**FIG. 221.16 Melioidosis orchitis and scrotal ulcer in a 49-year-old man.**

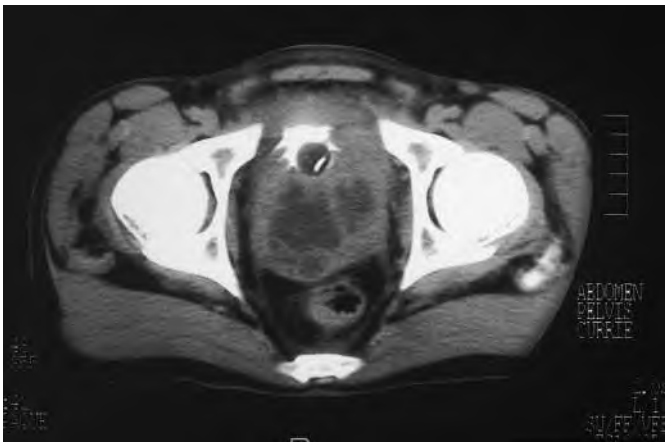

**FIG. 221.17 Melioidosis prostatic abscesses in a 31-year-old man who presented with fever and urinary retention.**

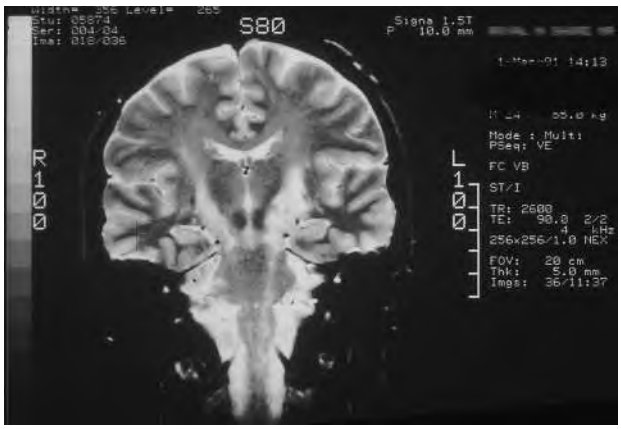

**FIG. 221.18 Melioidosis encephalomyelitis in a 24-year-old man.** Magnetic resonance imaging shows increased T2-weighted signal extending through the brainstem and into the spinal cord.

*pseudomallei* in an endemic region to onset of melioidosis in a nonendemic region was previously documented as being 62 years.[103] Importantly, this case was refuted by recent genotyping, which showed a sequence type not linked to Southeast Asia, the presumptive location of infection in the case report.[104] Cases of reactivated *B. pseudomallei* appear to be very uncommon, accounting for only 4% of cases in northern Australia. The vast majority of cases of melioidosis occur in the monsoonal wet seasons of the various endemic regions, supporting the concept that in endemic areas most patients with melioidosis have recent infections that appear with acute illness. Reactivation of melioidosis has been associated with influenza, other bacterial infections, and development of known melioidosis risk factors such as diabetes. What proportion of asymptomatic seropositive people actually have latent infection with the potential for reactivation is unknown.

## Laboratory Diagnosis

Definitive diagnosis of melioidosis requires a positive culture of *B. pseudomallei*.[105] Melioidosis must be considered in febrile patients in or returning from endemic regions to enable appropriate samples to be tested. *B. pseudomallei* readily grows in commercially available blood culture media, but it is not unusual for laboratories in nonendemic locations to misidentify the bacteria as a *Pseudomonas* spp. or other *Burkholderia* spp., especially because some commercial identification systems are poor at identifying *B. pseudomallei*.[106] Experience is accumulating with identification of *B. pseudomallei* by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems.[107] Culture from nonsterile sites increases the likelihood of diagnosis but can be problematic. The rate of successful culture is increased if sputum, throat swabs, ulcer or skin lesion swabs, and rectal swabs are placed into Ashdown medium, a colistin-containing liquid transport broth that results in the selective growth of *B. pseudomallei,* or plated onto Ashdown agar or a commercial *B. cepacia* medium.[108,109] Addition of ampicillin, norfloxacin, and polymyxin B to Ashdown medium has been studied as a selective medium to enhance recovery of *B. pseudomallei* from heavily contaminated specimens including stool.[110] *B. pseudomallei* can be identified by combining the commercial API 20 NE or 20 E biochemical kit with a simple screening system involving Gram stain, oxidase reaction, typical growth characteristics, and resistance to certain antibiotics.[111]

There are a variety of locally developed antigen and DNA detection techniques used in endemic regions for early identification of *B. pseudomallei* in culture media and patient blood or urine, but these are not yet widely available.[111,112,113,114,115] IHA, various enzyme-linked immunosorbent assays (ELISAs), and other serologic assays are available.[114,116,117] In endemic areas, their usefulness is limited by high rates of background antibody positivity. In acute septicemic melioidosis, IHA and ELISA are often initially negative, but repeat testing may show seroconversion. Positive IHA or ELISA in a tourist returning from a region where melioidosis is endemic is useful in supporting the possibility of melioidosis, but definitive diagnosis still requires a positive culture.

## Therapy

*B. pseudomallei* is characteristically resistant to penicillin, ampicillin, first-generation and second-generation cephalosporins, gentamicin, tobramycin, and streptomycin. Before 1989, conventional therapy for melioidosis consisted of a combination of chloramphenicol, trimethoprim-sulfamethoxazole (TMP-SMZ), doxycycline, and sometimes kanamycin, given for 6 weeks to 6 months.[6,118] However, there were also reports of the successful use of TMP-SMZ alone and tetracycline or doxycycline alone. These conventional antibiotics are bacteriostatic rather than bactericidal, and in vitro studies have shown various combinations to be antagonistic.

Subsequent studies have shown *B. pseudomallei* to be susceptible to various β-lactam antibiotics, especially ceftazidime, imipenem, meropenem, piperacillin, amoxicillin-clavulanate, ceftriaxone, and cefotaxime, with various degrees of bactericidal activity. Table 221.3 summarizes recommended antibiotic treatment[1,119]

### Initial Intensive Therapy

The most important therapeutic study for melioidosis was an open-label randomized trial in Thailand comparing ceftazidime (120 mg/kg/day) with conventional therapy, which showed that ceftazidime is associated with a 50% lower overall mortality in severe melioidosis.[118] Ceftazidime then became the drug of choice for initial intensive therapy for melioidosis. Another study from Thailand showed similar results when ceftazidime was used in combination with TMP-SMZ.[120] Two randomized controlled trials in Thailand studied whether TMP-SMZ

**TABLE 221.3 Antibiotic Therapy for Melioidosis**

**Initial Intensive Therapy (Minimum of 10–14 Days; see Table 221.4)**

Ceftazidime (50 mg/kg, up to 2 g) q6h
*or*
Meropenem (25 mg/kg, up to 1 g) q8h
*or*
Imipenem (25 mg/kg, up to 1 g) q6h
Any one of the three may be combined with TMP-SMZ (6/30 mg/kg, up to 320/1600 mg) q12h (recommended for neurologic, cutaneous, bone, joint, and prostatic melioidosis)

**Eradication Therapy (Minimum of 3 Months; see Table 221.4)**

TMP-SMZ (6/30 mg/kg, up to 320/1600 mg) q12h

*TMP-SMZ,* Trimethoprim-sulfamethoxazole.

**TABLE 221.4 Duration of Antibiotic Therapy for Melioidosis**

| ANTIBIOTIC DURATION–DETERMINING FOCUS | | MINIMUM INTENSIVE PHASE DURATION (WEEKS)[a] | ERADICATION PHASE DURATION (MONTHS) |
|---|---|---|---|
| Skin abscess | | 2 | 3 |
| Bacteremia with no focus | | 2 | 3 |
| Pneumonia | Without lymphadenopathy[b] or ICU admission | 2 | 3 |
| | With either lymphadenopathy[b] or ICU admission | 4 | 3 |
| Deep-seated collection[c] | | 4[d] | 3 |
| Osteomyelitis | | 6 | 6 |
| CNS infection | | 8 | 6 |
| Arterial infection[e] | | 8[d] | 6 |

[a]Clinical judgment to guide prolongation of intensive phase if improvement is slow or if blood cultures remain positive at 7 days.
[b]Defined as enlargement of any hilar or mediastinal lymph node to greater than 10-mm diameter.
[c]Defined as abscess anywhere other than skin, lungs, bone, CNS, or vasculature; septic arthritis is considered a deep-seated collection.
[d]Intensive phase duration is timed from date of most recent drainage or resection where culture of the drainage specimen or resected material grew *Burkholderia pseudomallei* or where no specimen was sent for culture; clock is not reset if specimen is culture-negative.
[e]Most commonly manifesting as mycotic aneurysm.
*CNS,* Central nervous system; *ICU,* intensive care unit.

added to ceftazidime is superior to ceftazidime alone.[121] Although the addition of TMP-SMZ conferred no survival benefit, the excellent tissue penetration of TMP-SMZ is the rationale for recommending combination therapy in neurologic, cutaneous, bone and joint, and prostatic melioidosis.

After initial favorable reports of use of amoxicillin-clavulanate, another randomized comparative trial in Thailand showed that initial therapy with high-dose intravenous amoxicillin-clavulanate is as effective as ceftazidime in preventing deaths in patients with severe melioidosis.[122] However, when amoxicillin-clavulanate was continued as eradication therapy (see "Subsequent Eradication Therapy"), treatment failure was more frequent.

The carbapenems imipenem and meropenem have the lowest minimal inhibitory concentrations against *B. pseudomallei.* Furthermore, in vitro time-kill studies to measure the rate of bacterial killing showed the carbapenems to perform better against *B. pseudomallei* than ceftazidime.[123,124] High-dose imipenem was shown in another comparative trial from Thailand to be at least as effective as ceftazidime for severe melioidosis, with no differences in mortality between the groups and with fewer treatment failures in patients given imipenem.[125] Observational data from Australia suggested that meropenem produces better outcomes in severe melioidosis than ceftazidime, which led to the recommendation that meropenem be the drug of choice for melioidosis septic shock.[126,127]

The duration of initial intensive therapy should be at least 10 to 14 days, with longer treatment required for critically ill patients or for patients with extensive pulmonary disease, deep-seated collections or organ abscesses, osteomyelitis, septic arthritis, and neurologic melioidosis. Even with the newer regimens, the therapeutic response can be slow, with median time to defervescence up to 9 days and longer times seen in patients with deep-seated abscesses.

A more recent analysis of outcomes of therapy based on duration of the intravenous phase of therapy supports recommending a longer minimum intensive phase duration for many cases of melioidosis.[128] The current Australian recommendations for the duration of initial intensive intravenous therapy are listed in Table 221.4.

Ceftazidime infusions (6 g over 24 hours, adult dose) through a peripherally inserted central catheter (PICC line) using an elastomeric infusion device (Baxter, Sydney, Australia) have enabled early hospital discharge for in-home therapy.[129] The absence of any postantibiotic effect with ceftazidime gives such a continuous infusion a theoretical advantage over intermittent dosing.

## Subsequent Eradication Therapy

After initial intensive therapy using ceftazidime, imipenem, or meropenem, possibly in combination with TMP-SMZ, subsequent eradication therapy is considered necessary for preventing recrudescence or later relapse of melioidosis. Earlier molecular typing of isolates from patients with recurrent melioidosis showed that most cases were true relapses from failed eradication rather than new infection.[83] However, a more recent study from northern Australia has documented that over the past decade, recurrent melioidosis has become very uncommon, and molecular typing of isolates from recurrent melioidosis shows a reversal of attribution for recurrent melioidosis from predominantly relapse to predominantly reinfection. The decrease in relapse cases was largely attributed to longer duration of the initial intravenous therapy for many of the patients with more severe disease.[130]

Reasons for failure of eradication therapy include the following:

1. One important factor responsible for recrudescences or relapses of melioidosis is poor compliance with eradication therapy.
2. Relapses were found to be 4.7 times (95% CI, 1.6–14.1) more common in patients with severe disease than in patients with localized melioidosis.[131] This emphasizes the importance of a sufficiently long duration of preceding intravenous intensive therapy. Positive blood cultures and multifocal disease were also associated with relapse.[83]
3. Use of ceftazidime in initial intensive therapy was also associated with a halving of relapses.[131]
4. Duration of eradication therapy is also crucial; relapses after oral therapy of 8 weeks or less are more likely than if eradication therapy is given for longer than 12 weeks.[83,131]
5. The choice of agents for eradication therapy is important. Both amoxicillin-clavulanate and oral quinolones (ciprofloxacin or levofloxacin) have been found to be less effective in preventing relapse than previous conventional eradication therapy with chloramphenicol (given usually only for the first 4–8 weeks), TMP-SMZ, and doxycycline.[83] Amoxicillin-clavulanate is recommended for eradication in patients in the first trimester of pregnancy, patients intolerant of TMP-SMZ, or patients with a *B. pseudomallei* isolate confirmed as resistant to TMP-SMZ, with dosing guidelines published.[132] Quinolones should not be considered as first-line agents for melioidosis, with in vitro susceptibility testing generally showing resistance or intermediate results. A trial of eradication therapy involved a comparison of doxycycline alone versus conventional chloramphenicol (first 4 weeks only), TMP-SMZ, and doxycycline combination therapy.[133] Relapses were significantly more common in the doxycycline alone group, resulting in a recommendation that doxycycline not be used alone as first-line eradication therapy.

A randomized trial found no benefit in adding chloramphenicol to TMP-SMZ plus doxycycline for the eradication phase.[134] It has been suggested that TMP-SMZ is the critical component for eradication

therapy, and prospective studies in Australia using TMP-SMZ alone have supported this because relapses occurred almost exclusively in noncompliant patients. A randomized, multicenter, double-blind trial in Thailand confirmed that it is not beneficial to add doxycycline to TMP-SMZ and that TMP-SMZ alone is generally the eradication therapy of choice for melioidosis.[135] Interpretation of disk diffusion sensitivity testing has been problematic for TMP-SMZ, and agar dilution methods have confirmed that the vast majority of *B. pseudomallei* isolates are sensitive to TMP-SMZ. Studies from Thailand, Laos, and Cambodia have confirmed very low rates of resistance to TMP-SMZ, reversing prior perceptions of higher resistance rates in Southeast Asia than in Australia.[136,137] Therefore eradication therapy with TMP-SMZ alone can now be considered the global recommendation. Current Australian recommendations for the duration of oral eradication therapy are listed in Table 221.4.[128]

### Adjunctive Therapy

Surgical drainage of large abscesses is indicated, but this is usually not necessary or possible for multiple small abscesses in the spleen and liver. Parotid abscesses require careful incision and drainage. Prostatic abscesses can often be drained under ultrasound guidance using a rectal probe, with transurethral resection reserved for failures of the simpler procedure.

State-of-the-art intensive care management has resulted in decreased mortality in patients with melioidosis septic shock. The possible primary role of neutrophil function in containing *B. pseudomallei* has led to empirical use of granulocyte colony-stimulating factor (G-CSF) in patients with strictly defined septic shock, with observational data from Australia showing a significant improvement in survival with G-CSF.[138] Nevertheless, a randomized controlled trial in Thailand has shown no survival benefit of G-CSF in that location.[139]

### Prevention

Primary prevention involves education in endemic areas about minimizing exposure to wet season soils, surface water, and potential aerosols during windy monsoonal rains, especially for patients with diabetes and patients on immunosuppressive therapy, most importantly high-dose or prolonged corticosteroids. Footwear and gloves while gardening are recommended in northern Australia, but preventing occupational exposure in rice farmers may be unrealistic in Southeast Asia. Patients with cystic fibrosis should consider avoiding travel to high-risk areas.

Laboratory-acquired infections, person-to-person spread, and zoonotic infection all are very uncommon, but secondary prophylaxis with TMP-SMZ, doxycycline, or amoxicillin-clavulanate could be considered for exceptional circumstances, especially if the exposed person is diabetic or has other risk factors for melioidosis. Guidelines for management of accidental laboratory exposure have been published.[119,140] Isolation of patients is recommended only for patients with severe suppurative pneumonia with productive sputum.

Concerns of possible bioterrorism using the bacterium or its virulence components in genetically engineered constructs and of exposure of military personnel to *B. pseudomallei* have driven funding for research. Development of a melioidosis vaccine could also have substantial benefits for people living in endemic regions and for commercial livestock, although cost will be a major impediment to availability. Preliminary studies have included various conjugate, live-attenuated, and heterologous vaccine candidates.[1,141,142]

## GLANDERS

Glanders is a highly communicable disease of solipeds (horses, donkeys, and mules) that is caused by *B. mallei*. It can be transmitted to other animals and to humans.

### History

Glanders was described by Hippocrates and has long been recognized as an occupational risk for horse handlers, veterinarians, equine butchers, and laboratory workers. Together with anthrax, glanders was involved in the first modern use of microbes as weapons when German agents targeted horses in the United States, Romania, Spain, Norway, and Argentina between 1915 and 1918.[143]

### Etiology

*B. mallei* is a small, gram-negative, oxidase-positive, aerobic bacillus. In contrast to *B. pseudomallei,* it is nonmotile. It is a host-adapted pathogen that, in contrast to *B. pseudomallei,* does not persist in the environment outside its equine host. The *Burkholderia* genome projects and multilocus sequence typing have supported the idea that *B. mallei* evolved in animals from the environmental pathogen *B. pseudomallei.*[144,145]

### Epidemiology, Transmission, and Pathogenesis

With quarantine and other control measures, glanders has been eradicated from most countries. Enzootic foci continue in the Middle East, Asia, Africa, and South and Central America, with increasing outbreaks in some locations over the last decade. In addition to disease in equines, glanders has occurred in cats and other carnivores eating infected horse meat. Inhalation and percutaneous inoculation also occur. Discharges from the horse respiratory tract and skin are highly infectious. *B. mallei* has much greater potential for zoonotic transmission than *B. pseudomallei,* and the risk of laboratory-acquired infection also appears to be greater for *B. mallei.*[146,147] The incubation period can range from 1 to 2 days (e.g., with inhalation) to many months, and as occurs with melioidosis, reactivation from a latent focus after many years has been described.

There are many parallels with the pathogenesis of *B. pseudomallei,* with studies showing the *B. mallei* extracellular polysaccharide capsule to be a critical determinant of virulence.[70,148,149] There is differential susceptibility among animals, and although it is likely that diabetic patients are more susceptible to infection and disease progression with *B. mallei,*[147] the human host risk factors are less well defined than for melioidosis.

### Clinical Manifestations

Knowledge of the disease in horses is useful for understanding the potential for zoonotic transmission to humans. In acute glanders in horses, fever is accompanied by necrotic ulcers and nodules in the nasal passages that result in copious, infectious, sticky yellow discharges. Neck and mediastinal lymph nodes are enlarged, and pneumonia with nodular abscesses and dissemination to internal organs can accompany the progressive deterioration. In cutaneous glanders (known as farcy), nodular lymphatic or skin abscesses (0.5–2.5 cm) occur and ulcerate, discharging infectious, oily yellow pus.[150]

Human glanders, similar to melioidosis, can be acute or chronic, with mode of infection, inoculating dose, and host risk factors determining the clinical course. With inhaled organisms (respiratory inoculation), acute febrile illness with ulcerative necrosis of the tracheobronchial tree can occur, with mucopurulent discharge involving the nose, lips, and eyes. Lobar or bronchial pneumonia, neck and mediastinal lymphadenopathy, pustular skin lesions, and septicemia with dissemination to internal organs can follow.[151] Historically, without antibiotics, death within 10 days usually occurred, but a more chronic pneumonic illness was also recognized after inhalation of *B. mallei.*[146]

After percutaneous inoculation, local skin nodules that can suppurate and regional lymphadenopathy occur, often accompanied by fever, rigors, and malaise.[147,151] Regional lymphadenopathy is much more common than with melioidosis. Lymphatic tract nodules and suppurating abscesses in the lymph nodes are common after several weeks in untreated cases. Dissemination at 1 to 4 weeks can result in infection in almost any tissue, with spleen and liver abscesses, pneumonia, lung abscesses, pleural nodules, and multiple subcutaneous and muscle abscesses all being common (Fig. 221.19). Central nervous system infection can also occur.

### Laboratory Diagnosis

Definitive diagnosis of glanders requires a positive culture of *B. mallei.* Blood, exudates, and pus from abscesses should be cultured on standard media. Organisms are often very scanty in exudates and pus and are morphologically indistinguishable from *B. pseudomallei* organisms. As occurs with *B. pseudomallei,* some commercial identification systems may misidentify *B. mallei* as a *Pseudomonas* spp., and 16S ribosomal RNA gene–sequenced analysis or a *B. mallei*–specific polymerase chain reaction assay may be required for confirmation.[147] Currently available complement fixation tests and ELISA serologic assays cannot distinguish

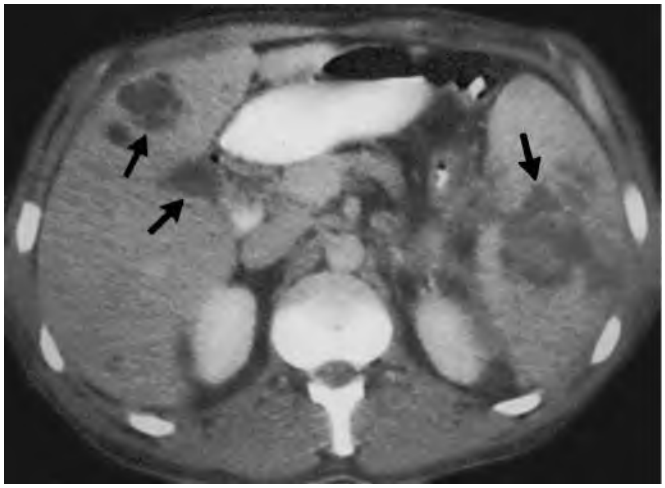

**FIG. 221.19 Computed tomography scan of a patient with laboratory-acquired glanders showing abscesses *(arrows)* in the liver and spleen.** *(From Srinivasan A, Kraus CN, DeShazer D, et al. Glanders in a military research microbiologist.* N Engl J Med. *2001;345:256–258.)*

*B. mallei* from *B. pseudomallei,* but new assays based on recombinant proteins show promise in distinguishing the two.[152] The mallein skin test has been used extensively in animal control programs and has been modified for human diagnosis but has poor specificity.[146]

## Therapy

The antibiotic susceptibility profile of *B. mallei* resembles that of *B. pseudomallei* except that gentamicin and newer macrolides (e.g., clarithromycin, azithromycin) are active against *B. mallei,* but not *B. pseudomallei.*[153] Although response to treatment with older regimens was often slow, rapid improvement occurred in a US military researcher with laboratory-acquired infection who was treated with imipenem and doxycycline.[147] This was the first reported case of glanders in the United States in more than 50 years. Recommended treatment and duration are the same as for melioidosis.[119]

## Prevention

Prevention depends on control of glanders in the equine species and strict precautions to prevent laboratory-acquired infection.[146,147] In contrast to the case with melioidosis, isolation of all infected persons is recommended to prevent person-to-person spread. Guidelines for the management of accidental laboratory exposure have been published.[119,140] As with melioidosis, much research is being done toward a vaccine to prevent disease in humans.[154,155]

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*The complete reference list is available online at Expert Consult.*

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# 222 *Acinetobacter* Species

Sarah Hochman and Michael Phillips

## SHORT VIEW SUMMARY

### Definition and Epidemiology

- *Acinetobacter* species are ubiquitous in soil and water, and are increasingly recognized to infect animals and ectoparasites, raising potential new sources of human exposure.
- *Acinetobacter baumannii* and the closely related and phenotypically indistinguishable *Acinetobacter pittii* and *Acinetobacter nosocomialis* cause the bulk of human infections and typically are acquired in the health care setting.
- *Acinetobacter* species readily incorporate multiple resistance mechanisms, and pan-resistant strains have established within the health care setting.

### Therapy

- Treatment is based on susceptibility testing.
- Bacteriophages show promise as a novel form of therapy.

### Prevention

- Prevention of *Acinetobacter* transmission in health care settings requires a multifactorial approach with environmental disinfection and hand hygiene as the cornerstone.
- The emergence of *A. baumannii* strains resistant to essentially all potent antimicrobial agents, coupled with the dearth of antibiotics in development, constitutes a significant public health threat.
- Vaccines, immunotherapy, or both will be important measures in the era of pan-resistant *Acinetobacter* strains.

*Acinetobacter,* an aerobic, catalase-positive, oxidase-negative, gram-negative coccobacillus, was first described in 1911, but the initial description of the taxonomy of this diverse species was not published until 1986.[1,2] Ubiquitous in nature, the 54 species of the genus *Acinetobacter* are associated with a specific ecologic niche that shapes their genomic contents.[3–6] (Table 222.1) *Acinetobacter baumannii* is the most virulent species and causes the bulk of human infections, but *Acinetobacter pittii*, *Acinetobacter nosocomialis, Acinetobacter lwoffii,* and *Acinetobacter radioresistens* are also significant nosocomial pathogens.[5] In the late 1980s, *A. baumannii* emerged as an important human pathogen exhibiting increased antimicrobial resistance.[7,8] Whole-genome sequencing analysis has suggested that the rapid spread of multidrug-resistant *A. baumannii* was associated with the ability to incorporate virulence and resistance determinants[9] (Fig. 222.1). The establishment of multidrug-resistant *A. baumannii* within the health care ecosystem has a tremendous cost, both financially and on patient safety. Among the gram-negative pathogens causing bacteremia, *A. baumanii* exhibits the highest rate of nonsusceptibility, with over 18% of isolates resistant to all first-line agents, including the carbapenems, β-lactams, and fluoroquinolones.[10] In the United States, over 12,000 *Acinetobacter* infections are estimated to occur annually, causing over 1300 deaths and costing society $1.6 billion dollars.[11] When compared to other multidrug-resistant gram-negative bacteria and methicillin-resistant *Staphylococcus aureus*, *Acinetobacter* infections had the highest risk for mortality at 30 and 90 days after isolation from culture.[12] These statistics, coupled with a paucity of potent antimicrobials in phase II or III of development, emphasize the importance of infection prevention efforts and the need to develop novel therapeutics and vaccines.

## EPIDEMIOLOGY

### Health Care–Associated Infections

Health care–associated infections represent the most substantial public health impact of *Acinetobacter,* given the rapid spread of strains resistant to all first-line antimicrobials. The application of molecular typing methods has revealed that a limited number of widespread clonal lineages of *A. baumannii* are responsible for hospital outbreaks worldwide.[13–15] Although increasing globally, the prevalence of carbapenem-resistant *A. baumannii* is decreasing over time in developed countries, suggesting that patients presenting for care after travel from areas with higher endemic rates should be considered for screening.[16–23] *A. baumannii* causes the bulk of health care–associated infections due to *Acinetobacter,* but a variety of species, including *A. pittii, A. nosocomialis, A. lwoffii,* and *Acinetobacter ursingii,* are emerging as nosocomial pathogens, especially in immunocompromised hosts.[3,5,24] Ventilator-associated pneumonia is the most frequent health care–associated *A. baumannii* infection, implicated in 3% to 7% of cases.[25,26] Among patients requiring mechanical ventilation for more than 5 days, the frequency of *Acinetobacter* increases dramatically, accounting for 26% of respiratory infections in one series.[27] Other nosocomial manifestations of *Acinetobacter* include bloodstream infections associated with intravascular catheters, surgical site infections, urinary tract infections, meningitis after neurosurgery, and soft tissue infections after burns.[5,25,28–30]

The factors that promote the emergence and transmission of *A. baumannii* in health care settings include hospitalization of patients at high risk for colonization, such as long-term care residents; breaches in environmental cleaning and disinfection; and antibiotic utilization, especially third-generation cephalosporins, fluoroquinolones, or carbapenems.[8,31–36] The ability of *Acinetobacter* species to survive for weeks on surfaces within the hospital environment leads to prolonged outbreaks, and patient movement between health care facilities without the intervention of adequate communication results in regional spread.[37–39] Essentially any surface within a patient care area can become contaminated with *Acinetobacter* and serve as a reservoir for ongoing transmission; these include sinks, faucets, humidifiers, hydrotherapy pools, curtains, pillows, and bedrails, as well as equipment such as supply carts, infusion pumps, and equipment control touch pads.[40–45] Patients with either recent or remote history of infection can remain colonized and able to contaminate their surrounding environment.[40]

Transmission of *Acinetobacter* within the health care setting occurs after lapses in proper hand hygiene, and failure to disinfect mobile medical equipment and surfaces within patient care areas.[46–48] Units with multiple-bedded rooms and susceptible patients, such as neonatal intensive care units (ICUs), are at high risk for outbreaks.[49–52] Procedures that result in a spray of contaminated fluids, such as pulsatile lavage of wounds or bronchoscopy, may also lead to heavy environmental contamination and transmission.[53,54] In addition to contaminated surfaces,

**TABLE 222.1 Named *Acinetobacter* Species**

| SPECIES | TYPICAL HABITAT |
|---|---|
| *A. albensis* | Water, soil |
| *A. antitratus* | Animals |
| *A. antiviralis* | Plants |
| *A. baumannii* | Water, soil, humans, animals, food |
| *A. baylyi* | Water |
| *A. bereziniae* | Soil, food |
| *A. bohemicus* | Water, soil |
| *A. bouvetii* | Water |
| *A. brisouii* | Soil |
| *A. calcoaceticus* | Water, soil, humans, animals, food |
| *A. calcoaceticus–A. baumannii* complex | Water, soil |
| *A. gerneri* | Water |
| *A. grimontii* | Water |
| *A. guangdongensis* | Soil |
| *A. guillouiae* | Food |
| *A. gyllenbergii* | Food |
| *A. haemolyticus* | Soil, humans |
| *A. indicus* | Soil |
| *A. johnsonii* | Water, soil, humans, animals, food |
| *A. junii* | Water, soil, humans |
| *A. kookii* | Soil |
| *A. kyongiensis* | Water |
| *A. lwoffii* | Water, soil, humans, animals, food |
| *A. nosocomialis* | Soil, food |
| *A. oleivorans* | Plants, soil |
| *A. pakistanensis* | Water |
| *A. parvus* | Soil, food |
| *A. pittii* | Soil, food |
| *A. populi* | Plants |
| *A. puyangensis* | Plants |
| *A. qingfengensis* | Plants |
| *A. radioresistens* | Soil, animals, food |
| *A. rudis* | Water |
| *A. schindleri* | Animals, soil |
| *A. seifertii* | Food |
| *A. seohaensis* | Water |
| *A. soli* | Soil, food |
| *A. tandoii* | Water, soil |
| *A. tjernbergiae* | Water |
| *A. towneri* | Water |
| *A. ursingii* | Humans, food |
| *A. venetianus* | Water |

*Modified from Adewoyin MA, Okoh AI. The natural environment as a reservoir of pathogenic and non-pathogenic* Acinetobacter *species.* Rev Environ Health. *2018;33:265–272.*

airborne particles are believed to play a role in transmission of *Acinetobacter,* either by spread through open units with multiple beds or through contamination of internal air filters of medical equipment.[55–57] An increase in health care–associated *Acinetobacter* infections during warmer, more humid months has been reported, potentially due to contamination of air handling systems.[58,59]

The integration of whole-genome sequencing with epidemiologic data such as patient movement and health care environment exposures is needed to determine transmission routes in an outbreak, and should become the standard.[60] In order to appropriately utilize whole-genome sequencing in an outbreak, an understanding of the endemic strains within a facility is needed.[61]

### Community-Associated Infections

*Acinetobacter* pneumonia is a rare but serious cause of community-acquired pneumonia in tropical regions during the summer months, and often presents with respiratory failure and shock.[62–66] Community-onset bacteremia is typically associated with respiratory infections, and is also associated with a worse outcome compared with hospital-onset infections.[67] Community-acquired *Acinetobacter* meningitis in patients without underlying medical compromise has been rarely reported; outcomes in those who received prompt therapy were favorable.[68,69] *A. baumannii* skin colonization and invasive soft tissue infections have been associated with warfare, natural disasters, and societal disruptions.[70–73] The environmental source of these community infections, which typically present in tropical or warm regions, is unknown. In addition to soil, there is an increasing appreciation for the potential role of contaminated food, infected head and body lice, colonized pets or other animals, and hospital wastewater as environmental reservoirs of *Acinetobacter.*[14,74–87]

## DIAGNOSIS

*Acinetobacter* is readily isolated with standard culture media, but differentiation of species based on phenotype alone is difficult, leading to the term *Acinetobacter calcoaceticus–Acinetobacter baumannii complex.*[88] The use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry allows rapid identification of *Acinetobacter* species and of the presence of resistance mechanisms. Once species are identified, a rapidly expanding number of polymerase chain reaction assays are commercially available to identify the presence of β-lactamase and carbapenemase genes.[89,90] The use of colorimetric assays and quantitative real-time polymerase chain reaction has also been used to detect antimicrobial resistance in *A. baumannii.*[91–93] The presence of heteroresistance to carbapenems has been identified in *A. baumannii,* raising the potential for breakthrough or relapsed infection.[94,95]

## CLINICAL MANIFESTATIONS

*Acinetobacter* is a leading cause of ventilator-associated pneumonia, with increased mortality rates seen in infections caused by carbapenem-resistant strains and patients receiving initial inappropriate therapy.[96,97] When *Acinetobacter* is isolated from a pulmonary specimen, differentiation between colonization and pneumonia is critically important to avoid unnecessary antibiotic treatments with the attendant risk of emergence of toxicity as well as antimicrobial resistance.[98] The measurement of volatile organic compounds may prove to be a useful diagnostic tool and allow for differentiation between colonization and invasive infection, although this method remains in the early stages of investigation.[99] *A. baumannii* was independently associated with increased mortality compared to *A. nosocomialis* in patients with health care–associated pneumonia complicated by bacteremia, in spite of their close genetic relatedness.[100]

*Acinetobacter* species account for 1% to 2% of all bloodstream infections and are typically associated with intravascular devices or pneumonia, with the majority caused by *A. baumannii,* followed by *A. nosocomialis* and *A. pittii.*[101,102] The mortality associated with *A. baumannii* bacteremia is the highest, followed by *A. nosocomialis* and *A. pittii* bacteremia; other species such as *A. lwoffii* and *Acinetobacter junii* have low bacteremia-associated mortality.[101,103–106]

*Acinetobacter* causes about 1% of urinary tract infections, most of which are caused by strains with the ability to form biofilms on urinary catheters.[25,107] Outbreaks of *A. baumannii* meningitis in postsurgical neurosurgery patients secondary to breaches in infection prevention measures have been reported.[28,108,109] Community-associated *Acinetobacter* meningitis in immunocompetent patients without surgical procedures have rarely been reported.[68,69] *A. baumannii* skin and soft tissue infections after burns and natural and wartime traumatic injuries occur and can be related to exposure at the time of injury or to exposure after

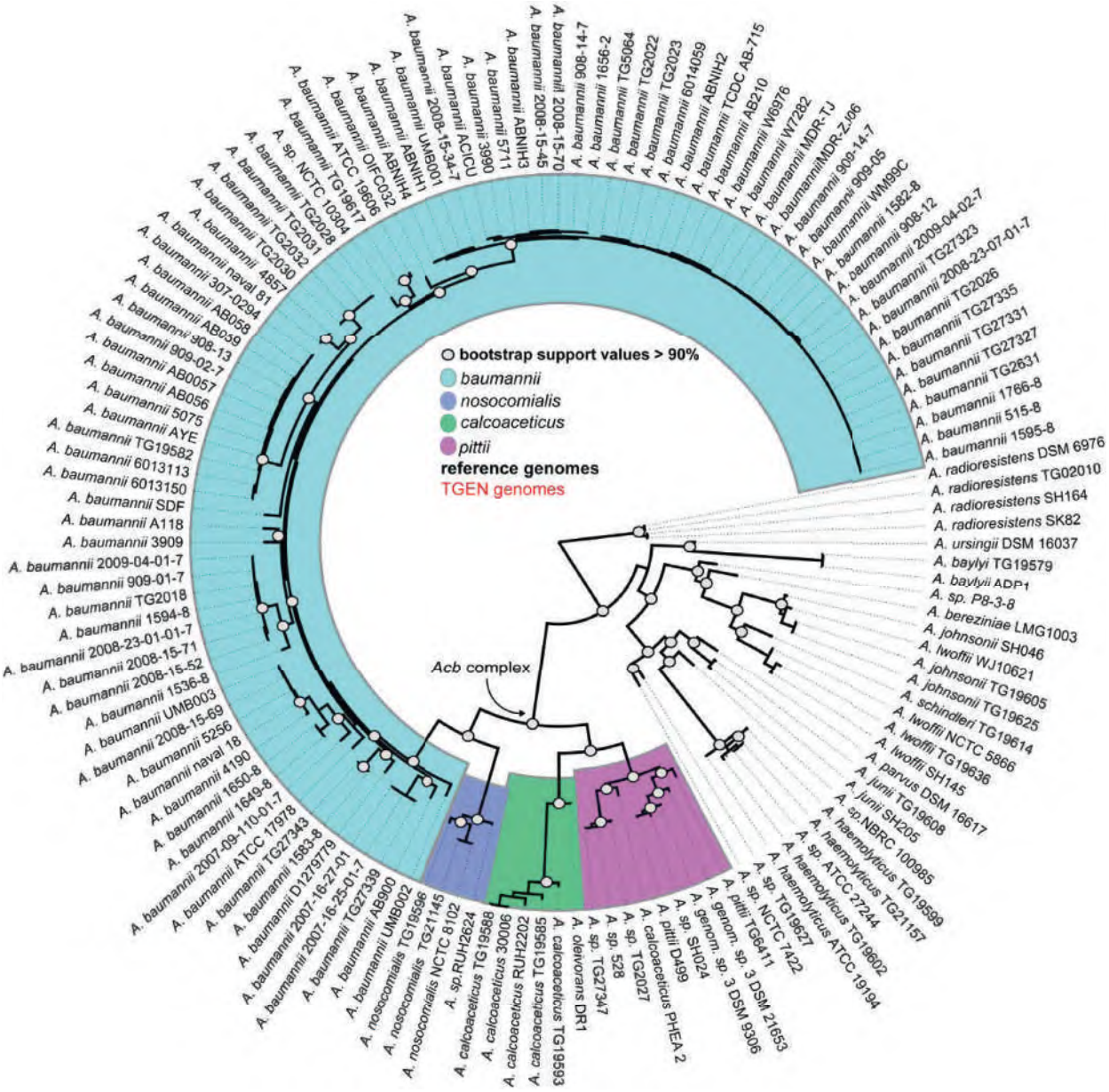


**FIG. 222.1 Whole-genome phylogeny of 136 sequenced genomes in the genus *Acinetobacter.*** The phylogeny was inferred with FastTree2 on a single nucleotide polymorphism (SNP) matrix alignment calculated with kSNP and filtered with noisy. The phylogeny was rooted with *A. radioresistens.* Genomes in the *Acinetobacter calcoaceticus-baumannii* (Acb) complex are colored by clade. *(From Sahl JW, Gillece JD, Schupp JM, et al. Evolution of a pathogen: a comparative genomics analysis identifies a genetic pathway to pathogenesis in* Acinetobacter. PLoS One. *2013;8:e54287.)*

hospitalization.[29,70,71,110,111] High rates of asymptomatic nasal colonization have been identified in long-term care facilities, among elderly residents and the health care workers who care for them.[112] Cocolonization with *Neisseria* species may result in prolonged colonization with *A. baumannii,* suggesting that microbial interactions may play a role in transmission.[113]

## PATHOGENESIS AND ANTIMICROBIAL RESISTANCE

The pathogenicity of *A. baumannii* relates to its ability to colonize and form biofilm on mucosal surfaces and medical devices, to survive in iron-limited environments within the host, and to acquire foreign genetic material to enhance survival and increase resistance to antimicrobial agents.[114–116] An essential precursor to biofilm formation is the generation of pili on the cell surface, leading to adhesion.[117] The production of *Acinetobacter* biofilm is controlled by environmental conditions, and a favorable milieu results in the overexpression of more than 1600 genes compared to 55 in planktonic bacteria, leading to important changes in cell metabolism, motility, iron acquisition, and quorum sensing.[118] Specific genes regulating the virulence of the bacteria, such as those that regulate creation of pili, motility, and formation of biofilm, have been described.[117,119,120] Further assessment of clinical *A. baumannii* isolates reveals a correlation between antibiotic-resistant phenotypes and the ability to form biofilms, and specific strains are associated with increased mortality.[121–123] Antibiotic exposure plays an important role in occurrence of antibiotic-resistant *A. baumannii,* especially exposure to carbapenems.[124] The spontaneous mutation rates of strains of antibiotic-resistant *A. baumannii* vary widely, but rare isolates with thousandfold higher mutation rates raise concern for heteroresistance and treatment failure.[125] Neutrophils, recruited by natural killer cells, play the predominant role in host immune response to *Acinetobacter* infection.[126,127] Host factors, such as serum albumin and vitamin D deficiency, may play a role in patient mortality.[128,129]

*Acinetobacter* is well known for its multitude of antimicrobial resistance mechanisms, which are associated with an increase in genome size[9] (Fig. 222.2). Genomic analysis has shown that pathogenic strains of *A. baumannii* contain genes clustered on resistance islands, whose

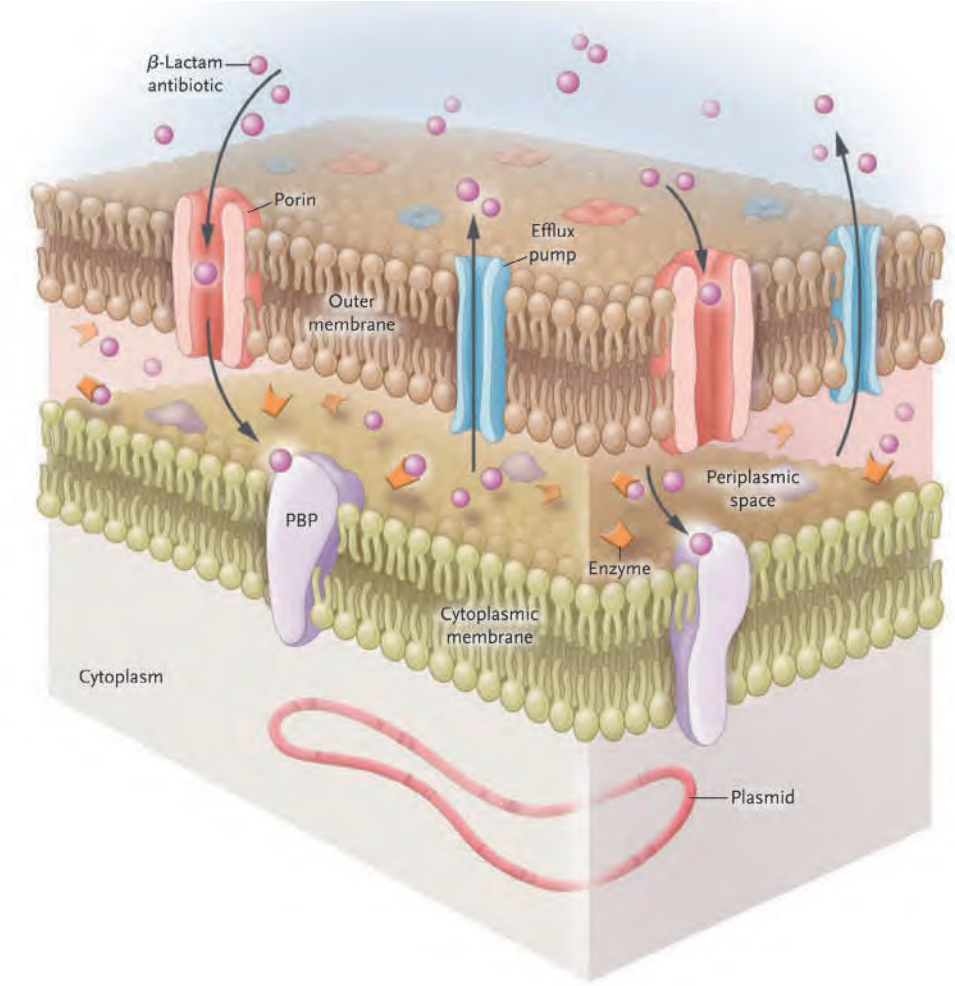


**FIG. 222.2** ***Acinetobacter*** **resistance mechanisms.** *Acinetobacter,* like other gram-negative bacteria, has an outer membrane and a cytoplasmic membrane, between which (the periplasmic space) β-lactamases (carbapenemases, Ambler class C β-lactamases, and extended-spectrum β-lactamases) reside. Penicillin-binding proteins (*PBPs*), located at the level of the cytoplasmic membrane, constitute the final targets of β-lactam antibiotics. To bind to these targets, antibiotics must traverse the outer membrane through porin channels (outer membrane proteins) into the periplasmic space. Once in the periplasmic space, β-lactam antibiotics bind to PBPs or are actively expelled from the bacterial structure through efflux pumps. *Acinetobacter* can harbor integrons and transposons, genetic elements on the bacterial chromosome or on plasmids, that can carry multiple cassettes with resistant genes (e.g., extended-spectrum β-lactamases and metallo-β-lactamases). *(From Munoz-Price LS, Weinstein RA.* Acinetobacter *infection.* N Engl J Med. *2008;358:1271-1281.)*

structure may facilitate the acquisition of resistance mechanisms from other species of bacteria.[130,131] Insertion sequences such as IS*Aba1* within the *Acinetobacter* genome promote the expression of neighboring genes and result in the overexpression of several key resistance mechanisms. Additionally, it has been postulated that the ability of *Acinetobacter* to acquire resistance determinants more effectively than other bacteria may be due to the close association of several *Acinetobacter* species to the soil and water environment, which contains a large reservoir of resistance genes.[132]

*Acinetobacter* exerts much of its antibiotic resistance through the expression of β-lactamases. Group 1 AmpC β-lactamases are chromosomally encoded cephalosporinases that hydrolyze penicillins and first-, second-, and third-generation cephalosporins, including ceftazidime, cefotaxime, and ceftriaxone. Rates of hydrolysis of fourth-generation cephalosporins, such as cefepime, and carbapenems by AmpC enzymes are low.[133]

AmpC is not inducible in *A. baumannii* as it is in some Enterobacteriaceae, but the presence of the promoter sequence IS*Aba1* increases expression of this enzyme.[134,135] Groups 2b and 2c Ambler class A β-lactamases are encoded on genes carried by large plasmids, and confer resistance to penicillins and narrow-spectrum cephalosporins.[136,137] The emergence and rapid worldwide spread of strains containing the group 2be Ambler class A extended-spectrum β-lactamases (ESBLs) in the 1980s represented the first, rapid global spread of multidrug-resistant *A. baumannii.* These ESBLs exhibit hydrolytic activity over penicillins and all cephalosporins, thereby leading to the use of carbapenems as treatment for significant *Acinetobacter* infection; this resulted in the subsequent emergence of the group 2d Ambler class D oxacillinases. This class of oxacillinases confers resistance to carbapenems, and has resulted in a marked increase in carbapenem resistance due to the widespread distribution of the successfully spreading international clonal complexes (ICCs), ICC-I, ICC-II, and ICC-III.[138–142] The episodic emergence of clades distinct from the three ICCs associated with high virulence represents an additional threat to public health.[143] Insertion sequence IS*Aba1* is a promoter for genes encoding oxacillinases in *Acinetobacter.*[144,145] The oxacillinase bla (OXA-51-like) is intrinsic and chromosomal, while the acquired OXA subclasses, called carbapenem-hydrolyzing class D β-lactamases, are found both on the chromosome and on plasmids, and include the ubiquitous 23-like oxacillinase as well as many others.[138,146,147] The group 3 Ambler class B metallo-β-lactamases imipenemase (IMP), Verona integron–encoded (VIM), and New Delhi 1 (NDM-1) and New Delhi 2 (NDM-2) are a less frequent cause of carbapenem resistance but continue to spread worldwide.[148–151]

The second most important determinant of drug resistance in *Acinetobacter* is the presence of efflux pumps, which confer resistance to β-lactam antibiotics, chloramphenicol, macrolides, tetracyclines,

tigecycline, aminoglycosides, polymyxin, and certain antiseptics. Efflux pumps in the resistance-nodulation-division (RND) family are located on chromosomes and are overexpressed after genetic mutation and induced by tigecycline.[152,153] The predominant pump expressed in this family is AdeABC, although others have been described.[154] Non-RND efflux systems seen in *Acinetobacter* are encoded by mobile genetic elements and also play a role in antimicrobial resistance.[153] Antiseptic resistance is also encoded by efflux pumps; a small multidrug resistance (SMR) efflux pump may confer quaternary ammonium resistance, and RND efflux pumps result in biocide resistance.[155,156]

Aminoglycoside resistance in *Acinetobacter* species is determined by the presence of aminoglycoside-modifying enzymes (AMEs). These enzymes either phosphorylate, acetylate, or adenylate aminoglycoside molecules and decrease their binding affinity to the ribosomal subunit.[157] AMEs are encoded on genetically mobile elements, especially class 1 integrons, which frequently also contain genetic elements for ESBLs and metallo-β-lactamases and are reported to cause outbreaks with high mortality[158] (Fig. 222.3).

The expression of porins modifies the ability of antimicrobials to permeate the outer membrane of the bacterial cell wall; OmpAab is the principal outer membrane protein (Omp) in *Acinetobacter* and confers resistance to β-lactams and carbapenems. OmpAab has been characterized in *A. radioresistens, A. junii,* and *A. baumannii.*[159,160]

*Acinetobacter* resistance to fluoroquinolones is multifactorial. Mutations in the quinolone resistance-determining regions (QRDRs) lower fluoroquinolone binding to bacterial DNA gyrase and topoisomerase IV; when combined with upregulation of the AdeABC efflux pump, quinolone resistance results.[161,162]

Sulbactam, a class A β-lactamase inhibitor, has inherent antibacterial activity against *Acinetobacter* species via binding to penicillin-binding proteins. Mutations of these penicillin-binding proteins, plus additional mechanisms resulting in the overexpression of β-lactamases, likely result in sulbactam resistance.[163,164]

Resistance to the polymyxins, which include colistin and polymyxin B, is associated with both mutations in the genes encoding the two-component polymyxin regulatory system PmrA and PmrB, as well as a reduction in lipopolysaccharides in the *Acinetobacter* cell wall, resulting in less negative charge and loss of antimicrobial affinity.[165,166] The emergence of colistin resistance has resulted in pan-resistant *A. baumannii,* with evidence of regional transmission.[167]

## TREATMENT

The selection of empirical and targeted therapy for *Acinetobacter* is driven by patient risk for multidrug-resistant strains, local epidemiology, site of infection, and results of antibiotic susceptibility testing. Before initiating antibiotic therapy, the clinician must determine whether the *Acinetobacter* isolated from culture represents invasive infection or colonization; this will help to reduce overutilization of antibiotics and minimize the subsequent risk of multidrug-resistant *Acinetobacter* infection.[34]

### β-Lactam Antibiotics

β-Lactam antibiotics are the drugs of choice for susceptible *Acinetobacter* infections, as they are rapidly bactericidal and have a wide volume of distribution with appropriate dosing. *Acinetobacter* species frequently contain intrinsic β-lactamases that inactivate first- and second-generation cephalosporins and penicillins; however, if sensitivity testing indicates susceptibility, third- and fourth-generation cephalosporins, such as cefepime, ceftriaxone, and cefotaxime, are useful agents.[168] The widespread distribution of ESBLs has led to heavy utilization of carbapenem for hospital-acquired *Acinetobacter* infections, with resultant worldwide emergence of carbapenem-resistant strains.[138,169,170] Cefiderocol, a novel siderophore antibiotic composed of a cephalosporin molecule with a catechol moiety, is stable against all classes of β-lactamases, including carbapenemases, and offers promise of effective therapy for extensively resistant strains of *Acinetobacter.*[171–173]

### β-Lactamase Inhibitors

With the widespread distribution of carbapenem-resistant *Acinetobacter,* the use of β-lactamase inhibitors should be considered as a therapeutic option, based on susceptibility testing. Sulbactam has the highest activity of the β-lactamase inhibitors, and when used alone or combined with ampicillin is effective for invasive *Acinetobacter* infections, including pneumonia, bloodstream infections, and meningitis.[174–177] Sulbactam is equally effective in treating pneumonia and bacteremia compared to carbapenems, tigecycline, colistin, and polymyxin B.[178–181] A dosage of at least 4 g of sulbactam per day in divided doses is recommended for most infections in adults, although dosages as high as 9 g in divided doses have been used.[179]

### Aminoglycosides

Although susceptibility testing may show that isolates of *Acinetobacter* are susceptible to aminoglycosides, the use of these agents is limited due to low penetration into the lungs and central nervous system, concern that automated tests for *Acinetobacter* susceptibility to aminoglycosides may be inaccurate, and the detection of heteroresistant strains leading to concerns of treatment failure.[182,183]

### Tigecycline

Tigecycline, a member of the glycylcycline class of antibiotics, has been successfully used to treat carbapenem-resistant *Acinetobacter* infections, but these results are based on observational studies, and the drug is frequently given in combination with other antibiotics.[184,185] Additional concerns include the large volume of distribution and low serum concentration of tigecycline, which preclude its use for bloodstream infections; reports of the development of resistance while on therapy; and increased mortality when used in combination with colistin.[186–188]

### Polymyxins

Colistin and polymyxin B are frequently included in the treatment of carbapenem-resistant *Acinetobacter* infections. Although the optimal dosing of these antibiotics to treat *Acinetobacter* infection has not been determined by randomized trials, evidence suggests that a loading dose, followed by high doses with longer dosing intervals, may improve outcomes[189–191] (see Chapter 32). Colistin heteroresistance in *A. baumannii* may emerge during therapy, but resistant strains appear to be associated

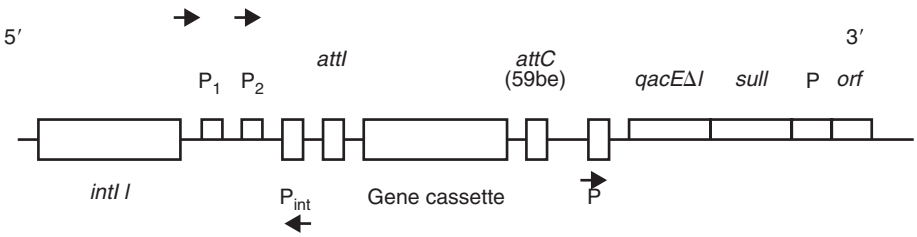


**FIG. 222.3 Simplified representation of a class 1 integron.** As pictured, integrons contain components of a site-specific recombination system that recognizes and captures mobile gene cassettes. Gene cassettes can be antibiotic resistance genes followed by a repeat sequence called a 59-bp element (*59be*) or *attC*. In *Acinetobacter baumannii,* gene cassettes may contain β-lactamase genes (e.g., *bla*IMP-2, *bla*IMP-4, *bla*VIM-1, and *bla*OXA). *attC,* Sequence in the gene cassette recognized by the integrase; *attI,* integration site; *orf,* open reading frame; *P,* promoter; *P1,* promoter for the gene cassette; *P2,* second promoter; *Pint,* promoter for the integrase; *qacEΔ1,* partially deleted gene that encodes resistance to a quaternary ammonium compound; *sul1,* gene for sulfonamide resistance. *(From Bonomo RA, Szabo D. Mechanisms of multidrug resistance in* Acinetobacter *species and* Pseudomonas aeruginosa. Clin Infect Dis. *2006;43:S49-S56.)*

with decreased fitness and virulence.[192,193] Innate colistin resistance is common in certain *Acinetobacter* species such as *A. junii.*[194]

## Combination Treatment

Although reports of the various combinations of colistin, rifampin, carbapenems, sulbactam, minocycline, and tigecycline show promising results, prospective randomized trials are few.[195,196] One multicenter randomized trial allocated patients with carbapenem-resistant *A. baumannii* infection to colistin alone or colistin plus rifampicin. Although microbiologic eradication was significantly shorter in the combination arm, no differences in infection-related death or length of hospitalization were observed.[197] Other studies have failed to show any benefit for combination therapy and may result in increased mortality.[188,196,198]

Given limited treatment choices, the clinician should consider optimization of currently available antimicrobials by using prolonged infusion of β-lactam antibiotics for strains with intermediate susceptibility and local instillation of colistin (e.g., intrathecal or intraventricular for central nervous system infections) when the intravenous formulation provides low tissue concentration at the site of infection.[199] Extended infusion of β-lactam antibiotics allows for maximized time of drug serum concentration above minimal inhibitory concentration for the *Acinetobacter* isolate by increasing the dose and shortening the dosing interval.[200] Continuous infusion results in higher mean trough levels of β-lactams, which may result in better clinical outcomes for less susceptible *Acinetobacter* infections.[201] High-dose colistin with an extended dosing interval may provide benefit in carbapenem-resistant *Acinetobacter* infections, but the data are scant and inconsistent.[196,202] Intrathecal colistin is effective for treatment of drug-resistant *Acinetobacter* central nervous system infection.[199,203] Several studies suggest aerosolized colistin is safe and possibly efficacious for treatment of pneumonia caused by resistant *Acinetobacter* species.[204,205] Unfortunately, not many antibiotics with potency against highly resistant strains of *Acinetobacter* are in phase II or phase III of development.[206]

The use of bacteriophages to treat antimicrobial-resistant *Acinetobacter* infections has shown promise in animal studies, but human trials are needed.[207–209] Finally, development of vaccines is also evolving and provides hope for future options to prevent these infections.[210–214]

# PREVENTION

Given the ability of *Acinetobacter* to survive on surfaces for weeks under dry conditions, hand hygiene and the routine disinfection of medical equipment and surfaces touched by patients and staff are the basic but essential steps to prevent transmission in the health care setting.[215–220] Factors that further increase the risk of *Acinetobacter* transmission include high colonization pressure and multiple beds within the same room.[221,222] In addition to emphasizing hand hygiene and cohorting of patients, environmental cleaning by effective disinfectants with appropriate contact times is critical to curb outbreaks.[38,47,223] Multidrug-resistant strains of *A. baumannii* expressing genes encoding porin mutations and efflux pumps resulting in reduced disinfectant efficacy have been reported; however, such strains may have reduced environmental fitness.[224] The ability of *Acinetobacter* to form biofilms on surfaces is an important step for their survival in the environment. Polycarbonate, a material frequently found in health care equipment and surfaces, allows *Acinetobacter* to form biofilm mass more readily than other materials.[225] Measuring the adequacy of the cleaning and disinfection process is important; use of an adenosine triphosphate bioluminescence assay improves performance and is helpful useful to evaluate cleaning protocols.[226,227] Environmental cultures also can play a role in outbreak evaluation, but the location and frequency of sampling may affect results.[228] Moist locations such as drains and sinks may also harbor *Acinetobacter* and serve as a source of infection; routine disinfection of drains or even removal of sinks may be required to halt outbreaks.[229–231]

Evidence of ongoing transmission should prompt consideration of alternative sources of contamination via atypical routes; air sampling in ICUs has yielded *Acinetobacter,* and fans within medical devices and ventilation systems have been implicated in transmission.[55–57,232,233] Stethoscopes may also become contaminated with *Acinetobacter* and potentially transmit infection.[234] Artificial nails on health care workers' hands are known to harbor a multitude of pathogens, including *Acinetobacter,* and should be banned from health care settings.[235,236] Additional vectors, such as contaminated external surfaces of mobile medical equipment and unused medical supplies, may play a role in transmission.[40,237] Whole-genome sequencing has proven to be a useful tool to determine the epidemiologic risk factors associated with infection.[60,238]

Good communication between health care facilities is required to prevent outbreaks due to transfer of patients colonized with multidrug-resistant *Acinetobacter.*[39] Surveillance cultures to identify patients with asymptomatic *Acinetobacter* colonization have a sensitivity between 55% and 89% depending on site and method of sampling, and may be considered when patients with epidemiologic risk factors, such as previous exposure to health care settings in countries with a high prevalence of multidrug-resistant *Acinetobacter.*[22,23] Those colonized with *Acinetobacter* upon admission to the ICU are at significantly higher risk of subsequent infection, and should prompt prevention strategies such as daily application of topical chlorhexidine gluconate.[239–242] Contact isolation precautions, composed of placing the patient in a single-bed room, dedicated equipment, and the routine use of gowns and gloves by health care workers during patient care, are also employed to reduce transmission from infected and colonized patients. *A. baumannii* is more likely to contaminate health care workers' gloves or gowns compared to other pathogens, especially after wound dressing and manipulating endotracheal tubes.[243–245] Health care workers caring for patients with *Acinetobacter* must follow standard precautions, as occupational acquisition of *Acinetobacter* is rarely reported.[246] Although antibiotic cycling has not proven effective, judicious use of antibiotics, particularly carbapenems, reduces the risk of multidrug-resistant *Acinetobacter* infection in ICU patients.[35,36,247]

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*The complete reference list is available online at Expert Consult.*

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# 223 *Salmonella* Species

*David A. Pegues and Samuel I. Miller*

## SHORT VIEW SUMMARY

**Definition**
- Salmonellosis includes gastroenteritis and other infections caused by nontyphoidal *Salmonella* (NTS).

**Epidemiology**
- There are approximately 1.2 million cases of NTS infection annually in the United States.
- Nontyphoidal salmonellosis is associated with diverse reservoirs, including fresh and prepared food items and other animal sources.

**Microbiology**
- There are more than 2500 *Salmonella* serotypes.
- Strains with multidrug resistance and decreased susceptibility to fluoroquinolones are increasingly prevalent.

**Diagnosis**
- Freshly passed stool should be plated directly on MacConkey agar or more selective media.
- Obtain blood cultures for patients suspected of bacteremia or vascular infection.
- Serogrouping is performed with commercially available antisera.

**Therapy**
- Antimicrobial therapy is not indicated for uncomplicated *Salmonella* gastroenteritis and prolongs the duration of fecal carriage.
- Ceftriaxone or fluoroquinolones should be administered empirically for treatment of severe gastroenteritis or when occurring in high-risk patients and for directed therapy of bacteremia or focal infections with NTS.

**Prevention**
- Control of foodborne outbreaks of NTS depend on a coordinated public health response and identification of controllable hazards from the farm to the table.

Salmonellae are named for the pathologist Salmon, who was involved in the first isolation (by Theobald Smith) of *Salmonella choleraesuis* from the porcine intestine.[1] *Salmonella* are effective commensals and pathogens that cause a spectrum of diseases in humans and animals, including domesticated and wild mammals, reptiles, birds, and insects. Some *Salmonella* serotypes, such as *Salmonella enterica* Typhi, *Salmonella* Paratyphi, and *Salmonella* Sendai, are highly adapted to humans and have no other known natural hosts, whereas others, such as *Salmonella* Typhimurium, have a broad host range and can infect a wide variety of animal hosts and humans. Some *Salmonella* serotypes, such as Dublin (cattle) and Arizonae (reptiles), are mostly adapted to an animal species and only occasionally infect humans. The widespread distribution of *Salmonella* bacteria in the environment, their increasing prevalence in the global food chain, and their virulence and adaptability have an enormous medical, public health, and economic impact worldwide. Salmonellae have been important organisms for the development of scientific knowledge. During the 1920s to 1940s, Kaufmann and White[2] pioneered the study of antibody interactions with the bacterial surface that resulted in agglutination assays that are the basis of serotyping today. In 1952 Zinder and Lederberg,[3] using *S.* Typhimurium, discovered the principle of genetic transduction, the transfer of genetic information from one cell to another by a virus particle (bacteriophage P22). In 1973 Ames and coworkers[4] developed the widely used Ames test, which uses *S.* Typhimurium auxotrophic mutants to test the mutagenic activity of chemical compounds. Over the last 25 years many of the important principles by which bacterial pathogenic mechanisms and host responses result in disease have been elucidated by studying salmonellae in animal and tissue culture models of mammalian infection.

## CLASSIFICATION AND TAXONOMY

*Salmonella* is a genus of the family of Enterobacteriaceae. Before 1983 the existence of multiple *Salmonella* spp. was taxonomically accepted. Currently, as a result of experiments indicating a high degree of DNA similarity, the genus *Salmonella* is separated into two species: *Salmonella enterica*, which contains six subspecies (I, II, IIIa, IIIb, IV, and VI), and *Salmonella bongori*, which was formerly subspecies V. *S. enterica* subspecies I contains almost all the serotypes pathogenic for humans, except for the uncommon human infections with subspecies IIIa and IIIb, which were formerly designated by the genus *Arizonae*.

Members of the seven *Salmonella* spp. can be serotyped into one of more than 2500 serotypes (serovars) according to antigenically diverse surface structures: somatic O antigens (the carbohydrate component of lipopolysaccharide [LPS]) and flagellar (H) antigens (Table 223.1).[5] The name usually refers to the location where the *Salmonella* serotype was first isolated. According to the current *Salmonella* nomenclature system in use at Centers for Disease Control and Prevention (CDC) and World Health Organization laboratories, the full taxonomic designation *Salmonella enterica* subsp. *enterica* serotype Typhimurium can be shortened to *Salmonella* serotype Typhimurium or *Salmonella* Typhimurium.[6] The authors have chosen to use the abbreviated form in this chapter and will omit the "serotype," for example, designating "*Salmonella* serotype Typhimurium" as "*Salmonella* Typhimurium."

## THE GENOME

The genome sequences of ≈8000 *S. enterica* strains, including *S.* Typhi; *S.* Paratyphi A, B, and C; and numerous nontyphoidal serotypes, are available in GenBank. The salmonellae genomes contain approximately 4.7 to 5.2 million base pairs, with approximately 4500 to 5400 coding sequences. Comparing sequence diversity by multilocus sequence typing suggests *S.* Typhi emerged from the *S. enterica* common ancestor around 50,000 years ago.[7] *S.* Typhi and *S.* Paratyphi A are closely related to each other but not to other *S. enterica* serotypes, and their host restriction to humans is related to loss of gene function through pseudogene formation and gene deletion.[8,9] Next-generation sequencing combined with traditional epidemiologic investigation permits a greater understanding of salmonellae evolution and spread. For example, whole-genome sequencing found that two closely related highly invasive strains of *S.* Typhimurium have recently emerged (late 20th century) and spread across sub-Saharan Africa temporally and geospatially, associated with the human immunodeficiency virus (HIV) pandemic, likely facilitated by the rapid expansion and mobility of a susceptible host population.[10] Of interest, these strains have undergone some genome reduction, similar to what has been seen in *S.* Typhi, possibly as a result of greater restriction to human hosts. However, in contrast to the speculation that these strains may have resulted in greater virulence or propensity to bacteremia,

**TABLE 223.1 *Salmonella* Species, Subspecies, and Serotypes and Their Usual Habitats**

| *SALMONELLA* SPECIES AND SUBSPECIES | NO. OF SEROTYPES WITHIN SUBSPECIES | USUAL HABITAT |
|---|---|---|
| *S. enterica* subsp. *enterica* (I) | 1531 | Warm-blooded animals |
| *S. enterica* subsp. *salmae* (II) | 505 | Cold-blooded animals and the environment[a] |
| *S. enterica* subsp. *arizonae* (IIIa) | 99 | Cold-blooded animals and the environment[a] |
| *S. enterica* subsp. *diarizonae* (IIIb) | 336 | Cold-blooded animals and the environment[a] |
| *S. enterica* subsp. *houtenae* (IV) | 73 | Cold-blooded animals and the environment[a] |
| *S. enterica* subsp. *indica* (VI) | 13 | Cold-blooded animals and the environment[a] |
| *S. bongori* (V) | 22 | Cold-blooded animals and the environment[a] |
| Total | 2579 | |

[a]Isolates of all species and subspecies have occurred in humans.
*Modified from Grimont PAD, Weill F-X.* Antigenic Formulae of the Salmonella Serovars 2007. *9th ed. WHO Collaborating Centre for Reference and Research on Salmonella. https://www.pasteur.fr/sites/default/files/veng_0.pdf.*

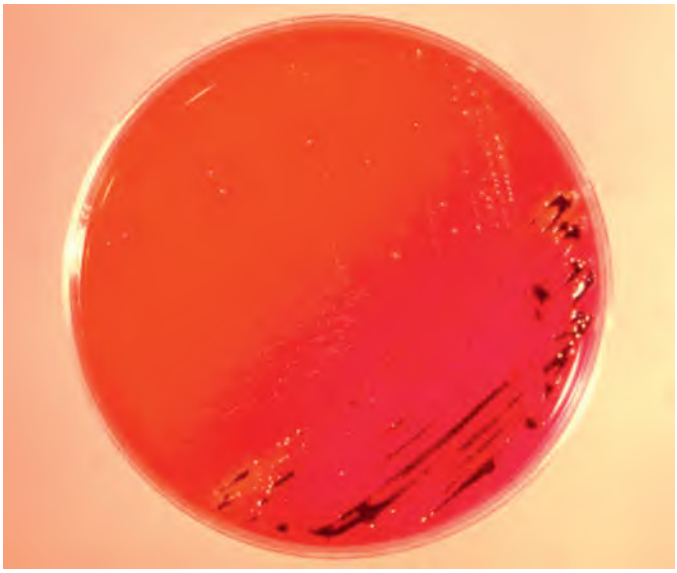

**FIG. 223.1 Colonial growth pattern of *Salmonella* Arizonae grown on xylose-lysine-deoxycholate agar.** Salmonellae metabolize thiosulfate to produce hydrogen sulfide, which leads to the formation of colonies with black centers and allows them to be differentiated from the similarly colored *Shigella* colonies.

if anything these strains appear less fit for resistance to innate immunity, and most usually cause simple gastrointestinal illness.[11]

## MICROBIOLOGY

Salmonellae are gram-negative, non–spore-forming, facultatively anaerobic bacilli that measure 2 to 3 by 0.4 to 0.6 µm in size. Like other Enterobacteriaceae, they produce acid on glucose fermentation, reduce nitrates, and do not produce cytochrome oxidase.[12] All organisms, except *S.* Gallinarum-Pullorum, are motile as a result of peritrichous flagella, and most do not ferment lactose. However, approximately 1% of organisms can ferment lactose and therefore may not be detected if only MacConkey agar or other semiselective media are used to identify *Salmonella* based on colorimetric assay for fermentation of lactose. The differential metabolism of sugars can be used to distinguish many *Salmonella* serotypes; serotype Typhi is the only organism that does not produce gas on sugar fermentation.[12]

Freshly passed stool is preferred for the isolation of *Salmonella* and should be plated directly onto agar plates. Low-selective media, such as MacConkey agar and deoxycholate agar, and intermediate-selective media, such as *Salmonella-Shigella,* xylose-lysine-deoxycholate (Fig. 223.1), or Hektoen enteric agar, are widely used to screen for both *Salmonella* and *Shigella* spp. Selective chromogenic media, such as CHROMagar *Salmonella* (DRG International, Springfield, NJ), are more specific than other selective media, reduce the need for confirmatory testing and time to identification, and increasingly are used for the primary isolation and presumptive identification of *Salmonella* from clinical stool specimens.[13] Stool specimens can be directly inoculated into selenite enrichment broth before plating on primary media to facilitate the recovery of low numbers of organisms.[13] Highly *Salmonella*-selective media, such as selenite with brilliant green, should be reserved for use in stool cultures of suspected carriers and under special circumstances, such as outbreaks. Bismuth sulfite agar, which contains an indicator of hydrogen sulfite production and does not contain lactose, is preferred for the isolation of *S.* Typhi and can be used for the detection of the 1% of *Salmonella* strains (including most *Salmonella* serogroup C strains) that ferment lactose.[14] After primary isolation, possible *Salmonella* isolates can be tested in commercial identification systems or inoculated into screening media, such as triple-sugar–iron and lysine-iron agar.

Direct detection of enteric pathogens from stool specimens by DNA-based syndrome panels is increasingly used by clinical laboratories to allow providers to rapidly identify the cause of gastroenteritis. To ensure that outbreaks of similar organisms are detected and investigated, all specimens that test positive for nontyphoidal *Salmonella* (NTS) by culture-independent diagnostic testing and for which isolate submission is requested or required under public health reporting rules should be cultured in the clinical laboratory or at a public health laboratory.

Isolates with typical biochemical profiles for *Salmonella* should be serogrouped with commercially available polyvalent antisera or sent to a reference or public health laboratory for complete serogrouping. Salmonellae are serogrouped according to their polysaccharide O (somatic) antigens, Vi (capsular) antigens, and H (flagellar) antigens according to the Kauffman-White scheme. The Vi antigen is a heat-labile capsular homopolymer of *N*-acetylgalactosaminouronic acid that is used for the identification of *S.* Typhi strains and on occasion other *Salmonella* serotypes by slide agglutination.[15] In *S.* Typhi and *S.* Paratyphi C the polysaccharide Vi antigen can inhibit O-antigen agglutination because it is so abundant, and boiling is required to inactivate Vi antigen and to detect O antigen. Most antigenic variability occurs in the O antigen, which is composed of chains of oligosaccharide attached to a core oligosaccharide that is linked covalently to lipid A.

Although serotyping of all surface antigens can be used for formal identification, most laboratories perform a few simple agglutination reactions that differentiate specific O antigens into serogroups, designated as groups A, B, $C_1$, $C_2$, D, and E *Salmonella.* Strains in these six serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Although this grouping is useful in epidemiologic studies and can be used to confirm genus identification, it cannot identify whether the organism is likely to cause enteric fever because considerable cross-reactivity occurs among serogroups. For example, *S.* Enteritidis, and *S.* Typhi are both group D, and *S.* Typhimurium and *S.* Paratyphi B are both group B.

Genotyping methods frequently are used for epidemiologic purposes to differentiate strains of common *Salmonella* serotypes. These methods include ribotyping, pulsed-field gel electrophoresis, insertion sequences analysis, polymerase chain reaction–based fingerprinting, multilocus sequence typing, and increasingly whole-genome sequencing.

## EPIDEMIOLOGY

In many countries the incidence of human *Salmonella* infections has increased markedly in recent decades, although good population-based surveillance data are mostly lacking, especially from sub-Saharan Africa.

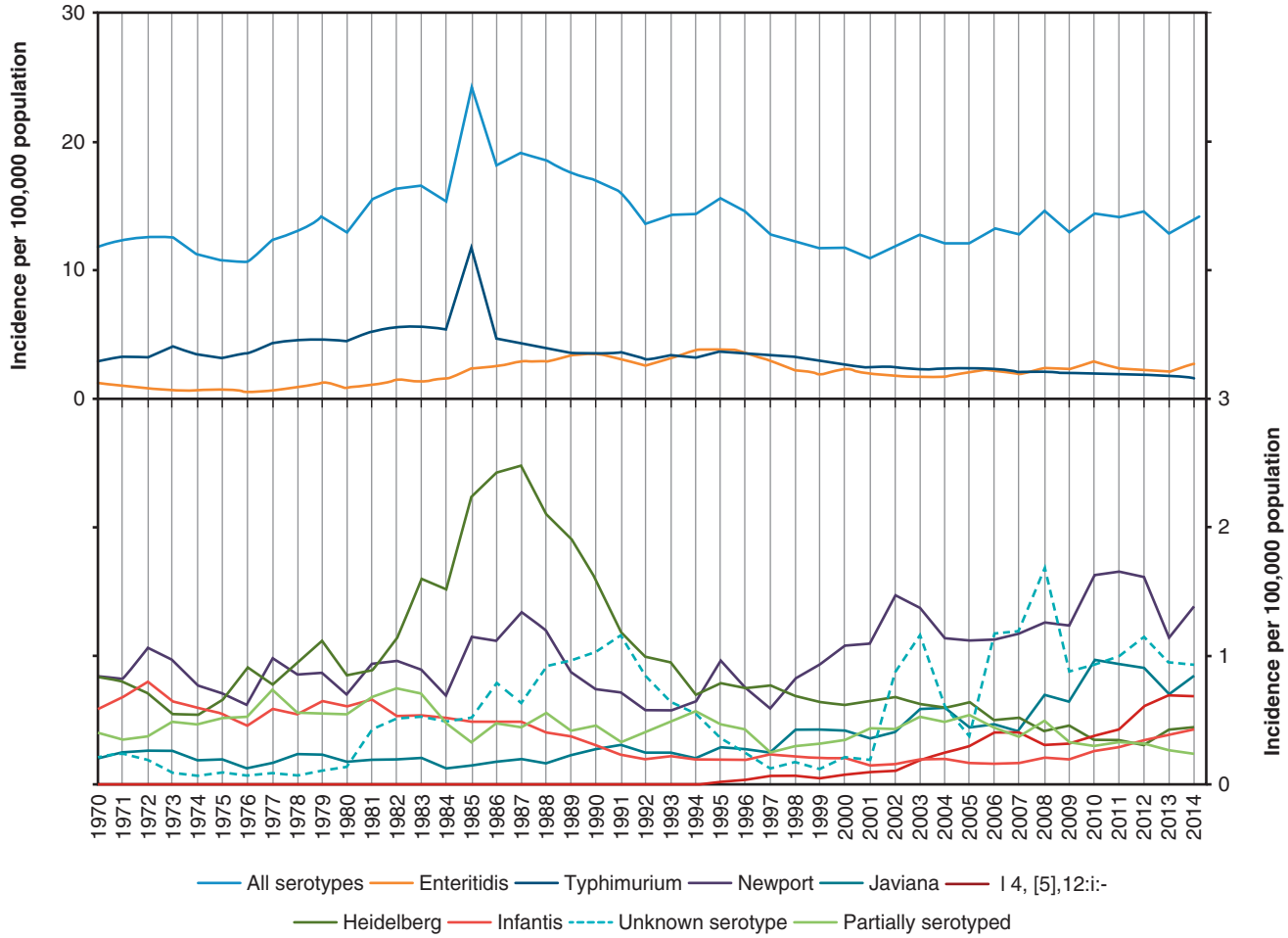


**FIG. 223.2 Incidence rate of culture-confirmed human *Salmonella* infection reported to Laboratory-Based Enteric Disease Surveillance System, including all serotypes and individual serotypes with ≥1000 infections reported in 2014, by year, United States, 1970–2014.** *(From Centers for Disease Control and Prevention [CDC]. National* Salmonella *Surveillance Annual Report, 2014. Atlanta, GA: US Department of Health and Human Services, CDC; 2017.* https://www.cdc.gov/nationalsurveillance/salmonella-surveillance.html.*)*

In the United States NTS species cause an estimated 1.2 million cases of foodborne illness each year, second only to noroviruses, and are associated with an estimated hospitalization rate of 2.7% and death rate of 0.5%.[16] In the United States the incidence rate of NTS infection has remained relatively stable in the last 20 years and continues to be driven largely by *S.* Typhimurium and *S.* Enteritidis[3] (Fig. 223.2).[17] In 2016 the incidence rate of salmonellosis (16.60/100,000 population) was second only to *Campylobacter* (17.43/100,000 population) among nine potentially foodborne diseases under active surveillance.[18] In comparison, during 2010–14, reported NTS incidence rates ranged between 21.4 to 25.7 per 100,000 population in the European Union.[19] Globally, *S.* Typhimurium (43.5%) and *S.* Enteritidis (17.1%) are the most common *Salmonella* serotypes, with large differences observed in the serotype distribution between regions but lesser differences between countries within the same region.[20] The incidence of NTS infection is highest during the rainy season in tropical climates and during the warmer months in temperate climates, coinciding with the peak in foodborne outbreaks.[21]

Unlike *S.* Typhi and *S.* Paratyphi, whose only reservoir is humans, NTS can be acquired from multiple animal reservoirs. Transmission of NTS to humans can occur by many routes, including consumption of food animal products, especially eggs, poultry, undercooked ground meat, dairy products, fresh produce contaminated with animal waste, contact with animals or their environment, and contaminated water. During the 1980s and 1990s, *S.* Enteritidis associated with shell eggs emerged as the predominant *Salmonella* serotype and source of foodborne disease in the United States and some other countries.[22] In the United States the rate of reported *S.* Enteritidis isolates increased from 0.6 per 100,000 population in 1976 to a high of 3.9 per 100,000 in 1994.[23] As a result of intensive surveillance and control effort, including egg farm management practices, such as rodent control and vaccination of young hens, egg quality-assurance programs on farms, egg refrigeration during storage and transport, and consumer education, the incidence of *S.* Enteritidis infection has declined in the United States and other developed countries[24] (see Fig. 223.2). However, outbreaks of *S.* Enteritidis infection associated with shell eggs continue to occur. In 2010 a national outbreak of *S.* Enteritidis infection resulted in more than 1900 reported illnesses and the recall of 500 million eggs.[25] Infection localizes to the ovaries and upper oviduct tissue and is transmitted to the forming egg before shell deposition, resulting in contamination of the albumen and yolk. Although cooking eggs until all liquid yolk is solidified kills *S.* Enteritidis, the use of pasteurized egg products remains the safest alternative for institutions and the general public.

Transmission of *S.* Enteritidis from farm to farm may be facilitated by contaminated chicken manure, insects, and rodents and by ingestion of feed contaminated with mouse droppings because *S.* Enteritidis strains cultured from the spleens of mice caught on farms have enhanced ability to contaminate eggs.[26,27] The loss of cross-immunity resulting from culling chickens infected with *S.* Gallinarum and *S.* Pullorum in the United States and United Kingdom also may have contributed to the emergence of *S.* Enteritidis.[28]

*Salmonella* live in the intestines of most food animals, and contamination of raw poultry and meat products can occur during slaughter and processing. Retail ground poultry and meat are at high risk of contamination with *Salmonella*, including with antimicrobial-resistant strains.[29] In 2013 18.0% of ground chicken, 15.0% of ground turkey, and 1.6%

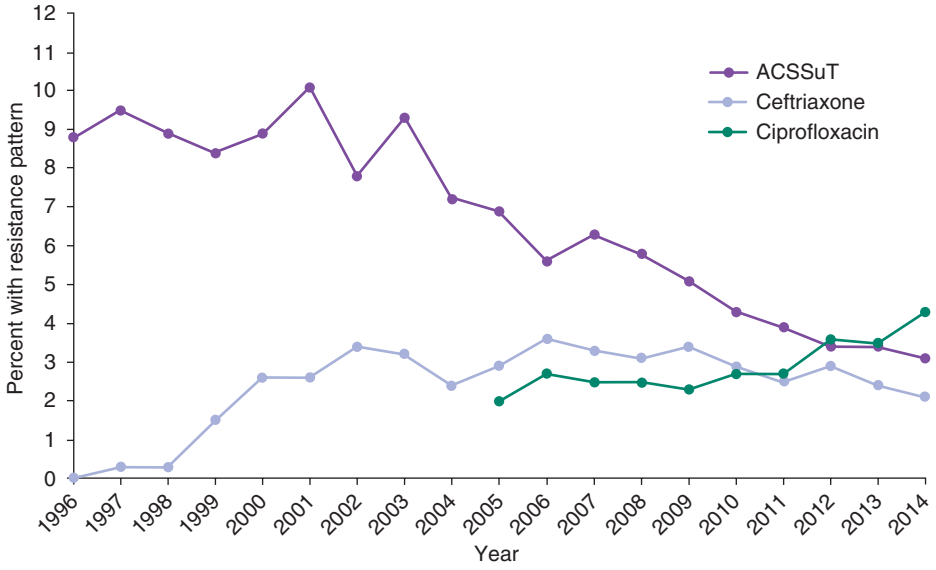


**FIG. 223.3 Resistance patterns of nontyphoidal *Salmonella* isolates, United States, 1996–2014.** *ACSSuT,* Resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline; *ceftriaxone,* minimal inhibitory concentration (MIC) > 4 μg/mL; *ciprofloxacin,* MIC > 0.12 μg/mL. *(From Centers for Disease Control and Prevention.* National Antimicrobial Resistance Monitoring System [NARMS] 2014 Human Isolates Surveillance Report. https://www.cdc.gov/narms/reports/index.html.*)*

of ground beef specimens sampled by the US Department of Agriculture tested positive for *Salmonella.*[30] Although raw chicken carcasses and other meats are less commonly contaminated with *Salmonella* than is ground poultry, cross-contamination of food items from handling of raw chicken and inadequate hand hygiene are risks for sporadic salmonellosis in the home.[31] There is considerable mismatch between animal and human *Salmonella* serotypes, suggesting that the risk of transmission is not equal for all food products and serotypes.[32]

Changes in food consumption and the rapid growth of international trade in agricultural food products and increasing use of manufacturing technologies have facilitated the dissemination of new *Salmonella* serotypes associated with fresh fruits and vegetables. Human or animal feces may contaminate the surface of fruits and vegetables and may not be removed by washing. Recent multistate foodborne outbreaks of salmonellosis in the United States associated with fresh produce include papayas—multiple serotypes, cantaloupe—multiple serotypes, pistachios—*S.* Montevideo, cucumbers—*S.* Poona, alfalfa sprouts—multiple serotypes, bean sprouts—*S.* Enteritidis, and tomatoes—multiple serotypes. Tomatoes can internalize *Salmonella* when immersed in water, and contamination on the tomato or melon surface can be transferred to the interior when it is cut.[33] Sprout seeds can become contaminated before sprouting, and soaking seeds with 20,000 parts per million calcium hypochlorite or other disinfectant can reduce but does not eliminate the risk of sprout-associated illness.[34] Recent salmonellae outbreaks have been associated with peanut products, including peanut butter and paste used as a food additive. Salmonellae appear capable of colonizing and adhering to the nut and can be present in raw nuts and, if inadequate processing and or roasting has occurred, in nut-related products.[35–37]

Manufactured food items pose an enormous potential hazard of foodborne salmonellosis in developed countries because of their centralized production and wide-scale distribution. Both pasteurized and unpasteurized milk and milk products, including ice cream and powdered infant formula, have been recognized as sources of *Salmonella* infections.[38,39]

Salmonellosis associated with exotic pets is a resurgent public health problem, especially from exposure to reptiles, including turtles, iguanas, lizards, and snakes, and from amphibians such as aquatic frogs.[40] Of all *Salmonella* serotypes, 40% have been cultured predominantly from reptiles and are rarely found in other animals or humans. Based on extrapolation from population-based surveillance, 6% of all sporadic *Salmonella* infections and 11% among persons younger than 21 years are attributable to contact with reptiles or amphibians.[40] The recognition of pet turtle–associated salmonellosis led to the banning of shipment of small pet turtles in the United States in 1975 and in several countries, but small turtles continue to be sold illegally and pose a health risk, especially to children.[41] Exposure to pet birds, live poultry, such as chicks and ducklings, pet rodents and hedgehogs, dogs and cats, and to pet food and pet treats made from animal parts are other reported sources of human salmonellosis, including infection with multidrug-resistant (MDR) strains.[42,43]

Multidrug resistance among human NTS isolates is increasing in both developing and developed countries.[3,44–46] A diversity of transferable resistance plasmids have been identified from MDR NTS strains and contribute to the conjugative transfer of resistance between enteric bacterial species.[47] Of particular concern has been the worldwide emergence in the 1990s of a distinct strain of MDR *S.* Typhimurium, characterized as definitive phage type 104 (DT104), that is resistant to at least five antimicrobials—ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (R-type ACSSuT).[48] All DT104 strains contain a chromosome- and integron-encoded β-lactamase (PSE-1) that appears to have been acquired from plasmids in *Pseudomonas* spp. The DT104 strain has broad host reservoirs, and its widespread clonal dissemination in domestic livestock, especially among beef and dairy cattle, likely was promoted by use of antimicrobials on farms for therapeutic uses and for growth enhancement.[49] In the United States the proportion of NTS with the ACSSuT phenotype has been decreasing since the early 2000s (Fig. 223.3). In 2014 resistance to at least ACSSuT was reported in 3.1% of NTS, including 14.5% of *S.* Typhimurium and 9.9% of *S.* Heidelberg isolates.[46] Acquisition of *S.* Typhimurium DT104 is associated with exposure to ill farm animals and to a variety of meat products, including raw or undercooked ground beef.[49] Infection with DT104 is associated with increased risk of bloodstream infection and hospitalization compared with infection with susceptible strains, likely reflecting inappropriate empirical antimicrobial therapy.[50]

Outbreaks and sporadic cases of NTS resistant to third-generation cephalosporins have been reported, and international travel and adoption may have contributed to the global spread.[51] Resistance is most commonly mediated by a transferable plasmid containing the *ampC* ($bla_{CMY}$) gene, although other extended-spectrum β-lactamases have been described. The $bla_{CMY}$ gene is probably acquired by horizontal genetic transfer from *Escherichia coli* strains in food-producing animals and linked to the widespread use of the veterinary cephalosporin ceftiofur.[52] In 2014 2.1% of NTS isolates from humans in the United States were ceftriaxone resistant (minimum inhibitory concentration [MIC] ≥ 4 μg/mL).[46]

Ceftriaxone resistance is more common among NTS isolated from blood than stool and is associated with invasive infection and high case-fatality rates among African children, although most cases have concomitant malnutrition, HIV, and malaria.[53,54]

An MDR strain of *S.* Newport (MDR-AmpC), with decreased susceptibility to ceftriaxone (MIC > 2 µg/mL) and resistance to eight other human antimicrobials and ceftiofur, has emerged in the United States.[55] In 2014 MDR-AmpC was detected in 1.2% of all US NTS and 3.0% of *S.* Newport isolates.[46] Risk factors for infection with MDR-AmpC *S.* Newport include consumption of uncooked ground beef, runny eggs or omelets, and recent exposure to an antimicrobial to which the strain is resistant.[56] More recently, carbapenemase-producing NTS have been reported in Europe, North Africa, and southern Asia.

Over the last decade, strains of NTS with decreased susceptibility to ciprofloxacin (MIC 0.12–0.5 µg/mL) or ciprofloxacin resistance (MIC ≥1 µg/mL) have emerged and have been associated with delayed response and treatment failure.[57] In 2014 4.3% of NTS isolates in the United States had a ciprofloxacin MIC ≥0.12 (see Fig. 223.3), and the proportion is higher in Europe (≈6%).[46] These strains have diverse resistance mechanisms, including single and multiple mutations in the DNA gyrase genes *gyrA* and *gyrB* and mutations in the chromosomally encoded quinolone resistance-determining region and plasmid-encoded quinolone resistance genes that are not reliably detected by nalidixic acid susceptibility testing or standard ciprofloxacin disk diffusion.[57] Because commercial test systems do not contain ciprofloxacin concentrations sufficiently low to allow use of this breakpoint, laboratories need to determine the ciprofloxacin MIC by Etest or another alternative method.

In Taiwan in 2000 a high-level ciprofloxacin-resistant strain of *S.* Choleraesuis caused a large outbreak of invasive infections that was linked to the use of enrofloxacin in swine feed.[44] On the basis of increased prevalence of nalidixic acid–resistant *Salmonella* and fluoroquinolone-resistant *Campylobacter* spp. in humans, the US Food and Drug Administration withdrew approval of the use of fluoroquinolones in poultry in 2005.

Although health care–associated salmonellosis is infrequent, such infections have been associated with MDR strains, sustained transmission, and substantial morbidity and mortality.[58,59] The most frequently reported route of transmission of NTS in health care facility–associated outbreaks is foodborne.[60] Although less common, transmission of *Salmonella* from patients to health care providers has been associated with phlebotomy, handling soiled linen, noncompliance with barrier precautions, and fecally incontinent residents.[61,62] However, the risk of transmission from health care providers to patients appears to be low if infection control measures, including hand hygiene, correct use of personal protective equipment, and routine disinfection of patient-care equipment, are observed.[63] In contrast, the risk of nosocomial transmission to neonates and infants from acutely or chronically infected family members appears higher.[64] Neonates are at high risk for fecal-oral transmission of *Salmonella* because of relative gastric achlorhydria and the buffering capacity of ingested breast milk and formula. High-iron infant formula may further increase the risk of infant salmonellosis compared with breastfeeding.[65] Contaminated enteral feeding and crowding also have been associated with nosocomial transmission among pediatric patients.[66] Control of outbreaks in daycare centers may be difficult because of the need for frequent diaper changing and the higher rate and longer duration of convalescent carriage seen in the preschool-age group.[67]

Residents of nursing homes are at increased risk of foodborne salmonellosis and more severe morbidity and mortality because of poor infection control compliance and presence of comorbid illnesses, acid-suppressing medications, and waning immunity.[58,68]

## PATHOGENESIS

*Salmonella* infections begin with the ingestion of bacteria in contaminated food or water. Estimates of the infectious dose vary substantially and depend on the method of determination. In studies involving administration of laboratory *Salmonella* strains to healthy human volunteers, the median dose required to produce disease was approximately $10^6$ bacteria.[69] In contrast, investigations of point-source outbreaks suggest that as few as 200 bacteria may produce nontyphoidal gastroenteritis in many of those exposed and that the ingested dose is an important determinant of incubation period and disease severity. Discrepancies in these results may stem from use of strains attenuated by in vitro passage in the challenge experiments and from variation in disease susceptibility in the general population. Gastric acidity represents the initial barrier to *Salmonella* colonization and conditions or medications, including antacids, H2 blockers, and proton pump inhibitors, that increase gastric pH increase susceptibility to infection. On exposure to acid in vitro, salmonellae display an adaptive acid tolerance response that probably facilitates bacterial survival in the stomach and passage to the small intestine.[70]

### Interactions With Intestinal Epithelium and Induction of Enteritis

Salmonellae must evade host antimicrobial factors secreted into the intestinal lumen, including antimicrobial peptides, bile salts, and secretory immunoglobulin A, and traverse a protective mucous barrier before encountering intestinal epithelial cells.[71,72] Salmonellae express an array of distinct fimbriae that contribute to tight adherence to intestinal epithelial cells in culture. It is necessary to delete multiple fimbriae synthesis genes to prevent infection in animal models, suggesting that functional redundancy exists.[73] Microscopy reveals that salmonellae invade intestinal epithelial cells by a morphologically distinct process termed *bacteria-mediated endocytosis* (Fig. 223.4).[74] Shortly after bacteria adhere to the apical epithelial surface, profound cytoskeletal rearrangements occur in the host cell, disrupting the normal epithelial brush border and inducing formation of membrane ruffles that reach out and enclose adherent bacteria in large vesicles. This process resembles the membrane ruffling and macropinocytosis induced in many cell types by growth factors and is functionally distinct from receptor-mediated endocytosis, the mechanism by which many other pathogens enter nonphagocytic cells. After the bacteria internalize, a fraction of the *Salmonella*-containing vesicles transcytose to the basolateral membrane, and the apical epithelial brush border reconstitutes. The epithelial cell type that serves as the principal portal for *Salmonella* invasion remains uncertain. In the mouse enteric fever model salmonellae preferentially adhere to and enter the specialized microfold cells (M cells) that overlie lymphoid tissue within Peyer patches.[75] In bovine and rabbit models of enteritis, however, salmonellae do not appear to interact preferentially with M cells but, instead, adhere to and invade intestinal enterocytes diffusely.[76] It is possible that M cells are the principal portal of entry in the enteric fever syndrome and that generalized invasion of enterocytes plays a greater role in the enteritis induced by NTS serotypes.

Salmonellae encode a type III secretion system (T3SS) within *Salmonella* pathogenicity island 1 (the SPI-1 T3SS), which is required for

**FIG. 223.4 Scanning electron micrograph showing *Salmonella* Typhimurium entering a HEp-2 cell through bacteria-mediated endocytosis.** Membrane ruffles extend from the cell surface, enclosing and internalizing adherent bacteria. *(From Ohl ME, Miller SI. Salmonella: a model for bacterial pathogenesis.* Annu Rev Med. *2001;52:259–274.)*

bacteria-mediated endocytosis and intestinal epithelial invasion. T3SSs are complex macromolecular machines that have evolved to subvert host cell function through the translocation of virulence proteins directly from the bacterial cytoplasm into the host cell (see Chapter 1 for an overview). *Salmonella* mutants lacking a functional SPI-1 T3SS do not invade epithelial cells in tissue culture and are severely attenuated in animal models of infection after oral administration.[76] In the past decade, considerable attention has focused on identifying the virulence proteins translocated into epithelial cells by the SPI-1 T3SS and delineating the host cell processes these proteins target. At least five translocated proteins are essential for efficient invasion of cultured epithelial cells, although invasion in animal tissues may be more complicated and diverse.[77]

Two SPI-1 translocated proteins, SipC and SipA, promote membrane ruffling and *Salmonella* invasion through direct interactions with the actin cytoskeleton. The SipC protein inserts into the host cell plasma membrane and forms part of a protein complex that allows translocation of additional SPI-1 virulence proteins directly into the host cell cytoplasm.[78] SipC also directly nucleates actin polymerization at the site of *Salmonella* attachment and stimulates actin filament bundling.[79] The SipA protein further enhances actin polymerization through stabilization of actin filaments and reduction of the critical concentration for polymerization.[80] SipA mutants invade epithelial cells less efficiently than wild-type bacteria and induce disorganized, diffuse ruffling in host cells, in contrast to the localized ruffling induced around wild-type bacteria.

Additional SPI-1 translocated proteins contribute to *Salmonella* invasion by targeting members of the Rho family of monomeric guanosine triphosphate (GTP)-binding proteins (G proteins). Rho family members, including Cdc42, Rac, and Rho, regulate the structure and dynamics of the actin cytoskeleton and are required for formation of the membrane ruffles that mediate *Salmonella* internalization. The SPI-1 translocated proteins SopE and SopE2 directly activate Rac1 and Cdc42 in vitro by acting as guanosine diphosphate/GTP exchange factors (GEFs) and induce membrane ruffling and macropinocytosis after microinjection into epithelial cells.[81] SopB is an additional SPI-1 translocated protein that targets inositol phosphate signaling within the host cell by acting as an inositol polyphosphatase.[82] Among other effects, this activity indirectly stimulates Rho GTPases and promotes membrane ruffling.[83] This may be an important pathway for the bacteria to enter human cells, as recent data suggest that a polymorphism in VAC14, a regulator of phosphoinositide can alter entrance of bacteria into host cells and determines host susceptibility to *S.* Typhi in Viet Nam.[84]

Recent data suggest that only Rac1 and RhoG are essential for the effects of SopE, SopE2, and SopB.[85] Although mutation of *sopB, sopE,* or *sopE2* alone does not impact invasion, combined deletion of these three genes leads to a severe reduction in epithelial cell invasion.[83] Such functional redundancy among translocated proteins is an emerging theme in a variety of T3SSs. Overall, available data indicate that SipA and SipC act in concert with downstream cellular effectors of activated Rho GTPases to initiate and spatially direct the actin rearrangements that lead to *Salmonella* internalization.

Studies in mice indicate that salmonellae may also cross the intestinal epithelial border by an SPI-1–independent process involving host dendritic cells.[86,87] These cells express tight junction proteins and can intercalate between intestinal epithelial cells and access the intestinal lumen without disrupting epithelial integrity. In this manner, dendritic cells may internalize bacteria in the intestinal lumen and subsequently carry these bacteria to distant sites as they undergo their physiologic migration to lymphoid tissues. The diversity of mechanisms used by salmonellae to cross the intestinal barrier indicates the importance of this mechanism to its lifestyle within mammals.

In addition to invasion of intestinal epithelial cells, *Salmonella* serotypes clinically associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen. The SPI-1 T3SS is also required for these responses in tissue culture and animal models of enteritis. Specifically, *Salmonella* strains unable to deliver any SPI-1 virulence proteins, as a result of mutations in the secretion apparatus, fail to induce fluid secretion or neutrophil accumulation in ligated bovine ileal loops and do not cause gastroenteritis in calves.[88] In tissue culture models of enteritis translocation of SPI-1 proteins into intestinal epithelial cells leads to synthesis and polarized secretion of inflammatory mediators and neutrophil chemokines, including interleukin-8 (IL-8).[89]

Several SPI-1 translocated proteins that contribute to intestinal inflammation and fluid secretion have been identified. Stimulation of Rho GTPase signaling by SopE and SopE2 also leads to activation of microtubule-associated protein kinase pathways and movement of the proinflammatory transcription factor nuclear factor kappa B (NF-κB) to its site of action in the nucleus.[81] In addition to its role in invasion, the inositol polyphosphatase activity of SopB leads to accumulation of D-myoinositol-1,4,5,6-tetrakisphosphate in epithelial cells.[90] The increased concentration of this compound ultimately leads to an increase in cellular basal chloride secretion, with associated fluid flux. The SPI-1 translocated proteins SopA and SopD also contribute to intestinal secretory and inflammatory responses in ligated ileal loops, but the molecular basis of these effects remains unclear. Many other effector proteins that are delivered by the T3SS apparatus may also effect these or similar pathways with different targets. Individual nontyphoidal salmonellae have a diverse complement of effector proteins; for instance, many strains do not have SopE2. The association of specific effector proteins could alter the pathogenicity of specific strains and their emergence in humans from animal reservoirs.[91]

After *Salmonella* invasion, intestinal inflammation may also result from activation of the innate immune system through stimulation of proinflammatory receptors present on phagocytes and the basolateral surface of intestinal epithelia. This includes activation of Toll-like receptor 4 (TLR4) by LPS and TLR5 by bacterial flagellin.[92] The cytosolic surveillance pathway is also activated by the translocation of flagellin into the cytoplasm by the T3SS and its recognition by the inflammasome through the IL-1β converting enzyme protease-activating factor (IPAF), or NLRC4, pathway. This pathway results in the secretion of IL-1β, an important proinflammatory cytokine.[93,94] Intestinal inflammation probably contributes to fluid secretion and diarrhea through disruption of the epithelial barrier and increased water flux by an exudative mechanism. In contrast to the neutrophilic inflammation and gastroenteritis induced by NTS strains, *S.* Typhi induces monocytic inflammation in the human intestine and produces significantly less, if any, diarrhea.[95] The molecular basis of this difference in the host response remains unknown. One possibility is the presence of the Vi polysaccharide capsule in most strains of *S.* Typhi that can prevent recognition of LPS by TLR4.[96]

Several studies demonstrate that salmonellae also use the SPI-1 T3SS to deliver proteins that downregulate the host inflammatory response associated with *Salmonella* invasion. The SptP protein inactivates Rho GTPase signaling by acting as a GTPase-activating protein (RhoGAP).[97] This directly opposes the activity of SopE and SopE2 and reduces membrane ruffling and proinflammatory signaling after bacterial invasion. In addition, the SspH1 ubiquitin ligase and AvrA proteins inhibit NF-κB activation and related host cell cytokine synthesis.[98,99] These SPI-1 translocated proteins may promote bacterial persistence in the host by maintaining host cell integrity and allowing evasion of the host immune response. The presence of SPI-1 translocated proteins with opposing molecular actions (e.g., SopE and SptP) suggests that there may be temporal ordering of protein function, with initial activity of SPI-1 proteins associated with invasion and proinflammatory signaling and subsequent activity of antiinflammatory proteins. This dampening of the inflammatory response attributed to multiple bacterial effector proteins may contribute to the long period of relative asymptomatic colonization of the intestinal tract typical of NTS infection.

After inflammation is generated, an important component of salmonellae survival during gastroenteritis and its continued colonization of the intestinal tract after the resolution of disease involves the organisms' use of the sulfur-containing compound tetrathionate as an electron acceptor to promote energy metabolism in a microaerobic environment.[100] The intestinal microbiota generate toxic hydrogen sulfide gas through their metabolism, and intestinal epithelia detoxify this gas to thiosulfate. On the induction of inflammation by salmonellae and recruitment of neutrophils, reactive oxygen radicals convert the thiosulfate to tetrathionate, which only salmonellae can use for microaerophilic-based respiration to generate energy. This allows the organism to outcompete with commensals and effectively colonize the intestinal tract. The growth

advantage to salmonellae in the host conferred by tetrathionate respiration explains the utility of tetrathionate enrichment broth in the identification of salmonellae. Of interest, this process has been lost in typhoidal salmonellae that are inefficient colonizers of the intestinal tract.

## Interactions With Macrophages and Systemic Infection

After crossing the epithelial barrier, salmonellae encounter and enter macrophages present in the submucosal space and Peyer patches. Macrophage invasion may occur through bacteria-mediated macropinocytosis or through phagocytosis directed by several receptors present on the macrophage. Available data in both human infection and animal models of disease indicate that the ability of *Salmonella* to survive and replicate within macrophages is essential for dissemination within the host and induction of systemic disease. In persons with enteric fever and positive blood cultures, the majority of organisms are contained within the mononuclear fraction.[101] Furthermore, the ability of *Salmonella* mutants to replicate within macrophages in tissue culture correlates with ability to produce systemic disease in the mouse typhoid model, and microscopic examination of infected mouse liver and spleen demonstrates that the majority of organisms are located within macrophages.[102,103] Although residence within the macrophage shields the bacterium from effectors of humoral immunity, it also exposes the bacterium to the microbicidal and nutrient-poor environment of the phagosome. Within the host, salmonellae induce the expression of numerous genes that allow evasion of these antimicrobial defenses.

Once in the intracellular environment, the bacteria persist within a vacuolar compartment that endures for hours to days. Salmonellae can survive within a compartment that fuses with lysosomes, and hence inhibition of phagosome fusion with lysosomes is unlikely to be a major pathogenic strategy of salmonellae. The vacuole acidifies, although its acidification may be delayed. Resistance to a variety of vacuolar bactericidal activities is essential to pathogenesis, including resistance to antimicrobial peptides, nitric oxide, and oxidative killing. This is supported by experiments demonstrating that *S.* Typhimurium mutants sensitive to these compounds are less virulent for mice and that mice deficient in these activities are more susceptible to *S.* Typhimurium.[104]

*Salmonella* senses the acidic environment of the *Salmonella*-containing vacuole (SCV) and activates a variety of regulatory proteins required for *Salmonella* adaptation to the intracellular environment for replication within host cells. The best studied of these is the PhoP/PhoQ two-component regulatory system. The PhoP/PhoQ system senses the intracellular environment and regulates transcription of more than 200 genes, some of which are required for survival within macrophages. PhoQ acts as the sensor protein for the phagosome environment by sensing acidic pH and antimicrobial peptides to activate gene expression.[105,106,107] Activation of the PhoP/PhoQ and other regulons leads to widespread modifications in the protein and LPS components of the bacterial inner and outer membranes.[108] As many as 900 to 1000 genes are induced in response to the phagosome environment, including many involved in remodeling of the cell surface to resist host cell killing mechanisms.[109] These surface modifications confer resistance to antimicrobial factors within the phagosome, including antimicrobial peptides, oxygen, and nitrogen radicals. PhoP/PhoQ-regulated LPS modifications include addition of aminoarabinose, ethanolamine, palmitate, and 2-hydroxymyristate to lipid A, thus altering the charge density and fluidity of the outer membrane and discouraging antimicrobial peptide insertion in the membrane.[108] Cell surface polysaccharide is also dramatically altered.[108] In addition, PhoP/PhoQ-regulated modifications in lipid A structure produce an LPS molecule with significantly less proinflammatory signaling activity and repress flagellin synthesis, which may facilitate bacterial survival within host tissues.[108] PhoP/PhoQ mutants of *S.* Typhi are avirulent in humans and are promising live typhoid vaccine candidates.[110] *S.* Typhi also modifies its surface through synthesis of the Vi capsule, a polysaccharide structure that confers resistance to phagocytosis by neutrophils and killing by complement, reduces recognition of LPS, and promotes survival within human macrophages.[111]

Another strategy for intracellular survival of Salmonellae is to slow its growth through specific mechanisms. Strains with slower growth are termed "persisters" because they have greater resistance to antimicrobials as a result of growth slowing. These persisters do not have mutations but move to a nongrowing state as a result of use of toxin-antitoxin modules that can inhibit protein translation by acetylation of transfer RNA molecules.[112,113]

*Salmonella* has a second T3SS that is necessary for survival in the macrophage and for establishment of systemic infection.[114] Proteins delivered by both T3SSs are important for intracellular survival. SipA delivered by SPI-1 persists on the phagosome membrane, where it promotes intracellular survival.[115] Encoded on SPI-2 is an additional T3SS that is adapted to be expressed by intracellular bacteria and translocates proteins across the membrane of the SCV into the macrophage cytosol. SPI-2 translocated proteins are hypothesized to alter trafficking to the SCV to promote bacterial growth such that useful nutrients are routed to the SCV. Most remarkably, salmonellae alter the phagosome to tubulate in a mechanism that requires SPI-2 translocated proteins. Such tubulation has been correlated with virulence because SPI-2 translocated proteins implicated in this process are required for phagosome tubulation to occur. Phagosome tubulation is dynamic and rapid and appears to be dependent on the recruitment of microtubule motors, the activation of small GTPases, and membrane lipid alteration. The mechanism by which tubulation of the phagosome promotes virulence is unknown, but it could allow bacteria or their products to specifically traffic within the phagosome to different cellular localizations to promote nutrient acquisition or cell-to-cell spread.

SPI-2 and its proteins are essential for the *S.* Typhimurium phagosome to migrate away from the nucleus after phagocytosis.[116] Several SPI-2 translocated proteins, including SifA, SifB, SseJ, SopD2, PipB, and PipB2, localize to the surface of the SCV and either contribute to tubulation or other alterations of the phagosome.[104] This also may involve manipulation of GTPases and the microtubule network, as SifA binds RhoA and a host protein, Skp, which associates with the microtubular network, and SseJ is a RhoA-dependent glycerol cholesterol transferase that alters phagosome lipids enzymatically and could alter phagosome tubulation or trafficking.[117,118] The ubiquitin ligase SspH2 and the effector SseI, which modulates host cell migration, both localize to the phagosome and to the apical cell surface membrane of polarized epithelial cells through S-palmitoylation.[119,120] Other SPI-2 translocated proteins interact with the actin cytoskeleton surrounding the SCV and probably contribute to remodeling of vacuole-associated actin networks.[121,122] SpvB is a *Salmonella* virulence protein that is secreted into the macrophage cytoplasm, possibly by the SPI-2 T3SS, and adenosine diphosphate ribosylates monomeric actin (G-actin), thus promoting disassembly of actin networks around the vacuole.[122,123]

Other SPI-2 effector proteins alter ubiquitination by functioning as ubiquitin ligases or deubiquitinases.[124,125] Although the molecular targets of their enzymatic activity are not known, currently, the mechanism by which ubiquitin ligases compete with mammalian enzymes has been shown to be by co-opting intermediates in the mammalian ubiquitin pathway.[126] Other proteins appear to localize to the Golgi apparatus, possibly to promote secretory traffic to the SCV.[104,127]

Many other bacterial factors are required for full virulence, including those required for synthesis of essential nutrients and iron acquisition and the virulence plasmids found in many NTS serotypes. The virulence plasmids of *S.* Typhimurium, *S.* Dublin, *S.* Choleraesuis, and *S.* Enteritidis all contain an 8-kilobase region that promotes dissemination beyond the intestine in animal models and bacteremia in humans.[128] This region encodes the SpvB protein and several other proteins of unknown function.

## Host Response and Immunity

The innate immune system senses invasive *Salmonella* infections by using receptors that recognize conserved elements of bacterial structure. This includes recognition by plasma membrane and phagosomal membrane TLRs and cytoplasmic recognition receptors or the nucleotide oligomerization domain–like receptors (NOD-like): LPS by TLR4, bacterial lipoproteins by TLR2, flagellin by TLR5, flagellin by a signaling system that includes IPAF, and peptidoglycan by NOD1 and NOD2.[81,92,129] Activation of these receptors on phagocytes and epithelia leads to synthesis of cytokines that orchestrate the inflammatory response and instruct the subsequent antigen-specific immune response. Mice lacking a