



Macrolide resistance in *Pseudomonas aeruginosa*: implications for practice

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New research gives us reason for concern regarding long-term macrolide use <http://ow.ly/g4Ap30b3lp3>

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Long-term low-dose macrolide use is rapidly becoming a standard treatment for a range of inflammatory lung diseases. In cystic fibrosis (CF), the 2014 annual report of the European Cystic Fibrosis Registry [1] shows that the use of macrolides is widespread, ranging from 10% of all CF patients in Austria, 38% in Spain, and 41% in the UK, to 44% in France, 48% in the Netherlands and 62% in Moldova. Macrolides are therefore one of the most frequently used therapies for CF in clinical practice [2]. In bronchiectasis not due to CF, data from the European Bronchiectasis Registry show that approximately 20% of patients are treated with macrolides, increasing to more than 30% in specialist European centres [3–5]. In chronic obstructive pulmonary disease (COPD), long-term macrolide therapy is not yet widespread, but is likely to become more so with the release of the updated 2017 Global Strategy for the Diagnosis, Management and Prevention of COPD (GOLD) report that now recommends long-term low-dose macrolide therapy to prevent exacerbations in patients with GOLD type-D COPD and frequent exacerbations despite triple-inhaled bronchodilator and corticosteroid therapy [6].

The inflammation in CF, bronchiectasis and many patients with COPD is neutrophilic and macrolides represent one of the few treatment options that are able to combat neutrophilic inflammation [7]. Nevertheless, the mechanisms of macrolide benefit are hotly debated. Macrolides (azithromycin in particular) have been reported to reduce neutrophilic inflammation, reduce the production of pro-inflammatory cytokines, regulate mucus production, and inhibit the expression of virulence factors by bacteria including *Pseudomonas aeruginosa* [8–10]. A Cochrane review of long-term low-dose macrolide treatment in CF has shown improvements in lung function (approximately 4% in forced expiratory volume in 1 s (FEV₁)), a reduced frequency of exacerbations, and an increase in patient weight over 6 months of treatment (there was an absence of clear data beyond 6 months) [11, 12]. In bronchiectasis, 3 small but conclusive trials showed a reduction in exacerbations of around 50% with associated improvements in quality of life [13, 14].

Existing evidence suggests that macrolides may be most effective in patients with *P. aeruginosa* and this is where the bulk of evidence exists in CF [11–14]. In bronchiectasis, only the randomised BLESS trial (of erythromycin *versus* a placebo) examined possible response modifiers and showed a trend towards greater benefit in patients with baseline *P. aeruginosa* [14]. The apparently excellent response to macrolides of patients with *P. aeruginosa* has been one of the primary justifications for believing that the anti-inflammatory effects of macrolides are more important than their antimicrobial effects [15]. *P. aeruginosa* is considered intrinsically resistant to macrolides, demonstrating minimum inhibitory concentrations (MICs) of >256 mg·L⁻¹ in standard growth media [16]. It uses multidrug-efflux systems to

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efficiently remove macrolides from the cell [16] and hence guidelines may suggest the use of inhaled antimicrobials for *P. aeruginosa* infection in preference to macrolides, despite their greater ease of use [17]. However, in 2012, BUYCK *et al.* [16] demonstrated that macrolides may kill *P. aeruginosa in vitro* under physiological conditions similar to those in the airway. Increased permeability of the *P. aeruginosa* outer membrane is observed when exposed to serum, bronchoalveolar lavage (BAL) fluid or RPMI 1640 cell culture medium. Under these conditions, sufficient antibiotic may enter the cell to inhibit synthesis of outer membrane porins and efflux transporters, allowing macrolides to become effective antibacterials [16].

The realization that information from standard microbiological testing can be misleading is a familiar one in the field of chronic airway disease. It is well known that using MICs determined for serum is unhelpful when dealing with organisms in the lung, particularly when inhaled antimicrobials are used that achieve concentrations far above those in serum [18]. Populations of *P. aeruginosa* are heterogeneous such that different colonies may be resistant or sensitive to antibiotics [19] and, consequently, patients may respond to treatment with antibiotics that they are “resistant” to *in vitro*. To this we can now add the fact that macrolides (and potentially other antibiotics) may show entirely different sensitivity results depending on the growth media used.

Expanding on this previous work, this issue of the *European Respiratory Journal* features a manuscript by MUSTAFA *et al.* [20] wherein they investigate the potential for *P. aeruginosa* to develop resistance to macrolides *in vivo*. After all, it is logical that if macrolides have an antimicrobial effect against *P. aeruginosa*, the organism will be able to develop resistance. MUSTAFA *et al.* [20] used 333 clinical *P. aeruginosa* isolates obtained from patients with CF cared for at four centres in Belgium, France, Germany and the UK, respectively. They demonstrated the presence of macrolide-resistance in these “CF isolates” in eukaryotic growth media, which were not present in healthcare-associated pneumonia isolates where patients had not been exposed to chronic macrolide usage. Resistance mechanisms for macrolides, across multiple species of bacteria, are usually due to efflux pumps or modification of the ribosomal binding site of the antibiotic. In this study, MUSTAFA *et al.* [20] demonstrated that efflux-pump inhibitors did not reverse macrolide resistance in the CF isolates. Furthermore, sequencing of the 23S rRNA gene subsequently demonstrated six different mutations in the ribosomal target of the macrolides which, when transformed into a susceptible isolate, resulted in induced resistance to azithromycin. These data confirm that *P. aeruginosa* can acquire mutations in the 23S rRNA gene rendering it nonsusceptible to macrolides. A direct relationship between chronic azithromycin use by patients and macrolide resistance in CF was observed that was statistically significant for the Liverpool epidemic strain [20]. This final data is not unexpected, but it is important in demonstrating that chronic antibiotic use is likely to drive mutations in *P. aeruginosa* in real life.

The clinical implications of this experimental work are important. It can no longer be assumed that macrolide effectiveness in *P. aeruginosa* is due to an anti-inflammatory mechanism and, if macrolide effects are partially or wholly due to antimicrobial activity, the potential for the development of resistance could mean a loss of macrolide effectiveness over time. Our ability to detect this has been limited to date, since the majority of CF studies are 6 months in duration and the longest studies in bronchiectasis and COPD are 12 months in duration [12–15]. Preliminary evidence from CF suggests that macrolides may indeed lose effectiveness with time. SAMSON *et al.* [21] studied 68 patients who showed an initial reduction in exacerbations over the first 12 months of azithromycin treatment, followed by an increase in exacerbations back to baseline levels after 12 months. This is a problem that is not going to go away and which presents us with a key challenge for future work involving macrolides. The adult CF population in Europe is projected to rise by up to 75% by the year 2025 due to improved survival, while the prevalence of bronchiectasis not due to CF has increased by more than 30% in the past decade and is set to rise further [22–24]. These factors, along with the recent GOLD recommendations for COPD, mean that we are likely to see a continued rise in the use of long-term macrolide therapy. We need longer studies to determine if ongoing treatment with macrolides presents ongoing benefits, or if potential resistance results in loss of effectiveness. If the latter is true, patients could potentially discontinue therapy after 12 months with resulting benefits in terms of treatment burden and side-effects. Alongside this research priority, recent publications in the *European Respiratory Journal* regarding priorities in COPD and bronchiectasis have highlighted the need to identify which patients benefit most from macrolide treatment [25, 26]. The potential for macrolides to cause resistance has to be taken seriously alongside other adverse effects such as drug–drug interactions, cardiovascular effects, hearing loss and the risk of inducing macrolide resistance in nontuberculous mycobacteria [27]. Recent evidence suggests that macrolide co-administration may antagonise the effect of inhaled tobramycin in CF, potentially through a different antibiotic resistance mechanism (the upregulation of efflux pumps as described by NICHOLS *et al.* [28]). This calls for careful selection of patients to receive macrolides. For example, existing data suggest that smokers respond poorly to macrolides in COPD; however, few other predictors have been identified [29, 30]. This should be

addressed with new studies or secondary analyses of existing datasets. Macrolides are relatively unique among respiratory therapies and, while all therapies present a risk of side-effects to the individual, macrolides also present a threat to society by the spread of antimicrobial resistance. Further studies such as those by Mustafa *et al.* are needed if we are to understand the extent and clinical implications of the development of macrolide-induced resistance.

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