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Evolution of high-level daptomycin resistance in *Enterococcus faecium* during daptomycin therapy is associated with limited mutations in the bacterial genome

Theodoros Kelesidis[1], Ryan Tewhey[2,3] and Romney M. Humphries[4]*

[1]Division of Infectious Diseases, UCLA David Geffen School of Medicine, Los Angeles, CA, USA; [2]Department of Organismic and Evolutionary Biology, Center for Systems Biology, Harvard University, Cambridge, MA, USA; [3]The Broad Institute of the Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA; [4]Department of Pathology and Laboratory Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA, USA

*Corresponding author. Tel: +1-310-794-3614; Fax: +1-310-794-2765; E-mail: rhumphries@mednet.ucla.edu

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Sir,

Treatment options for vancomycin-resistant *Enterococcus* (VRE) infections are limited. Daptomycin, a lipopeptide with *in vitro* bactericidal activity against VRE,[1] is commonly used to treat such infections. Over the past 5 years an increasing number of reports have documented the emergence of daptomycin-non-susceptible *Enterococcus* in patients following treatment with daptomycin,[1–3] but little is known about how quickly daptomycin non-susceptibility may emerge *in vivo*. We describe a case of high-level daptomycin resistance (MIC = 256 mg/L) in *Enterococcus faecium* following only 9 days of exposure to daptomycin and associated with limited mutations in the bacterial genome. All protocols were approved by the UCLA Institutional Review Board.

A woman with a history of thyroid carcinoma, status post-thyroidectomy, morbid obesity and hypertension was diagnosed with a pancreatic tumour and underwent an elective Whipple resection at our medical centre. Her postoperative course was complicated by persistent fevers, worsening leucocytosis and renal failure, for which she underwent haemodialysis. She was started on piperacillin/tazobactam and initial blood, sputum and urine cultures were negative for bacterial growth. She was also found to have persistent hypocalcaemia secondary to hypoparathyroidism (Figure S1, available as Supplementary data at *JAC* Online). A CT scan of the abdomen and pelvis on postoperative day 10 showed a new 1.8 cm fluid collection that was concerning for abscess formation. After a 14 day course of intravenous piperacillin/tazobactam she was started on intravenous vancomycin, ciprofloxacin and metronidazole. Repeat CT of the abdomen and pelvis with intravenous contrast on postoperative day 17 revealed multiple intra-abdominal fluid collections. Intra-abdominal drains were placed on postoperative day 19 and vancomycin-resistant *E. faecium* susceptible to daptomycin (MIC = 2 mg/L, isolate 11-9-40) and *Candida albicans* were isolated by the laboratory from peripancreatic fluid submitted for bacterial culture. She was started on intravenous 6 mg/kg of daptomycin every 48 h, cefepime and metronidazole. She was placed on total parenteral nutrition (TPN) as a nutritional supplement to her oral intake on postoperative day 21 and a high-calcium bath dialysis regimen. As a result, she developed hypercalcaemia during the next 4 days (Figure S1, available as Supplementary data at *JAC* Online) that resolved after adjustment of the calcium content in the TPN fluid. Urine collected on postoperative day 29 revealed 50 white blood cells/μL, 45 squamous epithelial cells/μL and grew 100 000 cfu/mL daptomycin-non-susceptible *E. faecium* (daptomycin MIC >256 mg/L, isolate 11-9-43). On postoperative day 35, a daptomycin-non-susceptible *E. faecium* (daptomycin MIC = 32 mg/L, isolate 11-9-38) was again isolated from intra-abdominal fluid submitted for bacterial culture. The patient was switched to intravenous tigecycline and caspofungin, her fever improved and she remained afebrile until discharge. A repeat CT scan prior to discharge demonstrated a stable appearance of intra-abdominal fluid collections.

Susceptibility testing was performed on all three isolates using the CLSI reference standard broth microdilution (BMD) method[4] in cation-adjusted Mueller–Hinton broth, supplemented with 50 mg/L of calcium for daptomycin testing, at the time of bacterial isolation. MICs were confirmed by BMD and Etest (bioMérieux, Durham, NC, USA) on Mueller–Hinton agar (BBL, Lenexa, KS, USA) directly prior to whole-genome sequencing (WGS). A daptomycin MIC >4 mg/L was considered to indicate non-susceptibility.[5] WGS of the three isolates was performed using an Illumina HiSeq 2000 sequencer, as described previously.[6]

Surprisingly few nucleotide polymorphisms were identified between the sequenced genomes of the three isolates (Table 1). Only four single-nucleotide variants (SNVs) were identified between the daptomycin-susceptible (11-9-40) and daptomycin-non-susceptible (11-9-43 and 11-9-38) isolates. Both daptomycin-non-susceptible isolates harboured an SNV in the cardiolipin gene, although at discrete loci (Table 1). No consistent mutation in *cls* has been associated with daptomycin non-susceptibility in *Enterococcus*, but *cls* polymorphism has been described in all daptomycin-non-susceptible *E. faecium*[6] and *Enterococcus faecalis*[7,8] to date, confirming the role of this

Table 1. Genetic characteristics of daptomycin-non-susceptible *E. faecium* as compared with parent daptomycin-susceptible strain 11-9-40

	Isolate					
	11-9-40		11-9-43		11-9-38	
Day isolated	1		9		14	
Source	peripancreatic fluid		urine		peripancreatic fluid	
Daptomycin MIC (mg/L)	2		>256		32	
	Mutations identified by WGS, as compared with 11-9-40					
Gene	nucleotide	predicted amino acid	nucleotide	predicted amino acid	nucleotide	predicted amino acid
Cardiolipin synthetase	WT	—	A38C	N12T	G177T	K59T
Hypothetical protein	WT	—	C109T	nonsense mutation	C109T	nonsense mutation
Dihydrodipicolinate reductase	WT	—	WT	—	deletion of A356	frameshift
Membrane protein	WT	—	339 bp deletion	—	339 bp deletion	
Aspartate semi-aldehyde dehydrogenase	WT	—	G416T	A139D	WT	—

WT, wild-type.

phospholipase in daptomycin resistance. We have now noted the same SNV, A38C, in two additional *E. faecium* with high-level daptomycin MICs (e.g. 192 and >256 mg/L, data not shown). Retrospective analysis of these isolates determined they were not clonally related, as described previously.[2]

Both daptomycin-non-susceptible *Enterococcus* harboured a nonsense mutation in a hypothetical protein and a 339 bp deletion in a predicted membrane protein of unknown function. Isolate 11-9-43 harboured an SNV in a gene predicted to encode an aspartate semi-aldehyde dehydrogenase not found in 11-9-38, whereas 11-9-38 harboured a frameshift mutation in a dihydrodipicolinate reductase gene not present in 11-9-43. It is unclear if these mutations contributed to the daptomycin-non-susceptible phenotype of these two isolates, but these genes have not been found by other studies and may relate to plasticity in the enterococcal genome. The WGS data also do not provide insight into differences in gene expression levels between the isolates, which may also contribute to differences in daptomycin MIC.

Similar to our previous findings regarding risk factors associated with the development of daptomycin-non-susceptible *Enterococcus*, this patient was immunocompromised, had recent surgery, an intra-abdominal pathological process, presence of a nidus of daptomycin-non-susceptible *Enterococcus* infection and exposure to antibiotics associated with the development of VRE, including third-generation cephalosporins and anti-anaerobic agents.[1–3] Several factors may have contributed to the development of daptomycin-non-susceptible *Enterococcus* in this patient. Three clinical events occurred during the time period between isolation of an *E. faecium* with a daptomycin MIC of 2 mg/L and one with an MIC of 256 mg/L: the patient was exposed to very high concentrations of cefepime, had significant fluctuations in calcium levels and experienced renal failure. Cephalosporins have been known to be associated with the development of VRE, but further case–control studies need to establish whether they can also be associated with the development of daptomycin-non-susceptible *Enterococcus*. Similarly, although daptomycin has a calcium-dependent mechanism of action and the *in vitro* calcium concentration of the test medium significantly impacts the daptomycin MIC, the impact of systemic calcium levels on the development of daptomycin-non-susceptible *Enterococcus in vivo* remains unknown. A 2-fold reduction in calcium concentration (e.g. 25 mg/L versus 50 mg/L) results in a 2-fold increase in the *Enterococcus* daptomycin MIC.[9] The patient's corrected serum calcium ranged from 65.2 to 116.4 mg/L in the month before isolation of daptomycin-non-susceptible *Enterococcus*, both significantly higher than those used for *in vitro* testing. This chronic severe hypocalcaemia may have led to an even lower calcium concentration at the infectious focus (abscesses), which caused a loss of daptomycin activity.[10] Patients with end-stage renal disease like our patient have a larger volume of distribution for daptomycin and lower C_{max} compared with those achieved in healthy volunteers.[11] Thus, the concentrations of daptomycin used in our patient (6 mg/kg every 48 h) may have been relatively low and may induced rapid development of daptomycin non-susceptibility.

The acquisition and emergence of daptomycin resistance among enterococci poses both treatment and infection control challenges. Clinicians should be vigilant that rapid emergence

of daptomycin-non-susceptible *Enterococcus* may occur in the setting of disorders of calcium homeostasis, relatively low doses of daptomycin and end-stage renal disease, which may be associated with limited mutations in the bacterial genome.

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Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://mc.manuscriptcentral.com/jac).

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Spread of NDM-2-producing *Acinetobacter baumannii* in the Middle East

P. Espinal[1], L. Poirel[2], Y. Carmeli[3], M. Kaase[4], T. Pal[5], P. Nordmann[2] and J. Vila[1]*

[1]Department of Clinical Microbiology, Hospital Clinic, CRESIB/IDIBAPS, School of Medicine, University of Barcelona, Barcelona, Spain; [2]Service de Bactériologie-Virologie, INSERM U914 'Emerging Resistance to Antibiotics', Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, K.-Bicêtre, France; [3]Division of Epidemiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [4]National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria, Department of Medical Microbiology, Ruhr-University, Bochum, Germany; [5]Department of Microbiology and Immunology, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

*Corresponding author. Tel: +34932275522; Fax: +34932279372; E-mail: jvila@ub.edu

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Sir,

Resistance of *Acinetobacter baumannii* isolates to β-lactams and particularly to carbapenems is on the rise. Carbapenem resistance in *A. baumannii* due to the production of metallo-β-lactamases (MBLs) such as New Delhi MBL (NDM) is increasingly being reported in different parts of the world. To date, two types of NDM (NDM-1 and NDM-2) have been described in *A. baumannii*[1–7] and in other *Acinetobacter* species.[8–11]

Five previously described NDM-2-producing *A. baumannii* isolates recovered from Egypt, Israel and the United Arab Emirates (UAE) were included in the study.[2,3,7] The first corresponded to an *A. baumannii* ML isolate obtained in October 2010 in Germany from a patient who had been hospitalized in Cairo.[3] The other four isolates comprised two (AB1 and AB2) clonally related organisms recovered in an Israeli rehabilitation centre in mid-2009[2] and two recovered 4 months apart in 2009 from the same patient in Al Ain in the UAE.[7] All the isolates were confirmed as *A. baumannii* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and resistance to carbapenems was confirmed by Etest (AB bioMérieux, Solna, Sweden). Results were interpreted according to CLSI guidelines.[12] Although all the isolates had previously been shown to belong to sequence type 103 by multilocus sequence typing according to the Pasteur system (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html), in this study we performed further epidemiological characterization using PFGE and plasmid profiles[13] to confirm they belonged to the same clone.