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Article

# Association between Macrolide Use in Food Animals and Macrolide Resistance in *Mycoplasma pneumoniae*: A Global Ecological Analysis

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**Abstract:** In the current study we test the hypothesis that the consumption of macrolides in food-animals is positively associated with the prevalence of macrolide resistance in *M. pneumoniae* at a country-level. The prevalence of *M. pneumoniae* macrolide resistance was positively associated with the consumption of macrolides for both food animals (Rho = 0.66; P=0.007) and humans (Rho = 0.54; P=0.040). Linear regression analysis revealed that macrolide consumption in food animals but not humans was borderline significantly associated with macrolide resistance (coef. 5547 [95% CI -596-11691] and coef. 0.006 [95% CI -0.008-0.020], respectively).

**Keywords:** macrolide resistance; mycoplasma pneumoniae

## Introduction

It is well known that macrolide resistance in *Mycoplasma pneumoniae* is primarily due to point mutations in domain V of the 23SrRNA gene at positions 2958,2059 and 2611 [1]. What is less clear is what underpins the global variations in macrolide resistance in *Mycoplasma genitalium*? Various studies have clearly established that exposure to macrolides is a primary driver of macrolide resistance in a number of bacterial species including the related species, *Mycoplasma genitalium* [2-6]. This has included ecological type studies that have established that population-level consumption of macrolides is positively associated with the prevalence of macrolide resistance in a number of these species [4, 5, 7].

Macrolide consumption in humans may, however, not be the only driver of macrolide resistance. This is evident if we consider the explosive emergence of macrolide resistance in various bacterial species in Asia, particularly in China. Despite relatively low population-level macrolide consumption in China, the prevalence of macrolide resistance among *Streptococcus pneumoniae* isolates was 74% in 2001 and increased to 96% by 2009. Similarly, a high prevalence of macrolide resistance has been found in other bacteria, including *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, *Treponema pallidum*, and group B streptococci [8-10]. Macrolide consumption in China in the year 2000 was less than 20% of the median consumption in European countries with available data [11, 12].

One alternative source of macrolide exposure could be the low doses of macrolides that are allowed in food. Previous research has shown that very low concentrations of antimicrobials can select for AMR, with the minimum selection concentration (MSC) being the lowest concentration of an antimicrobial that can select for AMR [13]. For example, the *Escherichia coli* ciprofloxacin MSC has been found to be approximately 240-fold lower than the MIC, and close to concentrations of quinolones detected in food residues in countries with high consumption of quinolones in food-animals [13]. Ecological studies have also found a positive association at country level between quinolone use in food animals and ciprofloxacin resistance in a number of gram-negative bacteria [14].

Only a single MSC has been experimentally established for macrolides. This is the erythromycin MSC for *Escherichia coli* which was found to be  $<0.2 \mu\text{g}/\text{mg}$  [13, 15]. Country-level ecological studies in Europe have found that there is a positive association between macrolide resistance in *Campylobacter* spp. in humans and macrolide use in animal husbandry [16]. Likewise, a systematic review found that interventions to limit macrolide use in food animals resulted in a decline of macrolide resistance in both humans and animals [17]. A single study found a positive correlation between the intensity of use of macrolides for food producing animals and the prevalence of macrolide resistance in *S. pneumoniae* [18].

In the current study we test the hypothesis that the consumption of macrolides in food-animals is positively associated with the prevalence of macrolide resistance in *M. pneumoniae* at a country-level.

## Methods

### Data

#### Prevalence of Macrolide Resistance

The prevalence of macrolide resistance by country for *M. pneumoniae* was obtained from a systematic review of the topic. Where countries were represented by multiple studies we used the median prevalence of resistance and we used the median year of all the studies for the year that this resistance estimate was obtained from.

#### Macrolide use in Food-Producing Animals

Country-level consumption of macrolides for the purposes of animal food production in the year 2013 was extracted from a systematic review that was performed by Van Boeckel et al. [19]. For 38 countries in the year 2013, Van Boeckel et al. estimated the volume of antimicrobials (in kilograms) consumed for each class of antimicrobial. They included four different categories of animals in this data: pigs, cattle, chickens and small ruminants. They then calculated for the year 2013, the number of kilograms of macrolides used in animal food production/population correction unit (PCU - a kilogram of animal product). The total tonnage of food producing animals produced per year per country was obtained from Food and Agriculture Organization (FAO) estimates. (<http://www.fao.org/faostat/en/?#data/>). This data was obtained for the year 2013.

#### Macrolide Consumption in Humans

National macrolide drug consumption was obtained from IQVIA. The data for the year prior to the year used for macrolide resistance prevalence estimates was used. IQVIA uses national sample surveys to obtain this data. IQVIA reports these antimicrobial consumption estimates as the number of defined daily doses (a dose is classified as a pill, capsule, or ampoule) per 1000 population per year [12].

### Statistical Analyses

We assessed the association between country-level macrolide resistance in *M. pneumoniae* and macrolide consumption using Spearman's correlation. Linear regression analysis was used to assess the association of macrolide consumption in both humans and food animals and macrolide resistance in *M. pneumoniae*. Statistical analyses were conducted using Stata v16.1 (StataCorp, LLC College Station, Texas).

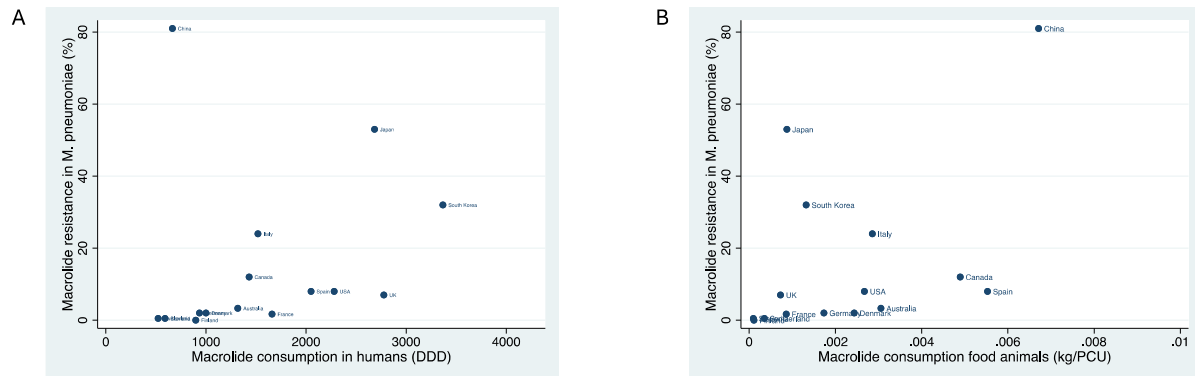
## Results

Data for the prevalence of *M. pneumoniae* macrolide resistance was available from 22 countries. Of these 22 countries, concomitant data was available for macrolide consumption for food animals and humans for 15 countries (Table 1).

**Table 1.** National prevalence of resistance to macrolides in *Mycoplasma pneumoniae*, consumption of macrolides in food animals (kilograms of macrolides used in animal food production/PCU) and macrolide consumption in humans (defined daily doses/1000 inhabitants per year, DID).

	2014	3.3	.0030558	1318
	2013	12	.0048947	1431
	2014	81	.0067121	665
	2018	0	.	.
	2017	19	.	.
	2012	2	.0024353	1000
	2019	0	.0001106	898
	2007	1.7	.0008601	1660
	2015	2	.0017334	935
	2017	25	.	.
	2011	30	.	.
	2015	24	.0028562	1519
	2013	53	.0008748	2684
	2020	1	.	.
	2017	13	.	.
	2015	.5	.0000965	591
	2017	32	.0013214	3367
	2020	8	.0055277	2050
	2014	.5	.0003538	522
	2017	80	.	.
	2013	7	.000727	2777
	2015	8	.0026729	2281

The prevalence of *M. pneumoniae* macrolide resistance was positively associated with the consumption of macrolides for both food animals (Rho = 0.66; P=0.007) and humans (Rho = 0.54; P=0.040; Figure 1). Linear regression analysis revealed that macrolide consumption in food animals but not humans was borderline significantly associated with macrolide resistance (coef. 5547 [95% CI -596-11691] and coef. 0.006 [95% CI -0.008-0.020], respectively).



**Figure 1.** Scatter plots of the association between macrolide resistance in *M. pneumoniae* (%) and A) macrolide consumption in humans (DDD) and B) macrolide consumption in food animals (kg/PCU).

## Discussion

A positive association was found between both macrolide consumption in food animals and humans and the prevalence of macrolide resistance in *M. pneumoniae*. In multivariate testing the association between macrolides used for animals was stronger than those used for humans and macrolide resistance. This stronger association was driven to a large extent by China which had a high prevalence of macrolide resistance, high consumption in food animals but low consumption in humans.

We did not however control for a large number of other variables which may have confounded our analyses [20]. The sample size for the study was limited. We did not adjust for the differences in study methodology between countries in sample collection and determination of macrolide resistance. The study is also ecological and thus susceptible to the ecological inference fallacy.

These findings do however suggest that macrolides used for food animals may play a role in macrolide resistance in *M. pneumoniae*. These findings build on the similar findings of those found for *S. pneumoniae* [18]. They suggest the need for further experimental work to ascertain if the consumption of concentrations of macrolides detected in food could induce macrolide resistance in bacteria such as *M. pneumoniae*.

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**Data availability:** The data is available from the sources described in the methods.

**Conflicts of Interest:** The author declares that he has no competing interests.

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