

Risk factors for carbapenem-resistant *Acinetobacter baumannii* blood stream infections in a neonatal intensive care unit, Delhi, India

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Abstract

Introduction: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) infection is being increasingly observed and is associated with significant morbidity and mortality in newborns.

In this study, we determined the epidemiology, risk factors, and outcomes of blood stream infection (BSI) caused by CRAB in neonates.

Methodology: The clinical charts of neonates who developed *Acinetobacter baumannii* BSI in the period between 1 January 2010 and 31 December 2012 were reviewed.

Results: During the study period, 65 neonates developed *Acinetobacter baumannii* BSI; 33 were CRAB at an incidence of 0.50 case per 1,000 patient-days. Compared with carbapenem-sensitive *Acinetobacter baumannii* (CSAB), patients with CRAB BSI had significantly higher prior antimicrobial use, longer duration of ventilation, and late isolation of organisms. Feeding with expressed breast milk was protective. All isolates of *Acinetobacter baumannii* were sensitive to colistin and tigecycline. The all-cause mortality rates were 27.3% in CRAB and 9.4% in CSAB BSI, respectively ($p = 0.074$).

Conclusions: Neonatal BSI caused by CRAB was not common but caused high mortality. Feeding with breast milk was protective. Lack of effective antibiotics was the major challenge in treating these patients.

Key words: sepsis; colistin; neonates; multiresistant organism; health care associated infections; nosocomial sepsis

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Introduction

Acinetobacter species are glucose non-fermentative Gram-negative coccobacilli that have in recent years emerged as an important cause of healthcare-associated infections (HAI) [1]. The broad-spectrum β -lactam antibiotics – carbapenems – were introduced by 1985 and since then have been the most important agents for the treatment of infections caused by multidrug-resistant (MDR) *A. baumannii*. Surveillance studies indicate that the percentage of carbapenem-resistant isolates has gradually increased over the last ten years in Europe, North America, and Latin America [1]. Numerous outbreaks of carbapenem-resistant *Acinetobacter baumannii* (CRAB) have been reported from hospitals in both developed and developing countries, including India [1].

The prevalence of *A. baumannii* bacteremia in neonates has been reported to be 0.2%–6.9% [2-5]. Fourteen percent of early-onset sepsis (EOS) [6] and 9% of late-onset sepsis (LOS) in newborns has been

reported to be due to *A. baumannii* species [7]. The prevalence of CRAB bacteremia has varied among the neonatal intensive care unit (NICU) populations across each geographic region and has been reported to be as high as 62% (5/8) in Southeast Asia [8] and up to 33.3% (4/12) in South Asia [9].

There have been very limited reports of risk factors and outcomes of CRAB BSI in the NICU in non-outbreak settings. In the present study, we report the epidemiology, risk factors, and outcomes of CRAB BSI in neonates.

Methodology

This study was a retrospective chart review.

Setting and study population

Kalawati Saran Children Hospital is a 450-bed academic tertiary care center attached to Lady Hardinge Medical College in New Delhi. The neonatal ward is a level III 35-bed NICU and level II 25-bed nursery with 900 to 1,000 admissions per year. The

unit is staffed by a faculty of five neonatologists and twenty-two residents. Only intramural babies are admitted. All newborns admitted to the NICU between 1 January 2010 and 31 December 2012 (36 months) whose blood cultures grew *A. baumannii* were included.

Blood culture

Blood samples were collected in BacT/Alert FA bottles (bioMerieux, Marcy l'Etoile, France) and specimens were incubated using the automated BacT/Alert system (bioMerieux, Marcy l'Etoile, France). Bacterial isolates were then plated onto blood agar, chocolate agar, and MacConkey agar plates. *A. baumannii* organisms were identified by conventional biochemical analysis. The genus and species were presumptively identified by a conventional manual technique and the Vitek card system (bioMerieux, Marcy l'Etoile, France). Susceptibility testing procedures of *A. baumannii* isolates were performed using the Clinical and Laboratory Standards Institute 2009 guidelines [10]. Resistance to imipenem and meropenem was defined at a minimal inhibitory concentration of ≥ 16 mcg/mL [10].

Definitions

A. baumannii BSI was defined as positive culture from one or more blood specimens. CRAB was defined as an *A. baumannii* isolate having *in vitro* resistance to imipenem and/or meropenem, whereas CSAB was defined as an *A. baumannii* isolate that was susceptible to both imipenem and meropenem. Pandrug resistance was defined as an *A. baumannii* isolate that was resistant to all cephalosporins, carbapenems, aminoglycosides, quinolones, and colistin. The outcomes of the patient were defined as survived or died either related or unrelated to bacteremia.

Data collection

Medical records, including charts, daily flow sheets, and laboratory and radiographic reports, were reviewed by one of the investigators. Data on patient demographics, underlying diseases, medications, central venous and arterial catheters, invasive procedures, ventilator use, and EOS or LOS in the NICU were recorded. Medications recorded included surfactant, total parenteral nutrition (TPN), and prior and current antibiotics.

Data analysis

Data analysis was performed using SPSS Version 20.0 (SPSS, Chicago, USA). Univariate logistic regression analysis was performed. Variables that were significant in univariate analysis or that had *a priori* clinical significance were entered into a backward step wise regression model in multivariate analysis. All p values were two tailed and values $< .05$ were taken as significant. Adjusted odds ratios (aOR) and 95% confidence intervals (CIs) were calculated for significant variables.

Ethical clearance

Ethical clearance was obtained from the Institutional Ethics Committee for Human Research, Lady Hardinge Medical College and Associated Hospitals.

Results

During the three-year period, there were 31,638 deliveries and 2,969 admissions to the NICU with an annual average of 10,546 live births and 956 admissions. There were 474 newborns who had organisms isolated from blood during the three-year period. Sixty-five neonates (13.7%) developed *A. baumannii* BSI; 33 (7%) of these were CRAB at an incidence of 0.5 case per 1,000 patient-days. The annual isolation of *A. baumannii* BSI increased from 1.6% to 2.6% and CRAB BSI from 0.75% to 1.33% during the study period (Figure 1). Isolation rates for CRAB were uniform throughout all the study months, and no outbreak was seen (Figure 2).

Characteristics and outcomes of CRAB BSI versus CSAB BSI are shown in Table 1. In univariate analysis, factors significantly associated with risk of CRAB BSI were central line (umbilical venous and/or umbilical arterial), ventilator support, ventilation exceeding seven days, isolation after more than seven days following admission, duration of hospital stay over seven days, prior antibiotic use, and lack of feeding the baby with expressed breast milk (EBM). In multivariate analysis (Table 2), the factors found to be significantly associated with increased risk of CRAB BSI were duration of ventilation, prior antimicrobial use, and day of isolation post admission. Feeding the baby with expressed breast milk (EBM) was significantly protective.

Figure 1. Annual isolations of *A. baumannii* and CRAB

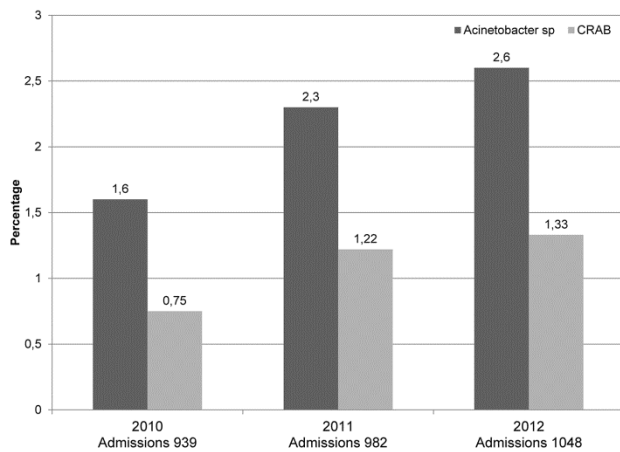


Figure 2. Quarterly breakup of *A. baumannii* isolation

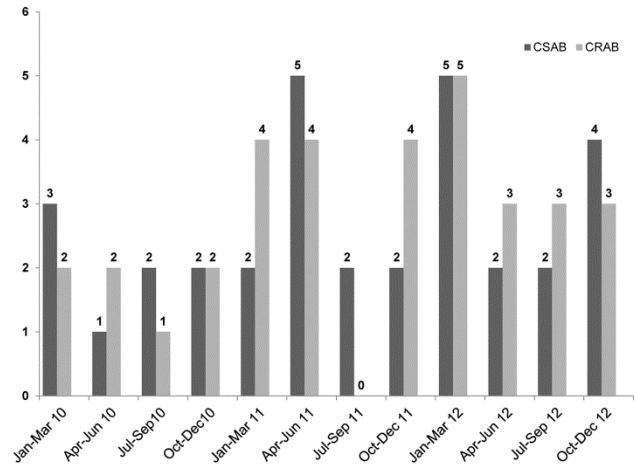


Table 1. Risk factors and outcomes of CRAB versus CSAB

Characteristic	CRAB (33) N (%)	CSAB (32) N (%)	P value	Odds Ratio	Confidence Interval (95%)
Gestation in weeks; mean (SD)	32.73 (4.3)	34.53 (3.3)	.067	0.88	0.77–1.00
Birth weight in grams; mean (SD)	1,500 (678)	1,706 (585)	.194	0.99	0.99–1.00
Male sex	18 (54.5)	17 (53.1)	.909	0.94	0.35–2.50
Age in days; mean (SD)	17.88(5.4)	15.59 (4.9)	.082	1.09	0.98–1.20
Pneumonia	20 (60.6)	4 (12.5)	.001	10.76	3.05–37.92
Central line	15 (45.5)	7 (21.9)	.048	2.97	1.00–8.78
UAC ^a	8 (24.2)	1 (3.1)	.036	9.92	1.16–84.70
UVC ^b	15 (45.5)	6 (18.8)	.025	3.61	1.17–11.08
Ventilatory support	17 (51.5)	7 (21.9)	.016	3.79	1.28–11.18
Ventilation >7 days	16 (48.5)	2 (6.9)	.001	14.11	2.89–68.94
Day of isolation >7 days	28 (84.8)	19 (59.4)	.026	3.83	1.17–12.52
Prior antibiotic use	25 (75.8)	7 (21.9)	.001	11.16	3.51–35.45
TPN use	5 (15.2)	6 (18.8)	.699	0.77	0.21–2.84
Surfactant	6 (18.2)	3 (9.4)	.312	2.14	0.48–9.45
Duration of stay > 7 days	20 (60.6)	7 (21.9)	.002	5.49	1.84–16.35
Received EBM	11 (33.3)	25 (78.1)	.001	0.14	0.04–0.42
Mortality	9 (27.3)	3 (9.4)	.074	3.62	0.88–14.91

^a umbilical arterial line; ^b umbilical venous line

Table 2. Multivariate analysis of risk factors between CRAB and CSAB

Characteristic	P value	aOdds Ratio	Confidence Interval (95%)
Ventilation > 7 days	.011	17.88	1.93–165.54
Day of isolation > 7 days	.025	8.37	1.30–53.60
Prior antibiotic use	.001	26.04	3.51–35.45
Received EBM	.003	0.05	0.01–0.35

Table 3. Sensitivity of *A. baumannii* to various antibiotics

Antibiotic	CSAB		CRAB	
	Number tested	number (%) sensitive	Number tested	number (%) sensitive
Ampicillin	32	0 (0)	33	0 (0)
Gentamicin	32	13 (40.6)	33	0 (0)
Amikacin	32	22 (68.8)	33	2 (6.1)
Chloremphenicol	32	0 (0)	33	0 (0)
Cefepime	32	17 (53.1)	33	5 (15.2)
Colistin	32	32 (100)	33	33 (100)
Tigecycline	32	32 (100)	33	33 (100)
Piperacillin/Tazobactam	18	13 (72)	21	4 (19)
Ciprofloxacin	32	21 (66)	19	0 (0)
Levofloxacin	17	8 (47)	21	0 (0)
Ceftazidime	14	2 (14)	14	0 (0)
Ceftriaxone	15	1 (6)	11	0 (0)

Antimicrobial susceptibilities of CSAB and CRAB isolates are shown in Table 3.

The all-cause mortality rate was 27.3% for CRAB versus 9.4% for CSAB, but the difference was not statistically significant ($p = 0.074$).

Discussion

Results showed that the background rate of CRAB BSI among the newborns was very low (0.5 episode per 1,000 patient-days) and comparable to that reported in adults and neonates [11,12]. Approximately 60% of *A. baumannii* BSI in our study were due to CRAB.

There have been limited reports of pandrug-resistant *A. baumannii* in neonates. One study from Taiwan reported a neonate who developed pandrug-resistant *A. baumannii* [13]. None of the *Acinetobacter* isolates in our study were pandrug resistant.

Most studies that evaluated CRAB BSI epidemiology were performed in adult populations. Risk factors considered included presence of central venous line (CVL), respiratory infection, diabetes mellitus or hematological malignancy as underlying disease, previous use of cefepime or carbapenems, and use of total parenteral nutrition [14-16]. Risk factors associated with CRAB infection among pediatric populations have been reported as case series only, and these have shown that previous surgery, prolonged tracheal intubation and mechanical ventilation for more than 10 days, previous carbapenems or aminoglycoside use, and length of stay in the pediatric intensive care unit (PICU) were significantly associated with CRAB infections [17].

A case series on a CRAB outbreak among six neonates revealed that all had extremely low birth weight (LBW), had CVL, and had received broad-spectrum antibiotics before culture positivity [18].

In a study by Thatrimontrichai *et al.* [19], out of the total 368 episodes of BSI, *A. baumannii* was isolated in 52 (14.1%) cases. Fourteen (26.9%) of these were CRAB. The crude mortality in the CRAB group was significantly higher compared to the CSAB group (42.9% versus 13.2%). There was no statistical difference between CRAB and CSAB as far as severity of illness, history of CVL use, and ventilator support was concerned. Neonates with CRAB BSI were more likely to have LBW. In another retrospective analysis involving NICU admissions, the authors could identify 22 patients with *A. baumannii* BSI, of which 13 (59%) were CRAB. Patients with CRAB had lower gestational age, LBW, and were more likely to have received assisted ventilation. Male

sex, apgar score at one and five minutes, age of infection onset, length of hospitalization, use of central catheter devices, TPN, and mechanical ventilation were comparable in the CRAB and CSAB groups [12].

Mechanical ventilation has been described as a risk factor for highly resistant *A. baumannii* in adult patients [20]. In addition, mechanical ventilation also significantly increases the rate of acquired pneumonia and late-onset septicemia in infants with very low birth weight [21]. These findings concurred with our results, which showed that mechanical ventilation lasting more than seven days was a significant risk factor for CRAB BSI.

Low gestational age and birth weight were not associated with CRAB BSI, which contradicts results of earlier studies [12,19]. Newborns with LBW were more likely to get bacteremia [12,22] because of their immature innate immunity. They were also at risk for nosocomial infections because of their prolonged hospital stay. Whether this increases their risk of acquiring drug resistant organisms (*e.g.*, CRAB) is debatable.

Central catheterization (both arterial and venous) was associated with acquisition of CRAB strains, but this was not an independent risk factor on multivariate analysis. Similar findings were reported by Nakwan *et al.* [12].

Our results showing that the CRAB BSI group had prolonged ventilation, late isolation, and prior antibiotic use are in agreement with most of the published literature in adults and children [12,18-19]. Probably several factors work together to maintain the presence of MDR *Acinetobacter* sp. in health care settings; these include presence of susceptible patients, presence of patients already colonized or infected with the organism, selective pressure from antimicrobial use, and inadequate compliance with infection control procedures [23], a common situation in most NICUs.

Feeding expressed breast milk to the baby reduced the odds of the baby acquiring CRAB sepsis (aOR 0.14, CI 0.04-0.42). Early feeding with human milk has been considered to be an important prevention strategy for nosocomial infections in NICUs [24]. A study on beta-lactam-resistant aerobic commensal fecal flora of newborns showed that feeding with tube feed was protective during the hospital stay and that failure to feed with breast milk was a risk factor for colonization with extended-spectrum beta-lactamase-producing bacteria after discharge [25]. To reduce mortality and morbidity due to infection caused by resistant microorganisms colonizing the infant's intestines, protection of normal non-pathogenic

bacterial flora is important. This can be provided by feeding neonates with breast milk.

Reported mortality of CRAB BSI among neonates is around 47% [26] to 49.2% [19]. An earlier study that included 13 neonates showed similar all-cause mortality between patients with CRAB and CSAB bacteremia (54% versus 11%, $p = 0.07$) [12]. In another study, the authors were unable to find any significant association between *Acinetobacter* BSI and mortality [26]. Mortality of babies with CRAB BSI in our study was 27.3%, which is much lower than what has been previously reported. We did not find significant differences in all-cause mortality between neonates with CRAB BSI and CSAB BSI.

Treatment options

CRAB isolates are usually resistant to all classes of antimicrobials. Resistance against carbapenem is in itself considered sufficient to define the isolate as highly resistant [27]. Increasing antimicrobial resistance leaves few therapeutic options, and there are no well-designed clinical trials to compare treatment regimens for MDR *Acinetobacter* infection. Carbapenems remain the treatment of choice if isolates retain susceptibility to either imipenem or meropenem. Susceptibility testing of imipenem does not predict susceptibility to meropenem or vice versa [28]. Tigecycline, a relatively new glycylcycline agent, has bacteriostatic activity against MDR *Acinetobacter* species [29]. This drug is not licensed for use in children under 18 years of age.

Given limited therapeutic options, clinicians have reverted to the use of polymyxin B or polymyxin E (colistin) for MDR *Acinetobacter* infections [28]. All our CRAB strains were sensitive to colistin and tigecycline. Observational studies have reported colistin cure or improvement rates of 57%–77% among severely ill patients with MDR *Acinetobacter* infections, including pneumonia, bacteremia, sepsis, intra-abdominal infections, and CNS infections [28]. Colistin is becoming a promising therapeutic option and has been used successfully by the intravenous route for treatment of CRAB infection in critically ill neonates and children [30].

Limitations

This study has several limitations. Because of its retrospective nature, we were not able to assess all the confounding variables and were limited by the completeness of documentation by the treating neonatologists. Moreover, we did not perform

surveillance and molecular studies to assess the source of infection.

Conclusions

Despite being an organism with low virulence, MDR *Acinetobacter* infection poses a formidable threat to neonates. The cause of many outbreaks, this organism is increasingly being recognized as endemic in NICU settings. Resistance to antimicrobials is increasing. Although the mortality attributed to MDR *Acinetobacter* infections is debatable, these infections are clearly associated with increased morbidity. Given the lack of good therapeutic options, more research and greater emphasis on the prevention of HAI due to MDR *Acinetobacter* infection are essential. As of now, colistin may be the most useful agent active against CRAB infections. With a lack of data of CRAB BSI epidemiology among the NICU population, this study provides some insight into CRAB prevention and control.

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