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CONCISE ARTICLE

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Macrolide use identified as risk factor for macrolide-resistant *Streptococcus pneumoniae* in a 17-center case-control study

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Abstract The objective of the case-control study presented here was to examine the risk factors for macrolide-resistant *Streptococcus pneumoniae*. As part of a 44-center U.S. surveillance study, 1,817 unique isolates of *S. pneumoniae* were collected from November 2002 through April 2003. Seventy-five randomly selected macrolide-resistant isolates (cases) were each matched with one susceptible control. Macrolide use in the 6 weeks prior to sample collection was reported for seven cases and one control. The final conditional logistic regression model identified two statistically significant variables: a history of alcohol abuse was protective, while macrolide use in the 6 weeks prior to sample collection was a significant risk factor for macrolide-resistant *S. pneumoniae*. Macrolide resistance was associated with use of any antibiotic during the prior 6 weeks, and was most strongly associated with previous macrolide use.

Keywords *Streptococcus pneumoniae* · Macrolide resistance · Case-control study

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Introduction

Streptococcus pneumoniae is the most significant bacterial cause of outpatient respiratory tract infection in the USA, and pneumococcal infections are among the leading causes of illness and death worldwide. The emergence of antimicrobial resistance in this pathogen during the past 15 years has substantially complicated the effective management of these infections with oral antimicrobial agents. In a number of studies, increasing macrolide use has been associated with the development of macrolide-resistant *S. pneumoniae* [1–6]. We performed a case-control study to examine the association of several risk factors, including recent antibiotic use, with macrolide-resistant *S. pneumoniae*.

Materials and methods

As part of a 44-center U.S. respiratory pathogen surveillance study (GRASP), 1,817 unique clinical isolates of *S. pneumoniae* were collected from November 2002 through April 2003 [7]. Macrolide nonsusceptibility (erythromycin MIC $\geq$ 0.5 μg/ml, as determined by broth microdilution) was present in 536 (29.5%) isolates. Seventy-five macrolide-resistant cases were randomly selected from 222 isolates provided by the 17 hospitals that agreed to participate in this follow-up study. One macrolide-susceptible control per case, matched for age group (birth to 5, 6–20, 21–64, and $\geq$ 64 years), specimen source (ear, eye, blood, cerebrospinal fluid/sterile body fluid, lower respiratory tract, sinus, other), geographic location (U.S. Bureau of Census region), and time of entry into the study (study year), was then randomly selected. If patient data were unavailable for a selected isolate, another eligible case or control, as necessary, was chosen.

Participating centers performed medical record reviews. History of antimicrobial use was assessed for two time periods: 6 weeks and the remaining 12 months (minus the initial 6-week period) prior to collection of the isolate. The antibiotic(s) administered, as well as indication for use and start/stop dates (for the 6-week period) and approximate

dates of administration (for the 12-month period), were requested. Demographic variables collected included age, gender, place of residence, and whether the patient or a family member was in child daycare. Current episode variables included date and source of specimen, inpatient or outpatient location, dates of admission and discharge where applicable, infection syndrome associated with the isolate, and whether the patient died within 28 days. Medical history variables included 12-month history of hospitalization, presence of underlying diseases or immunosuppression,

Table 1 Characteristics of 75 case patients infected or colonized with macrolide-resistant *S. pneumoniae* and 75 matched control patients infected or colonized with macrolide-susceptible *S. pneumoniae*

Variable	Case patients[a]	Control patients[a]	*p* value
Mean age in years	40.9±27.5 (44.8)	42.1±28.3 (46.9)	0.732
Age group			0.872
0–5 year	18 (24.0)	18 (24.0)	
6–20 year	3 (4.0)	3 (4.0)	
21–64 year	33 (44.0)	36 (48.0)	
≥65 year	20 (26.7)	18 (24.0)	
Specimen source			1.0
Ear	5 (6.7)	5 (6.7)	
Eye	3 (4.0)	3 (4.0)	
Blood	13 (17.3)	14 (18.7)	
CSF/sterile body fluid	3 (4.0)	3 (4.0)	
Lower respiratory tract	41 (54.7)	42 (56.0)	
Other/upper respiratory tract	5 (6.7)	4 (5.3)	
Sinus	5 (6.7)	4 (5.6)	
Census region			0.802
New England	2 (2.7)	4 (5.3)	
Middle Atlantic	11 (14.7)	10 (13.3)	
South Atlantic	3 (4.0)	5 (6.7)	
East North Central	25 (33.3)	24 (32.0)	
East South Central	8 (10.7)	5 (6.7)	
West North Central	16 (21.3)	17 (22.7)	
West South Central	2 (2.7)	2 (2.7)	
Mountain	1 (1.4)	4 (5.3)	
Pacific	7 (9.3)	4 (5.3)	
Female	35 (46.7)	26 (34.7)	0.135
Residence			0.009
Private	74 (98.7)	66 (88)	
Chronic care or homeless/transient facility	1 (1.3)	9 (12)	
Patient/family member in daycare			1.0
Yes	4 (5.3)	4 (5.3)	
No or unknown	71 (94.7)	71 (94.7)	
Inpatient	45 (60.0)	57 (76.0)	0.103
Death	5 (6.7)	11 (14.7)	0.119
Hospitalization in past 12 months	27 (36.0)	23 (30.7)	0.763
Chronic underlying disease	59 (78.7)	61 (81.3)	0.683
COPD	16 (21.3)	24 (32.0)	0.014
Immunosuppression	23 (30.7)	20 (26.7)	0.618
Asplenic	1 (1.3)	2 (2.7)	0.673
Alcohol abuse	4 (5.3)	13 (17.3)	0.068
History of infection in past 12 months	36 (48.0)	26 (34.7)	0.097
Antibiotics received in past 12 months	26 (34.7)	22 (29.3)	0.484
Antibiotics received in past 6 weeks	30 (40.0)	18 (24.0)	0.036
Macrolide	7 (9.3)	1 (1.3)	0.051
Other	16 (21.3)	12 (16.0)	
None	52 (69.3)	62 (82.7)	

[a]Number (%) of patients or mean value±standard deviation (median)
COPD chronic obstructive pulmonary disease

asplenia, whether the patient had an 'identified problem with alcohol abuse', and any infections diagnosed in the previous 12 months.

Analytic variables for antimicrobial exposure were created several different ways. Antimicrobial exposure, regardless of class or duration, was first considered as a binomial variable for both time periods. The number of antibiotic classes (macrolides, cephalosporins, fluoroquinolones, penicillins, other) taken in the past 6-week or 12-month period was also analyzed. Finally, antimicrobial exposure was categorized as macrolides versus other antimicrobials versus none.

Potential risk factors for macrolide-resistant *S. pneumoniae* were identified by univariate analysis. Student's *t* test was used to compare continuous variables and chi-square or Fisher's exact test was used to compare categorical variables. Variables with a p value of <0.1 on univariate analysis were candidates for multivariate analysis. A backwards, stepwise, conditional, logistic regression analysis was used; interaction terms were added to all models when appropriate. Analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA). Institutional review board approval was obtained from the University of Iowa, as well as from each of the participating centers.

Results and discussion

Penicillin resistance was present in 81% of cases and 15% of controls. Low-level macrolide resistance (efflux phenotype) was present in 63% of cases, while the MLS_B phenotype (erythromycin MIC ≥32 μg/ml and clindamycin MIC ≥8 μg/ml) appeared in 37% of cases. Case and control patients were similar in terms of age, specimen source of isolate, and geographic region, which was to be expected, since subjects were matched according to these variables (Table 1). In both groups, isolation of *S. pneumoniae* was associated with a similar proportion of infection syndromes, including acute exacerbation of COPD (five cases, five controls), pneumonia with or without bacteremia (41 vs 44), and asymptomatic colonization of the respiratory tract (13 vs 10) (data not shown). Bacteremia occurred at similar rates among case and control patients (9 vs 13 episodes). The median duration of hospital stay was longer among case patients (10.0 days, standard deviation 14.7) but not significantly different than it was for control patients (7.0 days, standard deviation 19.1).

Potential risk factors for infection or colonization with macrolide-resistant *S. pneumoniae* are shown in Table 1. Case patients were significantly more likely to reside in a private home as compared to a chronic care or homeless/transient facility (p=0.026), and to have received antibiotics in the past 6 weeks (p=0.036). Whether this finding is related to more macrolide use in the general community as compared to chronic care/homeless facilities is unknown. COPD was significantly more common among control patients (p=0.014). Three additional variables approached statistical significance: (a) cases were more likely to have received a macrolide as compared to another antibiotic or no antibiotic (p=0.051), (b) they were more likely to have had a history of infection in the past 12 months (p=0.097), and (c) controls were more likely to have a history of alcohol abuse (p=0.068).

Macrolide use in the 6 weeks prior to sampling was reported in seven cases and one control. Five of these eight subjects were between 21 and 64 years of age, six subjects had lower respiratory tract infections, with one having concomitant bacteremia, and five having underlying medical conditions including COPD (n=4), asthma (n=2) and hypertension (n=4). All eight subjects had a history of respiratory tract infection in the 6 weeks prior to obtaining the isolate. Azithromycin was administered to five of the seven cases, while the remaining two cases took erythromycin. The duration of macrolide therapy was short with a mean duration of 4 days (median 3.0 days, range 1–13). The single control patient with a history of macrolide use took azithromycin for sinusitis but the duration of therapy was unknown.

The final conditional logistic regression model included two variables (Table 2). Macrolide resistance in our study was associated with antibiotic use during the prior 6 weeks, and was most strongly associated with previous use of macrolides, in particular azithromycin. Specifically, previous azithromycin use was more commonly associated with macrolide resistance than was erythromycin use. Clarithromycin was not used by any of the cases or controls. These findings are consistent with the results of multiple other studies using a variety of methodologies [3–6, 8]. The protective effect of a history of alcohol abuse was unexpected, and the mechanism of protection is not obvious.

Table 2 Summarized results of conditional logistic regression analyses

Variable	Odds ratio	Confidence interval	p value
Patient with identified alcohol abuse			
Yes vs no	0.9998	0.9997,1.0000	0.0117
Antimicrobial use in 6 weeks prior to obtaining the specimen			
Any macrolide vs no antibiotic	1.0003	1.0001,1.0004	<0.0001
Any macrolide vs other antibiotic	1.0002	1.0001,1.0004	0.0027
Other antibiotic vs no antibiotic	1.0000	0.9999,1.0002	0.4557

The reference, or control, group chosen for case-control studies of antimicrobial-resistant bacteria has been shown to influence the results of outcome studies [9, 10]. We chose to use controls infected with susceptible *S. pneumoniae* rather than uninfected individuals because our goal was to identify risk factors for macrolide resistance developing in previously susceptible strains.

A history of alcohol use was shown to have a very small but significant protective effect on the risk of developing macrolide-resistant *S. pneumoniae*. Our data do not allow us to rule out the existence of an unmeasured confounding variable, but this effect remained significant even though potential confounders and interaction terms available in our data set were examined. For example, more controls than cases were homeless, which potentially could be associated with higher levels of alcohol abuse. Nonetheless, when the analysis was corrected for antibiotic use and alcohol abuse, neither the place of residence variable nor an interaction term between residence and alcohol abuse was independently associated with macrolide resistance. Alcohol abuse has been found to be associated with penicillin resistance in *S. pneumoniae* [11, 12], but its association with macrolide resistance has not been studied, perhaps because many of these analyses are based on pediatric populations. This association should be tested in additional studies with larger numbers of patients, as the real biological significance of this observation is unknown.

This study has several limitations. We relied, of necessity, on the antibiotic history present in the patient's hospital record, which potentially could have been incomplete. It is unlikely that either cases or controls had more or less complete information. Therefore, incomplete data should have biased these results towards the null hypothesis. All patients enrolled in this study had *S. pneumoniae* isolated from culture. This limits the applicability of the findings to individuals who are known or suspected to be infected or colonized with *S. pneumoniae*.

In conclusion, in this case-control study, macrolide use compared to no antibiotic use during the 6 weeks preceding sample collection had a more significant association with macrolide resistance than did either macrolide use compared to other antibiotic use, or other antibiotics compared to no antibiotic use. In addition, most case patients with a history of macrolide use had received azithromycin, which is significantly less potent than clarithromycin [13, 14]. It is possible that the limited potency of azithromycin is in part responsible for driving macrolide resistance with *S. pneumoniae*, as has been suggested by others [8]. Additional studies that include larger numbers of patients exposed to the other macrolides are required to confirm this hypothesis.

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All data and experiments comply with the current laws of the USA.

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