

RESEARCH ARTICLE

## Genetic Relationship of Multi-Resistant *Acinetobacter baumannii* Isolates in Kingdom of Saudi Arabia

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### Abstract

In the last two decades, there has been a remarkable rise in the instances of nosocomial infections associated with antibiotic-resistant *Acinetobacter baumannii*. The aimed of this study to determine the antibiotic resistance patterns and genetic relationship among isolates of carbapenem-resistant *A. baumannii* (CRAB) isolated from the clinical specimens of various inpatients. A total of sixty clinical isolates of *A. baumannii* were collected during February to September 2014 from King Fahd Hospital of the University (KFHU) in Khobar, Saudi Arabia. Antimicrobial susceptibility was tested using the Vitek 2 system and the minimum inhibitory concentrations (MICs) were estimated following the guidelines of the Clinical and Laboratory Standards Institute. Molecular epidemiological analysis of carbapenem-resistant *A. baumannii* (CRAB) was carried out by using enterobacterial repetitive intergenic consensus (ERIC-PCR). All of tested clinical isolates of *A. baumannii* in this study were associated with nosocomial infections and the highest rates of *A. baumannii* isolates were from wound swabs (23.3%) and transtracheal aspiration (20%). All of the 60 analyzed strains of *A. baumannii* in this study were classified as extensively drug-resistant (XDR), and the rates of antibiotic resistance against imipenem and meropenem were 93.3% and 96.6% respectively, while just 6.6% of strains were resistant to tigecycline. All 60 XDR *A. baumannii* isolates were sensitive only to colistin. The genotypic analysis of CRAB was performed by enterobacterial repetitive intergenic consensus (ERIC-PCR). ERIC-PCR able to discriminate the CRAB strains into four distinct clusters (A, B, C, and D) with genetic similarity ranged from 82.5 to 100%.

**Keywords:** Carbapenem, Extensively Drug Resistant, Enterobacterial Repetitive Intergenic Consensus, ERIC-PCR, Saudi Arabia, *Acinetobacter baumannii*.

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## INTRODUCTION

*Acinetobacter baumannii* is an encapsulated, catalase-positive, non-motile, Gram-negative coccobacilli bacterium<sup>1</sup>. This species is widely distributed in the environment and can be found in water, soil, and, occasionally, in human skin and throat as a normal microbiota. The presence of this species in the environment suggests an ability to persist desiccation and survive in the environment for long periods<sup>2</sup>. This phenomenon of adverse environmental persistence has increased the instance of hospital infections. Moreover, *A. baumannii* has the ability to adhere to hospital equipment, patients' skin, and on the hands of doctors and nurses<sup>3,4</sup>. This species is responsible for numerous infections associated with skin, bloodstream, burns patients, and respiratory and urinary tract infections<sup>1,5</sup>. The use of mechanical ventilation and burn patients admitted to intensive care units (ICUs) are risk factors for acquiring infection with this species<sup>6,7</sup>. *A. baumannii* is considered among the most flourishing bacterial pathogens in acquiring antibiotic-resistant genes and has developed resistance to most of the available therapeutic antibiotics<sup>8</sup>. This species has multiple antibiotic resistance mechanisms, such as  $\beta$ -lactamases, aminoglycoside enzymes, altered target sites, efflux pump mechanisms, and permeability defects. Therefore, these mechanisms play a significant role in decreasing the therapeutic action of available antibiotics for the treatment of infections associated with *A. baumannii*<sup>9,10</sup>.

Multi-resistant, extensive-resistant, and pan-drug resistant (MDR, XDR, and PDR) *A. baumannii* strains are on the rise worldwide and present infection control and treatment challenges for clinicians and clinical microbiologists<sup>11-13</sup>. The evolution and spread of *A. baumannii* resistant to most of the available antimicrobial agents pose problems for future management since the pathogen plays a role in nosocomial infections. Currently, the management of MDR, XDR, and PDR *A. baumannii* infections poses serious clinical and epidemiological challenges<sup>14</sup>. The problem of antimicrobial resistance is of international concern because *A. baumannii* strains can display resistance to most available antibiotics, making treatment of these infections complicated<sup>15</sup>. The Centers for Diseases Control and Prevention (CDC)

has classified MDR *A. baumannii* as a serious threat pathogen<sup>9</sup>. This species is also categorized by the Infectious Diseases Society of America (IDSA) as a superbug and is one of the six significant "ESKAPE" pathogens, which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species "ESKAPE"<sup>16</sup>. The current study aimed to determine the antibiotic resistance patterns in isolates of *A. baumannii* from various inpatients' clinical specimens and to determine the genetic relationship among the isolates of carbapenem-resistant *A. baumannii* (CRAB) isolated in King Fahd Hospital of the University (KFHU) from February to September 2014 in Khobar, Saudi Arabia.

## MATERIALS AND METHODS

### Ethics Statement

The ethics committee of the Imam Abdulrahman Bin Faisal University approved this study (IRB -2017-01-203).

### Bacterial Isolates

From February to September 2014, a total of 60 *A. baumannii* clinical isolates were analyzed from a 450-bed teaching hospital. The selection criteria for isolates were only carbapenem-resistance and meeting the definition of XDR strains using the definition criteria described by Magiorakos et al. (2012)<sup>12</sup>. All hospital isolates of *A. baumannii* were identified by the Vitek 2 automatic system and further confirmed using the API 20 NE system (BioMérieux, France).

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was tested using the Vitek 2 system (BioMérieux, France) and the minimum inhibitory concentrations (MICs) were estimated following the guidelines of the Clinical and Laboratory Standards Institute (17). The antibiotics tested in this study were ampicillin/sulbactam (AM/SUL), amikacin (AK), cefepime (FEP), ceftazidime (CAZ), ciprofloxacin (CIP), colistin (CL), gentamicin (GM), imipenem (IMP), meropenem (MEP), piperacillin/tazobactam (PIP/TAZ), tobramycin (TOB) and tigecycline (TIG).

### DNA Extraction

Isolates of *A. baumannii* were grown in Luria Bertani (LB) broth and incubated in a thermal shaker at 37°C for 24 h. Incubated LB broth (1.5 ml) was then harvested and centrifuged

for 1 min at 10,000 rpm, and the obtained pellet was suspended in 700 µl of deionized water and boiled at 100°C for 15 min to lyse the cells and free the DNA. The extracted DNA for all isolates was immediately stored at -20°C and used as a template for ERIC-PCR.

#### Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction

The genetic relationship between 56 carbapenem-resistant isolates was determined using the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) method as described elsewhere<sup>18</sup>.

#### ERIC-PCR Data Analysis

The DNA polymorphism patterns were obtained by electrophoresis separation and were analyzed using GelJ software<sup>19</sup>. The DNA fingerprints for all gel images were calculated using the Dice coefficient. The unweighted pair-group method using arithmetic averages (UPGMAs) was used to construct the phylogenetic tree for CRAB isolates. The clonal relationship was constructed based on the similarity matrix, and a dendrogram was generated by ERIC fingerprints among the isolates. Isolates in the clustered dendrogram exceeding 85% similarity are were considered clonally related.

**Table 1.** The distribution of *A. baumannii* in clinical specimens

Specimen	Male	Female	Total
Abscess	0	1	1
Blood	4	0	4
Intravenous catheter tip	3	0	3
Nasal Swab	1	0	1
Peritoneal fluid	2	0	2
Pleural fluid	1	1	2
Rectal swab	3	3	6
Skin swab	1	0	1
Sputum	2	2	4
Throat swab	4	1	5
Transtracheal aspiration	7	5	12
Urine	5	0	5
Wound swab	6	8	14
Total	39	21	60

There was no association between gender and specimen ( $p = 0.22$ ).

#### Statistical analysis

The Chi-square tests and other analyses were carried out using MS Excel 2007 (Microsoft, Redmond, WA, USA) and PAST statistical software (version 2.04), as described by Hammer<sup>20</sup>.

#### RESULTS

A total of 60 isolates of *A. baumannii* were isolated from various clinical specimens of inpatients admitted to the KFHu (Table 1). The highest rates of *A. baumannii* isolation were associated with wound swab (23.3%) and transtracheal (20%) specimens. According to the chi-square goodness-of-fit test, there was no association between gender and specimen ( $P = 0.22$ ). Most of the *A. baumannii* strains were isolated from adult patients (Table 2).

All of the analyzed strains of *A. baumannii* in this study were classified as XDR, and no PDR isolate was detected (Table 3). The percentage of antibiotic resistance and the MICs of the *A. baumannii* are shown in (Fig. 1) and (Table 3). Evaluating all of the major classes of antibiotics, we found that all of the isolates were susceptible to colistin (Table 3). However, 93.3% and 96.6% of strains were resistant to imipenem and meropenem respectively (Table 3). Just 6.6% of

**Table 2.** The distribution of *A. baumannii* strains in different age groups

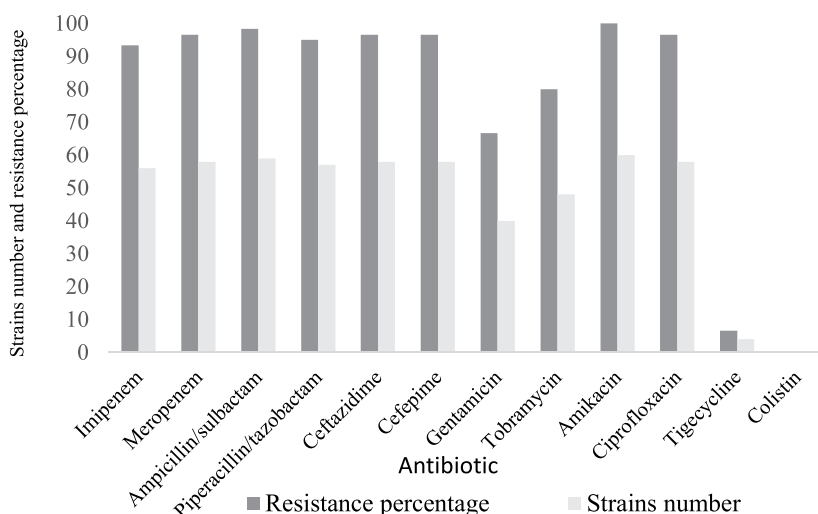
Specimen	0–25 years	26–55 years	56 and above
Abscess	0	1	0
Blood	0	3	1
Intravenous catheter tip	0	0	3
Nasal Swab	0	1	0
Peritoneal fluid	0	1	1
Pleural fluid	0	0	2
Rectal swab	1	4	1
Skin swab	0	0	1
Sputum	0	2	2
Throat swab	1	3	1
Transtracheal aspiration	0	3	9
Urine	0	1	4
Wound swab	2	6	6
Total (60)	4	25	31

There was no association between months and drug ( $p = 0.537$ ).

strains were resistant to tigecycline (Fig. 1) (Table 3). All the study isolates were resistant to amikacin, with similar MIC values (all > 16 µg/mL) (Table 3).

Overall, the highest rates of nosocomial XDR of *A. baumannii* isolates at our hospital were reported between February to September 2014, while the highest number of CRAB isolates were reported between May to July 2014 (Table 4). According to the chi-square goodness-of-fit test, there was no association between month of isolation and drug resistance ( $p = 0.999$ ).

The ERIC-PCR technique was used for the molecular typing of 56 CRAB strains. The banding patterns consisted of 5 to 14 fragments per strain. The apparent molecular sizes of the band fragments ranged from 200 to 3,900 bp (Fig. 2). The ERIC dendrogram revealed four distinct clusters (A, B, C, and D) with genetic similarity ranging from 82.5 to 100% (Fig. 3). These clustered strains were recovered from clinical inpatients specimens throughout the four months (May, June, July, and August), raising concerns about the



**Fig. 1.** Percentage of antibiotic resistance in *A. baumannii* strains.

**Table 3.** Minimum inhibitory concentrations breakpoint for an extensively drug-resistant strain of *A. baumannii*

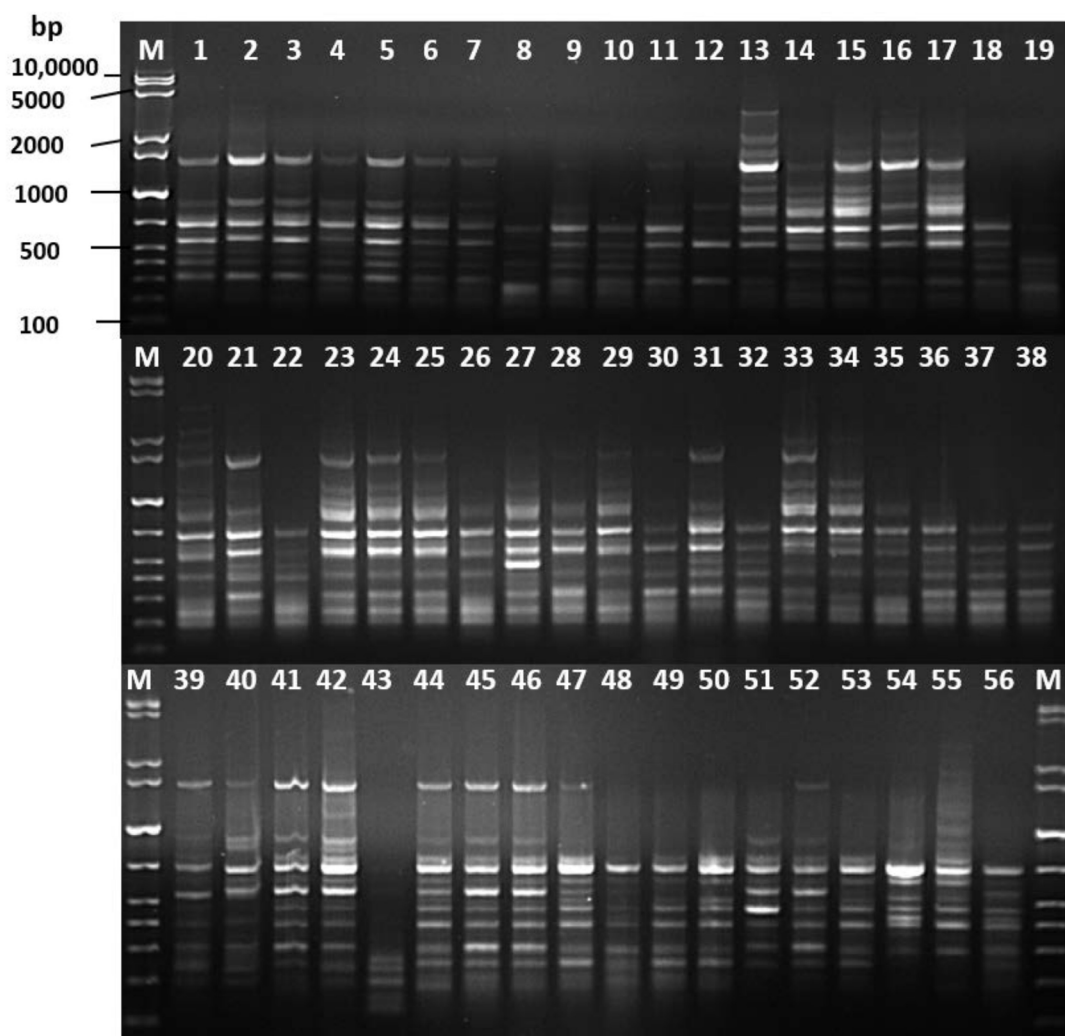
Antibiotic	MIC breakpoint (µg/mL)		
	< 1	Between 2 and 8	> 16
Imipenem	4	0	56
Meropenem	2	0	58
Ampicillin/sulbactam	0	5	55
Piperacillin/tazobactam	0	2	58
Ceftazidime	0	2	58
Cefepime	0	2	58
Gentamicin	15	5	40
Tobramycin	10	2	48
Amikacin	0	0	60
Ciprofloxacin	2	58	0
Tigecycline	18	42	0
Colistin	60	0	0

There was no association between MIC and drug ( $p < 0.001$ ).

**Table 4.** The resistance rates of nosocomial *A. baumannii* strains between February and September 2014

Drugs	Months (n)		
	Feb-March (6)	May-July (42)	Aug-Sept (12)
Imipenem	5	40	11
Meropenem	6	41	11
Ampicillin/sulbactam	6	42	11
Piperacillin/tazobactam	6	41	10
Ceftazidime	6	41	11
Cefepime	6	41	11
Gentamicin	4	29	7
Tobramycin	5	36	7
Amikacin	6	42	12
Ciprofloxacin	6	41	11
Tigecycline	1	2	2
Colistin	0	0	0

There was no association between the months and drug ( $p = 0.999$ ).



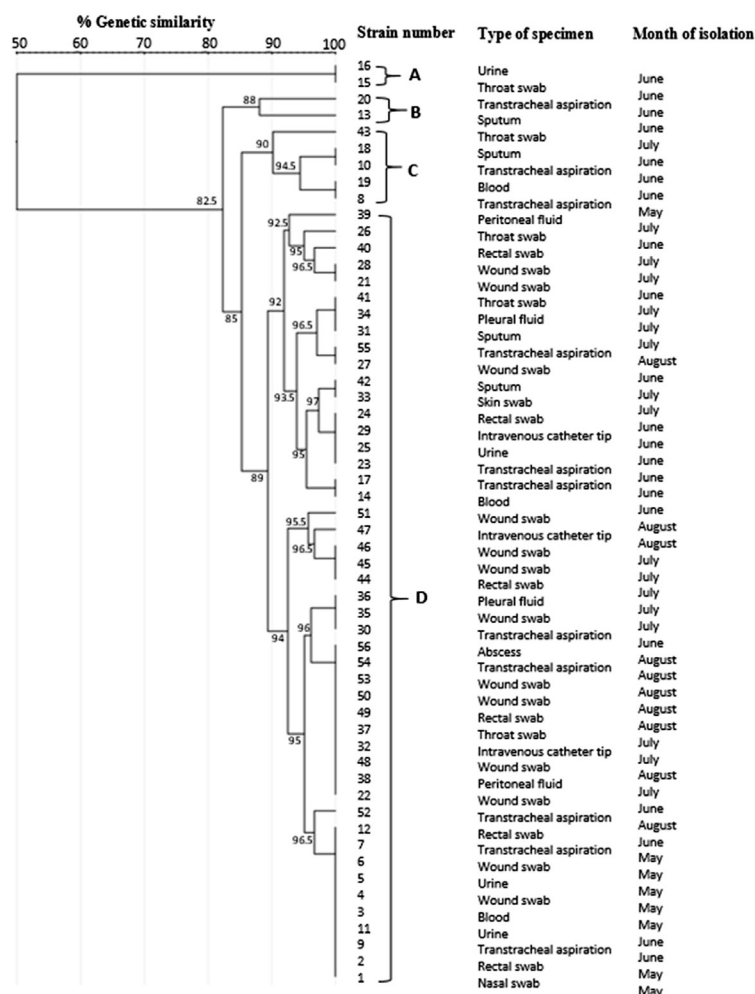
**Fig. 2.** DNA fingerprint analysis of the 56 carbapenem-resistant *Acinetobacter baumannii* by ERIC-PCR. M: GelPilot 1 Kb Plus ladder.

potential persistence of the epidemic clonal strain. Among the four clusters, Cluster D accounted for 47 (84%) of the isolates, suggesting a high genetic relatedness among the CRAB genotype carrying strains (Fig. 3).

The strains of Cluster D were highly genetically similar, raising the possibility of cross-transmission within some of the hospital wards, including ICU wards. The other three minor clusters (A, B, and C) account for 16% of the recovered isolates. Our findings demonstrate that the ERIC-PCR method is a rapid, simple typing technique with a level of discrimination equivalent to that of pulsed-field gel electrophoresis (PFGE).

## DISCUSSION

*A. baumannii* has been implicated in many types of nosocomial infection in healthcare settings<sup>19</sup>. Recently, the number of studies documenting epidemics of CRAB outbreaks in hospital ICUs has increased<sup>21,22</sup>. An increased rate has been reported in Saudi hospital ICUs, particularly among immunocompromised patients<sup>23,24</sup>. In this study, all of the *A. baumannii* isolates associated with inpatient infections were classified and identified as XDR (Table 3). The overall resistance rate of *A. baumannii* to the tested antibiotics was 84.2%. The antibiotic susceptibility testing revealed that 56 (93.3%) *A.*



**Fig. 3.** Dendrogram based on clusters analysis of ERIC-PCR fingerprint data illustrating the relationships among the carbapenem-resistant *Acinetobacter baumannii* strains. The genetic similarities between the fingerprints were calculated using the Dice coefficient (optimization, 1%; position tolerance, 1%), and the fingerprints were grouped according to their similarities using the unweighted pair-group method using arithmetic averages method.

*baumannii* isolates were carbapenem-resistant. Thus, this reported rate of resistance is similar to those reported in other countries, as discussed elsewhere<sup>21,22</sup>. Our data is in line with other studies conducted in Saudi Arabia, and the Gulf Cooperation Council States have reported a significantly increased rate of CRAB isolates<sup>23-25</sup>. We detected four strains (6.6%) resistant to tigecycline (Fig. 1). Several studies have reported an increased resistance to tigecycline, which has created a *therapeutic challenge* for clinicians, as discussed elsewhere<sup>26-28</sup>.

DNA-based molecular epidemiology typing techniques are important tools to investigate

and track outbreaks of bacterial strains and to control the spread of bacterial isolates associated with nosocomial infections in healthcare settings<sup>29</sup>. In this study, we used ERIC-PCR to determine the genetic relatedness of 56 CRAB isolates. By this approach, we detected an increased rate of detection of CRAB isolates between May and August 2014 (Table 4). Interestingly, based on dendrogram cluster analysis of ERIC-PCR fingerprint data, a single genetically-related cluster (Cluster D) was shown to account for 47 of the 56 (84%) CRAB isolates. This is likely due to the spread of clonally related CRAB isolates, which were responsible for nosocomial infections



between May and August 2014. Molecular typing can be used as an epidemiological method to type bacterial strains and thus monitor and track the spread of major nosocomial infections pathogens<sup>30</sup>. Rapid molecular typing methods have become popular in clinical microbiology research laboratories and play a significant role in decontamination and controlling the spread of infection in hospitals<sup>30</sup>. The inclusion of molecular typing techniques to determinate microbial clonality as part of routine infection control programs within healthcare settings will be of medical and economic benefit<sup>31</sup>.

## CONCLUSION

Here we detected a rapid increase in the isolation of XDR *A. baumannii* and CRAB infections at our hospital between May and August 2014. ERIC-PCR was shown to be useful for typing XDR *A. baumannii* and CRAB, thus providing tools for epidemiological and clinical follow-up studies. The isolation of similar clones (> 90% genetic similarity) from different specimens within a single hospital suggests horizontal transmission, and it is possible that these related isolates of XDR *A. baumannii* and CRAB belonged to a single bacterial clone circulating through the hospital setting. The frequency of isolation of these drug-resistant bacteria is a reminder of the importance of stewardship surveillance for resistant bacteria in the hospital settings to prevent their spread and epidemic, and the importance of strict implementation of infection control guidelines to prevent circulation through the hospital setting. This study established baseline evidence of clonal dissemination of closely related CRAB at our hospital, indicating the need for further surveillance.

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