International Journal of Antimicrobial Agents 36 (2010) 430–435

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Extensively drug-resistant *Acinetobacter baumannii*: risk factors for acquisition and prevalent OXA-type carbapenemases—a multicentre study

Yoon Soo Park[a,1], Hyukmin Lee[b,1], Kkot Sil Lee[c], Seung Sik Hwang[d], Yong Kyun Cho[a], Hyo Youl Kim[e], Young Uh[f], Bum Sik Chin[g], Sang Hoon Han[g], Seok Hoon Jeong[h], Kyungwon Lee[h], June Myung Kim[g,*]

[a] *Department of Internal Medicine, Gachon University Gil Hospital, 1198 Guwol-dong, Namdong-gu, Incheon 405-706, South Korea*
[b] *Department of Laboratory Medicine, Kwandong University College of Medicine, 697-24 Hwajung-dong, Dukyang-gu, Goyang 412-270, South Korea*
[c] *Department of Internal Medicine, Kwandong University College of Medicine, 697-24 Hwajung-dong, Dukyang-gu, Goyang 412-270, South Korea*
[d] *Department of Social and Preventive Medicine, Inha University School of Medicine, 7-206 Shinheung-dong, Jung-gu, Incheon 400-700, South Korea*
[e] *Division of Infectious Diseases, Wonju Christian Hospital, Yonsei University Wonju College of Medicine, 162 Ilsan-dong, Wonju 220-701, South Korea*
[f] *Department of Laboratory Medicine, Wonju Christian Hospital, Yonsei University Wonju College of Medicine, 162 Ilsan-dong, Wonju 220-701, South Korea*
[g] *Department of Internal Medicine, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-750, South Korea*
[h] *Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-750, South Korea*

ARTICLE INFO

Article history:
Received 23 March 2010
Accepted 30 June 2010

Keywords:
Risk factor
Extensive drug resistance
Acinetobacter baumannii
OXA carbapenemase

ABSTRACT

In this study, we investigated the risk factors for and carbapenem resistance mechanisms of extensively drug-resistant *Acinetobacter baumannii* (XDR-AB). Isolates of XDR-AB were collected from seven tertiary care hospitals in South Korea. A case–control study for risk factor analysis was performed and the presence of the metallo-β-lactamase (MBL) and OXA genes was examined. The control group consisted of adult inpatients receiving care from the same hospital. XDR-AB were isolated from 26 patients who were studied for risk factor analysis. Third-generation cephalosporin use [odds ratio (OR) = 9.6, 95% confidence interval (CI) 1.3–171.3; $P = 0.02$] and Acute Physiology and Chronic Health Evaluation (APACHE) II score (OR = 1.2, 95% CI 1.1–1.5; $P = 0.004$) were identified as risk factors for acquisition of XDR-AB. Pulsed-field gel electrophoresis (PFGE) showed clonal epidemic isolates in hospitals. MBLs were not detected, and all 30 XDR-AB isolates had upregulated OXA-type carbapenemase genes. These results suggest that third-generation cephalosporin use and disease severity are associated with XDR-AB acquisition amongst typical adult inpatients. This study also points to intrahospital spread of XDR-AB. Upregulated OXA-type carbapenemases are prevalent in XDR-AB founded in South Korean hospitals.

1. Introduction

Acinetobacter baumannii has emerged as a significant infectious agent in hospitals worldwide [1]. The organism's ubiquity in nature and its ability to persist for extended periods on surfaces allow for frequent outbreaks and its existence as an endemic pathogen in hospitals [1,2].

Along with its ubiquitous characteristics, another threat associated with *A. baumannii* is its potential to acquire antimicrobial resistance genes rapidly, leading to multidrug resistance [1]. Antimicrobial resistance amongst *A. baumannii* has increased substantially in the past decade [3].

Although carbapenems generally represent the last resort in treating life-threatening infections caused by *A. baumannii*, carbapenem resistance due to OXA-type (class D) carbapenemases is increasing [4–6]. Recently, strains demonstrating resistance to all commercially available antimicrobial agents have also been reported, making treatment of these infections difficult and in some cases impossible [7,8].

Definitions of multidrug-resistant (MDR) *A. baumannii* vary [9] and the term 'extensive drug resistance', designating resistance to all but one or two classes of antimicrobial agents, was recently proposed [10]. Although there have been numerous reports of risk factor analysis for *A. baumannii*, risk factors for the acquisition of extensively drug-resistant *A. baumannii* (XDR-AB) have not been elucidated.

* Corresponding author. Tel.: +82 2 2228 1946; fax: +82 2 393 6884.
E-mail address: jmkim@yuhs.ac (J.M. Kim).
[1] These two authors contributed equally to this work.

0924-8579/$ – see front matter © 2010 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2010.06.049

The aim of this multicentre study was to identify risk factors for the acquisition of XDR-AB in South Korean hospitals. Carbapenem resistance mechanisms of XDR-AB in South Korea were also examined.

2. Materials and methods

2.1. Study site, subjects and design

Seven tertiary care hospitals located in four geographically distinct areas of South Korea (Seoul, Gyeonggi, Gangwon and Busan) participated in the study. Clinical samples received from June to November 2007 by the microbiology laboratories of these hospitals were studied. *Acinetobacter baumannii* isolates showing resistance or intermediate susceptibility to piperacillin, piperacillin/tazobactam (PIP/TAZ), ampicillin/sulbactam (SAM), ceftazidime, cefotaxime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin and/or trimethoprim/sulfamethoxazole (SXT) were considered as XDR-AB and were included for further study.

Risk factors for acquisition of XDR-AB in hospitalised patients were investigated in a case–control study. The base population for the case–control study consisted of all inpatients at the participating centres. Case patients included adult patients (>18 years) who had nosocomial isolation of XDR-AB in clinical cultures. The microbiology laboratory database was electronically searched to identify all XDR-AB-positive clinical cultures of specimens obtained from patients admitted to the hospital during the study period. Patients who had XDR-AB isolates and who recovered within 48 h of admission were excluded from the study. The control group consisted of adult inpatients receiving care from the same hospital from which case patients were receiving care during the study period. Patients in the control group did not have XDR-AB isolated during their hospital stay. For each case patient with XDR-AB who was selected, two control patients were randomly chosen. Patients who were ≤18 years old and admitted for <48 h were excluded from the control group.

Variables analysed as possible risk factors included age, sex, associated diseases or co-morbid conditions, Charlson score [11], length of Intensive Care Unit (ICU) stay, surgery, length of hospital stay before outcome of interest (time at risk; for case patients, from admission to nosocomial isolation of XDR-AB; and for controls, complete length of hospital stay), and severity of illness as calculated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score. Presence of a central venous catheter, arterial catheter, urinary catheter, mechanical ventilation or nasogastric tube was documented as well as all antimicrobial therapy administered before the outcome of interest was ascertained.

2.2. Microbiological studies

2.2.1. Bacterial isolates and susceptibility assays

Only the first isolate per case patient was studied. All isolates were sent to a reference laboratory (Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, South Korea), where the minimum inhibitory concentrations (MIC) of antimicrobial agents (piperacillin, PIP/TAZ, SAM, ceftazidime, cefotaxime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin and SXT) were determined by the agar dilution method [12]. Resistance to colistin and tigecycline was not included in the definition of XDR-AB.

2.2.2. Detection of metallo-β-lactamases (MBLs)

Carbapenemase and MBL production were determined by the modified Hodge test and the double-disk synergy test (DDST), respectively, as previously reported [13,14]. Briefly, a 10 μg imipenem disk (BBL, Cockeysville, MD) and a 10 μg of 0.5 M ethylene diamine tetra-acetic acid (EDTA) disk were placed 10 mm apart from edge to edge. Following overnight incubation, the presence of an enlarged zone of inhibition was interpreted as EDTA–imipenem DDST-positive.

Table 1
Primers used for detection of carbapenemases.

Primer	Sequence (5′ → 3′)	Target
IS*Aba1*F	CAC GAA TGC AGA AGT TG	IS*Aba1*
IS*Aba1*R	CGA CGA ATA CTA TGA CAC	
OXA23-F	GAT CGG ATT GGA GAA CCA GA	$bla_{\text{OXA-23}}$
OXA23-R	ATT TCT GAC CGC ATT TCC AT	
OXA24-F	GGT TAG TTG GCC CCC TTA AA	$bla_{\text{OXA-24}}$
OXA24-R	AGT TGA GCG AAA AGG GGA TT	
OXA51-F	TAA TGC TTT GAT CGG CCT TG	$bla_{\text{OXA-51}}$
OXA51-R	TGG ATT GCA CTT CAT CTT GG	
OXA58-F	AAG TAT TGG GGC TTG TGC TG	$bla_{\text{OXA-58}}$
OXA58-R	CCC CTC TGC GCT CTA CAT AC	

2.2.3. Detection of OXA genes and insertion sequences

Detection of the four groups of OXA carbapenemase genes ($bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-58}}$) was carried out using a multiplex assay (Table 1) [15,16]. Templates for polymerase chain reaction (PCR) amplification in the clinical isolates were made of whole cell lysate by boiling the colonies. Amplification conditions were as follows: initial denaturation at 94 °C for 5 min, then 30 cycles at denaturation at 94 °C for 25 s, annealing at 52 °C for 40 s and extension at 72 °C for 50 s, and finally extension at 72 °C for 6 min. The insertion sequence IS*Aba1* upstream of the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes was sought using combinations of the IS*Aba1*F primer and the OXA23-R/OXA51-R primer. Amplification conditions for the IS*Aba1*F/OXA23-R were initial denaturation at 95 °C for 5 min, then 35 cycles at 95 °C for 45 s, 56 °C for 45 s and 72 °C for 3 min, and finally 72 °C for 5 min. The same conditions were used for the IS*Aba1*F/OXA51-R, except at an annealing temperature of 58 °C.

2.2.4. Pulsed-field gel electrophoresis (PFGE)

For PFGE analysis, *Sma*I-digested genomic DNA of XDR-AB was prepared according to the manufacturer's instructions (Bio-Rad, Hercules, CA). Fragments were separated for 20 h at 6 V/cm at 11 °C using a CHEF-DR II System (Bio-Rad) with initial and final pulse times of 0.5 s and 30 s, respectively [17]. The pattern was analysed visually and with Fingerprinting II software (Bio-Rad).

2.3. Statistical analysis

All statistical analyses were performed using STATA software package v.10.0 (StataCorp, College Station, TX). Unadjusted analyses were performed separately for each of the variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for binomial variables. *P*-values were calculated by Fisher's exact test for categorical variables and by Student's *t*-test or Mann–Whitney *U*-test for continuous variables. Variables for which the *P*-value was <0.05 in unadjusted analysis were included in the multiple logistic regression model adjusting for other covariates. When the estimate of the coefficient was zero or extreme, exact logistic regression was used to obtain a median unbiased estimate. All tests were two-tailed and a *P*-value of <0.05 was considered significant in the multivariate model.