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LETTERS

Macrolide-resistant *Mycoplasma pneumoniae* in paediatric pneumonia

To the Editors:

Mycoplasma pneumoniae is one of the most common causes of bacterial community-acquired pneumonia (CAP) in paediatrics, and can lead to severe and long-lasting disease [1]. Macrolides are usually considered the first-choice antimicrobials for *M. pneumoniae* CAP in children because the alternatives (*i.e.* fluoroquinolones and tetracyclines) are not approved for use in the first years of life [2]. Recent studies from Japan and China have shown macrolide resistance in up to 80% of *M. pneumoniae* strains [3, 4], but it has been detected in relatively few cases in the USA, France and Germany, and not at all in other European countries [5–7].

The mechanism of *M. pneumoniae* macrolide resistance is related to point mutations in domain V of the 23S rRNA gene of *M. pneumoniae* and macrolide resistance is usually detected at disease onset [4]. We here describe the first case of macrolide-resistant *M. pneumoniae* detected during treatment with clarithromycin in an otherwise healthy child with CAP.

An otherwise healthy 6-yr-old girl with an unremarkable medical history who had never travelled abroad was admitted in Bari, Italy, after suffering from a dry cough for 3 days with fever up to 40.5°C, accompanied by increasing malaise and dyspnoea. Upon admission, she was severely ill, with a high temperature, lethargy, an increased respiratory rate (60 breaths·min^{-1}), tachycardia and normal blood pressure. Room air oximetry revealed 85% oxygen saturation, whereas arterial gas sampling showed severe hypoxaemia (50 mmHg) with hypocapnia and a normal pH. Other routine blood examinations revealed neutrophilia with increased C-reactive protein levels and a high erythrocyte sedimentation rate. A physical examination revealed diffuse crackles with reduced vesicular sounds on both lungs, and chest radiography showed an interstitial pattern with multiple ''ground-glass'' infiltrates. Blood, nasopharyngeal swab and sputum samples were immediately obtained for serology, real-time PCR, and respiratory virus and bacterial cultures. High-flow oxygen (8 L·min^{-1}) was necessary to maintain saturation above 90%, and the girl also received nebulised albuterol, intravenous antibiotics (ampicillin/sulbactam 150 mg·kg^{-1}·day^{-1} *i.v.* in three doses and clarithromycin 7.5 mg·kg^{-1}·day^{-1} *i.v.* in two doses) and cristalloids.

Table 1 shows the clinical and laboratory findings upon admission and during the course of hospitalisation. The results of microbiological tests of the samples obtained at admission (available over the following 3 days) were negative for respiratory syncytial virus, adenovirus, influenza viruses, parainfluenza viruses, human metapneumovirus, human bocavirus, coronaviruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Chlamydophila pneumoniae* and *Legionella pneumophila*. However, serology and nasopharyngeal swab real-time PCR (performed at day 0 and available after 3 days) were positive for acute *M. pneumoniae* infection (specific immunoglobulin (Ig)M 1:300 and specific IgG 1:800; real-time PCR positive for *M. pneumoniae* DNA).

Because of the persistence of severe hypoxaemia and the girl's poor general condition, she was transferred to an intensive care unit (ICU) for monitoring after 4 days. A chest computed tomography (CT) scan performed in the ICU showed further progression of the lung infiltration with multiple bilateral areas of consolidation in both lungs and diffuse interstitial infiltration. The same supportive therapy was continued with the administration of clarithromycin (7.5 mg·kg^{-1}·day^{-1} *i.v.* in two doses), meropenem (100 mg·kg^{-1}·day^{-1} *i.v.* in three doses) and teicoplanin (10 mg·kg^{-1}·day^{-1} *i.v. o.d.*) instead of ampicillin/sulbactam. A broad-spectrum antibiotic therapy was continued despite the detection of only *M. pneumonia*e because of the severity of the disease. Further microbiological tests of blood, nasopharyngeal swab and sputum samples continued to show *M. pneumoniae* DNA on the nasopharyngeal swab and no other pathogen.

Despite the broad-spectrum antibiotic therapy, the child remained severely ill and, on day 10, nasopharyngeal aspiration was repeated and a bronchoalveolar lavage sample was obtained: real-time PCR revealed *M. pneumoniae* DNA in both samples.

Given the failure of 10 days of *i.v.* clarithromycin therapy and the persistence of a positive *M. pneumoniae* PCR, a macrolide-resistant *M. pneumoniae* infection was hypothesised and clarithromycin was replaced by ciprofloxacin (40 mg·kg^{-1}·day^{-1} in two doses). The child's clinical condition subsequently improved, as did the results of routine and radiological examinations. She became oxygen-independent after 15 days, and was discharged 20 days after beginning ciprofloxacin (*i.e.* 30 days after admission) with a negative *M. pneumoniae* PCR and no antibiotic therapy. No ciprofloxacin-related adverse event was observed during the following 3 months, at the end of which a chest CT scan appeared normal.

DNA extracted from the respiratory samples using a Nuclisens EasyMAG automated extraction system (Biomeriéux, Craponne, France) was pretested by means of real-time PCR targeting a conserved inter-repetitive region of the P1 gene of M. pneumoniae [8], and the *M. pneumoniae*-positive samples were characterised in known subtypes and variants by sequencing a variable part of the RepMP2/3 repetitive element of the P1 gene as recently described [9]. Briefly, the nested PCR procedure amplifies a part of RepMP2/3 that shows characteristic

TABLE 1 Clinical and laboratory findings in a 6-yr-old girl with community-acquired pneumonia and clarithromycin-induced *Mycoplasma pneumoniae* macrolide resistance

Characteristic	Day 0	Day 4	Day 10	Day 15	Day 25	Day 30
Axillary temperature[#] °C	40.5	39.0	38.3	Apyrexia	Apyrexia	Apyrexia
Respiratory rate breaths·min^{-1}	60	55	52	45	25	24
S_{p,O_2}[¶] %	85	88	85	90	95	98
Heart rate beats·min^{-1}	135	139	140	110	82	76
Blood pressure mmHg	90/60	93/61	88/58	91/62	95/65	90/60
Chest radiography	Multiple ''ground-glass'' infiltrates	NE	''Ground-glass'' opacifications	NE	Significantly improved	NE
Chest CT scan	NE	Bilateral infiltrates, with ''ground-glass'' opacifications	NE	NE	NE	NE
White blood cell count cells·μL^{-1}	10100	NE	11860	11280	4900	6790
Neutrophils %	79	NE	82	84	41	60
CRP mg·dL^{-1}	14.2	7.1	7.1	3.9	0.8	<0.3
ESR mm first hour	53	NE	48	NE	NE	11
Bacterial findings	*M. pneumoniae* DNA in nasopharyngeal swab	*M. pneumoniae* DNA in nasopharyngeal swab	*M. pneumoniae* DNA in nasopharyngeal swab and BAL	*M. pneumoniae* DNA in nasopharyngeal swab	*M. pneumoniae* DNA in nasopharyngeal swab	Negative
Genotype	Variant 2a	Variant 2a	Variant 2a	Variant 2a	Variant 2a	NE
Mutation of 23S rRNA	None	None	A2063G	A2063G	A2063G	NE
Viral findings	Negative	Negative	Negative	Negative	Negative	Negative
Antibiotic therapy	Ampicillin/sulbactam and clarithromycin	Meropenem and teicoplanin and clarithromycin	Meropenem and teicoplanin and clarithromycin	Meropenem and teicoplanin and ciprofloxacin	Ciprofloxacin	End of ciprofloxacin
Supportive O_2 therapy L·min^{-1}	8	6	6	4	NR	NR

S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry; CT: computed tomography; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; rRNA: ribosomal RNA; BAL: bronchoalveolar lavage; NE: not evaluated; NR: not required. [#]: peak day value; [¶]: in room air.

conserved differences in the sequences of the known subtypes and variants of *M. pneumoniae*; sequencing the PCR product allows the reliable classification of the detected strains. Mutations of the 23S rRNA gene of *M. pneumoniae* at positions relevant for most cases of macrolide resistance (2,063 and 2,064) were detected in a culture-independent manner as recently described [7]. Briefly, an aliquot of the product of the nested PCR amplifying a part of the 23S rRNA gene was used as a template for real-time PCR, and the putative mutations at positions 2,063/2,064, suggested by melting peak analysis were confirmed by means of sequence analyses, which also revealed the occurrence of mutations at position 2,617. Molecular subtyping showed a variant 2a in all of the investigated *M. pneumoniae*-positive samples, and an A-G transition at nucleotide 2,063 was found in the samples collected on days 10, 15 and 25, but not in those collected on day 0 and 4 (table 1).

This is the first case report showing the detection of macrolide-resistance in *M. pneumoniae* not at admission but during treatment with a macrolide (clarithromycin). The child did not respond to *i.v.* clarithromycin administered at the usually recommended doses, but showed clinical resolution with ciprofloxacin. After 10 days of therapy (but not upon admission), it was found that the *M. pneumoniae* DNA had a mutation in the 23S rRNA gene associated with *in vitro* resistance to macrolides. This is important because there has recently been a surge in macrolide resistance among clinical isolates of *M. pneumoniae* in various parts of the world. However, children in Europe are not usually involved and there is no report of resistance arising during the course of macrolide treatment.

Our case offers some insights into problems relating to the clinical significance of macrolide-resistant *M. pneumoniae* and the antibacterial treatment of the resulting infections. First of all, it supports the hypothesis that the emergence of macrolide-resistant strains may also be related to drug administration as the mutation was only found after 10 days of clarithromycin treatment and the patient had no known predisposing factor related to the development of macrolide resistance (including immunosuppressive therapy). It is possible that this mechanism of resistance induction has been rarely reported because most previous studies have only considered samples obtained once during the course of the disease. An alternative intriguing explanation could be that the 23S rRNA mutation was already present in the *M. pneumoniae* population (at low levels) within the patient before the administration of antibiotics. The treatment of the patient with clarithromycin may have selected for outgrowth of those bacteria from the population that carried

the 23S rRNA mutation. According to this last hypothesis, the PCR results may only represent the most prominent genotype of the *M. pneumoniae* population and could explain why only a ''non-resistant'' genotype was found at day 0 and 4, and only a ''resistant genotype'' was found at day 10.

Moreover, our case confirms that the *M. pneumoniae* DNA with the A2,063G mutation can be associated with severe disease even when it infects otherwise healthy children. Although macrolide-resistant *M. pneumoniae* strains have been associated with prolonged fever in children [3], there are still no published data indicating that this resistant variant is inherently more pathogenic in any human host. Additionally, the appearance of macrolide resistance has been described in subtypes 1 and 2, which are characterised by sequence differences in all RepMP2/3, RepMP4 and RepMP5 repetitive elements [9]. Although the rate of variant 2a strains has increased in Europe [10], this is the first detection of macrolide resistance in this genotype.

Finally, the optimal treatment for a serious infection caused by macrolide-resistant *M. pneumoniae* remains uncertain. Pharmacokinetics and *in vitro* data indicate that fluoroquinolones and tetracyclines are potential options, but both are contraindicated in children. We administered ciprofloxacin, which slowly led to a favourable clinical and microbiological response without any adverse events. This suggests that the potential benefits of this drug could be considered in the management of serious infections caused by macrolide-resistant *M. pneumoniae*. Another possibility is to verify whether high clarithromycin doses improve its antimicrobial efficacy and lead to a significantly greater improvement in the markers of disease severity without increasing the incidence or severity of adverse events. It has also been shown in a gnotobiotic mouse model mono-associated with macrolide-resistant *M. pneumoniae* that clarithromycin decreased the number of macrolide-sensitive and -resistant *M. pneumoniae* by modulating pulmonary inflammation [11].

In conclusion, our case shows that an A-G transition at position 2,063 of the 23S rRNA gene of *M. pneumoniae* can be detected during adequate treatment with clarithromycin and seems to be associated with treatment failure. It also highlights the fact that local surveillance using molecular methods may be important in determining the prevalence of macrolide resistance among *M. pneumoniae* strains. Furthermore, in the case of macrolide failure during the course of treatment, the presence of macrolide resistance needs to be assessed in order to optimise the antibiotic strategy rapidly.

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