

# Bacterial Cognition and Consensual Pathogenesis: The Microbiology of Intensive Care

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Most Intensive Care Units have a close working relationship with the Clinical Microbiologist or Infectious Diseases specialist. This is largely driven by the provision and interpretation of culture-based data from relevant clinical samples, as well as the difficulty and complexity of infection management in the critically ill. It is therefore somewhat disconcerting that much of the routine diagnostic yield from the microbiology laboratory is perceived as unhelpful.<sup>1</sup> While information content and delivery clearly can be improved, new understandings in bacteriology may help us to better manage the host-pathogen relationship. In this review, we revisit some traditional paradigms and look at new approaches to Intensive Care microbiology.

The human host environment varies enormously, and is subject to constant monitoring in the critically ill patient. Extracellular fluid distribution, protein binding, tissue perfusion and changing drug metabolism and elimination during shock and resuscitation are familiar variables. Local factors such as antibiotic penetration and activity in abscess cavities or ischaemic tissues are also factors we must consider when planning antibiotic therapy for sepsis. We will not deal with these topics further, but they are important to contextualise the discussion which follows, since changes in the host environment directly affect the behaviour of the invading pathogen and its response to therapy.

An anthropocentric view of microbiology has served us well for many years. We have traditionally defined medically important bacteria on the basis of criteria articulated by Robert Koch in the late nineteenth century for tuberculosis,<sup>2</sup> and the introduction of specific therapies against agents such as *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* are milestones in modern medicine. In general terms, Koch's postulates for defining the causative link of organism to disease state that the organism must be present in every case of the disease, and be isolated in pure culture from infected patients. The pure isolate must cause the disease when introduced into a new host, and be isolated again from that secondary host. The concept of the bacterial pathogen as a single species invader, armed with toxins and specific resistances to antibiotics and/ or immune defences such as phagocytes, has been

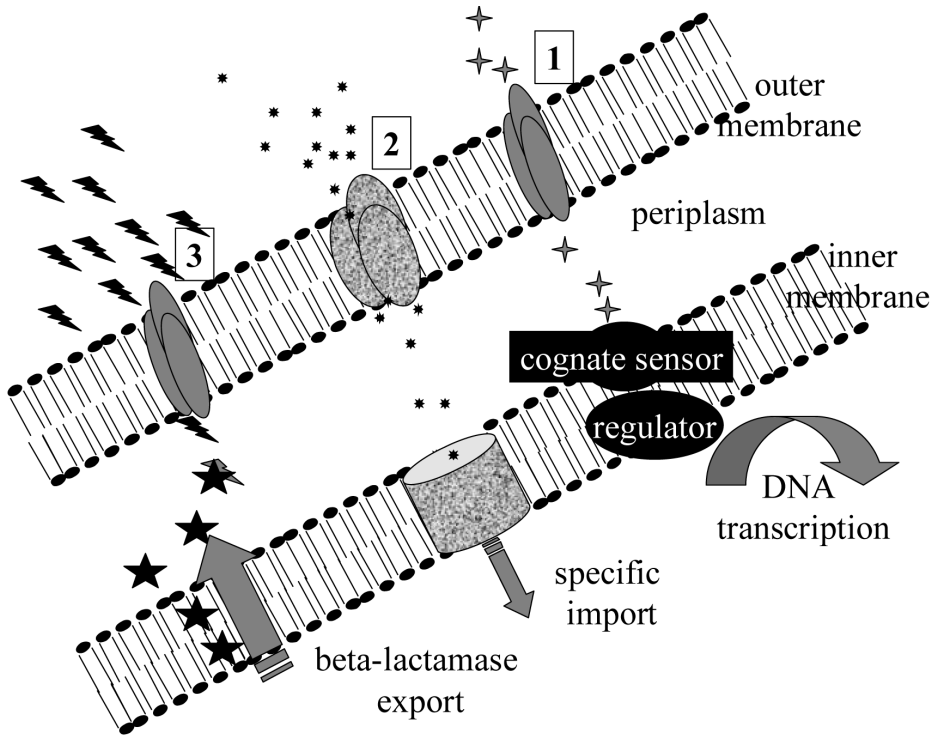
further modified to acknowledge that we cannot isolate every relevant pathogen in cell-free culture and that many organisms are diagnosable now only by culture-independent methods.<sup>3</sup>

### **Environmental sensing from the periplasmic space (Figure 1)**

The Gram-negative/Gram-positive divide reflects basic physical differences in bacteria. In general terms, the envelope of the Gram-negative organism (e.g. “enterics” such as *E. coli* and *Klebsiella spp.*, and “non-fermenters” such as *P. aeruginosa* and *Acinetobacter spp.*) is a much more sophisticated structure than that of the Gram-positive organism (e.g. *S. aureus*, *S. pneumoniae*). The Gram-positive envelope, designed to withstand physical stress, desiccation and environmental insult, simply comprises a cytoplasmic membrane surrounded by a thick layer of peptidoglycan, giving physical strength to a typically spherical organism. The Gram-negative envelope is dual, with a unique hydrophobic outer membrane. Transduction of energy to this is difficult and access through it is controlled by membrane channels (porins). These porins may allow facilitated diffusion for desirable nutrients or act as simple aqueous channels of relatively fixed diameter and charge. The expression and assembly of these channels are actively regulated, to alter overall “porosity” of the outer membrane, which in certain Gram-negative organisms such as *Acinetobacter spp.* (relatively tolerant of drying) may be several logs less than that of, say, *E. coli*. This allows rapid downregulation of porins to confer extreme antibiotic resistance at minimal biological cost,<sup>4</sup> and is also reflected in greater tolerance of drying and, therefore, enhanced survival on environmental surfaces and fomites.

The other practical consequence is the tiny but critically important area between the inner and outer membranes: the periplasmic space. This is protected by the hydrophobic Gram-negative outer membrane, and houses a number of important structures which give the Gram-negative bacteria unique advantages. A primary example is the array of delicate two-component sensor-regulator systems which “sense” the environment. Conformational change in cognate sensor proteins, upon binding relevant substrate, is transduced to the paired regulatory protein responsible for adjusting DNA transcription of messenger molecules in the cell. The sensed molecule thus triggers a relevant reaction or cascade of responses. The periplasm also provides a sheltered space for assembly of specialised structures such as protein adhesins, while enzymes such as beta-lactamases need not be highly active nor produced in large amounts to be effective, but can be contained in high concentration where they are most needed.

The inner (cytoplasmic) membrane is an active structure, easily energised and supplied with amino acids, and is the site for macromolecular assemblies such as transporter pumps and flagellar motors. Environmental sensing from the periplasm permits chemotaxis down specific concentration gradients, and regulatory systems facilitate adjustment to a new environment. *V. cholerae*, for example, senses certain specific sugars and amino acids, pH and temperature and, through a cascade of regulatory networks, switches off motility and switches on adhesins and toxin formation when the time is right.<sup>5</sup> This allows the organism to be vigorously motile in the mucus layer of the colon and the rice water stool shed into the environment, but to grow in adherent microcolonies utilising specific adhesins when in the gut. Tangled pilin bundles around the flagellar shaft illustrate the incompatibility of these systems when the organism is forced *in vitro* to simultaneously express both adhesion and

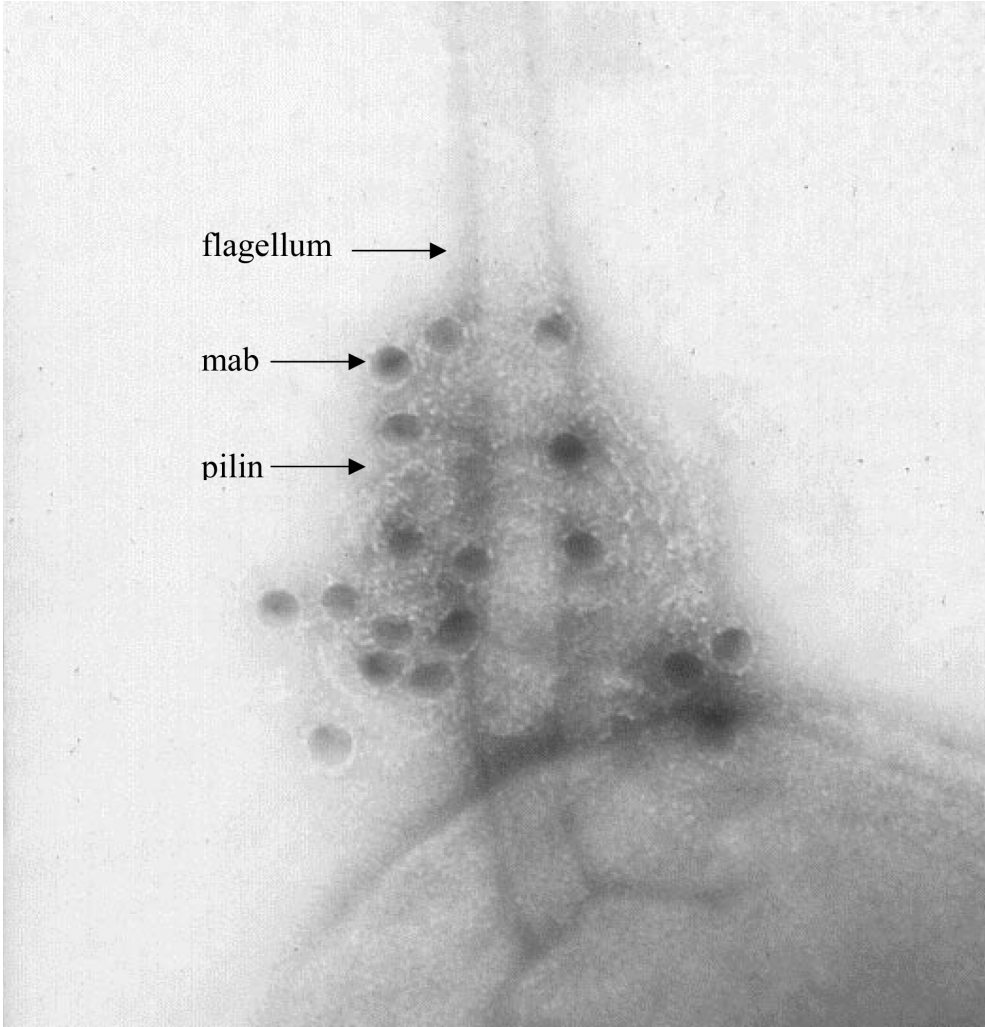


**Figure 1.** The periplasmic space. Sensing of the environment within the periplasm is by interaction with cognate sensor/s (1), which mediate DNA transcription via their paired regulatory protein. Specific porins may facilitate diffusion of desirable substances (2) into the periplasmic space for importation. Beta-lactamases are exported into the periplasm (3), to attack antibiotics which have got through the porin channels.

motility phenotypes (Figure 2). Bacteria are thus continually niche-adapting in real time, with a highly flexible repertoire of responses often poorly reflected by the *in vitro* phenotype reported from the diagnostic Microbiology laboratory.

### **Cognition and consensual pathogenesis: quorum sensing in bacteria**

The idea of bacteria as cognisant beings is fundamental to current concepts of bacterial pathogenesis. A truly cognisant being is not only able to sense its environment and adapt immediately, but is able to communicate with peers to coordinate action to mutual advantage. In bacteria this is referred to as quorum sensing.<sup>6</sup> Small secreted molecules send messages between bacteria, allowing them to co-ordinate responses in newly arriving cells. In its original form, this local signalling was recognised as population-density sensing, and it is now known to be widespread in eubacteria. These signals are complex and variable, and may also be recognised by other organisms. Signals may even be used as disinformation networks, to alter behaviour of arriving competing species so as to suit the incumbents. Many systems coexist in a single organism and coordinate sophisticated responses to like and competing organisms in real time.<sup>5,6</sup> In our cholera example, the bacillus is vigorously motile until it reaches the



**Figure 2.** 10 nm Au particle-labelled monoclonal antibodies (mab) can be seen decorating adhesin fibres (pilin) tangled around the polar flagellum of a cholera bacillus forced to simultaneously express both adhesion and motility phenotypes *in vitro*.

mucosa, where it stops and develops a microcolony. Once high population density is sensed, expression of cholera toxin causes the gut to flush the amplified microcolonies out to begin the cycle again.<sup>7</sup>

#### **Antibiotic sensitivity is context-specific**

Bacterial populations thus wax and wane in relatively short growth cycles. Clinically apparent bacterial infection can be visualised as a continuum from rapid replication to extinction and it is helpful to think in terms of the two extremes of the growth curve. An organism which is not actively growing and dividing is less susceptible to antibiotics, which characteristically target the machinery on which these processes are dependent.

The minimal inhibitory concentration (MIC) is the antibiotic concentration required to prevent exponential growth of the organism in question. It may be two or three logs higher for some drugs (e.g. aminoglycosides) in the stringent or stationary phase, than that which is derived in the diagnostic laboratory for bacteria in optimal growth conditions. It becomes easier to understand why early bacteraemic Staphylococcal endocarditis responds effectively to a combination of beta-lactam (cell wall-active) and aminoglycoside (protein synthesis inhibition) therapy for a few days, with rapid sterilisation of the bloodstream,<sup>8</sup> but why weeks of therapy with a cell-wall active agent are subsequently needed to prevent relapse (Figure 3).

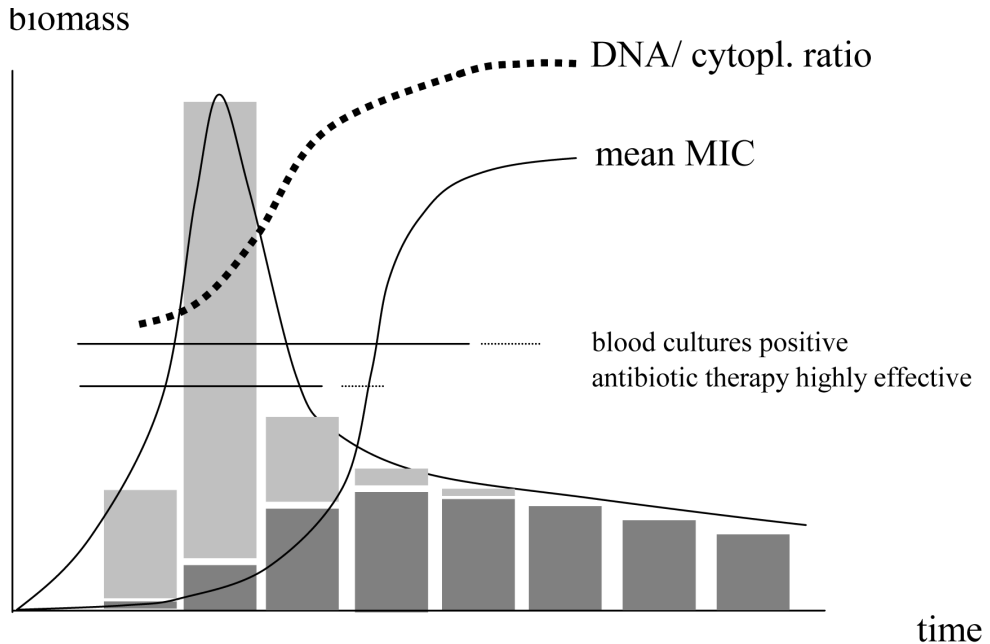


Figure 3. Hypothetical schema of the transition from vegetative growth to stringent state, with impact upon blood culture positivity and antibiotic susceptibility. During initial rapid replication, vegetative organisms (light grey) spread through the bloodstream and are readily cultured. The sensitive (vegetative) biomass quickly dies, and stringent state organisms (dark grey) become the dominant surviving biomass. Stringent state organisms have higher DNA/ cytoplasmic ratios, reflecting reductive division to preserve DNA mass at the expense of cytoplasmic activity (and thus, antibiotic sensitivity).

Likewise, infections of prosthetic material or the poorly vascularised long bone of an adult with chronic osteomyelitis is largely a stringent-state infection. This term means, as it implies, an adaptation to stressful and/or nutrient-limited environments. Reductive division to conserve DNA at the expense of cytoplasmic material and activity is driven by similar processes in Gram-positive and Gram-negative bacteria.<sup>9</sup> At its extreme, this can be seen as the sporulation response in organisms with that capacity (e.g. Clostridia) and the viable non-cultivable state in Gram-negative organisms, which is a normal response to physiologic stress.<sup>10</sup> In the stressed or stringent, most of the organisms in the population are very slowly growing and slowly dividing and so

relatively insensitive to growth and replication inhibitors. Bacteria have evolved to live on the brink of survival, and to launch into explosive growth (and a frenzy of genetic exchange, in many cases) when the opportunity presents itself. We see this at the bedside in an overwhelming infection, as clinical sepsis, which is exquisitely sensitive to appropriately targeted growth and replication inhibitors (antibiotics). Reversion to "ticking-over" (and less antibiotic-sensitive) growth is actually the more usual state.

Importantly, the common site for this stressed- or stringent-state growth is on surfaces which provide moisture and warmth but little nutrients. In a clinical environment, this can be the endotracheal tube, intravesical and intravascular catheters and so on. This helps to explain why growth of a microorganism from a prosthesis or catheter often cannot be eradicated by the antibiotic to which it seems exquisitely sensitive *in vitro*.<sup>11</sup> A dependent patient in the operating theatre or the Intensive Care Unit has a number of such ecological niches which can be opportunistically colonised, with the formation of a biofilm. Surgical and supportive care devices not only seed infection, as they are usually simultaneously breaching anatomical defences, but provide a reservoir of organisms which may be subjected to repeated or ongoing antibiotic selection pressure and are often competent for genetic exchange. Close similarities between the type of adhesins needed for biofilm formation<sup>12</sup> and those associated with specific DNA uptake<sup>13</sup> are probably no coincidence.

The biofilm growth characteristic of inert surface infection (e.g. catheter infection) is a normal adaptation for many Gram-negative bacteria and, when conditions are optimal, may be logarithmic and therefore quite antibiotic-sensitive. However, the biofilm is a loose three-dimensional structure with aqueous channels designed to optimise nutrient access in a nutrient-poor environment; it is an efficient system for stressed organisms and often supports stringent growth.<sup>11</sup> Secondly, many biofilms are associated with exopolysaccharide secretion, producing antiphagocytic barriers which protect the organisms directly (e.g. *Strep. viridans* in endocarditis). Finally, biofilms may form along with adherent fibrin or blood clot (e.g. endovascular catheter lumens), making antibiotic delivery to the organism more difficult again. All of these factors contribute to the difficulty of eradicating biofilm infections in the clinical environment.

### **Reconsidering the species concept in bacteria: the floating genome**

It is also important to remember that bacterial genomes are uniquely fluid. Small subunit (16S) ribosomal (rDNA) sequences have long been considered the most significant and reliable genotypic basis for bacterial speciation. However, this belief is undermined by work suggesting a role for horizontal gene exchange in generating intra-species diversity in bacteria,<sup>14,15</sup> and by recognition of the importance of mobile genetic elements such as gene cassettes in (especially Gram-negative) bacteria. This complexity has led to calls for complete review of the notion of species, which has translated poorly from Mendelian concepts of eukaryotic evolution.<sup>16</sup> The bacterial genome is both uniquely tolerant of extrachromosomal material, and uniquely flexible in genomic structure and content. A bacterial genome of between two and six million base pairs is not only several logs larger than a simple viral genome (e.g. around ten thousand base pairs for HIV or about twice that size for human coronavirus), but may house literally as many as hundreds of copies of extrachromosomal plasmids with more genetic information than the average virus in each plasmid. In addition to this huge increase in complexity, bacteria have a unique ability to exchange these extra-

chromosomal vehicles, to pick up DNA from outside the cell, and to integrate and excise DNA for biological advantage. This DNA pool is the “floating genome”,<sup>17</sup> the smallest common component of which is the integron, a gene capture and management system which is apparently unique to and ubiquitous in bacteria.<sup>18, 19</sup>

The floating genome can be considered as a genetic pool shared between bacteria, flux within which is determined by the transferability of the DNA, and the competence of various bacteria to receive it. Some of this genetic material is specifically packaged in plasmids or bacteriophages, which use a variety of mechanisms for entry, replication, and transmission. Their ability to move from one bacterium to another is termed the “host range”. Some vehicles (e.g. bacteriophages) may be extremely specific and confer unique virulence characteristics,<sup>20</sup> while others (e.g. “broad host-range” plasmids) may be highly promiscuous and can be picked up and transmitted by distantly related bacteria.

Bacteria which are able to survive antibiotic attack are usually swimming in a soup of DNA released by lysis of susceptible organisms, and thus exposed to potentially useful genetic material if competent to take it up. The highly dependent surgical or ICU patient usually has a greatly increased burden of microbes in the oropharynx, upper respiratory tract, wound sites and drains, monitoring devices etc, and the intensity and complexity of microbial interactions are greatly increased over that ordinarily seen in a healthy individual with all anatomical defences (such as skin, airway, urogenital tract) intact.

### **Getting it right: the essential tension in prescribing**

The necessity to get it right first time in sepsis is well established, with worse outcomes in bacteraemia and ventilator-associated pneumonia as a result of delays in appropriate antibiotic therapy.<sup>21-23</sup> Empiric broad spectrum antibiotics is the mainstay of therapy in the critically ill patient with suspected infection,<sup>1</sup> since only a minority of prescriptions are informed by a timely and relevant microbiological diagnosis.<sup>24-26</sup> Antibiotic usage is well documented to be associated with emergence of specific resistance,<sup>27-30</sup> and reduced usage of an antibiotic in the ICU setting has been shown to be associated with reduced resistance to that antibiotic in subsequently cultured isolates over a period of months.<sup>30</sup> Attempts to “cycle” antibiotics in an effort to reduce selection for resistance have met with some success,<sup>30, 31</sup> but only cautious acceptance as yet.<sup>32, 33</sup> Part of this caution reflects our imperfect understanding of the processes underlying resistance development and transmission, and the failure of traditional teaching in microbiology to provide us with more relevant paradigms.

Most Australian intensivists state that they need to cover 95% or more of the possible pathogens in critically ill patients with serious infection.<sup>1</sup> Protocol-driven prescribing is thus widely adopted as the primary principle, based on local and regional data about appropriate targeting (organisms, sensitivities). The competent prescriber for critically ill patients is therefore always trying to reconcile an inherent conflict between the need to “get it right first time” and the need to minimise the selection for resistance. There is a need for better quality information at the bedside if we are to manage this paradox more successfully in the future.

The majority (typically, around 70%) of Intensive Care Unit patients are on broad spectrum beta-lactam antibiotics such as cephalosporins and antipseudomonal penicillins,<sup>24</sup> often in combination with aminoglycosides and other classes. Antibiotic susceptibility profiles are often quite specific to a Unit, even within the same hospital

department.<sup>35</sup> When surveyed directly, most intensivists in Australia agree that antibiotic resistance is a problem and that local profiles are essential, but less than 40% state that they actually use local laboratory data to guide their decision-making. A significant percentage find “expert” advice from Infectious Diseases and Microbiology colleagues to be completely unhelpful.<sup>1</sup> Decision support systems are of proven value, with objective criteria applied in the scoring of ventilator-associated pneumonia having been shown to be effective in reducing antibiotic use without compromising efficacy.<sup>35</sup>

### Where do we go from here?

Thus while the use of bedside information systems is increasingly popular, it is heavily dependent on the information quality itself, and the models we use to interpret the data. It may be that we should be looking at different data altogether. If much of what we do in Intensive Care is empiric, and protocols need to be based on resistance potential and selection pressures, then perhaps we need to be using existing molecular techniques as Infection Control tools. Paired with a knowledge of the vehicles of genetic flux (the plasmids and transposons, and even the bacteria themselves), twice-weekly surveillance of representative sites such as the bronchial tree (e.g. via non-bronchoscopic BAL),<sup>36</sup> and perineum, wounds, drains and “usual” sites (sterile sites such as blood and CSF) when indicated, augmented by molecular tools to examine mobile resistance elements, should provide a relevant local census of the microflora and its resistance potential. Ultimately, advances such as real-time PCR and microarray (gene chip) technology, might even allow relevant patient-specific studies. Improved understandings of bacteria and how they respond to selection pressure and niche modification in the clinical context should promote better husbandry, not only of the microflora in an individual patient but the entire ecosystem in which we all participate.

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