287	R	R	R	R	R	R	R	R	R	S
27	5781	R	R	R	R	R	R	R	R	R	S
28	5617	R	R	R	R	S	R	R	R	R	S
29	5A004	S	R	R	R	R	R	R	R	R	S
30	5A017	R	R	R	R	R	R	R	R	R	S
31	5A027	R	R	R	R	R	R	R	R	R	S
32	5837	R	R	R	R	R	R	R	R	R	S
33	5A012	R	R	R	R	R	R	R	R	R	S
34	5A055	R	R	R	R	R	R	R	R	R	S
35	5A047	R	R	R	R	R	R	R	R	R	S
36	5A049	R	R	R	R	R	R	R	R	R	S

AMK, amikacin; GEN, gentamicin; TIM, ticarcillin-clavulanic acid; PIP, piperacillin; IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; CAZ, ceftazidime; CPM, cefepime; PMB, polymyxin B; *A. baumannii*, *Acinetobacter baumannii*.

patterns, including five clonally related groups. A 96.5% similarity line was obtained by DiversiLab software to represent the best match between pattern assignments and the dendrogram (Fig. 1). The following differences in rep-PCR patterns were observed between ST69 and ST92: band pattern 1 [2 isolates (ST69)], band pattern 2 [2 isolates (ST69)], band pattern 3 [5 isolates (ST69), 1 isolate (ST92)], band pattern 4 [3 isolates (ST69), 1 isolate (ST92)], band pattern 5 [1 isolate (ST69), 13 isolates (ST92)], band pattern 6 [4 isolates (ST92)], band pattern 7 [3 isolates (ST69)], and band pattern 8 [1 isolate (ST69)] (Table 2).

DISCUSSION

A. baumannii is a rapidly emerging pathogen in hospitals, frequently causing nosocomial outbreaks in ICUs. Several hospital outbreaks caused by MDR *A. baumannii* clones

Table 2. Genotyping Results of Class D ß-Lactamase and Multilocus Sequence Typing (MLST) in the 36 Carbapenem-Resistant *A. baumannii* Isolates

No.	Yr	Isolates	Specimen	Province	Carbapenemase genes		Allele types for MLST genes	
					IS-OXA23 like	*IS-OXA51 like*	STs	*alleles*
1	2004	5303	CSF	Jeju	-	○	92	(1-3-3-2-2-7-3)
2	2005	5A037	Wound	Gyeonggi	-	○	69	(1-46-3-2-2-58-3)
3	2005	5A035	Wound	Daegu	-	○	69	(1-46-3-2-2-58-3)
4	2005	5A067	Wound	Daegu	-	○	69	(1-46-3-2-2-58-3)
5	2006	6A115	Sputum	Seoul	-	○	69	(1-46-5-2-2-58-3)
6	2006	6A030	Wound	Incheon	-	○	69	(1-46-3-2-2-58-3)
7	2006	6A032	Wound	Incheon	-	○	69	(1-46-3-2-2-58-3)
8	2006	6A109	Sputum	Gyeongbuk	○	-	69	(1-46-3-2-2-58-3)
9	2005	5A114	Pus	Gyeongbuk	-	○	92	(1-3-3-2-2-7-3)
10	2005	5A127	Sputum	Gangwon	-	○	92	(1-3-3-2-2-7-3)
11	2005	5A042	Wound	Daegu	○	○	92	(1-3-3-2-2-7-3)
12	2006	6A018	Sputum	Busan	-	○	92	(1-3-3-2-2-7-3)
13	2006	6A076	Sputum	Incheon	-	○	92	(1-3-3-2-2-7-3)
14	2006	6A111	Wound	Gyeongnam	-	○	92	(1-3-3-2-2-7-3)
15	2006	6A107	Wound	Jeonbuk	-	○	92	(1-3-3-2-2-7-3)
16	2004	5620	Wound	Daegu	-	○	69	(1-46-3-2-2-58-3)
17	2005	5A023	Pus	Gangwon	○	-	92	(1-3-3-2-2-7-3)
18	2004	5345	Fluid	Gyeonggi	-	○	92	(1-3-3-2-2-7-3)
19	2004	5821	Other	Gyeongnam	-	○	69	(1-46-3-2-2-58-3)
20	2005	5A010	Wound	Gyeongnam	○	-	69	(1-46-3-2-2-58-3)
21	2006	6A061	Sputum	Gyeonggi	-	○	92	(1-3-3-2-2-7-3)
22	2005	5A031	Wound	Daegu	○	○	92	(1-3-3-2-2-7-3)
23	2005	5A018	Wound	Daegu	○	○	92	(18-3-3-2-2-7-3)
24	2004	5621	Pus	Seoul	-	○	92	(1-3-3-2-2-7-3)
25	2005	5A022	Wound	Daegu	-	○	92	(1-3-3-2-2-7-3)
26	2004	5287	Sputum	Incheon	-	○	92	(1-3-3-2-2-7-3)
27	2004	5781	Wound	Gyeongnam	-	○	92	(1-3-3-2-2-7-3)
28	2004	5617	Other	Gyeongbuk	-	○	92	(1-3-3-2-2-7-3)
29	2005	5A004	Other	Gyeongbuk	○	-	92	(1-3-3-2-2-7-3)
30	2005	5A017	Wound	Gyeongnam	○	-	69	(1-46-3-2-2-58-3)
31	2005	5A027	Wound	Seoul	○	-	69	(1-46-3-2-2-58-3)
32	2004	5837	Wound	Gyeongbuk	○	-	69	(1-46-3-2-2-58-3)
33	2005	5A012	Wound	Seoul	○	-	69	(1-46-3-2-2-58-3)
34	2005	5A055	Wound	Seoul	○	-	69	(1-46-3-2-2-58-3)
35	2005	5A047	Wound	Seoul	○	-	69	(1-46-3-2-2-58-3)
36	2005	5A049	Wound	Seoul	○	-	69	(1-46-3-2-2-58-3)

STs, sequence types; *A. baumannii, Acinetobacter baumannii*, CSF, cerebral spinal fluid.

have been described in Europe and worldwide. The incidence of carbapenem-resistant *A. baumannii* has increased worldwide since its first emergence in New York in 1991.[14] Despite the high ratio of carbapenem-resistant *A. baumannii* isolates in Korean tertiary hospitals,[15] there have been no reports about the antimicrobial susceptibility, resistance mechanisms or molecular epidemiology of carbapenem-resistant *A. baumannii* in Korean non-tertiary hospitals. In this study, we used MLST and rep-PCR to investigate the clonality of carbapenem-resistant *A. baumannii* isolates in non-tertiary hospitals throughout various regions of Korea. In MLST, two distinctive clones, ST92 and its double-locus variant ST69, were identified among 36 carbapenem-resistant *A. baumannii* isolates. Our results corresponded well with those of an earlier study which reported that ST92 (ST22 was redesignated ST92 after an update of allelic profiles at the database website http://pubmist.org) may contribute to the high carbapenem resistance rates of *Acinetobacter*

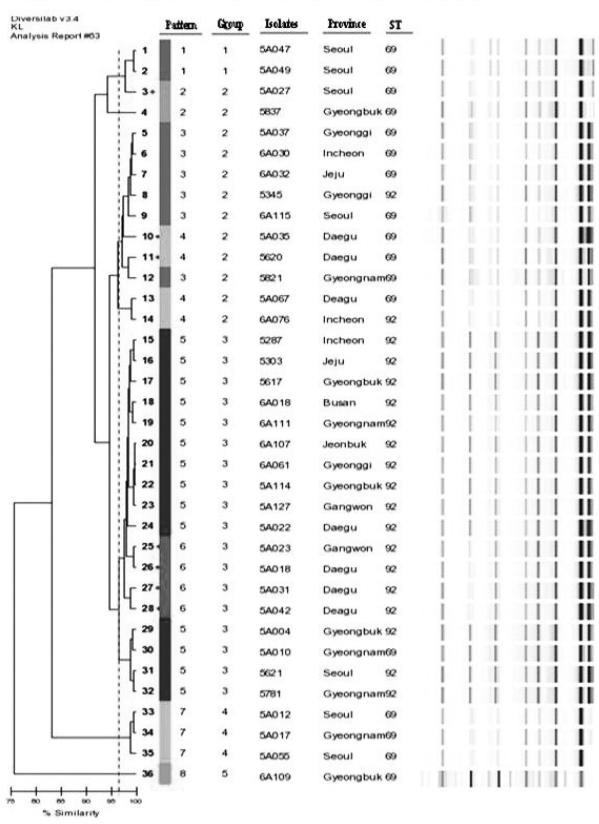

Fig. 1. Rep-PCR banding patterns and STs of 36 carbapenem resistant *A. baumannii*.

isolates in Korean university hospitals.[7] Wide dissemination of non-carbapenem-susceptible ST92 isolates has also been described in China and Korea.[16] In addition, Park, et al.[8] demonstrated that ST69 was another major carbapenem-resistant clone found in tertiary care hospitals in Korea. ST28, however, was not identified in this study, which was previously indicated as a major carbapenem-resistant clone found in Korea.[8] There were no distinctive characteristics in regards to *bla*OXA type in the two major clones, ST92 and ST69, as shown in Table 2. Because both clones were found in most regions, they may be distributed ubiquitously throughout Korea. Most of the isolates of the ST92 clone (17/19, 89.5%) were clustered into three rep-PCR band groups. The ISA*ba1*-activated OXA-23-like β-lactamase gene was found in the isolates of ST69 (9 isolates) and ST92 (5 isolates).

In summary, we investigated the antimicrobial resistance and clonality of carbapenem-resistant *A. baumannii* isolates in non-tertiary hospitals throughout Korea. Carbapenem-resistant *A. baumannii* isolates in Korea may be due to two major clones, ST69 and ST92, which have disseminated nationwide in not only tertiary hospitals but also in non-tertiary hospitals throughout Korea.

ACKNOWLEDGEMENTS

This study was supported by an intramural research fund of the Korea Centers for Disease Control and Prevention. 2007-N44002-00.

REFERENCES

1. Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med 2008;358:1271-81.
2. Poirel L, Lebessi E, Héritier C, Patsoura A, Foustoukou M, Nordmann P. Nosocomial spread of OXA-58-positive carbapenem-resistant Acinetobacter baumannii isolates in a paediatric hospital in Greece. Clin Microbiol Infect 2006;12:1138-41.
3. Garnacho-Montero J, Amaya-Villar R. Multiresistant Acinetobacter baumannii infections: epidemiology and management. Curr Opin Infect Dis 2010;23:332-9.
4. Kwon KT, Oh WS, Song JH, Chang HH, Jung SI, Kim SW, et al. Impact of imipenem resistance on mortality in patients with Acinetobacter bacteraemia. J Antimicrob Chemother 2007;59:525-30.
5. Lee JH, Choi CH, Kang HY, Lee JY, Kim J, Lee YC, et al. Differences in phenotypic and genotypic traits against antimicrobial agents between Acinetobacter baumannii and Acinetobacter genomic species 13TU. J Antimicrob Chemother 2007;59:633-9.
6. Walther-Rasmussen J, Høiby N. OXA-type carbapenemases. J Antimicrob Chemother 2006;57:373-83.
7. Park YK, Lee GH, Baek JY, Chung DR, Peck KR, Song JH, et al. A single clone of Acinetobacter baumannii, ST22, is responsible for high antimicrobial resistance rates of Acinetobacter spp. isolates that cause bacteremia and urinary tract infections in Korea. Microb Drug Resist 2010;16:143-9.
8. Park YK, Choi JY, Jung SI, Park KH, Lee H, Jung DS, et al. Two distinct clones of carbapenem-resistant Acinetobacter baumannii isolates from Korean hospitals. Diagn Microbiol Infect Dis 2009;64:389-95.
9. La Scola B, Gundi VA, Khamis A, Raoult D. Sequencing of the rpoB gene and flanking spacers for molecular identification of Acinetobacter species. J Clin Microbiol 2006;44:827-32.
10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20. Wayne, PA, USA: CLSI; 2010.
11. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodríguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii. J Clin Microbiol 2005;43:4382-90.
12. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol 2004;186:1518-30.
13. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006;27:351-3.
14. Manikal VM, Landman D, Saurina G, Oydna E, Lal H, Quale J. Endemic carbapenem-resistant Acinetobacter species in Brooklyn, New York: citywide prevalence, interinstitutional spread, and rela-

tion to antibiotic usage. Clin Infect Dis 2000;31:101-6.
15. Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of Acinetobacter baumannii isolates from Korea. J Antimicrob Chemother 2007;60:1163-7.
16. Lee Y, Lee J, Jeong SH, Lee J, Bae IK, Lee K. Carbapenem-non-susceptible Acinetobacter baumannii of sequence type 92 or its single-locus variants with a G428T substitution in zone 2 of the rpoB gene. J Antimicrob Chemother 2011;66:66-72.