JCM
Journals.ASM.org

# Molecular Epidemiology of Carbapenem-Resistant *Acinetobacter baumannii* Isolates in the Gulf Cooperation Council States: Dominance of OXA-23-Type Producers

**Hosam M. Zowawi,[a,b,c] Anna L. Sartor,[a] Hanna E. Sidjabat,[a] Hanan H. Balkhy,[b,c,d] Timothy R. Walsh,[a,e] Sameera M. Al Johani,[b,f] Reem Y. AlJindan,[g] Mubarak Alfaresi,[h,i] Emad Ibrahim,[j] Amina Al-Jardani,[k] Jameela Al Salman,[l,c] Ali A. Dashti,[m] Khalid Johani,[n,o] David L. Paterson[a]**

The University of Queensland, UQ Centre for Clinical Research, Herston, Queensland, Australia[a]; King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia[b]; World Health Organization Collaborating Centre for Infection Prevention and Control and Gulf Cooperation Council Center for Infection Control, Riyadh, Saudi Arabia[c]; King Abdullah International Medical Research Centre, Riyadh, Saudi Arabia[d]; Department of Medical Microbiology and Infectious Diseases, School of Medicine, Cardiff University, Heath Park, Cardiff, United Kingdom[e]; Microbiology, Department of Pathology and Lab Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia[f]; University of Dammam, College of Medicine, Dammam, Saudi Arabia[g]; Zayed Military Hospital, Clinical Microbiology Department, Abu Dhabi, United Arab Emirates[h]; Pathology and Laboratory Medicine Department, Sheikh Khalifa General Hospital, Umm Al Quwain, United Arab Emirates[i]; Hamad Medical Cooperation, Clinical Microbiology Department, Doha, Qatar[j]; The Royal Hospital, Medical Microbiology Department, Muscat, Oman[k]; Samlaniya Medical Complex, Infectious Diseases Unit, Manama, Bahrain[l]; Medical Laboratory Department, Faculty of Allied Health Sciences, Health Science Center, Kuwait University, Kuwait City, Kuwait[m]; Division of Microbiology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia[n]; Surgical Infection Research Group, Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia[o]

**The molecular epidemiology and mechanisms of resistance of carbapenem-resistant *Acinetobacter baumannii* (CRAB) were determined in hospitals in the states of the Cooperation Council for the Arab States of the Gulf (Gulf Cooperation Council [GCC]), namely, Saudi Arabia, United Arab Emirates, Oman, Qatar, Bahrain, and Kuwait. Isolates were subjected to PCR-based detection of antibiotic resistance genes and repetitive sequence-based PCR (rep-PCR) assessments of clonality. Selected isolates were subjected to multilocus sequence typing (MLST). We investigated 117 isolates resistant to carbapenem antibiotics (either imipenem or meropenem). All isolates were positive for OXA-51. The most common carbapenemases were the OXA-23-type, found in 107 isolates, followed by OXA-40-type (OXA-24-type), found in 5 isolates; 3 isolates carried the IS*Aba1* element upstream of $bla_{\text{OXA-51-type}}$. No OXA-58-type, NDM-type, VIM-type, or IMP-type producers were detected. Multiple clones were detected with 16 clusters of clonally related CRAB. Some clusters involved hospitals in different states. MLST analysis of 15 representative isolates from different clusters identified seven different sequence types (ST195, ST208, ST229, ST436, ST450, ST452, and ST499), as well as three novel STs. The vast majority (84%) of the isolates in this study were associated with health care exposure. Awareness of multidrug-resistant organisms in GCC states has important implications for optimizing infection control practices; establishing antimicrobial stewardship programs within hospital, community, and agricultural settings; and emphasizing the need for establishing regional active surveillance systems. This will help to control the spread of CRAB in the Middle East and in hospitals accommodating transferred patients from this region.**

*Acinetobacter baumannii* is a major pathogen associated globally with hospital-acquired infections (HAIs). It was found that 26.5% of ventilator-associated pneumonias in Riyadh, Saudi Arabia, between 2005 and 2009 were caused by *Acinetobacter* spp. (1). The success of this pathogen is partially due to the high prevalence of a multidrug-resistant phenotype that *A. baumannii* now demonstrates (2). In the Middle East, particularly in states of the Cooperation Council for the Arab States of the Gulf (Gulf Cooperation Council [GCC]; i.e., Saudi Arabia, United Arab Emirates, Oman, Kuwait, Qatar, and Bahrain), the prevalence of carbapenem-resistant *A. baumannii* (CRAB) has increased dramatically over the last decade (3). This high prevalence limits treatment options, which can lead to increased morbidity and mortality due to infections caused by CRAB.

The phenotypic resistance characteristics of CRAB are mainly due to the expression of class D carbapenemases, called oxacillinases. Moreover, plasmid-mediated metallo-β-lactamases (MBL) have been associated with the resistance phenotype (2). The existence of IS*Aba1* elements upstream of the $bla_{\text{OXA-51-type}}$ gene is also associated with the carbapenem resistance phenotype in *A. baumannii* by overexpressing the intrinsic OXA-51 carbapenemase (4). Previous reports on isolates from the GCC states show that the carbapenem resistance phenotype in *A. baumannii* is often due to the expression of OXA enzymes, particularly OXA-23 (3). However, MBL-encoding genes, including the recently

**Received** 25 September 2014 **Returned for modification** 28 October 2014
**Accepted** 4 January 2015

**Accepted manuscript posted online** 7 January 2015

**Citation** Zowawi HM, Sartor AL, Sidjabat HE, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, Alfaresi M, Ibrahim E, Al-Jardani A, Al Salman J, Dashti AA, Johani K, Paterson DL. 2015. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* isolates in the Gulf Cooperation Council states: dominance of OXA-23-type producers. J Clin Microbiol 53:896–903. doi:10.1128/JCM.02784-14.

**Editor:** P. H. Gilligan

Address correspondence to Hosam M. Zowawi, h.zowawi@uq.edu.au.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.02784-14.



doi:10.1128/JCM.02784-14

emerged New Delhi metallo-β-lactamase (NDM), have been increasingly reported in *Acinetobacter* spp. isolated from different parts of the world (5–7).

Due to the geographic location of the GCC states and the ethnic relationships of residents, heavy travel occurs between the GCC states and the Indian subcontinent, where NDM enzymes are widespread. The current socioeconomic structure of the GCC states relies heavily on an international workforce. For example, about 37% of the total population of the GCC states are nonnational expatriates, mainly from the Indian subcontinent (8). Saudi Arabia receives more than 1.5 million international pilgrims from all over the world to perform Hajj (9). This exemplifies the heavy travel occurring in the GCC region, and travel is known to be a risk factor for spreading/acquiring antimicrobial-resistant bacteria (3).

As one of many desperately needed first steps to control the spread of CRAB, we aimed to determine the molecular genetics of CRAB in the GCC states. To our knowledge, no region-wide study on the molecular genetics of CRAB has been undertaken. For this reason, we have performed a "snapshot" analysis of the molecular epidemiology of CRAB in the states of the Gulf Cooperation Council.

## MATERIALS AND METHODS

**Bacterial isolates.** Between July 2011 and January 2013, *Acinetobacter* spp. were collected from seven participating institutes across the GCC states (two hospitals in Saudi Arabia from Riyadh and Khobar and one hospital each from United Arab Emirates [UAE], Kuwait, Qatar, Oman, and Bahrain) (Table 1). These hospitals are part of a region-wide collaborative study on multidrug-resistant Gram-negative bacilli (10). *Acinetobacter* spp. were identified and tested for their susceptibility to a panel of antimicrobials using semiautomated systems in each clinical microbiology laboratory (Table 1). Isolates were included on the basis of showing decreased susceptibility to imipenem (MIC, ≥8 μg/ml) or meropenem (MIC, ≥8 μg/ml) using CLSI breakpoints. Only one isolate per patient was included, and isolates originated from a range of clinical specimens.

Isolates were sent to the research laboratory at the University of Queensland Centre for Clinical Research (UQCCR).

**Antibiotic susceptibility testing.** All isolates underwent disk diffusion susceptibility testing following the methodology and the updated breakpoint defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (11) for the following antimicrobial agents: imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), and sulfamethoxazole-trimethoprim (23.75 /1.25 μg). The updated breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI) (12) were also used to assess disk diffusion susceptibility for ceftazidime (30 μg), tetracycline (30 μg), ticarcillin-clavulanic acid (75/10 μg), piperacillin-tazobactam (100/10 μg), and ampicillin-sulbactam (10/10 μg), since these antibiotics do not have EUCAST breakpoints available for *Acinetobacter* spp.

**PCR for carbapenemase genes.** Genomic DNA was extracted using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories) as recommended by the manufacturer. Species identification was performed using *gyrB* multiplex PCR as previously described (13). Detection of the intrinsic carbapenemase-encoding gene $bla_{\text{OXA-51-type}}$ was performed using standard PCR based on the primers listed in Table 2 (14). The samples were also screened for the other major groups that confer clinically relevant resistance to carbapenems, i.e., $bla_{\text{OXA-23-type}}$, $bla_{\text{OXA-40-type (24-type)}}$, $bla_{\text{OXA-58-type}}$ (14, 15), as well as for the $bla_{\text{NDM-type}}$, $bla_{\text{IMP-type}}$, and $bla_{\text{KPC-type}}$ in a multiplex reaction (16, 17) and for $bla_{\text{VIM-type}}$ in a single PCR (18) (Table 2). Isolates not carrying another carbapenemase gene apart from $bla_{\text{OXA-51-type}}$ were subjected to PCR screening for the IS*Aba1* element upstream of $bla_{\text{OXA-51-type}}$ (19) and class 1 integron (*intI1*) as

**TABLE 1** Summary of CRAB clinical isolates in the GCC states

| Location | Hospital | Hospital category | No. of beds | Semiautomated system used for species identification and antibiotic sensitivity[a] | No. of carbapenem-resistant *A. baumannii* isolates | No. (%) of carbapenem resistance mechanisms[b] | | | No. of isolates carrying IS*Aba1* upstream of $bla_{\text{OXA-51-type}}$[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $bla_{\text{OXA-51-type}}$ | $bla_{\text{OXA-23-type}}$ | $bla_{\text{OXA-40-type}}$ | |
| Riyadh, Saudi Arabia | King Abdulaziz Medical City | Tertiary and academic | 1,000 | Vitek II | 49 | 49 (100) | 47 (96) | 0 | 0 |
| Khobar, Saudi Arabia | King Fahad University Hospital | Tertiary and academic | 450 | Vitek II | 31 | 31 (100) | 28 (90) | 0 | 3 |
| Abu Dhabi, United Arab Emirates | Sheikh Zayed Military hospital | Tertiary | 365 | Vitek II | 8 | 8 (100) | 8 (100) | 0 | NT[d] |
| Kuwait, Kuwait | Al-Ameri Hospital | Tertiary | 398 | Vitek II | 8 | 8 (100) | 8 (100) | 0 | NT |
| Muscat, Oman | The Royal Hospital | Tertiary and teaching | 750 | Phoenix | 5 | 5 (100) | 5 (100) | 0 | NT |
| Doha, Qatar | Hamad Medical Cooperation | Tertiary | >1,300 | Phoenix | 8 | 8 (100) | 8 (100) | 0 | NT |
| Manama, Bahrain | Salmaniya Medical Complex | Tertiary and teaching | 1,000 | | 8 | 8 (100) | 3 (38) | 5 (63) | NT |
| Total | | | | | 117 | 117 (100) | 107 (91) | 5 (4) | 3 |

[a] Vitek II, bioMérieux; Phoenix, Becton, Dickinson.
[b] All isolates were negative for $bla_{\text{OXA-58-type}}$, $bla_{\text{NDM-type}}$, $bla_{\text{KPC-type}}$, $bla_{\text{IMP-type}}$, and $bla_{\text{VIM-type}}$ PCR.
[c] Screening for IS*Aba1* element upstream of $bla_{\text{OXA-51-type}}$ was only performed on the five isolates with negative carbapenemase genes (except $bla_{\text{OXA-51-type}}$).
[d] NT, not tested because they were positive for either $bla_{\text{OXA-23-type}}$ or $bla_{\text{OXA-40-type}}$.

**TABLE 2** Sequence of primers used to screen for the carbapenemase-encoding genes, IS*Aba1* upstream of $bla_{\text{OXA-51-type}}$, and class 1 integron

| Primer | Target | Sequence (5′ to 3′) | Size (bp) | Annealing temperature (°C) | Reference |
|---|---|---|---|---|---|
| OXA-51-F | $bla_{\text{OXA-51}}$ | TAATGCTTTGATCGGCCTTG | 353 | 60 | 14 |
| OXA-51-R | | TGGATTGCACTTCATCTTGG | | | |
| OXA-23-F | $bla_{\text{OXA-23}}$ | GATGTGTCATAGTATTCGTCG | 1,065 | 52 | 15 |
| OXA-23-R | | TCACAACAACTAAAAGCACTG | | | |
| OXA-40-F | $bla_{\text{OXA-40 (24)}}$ | GGTTAGTTGGCCCCCTTAAA | 246 | 50 | 14 |
| OXA-40-R | | AGTTGAGCGAAAAGGGGATT | | | |
| OXA-58-F | $bla_{\text{OXA-58}}$ | AAGTATTGGGGCTTGTGCTG | 599 | 56 | 14 |
| OXA-58-R | | CCCCTCTGCGCTCTACATAC | | | |
| NDM-F | $bla_{\text{NDM}}$ | GCAGGTTGATCTCCTGCTTG | 203 | 55 | 16 |
| NDM-R | | ACGGTTTGGCGATCTGGT | | | |
| IMP-F | $bla_{\text{IMP}}$ | CTACCGCAGCAGAGTCTTTGC | 591 | 58 | 17 |
| IMP-R | | GAACAACCAGTTTTGCCTTACC | | | |
| KPC-F | $bla_{\text{KPC}}$ | ATCTGACAACAGGCATGACG | 452 | 55 | 16 |
| KPC-R | | GACGGCCAACACAATAGGTG | | | |
| VIM-F | $bla_{\text{VIM}}$ | GATGGTGTTTGGTCGCATA | 390 | 55 | 18 |
| VIM-R | | CGAATGCGCAGCACCAG | | | |
| IS*Aba1*-F | Upstream | CACGAATGCAGAAGTTG | >1,107 | 58 | 19 |
| OXA-51-R | | TGGATTGCACTTCATCTTGG | | | |
| HS317 | *intI1* | GAACCTTGACCGAACGC | Variable | 50 | 20 |
| HS320 | | AGTAAAGCCCTCGCTAG | | | |

previously described (20) (Table 2). PCRs were carried out using GoTaq green master mix (Promega, USA).

**Clonal analysis of carbapenem-resistant *A. baumannii*.** Genetic relatedness among CRAB isolates from the GCC states was determined by rep-PCR–based typing using the DiversiLab system (bioMérieux, Oakleigh, Australia). DNA fragment patterns were analyzed using the Kullback-Leibler statistical method to determine clonal relationships and to create the dendrogram with a 95% cutoff. Isolates were considered related and defined as rep-PCR clusters if they were ≥95% similar (21, 22).

Representative isolates, determined by DiversiLab rep-PCR clusters, from the six states were also analyzed by multilocus sequence typing (MLST). Genotyping by MLST was performed as previously described (23), using the seven housekeeping genes *glt*A, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*. Analyses of the allele sequences and ST were performed through the *A. baumannii* MLST website (http://pubmlst.org/abaumannii).

**Clinical data collection.** Clinical data included in this study for each patient identified as infected or colonized with CRAB were collected by the participating institutions. A concise one-page questionnaire was used to collect demographic data, the clinical source of the isolates, microbiology results, antibiotic exposure, travel within the last 6 months, and medical history. Hospital-acquired infections were defined by a positive microbiology culture from an infection in patients who were hospitalized for ≥48 h. Patients transferred from another hospital had their hospital stay duration calculated from the date of the first hospital admission (24). Hospital-acquired colonization was defined by a positive microbiology culture of surveillance sampling and not associated with the clinical manifestation of an infection from patients who had been hospitalized for ≥48 h. Health care-associated infections were classified as infections occurring within 48 h after admission in patients who were hospitalized during the previous 90 days; who received hemodialysis, intravenous medication, or home wound care in the 30 days before the infection; or who were residents of nursing homes or long-term-care facilities. Otherwise, cases were considered to be community acquired (24).

**Human ethics.** The University of Queensland granted human ethics clearance to conduct this project (2011000474). Permission from King Abdulaziz Medical City, Ministry of National Guard-Health Affairs, Saudi Arabia, was granted to conduct the regionwide collaborative study on multidrug-resistant Gram-negative bacilli.

## RESULTS

**Bacterial isolates and carbapenem susceptibility.** A total of 117 nonrepetitive isolates nonsusceptible to imipenem and/or meropenem were further assessed. The numbers of CRAB isolates found in each participating hospital were as follows: Riyadh, Saudi Arabia, 49; Khobar, Saudi Arabia, 31; Kuwait, 8; United Arab Emirates, 8; Oman, 5; Qatar, 8; Bahrain, 8 (Table 1). Carbapenem coresistance to sulfamethoxazole-trimethoprim (93%), gentamicin (95%), amikacin (83%), ciprofloxacin (98%), tetracycline (68%), ticarcillin-clavulanic acid (100%), piperacillin-tazobactam (100%), and ampicillin-sulbactam (74%) was found for the 117 CRAB isolates.

**Carbapenemase-encoding genes.** All 117 isolates were positive for $bla_{\text{OXA-51-type}}$. The clinically relevant carbapenemase-encoding gene $bla_{\text{OXA-23-type}}$ was found in 107 isolates (91%). As a breakdown, 47 (96%) of the CRAB isolates from Riyadh, Saudi Arabia, 28 (90%) from Khobar, Saudi Arabia, 3 (38%) from Bahrain, and all from Oman ($n = 5$), Kuwait ($n = 8$), Qatar ($n = 8$), and UAE ($n = 8$) carried $bla_{\text{OXA-23-type}}$. Five isolates from Bahrain carried $bla_{\text{OXA-40-type}}$ genes. None of the isolates had a positive PCR result for $bla_{\text{OXA-58-type}}$, $bla_{\text{NDM-type}}$, $bla_{\text{KPC-type}}$, $bla_{\text{IMP-type}}$, or $bla_{\text{VIM-type}}$. Five (4%) of the CRAB isolates did not give positive PCR results for any of the tested carbapenemase-encoding genes except $bla_{\text{OXA-51-type}}$. None of these five isolates carried the class 1 integron, but 3 isolates (2.8%) (from Khobar, Saudi Arabia) carried the IS*Aba1* element upstream of $bla_{\text{OXA-51-type}}$, which is known to mediate the carbapenem resistance phenotype (4). Two isolates (1.7%) (from Riyadh, Saudi Arabia) remained negative for all the tested carbapenemase genes.

**Genotyping and clonality.** A total of 16 DiversiLab rep-PCR clusters (clusters A to P) and 11 singletons were identified among the 117 study isolates (Table 3; see also Fig. S1 in the supplemental material). The main cluster (B) included 53 of the 117 isolates (45%) and represented isolates from all six locations (five states). The rep-PCR analysis showed that 11 isolates had unique banding

**TABLE 3** Clustering results based on rep-PCR patterns of 117 carbapenem-resistant *A. baumannii* isolates from the GCC states[a]

| Cluster or singleton | No. of isolates | | | Sequence type |
|---|---|---|---|---|
| | Total | By location | By carbapenemase gene[b] | |
| Clusters | | | | |
| A | 8 | Riyadh, Saudi Arabia, 8 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND[c] |
| B | 53 | Riyadh, Saudi Arabia, 22 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$, 21; $bla_{\text{OXA-51-type}}$, 1 | ST195; clustered with ST195 and ST208[d] |
| | | Khobar Saudi Arabia, 19 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$, 18; IS*Ab1* upstream of $bla_{\text{OXA-51-type}}$, 1 | Novel ST; clustered with novel ST and ST208 |
| | | Kuwait, 6 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST208; clustered with novel ST and ST195[d] |
| | | Bahrain, 2 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-40-like}}$ | ST208; clustered with ST208 |
| | | Oman, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST195; clustered with ST195 |
| | | UAE, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Clustered with ST195 |
| C | 3 | Bahrain, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-40-like}}$ | ND |
| D | 2 | Riyadh, Saudi Arabia, 2 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| E | 3 | Riyadh, Saudi Arabia, 2 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$, 1; $bla_{\text{OXA-51-type}}$, 1 | ST499; clustered with ST499 |
| | | Kuwait, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Clustered with ST499 |
| F | 2 | UAE, 2 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| G | 5 | Riyadh, Saudi Arabia, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST450 |
| | | Khobar, Saudi Arabia, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Clustered with ST450 |
| | | UAE, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | |
| H | 3 | Bahrain, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST452; clustered with ST452 |
| I | 4 | Riyadh, Saudi Arabia, 4 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Novel ST; clustered with novel ST |
| J | 3 | Riyadh, Saudi Arabia, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| K | 8 | Qatar, 6 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST299; clustered with ST299 |
| | | Kuwait, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Clustered with ST299 |
| | | UAE, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Clustered with ST299 |
| L | 2 | Qatar, 2 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST299; clustered with ST299 |
| M | 3 | Khobar, Saudi Arabia, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | Novel ST; clustered with novel ST |
| N | 3 | Khobar, Saudi Arabia, 3 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST436; clustered with ST436 |
| O | 2 | Oman, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| | | Riyadh, Saudi Arabia, 1 | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| P | 2 | Khobar, Saudi Arabia, 2 | IS*Ab1* upstream of $bla_{\text{OXA-51-type}}$ | ND |
| Singletons | | | | |
| 1 | | Riyadh, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ST195 |
| 2 | | UAE | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 3 | | Riyadh, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 4 | | UAE | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 5 | | Riyadh, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 6 | | UAE | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 7 | | Khobar, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 8 | | Oman | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 9 | | Riyadh, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 10 | | Riyadh, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |
| 11 | | Riyadh, Saudi Arabia | $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ | ND |

[a] For a full dendrogram, see Fig. S1 in the supplemental material.
[b] No number indicates that all isolates were the same type(s).
[c] ND, not determined.
[d] There is only a single allele difference between sequence types ST195 and ST208.

patterns. These singletons were unrelated to the remaining isolates, representing various locations, except for singleton 1, which was only slightly less than 95% similar to cluster A isolates.

Well-defined clusters by location were seen in clusters A, C, F, H, M, N, and P (Table 3), whereas the remaining rep-PCR clones included isolates from two or more locations. Isolates harboring $bla_{\text{OXA-23-type}}$ genes were scattered throughout the rep-PCR patterns, except for clusters K, L, and M, in which all of the isolates carried $bla_{\text{OXA-51-type}}$ and $bla_{\text{OXA-23-type}}$ genes.

Seven different sequence types (ST195, ST208, ST229, ST436, ST450, ST452, and ST499) and three novel STs were assigned to the 15 representative isolates. ST195 isolates clustered with ST208 within the same cluster; note that there is only a single allele difference between these two sequence types, which represents a true single locus variant. ST195, ST208, and ST436 fall under the widely disseminated clonal complex CC92 (also known as international clone number 2), while ST229 is under CC110 (also known as international clone number 3). We found good correlation between the sequence types and the rep-PCR clusters.

**Clinical data.** The clinical data are summarized in Table 4. Demographic and clinical data were successfully retrieved for 100 patients (85%) as follows: Riyadh, Saudi Arabia, 49; Khobar,

**TABLE 4** Demographic and clinical characteristics of 100 patients with carbapenem-resistant *A. baumannii*

| Characteristic | No. of patients |
|---|---|
| Age (yr) | |
| 0–7 | 4 |
| 7–18 | 5 |
| 18–30 | 23 |
| 30–50 | 19 |
| 50–60 | 11 |
| 60–70 | 13 |
| >70 | 25 |
| Gender | |
| Male | 68 |
| Female | 32 |
| Residency status | |
| Local[a] | 79 |
| Resident | 20 |
| No data | 1 |
| Source | |
| Blood | 18 |
| Sputum | 22 |
| Swab | 39 |
| Urine | 6 |
| Other[b] | 15 |
| Initial medical condition | |
| Burns | 6 |
| Community acquired pneumonia | 5 |
| Hospital acquired pneumonia | 8 |
| Motor vehicle accident | 17 |
| Hemodialysis | 3 |
| Other | 30 |
| No data | 31 |
| Infection category | |
| Health care-associated infection | 24 |
| Hospital acquired | |
| Infection | 53 |
| Colonization | 7 |
| Community-acquired infection | 8 |
| No data | 8 |
| Antibiotic exposure before isolation | |
| Yes | 87 |
| 1–2 days | 34 |
| 3–4 days | 31 |
| 5–6 days | 9 |
| ≥7 days | 3 |
| No data | 10 |
| No | 12 |
| No data | 1 |
| Previous medical history | |
| Outpatient clinic or emergency room | |
| ≤30 days | 14 |
| 1–6 mo | 24 |
| 6–12 mo | 18 |
| ≥12 mo | 21 |
| Hemodialysis | |
| ≤30 days | 14 |
| 1–6 mo | 9 |
| 6–12 mo | 4 |
| ≥12 mo | 5 |
| Surgical procedure | |
| ≤30 days | 24 |
| 1–6 mo | 18 |
| 6–12 mo | 5 |
| ≥12 mo | 14 |
| Hospital admission | |
| ≤30 days | 46 |
| 1–6 mo | 39 |
| 6–12 mo | 13 |
| ≥12 mo | 23 |
| Recent overseas medical treatment | |
| Yes | 4 |
| Bangladesh | 1 |
| India | 1 |
| Singapore | 1 |
| Thailand | 1 |
| No | 45 |
| Never | 1 |
| No data | 50 |

[a] Citizens of the designated state.
[b] Includes respiratory specimens and body fluids.

Saudi Arabia, 30; United Arab Emirates, 8; Oman, 5; Bahrain, 8. We found that 25 of the patients were ≥ 70 years old, and the second group ($n = 23$) was between 18 and 30 years old (Table 4). Most of the identified patients were male ($n = 68$) and local citizens ($n = 79$). CRAB isolates were mainly isolated from swab specimens ($n = 39$) and sputum ($n = 22$), and blood ($n = 18$) samples (Table 4). Note that 24% of the isolates represented health care-associated infections, while 53% were associated with hospital-acquired (nosocomial) infections and 7% were colonizing hospital patients. Eight percent of the isolates were classified as community acquired, and we did not categorize the last 8% of isolates due to lack of data. Antibiotics were administered to 87 patients before the isolation of CRAB. Overseas medical treatment information was not collectable for 50% of the patients. Four patients had recently traveled to Bangladesh, India, Singapore, or Thailand for medical proposes. An isolate from a UAE patient who recently traveled to Bangladesh for medical purposes clustered with another isolate from a UAE patient with no recent travel (cluster F) (Table 3; see also dendrogram in Fig. S1 of the supplemental material). Interestingly, a UAE isolate obtained from a patient who recently traveled to Singapore clustered with a group of isolates from Saudi Arabia, Oman, and Kuwait (cluster B) (Table 3). However, isolates from patients who recently traveled to India and Thailand did not show similarity to any another isolate tested (singletons 2 and 6, respectively) (Table 3). The remaining 46 patients did not receive overseas medical treatment.

## DISCUSSION

We described the molecular genetics of CRAB isolates from patients in selected GCC hospitals. We found that OXA-23-type was the major carbapenemase mechanism responsible for the resistance phenotype. This finding is similar to data previously reported from the Gulf region (3, 25, 26) and neighboring Egypt (27). OXA-23-type contributes to carbapenem resistance in *A.*

*baumannii* in many other parts of the world (2) and has been associated with outbreaks in Spain (28), Italy (29), and the United States (30). It is important to note that outbreaks have occurred as a result of the international transfer of patients (31). This represents a risk factor to hospitals in countries where CRAB is not endemic that receive patients from countries with a high prevalence of CRAB.

Epidemiological tools are important in developing effective strategies for monitoring CRAB. We utilized rep-PCR typing using the DiversiLab system and MLST typing, as these methods demonstrated validity in comparing geographically diverse groups of clinical isolates (32). In this study, we found a correlation between the carbapenemase gene profile and rep-PCR typing together with MLST results. We also found that several large clusters of indistinguishable isolates that produce dominant OXA-23-type enzymes are not only circulating within hospitals of the GCC states but also across borders. This includes the internationally disseminated ST208 and ST195, which belong to clonal complex 92 (33–36). This finding suggests that certain strains of CRAB have been prevalent in some Gulf region hospitals for an extended period. It also points to the need for optimizing infection control practices to avoid cross transmission and potential outbreaks. Lastly, this finding highlights the unanswered question regarding the source of *A. baumannii* and how certain strains found their way into the hospital environment.

We detected OXA-40-type–producing CRAB in five isolates in only a single hospital in Bahrain. Three of the isolates were indistinguishable, but two were quite diverse (Table 3). OXA-40 producers have been identified in Europe and the United States (2). However, to our knowledge, this is the second report of OXA-40 in Bahrain and the third from the GCC region (3). Three isolates from Saudi Arabia had an IS*Aba1* element upstream of $bla_{\text{OXA-51-type}}$. This carbapenem resistance mechanism has been described in Saudi Arabia (37, 38). These findings of sporadic resistance mechanisms might indicate a slow change in the molecular epidemiology of CRAB in the Gulf region.

No isolate was found to produce OXA-58, NDM, VIM, IMP, or KPC. This is in agreement with previous work reported on CRAB in the Gulf region (3). However, genes of the NDM type carried by CRAB have been reported from the Indian subcontinent (39, 40), Asia (41), Lebanon (42), and Europe (5). A related outbreak with five cases was reported from France in an intensive care unit (ICU) where the index patient was transferred from Algeria (43). It is believed that NDM-1 occurred in *Acinetobacter* spp. before becoming prevalent among *Enterobacteriaceae* (39). Other metallo-β-lactamases, such as VIM and IMP, are less common in *A. baumannii* (2), although recent reports from India found VIM in 45% of tested CRAB isolates (44). KPC-producing *A. baumannii* were not known (2) until a report from Puerto Rico (45), and they were subsequently found in 4.3% of tested isolates from Puerto Rico (46). We did not search for $bla_{\text{GES}}$, although a recent report documented this β-lactamase in the Gulf region (26).

In this study, we found that the vast majority (84%) of tested CRAB isolates were associated with health care exposure. CRAB has been described in 23% of patients with ventilator-associated pneumonia in Riyadh (1, 47). An epidemiological study from Riyadh looked at the factors related to health care-associated infections caused by multidrug-resistant *A. baumannii* among a pediatric population and found that ICU and hospital admissions after burns increased the risk of acquiring related infections (48). A recent study identified patients at risk for bloodstream infection due to *A. baumannii-A. calcoaceticus* complex and mainly found that critically ill and interhospital transferred patients and patients who were heavily exposed to health care settings and invasive devices are at the highest risk (49).

In summary, we evaluated CRAB in hospitals from across Gulf Cooperation Council states. Although this is not a formal surveillance study, it is the first snapshot study to determine the molecular epidemiology of CRAB in the region. Investigating the epidemic situation within or across hospitals provides data to support policy making and practices in regard to infection control. Our findings of multiple large clusters of OXA-23-type–producing *A. baumannii* within a hospital and across countries have important implications in controlling the spread of CRAB in the Middle East and in hospitals receiving patients transferred from the region. Additionally, optimization of antibiotic stewardship in hospitals and community pharmacies and within the agricultural setting should be a priority for health agencies in the Gulf region.

## ACKNOWLEDGMENTS

The Surveillance of Antibiotic Resistant Gram Negative Bacilli in Saudi Arabia and the Gulf States (project no. IRBC/193/12) is supported by the Ministry of National Guard, Health Affairs, King Abdullah International Medical Research Centre, Saudi Arabia. H.M.Z. is academically sponsored by the government of Saudi Arabia to pursue postgraduate studies in the field of clinical microbiology and infectious diseases. T.R.W. is funded by the HEFC, British government.

We thank all the staff from the collaborating clinical microbiology laboratories across the GCC states and the active role of the GCC Center for Infection Control under the umbrella of the Ministry of National Guard Health Affairs. We also thank Wan Keat Yam and Moongaambikai Thangaveloo for helping to prepare the transport media.

D.L.P. has received honoraria for advisory board participation, not relating to this work, from AstraZeneca, Bayer, Cubist, Pfizer, and Merck.

## REFERENCES

1. **El-Saed A, Balkhy HH, Al-Dorzi HM, Khan R, Rishu AH, Arabi YM.** 2013. *Acinetobacter* is the most common pathogen associated with late-onset and recurrent ventilator-associated pneumonia in an adult intensive care unit in Saudi Arabia. Int J Infect Dis **17:**e696–e701. http://dx.doi.org/10.1016/j.ijid.2013.02.004.
2. **Peleg AY, Seifert H, Paterson DL.** 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev **21:**538–582. http://dx.doi.org/10.1128/CMR.00058-07.
3. **Zowawi HM, Balkhy HH, Walsh TR, Paterson DL.** 2013. Beta-lactamase production in key gram-negative pathogen isolates from the Arabian Peninsula. Clin Microbiol Rev **26:**361–380. http://dx.doi.org/10.1128/CMR.00096-12.
4. **Al-Agamy MH, Khalaf NG, Tawfick MM, Shibl AM, El Kholy A.** 2014. Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt. Int J Infect Dis **22:**49–54. http://dx.doi.org/10.1016/j.ijid.2013.12.004.
5. **Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, Schrenzel J, Nordmann P.** 2012. Dissemination of New Delhi metallo-beta-lactamase-1-producing *Acinetobacter baumannii* in Europe. Clin Microbiol Infect **18:**E362–E365. http://dx.doi.org/10.1111/j.1469-0691.2012.03928.x.
6. **Espinal P, Poirel L, Carmeli Y, Kaase M, Pal T, Nordmann P, Vila J.** 2013. Spread of NDM-2-producing *Acinetobacter baumannii* in the Middle East. J Antimicrob Chemother **68:**1928–1930. http://dx.doi.org/10.1093/jac/dkt109.
7. **Yang J, Chen Y, Jia X, Luo Y, Song Q, Zhao W, Wang Y, Liu H, Zheng D, Xia Y, Yu R, Han X, Jiang G, Zhou Y, Zhou W, Hu X, Liang L, Han L.** 2012. Dissemination and characterization of NDM-1-producing *Acinetobacter pittii* in an intensive care unit in China. Clin Microbiol Infect **18:**E506–E513. http://dx.doi.org/10.1111/1469-0691.12035.

8. **Kapiszewski A.** 2006. Arab versus Asian migrant workers in the GCC countries, UN/POP/EGM/2006/02. *In* United Nations Expert Group Meeting on International Migration and Development in the Arab Region, Beirut, Lebanon.
9. **Memish ZA.** 2010. The Hajj: communicable and non-communicable health hazards and current guidance for pilgrims. Euro Surveill **15:**19671. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19671.
10. **Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, Alfaresi M, Ibrahim E, Al-Jardani A, Al-Abri S, Al Salman J, Dashti AA, Kutbi AH, Schlebusch S, Sidjabat HE, Paterson DL.** 2014. Molecular characterization of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in the countries of the Gulf Cooperation Council: dominance of OXA-48 and NDM producers. Antimicrob Agents Chemother **58:**3085–3090. http://dx.doi.org/10.1128/AAC.02050-13.
11. **European Committee on Antimicrobial Susceptibility Testing (EUCAST).** 2014. Breakpoint tables for interpretation of MICs and zone diameters, version 4. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf
12. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
13. **Higgins PG, Lehmann M, Wisplinghoff H, Seifert H.** 2010. gyrB multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3. J Clin Microbiol **48:**4592–4594. http://dx.doi.org/10.1128/JCM.01765-10.
14. **Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM.** 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents **27:**351–353. http://dx.doi.org/10.1016/j.ijantimicag.2006.01.004.
15. **Afzal-Shah M, Woodford N, Livermore DM.** 2001. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother **45:**583–588. http://dx.doi.org/10.1128/AAC.45.2.583-588.2001.
16. **Lim S.** 2011. Development of multiplex PCR: β-lactamase genes and virulence determinants in *E. coli*.University of Queensland Centre for Clinical Research, Brisbane St Lucia, Australia.
17. **Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother **44:**891–897. http://dx.doi.org/10.1128/AAC.44.4.891-897.2000.
18. **Ellington MJ, Kistler J, Livermore DM, Woodford N.** 2007. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother **59:**321–322. http://dx.doi.org/10.1093/jac/dkl481.
19. **Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL.** 2006. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett **258:**72–77. http://dx.doi.org/10.1111/j.1574-6968.2006.00195.x.
20. **Sidjabat HE, Townsend KM, Hanson ND, Bell JM, Stokes HW, Gobius KS, Moss SM, Trott DJ.** 2006. Identification of $bla_{CMY-7}$ and associated plasmid-mediated resistance genes in multidrug-resistant *Escherichia coli* isolated from dogs at a veterinary teaching hospital in Australia. J Antimicrob Chemother **57:**840–848. http://dx.doi.org/10.1093/jac/dkl057.
21. **Hojabri Z, Pajand O, Bonura C, Aleo A, Giammanco A, Mammina C.** 2014. Molecular epidemiology of *Acinetobacter baumannii* in Iran: endemic and epidemic spread of multiresistant isolates. J Antimicrob Chemother **69:**2383–2387. http://dx.doi.org/10.1093/jac/dku045.
22. **Pasanen T, Koskela S, Mero S, Tarkka E, Tissari P, Vaara M, Kirveskari J.** 2014. Rapid molecular characterization of *Acinetobacter baumannii* clones with rep-PCR and evaluation of carbapenemase genes by new multiplex PCR in Hospital District of Helsinki and Uusimaa. PLoS One **9:**e85854. http://dx.doi.org/10.1371/journal.pone.0085854.
23. **Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F.** 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol **43:**4382–4390. http://dx.doi.org/10.1128/JCM.43.9.4382-4390.2005.
24. **Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, Lamm W, Clark C, MacFarquhar J, Walton AL, Reller LB, Sexton DJ.** 2002. Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med **137:**791–797. http://dx.doi.org/10.7326/0003-4819-137-10-200211190-00007.
25. **Aly M, Tayeb HT, Al Johani SM, Alyamani EJ, Aldughaishem F, Alabdulkarim I, Balkhy HH.** 2014. Genetic diversity of OXA-51-like genes among multidrug-resistant *Acinetobacter baumannii* in Riyadh, Saudi Arabia. Eur J Clin Microbiol Infect Dis **33:**1223–1228. http://dx.doi.org/10.1007/s10096-014-2068-0.
26. **Bonnin RA, Rotimi VO, Al Hubail M, Gasiorowski E, Al Sweih N, Nordmann P, Poirel L.** 2013. Wide dissemination of GES-type carbapenemases in *Acinetobacter baumannii* isolates in Kuwait. Antimicrob Agents Chemother **57:**183–188. http://dx.doi.org/10.1128/AAC.01384-12.
27. **Fouad M, Attia AS, Tawakkol WM, Hashem AM.** 2013. Emergence of carbapenem-resistant *Acinetobacter baumannii* harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. Int J Infect Dis **17:**e1252–1254. http://dx.doi.org/10.1016/j.ijid.2013.07.012.
28. **Merino M, Poza M, Roca I, Barba MJ, Sousa MD, Vila J, Bou G.** 2014. Nosocomial outbreak of a multiresistant *Acinetobacter baumannii* expressing OXA-23 carbapenemase in Spain. Microb Drug Resist **20:**259–263. http://dx.doi.org/10.1089/mdr.2013.0127.
29. **Dettori M, Piana A, Deriu MG, Lo Curto P, Cossu A, Musumeci R, Cocuzza C, Astone V, Contu MA, Sotgiu G.** 2014. Outbreak of multidrug-resistant *Acinetobacter baumannii* in an intensive care unit. New Microbiol **37:**185–191. http://www.newmicrobiologica.org/PUB/allegati_pdf/2014/2/185.pdf.
30. **Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, Ecker DJ, Adams MD, Toltzis P, Dul MJ, Windau A, Bajaksouzian S, Jacobs MR, Salata RA, Bonomo RA.** 2010. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. J Antimicrob Chemother **65:**1807–1818. http://dx.doi.org/10.1093/jac/dkq191.
31. **Ahmed-Bentley J, Chandran AU, Joffe AM, French D, Peirano G, Pitout JD.** 2013. Gram-negative bacteria that produce carbapenemases causing death attributed to recent foreign hospitalization. Antimicrob Agents Chemother **57:**3085–3091. http://dx.doi.org/10.1128/AAC.00297-13.
32. **Sabat AJ, Budimir A, Nashev D, Sa-Leao R, van Dijl J, Laurent F, Grundmann H, Friedrich AW, ESCMID Study Group of Epidemiological Markers (ESGEM).** 2013. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill **18:**20380. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20380.
33. **Adams-Haduch JM, Onuoha EO, Bogdanovich T, Tian GB, Marschall J, Urban CM, Spellberg BJ, Rhee D, Halstead DC, Pasculle AW, Doi Y.** 2011. Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States. J Clin Microbiol **49:**3849–3854. http://dx.doi.org/10.1128/JCM.00619-11.
34. **Deng M, Zhu MH, Li JJ, Bi S, Sheng ZK, Hu FS, Zhang JJ, Chen W, Xue XW, Sheng JF, Li LJ.** 2014. Molecular epidemiology and mechanisms of tigecycline resistance in clinical isolates of *Acinetobacter baumannii* from a Chinese university hospital. Antimicrob Agents Chemother **58:**297–303. http://dx.doi.org/10.1128/AAC.01727-13.
35. **Li Y, Pan C, Zhao Z, Zhao Z, Chen H, Lu W.** 2013. Effects of a combination of amlodipine and imipenem on 42 clinical isolates of *Acinetobacter baumannii* obtained from a teaching hospital in Guangzhou, China. BMC Infect Dis **13:**548. http://dx.doi.org/10.1186/1471-2334-13-548.
36. **Tada T, Miyoshi-Akiyama T, Shimada K, Shimojima M, Kirikae T.** 2014. Dissemination of 16S rRNA methylase ArmA-producing *Acinetobacter baumannii* and emergence of OXA-72 carbapenemase coproducers in Japan. Antimicrob Agents Chemother **58:**2916–2920. http://dx.doi.org/10.1128/AAC.01212-13.
37. **Alsultan AA, Hamouda A, Evans BA, Amyes SG.** 2009. *Acinetobacter baumannii*: emergence of four strains with novel $bla_{OXA-51-like}$ genes in patients with diabetes mellitus. J Chemother **21:**290–295. http://dx.doi.org/10.1179/joc.2009.21.3.290.
38. **Abdalhamid B, Hassan H, Itbaileh A, Shorman M.** 2014. Characterization of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in a tertiary care hospital in Saudi Arabia. New Microbiol **37:**65–73. http://www.newmicrobiologica.org/PUB/allegati_pdf/2014/1/65.pdf.
39. **Jones LS, Toleman MA, Weeks JL, Howe RA, Walsh TR, Kumarasamy KK.** 2014. Plasmid carriage of $bla_{NDM-1}$ in clinical *Acinetobacter bauman-*

*nii* isolates from India. Antimicrob Agents Chemother **58:**4211–4213. http://dx.doi.org/10.1128/AAC.02500-14.

40. **Sartor AL, Raza MW, Abbasi SA, Day KM, Perry JD, Paterson DL, Sidjabat HE.** 2014. Molecular epidemiology of NDM-1-producing *Enterobacteriaceae* and *Acinetobacter baumannii* isolates from Pakistan. Antimicrob Agents Chemother **58:**5589–5593. http://dx.doi.org/10.1128/AAC.02425-14.
41. **Zhang R, Hu YY, Yang XF, Gu DX, Zhou HW, Hu QF, Zhao K, Yu SF, Chen GX.** 2014. Emergence of NDM-producing non-*baumannii Acinetobacter* spp. isolated from China. Eur J Clin Microbiol Infect Dis **33:**853–860. http://dx.doi.org/10.1007/s10096-013-2024-4.
42. **Rafei R, Dabboussi F, Hamze M, Eveillard M, Lemarie C, Mallat H, Rolain JM, Joly-Guillou ML, Kempf M.** 2014. First report of $bla_{NDM-1}$-producing *Acinetobacter baumannii* isolated in Lebanon from civilians wounded during the Syrian war. Int J Infect Dis **21:**21–23. http://dx.doi.org/10.1016/j.ijid.2014.01.004.
43. **Decousser JW, Jansen C, Nordmann P, Emirian A, Bonnin RA, Anais L, Merle JC, Poirel L.** 2013. Outbreak of NDM-1-producing *Acinetobacter baumannii* in France, January to May 2013. Euro Surveill 18:pii=20547. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20547.
44. **Amudhan MS, Sekar U, Kamalanathan A, Balaraman S.** 2012. $bla_{IMP}$ and $bla_{VIM}$ mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India. J Infect Dev Ctries **6:**757–762. http://dx.doi.org/10.3855/jidc.2268.
45. **Robledo IE, Aquino EE, Sante MI, Santana JL, Otero DM, Leon CF, Vazquez GJ.** 2010. Detection of KPC in *Acinetobacter* spp. in Puerto Rico. Antimicrob Agents Chemother **54:**1354–1357. http://dx.doi.org/10.1128/AAC.00899-09.
46. **Robledo IE, Aquino EE, Vazquez GJ.** 2011. Detection of the KPC gene in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-based nosocomial surveillance study in Puerto Rico. Antimicrob Agents Chemother **55:**2968–2970. http://dx.doi.org/10.1128/AAC.01633-10.
47. **Balkhy HH, El-Saed A, Maghraby R, Al-Dorzi HM, Khan R, Rishu AH, Arabi YM.** 2014. Drug-resistant ventilator associated pneumonia in a tertiary care hospital in Saudi Arabia. Ann Thorac Med **9:**104–111. http://dx.doi.org/10.4103/1817-1737.128858.
48. **Balkhy HH, Bawazeer MS, Kattan RF, Tamim HM, Al Johani SM, Aldughashem FA, Al Alem HA, Adlan A, Herwaldt LA.** 2012. Epidemiology of *Acinetobacter* spp.-associated healthcare infections and colonization among children at a tertiary-care hospital in Saudi Arabia: a 6-year retrospective cohort study. Eur J Clin Microbiol Infect Dis **31:**2645–2651. http://dx.doi.org/10.1007/s10096-012-1608-8.
49. **Chopra T, Marchaim D, Johnson PC, Awali RA, Doshi H, Chalana I, Davis N, Zhao JJ, Pogue JM, Parmar S, Kaye KS.** 2014. Risk factors and outcomes for patients with bloodstream infection due to *Acinetobacter baumannii-calcoaceticus* complex. Antimicrob Agents Chemother **58:**4630–4635. http://dx.doi.org/10.1128/AAC.02441-14.