Molecular evidence for the absence of an association between Simkania negevensis and respiratory diseases

Hesham M. Al-Younes, Wael Al-Zereini and Nathir M. Obeidat

Abstract

Simkania negevensis has been implicated in respiratory diseases. This study aimed to unveil the aetiological role of this bacterium in community-acquired pneumonia (CAP) and bronchitis in Jordanian adults. Nasopharyngeal samples were collected from 98 CAP or bronchitis patients and 96 control individuals, and tested for Simkania nucleic acids using a PCR assay. The overall prevalence of the bacterial DNA in patients was markedly high and reached 57.1%. Intriguingly, Simkania DNA was detected in 62.5% of the nasopharyngeal swabs collected from apparently healthy controls (P>0.05). The DNA positivity in the bronchitis and CAP subgroups was 57.7% and 56.9%, respectively, percentages that are approximately comparable to the DNA positivity assessed for the entire patient population. Simkania is most likely not a causative agent of CAP or bronchitis, despite its remarkable high prevalence. This organism, in the nasopharynx, is potentially harmless to the host and may coexist in a commensal relationship.

Simkania negevensis, previously known as 'micro-organism Z' or 'Simkania Z', is a bacterium that was first described as a cell culture contaminant of unknown origin [1]. It belongs to the family Simkaniaeaceae within the order Chlamydiales and shares some biological features with members of the family Chlamydiaceae. It is an obligate intracellular microorganism and typically has a two-stage developmental cycle, consisting of infectious elementary bodies and replicative reticulate bodies [1]. Simkania negevensis appears to be transmitted via domestic water [2].

The seroprevalence of Simkania in different parts of the world revealed a range of 4.3% to approximately 80%, which is considered extremely variable [3–10]. Simkania negevensis has also been correlated with various clinical manifestations, such as pneumonia, exacerbation of chronic obstructive pulmonary disease (COPD), bronchiolitis and gastrointestinal infections [2, 4, 11–17]. Simkania DNA has been amplified from an aortic aneurysm [18]. Other studies, however, did not detect a link between Simkania and respiratory diseases [9, 19–21].

A previous investigation by our group confirmed a high level of S. negevensis infection seropositivity among Jordanian healthy volunteers from both sexes and of different ages [3]. The overall prevalence of anti-Simkania IgG and IgM antibodies was 58.4 and 24.8%, respectively [3]. The aim of the present case-control study was to uncover any causal role of Simkania in lower respiratory diseases based on the amplification of the bacterial nucleic acids from clinical samples collected from hospitalized Jordanian patients suffering from community-acquired pneumonia (CAP) or bronchitis.

Here, 98 adult patients aged between 17 and 92 years (mean age of 55 years ±18) who suffered from acute CAP or bronchitis and were hospitalized at the University of Jordan Hospital, Amman, Jordan, were enrolled. The diagnosis of CAP or bronchitis was made based on clinical, laboratory and radiographic findings. The control individuals were healthy blood donors, laboratory personnel and students at the Department of Biological Sciences, University of Jordan. The ages of the controls ranged from 18 and 87 years (mean age 40.8 years ±15). Control subjects were not eligible for this study if they had symptoms of respiratory tract infection or had received antibiotics within the 3 months prior to enrolment. This study was approved by the respective research committees in addition to the Ethics Committee of the University of Jordan Hospital. Signed informed consent was obtained from each individual who participated in this study. Nasopharyngeal samples were obtained from patients within 48 h of hospital admission and then stored at −70 °C until use. The sampling procedure was conducted as previously described [22]. Extraction of nucleic acids from 200 µl of each nasopharyngeal sample was followed by a PCR assay with specific Simkania primers.
sample was performed according to a protocol specific for the harvesting of DNA from body fluids, provided with the G-spin total DNA extraction kit (iNtRON Biotechnology). DNA was eluted in a final volume of 50 µl of a buffer provided with the kit, aliquoted and stored at −20 °C until analysed. DNA quantification was determined spectrophotometrically. Simkania DNA was tested by PCR using primers targeting a 311 bp fragment of the S. negevensis chaperonin-60 (cpn60) gene as described by Donati et al. [4]. The forward and reverse oligonucleotide primers were 5'-GAT GGA ACG ATG ACT GTT GAA-3’ and 5'-GCA CAA ACT TTT AGT CCT GC-3’, respectively. The cycling conditions were as follows: denaturation for 4 min at 94 °C, followed by 35 cycles, each of which consisted of a denaturation step for 1 min at 94 °C, annealing for 1 min at 55 °C, extension for 1 min at 72 °C and a final elongation step for 7 min at 72 °C. In every PCR run, negative and positive controls were included. The negative PCR controls consisted of all of the components of the amplification mixture, but with distilled water instead of the isolated DNA. Simkania nucleic acid, which served as a positive control, was extracted from purified intact S. negevensis strain Z (ATCC 1325) using a QIAamp DNA mini kit (Qiagen). The statistical analysis of the data obtained was determined using a z-test for percentage differences. A probability value (P) of <0.05 was considered statistically significant.

Table 1 illustrates the demographic characteristics of the study subjects, in addition to the main symptoms and clinical features of patients at hospital admission. Approximately 19 % of bronchitis patients and 90 % of CAP cases showed new pulmonary infiltrates when examined by chest X-ray. The bacterial DNA in nasopharyngeal samples was detected in 57.1 % (56/98) of the patient group suffering from either CAP or bronchitis, whereas 62.5 % (60/96) of the control group showed positive PCR results (Table 2). This elevated level of DNA detection rate in nasopharyngeal swabs from control individuals is insignificant when compared to that of the entire patient cohort (P>0.05). Collectively, these data suggest very strongly that S. negevensis is not an aetiological agent of bronchitis or CAP in Jordan. Analysis of data related to PCR-mediated DNA testing for each patient subgroup remarkably revealed comparable detection rates in bronchitis and CAP groups (Table 2). The prevalence for both patient subgroups was also similar to the overall DNA positivity reported for all patients examined in this study (57.1 %; 56/98). The differences between the DNA positivity obtained for each patient subgroup and that of the control group is not significant (P>0.05). These comparable rates strongly support the notion that S. negevensis does not play a role in the aetiology of bronchitis or CAP in Jordan.

To the best of our knowledge, this was the first controlled study to attempt to correlate S. negevensis with bronchitis and CAP in Jordanian nationals. The difference in DNA positivity between the controls and the entire patient cohort (62.5 % vs 57.1 %), although insignificant, may be partially related to variations in the ages of persons included. As presented in Table 1, analysis of their ages revealed that up to 76 % of the control subjects were younger than 50 years, while the percentage of patients who were less than 50 years old was only 34.7 %.

Intriguingly, the high detection rates for DNA in patients and healthy controls were almost consistent with the overall prevalence of anti-Simkania IgG (58.4 %) in Jordanian individuals [3]. Similarly, these rates are also comparable to those in many epidemiological investigations, which have detected relatively high seroprevalences for S. negevensis in healthy people, ranging from 35–80 % [5–8, 16, 18]. Collectively, these results indicate that this bacterium is highly endemic in Jordan, but it is not an effective causative agent of lower respiratory diseases in adults.

The possible association of Simkania with various respiratory diseases has been verified in a limited number of acute bronchiolitis and CAP cases, mainly in children [2, 11–14, 16, 17]. However, coinfections with respiratory viruses or CAP-associated bacterial agents were detected in many of these patients [11–13, 16]. Interestingly, several reports were contradictory and provided evidence for the absence

<table>
<thead>
<tr>
<th>Current diagnosis</th>
<th>No. examined</th>
<th>No. positive</th>
<th>% positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchitis</td>
<td>26</td>
<td>15</td>
<td>57.7</td>
</tr>
<tr>
<td>CAP</td>
<td>72</td>
<td>41</td>
<td>56.9</td>
</tr>
<tr>
<td>Apparently healthy controls</td>
<td>96</td>
<td>60</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Table 2. Findings of PCR assay targeting Simkania DNA in nasopharyngeal swabs from Jordanian controls and hospitalized subjects suffering from either bronchitis or CAP
of a correlation between *Simkania* and the pathogenesis of respiratory illnesses. For example, in a controlled study performed in the USA, Kumar and colleagues [19] found higher IgG prevalence in control subjects than in patients (children and adults) diagnosed with asthma, bronchiolitis or pneumonia. Similarly, 23 % of nasopharyngeal swabs collected from controls were PCR-positive, compared with 17 % of cases. In another study, serological assays only detected anti-*Simkania* IgM in 2 % of children suffering from asthma and 8 % of controls, while IgG was detected in 12 % of patients and 10 % of control subjects [9]. Recently, *Simkania* infection was not detectable by real-time PCR in nasopharyngeal specimens collected from children with CAP, bronchiolitis or asthma in Turkey [20]. Here, our findings correlate well with the available reports that exclude the existence of a correlation between *Simkania* and respiratory diseases. Overall, these controversial studies on the aetiology of *S. negevensis* in respiratory tract diseases may be related to geographical locations, ethnic variations, sample type and processing, and inconsistencies between the diagnostic assays utilized. An additional major factor contributing to this contradiction could be the lack of healthy controls in most studies that proposed a role for *S. negevensis* as a respiratory pathogen.

Several *in vitro* studies proposed a potential pathogenic capacity for *Simkania* (reviewed in [23]). For instance, *Simkania* could readily infect several cell lines in which apoptosis was inhibited and an inflammatory response was elicited. In specific cell lines, *Simkania* could establish persistent latent infection that was reactivated under certain conditions [23]. It is also documented that intracellular *Simkania* vacuole is closely associated with the endoplasmic reticulum and requires early retrograde transport for its optimal maturation and nutrient acquisition [24, 25]. Taken together, these investigations have provided valuable information about *Simkania*–host cell interaction, as well as the bacterial response to particular host environments when mimicked *in vitro*. However, the *in vivo* setting may be far more complex and significantly different. Thus, it is tempting to assume that *Simkania* may exist as a commensal non-pathogenic agent in the nasopharyngeal region. This hypothesis is supported by the high incidence of this bacterium in patients and healthy controls, as shown in this study, which is consistent with previous controlled reports that presented the absence of a link between *Simkania* and respiratory diseases [9, 19–21]. In summary, this study clearly shows the absence of a link between *S. negevensis* and acute infections of the lower respiratory tract, namely bronchitis and CAP. The analogous high detection rates of *Simkania* among controls and patients recorded here may provide strong evidence for the existence of this bacterium as a harmless commensal organism, at least in the nasopharynx of adults.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This work has been approved by the Ethical Committee of the University of Jordan Hospital. The subjects who participated in this work gave informed consent.

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