Comparison of \textit{Acinetobacter baumannii} Isolates from the United Kingdom and the United States That Were Associated with Repatriated Casualties of the Iraq Conflict

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\textit{Acinetobacter baumannii} is an increasingly important opportunistic pathogen that affects vulnerable groups of people, particularly patients in intensive care and burn units (5). It is usually associated with multiple antibiotic resistance, and few therapeutic agents remain that are effective against many strains of the organism (6, 20). Although \textit{Acinetobacter} species are commonly found in soil and water, the origin of the multiresistant outbreak strains of \textit{A. baumannii} in hospitals, which are often genetically similar, remains obscure (8, 14). \textit{Acinetobacter} infections have frequently been reported in trauma victims (1, 4, 7, 9, 12, 13, 15), but it is not clear whether these infections arose from environmental contamination at the scene or were acquired from the health care facilities in which these patients were subsequently treated.

Military and civilian casualties from the Iraq conflict have been repatriated to both the United Kingdom and the United States, and in both, \textit{Acinetobacter} colonizations and infections have been reported. There has been some concern that multidrug-resistant outbreak strains of \textit{A. baumannii} may have been introduced into hospitals via these casualties. In the United Kingdom, some 10 strains, consisting of outbreak and sporadic strains, have been associated with casualties returning from Iraq, although 3 of the outbreak strains have previously been found in United Kingdom hospitals (Table 1). From the United States, there has been a published report of 102 patients with \textit{A. baumannii} bacteremias (4) and a media report (9a) of some 280 cases of infection in army medical centers that had received patients injured in the Iraq/Kuwait region or in Afghanistan. Some of these infections had been reported from Landstuhl Regional Medical Center, in Germany, which is a U.S. military regional medical center for returning soldiers along the aeromedical evacuation route from Iraq/Kuwait to the United States and United Kingdom.

(A report of a United Kingdom nationwide survey of \textit{Acinetobacter} infections with possible links to Iraq, which includes some of the observations from this study, has been previously published (10).)

To identify if a common source(s) may be involved, 15 representative isolates of \textit{A. baumannii} from the outbreak investigation archive at Walter Reed Army Medical Center (WRAMC), a military tertiary care hospital in Washington, D.C., where many U.S. casualties from the war in Iraq receive care, were compared with United Kingdom isolates with links to Iraq received by the Laboratory of HealthCare Associated Infection (Table 1). Comparison was by pulsed-field gel electrophoresis (PFGE) of Apal-digested genomic DNA, as described previously (16). Class 1 integrase and \textit{bla}OXA-23 genes were detected using published methods (2, 11). Amplification and sequencing of the class 1 integron variable regions and determination of the number of copies of orfX were carried out as previously described (17). Integrons were compared by cassette array size and HaeIII restriction digestion pattern. Identification of outbreak strains as \textit{A. baumannii} was by amplified rRNA gene restriction analysis (19).

A dendrogram of the PFGE profiles is given in Fig. 1. The United Kingdom isolates included representatives of the “T strain,” first seen in May 2003, which has been strongly associated with casualties from Iraq in hospital H1. They also included representatives of OXA-23 clone 2, a carbapenem-resistant clone with \textit{bla}OXA-23, which was responsible for an outbreak in hospital H2 in late 2003, following admission of a civilian casualty from Iraq. However, this clone had previously been found in three other United Kingdom hospitals with no known connection with Iraq. In addition, a comparatively minor outbreak strain, associated with casualties from Iraq in two hospitals, was found. Profiles obtained of the U.S. isolates, collected between March and October 2003 from inpatients at...
WRAMC, Landstuhl Regional Medical Center, and a U.S. Army field hospital, Baghdad, Iraq, showed that these also included representatives of the T strain, OXA-23 clone 2, and the minor outbreak strain (referred to as H1AC-2, H3AC-1, or USAC-3) common to two hospitals in the United Kingdom. The remaining nine U.S. isolates, representing eight strains, were PCR negative for the class 1 integrase gene. Integrons are a characteristic of outbreak strains of A. baumannii and can be readily detected by integrase gene PCR; PCR-negative isolates are therefore likely to be sporadic (11, 17). These nine U.S. isolates represented different strains from those found in the United Kingdom. The United Kingdom isolates alone included representatives of the W strain, known to belong to European clone 1 (17). Isolates from both countries associated with Iraq

| Isolate groupa | Hospital | Date received (mo/yr) | PFGE result | Link with Iraq | Comments |
|----------------|----------|-----------------------|-------------|----------------|----------|
| Exchange isolates |          |                       |             |                |          |
| UK 1 H1          |          | 5/03                  | T strain    | From a patient transferred from Iraq | First isolate of T strain received by LHCAI; 1 of 5 isolates received (from 5 patients who served in Iraq) (UK 1 and 22–25), all representing different strains |
| UK 2 H1          |          | 7/03                  | T strain    | Patient in hospital H1 | Further representatives of the T strain from patients in hospital H1 |
| UK 3 H1          |          | 12/03                 | T strain    | Patient in hospital H1 | T strain linked directly with soldier returning from Iraq i.e., not acquired in hospital H1 |
| UK 4 H1          |          | 11/04                 | T strain    | From soldier who served in Iraq immediately on arrival at the hospital |          |
| UK 5 H1          |          | 12/04                 | T strain    | Patient in hospital H1 |          |
| UK 6 H1          |          | 7/03                  | W strain    | Patient in hospital H1 |          |
| UK 7 H1          |          | 12/04                 | W strain    | Patient in hospital H1 |          |
| UK 8 H1          |          | 1/05                  | W strain    | Patient in hospital H1 |          |
| UK 9 H1          |          | 7/03                  | H1AC-3b     | Patient who served in Iraq | PFGE profile similar to OXA-23 clone 2, but OXA-23 negative by PCR |
| UK 10 H1         |          | 9/04                  | H1AC-2      | Patient who served in Iraq | Represents same strain as H3AC-1 |
| UK 11 H3         |          | 12/03                 | H3AC-1      | Patient who served in Iraq | Represents same strain as H1AC-2 |
| UK 12 H2         |          | 10/03                 | OXA-23 clone 2 | Outbreak in hospital H2 associated with admission of civilian casualty from Baghdad | OXA-23 clone 2 previously found in 3 other UK hospitals, with the first isolate being seen in July 2003 |
| UK 13 H2         |          | 10/03                 | OXA-23 clone 2 | Patient in hospital H2 | Further representative of OXA-23 clone 2 from outbreak in hospital H2 |
| UK 14 H2         |          | 11/03                 | OXA-23 clone 2 | Patient in hospital H2 | Further representative of OXA-23 clone 2 from outbreak in hospital H2 |
| UK 15 H1         |          | 7/03                  | Uniqueb     | Patient in hospital H1 | Treated in military hospital before coming to UK |
| UK 16 H4         |          | 8/03                  | Uniqueb     | Associated with Iraq |          |
| UK 17 H5         |          | 10/03                 | Unique      | Iraqi patient injured in Basra from gas cooker explosion |          |
| UK 18 H6         |          | 11/03                 | Uniqueb     | Person who served in Iraq |          |
| UK 19 H6         |          | SE clone              |             | None |          |
| UK 20 H6         |          | OXA-23 clone 1        |             | None |          |
| Additional isolates |        |                       |             |                |          |
| UK 21 H7         |          | 4/03                  | Uniqueb     | Child with extensive burns from Basra |          |
| UK 22 H1         |          | 5/03                  | V strain    | Patient who served in Iraq | PFGE profile similar to, but distinct from, OXA-23 clone 2; OXA-23 negative by PCR |
| UK 23 H1         |          | 5/03                  | H1AC-2      | Patient who served in Iraq | Represents same strain as H3AC-1 |
| UK 24 H1         |          | 5/03                  | R strain    | Patient who served in Iraq | Strain previously found in hospital |
| UK 25 H1         |          | 5/03                  | W strain    | Patient who served in Iraq | Strain predicts Iraq conflict |

a Isolates UK 1 to UK 20 inclusive were included in an isolate exchange with WRAMC.
b This isolate was PCR negative for the class 1 integrase gene, indicating that it is sporadic (16).
c The strain was previously found in hospital H1; the clone has been widely found in Europe for decades (8).
d LHCAI, Laboratory of HealthCare Associated Infection.
were distinct from representatives of the SE (southeast) clone (UK 19) and OXA-23 clone 1 (UK 20), which are the most prevalent strains of *A. baumannii* in hospitals in the United Kingdom (16, 17).

To characterize these isolates further, the integrons in the United Kingdom and U.S. representatives of the outbreak strains were compared (Table 2). Most isolates of the T strain possessed a 3-kb integron. Isolate US 1, however, contained a 3.5-kb integron, which differed from the 3-kb integron (GenBank accession number AY922990) in having an additional copy of orfX. U.S. representatives of the remaining outbreak strains carried the same integron as the corresponding United Kingdom representatives. The integron cassette arrays in isolates of the T strain, SE clone, and OXA-23 clone 2 differ only in the number of copies of orfX and are associated with highly successful strains of *A. baumannii* (17).

Antibiotic susceptibilities of representatives of the outbreak strains common to isolates from both countries are given in

FIG. 1. Dendrogram of PFGE profiles of Apal-digested genomic DNA from isolates. United Kingdom isolates (UK 1 to UK 25) were from hospitals H1 to H7. Isolates from the United States (US 1 to US 15) were from an *Acinetobacter* outbreak investigation archive at WRAMC.
TABLE 2. Class 1 integrons found in isolates

| Isolate     | PFGE result | Approx size (5' CS to 3' CS) of PCR product (kb) | Integron cassette array |
|-------------|-------------|-------------------------------------------------|-------------------------|
| United Kingdom and U.S. isolates\(^d\) |             |                                                 |                         |
| UK 1        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| UK 2        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| UK 3        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| UK 4        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| UK 5        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| US 7        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| US 9        | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| US 13       | T strain    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| US 1        | T strain    | 3.5                                              | aacC1 orfX orfX orfX' aadA1a\(^c\) |
| UK 14       | OXA-23 clone 2 | 2.5                                       | aacC1 orfX orfX' aadA1a |
| US 12       | OXA-23 clone 2 | 2.5                                       | aacC1 orfX orfX' aadA1a |
| UK 23       | H1AC-2\(^b\) | 0.7                                              | aadB                    |
| UK 10       | H1AC-2\(^b\) | 0.7                                              | aadB                    |
| UK 11       | H3AC-1\(^b\) | 0.7                                              | aadB                    |
| US 8        | USAC-3\(^b\) | 0.7                                              | aadB                    |
| Other United Kingdom isolates |             |                                                 |                         |
| UK 6        | W strain    | 0.8                                              | aacA4                   |
| UK 8        | W strain    | 0.8                                              | aacA4                   |
| UK 22       | V strain    | 2.5                                              | aacC1 orfX orfX' aadA1a |
| UK 19       | SE clone    | 3.0                                              | aacC1 orfX orfX orfX' aadA1a |
| UK 20       | OXA-23 clone 1 | 2.3                                      | aacA4 catB8 aadA1 |

\(^a\) UK 12, UK 13, and UK 17 were PCR positive for the class 1 integrase gene, but integron cassette arrays failed to amplify.
\(^b\) Represents a single strain.
\(^c\) As in GenBank accession no. AY922990.
\(^d\) Comparison of integrons in United Kingdom and U.S. isolates of the same strain.
\(^e\) 5' CS and 3' CS are primers used to amplify integron cassette arrays (17).

Table 3. Representatives of the T strain were resistant to ampicillin, amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, cefoxitin, piperacillin, pipercillin-tazobactam, ciprofloxacin, gentamicin, and sulbactam; most United Kingdom isolates were susceptible to tobramycin and amikacin, but U.S. isolates were resistant. All the T strain isolates in this study were susceptible to carbapenems and colistin; however, some more recent isolates are carbapenem resistant (18). Isolates of OXA-23 clone 2 were resistant to all antibiotics tested, with the exception of amikacin, tobramycin (United Kingdom isolates only), minocycline, and colistin (all isolates). Isolates of the minor outbreak strain, referred to as H1AC-2, H3AC-1, or USAC-3, were also resistant to most antibiotics but susceptible to imipenem, meropenem, minocycline, sulbactam, and colistin.

The finding of strains that were common among the United Kingdom and U.S. isolates is consistent with a common origin. The fact that most isolates of the same strain also shared the same integron supports this. However, genetically similar isolates have often been found in geographically distant centers, perhaps because they have been independently selected from a common ancestor (8).

If a common origin for the outbreak strains is assumed, the source of these organisms is still a subject of some debate. Injured British and American soldiers had shared exposure to various medical facilities and modes of transport during their evacuation, and it is plausible that these may have been potential source(s). There are some claims that these infections may have originated from Iraqi soil, with a combination of acquisition from soil and selection by antibiotics having been involved (9a). However, A. baumannii has generally not been isolated from patients’ wounds immediately or very shortly after injury (21). Moreover, it seems unlikely that numerous individuals, injured at various sites, would all acquire the same outbreak strain in this way.

Of the three outbreak strains common to both the United Kingdom and U.S. isolates, it is the T strain that has been most strongly associated with these casualties and that has, in the United Kingdom at least, caused the most infections. The first known isolate of the T strain in the United Kingdom was from a casualty returning from Iraq. It has been isolated from at least one soldier immediately on admission to the United Kingdom hospital, ruling out the possibility that it was acquired in that hospital. This strain has all the characteristics of a highly successful outbreak strain (and indeed shares many characteristics with the SE clone, which is widespread in London and southeast England). Although this strain has affected other patients in the hospital, it appears to have remained confined to that area for over 2 years and has therefore not greatly contributed to an increase in Acinetobacter infections in the United Kingdom. Two isolates have, however, recently been found in a further United Kingdom hospital.

In conclusion, at least one outbreak strain of A. baumannii, responsible for further infections in the hospitals concerned, is associated with soldiers returning to the United States or United Kingdom from Iraq. The exact source(s) of the organism remains unclear. The results of detailed epidemiological investigations conducted by the U.S. military will be reported separately.
TABLE 3. MICs for representatives of outbreak strains of Acinetobacter baumannii common to the United Kingdom and U.S. isolates

| MIC (μg/l) | UK 12 United Kingdom OXA-23 clone 2/H11349 | UK 14 United Kingdom OXA-23 clone 2/H11349 | US 12 United States OXA-23 clone 2/H11349 | UK 3 United Kingdom T strain/H11349 | US 1 United States T strain/H11349 | UK 10 United Kingdom H1AC-2 | US 8 United States USAC-3/H11349 |
|-----------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------|-------------------------------------|-------------------------------|----------------------------------|
| AMP       | 0.5                                         | 1                                           | 0.5                                         | 0.5                                   | 0.5                                 | 0.5                           | 0.5                              |
| AMC       | >32                                         | >32                                         | >32                                         | >32                                   | >32                                 | >32                           | >64                              |
| CTX       | >16                                         | >16                                         | >16                                         | >16                                   | >16                                 | >16                           | >16                              |
| FOX       | 4                                           | 4                                           | 4                                           | 4                                     | 4                                   | 4                             | 4                                |
| PIP       | >32                                         | >32                                         | >32                                         | >32                                   | >32                                 | >32                           | >32                              |
| TZP       | >16                                         | >16                                         | >16                                         | >16                                   | >16                                 | >16                           | >16                              |
| IPM       | >16                                         | >16                                         | >16                                         | >16                                   | >16                                 | >16                           | >16                              |
| MEM       | >16                                         | >16                                         | >16                                         | >16                                   | >16                                 | >16                           | >16                              |
| TOB       | >16                                         | >16                                         | >16                                         | >16                                   | >16                                 | >16                           | >16                              |
| GEN       | 8                                           | 8                                           | 8                                           | 8                                     | 8                                   | 8                             | 8                                |
| MIN       | 1                                           | 1                                           | 1                                           | 1                                     | 1                                   | 1                             | 1                                |

MICs were determined by agar dilution using the British Society for Antimicrobial Chemotherapy (BSAC) methodology (3). Antibiotic abbreviations are as follows: AMP, ampicillin; AMC, amoxicillin-clavulanic acid; ATM, aztreonam; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; FOX, cefoxitin; PIP, piperacillin; TZP, piperacillin-tazobactam; IPM, imipenem; MEM, meropenem; TOB, tobramycin; AMK, amikacin; MIN, minocycline. Resistance is indicated by boldface. Breakpoints (mg/liter) are in parentheses following the drug names. No BSAC breakpoint; Clinical and Laboratory Standards Institute breakpoint quoted. NT, not tested.