ACINETOBACTER BAUMANNII SURVIVES IN SOIL FOR OVER A YEAR

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Introduction
The acute community-acquired human infections with Acinetobacter baumannii [1] suggest a source of this pathogen outside hospital settings. Due to the limited number of attempts, only two studies reported detection of clinically relevant A. baumannii in soils influenced by human solid waste [2,3]. In vitro A. baumannii can endure a wide range of environmental conditions such as desiccation, pH, and temperature [4], suggesting the possibility of long-term persistence in soils. The survival of clinically relevant A. baumannii isolates that express resistance to last-resort antibiotics in autochthonous soils poses a concern, and remains to be determined. This study investigated the long-term survival of A. baumannii in soil, to evaluate the soil as a possible environmental reservoir of A. baumannii, prediction of A. baumannii behaviour in soil, and their potential consequences.

Materials and methods
Three carbapenem-resistant isolates of A. baumannii, having extensive drug-resistant (XDR) or pandrug-resistant (PDR) profile (Table 1) were tested. Two environmental isolates (EF7 and EF8) were recovered from effluent of the Zagreb wastewater treatment plant [5]. One clinical isolate (OB4138) was recovered from the patient suffering from hospital-acquired pneumonia at the Special Hospital for Pulmonary Diseases in Zagreb [6]. Sterilized and fresh red soil of pH 8.43 from Istra, Croatia was chosen for the experiment. Isolates were separately suspended in the autoclaved commercial spring water. These suspensions were used to adjust the moisture of the soil to maximum water holding capacity and simultaneously to supplement the soil with 6.7×10^3 log CFU/g of A. baumannii. Inoculated soils (Fig. 1) were left to dry in the dark at 22°C naturally, following drying in the desiccator when the soil moisture dropped to 5%. The soil moisture was measured gravimetrically by drying at 105°C to constant weight. Number of viable A. baumannii was measured after vortexing one g of soil in sterile physiological solution, following dilution of samples, inoculation onto selective CHROMagar Acinetobacter medium supplemented with CR102, and incubation at 42°C/24h. In fresh soil, additionally number of total heterotrophic bacteria was measured by the inoculation of samples onto nutrient agar and incubation at 22°C/72h. Soil subsample was fixed in 2.5% glutaraldehyde, following the standard preparation for scanning electron microscopy (SEM). Sample was examined at low voltage (0.5 kV) with a Zeiss Ultra PLUS FEG SEM.

Results
In both sterilized (Fig. 2) and fresh soil (Fig. 3), A. baumannii isolates slightly multiplied when the water content ranged from 32-16 wt%. A drop of soil moisture from 16-5 wt% was accompanied by a sharp decrease in viable A. baumannii. At soil moisture below 5 wt%, viable A. baumannii were maintained during more than one year monitoring in sterilized (final abundance 3.6 log CFU/g after 478 days), as well in fresh (final abundance 2.6±0.2 log CFU/g after 352 days) soil. SEM confirmed the presence of A. baumannii biofilm that developed on the soil particles (Fig. 4).

Conclusions
• Extensively- and pandrug-resistant A. baumannii remain viable in soil for over a year.
• This suggest the soil as a potential source of clinically relevant A. baumannii isolates which poses a threat to people that come into contact with the soil.

Acknowledgement
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References

Table 1. Origin, MIC values of tested antibiotics*, and antibiotic resistance profile for three tested isolates of A. baumannii.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>MEM</th>
<th>IMI</th>
<th>CIP</th>
<th>UVX</th>
<th>TOB</th>
<th>GEN</th>
<th>AMK</th>
<th>MIN</th>
<th>SAM</th>
<th>TIM</th>
<th>SXT</th>
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<td>wastewater</td>
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<td>&gt;8</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>B</td>
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<td>&gt;128</td>
<td>&gt;256</td>
<td>≥32</td>
<td>PDR</td>
</tr>
<tr>
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<td>≤16</td>
<td>≤16</td>
<td>≤16</td>
<td>B</td>
<td>≤16</td>
<td>≤128</td>
<td>≤256</td>
<td>≤0.5</td>
<td>XDR</td>
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<tr>
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<td>≥16</td>
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<td>8</td>
<td>128</td>
<td>&gt;256</td>
<td>&gt;512</td>
<td>≥128</td>
<td>XDR</td>
</tr>
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</table>

Fig. 1. Soil artificially contaminated with A. baumannii.

Fig. 2. Survival of A. baumannii (isolate EF8) in sterilized soil adjusted initially to maximum water holding capacity.

Fig. 3. Survival of three isolates of A. baumannii in fresh soil containing the native population of heterotrophic bacteria and adjusted initially to maximum water holding capacity.

Fig. 4. Biofilm of A. baumannii Isolate EF8) developed on the particles of sterilized soil after 6 days of contact.