Reviewer's report

**Title:** Gene and cytokine profile analysis of macrolide-resistant Mycoplasma pneumoniae infection in Fukuoka, Japan

**Version:** 2  **Date:** 10 October 2013

**Reviewer:** Cecile Bebear

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This is the description of an outbreak of macrolide-resistant M. pneumoniae infection in Fukuoka, Japan. A high prevalence of macrolide resistance in M. pneumoniae in Japan has been described in many articles. The new findings presented in this manuscript are the description of an outbreak of macrolide-resistant M. pneumoniae in outpatient settings with a high percentage of a rarely-reported mutation, A2063T. However, this mutation has already been recently described in several outbreaks in Japan.

According to that, the manuscript could be shortened to a Note format.

**Major comments**

1. The authors have to consider the small surge described in Fukuoka in the context of the worldwide epidemic of M. pneumoniae infection described in several European countries, Israel and Japan. Line 68, they cite 6 Japanese papers over 8 to describe the worldwide macrolide resistance of M. pneumoniae. They can cite more papers worldwide and less Japanese papers.

2. The authors reported an outbreak of macrolide-resistant M. pneumoniae with the mutation A2063G rarely described. It should be very interesting to determine if this outbreak is clonal or multiclonal using a discriminant molecular typing method such as the MLVA method published by Degrange et al., used in several papers worldwide.

3. In the introduction (lines 80-81) and in the discussion (lines 293-294), the authors should mention the description of several real-time PCR and pyrosequencing assays to look for 23S rRNA mutations directly from respirator specimens. These methods allow a rapid diagnosis of such resistance and are much more specific than an increase of interleukines or interferons to screen for macrolide resistance.

**Specific comments**

- MIC determination, lines 134-136: the MICs cannot have been determined using the CLSI recommendations because theses recommendations have been published in 2011. The references (2,5) cited for the method are from papers published in 2001 and 2004.

- Table 1 should be deleted and data inserted in the text.
- Tables 2 and 3 grouped in a same table.
- Table 3: the isolates harboring the same MICs and the same mutations could be grouped into one single line in the table.

How can the authors explain the MIC differences for CLI and AZM for isolates 1 and 7 and the other isolates which harbor the same A2063G mutation? Did the authors look at mutations in proteins L4 and L22 and in domain II of 23S rRNA for these isolates? To date no ribosomal protein mutation was reported in clinical strains of M. pneumoniae but some were previously described in mutants of M. pneumoniae obtained in vitro.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests' below.