



Presence of carbapenem-resistant bacteria in soils affected by illegal waste dumps

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ABSTRACT

The carbapenem-resistant bacteria (CRB) are currently at the top of the WHO priority list of bacteria that pose the greatest threat to human health. Considering that soil is one of the important environments for the emergence of antibiotic-resistant bacteria, we isolated and quantified cultivable CRB in soils across Croatia, including ones affected by illegal dumps.

We cultivated CRB at two temperatures, distinguishing between the intrinsically resistant CRB (37°C, mostly *Stenotrophomonas* spp.) and the ones that are presumably human-associated and clinically relevant (42°C, *Acinetobacter* sp., *Enterobacteriaceae*, *Burkholderia* spp.).

Our study demonstrated that distinguishing between the two offers a better insight into the diversity of CRB in the environment. The ones cultivated at 37 °C were found in almost all soil samples, while the presumably clinically relevant ones were absent from uncontaminated pasture and grassland, indicating that human-associated CRB are unlikely to be found in soils spared from anthropogenic influence.

MATERIALS AND METHODS

Soil samples were taken at 14 locations (eight illegal dump sites, one coke factory, one thermal power plant, two arable lands, one pasture, and a grassland). Used methodology is based on cultivation of environmental samples on 37 and 42°C in parallel. The latter suppresses the growth of *Stenotrophomonas* sp. which are intrinsically carbapenem-resistant and ubiquitous in soil, and therefore dominant in environmental/soil samples cultivated at 37°C. Cultivation at 42°C allows the detection of other CRB that are usually present in far smaller numbers than *Stenotrophomonas* sp.. This methodology has been successfully used in our previous studies where CRB were monitored in wastewater treatment plant and now it was, to best of our knowledge, implemented for soil samples for the first time.

The CRB were cultivated on selective CHROMagar™ Acinetobacter medium (CHROMagar, Paris, France). With addition of CR102 supplement, the growth of carbapenem-resistant *Acinetobacter* sp. and other carbapenem-resistant Gram-negative bacteria, belonging mostly to the *Enterobacteriaceae*, *Pseudomonas* spp., and *Stenotrophomonas* genera is allowed.

Incubating the samples at 37 and 42°C, respectively, enabled us to identify CRB with acquired resistance, that are normally present in smaller numbers and shaded by large numbers of intrinsically resistant *Stenotrophomonas* sp. and/or other species.

Intrinsically resistant CRB were found in every soil sample, including the uncontaminated pasture and grassland (log 2.4-5.3 CFU g⁻¹) confirming that CRB with intrinsic resistance are abundant in soil. Presumably clinically relevant CRB, with acquired resistance, were found in most dump sites (log 1.2 – 4.7 CFU g⁻¹), but not in uncontaminated pasture and grassland, recognizing illegal dump sites as potential sites of dissemination of antibiotic-resistant bacteria to the environment.



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Species	No of isolates	No	No
<i>Acinetobacter baumannii</i>	3	<i>Enterobacter asburiae</i>	1
<i>Acinetobacter</i> sp.	6	<i>Enterobacter cloacae</i>	3
<i>Burkholderia ambifaria</i>	12	<i>Enterobacter ludwigii</i>	1
<i>Burkholderia multivorans</i>	2	<i>Enterobacter</i> sp.	2
<i>Burkholderia</i> sp.	2	<i>Escherichia coli</i>	3
<i>Cupriavidus gilardii</i>	3	<i>Escherichia</i> sp.	1
<i>Cupriavidus respiraculi</i>	5	<i>Ochrobactrum intermedium</i>	3
		<i>Pediococcus</i> sp.	1
		<i>Providencia</i> sp.	1
		<i>Providencia stuartii</i>	1
		<i>Pseudomonas putida</i>	1
		<i>Sphingobacterium thalophilum</i>	1

List of bacteria with acquired resistance to carbapenems randomly isolated after cultivation at 42°C. Bacteria isolated after cultivation at 37°C were intrinsically resistant to carbapenems with 10 isolates of *Stenotrophomonas* sp. and one isolate of *Elizabethkingia meningoseptica*.

An example of CHROMagar Acinetobacter™ plate (without CR102 supplement for cultivation of carbapenem-resistant bacteria) inoculated with wastewater sample after incubation at 37°C for 48 hours. The blue colonies are usually *Enterobacteriaceae* and red colonies are *Stenotrophomonas* sp., *Acinetobacter* sp., *Pseudomonas* sp. or other Gram-negative bacteria.

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