Infection, Genetics and Evolution 19 (2013) 219–222

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* sequence type 191 in a Korean hospital

Mamata Gurung [1], Jae Su Rho [1], Yoo Chul Lee [1], Hyung Sun Kim, Sun Young Moon, Byung Hyuk Yu, Joo Hyung Lee, Cheol Woo Jang, Seung Yeon Chung, Il Hwan Cho, Hyeon Sook Jee, Seung Won Choi, Ji Young Heo, Je Chul Lee *

Department of Microbiology, Kyungpook National University School of Medicine, Daegu 700-422, South Korea

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form 11 July 2013
Accepted 15 July 2013
Available online 22 July 2013

Keywords:
Carbapenem
Clonal spread
Sequence type
Extended-spectrum β-lactamase
Multi-drug resistance

ABSTRACT

Emergence and spread of specific carbapenem-resistant *Acinetobacter baumannii* (CRAB) clones cause a serious therapeutic problem. This study was aimed to investigate the clonal diversity and genetic basis of antimicrobial resistance among the 69 CRAB isolates from 2009 to 2010 in a Korean hospital. All CRAB isolates were found to be sequence type (ST) 2 using the Institute Pasteur's multilocus sequence typing (MLST) scheme, but classified into two sequence groups and nine pulsotypes. Fifty-six CRAB isolates belonging to two main pulsotypes were found to be ST191 using the Bartual's MLST scheme. All CRAB isolates showed an extensively drug-resistant phenotype. The bla_{OXA-51}/bla_{OXA-23}, bla_{AmpC}/bla_{PER-1} and *armA* genes were largely responsible for resistance to carbapenems, extended-spectrum β-lactams and aminoglycosides, respectively. The first CRAB strains identified in 2005 in this hospital were found to be ST2 using the Institute Pasteur's MLST scheme, but showed ST353 using the Bartual's MLST scheme and different pulsotypes from the CRAB isolates from 2009 to 2010. In conclusion, this is the first report of emergence and spread of *A. baumannii* ST191 in Korea, as well of the genetic basis of its antimicrobial resistance.

1. Introduction

Acinetobacter baumannii is a Gram-negative, lactose non-fermenting organism which is increasingly becoming a major nosocomial pathogen worldwide. Clinical significance of *A. baumannii* has increased due to its ability to develop antimicrobial resistance by intrinsic and acquired mechanisms, making it a significant threat in the current antibiotic era (Dijkshoorn et al., 2007). Carbapenems are the most effective β-lactam antibiotics against Gram-negative bacteria and are widely used for treatment of life-threatening multi-drug resistant *A. baumannii* infections (Dijkshoorn et al., 2007). However, extensive use of carbapenems has led to the emergence of carbapenem-resistant *A. baumannii* (CRAB) worldwide. Resistance of *A. baumannii* to carbapenems is primarily mediated by Ambler's class D OXA-type β-lactamases that possess carbapenemase activity and metallo-β-lactamases (Livermore and Woodford, 2006).

Resistance of *A. baumannii* isolates to imipenem dramatically increased from 16% in 2003–2007 to 78% in 2008–2010 in a single tertiary hospital in South Korea (Park et al., 2012). *A. baumannii* clonal complex 92 corresponding to the worldwide clone was the main clone in South Korea during the last decade, but the predominant sequence types (STs) have been changing over time: ST92 was the most predominant until 2007, whereas single locus variants (slv) of ST92 such as ST75 and ST138 were found to be main clones among the CRAB isolates from 2008 to 2010 (Lee et al., 2011; Park et al., 2009; Park et al., 2012). Moreover, antimicrobial susceptibility profiles and resistance determinants were different between ST92 and newly emerged STs. *A. baumannii* ST75 and ST138 clones, whichpossessed the $bla_{OXA-23-like}$ gene, were resistant to carbapenems. In contrast, *A. baumannii* ST92 clone rarely carried the $bla_{OXA-23-like}$ gene and only 20% were resistant to carbapenems in South Korea (Park et al., 2012). Our group screened the CRAB among the clinical *A. baumannii* isolates from 2005 in a tertiary hospital, located in Daegu, South Korea and identified only 5 CRAB isolates (Lee et al., 2007). Thereafter, carbapenem-resistance of *A. baumannii* isolates increased up to approximately 40% during 2009–2010 in the same hospital. With regard to the sharp increase of CRAB isolates, the aim of was to investigate the epidemiological traits of these strains from 2009 to 2010 and also examine the genetic basis of their antimicrobialresistance.

* Corresponding author. Address: Department of Microbiology, Kyungpook National University School of Medicine, 101 Dongin-dong-2 ga, Jung-gu, Daegu 700-422, Republic of Korea. Tel.: +82 53 420 4844; fax: +82 53427 5664.
E-mail address: leejc@knu.ac.kr (J.C. Lee).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.meegid.2013.07.016

2. Materials and methods

2.1. Bacterial isolates

A total of 69 non-duplicate clinical CRAB isolates from Kyungpook National University Hospital in Daegu, South Korea, between February 2009 and June 2010 were used in this study. The CRAB isolates were obtained from a Kyungpook National University Hospital-Culture Collection for Pathogens (KNUH-CCP). Five CRAB isolates from 2005 were also used (Lee et al., 2007). The isolates were phenotypically identified at the genus *Acinetobacter* using Vitek 2 system with a GN card (bioMerieux, Marcy l'Etoile, France) and their genomic species were identified by polymerase chain reaction (PCR) of tri-locus sequence typing (Turton et al., 2007; Towner et al., 2008).

2.2. Antimicrobial susceptibility test

The minimum inhibitory concentrations (MICs) to antimicrobial agents were determined by the agar dilution method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) (2010). The antimicrobial agents were selected based on the guidelines of international standard definitions for acquired resistance in *Acinetobacter* spp. proposed by Magiorakos et al. (2012). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

2.3. Detection of antimicrobial resistance genes

Genes coding for 16S rRNA methylases were sought by PCR using primers specific for *armA*, *rmtA*, *rmtB*, *rmtC* and *rmtD* (Cho et al., 2009). Similarly, *aphA1*, *aphA6*, *aadA1*, *aadB*, *aacA4*, *aacC1* and *aacC2* genes encoding aminoglycoside-modifying enzymes (AMEs) were detected by PCR (Cho et al., 2009). The primer sets specific for bla_{AmpC}, $bla_{\text{PER-1}}$, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$, $bla_{\text{IMP-1}}$ and $bla_{\text{VIM-2}}$ were used to detect genes for resistance to β-lactam agents (Corvec et al., 2003; Jeon et al., 2005; Héritier et al., 2006; Lin et al., 2010). The presence of IS*Aba1* inserted upstream of $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$ and bla_{AmpC} was sought by PCR as previously described (Lin et al., 2010).

2.4. Pulsed-filed gel electrophoresis (PFGE)

PFGE was performed as previously described (Lee et al., 2007). The banding patterns were analyzed with GelCompar II software (Applied Maths, Kortijk, Belgium) to produce a dendrogram.

2.5. PCR-based sequence groups and multilocus sequence typing (MLST)

A PCR-based sequence group of CRAB isolates was determined through a PCR for *ompA*, *csuE* and $bla_{\text{OXA-51-like}}$ genes (Turton et al., 2007; Towner et al., 2008). Two different MLST analysis, the Institute Pasteur's MLST scheme (Diancourt et al., 2010) and the Bartual's MLST scheme (Bartual et al., 2005), were performed to identify STs using tools in the web site http://www.pasteur.fr/Abaumannii.html and http://pubmlst.org/abaumannii, respectively.

3. Results

3.1. Characteristics of CRAB isolates

A total of 69 CRAB isolates were obtained from hospitalized patients admitted to 13 different wards. The CRAB isolates were originated from blood ($n = 33$), wound ($n = 12$), sputum ($n = 9$), ascitic fluids ($n = 8$), pus ($n = 4$) and other specimens ($n = 3$). Of the 18 antimicrobial agents tested among 9 antimicrobial categories, all CRAB isolates were resistant to 14 agents, and 65 and 67 isolates were resistant to amikacin and tobramycin, respectively. Resistance to colistin and minocycline was found in one isolate, respectively. All CRAB isolates were considered to be extensively drug-resistant (XDR) strains in terms of non-susceptible to at least one agent in all but two or fewer antimicrobial categories (Magiorakos et al., 2012).

3.2. Clonal relatedness of CRAB isolates from 2009 to 2010

All CRAB isolates were assigned to ST2 using the Institute Pasteur's MLST scheme. Sixty-nine CRAB isolates were classified into 9 arbitrary pulsotypes based on similarity value of 0.85 (Fig. 1). Among them, pulsotypes H and I were found to be the most predominant, which accounted for 27 and 29 isolates, respectively. Sixty-six CRAB isolates that showed pulsotypes A, B, D, E, F, H and I were found to be sequence group 1. The remaining three CRAB isolates that showed pulsotypes C and G were found to be sequence group 4 (Towner et al., 2008). The CRAB isolates belonging to pulsotypes A, D, F, H and I were subjected to perform MLST analysis using the Bartual's scheme and were found to be ST208, ST353, ST208, ST191 and ST191, respectively.

3.3. Clonal relatedness between the CRAB isolates from 2009 to 2010 and 2005

We previously identified 5 CRAB isolates from 2005 in the study hospital (Lee et al., 2007). To determine whether CRAB isolates from 2009 to 2010 were clonally related to those from 2005, pulsotypes, sequence groups and STs were determined in the 5 CRAB isolates from 2005. They were found to be of sequence group 1 and ST2 using the Institute Pasteur's MLST scheme, but were assigned to ST353 using the Bartual's MLST scheme. PFGE analysis showed that the CRAB isolates from 2005 were not clonally related to those from 2009 to 2010 (Fig. 1).

3.4. Genetic basis of XDR in the CRAB isolates

PCR was performed to determine the genetic basis for resistance against carbapenems, extended-spectrum β-lactams and aminoglycosides. All CRAB isolates from 2009 to 2010 carried intrinsic $bla_{\text{OXA-51}}$ and bla_{AmpC} and acquired $bla_{\text{OXA-23}}$. IS*Aba1* was inserted upstream of these genes. The $bla_{\text{PER-1}}$ gene was detected in 9 isolates. The 16S rRNA methylase gene *armA* was identified in 58 out of 69 isolates showing a high-level resistance to aminoglycosides (MICs, $\geqslant 1024$ μg/ml). Four different AME genes, *aacA4* ($n = 66$), *aacC1* ($n = 56$), *aphA6* ($n = 11$) and a*phA1* ($n = 6$), were identified (Table 1). The combination of $bla_{\text{OXA-23}}$, *armA*, *aacA* and *aacC1* was the most prevalent and 41 CRAB isolates co-carrying $bla_{\text{OXA-23}}$, *armA*, *aacA4* and *aacC1* belonged to arbitrary pulsotypes H and I. There was no significant difference in resistance rates, MIC_{50} and MIC_{90} between the different pulsotypes of CRAB isolates. Five CRAB isolates from 2005 showed a XDR phenotype. Of the five isolates from 2005, the $bla_{\text{OXA-23}}$, *aacA4* and *aacC1* were detected in 5, 5 and one isolate, respectively. However, the $bla_{\text{PER-1}}$ and *armA* were not found among the isolates from 2005.

4. Discussion

The present study demonstrated that the prevalence of CRAB in South Korea was due to the clonal spread of CRAB ST191 and that *A. baumannii* ST191, slv (*gpi*) of ST92, was first detected as an epidemic clone in this country. The carriage of $bla_{\text{OXA-51}}$/$bla_{\text{OXA-23}}$, bla_{AmpC}/$bla_{\text{PER-1}}$ and *armA* was largely responsible for resistance