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Brief communication (Original)

Clinical features, risk factors, and outcome of carbapenem-resistant *Acinetobacter baumannii* bacteremia in a Thai neonatal intensive care unit

Narongsak Nakwan[a,b], Jeerawan Wannaro[b], Narongwit Nakwan[c], Wichian Patungkalo[d], Kulkanya Chokephaibulkit[e]

[a]Neonatal Intensive Care Unit, [b]Department of Pediatrics, [c]Department of Medicine, [d]Division of Microbiology, Hat Yai Medical Education Center, Hat Yai Hospital, Songkhla 90110, [e]Division of Infectious Diseases, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) infection have been increasingly observed and are associated with mortality in neonatal population.
Objective: We determined risk factors for and outcome of bacteremia caused by CRAB in neonates.
Methods: The clinical data of neonates who developed *A. baumannii* bacteremia from January 2005 to December 2010 were retrospectively reviewed.
Results: During the study period, 22 neonates developed *A. baumannii* bacteremia, 13 were CRAB at an incidence of 0.5 case/1000 patients-day. Compared with carbapenem-sensitive *A. baumannii* (CSAB), patients with CRAB bacteremia had lower birth weight, lower gestational age and were more often receiving mechanical ventilation at the onset of bacteremia. Most of the CSAB isolates were susceptible to cefoperazone/sulbactam (89%) and aminoglycosides (50-75%). In contrast, CRAB strains were all resistant to cephalosporins, carbapenems, quinolones, with 39% susceptible to cefoperazone/sulbactam and 8% susceptible to amikacin. Most cases of CRAB bacteremia were treated with cefoperazone/sulbactam or meropenem and in some cases, with the addition of colistin. The all cause morality rates were 54% in CRAB and 11% in CSAB bacteremia, respectively ($p = 0.07$).
Conclusions: Neonatal bacteremia caused by *A. baumannii* was not common but caused high mortality, particularly from CRAB. Lack of effective antibiotics was the major challenge in treating these patients.

Keywords: *Acinetobacter baumannii*, bacteremia, carbapenem-resistant *Acinetobacter baumannii*, colistin, neonates

Nosocomial infections with extensive drug resistant (XDR) gram-negative organisms, particularly strains of *Acinetobacter baumannii*, have become a significant problem in the neonatal intensive care units (NICU) [1-5]. Infection from this bacterium can cause sepsis, pneumonia, wound infection, urinary tract infection, peritonitis, and death [6]. In critical care setting, carbapenems has been one of the most effective antibiotics for treatment of these severe hospital acquired infections. Most carbapemen-resistant gram negative bacilli were not susceptible to other antibiotics and became a challenge. Carbapenem-resistant *A. baumannii* (CRAB) have been now also been increasingly reported as the cause of outbreaks of nosocomial infections in critical care settings [7-10].

There have been very limited reports of clinical features and outcome of CRAB bacteremia in the NICU setting [1, 2]. In the present study, we report the clinical, bacteriological features, and outcomes of *A. baumannii* bacteremia in neonates, as well as the factors associated with acquiring CRAB bacteremia compared to carbapenem-sensitive *A. baumannii* (CSAB).

Correspondence to: Narongsak Nakwan, MD, Neonatal Intensive Care Unit, Department of Pediatrics, Hat Yai Medical Education Center, Hat Yai Hospital, Songkhla 90110, Thailand. E-mail: nnakwan@hotmail.com

Material and methods

Study design and population

We retrospectively reviewed the medical

records of all neonates diagnosed with *A. baumannii* bacteremia in the NICU at Hat Yai Hospital which is a level III 12-bed unit in southern Thailand, from January 2005 to December 2010. We collected clinical and laboratory characteristics, the sensitivities profile of the isolates and outcomes. The study was approved by the Hat Yai Hospital Ethics Committee.

Blood cultures were obtained routinely from all neonates suspected of sepsis. Blood samples were collected in BacT/Alert FA bottles (bioM□rieux, Durham, USA) and specimens were incubated using the automated BacT/Alert system (bioM□rieux, Marcy l'Etoile, France). Bacterial isolates were then plated onto blood agar plates, chocolate agar plates, and MacConkey agar plates. They were incubated at 35°C and examined for growth at 24 hours. *Acinetobacter baumannii* organisms were identified by conventional biochemical analysis. The genus and species were identified by Gram staining, cell and colony morphology, negative oxidase, positive lactose and glucose fermentation, absence of motility, negative catalase reaction, and non-hemolysis of sheep blood. Routine laboratory susceptibility testing procedures of *A. baumannii* isolates for commonly used antibiotics were performed by Kirby-Bauer disk-diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines in 2010 [11]. The drugs tested were penicillins (piperacillin/tazobactam, amoxicillin/clavulanate, and cefoperazone/sulbactam), cephalosporins (ceftriaxone, ceftazidime, and cefepime), carbapenems (imipenem, and meropenem), quinolones (ciprofloxacin, and levofloxacin), and aminoglycosides (amikacin, gentamicin, and netilmicin). Colistin disks were not available to test except in the most recent case.

Acinetobacter baumannii bacteremia was defined as positive culture from one or more blood specimens. CRAB was defined in vitro resistance to imipenem and meropenem. The outcomes of the patient were defined as survived, died related, or died unrelated to bacteremia. XDR-AB was defined as an *A. baumannii* isolate that was resistant to all cephalosporins, carbapenems, aminoglycosides, quinolones, and ceperazone/sulbactam.

Statistical Analysis

Data were evaluated using descriptive statistics. The univariate analysis for factors associated CRAB bacteremia compared to CSAB bacteremia were performed using Mann-Whitney U test for continuous variables, and Chi-square or Fisher exact test for comparisons of categorical data. A *p*-value of <0.05 was considered statistically significant. Data analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, IL).

Results

During the six-year period, there were 41,452 live-birth infant deliveries at this institute with an average annual 6,908 live births, and 4,087 infants admitted to NICU with an average annual admitted number of 681 infants. Twenty-two patients with *A. baumannii* bacteremia were identified, accounting for a rate of 0.5 episode per 1000 patients-day. Of these, nine (41%) were caused by CSAB and 13 (59%) were CRAB. Characteristics and outcomes of the 22 patients are in **Table 1**. The median gestational age and median birth weights were 32 days (range: 26-40), and 1,375g (range: 760-4,060), respectively. The median age of onset of *A. baumannii* bacteremia was nine days (range: 2-37). The median length of NICU stay was 35 days (range: 2-105).

The clinical presentations of *A. baumannii* bacteremia were alteration of body temperature (59% fever, 27% hypothermia), irritability and lethargy (68%), respiratory symptoms (11%), feeding intolerance (32%), and cardiovascular dysfunction (5% bradycardia and tachycardia, 18% hypotension). Compared to those with CSAB, patients with CRAB bacteremia had lower gestational age (31 vs. 36 weeks, $p = 0.01$), lower birth weight (1,285 vs. 2,090 g, $p = 0.01$) and were likely to have received assist ventilation at bacteremia onset (85% vs. 22%, $p < 0.01$). There were no significant differences between the patients with CSAB and those with CRAB bacteremia with regards to demographic characteristics, length of NICU stay, clinical presentation, and laboratory results (**Table 2**).

Antimicrobial susceptibilities of CSAB and CRAB isolates are in **Table 3**. Most of CSAB isolates were susceptible to cefoperazone/sulbactam and aminoglycosides, with half of susceptible to quinolones. In contrast, all of the CRAB strains were resistant to aminoglycosides, cephalosporins, carbepemens, and quinolones, but 39% were still susceptible to cefoperazone/sulbactam and 8% were susceptible to amikacin. There was only one isolate (case 22) that was tested for colistin susceptibility and found to be sensitive at minimal inhibitory concentration (MIC) of 0.38 μg/mL.

Table 1. Demographic and clinical characteristics of 22 infants of *Acinetobacter baumannii* bacteremia.

Cases	Sex	GA (weeks)	BW (gram)	Apgar at 1 &5 minute	Age of infection onset (day)	Underlying disease	Bacteria (selective susceptibility)	Other diagnosis	Prior antibiotic	Treatment and duration of antibiotics therapy	Infection Outcome
1	M	33	1,550	7, 9	10	RDS	CSAB	None	Ceto+amik	Mero x 21 days	Survived
2	M	35	2,115	8, 9	5	None	CSAB	None	None	Cefo+amik x 10 days	Survived
3	M	31	1.100	6, 7	4	RDS	CSAB	None	Ampi+gent	Cep/sul+amik x 14 days	Survived
4	F	40	4,060	4, 9	3	MAS	CSAB	None	None	Cefta x 14 days	Survived
5	F	31	1,330	2, 5	19	RDS	CSAB	VAP	None	Cep/sul+metro x 2 days	Died
6	F	36	2,065	9, 10	28	SGA	CSAB	NEC	Cefo+metro	Cep/sul x 14 days	Survived
7	M	30	1,370	8, 9	6	RDS	CSAB	None	Ampi+gent	Imip x 14 days	Survived
8	M	39	3,490	9,9	27	None	CSAB	None	None	Imip X 10 days	Survived
9	M	38	2,770	8, 8	13	TTNB, PPHN	CSAB	None	None	Vanco+Mer x 14 day	Survived
10	F	33	1,640	8, 9	3	RDS	CRAB	None	Ceto+amik	Cep/sul x 14 days	Survived
11	M	33	1,380	NA	37	RDS	CRAB	None	Imip	Imip x 7 days	Died
12	F	30	1,300	3, 5	2	RDS	CRAB	None	Ampi+gent	Sul x 2 days	Died
13	F	32	1,470	9, 9	12	RDS	CRAB	None	Ampi+gent	Sul x 14 days	Survived
14	M	35	1,690	1, 2	8	RDS	CRAB	VAP	Ampi+gent	Sul x 14 days	Survived*
15	M	28	970	2, 8	5	RDS	CRAB (XDR)	VAP	Ampi+gent	Cep/sul x 1 day	Died
16	M	26	950	6, 8	7	RDS	CRAB (XDR)	None	Cefo+vanco	Mero+colis x 14 days	Survived
17	F	29	760	7, 8	3	Pneu	CRAB (XDR)	VAP	Ampi+gent	Cep/sul+amik x 3 days	Died
18	M	31	1,545	9, 9	11	RDS	CRAB (XDR)	None	Cefo+amik	Mero+vanco x 14 days	Survived
19	F	28	760	4, 5	4	RDS	CRAB (XDR)	Skin abscess	Ampi+gent	Mero+vanco+colis x 7 days	Died
20	M	28	945	7, 8	25	RDS	CRAB (XDR)	None	None	Mero+vanco+colis x 14 days	Survived
21	M	33	1,270	4, 5	15	RDS, Pneu	CRAB (XDR)	VAP	Vanco+Mer	Cep/sul x 7 days	Died
22	M	29	1,010	8, 8	20	RDS	CRAB (XDR)	None	Cefta+amik	Mero+colis X 14 days	Survived

GA=Gestation age, BW=birth weight, RDS=respiratory distress syndrome, MAS=meconium aspiration syndrome, Pneu=pneumonia; SGA, small for gestational age, TTNB=transient tachypnea of the newborn, PPHN=persistent pulmonary hypertension of the newborn, CSAB=carbapenem-sentitive *Acinetobacter baumannii*, CRAB=carbapenem-resistant *Acinetobacter baumannii*, XDR=extensive drug-resistant, VAP=ventilator-associated pneumonia; NEC, necrotizing enterocolitis, Ampi=ampicillin, Amik=amikacin, Gent=gentamicin, Cefo=cefotaxime, Cefta=ceftazidime, Vanco=vancomicin, Mero=meropenem, Imip=imipenem, Cep/sul=ceperazone/sulbactam, Metro=metronidazole, Colis=colistin

*Case 14 died with unrelated with CRAB bacteremia, due to pneumothorax.

Table 2. Comparison of demographic and clinical characteristics of the infants with *A. baumannii* bacteremia with or without carbapenem resistance.

Variables	CSAB (n=9)	CRAB (n=13)	p-value
Gestation age (weeks), median (range)	36 (30-40)	31 (26-35)	*0.01*
≤27 weeks	0	1 (7%)	1.00
28-34 weeks	4 (44%)	11 (86%)	0.07
≥35 week	5 (56%)	1 (7%)	*0.02*
Birth weight (g), median (range)	2,090 (1,100-4,060)	1,285 (760-1,690)	*0.01*
≤999 gm	0	5 (38%)	0.05
1,000-1,499 gm	3 (33%)	5 (38%)	1.00
≥1,500 gm	6 (67%)	3 (24%)	0.08
Male, n (%)	6 (67%)	8 (62%)	1.00
Apgar Score at 1 min, median (range)	8 (4-9)	6 (1-9)	0.35
Apgar Score at 5 min, median (range)	9 (4-10)	8 (2-9)	0.08
Outborn, n (%)	0	3 (23.1%)	0.24
Age of infection onset (days), median (range)	10 (3-28)	8 (2-37)	0.66
≤7 days of age	4 (44%)	6 (46%)	1.00
Length of hospitalization (days), median (range)	36 (13-57)	34 (2-105)	0.97
Clinical presentations			
Fever	5 (56%)	8 (62%)	1.00
Hypothermia	2 (22%)	4 (31%)	1.00
Irritability/lethargy	4 (44%)	11 (85%)	0.07
Apnea, tachypnea, dyspnea	3 (33%)	8 (62%)	0.39
Feeding difficulty	4 (44%)	3 (23%)	0.38
Bradycardia, tachycardia	0	1 (8%)	1.00
Hypotension	2 (22%)	2 (15%)	1.00
Investigations at bacteremia onset			
White blood cells count (cells/cu.mm)	11,200 (3,800-32,000)	14,400 (3,400-316,000)	0.83
Hemoglobin (gm/dL)	15.6 (7.6-19)	11.0 (9.2-13.8)	0.32
Hematocrit (%)	46.9 (21-58)	32.0 (26.7-38.9)	0.26
Platelets count (cells/cu.mm)	158,000 (50,000-602,000)	90,500 (29,000-183,000)	0.11
Risk factors			
Central catheter devices	3 (33%)	10 (77%)	0.08
Total parenteral nutrition	6 (67%)	9 (69%)	1.00
H_2-blocker	2 (22%)	4 (31%)	1.00
Previous blood stream infection	0	2 (15%)	0.49
Receiving of mechanical ventilation	2 (22%)	11 (85%)	*< 0.01*
Recent ventilator-associated pneumonia	2 (22%)	5 (38%)	0.65
Outcomes			
Bacteremia mortality	1 (11%)	6 (46%)	0.17
All cause mortality	1 (11%)	7 (54%)	0.07

CSAB=carbapenem-sensitive *Acinetobacter baumannii*, CRAB=carbapenem-resistant *Acinetobacter baumannii*

Table 3. Antibiotic susceptibility profiles by disk diffusion test of CRAB and CSAB

Antibiotics	No. (%) of isolates susceptible	
	CSAB (%)	CRAB (%)
Gentamicin	6/8 (61)	0/13 (0)
Amikacin	4/8 (50)	1/13 (8)
Netilmicin	6/8 (75)	0/13 (0)
Ceftriaxone	2/6 (33)	0/13 (0)
Cetazidime	5/9 (56)	0/13 (0)
Cefepime	3/5 (60)	0/13 (0)
Ceperazone/sulbactam	8/9 (89)	5/13 (39)
Imipenem	7/8 (88)	0/13 (0)
Meropenem	5/6 (83)	0/9 (0)
Ciprofloxacin	5/9 (56)	0/13 (0)
Levofloxacin	1/2 (50)	0/8 (0)
Piperacillin/tazobactam	0/1 (0)	0/4 (0)
Colistin*	NA	1/1 (100)

CSAB=carbapenem-sensitive *Acinetobacter baumannii*, CRAB=carbapenem-resistant *Acinetobacter baumannii*
*Minimal inhibitory concentration by E-test: 0.38 μg/mL

The most commonly used antimicrobial agents before the onset of *A. baumannii* bacteremia were ampicillin (8), gentamicin (8), cefotaxime (5), and amikacin (4) as can be seen in **Table 1**. The antimicrobial agents used in the patients with CSAB were all confirmed susceptible in vitro, including carbapenems (4), cefoperazone/sulbactam (3), third generation cephalosporins (2), and amikacin (2). Of the 13 patients with CRAB, nine cases (69%) received the antibiotic that was not effective in vitro, including meropenem in five cases (56%), cefoperazone/sulbactam in three cases (33%), and imipenem in one case (11%). Three of them (case 16, 20, and 21) also received colistin without susceptibility testing. Of the 13 infants who had central venous catheters at the onset (3 CSAB and 10 CRAB), only two patients had the catheter removed.

The all cause mortality rate for *A. baumannii* bacteremia was 36% (8/22). Of the 13 patients with CRAB bacteremia, six died related to the infection, and one died unrelated from severe pneumothorax (case 14). Of the seven infants who received non-susceptible antibiotics without colistin treatment, only one (case 18) survived. This infant did not have a central venous catheter. In CSAB group, only one patient died from the bactermic episode despite effective antibiotic therapy. There was no statistically significant difference in all cause mortality between patients those of CRAB and CSAB bacteremia (54% vs. 11%, $p = 0.07$, respectively).

In univariate analysis, comparing with patients who survive the bacteremic episode, the patients who died had less gestational age (30 vs. 33 weeks, $p = 0.03$), lower birth weight (1,120 vs. 1,595 g, $p < 0.01$), lower Apgar scores at one and five minutes (at one minute: 4 vs. 8, $p = 0.01$, at five minute: 5 vs. 9, $p = 0.07$), and lower platelets counts (69,500 vs. 158,000 cells/cu.mm, $p = 0.01$), respectively. In addition, patients who died were more likely to use histamine-2 receptor blockers (71% vs. 7%, $p = 0.04$). In multivariate analysis, there was no detectable factor found associated with acquiring CRAB infections or mortality.

Discussion

Our data showed that the background rate of *A. baumannii* bacteremia among newborn infants was very low (0.5 episode per 1000 patients-day) and similar to an adult study of Le Hello et al with a rate of 0.48 episode per 1000 patients-day [10]. Approximately 60% of *A. baumannii* bacteremia in our report were CRAB. A higher mortality in patients with CRAB bacteremia was found, but was not significantly different from CSAB bacteremia, probably due to small sample size.

Although invasive infections from XDR-AB have been reported increasingly in recent years, there have been limited reports in neonates. One study from Taiwan, reported a seven-day old female neonate who developed pandrug resistant *A. baumannii* (PDR-

AB) bacteremia in the NICU [1]. The infection was successfully treated with ampicillin/sulbactam and the outbreak of PDR-AB was controlled by vigilance, prompt intervention and strict adherence to hand hygiene protocol.

We found that lower gestational and birth weight were factors associated with infections caused by carbapenem resistant strains. This can be explained by the fact that our preterm infants were more likely to have weaker immune systems, leading to more susceptibility to systemic infectious. This finding is similar to that reported in a previous study which found that patients who had lower birth weight were more likely to get bacteremia [12]. Mechanical ventilation has been described as a risk factor for highly resistant *A. baumannii* in adult patients [13]. In addition, mechanical ventilation also significantly increases the rate of acquired pneumonia and late-onset septicemia in very low birth weight infants [14]. These findings concurred with our results that found mechanical ventilation a risk factor of CRAB bacteremia. We also found that central venous catheterization tended to be associated with acquisition of carbapenem resistant strains but this was not statistically significant. In addition, our study found that gestational age, birth weight, Apgar scores at one and five minutes, platelets counts, and receipt of histamine two receptor blocker were factors associated with mortality, similar to previous studies result [15]. The multivariable analysis, however, was inconclusive, probably due to the small sample size.

Effective antimicrobial therapy is crucial for the survival of critically ill neonates with CRAB. In this study, we found that ceperazone/sulbactam and carbapenems were the most effective antibiotics against CSAB organisms. While ceperazone/sulbactam was the only agent tested found to be active against some CRAB strain (39%). Colistin has been a promising therapeutic option in the treatment of infections caused by XDR-AB. Hello et al reported 50 CRAB isolates from 50 adult patients and all were susceptible to colistin [10]. There has been limited clinical evidence in the neonatal population. Colistin has good in vitro efficacy against XDR-AB, and has been used successfully by intravenous or aerosol route for treatment of XDR-AB infection in critically ill neonates and children [16-17]. Unfortunately, most of the isolates from our patients did not receive colistin susceptibility test because it was not available until recently. From July 2010 to September 2010, however, our hospital performed colistin susceptibility tests by E-test in 83 XDR-AB isolates and found that 96% were susceptible (unpublished data). It could be assume that CRAB cases from our study probably were susceptible to colistin. Three (case 16, 20, and 21) of eight surviving patients with CRAB bacteremia were successfully treated with intravenous colistin. However, one preterm patient (case 18) with CRAB bacteremia was successfully treated with meropenem and vancomycin without colistin. This suggested other factors influenced outcome, not only in vitro drug susceptibility data. Using tigecycline as antimicrobial agent for XDR-AB infection treatment has been reported in adult patients [18-19], but not in the pediatric population. This was due to the potential side effects especially on bone growth.

This study has several limitations. It is a small sample size and retrospective study and may contain incomplete information. There was no in vitro susceptibility data for colistin. The mortality found in this report was higher than in other previous study [2]. This could be the affect of low rate of central venous catheter removal after bacteremic onset. Moreover, we did not perform molecular studies to assess the source of infection. In response to the emergence of CRAB, we investigated the environmental source and the mode of transmission during time of outbreak but was unable to find an answer.

In conclusion, this study suggested that *A. baumannii* bacteremia in neonates was not common but had a high mortality and challenged treatment especially for CRAB. Ceperazone/sulbactam and colistin may be the most useful agents active against CRAB infection in our setting. Effective infection control measures were needed to prevent spreading of the outbreak. However, larger prospective study is warranted to provide risk factors of CRAB bacteremia development in the NICU setting.

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