Commentary

70th Anniversary Collection for the Microbiology Society: *Journal of Medical Microbiology*

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In the last 70 years, we have seen a radical change in our perception and understanding of the microbial world. During this period, we learned from Woese and Fox that there exists a third kingdom called ‘Archea’ based on the phylogenetic studies of the 16S rRNA that revolutionized microbiology (Woese & Fox, 1977; Woese et al., 1978). Furthermore, we were forced to reckon with the fact that Koch and Pasteur’s way of growing cells in test-tubes or flasks planktonically does not necessarily translate to the real-life scenario of bacterial lifestyle, where they prefer to live and function as a closely knit microbial community called biofilm. Thanks are due to Costerton, who led the crusade on the concept of biofilms and expanded its scope of inquiry, which forced scientists and clinicians worldwide to rethink how we evaluate and apply the data. Then progressively, disbelief turned into belief, and now it is universally accepted that the micro-organisms hobnob with the members of their community to communicate and coordinate their behaviour, especially in regard to growth patterns and virulence traits via signalling molecules. Just when we thought that we were losing the battle against bacteria, antimicrobials were discovered. We then witnessed the rise and fall of antibiotics and the development of antibiotic resistance. Due to space and choice limitation, we will focus on the three areas that caused this major paradigm shift (i) antimicrobial resistance (AMR), (ii) biofilm and (iii) quorum sensing (QS), and how the *Journal of Medical Microbiology* played a major role in advancing the shift.

Antimicrobial resistance (AMR)

It is notable that this 70th anniversary collection for the journal coincides with the 70th anniversary of the Nobel Prize to Florey and Chain for their work on penicillin, a β-lactam antibiotic. It was first used to treat a patient in 1942, which revolutionized medicine and ushered in the age of antibiotics. Yet, resistance mechanisms that could inactivate penicillin, the β-lactamases, were already present among bacteria (Abraham & Chain, 1940). As has been uncovered over the years, the origins of most drug-specific antibiotic-resistance mechanisms are genetically encoded functions that predate the introduction of the antibiotics. These mechanisms have been derived from the antibiotic-producing organisms, from the pathogens themselves or from commensals or environmental bacteria. Selective pressure by antibiotics has led to horizontal transmission of these resistance genes and further optimization (evolution) of the mechanisms (Davies, 1994; Davies & Davies, 2010).

The *Journal of Medical Microbiology* has published many insightful reviews on AMR, and important laboratory and epidemiological studies of the prevalence and spread of specific resistance mechanisms. A 1973 paper (Anderson et al., 1973) presented a study of antibiotic-resistance transfer in the intestines of human volunteers, finding that resistance-factors (R-factors) were successfully transferred when patients were treated with antibiotics, and not in the absence of treatment. A later review of plasmid-mediated, horizontally transmitted, enzymic resistance to ampicillin and trimethoprim was the subject of an Oakley Lecture (Amyes, 1989). This lecture acknowledged the growing numbers of resistance determinants to each antibiotic, but emphasized evolutionary patterns showing that the enzymes fell into a small number of groups. The metallo-β-lactamases,
which can hydrolyse the carbapenems, were flagged more than 20 years ago as an emerging medical challenge (Payne, 1993). That challenge was realized recently with the advent of the NDM-1 metallo-β-lactamase. A recent review used the rapid global spread of the NDM-1 β-lactamase as an example – and a warning – of the looming public-health problem of antibiotic resistance that may only be controlled through international cooperation (Johnson & Woodford, 2013).

It is not only transmissible factors that are responsible for AMR. Especially in Gram-negative pathogens, intrinsic impermeability of the cell envelope and efflux mechanisms also plays a role. Mutational changes in these factors can lead to decreased susceptibility. *Pseudomonas aeruginosa* is a particularly problematic pathogen because it utilizes a variety of these mechanisms to put up a formidable barrier, limiting greatly the number of classes of chemotherapeutics that can overcome those mechanisms, to β-lactams, aminoglycosides, fluoroquinolones and colistin. A recent review in the *Journal of Medical Microbiology* described this phenomenon (Strateva et al., 2014). In addition to permeability barriers and efflux, certain antibiotics, such as fluoroquinolones, can select mutations in the genes encoding their molecular targets. For the commonly used systemic monotherapeutic agents, resistance does not occur via single mutations in the target as they are generally not single proteins but are, rather, the products of multiple genes or a pathway (Silver, 2007). It is clear that new antimicrobial therapies are needed to bolster the diminishing antibacterial armamentarium, but this is a difficult undertaking (Silver, 2011). AMR has become a real and proximate threat that requires constant monitoring – and the *Journal of Medical Microbiology* remains at the forefront of the undertaking.

**Biofilm**

The notion that micro-organisms, in nature, exist as sessile, systematized, multicellular communities was first described in the late 17th century by Van Leeuwenhoek. He examined plaque samples from his own teeth and found them to be a complex aggregation of micro-organisms, which he then referred to as ‘animalcules’. Even though the biofilm mode of bacterial existence was known for several centuries, the concept was not promulgated until the late 20th century. The late 1980s and 1990s saw an increased recognition of the fact that bacteria in their natural habitat survive and function as organized communities, encapsulated within a polymeric matrix – a crusade led by Costerton, the pioneer and father of biofilm research (Costerton et al., 1987; Lappin-Scott et al., 2014).

In the years that followed, *P. aeruginosa* emerged as the model organism to glean information on the various aspects of the biofilm mode of bacterial lifestyle. Our appreciation for this lifestyle was facilitated by the advent of non-destructive investigation techniques, especially the use of live-monitor systems with confocal laser scanning microscopy. This provided an unprecedented ability to see (and almost feel) the beautiful architectural formations held together by an extracellular matrix. This also led us to question many aspects of biofilm that were accepted as a fact, especially where repetition has led to persuasion – the illusory truth effect. At that point, it was well established that *P. aeruginosa* secretes alginate, a complex exopolysaccharide made of guluronic and mannuronic acid that provides selective advantage for *P. aeruginosa* survival in the lungs of patients with cystic fibrosis (Song et al., 2003). The scientific community was persuaded that alginate was the matrix in *P. aeruginosa* biofilms. The *Journal of Medical Microbiology* was bold enough to publish the paper by Stapper et al. (2004) that questioned the ‘illusion of truth’. Notwithstanding the importance of alginate in infection, this paper alluded to the potential contribution of other polysaccharides, which turned out to be Psl and Pel polysaccharides (Ma et al., 2012; Jennings et al., 2015).

Micro-organisms coexist in a complex society that includes bacteria, fungi, archaea and viruses, forming the multifaceted polymicrobial or mixed-species biofilm communities. This provides the communities with a competitive advantage such as AMR, metabolic cooperation, quorum sensing (QS) and many other synergies. It is only in the last few years that we have begun to fathom the complex nature of the mixed-species biofilm phenotype and its physiological role during infection. Adam et al. (2002) presented a study on mixed fungal–bacterial biofilms using *Candida albicans* and *Staphylococcus epidermidis*, organisms that are frequently implicated in catheter-associated infections. Their findings highlighted the coexistence of different microbial species, and their interdependence in biofilm formation and AMR. In a follow-up study, the same group focused on the composition of *Candida’s* polymeric matrix, which contains carbohydrates, proteins, hexosamine, phosphorous and uronic acid, and its importance in promoting AMR (Al-Fattani & Douglas, 2006). Their work provided a critical insight into the complex biofilm phenotype. Following a series of articles in the journal, Wilson published an insightful review that highlighted the antimicrobial sensitivity in oral biofilms (Wilson, 1996). This review summarized studies pertaining to the susceptibility of oral bacteria to antimicrobials, based on the type of biofilm models used. The author also emphasized the need for research in studying the resistance profile of oral biofilm communities, which can be crucial in developing an efficient therapeutic regimen. The *Journal of Medical Microbiology* has been instrumental in advancing biofilm research by publishing these studies, the first of their kind to focus on the mixed-species biofilm and its contribution to AMR.

More importantly, the recognition of mixed biofilms in nature was the precursor to the microbiome studies that were facilitated by metagenomic analyses. Now, one could look at the potential relationship between groups of organisms within a single niche that makes a bacterial social network (Fernandez et al., 2015). This was clearly
facilitated by metagenomic analysis, heralded by PCR, that has become a breastwork in all laboratories, replacing traditional techniques. This has revolutionized how we view ourselves, realizing that the number of bacteria exceeds the number of human cells by 10:1. The ease with which DNA is being sequenced and the rapid growth of DNA databases, and the development of eubacterial-specific, fungal-specific and species-specific primers, will enable rapid detection of micro-organisms that will become a mainstay in clinical laboratories. Although we borrowed the knowledge of soil microbiologists who developed bacterial-specific primers over 20 years ago, we have to thank the human microbiome project (HMP) launched in 1990, which truly contributed to the refinement of many bacterial-specific DNA primers (Jaric et al., 2013). The Journal of Medical Microbiology contributed to this revolution by publishing two articles, one that focused on fungal-specific (Makimura et al., 1994) and the other on bacterial-specific (Harris & Hartley, 2003) analyses. The latter compared molecular diagnostics with standard culture techniques and offered a broad-range 16S rDNA PCR optimized to obtain the highest level of sensitivity for the detection of bacteria in clinical specimens. The future of rapid detection and diagnosis of infection truly rests on adopting molecular tools as part of routine clinical work.

The HMP studies have contributed significantly to our knowledge of the human microbiome (Turnbaugh et al., 2007). It is now known that the human gastrointestinal tract alone harbours a diverse array of microbes that are critical to host nutrition, regulation of intestinal angiogenesis and development of the immune response. Alterations in the composition of the intestinal microbiota have been associated with various disease states (Larson & Welch, 1993; Masseret et al., 2001). A leading example of this is Clostridium difficile-associated diarrhoea (CDAD), an infection caused by the Gram-positive C. difficile. CDAD occurs primarily in patients whose colonic microorganisms have been functionally altered by antibiotic therapy (Kelly & LaMont, 2008). C. difficile was identified in 1935 in a stool sample from a healthy neonate (Hall & O'Toole, 1935). However, it was not until 1976, just over four decades after its identification, that it was successfully cultured using reinforced clostridial medium (Hafiz & Oakley, 1976). The Journal of Medical Microbiology took the lead in publishing the findings at a time when C. difficile was still considered a human commensal and was not associated with any disease. It was later in 1978 that C. difficile was first reported to be a human pathogen (Larson et al., 1978). In recent years CDAD has become rampant, with morbidity and mortality increased over decades past (Kelly & LaMont, 2008; Britton & Young, 2014). Colonic dysbiosis due to the use of antibiotics favours C. difficile germination and growth, as it is resistant to most of the antibiotics (Knecht et al., 2014; Theriot et al., 2014). Following the HMP, many studies have targeted alternative therapeutic regimens against C. difficile, including faecal transplantation. The Journal of Medical Microbiology has always been in the vanguard, even before the HMP published data pertaining to the faecal microbial load (Stephen & Cummings, 1980) and the effect of C. difficile infection on the faecal microbiome (Hopkins & Macfarlane, 2002).

**Quorum sensing (QS)**

In the 1960s and 70s, scientists decried the notion that bacteria have the ability to communicate. In the 1990s this notion was christened as QS, by which bacteria can sense bacterial numbers (cell density), integrate and process the environmental cues, and coordinately modify their behaviour by expressing target genes (Nealson & Hastings, 1979; Greenberg, 2003). Now, ample evidence exists for inter-species, inter-genera and inter-kingdom communications using largely diffusible small molecules called quorumones or autoinducers (Williams, 2007). In Gram-negatives, these molecules are largely made of small chemicals, whereas Gram-positives tend to use oligopeptides. These signalling molecules interact with cognate receptors. It became clear that bacteria employ QS to coordinate the expression of virulence factors critical for the initiation and establishment of infection. It was also shown that the QS molecules themselves acted as virulence factors by being potent stimulators of multiple eukaryotic cells (Smith & Iglewski, 2003). Subsequently, a flurry of activities began in this area with everyone looking to link their pet system to QS. A significant publication in Science using the P. aeruginosa PAO1, which had seen many passages and its isogenic QS mutants, argued for the complete dependence on QS for biofilm formation (Davies et al., 1998). Once again, the Journal of Medical Microbiology led the way in debunking this story. In 2007, Schaber and colleagues were able to isolate QS-deficient clinical isolates and demonstrate their ability to form biofilms, although the quality of those biofilms varied (Schaber et al., 2007). This seriously questioned whether QS was critical for biofilm formation and infection. In fact, recent work by Greenberg’s team showed that within a colonizing community, one could have social cheaters who can coexist and that QS plays a role in policing this cooperation (Wang et al., 2015).

With the emergence of AMR it seems natural that research turn towards finding anti-QS molecules as an alternative therapy. With the first report of furanones as anti-QS molecules (Manefield et al., 1999), there was a slew of articles that focused on everything from natural products to synthetic compounds. Once again, the opportunistic pathogen P. aeruginosa became the protagonist in the search for anti-QS molecules, but investigations also included Gram-positives such as Staphylococcus spp. (Nostro et al., 2007). Some researchers randomly looked at herbs, including ancient remedies such as ginseng (Schnepf et al., 2011), and others took an ethnobotanic approach by looking at natural products that are used by local communities for medicinal value (Adonizio et al., 2006). The latter identified various medicinal plants that had anti-QS activities which attenuated bacterial pathogenic virulence.
factor production, and could rescue *Caenorhabditis elegans* from *P. aeruginosa*-induced death (Adonizio et al., 2008). However, with over 15 years of searching, an anti-QS panacea has not emerged. This is, in part, due to the ability of the QS and the anti-QS molecules to elicit an immune response that can lead to inflammation.

Though the focus has largely been on looking at the infecting organism for drug targets, we tend to forget that the host contributes to bacterial shenanigans. It was well known that in the lungs of cystic fibrosis patients, *P. aeruginosa* lives in a novel biofilm lifestyle as aggregates (Sriramulu et al., 2005) surrounded by ‘frustrated’ phagocytes (Høiby et al., 1993), and accumulates mutations (Oliver et al., 2000). However, it was in the late 1990s that the role of neutrophils in the critical phenotypic conversion contributing to poor prognosis for the patient was demonstrated (Mathee et al., 1999). That study suggested anti-oxidants and anti-inflammatory agents as potential therapeutic measures. In 2009, a paper by Parks and colleagues not only explored the role of neutrophils in the quality and quantity of biofilm, but also proposed neutrophil necrosis as a potential drug target (Parks et al., 2009).

In this 70-year journey, the *Journal of Medical Microbiology* has been one of the torch bearers in advancing scientific research by critically reviewing and publishing articles related to the diverse medical microbiology field. The journal has never shied away from thinking outside the box, allowing it to embrace new viewpoints. This will substantially benefit basic research and will help in solving the mysteries of the microbial world, whether they be antibiotic resistance, biofilm formation, QS or the more advanced microbiome studies.

## Highlighted articles


## Further reading

- **Britton, R. A. & Young, V. B. (2014).** Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. *Gastroenterology* 146, 1547–1553.


