# Advancing Technologies for the Diagnosis and Management of Infections

Daithi S. Heffernan, MD, AFRCSI[a,*], Elizabeth D. Fox, MD[b]

**KEYWORDS**

• Infections • Diagnosis • Management • Advancing technologies

**KEY POINTS**

- No longer limited to culture-based methods of pathogen detection and characterization, or standard antimicrobial therapies, surgeons now have exciting new options for the diagnosis, treatment, and prophylaxis of surgical infections.
- Infections remain a significant problem among surgical patients. As technological advances continue, our understanding of the host pathogen interaction, especially in the era of computer-based modeling, will grow exponentially, expediting many research endeavors.

## INTRODUCTION

The history of surgical infections spans the ages from the tenets of the ancients encompassing tumor, rubor, dolor, and calor, through the beginnings of germ theory in the sixteenth century, into Fleming's earliest days of penicillin, arriving at the gates of Hades with the current epidemic of antimicrobial resistance. As the field has changed over the centuries, so too have diagnostic and therapeutic modalities. Technologies once exclusively found in cutting-edge laboratories are now being used as point-of-care testing and have expanded our ability to identify resistant organisms. Furthermore, although oral and parenteral antibiotics remain a mainstay of antimicrobial therapy for infections, novel technologies are expanding the therapeutic and prophylactic options available to clinicians. Enhanced delivery of antibiotics, local release of immunomodulatory and antimicrobial substances, and alterations in local physical and chemical properties are emerging as attractive alternatives to standard antibiotic therapy. Herein, the authors explore the emerging technologies that are poised to change surgical infections.

[a] Division of Trauma and Surgical Critical Care, Department of Surgery, Alpert Medical School Brown University, Rhode Island Hospital, 435 APC Building, 593 Eddy Street, Providence, RI 02903, USA; [b] Department of Surgery, Alpert Medical School Brown University, Rhode Island Hospital, 429 APC Building, 593 Eddy Street, Providence, RI 02903, USA
* Corresponding author.
*E-mail address:* Dheffernan@Brown.edu

Surg Clin N Am 94 (2014) 1163–1174
http://dx.doi.org/10.1016/j.suc.2014.08.013 **surgical.theclinics.com**
0039-6109/14/$ – see front matter 

## DIAGNOSIS

Microbial culture has long been the leading method of pathogen isolation and identification. However, it relies on highly skilled technicians and significant expenditures of time and money. Culture techniques may require up to 72 hours of incubation. Subsequent antibiotic susceptibility requires an additional 24 to 48 hours. Despite advances in culture techniques, there are still several critical shortcomings. In the context of multidrug-resistant organisms, the prolonged time to detect these resistance profiles leaves patients with prolonged durations of inappropriate antimicrobial coverage. Further, many of the current techniques fail to detect fastidious organisms or, in the case of a patient with recent exposure to antibiotics, results are reported as negative despite the presence of a virulent organism. Furthermore, there is an increasing need for rapid testing. Any delay in diagnosis and initiation of appropriate antimicrobial agents for an infection is well known to negatively affect outcomes. Kumar and colleagues[1] reported a 7.6% decrease in survival for every hour effective antibiotic therapy is delayed following the onset of sepsis shock. New diagnostic technologies have the potential to address many of the limitations of current culture-based technology with high automatization potential, low cost, and rapid return of results.

### *Polymerase Chain Reaction*

Polymerase chain reaction (PCR) is one of the earliest modern techniques for diagnosing infection, and its use in infections was first described in 1987.[2] PCR has been used in the diagnosis of infections, such as human immunodeficiency virus, *Clostridium difficile*, and in screening for methicillin-resistant *Staphylococcus aureus* (MRSA) carriage.[3] PCR relies on the amplification of nucleic acids. In the case of universal PCR, the 16S ribosomal RNA, common to all bacteria, is amplified. This amplification is then followed by DNA sequencing of the amplification products, probe hybridization techniques, or immunoassays. Specific PCR, however, relies on primers complementary to known DNA sequences of predefined bacteria to verify its presence. When compared with conventional culture techniques, PCR has been shown capable of diagnosing bacterial endophthalmitis in patients with prior exposure to antibiotics.[4] PCR processing times continue to shorten, including assays, such as SeptiFast (Roche Diagnostics, Indianapolis, IN), allowing for rapid adjustment of antimicrobial therapy.[5,6]

### *Next-Generation Sequencing*

Next-generation sequencing (NGS) integrates a variety of technologies to identify microbial DNA. First-generation (Sanger) genomic sequencing uses nucleotide chain termination and requires specific primers. NGS creates a variety of DNA fragments that then undergo parallel sequencing, massive parallel sequencing. This sequencing typically involves *whole-genome sequencing* of bacterial isolates permitting sequencing of an entire genome in less than a day. Organisms relevant to surgical practice, such as MRSA, *Escherichia coli, C difficile*, and carbapenem-resistant *Klebsiella pneumoniae* have been identified using this technology.[7] Despite the complexity and the ability to achieve higher throughput with greater characterization, the cost of NSG is comparable with the Sanger method.[8] PathoQuest and Pathogenica are currently involved in clinical trials to evaluate NGS in clinical microbiologic diagnostics. Beyond pathogen identification, this technique can assess for antibiotic resistance traits,[9,10] virulence factors, and genetically distinct isolates of tuberculosis.[11]

### *Microarray Analysis*

Microarray analysis offers high yield on a large scale allowing the detection and analysis of a large number of microbial genes, including specific strains of a bacterium with their virulence and resistance genes. Oligonucleotide probes are bound and immobilized on a microchip in a predefined array. A pathogen's nucleic acids are labeled with a fluorescent. These labeled nucleic acids are hybridized to the complementary immobilized probe on the array. This hybridization is then measured with a fluorescent scanner or cytometer. Advances in array techniques include the use of in situ synthesis on a quartz wafer surface, high- versus low-density arrays, or liquid-based suspensions. Microbial diagnostic microarrays have been used to characterize various *E coli*, *S aureus,* and *Pseudomonas aeruginosa* isolates[12] as well as to identify specific functional genes responsible for toxin production, virulence, and resistance.[13,14] Microarray analysis has further been able to detect subtle differences in coagulase profile among these *S aureus* isolates.[15]

### *Bacteriophages*

Bacteriophages are bacterial viruses that recognize their target host bacteria and inject their genetic material into them. Following this, the bacteriophage uses the bacteria to rapidly reproduce within the host bacteria, which ultimately kills the host bacteria releasing large quantities of bacteriophage progeny. This production of large numbers of rapidly produced bacteriophage progeny is thereby used as a secondary detector of the presence of the offending bacteria. Advances to this technology use the reporter labeling or luciferase assay.

Because bacteriophages are specific to a particular organism, bacteriophage diagnostics use this specificity to secondarily detect the presence of a bacterium. The speed of reproduction allows for near real-time detection of the infecting bacteria. Unlike PCR, which cannot distinguish between live and dead cells, bacteriophage technology relies on the presence of a live host bacterium capable of reproduction. If used in the presence of an antimicrobial agent, reproduction and, therefore, detection of the bacteriophage is possible only if the bacterium is resistant to the antimicrobial agent. The speed of production of bacteriophage progeny in a resistant organism, therefore, allows for earlier detection of a resistant bacterium. Advances in phage technology in multidrug-resistant tuberculosis[16] are now being applied across a variety of surgically relevant resistant organisms, including the KeyPath assay (MicroPhage, Longmont, CO),[17,18] in multidrug-resistant *S aureus* infections. Incubation with cefoxitin (a methicillin surrogate) provides further information about the presence of MRSA. The MicroPhage system yielded results 30 hours sooner than conventional microbiological methods. Bacteriophage technology has been used for both treatment and diagnostic applications *(see later discussion)*.

### *Microfluidics*

Microfluidics technology involves microscopic analysis of droplets of fluid, often incorporating a variety of techniques, including microscale PCR,[19] flow cytometry, and immunoassays.[20] Bacteria that are difficult to culture are particularly attractive targets for this technology. Difficult-to-culture microbes, including *Mycoplasma pneumoniae*, can be detected with equal sensitivity using Microfluidics-based techniques but in less than half the time of conventional PCR-based methods.[21] This microscale technology is particularly attractive for detection of infections in resource-limited developing countries.[20]

### *Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry compares a pathogen's mass spectrometry against a database of spectrometry profiles

from known pathogens.[10,22] Several groups have shown that the combination of PCR with mass spectrometry can accurately identify pathogens from whole blood and surgical site cultures. Examples include gene analysis for resistance profiles (MRSA) with results as quickly as 6 to 8 hours.[23,24] Using combination mass spectrometry/PCR techniques, Jacovides and colleagues[24] detected pathogens in revision arthroplasties from patients in whom the hardware failure was originally thought to be aseptic. The investigators proposed that this combination technology has the potential to identify previously undiagnosed and subclinical infections.

### *On the Horizon*

In vivo testing would allow for either pathogen detection or exclusion without the need for removal of such potentially noninfected devices. Paredes and colleagues[25] reported a "smart central venous catheter" prototype that uses a microelectrode to assess electrical impedance characteristics related to bacterial biofilm formation. The device is capable of detecting biofilm-related bioimpedance changes within 10 hours of the appearance of bacteria. A promising application of in vivo detection would be in immune-compromised patients who do not develop the typical infected profile of fever or leukocytosis.

## TREATMENT AND NANOTECHNOLOGY

Nanotechnology is the study and application of nanoscale particles. By definition, nanoparticles measure in the 1- to 100-nm range. Nanoscale substances potentially exhibit properties that differ chemically and biologically from the properties seen at the macroscale level. Nanotechnology in medicine uses these specific nanoscale-related alterations in properties to significantly improve drug delivery, overcome antibiotic resistance, and decrease the rates of implantable device-related infections. The antibacterial properties of silver are well recognized. However, decreasing the size of silver particles to nanoparticle range, the antibacterial activity is significantly increased. Several other metals, including magnesium and iron, exhibit bactericidal activity against *E coli* or *S aureus* when in nanoparticle size.

A vast number of compounds have been explored under the umbrella of nanotechnology. These compounds include liposomes (vesicles composed of a lipid bilayer), polymeric microspheres, and metal ion compounds, such as silver, zinc, and gold.[26] The surface of implantable devices as diverse as endotracheal tubes and prosthetic joints can undergo nanoscale modifications, leading to alterations in characteristics such as roughness, hydrophobicity, and charge. These changes are largely targeted toward achieving a particular concentration, bioactivity, or conformation of a compound that is needed to be adsorbed onto a material surface.[27]

### *Drug Design*

Antimicrobial therapeutics often belonged to limited categories: agents affecting bacterial cell wall synthesis, protein synthesis, and nucleic acid synthesis and modification. The epidemic of antimicrobial resistance drives technological advances in drug design and delivery.

The pore-forming toxins, drivers of the pathogenesis of *S aureus, Streptococcus pneumoniae,* and *E coli* infections, offer a potential therapeutic target. Nanosponges have been shown to effectively bind alpha-toxin (*S aureus*) and streptolysin O (*S pyogenes*), reducing cellular exposure to the toxins.[28] In these sponges, the polymeric core is coated with a red blood cell bilayer. This combination is capable of nonspecifically absorbing a wide variety of toxins. Prior immunologic approaches to

toxins rely on individualized knowledge of, and isolation of antisera from, a specific infecting organism. The Nanosponge could obviate the diagnosis of the exact infecting organism.

Photodynamic antimicrobial therapy, which synergizes visible light and photosensitizing drugs, has apparent effects independent of the specific infecting organism and has shown promise in localized skin and soft tissue infections. Diverse bacteria and fungi often colonize chronic lower extremity wounds, and treatment is limited by poor tissue penetration of antibiotics. Morley and colleagues[29] applied PPA904, a phenothiazine photosensitizer, coupled with phototherapy to chronic leg wounds in a placebo-controlled study. Immediate bacterial count reduction was noted in the treatment arm. This effect, however, was lost at 24 hours. Further studies are underway to determine if sustained eradication can be achieved with repeated dosing. Intriguingly, despite the lack of bacterial eradication, improved wound healing was noted in the PPA904 group at 3 months, indicating a potential immunomodulatory role beyond an antimicrobial effect.

Naturally occurring host defense peptides (HSPs), also called *antimicrobial peptides*, are components of the innate immune response in humans that have direct broad-spectrum antimicrobial effects.[30] They are also capable of activity against viruses and even cancer cells. These small naturally produced molecules are also capable of immunomodulation via their chemotactic properties, ability to alter gene expression, and modulation of inflammatory cytokines release.[31] The two most studied are $\alpha$- and $\beta$-defensins. Two such peptides, LL-37 and human beta-defensin, demonstrate antimicrobial activity against numerous gram-positive and gram-negative bacterial as well as fungi. Ongoing work is aimed at creating antimicrobial peptide elicitors, which are chemical or biological agents that enhance HSP expression.[31,32] A recently completed clinical trial (NCT01211470) found that the defensin peptide mimetic PMX 30063 (PolyMedix) showed high clinical response rates in the treatment of methicillin sensitive *Staphylococcus aureus* and MRSA-related acute skin and soft tissue infections.

Pleuromutilins represent a new class of antimicrobial agents. First discovered in 1959,[33] pleuromutilins exhibit unique bacterial protein synthesis inhibition by selectively binding to peptidyl transferase center of the 50S ribosome subunit. Retapamulin was the first approved topical pleuromutilin, and BC-3781 recently completed a phase II trial demonstrating systemic efficacy. Pleuromutilins demonstrate potency against methicillin-sensitive and methicillin-resistant *S aureus*, beta-hemolytic *Streptococci*, and *Haemophilus influenzae*.[34,35]

### *Treatment*

Bioavailability of antimicrobial agents can be increased through variations in nanoparticle formulations, including solid lipid nanoparticle formulations, nanosuspensions, and liposomal-mediated drug delivery. Directing nanoparticles to specific sites of infection reduces exposure of normal tissues and flora to the antimicrobial agent. Nanoparticle modification has improved isoniazid and rifampin delivery decreasing systemic toxicity.[36] Many of these processes require nanoparticle recognition of an epitope (a part of molecule or protein) found in the bacterial biofilm. Suci and colleagues[37] demonstrated that viral nanoparticles coated with antibodies to staphylococcal protein A (a surface protein and virulence factor) were markedly better able to target *S aureus* biofilms.

Nanotechnology offers potential in overcoming antimicrobial resistance.[38] Fayaz and colleagues[39] demonstrated that gold nanoparticles coated with vancomycin were capable of inhibiting vancomycin-resistant *S aureus*. Intriguingly, gold-vancomycin nanoparticles also exerted antibacterial activity against *E coli*, an

organism not normally susceptible to vancomycin given the drug's inability to penetrate gram-negative bacteria.[39,40] Gold particles are hypothesized to alter binding properties leading to the antimicrobial efficacy, a process called polyvalent inhibition.[41]

Beyond diagnostic applications *(*see later discussion*)*, bacteriophages demonstrate antimicrobial properties. Phages may have lytic properties wherein products of phages replication are capable of bacterial cell wall destruction, leading to bacteriolysis. Phages are further capable of producing large quantities of antimicrobial molecules or toxins, thereby potentially rapidly controlling microbial growth without the use of antibiotics. Biofilm formation reduction was noted within several hours of incubation with a bacteriophage directed against uropathogenic *E coli*.[42]

Bacteriophages have also been used for infection prophylaxis when conjugated to polymeric surfaces, such as polytetrafluoroethylene, preventing the growth of bacteria. The targeted specificity of each bacteriophage to a specific bacterium limits the activity spectrum to individual organisms, unlike the broad-spectrum activity of many currently available antibiotics.[43]

## PROPHYLAXIS

Indwelling device-related infections, such as those associated with Foley catheters and central lines, greatly contribute to hospital-associated morbidity. A recent survey from the Centers for Disease Control and Prevention found that pneumonia, urinary tract infections (UTIs), and bloodstream infections are among the most common nosocomial infections, accounting for 44.6% of all health care–associated infections.[44] A total of 39.1% of pneumoniae were associated with endotracheal tubes, 67.7% of UTIs were associated with catheter use, and 84% of bloodstream infections were associated with central venous line usage. These infections can be particularly difficult to treat because of biofilm formation. Nanoparticles incorporating a variety of molecules, including metals, peptides, and immunoglobulins, are able to alter biofilm physical characteristics, such as charge and surface topography.[27,45] Additionally, some nanoparticles can cause production of antimicrobial reactive oxygen and nitrogen species by leukocytes in response to an infection.[46]

Antimicrobial-coated indwelling devices are gaining acceptance, with up to 45% of hospitals using such technology.[47] Endotracheal (ET) tubes remain an important target for prevention of hospital-acquired pneumonia. In the NASCENT (North American Silver-Coated Endotracheal Tube) trial, patients with silver-coated ET tubes had a 36% relative risk reduction for development of ventilator-associated pneumonia.[48] Machado and colleagues[49] demonstrated that nanomodification of ET tubes, undertaken by enzymatically roughened ET tubes, was associated with a 1.5 log reduction in colony-forming units of *S aureus* compared with standard ET tubes.

Central venous catheters (CVCs) similarly are often causatively associated with nosocomial infections. Aggregate analyses of multiple randomized control trials of CVCs impregnated with silver sulfadiazine and chlorhexidine note a 40% reduction in CVC-related blood stream infections.[50–52] Using DNA subtyping to confirm infection, Maki and colleagues[53] noted a 5-fold reduction in blood stream infections with the use of silver sulfadiazine– and chlorhexidine-coated CVCs. Antimicrobial coating of CVCs with minocycline-rifampin has also been shown to reduce catheter colonization and blood stream infection.[54] Roe and colleagues[55] demonstrated that silver coating of central venous catheters can inhibit growth of a variety of common pathogenic bacteria, including *S aureus*, *E coli*, and *Candida albicans*, and reduce biofilm formation. Advances in this technology include the addition of platinum into the catheter facilitating local release of silver ions.

Indwelling urinary catheters account for most of the nosocomial UTIs. Silicon quaternary ammonium salt can form a positively charged film on urinary catheters. When applied to catheters before insertion followed by twice-daily application to both the urethral orifice and catheter, rates of UTIs are significantly lowered.[56] In a preclinical study, urinary catheters modified with a nitric oxide and acetic acid impeded bacterial growth and biofilms formation from clinically significant organisms, including *P aeruginosa, K pneumoniae,* and *Enterococcus faecalis*.[46]

Orthopedic implant infections are associated with significant morbidity. Nanotechnology has been used to alter the surfaces of joint prostheses by means such as impregnation of chlorhexidine or covalently immobilizing antibiotics, such as vancomycin.[57] Further, the use of immunomodulatory molecules has been reported.[58] Nanoscale coating of implants with the macrophage migration and activation modulators monocyte chemoattractant protein-1 and interleukin-12 has been demonstrated to lower infection rates and improve bone healing.[58] Bone grafting, which involves implanting devitalized bone, is associated with infection rates of up to 15%.[59] A fibrin gel mix can be created by impregnating the bone graft with vancomycin-alginate beads. Chang and colleagues[60] demonstrated that this antibiotic-impregnated bone graft was capable of bactericidal activity.

Surgical site infections (SSIs) represent a particular challenge for the surgeon. Technological advances aimed at this problem range from antimicrobial-coated sutures to skin sealants and biological dressings. Data from meta-analyses on antimicrobial-impregnated sutures remain discordant, with Edmiston and colleagues[61] demonstrating a reduction and Chang and colleagues[62] showing no significant benefit in superficial SSIs. Topical sealants achieve antimicrobial effects by preventing migration of skin flora into the incision and preventing recolonization of these spaces. Dohmen and colleagues[63] demonstrated a 76% relative risk reduction in cardiac surgery SSIs using a cyanoacrylate-based polymers (IntéguSeal, Kimberly-Clark Health Care, Roswell, GA). A Cochrane review found a significant reduction in the rates of SSIs using antimicrobial sealants, although enthusiasm for this technology remains limited.[64] Fowler and colleagues[65] assessed preoperative vaccination against *S aureus*. Not only did vaccines not reduce rates of wound infections but rather, among those who did develop an *S aureus* infection, mortality was higher among those who had been vaccinated.

## COMPUTATIONAL MODELING

Many surgical infection dicta tend to be linear and sequential. Patient factors such as being immunocompromised may allow nonvirulent organisms to prove fatal. Conversely in immunocompetent patients, intrinsic organism factors, such as virulence or antimicrobial resistance, dictate outcomes. However, this sequential thinking does not take into account the multidimensionality and complexity of the biological systems that are truly occurring in both patients and the microbe. Patients are not genetically identical; treatments vary widely by practitioner, and inflammatory responses to bacteria range from local wound infections to profound inflammatory responses, tissue destruction, and organ failure. These changes in host cells and tissues may induce changes in the bacteria during the course of an infection. Hypophosphatemia can induce gene transcription for motility and virulence factors in pseudomonas, potentially leading to a necrotizing infection. Replete phosphorus will stop such gene transcription.[66] Such complexity quickly outstrips our traditional modeling used to analyze these findings.[67]

Dynamic computational modeling and simulation has been demonstrated to be a useful approach in integrating mechanistic knowledge and bridging different scales

of biological organization (genes → molecules → cells → cell population/tissue → organs → entire patient). The resulting models can aid in the analysis of overall system behavior as well as serve as experimental objects to be used alongside traditional biological models.[68] Advances in technology have created virtual bench spaces and computer-simulated experiments. Agent-based modeling (ABM)[69] uses computer simulation to allow repeated alteration and manipulation of the complex biological systems. Computer-based models are built on our existing understanding of how either the pathogen or the patients change in response to small and incremental changes in the environment.

Many traditional experimental approaches follow a standard logistic of altering only one factor at a time and following a limited number of measurable outcomes in a linear fashion. However, ABM allows for very rapid simultaneous manipulation of multiple facets of an experiment, creating the complex reality that exists wherein the pathogen may alter the host, which in turn feeds back to alter the pathogen. These experiments include alterations in microbe virulence,[70] tissue environment including electrolyte disturbances, alterations in circulation or nutrient flow and patient immune response, both cytokine and cellular alterations,[71] as well as a better understanding of the mechanisms of antimicrobial resistance.[72] Key findings from these computer-generated experiments can then be confirmed with traditional bench-based experimentation in a much more time- and resource-efficient manner.

ABM and computational modeling have led to fresh insights into the role bacteria play in enteric anastomotic breakdown[73] as well as improved delineation of the pathogenesis of necrotizing enterocolitis. Arciero and colleagues[74] assessed the optimal time to administer probiotics by varying a wide range of factors spanning from organism virulence, the infant's potential cytokine responses, type of feeding administered, and even the route of birth of the infant. Kim and colleagues[75] used ABM to assess the complex interaction between oxidative stress, toll-like receptor-4 activation, and mucus barrier dysfunction in the pathogenesis of necrotizing enterocolitis.

In essence, advances in technology have connected complex biological patterns with clinical outcomes through the use of advanced computing and analytical methods. It is potentially possible that all animal-based experimentation will need to be grounded in ABM to circumvent the need for exhaustive animal or human experimentation.

## SUMMARY

Infections remain a significant problem among surgical patients. As technological advances continue, our understanding of the host-pathogen interaction, especially in the era of computer-based modeling, will grow exponentially, expediting many research endeavors. No longer limited to culture-based methods of pathogen detection and characterization, or standard antimicrobial therapies, surgeons now have exciting new options for the diagnosis, treatment, and prophylaxis of surgical infections.

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