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Commentary

Emergence of Microbial Resistance During Hospitalization

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Legionellosis is an acute pneumonia that can be fatal particularly in immunocompromised patients, if not treated promptly. The pre-eminent causative agent, *Legionella pneumophila*, like other *Legionella* species, is an inhabitant of aquatic biotopes and of man-made water systems, including also home drinking water sources, where it can survive and replicate intracellularly in free-living amoebae and protozoa (Pedro-Botet et al., 2002; Taylor et al., 2009). Occasionally, humans can be infected by inhaling contaminated aerosols, with *Legionella* reaching the alveoli of the lungs, where it is engulfed by macrophages. In contrast to most bacteria which are destroyed there, *Legionella* can proliferate inside a *Legionella*-containing-vacuole, similarly to what occurs in amoebae, eventually killing the macrophage and giving rise to the disease (Isberg et al., 2009). Antibiotic therapy with macrolides or fluoroquinolones is the first choice in legionellosis and is usually effective in killing the bacteria and curing most patients, though treatment failure or relapses may occur (Roig and Rello, 2003).

Human to human transmission has not been reported for *L. pneumophila*, and the bacteria that proliferate in legionellosis patients will disappear or die with the patient. Chances for them to reach the natural environment are negligible. For this reason, the development of antibiotic resistance in patients with persisting or relapsing *Legionella* infections has not been considered an issue, though it is well known that the same antibiotics used in the clinic will select for mutants in *in-vitro* experiments (Roig and Rello, 2003).

The results obtained by Lubana Shadoud and colleagues (Shadoud et al., 2015) cast now some doubts on this conclusion, as they show the emergence of fluoroquinolone resistance *in vivo* in two patients hospitalized for *Legionella* infection. By taking advantage of the characterization of the genetic locus for quinolone resistance and its mutational pathway (Almahmoud et al., 2009), the authors have devised and standardized a specific qPCR assay to detect fluoroquinolone resistance mutations. The assay was applied to lower respiratory samples of 82 legionellosis patients, four of which displayed mutations that were confirmed by next generation sequencing. More important, in two patients it could be shown that the mutated forms were progressively replacing the wild-type DNA during the antibiotic treatment, suggesting *in vivo* selection of fluoroquinolone resistant mutants. It is worth mentioning that only few mutations in a small region of the quinolone resistance locus could be analyzed, leading the authors to conclude that the incidence of *in vivo* selection of fluoroquinolone resistance might be much higher. Did the mutations arise during the antibiotic treatment? It is more likely that both patients were already infected with a heterogeneous *Legionella* population containing the resistant alleles at almost undetectable level, yet few days of fluoroquinolone treatment were sufficient to rapidly select the mutant population.

These findings are important because they show that selection for fluoroquinolone resistance may be induced by the antibiotic treatment, with worsening of the disease, as it was the case for the two patients, although it could be objected that two cases are statistically not significant. Decades ago in a similar study, but with totally different assay, no resistant mutants were isolated in 98 clinical samples (Onody et al., 1997). On the other hand, an independent case of fluoroquinolone-resistant *L. pneumophila* isolated from a clinical specimen was reported recently (Bruin et al., 2014). Thus, taken together these results sound as an alarm-bell that lack of clinical response during treatment with quinolone could be due to selection of resistant mutants of *L. pneumophila*, and this should be taken in consideration for a more appropriate treatment. The results also justify the practice of combined antibiotic treatment with macrolide and quinolone in severe *Legionella* cases. Further systematic investigations, not only for Legionnaire's disease but also for other infectious diseases caused by pathogens with strict intracellular lifestyles, are needed in order to shed more light on the incidence of antibiotic resistance during antibiotic therapy.

Disclosure

The author declared no conflicts of interest.

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