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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 Istria, 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±0.3 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.

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

[a] carbapenems (MEM-meropenem, IMI-imipenem), fluoroquinolones (CIP-ciprofloxacin, LVX-levofloxacin), aminoglycosides (TOB-tobramycin, GEN-gentamicin, AMK-amikacin), tetracyclines (MIN-minocycline), penicillins/β-lactamase inhibitors (SAM-ampicillin/sulbactam, TIM-ticarcillin/clavulanic acid), SXT- trimethoprim/sulfamethoxazole, CST-colistin. [R] - resistant, [I] - intermediate according to EUCAST or CLSI criteria.

Isolate	Origin	MEM	IMI	CIP	LVX	TOB	GEN	AMK	MIN	SAM	TIM	SXT	CST	Resistance profile
EF7	wastewater effluent	>16[R]	>16[R]	>4[R]	>8[R]	>16[R]	>16[R]	>64[R]	8[I]	>32[R]	>128[R]	>320[R]	20[R]	PDR
EF8		≥16[R]	≥16[R]	≥4[R]	≥8[R]	≥16[R]	≥16[R]	8[S]	≥16[R]	≥32[R]	≥128[R]	≤20[S]	≤0.5[S]	XDR
OB4138	bronchial aspirate	≥16[R]	≥16[R]	>4[R]	8[R]	>16[R]	>16[R]	>64[R]	>16[R]	16[I]	128[R]	<20[S]	≤0.5[S]	XDR

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

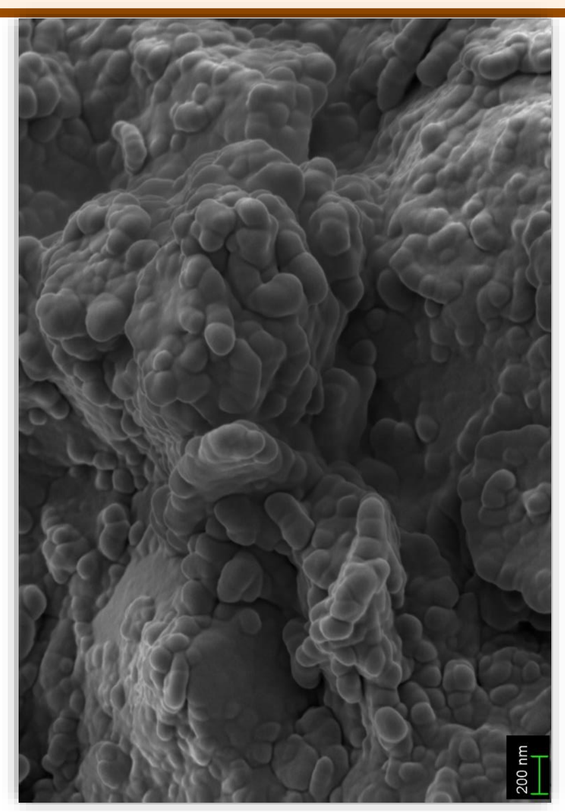

Fig. 4. Biofilm of *A. baumannii* (isolate EF8) developed on the particles of sterilized soil after 6 days of contact.

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).

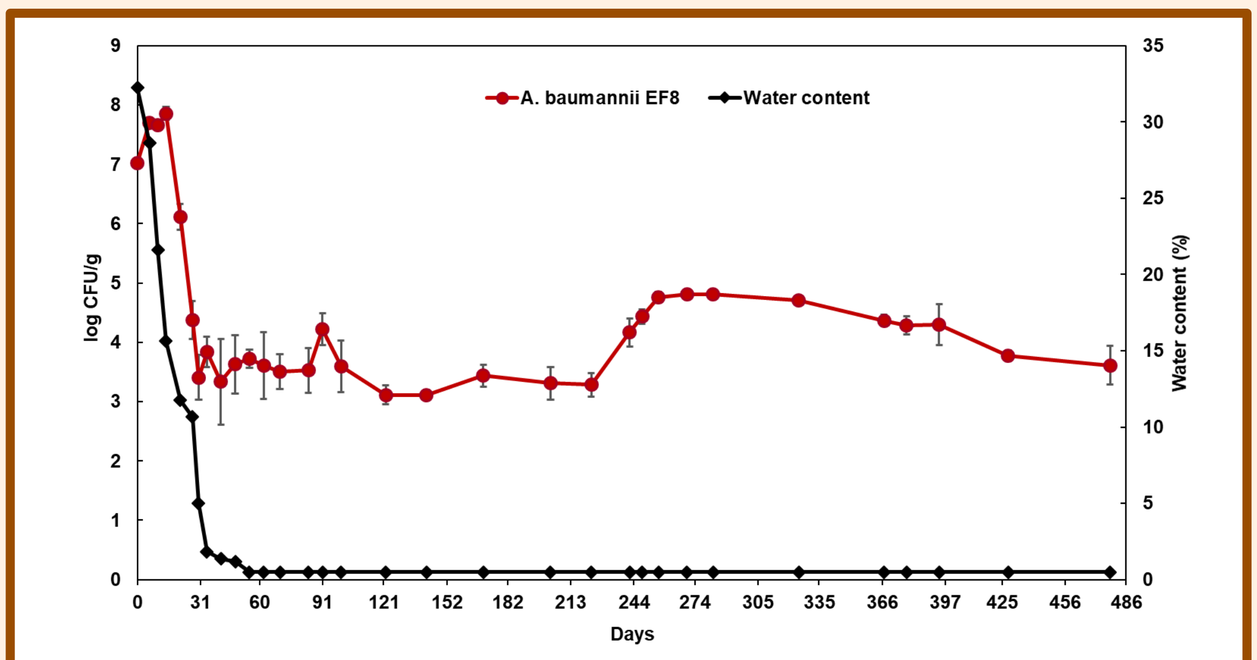

Fig. 2. Survival of *A. baumannii* (isolate EF8) in sterilized soil adjusted initially to maximum water holding capacity.

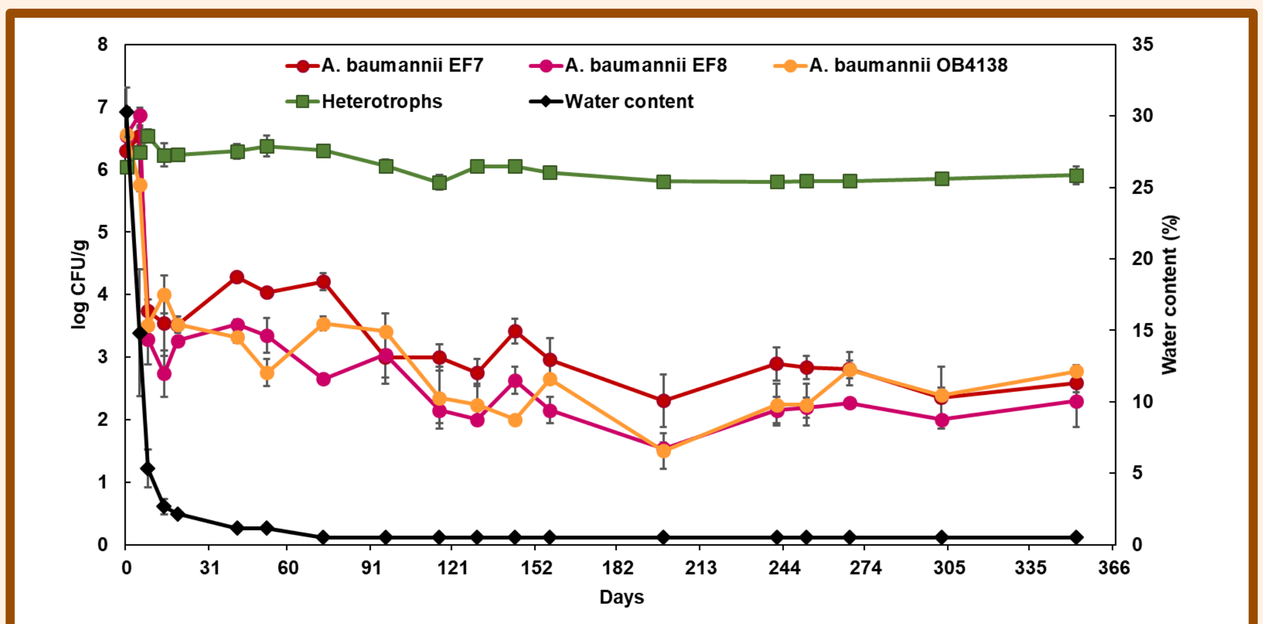

Fig. 3. Survival of three isolates of *A. baumannii* in fresh soil containing the native population of heterotrophic bacteria and adjusted initially to maximum water holding capacity.

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.

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HrZZ Croatian Science Foundation

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