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Short Communication

First detection and characterization of macrolide-resistant *Mycoplasma pneumoniae* strains in Cuba

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ABSTRACT

Objectives: Mycoplasma pneumoniae is a common cause of community-acquired pneumonia in humans. Treatment of infections can be complicated by the occurrence of macrolide resistant strains. The study was conducted to evaluate the presence of resistant strains in Cuba and to determine the corresponding genotypes.
Methods: DNA of *M. pneumoniae* isolates and positive respiratory tract specimens collected in the years 2012 and 2017 were tested for resistance-associated mutations of 23S rRNA. In addition, strain types (P1 and MLVA) were determined.
Results: Macrolide resistance mutations were confirmed in 5 out of 27 strains (18.5%). Whereas both P1 subtypes 1 and 2 as well variants V2a and V2c were identified, only two MLVA types (4/5/7/2 and 3/5/6/2) could be found.
Conclusions: During both sampling years, circulation of macrolide resistant strains was demonstrated. No association of resistance with a particular P1/MLVA type was found. Future longitudinal sampling to monitor prevalence of macrolide resistance of *M. pneumoniae* is recommended to verify the resistance pattern of this important pathogen of human respiratory tract infections.

Introduction

Mycoplasma pneumoniae can induce upper and lower respiratory tract infections in humans of all ages, particularly in children and young adults. Although mild tracheobronchitis is the common clinical manifestation, pneumonia is the clinically most important illness associated with *M. pneumoniae* infections. During epidemic peaks reported every three to seven years, up to 40% of cases of community-acquired pneumonia are attributed to this pathogen. Since the cell wall-less mycoplasmas are intrinsically resistant to all betalactam antibiotics, tetracyclines, fluoroquinolones, and macrolides are the only effective options for the treatment of severe infections due to *M. pneumoniae*. With respect to side effects and low inhibitory concentrations, macrolides are the first-line antibiotics and the only recommended treatment for pediatric patients (Waites et al., 2017). In the last years, incidence of macrolide resistant *M. pneumoniae* clinical isolates has been determined resulting in varying rates in different geographic regions (Pereyre et al., 2016). Resistance based on mutations of domain V of the single copy of 23S rRNA of *M. pneumoniae* mainly at positions 2063/2064 (*Escherichia coli* numbering). Recently, a correlation between macrolide resistance and distinct multiple-locus variable-number tandem repeat analysis (MLVA) types have been discussed (Ho et al., 2015) making the further molecular characterization of strains epidemiologically interesting. Thus, the aim of this study was not only to investigate the presence of macrolide resistance mutations but also the corresponding P1/MVLA types in Cuban samples.

Methods

During the year 2012, twenty one *M. pneumoniae* clinical isolates were cultivated from respiratory tract materials (pharyngeal swabs) of children with pneumonia (age: between 8 months and 14 years). In addition, six *M. pneumoniae* positive samples from adults with acute respiratory infections were included. These samples were collected in 2017 and *M. pneumoniae* was identified by real-time PCR.

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DNA from positive patient samples and isolates was extracted with the QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. P1 typing was carried out by analysis of sequence differences in copies of the repetitive elements RepMp4 and RepMp2/3 located in the main P1 adhesin of *M. pneumoniae*. MLVA types were determined by counting the number of repeats in the four recommended variable number of tandem repeat (VNTR) loci (MPN13-16). Using previously described PCR methods (Dumke et al., 2015), parts of 23S rRNA and the P1 adhesin as well the genome regions around the VNTR loci were amplified by nested PCR and sequenced.

Results

M. pneumoniae strains with macrolide resistance mutation were found in both investigated years (Table 1). Overall, 18.5% (5/27) of the analyzed samples demonstrated resistance-associated mutations. Sequencing confirmed three A to G transitions at position 2063 and two mutations at position 2064 (A to G). Mutations of position 2067 und 2617 were not detected in any of the samples. Genotyping based on sequence differences in the repetitive elements of the immunodominant P1 adhesin resulted in occurrence of the main subtypes 1 (48% of tested samples including three resistant strains) and 2 (7%) as well as the subtype 2 variants V2a (18%; one resistant strain) and V2c (26%; one resistant strain), respectively. Using MLVA typing, only two types were found: 3/5/6/2 (52%; two resistant strains) and 4/5/7/2 (48%; three resistant strains). All P1 subtype 1 strains belong to MLVA type 4/5/7/2 and the P1 subtype 2 strains (including the variants) are MLVA type 3/5/6/2.

Discussion

The results of this study show the presence of macrolide resistant *M. pneumoniae* in Cuba. According to the fastidious and long-term growth of mycoplasmas, many reports described the prevalence of macrolide resistance by molecular approaches. The correlation of mutations at positions 2063 or 2064 of 23S rRNA with a broad-range phenotypic resistance to macrolides was confirmed in previous studies (Pereyre et al., 2016). The detected A to G transitions are the most common mutations reported internationally. In general, alarming rates of macrolide resistance up to 100% were measured in Asia whereas significantly lower percentages (0 to <20%) have been demonstrated in European countries (Pereyre et al., 2016). Rates between 4 and 13% were detected in Canada (Eshaghi et al., 2013) and in the United States (Diaz et al., 2015b; Zheng et al., 2015). Little is known about the situation in the Caribbean, Middle and South America. A recent report from Colombia did not find strains with macrolide resistance mutation in children with community-acquired pneumonia caused by *M. pneumoniae* (Copete et al., 2018). The rate of 18% indicated a situation in Cuba which is comparable with data from North America.

Interestingly, further genotyping of the strains in the present study resulted in a higher number of P1 types in comparison with MLVA types. Typing by MLVA using four VNTR loci was described as more discriminatory in comparison with P1 typing (Brown et al., 2015). Our results confirm an epidemiological situation with very low variability of MLVA types emphasizing the further importance of P1 characterization. As reported in other studies (Diaz et al., 2015a), main P1 subtypes 1 and 2 (including variants) strains are correlated with MLVA types 4/5/7/2 and 3/5/6/2. According to the limited number of investigated strains, a clear shift of dominating P1 and MLVA types in the different time periods 2012 and 2017 was not observed in this study. However, the results of the present study demonstrate a correlation between P1 and MLVA type but no association between macrolide resistance mutation and P1/MLVA type.

Despite the limitations of the present study, especially the relatively low number of available samples and missing information about prescription of antibiotic during course of infections, the results should induce clinicians in Cuba to keep the occurrence of macrolide resistance in mind. This is especially the case in patients with confirmed *M. pneumoniae* infection showing no response to adequate antibiotic treatment. For final conclusions about the prevalence of resistant strains, more extensive studies are needed.

Table 1
Genotyping results of clinical isolates and *M. pneumoniae* positive samples in Cuba.

Sample	Year of sampling	Type of sample	Macrolide resistance mutation	P1 type	MLVA type
M.p 2115	2012	Isolate	None	ST1	4/5/7/2
M.p 1	2012	Isolate	None	ST1	4/5/7/2
M.p 2	2012	Isolate	None	ST1	4/5/7/2
M.p 3	2012	Isolate	None	ST1	4/5/7/2
M.p 4	2012	Isolate	None	ST1	4/5/7/2
M.p 5	2012	Isolate	2063: A to G	ST1	4/5/7/2
M.p 6	2012	Isolate	2063: A to G	ST1	4/5/7/2
M.p 7	2012	Isolate	None	ST1	4/5/7/2
M.p 8	2012	Isolate	None	ST2	3/5/6/2
M.p 10	2012	Isolate	None	V2a	3/5/6/2
M.p 11	2012	Isolate	2064: A to G	V2a	3/5/6/2
M.p 12	2012	Isolate	None	ST2	3/5/6/2
M.p 13	2012	Isolate	None	ST1	4/5/7/2
M.p 14	2012	Isolate	None	V2a	3/5/6/2
M.p 15	2012	Isolate	2064: A to G	V2c	3/5/6/2
M.p 16	2012	Isolate	None	V2c	3/5/6/2
M.p 17	2012	Isolate	None	V2a	3/5/6/2
M.p 2178	2012	Isolate	None	ST1	4/5/7/2
M.p M10B	2012	Isolate	None	V2a	3/5/6/2
M.p 2182R	2012	Isolate	None	ST1	4/5/7/2
M.p 25	2012	Isolate	None	ST1	4/5/7/2
12	2017	Clinical sample	None	V2c	3/5/6/2
20	2017	Clinical sample	None	V2c	3/5/6/2
486	2017	Clinical sample	2063: A to G	ST1	4/5/7/2
267	2017	Clinical sample	None	V2c	3/5/6/2
119	2017	Clinical sample	None	V2c	3/5/6/2
235	2017	Clinical sample	None	V2c	3/5/6/2

Conflict of interest statement

There are no conflicts of interest to disclose.

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Ethical approval

This study was approved by the ethical committee of Tropical Medicine Institute "Pedro Kourí" (**CEI-IPK 33-12**).

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