A case-control study to identify predictors of 14-day mortality following carbapenem-resistant *Acinetobacter baumannii* bacteraemia

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Abstract

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is an increasingly common nosocomial pathogen. We sought to identify clinical and microbiological predictors of 14-day mortality among patients with CRAB bacteraemia. This case-control study included all adult patients in one Israeli hospital with CRAB on blood culture between July 2008 and June 2011. Cases were defined as patients who died within 14 days of bacteraemia onset and controls as patients who survived over 14 days. Sequence-typing of the $bla_{\text{OXA-51-like}}$ gene and REP-PCR identified CRAB clone groups. Logistic regression was performed to analyze predictors of 14-day all-cause mortality. To correct for differences in treatment onset, Cox regression was used to examine the effect of receiving an active antibiotic. Eighty-three cases and 89 controls were included. Six major CRAB clone groups were identified, with 14-day mortality ranging from 17 to 66%. Independent predictors of 14-day mortality were severity of illness (OR = 1.38 for each 1-point increase in Sequential Organ Failure Assessment (SOFA) score; 95% CI, 1.21, 1.56), independence in activities of daily living (ADL) on admission (OR = 3.40; 95% CI, 1.20, 9.67, for fully dependent vs. independent), surgery before bacteraemia (OR = 0.25; 95% CI, 0.11, 0.59) and clone group (OR = 7.76; 95% CI, 2.52, 23.85, for the most virulent group vs. the reference group). In the multivariate Cox model using a propensity score to adjust for SOFA, clone, ADL and surgery, active antibiotic treatment was protective (HR = 0.30; 95% CI, 0.15, 0.60). Differences in virulence between CRAB clones may partly explain heterogeneous results in previous studies of mortality following CRAB infection.

Keywords: bacteraemia, carbapenem-resistant *Acinetobacter baumannii*, molecular epidemiology, mortality
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Introduction

Acinetobacter spp. were in the past frequently ignored as commensals with relatively low virulence. However, *A. baumannii*'s impressive ability to acquire resistance mechanisms, survive for prolonged periods in the hospital environment and cause nosocomial outbreaks has alerted clinicians to the emergence of a potentially dangerous pathogen [1–3].

Surveillance data collected globally reveal increased resistance among *Acinetobacter* spp. isolates over the past decade, with resistance to carbapenems of up to 88% in Europe [4] and 85% in Latin America [5]. Data from our hospital in Israel show that resistance of *A. baumannii* to carbapenems increased from 52% in 2009 to 70% in 2012.

Four *Acinetobacter* genospecies, *A. calcoaceticus*, *A. baumannii*, *A. pittii* and *A. nosocomialis*, are phenotypically similar and are referred to as the *A. calcoaceticus* – *A. baumannii* complex. *A. calcoaceticus* is an environmental species, while the three latter are clinically relevant. Simple phenotypic tests do not permit unambiguous identification of these closely related species and molecular methods are usually needed [1]. *A. baumannii* can be specifically identified by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species [6,7].

Because *A. baumannii* typically affects critically ill patients whose prognosis is influenced not only by infection, it is challenging to determine the true clinical impact of *A. baumannii*

infection and CRAB specifically [1,2]. Numerous studies have investigated risk factors for adverse outcomes, but limitations such as failure to distinguish between *A. baumannii* colonization and infection, failure to adjust for confounders such as severity of illness and co-morbid conditions, and suboptimal species identification, make it difficult to draw definitive conclusions [1].

The aim of the present study was to identify predictors of 14-day mortality among patients with carbapenem-resistant *A. baumannii* (CRAB) bacteraemia. We sought to determine factors associated with patient characteristics, the antibiotic treatment given and *A. baumannii* clone type.

Methods

Study setting and participants

This case-control study was performed at Tel-Aviv Sourasky Medical Center, a 1300-bed tertiary care teaching hospital in Israel. The sample included all patients aged 18 or older who had a peripheral blood culture positive for CRAB, and the isolate was preserved in the laboratory between July 2008 and June 2011. Only the first bacteraemic episode from each patient was considered. Cases were defined as patients who died within 14 days of bacteraemia onset and controls as patients who survived for more than 14 days. The protocol was approved by the hospital's Institutional Review Board.

Data collection

Data from the patients' medical records and from hospital computerized databases were recorded. Data collected included age, sex, admission from home vs. from an institution, level of independence in activities of daily living (ADL), number of hospitalizations in the previous year, and known *A. baumannii* carriage in the previous year. Co-morbidities were measured according to the Charlson co-morbidity score (CCS) [8]. The following data were collected regarding the current hospitalization: diagnosis on admission, time from admission to bacteraemia onset, number of surgeries, ICU stay, presence of a central venous catheter, dialysis, source of infection, antibiotic treatments, and positive blood culture with a different organism within 2 days of the CRAB blood culture. Two different scoring systems were used to quantify the severity of illness in the 48 h before the onset of bacteraemia: the Sequential Organ Failure Assessment (SOFA) score [9] and the Pitt Bacteremia Score (PBS) [10].

Definitions

The outcome of interest was death from any cause within 14 days of bacteraemia onset, defined as the date on which the first blood culture positive for CRAB was obtained. We chose 14-day mortality as the endpoint to allow adequate ascertainment of treatment response. We reasoned that in critically ill patients, 30-day mortality is too long, as there are many competing causes of death, and 7 days is too short a time to witness a response to treatment. An infection was considered nosocomial if it developed more than 3 days after admission. Patients were defined as immunosuppressed if they had HIV or AIDS, were post-transplant, had received chemotherapy within 6 weeks, had received systemic steroids (equivalent to or higher than 20 mg of prednisone) for 2 weeks or other immunosuppressive agents within 2 weeks before hospitalization, or had an absolute neutrophil count of $<500/\mu$L on admission. Source of infection was determined based on guidelines issued by the Centers for Disease Control and Prevention [11]. If no other source could be identified, the bacteraemia was defined as primary. An infection was defined as polymicrobial if another organism was detected by blood culture within 2 days of the CRAB blood culture. Antibiotic treatment was defined as active if the laboratory reported that the isolate was susceptible to the drug given; the dose, frequency or length of treatment were not considered.

Microbiological methods

Identification of isolates to the level of the *A. baumannii* complex was performed using the VITEK 2 automated system (bioMérieux, Inc., Durham, NC, USA). Susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Isolates were considered carbapenem-resistant if they were non-susceptible to imipenem or meropenem. Isolates with intermediate resistance were regarded as resistant.

All isolates were re-grown from storage and were subjected to PCR for detection of the $bla_{\text{OXA-51-like}}$ gene [6]. Detection of the $bla_{\text{OXA-51-like}}$ gene was regarded as a positive identification of *A. baumannii* to the species level.

Single locus amplification and sequence-typing of the $bla_{\text{OXA-51-like}}$ gene has demonstrated concordance with MLST typing and has the advantage that typing is based on a single gene [13,14]. Sequencing of the $bla_{\text{OXA-51-like}}$ gene was performed for all isolates. The primer pair 5′-CTAATAAT TGATCTACTCAAG-3′ (forward) and 5′-CCAGTGGATG GATGGATAGATTATC -3′ (reverse) was used to amplify the $bla_{\text{OXA-51-like}}$ gene [15]. The PCR products were outsourced for sequencing (HyLabs, Rehovot, Israel) and analyzed by DNAMAN software (Lynnon Corporation, Pointe-Claire, Quebec, Canada) and BLAST query (NCBI).

In addition, to improve discrimination within $bla_{\text{OXA-51-like}}$ sequence types, we used repetitive extragenic palindromic PCR (REP-PCR), a fingerprinting method that is based on the