Global Prevalence, Genetics and Clonal Relatedness of Macrolide Resistance in *Moraxella catarrhalis* (2010-2012)

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ABSTRACT

Objective: To evaluate the global prevalence of macrolide resistance (MAC-R) in *Moraxella catarrhalis* during 2010 to 2012 and to determine the genetic basis of resistance and clonal relatedness of strains. MAC-R is generally <1% in most regions of the world but is known to be higher in the Asia Pacific region, especially in China.

Methods: Susceptibility testing was performed using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology on 2,366 isolates isolated in 2010 (853 isolates), 2011 (771 isolates) and 2012 (742 isolates). Isolates were from 169 medical centers in 35 countries in the European Union (671 isolates), United States (USA; 1214 isolates), Latin America (117 isolates), and Asia-Pacific region (APAC; 364 isolates). A total of 21 isolates were non-susceptible to clarithromycin (≥2 mg/L, CLSI M45-A2 criteria) and 20 of these strains were available for further testing (one isolate from UK could not be recovered from storage). Known methylase- and efflux-encoding genes were investigated using PCR. Ribosomal protein L4 and L22 genes and 23S rRNA (each of 4 alleles) were sequenced. Clonality was assessed by PFGE using SpeI.

Results: Overall, 21/2366 (0.9%) of isolates were macrolide resistant. In China, 17/105 (16.2%) of isolates were macrolide resistant with the four remaining isolates found in Korea, New Zealand (NZ), UK and USA. No isolates were positive for the methylase or efflux genes tested. The most prevalent resistance mechanism (15 isolates) was A2058T mutation in all 4 23S rRNA gene alleles in 6 cities in China (13 isolates) and one isolate in Korea. PFGE patterns showed diverse clonality but with some isolates being identical within and between cities. A2058T (4 alleles) was associated with high level clarithromycin (512-2048 mg/L) and azithromycin (128-512 mg/L) resistance. Three isolates (all same city in China with identical PFGE patterns) possessed A2059T 23S rRNA gene mutations (4 alleles) combined with an L22 K68T amino acid substitution and demonstrated low-level MAC-R (clarithromycin and azithromycin ≤4 mg/L). One strain from USA had a P87_R88 RAMP insertion in L22 with low-level MAC-R and one strain from NZ had A2058T (4 alleles) combined with L22 K68T and showed high-level MAC-R.

Conclusions: Overall MAC-R in *M. catarrhalis* was low (0.9%) but much higher in the APAC region, primarily due to high MAC-R (16.2%) from multiple sites in China. The majority of the Chinese isolates and the Korean isolate demonstrated high level MAC-R and had a common mechanism of resistance (A2058T in all 4 alleles) and some evidence of clonality between and within cities. Other mutations were associated with low-level MAC-R and were either clonal (the 3 Chinese isolates) or not related to each other or any of the other strains (the NZ and USA single isolates).

INTRODUCTION

Moraxella catarrhalis is an exclusive human pathogen and resides in the respiratory tract. It is the causative organism in several human diseases (approximate % of cases); otitis media in children (15-20%), exacerbations of chronic obstructive pulmonary disease (10%), sinusitis (20%), and pneumonia (infrequent cases).

M. catarrhalis is usually resistant (>90%) to ampicillin and other penicillins, due to the presence of BRO-1 (common) or BRO-2 (less common) β-lactamases. Resistance to other therapeutically useful agents, such as extended-spectrum cephalosporins, macrolides, trimethoprim/sulphamethoxazole, tetracyclines and fluoroquinolones, is very rare (<1% reported in several regions of the world).

We previously reported on increasing macrolide resistance (up to 7.6%) in the Asia-Pacific region, specifically China, in isolates collected during 2009 to 2011 (Flamm RK, *et al.* 2012). In this study, we examined macrolide resistance in global isolates collected during 2010 to 2012 and determined the macrolide resistance mechanisms associated with this resistance. In addition, we investigated the genetic relatedness of these strains.

MATERIALS AND METHODS

- Susceptibility testing was performed and interpreted using Clinical and Laboratory Standards Institute (CLSI; M45-A2) broth microdilution methodology on 2,366 isolates isolated in 2010 (853 isolates), 2011 (771 isolates) and 2012 (742 isolates). Isolates were from 169 medical centers in 35 countries in the European Union (671 isolates), United States (USA; 1214 isolates), Latin America (117 isolates), and Asia-Pacific region (APAC; 364 isolates). Minimum inhibitory concentration (MIC) testing was performed using fresh frozen broth microdilution panels produced by JMI Laboratories (North Liberty, IA, USA).
- Isolates were identified by the submitting laboratories and confirmed by JMI Laboratories using standard bacteriologic algorithms and methodologies. Identification was confirmed by MALDI-TOF (Bruker, Billerica, MA, USA) for all 21 strains described in this study.
- Macrolide resistance was defined as non-susceptible to clarithromycin (≥2 mg/L, CLSI M45-A2 criteria).
- Known methylase- and efflux-encoding genes, and the β-lactamase genes *bro*-1 and *bro*-2, were investigated using PCR. Ribosomal protein L4 and L22 genes and 23S rRNA (each of 4 alleles) were amplified by PCR and sequenced. Clonality was assessed by PFGE using SpeI.

RESULTS

- Overall, 21/2366 (0.9%) of isolates were macrolide resistant. In China, 17/105 (16.2%) of isolates were macrolide resistant with the four remaining isolates found in Korea, New Zealand (NZ), UK and USA. Susceptibility rates to levofloxacin, tetracycline and trimethoprim/sulfamethoxazole were 99.9%, 99.6% and 95.5%, respectively.
- None of the 21 macrolide-resistant strains were positive for the methylase-encoding genes *erm*(A33TR), *erm*(B), *erm*(34), *erm*(38ML), *erm*(F), *erm*(K), *erm*(CTG), *erm*(D), *erm*(GM), *erm*(Q), *erm*(X).
- None of the 21 macrolide-resistant strains were positive for the efflux-encoding genes *mef*(A), *car*(A), *mac*(A), *mac*(B), *lsa*(A), *lsa*(B), *imr*(A), *vga*(A), *vga*(B) *msr*(A), *msr*(D), *mre*(A).
- The most prevalent resistance mechanism (15 isolates) was A2058T mutation in all 4 23S rRNA gene alleles in 6 cities in China (13 isolates) and one isolate in Korea. PFGE patterns in this group showed diverse clonality but with some isolates being identical within and between cities. All 15 isolates were positive for *bro*-1 (**Table 1**).
- Three isolates (all same city in China with identical PFGE patterns) possessed A2059T 23S rRNA gene mutations (4 alleles) combined with an L22 K68T amino acid substitution and demonstrated low-level MAC-R (clarithromycin and azithromycin ≤4 mg/L). All three isolates were positive for *bro*-1 (**Table 1**).
- One strain from USA had a P87_R88 RAMP insertion in L22 with low-level MAC-R and one strain from NZ had A2058T (4 alleles) combined with L22 K68T and showed high-level MAC-R. The *bro*-2 gene was detected in both of these strains. One isolate from the UK could not be recovered from storage and therefore resistance mechanisms and clonality could not be investigated.

CONCLUSIONS

- Overall global macrolide resistance in *M. catarrhalis* was low, but much higher in the Asia-Pacific region, primarily from multiple sites in China.
- The majority of the Chinese isolates and the Korean isolate demonstrated high-level macrolide resistance and had a common mechanism of resistance (ribosomal 23S A2058T in all 4 alleles) and some evidence of clonality between and within cities.
- Other mutations were associated with low-level macrolide-resistance and were either clonal or not related to each other or any of the other strains.

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Table 1. Geographic, phenotypic, and molecular characteristics of 21 macrolide resistant *M. catarrhalis* isolated globally during 2012 to 2012.

						23S rRNA gene mutations[c]				Ribosomal proteins[d]		MIC (mg/L)[e]		
Isolate No.	Year	Country	City	PFGE[a]	BRO[b]	allele 1	allele 2	allele 3	allele 4	*Rpl*D (L4)	*Rpl*V (L22)	AZI	CLA	CLI
16139	2010	China	Peking	E	1	A2059T	A2059T	A2059T	A2059T	wt	K68T	2	4	128
16356	2010	China	Beijing	F2	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	512
16361	2010	China	Beijing	E	1	A2059T	A2059T	A2059T	A2059T	wt	K68T	1	2	64
16362	2010	China	Beijing	E	1	A2059T	A2059T	A2059T	A2059T	wt	K68T	4	4	64
42970	2010	Korea	Seoul	F2	1	A2058T	A2058T	A2058T	A2058T	wt	wt	512	1024	512
29960	2011	China	Beijing	N	1	A2058T	A2058T	A2058T	A2058T	wt	wt	128	512	1024
29961	2011	China	Beijing	N	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	512	512
30001	2011	China	Zhengzhou	G	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	1024
30037	2011	China	ShenYang	F	1	A2058T	A2058T	A2058T	A2058T	wt	wt	512	1024	1024
30038	2011	China	ShenYang	L	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	512
30039	2011	China	ShenYang	K	1	A2058T	A2058T	A2058T	A2058T	wt	wt	512	2048	1024
30164	2011	China	Peking	O	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	512
30205	2011	China	Jilin	L1	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	2048	512
30206	2011	China	Jilin	J	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	1024
30209	2011	China	Jilin	J	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	1024
30214	2011	China	Wuhan	M	1	A1986C, G1987A, A2058T, A2105G, T2113C, T2131A, C2160T, A2184C	A1986C, G1987A, A2058T, A2105G, T2113C, T2131A, C2160T, A2184C	A1986C, G1987A, A2058T, A2105G, T2113C, T2131A, C2160T, A2184C	A1986C, G1987A, A2058T, A2105G, T2113C, T2131A, C2160T, A2184C	wt	wt	512	1024	512
35450	2012	UK	Leeds	NA[f]	NA	NA	NA	NA	NA	NA	NA	0.06	2	NA
16790	2012	USA	Kansas City	I	2	wt	wt	wt	wt	wt	P87_R88insRAMP	4	4	2
52183	2012	New Zealand	Auckland	B	2	A2058T	A2058T	A2058T	A2058T	wt	K68T	512	512	1024
53268	2012	China	Wuhan	F1	1	A2058T	A2058T	A2058T	A2058T	wt	wt	256	1024	1024
53269	2012	China	Wuhan	F	1	A2058T	A2058T	A2058T	A2058T	wt	wt	512	1024	1024

a. PFGE = Pulsed-field gel electrophoresis pattern; letter designations indicate distinct patterns and numbered patterns of the same letter represent subtypes
b. BRO = presence of *bro* β-lactamase; 1 = *bro*-1, 2 = *bro*-2
c. Nucleotide mutations at alleles 1 to 4 of the 23S rRNA gene (there are 4 copies of this gene in *M. catarrhalis*)
d. Amino acid substitutions in ribosomal proteins L4 and L22
e. AZI = azithromycin; CLA = clarithromycin; CLI = clindamycin
f. NA = not available for testing (i.e. did not survive storage)