

Review Article

Acinetobacter: A War Zone in the Hospital

ABSTRACT

Acinetobacter baumannii has emerged as a significant hospital pathogen because of the increasing number of infections and the global spread of strains with resistance to multiple antibiotic classes. An important contribution to the epidemiology of infections with *A. baumannii* is the return of military personnel who have fought in Iraq or Afghanistan. In spite of its clinical relevance, until recently, there has been limited scientific data regarding the microbiological and pharmacological aspects of this organism. The availability of complete genome sequences, molecular tools for manipulating the bacterial genome, and animal models of infection facilitated the identification of factors that play a role in *A. baumannii* persistence and infection. This review summarizes the currently available data on the microbiology of *A. Baumannii* and its clinical and pharmacotherapeutic implications on healthcare.

Keywords: *Acinetobacter baumannii*; pathogen transmission; drug resistance; nosocomial; microbiology; molecular diagnostics

INTRODUCTION

A.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 most sinister organisms threatening the current antibiotic era.^{1,2} It has also attained notoriety by causing crippling infections in soldiers at war in Iraq, earning it the epithet “Iraqibacter”.³ The genus was first described in 1911 and *A.baumannii* is just one of the many *Acinetobacter* species that can cause disease in humans, but it accounts for approximately 80% of all reported *Acinetobacter* infections.^{1,2} Acting in synergy with its 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.^{1,2,3}

Significant advances have been made in our understanding of this organism. In the present review, we describe these advances and also attempt to provide an appraisal of the relevant microbiological characteristics and clinical implications of *A. baumannii*.

EPIDEMIOLOGY

Since the early 1980s, hospital outbreaks of *A. baumannii* infections in Europe, mainly in England, France, Germany, Italy, Spain, and The Netherlands have been investigated using molecular epidemiological typing methods. Transmission of such strains has been observed between hospitals, most probably via transfer of colonized patients.^{2,4,5} 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.^{6,7} Intercontinental spread of multidrug-resistant *A.baumannii* has also been described between Europe and other countries as a consequence of airline travel.^{6,7}

National surveillance studies in North America have demonstrated significant trends in the emergence of multidrug-resistant *Acinetobacter* strains.^{2,5} Nosocomial *Acinetobacter* infections have some seasonal variation in the United States, with an unexplained upswing in late summer months.^{2,8} Rates of nonsusceptibility to meropenem, imipenem, ceftazidime, piperacillin-tazobactam, ciprofloxacin, and gentamicin in Latin America are among the highest in the world.^{8,9}

Numerous outbreaks of pandrug-resistant *A. baumannii* have been documented in Asian and Middle Eastern hospitals and a variety of carbapenemases have been described to originate there.^{2,3,9-11} Outbreaks of carbapenem-resistant *A. baumannii* have also occurred in French Polynesia.¹¹ Most recently, reduced susceptibility to tigecycline of multidrug-resistant *A. baumannii* strains has been described in Australia.^{2,12}

A. baumannii and War

An important contribution to the epidemiology of infections with *A. baumannii* is the return of military personnel who have fought in Iraq or Afghanistan.^{2,3,13} An increase in infections with *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.^{1,2,13,14} 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. Rather, multiple *A. baumannii* isolates were cultured from a range of inanimate surfaces in field hospitals and were genotypically linked to patient isolates.^{1,2,13,14} The reservoir for these infections is not well known and may be different for each treatment facility, but the evidence strongly suggests the high numbers of wounded soldiers and the transfer of these soldiers from one treatment facility to another aids the transmission and the growing resistance of *A. baumannii*.^{1,2,13,14}

HISTORY

In 1911, Martinus Willem Beijerinck, a Dutch microbiologist, described an organism named *Micrococcus calcoaceticus* that was isolated from soil by enrichment.² The current genus designation, *Acinetobacter*, was proposed by Brisou and Prevot in 1954 to separate the nonmotile from the motile microorganisms within the genus *Achromobacter*.¹⁵ In 1986, Bouvet and Grimont, delineated 12 DNA groups of *Acinetobacter* using DNA-DNA hybridization and proposed 4 new species.³ The genus *Acinetobacter*, comprises *A.calcoaceticus*, *A.baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU, these 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.¹⁶ This group of organisms comprises the vast majority of both community-acquired and nosocomial infections.

MICROBIOLOGY

Acinetobacter baumannii is a gram-negative, strictly aerobic, nonfermenting, nonfastidious, nonmotile, catalase-positive, oxidase-negative coccobacillus harboring a number of effective virulence factors.² These factors include the attachment to and persistence on solid and dry surfaces, the ability to obtain essential nutrients such as iron, the adhesion to and subsequent destruction of epithelial cells, and the ability to produce gelatinases and proteinases that damage host tissues.¹⁷ Members of the genus *Acinetobacter* are considered ubiquitous organisms. *Acinetobacters* can be recovered after enrichment culture from virtually all samples obtained from soil or surface water.^{2,4} *Acinetobacters* are part of the human skin flora. Up to 43% of nonhospitalized individuals were found to be colonized with these organisms.¹⁸

Acinetobacter baumannii has the added ability to colonize the skin of patients or healthy individuals without causing illness. It also has the ability to form biofilms, which may play a role in the process of colonization. A biofilm is an aggregate of microorganisms in which the cells adhere to one another or to a surface in a self-produced matrix of extracellular DNA, proteins, and polysaccharides.^{4,18,19} The cells forming the biofilm are morphologically, metabolically, and physiologically different from their planktonic counterparts. Biofilms help the bacteria resist disinfection while also allowing the participating cells to trade resistance genes, further facilitating the persistence of the pathogen.^{4,19}

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 which resemble those of *Enterobacteriaceae*, with a colony diameter of 1.5 to 3 mm after overnight culture.^{2,4} There is no single metabolic test distinguishes acinetobacters from other similar nonfermenting gram-negative bacteria. A reliable method for 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 *trpE27*, a naturally transformable tryptophan auxotroph recently identified as *A. baylyi*, to be transformed by crude DNA of any *Acinetobacter* species to a wild-type phenotype.²⁰

Molecular methods that have been developed and validated for identification of acinetobacters include amplified 16S rRNA gene restriction analysis (ARDRA) (high-resolution fingerprint analysis by amplified fragment length polymorphism (AFLP), ribotyping, tRNA spacer fingerprinting, and sequencing of the *rpoB* (RNA polymerase-subunit) gene and its flanking spacers. ARDRA and AFLP analysis are currently the most widely accepted and validated reference methods for species identification of acinetobacters.^{2,21,22}

VIRULENCE FACTORS & CLINICAL IMPACT

Virulence mechanisms in *A. baumannii* include siderophore-mediated iron acquisition systems, biofilm formation, adherence and OMP function, and the *A. baumannii* LPS (152, 222, 292).^{2,19,23,24} In order for *A. baumannii* to thrive in the iron-deficient environment of a human host, it secretes lowmolecular- mass ferric binding compounds, or siderophores.²³ The ability of *A. baumannii* to adhere to and form biofilms on inanimate objects and surfaces may explain its success in the hospital environment. Adherence of *A. baumannii* to human bronchial epithelial

cells and erythrocytes has also been demonstrated, with similar pilus like structures appearing important for adherence.^{19,23,24} After adherence to human cells, it appears that *A. baumannii* can induce apoptosis via an OMP (Omp38).² This protein appears to localize to the mitochondria, leading to both caspase-dependent and -independent pathways of apoptosis. *A. baumannii* LPS has been identified as the major immunostimulatory factor which has also demonstrated potent endotoxic potential, stimulating the proinflammatory cytokines interleukin-8 and tumor necrosis factor alpha.²⁶ Humoral immune responses have also been described for *Acinetobacter* infection, with antibodies being targeted toward iron-repressable OMPs and the O polysaccharide component of LPS.²

MECHANISMS OF ANTIBIOTIC RESISTANCE

The ability of *A. baumannii* to acquire antibiotic resistance mechanisms has allowed this organism to persist in hospital environments and has facilitated the global emergence of MDR strains. β -lactam resistance in *A. baumannii* is due to its ability of enzymatic degradation by β -lactamases. *A. baumannii* strains possess chromosomally encoded Amp C cephalosporinases, also known as *Acinetobacter*-derived cephalosporinases (ADCs).^{4,27,28} The key determinant regulating overexpression of this enzyme in *A. baumannii* is the presence of an upstream IS element known as *ISAbal*.²⁹ They encode a transposase and therefore are mobile. Second, they can contain promoter regions that lead to overexpression of downstream resistance determinants. Unlike the OXA-type enzymes, MBLs are most commonly found within integrons, which are specialized genetic structures that facilitate the acquisition and expression of resistance determinants. Other ESBLs identified in *A. baumannii* include TEM-92, TEM-116, SHV-12, CTX-M-2, CTX-M-43, TEM-1 and TEM-2.^{30,31} 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).

Resistance, has also been ascribed to nonenzymatic mechanisms, including changes in outer membrane proteins (OMPs), multidrug efflux pumps, and alterations in the affinity or expression of penicillin-binding proteins.^{2,4,24,31-33} The presence of genes coding for aminoglycoside-modifying enzymes has been noted in multidrug-resistant *A. baumannii* strains.^{4,34,35} This emerging resistance mechanism impairs aminoglycoside binding to its target site and confers high-level resistance to all clinically useful aminoglycosides. Modifications to DNA

gyrase or topoisomerase IV through mutations in the *gyrA* and *parC* genes have been described for *A. baumannii*.³⁶ These mutations interfere with target site binding. Similar to aminoglycosides, many quinolones are also substrates for multidrug efflux pumps.^{2,36}

DEFINITIONS & TRANSMISSION MULTI-DRUG RESISTANCE

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).^{2,37} Pan-drug resistance is often defined as resistance to all antimicrobials that undergo first-line susceptibility testing that have therapeutic potential against *A. baumannii*. *A. baumannii* has the ability to live on dry environmental surfaces in an ICU for up to 13 days—10 days more than other gram-negative bacteria. Studies have shown similar abilities by *A. baumannii* in humid conditions and on bed rails.^{4,37} It is this ability to survive for long periods coupled with its ability to demonstrate a number of antimicrobial resistance genes that have made *A. baumannii* a successful hospital pathogen.³⁷

CLINICAL PICTURE

Hospital-Acquired Pneumonia

In most institutions, the majority of *A. baumannii* isolates are from the respiratory tracts of hospitalized patients. Between 5 and 10% of cases of ICU-acquired pneumonia are due to *A. baumannii*.^{8,38} Typically, patients with *A. baumannii* infections have had prolonged ICU stays, although in outbreak situations, earlier acquisition of infection may occur.^{8,38}

Community-Acquired Pneumonia

Community-acquired pneumonia due to *A. baumannii* has been described for tropical regions of Australia and Asia.³⁹ The disease most typically occurs during the rainy season among people with a history of alcohol abuse.⁸ It is characterized by a fulminant clinical course, secondary bloodstream infection, and mortality rate of 40 to 60%.⁴⁰

Bloodstream Infection

A. baumannii is a more common cause of ICU-acquired bloodstream infection than of non-ICU-ward infection (1.6% versus 0.9% of bloodstream infections). *A. baumannii*

bloodstream infection had the third highest crude mortality rate in the ICU, exceeded only by *P. aeruginosa* and *Candida* sp. infections.^{1,37}

Battlefield Trauma

A. baumannii may occasionally cause skin/soft tissue infections outside of the military population. It is a well-known pathogen in burn units and may be difficult to eradicate from such patients.^{1,2} *A. baumannii* is commonly isolated from wounds of combat casualties from Iraq and Afghanistan.⁴¹⁻⁴³ It is the most commonly isolated organism (32.5% of cases) in combat victims with open tibial fractures.⁴¹

UTI

A. baumannii is an occasional cause of UTI, being responsible for just 1.6% of ICU-acquired UTIs. Typically, the organism is associated with catheter-associated infection or colonization.^{2,41}

Meningitis

Nosocomial, postneurosurgical *A. baumannii* meningitis is an important entity. Typical patients have undergone neurosurgery and have an external ventricular drain.⁴⁴ Mortality may be as high as 70%, although the cause of mortality may often difficult to discern.⁴⁴

Other Manifestations

A small number of case reports of *Acinetobacter* endocarditis exist.⁴⁵⁻⁴⁷ Most cases have involved prosthetic valves. *Acinetobacter* may cause endophthalmitis or keratitis, sometimes related to contact lens use or following eye surgery.⁴⁵⁻⁴⁸

INFECTION CONTROL PERSPECTIVE

Three factors lead to the persistence of *A. baumannii* in the hospital environment, namely resistance to major antimicrobial drugs, resistance to desiccation, and resistance to disinfectants. The overall mean survival time is 27 days, with a range of 21 to 33 days.^{1,2,4} 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.^{2,8,37} Recommendations for all health care facilities include improvement of hand hygiene, use of contact precautions until the patient tests culture-negative for the target organism, active surveillance cultures, education of hospital personnel, improved environmental cleaning, and better communication about patients with these infections to not just

personnel within the facility but also between facilities. Lower rates of infection have been reported when the rooms and equipment were cleaned completely and more frequently.^{1,2,4,5,37} Bleach solutions and other disinfectants should be used in rooms and on equipment often and thoroughly to effectively control transmission of *A. baumannii*. To control the spread of *A. baumannii* in the hospital, potential reservoirs of the organism and the modes of transmission need to be identified.^{1,2,4,5,37}

Hospital Outbreaks and Control Measures

Infection control interventions during an *A. baumannii* outbreak include, molecular epidemiologic investigations to determine if a clonal outbreak strain is present. Environmental cultures should be used to determine if a common environmental source is present. Enhanced environmental cleaning should be performed in order to eliminate the organism from the peripatient environment. Enhanced isolation procedures, aimed at optimizing contact isolation and improving hand hygiene, should be implemented. Antibiotic management processes should be used to ensure that “at-risk” antibiotics are not being used excessively. Three classes of antibiotics which have been implicated in the emergence of multi-drug resistance are broad-spectrum cephalosporins, carbapenems, and fluoroquinolones.^{1,2,4,5,37} Monitoring adherence to such infection control interventions is also important.^{1,2,4,5,37}

LABORATORY DIAGNOSTIC TECHNIQUES

Acinetobacter can be cultured on routine laboratory media due to its ability to use various sources of nutrition and will grow at 44°C.¹⁵ Standard laboratory techniques will identify the genus but may not identify the species.³ Phenotypic typing systems based on biochemical profiles (biotyping), antibiotic susceptibility patterns, serological reactions (serotyping), phage typing, and protein profiles have largely been replaced by a multitude of molecular typing systems, including, in historical order, plasmid profiling; ribotyping; PFGE; randomly amplified polymorphic DNA analysis; repetitive extragenic palindromic sequence-based (REP) PCR; AFLP analysis, a high-resolution genomic fingerprinting method; integrase gene PCR; infrequent-restriction-site PCR; and most recently, MLST and multilocus PCR–ESI-MS.^{2,4,49-53} Pulsed-field gel electrophoresis (PFGE) and 16s rRNA sequencing are two commonly used molecular methods. Pulsed-field gel electrophoresis is a type of gel electrophoresis used to separate large molecules of DNA up to 2000 kb. A second molecular method employed in

bacterial identification is 16s ribosomal DNA (rDNA) sequencing.⁵⁴ In this method, DNA is isolated from bacterial colonies and amplified with primers selective for the 16s rDNA. The rDNA can then be sequenced and results compared to *Acinetobacter* genotypes on public-domain sequence databases.⁵⁴ These molecular methods are not only useful in identifying causes of bacterial infection, but they can aid a hospital in its infection control measures, highlighting areas of weakness, and can identify the source of hospital-acquired infections.⁵⁴ Plasmid analysis is used successfully for epidemiological typing of *A. baumannii* strains, and plasmid profiling is one of the methods that have been applied to study the epidemiology of *Acinetobacter* species outside the *A. baumannii* group.^{4,55}

THERAPEUTIC STRATEGIES FOR ACINETOBACTER BAUMANNII INFECTION

A. baumannii is one of the “red alert” pathogens that greatly threaten the utility of our current antibacterial armamentarium.⁵⁶ 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.

Existing Antimicrobial Agents

Therapy should be based on the results of adequately performed antimicrobial susceptibility testing. Carbapenems have been thought of as the agent of choice for serious *A. baumannii* infections. However, the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance.^{1,2,4,57}

Sulbactam

Unlike clavulanic acid and tazobactam, it has clinically relevant intrinsic antimicrobial activity against *Acinetobacter* and *Bacteroides* spp., mediated by its binding to penicillin-binding protein 2.^{58,59} Despite the absence of randomized clinical trials, sulbactam has shown promising results against *A. baumannii* strains with various susceptibility profiles. The use of a sulbactam-containing regimen for milder infections may be an appropriate strategy in limiting excessive carbapenem use.^{56,58}

Polymyxins.

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.⁶⁰ 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%.^{1,4,56,60-62} Nebulized colistin is increasingly being used in an attempt to minimize systemic toxicity and improve drug deposition at the site of infection.^{1,4,56,60-62} Rates of clinical cure appeared superior with supplemental nebulized colistin. 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 infections, intrathecal or intraventricular colistin has been utilized. Of concern, rates of resistance to the polymyxins have recently been reported to be as high as 3.2% for multi drug resistant *A. baumannii*.⁶⁰⁻⁶²

Tigecycline

It is a semisynthetic derivative of minocycline, which inhibits the 30S ribosomal subunit, but its unique feature is its ability to evade the major determinants of tetracycline resistance, i.e., the efflux pumps and the determinants that provide ribosomal protection.^{2,56,63} No difference was observed for all tigecycline combinations, including combinations with amikacin, meropenem, imipenem ciprofloxacin, levofloxacin, ampicillin-sulbactam, rifampin, carbapenem, and the next generation of cephalosporins with activity against MRSA, ceftobiprole and ceftaroline.^{2,56,63}

Combination Therapy

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.⁶⁴ The studies involving combination therapy with either sulbactam or the polymyxins form the 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.^{56,64-67} The results for quinolone combination therapy are varied, with reduced efficacy being described when ciprofloxacin was used for ciprofloxacin-resistant *A. baumannii*, as well as a lack of enhanced activity with levofloxacin combined with imipenem or amikacin.^{56,67}

FUTURE THERAPEUTIC CONSIDERATIONS

Newer therapeutic options can be divided into those that inhibit a currently recognized mechanism of resistance or those that have a novel mechanism of action. Cationic antimicrobial peptides that are capable of inhibiting both aminoglycoside phosphotransferases and acetyltransferases have been described.⁶⁸ The importance of multidrug efflux pumps in *A. baumannii* is recognized, with tigecycline identified as a substrate of the RND-type pump AdeABC.^{56,69} Eukaryotic antimicrobial peptides are cationic peptides which 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.⁷⁰

Bacterial conjugation is a novel antimicrobial strategy resulting in antibacterial gene transfer.⁷¹ This approach uses attenuated *E. coli* as a vector for a conjugative plasmid carrying bactericidal genes that disrupt protein synthesis. Topical agents that may be effective for environmental cleaning of *A. baumannii*, including highly charged copper-based biocides.⁷² Such agents have broad-spectrum activity, including activity against *Clostridium difficile* spores.

Other innovative therapeutic avenues against *A. baumannii* include the use of bacteriophage treatment, improvement in host response via passive or active immunization, and modification of bacterial virulence by inhibition of quorum sensing, other bacterial secretion systems, or LPS biosynthesis.^{56,72-79}

CONCLUSIONS

Significant advances have been made in our understanding of *A. baumannii* over recent years, but many unanswered questions still remain. With the advent of whole-genome sequencing there have been important insights into the genetic complexity and agility of this fascinating organism. Its wide array of drug resistance determinants and ability to effectively regulate these according to selective environmental pressures clearly demand respect. The evidence suggests that hospital acquired *A. baumannii* infections prolong the lengths of hospital stays and subsequent health care costs. Despite the majority of *A. baumannii* strains still being susceptible to carbapenems, most institutions are faced with the challenging issue of pandrug resistance. An understanding of the role of combination therapy for multidrug- or pandrug-resistant *A. baumannii* infections is critical. New therapeutics are clearly needed, and clinicians, microbiologists, and scientists must

work in tandem as a team to reevaluate our approach to antimicrobial drug development, as novel targets will no doubt provide the most reward for the afflicted patients

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REFERENCES

1. Towner KJ. Acinetobacter: An old friend, but a new enemy. *J Hosp Infect.* 2009;73:355-363.
2. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: Emergence of a Successful Pathogen *Clin. Microbiol. Rev.* 2008; 21(3):538–582
3. Davis KA, Moran KA, McAllister CK, et al. Multidrug-resistant Acinetobacter extremity infections in soldiers. *Emerg Infect Dis.* 2005;11:1218-1224.
4. Bergogne-Berezin E, Towner KJ. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin. Microbiol. Rev.* 1996;9:148–165.
5. Fournier PE, Richet H. The epidemiology and control of Acinetobacter baumannii in health care facilities. *Clin. Infect. Dis.* 2006;42:692–699.
6. Schulte BC, Goerke P, Weyrich S, et al. Clonal spread of meropenem-resistant Acinetobacter baumannii strains in hospitals in the Mediterranean region and transmission to south-west Germany. *J. Hosp. Infect.* 2005;61:356–357.
7. Peleg AY, Bell JM, Hofmeyr A, Wiese P. Inter-country transfer of gram-negative organisms carrying the VIM-4 and OXA-58 carbapenem-hydrolysing enzymes. *J. Antimicrob. Chemother.* 2006;57:794–795
8. McDonald LC, Banerjee SN, Jarvis WR, et al. Seasonal variation of Acinetobacter infections: 1987–1996. *Clin. Infect. Dis.* 1999;29:1133–1137
9. Unal S, Garcia-Rodriguez JA. Activity of meropenem and comparators against Pseudomonas aeruginosa and Acinetobacter spp. isolated in the MYSTIC Program, 2002–2004. *Diagn. Microbiol. Infect. Dis.* 2005;53:265–271.
10. Koh TH, Sng LH, Wang GC, Hsu LY, Zhao Y. IMP-4 and OXA beta-lactamases in Acinetobacter baumannii from Singapore. *J. Antimicrob. Chemother.* 2007;59:627–632.

11. Naas T, Levy M, Hirschauer C, Marchandin H, Nordmann P. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. *J. Clin. Microbiol.* 2005;43:4826–4829.
12. Iredell J, Thomas L, Power D, Mendes E. Tigecycline resistance in Australian antibiotic-resistant gram-negative bacteria. *J. Antimicrob. Chemother.* 2007;59:816–818.
13. Davis KA, Moran KA, McAllister CK, Gray PJ. Multidrug-resistant *Acinetobacter* extremity infections in soldiers. *Emerg. Infect. Dis.* 2005;11:1218–1224
14. Scott P, Deye G, Srinivasan A, et al. An outbreak of multidrug-resistant *Acinetobacter baumannii*-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. *Clin. Infect. Dis.* 2007;44:1577–1584.
15. Brisou J, Prevot AR. Studies on bacterial taxonomy X. The revision of species under *Achromobacter* group. *Ann. Inst. Pasteur (Paris)* 1954;86:722–728.
16. Gerner-Smidt P. Ribotyping of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex. *J. Clin. Microbiol.* 1992;30:2680–2685.
17. Van Looveren M, Goossens H; ARPAC Steering Group. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clin Microbiol Infect.* 2004;10:684-704
18. Seifert H, Dijkshoorn L, Gerner-Smidt P, Pelzer N, Tjernberg I, Vaneechoutte M. Distribution of *Acinetobacter* species on human skin: comparison of phenotypic and genotypic identification methods. *J. Clin. Microbiol.* 1997;35:2819–2825.
19. Rajamohan G, Srinivasan VB, Gebreyes WA. Biocide-tolerant multidrug-resistant *Acinetobacter baumannii* clinical strains are associated with higher biofilm formation. *J Hosp Infect.* 2009;73:287-289
20. Vaneechoutte M, Young DM, Ornston LN, et al. Naturally transformable *Acinetobacter* sp. strain ADP1 belongs to the newly described species *Acinetobacter baylyi*. *Appl. Environ. Microbiol.* 2006;72:932–936
21. Vaneechoutte M, Dijkshoorn L, Tjernberg I, et al. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. *J. Clin. Microbiol.* 1995; 33:11–15.
22. La Scola B, Gundi VA, Khamis A, Raoult D. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. *J. Clin. Microbiol.* 2006;44:827–832.

23. Dorsey CW, Tomaras AP, Connerly PL, et al. The siderophore-mediated iron acquisition systems of *Acinetobacter baumannii* ATCC 19606 and *Vibrio anguillarum* 775 are structurally and functionally related. *Microbiology* 2004;150:3657–3667
24. Siroy A, Cosette P, Seyer D, et al. Global comparison of the membrane subproteomes between a multidrug-resistant *Acinetobacter baumannii* strain and a reference strain. *J. Proteome Res.* 2006;5:3385–3398.
25. Gospodarek E, Grzanka A, Dudziak Z, Domaniewski J. Electron-microscopic observation of adherence of *Acinetobacter baumannii* to red blood cells. *Acta Microbiol. Pol.* 1998;47:213–217.
26. Erridge C, Moncayo-Nieto OL, Morgan R, Young M, Poxton IR. *Acinetobacter baumannii* lipopolysaccharides are potent stimulators of human monocyte activation via Toll-like receptor 4 signalling. *J. Med. Microbiol.* 2007;56:165–171.
27. Hujer KM, Hamza NS, Hujer AM, et al. Identification of a new allelic variant of the *Acinetobacter baumannii* cephalosporinase, ADC-7 beta-lactamase: defining a unique family of class C enzymes. *Antimicrob. Agents Chemother.* 2005;49:2941–2948.
28. Hujer KM, Hujer AM, Hulten EA, et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. *Antimicrob. Agents Chemother.* 2006;50:4114–4123.
29. Heritier C, Poirel L, Nordmann P. Cephalosporinase overexpression resulting from insertion of ISAbal in *Acinetobacter baumannii*. *Clin. Microbiol. Infect.* 2006;12:123–130.
30. Ruiz M, Marti S, Fernandez-Cuenca F, Pascual A, Vila J. Prevalence of IS(Aba1) in epidemiologically unrelated *Acinetobacter baumannii* clinical isolates. *FEMS Microbiol. Lett.* 2007;274:63–66.
31. Naiemi NA, Duim B, Savelkoul PH, et al. Widespread transfer of resistance genes between bacterial species in an intensive care unit: implications for hospital epidemiology. *J. Clin. Microbiol.* 2005;43:4862–4864.
32. Obara M, Nakae T. Mechanisms of resistance to beta-lactam antibiotics in *Acinetobacter calcoaceticus*. *J. Antimicrob. Chemother.* 1991;28:791–800.

33. Fernandez-Cuenca F, Martinez-Martinez L, Conejo MC, et al. Relationship between beta-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 2003;51:565–574.
34. Lee K, Yum JH, Yong D, et al. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrob. Agents Chemother.* 2005;49:4485–4491
35. Zhou H, Pi BR, Yang Q, et al. Dissemination of imipenem-resistant *Acinetobacter baumannii* strains carrying the ISAbal blaOXA-23 genes in a Chinese hospital. *J. Med. Microbiol.* 2007;56:1076–1080.
36. Vila J, Marti S, Sanchez-Cespedes J. Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 2007;59:1210–1215.
37. Abbo A, Navon-Venezia S, Hammer-Muntz O, et al. Multidrug-resistant *Acinetobacter baumannii*. *Emerg. Infect. Dis.* 2005;11:22–29.
38. Garnacho-Montero J, Ortiz-Leyba C, Fernandez-Hinojosa E, et al. *Acinetobacter baumannii* ventilator-associated pneumonia: epidemiological and clinical findings. *Intensive Care Med.* 2005;31:649–655
39. Gottlieb T, Barnes DJ. Community-acquired *Acinetobacter* pneumonia. *Aust. NZ. J. Med.* 1989;19:259–260.
40. Leung WS, Chu CM, Tsang KY, et al. Fulminant community-acquired *Acinetobacter baumannii* pneumonia as a distinct clinical syndrome. *Chest* 2006;129:102–109.
41. Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. *Clin. Infect. Dis.* 2007;45:409–415.
42. Yun, HC, Murray CK, Roop SA, et al. Bacteria recovered from patients admitted to a deployed U.S. military hospital in Baghdad, Iraq. *Mil. Med.* 2006;171:821–825.
43. Whitman TJ. Infection control challenges related to war wound infections in the ICU setting. *J. Trauma* 2007;62:S53.
44. Metan G, Alp E, Aygen B, Sumerkan B. *Acinetobacter baumannii* meningitis in post-neurosurgical patients: clinical outcome and impact of carbapenem resistance. *J. Antimicrob. Chemother.* 2007;60:197–199

45. Menon T, Shanmugasundaram S, Nandhakumar B, Nalina K, Balasubramaniam. Infective endocarditis due to *Acinetobacter baumannii* complex—a case report. *Indian J. Pathol. Microbiol.* 2006;49:576–578.
46. Levy J, Oshry T, Rabinowitz R, Lifshitz T. *Acinetobacter* corneal graft ulcer and endophthalmitis: report of two cases. *Can. J. Ophthalmol.* 2005;40:79–82.
47. Lindbohm N, Moilanen JA, Vesaluoma MH, Tervo TM. *Acinetobacter* and *Staphylococcus aureus* ulcerative keratitis after laser in situ keratomileusis treated with antibiotics and phototherapeutic keratectomy. *J. Refract. Surg.* 2005;21:404–406.
48. Kau HC, Tsai CC, Kao SC, Hsu WM, Liu JH. Corneal ulcer of the side port after phacoemulsification induced by *Acinetobacter baumannii*. *J. Cataract Refract. Surg.* 2002;28:895–897.
49. Seifert H, Boullion B, Schulze A, Pulverer G. Plasmid DNA profiles of *Acinetobacter baumannii*: clinical application in a complex endemic setting. *Infect. Control Hosp. Epidemiol.* 1994;15:520–528.
50. Snelling AM, Gerner-Smidt P, Hawkey PM, et al. Validation of use of whole-cell repetitive extragenic palindromic sequence-based PCR (REP-PCR) for typing strains belonging to the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex and application of the method to the investigation of a hospital outbreak. *J. Clin. Microbiol.* 1996;34:1193–1202.
51. Koeleman JG, Stoof J, Biesmans DJ, Savelkoul PH, Vandenbroucke-Grauls CM. Comparison of amplified ribosomal DNA restriction analysis, random amplified polymorphic DNA analysis, and amplified fragment length polymorphism fingerprinting for identification of *Acinetobacter* genomic species and typing of *Acinetobacter baumannii*. *J. Clin. Microbiol.* 1998;36:2522–2529.
52. Bartual SG, Seifert H, Hippler C, et al. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *J. Clin. Microbiol.* 2005;43:4382–4390.
53. Ecker JA, Massire C, Hall TA, et al. Identification of *Acinetobacter* species and genotyping of *Acinetobacter baumannii* by multilocus PCR and mass spectrometry. *J. Clin. Microbiol.* 2006;44:2921–2932.

54. Misbah S, Hassan H, Yusof MY, et al. Genomic species identification of *Acinetobacter* of clinical isolates by 16S rDNA sequencing. *Singapore Med J.* 2005;46:461-464.
55. Seifert H, Schulze A, Baginski R, Pulverer G. Plasmid DNA fingerprinting of *Acinetobacter* species other than *Acinetobacter baumannii*. *J. Clin. Microbiol.* 1994;32:82–86.
56. Talbot GH, Bradley J, Edwards JE, et al. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2006; 42:657–668.
57. Bou G, Cervero G, Dominguez MA, Quereda C, Martinez-Beltran J. Characterization of a nosocomial outbreak caused by a multiresistant *Acinetobacter baumannii* strain with a carbapenem-hydrolyzing enzyme: high-level carbapenem resistance in *A. baumannii* is not due solely to the presence of beta-lactamases. *J. Clin. Microbiol.* 2000; 38:3299–3305
58. Rodriguez-Hernandez MJ, Cuberos L, Pichardo C, et al. Sulbactam efficacy in experimental models caused by susceptible and intermediate *Acinetobacter baumannii* strains. *J. Antimicrob. Chemother.* 2001;47:479–482.
59. Williams JD. β -Lactamase inhibition and in vitro activity of sulbactam and sulbactam/cefoperazone. *Clin. Infect. Dis.* 1997;24:494–497.
60. Rodriguez-Hernandez MJ, Jimenez-Mejias ME, Pichardo C, et al. Colistin efficacy in an experimental model of *Acinetobacter baumannii* endocarditis. *Clin. Microbiol. Infect.* 2004;10:581–584.
61. Pereira GH, Muller PR, Levin AS. Salvage treatment of pneumonia and initial treatment of tracheobronchitis caused by multidrug-resistant gram-negative bacilli with inhaled polymyxin B. *Diagn. Microbiol. Infect. Dis.* 2007;58:235–240.
62. Kwa AL, Loh C, Low JG, Kurup A, Tam VH. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Clin. Infect. Dis.* 2005;41:754–757
63. Fluit AC, Florijn A, Verhoef J, Milatovic D. Presence of tetracycline resistance determinants and susceptibility to tigecycline and minocycline. *Antimicrob. Agents Chemother.* 2005;49:1636–1638.
64. Chait R., Craney A, Kishony R. Antibiotic interactions that select against resistance. *Nature* 2007;446:668–671.

65. Chang SC, Chen YC, Luh KT, Hsieh WC. In vitro activities of antimicrobial agents, alone and in combination, against *Acinetobacter baumannii* isolated from blood. *Diagn. Microbiol. Infect. Dis.* 1995;23:105–110
66. Ermertcan S, Hosgor M, Tunger O, Cosar G. Investigation of synergism of meropenem and ciprofloxacin against *Pseudomonas aeruginosa* and *Acinetobacter* strains isolated from intensive care unit infections. *Scand. J. Infect. Dis.* 2001;33:818–821.
67. Joly-Guillou ML, Wolff M, Farinotti R, Bryskier A, Carbon C. In vivo activity of levofloxacin alone or in combination with imipenem or amikacin in a mouse model of *Acinetobacter baumannii* pneumonia. *J. Antimicrob. Chemother.* 2000;46:827–830.
68. Boehr DD, Draker KA, Koteva K, et al. Broad-spectrum peptide inhibitors of aminoglycoside antibiotic resistance enzymes. *Chem. Biol.* 2003;10:189–196.
69. Peleg AY, Adams J, Paterson DL. Tigecycline efflux as a mechanism for nonsusceptibility in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2007;51:2065–2069.
70. Saugar JM, Rodriguez-Hernandez MJ, de la Torre BG, et al. Activity of cecropin A-melittin hybrid peptides against colistin-resistant clinical strains of *Acinetobacter baumannii*: molecular basis for the differential mechanisms of action. *Antimicrob. Agents Chemother.* 2006;50:1251–1256.
71. Shankar R, He LK, Szilagyi A, Muthu K, et al. A novel antibacterial gene transfer treatment for multidrug-resistant *Acinetobacter baumannii* induced burn sepsis. *J. Burn Care Res.* 2007;28:6–12.
72. Gant VA, Wren MW, Rollins MS, et al. Three novel highly charged copper-based biocides: safety and efficacy against healthcare-associated organisms. *J. Antimicrob. Chemother.* 2007;60:294–299
73. Wang J, Hu B, Xu M, et al. Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant *Pseudomonas aeruginosa*. *Int. J. Mol. Med.* 2006;17:309–317
74. Brockstedt DG, Bahjat KS, Giedlin MA, et al. Killed but metabolically active microbes: a new vaccine paradigm for eliciting effector T-cell responses and protective immunity. *Nat. Med.* 2005;11:853–860.

- 555 75. DiGiandomenico A, Rao J, Harcher K, et al. Intranasal immunization with heterologously
556 expressed polysaccharide protects against multiple *Pseudomonas aeruginosa* infections.
557 Proc. Natl. Acad. Sci. USA 2007;104:4624–4629
- 558 76. Doring G, Meisner C, Stern M. A double-blind randomized placebo-controlled phase III
559 study of a *Pseudomonas aeruginosa* flagella vaccine in cystic fibrosis patients. Proc. Natl.
560 Acad. Sci. USA 2007;104:11020–11025.
- 561 77. Muh U, Schuster M, Heim R, et al. Novel *Pseudomonas aeruginosa* quorum-sensing
562 inhibitors identified in an ultra-high-throughput screen. Antimicrob. Agents Chemother.
563 2006;50: 3674–3679.
- 564 78. Gonzalez JE, Keshavan ND. Messing with bacterial quorum sensing. Microbiol. Mol.
565 Biol. Rev. 2006;70:859–875.
- 566 79. Baron C, Coombes B. Targeting bacterial secretion systems: benefits of disarmament in
567 the microcosm. Infect. Disord. Drug Targets 2007;7:19–27.
568