Acinetobacter baumannii: Emergence of a Successful Pathogen

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INTRODUCTION

The genus known as Acinetobacter has undergone significant taxonomic modification over the last 30 years. Its most important representative, Acinetobacter baumannii, has emerged as one of the most troublesome pathogens for health care institutions globally. Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to upregulate or acquire resistance determinants, making it one of the organisms threatening the current antibiotic era. A. baumannii strains resistant to all known antibiotics have now been reported, signifying a sentinel event that should be acted on promptly by the international health care community. Acting in synergy with this emerging resistance profile is the uncanny ability of A. baumannii to survive for prolonged periods throughout a hospital environment, thus potentiating its ability for nosocomial spread. The organism commonly targets the most vulnerable hospitalized patients, those who are critically ill with breaches in skin integrity and airway protection. As reported from reviews dating back to the 1970s (199), hospital-acquired pneumonia is still the most common infection caused by this organism. However, in more recent times, infections involving the central nervous system, skin and soft tissue, and bone have emerged as highly problematic for certain institutions.

Interest in Acinetobacter, from both the scientific and public community, has risen sharply over recent years. Significant advances have been made in our understanding of this fascinating organism since it was last reviewed in this journal in 1996 (28). In the present review, we describe these advances and also provide a comprehensive appraisal of the relevant microbiological, clinical, and epidemiological characteristics of A. baumannii, the most clinically relevant species. The epidemiology, clinical impact, and resistance mechanisms of Acinetobacter species outside the A. baumannii group are not covered in this review.

MICROBIOLOGY

Historical Perspective of the Genus Acinetobacter

The history of the genus Acinetobacter dates back to the early 20th century, in 1911, when Beijerinck, a Dutch microbiologist, described an organism named Micrococcus calcoaceticus that was isolated from soil by enrichment in a calcium-acetate-containing minimal medium (24). Over the following decades, similar organisms were described and assigned to at least 15 different genera and species, including Diplococcus mucosus (587), Micrococcus calcoaceticus (24), Alcaligenes haemolygens (228), Mima polymorpha (117), Moraxella lwoffi (14), Herellea vaginicola (116), Bacterium anitratum (485), Moraxella lwoffi var. glucidolytica (434), Neisseria winogradskyi (323), Achromobacter anitratus (60), and Achromobacter mucosus (352). For a comprehensive review of the history of the genus, the reader is referred to the work of Henriksen (228).

The current genus designation, Acinetobacter (from the Greek akinetos [akinetos], i.e., nonmotile), was initially proposed by Brisou and Prevot in 1954 to separate the nonmotile from the motile microorganisms within the genus Achromobacter (61). It was not until 1968 that this genus designation became more widely accepted (21). Baumann et al. published a comprehensive survey and concluded that the different species listed above belonged to a single genus, for which the name Acinetobacter was proposed, and that further subclassification into different species based on phenotypic characteristics was not possible (21). These findings resulted in the official acknowledgment of the genus Acinetobacter by the Subcommittee on the Taxonomy of Moraxella and Allied Bacteria in 1971 (324). In the 1974 edition of Bergey’s Manual of Systematic Bacteriology (312), the genus Acinetobacter was listed, with the description of a single species, Acinetobacter calcoaceticus (the type strain for both the genus and the species is A. calcoaceticus ATCC 23055) (24). In the “Approved List of Bacterial Names,” in contrast, two different species, A. calcoaceticus and A. lwoffi, were included, based on the observation that some acinetobacters were able to acidify glucose whereas others were not (512). In the literature, based on the same properties, the species A. calcoaceticus was subdivided into two subspecies or biovars, A. calcoaceticus bv. anitratus (formerly called Herellea vaginicola) and A. calcoaceticus bv. lwoffi (formerly called Mima polymorpha). These designations, however, were never officially approved by taxonomists.

Current Taxonomy

The genus Acinetobacter, as currently defined, comprises gram-negative, strictly aerobic, nonfermenting, nonfastidious, nonmotile, catalase-positive, oxidase-negative bacteria with a DNA G+C content of 39% to 47%. Based on more recent taxonomic data, it was proposed that members of the genus Acinetobacter should be classified in the new family Moraxellaceae within the order Gammaproteobacteria, which includes the genera Moraxella, Acinetobacter, Psychrobacter, and related
organisms (466). A major breakthrough in the long and complicated history of the genus was achieved in 1986 by Bouvet and Grimont, who—based on DNA-DNA hybridization studies—distinguished 12 DNA (hybridization) groups or genospecies, some of which were given formal species names, including A. baumannii, A. calcoaceticus, A. haemolyticus, A. johnsonii, A. junii, and A. hovfii (51). Work done by Bouvet and Jeanjean, Tjernberg and Ursing, and Nishimura et al. (53, 401, 542) resulted in the description of further Acinetobacter genomic species, including the named species A. radioresistens, which corresponds to Acinetobacter genomic species 12 described previously by Bouvet and Grimont (51). Some of the independently described (genomic) species turned out to be synonyms, e.g., A. hovfii and Acinetobacter genomic species 9 or Acinetobacter genomic species 14, described by Bouvet and Jeanjean (14BJ), and Acinetobacter genomic species 13, described by Tjernberg and Ursing (13TU). More recently, 10 additional Acinetobacter species were described, including 3 species of human origin, A. parus, A. schindleri, and A. ursingii (392, 393), and 7 species isolated from activated sludge (recovered from sewage plants), namely, A. baylyi, A. bouvetii, A. grimontii, A. tjernbergiae, A. towneri, A. tandoii, and A. germeri (72), increasing the actual number of validly described (genomic) species to 31, of which 17 have been given valid species names (Table 1). It has to be noted, however, that some of the recently described environmental Acinetobacter species included only one or a few strains at the time of publication (72).

Four of the above listed species, i.e., A. calcoaceticus, A. baumannii, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU, are very closely related and difficult to distinguish from each other by phenotypic properties. It has therefore been proposed to refer to these species as the A. calcoaceticus-A. baumannii complex (189, 191). However, this group of organisms comprises not only the three most clinically relevant species that have been implicated in the vast majority of both community-acquired and nosocomial infections, i.e., A. baumannii, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU (see below), but also an environmental species, A. calcoaceticus, that has frequently been recovered from soil and water but has, to our knowledge, never been implicated in serious clinical disease. Therefore, since it is the environmental species that has given its name to the complex, the designation A. calcoaceticus-A. baumannii complex may be misleading and not appropriate if used in a clinical context.

### Species Identification

Acinetobacters may be identified presumptively to the genus level as gram-negative, catalase-positive, oxidase-negative, nonmotile, nonfermenting coccobacilli. They are short, plump, gram-negative rods that are difficult to destain and may therefore be misidentified as either gram-negative or gram-positive cocci (hence the former designation Mimae). Acinetobacter species of human origin grow well on solid media that are routinely used in clinical microbiology laboratories, such as sheep blood agar or tryptic soy agar, at a 37°C incubation temperature. These organisms form smooth, sometimes mucoid, grayish white colonies; colonies of the A. calcoaceticus-A. baumannii complex resemble those of Enterobacteriaceae, with a colony diameter of 1.5 to 3 mm after overnight culture, while most of the other Acinetobacter species produce smaller and more translucent colonies. Unlike the Enterobacteriaceae, some Acinetobacter species outside the A. calcoaceticus-A. baumannii complex may not grow on McConkey agar. Isolates of the species A. haemolyticus and several other currently not-well-defined species, such as Acinetobacter genomic species 6, 13BJ, 14BJ, 15BJ, 16, and 17, may show hemolysis on sheep blood agar, a property that is never present in Acinetobacter isolates belonging to the A. calcoaceticus-A. baumannii complex. Unfortunately, no single metabolic test distinguishes acinetobacters from other similar nonfermenting gram-negative bacteria. A reliable method for unambiguous identification of acinetobacters to the genus level is the transformation assay of Juni, which is based on the unique property of mutant Acinetobacter strain BD413 tpPE27, a naturally transformable tryptophan auxotroph recently identified as A. baylyi (574), to be transformed by crude DNA of any Acinetobacter species to a wild-type phenotype (281). For the recovery of acinetobacters from environmental and clinical specimens (e.g., skin swabs to detect skin colonization), enrichment culture at low pH in a vigorously aerated liquid mineral medium supplemented with acetate or another suitable carbon source and with nitrate as the nitrogen source has proven useful (20). To

### TABLE 1. Delineation of Acinetobacter genomic species

<table>
<thead>
<tr>
<th>Species</th>
<th>Genomic species</th>
<th>Type or reference strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td>2</td>
<td>ATCC 19606T</td>
<td>51, 542</td>
</tr>
<tr>
<td>A. baylyi</td>
<td>3</td>
<td>ATCC 19606T</td>
<td>51, 542</td>
</tr>
<tr>
<td>A. bouvetii</td>
<td>4</td>
<td>DSM 14961T</td>
<td>72</td>
</tr>
<tr>
<td>A. calcoaceticus</td>
<td>5</td>
<td>DSM 14961T</td>
<td>72</td>
</tr>
<tr>
<td>A. johnsonii</td>
<td>6</td>
<td>DSM 14961T</td>
<td>72</td>
</tr>
<tr>
<td>A. ursingii</td>
<td>7</td>
<td>DSM 14961T</td>
<td>72</td>
</tr>
</tbody>
</table>

* Unless indicated otherwise, genomic species delineation is according to Bouvet and Grimont (51) and Bouvet and Jeanjean (53). BJ, Bouvet and Jeanjean; TU, Tjernberg and Ursing.

* "A. venetiarius" is found in marine water but does not yet have formal species status.
facilitate the isolation of acinetobacters from mixed bacterial populations, Leeds Acinetobacter medium was proposed (260).

Of the few methods that have been validated for identification of Acinetobacter species, DNA-DNA hybridization remains the reference standard (51). The phenotypic identification scheme proposed by Bouvet and Grimont in 1986 is based on 28 phenotypic tests (51). This identification scheme was refined in 1987 by the same authors and includes growth at 37°C, 41°C, and 44°C; production of acid from glucose; gelatin hydrolysis; and assimilation of 14 different carbon sources (52). While this simplified identification scheme allows discrimination between 11 of the 12 genomic species initially described (51) and correctly identified to the species level 95.6% of 136 Acinetobacter isolates recovered from human skin samples (495), it does not permit identification of the more recently described (genomic) species. In particular, the closely related and clinically most relevant species A. baumannii and Acinetobacter genomic species 13TU cannot be distinguished, while A. calcoaceticus and Acinetobacter genomic species 3 can only be separated by their growth properties at different temperatures (191). Unfortunately, simple phenotypic tests that are commonly used in routine diagnostic laboratories for identification of other bacterial genera to the species level are unsuitable for unambiguous identification of even the most common Acinetobacter species.

Both DNA-DNA hybridization and the phenotypic identification system of Bouvet and Grimont are laborious and far from being suitable for routine microbiology laboratories. In fact, these methods are available in only a few reference laboratories worldwide. Molecular methods that have been developed and validated for identification of acinetobacters include amplified 16S rRNA gene restriction analysis (ARTRA) (572; for an evaluation of ARTRA, see reference 127), high-resolution fingerprint analysis by amplified fragment length polymorphism (AFLP) (258, 392), ribotyping (189), tRNA spacer fingerprinting (146), restriction analysis of the 16S-23S rRNA intergenic spacer sequences (131), sequence analysis of the 16S-23S rRNA gene spacer region (79), and sequencing of the rpoB (RNA polymerase β-subunit) gene and its flanking spacers (310). ARDRA and AFLP analysis are currently the most widely accepted and validated reference methods for species identification of acinetobacters, with a large library of profiles available for both reference and clinical strains, while tRNA fingerprinting, though generally also suitable for species identification, does not discriminate between A. baumannii and Acinetobacter genomic species 13TU. Both ribotyping and sequence analysis of the 16S-23S rRNA gene spacer region were found to discriminate between species of the A. calcoaceticus-A. baumannii complex but have not been applied to other Acinetobacter species, and sequencing of the rpoB gene, although very promising, awaits further validation. All of these methods have contributed to a better understanding of the epidemiology and clinical significance of Acinetobacter species during recent years, but they are too laborious to be applied in day-to-day diagnostic microbiology, and their use for the time being is also confined mainly to reference laboratories.

More recent developments include the identification of A. baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species (559), PCR-electrospray ionization mass spectrometry (PCR–ESI-MS) (145), and a simple PCR-based method described by Higgins et al. (234) that exploits differences in their respective gyrB genes to rapidly differentiate between A. baumannii and Acinetobacter genomic species 13TU. Promising results with matrix-assisted laser desorption ionization–time-of-flight MS have been obtained for species identification of 552 well-characterized Acinetobacter strains representing 15 different species (496). Matrix-assisted laser desorption ionization–time-of-flight MS allows for species identification in less than 1 hour, but it requires expensive equipment and needs further evaluation.

Species identification with manual and semiautomated commercial identification systems that are currently used in diagnostic microbiology, such as the API 20NE, Vitek 2, Phoenix, and MicroScan WalkAway systems, remains problematic (33, 35, 244). This can be explained in part by their limited database content but also because the substrates used for bacterial species identification have not been tailored specifically to identify acinetobacters. In particular, the three clinically relevant members of the A. calcoaceticus-A. baumannii complex cannot be separated by currently available commercial identification systems; in fact, A. baumannii, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU are uniformly identified as A. baumannii by the most widely used identification systems. In referring to these species, it therefore seems appropriate to use the term A. baumannii group instead of A. calcoaceticus-A. baumannii complex. This reflects the fact that A. baumannii, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU share important clinical and epidemiological characteristics (124, 335, 498) and also eliminates the confusion resulting from inclusion of an environmental species, A. calcoaceticus (see above). However, since the vast majority of studies that have addressed epidemiological and clinical issues related to Acinetobacter have not employed identification methods that allow for unambiguous species identification within the A. baumannii group, the designation A. baumannii in this review, if not stated otherwise, is used in a broader sense to also accommodate Acinetobacter genomic species 3 and 13TU.

The need for species identification of acinetobacters in routine clinical laboratories has been questioned by some researchers (191). From a clinical and infection control point of view, however, it is necessary to distinguish between the A. baumannii group and acinetobacters outside the A. baumannii group since the latter organisms rarely have infection control implications. In addition, these organisms are usually susceptible to a range of antimicrobials, and infections caused by these organisms are most often benign. From a research perspective, in contrast, clinical studies using proper methods for species identification of acinetobacters, including those within the A. baumannii group, are mandatory to increase our knowledge of the epidemiology, pathogenicity, and clinical impact of the various species of this diverse genus.

**Natural Habitats**

Members of the genus Acinetobacter are considered ubiquitous organisms. This holds true for the genus Acinetobacter, since acinetobacters can be recovered after enrichment culture from virtually all samples obtained from soil or surface water (20). These earlier findings have contributed to the common
misconception that A. baumannii is also ubiquitous in nature (171). In fact, not all species of the genus Acinetobacter have their natural habitat in the environment. However, a systematic study to investigate the natural occurrence of the various Acinetobacter species in the environment has never been performed.

Most Acinetobacter species that have been recovered from human clinical specimens have at least some significance as human pathogens (493, 502). Acinetobacters are part of the human skin flora. In an epidemiological survey performed to investigate the colonization of human skin and mucous membranes with Acinetobacter species, up to 43% of nonhospitalized individuals were found to be colonized with these organisms (495). The most frequently isolated species were A. lwoffii (58%), A. johnsonii (20%), A. junii (10%), and Acinetobacter genomic species 3 (6%). In a similar study, a carrier rate of 44% was found for healthy volunteers, with A. lwoffii (61%), Acinetobacter genomic species 15B1 (12%), A. radioresistens (8%), and Acinetobacter genomic species 3 (5%) being the most prevalent species (31). In patients hospitalized on a regular ward, the carriage rate of Acinetobacter species was even higher, at 75% (495). Dijkshoorn et al. found 53% of medical students and new nurses to be colonized with Acinetobacter species 11 predominating (126). In contrast, A. baumannii, the most important nosocomial Acinetobacter species, was found only rarely on human skin (0.5% and 3% in references 31 and 495, respectively) and in human feces (0.8%) (126), and Acinetobacter genomic species 13TU was not found at all (31, 126, 495). More recently, Griffith et al. investigated the nares of healthy U.S. soldiers and did not find acinetobacters at all, but they did not use enrichment culture to increase the recovery rate (211). In a subsequent study, Griffith et al. did not detect skin carriage of the A. calcoaceticus-A. baumannii complex among a representative sample of 102 U.S. Army soldiers deployed in Iraq, but again, they performed cultures without enrichment and with an extremely long transport time that may have contributed to this finding (212). Notably, in tropical climates, the situation may be different. In Hong Kong, Chu et al. found 53% of medical students and new nurses to be colonized with acinetobacters in summer versus 32% in winter (91). Such a seasonal variability in skin colonization may contribute to the seasonal variation seen in the prevalence of A. baumannii in clinical samples (360). Acinetobacter genomic species 3 (36%), Acinetobacter genomic species 13TU (15%), Acinetobacter genomic species 15TU (6%), and A. baumannii (4%) were the most frequently recovered species, while A. lwoffii, A. johnsonii, and A. junii were only rarely found.

Although various Acinetobacter species have been isolated from animals and A. baumannii was occasionally found as an etiologic agent in infected animals (173, 571), the normal flora of animals has never been studied systematically for the presence of acinetobacters. Of note, A. baumannii was recovered from 22% of body lice sampled from homeless people (311). It has been speculated that this finding might result from clinically silent bacteremia in these people; the clinical significance of this observation, however, is not yet clear.

The inanimate environment has also been studied for the presence of acinetobacters. Berlau et al. investigated vegetables in the United Kingdom and found that 30 of 177 vegetables (17%) were culture positive for Acinetobacter (32). Interestingly, A. baumannii and Acinetobacter genomic species 11 (each at 27%) were the predominant species, followed by A. calcoaceticus and Acinetobacter genomic species 3 (each at 13%), while Acinetobacter genomic species 13 was found only once. In Hong Kong, 51% of local vegetables were culture positive for Acinetobacter species, the majority of which were Acinetobacter genomic species 3 (75%), but one sample grew A. baumannii (245). Hoang et al. found acinetobacters in 22 of 60 soil samples in Hong Kong, and the most frequent species were Acinetobacter genomic species 3 (27%) and A. baumannii (23%), with only one sample yielding A. calcoaceticus (245). In an unpublished study from Germany, 92 of 163 samples (56%) from soil and surface water yielded acinetobacters, and A. calcoaceticus, A. johnsonii, A. haemolyticus, and Acinetobacter genomic species 11 were found most frequently. Only a single sample yielded A. baumannii, three samples were positive with Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU was not found at all in soil and water (H. Seifert, personal communication). Some recently described Acinetobacter species, i.e., A. baylyi, A. bouvetii, A. grimontii, A. tjernbergiae, A. toweri, and A. tandoii, that were isolated from activated sludge are obviously environmental species and have, as yet, never been found in humans (72). In contrast, two other recently described species, A. schindleri and A. ursingii, have been recovered only from human specimens, while A. parvus was found in humans and was also cultured from a dog (138, 392, 393).

In conclusion, although available data derive from only a few studies, some Acinetobacter species indeed seem to be distributed widely in nature, i.e., A. calcoaceticus is found in water and soil and on vegetables; Acinetobacter genomic species 3 is found in water and soil, on vegetables, and on human skin; A. johnsonii is found in water and soil, on human skin, and in human feces; A. lwoffii and A. radioresistens are found on human skin; and Acinetobacter genomic species 11 is found in water and soil, on vegetables, and in the human intestinal tract. At least in Europe, the carrier rate of A. baumannii in the community is rather low. Also, although it has been found in soil samples in Hong Kong and on vegetables in the United Kingdom, A. baumannii does not appear to be a typical environmental organism. Existing data are not sufficient to determine if the occurrence of severe community-acquired A. baumannii infections that have been observed in tropical climates (8, 325, 591) may be associated with an environmental source. Acinetobacter genomic species 13TU was found on human skin in Hong Kong but not in Europe. Also, it has not been identified in the inanimate environment. Thus, the natural habitats of both A. baumannii and Acinetobacter genomic species 13TU still remain to be defined.

**MECHANISMS OF ANTIBIOTIC RESISTANCE**

The wide array of antimicrobial resistance mechanisms that have been described for A. baumannii is impressive and rivals those of other nonfermentative gram-negative pathogens (Table 2) (426, 443). The rapid global emergence of A. baumannii strains resistant to all β-lactams, including carbapenems, illustrates the potential of this organism to respond swiftly to changes in selective environmental pressure. Upregulation of
innate resistance mechanisms and acquisition of foreign determinants are critical skills that have brought \textit{A. baumannii} great respect. Despite the absence of data on the genetic competence of \textit{A. baumannii}, other \textit{Acinetobacter} spp., in particular \textit{A. baylyi}, are highly competent and recombinogenic (16, 574). A recent study by Fournier et al. typifies the genetic agility and broad resistance armamentarium of \textit{A. baumannii} (172). After performing whole-genome sequencing of a clinical epidemic \textit{A. baumannii} strain found in France (AYE), an 86-kb resistance island, one of the largest to be described thus far, was identified (AbaR1). Of the 88 predicted open reading frames (ORFs) within this genomic region, 82 were predicted to have originated from other gram-negative organisms, such as \textit{Pseudomonas} sp., \textit{Salmonella} sp., and \textit{Escherichia coli}. Furthermore, the G+C content of this region was 52.8%, compared to 38.8% for the remaining chromosome, indicating a likely foreign source. Overall, 52 resistance genes were identified, and surprisingly, 45 (86.5%) were localized to the AbaR1 resistance island (172). The genetic surroundings of these resistance determinants provided more evidence for genetic promiscuity, with an array of broad-host-range mobile genetic elements identified, including three class 1 integrons, transposons, and insertion sequence (IS) elements. Interestingly, no plasmid markers were identified in this resistance hot spot, and of the three plasmids found within the AYE strain, none contained any known resistance marker (172). Compared to a susceptible \textit{A. baumannii} strain from the same geographic region (SDF), a similar structure was identified (AbaG1) in the homologous ATPase-like ORF, but it was devoid of resistance determinants (172). To assess whether this hot spot is conserved among \textit{A. baumannii} strains, a further 22 clinical strains were screened. Seventy-seven percent had an intact ATPase ORF yet also had a multidrug resistance phenotype (172), indicating that resistance determinants can be inserted into other areas of the genome. Similarly, the recently published genome sequence of \textit{A. baumannii} ATCC 17978 demonstrated a wide array of resistance markers but only one within the homologous location to that described by Fournier et al. (514), again illustrating the genetic flexibility of this pathogen.

### 1. Beta-Lactams

**Enzymatic mechanisms.** The most prevalent mechanism of beta-lactam resistance in \textit{A. baumannii} is enzymatic degradation.
by β-lactamases. However, in keeping with the complex nature of this organism, multiple mechanisms often work in concert to produce the same phenotype (47, 165, 446).

Inherent to all *A. baumannii* strains are chromosomally encoded AmpC cephalosporinases (49, 249, 250, 427, 468), also known as *Acinetobacter*-derived cephalosporinases (ADCs) (249). Unlike that of AmpC enzymes found in other gram-negative organisms, inducible AmpC expression does not occur in *A. baumannii* (49, 233). The key determinant regulating overexpression of this enzyme in *A. baumannii* is the presence of an upstream IS element known as IS*Aba1* (described below) (106, 233, 468, 492). The presence of this element highly correlates with increased AmpC gene expression and resistance to extended-spectrum cephalosporins (106, 468). Cefepime and carbapenems appear to be stable in response to these enzymes (249).

Extended-spectrum β-lactamases (ESBLs) from the Ambler class A group have also been described for *A. baumannii*, but assessment of their true prevalence is hindered by difficulties with laboratory detection, especially in the presence of an AmpC. More recent focus has been on VEB-1, which disseminated throughout hospitals in France (clonal dissemination) and was also recently reported from Belgium and Argentina (VEB-1a) (71, 381, 382, 417, 442); PER-1, from France, Turkey, Belgium, Romania, Korea, and the United States (250, 381, 385, 439, 565, 611); and PER-2, from Argentina (417). Interestingly, *bla*VEB-1 was found to be integron borne (class 1) yet encoded on the chromosome (442). This integron was identical to that identified in *Pseudomonas aeruginosa* in Thailand (197) and was also associated with an upstream IS element (IS*26*), indicating the possible origin and mechanism of spread to *A. baumannii* (442). *bla*PER-1 is either plasmid or chromosomally encoded and also has an upstream IS element (IS*Pa12*) that may enhance its expression (438). Other ESBLs identified in *A. baumannii* include TEM-92 and -116 (148, 387), from Italy and The Netherlands, respectively, and SHV-12 from China and The Netherlands (248, 387). Also, CTX-M-2 and CTX-M-43 have been described from Japan and Bolivia, respectively (76, 386). Narrow-spectrum β-lactamases, such as TEM-1 and TEM-2, are also prevalent in *A. baumannii* (111, 250, 579), but their current clinical significance is limited given the potency of other resistance determinants.

Of the β-lactamases, those with carbapenemase activity are most concerning and include the serine oxacillinases (Ambler class D OXA type) and the metallo-β-lactamases (MBLs) (Ambler class B) (443, 447, 589). Thus far, the Ambler class A carbapenemases (KPC, GES, SME, NMC, and IMI) have not been described for *A. baumannii* (447). For a detailed review of carbapenemases in *A. baumannii*, readers are referred to an excellent review by Poirel and Nordmann (443), and for carbapenemases in general, see the work of Queenan and Bush (447).

A summary of OXA-type enzymes in *A. baumannii* is shown in Fig. 1. The first identified OXA-type enzyme with carbapenemase activity is the serine oxacillinase (Ambler class D OXA type) and the metallo-β-lactamases (MBLs) (Ambler class B) (443, 447, 589). Thus far, the Ambler class A carbapenemases (KPC, GES, SME, NMC, and IMI) have not been described for *A. baumannii* (447). For a detailed review of carbapenemases in *A. baumannii*, readers are referred to an excellent review by Poirel and Nordmann (443), and for carbapenemases in general, see the work of Queenan and Bush (447).

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FIG. 1. Summary of the distribution and genetic context of the OXA-type enzymes in *Acinetobacter baumannii*. The arrows and corresponding percentages represent the degrees of amino acid homology between the enzyme clusters. The enzyme clusters within large circles signify the acquired enzyme types, in contrast to the naturally occurring OXA-51 cluster within the large square.
enem-hydrolyzing activity was from a clinical *A. baumannii* strain isolated in 1985 from Edinburgh, Scotland (418). This plasmid-encoded resistance determinant (initially named ARI-1) was found to be transferable, and the gene was later sequenced and named *bla*<sub>OXA-23</sub> (132, 482). This enzyme type now contributes to carbapenem resistance in *A. baumannii* globally (46, 99, 107, 110, 250, 264, 265, 298, 358, 384, 556, 585, 619). OXA-27 and OXA-49 are closely related enzymes that make up the *bla*<sub>OXA-23</sub> gene cluster in *A. baumannii* (3, 65) (Fig. 1). Two other acquired OXA-type gene clusters with carbapenemase activity have been described, including the *bla*<sub>OXA-24</sub>-like (encoding OXA-24, -25, -26, and -40) (3, 50, 70, 114, 230, 342, 344) and the *bla*<sub>OXA-53</sub>-like (36, 42, 98, 196, 421, 440, 441, 445, 550, 564, 617) carbapenemase genes. The crystal structure of OXA-24 was recently described and provides important insights for future drug development toward this emerging class of carbapenemases (477). *bla*<sub>OXA-53</sub> was identified more recently and, similar to *bla*<sub>OXA-23</sub>, is often plasmid mediated (441), which may explain its widespread distribution (98, 358, 421). *bla*<sub>OXA-53</sub> has also been identified in *A. junii* from Romania and Australia (358, 423). The final gene cluster, *bla*<sub>OXA-51</sub>-like genes (encoding OXA-51, -54, -64, -65, -66, -68, -69, -70, -71, -78, -79, -80, and -82), is unique in that it is naturally occurring in *A. baumannii*, hence its chromosomal location and prevalence (66, 98, 231, 250, 559, 564, 606, 619). Similar to other class D enzymes, its product has a greater affinity for imipenem than for meropenem (66, 230). Its role in carbapenem resistance appears to be related to the presence of IS<sub>AbA1</sub> (558). In the absence of this element, cloning studies have shown the presence of *bla*<sub>OXA-51</sub> (558) (Fig. 1). Interestingly, certain IS elements, especially IS<sub>AbA1</sub>, appear relatively unique to *A. baumannii* (491). As described in this section, IS elements are also important for the expression of resistance to other antibiotics in *A. baumannii* (438, 442, 455, 468, 469).

Despite MBLs being less commonly identified in *A. baumannii* than the OXA-type carbapenemases, their hydrolytic activities toward carbapenems are significantly more potent (100- to 1,000-fold) (443). These enzymes have the capability of hydrolyzing all β-lactams (including carbapenems) except the monobactam aztreonam, which may assist in laboratory detection. Of the five MBL groups described to date (589), only three have been identified in *A. baumannii*, including IMP (89, 104, 113, 179, 246, 265, 298, 316, 402, 471, 506, 530, 544, 618), VIM (316, 335, 551, 606, 615), and SIM (320) types. Several geographic regions, such as Spain, Singapore, Greece, and Australia, have shown the presence of both OXA- and MBL-type enzymes in the same strains (70, 298, 423, 551). Unlike the OXA-type enzymes, MBLs are most commonly found within integrons, which are specialized genetic structures that facilitate the acquisition and expression (via a common promoter) of resistance determinants. Most acquired MBL genes in *A. baumannii* have been found within class 1 integrons, often containing an array of resistance gene cassettes, especially those encoding aminoglycoside-modifying enzymes (246, 320, 458, 551, 618). Not surprisingly, *A. baumannii* strains carrying integrons have been found to be significantly more drug resistant than strains without integrons (216). The clinical significance of this unique genetic structure is that overuse of one antimicrobial may lead to overexpression of multiple resistance determinants as a consequence of a common promoter. In isolation, integrons are not mobile and therefore are embedded within plasmids or transposons that act as the genetic vehicles for resistance dissemination. For a detailed review of MBLs, readers are referred to the work of Walsh et al. (589).

**Nonenzymatic mechanisms.** β-Lactam resistance, including carbapenem resistance, has also been ascribed to nonenzymatic mechanisms, including changes in outer membrane proteins (OMPs) (47, 108, 119, 165, 209, 336, 380, 446, 510, 511), multidrug efflux pumps (232, 236, 347), and alterations in the affinity or expression of penicillin-binding proteins (165, 188, 406, 510). Relative to other gram-negative pathogens, very little is known about the outer membrane porins of *A. baumannii*. Recently, the loss of a 29-kDa protein, also known as CarO, was shown to be associated with imipenem and meropenem resistance (336, 380, 511). This protein belongs to a novel family of OMPs found only in members of the *Moraxella* family of the class *Gammaproteobacteria* (380). No specific imipenem-binding site was found in CarO (511), indicating that this porin forms nonspecific channels. A second protein, known as Omp25, was identified in association with CarO, but it lacked pore-forming capabilities (511). The loss of the CarO porin in imipenem-resistant *A. baumannii* appears to be secondary to *carO* gene disruption by distinct insertion elements (380). Clinical outbreaks of carbapenem-resistant *A. baumannii* due to porin loss, including reduced expression of 47-, 44-, and 37-kDa OMPs in *A. baumannii* strains endemic to New York City (446) and reduced expression of 22- and 33-
kDa OMPs in association with OXA-24 in Spain (47), have been described. Other identified OMPs relevant to β-lactam resistance include the heat-modifiable protein HMP-AB (209), which is homologous to OmpA of *Enterobacteriaceae* and OmpF of *P. aeruginosa* (580); a 33- to 36-kDa protein (94, 119); a 43-kDa protein which shows significant homology to OprD from *P. aeruginosa* (141); and OmpW, which is homologous to OmpW proteins found in *E. coli* and *P. aeruginosa* (510, 580). Interestingly, when comparative proteomic studies were performed between a multidrug-resistant *A. baumannii* strain and a reference strain, no difference in expression was identified for Omp33/36 or OprD, but CarO expression and the structural isoforms of OmpW were different (510). Further studies are still required to elucidate the significance of these porins and their overall prevalence in multidrug-resistant *A. baumannii*.

As represented by Fournier et al., the genome of a multidrug-resistant *A. baumannii* strain encodes a wide array of multidrug efflux systems (172). The resistance-nodulation-division (RND) family-type pump AdeABC is the best studied thus far and has a substrate profile that includes β-lactams (including carbapenems) (232, 236), aminoglycosides, erythromycin, chloramphenicol, tetracyclines, fluoroquinolones, trimethoprim, and ethidium bromide (232, 236, 347, 356, 397, 420, 469). Similar to other RND-type pumps, AdeABC has a three-component structure: AdeB forms the transmembrane component, AdeA forms the inner membrane fusion protein, and AdeC forms the OMP. AdeABC is chromosomally encoded and is normally regulated by a two-component system with a sensor kinase (AdeS) and its associated response regulator (AdeR) (356). Point mutations within this regulatory system have been associated with pump overexpression (356), but such mutations are not necessary (420, 469). Most recently, disruption of the adeS gene by the IS element IS*aba1* was identified (469). Insertional inactivation of the transmembrane component of the pump, encoded by adeB, led to loss of pump function and multidrug resistance (347). However, this was not the case with inactivation of the gene encoding the OMP, adeC, suggesting that AdeAB may be able to recruit other OMPs to form a functional tripartite complex (356). Other RND-type pumps have been described for different *Acinetobacter* genomic species (82, 90).

### Aminoglycosides

As mentioned above, the presence of genes coding for aminoglycoside-modifying enzymes within class 1 integrons is highly prevalent in multidrug-resistant *A. baumannii* strains (246, 320, 395, 458, 503, 551, 556, 618). All of the major enzyme classes have been described, including acetyltransferases, nucleotidytransferases, and phosphotransferases (250, 395). More recently, 16S rRNA methylation has been described for *A. baumannii* (*armA*) strains from Japan, Korea, and the United States (129, 314, 608). This emerging resistance mechanism impairs aminoglycoside binding to its target site and confers high-level resistance to all clinically useful aminoglycosides, including gentamicin, tobramycin, and amikacin (130). Interestingly, the genetic surroundings of *armA* appear very similar across gram-negative organisms, as it is plasmid borne and within a transposon (Tn*348*) (129).

Apart from the AdeABC efflux pump, which less effectively transports amikacin and kanamycin due to their more hydrophilic nature (347), aminoglycosides (gentamicin and kanamycin) are also substrates of the recently described AbeM pump, a member of the multidrug and toxic compound extrusion (MATE) family (525).

### Quinolones

Modifications to DNA gyrase or topoisomerase IV through mutations in the *gyrA* and *parC* genes have been well described for *A. baumannii* (220, 236, 504, 581, 582). These mutations interfere with target site binding. Similar to aminoglycosides, many quinolones are also substrates for multidrug efflux pumps (456), including the RND-type pump AdeABC (236, 347) and the MATE pump AdeM (525). Thus far, plasmid-mediated quinolone resistance, mediated by *qnr* genes, has not been reported for *A. baumannii*.

### Tetracyclines and Glycycyclines

Resistance to tetracyclines and their derivatives can be mediated by efflux or ribosomal protection (169). Tetracycline-specific efflux pumps include those encoded by the *tet(A)* to *tet(E)* determinants, most often found within gram-negative organisms, and the *tet(K)* determinant found in *Staphylococcus aureus*. Thus far, the *tet(A)* and *tet(B)* determinants have been described for *A. baumannii* (217, 455, 457). *tet(A)* was found within a transposon similar to Tn*1721*, in association with an IS element (455). *tet(A)* confers resistance to tetracycline but not minocycline, an agent with greater activity against *A. baumannii*. Ribosomal protection is mediated by the *tet(M)* and *tet(O)* determinants, with *tet(M)* being described rarely for *A. baumannii* (457). Interestingly, this *tet(M)* determinant was identical to that described for *S. aureus* (457).

Apart from tetracycline-specific efflux pumps, this class of antimicrobials is also susceptible to efflux by the multidrug efflux systems, such as the AdeABC pump (347). Importantly, tigecycline, which is the first of a new class of modified tetracycline antimicrobials known as glycycyclines, is also a substrate for this emerging efflux system (420, 469). By performing real-time PCR with the *adeB* gene in clinical and laboratory exposed isolates with increased MICs of tigecycline, increased *adeB* gene expression was identified (420). It was of concern that the rise in MIC of tigecycline occurred rapidly with in vitro passage, suggesting that the expression of this multidrug efflux pump can be upregulated swiftly in response to selective pressure. The role of the AdeABC efflux pump in reduced susceptibility to tigecycline was confirmed by insertional inactivation of the *adeB* gene, which led to a significant drop in the MIC of tigecycline (4 μg/ml to 0.5 μg/ml) (469). These data suggest that caution should be used in considering tigecycline treatment for *A. baumannii* infection in sites where drug levels may be suboptimal, such as the bloodstream (424).

### Polymyxins

Despite recent reports demonstrating increasing in vitro resistance and heteroresistance to the polymyxins in *A. baumannii* (177, 334), the mechanism of resistance remains unknown.
TABLE 3. Comparison of EUCAST, CLSI, and BSAC breakpoints for various antibiotics versus *Acinetobacter* spp.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Breakpoints for susceptibility/ resistance (µg/ml)</th>
<th>EUCAST*</th>
<th>CLSI*</th>
<th>BSAC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem, meropenem</td>
<td></td>
<td>2/8</td>
<td>16/16</td>
<td>2/8</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td></td>
<td>1/1</td>
<td>1/4</td>
<td>1/1</td>
</tr>
<tr>
<td>Levofloxacine</td>
<td></td>
<td>1/2</td>
<td>2/8</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
<td>8/16</td>
<td>64/64</td>
<td></td>
</tr>
<tr>
<td>Gentamicin, tobramycin</td>
<td></td>
<td>4/4</td>
<td>4/16</td>
<td>4/4</td>
</tr>
<tr>
<td>Nalidixic</td>
<td></td>
<td>4/4</td>
<td>8/32</td>
<td></td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td></td>
<td>8/32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td></td>
<td>16/128</td>
<td>16/16</td>
<td></td>
</tr>
<tr>
<td>Ticarcillin-clavulanate</td>
<td></td>
<td>16/128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazidine, cefepime</td>
<td></td>
<td>8/32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone, cefotaxime</td>
<td></td>
<td>8/64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymyxin B, colistin</td>
<td></td>
<td>2/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td></td>
<td>2/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline, minocycline, tetracycline</td>
<td></td>
<td>4/16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td></td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For EUCAST and BSAC breakpoints, susceptibility is defined by a MIC equal to or lower than the first number and resistance is defined by a MIC greater than the second number.

** For CLSI breakpoints, susceptibility is defined by a MIC equal to or lower than the first number and resistance is defined by a MIC equal to or greater than the second number.

It has previously been shown that reduced binding to the lipopolysaccharide (LPS) target site can lead to resistance in *E. coli*, *Salmonella* spp., and *P. aeruginosa* (100, 431). Also, changes in OMPs causing reduced susceptibility to polymyxins have been described for *P. aeruginosa* (400, 614).

Other Antibiotics

The prevalence of trimethoprim-sulfamethoxazole resistance in *A. baumannii* is high in many geographic regions (216, 575). As discussed above, integrons are very common among strains of *A. baumannii* that have a multidrug resistance phenotype. The 3′-conserved region of an integron most commonly contains a *gac* gene fused to a *sul* gene, conferring resistance to antiseptics and sulfonamides, respectively (589). Consequently, sulfonamide resistance has been shown to be highly predictive of integron-carrying strains of *A. baumannii* (83, 216). Similarly, genes coding for trimethoprim (dldr) and chloramphenicol (cat) resistance have also been reported within integron structures in *A. baumannii* (216, 246, 320, 551). Efflux may also contribute to resistance against these agents (525).

ANTIBIOTIC SUSCEPTIBILITY TESTING FOR THE CLINICAL MICROBIOLOGY LABORATORY

Breakpoints for Various Antibiotics and *A. baumannii*

It is noteworthy that the major organizations that determine breakpoints (CLSI and the European Committee on Antimicrobial Susceptibility Testing [EUCAST]) have different breakpoints for many of the key antibiotics used in the therapy of *A. baumannii* infections (for example, carbapenems, fluoroquinolones, and aminoglycosides) (Table 3). At the time of this writing, no EUCAST breakpoints exist for penicillins, cephalosporins, polymyxins, tetracyclines, or trimethoprim-sulfamethoxazole versus *A. baumannii*. Breakpoints for tigecycline versus *A. baumannii* are not available via EUCAST, CLSI, or the FDA.

Issues for Antibiotic Susceptibility Testing of *A. baumannii*

CLSI recommends that MICs for antibiotics versus *Acinetobacter* spp. be determined in broth, using cation-adjusted Mueller-Hinton broth, or on agar, using Mueller-Hinton agar (97). Disk diffusion should also be performed using Mueller-Hinton agar (97). Swenson and colleagues assessed these CLSI-recommended methods and identified several problems in testing β-lactam antibiotics (529). First, very small colonies or a star-like growth was frequently observed in wells containing high concentrations of β-lactam antibiotics. This apparent growth beyond a more obvious end point makes determining an MIC by broth microdilution methods quite difficult. Second, there were many discrepancies between results obtained by broth microdilution and those obtained by disk diffusion. Very major errors (susceptible according to disk diffusion but resistant according to broth microdilution) occurred with ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidine, and cefepime. In the absence of human or animal model data, it is impossible to determine which testing method is more clinically relevant. Finally, interlaboratory variations in susceptibility testing results were frequent, especially for cefepime (529). In contrast to the findings with these β-lactams, there was little MIC and zone diameter discrepancy for carbapenems, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole (529).

The specific issue of in vitro testing of β-lactam–β-lactamase inhibitor combinations has been assessed by Higgins et al. (235). CLSI guidelines for testing piperacillin-tazobactam and ticarcillin-clavulanic acid require fixed concentrations of 4 µg/ml (tazobactam) and 2 µg/ml (clavulanic acid) (97). In contrast, CLSI guidelines for testing ampicillin-sulbactam require a ratio of ampicillin to sulbactam of 2:1 (97). Higgins et al. showed that the in vitro results for β-lactam–β-lactamase inhibitor combinations against *A. baumannii* are determined mainly by the activity of the inhibitors alone and therefore influenced by whether a fixed ratio of β-lactam to inhibitor or a fixed concentration of inhibitor is used (235). Therefore, it is doubtful that current testing of piperacillin-tazobactam or ticarcillin-clavulanic acid achieves clinically meaningful results, and we recommend that these drugs not be tested for susceptibility versus *A. baumannii*. The situation with disk diffusion testing is also problematic. Owing to the methodologic problems described above, we discourage the use of disk diffusion testing for all of the β-lactam–β-lactamase inhibitor combinations versus *A. baumannii*.

Semiautomated methods, such as those for the Vitek 2, Microscan, and BD Phoenix systems, are commonly used for antimicrobial susceptibility testing by clinical microbiology laboratories. Unfortunately, there is limited information about the performance of these methods against *A. baumannii*. Studies from the 1990s with an early Vitek system showed that numerous isolates were reported as resistant to imipenem by Vitek but typically were susceptible to imipenem when tested by broth and agar dilution (552). In view of this report and a
We urge caution in applying tigecycline breakpoints defined for the Enterobacteriaceae to A. baumannii for several reasons. Breakpoints are established with knowledge of the wild-type susceptibility of the organism to the antibiotic, the pharmacokinetics and pharmacodynamics of the antibiotic, and clinical data with respect to serious infections with the organism treated with the antibiotic (554). Clearly, wild-type susceptibilities and clinical responses may be organism specific. This has led to the situation, for example, whereby the FDA breakpoint for susceptibility of enterococci to tigecycline is ≤0.25 μg/ml while that for Enterobacteriaceae is ≥2 μg/ml (http://www.wyeth.com/content/showlabeling.asp?id=474 [accessed 2 August 2007]). There are no data available to make such distinctions for tigecycline and A. baumannii. EUCAST notes that “there is insufficient evidence that the species in question is a good target for therapy with the drug” (http://www.srga.org/eucastwt/MICTAB/MICtigecycline.htm [accessed 2 August 2007]). Furthermore, there is a difference in tigecycline breakpoints for Enterobacteriaceae between different breakpoint setting organizations (FDA versus EUCAST), and no breakpoints have been set by CLSI. Finally, the mean maximum blood concentration of tigecycline is 0.63 μg/ml after administration of a 100-mg intravenous loading dose followed by 50 mg every 12 h, so it would seem prudent not to report bloodstream isolates of A. baumannii with tigecycline MICs of >0.5 μg/ml as susceptible (424). Indeed, it is for this reason that we suggest that an MIC-based method of antibiotic susceptibility testing (rather than disk diffusion testing) be performed for tigecycline for bloodstream isolates of A. baumannii. The British Society for Antimicrobial Chemotherapy (BSAC) has established tentative tigecycline breakpoints for Acinetobacter spp., as follows: MICs of ≤1 μg/ml, susceptible; MIC of 2 μg/ml, intermediate; and MICs of >2 μg/ml, resistant (www.bsac.org.uk/_db_/documents/version_6_1.pdf [accessed 2 August 2007]). Pending further information, we recommend using these breakpoints for infection sites other than blood.

Unlike EUCAST and BSAC, the CLSI has established breakpoints for colistin and polymyxin B versus A. baumannii (97). These are as follows: MICs of ≤2 μg/ml, susceptible; and MICs of ≥4 μg/ml, resistant. Testing of A. baumannii susceptibility to colistin or polymyxin B should be performed by a method enabling determination of the MIC, such as broth dilution (178, 276). Using agar dilution, MICs of colistin may be 1 dilution higher than those of polymyxin B for some organisms (238). We recommend that institutions test the susceptibility of the polymyxin that is used in clinical practice at their institution. It is important that although colistin methanesulfonate (CMS; also known as colistimethate) is used in intravenous formulations of “colistin,” the human formulation should not be used for susceptibility testing (332). This is for several reasons. First, CMS is an inactive prodrug of colistin (27). Second, in determining MICs in broth during overnight incubation at 35°C, hydrolysis of CMS to colistin occurs via a series of partly methanesulfonated intermediates; the killing characteristics of this mixture change over time during incubation, leading to potentially unpredictable results (332). Thus, dilution-based testing should always be done with colistin sulfate (obtained, for example, from chemical supply companies such as Sigma-Aldrich), not with the intravenous “colistin” formulation obtained from a hospital pharmacy.

Subsequent evaluation showing that carbapenem testing difficulties existed for Vitek 2 in examining the susceptibility of Enterobacteriaceae and P. aeruginosa (523), some authors advocate confirmation of Vitek-determined carbapenem resistance (195). An “all-in-one plate” for this purpose has been described, in which susceptibility to imipenem and meropenem is confirmed by disk diffusion and the MIC of colistin is determined on the same plate by Etest (195). In general, however, the Vitek 2 system does appear to be reliable, in comparison to reference broth microdilution methods, for assessing susceptibility of A. baumannii to imipenem and other commonly used antibiotics (279). In evaluations of small numbers of A. baumannii group strains, the BD Phoenix automated microbiology system did not give very major errors in susceptibility testing compared to reference methods (133, 149, 176, 362, 521).

Susceptibility testing of the polymyxins and tigecycline against A. baumannii warrants specific mention because these antibiotics are often utilized for serious infections with multidrug-resistant A. baumannii. As mentioned above, the FDA, CLSI, and EUCAST have established no breakpoints for interpretation of antibiotic susceptibility testing of tigecycline versus A. baumannii. This has resulted in immense confusion as to appropriate methods for performing and interpreting antibiotic susceptibility testing for this drug-organism combination. In the product information for tigecycline (http://www.wyeth.com/content/showlabeling.asp?id=474 [accessed 2 August 2007]), it is recommended in general for tigecycline susceptibility testing that disk diffusion testing (with paper disks impregnated with 15 μg/ml tigecycline) or broth, agar, or broth microdilution methods be used. MICs must be determined with testing medium that is fresh (that is, ≤12 h old) (54, 243, 429). When tested in freshly prepared media (<12 h old), tigecycline was 2 to 3 dilutions more active than when it was tested in “aged” media. Media stored under anaerobic conditions or supplemented with the biocatalytic oxygen-reducing reagent Oxarze resulted in MICs similar to those obtained with fresh medium (54, 429). Tigecycline is stable in MIC trays that are prepared with fresh broth and then frozen. Therefore, the laboratory can thaw the preprepared MIC plates on the day of use and retain accuracy in MIC measurements (54).

Questions have arisen regarding the reliability of disk diffusion or Etest determination of tigecycline susceptibility testing versus A. baumannii (278, 538). In one study, Etest MICs were typically fourfold higher than those determined by broth microdilution (538). However, others have found good correlation between tigecycline MIC determinations by Etest versus reference broth microdilution methods, although the numbers of Acinetobacter isolates in these studies were small (44, 242).

The utility of the Vitek 2, Microscan, or BD Phoenix system for susceptibility testing of A. baumannii versus tigecycline has not yet been reported. With regard to disk diffusion testing, Jones and colleagues extrapolated FDA breakpoints for tigecycline versus Enterobacteriaceae to 103 Acinetobacter strains and found that approximately 20% of strains would appear “falsely intermediate” by disk diffusion testing in comparison to broth microdilution testing (278). Suggestions have been made to utilize an inhibition zone diameter of ≥16 mm (278) or ≥13 mm (538) as an indicator of A. baumannii susceptibility to tigecycline.
A number of studies have assessed the performance of Etest for determination of colistin susceptibility (13, 346, 536). Although agreement between MICs within one twofold dilution obtained by Etest and broth microdilution is rather low, categorical concordance is 87% to ≥95% (13, 346, 536). In one evaluation, there was 100% categorical agreement between agar dilution and Vitek 2 testing for colistin susceptibility, but no colistin-resistant isolates were tested (534). Inherent properties of the polymyxins make disk diffusion testing difficult, and we do not recommend it as a means of assessing susceptibility of *A. baumannii* to colistin (346, 533). The polymyxins are large polypeptides and diffuse poorly in agar, resulting in small zones of inhibition. Subsequently, this results in poor categorical differentiation of susceptible and resistant isolates. Use of higher concentrations of the polymyxin in the disks does not appear to improve the accuracy of test results (533).

**Clinical Laboratory Detection of Carbapenemases**

As described above, a variety of β-lactamas produced by *A. baumannii* are capable of hydrolyzing carbapenems. These “carbapenemases” were recently reviewed in detail in this journal by Queenan and Bush (447). *Acinetobacter* isolates that express these enzymes but which have carbapenem MICs in the susceptible range have been described, but these appear to be uncommon (174). Phenotypic tests for evaluating the presence of serine carbapenemases (OXA type) in *A. baumannii* have not yet been described. The most frequently used methods for detecting MBLs have been disk approximation methods comprising imipenem and imipenem plus EDTA (174, 290, 318). Others have used 2-mercaptoethane acid for this purpose (12). An Etest MBL strip has been developed and, in published reports, has been shown to be reliable for detecting IMP- and VIM-type MBLs in *A. baumannii* (319, 589). Apparently, false-positive results were seen for isolates producing OXA-23 but lacking genes encoding IMP and VIM (490). These investigators did not seek other MBLs, however. It is also noteworthy that since the lowest concentrations of imipenem with and without EDTA on the Etest MBL strip are 1 and 4 µg/ml, respectively, the strip cannot be used in the evaluation of an isolate with an imipenem MIC of <4 µg/ml (609).

**Role of the Clinical Microbiology Laboratory in Providing Surveillance for Multidrug-Resistant *A. baumannii***

Surveillance for patients colonized with multidrug- or pandrug-resistant *A. baumannii* may be considered for infection control purposes. There are few data at present on which to base recommendations. Culture of samples from the nostrils, pharynx, skin, and rectum of patients with recent clinical cultures of *A. baumannii* was thought to have poor sensitivity (<25% for any one site) when samples were plated onto MacConkey agar plates containing 8 µg/ml ceftazidime and 2 µg/ml amphotericin (355). Further studies are required to define the most effective methods for screening *A. baumannii* carriage in hospitalized patients and to determine the impact of such screening on infection rates and containment of this problematic organism.

**DEFINITIONS OF MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII**

Unfortunately, problems exist in evaluating previously published literature on the epidemiology of multidrug-resistant *A. baumannii*. Most surveillance studies indicate the percentages of isolates susceptible (or resistant) to a variety of antibiotics. However, few assess the percentage resistant to multiple antibiotics. Furthermore, when such assessments have occurred, a variety of definitions of multidrug resistance in *A. baumannii* have been utilized. This has clearly hindered comparison of the epidemiology of multidrug-resistant *A. baumannii* in different regions of the world, and we encourage the development of guidelines to unify the approach to these definitions.

For the purposes of this review, the following definitions are used. Multidrug resistance is resistance to more than two of the following five drug classes: antipseudomonal cephalosporins (ceftazidime or cefepime), antipseudomonal carbapenems (imipenem or meropenem), ampicillin-sulbactam, fluoroquinolones (ciprofloxacin or levofloxacin), and aminoglycosides (gentamicin, tobramycin, or amikacin). It needs to be acknowledged that susceptibility testing of β-lactam–β-lactamase inhibitor combinations is highly problematic and that laboratories may not test piperacillin–tazobactam or ticarcillin–clavulanate versus *A. baumannii*. Despite “pan-β” meaning “all,” pandrug resistance is often defined as resistance to all antimicrobials that undergo first-line susceptibility testing that have therapeutic potential against *A. baumannii*. This would include all β-lactams (including carbapenems and sulbactam [MICs of >4 µg/ml]), fluoroquinolones, and aminoglycosides. However, with the increased use of the polymyxins and possibly tigecycline, this definition will likely have to encompass these other agents.

**GLOBAL EPIDEMIOLOGY OF ACINETOBACTER BAUMANNII**

**Europe**

*A. baumannii* infections have been a substantial clinical issue in many parts of Europe (Fig. 2) (575). Since the early 1980s, hospital outbreaks of *A. baumannii* infections in Europe, mainly in England, France, Germany, Italy, Spain, and The Netherlands (28, 171, 584), have been investigated using molecular epidemiological typing methods. In the majority of cases, one or two epidemic strains were detected in a given epidemiological setting. Transmission of such strains has been observed between hospitals, most probably via transfer of colonized patients (112, 557, 569). Spread of multidrug-resistant *A. baumannii* is not confined to hospitals within a city but also occurs on a national scale. Examples are the spread of the so-called Southeast clone and the Oxa-23 clones 1 and 2 in Southeast England (99, 557), the dissemination of a multidrug-resistant *A. baumannii* clone in Portugal (112), the interhospital spread of a VEB-1 ESBL-producing *A. baumannii* clone from a total of 55 medical centers in northern and southeastern France (382), and the spread of an amikacin-resistant *A. baumannii* clone observed in nine hospitals in various regions in Spain (583). International transfer of colonized patients has led to the introduction and subsequent epidemic spread of multidrug-resistant *A. baumannii* strains from Southern into Northern European countries, such as Belgium and Germany.
Intercontinental spread of multidrug-resistant *A. baumannii* has also been described between Europe and other countries as a consequence of airline travel (383, 421). These events highlight the importance of appropriate screening and possible isolation of patients transferred from countries with high rates of drug-resistant organisms.

In addition to these interinstitutional outbreaks, three international *A. baumannii* clones (the so-called European clones I, II, and III) have been reported from hospitals in Northern Europe (including hospitals in Belgium, Denmark, the Czech Republic, France, Spain, The Netherlands, and the United Kingdom) as well as from hospitals in southern European countries, such as Italy, Spain, Greece, and Turkey (123, 394, 570), and in Eastern Europe (606). Initially detected by AFLP clustering at a similarity level of \( >80\% \), the epidemiological relationship of these clones was confirmed by ribotyping (394, 570), pulsed-field gel electrophoresis (PFGE) (570), and most recently, multilocus sequence typing (MLST) (18). In contrast to the aforementioned multisite outbreaks, no epidemiological link in time or space could be established between the outbreaks of the European clones in different medical centers, and the actual contributions of these three widespread clones to the overall burden of epidemic *A. baumannii* strains remain to be determined.

Carbapenem resistance in *A. baumannii* is now an issue in many European countries. Information on the prevalence of carbapenem resistance in various European countries is difficult to obtain, but it appears from the outbreak literature that carbapenem resistance rates are highest in Turkey, Greece, Italy, Spain, and England and are still rather low in Germany and The Netherlands. Carbapenem resistance in Eastern Europe appears to be increasing (128, 606). Rates appear to be lowest in Scandinavia, although sporadic isolates have been reported from patients transferred from elsewhere, including victims of the Indian Ocean tsunami (284). In an industry-supported surveillance report (MYSTIC) from 48 European hospitals for the period 2002–2004, just 73.1% of isolates were susceptible to meropenem and 69.8% were susceptible to imipenem (560). Susceptibility to other antibiotics was also very low, with 32.4%, 34.0%, and 47.6% being susceptible to ceftazidime, ciprofloxacin, and gentamicin, respectively (560). *A. baumannii* isolates resistant to the polymyxins have been detected in Europe, although at present these remain rare (26, 74, 140, 177, 182, 229, 568). For a detailed review of phenotypic resistance in *Acinetobacter* spp. throughout Europe, readers are referred to an excellent review by Van Looveren and Goossens (575).

**North America**

There is a long history of multidrug-resistant *A. baumannii* infections occurring in the United States. In 1991 and 1992, outbreaks of carbapenem-resistant *A. baumannii* were observed in a hospital in New York City (200). This followed an outbreak of infections due to ESBL-producing *Klebsiella pneumoniae* during which use of imipenem increased substantially.

![FIG. 2. Countries that have reported an outbreak of carbapenem-resistant *Acinetobacter baumannii*. Red signifies outbreaks reported before 2006, and yellow signifies outbreaks reported since 2006.](http://cmr.asm.org/)
(365, 450). The organisms in this outbreak were multidrug resistant, retaining susceptibility only to polymyxins and ampicillin-sulbactam (200). Numerous other hospitals in New York City also had clonal outbreaks of multidrug- or pandrug-resistant \textit{A. baumannii} (143, 200, 277, 308, 309, 348, 351, 446), and similar outbreaks have frequently been reported from many other regions of the United States (277, 342, 359, 522, 549, 604). National surveillance studies have demonstrated significant trends in the emergence of multidrug-resistant \textit{Acinetobacter} strains (187). For example, data from the National Nosocomial Infection Surveillance system collected from 1986 to 2003, involving many hospitals throughout the United States, showed significant increases in \textit{Acinetobacter} strains resistant to amikacin (5% to 20%; \( P < 0.001 \)), ceftazidime (25% to 68%; \( P < 0.001 \)), and imipenem (0% to 20%; \( P < 0.001 \)) (187).

In a more recent industry-supported surveillance study including isolates of \textit{Acinetobacter} spp. collected between 2004 and 2005 from 76 centers throughout the United States, only 60.2% were susceptible to imipenem (218). A further industry-supported surveillance study including isolates of \textit{Acinetobacter} spp. from 15 centers throughout the United States reported improved carbapenem and aminoglycoside susceptibilities in 2005 compared with those in 2004 (454). However, rates of nonsusceptibility were still substantial, as follows: 10% to 15% for carbapenems, 35% to 40% for ceftazidime/cefepime, 10% to 30% for aminoglycosides, and 35% to 40% for ciprofloxacin/levofloxacin (454). The MIC\(_{50}\) and MIC\(_{90}\) of tigecycline for \textit{A. baumannii} isolates collected from the United States between 2004 and 2005 were 0.5 \( \mu \)g/ml and 1 \( \mu \)g/ml, respectively, with an MIC range of 0.03 \( \mu \)g/ml to 8 \( \mu \)g/ml (588). The MIC\(_{90}\) for multidrug-resistant strains collected in the same time period was 2 \( \mu \)g/ml (237), which is more consistent with that reported from individual centers. Global surveillance data for susceptibility to polymyxin B have reported an MIC\(_{50}\) of \( \leq 1 \) \( \mu \)g/ml and an MIC\(_{90}\) of 2 \( \mu \)g/ml against 2,621 \textit{Acinetobacter} sp. isolates from four major geographic regions (Asia-Pacific, Europe, Latin America, and North America) (177). The rate of polymyxin B-nonsusceptible \textit{Acinetobacter} spp. in North America was reported to be 1.7% (177). This compares with 1.9%, 2.7%, and 1.7% in the Asia-Pacific region, Europe, and Latin America, respectively (177). Overall, 2.8% and 3.2% of carbapenem-resistant and multidrug-resistant \textit{Acinetobacter} spp., respectively, were resistant to polymyxin B (177).

It is clear that more attention is being paid to \textit{A. baumannii} infections in the United States now than at any time in the past. This may reflect increased recognition of pandrug-resistant strains. There are some data to suggest that the proportion of intensive care unit (ICU)-acquired pneumonia cases being found to be due to \textit{A. baumannii} is actually increasing. In a review from the CDC, 7% of ICU-acquired pneumonias were due to \textit{Acinetobacter} in 2003, compared to 4% in 1986 (\( P < 0.001 \)) (187). The proportion of urinary tract infections (UTIs) and skin/soft tissue infections due to \textit{Acinetobacter} also significantly increased during this period (187). There is some evidence that nosocomial \textit{Acinetobacter} infections have some seasonal variation in the United States, with an unexplained upswing in late summer months (360).

An important contribution to the epidemiology of infections with \textit{A. baumannii} in the United States is the return of military personnel who have fought in Iraq or Afghanistan (75, 115, 376, 489, 555). An increase in infections with \textit{A. baumannii} was first observed in U.S. military personnel in March 2003, soon after combat operations commenced in Iraq. Most injured military personnel were first treated at field hospitals before being evacuated to the Landstuhl Regional Medical Center (Germany) or the Walter Reed Army Medical Center (United States) (489). Most of these infections were detected at or soon after admission to these institutions. In a careful outbreak investigation, it was determined that neither preinjury skin colonization nor introduction of the organism from soil at the time of traumatic injury was the source of infection (376, 489).

Rather, multiple \textit{A. baumannii} isolates were cultured from a range of inanimate surfaces in field hospitals and were genotypically linked to patient isolates (489). Typically, these isolates were multidrug resistant, being resistant to fluoroquinolones, cephalosporins, and piperacillin-tazobactam. Just 10% were nonsusceptible to carbapenems (489). However, in a paper by Hawley et al., the rate of non-imipenem-susceptible \textit{A. baumannii} was 37% for injured deployed military personnel (224). Such rates are more consistent with those seen in Europe than in the United States. The MIC\(_{90}\) of tigecycline for these strains was 8 \( \mu \)g/ml (224). Hujer and colleagues found that carbapenem-resistant isolates from patients at Walter Reed Army Medical Center typically produced OXA-23 or OXA-58 carbapenemase (250). Interestingly, in a study comparing the clonal relatedness of \textit{A. baumannii} strains from injured military personnel from the United States with that of strains from the United Kingdom, the main outbreak strains were indistinguishable (555). This provides further support that \textit{A. baumannii} acquisition is occurring in the field hospitals.

A comparable situation with Canadian soldiers injured in Afghanistan and British soldiers injured in Iraq has recently been reported (274, 540, 555). Outbreaks of multidrug-resistant \textit{A. baumannii} in Canadian civilian hospitals appear to be less common than those in comparable institutions in the United States but have certainly still been reported (509).

**Latin America**

Rates of nonsusceptibility to meropenem, imipenem, ceftazidime, piperacillin-tazobactam, ciprofloxacin, and gentamicin in Latin America appear to be among the highest in the world (560). For example, just 71% of isolates were susceptible to meropenem or imipenem in an assessment from a surveillance program in the period 2002–2004 (560). In a surveillance study involving Argentina, Brazil, Chile, and Colombia from 1997 to 2001, resistance rates were highest in Argentina, but no countries were spared multidrug-resistant isolates (543). As described previously, a variety of carbapenemases have been identified in \textit{A. baumannii} isolates in Latin America, including IMP-1 and IMP-6 in Brazil (179, 471, 544), OXA-23 in Brazil and Colombia (110, 585), and OXA-58 in Argentina (98). Interestingly, the SPM- and VIM-type MBLs, which are widespread in Brazil (SPM) and other parts of Latin America (VIM) in \textit{P. aeruginosa} strains, have not yet been reported for \textit{A. baumannii} strains in these regions, to our knowledge.
Africa

Data on the extent of antibiotic resistance in Acinetobacter baumannii in Africa are largely limited to South Africa at the present time, although there are scattered reports from other countries (255, 391, 475). Brink and colleagues have shown that about 30% of Acinetobacter baumannii bloodstream isolates in South Africa are carbapenem resistant, >40% are resistant to cefepime and piperacillin-tazobactam, and >30% are resistant to ciprofloxacin and levofloxacin (59). Such resistant strains are endemic in some units (for example, burns and ICUs) and have been spread from institution to institution (354).

Asia and the Middle East

Numerous outbreaks of pandrug-resistant Acinetobacter baumannii have been documented in Asian and Middle Eastern hospitals (Fig. 2), and a variety of carbapenemases have been described to originate there (2, 3, 78, 186, 264, 265, 293, 298, 317, 320, 419, 610). Rates of nonsusceptibility in SENTRY isolates (2001–2004) exceeded 25% for imipenem and meropenem, 40% for cefepime and ceftazidime, 40% for ampicillin-sulbactam, 35% for amikacin, and 45% for ciprofloxacin (177). Unfortunately, resistance to tigecycline (389) and polymyxin B (177, 293) already exists in this region.

Australia and Pacific Islands

Initial reports of Acinetobacter baumannii from Australia came from the Northern Territory, where community-acquired infections are well described (8, 9). Such infections have a vastly different epidemiology from that seen in hospital-acquired infections, with male gender, age of >45 years, Aboriginal ethnic background, cigarette smoking, alcoholism, diabetes mellitus, and chronic obstructive airway disease being important risk factors (9). Also, these community-acquired strains are significantly more susceptible to antimicrobials (9). Throat carriage and microaspiration may be involved in the pathogenesis of these infections (8).

The first described Australian outbreak of hospital-acquired Acinetobacter baumannii was in Western Australia (460). These isolates were resistant to gentamicin, cephalexin, and ticarcillin, with some isolates also being resistant to ciprofloxacin. Molecular epidemiological analysis identified that 11% of staff hand samples were positive for the same strain of Acinetobacter baumannii as that causing patient infection (460). More recently, outbreaks of Acinetobacter baumannii have affected other major cities along the eastern seaboard of Australia, including Brisbane, Sydney, and Melbourne (422, 437, 566). Unfortunately, these outbreaks have involved carbapenem-resistant strains of Acinetobacter baumannii, with OXA-23 contributing to this phenotype (566). As seen in other countries, strains within institutions are often clonally related (422). Also, interhospital spread of multidrug-resistant Acinetobacter baumannii strains has likely occurred in certain cities (435). Outbreaks of carbapenem-resistant Acinetobacter baumannii have also occurred in French Polynesia (384). Most recently, reduced susceptibility to tigecycline of multidrug-resistant Acinetobacter baumannii strains has been described in Australia (254).

CLINICAL MANIFESTATIONS OF ACINETOBACTER BAUMANNII INFECTIONS

In the vast majority of publications on the clinical manifestations of Acinetobacter infections, the methods used for species identification were not appropriate according to current standards (see above). However, with an acceptable level of uncertainty, we can assume that what has been published on nosocomial Acinetobacter infection in general, or on Acinetobacter infection in particular, is indeed applicable to Acinetobacter baumannii. Case reports or small series on clinical manifestations of infections caused by Acinetobacter infections outside the Acinetobacter baumannii group should be interpreted with caution if (semi-)automated methods for species identification were employed.

Hospital-Acquired Pneumonia

In most institutions, the majority of Acinetobacter baumannii isolates are from the respiratory tracts of hospitalized patients. In many circumstances, it is very difficult to distinguish upper airway colonization from true pneumonia. There is no doubt, however, that true ventilator-associated pneumonia (VAP) due to Acinetobacter baumannii occurs. In large surveillance studies from the United States, between 5 and 10% of cases of ICU-acquired pneumonia were due to Acinetobacter baumannii (187). However, it is highly likely that in certain institutions, the proportion of ICU-acquired pneumonia due to Acinetobacter baumannii is much higher. Typically, patients with Acinetobacter baumannii infections have had prolonged ICU stays (184), although in outbreak situations, earlier acquisition of infection may occur.

Community-Acquired Pneumonia

Community-acquired pneumonia due to Acinetobacter baumannii has been described for tropical regions of Australia and Asia (8, 9, 39, 205, 325). The disease most typically occurs during the rainy season among people with a history of alcohol abuse and may sometimes require admission to an ICU (8). It is characterized by a fulminant clinical course, secondary bloodstream infection, and mortality rate of 40 to 60% (325). The source of infection may be throat carriage, which occurs in up to 10% of community residents with excessive alcohol consumption (8).

Bloodstream Infection

In a large study of nosocomial bloodstream infection in the United States (1995–2002), Acinetobacter baumannii was the 10th most common etiologic agent, being responsible for 1.3% of all monomicrobial nosocomial bloodstream infections (0.6 bloodstream infection per 10,000 admissions) (597). Acinetobacter baumannii was a more common cause of ICU-acquired bloodstream infection than of non-ICU-ward infection (1.6% versus 0.9% of bloodstream infections, respectively, in those locations). Crude mortality overall from Acinetobacter baumannii bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU. Acinetobacter baumannii bloodstream infection had the third highest crude mortality rate in the ICU, exceeded only by P. aeruginosa and Candida sp. infections. Acinetobacter baumannii infections were the latest of all bloodstream infections to occur during hospitalization, occurring a mean of 26 days from the time of hospital admission (597). It is therefore not certain if the high crude mortality...
rate represents its occurrence in patients with ongoing underlying critical illness or whether the organism does have significant attributable mortality (see below). Sources of bloodstream infection were not described in the study mentioned above but are typically line related or attributed to underlying pneumonia, UTI, or wound infection (501). It is notable that 102 patients had bloodstream infections at sites treating U.S. military members injured in Iraq or Afghanistan from 1 January 2002 and 31 August 2004 (75). The sites of origin of these infections were not described in this report.

Traumatic Battlefield and Other Wounds

_A. baumannii_ may occasionally cause skin/soft tissue infections outside of the military population. The organism caused 2.1% of ICU-acquired skin/soft tissue infections in one assessment (187). It is a well-known pathogen in burn units and may be difficult to eradicate from such patients (549). However, its contribution to poor outcome in burn patients is debated (5, 600). _A. baumannii_ is commonly isolated from wounds of combat casualties from Iraq or Afghanistan (270, 376, 428, 489, 595, 616). It was the most commonly isolated organism (32.5% of cases) in one assessment of combat victims with open tibia fractures (270). However, it appears to be of low pathogenicity at this site—after initial treatment, the organism was never isolated from follow-up cultures in any of the patients with open tibia fractures and did not appear to contribute directly to persistent nonunion or need for amputation (270).

UTI

_A. baumannii_ is an occasional cause of UTI, being responsible for just 1.6% of ICU-acquired UTIs in one study (187). Typically, the organism is associated with catheter-associated infection or colonization. It is not usual for this organism to cause uncomplicated UTI in healthy outpatients.

Meningitis

Nosocomial, postneurosurgical _A. baumannii_ meningitis is an increasingly important entity. The microbial epidemiology of nosocomial meningitis is evolving to include more gram-negative pathogens (58, 142, 414, 507), so it is not surprising that multidrug-resistant _A. baumannii_ is among the pathogens implicated (364, 399, 404, 408). Typical patients have undergone neurosurgery and have an external ventricular drain (363). Mortality may be as high as 70%, although the cause of mortality is often difficult to discern (363).

Other Manifestations

A small number of case reports of _Acinetobacter_ endocarditis exist (361, 407, 461, 520, 567). Most, but not all, cases have involved prosthetic valves. _Acinetobacter_ spp. may cause endophthalmitis or keratitis, sometimes related to contact lens use or following eye surgery (105, 287, 329, 338). A single case report exists of a Shiga toxin-producing _A. haemolyticus_ strain, which was associated with bloody diarrhea in a 3-month-old infant (213). Note that precise species identification remains an issue in these reports.

**CLINICAL IMPACT OF ACINETOBACTER BAUMANNII INFECTION**

Given the predilection of _A. baumannii_ to colonize and infect critically ill patients, who often have a poor prognosis irrespective of secondary infective complications, it has been challenging to determine the true clinical impact of this pathogen, and much debate still exists in the literature (154, 160, 528). Unfortunately, significant methodological heterogeneity exists between studies (Table 4); thus, it has been difficult to formulate conclusions. Most studies utilize a matched cohort or case control study design, but the definitions used for a case and the comparative control group are clearly diverse among studies. For example, the definitions of a case include patients with _A. baumannii_ infection only (5, 183, 528), infection and colonization (1, 341, 345, 437), infection of one site (40, 183, 215, 306, 600), or infection of multiple sites (1, 5, 528). Patients with polymicrobial infections were also allowed in some studies (40, 600). However, it is the extreme diversity in controls that really distinguishes one study from another, as controls included patients with no _A. baumannii_ infection or colonization but infection with other organisms allowed (183), no _A. baumannii_ infection only but colonization allowed (600), no infection with drug-resistant _A. baumannii_ but infection with susceptible isolates allowed (1, 437), or no specific infection type, such as bacteremia, with any organism allowed (40). Also, the rigor of matching for severity of illness and comorbid conditions varies, and thus important confounding factors cloud some of the literature, and the quality of species identification in many studies is suboptimal, which may also affect outcomes. At this point, despite the influx of such studies over recent times, we still feel that the issue of attributable mortality remains unsettled. For example, in a recent study performed by the CDC, which involved thorough adjustment of important confounding variables and used clear definitions for comparison groups, there was no significant increase in mortality between those infected with multidrug-resistant _A. baumannii_ and those with no infection (odds ratio [OR], 6.6; 95% confidence interval [95% CI], 0.4 to 108.3) (528). However, hospital and ICU lengths of stay were significantly longer in the former group. A comparison between all those with infection, regardless of susceptibility profile, and those without infection was not performed. These results are supported by several studies (5, 40, 183, 341) yet contrast with many others (1, 181, 215, 306, 345), including those that primarily assessed the clinical impact of multidrug or carbapenem resistance on patient outcomes (306, 437).

Interestingly, when outcomes from _A. baumannii_ bacteremia were compared directly to those for patients who had bacteremia with other gram-negative organisms, including _Klebsiella pneumoniae_, a significant increase in mortality was noted for _A. baumannii_ (266, 462). A further study showed a significant increase in mortality with multidrug-resistant _A. baumannii_ colonization or infection compared to that with multidrug-resistant _Pseudomonas aeruginosa_ colonization or infection, using a Kaplan-Meier analysis (198). However, none of these studies used a formal, standardized method to adjust for severity of illness or comorbidities, such as an APACHE, McCabe, or Charlson score (81, 322). Whether the disparity between studies can be explained purely by methodological...
<table>
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<td>Abbo et al. (1)</td>
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<td>McCabe Score</td>
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<td>6 (P = 0.057)</td>
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<td>(i) 6.6 (P = 0.04; adjusted OR, 2.1 [10.0–4.3]) (ii) 8.9 (P &lt; 0.01; adjusted OR, 2.5 [1.2–5.4])</td>
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<td>(ii) 6 (P &lt; 0.01; adjusted OR, 4.2 [15–11.6])</td>
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<td>Playford et al. (437)</td>
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<td>Apache II score</td>
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<td>(i) 30 (P = NA)</td>
<td>(i) 15 (P = NA)</td>
</tr>
<tr>
<td>Garcia-Garmendia et al. (181)</td>
<td>Prospective, matched case control study</td>
<td>Patients with infection (48) or colonization (27) with A. baumannii</td>
<td>Patients without infection or colonization with A. baumannii (75)</td>
<td>Apache II score</td>
<td>For those with infection, 43% (P &lt; 0.01); risk rate of death, 4.0 (1.9–8.3)</td>
<td>NA</td>
<td>13 (P &lt; 0.01)</td>
</tr>
<tr>
<td>Blot et al. (40)</td>
<td>Retrospective matched cohort study</td>
<td>Patients with A. baumannii bacteremia (45)</td>
<td>Patients with no evidence of bacteremia from any cause (90)</td>
<td>Apache II score</td>
<td>7.8% (P = 0.38); adjusted HR, 0.96 (0.67–1.38)</td>
<td>NA</td>
<td>5 (P = 0.04)</td>
</tr>
<tr>
<td>Garnacho et al. (183)</td>
<td>Retrospective matched case control study</td>
<td>Patients with A. baumannii VAP (60)</td>
<td>Patients without any type of A. baumannii infection (may have had infection with other organisms) (60)</td>
<td>Apache II score</td>
<td>11.7% (P = 0.17)</td>
<td>NA</td>
<td>−1.3 days (P = nonsignificant)</td>
</tr>
<tr>
<td>Albrecht et al. (5)</td>
<td>Retrospective cohort study involving burn patients</td>
<td>Patients with A. baumannii infection (59)</td>
<td>(i) Patients colonized with A. baumannii (52); (ii) Patients without infection or colonization with A. baumannii (691)</td>
<td>Injury severity score</td>
<td>For infected patients vs noninfected patients, 14.3% (unadjusted P &lt; 0.01); adjusted P = 0.65</td>
<td>(i) 12.3 (P &lt; 0.05) 6; (ii) 41.8 (P &lt; 0.05)</td>
<td>(i) 15.9 (P &lt; 0.05) 6; (ii) 26.7 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Grupper et al. (215)</td>
<td>Retrospective matched cohort study</td>
<td>Patients with Acinetobacter bacteremia (52)</td>
<td>Patients without Acinetobacter bacteremia (52)</td>
<td>McCabe score</td>
<td>36.5% (unadjusted P &lt; 0.01); adjusted HR, 4.01 (1.29–1253)</td>
<td>5 (P = 0.06)</td>
<td>NA</td>
</tr>
</tbody>
</table>
A. BAUMANNII, A SUCCESSFUL PATHOGEN

Relative to other gram-negative organisms, such as *P. aeruginosa*, very little is known about the host-pathogen interactions involving *A. baumannii*. Recent whole-genome sequencing studies involving *A. baumannii* have demonstrated not only a vast array of antibiotic drug resistance determinants but also many pathogenicity islands (172, 514). Of relevance, a significant number of identified genes encoding resistance to antibiotics, heavy metals, and antiseptics likely originated in other highly pathogenic organisms, including *Pseudomonas* spp., *Salmonella* spp., and *E. coli* (172). This implies that genetic transfer of virulence determinants may also be possible. After performing random mutagenesis of an *A. baumannii* ATCC strain (17978), Smith et al. were able to identify several mutants in six different pathogenicity islands with attenuated virulence, as determined by the nonmammalian models *Caenorhabditis elegans* and *Dictyostelium discoideum* (514). The relevant mu-
tated genes encoded transcription factors, multidrug efflux transport systems, and a urease. Unfortunately, the virulence of these mutants was not assessed in a mammalian model. When the genome of \textit{A. baumannii} was compared to that of the nonpathogenic species \textit{A. baylyi}, 28 gene clusters were unique to \textit{A. baumannii}, with 16 having a potential role in virulence (514). One of the most interesting of these was a 133,740-bp island that contained not only transposons and integrases but also genes homologous to the \textit{Legionella/Coxiella} type IV virulence/secretion systems (514). Other relevant genes included those involved in the cell envelope, pilus biogenesis, and iron uptake and metabolism. An earlier study, which first described the applicability of transposon mutagenesis to \textit{A. baumannii}, identified several mutants with altered metabolic and global regulatory functions, including a gacS-like gene encoding a sensor kinase that is important for the regulation of virulence determinants in other gram-negative organisms, such as \textit{Pseudomonas} (291, 577), as well as attachment or biofilm mutants (136). Such broad-based genetic approaches are critical for the future identification of novel virulence factors in \textit{A. baumannii}.

The study of more specific virulence mechanisms in \textit{A. baumannii} has focused on siderophore-mediated iron acquisition systems (135, 137, 144, 607), biofilm formation (545, 578), adherence and OMP function (315, 510), and the \textit{A. baumannii} LPS (152, 222, 292). In order for \textit{A. baumannii} to thrive in the iron-deficient environment of a human host, it secretes low-molecular-mass ferric binding compounds, or siderophores (135, 137). Interestingly, the expression of these elements can vary greatly between clinical strains of \textit{A. baumannii} (135, 607), and these elements have structural and functional similarities to a siderophore produced by the fish pathogen \textit{Vibrio anguilinarum} (137), a potential origin of this critical virulence mechanism. The ability of \textit{A. baumannii} to adhere to and form biofilms on inanimate objects and surfaces may explain its success in the hospital environment. Tomaras et al. demonstrated that biofilm formation in \textit{A. baumannii} is phenotypically associated with exopolysaccharide production and pilus formation (545). Through random mutagenesis and genetic complementation, a gene encoding a protein highly similar to that encoded by the \textit{Vibrio parahaemolyticus} csuE gene was identified as a key factor in pilus and biofilm formation (545). Further sequence analysis identified a csu polycistronic operon involving five genes, some of which are homologous to genes that encode proteins related to chaperones and involved in pilus assembly in other gram-negative bacteria (545). Adherence of \textit{A. baumannii} to human bronchial epithelial cells and erythrocytes has also been demonstrated, with similar pilus-like structures appearing important for adherence (204, 315). Interestingly, considerable variation in quantitative adherence was observed between strains, including greater adherence of strains from European clone II than from clone I (315). However, no difference between outbreak and nonoutbreak strains was observed. After adherence to human cells, it appears that \textit{A. baumannii} can induce apoptosis via an OMP (Omp38) (84). This protein appears to localize to the mitochondria, leading to both caspase-dependent and -independent pathways of apoptosis (84). However, it is not the only factor involved, as an Omp38 mutant caused incomplete attenuation of cell death (84). Finally, quorum sensing has been shown to regulate a wide array of virulence mechanisms in many gram-negative organisms, particularly \textit{P. aeruginosa}. Up to four different quorum-sensing signal molecules have been identified in \textit{Acinetobacter}, indicating that this may be a central mechanism for autoinduction of multiple virulence factors (203, 271).

Apart from biofilm formation, exopolysaccharide production is also thought to protect bacteria from host defenses (271). Several recent studies have described the innate immune response to \textit{A. baumannii} and the importance of Toll-like receptor (TLR) signaling (152, 292). In a mouse pneumonia model, TLR4 gene-deficient mice had increased bacterial counts, increased bacteremia, impaired cytokine/chemokine responses, and delayed onset of lung inflammation compared to wild-type mice (292). \textit{A. baumannii} LPS was identified as the major immunostimulatory factor (292). This was further illustrated by the attenuated effects of \textit{A. baumannii} on mice deficient in CD14, an important molecule that enables LPS binding to TLR4 (292). These findings were recently confirmed using human cells, but in contrast to the mouse model, TLR2 was also identified as an important signaling pathway (152). The latter study also demonstrated the potent endotoxic potential of \textit{A. baumannii} LPS, which stimulated the proinflammatory cytokines interleukin-8 and tumor necrosis factor alpha equally to the stimulation by \textit{E. coli} LPS at similar concentrations (152). These studies suggest that \textit{A. baumannii} endotoxin may incite a strong inflammatory response during infection.

Humoral immune responses have also been described for \textit{Acinetobacter} infection, with antibodies being targeted toward iron-repressible OMPs and the O polysaccharide component of LPS (513). Of interest, a more recent study showed that mouse-derived monoclonal antibodies directed at \textit{A. baumannii} OMPs expressed in an iron-depleted environment have bactericidal and opsonizing in vitro activity (201). These antibodies were also able to block siderophore-mediated iron uptake (201).

**INFECTION CONTROL PERSPECTIVE**

Why Is \textit{A. baumannii} a Persistent Hospital Pathogen?

There are three major factors possibly contributing to the persistence of \textit{A. baumannii} in the hospital environment, i.e., resistance to major antimicrobial drugs, resistance to desiccation, and resistance to disinfectants. Resistance to antibiotics may provide certain \textit{A. baumannii} strains with a selective advantage in an environment, such as the modern ICU, where microorganisms are confronted with extensive exposure to antimicrobials. Several researchers have observed that resistance rates in epidemic \textit{A. baumannii} strains are significantly higher than those in sporadic \textit{A. baumannii} strains (123, 227, 262, 297). Resistance to the fluoroquinolones in particular was associated with epidemic behavior (227, 262). Villers et al. identified previous therapy with a fluoroquinolone as an independent risk factor for infection with epidemic \textit{A. baumannii}, and it appeared that the selection pressure caused by the indiscriminate use of fluoroquinolones was responsible for the persistence and epidemic spread of multidrug-resistant \textit{A. baumannii} clones for at least 5 years (586). The recently observed increase in carbapenem-resistant \textit{A. baumannii} strains was associated almost exclusively with hospital outbreaks (99, 351, 354). It has
been suggested that any clinical A. baumannii isolate with resistance to multiple antibiotics indicates a potential nosocomial outbreak strain (297).

To assess the desiccation tolerance of A. baumannii, Jawad et al. compared the survival times on glass coverslips of 22 strains isolated from eight well-defined hospital outbreaks with the survival times of 17 sporadic strains. The overall mean survival time was 27 days, with a range of 21 to 33 days (262). Of note, there were no differences in survival times between outbreak and sporadic strains; all investigated A. baumannii strains had the ability of long-time survival on dry surfaces and therefore an increased potential for epidemic spread. It has also been shown that A. baumannii strains survive desiccation far better than do other Acinetobacter species, such as A. johnsonii, A. junii, and A. lwoffi (261, 377). This, together with their greater susceptibility to commonly used antimicrobials, may explain why Acinetobacter strains belonging to these species have been implicated only very rarely in hospital outbreaks. The majority of A. baumannii strains had survival times that were considerably longer than those found for Escherichia coli and other Enterobacteriaceae but similar to those observed for Staphylococcus aureus. These observations, as well as the previously suggested airborne spread of Acinetobacter spp. in hospital wards (6, 34), may explain the occurrence of repeated outbreaks after incomplete disinfection of contaminated dry surfaces.

Prolonged survival of A. baumannii in a clinical setting, i.e., on patients’ bed rails, has been found to be associated with an ongoing outbreak in an ICU and illustrates that dry vectors can be secondary reservoirs where A. baumannii can survive (73). Concern has been growing regarding the potential of antibiotic and disinfectant co-resistance in clinically important bacteria. Reduced susceptibility of methicillin-resistant S. aureus (MRSA) versus methicillin-susceptible S. aureus to chlorhexidine and quaternary ammonium compounds was reported (527), and MRSA strains with low-level resistance to triclosan have emerged (57). Similar observations were made in gram-negative bacteria, such as Pseudomonas aeruginosa (539). It has been speculated that resistance to disinfectants may contribute to the epidemicity of the organism in a clinical setting, but to our knowledge, the association of resistance to biocides and the propensity for epidemic spread has never been studied systematically. Wisplinghoff et al. recently compared the in vitro activities of various disinfectants, such as propanol, mephen-trominum ethylsulfate, polyvinylpyrrolidone-iodine, triclosan, and chlorhexidine, against sporadic and epidemic A. baumannii strains by using a broth macrodilution method (601). They concluded that resistance to currently used disinfectants is probably not a major factor favoring the epidemic spread of A. baumannii, since all disinfectants inhibited growth of all A. baumannii isolates when concentrations and contact times recommended by the respective manufacturer were used. However, with most of the disinfectants tested, a substantial number of viable bacteria remained if contact times were <30 s or if diluted agents were used, as may occur in day-to-day clinical practice. No significant differences in susceptibility between outbreak-related and sporadic strains were observed under these conditions. Minor deviations from the recommended procedures leading to decreased concentrations or exposure times may play a role in nosocomial cross-transmission, but larger studies using additional methods would be required to confirm these findings.

**Molecular Epidemiologic Techniques**

To control the spread of A. baumannii in the hospital, it is necessary to identify potential reservoirs of the organism and the modes of transmission. To distinguish the outbreak strain from epidemiologically unrelated acinetobacters, comparison of isolates at the subspecies level is required, and the methods used for this purpose are designated epidemiological typing methods. Phenotypic typing systems based on biochemical profiles (biotyping), antibiotic susceptibility patterns, serological reactions (serotyping), phage typing, and protein profiles (for a comprehensive review of these techniques, the reader is referred to the work of Bergogne-Berezin and Towner [28]) have largely been replaced by a multitude of molecular typing systems, including, in historical order, plasmid profiling (221, 494); ribotyping (62, 123, 189, 498); PFGE (48, 206, 498); randomly amplified polymorphic DNA analysis (208, 214, 295); repetitive extragenic palindromic sequence-based (REP) PCR (252, 516); AFLP analysis, a high-resolution genomic fingerprinting method (123, 257, 295); integrase gene PCR (296); infrequent-restriction-site PCR (612); and most recently, MLST (18) and multilocus PCR–ESI-MS (145).

**Plasmid Analysis.** The majority of Acinetobacter species contain indigenous plasmids. Plasmid analysis has been used successfully for epidemiological typing of A. baumannii strains (221, 396, 494), and plasmid profiling is one of the few methods that have also been applied to study the epidemiology of Acinetobacter species outside the A. baumannii group (495, 499, 502). Even though the method is fairly robust, interpretation of results must include the consideration that many plasmids are easily transferable and may be gained or lost, and this contributed to the replacement of plasmid profiling by more robust molecular methods for epidemiological studies of acinetobacters.

**Ribotyping.** Ribotyping was developed primarily to identify acinetobacters, in particular strains of the A. calcoaceticus-A. baumannii complex, to the species level (189). This method—using EcoRI, Clal, and SalI for restriction of purified chromosomal DNA, followed by electrophoresis, blotting, and hybridization with a digoxigenin-11-UTP-labeled cDNA probe derived from E. coli 16S rRNA—also has been used to type strains in several studies investigating the epidemiology of acinetobacters (210, 211, 394). However, the discriminatory power of ribotyping is limited, and PFGE (see below) and other methods are less labor-intensive and more discriminatory (498, 508). More accurate typing results with a discriminatory power comparable to that of PFGE have been obtained using an automated ribotyping system (RiboPrinter; DuPont Qualicon, Wilmington, DE) (62, 453, 508). Automated ribotyping generates typing results more rapidly than PFGE does, but it is expensive and requires specialized equipment that is available in only a few laboratories that perform high-throughput molecular epidemiology investigations.

**PFGE.** Even in the face of sequence-based methods that are now available and are challenging PFGE as the gold standard for typing of many bacterial species, for Acinetobacter PFGE still remains the reference method of choice. It is a rather
laborious method that requires several days before generating a typing result, but the necessary equipment is now standard not only in most reference laboratories but also in hospital-based laboratories. Generally, ApaI and/or SmaI is used for restriction of intact chromosomal DNA (48, 206, 498). The resulting chromosomal fragments are separated by electrophoresis, and fingerprint profiles are compared visually or using specialized computer programs that also allow the storage of profiles in a database. As with other so-called comparative typing systems that are based mainly on a side-by-side comparison of molecular fingerprint patterns of a limited number of strains, interlaboratory comparison has always been a problem with PFGE, but a recent study has shown that with sufficient standardization of protocols interlaboratory reproducibility can be achieved (497). This approach would permit the recognition of epidemic strains and the early detection of multihospital or nationwide outbreaks, particularly if cases are geographically separated. As seen with other species, the discriminatory power of PFGE may be too high for large-scale epidemiologic and population studies, but the potentially greater value of newer methods, such as MLST or PCR–ESI-MS (see below), remains to be demonstrated for Acinetobacter.

**PCR-based typing methods.** Randomly amplified polymorphic DNA PCR, involving amplification of random fragments of genomic DNA with single primers with an arbitrary sequence, has been used successfully to assess the strain relatedness of Acinetobacter isolates (208). An alternative approach, referred to as REP-PCR (48, 516), uses consensus primers for the highly conserved REP sequences to amplify intervening sequences located between these DNA motifs. Both methods do not require specialized equipment and are fast, easy, and low-cost methods that allow grouping of A. baumannii strains with various degrees of genotypic relatedness. The discriminatory power of these methods, however, is inferior to that of PFGE. Interlaboratory reproducibility of PCR-generated fingerprints was demonstrated in one study, using four different primers (DAF4, ERIC-2, M13, and REP1 plus REP2) and a highly standardized protocol (214), but these findings could not be confirmed in later studies (L. Dijkshoorn, L. Dolzani, and H. Seifert, unpublished data). Huys et al. (252) recently used REP-PCR fingerprinting with a (GTG)_5 primer to distinguish members of the pan-European multidrug-resistant A. baumannii clone III (570) from the known clones I and II (123). In general, PCR-based typing methods allow for a quick estimate of epidemiological relatedness in a defined setting (599, 605), but they are not suited for large-scale comparative epidemiological studies. It remains to be shown if more rigorous standardization and automation of REP-PCR, such as by use of a DiversiLab system (bioMérieux), which includes a microfluidics-based detection system, will allow bacterial strain typing with an increased interlaboratory reproducibility (226).

**AFLP analysis.** AFLP analysis was established in the 1990s. It is a highly sensitive DNA fingerprinting method by which DNA is digested with restriction enzymes, followed by selective amplification, electrophoretic separation of fragments, and visualization. It is a rather cumbersome and expensive method that is usually performed in a semiautomated procedure, with laser detection of fragments on a sequencing platform. The resulting complex profiles are digitized and usually analyzed with appropriate software. Apart from being a powerful tool in bacterial taxonomy (256, 392), this high-resolution fingerprinting method has also been found to be useful for the characterization of Acinetobacter strains at the subspecies level and for outbreak investigation (123, 128, 257, 258, 295, 570, 605). Even though AFLP analysis is a relatively robust method, it requires a high level of standardization and extensive experience in interpretation of banding patterns even if sophisticated computer programs are available to aid in pattern analysis. Therefore, this method is restricted to reference laboratories and not suited for routine epidemiological analyses. In addition, data are not readily transportable between laboratories, mainly due to a lack of reproducibility when different sequencing platforms are used. Although clustering obtained with AFLP analysis compared well to PFGE-derived clustering in small-scale studies (109, 508, 570), a detailed and comprehensive side-by-side comparison of these two typing methods has never been performed.

**MLST.** MLST is a highly discriminative typing method that has been applied to a variety of bacterial pathogens, such as Neisseria meningitidis (349), Streptococcus pneumoniae (164), and S. aureus (150). The MLST scheme that was recently developed for A. baumannii by Bartual and coworkers is based on 305- to 513-bp sequences of the conserved regions of the following seven housekeeping genes: gltA, gyrB, gdhB, recA, cnp60, gpi, and rpoD (18). It can also be applied to Acinetobacter genomic species 13TU isolates (598). The currently available MLST data are in good concordance with typing results generated by PFGE and AFLP analysis (18). Thus far, the system has been used with only a limited number of A. baumannii strains, mainly from Spanish and German hospital outbreaks, and requires further evaluation.

The discriminatory power of the currently proposed MLST system is comparable to that of both PFGE and AFLP analysis. However, MLST is expensive and laborious and therefore not suited for routine outbreak analysis or other limited-scale analysis of the epidemiology of A. baumannii. It remains to be determined if this typing scheme is appropriate for the study of the population structure of A. baumannii and perhaps other Acinetobacter species, as shown successfully when this method was applied to other microorganisms. To date, MLST is one of the few so-called library typing systems used for the epidemiological study of A. baumannii, i.e., a typing system where typing data are translated into a numerical code that can be obtained in an identical manner at different laboratories by using the same protocol. It provides a portable method that may be suitable for global epidemiologic study and allow the recognition of epidemic, multiresistant, and virulent A. baumannii clones and the monitoring of their national and international spread.

**PCR–ESI-MS.** PCR–ESI-MS is a form of high-throughput MLST that can be used for species identification of A. baumannii as well as Acinetobacter genomic species 3 and 13TU and, in addition, to determine clonality (145). The conserved regions of six bacterial housekeeping genes (acrE, adk, efp, mutY, fumC, and ppa) are amplified from each isolate, amplification products are then desalted and purified, and the mass spectra are determined. The system was established using 267 Acinetobacter isolates collected from infected and colonized soldiers and civilians involved in an outbreak in the military...
health care system associated with the war in Iraq, previously characterized outbreaks in European hospitals, and culture collections. A good correlation with PFGE typing was observed. As a major advantage, the PCR-ESI-MS genotyping method appears to be very fast (taking only 4 h), providing typing results on a time scale not achievable with most other systems. Further evaluation of this method is clearly warranted.

**Hospital Outbreaks and Control Measures**

The propensity for outbreaks of multidrug-resistant *A. baumannii* has been demonstrated clearly. Only one or two strain types were found in the majority of more than 20 outbreaks assessed, using PFGE or PCR-based typing methods to assess clonality (584). In New York City, two strain types accounted for >80% of carbapenem-resistant isolates. Among six pan-drug-resistant isolates, three separate ribotypes were identified (446). This clearly demonstrates the importance of infection control interventions in response to outbreaks of multidrug-resistant *A. baumannii* infections.

The following infection control interventions are appropriate with regard to *A. baumannii* outbreaks. (i) Molecular epidemiologic investigations should be conducted to determine if a clonal outbreak strain is present (as described above). (ii) Environmental cultures should be used to determine if a common environmental source is present. If such a source is found, it should be removed from the patient care setting. Numerous potential sources have been identified in prior studies, including ventilator tubing, suction catheters, humidifiers, containers of distilled water, urine collection jugs, multidose vials of medication, intravenous nutrition, moist bedding articles, inadequately sterilized reusable arterial pressure transducers, and computer keyboards (171, 390, 563, 584). A high-profile outbreak arose from pulsatile lavage wound treatment, a high-pressure irrigation treatment used to debride wounds (353). (iii) Enhanced environmental cleaning should be performed in order to eliminate the organism from the peripatient environment. (iv) Enhanced isolation procedures, aimed at optimizing contact isolation (usage of gloves and gowns when dealing with colonized patients or their environment) and improving hand hygiene, should be implemented. In some circumstances, cohorts of patients or staff are used, but optimally patients should be nursed in single rooms with a dedicated nurse. This will be impractical in many settings. (v) Antibiotic management processes should be used to ensure that “at-risk” antibiotics are not being used excessively. Optimally, a case-case control study should be performed to determine which antibiotics truly do increase the risk of multidrug-resistant *A. baumannii* strains (288). Numerous studies have assessed antibiotic risk factors for infection with multidrug-resistant *A. baumannii*, although only a few have examined risk factors for emergence of pan-drug resistance. Although exposure to any antibiotic active against gram-negative bacteria has been associated with the emergence of multidrug-resistant *A. baumannii* (359), three classes of antibiotics have been implicated most frequently, including broad-spectrum cephalosporins (71, 251, 309, 483), carbapenems (103, 120, 321), and fluoroquinolones (586).

A number of investigators have demonstrated that interventions such as those described above can be effective in the control of *A. baumannii* infections (78, 109, 121, 390, 435, 594). In some cases, despite these efforts, ongoing cases of multidrug-resistant *A. baumannii* infection continue to occur. Monitoring adherence to such infection control interventions is also important. Although health care worker hand carriage with *Acinetobacter* is typically transient, it may be more prolonged in individuals with damaged skin (22, 247). In some scenarios, closure of wards to new admissions needs to be undertaken (435). Some authors have suggested that eradication of colonization be performed by techniques such as selective digestive tract decolonization or use of topical or aerosolized polymyxins (200, 563). However, we are hesitant to recommend these interventions due to the possible risks of polymyxin-resistant organisms. Rather, we would prefer greater assessment for colonized patients, greater attention to environmental decontamination, and improved hand hygiene as a means for prevention of patient-to-patient transfer. Further studies are still required to define the efficacy of these infection control interventions in the prevention of *A. baumannii* dissemination.

**THERAPEUTIC STRATEGIES FOR ACINETOBACTER BAUMANNII INFECTION**

The wide array of intrinsic and acquired resistance determinants that have emerged in *A. baumannii* have justifiably brought it great scientific attention. As determined by the Infectious Diseases Society of America, *A. baumannii* is one of the “red alert” pathogens that greatly threaten the utility of our current antibacterial armamentarium (531). Prior to the 1970s, it was possible to treat *Acinetobacter* infections with a range of antibiotics, including aminoglycosides, β-lactams, and tetracyclines (28). However, resistance to all known antibiotics has now emerged in *A. baumannii* (153, 309), thus leaving the majority of today’s clinicians in unfamiliar territory. Compounding the problem is the large number of pharmaceutical companies that have abandoned antibiotic drug discovery and development, driven primarily by the risks of poor financial returns relative to those for more lucrative classes of drugs (519, 593). The dearth of antibiotics, especially for gram-negative organisms, has recently stimulated attention from major research and governing bodies (388, 531). Unfortunately, at this stage, very little is in the therapeutic pipeline (459), and the new agents with activity against gram-negative organisms are all modifications of existing classes. Novel antibiotic targets and mechanisms of action are urgently required.

Given the current therapeutic environment, optimizing the use of existing antimicrobials is critical. To achieve this goal, a thorough understanding of the pharmacokinetic and pharmacodynamic parameters that predict maximal drug efficacy yet minimize the evolution of drug resistance, as well as an evidence-based approach to therapeutic strategies for highly drug-resistant strains, is required. The following section concentrates on the available in vitro, animal, and human data to assist the reader in the management of infections due to highly drug-resistant *A. baumannii*.

**Existing Antimicrobial Agents**

Given the range and diversity of resistance determinants in *A. baumannii*, therapy should be based on the results of ade-
quately performed antimicrobial susceptibility testing. The details and important pitfalls of such testing are described above. Antibiotic selection for empirical therapy is challenging and must rely on recent institutional-level susceptibility data. Time to effective therapy clearly impacts patient outcomes (253, 299), and this may include patients with *A. baumannii* infection (159, 306). Thus far, carbapenems have been thought of as the agents of choice for serious *A. baumannii* infections. However, although these drugs are still active against the vast majority of *A. baumannii* strains worldwide, the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance, as described above, often working in concert (47, 446). This concerning phenotype, challenging one of our most potent last lines of defense, is unfortunately being described increasingly (167, 294, 372, 405, 602) and, most importantly, are in keeping with results from clinical human studies (85, 93, 102, 263, 515, 561, 603).

Urban et al. performed a small study assessing the clinical efficacy of sulbactam during an outbreak of *A. baumannii* resistant to carbapenems, aminoglycosides, and other β-lactams (561). Of the 10 patients who received ampicillin-sulbactam for >3 days, 9 clinically responded, with many achieving microbiological eradication (561). These results were supported by a further noncomparative study from Spain, which showed that 29/41 (95%) patients with non-life-threatening *A. baumannii* infections were cured or clinically improved with ampicillin-sulbactam or sulbactam alone (102). All isolates were multiresistant but were susceptible to imipenem, sulbactam, and polymyxins. As pointed out in that study, the use of a sulbactam-containing regimen for milder infections may be an appropriate strategy in limiting excessive carbapenem use. More supportive data are now available on the efficacy of sulbactam in treating serious *A. baumannii* infections. Wood et al. performed a retrospective comparative trial involving patients with *A. baumannii* VAP and found that treatment successes were similar between those who received ampicillin-sulbactam (*n*= 14) and those who received imipenem-cilastatin (*n*= 63) (93% versus 83%, respectively; *P* > 0.05) (603). Microbiological eradication appeared to be superior in the ampicillin-sulbactam group; however, this was not statistically significant. Ampicillin-sulbactam was primarily prescribed for patients infected with sulbactam-susceptible, non-imipenem-susceptible organisms. Interestingly, significantly more patients in the ampicillin-sulbactam group received combination therapy (50% versus 14%; *P* = 0.01), most commonly with an aminoglycoside. No outcome differences were observed between these subgroups, but the small sample size limits this analysis.

An equivalent efficacy of sulbactam to that of imipenem has also been shown for treatment of *A. baumannii* bloodstream infection (85, 93, 263), with one study demonstrating a reduction in pharmaceutical costs (263). In a further study, treatment of highly drug-resistant *A. baumannii* bacteremia (susceptible only to sulbactam and polymyxin E) with ampicillin-sulbactam was comparable to treatment of more susceptible strains with other therapies, including imipenem, aminoglycosides, and quinolones (515). These data indicate that when *A. baumannii* is susceptible to sulbactam, this agent is probably as efficacious as any other.

Small observational series have shown encouraging results with ampicillin-sulbactam for the treatment of nosocomial *A. baumannii* meningitis (268, 328), a problematic entity of increasing significance. However, failures have been reported (268, 328). The cerebrospinal fluid (CSF) penetration of sulbactam in patients with inflamed meninges is reported to be 2 to 32% of serum levels (170), with peak serum levels reaching approximately 42 to 60 mg/liter after an intravenous (i.v.) dose of 1 g (170, 366). Thus, assuming that the susceptibility breakpoint for sulbactam is ≤4 µg/ml, inadequate dosing may explain the variable clinical response. For serious *A. baumannii* infections, we recommend dosing of at least 6 g/day of sulbactam in divided doses, assuming normal renal function. Recently, dosing of up to 12 g/day was reported for the treatment of hospital-acquired pneumonia caused by sulbactam-resistant *A. baumannii*, without major adverse effects; however, outcomes were similar in those who received 9 g/day (38).

The clinical benefits of combination therapy with sulbactam compared with sulbactam monotherapy are not yet clear. Much of the data comes from in vitro studies using checkerboard or time-kill analyses and shows enhanced activity when sulbactam is combined with cefepime (472, 548), imipenem (86, 518), meropenem (289, 294), amikacin (481), rifampin (11), and ticarcillin-clavulanate (272). In a mouse pneumonia model, the combination of ticarcillin-clavulanate, sulbactam, and rifampin appeared most efficacious toward a carbapenem- and sulbactam-resistant *A. baumannii* strain (rifampin MIC of 4 mg/liter); however, such effects were lost when a strain that...
was less susceptible to rifampin (MIC, 8 mg/liter) was tested (602). Interestingly, combination therapy can prevent the emergence of rifampin resistance (412, 602). Another mouse model, using an *A. baumannii* strain with reduced susceptibility to meropenem and sulbactam (MICs, 8 mg/liter and 8 mg/liter, respectively), showed a statistically significant improvement in mouse survival with the combination compared to that with each agent alone (294). Human data are very limited and lack respective), showed a statistically significant improvement in outcome for patients receiving colistin and ampicillin-sulbactam compared to those given a carbapenem alone (30.8% versus 58.3%, respectively), but no patient matching was performed (303). Such data are intriguing and require further clinical evaluation in humans.

Despite the shortcomings of these studies, the results suggest that sulbactam should be considered as a therapeutic option for mild to severe *A. baumannii* infections caused by sulbactam-susceptible organisms. Unfortunately, sulbactam resistance is common in certain geographic areas, and this phenomenon will no doubt increase over time.

**Polymyxins.** The emergence of *A. baumannii* strains resistant to all routinely tested antimicrobials has led to the necessary revival of the polypeptide antibiotics known as the polymyxins (colistin or polymyxin E and polymyxin B). These positively charged antimicrobial peptides were discovered in 1947 (524), originating from *Bacillus polymyxa* (300). They target the anionic LPS molecules in the outer cell membranes of gram-negative bacteria, leading to interactions between the inner and outer cell membranes, with associated lipid exchange, membrane disturbance, osmotic instability, and eventual cell death (95, 156). There are two commercially available forms of colistin, namely, colistin sulfate for oral and topical use, and CMS, also known as sodium colistin methanesulfonate or colistin sulfomethate sodium, for parenteral use (332). Both forms are available for nebulization. Given that the polymyxins were discovered over 50 years ago, they were never subjected to the rigorous drug development process that we now expect of more contemporary antimicrobials. Thus, our understanding of the critical pharmacological parameters that govern dosing for maximal efficacy and minimal toxicity is poor. As a consequence, confusion exists among clinicians and in the literature regarding formulations, nomenclature, and dosing (157, 331, 332). Such information is urgently required before we mistakenly jeopardize this valuable antimicrobial (10, 153, 334, 452). For a detailed review of the pharmacology of the polymyxins, readers are referred to the work of Li et al. (332). For the remainder of this section, colistin is used to mean all formulations of CMS.

In vitro, colistin demonstrates concentration-dependent bactericidal activity against *A. baumannii* strains with various susceptibility profiles, as determined by time-kill analyses (372, 411, 465). However, significant regrowth has been observed at 24 h for strains characterized as having heterogeneous resistance to colistin (411). Presumably, the subpopulations with higher MICs flourish under selection pressure. Whether this phenomenon is clinically relevant has yet to be determined. Conflicting results have been reported for the postantibiotic effect of colistin (411, 436), a parameter often used to assist in optimizing dosing. Thus far, animal models have shown mixed results for colistin efficacy (372, 415, 465) and serve to remind us of the potential limitations of extrapolating such data to clinical management. Interestingly, the efficacy of colistin at reducing the bacterial burden in the lungs was poor in a murine pneumonia model (direct upper airway inoculation) (372), in contrast to excellent activity in a rat thigh infection model that led to hematogenous dissemination of the lungs (415). This may be explained by varied colistin penetration into lung compartments or a modification of activity in the epithelial lining fluid.

The cumulative human data on the efficacy and toxicity of colistin for treating *A. baumannii* infection in the modern era are represented in Table 5. Overall, the efficacy of the drug has been highly encouraging in both adult and pediatric populations, with favorable or curative responses ranging from 57% to >80% (158, 185, 207, 241, 283, 301, 327, 357, 369, 409, 451, 517). In several recent prospective cohort studies across a range of infection types, the outcomes for patients receiving definitive therapy with colistin for colistin-only-susceptible organisms were similar to those for patients receiving combinations of other antibiotics (predominantly carbapenems with or without other antibiotics) for more susceptible organisms (185, 451). Interestingly, despite the majority of patients in the colistin groups receiving inactive empirical therapy compared to the noncolistin groups, outcomes were similar (185, 451). Most recently, a further prospective cohort study reported a statistically significant improvement in outcome for patients receiving colistin compared with those receiving other antibiotics (301). However, all patients were infected with isolates susceptible only to colistin, and therefore many patients in the noncolistin group received definitive therapy with inactive antimicrobials (at least 40%) (301).

Nebulized colistin is increasingly being used in an attempt to minimize systemic toxicity and improve drug deposition at the site of infection (158, 219, 305, 339, 367, 425). Much of the data on nebulized colistin originates from patients with cystic fibrosis who are colonized or infected with *Pseudomonas aeruginosa* (29, 240). However, the literature is expanding outside this patient population (Table 6). Thus far, no prospective comparative study has been performed to assess the efficacy of nebulized colistin for *A. baumannii* infection. In a retrospective case series by Kwa et al., 18 of 21 (86%) patients who received nebulized colistin for hospital-acquired pneumonia caused by *A. baumannii* or *P. aeruginosa* (resistant to all antimicrobials except polymyxins) had favorable clinical and microbiological responses (305). None of these patients received parenteral colistin, but most were receiving other antimicrobials for concomitant infections. One (5%) patient developed bronchospasm, and no cases of nephro- or neurotoxicity were reported (305). Similar, encouraging results were reported by Michalopoulos et al., who compared the outcomes for 8 patients who received supplemental nebulized colistin combined with parenteral antibiotics with those for 45 patients who received parenteral colistin only (367). Rates of clinical cure appeared superior with supplemental nebulized colistin, but the patient numbers were small and limit statistical comparisons (Table 6).

The largest series thus far, by Berlana et al., included 71 patients who received nebulized colistin for presumed pneumonia caused by more susceptible *A. baumannii* and *P. aeruginosa* strains (30). Conclusions from this study are difficult to ascer-
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design/series</th>
<th>Infection type (%)</th>
<th>No. of patients treated with colistin</th>
<th>No. of patients in comparator group (description)</th>
<th>Outcome</th>
<th>Nephrotoxicity (%)</th>
<th>Neurotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koomanachai et al. (301)</td>
<td>Prospective cohort study</td>
<td>Pneumonia (69), bacteremia (12), intra-abdominal (6), urinary tract (5), skin/soft tissue (6), sinus (1)</td>
<td>78 (71 infected with A. baumannii and 7 infected with P. aeruginosa)</td>
<td>15 (other antibiotics (12 infected with A. baumannii and 3 infected with P. aeruginosa))</td>
<td>Good clinical response, 80.8% for colistin group (C) vs 26.7% for noncolistin group (NC) (P &lt; 0.01); 30-day mortality, 46.2% for C vs 80% for NC (P = 0.03)</td>
<td>30.8 for C vs 66.7 for NC (P = 0.02)</td>
<td>Nil</td>
</tr>
<tr>
<td>Reina et al. (451)</td>
<td>Prospective cohort study</td>
<td>Pneumonia (53), bacteremia (16), urinary tract (18), other (catheter, central nervous system [CNS], peritonitis, wound) (13)</td>
<td>55 (36 infected with A. baumannii and 19 infected with P. aeruginosa)</td>
<td>130 (other antibiotics, predominantly carbapenems [81%]) (69 infected with A. baumannii and 61 infected with P. aeruginosa)</td>
<td>Improvement on day 6, 15% for C vs 17% for NC; in-hospital mortality, 29% for C vs 26% for NC (P = 0.2); microbiological eradication in pneumonia cases, 93% for C vs 94% for NC</td>
<td>Nil</td>
<td>NA</td>
</tr>
<tr>
<td>Garnacho-Montero et al. (185)</td>
<td>Prospective cohort study</td>
<td>Pneumonia (100)</td>
<td>21 (all infected with A. baumannii)</td>
<td></td>
<td>Cure, 57% for C vs 57% for NC; microbiological eradication, 66.7% (6.9 patients) for C vs 50% (24 patients) for NC; pneumonia-related mortality, 38% for C vs 36% for NC</td>
<td>24 for C vs 43 for NC</td>
<td>Nil</td>
</tr>
<tr>
<td>Kalkel et al. (283)</td>
<td>Prospective case series</td>
<td>Pneumonia (78), urinary tract (8), bacteremia (12), CNS (3)</td>
<td>78 (43 infected with A. baumannii and 35 infected with P. aeruginosa)</td>
<td>NA</td>
<td>Favorable response (77%)</td>
<td>9</td>
<td>One patient developed diffuse muscular weakness in the ICU</td>
</tr>
<tr>
<td>Falagas et al. (158)</td>
<td>Prospective case series</td>
<td>Pneumonia (30), urinary tract (26), wound (15), peritonitis (11), abdominal (4), cellulitis (4), osteomyelitis (4), catheter (6)</td>
<td>27 (12 infected with A. baumannii, 17 infected with P. aeruginosa, 5 infected with Kloebsie pneumonii, and 4 infected with Escherichia coli)</td>
<td>NA</td>
<td>Clinical response, 85%; all-cause mortality, 15%</td>
<td>7</td>
<td>NA</td>
</tr>
<tr>
<td>Goverman et al. (207)</td>
<td>Retrospective case series of pediatric burn patients</td>
<td>Bacteremia (100), pneumonia (21), wound (57), urinary tract (36)</td>
<td>14 (3 infected with A. baumannii and 11 infected with P. aeruginosa)</td>
<td>NA</td>
<td>Favorable response, 78.6%; mortality, 14.3%</td>
<td>14.3 (2/14 patients)</td>
<td>Nil</td>
</tr>
<tr>
<td>Holloway et al. (241)</td>
<td>Retrospective case series</td>
<td>Bacteremia (100), pneumonia (100), wound (57), urinary tract (36)</td>
<td>29 (all infected with A. baumannii)</td>
<td>NA</td>
<td>clinical cure, 76%; microbiological eradication (evaluable in 21 patients), 81%; crude mortality, 27%</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>Michalopoulos et al. (369)</td>
<td>Retrospective case series</td>
<td>Pneumonia (84), bacteremia (35), urinary tract (23), soft tissue (12), CNS (2)</td>
<td>43 (8 infected with A. baumannii and 35 infected with P. aeruginosa)</td>
<td>NA</td>
<td>Clinical cure or improvement, 75%; microbiological clearance, 67%; mortality, 28%</td>
<td>18.6 (8/43 patients)</td>
<td>Nil</td>
</tr>
<tr>
<td>Sobieszczyn et al. (517)</td>
<td>Retrospective case series</td>
<td>Pneumonia (100)</td>
<td>25 (29 episodes) (16 A. baumannii infections, 12 P. aeruginosa infections, and 1 Alcaligenes xylosoxidans infection)</td>
<td>NA</td>
<td>Favorable response, 76%; microbiological eradication (evaluable in 22 patients), 41%; end-of-treatment mortality, 21%</td>
<td>10</td>
<td>7 (seizure and weakness)</td>
</tr>
</tbody>
</table>
Renal failure not definitively defined; 37% had worsening of renal function during treatment.

Michalopoulos et al. (368) Retrospective case series Bacteremia (100) 1 (continuous i.v. infusion) (infected with A. baumannii) NA NA Good outcome, 58%; microbiological eradication (evaluable in 41 patients), 88%; mortality, 20%

Kasiakou et al. (367) Retrospective case series Fixation device-related orthopedic infections 2 NA Cure (100%) Nil Nil Microbiological eradication (evaluable in 29 patients), 93%; mortality, 37%

Markou et al. (409) Retrospective case series Pneumonia (65), bacteremia (39), infections) A. baumannii, 2 infected with P. aeruginosa, 20 polymicrobial infections, and 10 unidentified infections NA NA Nil Nil NA Clinical response, 73%; 30-day mortality, 42%

Levin et al. (327) Retrospective case series CNS (8), surgical site (7), skin (2), catheter (2), bone (1), pulmonary, 2 infections arising from respiratory tract (4) NA NA Nil Nil NA Clinical response, 73%

Ouderkirk et al. (357) Retrospective case series Pneumonia (58), catheter infections (35), skin (30), bone (24), orthopedic infections (23), surgical site (17), peritonitis (15), urinary tract (20), CNS (8), abdominal (8), surgical site (7), otitis media (2) A. baumannii, 2 infected with P. aeruginosa, 2 polymicrobial infections, and 10 unidentified infections NA NA Nil Nil NA Clinical response, 73%; 30-day mortality, 42%

As described, nosocomial meningitis is an increasingly important entity, with multidrug-resistant gram-negative pathogens being implicated as an etiology with greater frequency. To optimize therapy of these challenging infections, intrathecal or intraventricular colistin has been utilized (Table 7). This route of administration is not novel for the polymyxins and was implemented soon after its availability (96, 225, 546). Despite the majority of patients with A. baumannii meningitis receiving colistin both systemically and directly into the CSF, the latter alone may be adequate (25, 398, 576). Such a strategy avoids the unnecessary risks of nephrotoxicity. Thus far, intrathecal/ventricular colistin has been well tolerated, with rare reports of chemical meningitis that spontaneously resolved with either drug cessation or dose reduction (155, 398). In the absence of an external CSF drain, which is often the source of infection, repeated lumbar punctures are required, with the associated risk of introducing other pathogens. Several cases of A. baumannii meningitis have been cured with i.v. colistin only (267, 269, 327, 416), with reported levels in CSF of 1.25 mg/liter (CSF/serum ratio of 25%) (267, 269); however, failures have also been reported that eventually responded only to intrathecal/ventricular administration (7, 67, 286, 398, 526). Dosing varies greatly between studies, but according to the Infectious Diseases Society of America, 5 mg of polymyxin B or 10 mg of colistin daily is recommended for adults (553). Given the avail-
TABLE 6. Studies that primarily assessed the efficacy and/or toxicity of inhaled polymyxins with or without intravenous polymyxins

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Infection type (%)</th>
<th>No. of patients treated with colistin (delivery)</th>
<th>Comparator group</th>
<th>Outcome</th>
<th>Respiratory adverse events</th>
<th>Nephrotoxicity or neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michalopoulos et al. (367)</td>
<td>Retrospective cohort study</td>
<td>Pneumonia (100)</td>
<td>8 (supplemental nebulized colistin in conjunction with i.v. antibiotics) (seven infected with A. baumannii and one infected with P. aeruginosa)</td>
<td>45 patients receiving i.v. colistin</td>
<td>Clinical cure (88% in nebulized + i.v. group vs 67% in i.v.-only group) ($P = 0.67$), mortality (13% vs 24%, respectively) ($P = 0.41$)</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Kwa et al. (305)</td>
<td>Retrospective case series</td>
<td>Pneumonia (100)</td>
<td>21 (nebulized colistin) (17 infected with A. baumannii and 4 infected with P. aeruginosa)</td>
<td>NA</td>
<td>Favorable response (86%), microbiological eradication (86% [documented for 52% of cases]), mortality (all causes, 46.7%; related to pneumonia, 14.3%)</td>
<td>One (5%) patient developed bronchospasm</td>
<td>Nil</td>
</tr>
<tr>
<td>Berlana et al. (30)</td>
<td>Retrospective case series</td>
<td>Respiratory (89), urinary (9), blood (2.5), or CNS (2.5)</td>
<td>80 (68 received nebulized colistin only, 3 received nebulized and parenteral colistin, 2 received parenteral and intrathecal colistin, and 7 received parenteral colistin only) (69 infected with A. baumannii and 11 infected with P. aeruginosa)</td>
<td>NA</td>
<td>Mortality (18%), microbiological clearance (92%)</td>
<td>Nil</td>
<td>NA</td>
</tr>
</tbody>
</table>

* NA, not assessed.
able literature and the severity of this infection, we recommend the use of colistin directly into the CSF early for patients with carbapenem-resistant *A. baumannii* meningitis.

Many in vitro and animal studies support the role of combination therapy with colistin (Table 8). In particular, colistin in combination with a carbapenem and/or rifampin appears most promising (194, 239, 351, 373, 415, 518, 537, 541, 613). Understandably, in the presence of significant carbapenemase activity, as opposed to membrane-based changes (porins and efflux pumps) that are likely disrupted by the polymyxins, synergy with carbapenems may be lost (592). Unfortunately, very few human data are available to support these in vitro studies. Much of the clinical data on colistin efficacy comes from uncontrolled, retrospective case series (Table 5), which often include heterogeneous populations. In addition to colistin, the majority of patients received a potpourri of other antimicrobials, most commonly carbapenems but also quinolones, aminoglycosides, sulbactam, rifampin, and others (30, 158, 301, 305, 357, 367, 369, 409, 517). In the absence of a well-matched control group (i.e., a group receiving colistin alone), it is difficult to make conclusions about the potential benefits of combination therapy (374, 432, 517). More recently, Falagas et al. performed a retrospective cohort study, comparing patients who received colistin only with those who received colistin and meropenem (161). After adjusting for severity of illness, no difference in outcomes was observed. Whether combination therapy will protect colistin from the emergence of resistance is presently unknown, but an in vitro pharmacodynamic model suggests that this may be possible (302). Further human studies are warranted.

The most surprising feature of the colistin renaissance has been the low rates of nephrotoxicity, whose frequency is highly dependent on study definitions. Overall, however, the rates are significantly less than previously reported (168) and appear to be reversible with cessation of the drug (158, 301, 327). Prolonged administration of colistin (>4 weeks) without adverse effects has been reported (162). Patients with a history of renal impairment (301, 327, 369), those given concomitant nephrotoxins (301, 409, 517), and the elderly (409) are at greatest risk. Dosing should be adjusted in those with preexisting renal dysfunction (colymycin M parenteral package insert; Monarch Pharmaceuticals, Bristol, TN) and those receiving renal replacement therapy (333). As observed with acute renal failure in other clinical settings (19), this adverse outcome may be a predictor of increased mortality in patients receiving colistin (369, 409).

Of concern, rates of resistance to the polymyxins have recently been reported to be as high as 3.2% for multidrug-resistant *A. baumannii* strains (177), with higher rates reported in Korea (293). Resistance in other problematic gram-negative pathogens is also emerging (10, 177, 307). Such events signify the importance of prudent and cautious use of this class of antimicrobial as well as an urgent need to further understand its complex pharmacology.

### New Antimicrobials

A concerning void of new therapeutic options exists for *A. baumannii* infections. Of the recently licensed antimicrobials, tigecycline, a 9-t-butylglycylamido semisynthetic derivative of minocycline, has provided some hope, but clinical data are still limited. As with other tetracycline derivatives, tigecycline inhibits the 30S ribosomal subunit, but its unique feature is its ability to evade the major determinants of tetracycline resistance, i.e., the tet(A) to tet(E) and tet(K) efflux pumps and the tet(M) and tet(O) determinants that provide ribosomal protection (169, 430). Thus far, the in vitro activity of tigecycline against *A. baumannii* has been assessed largely by MIC testing. Most studies have reported an MIC<sub>50</sub> of 1 μg/ml and an MIC<sub>90</sub> of 2 μg/ml (229, 413, 473), but studies from Germany (500), Spain (37), and Israel (389) have reported MIC<sub>90</sub> of 4 μg/ml, 8 μg/ml, and 32 μg/ml, respectively, with the last being determined by Etest only. More recently, combination therapy with tigecycline has been studied using time-kill and Etest synergy methodology (476, 486). When it was tested against a non-carbapenem-susceptible *A. baumannii* strain, tigecycline alone allowed maximal killing at concentrations near the MIC, which was 1 μg/ml, with no benefit of using higher concentrations (486). Importantly, concentrations just below the MIC (0.5 μg/ml to 0.7 μg/ml), which are consistent with the mean maximum serum steady-state concentration achieved with standard dosing (0.63 μg/ml after a 100-mg i.v. loading dose followed by 50 mg i.v. twice/day) (410), caused significant regrowth at 24 h (486). No difference was observed for all tigecycline combina-

### Table 7. Studies that primarily assessed the efficacy and/or toxicity of intrathecal/ventricular polymyxins for CNS infection with *Acinetobacter baumannii*

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>No. of patients treated with colistin</th>
<th>Neurotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parhami et al. (416)</td>
<td>Retrospective case series</td>
<td>2 (all infected with <em>A. baumannii</em>)</td>
<td>Nil</td>
</tr>
<tr>
<td>Ng et al. (398)</td>
<td>Retrospective case series</td>
<td>5 (all infected with <em>A. baumannii</em>)</td>
<td>Chemical meningitis (60%)</td>
</tr>
<tr>
<td>Kasiakou et al. (286)</td>
<td>Case report</td>
<td>1 (five episodes [two <em>A. baumannii</em> episodes, two <em>P. aeruginosa</em> episodes, and one <em>Enterobacter cloacae</em> episode])</td>
<td>Nil</td>
</tr>
<tr>
<td>Al Shirawi et al. (7)</td>
<td>Case report</td>
<td>1 (infected with <em>A. baumannii</em>)</td>
<td>Nil</td>
</tr>
<tr>
<td>Bukhary et al. (67)</td>
<td>Case report</td>
<td>1 (infected with <em>A. baumannii</em>)</td>
<td>Nil</td>
</tr>
<tr>
<td>Fernandez-Viladrich et al. (166)</td>
<td>Retrospective case series</td>
<td>2 (all infected with <em>A. baumannii</em>)</td>
<td>Nil</td>
</tr>
<tr>
<td>Vasen et al. (576)</td>
<td>Case report</td>
<td>1 (infected with <em>A. baumannii</em>)</td>
<td>Nil</td>
</tr>
<tr>
<td>Benífa et al. (25)</td>
<td>Case report</td>
<td>1 (infected with <em>A. baumannii</em>)</td>
<td>Nil</td>
</tr>
</tbody>
</table>

<sup>a</sup> The outcome in all studies was 100% cure. None of the studies reported any nephrotoxicity.

<sup>b</sup> All studies were noncomparative, and only select case reports were included.
Human studies

Falgas et al. (161) Retrospective cohort study

Colistin (n = 14) vs colistin-meropenem (n = 57)

No synergy observed

Motaouakkil et al. (374) Retrospective case series (noncomparative)

Colistin + i.v. rifampin (n = 26)
(16 received nebulized colistin, 9 received i.v. colistin, and 1 received intrathecal colistin)

NA (favorable response in all)

Petrosillo et al. (432) Retrospective case series (noncomparative)

i.v. colistin + i.v. rifampin (n = 14) (five patients also received ampicillin-sulbactam)

NA (mortality rate of 50%)


tions, including combinations with amikacin, meropenem, imipenem, ciprofloxacin, levofloxacin, ampicillin-sulbactam, rifampin, and polymyxin B (476, 486). These data raise concerns about the use of tigecycline for bloodstream infections caused by organisms with MICs of > 1 μg/mL. Such concerns have now been illustrated in several clinical reports involving A. baumannii (424, 484). Subinhibitory concentrations may promote the rapid emergence of resistance, leading to clinical failure (420, 424). At this point, we would not recommend using tigecycline for A. baumannii bacteremia if another option is available. Conversely, the drug is concentrated in tissues, including the lung parenchyma (101), and thus its utility for treating A. baumannii pneumonia or other tissue-based infections warrants further evaluation. A recent retrospective series including 22 patients with VAP caused by multidrug-resistant A. baumannii showed that 18 patients had clinical resolution (484). However, only three of these patients received tigecycline monotherapy, with the remainder also receiving therapy with imipenem and/or colistin. Further clinical data on tigecycline’s efficacy in pneumonia are still awaited. The most common adverse effect of tigecycline is nausea (15, 147), often requiring concomitant antiemetic therapy and limiting dose escalation. However, this is less of a problem in an ICU setting, where most A. baumannii infections occur.

Other agents on the licensing horizon with activity against gram-negative organisms include doripenem, a new parenteral
carbapenem, and the next generation of cephalosporins with activity against MRSA, ceftobiprole and ceftaroline. At this point, none of these agents appear to have significant advantages over current antimicrobials for A. baumannii, but in vitro data for doripenem suggest a slight advantage over meropenem (175, 275, 378, 379). Clinical data for doripenem against A. baumannii are still awaited.

**Other Combination Therapy**

The use of combination therapy to treat multidrug- or pan-drug-resistant gram-negative organisms has become an area of great interest (449). This strategy aims to create an active combination out of two agents to which the organism tests nonsusceptible in the laboratory. Apart from trying to improve efficacy, combination therapy may also help to prevent the emergence of resistance when at least one agent is active in vitro (77, 412). Future studies assessing combination therapy for multidrug-resistant organisms should address this issue. The studies involving combination therapy with either sulbactam or the polymyxins that are described above form the major and most promising group. Other combinations have also been studied using in vitro techniques and animal models, including various combinations of quinolones, β-lactams, and/or amikacin (45, 80, 139, 280, 467). The results for quinolone combination therapy are varied, with reduced efficacy being described when ciprofloxacin was used for ciprofloxacin-resistant A. baumannii (151), as well as a lack of enhanced activity with levofloxacin combined with imipenem or amikacin in a mouse pneumonia model (273). Interestingly, enhanced activity was seen when aztreonam was tested in combination with other β-lactams against a select group of MBL-producing A. baumannii strains (474). Using a mouse pneumonia model, Montero et al. reported on the potential efficacy of imipenem and rifampin against a carbapenem- and rifampin-resistant A. baumannii strain (373). However, after performing a small preliminary clinical study, the same group suggested against such a combination for infection with A. baumannii isolates with a similar resistance pattern (470). Unfortunately, in some geographic regions, desperate therapeutic measures are required, including the use of multiple antibacterial agents that in isolation are predicted to have poor activity against the infecting A. baumannii strain, as determined by standardized laboratory testing.

**Pharmacokinetic/Pharmacodynamic Strategies**

Achieving pharmacodynamic target attainment with our existing antimicrobials is now, more than ever, critical in our therapeutic approach to drug-resistant A. baumannii infection. The use of Monte Carlo simulation, which is a stochastic modeling tool that combines pharmacokinetic parameters of antibiotics with population-based microbiological susceptibility data, has improved our understanding of appropriate antibiotic dosing. Apart from assisting in the optimization of drug efficacy (259, 304, 330, 340), this strategy may also help to prevent the evolution of drug resistance, as reported for P. aeruginosa (478, 532). The most promising data with regard to A. baumannii are the benefits of a prolonged infusion of meropenem (259, 330). The likelihood of achieving bactericidal target attainment (defined as a time above the MIC of at least 40% of the dosing interval) for organisms that have an MIC at the susceptibility breakpoint (4 µg/ml) increases from 64% to 90% as the infusion time of a 1-g dose is extended from 0.5 to 3 h (330). In a study involving patients with VAP, extending the meropenem infusion time to 3 h and increasing the dose to 2 g every 8 h provided concentrations in serum of >16 µg/ml for almost 60% of the time (259). Such data support the use of an extended meropenem infusion time for treating serious A. baumannii infections and highlight the benefits of an increased dose for nonsusceptible isolates. Thus far, the effects of such a dosing strategy on the clinical outcomes of patients with A. baumannii infection are unknown and should be a focus of future research.

**Future Therapeutic Considerations**

Despite the absence of new therapeutic options for A. baumannii in clinical studies, the activity in the preclinical arena is notable. Such agents can be divided into those that inhibit a currently recognized mechanism of resistance or those that have a novel mechanism of action. With regard to the former, attention has been directed toward new β-lactamase inhibitors, especially those targeting the Ambler class B MBLs (547), as well as toward inhibitors of aminoglycoside-modifying enzymes and multidrug efflux pumps. As mentioned above, MBLs have played an important role in the emergence of carbapenem resistance in A. baumannii, although they are less prevalent than the OXA-type enzymes. Their structure and catalytic mechanism, being zinc dependent, contrast with those of other serine β-lactamases, hence their stability toward current β-lactamase inhibitors. Agents that chelate the active Zn²⁺ site appear to be the most promising; however, several challenges exist (589). As a consequence of the significant differences in the active site architecture between MBL types, the ability to develop a pan-MBL inhibitor is problematic (589). Of most concern, MBLs have homologous mammalian enzymes (69, 487) and therefore increase the potential for significant toxicity. Despite these hurdles, the development of compounds that target the metalloenzymes continues (68). Thus far, inhibitors of both aminoglycoside-inactivating enzymes and multidrug efflux pumps have also been troubled by diverse targets, with bacteria often harboring multiple enzyme or pump types. More recently, cationic antimicrobial peptides that are capable of inhibiting both aminoglycoside phosphotransferases and acetyltransferases have been described (41). The importance of multidrug efflux pumps in A. baumannii is increasingly being recognized, with tigecycline recently identified as a substrate of the RND-type pump AdeABC (420, 469). Through large-scale in vitro screening, a range of efflux pump inhibitors have been identified, with the majority being plant alkaloids (433) but, more recently, also synthetic compounds (43). Unfortunately, progress has been slow, with agents such as phenyl-arginine-β-naphthylamide doing well when assessed in vitro but coming to demise due to toxicity concerns (433). Challenges are also anticipated as a result of the number of efflux systems often available in gram-negative organisms, thus leading to compensatory upregulation of noninhibited pumps. For a more detailed review of efflux inhibitors, readers are referred to references 282 and 343.
With advances in genomics, proteomics, and chemical biology, new strategies for pathogen control are being devised. Justifiably, significant attention is being directed toward eukaryotic antimicrobial peptides, which are ubiquitous elements of the innate immune response in a variety of invertebrate, plant, and animal species (64). These cationic peptides act primarily by disturbing the cell membranes and share a similar structure and charge profile with the polymyxins, but the final steps in pathogen lethality have been shown to be different (479, 480). This mechanistic difference is clinically attractive and is well illustrated by the susceptibility of polymyxin-resistant \textit{A. baumannii} strains to such peptides (480, 562). Bactericidal activity against \textit{A. baumannii}, using both in vitro (4, 193, 448, 479, 480, 562) and in vivo (56, 125) models, has been reported. Combination studies, as determined by fractional inhibitory indexes, demonstrated that magainin II acted synergistically with \(\beta\)-lactams against multidrug-resistant \textit{A. baumannii} but that four other peptides showed no synergy (192). More recently, modified peptides containing linear sequences of alternating acyl chains and cationic amino acids, known as oligo-AKs, were reported (448). In this study, the oligo-AK compound \(C_{12}K-7\alpha_8\) was compared to conventional antimicrobial peptides and standard antibiotics. It was found to have significant bactericidal activity, more so than imipenem and ciprofloxacin at 6× MIC, similar membrane binding properties to other peptides, no emergence of resistance after serial passages, and almost no toxicity toward human red blood cells (448). Furthermore, in a mouse peritoneal infection model using \textit{E. coli}, \(C_{12}K-7\alpha_8\) prevented mortality similar to imipenem and ciprofloxacin, whereas conventional peptides did not (448). Overall, antimicrobial peptides have demonstrated great potential and may provide a feasible alternative for treatment of \textit{A. baumannii} infections, including those caused by polymyxin-resistant \textit{A. baumannii} (480, 562). Toxicity and efficacy data from human studies are awaited.

Other novel antimicrobial strategies for multidrug-resistant \textit{A. baumannii} include the use of bacterial conjugation, resulting in antibacterial gene transfer (505). This highly innovative approach uses attenuated \textit{E. coli} as a vector for a conjugative plasmid carrying bactericidal genes that disrupt protein synthesis. While in \textit{E. coli} the plasmid is tightly repressed, once it is transferred into the target pathogen, derepression occurs. In vitro, the donor \textit{E. coli} cells led to killing of \textit{A. baumannii} (505). Using a murine burn sepsis model, a significant improvement in survival of animals that were treated with donor cells was observed, as well as inhibition of \textit{A. baumannii} growth in burn wounds (505). Novel topical agents that may be effective for environmental cleaning of \textit{A. baumannii}, including highly charged copper-based biocides, have recently been reported (180). Such agents have broad-spectrum activity, including activity against \textit{Clostridium difficile} spores; however, copper resistance was described when copper was used in animal feed (223). Other innovative therapeutic avenues, which have not yet been tested against \textit{A. baumannii} but have the potential for efficacy, include the use of bacteriophage treatment (590), improvement in host response via passive or active immunization (63, 122, 134, 350), and modification of bacterial virulence by inhibition of quorum sensing (202, 375), other bacterial secretion systems (17), or LPS biosynthesis (118). More broadly, deepening our understanding of the mechanisms of antimicrobial resistance gene transfer and the cellular responses (such as the SOS response) that lead to mutagenesis and eventual resistance (23, 92, 313) will provide unique targets that, when inhibited, may prevent the emergence and dissemination of resistance. The clinical utility of these agents will be an adjunct to other antimicrobials. Which of these new agents will successfully progress through the gauntlet of drug development is unknown, but it is reassuring to see the surge in interest.

CONCLUSIONS

Significant advances have been made in our understanding of \textit{A. baumannii} over recent years, but many unanswered questions still remain. With the advent of whole-genome sequencing, we have been able to gain important insights into the genetic complexity and agility of this fascinating organism. Its wide array of drug resistance determinants and its ability to effectively regulate these according to selective environmental pressures clearly demand respect. The global epidemiology of \textit{A. baumannii} is concerning for widespread dissemination, most often in a clonal manner within institutions or cities, and sometimes between countries. The evidence suggests that hospital-acquired \textit{A. baumannii} infections prolong the lengths of hospital stays and subsequent health care costs. However, the direct effects of \textit{A. baumannii} on mortality appear less well defined. Despite the majority of \textit{A. baumannii} strains still being susceptible to carbapenems, many institutions around the world are faced with the challenging issue of pandrug resistance. Concerns have been raised about the use of tigecycline for \textit{A. baumannii} infection, particularly for bacteremia, leaving colistin as the only therapeutic option for some. It is imperative that future research be directed toward understanding the pharmacokinetic and pharmacodynamic properties of the polymyxins in order for clinicians to optimize patient outcomes, minimize adverse effects, and prevent the emergence of secondary resistance. Our understanding of the role of combination therapy for patients with multidrug- or pandrug-resistant \textit{A. baumannii} infections is also critical. New therapeutics are clearly needed, and we as clinicians, microbiologists, and scientists must think broadly about our approach to antimicrobial drug development, as novel targets will no doubt provide the most reward for our afflicted patients.

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