

Acinetobacter

Acinetobacter (*ah-see-netto-BAK-ter*) :

Is a genus of Gram-negative bacteria belonging to the wider class of *Gammaproteobacteria*. *Acinetobacter* species are nonmotile and oxidase-negative, and occur in pairs under magnification.

They are important soil organisms, where they contribute to the mineralization of, for example, aromatic compounds. *Acinetobacter* are a key source of infection in debilitated patients in the hospital, in particular the species *Acinetobacter baumannii*.

Etymology

Acinetobacter is a compound word from scientific Greek [+ + ()], meaning 'non-motile-rod'. The first element *acineto-* is a somewhat baroque rendering of the Greek morpheme -; the more common transliteration in English is *akineto-*, as in *akinetic*.

Description

Species of the genus *Acinetobacter* are strictly aerobic nonfermentative Gram-negative bacilli. They show preponderantly a coccobacillary morphology on nonselective agar. Rods predominate in fluid media, especially during early growth.

The morphology of *Acinetobacter sp.* can be quite variable in gram-stained human clinical specimens, and cannot be used to differentiate *Acinetobacter* from other common causes of infection.

Most strains of *Acinetobacter*, except some of the *A. lwoffii* strain, grow well on MacConkey agar (without salt). Although officially classified as nonlactose-fermenting, they are often partially lactose-fermenting when grown on MacConkey agar. They are oxidase-negative, nonmotile, and usually nitrate negative.

Bacteria of the genus *Acinetobacter* are known to form intracellular inclusions of polyhydroxyalkanoates under certain environmental conditions (e.g. lack of elements such as phosphorus, nitrogen, or oxygen combined with an excessive supply of carbon sources).

Taxonomy

The genus *Acinetobacter* comprises 27 validly named and 11 unnamed (genomic) species.

However, because routine identification in the clinical microbiology laboratory is not (yet) possible, they are divided and grouped into three main complexes:

- *Acinetobacter calcoaceticus-baumannii* complex: glucose-oxidising nonhemolytic, (*A.baumannii* can be identified by OXA-51 typing)
- *Acinetobacter lwoffii*: glucose-negative nonhemolytic
- *Acinetobacter haemolyticus*: hemolytic

Identification

Different species of bacteria in this genus can be identified using Fluorescence-Lactose-Denitrification (FLN) to find the amount of acid produced by metabolism of glucose.

The other reliable identification test at genus level is chromosomal DNA transformation assay (CTA): In this assay, a naturally competent tryptophan auxotrophic mutant of *Acinetobacter baylyi*(BD4 trpE27) is transformed with the total DNA of a putative *Acinetobacter* isolate and the transformation mixture plated on a brain heart infusion agar (BHI). The growth is then harvested after incubation for 24 h at 30°C, plating on an *Acinetobacter* minimal agar (AMM), and incubating at 30°C for 108 h. Growth on the minimal agar medium indicates a positive transformation assay and confirms the isolate as a member of the genus *Acinetobacter*. *E. coli* HB101 and *A. calcoaceticus* MTCC1921T can be used as the negative and positive controls, respectively

Habitat

Acinetobacter are widely distributed in nature, and commonly occur in soil. They can survive on moist and dry surfaces, including in a hospital environment. Some strains have been isolated from foodstuffs. In drinking water, they have been shown to aggregate bacteria that otherwise do not form aggregates.

Description and Significance

Acinetobacter is a genus of opportunistic pathogens in the proteobacteria group, species of which are distributed in widespread, diverse habitats. It has garnered media attention because of an outbreak among soldiers in Iraq who contracted the species *Acinetobacter baumannii*. While it was initially thought that the bacteria had come from the Iraqi soil, it turns out that the bacteria were actually contracted in the military's evacuation chain. At least 280 people, mostly soldiers returning from the battlefield, were infected with the disease, and at least 5 (non-active-duty soldiers) died.

These bacteria can often be found as the cause of pneumonia in hospitalized patients, especially those dependent on ventilators in Intensive Care Units. The bacteria are most often contracted through the exposure of open wounds to contaminated soil. This makes it a problem during warfare (it was the second-leading cause of infection among troops during the Vietnam conflict) because of the high number of injuries caused by explosives, which can easily lead to dirtying exposed skin. In healthy humans, it is normal to have some amount of *Acinetobacter* on the skin surface; as many as 25% of healthy adults do in fact harbor these bacteria.

Genome Structure

While there is much information available on the types of infections *Acinetobacter* causes, relatively few studies have been performed on the bacteria's genetics. Because *Acinetobacters* are very resistant to antibiotics and are difficult to differentiate between species when isolated from patients, learning more about their DNA will help develop better drugs to control outbreaks of the infection.

Cell Structure and Metabolism

Acinetobacter cells are short, Gram-negative rods, measuring 1.0-1.5 by 1.5-2.5 microns during growth; they often become more coccoid during the stationary phase. Cells are found in pairs or small clusters; the groups form smooth, pale colonies on solid media. All species are strictly aerobic, catalase positive, and oxidase negative; it is the last test which is most used to distinguish *Acinetobacter* from other infective bacteria. These bacteria can use a varied selection of organic materials as sources of carbon.

Ecology

Acinetobacters are widely distributed in hospitals, where they pose the danger of transferring resistance to other hospital-inhabiting bacteria. They are found in soil, water, and in living organisms, where they may or may not be pathogenic. Up to 27% of hospital sink traps and 20% of hospital floor swabs have yielded isolates of *Acinetobacter*. The bacteria has been found to contaminate respirators and hospital air when there are colonized patients present, as well as nearby bed blankets and bed curtains. Currently studies are being performed on methods for hospital air purification in order to lower the prevalence of these pathogens among compromised patients..

Pathology

In healthy individuals *Acinetobacter* colonizes on the skin correlate with low incidence of allergies; *Acinetobacter* is thought to be allergy-protective.

The species *A. baumannii* is the second-most-commonly-isolated nonfermenting bacterium in humans. In immunocompromised individuals, several *Acinetobacter* can cause life-threatening infections. Such species also exhibit a relatively broad degree of antibiotic resistance.

Acinetobacter is frequently isolated in nosocomial infections, and is especially prevalent in intensive care units, where both sporadic cases as well as epidemic and endemic occurrence is common. *A. baumannii* is a frequent cause of nosocomial pneumonia, especially of late-onset ventilator associated pneumonia. It can cause various other infections including skin and wound infections, bacteremia, and meningitis, but *A. lwoffii* is mostly responsible for the latter. *A. baumannii* can survive on the human skin or dry surfaces for weeks.

Epidemiologic evidence indicates that *Acinetobacter* biofilms play a role in infectious diseases such as periodontitis, bloodstream infections, and urinary tract infections, because of the bacteria's ability to colonize indwelling medical devices (such as catheters). Antibiotic resistance markers are often plasmid-borne, and plasmids present in *Acinetobacter* strains can be transferred to other pathogenic bacteria

via horizontal gene transfer. The ability of *Acinetobacter* species to adhere to surfaces, to form biofilms, and to display antibiotic resistance and gene transfer motivates research into the factors responsible for their spread.

Since the start of the Iraq War, more than 700 U.S. soldiers have been infected with *A. baumannii*. Four civilians undergoing treatment for serious illnesses at Walter Reed Army Medical Center in Washington, D.C. contracted *A. baumannii* infections and died. At Landstuhl Regional Medical Center, a U.S. military hospital in Germany, another civilian under treatment, a 63-year-old German woman, contracted the same strain of *A. baumannii* infecting troops in the facility and also died. These infections appear to have been hospital-acquired. Based on genotyping of *A. baumannii* cultured from patients prior to the start of the Iraq War, one can presume that it is likely the soldiers contracted the infections while hospitalized for treatment in Europe.

Treatment

Acinetobacter species are innately resistant to many classes of antibiotics, including penicillin, chloramphenicol, and often aminoglycosides. Resistance to fluoroquinolones has been reported during therapy, which has also resulted in increased resistance to other drug classes mediated through active drug efflux. A dramatic increase in antibiotic resistance in *Acinetobacter* strains has been reported by the CDC and the carbapenems are recognised as the gold-standard and treatment of last resort. *Acinetobacter* species are unusual in that they are sensitive to sulbactam; sulbactam is most commonly used to inhibit bacterial beta-lactamase, but this is an example of the antibacterial property of sulbactam itself

\In November, 2004, the CDC reported an increasing number of *A. baumannii* bloodstream infections in patients at military medical facilities in which service members injured in the Iraq/Kuwait region during Operation Iraqi Freedom (OIF) and in Afghanistan during Operation Enduring Freedom (OEF) were treated.^[8] Most of these were multidrug-resistant. Among one set of isolates from Walter Reed Army Medical Center, 13 (35%) were susceptible to imipenem only, and two (4%) were resistant to all drugs tested. One antimicrobial agent, colistin (polymyxin E), has been used to treat infections with multidrug-resistant *A. baumannii*; however, antimicrobial susceptibility testing for colistin was

not performed on isolates described in this report. Because *A. baumannii* can survive on dry surfaces for up to 20 days, they pose a high risk of spread and contamination in hospitals, potentially putting immune-compromised and other patients at risk for drug-resistant infections that are often fatal and, in general, expensive to treat.

Reports suggest that this bacteria is susceptible to phage therapy. Gene-Silencing antisense oligomers in a form called PPMOs (peptide-conjugated phosphorodiamidate morpholino oligomers) have also been reported to inhibit growth in tests carried out in animals infected with antibiotic-resistant *A. baumannii*.

Acinetobacter baumannii has emerged as a major cause of healthcare-associated infections. It commonly presents resistance to multiple antimicrobial agents, occasionally including carbapenems and polymyxins, and hence, it is considered the paradigm of multidrug-resistant (MDR) or pandrug-resistant (PDR) bacterium. MDR *A. baumannii* is a rapidly emerging pathogen, especially in the intensive care setting, causing infections including bacteremia, pneumonia/ventilator-associated pneumonia (VAP), meningitis, urinary tract infection, central venous catheter-related infection, and wound infection. The antibiotics that are usually effective against *A. baumannii* infections include carbapenems, polymyxins E and B, sulbactam, piperacillin/tazobactam, tigecycline and aminoglycosides. Carbapenems (imipenem, meropenem, doripenem) are the mainstay of treatment for *A. baumannii*, though carbapenem-resistant *Acinetobacter* strains have increasingly been reported worldwide in recent years. However, although well-designed trials of new therapeutic approaches are certainly required, the most important factor necessary to guide clinicians in their choice of empirical or targeted therapy should be knowledge of the susceptibility patterns of strains present in their own geographical area.

Natural transformation

Bacterial transformation involves the transfer of DNA from a donor to a recipient bacterium through the intervening liquid medium. Recipient bacteria must first enter a special physiological state termed competence to receive donor DNA (see Natural competence). *Acinetobacter calcoaceticus* is induced to become competent for natural transformation by dilution of a stationary culture into fresh nutrient medium

Competence is gradually lost during prolonged exponential growth and for a period after entrance into the stationary state. The DNA taken up may be used to repair DNA damage or as a means to exchange genetic information by horizontal gene transfer. Natural transformation in *A. calcoaceticus* may protect against exposure to DNA damaging conditions in the natural environment of these bacteria, as appears to be the case for other bacterial species capable of transformation

MECHANISMS OF RESISTANCE

Resistance mechanisms that are expressed frequently in nosocomial strains of acinetobacter include β -lactamases, alterations in cell-wall channels (porins), and efflux pumps (Potential Mechanisms of Antimicrobial Resistance in Acinetobacter.). *A. baumannii* can become resistant to quinolones through mutations in the genes *gyrA* and *parC* and can become resistant to aminoglycosides by expressing aminoglycoside-modifying enzymes.

AmpC β -lactamases are chromosomally encoded cephalosporinases intrinsic to all *A. baumannii*. Usually, such β -lactamases have a low level of expression that does not cause clinically appreciable resistance; however, the addition of a promoter insertion sequence, *ISAbal*, next to the *ampC* gene increases β -lactamase production, causing treatment-limiting resistance to cephalosporins. Although porin channels in *A. baumannii* are poorly characterized, it is known that reduced expression or mutations of bacterial porin proteins can hinder passage of β -lactam antibiotics into the periplasmic space, leading to antibiotic resistance.

Overexpression of bacterial efflux pumps can decrease the concentration of β -lactam antibiotics in the periplasmic space. To cause clinical resistance in acinetobacter, efflux pumps usually act in association with overexpression of *AmpC* β -lactamases or carbapenemases. In addition to removing β -lactam antibiotics, efflux pumps can actively expel quinolones, tetracyclines, chloramphenicol, disinfectants, and tigecycline.

Clinically most troubling have been acinetobacter's acquired β -lactamases, including serine and metallo- β -lactamases, which confer resistance to carbapenems. Acquired extended-spectrum β -lactamase carriage occurs in acinetobacter but is not as widespread as in *Klebsiella pneumoniae* or *Escherichia coli*.

A recent report described a "resistance island" containing 45 resistance genes within the acinetobacter genome. Resistance islands comprise one

or more virulence genes located in a mosaic distribution within a large genomic region.

Currently, the term “multidrug resistance” in reference to acinetobacter does not have a standard definition. It is sometimes used to denote resistance to three or more classes of drugs that would otherwise serve as treatments for acinetobacter infections (e.g., quinolones, cephalosporins, and carbapenems). The term “panresistance” has been used to describe strains of acinetobacter that are resistant to all standard antimicrobial agents tested (except colistin).

Multidrug Resistant *Acinetobacter*

Management of multidrug-resistant *Acinetobacter* spp. infections is a great challenge for physicians and clinical microbiologists. Its ability to survive in a hospital milieu and its ability to persist for extended periods of time on surfaces makes it a frequent cause for healthcare-associated infections and it has led to multiple outbreaks. It causes a wide spectrum of infections that include pneumonia, bacteremia, meningitis, urinary tract infection, and wound infection.

Definitions

Definitions of multidrug-resistant *Acinetobacter* species vary when referring to a wide array of genotypes and phenotypes. Different terms like ‘multidrug resistant (MDR)’, ‘extensive drug resistant (XDR),’ and ‘pandrug resistant (PDR)’ have been used with varied definitions to describe the extent of antimicrobial resistance among *Acinetobacter* spp. However, to date, unlike *Mycobacterium tuberculosis*, internationally, there are no accepted definitions for the extent of resistance in the bacteria. Arbitrarily used terms have thus caused great confusion making it difficult for the available literature to be analyzed

EPIDEMIOLOGY

Historically, acinetobacter has been a pathogen of hot and humid climates, where it has been a major cause of infections, particularly in intensive care units (ICUs), and sometimes a cause of community-acquired pneumonia. Acinetobacter was cited as the cause of 17% of cases of ventilator-associated pneumonias in a Guatemalan ICU — second only to pseudomonas, which caused 19% of cases — years before becoming a concern in ICUs in the United States. Over the past two

decades, acinetobacter infections have become an increasingly common nosocomial problem in temperate climates.

Health Care–Associated Infections

Most information about health care–associated acinetobacter infections is based on outbreak investigations. Infections with *A. baumannii* tend to occur in debilitated patients, mostly in ICUs. Residents of long-term care facilities, particularly facilities caring for ventilator-dependent patients, are at increased risk. In addition to a stay in the ICU, risk factors for colonization and infection are recent surgery, central vascular catheterization, tracheostomy, mechanical ventilation, enteral feedings, and treatment with third-generation cephalosporin, fluoroquinolone, or carbapenem antibiotics.

Acinetobacter outbreaks have been traced to common-source contamination, particularly contaminated respiratory-therapy and ventilator equipment, to cross-infection by the hands of health care workers who have cared for colonized or infected patients or touched contaminated fomites, and to the occasional health care worker who carries an epidemic strain. Once introduced into a hospital, acinetobacter often has an epidemiologic pattern of serial or overlapping outbreaks caused by various multidrug-resistant strains, with subsequent endemicity of multiple strains and a single endemic strain predominating at any one time. Prolonged colonization — for up to 42 months and affecting 17% of patients in one study — may contribute to the endemicity of *A. baumannii* after an outbreak.

Dramatic multihospital outbreaks have been described in Brooklyn, Chicago, northwestern Indiana, Detroit, and cities in Europe, South America, Africa, Asia, and the Middle East. A single-strain outbreak — monoclonal, as identified by molecular typing — of carbapenemase-producing (OXA-40) acinetobacter was described recently in Chicago and neighboring northwestern Indiana. Since 2005, at least five hospitals, three long-term care facilities, and more than 200 patients have been affected by this outbreak. In a French multicity, monoclonal outbreak of multidrug-resistant *A. baumannii*, 290 isolates were collected in 53 hospitals from April 2003 to June 2004. The epidemic strain harbored an extended-spectrum β -lactamase known as VEB-1. Most infected patients were in ICUs, medical wards, or long-term care facilities.

The occurrence of monoclonal outbreaks in multiple hospitals suggests interinstitutional spread, presumably by movement of patients or personnel, or exposure to common-source contamination of food or equipment. Such outbreaks highlight the importance of ongoing

surveillance, interfacility communication, and measures to prevent the introduction of acinetobacter into, and the spread from, nursing homes.

Seasonal Variation

Since 1974, the CDC has noted higher rates of nosocomial acinetobacter infections in the summer than in other seasons.^{30,31} McDonald and colleagues evaluated 3447 acinetobacter infections in adults and children in ICUs that were reported to the CDC between 1987 and 1996; infection rates were approximately 50% higher from July to October than at other times of the year.³¹ Possible explanations include warmer, more humid ambient air, which favors growth of acinetobacter in its natural habitats, and potentially preventable environmental contaminants, such as condensate from air-conditioning units, which has been implicated as a cause of epidemic acinetobacter infections.

Community-Acquired Infections

Community-acquired infections with acinetobacter have been reported in Australia and Asia. These infections were characterized by pharyngeal carriage of the organism, aggressive pneumonia, and high case fatality rates and were linked to alcoholism and cancer. The reason for the higher prevalence of acinetobacter infections in certain geographic areas is not known, but it may be due in part to differences in temperature and humidity that influence colonizing bacteria.

In the United States, community-acquired infections are rare. In 1979, *A. baumannii* pneumonias occurred in three foundry employees who worked within meters of each other. Postmortem evaluations in two of the patients showed severe underlying pneumoconiosis. *A. baumannii* was isolated from foundry air, but the source was not identified.

TREATMENT

Infections caused by antibiotic-susceptible acinetobacter isolates have usually been treated with broad-spectrum cephalosporins, -lactam- -lactamase inhibitor combinations (e.g., a combination that includes sulbactam, a drug marketed only in combination intravenous products in the United States), or carbapenems (e.g., imipenem or meropenem, although there are reports of discordant susceptibility to carbapenems⁵⁴), used alone or in combination with an aminoglycoside. The duration of treatment is generally similar to that for infections caused by other gram-negative bacilli, is largely empirical, and depends mostly on the site of infection.

For infections caused by multidrug-resistant isolates, antibiotic choices may be quite limited; the most active agents in vitro are the polymyxins — polymyxin B and polymyxin E (colistin). Polymyxins are cationic

detergents that disrupt bacterial cytoplasmic membranes, causing leakage of cytoplasmic contents.⁵⁸ Clinicians abandoned polymyxins in the 1960s and 1970s, prompted by problems of nephrotoxicity and neurotoxicity (mostly paresthesias). The emergence of multidrug-resistant gram-negative bacilli has brought polymyxins back into use during the past few years; recent studies show less toxicity, possibly because of lower doses, different drug formulations, and careful ICU monitoring.⁵⁹ Current nephrotoxicity rates range up to 36%, and neurotoxicity is now uncommon. The main side effect of inhaled colistin — used in the past for prevention and more recently for treatment of ventilator-associated pneumonia — is bronchoconstriction. Recently, in vitro studies have suggested colistin heteroresistance in some phenotypically susceptible acinetobacter strains,^{60,61} but the clinical importance of this phenomenon is unknown.

Tigecycline, a new glycylcycline antibiotic, is another drug that has been active in vitro and clinically against some multidrug-resistant strains of *A. baumannii* however, development of resistance to tigecycline has been reported recently. In addition, in some outbreaks of acinetobacter infections, most isolates were not susceptible to tigecycline. Only limited conclusions can be drawn from studies of resistant acinetobacter infections

Examples of Treatment Regimens and Outcomes of Infections Due to Multidrug-Resistant *Acinetobacter baumannii*.). These studies have been mostly retrospective, small case series that often included a mix of patients with infections at different sites, and in some of the studies, combined outcomes were reported for grouped cases of multidrug-resistant bacteria. In many series, intravenous colistin has shown success rates of 50% or more for the treatment of pneumonia, but a success rate of only 25% was reported in one series of 20 cases. Others used inhaled colistin as monotherapy in 17 patients with acinetobacter pneumonia and reported clinical improvement in 57.1%.

Data on the treatment of bloodstream infections are even more limited. During the acinetobacter outbreak in Chicago and northwestern Indiana, 81 bloodstream infections were treated. In two thirds of the cases, only a single blood culture was positive; in 25% of patients, vascular catheters were changed before the first negative culture result was obtained, suggesting aborted catheter-related infections. Active antibiotic therapy was never given in 49% of the cases or was started only after blood cultures became negative in 22% of the cases. These data support the notion that in some cases acinetobacter bacteremia may represent specimen contamination.

Intravenous or intrathecal colistin has been used successfully for the treatment of central nervous system infections caused by acinetobacter. Intravenous administration of the drug results in moderate penetration of inflamed meninges, with cerebrospinal fluid levels that are approximately 25% of serum levels.

When faced with infections due to multidrug-resistant bacteria, clinicians frequently use combinations of antibiotics. In vitro studies have demonstrated either synergy or additive effects when polymyxins were used with imipenem, rifampin, or azithromycin against multidrug-resistant acinetobacter successfully treated ventilator-associated pneumonias or bloodstream infections with the combination of colistin and rifampin. Clinical use of rifampin with imipenem for carbapenem-resistant acinetobacter infections has been less successful

INFECTION CONTROL

The primary goals for the control of multidrug-resistant acinetobacter infection are recognizing its presence in a hospital or long-term care facility at an early stage, controlling spread aggressively, and preventing the establishment of endemic strains. Control measures are based almost entirely on experiences from outbreaks of acinetobacter infection and generally address the organism's major epidemic modes of transmission (Reservoirs, Sources, and Transmission Patterns for Acinetobacter in Health Care Facilities.) and the excessive use of broad-spectrum antibiotics

Control is most successful when a common source is identified and eliminated. A review of hospital outbreaks showed that had a common source: outbreaks with predominantly respiratory tract infections and with predominantly bloodstream or other infections were controlled by removal or disinfection and sterilization of contaminated ventilator (or related) equipment or contaminated moist fomites. In a single-hospital, multi-ICU outbreak of ventilator-associated pneumonia, *A. calcoaceticus* was cultured from 18% of reusable ventilator circuits after pasteurization and from the hands of the four health care workers — one of whom was persistently colonized — who assembled circuits; both disinfection failure and recontamination of circuits by colonized workers during handling probably caused the outbreak. Nevertheless, multidrug-resistant acinetobacter has remained largely susceptible to disinfectants and antiseptics; occasional reports of disinfectant failure are more likely to represent the failure of personnel to follow cleaning procedures than disinfectant resistance.

Aggressive cleaning of the general environment has been the next most frequent outbreak intervention,²² reflecting the concern that

acinetobacter's ability to survive for weeks on wet or dry surfaces facilitates nosocomial transmission.⁹ A review of 1561 hospital epidemics reported over the past 40 years noted that closure, typically for cleaning, was considered necessary for outbreak control in 22.9% of 105 units affected by acinetobacter, as compared with 11.7% affected by other pathogens. An outbreak attributed to dissemination of acinetobacter by high-pressure lavage of wounds demonstrated the effect of extensive environmental contamination on the risk of cross-infection.²⁶ Because multiple measures are usually introduced simultaneously, it has been difficult to assess the independent effect of cleaning. However, in one ICU outbreak, failure to maintain a low level of environmental contamination by *A. baumannii* correlated with an increased risk of patient colonization.

When neither common sources nor environmental reservoirs are identified, control has depended on active surveillance and contact isolation for colonized and infected patients, improvements in the hand hygiene of health care workers (generally the hardest measure to implement), and aseptic care of vascular catheters and endotracheal tubes. A few reports credit outbreak control to reduced prescribing of broad-spectrum antibiotics, such as fluoroquinolones or carbapenems.²² Because antibiotic exposure is often a risk factor for an outbreak, these findings are plausible; however, use of multiple interventions and historical controls complicates interpretation of these studies. Finally, patient decolonization — by skin cleansing with chlorhexidine or the use of polymyxin topically, orally, or by aerosol — has been an occasional adjunctive control measure that warrants evaluation.