Nosocomial Infection by Sequence Type 357 Multidrug-Resistant Acinetobacter baumannii Isolates in a Neonatal Intensive Care Unit in Daejeon, Korea

Ji Youn Sung, Ph.D., Sun Hoe Koo, M.D., Hye Hyun Cho, M.S., and Kye Chul Kwon, M.D.

Department of Biomedical Laboratory Science, Far East University, Eumseong; Department of Laboratory Medicine, College of Medicine, Chungnam National University, Daejeon; Department of Biomedical Laboratory Science, Jeonju Kijeon College, Jeonju, Korea

Acinetobacter baumannii is an important microorganism responsible for a number of nosocomial outbreaks, in particular, in intensive care units (ICUs). We investigated a nosocomial infection caused by multidrug-resistant (MDR) A. baumannii in a neonatal intensive care unit (NICU) in Korea. A. baumannii isolates were characterized using Etest (AB Biodisk, Sweden), two multiplex PCR assays, and multilocus sequence typing (MLST) scheme. PCR and PCR mapping experiments were performed for detecting and characterizing the determinants of antimicrobial resistance. Eight strains isolated from an NICU belonged to European (EU) clone II and revealed only one sequence type (ST), namely, ST357. All the isolates were susceptible to imipenem but were resistant to amikacin, gentamicin, ceftazidime, cefepime, and ciprofloxacin. To the best of our knowledge, this is the first report of a nosocomial infection in an NICU in Korea caused by ST357 MDR/carbapenem-susceptible A. baumannii strains. This result demonstrates that nosocomial outbreaks of MDR/carbapenem-susceptible strains as well as MDR/carbapenem-resistant isolates may occur in NICUs.

Key Words: Acinetobacter baumannii, Neonatal intensive care unit, Nosocomial infection, Carbapenem
February 2012 (Table 1). *A. baumannii* strains were identified using the Vitek 2 automated instrument ID system (BioMérieux, Marcy l’Etoile, France) and by sequencing the partial rpoB housekeeping gene, as described previously [6].

The minimum inhibitory concentrations (MICs) for *A. baumannii* isolates of amikacin, gentamicin, ceftazidime, cefepime, imipenem, and ciprofloxacin were determined using Etest (AB Biodisk, Solna, Sweden). The results obtained were interpreted as per the criteria approved by the CLSI guidelines [7]. *Escherichia coli* ATCC 25922 was used as a reference strain.

Whole cell (genomic) DNA for PCR templates was obtained from each target strain by using a genomic DNA purification kit (SolGent, Daejeon, Korea), according to the manufacturer’s instructions. Two multiplex PCR assays were used as previously described [8] to identify members of European (EU) clones I and II. The Oxford multilocus sequence typing (MLST) scheme [9], which uses 7 housekeeping genes (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD24*), was used to determine the sequence types (STs). An ST number was assigned by comparing the allele sequences to those in the MLST database (http://pubmlst.org/abaumannii/). Epidemiological typing of isolates was performed by repetitive extragenic palindromic sequence (REP)-PCR [10].

All *A. baumannii* isolates were subjected to PCR and sequencing assays for detecting the determinants of antimicrobial resistance and for identifying the mutations associated with fluoroquinolone resistance. Specific primers and PCR conditions for detection of antimicrobial resistance determinants were as described in a previous study [11]. The amplicons were purified with a PCR purification kit (SolGent) and sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3730XL DNA analyzer (PE Applied Biosystems).

A total of 8 *A. baumannii* isolates were identified on the basis of their rpoB gene analysis. Although all the isolates were susceptible to imipenem, they showed resistance to amikacin, gentamicin, ceftazidime, cefepime, and ciprofloxacin (Table 2). These isolates belonged to EU clone II and carried allele 66 of

| Table 1. Clinical characteristics of 8 patients infected with multidrug-resistant *A. baumannii* strains isolated in a neonatal intensive care unit |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Isolate | Date of admission | Date of isolation | Specimen | GA at birth (weeks) | Impression and diagnosis |
| C10 | 2011/Sep | 2011/Oct | Respiratory tract exudate | 38 | Perinatal distress, birth asphyxia |
| C51 | 2011/Sep | 2011/Nov | Other | 35 | Premature birth, perinatal distress, birth asphyxia |
| C64 | 2011/Nov | 2011/Nov | Respiratory tract exudate | 34 | Premature birth |
| C81 | 2011/Dec | 2011/Dec | Eye discharge | 27 | Premature birth |
| C84 | 2011/Dec | 2012/Jan | Respiratory tract exudate | 25 | Premature birth |
| C85 | 2011/Dec | 2012/Jan | Respiratory tract exudate | 27 | Premature birth, RDS grade III–IV |
| C132 | 2012/Jan | 2012/Jan | Urine | 27 | Premature birth, RDS grade II |
| C147 | 2012/Jan | 2012/Feb | Wound exudate | 27 | Premature birth, RDS grade II |

Abbreviations: GA, gestational age; RDS, respiratory distress syndrome.

| Table 2. Properties of multidrug-resistant *A. baumannii* strains isolated from a neonatal intensive care unit |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Isolate | ST | Minimum inhibitory concentration (μg/mL) | Antimicrobial resistance determinants |
| | | AMK | GEN | CAZ | FEP | IPM | CIP | AMEs & 16S rRNA methylase | gyrA/parC* mutation |
| C10 | 357 | >256 | >1,024 | >256 | 64 | 1.0 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C51 | 357 | >256 | >1,024 | >256 | 64 | 1.5 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C64 | 357 | >256 | >1,024 | >256 | 32 | 1.5 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C81 | 357 | >256 | >1,024 | >256 | 32 | 1.0 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C84 | 357 | >256 | >1,024 | >256 | 64 | 1.0 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C85 | 357 | >256 | >1,024 | >256 | 64 | 1.5 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C132 | 357 | >256 | >1,024 | >256 | 64 | 1.5 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |
| C147 | 357 | >256 | >1,024 | >256 | 128 | 1.5 | >32 | aac(6’)-Ib, aph(3’)-Ia, armA | +/+ |

*Indicates sense mutations at the 83rd residue (serine to leucine) in gyrA and at the 80th residue (serine to leucine or tryptophan) in parC.

Abbreviations: ST, sequence type; AMK, amikacin; GEN, gentamicin; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; CIP, ciprofloxacin; AMEs, aminoglycoside-modifying enzymes.
the intrinsic blaOXA-51-like genes, which agrees with their assignment to EU clone II. They were all identified as ST357 (1-12-3-2-2-145-3) by MLST analysis. To determine the clonality of all 8 A. baumannii isolates, REP-PCR was carried out using genomic DNA. They all displayed the same REP-PCR type (Fig. 1). Our results suggest that ST357 A. baumannii strains isolated during instances of nosocomial infections or colonization in the NICU of the university hospital in Daejeon, Korea, belonged to EU clone II. Nosocomial infections or colonization due to ST357 A. baumannii isolates have not been detected in Korea previously. Thus, to the best of our knowledge, this is the first report of ST357 MDR/carbapenem-susceptible A. baumannii strains causing NICU nosocomial infections in Korea. This result suggests that not only MDR/carbapenem-resistant strains but also MDR/carbapenem-susceptible isolates act as major causes of nosocomial outbreaks, especially in the NICU, in Korea.

To define the genetic basis of MDR A. baumannii isolates by detecting antimicrobial resistance determinants, PCR and PCR mapping experiments were performed. All 8 A. baumannii isolates had the combination of AMEs encoded by aac(6’)-Ib/aph(3’)-la and 16S rRNA methylase encoded by armA. They showed high-level resistance to amikacin (MIC ≥256 mg/L) and gentamicin (MIC ≥1,024 mg/L). Unlike AMEs, which varies in their substrate ranges, 16S rRNA methylases confer high-level resistance to almost all clinically important aminoglycosides [12, 13]. In addition, they all had sense mutations at the 83rd residue (serine to leucine) in gyrA and at the 80th residue (serine to leucine or tryptophan) in parC and had high-level ciprofloxacin resistance (MIC90 ≥32 mg/L). A major mechanism of fluoroquinolone resistance in gram-negative bacteria involves changes in the structure of DNA gyrase and DNA topoisomerase IV [14]. Notably, amino acid substitutions in both the GyrA and ParC polypeptides are consistent with a high-level fluoroquinolone-resistant phenotype [15]. In this study, all 8 isolates harbored sense mutations in both gyrA and parC and showed high-level resistance to ciprofloxacin. In contrast, carbapenem-resistance genes, including metallo-β-lactamase genes and blaOXA-23-like, blaOXA-48-like, and blaOXA-58-like genes, were not found in this study.

Carbapenemens are frequently the drug of choice to treat A. baumannii infections, and carbapenem resistance is, in itself, sufficient to define a highly resistant phenotype. Although nosocomial infections and colonization due to MDR A. baumannii have been reported worldwide, previous studies were focused primarily on carbapenem-resistant strains [12, 16]. Consequently, MDR/carbapenem-susceptible isolates have been rarely recovered worldwide. In our study, ST357 MDR/carbapenem-susceptible strains were isolated from the NICU. Our results emphasize that investigations should be performed not only on carbapenem-resistant but also on carbapenem-susceptible MDR A. baumannii isolates for studying the nosocomial infections and outbreaks caused by this organism.

**Authors’ Disclosures of Potential Conflicts of Interest**

No potential conflicts of interest relevant to this article were reported.

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