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Major Article

An outbreak of bloodstream infection due to extensively resistant *Acinetobacter baumannii* among neonates

Aysegul Ulu-Kilic MD ^{a,*}, Aycan Gundogdu PhD ^b, Fatma Cevahir ICN ^c, Huseyin Kilic PhD ^b, Tamer Gunes MD ^d, Emine Alp MD, PhD ^a

^a Faculty of Medicine, Department of Infectious Diseases, Erciyes University, Kayseri, Turkey

^b Faculty of Medicine, Department of Clinical Microbiology, Erciyes University, Kayseri, Turkey

^c Faculty of Medicine, Infection Control Committee, Erciyes University, Kayseri, Turkey

^d Faculty of Medicine, Department of Pediatrics, Erciyes University, Kayseri, Turkey

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Background: Extensively resistant *Acinetobacter baumannii* has emerged and spread worldwide as a significant cause of health care-associated infections and outbreaks. It also causes life-threatening infections among neonates, including bacteremia. The aim of this study was to investigate an outbreak of *A baumannii* bacteremia (ABB) among neonates.

Materials and methods: A retrospective, case-control study was conducted from July 2014 to July 2015 in a neonatal intensive care unit (NICU). Risk factors associated with ABB in univariate and multivariate analysis with logistic regression was performed. Molecular typing by pulsed field gel electrophoresis was used to confirm relatedness of bacteremic *A baumannii* strains.

Results: During the 5-year period (2011-2016), 68 patients in our NICU were diagnosed with BSI due to *A baumannii*. The case-control study included 41 case patients within the outbreak caused by a major epidemic clone and 108 control patients. Risk factors (by univariate analysis) associated with ABB were intubation, 14-day mortality, and use of peritoneal dialysis and an umbilical catheter. Multivariate analysis identified 14-day mortality (odds ratio, 5.75; 95% confidence interval, 2.58-12.79) and umbilical catheter use (odds ratio, 2.44; 95% confidence interval, 1.1-5.4) as independent risk factors for ABB.

Conclusions: This outbreak of bacteremia due to resistant *A baumannii* affected 41 infants and was associated with 58% mortality. Control of the outbreak was achieved by implementing long-term sustained infection control measures within the unit.

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Extensively resistant *Acinetobacter baumannii* (XDR-AB) has emerged and spread worldwide as a significant cause of health care-associated infections and outbreaks, particularly in intensive care units (ICUs). It also causes life-threatening infections among neonates, including pneumoniae, meningitis, and bacteremia. The morbidity and mortality rates are high due to the limited therapeutic alternatives because the isolates are resistant to carbapenems.¹

Several outbreaks of *A baumannii* infections in neonatal intensive care units (NICUs) have been reported to date.²⁻⁸ Some sources of the related outbreaks have been traced to the environment (eg, mattresses, medical equipments, tap water, and floor) because *A baumannii* is able to persist on such surfaces for long periods of time. It has been observed that the presence of multidrug resistance

contributes to the survival of bacteria on inanimate surfaces.^{1,9,10} Moreover, it has also been shown that NICU outbreaks have been linked to cross-transmission among patients due to lapses in infection control measures.²

In this study, we investigated an outbreak of bloodstream infections (BSIs) due to XDR-AB in a NICU. A case-control study was performed to determine the risk factors associated with the outbreak. The molecular study on outbreak strains was conducted using pulsed field gel electrophoresis (PFGE). The clonal relationship of these strains with the major epidemic XDR-AB clones of our adult hospital¹¹ was also investigated.

MATERIAL AND METHODS

Patients and settings

This study was conducted in Erciyes University Hospital, a tertiary care center with a 1,300-bed capacity in central Turkey.

* Address correspondence to Aysegul Ulu-Kilic, MD, Department of Infectious Diseases, Faculty of Medicine, Erciyes University, Talas Blvd, Kayseri, Turkey.

E-mail address: draysegululu@yahoo.co.uk (A. Ulu-Kilic).

Conflicts of interest: None to report

The pediatric hospital is located in a separate building (205-bed capacity) with 154,680 pediatric admissions and 1,653 ICU admissions annually.

Our NICU has 18 beds with an admission of approximately 250 newborn infants annually. The unit supports a level-3 care for critically ill patients, has 1 isolation room for every 9 beds, and 1:5 nurse-to-patient ratio. The distances between the beds are >1.5 m in 1 unit. There are 8 nonhand-operated sinks. Hand hygiene is performed using a 70% alcohol-based disinfecting solution provided next to each bed and chlorhexidine-containing soap is provided for each sink. A total of 35 health care workers (HCWs) work in 3 shifts, including 3 neonatologists, 2 pediatricians, 26 nurses, and 4 cleaners.

Milk formulas and total parenteral nutrition are prepared in a specifically dedicated kitchen outside the NICU. The solutions are prepared by 2 kitchen employees. Umbilical catheter insertion is performed in the NICU, whereas peritoneal dialysis catheterization is performed in an operating room. Central venous catheter insertion is performed in an interventional radiology unit located in the adult hospital. The interventional radiology room serves both adult and pediatric patients.

First-line empiric treatment for neonates with suspected sepsis consists of meropenem plus vancomycin.

Identification and susceptibility testing

A baumannii strains isolated from the blood samples of neonates ($n = 41$) and adults in our ICUs ($n = 22$) between 2014 and 2015 were included in this study. Identification and antimicrobial susceptibility testing of each isolate were conducted by following conventional methods and by running the Vitek-2 automatized system (bioMérieux, Marcy-l'Étoile, France). The blood samples were incubated employing the BACTEC 9249 (Becton Dickinson) blood culture system up to 5 days. After the initial incubating period, cultured blood samples were inoculated in 5% sheep blood agar and in eosin methylene blue agar, and incubated for 24–48 hours for the purification of possible *A baumannii* strains. The Vitek-2 automatized system was used in the identification of lactose-negative colonies reproducing in hektoen enteric agar. The data interpretation was done in accordance with the Clinical and Laboratory Standards Institute breakpoints.¹² A colistin minimum inhibitory concentration was determined using Vitek-2 and confirmed by E-test. XDR characteristics were defined as the isolate nonsusceptible to at least 1 agent in all but 2 or fewer in ≥ 6 antimicrobial categories.¹³ One isolate per patient (per episode) was included in the study.

In addition to the clinical isolates, environmental sampling for different equipment and surfaces of the NICU, the interventional radiology room, and the NICU kitchen were applied. For this purpose, 30 samples were taken with sterile premoistened FLOQSwabs (Copan Diagnostics, Murrieta, CA). The sampled swabs were incubated in tripticase soy broth for 24 hours first and then cultured in eosin methylene blue agar. The above procedures were followed for the lactose-negative colonies reproducing in hektoen enteric agar.

Molecular assays for the collected strains

Genetic relatedness of the 66 *A baumannii* strains belonging to the neonates ($n = 37$), adults ($n = 22$), environmental sources ($n = 3$), and endemic clones of the hospital ($n = 4$) circulating in our adult hospitals was determined by PFGE using a CHEF DR III system (Bio-Rad, Hercules, CA). *Apal* restriction enzyme was used for the pulsotyping of *A baumannii* strains. The data were interpreted according to the criteria proposed by Tenover et al.¹⁴ The genetic relatedness among the isolates was assessed by the unweighted-pair group method using Bionumerics version 6.01 (AppliedMaths,

Sint-Martens-Latem, Belgium). Hierarchical clustering cutoff was set to 80% similarity to identify the pulsotypes. A pulsotype was defined as a unique electrophoretic banding pattern.

Case-control study

A retrospective case-control study was conducted for the neonates hospitalized between July 2014 and July 2015 in our NICU. Cases were defined as the neonates with BSI caused by *A baumannii*. Controls were defined as the neonates hospitalized in NICU during the same time period from whom *A baumannii* was not isolated from any clinical specimens. The risk factors associated with BSI due to *A baumannii* were detected using univariate statistical analysis and multivariate logistic regression.

Patients were assessed by reviewing the patient files, the hospital electronic records, and the Infection Control Committee reports. The obtained data included age, sex, birth weight, prematurity, invasive procedures, and antibiotic use.

BSIs and catheter-related BSIs were defined according to Centers for Disease Control and Prevention National Healthcare Safety Network surveillance definitions.¹⁵ Rates of nosocomial infections and BSIs are given as total number of episodes per 1,000 ICU patient-days. Catheter-related BSI rates were calculated by dividing the number of catheter-related BSIs by the total number of catheter-days and multiplying the result by 1,000. Infants born before 37 weeks' gestation were considered premature and birth weight <2,500 g was considered low birth weight.

Infection prevention and control interventions

Once the outbreak was identified, patients with *A baumannii* infection were cohorted in isolation rooms or separate areas within the NICU. Contact isolation was performed for all case patients. Due to limited number of HCWs in this unit, cohorting personnel was unsatisfactory. The NICU was not completely closed for admissions, but transfers were limited. Multiple environmental samples were collected immediately, including ventilator circuits, monitors, hand disinfectants, and surfaces in the NICU; kitchen equipment such as formula bottles, trays, sink tabs, sink drains, and formula preparation dishes; and the interventional radiology room surfaces. After sampling, environmental decontamination was performed for all areas of patient care and the kitchen.

Low compliance of clinicians, particularly to maximum sterile barrier precautions during catheter insertion, was observed. Thus, education for catheter insertion and standards of catheter care was provided to them. To improve hand hygiene compliance, infection control nurses reviewed the availability of hand disinfectants and antiseptics. All levels of staff (ie, clinicians, nurses, and cleaners) were educated to revise for cleaning and disinfection procedures. The Infection Control Committee also reviewed the procedures for the preparation, handling, and storage of milk formulas and total parenteral nutrition solutions.

The empirical antibiotic therapy for neonates with suspected sepsis was revised by including colistin administration for resistant *A baumannii* strains with meropenem and vancomycin.

Statistical analysis

The statistical analysis was performed using SPSS software version 15 (IBM-SPSS Inc, Armonk, NY). The χ^2 test was used for the categorical variables. Mann-Whitney *U* test was used to determine the differences between the 2 groups. Univariate and multiple binary logistic regression analyses (backward Wald) were performed to analyze the effects of variables. Gestational age, peritoneal dialysis, mechanical ventilation, umbilical catheter use, and mortality were

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