

Introduction

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Abstract This introductory chapter discusses the problem of drug resistance and persistent medical biofilm infections, emphasizing the need for alternative approaches to the prevention and treatment of biofilm infections. Such alternative approaches are described in subsequent chapters, culminating with clinical studies that describe treating otherwise untreatable wound infections with the aid of antibiofilm approaches.

1 The Problem: Untreatable Bacterial Infections^{1–4}

The discovery of penicillin by Fleming in 1929 opened the era of antimicrobial chemotherapy, which has saved millions of lives by bringing many serious bacterial infections under control (Drews 2000; Fleming 1929). However, this medical miracle is being eroded by the emergence and spread of bacterial drug resistance. This problem has become a serious global issue. For instance, *Staphylococcus aureus* and *S. epidermidis* are leading causes of hospital-acquired infections, and the mortality associated with *S. aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials (Lowy 2003). Of the 2 million nosocomial infections each year, staphylococci cause over 90 000 deaths a year in the United

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States alone (Lowy 2003). The first effective antibiotic against *S. aureus*, penicillin, became available in the 1940s. Soon after, the bacteria evolved resistance to penicillin, and by the late 1950s, 50% of all *S. aureus* strains were resistant. Today, fewer than 10% of *S. aureus* infections can be cured with penicillin. The next weapons against *S. aureus*, methicillin and cephalosporins, became available in the 1960s and 1970s. By the late 1970s, some strains (2%) of *S. aureus* had evolved resistance to these drugs. Today, as much as 70% of *S. aureus* isolated from U.S. hospitals are resistant to methicillin (Fig. 1). The last effective defense against methicillin-resistant *S. aureus* (MRSA) is vancomycin. However, the increasing use of vancomycin has set the stage for the evolution of vancomycin-resistant *S. aureus* (VRSA) (Lowy 2003; Appelbaum et al. 2006). Over the past 20 years, MRSA infections have been limited primarily to patients in hospitals or long-term-care facilities. However, recent reports of “community-acquired” MRSA infections are alarming.

The same trend is observed for *S. epidermidis*. A study of hundreds of clinical *S. epidermidis* isolates derived from clinical orthopedic infections associated with prosthetic devices indicated that 37–38% were resistant to beta-lactams such as oxacillin and imipenem, while resistance to penicillin, ampicillin, cefazolin, and cefamandole was consistently observed in over 80% of the strains. Forty-one percent were resistant to erythromycin, 16% to clindamycin, 10% to chloramphenicol, 23% to sulfamethoxazole, and 26% to ciprofloxacin (Arciola et al. 2005).

Another example is *Pseudomonas aeruginosa* infections. *P. aeruginosa* is the fourth most commonly isolated nosocomial pathogen, accounting for 10% of all hospital-acquired infections. The gram-negative bacterium *P. aeruginosa* is adept at infecting many different organs and tissues. Because it causes disease primarily in persons whose health is compromised in some manner, it is considered an opportunistic pathogen. Mechanical ventilation, for instance, predisposes patients to pneumonia caused by *P. aeruginosa*. Likewise, the presence of a urinary catheter is associated with an increased risk of urinary tract infections. Patients with cancer who have neutropenia resulting from chemotherapy or hematologic malignan-

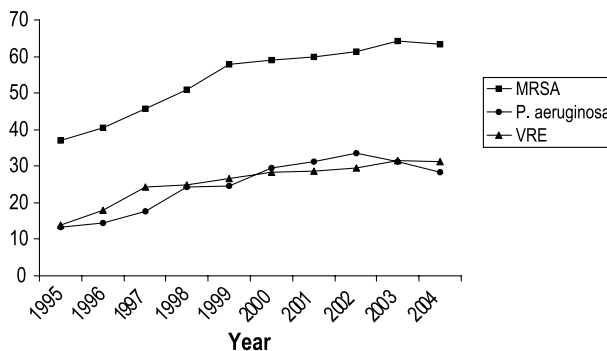


Fig. 1 Percentage of nosocomial infections caused by methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and fluoroquinolone-resistant *P. aeruginosa* in intensive-care patients in the United States in 1995–2004 (data source: National Nosocomial Infections Surveillance)

cies are prone to bacteremia, and burn patients often experience wound infections. Although each of these infections is most often categorized as hospital-acquired, *P. aeruginosa* frequently causes community-acquired infections in patients with cystic fibrosis (Hauser and Sriram 2005). *P. aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed; for example, fluoroquinolone-resistant *P. aeruginosa* strains have risen from 14% to 25% in the last 10 years (Fig. 1).

The rapid development of antimicrobial resistance could eventually lead to failure of most, if not all, of the currently available antibiotics. Hence, it poses a great threat to the economy and public health. While the problem is partially caused by overuse of antibiotics, it is also due to the inhibitory mechanisms of presently available antimicrobials. Most of these drugs were discovered for growth inhibition of individual cells in growing cultures—that is, in planktonic conditions (Stewart and Costerton 2001). However, the vast majority of bacteria exist within bacterial communities, otherwise known as biofilms (see below). The biofilm mode of growth plays an important role in antimicrobial resistance: Biofilm cells are up to 1000 times less susceptible to environmental stresses and disinfection treatments than planktonic (free-swimming) cells (Hoyle and Costerton 1991; LeChevallier et al. 1988). Whereas the planktonic cells are easily eliminated, the biofilm cells can survive and therefore provide a source of recontamination in both medical and engineering environments.

In clinical settings, biofilms are believed to be a common cause of persistent infections. The ability of biofilm-forming bacteria, such as *S. aureus*, *S. epidermidis*, and *P. aeruginosa*, to establish sessile communities on inert surfaces of medical devices or on dead as well as living tissue is now being recognized as a major problem (Costerton et al. 1999). Growing in biofilms, bacteria are protected against antibodies, leukocytes, and antibiotics. In addition, biofilms may spawn systemic infections by sloughing of planktonic bacteria, leading to dissemination, bacteremia, sepsis, and death.

2 Biofilm^{3,4}

Costerton et al. (1999) proposed a basic definition of biofilm as “a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface.” The matrix components can be exopolysaccharides, proteins, nucleic acids, or other substances (referred to as extrapolymeric substances, or EPS) that are believed to provide the cells with an array of advantages as compared to planktonic cells (Costerton et al. 1987, 1999; Anwar et al. 1990; Matz et al. 2004). This is important, especially in the clinical context, where it is estimated that about 60% of all microbial infections involve bacterial biofilms (Lewis 2001). (Refer to the case studies in the chapter *Clinical Wound Healing Using Signal Inhibitors*.)

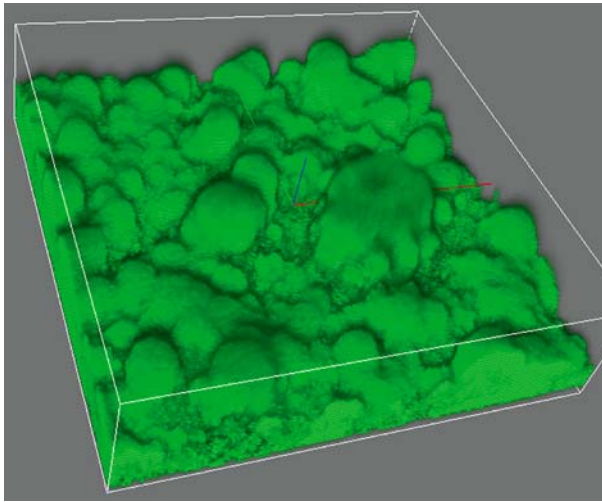


Fig. 2 Biofilm formed by Gfp-tagged *P. aeruginosa* in a continuous flow cell

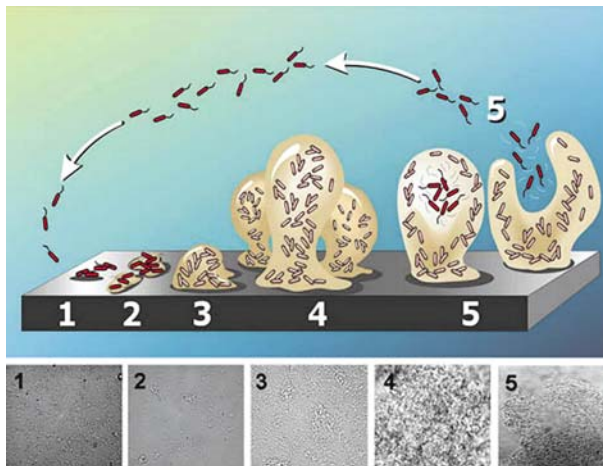


Fig. 3 Development of a *P. aeruginosa* biofilm. (1) Initial attachment. (2) Bacterial adherence. (3) Microcolony formation. (4) Biofilm maturation and development of three-dimensional structures. (5) Release/sloughing of cells able to form new biofilms. Reprinted, with permission, from the Annual Review of Microbiology, Volume 56 © 2002 by Annual Reviews www.annualreviews.org

Biofilms are not homogenous layers of cells; they are highly heterogeneous because they are comprised of patches of cells that are interspersed in the EPS matrix, which itself varies in density. This creates open areas where water channels are formed, allowing nutrients to enter the lower layers of the biofilm and, in addition, allowing waste products to be removed (Davey and O'Toole 2000; Dunne 2002). The bacteria found in a biofilm can either be of one species or it can, depending on the environment, be composed of multiple species.

In vitro biofilm formation by *P. aeruginosa* is one of the most intensively studied cases. After initial attachment of *P. aeruginosa* to a surface, microcolonies are formed, which in turn can grow to larger structures such as towers and mushrooms (Figs. 2 and 3). Recent analysis based on transcriptomics revealed that biofilm cells express their genes in a pattern that differs from that expressed by most stages of growth of planktonic bacteria, and the bulk of biofilm cells, even in the early stages, express genes in a pattern that is reminiscent of gene expression seen in the early stationary phase of planktonic cells (Hentzer et al. 2005). Although the experimental conditions would differ in the various experiments, the existence of a specific biofilm program would always require a core set of genes to be expressed, regardless of the experimental conditions. To date, transcriptomic studies such as of *P. aeruginosa* biofilms have not delivered such an outcome, and it strongly suggests that multiple pathways exist by which a biofilm can be built. Regardless, what is becoming evident is that bacterial cell-to-cell communication is required for a successful biofilm to form in vivo; this is discussed in subsequent chapters.

3 Resistance to Antibiotics¹⁻⁴

3.1 Inherent Bacterial Resistance to Antibiotics

P. aeruginosa and *S. aureus* will be used here as examples of antibiotic resistance. Several factors contribute to the antibiotic resistance of *P. aeruginosa*. It appears that the bacterium has an intrinsic resistance conferred by lowered permeability of the outer membrane as well as efflux pumps that rapidly shuttle many different compounds out of the cell (Hancock 1998; Lee et al. 2000). Five different efflux systems have been identified in *P. aeruginosa*, but the sequence analysis by Stover et al. (2000) suggests that there may be up to 30. The identified systems include the MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexJK-OprM, and MexXY-OprM systems (Adewoye et al. 2002). The highly homologous efflux pump proteins consist of a cytoplasmic-membrane-associated drug-proton antiporter, a membrane channel-forming protein, and a periplasmic fusion protein. The pumps have broad specificity and transport varying molecules, including dyes, detergents, antibiotics, organic solvents, and secondary metabolites and signaling molecules such as *N*-acyl homoserine lactone (AHLs) (Poole and Srikumar 2001). The antibiotics to which the multidrug efflux pumps confer resistance include chloramphenicol, gentamicin, trimethoprim, imipenem, and tetracycline as well as other quinolones, macrolides, and beta-lactams (Kohler et al. 1997, 1999; Yoneyama et al. 1997; Pumbwe and Piddock 2000). Other compounds also affected by the action of the pumps include the heavy metal vanadium (Aendekerk et al. 2002). In addition, *P. aeruginosa* (and staphylococci; see below) produce beta-lactamases encoded on the chromosome, conferring enhanced resistance to beta-lactam-based antibiotics such as imipenem (Bagge et al. 2002).

Control of Biofilm Infections by Signal Manipulation

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