We should be measuring genomic bacterial load and virulence factors

Thiago Lisboa, MD; Grant Waterer, MD, PhD; Jordi Rello, MD, PhD

Objective: **To describe relevant pathogen-related characteristics and their impact on sepsis pathogenesis and prognosis.**

Data Source: **Current literature regarding genomic bacterial load and virulence factors, with an emphasis on the impact of these factors on pathophysiology and prognosis of sepsis.**

Data Extraction and Synthesis: **The current paradigm on sepsis pathophysiology and management overlooks aspects concerning the nature and characteristics of the infecting pathogen. Our findings suggest that evaluation of genomic bacterial load might be useful to assess severity and predict prognosis in septic patients; its use during treatment for monitoring clinical response is another interesting potential application. Virulence factors identification might help to develop pathogen-specific therapeutic strategies for higher-risk septic patients.**

Conclusions: **The recognition of the importance of quantifying the pathogen has major clinical implications and will open up a new field of exploration of therapies targeted at anticipating development and appropriate treatment in severe sepsis. The improved detection and understanding of bacterial virulence factors may lead to specific therapies. (Crit Care Med 2010; 38[Suppl.]:S656–S662)**

KEY WORDS: genomic bacterial load; virulence; pathogenesis; sepsis; infection; pathogen

The current paradigm on sepsis pathophysiology and management highlights the importance of host response and overlooks aspects concerning the nature and characteristics of the infecting pathogen. Standard sepsis definitions do not focus on the site of infection or the causative pathogen. Thus, sepsis is often considered as a single entity, with little or no reference to the causative agent or the anatomical focus on infection (1). Clinically, the nature of the organism is critical in that many possess specific characteristics that have considerable prognostic significance. Specific virulence factors influence the manifestations, morbidity, and mortality of sepsis (1–3). Nonetheless, availability of new molecular techniques that allow quantitation of genomic bacterial load raised interest on the effects of different levels of bacterial burden on clinical evolution and prognosis (1, 2). Here, we will discuss why we should assess genomic bacterial load and the expression of virulence factors in septic patients, highlighting the importance of pathogen-specific characteristics in addition to the current paradigm of host response on the pathophysiology, clinical evolution, and prognosis of sepsis.

From the Critical Care Department (TL), Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; School of Medicine and Pharmacology (GW), University of Western Australia, Perth, Australia; Critical Care Department (JR), Hospital Vall d'Hebron, Universidad Autonoma de Barcelona, Fundació Institut de Recerca de l'Hospital Vall d'Hebron, Barcelona, Spain.

Supported, in part, by CIBERes (06/06/36), Agència de Gestió d'Ajuts Universitaris I de Recerca (2009/SGR/1226), and Fondo de Investigaciones Sanitarias de la Seguridad Social (08/0452 and 04/1500).

The authors have no potential conflicts of interest to disclose.

For information regarding this article, E-mail: jrello.hj23.ics@gencat.cat

DOI: 10.1097/CCM.0b013e3181f2453a

Quantitative molecular diagnosis and sepsis etiology

The ideal microbiological testing for patients with sepsis is still a matter of debate. Unfortunately, a specific etiological agent is determined in the minority of septic patients, even when the best available diagnostic methods are used. Nucleic acid amplification methods have the potential to improve the timeliness, sensitivity, and accuracy of the tests used to detect respiratory pathogens (4, 5). Advances in real-time amplification systems, multiplex analysis, and liquid-bead arrays have been key to the development of individual-pathogen and multipathogen panel approaches to diagnosis of sepsis (4).

Currently, microbiological testing in sepsis still relies on blood cultures, microscopic examination, and culture of specimens from the suspected focus on infection (6). By nature, microbiological culture is a slow process. Typically, culture-based procedures detect bacterial pathogens within 12–48 hrs, but fastidious pathogens such as yeasts can take longer. By conventional methods, microbial identification and drug susceptibility profiles require a further testing time of 6–24 hrs after isolation. Faster identification of microbial pathogens is achievable by using mass spectrometry (7). False-negative results do occur with blood cultures because of low microbial inoculum or growth inhibition by residual antibiotics in the sample. Therefore, faster and more sensitive diagnostic tests are needed to better target antibiotic therapy of bacterial and fungal infections and improve the management of patients with sepsis.

Culture of viable pathogens from the bloodstream seems intuitively superior to the presence of pathogen deoxyribonucleic acid (DNA) and is advocated with high priority in guidelines for the initial diagnostic workup for sepsis. However, the potential of blood culture to improve clinical decision making is hampered by a high proportion of negative results, and even if positive, the results are frequently obtained too late to affect the unfavorable outcome associated with inadequate empirical antimicrobial therapy (4). Molecular tools potentially offer a significant decrease in time requirements along with increased sensitivity compared with blood culture.

Molecular diagnostics hold much promise for the detection of common and atyp-

ical bacterial pathogens causing sepsis. Analysis can be completed in hours, rather than days, for the detection of typical pathogens and in weeks for the detection of atypical pathogens. This approach eliminates concerns about decreased organism viability associated with transport of specimens and the effects of previous antibiotic therapy. Real-time polymerase chain reaction (PCR) panels that include the common pathogens could substantially increase the diagnostic yield in clinical practice (8).

Recently Bloos et al (5) reported a multicenter prospective cohort study comparing PCR on blood samples to blood cultures in 142 surgical intensive care unit patients with severe sepsis, 54.5% of whom had microbiologically documented infection. The same tests were performed in a control group of 63 postoperative patients without signs of infection. Secondary end points included organ dysfunction scores, biological markers of systemic inflammation, and intensive care unit mortality. Whereas control patients showed only 4.5% positive PCR and 3.6% positive blood culture results, septic patients displayed 34.7% positive PCR tests and 16.5% positive blood cultures. An interesting concept derived was that PCR-positive episodes were associated with more severe disease, as indicated by significantly greater sequential organ failure assessment scores and increased interleukin-6 levels, even in patients with negative blood cultures. This association suggests either that microbial DNA, namely, a Toll-like receptor 9 ligand (9), contributed to the initiation of systemic inflammation in human sepsis or that a DNA amplicon indicates a causative rather than a colonizing or contaminating pathogen. This hypothesis is supported by the very high mortality rates in patients with fungal or polymicrobial sepsis. Bacterial and fungal DNAs are increasingly recognized as potent triggers of the host response. Consistently, amplicons indicating the presence of microbial DNA, even in the absence of viable bacteria, and its load on blood might indicate a role in the development of multiple organ dysfunction in the critically ill.

PCR-based pathogen DNA detection is biologically meaningful and clinically relevant in severely septic patients even in the absence of cultivable bacteria or fungi in blood, warranting investigation of its usefulness to guide antimicrobial therapy. Several techniques are being developed that accelerate the identification of infecting organisms detecting nucleotide sequences specific to pathogens in blood after standard culture. It is theoretically possible to undertake PCR-based amplification of sufficient magnitude to detect low copy numbers of DNA sequences, thereby eliminating the requirement for an initial period of standard culture (10). The utility of these techniques is currently limited by difficulties in differentiating contaminants and nonliving or degraded bacteria from clinically relevant isolates (1). An additional complicating factor is that increasing the number of pathogens that an assay can detect (multiplexing) almost always leads to some compromises in the primers, probes, and conditions that reduce the overall sensitivity for each organism. This is a major reason why multiplexed PCR assays have generally struggled to exceed the sensitivity of blood cultures in patients with sepsis (6, 11, 12).

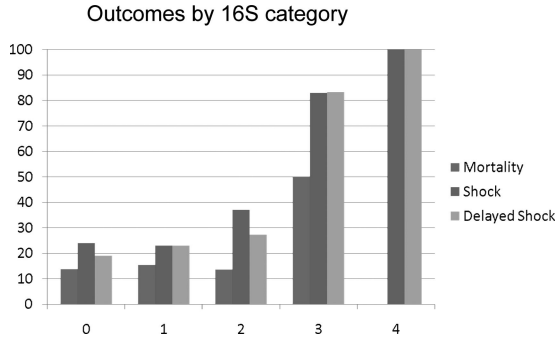

Figure 1. Outcomes according to 16S category in pneumococcal pneumonia patients.

The quantitative real-time PCR technique may provide a valuable diagnostic tool allowing the rapid detection of bacteria. There is no doubt about the fact that nucleic acid amplification techniques can overcome some of the problems associated with culture. They do not rely on viability of the organisms, thus making the storage and transport of samples a less likely source of error. Nucleic acid amplification techniques also offer exquisite sensitivity and speed, and real-time-based platforms also allow for quantification and bacterial load assessment (13).

Bacterial load and disease severity

Using microbiological quantitative load measurement is not a new concept in clinical practice. Human immunodeficiency virus type 1 ribonucleic acid quantification or viral load measurements are routinely used in the management of persons infected with human immunodeficiency virus type 1. Studies have shown human immunodeficiency virus type 1 ribonucleic acid levels to be a predictor of the time to progression to acquired immunodeficiency syndrome and death that is independent of CD4 cell count (14, 15). Viral load measurements are also useful in determining when to initiate antiretroviral therapy and in monitoring the response to such therapy. To determine whether changes in viral load represent clinically significant changes in viral replication, both intra-assay and biological variation need to be considered. Following this same rationale, bacterial load could also be used in order to assess severity, monitor microbiological response to therapy, and evaluate prognosis in septic patients. A few studies have tried this new approach in critically ill septic patients.

Our group investigated the relationship between pneumococcal bacterial load and clinical outcomes (16). It described an association between a high quantitative bacterial genomic load of *Streptococcus pneumoniae* in blood samples and increased mortality (Fig. 1). It also reveals an association between bacterial load and severity of disease, by the development of septic shock or the need for mechanical ventilation (MV). The implication of these findings is that a sample obtained for bacterial load measurement will provide valuable prognostic information. Also, these findings reveal some insight into the mechanisms that underlie the risk factors associated with death and shock in patients with pneumococcal pneumonia. Data demonstrate that patients with community-acquired pneumonia with >103 copies/mL of *S. pneumoniae* DNA in their blood at the time of emergency department presentation are an easily and specifically defined high-risk group for septic shock, the need for MV, and death. Furthermore, the detection of bacterial load was nearly twice as sensitive as blood cultures for the detection of pneumococcal bacteremia. The

strong correlation between clinical outcomes and *S. pneumoniae* bacterial load might modify the current paradigm of management for patients with pneumococcal community-acquired pneumonia. Invasive pneumococcal disease is associated with a high mortality despite antimicrobial therapy (17), and these findings are consistent with those of a smaller study (18) in children with invasive pneumococcal disease. Aside from the significant implications for therapy in patients with pneumococcal community-acquired pneumonia, these findings open up new insights into the biology of sepsis. The prevailing sepsis paradigm suggests that the primary driver is an excessive host inflammatory response to bacterial pathogens (19). After these data, we should consider that, at least in patients with pneumococcal pneumonia, a major determining factor for shock and organ failure development was bacterial load in the bloodstream. Intensity, and not only the presence of bacteremia in patients with pneumococcal pneumonia, is associated with poor outcome.

Another setting where such hypothesis was assessed was in meningococcal disease. Previous studies, mainly involving small pediatric samples, have demonstrated a correlation between bacterial load and death (20–22). Brandtzaeg et al (22) revealed a clear relationship between lipopolysaccharide concentrations in blood and cerebral spinal fluid specimens and the severity of meningococcal septicemia and meningitis and also with levels of plasma proinflammatory markers, such as tumor necrosis factor and interleukin-1. It can be suggested that a primary determinant for septic injury in patients with meningococcal disease is failure of the hosts' innate immunity to limit bacterial replication in sterile sites.

Among patients who survive, a high bacterial load was associated with loss of limbs, digits, or skin/soft tissue; with renal failure requiring hemodialysis; and with prolonged hospitalization. It also reveals that the influence of host factors (e.g., age and interleukin-1 genotype) on mortality is independent of an effect upon the intensity of bacteremia (23). In patients with meningococcemia, the proliferation of bacteria correlates with the production of lipopolysaccharide (22, 24), complement activation (25, 26), and cytokine or chemokine disruption, all of which affect the severity of disease and the likely clinical outcome (23, 27).

The observation that bacterial load measurements are unaffected by delay in sample submission or by the administration of antibiotics before hospital admission is relevant to the interpreting clinician. The implication of these findings is that a sample obtained for bacterial load measurement will provide valuable prognostic information. It also reveals some insight into mechanisms that underlie risk factors associated with death in patients with meningococcal disease (23).

The magnitude of bacteremia, as based on quantitative blood cultures, relates to the severity of *S. pneumoniae* infection (28). A high bacterial count in blood of children with bacteremia is associated with an increased risk of the development of more serious disease (29). Also, a shorter time to positivity of blood cultures, supposedly reflecting a higher initial bacterial load, is associated with a higher severity of disease (30). This relation between bacterial concentration and the severity of disease may also hold true for the level of *S. pneumoniae* DNA in blood.

Introduction of the genomic bacterial load enables clinical prediction of *S. pneumoniae* infection and helps to produce insight in the clinical interpretation of a positive DNAemia. Peters et al (31) described that 77% of patients with bacteremia had *S. pneumoniae* DNA detected from whole-blood samples. They also described a relationship between genomic bacterial load and several clinical and laboratory markers of the severity of disease: systemic inflammatory response syndrome and mental status alteration on admission and the level of C-reactive protein. Also, genomic bacterial load tended to be higher in patients with invasive disease and in those with a pneumonia severity index class higher than two. This confirms earlier findings in which higher *S. pneumoniae* genomic bacterial load was reported in children with meningitis compared with those with pneumonia, and in nonsurvivors vs. survivors (32).

This suggests that monitoring of the course of genomic bacterial load during treatment is another interesting potential application (33, 34). Like CRP, the course of genomic bacterial load might be indicative of response to treatment or the development of complicated disease (35). Important determinants for the potential application and clinical utility of genomic bacterial load will be the performance and turnaround time of the assay, logistics, organization of the laboratory services, and costs. These might represent important limitations on the applicability of such strategy on clinical practice.

An additional consideration opened up by our new understanding of the relationship between bacterial load and outcome is the possibility that antibiotic therapy itself may be responsible for the onset of severe sepsis. In patients with high bacterial loads, it is quite possible that rapid bacterial lysis from β-lactam antibiotics could initiate a massive antigen release and subsequent overwhelming inflammatory response. This may explain the well-recognized clinical phenomenon of patients developing shock some hours after presentation to hospital despite appropriate antibiotic therapy. If the hypothesis of antigen release due to bacterial lysis is proven to be true, and there is substantive evidence of antibiotics causing increases in the release of key sepsis-inducing cytokines such as tumor necrosis factor (36, 37), then our entire treatment strategy may need to be changed based on assessment of bacterial load. However, such strategies should be assessed prospectively and better evidence provided before changing clinical practice.

Importance of virulence factors

Bacterial virulence factors enable a host to replicate and disseminate within a host in part by subverting or eluding host defenses. The use of genomic techniques has led to the identification of new virulence factors that may serve as targets for new therapies (1, 38).

It is both timely and appropriate to refocus attention on targeting the bacteria. Although we now have potent molecular tools for identifying novel virulence determinants, they must be evaluated by complementary *in vitro* and *in vivo* studies and supported by relevant clinical observations to assess the appropriate impact on therapy. Identification of such virulence factors could help to develop specific therapeutic strategies for higher-risk septic patients (1, 2, 38). We here discuss specific virulence factors expressed by *S. pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

S. pneumoniae

It has been demonstrated that numerous protein virulence factors are involved in the pathogenesis of pneumococcal disease. An important related finding from

experimental animal models is that noncapsulated strains of pneumococci are protective against capsulated ones (39). Hence, new vaccine designs are focused on the surface proteins (e.g., PspA and PspC) and on the cytolysin, pneumolysin. Some antibiotics, especially macrolides, have demonstrated *in vitro* effects inhibiting the action of some virulence factors such as pneumolysin (40). This finding allows some insights regarding the usefulness of such drugs in septic patients, in particular those with community-acquired pneumonia.

The polysaccharide capsule has been considered the primary virulence factor of *S. pneumoniae* and is a major determinant in antibody accessibility to surface antigens. The pneumococcal capsule has a modular structure that facilitates the exchange of specific genes between serotypes. Depending on the site of infection, the combination of capsule type and genetic background is important in determining virulence (39). The pneumococcal intrastrain phenotypic variants (opaque and transparent) are evident in colonies growing in solid transparent media, such as trypticase soy agar, and are associated with both virulence and capsule expression. The transparent phenotype has less capsular polysaccharide, which exposes adhesive molecules; hence, the pathogen is able to bind closely to epithelial cells. In addition to the capsule, interactions between bacteria and host involve extracellular and intracellular virulence factors that are expressed by the pneumococci (39). Current research is focused on developing vaccines based on protein antigens common to all pneumococcal types. PspA displays high-quality protection in animal models (39, 41, 42) and is immunogenic in humans (43). This protein has a high polymorphism attributable to immunologic selection because it is readily accessible to antibodies. In contrast, pneumolysin is a highly conserved antigen capable of stimulating protective immunity and is an excellent vaccine candidate (38, 44).

S. aureus

The armamentarium of virulence factors of *S. aureus* is extensive, with both structural and secreted products playing a role in the pathogenesis of infection (45). Two noteworthy features of staphylococci are that a virulence factor may have several functions in pathogenesis and that multiple virulence factors may perform the same function. In establishing an infection, *S. aureus* has numerous surface proteins, called *microbial surface components recognizing adhesive matrix molecules* (MSCRAMMs), that mediate adherence to host tissues. It appears to play a key role in initiation of endovascular infections, bone and joint infections, and prosthetic device infections (45). Different *S. aureus* strains may have different constellations of surface components and so may be predisposed to causing certain kinds of infections. *S. aureus* can form biofilms on host and prosthetic surfaces, enabling it to persist by evading host defenses and antimicrobials (46). The ability to form and reside in biofilms is one reason why prosthetic device infections, for example, can be so difficult to eradicate without removal of the device. *S. aureus* is also able to form small colony variants, which may contribute to persistent and recurrent infection. *S. aureus* has many other characteristics that help it evade the host immune system during an infection. Its main defense is the production of an antiphagocytic microcapsule (most clinical isolates produce type 5 or 8) (45). During infection, *S. aureus* produces numerous enzymes, such as proteases, lipases, and elastases, which enable it to invade and destroy host tissues and metastasize to other sites. Unlike the structural components noted earlier, these antigens can produce a sepsis-like syndrome by initiating a "cytokine storm." Some strains also produce epidermolysins or exfoliative toxins capable of causing scalded skin syndrome or bullous impetigo (47). Different strains may contain different adhesins or toxins or may differ in their ability to produce biofilms and resist phagocytosis. The distribution of some virulence factors is related to clonal type, whereas the presence of others is unrelated to genetic background (48). In this regard, it is important to note that there is limited information on the expression of these genes during infection.

There is a strong epidemiologic association between Panton-Valentine leukocydin (PVL) and the emergence of community-acquired methicillin-resistant *S. aureus* infections (45). PVL is uncommonly found in methicillin-sensitive *S. aureus* and hospital-acquired methicillin-resistant *S. aureus* isolates. This may cause cells such as neutrophils to release inflammatory enzymes and cytokines (sublytic concentrations of PVL also appear to induce the release of these substances) (49, 50). PVL also appears to induce apoptosis of neutrophils via a mitrochondrial pathway at lower concentrations, whereas, at higher concentrations, PVL induces necrosis (51). Given this evidence and the strong epidemiologic association between PVL-containing community-acquired methicillin-resistant *S. aureus* strains and necrotizing pneumonia and skin and soft tissue infections, it is plausible that PVL is partly responsible for the enhanced virulence of community-acquired methicillin-resistant *S. aureus* (other leukocidins may also play a role). However, recent studies comparing the virulence of PVL-positive and PVL-negative strains have had conflicting results (45).

P. aeruginosa

Interactions between *P. aeruginosa* virulence factors and the host immune response dictate the severity and type of infection. Depending on the environmental conditions and the immune status of the host, *P. aeruginosa* can be a quiescent colonizer, a cause of chronic infection, or a highly virulent invader during acute infections (52, 53). Bacterial surface factors, such as flagella, pili, and lipopolysaccharide, as well as active processes, such as the secretion of toxins, biofilm formation, and quorum sensing, are virulence determinants that impact the outcome of *P. aeruginosa* infections (52, 53). Interaction with the host immune system via soluble and cell surface receptors (e.g., Toll-like receptors) controls signaling molecules (e.g., cytokines) and modulates the host response, which impacts disease severity both by influencing the rate of bacterial clearance and by causing collateral damage to host tissues (54–56).

P. aeruginosa secretes a number of toxins into the extracellular environment, but one set of toxins is injected directly into host cells. This occurs through a macromolecular syringe called a *type 3 secretion system* (57). The secreted toxins themselves are referred to as *effector proteins* and four of them have been identified to date: ExoS, ExoT, ExoU, and ExoY. The first three have been closely linked to virulence. Translation of our knowledge of the *P. aeruginosa* type 3 secretion system to the clinical setting is crucial for evaluating the potential of type 3 secretion neutralizing strategies as effective therapies. For example, the pres-

ence of a functional type 3 secretion system was associated with bacterial persistence in the lungs (and, therefore, perhaps clinical recurrence), higher relapse rates, and increased mortality in patients with acute respiratory infections caused by *P. aeruginosa* (58, 59). Furthermore, secretion of type 3 proteins was associated with increased mortality in patients with a high bacterial burden in respiratory secretions but who failed to meet clinical criteria for the diagnosis of ventilator-associated pneumonia (60). Efforts have also been directed at developing inhibitors that block other aspects of the *P. aeruginosa* type 3 secretion system, and specific active and passive immunization appears to have both pathogenic and prognostic significance in human infections.

In quorum sensing, small compounds called *autoinducers* are released by bacteria into the environment. Autoinducer concentrations are then sensed by neighboring bacteria to infer the density of the local bacterial population and to regulate gene expression accordingly. Numerous approaches have been used successfully to inhibit quorum sensing in culture and *in vivo* model systems. Further investigations are necessary to determine whether these approaches will prove efficacious in inhibiting quorum sensing in human infections (52).

Biofilms are bacterial cities, highly organized, microbial communities encased in a polysaccharide matrix and attached to a surface (61). When that surface is a surgical implant, endotracheal tube, catheter, or the airways of individuals with cystic fibrosis, biofilms become a medical problem. They are highly resistant to antimicrobial agents, and their resistance occurs by a number of mechanisms that are now becoming clear. Given their importance in *P. aeruginosa* pathogenesis, biofilms have been an obvious target for efforts aimed at therapeutic interventions. One approach has been to block the earliest step in biofilm formation: bacterial attachment. For example, coating endotracheal tubes with silver was associated with a delay in bacterial colonization, a reduction in bacterial burdens in mechanically ventilated patients, and a reduction in the incidence of ventilator-associated pneumonia (62, 63).

The flagellum of *P. aeruginosa* is required for swimming motility but also plays crucial roles in biofilm dispersal and adhesion to the surface of host cells (52). The significance of these processes in pathogenesis is underscored by the loss of virulence of nonflagellated mutants in models of acute infection (64, 65). Although 40% of *P. aeruginosa* isolates from patients with cystic fibrosis do not produce flagella, this virulence factor is still thought to be necessary for the initial infection of these patients (66). Animal models have demonstrated that antibodies against the flagellum, induced by either active or passive immunization, are protective (52). Prevention of *P. aeruginosa* lung infection by immunization against flagellar antigens might therefore be a suitable adjunctive therapy in individuals with cystic fibrosis. A recent randomized placebo-controlled trial of 483 patients with cystic fibrosis found a 34% reduction in infection episodes over a 2-yr period in those immunized with a bivalent flagella vaccine (67).

Future directions

The next 5 years promise to be a very exciting time in sepsis research. A key development will be improving analysis platforms to rapidly detect pathogens in clinical specimens, particularly blood. The time taken to produce results and the cost per sample should decrease with improvements in technology, enabling a rapid translation into clinical practice. The recognition of the importance of not just determining the presence of bacteria, but also quantifying the number of bacteria has major clinical implications and will open up a whole new field of exploration of therapies targeted at preventing severe sepsis, rather than our current approach of addressing the consequences once they have already occurred. Improved detection and understanding of key bacterial virulence factors also may lead to therapies with specific inhibitors. Although it has taken more than a decade to arrive, the promise that molecular testing has always shown may finally be delivered.

REFERENCES

1. Gao H, Evans TW, Finney SJ: Bench-to-bedside review: Sepsis, severe sepsis and septic shock - does the nature of the infecting organism matter? *Crit Care* 2008; 12:212
2. Cohen J: Diagnosing sepsis: Does the microbiology matter? *Crit Care* 2008; 12:145
3. Llewelyn MJ, Cohen J: Tracking the microbes in sepsis: Advancements in treatment bring challenges for microbial epidemiology. *Clin Infect Dis* 2007; 44:1343–1348
4. Struelens MJ: Detection of microbial DNAemia: Does it matter for sepsis management? *Intensive Care Med* 2010; 36:193–195
5. Bloos F, Hinder F, Becker K, et al: A multicenter trial to compare blood culture with polymerase chain reaction in severe human sepsis. *Intensive Care Med* 2010; 36:241–247
6. Dark PM, Dean P, Warhurst G: Bench-to-bedside review: The promise of rapid infection diagnosis during sepsis using polymerase chain reaction-based pathogen detection. *Crit Care* 2009; 13:217
7. Seng P, Drancourt M, Gouriet F, et al: Ongoing revolution in bacteriology: Routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009; 49: 543–551
8. Louie RF, Tang Z, Albertson TE, et al: Multiplex polymerase chain reaction detection enhancement of bacteremia and fungemia. *Crit Care Med* 2008; 36:1487–1492
9. Bauer S, Kirschning CJ, Häcker H, et al: Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci USA* 2001; 98: 9237–9242
10. Cleven BE, Palka-Santini M, Gielen J, et al: Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. *J Clin Microbiol* 2006; 44:2389–2397
11. Peters RP, van Agtmael MA, Danner SA, et al: New developments in the diagnosis of bloodstream infections. *Lancet Infect Dis* 2004; 4:751–760
12. Reier-Nilsen T, Farstad T, Nakstad B, et al: Comparison of broad range 16S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: A case-control study. *BMC Pediatr* 2009; 9:5
13. Apfalter P, Stoiser B, Barousch W, et al: Community-acquired bacteria frequently detected by means of quantitative polymerase chain reaction in nosocomial early-onset ventilator-associated pneumonia. *Crit Care Med* 2005; 33:1492–1498
14. O'Brien TR, Blattner WA, Waters D, et al: Serum HIV-1 RNA levels and time to development of AIDS in the Multicenter Hemophilia Cohort Study. *JAMA* 1996; 276: 105–110
15. O'Brien WA, Hartigan PM, Martin D, et al: Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. *N Engl J Med* 1996; 334: 426–431
16. Rello J, Lisboa T, Lujan M, et al: Severity of pneumococcal pneumonia associated with genomic bacterial load. *Chest* 2009; 136: 832–840
17. Parsons HK, Metcalf SC, Tomlin K, et al: Invasive pneumococcal disease and the potential for prevention by vaccination in the United Kingdom. *J Infect* 2007; 54:435–438
18. Carrol ED, Guiver M, Nkhoma S, et al: High pneumococcal DNA loads are associated with mortality in Malawian children with invasive

pneumococcal disease. *Pediatr Infect Dis J* 2007; 26:416–422
19. Cohen J: The immunopathogenesis of sepsis. *Nature* 2002; 420:885–891
20. Hackett SJ, Guiver M, Marsh J, et al: Meningococcal bacterial DNA load at presentation correlates with disease severity. *Arch Dis Child* 2002; 86:44–46
21. Lala HM, Mills GD, Barratt K, et al: Meningococcal disease deaths and the frequency of antibiotic administration delays. *J Infect* 2007; 54:551–557
22. Brandtzaeg P, Bjerre A, Øvstebø R, et al: Neisseria meningitidis lipopolysaccharides in human pathology. *J Endotoxin Res* 2001; 7:401–420
23. Darton T, Guiver M, Naylor S, et al: Severity of meningococcal disease associated with genomic bacterial load. *Clin Infect Dis* 2009; 48:587–594
24. Brandtzaeg P, Øvstebø R, Kierulf P: Quantitative detection of bacterial lipopolysaccharides in clinical specimens. *In:* Meningococcal Disease: Methods and Protocols. Pollard AJ (Ed). Totowa, NJ, Humana Press, 2001, pp 411–426
25. Brandtzaeg P, Høgåsen K, Kierulf P, et al: The excessive complement activation in fulminant meningococcal septicemia is predominantly caused by alternative pathway activation. *J Infect Dis* 1996; 173:647–655
26. Høgåsen K, Mollnes TE, Brandtzaeg P: Low levels of vitronectin and clusterin in acute meningococcal disease are closely associated with formation of the terminal-complement complex and the vitronectin-thrombin-antithrombin complex. *Infect Immun* 1994; 62:4874–4880
27. Bjerre A, Brusletto B, Høiby EA, et al: Plasma interferon-gamma and interleukin-10 concentrations in systemic meningococcal disease compared with severe systemic Gram-positive septic shock. *Crit Care Med* 2004; 32:433–438
28. Bell LM, Alpert G, Campos JM, et al: Routine quantitative blood cultures in children with Haemophilus influenzae or Streptococcus pneumoniae bacteremia. *Pediatrics* 1985; 76: 901–904
29. Sullivan TD, LaScolea LJ Jr, Neter E: Relationship between the magnitude of bacteremia in children and the clinical disease. *Pediatrics* 1982; 69:699–702
30. Peralta G, Rodríguez-Lera MJ, Garrido JC, et al: Time to positivity in blood cultures of adults with Streptococcus pneumoniae bacteremia. *BMC Infect Dis* 2006; 6:79
31. Peters RP, de Boer RF, Schuurman T, et al: Streptococcus pneumoniae DNA load in blood as marker of infection in patients with community-acquired pneumonia. *J Clin Microbiol* 2009; 47:3308–3312
32. Carrol ED, Guiver M, Nkhoma S, et al: High pneumococcal DNA loads are associated with mortality in Malawian children with invasive pneumococcal disease. *Pediatr Infect Dis J* 2007; 26:416–422
33. Peters RP, van Agtmael MA, Gierveld S, et al: Quantitative detection of Staphylococcus aureus and Enterococcus faecalis DNA in blood to diagnose bacteremia in patients in the intensive care unit. *J. Clin Microbiol* 2007; 45:3641–3646
34. Navarro E, Segura JC, Castaño MJ, et al: Use of real-time quantitative polymerase chain reaction to monitor the evolution of Brucella melitensis DNA load during therapy and post-therapy follow-up in patients with brucellosis. *Clin Infect Dis* 2006; 42:1266–1273
35. Póvoa, P, Coelho L, et al: Pilot study evaluating C-reactive protein levels in the assessment of response to treatment of severe bloodstream infection. *Clin Infect Dis* 2005; 40:1855–1857
36. Morrison DC, Bucklin SE: Evidence for antibiotic-mediated endotoxin release as a contributing factor to lethality in experimental gram-negative sepsis. *Scand J Infect Dis Suppl* 1996; 101:3–8
37. Holzheimer RG: Antibiotic induced endotoxin release and clinical sepsis: A review. *J Chemother* 2001; 1:159–172
38. Webb SAR, Kahler CM: Bench-to-bedside review: Bacterial virulence and subversion of host defences. *Crit Care* 2008; 12:234
39. Garcia-Suárez MM, Vázquez F, Méndez FJ: Streptococcus pneumoniae virulence factors and their clinical impact: An update. *Enferm Infecc Microbiol Clin* 2006; 24:512–517
40. Anderson R, Steel HC, Cockeran R, et al: Comparison of the effects of macrolides, amoxicilin, ceftriaxone, doxycycline, tobramycin, and fluoroquinolones on the production of pneumolysin by Streptococcus pneumoniae in vitro. *J Antibmicrob Chemother* 2007; 60:1155–1158
41. Gor DO, Ding X, Briles DE, et al: Relationship between surface accessibility for PpmA, PsaA, and PspA and antibody-mediated immunity to systemic infection by Streptococcus pneumoniae. *Infect Immun* 2005; 73: 1304–1312
42. Berry AM, Paton JC: Additive attenuation of virulence of Streptococcus pneumoniae by mutation of the genes encoding pneumolysin and other putative pneumococcal virulence proteins. *Infect Immun* 2000; 68:133–140
43. Nabors GS, Braun PA, Herrmann DJ, et al: Immunization of healthy adults with a single recombinant pneumococcal surface protein A (PspA) variant stimulates broadly cross-reactive antibodies to heterologous PspA molecules. *Vaccine* 2000; 18:1743–1754
44. Hausdorff WP, Feikin DR, Klugman KP: Epidemiological differences among pneumococcal serotypes. *Lancet Infect Dis* 2005; 5:83–93
45. Gordon RJ, Lowy FD: Pathogenesis of methicillin-resistant Staphylococcus aureus infection. *Clin Infect Dis* 2008; 46:S350–S359
46. Donlan RM, Costerton JW: Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 2002; 15: 167–193
47. Prévost G, Couppié P, Monteil H: Staphylococcal epidermolysins. *Curr Opin Infect Dis* 2003; 16:71–76
48. Peacock SJ, Moore CE, Justice A, et al: Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. *Infect Immun* 2002; 70:4987–4996
49. Hensler T, König B, Prévost G, et al: Leukotriene B4 generation and DNA fragmentation induced by leukocidin from Staphylococcus aureus: Protective role of granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF for human neutrophils. *Infect Immun* 1994; 62:2529–2535
50. König B, Köller M, Prévost G, et al: Activation of human effector cells by different bacterial toxins (leukocidin, alveolysin, and erythrogenic toxin A): Generation of interleukin-8. *Infect Immun* 1994; 62:4831–4837
51. Genestier AL, Michallet MC, Prévost G, et al: Staphylococcus aureus Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J Clin Inwest* 2005; 115: 3117–3127
52. Veesenmeyer JL, Hauser AR, Lisboa T, et al: Pseudomonas aeruginosa virulence and therapy: Evolving translational strategies. *Crit Care Med* 2009; 37:1777–1786
53. Sadikot RT, Blackwell TS, Christman JW, et al: Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. *Am J Respir Crit Care Med* 2005; 171:1209–1223
54. Kipnis E, Sawa T, Wiener-Kronish J: Targeting mechanisms of Pseudomonas aeruginosa pathogenesis. *Med Mal Infect* 2006; 36:78–91
55. Kurahashi K, Kajikawa O, Sawa T, et al: Pathogenesis of septic shock in Pseudomonas aeruginosa pneumonia. *J Clin Invest* 1999; 104:743–750
56. Lorenz E, Chemotti DC, Vandal K, et al: Toll-like receptor 2 represses nonpilus adhesin-induced signaling in acute infections with the Pseudomonas aeruginosa pilA mutant. *Infect Immun* 2004; 72:4561–4569
57. Frank DW: The exoenzyme S regulon of Pseudomonas aeruginosa. *Mol Microbiol* 1997; 26:621–629
58. Hauser AR, Cobb E, Bodi M, et al: Type III protein secretion is associated with poor clinical outcomes in patients with ventilator-associated pneumonia caused by Pseudomonas aeruginosa. *Crit Care Med* 2002; 30: 521–528
59. Roy-Burman A, Savel RH, Racine S, et al: Type III protein secretion is associated with death in lower respiratory and systemic Pseudomonas aeruginosa infections. *J Infect Dis* 2001; 183:1767–1774
60. Zhuo H, Yang K, Lynch SV, et al: Increased mortality of ventilated patients with endotracheal Pseudomonas aeruginosa without clinical signs of infection. *Crit Care Med* 2008; 36:2495–2503
61. Høiby N, Krogh Johansen H, Moser C, et al: Pseudomonas aeruginosa and the in vitro and in vivo biofilm mode of growth. *Microbes Infect* 2001; 3:23–35.

62. Kollef MH, Afessa B, Anzueto A, et al: Silver-coated endotracheal tubes and incidence of ventilator-associated pneumonia: The NASCENT randomized trial. *JAMA* 2008; 300: 805–813
63. Rello J, Kollef M, Diaz E, et al: Reduced burden of bacterial airway colonization with a novel silver-coated endotracheal tube in a randomized multiple-center feasibility study. *Crit Care Med* 2006; 34:2766–2772
64. Montie TC, Doyle-Huntzinger D, Craven RC, et al: Loss of virulence associated with absence of flagellum in an isogenic mutant of Pseudomonas aeruginosa in the burned-mouse model. *Infect Immun* 1982; 38: 1296–1298
65. Fleiszig SM, Arora SK, Van R, et al: FlhA, a component of the flagellum assembly apparatus of Pseudomonas aeruginosa, plays a role in internalization by corneal epithelial cells. *Infect Immun* 2001; 69: 4931–4937
66. Mahenthiralingam E, Campbell ME, Speert DP: Nonmotility and phagocytic resistance of Pseudomonas aeruginosa isolates from chronically colonized patients with cystic fibrosis. *Infect Immun* 1994; 62:596–605
67. Döring G, Meisner C, Stern M: A double-blind randomized placebo-controlled phase III study of a Pseudomonas aeruginosa flagella vaccine in cystic fibrosis patients. *Proc Natl Acad Sci USA* 2007; 104: 11020–11025