Differential impact of macrolide compounds in the selection of macrolide nonsusceptible *Streptococcus pneumoniae*

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Antimicrobial agents are used to treat patients with infectious diseases caused by organisms considered susceptible to the treatment drug. Antimicrobial resistance has reshaped our thinking with regard to the use of antibacterial compounds. In the earlier days of drug-resistant bacteria, the strategy for dealing with the problem was to find new drugs that remained active against the pathogen(s) and not negatively affected by that particular mechanism of resistance. Similar approaches would remain relevant today; however, there clearly appears to be fewer new antimicrobial drugs in development than there was several years ago. How then do we deal with drug-resistant bacteria, given that simply ignoring the problem is not an option? One approach may be to better understand the factors that contribute to resistance and whether the modification of these factors has an impact on slowing, reducing or reversing resistance trends. Such factors may include the propensity of a particular drug class or specific drug to be disproportionately associated with a resistance trend. Such arguments have been previously debated for fluoroquinolones and *Streptococcus pneumoniae* [1,2], but do similar data exist for the macrolides?

It has long been suggested that macrolide use precedes macrolide resistance and the use of long-acting macrolides are more likely to correlate with resistance development whereas short-acting compounds were not [3]. When compared with erythromycin, which has a relatively short half-life (2 h), both azithromycin and clarithromycin would be considered longer-acting agents due to their prolonged half-life. However, for azithromycin, the half-life is ten times longer than that of clarithromycin (60–70 vs. 6 h). Is there any evidence to suggest that despite the relatively extended half-lives of both compounds, the likelihood of selecting macrolide-resistant organisms is greater with azithromycin than it is with clarithromycin? The answer to this question appears to be yes! Several recent lines of evidence suggest that there may be a differential impact on macrolide resistance when organisms are exposed to azithromycin and clarithromycin with the former being more likely to be associated with resistance.

Blondeau and colleagues have applied minimum inhibitory concentration (MIC) and mutant prevention concentration (MPC) testing to more than 170 randomly collected unique clinical isolates of *S. pneumoniae* and found that 14 to 16% of the strains had MIC values of 1 µg/ml or more to azithromycin, clarithromycin and erythromycin – a finding consistent with current levels of macrolide nonsusceptibility in Canada [2,4–6]. When tested by MPC, the percentage of strains with MPCs of 1 ug/ml or more increased to 73% in the presence of azithromycin, 23% in the presence of clarithromycin ($p < 0.0001$ compared with azithromycin) and 33% in the presence of erythromycin ($p < 0.0001$ compared with azithromycin; $p = 0.03$ compared with clarithromycin). The MPC approach tests 10^9 colony-forming units (CFU) or greater of bacteria on agar plates containing the drug – an inoculum most likely to contain resistant subpopulations, and also a bacterial burden present during various human infectious diseases [7–10]. For MIC testing, 10^5 CFU/ml are tested as recommended by the Clinical and Laboratory Standards Institute (CLSI) – formerly the National Commmittee for Clinical Laboratory Standards (NCCLS). Challenging antimicrobial compounds against higher-density bacterial inocula is relevant, and more likely to provide a better understanding of the dynamics of heterogeneous bacterial populations containing mutants present during infection and when exposed to drug. While pulmonary drug concentrations for macrolides are higher than serum concentrations, the propensity for azithromycin to select for resistant subpopulations at a frequency statistically higher than either clarithromycin or erythromycin suggest, in this model, a differential impact within the macrolide class on

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resistance. Could a possible explanation of this observation relate to the substantially longer drug half-life of azithromycin, that may serve to prolong the time that drug concentrations remain within the mutant selection window (MSW)? The MSW is the drug concentration between the measured MIC and MPC values. Does prolonged exposure of bacteria to subinhibitory drug concentrations of bacteriostatic versus bactericidal agents contribute to the resistance selection process?

Vanderkooi and colleagues predicted antimicrobial resistance in invasive pneumococcal infections [11]. As the prevalence of multiantimicrobial resistance increases worldwide among clinical strains of *S. pneumoniae*, the recognition of risk factors that would identify those likely to have an antibiotic-resistant pathogen might assist in the selection of the most appropriate empirical therapy. This prospective study was carried out in Toronto, Canada and involved analysis of more than 3300 patients with invasive pneumococcal infection from 1995 to 2002.

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Following multivariate modeling, risk factors for infection with penicillin-resistant as opposed to penicillin-susceptible pneumococci included:

- Year of infection (odds ratio [OR]: 1.28; $p < 0.001$)
- Absence of chronic organ system disease (OR: 1.72; $p < 0.03$)
- Previous use of penicillin (OR: 2.47; $p < 0.006$)
- Previous use of trimethoprim/sulfamethoxazole (TMP/SMX) (OR: 5.97; $p < 0.001$)
- Previous use of azithromycin (OR: 2.78; $p < 0.05$)

Risk factors for infection with TMP/SMX-resistant *S. pneumoniae* included:

- Absence of chronic organ system disease (OR: 1.64, $p < 0.001$)
- Previous penicillin use (OR: 1.71; $p < 0.03$)
- Previous TMP/SMX use (OR: 4.73; $p < 0.001$)
- Previous azithromycin use (OR: 3.49; $p < 0.001$)

Risk factors for infection with *S. pneumoniae* that was macrolide resistant included:

- Previous use of penicillin (OR: 1.77, $p < 0.03$)
- Previous use of TMP/SMX (OR: 2.07; $p < 0.04$)
- Previous use of clarithromycin (OR: 3.93; $p < 0.001$)
- Previous use of azithromycin (OR: 9.93; $p < 0.001$)

Risk factors for infection with fluoroquinolones-resistant *S. pneumoniae* were:

- Previous use of fluoroquinolones (OR: 12.1; $p < 0.001$)
- Current residence in a nursing home (OR: 12.9; $p < 0.001$)
- Nosocomial acquisition of pneumococcal infection (OR: 9.94; $p < 0.003$)

In this study, 24 patients had received erythromycin therapy, compared with 67 receiving clarithromycin and 37 receiving azithromycin. According to the authors, azithromycin was consistently associated with an increased risk of resistance to agents from all classes except the fluoroquinolones. As such, they concluded that macrolides were not homogeneous with respect to antimicrobial resistance. For example, erythromycin use was not associated with infecting organisms that were resistant to any antimicrobial class. Clarithromycin use was associated with an increased likelihood of erythromycin resistance. Azithromycin use was associated with an increased risk of resistance to macrolides, penicillin and TMP/SMX in infecting strains. Indeed, over 50% of isolates recovered from patients with invasive pneumococcal strains who had received azithromycin during the 3-month period before infection were resistant to erythromycin. As some data have suggested this association may be related to a long half-life leading to sub-MIC blood and pulmonary drug levels, the selective pressure for resistance may be reduced if shorter-acting macrolides are used preferentially [12–15].

Kastner and Guggenbichler studied the impact that various macrolides had on the promotion of resistance in the oral flora of children [16]. Children were randomly assigned to receiving azithromycin, clarithromycin, erythromycin, roxithromycin or josamycin for respiratory tract infections. Throat swabs were collected for culture prior to treatment and weekly for 6 weeks. At 1 week post-treatment, 90% of children harbored macrolide-resistant strains in their oral flora. With the exception of azithromycin, the percentage of

patients colonized by resistant organisms decreased to 17% for clarithromycin, erythromycin and josamycin and to 33% for roxithromycin after 6 weeks. For the azithromycin group, 85% of patients remained colonized by macrolide-resistant organisms after 6 weeks and 11.6% suffered from reinfection. The authors argued that the long elimination half-life of azithromycin allows for subinhibitory serum and epithelial lining fluid (ELF) drug concentrations over a period of several weeks post-treatment and this may impact on the emergence of resistance. They concluded from the study that azithromycin therapy appears to place selective pressure on the infective and native flora of children, thereby promoting the carriage of macrolide-resistant strains.

Davidson and colleagues reported on macrolide-resistant *S. pneumoniae* and correlated the findings with azithromycin, clarithromycin and erythromycin use [17]. The study collected pneumococcal isolates from across Canada and followed standardized susceptibility testing, macrolide-resistant strains were genetically characterized to detect the presence of the *erm* or *mef* genes. Susceptibility data were correlated with macrolide usage that was normalized for the population.

According to the data summarized in **Figure 1**, the incidence of macrolide resistance with *S. pneumoniae* varied considerably in Canada in 2002; however, despite this, three distinct trends were recognized:

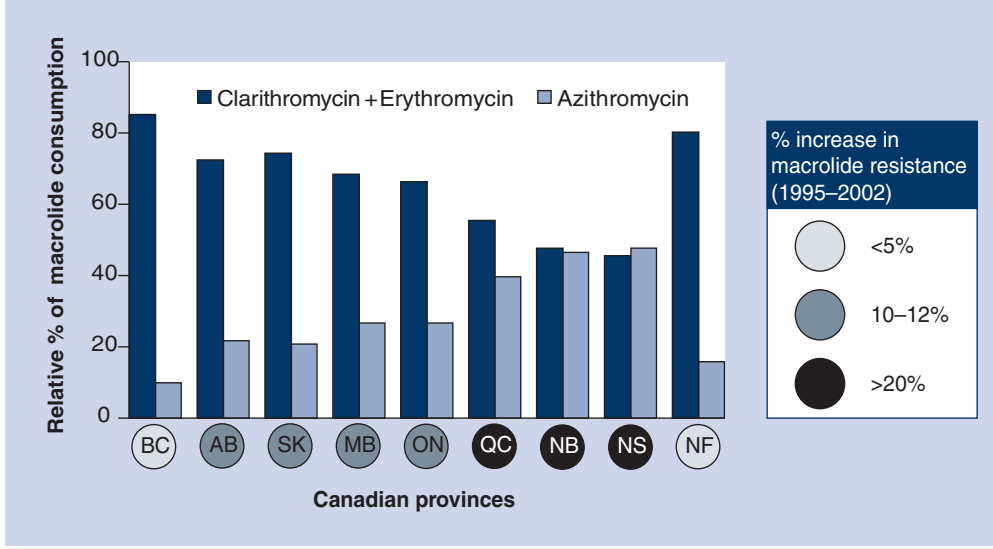
- The coastal provinces had resistance rates approximating 5%
- The prairie provinces and Ontario had resistance rates between 9 and 14%
- Quebec and the maritime provinces had resistance rates exceeding 20%

The following points regarding azithromycin consumption were summarized:

- Azithromycin consumption in the coastal provinces remained low at less than 20% of prescribed macrolides
- Azithromycin use accounted for more than 44% of all macrolides in the three provinces with the highest macrolide-resistance rates
- For the prairie provinces, azithromycin use accounted for 25 to 32% of macrolide use

There was no correlation identified between total macrolide consumption and the regional differences in macrolide resistance. According to data from Davidson and colleagues, regions with the lowest rates of macrolide resistance used significantly less azithromycin than other macrolides [17]. Provinces with the highest rates of macrolide resistance used more azithromycin than other macrolides. From this study, Davidson and colleagues concluded that azithromycin may have a greater propensity to select for macrolide-resistant *S. pneumoniae* compared with clarithromycin and erythromycin.

Figure 1. Correlation between macrolide use and rates of resistance in Canada.



AB: Alberta; BC: British Columbia; MB: Manitoba; NB: New Brunswick; NF: Newfoundland; NS: Nova Scotia; ON: Ontario; QC: Quebec; SK: Saskatchewan. Reproduced with permission from Davidson *et al. European Congress for Clinical Microbiology and Infectious Disease* (2003) (Poster).

In 2001, Doern commented on antimicrobial use and the emergence of antimicrobial resistance with *S. pneumoniae* in the USA [18]. He argued that, "the more potent an antimicrobial agent, the less likely it is to select for resistance." and, "within each class, potencies differ." Regarding macrolides, Doern indicated that azithromycin is consistently three- to four-times less active than clarithromycin for the pneumococcus based on *in vitro* MIC measurements. Additionally, peak serum drug levels of azithromycin following administration of standard doses are approximately a tenth of those achieved with clarithromycin.

"In its simplest terms, macrolide resistance would refer to any organism requiring more drug than the susceptibility breakpoint for inhibition."

Doern argued that serum levels are appropriate for pharmacodynamic analysis and, as such, azithromycin was inferior to clarithromycin in terms of *in vitro* activity and pharmacokinetics. To support his position, Doern cited studies by Diekema and colleagues and Leach and colleagues, indicating that azithromycin use was more likely to select for macrolide resistance than was clarithromycin [19,20]. In 2002, Edelstein responded to the arguments of Doern by indicating that the studies cited do not contain any data regarding the relative resistance emergence rates for azithromycin and clarithromycin, and that serum drug levels may not be the correct parameter to assess the pharmacodynamic behavior of azithromycin (in pneumonia) as it ignores solid evidence regarding the delivery of azithromycin by drug-containing neutrophils to the infection site [21].

In a subsequent rebuttal to the points raised by Edelstein, Doern cited studies by Ghaffar and colleagues and Gary and colleagues, showing the emergence of macrolide resistance in *S. pneumoniae* isolates following exposure of infected persons to azithromycin; however, as these studies were noncomparative, there was no attempt to assess the effect of clarithromycin exposure on the emergence of macrolide-resistant *S. pneumoniae* [22–24]. From studies published by Hyde and colleagues and Garcia-Rey and colleagues, associations were made between macrolide use and macrolide-resistant *S. pneumoniae* [25,26]. Doern indicated that these two studies suggest the higher probability of macrolide resistance following azithromycin therapy, as in the USA the vast majority of macrolide use in pediatric patients is azithromycin, and for the study conducted in Spain, the once-daily administered macrolide was presumably azithromycin. In the Spanish study, the administered macrolide had a 1.5-times greater statistical association with the emergence of macrolide-resistant *S. pneumoniae*, than the macrolides that were administered two- to three-times daily.

Doern suggested that the relative potency of azithromycin versus clarithromycin against *S. pneumoniae* may be related to the differential impact observed between azithromycin and clarithromycin on the selection of *S. pneumoniae* resistance to macrolides [22]. Edelstein argued that alveolar lining fluid drug concentrations may not be the correct parameter to assess azithromycin pharmacodynamics because of high drug concentrations delivered by drug-containing neutrophils [21]. To this point, Doern argued that for extracellular pathogens such as the pneumococcus, ELF drug concentrations are likely to be more relevant than intracellular drug concentrations [22]. Comparing clarithromycin with that of azithromycin suggests that ELF levels of clarithromycin are approximately 30-fold higher than those of azithromycin [27–29]. Thus, one can imagine that if drug concentrations within an infected compartment does not achieve or exceed the minimum amounts required to inhibit the growth of the infecting pathogen, then antimicrobial resistance could ensue if subinhibitory drug concentrations (perhaps within the MSW) promoted the selection and amplification of resistant bacterial subpopulations – particularly those likely to exist in high-density populations. Such a point has previously been argued from the author's laboratory, based on the MPC approach and the suggestion that the drug concentration shown to be therapeutic may, in fact, be the very drug concentrations that allows for the selective amplification of resistant subpopulations when the concentration is insufficient to inhibit the growth of resistant cells [2].

What does the data summarized above suggest and how should it be used? From *in vitro* clinical and drug-usage data, it suggests a differential impact of various macrolides for their propensity to select for macrolide resistance. Each study identifies azithromycin as being more frequently associated with macrolide resistance. In its simplest terms, macrolide resistance would refer to any organism requiring more drug than the susceptibility break point for inhibition. Despite these observations, the subcellular mechanism(s) of this differential impact remain undefined.

In the clinical world, drug approval is based on clinical trials showing noninferiority between an investigational compound and some appropriate compound that is already approved for the same indication. What would be the design of a clinical evaluation of a drug, if in addition to clinical outcome, microbiologic and pharmacologic outcomes were also made based on some defined criteria or break point? Should long-term societal consequences be considered? If a favorable clinical outcome and the selection of drug-resistant bacteria are capable of being two independent events, then which criteria should be used for drug approval? If in fact the patient gets better, should that be sufficient? Conversely, if selection of drug-resistant bacterial subpopulations occurs, is this acceptable given that a drug class could ultimately be compromised over time? Such a finding could shorten the life expectancy of a particular drug or drug class and deter future research efforts. In an ideal world, pathogen-specific therapy would be based on comprehensive and sensitive laboratory testing that would ensure the right drug for the task. Given the relative scarcity of new antimicrobial compounds being developed for the treatment of a variety of both community- and hospital-acquired infections, perhaps a greater appreciation of the factors leading to the selection of antimicrobial resistance, in addition to the prevention of the selection of antimicrobial-resistant subpopulations, should be reviewed more vigorously. Doern suggested that drug potency was of paramount importance to this whole equation [30]. Perhaps he is right.

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