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 (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 element upstream of blaOXA-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 (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-lactamas (MBL) have been associated with the resistance phenotype (2). The existence of ISAba1 elements upstream of the blaOXA-31-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
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 non-citizens. The current socioeconomic structure of the GCC states and the Indian subcontinent, where NDM enzymes have been reported (18).

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), ciprofloxacin (5 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 blaOXA-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., blaOXA-23-type, blaOXA-40-type (24-type), blaOXA-58-type (14, 15), as well as for the blalNDM-type, blalIMI-type, and blalVIM-type in a multiplex reaction (16, 17) and for blalKPC-type in a single PCR (18) (Table 2). Isolates not carrying another carbapenemase gene apart from blalOXA-51-type were subjected to PCR screening for the ISAb1 element upstream of blalOXA-51-type (19) and class 1 integron (intI1) as
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 gltA, gyrB, gdhB, recA, rpoD, gpi, and rpsL. 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 culture and susceptibility 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 Abdullah 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). Carbenem coreistance 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.

**Carbenemencoding genes.** All 117 isolates were positive for blaOXA-51-type. The clinically relevant carbapenem-encoding gene blaOXA-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 blaOXA-23-type. Five isolates from Bahrain carried blaOXA-40-type genes. None of the isolates had a positive PCR result for blaOXA-58-type, blaNDM-type, blaKPC-type, blaOXA-51MP-type, or blaOXA-23M-type. Five (4%) of the CRAB isolates did not give positive PCR results for any of the tested carbapenem-encoding genes except blaOXA-51-type. None of these five isolates carried the class 1 integron, but 3 isolates (2.8%) from Khobar, Saudi Arabia, carried the ISAba1 element upstream of blaOXA-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 carbapenem 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 patterns.
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, and H (Table 3), whereas the remaining rep-PCR clones included isolates from two or more locations. Isolates harboring blaOXA-23-type genes were scattered throughout the rep-PCR patterns, except for clusters K, L, and M, in which all of the isolates carried blaOXA-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 single locus variant. ST195, ST208, and ST436 fall under the national clone number 2), while ST229 is under CC110 (also known as international clone number 1). ST195 isolates clustered with ST208, ST229, and ST436; clustered with ST299; clustered with ST195 and ST208. ST195, ST208, and ST436 fall under the single locus variant. ST195, ST208, and ST436 fall under the national clone number 2), while ST229 is under CC110 (also known as international clone number 1). ST195 isolates clustered with ST208, ST229, and ST436; clustered with ST299; clustered with ST195 and ST208.

**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,
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 healthcare-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 purposes. 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.

### TABLE 4 (Continued)

<table>
<thead>
<tr>
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</tr>
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<td>≥12 mo</td>
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<td>Surgical procedure</td>
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<td>≤30 days</td>
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<td>1–6 mo</td>
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<td>46</td>
</tr>
<tr>
<td>1–6 mo</td>
<td>39</td>
</tr>
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<td>6–12 mo</td>
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</tr>
<tr>
<td>Never</td>
<td>1</td>
</tr>
<tr>
<td>No data</td>
<td>50</td>
</tr>
</tbody>
</table>

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 healthcare-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 purposes. 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.
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 these 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 ISAb11 element upstream of the blaOXA-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 the blaKPC 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.

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