Journal of Global Antimicrobial Resistance 15 (2018) 88–92

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Transmission of IMI-2 carbapenemase-producing Enterobacteriaceae from river water to human

Chrislène Laurens[a], Hélène Jean-Pierre[a], Patricia Licznar-Fajardo[b], Stefaniya Hantova[b], Sylvain Godreuil[c], Orianne Martinez[d], Estelle Jumas-Bilak[b,*]

[a] Laboratoire de bactériologie, CHU de Montpellier, Université de Montpellier, Montpellier, France
[b] HydroSciences Montpellier, Université de Montpellier, CNRS, IRD, Département d'Hygiène Hospitalière, CHU de Montpellier, Montpellier, France
[c] MIVEGEC, IRD, CNRS, Université de Montpellier, Laboratoire de bactériologie, CHU de Montpellier, Montpellier, France
[d] Département Anesthésie Réanimation A, CHU de Montpellier, Université de Montpellier, Montpellier, France

ARTICLE INFO

Article history:
Received 7 January 2018
Received in revised form 17 June 2018
Accepted 29 June 2018
Available online 6 July 2018

Keywords:
Bacteraemia
Carbapenemase-producing enterobacteria
Corbicula
Environmental reservoir
Gut microbiota
Pathobiome

ABSTRACT

Objectives: Carbapenemase-producing Enterobacteriaceae (CPE) are increasing worldwide in human infections. The role of rivers as reservoirs is highlighted, but transmission from the environment to humans is not documented. A human case of bacteraemia caused by IMI-2 carbapenemase-producing *Enterobacter asburiae* following massive river water exposure underwent microbiological investigations with the aim of deciphering the origin and mechanism of infection.
Methods: Clinical and environmental bacterial strains were compared by resistotyping and genomotyping using pulsed-field gel electrophoresis (PFGE). PFGE was also used to determine the location of the bla_{IMI-2} carbapenemase gene. The patient's microbiota and river bacterial communities were compared by fingerprinting using 16S rRNA gene PCR–temporal temperature gel electrophoresis.
Results: Enterobacter asburiae causing bacteraemia carried the same plasmidic bla_{IMI-2} gene as *E. asburiae* strains detected in river water 1 month later. Clinical and river strains displayed identical PFGE profiles. Community fingerprinting showed the persistence in the patient's microbiota of carbapenem-resistant bacteria, which were also autochthonous in the river community (*E. asburiae*, *Aeromonas veronii* and *Pseudomonas fluorescens*).
Conclusion: Here we have identified for the first time the presence of an IMI-2-producing *E. asburiae* in a river in the South of France and suggest transmission from the river to a human probably following intestinal translocation. General insights into transmission of CPE from the environment to humans are gained from this case. Considering the rapid spread of CPE in humans, the risk of transfer from an environmental reservoir to human microbiota should be thoroughly investigated at least by implementing environmental surveillance of carbapenem resistance.

1. Introduction

The emergence and spread of carbapenemase-producing Enterobacteriaceae (CPE) is of great concern as carbapenems are antibiotics of last-resort to treat serious infections. In most countries, human infections caused by CPE are increasing annually [1], whilst reports of carbapenemases in animal and environmental reservoirs are published more and more frequently [2]. Aquatic ecosystems that integrate xenobiotics, resistance genes, and allochthonous and autochthonous bacteria require particular attention with regard to the emergence and spread of CPE [3–6].

A recent study detected an average of 2.24 CFU/mL of imipenem-resistant bacteria, including Enterobacteriaceae, in riverine ecosystems [7]. Moreover, some carbapenemase-encoding genes found in clinically relevant species appear to have originated from bacteria in aquatic environments. The principal example is OXA-48 carbapenemase, which is prevalent in human infections and occurs naturally in *Shewanella* spp., a genus inhabiting natural waters and sediments [8]. Conversely, the release of high-risk CPE into the environment from clinical settings also occurs [9]. Several bacteria, such as Enterobacteriaceae and *Aeromonas*, can act as shuttles for resistance genes between the environment and humans because such bacteria can thrive in both habitats.

* Corresponding author. Present address: UMR 5569 HydroSciences, équipe Pathogènes Hydriques Santé Environnements, 15 Avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France.
E-mail address: estelle.bilak@umontpellier.fr (E. Jumas-Bilak).

https://doi.org/10.1016/j.jgar.2018.06.022
2213-7165/

IMI carbapenemases are less described in clinical settings than OXA-48, KPC, IMP, NDM and VIM carbapenemases [2] but are considered as emerging carbapenemases [10]. The IMI-1 carbapenemase gene was initially detected in the chromosome of a clinical strain of *Enterobacter cloacae* in 1984, a year prior to clinical use of imipenem [11]. The IMI-2 variant was first identified on plasmids from environmental strains of *Enterobacter asburiae* in rivers in the USA [3] and then from clinical isolates of *E. cloacae* [12], *Escherichia coli* [13] and *Klebsiella variicola* [10]. IMI-2-producing *E. asburiae* has been recently described as emerging in the Czech Republic [14].

A human case of bacteraemia caused by IMI-2 carbapenemase-producing *E. asburiae* after accidental near-drowning in a French river was submitted to clinical and environmental microbiology investigations with the aim of demonstrating the environmental origin of the infection and to decipher the mechanism of infection.

2. Materials and methods

2.1. Bacterial strains, culture and identification

Clinical strains were obtained during the patient's hospital follow-up. Ten *E. asburiae* strains isolated in the environment of the intensive care unit (ICU) where the patient was hospitalised were included. A total of 1 L of river water was filtered through 0.22-μm nitrocellulose membranes and bacteria were grown for 48 h at 37 °C on MacConkey agar and chromogenic Brilliance CRE agar (Thermo Fisher Scientific, Villebon-sur-Yvette, France) to select enterobacteria and carbapenemase-producers, respectively. Invasive freshwater molluscs of the genus *Corbicula* were also collected and were crushed and cultured on the same media.

Species and/or genus identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) and 16S rRNA gene sequencing. Specific identification within the *E. cloacae* complex was realised by testing esculin hydrolysis and by *hsp60* and *rpoB* gene sequencing [15].

2.2. Antimicrobial susceptibility testing and characterisation of carbapenemase

Antimicrobial susceptibility testing was performed by the disk diffusion assay and Etest (bioMérieux France, Craponne, France) on Mueller–Hinton agar according to the Antibiogram Committee of the French Society of Microbiology, 2016 (http://www.sfm-microbiologie.org/). Synergy of meropenem with the β-lactamase inhibitors boronic acid, dipicolinic acid and cloxacillin (Rosco Diagnostica, Taastrup, Denmark) was performed to determine the Ambler class of the carbapenemase. A microarray was used to detect KPC, VIM, NDM, IMP and OXA-48 genes (Check-MDR CT103 array; Check-Points B.V., Wageningen, The Netherlands). The bla_{IMI-2} gene was detected by PCR and was sequenced as previously described [3].

2.3. Pulsed-field gel electrophoresis (PFGE) and DNA hybridisation experiments

For strain comparison, genomic DNA was digested with *Xba*I or I-*Ceu*I [16] or S1 nuclease [17] and was subjected to PFGE for 22 h at 6 V/cm and 12 °C with initial and final pulse times of 5 s and 40 s using a CHEF-DR® II System (Bio-Rad, Marnes-la-Coquette, France). The location of the bla_{IMI-2} gene was determined by hybridisation with a digoxigenin-labelled bla_{IMI-2} probe [16] following Southern blotting of PFGE gels [16].

2.4. Temporal temperature gel electrophoresis of amplified 16S rRNA gene sequences (16S rRNA-PCR-TTGE)

DNA extracted from bacterial communities was amplified by V3 region 16S rRNA-based PCR using HDA1-GC and HDA2 as primers

Table 1
Summary of the clinical and microbiological status of the patient as described in the patient's medical record.

Day from admission	Clinical event	Bacteriology (sample)	Antimicrobial resistance	Treatment
D0	Multiple organ failure post-resuscitation			Amoxicillin/clavulanic acid 3 g/day
D2	Deterioration of pulmonary status	*Aeromonas veronii* (respiratory tract)	Imipenem Ticarcillin Amoxicillin *Wild-type resistance*	(considering *A. veronii* and *P. fluorescens* as colonising but not infecting)
		Pseudomonas fluorescens (respiratory tract)	Imipenem (MIC ≥ 32 μg/mL) Doripenem (MIC = 6 μg/mL) Meropenem (MIC = 8 μg/mL) *No associated resistance to cefepime, piperacillin, ceftazidime, fluoroquinolones or aminoglycosides*	
D5	Sepsis	*Enterobacter asburiae* (blood culture)	Amoxicillin Amoxicillin/clavulanic acid Cefalotin Cefoxitin Ticarcillin Piperacillin Imipenem (MIC ≥ 32 μg/mL) Ertapenem (MIC ≥ 32 μg/mL) *No associated resistance to aminoglycosides or fluoroquinolones*	Cefepime (4 g/day) for 15 days with amikacin (15 mg/kg/day) for 2 days and then ciprofloxacin (800 mg/day)
D8	ND	*Pseudomonas aeruginosa* (rectal swab)	Multidrug-resistant	
D26	Right-sided pneumonia	*P. aeruginosa* (respiratory tract)	Multidrug-resistant	Piperacillin/tazobactam (12/1.5 g/day) plus ciprofloxacin for 12 days
D50	Patient discharged from hospital			

MIC, minimum inhibitory concentration; ND, not determined.