*Brief Original Article*

# Genetic relatedness of clinical and environmental *Acinetobacter baumanii* isolates from an intensive care unit outbreak

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**Abstract**
Introduction: Determination of microbial genetic relatedness is important for improving efficiency of infection control measures during hospital outbreaks. This study aimed to analyze the clonal relationships of clinical and environmental *Acinetobacter baumannii* strains isolated during an outbreak in the intensive care unit (ICU) of a secondary care hospital in Saudi Arabia.
Methodology: Twelve clinical and fourteen environmental *A. baumannii* isolates identified during an outbreak in February 2013 in the 14-bed adult intensive care unit of a tertiary care hospital in Riyadh, Saudi Arabia, were studied. Bacterial identification and antimicrobial susceptibility testing were carried out using Microscan Walkaway 96 automated system. Determination of clonal diversity was investigated by repetitive-sequence-based polymerase chain reaction (rep-PCR) with the semi-automated DiversiLab system.
Results: The majority of the clinical isolates were from endotracheal tube aspirates (n = 9), one from a wound swab and two were from urine and sputum, respectively. Environmental isolates were from bed railings (n = 10) and with one each from curtain, stethoscope, computer mouse and telephone. Isolates were categorized into 6 clusters (Groups 1-6). Most of the isolates were associated with two clusters namely Groups 2 (n = 11) and 3 (n = 9). All isolates were multidrug resistant showing resistance to three or more classes of antibiotics. One clinical strain from Cluster 3 was resistant to colistin (MIC > 4ug/ml).
Conclusion: This outbreak was caused by two clonal groups of multidrug resistant *A. baumannii*. The presence of multiple environmental clones was suggestive of environmental source dissemination via healthcare workers within the ICU.

**Key words:** *Acinetobacter baumannii*; rep-PCR; DiversiLab; infection control.

## Introduction

*Acinetobacter baumannii* has emerged as an important opportunistic pathogen worldwide. These non-fermentative, Gram-negative bacteria commonly exist in nature and have the ability to survive for prolonged periods in dry environmental conditions. Infections in critically ill patients in intensive care units (ICU) are common and often associated with occurrence of outbreaks [1,2]. With the emergence of multidrug resistant strains, this organism continues to pose a significant threat in critical care units particularly in settings where successful clones have become endemic [3-6]. Determination of the genetic relatedness of isolates using molecular typing methods such as repPCR or pulsed field gel electrophoresis provide better understanding of epidemic dynamics and help to identify the determinants of transmission during an outbreak [4,7-9]. The automated rep-PCR assays on the DiversiLab system have been described as a reliable method for molecular analysis of nosocomial outbreaks providing rapid information useful for improving efficiency of outbreak infection control measures [9]. This is the first report from Saudi Arabia of the clonal relationships of clinical and environmental *A. baumannii* strains isolated during an outbreak and the implication for infection control in an endemic setting.

## Methodology

Twenty-six clinical and environmental isolates were collected in February 2013 during an outbreak of multi-drug resistant *A. baumannii* (MRAB) in the intensive care unit (ICU) of King Salman Hospital (Formerly Prince Salman Hospital), a secondary care hospital in Riyadh, Saudi Arabia. The environmental samples from the ICU were collected using sterile

amies transport medium (ATM) swabs moistened with sterile distilled water. Surveillance swabs were collected from hands of healthcare workers, commonly-shared equipment at nursing stations such as telephone sets, computer units including mouse and keyboards, surfaces of nursing station, crash carts of the unit, as well as specific patient dedicated equipment such as ventilators, syringe pumps, suction devices, electronic thermometers and stethoscopes. All clinical isolates obtained from patients in the ICU during the outbreak were also included in the study. Bacterial identification to species level and antimicrobial susceptibility testing were performed by routine laboratory methods in the Clinical Microbiology Laboratory using MicroScan WalkAway 96 automated system (Siemens Healthcare Diagnostic Inc, New York, USA) using NBC-42 panels. The breakpoint minimum inhibitory concentrations (MIC) were determined by the MicroScan WalkAway using the broth dilution method. The colistin MICs were also tested manually using Etest MIC gradient strips (bioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions and interpretive breakpoints were according to CLSI recommendations [10]. Molecular typing was carried out by repetitive-sequence-based polymerase chain reaction (rep-PCR) with the semi-automated DiversiLab system (bioMerieux) as previously described and in accordance with manufacturer guidelines [9]. Briefly, genomic DNA extraction was carried out using the Mo Bio UltraClean Microbial DNA Isolation Kit. The rep-PCR amplification was performed using the DiversiLab *Acinetobacter* DNA fingerprinting kit. Detection of amplified rep-PCR DNA fragment patterns was carried out using the microfluidic Lab Chips on the Agilent 2100 Bioanalyzer according to the manufacturer's instructions (bioMérieux). The amplified rep-PCR DNA fragment patterns were analyzed with the DiversiLab analysis software using the modified Kullback–Leibler statistical method.

## Results

A total of 26 isolates comprising of 12 clinical and 14 environmental isolates were identified during the outbreak. The clinical isolates were obtained from 10 patients and this included two patients who each yielded more than one *A. baumannii* isolate (three isolates were obtained from one individual and the second had two isolates). The majority of the clinical isolates were from endotracheal tube (ETT) aspirate (n = 6), and two each from tracheostomy tube (TT) aspirate, urine and sputum. The environmental isolates

**Figure 1.** Clonal distribution of clinical and environmental *A. baumanii* isolates using rep-PCR
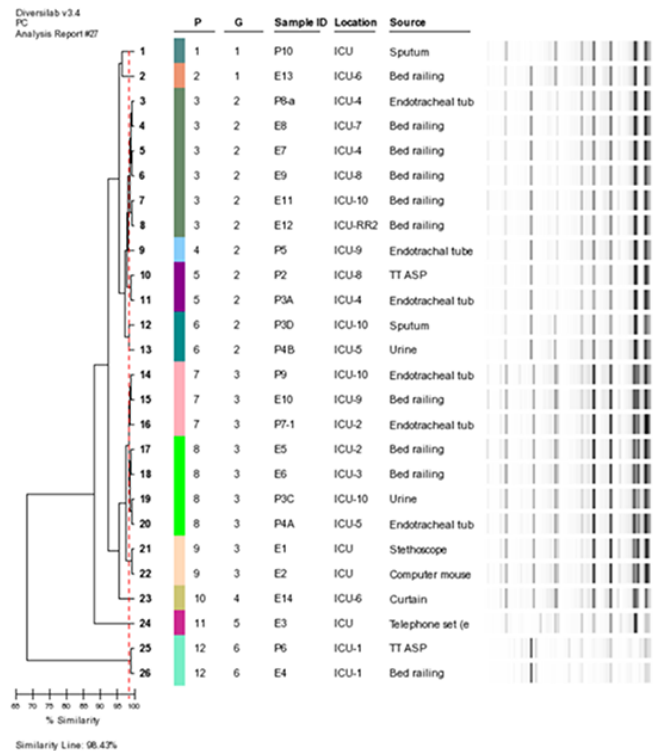


P3A, P3C and P3D were obtained from the same patient; P4A and P4B P4B were from the same patient; TT Asp: Tracheal tube aspirate

were from bed railings (n = 10) and one each from curtain, stethoscope, computer mouse and telephone.

Based on rep-PCR typing, isolates were categorized into 6 clusters (Groups 1-6) (Figure 1). Most of the isolates were associated with two clusters namely Groups 2 (n = 11) and 3 (n = 9). Groups 1 and 6 each had one clinical and one environmental isolate (Figure 1). The environmental isolate in Groups 6 was obtained from the bed railing of the same patient from whom the clinical isolate originated. Groups 4 and 5 each comprised of a single environmental isolate which were not identified in any patient. Three of the clinical isolates were from a single patient and were clustered in Group 2 (P3A and P3D) and Group 3 (P3C) (Figure 1). These three clinical isolates were obtained from different body sites on different days. In addition to showing resistance to three or more classes of antibiotics as shown in Table 1, all isolates exhibited resistance to meropenem (MIC > 8ug/ml). Table 1 shows the range of MIC for the antibiotics tested. One clinical isolate (P3C) was resistant to colistin (MIC > 4ug/ml).

**Table 1.** Minimum inhibitory concentrations and antimicrobial susceptibility pattern

| Antibiotic | MIC range | Interpretation |
| --- | --- | --- |
| Amikacin | >32 | Resistant |
| Amoxicillin/Clavulinic acid | >16/8 | Resistant |
| Ampicillin | >16 | Resistant |
| Cefazolin | >16 | Resistant |
| Cefepime | >16 | Resistant |
| Cefotaxime | >32 | Resistant |
| Cefoxitin | >8 | Resistant |
| Ceftazidime | >16 | Resistant |
| Cefuroxime | >16 | Resistant |
| Ciprofloxacin | >2 | Resistant |
| Colistin* | 4 - >4 | Sensitive / Resistant |
| Ertapenem | >4 | Resistant |
| Fosfomycin | >32 | Resistant |
| Gentamicin** | <=4 - >8 | Sensitive/ Resistant |
| Levofloxacin | 4 | Intermediate resistant |
| Meropenem | >8 | Resistant |
| Moxifloxacin | >1 | Resistant |
| Nitrofurantoin | >64 | Resistant |
| Norfloxacin | >8 | Resistant |
| Piperacillin | >64 | Resistant |
| Tobramycin*** | <=4 - >8 | Sensitive /Resistant |
| Trimethoprim/ Sulfamethoxazole | >2/38 | Resistant |

*One clinical isolate was colistin resistant (MIC>4ug/ml); **One clinical isolate was gentamicin sensitive with MIC $\leq$4ug/ml ; Three isolates were tobramycin resistant with (MIC>8ug/ml)

## Discussion

*A. baumannii* is a recognized opportunistic pathogens with the potential to cause outbreaks in the health care setting, particularly in debilitated patients in critical care units [11]. Reported literature from Saudi Arabia indicates that multidrug resistant *A. baumannii* of wide clonal diversity are circulating in healthcare facilities [12-14]. Additionally, emerging data over the past couple of years has shown an increasing trend of identification of carbapenem-resistant isolates habouring class D carbapenemase-encoding genes with predominance of OXA-23 [13,15,16]. Environmental contamination with *A. baumannii* particularly in critical care areas such as the ICU has been identified as an important risk factor for occurrence and sustenance of outbreaks [17-20]. Indeed, eradication of environmental contamination through effective terminal disinfection procedures has been shown to be a key infection control strategy for preventing further outbreaks [20]. In Saudi Arabia, there is a paucity of data on the level of environmental contamination with *A. baumannii* particularly under outbreak conditions. A 2001 report by Mah *et al.* did not identify any environmental reservoir of MRAB under outbreak conditions in an ICU in a tertiary care facility in Riyadh [21]. More recently only two environmental isolates were identified in a six-month

prospective epidemiological study conducted by El-Ageery *et al.* in the ICU of a tertiary care facility in the country [22]. Findings from these previous reports are in sharp contrast to the large number of environmental isolates observed in this study. Our finding indicates that attention to environmental screening and decontamination should be important components of the infection control protocol for *A. baumannii* outbreaks in our setting.

During an epidemic outbreak it is crucial to rapidly confirm or exclude the genetic relationship among isolates as this information can be critical in guiding infection control measures. Methods like the pulse field gel electrophoresis (PFGE) and multi locus sequence typing (MLST) are common bacterial typing methodologies. However, these methods, particularly PFGE are expensive, labour-intensive and require high technical skills. In addition, the wide inter-laboratory variation associated with PFGE makes comparison of data rather difficult. Although MLST is electronically portable, it has a low discriminating power in an outbreak situation [23]. In this study we have utilized the semiautomated DiversiLab repPCR system which is a rapid and reproducible system that has been shown to be very useful for the identification of clonal relationship particularly during an outbreak. Its discriminating power has been compared with other

genotyping methods for different pathogens and found to be comparable and higher in some instances [24]. The combination of the rep-PCR method with epidemiological investigation provides data on understanding the dynamics of the outbreak. With this approach, we have been able to identify the occurrence of two predominant clones (cluster group 2 and 3) as being responsible for this outbreak. These isolates were widely disseminated in the ICU environment including on a computer mouse and a stethoscope suggestive of a possible role of healthcare workers in the transmission of these clones within the ICU. It is also noteworthy that colistin resistance was observed within one of the predominant cluster being trafficked in the ICU. The occurrence and dissemination of pan-drug resistant *A. baumannii* particularly as part of an outbreak is of major concern. There were two environment isolates (Group 4 & 5) which although present in the ICU were not part of this clinical isolates responsible for this outbreak. Infection control measures advocated for control of *A. baumannii* outbreaks include handwashing, environmental decontamination and antibiotic stewardship. In this particular setting, our findings guided the recommendation for strict hand hygiene compliance and environmental decontamination for controlling the outbreak. In view of the emergence of colistin resistance, continued surveillance and judicial use of antibiotics were also advocated.

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**Conflict of interests:** No conflict of interests is declared.