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Characterization of daptomycin non-susceptible *Enterococcus faecium* producing urinary tract infection in a renal transplant recipient

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ABSTRACT

Objectives. Characterization of a urine isolate of daptomycin non-susceptible *Enterococcus faecium* recovered from a patient with kidney transplantation and no history of daptomycin exposure.

Methods. After isolation in a urine sample, identification of *E. faecium* was confirmed by amplification of the *E. faecium*-specific gene encoding D-alanyl-D-alanine ligase (*ddl*) and daptomycin susceptibility testing was performed by E-test on cation-adjusted Mueller-Hinton agar. In order to determine the genetic bases of daptomycin resistance, the open reading frames of five genes previously associated with daptomycin resistance in enterococci were sequenced.

Results. Substitutions in the response regulator LiaR (S19F) and cardiolipin synthase (R218Q) were identified.

Conclusions. To the best of our knowledge, this is the first characterization of emerging daptomycin resistance in *E. faecium* in a Spanish hospital in the absence of daptomycin exposure and in a renal transplant recipient.

Keywords: *Enterococcus faecium*, daptomycin non-susceptible, *liaR*, cardiolipin synthase

Caracterización de *Enterococcus faecium* no sensible a daptomicina produciendo infección del tracto urinario en un paciente trasplantado renal

RESUMEN

Objetivos. Presentamos la caracterización de un aislado de *Enterococcus faecium* no sensible a daptomicina, recuperado de una muestra de orina de un paciente con trasplante

de riñón e infección urinaria y sin antecedentes de exposición previa a daptomicina.

Métodos. Tras el aislamiento, la identificación de *E. faecium* fue confirmada por la amplificación del gen que codifica la región específica de la ligasa de la D-alanil-D-alanina (*ddl*) y la prueba de sensibilidad a daptomicina se realizó mediante E-test en agar Mueller-Hinton ajustado para cationes. Con el fin de determinar las bases genéticas de la resistencia a daptomicina, se secuenciaron las regiones de lectura abierta de cinco genes previamente asociados con la resistencia a daptomicina en enterococos.

Resultados. Se identificaron cambios en el promotor de LiaR (S19F) y la sintetasa de la cardiolipina (R218Q).

Conclusiones. Esta es la primera caracterización de un aislado clínico de *E. faecium* con resistencia a daptomicina en un hospital español, en ausencia de exposición previa y en un receptor de trasplante renal.

Palabras clave: *Enterococcus faecium*, daptomicina, *liaR*, cardiolipina sintetasa.

INTRODUCTION

Daptomycin has been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for skin and soft tissue infections and *Staphylococcus aureus* bacteremia. Although this antibiotic does not have an approved indication for multidrug-resistant enterococci, daptomycin has been extensively used against these organisms due to the paucity of other bactericidal options¹. However, reports of emergence daptomycin non-susceptibility during therapy of enterococci infections appear to be a problem with cases reported even in patients who have not received the antibiotic²⁻⁶. Here, we report the detection of a clinical isolate of daptomycin non-susceptible *Enterococcus faecium* (MIC=12 mg/L) in a 46-years-old woman with kidney transplantation in Spain.

PATIENTS AND METHODS

In May 2005, this woman started hemodialysis due to chronic terminal kidney failure (National Kidney Foundation

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stage 5) caused by chronic glomerulonephritis⁷. In October 2013, she was admitted to the Service of Nephrology of Virgen de las Nieves University Hospital (VNUH) for a cadaveric renal transplant of the right kidney. At admission, she showed arterial hypertension, non-rheumatic aortic failure with preserved ventricular function, atheromata in ascending aorta and aortic arch, and multinodular goiter. She underwent the surgical procedure without complications and was discharged from the hospital eight days after surgery with prednisone, tacrolimus, mycophenolate mofetil, cotrimoxazole, omeprazole, magnesium and acetylsalicylic acid. Three weeks after hospital discharge, the patient returned to the hospital with urinary complaints, and a spontaneous urine sample was sent to the Clinical Microbiology Laboratory of the VNUH for microbiological diagnosis⁸.

Study of the sample with the Sysmex UF-1000i system (TOA Medical Electronics, Kobe, Japan) revealed 44 white blood cells/ μl in non-centrifuged urine and absence of yeast, erythrocytes or squamous epithelial cells. Culture yielded $>10^4$ CFU/ml of a single microorganism (isolate 3076755) that was identified as *E. faecium* by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry and the MicroScan[®] system (Siemens Healthcare Diagnostics, Madrid, Spain). Confirmation of the identity of the microorganism was performed by PCR targeting the *ddl* gene encoding the *E. faecium* D-alanyl-D-alanine ligase, as described before⁹. Susceptibility testing yielded a DAP MIC >4 mg/L and resistance to fluoroquinolones, tetracyclines, nitrofurantoin, macrolides (and lincosamides) and high-level resistance to both streptomycin and gentamicin. The isolate was susceptible to fosfomicin (MIC ≤ 32 mg/L), glycopeptides (MIC ≤ 1 mg/L for vancomycin and for teicoplanin), linezolid (MIC=2 mg/L) and quinupristin-dalfopristin (MIC ≤ 0.5 mg/L). *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 served as controls for susceptibility testing.

RESULTS

Daptomycin MIC by E-test on cation-adjusted Mueller-Hinton agar¹⁰ was 12 mg/L. Of note, neither the recipient nor the donor had documented exposure to daptomycin or evidence in their medical records of daptomycin administration before or, in the case of the former, after transplantation. The patient was treated with intravenous fosfomicin and subsequent microbiological studies were negative.

Multilocus sequence typing (MLST) analysis¹¹ was performed to detect the housekeeping genes adenylate kinase (*adk*), ATP synthase alpha subunit (*atpA*), D-alanine-D-alanine ligase (*ddl*), glucose-6-phosphate dehydrogenase (*gdh*), glyceraldehyde-3-phosphate dehydrogenase (*gya*), phosphate ATP-binding cassette transporter (*pstS*), and phosphoribosylaminoimidazole carboxylase ATPase subunit (*purK*), revealing that the isolate belonged to ST117 (clonal cluster 17). In order to determine the genetic bases of daptomycin resistance, the open reading frames of five genes previously associated with daptomycin resistance in enterococci¹² were sequenced in their entirety including *i*) *liaFSR*, encoding a three-component regulatory system LiaFSR that is postulated to

orchestrate the cell envelope response to antibiotics and antimicrobial peptides^{13,14}, *ii*) *gdpD*, encoding a glycerophosphoryl-diester-phosphodiesterase that is involved in cell membrane phospholipid metabolism, and *iii*) *cls* encoding a cardiolipin synthetase that mediates synthesis of cardiolipin, an important phospholipid of bacterial cell membranes. A mutation was defined as a nucleotide change that resulted in an amino acid substitution that was not present in any other daptomycin-susceptible enterococci whose genomes are publicly available. Interestingly, two putative amino acid substitutions were found; *i*) a novel S19F in the response regulator LiaR which is located in the putative receiver domain of the protein¹⁵ and *ii*) R218Q in the predicted phospholipase domain of Cls that has been described before^{12,16}.

DISCUSSION

Enterococci are gram-positive, facultative anaerobes that commonly colonize gastrointestinal and genitourinary tracts. They are generally considered to be of low virulence but are associated with hospital-acquired infections, including UTI, bacteraemia, postsurgical wound infections, and gastrointestinal infections^{5,16}. *E. faecium* is frequently isolated in urine samples from UTI patients hospitalized in our Department of Nephrology, and phenotypes of resistance to beta-lactam antibiotics, fluoroquinolones, macrolides and of high-level resistance to aminoglycosides are commonly detected in these samples, although almost all ($>99.9\%$) of clinical *E. faecium* isolates remain susceptible to linezolid, glycopeptides, and daptomycin (data not shown).

Daptomycin, a cyclic lipopeptide with *in vitro* bactericidal activity against Gram-positive bacteria including multidrug-resistant enterococci acts by causing important changes in the biophysical properties of the cell membrane of the bacterial cell membrane altering cell division and cell wall synthesis¹⁷. Daptomycin is now widely used against severe multidrug-resistant enterococci (including VRE) infections, although it is not approved for these conditions^{5,18}. In our hospital, the utilization of daptomycin began in 2008 and has progressively increased over the past six years; however, daptomycin exposure was not identified as a risk factor in the present case, a phenomenon that has been reported previously⁶.

Daptomycin non-susceptible enterococci (DNSE) are emerging as important causes of healthcare-associated infection affecting patients with multiple comorbid conditions, patients exposed to antimicrobials in the previous three months (especially third-generation cephalosporins and metronidazole) or intraabdominal disease. There have been reports of DNSE in immunocompromised patients with severe chronic disease, as in the present case, and recent intraabdominal surgery^{3,5,6}. Interestingly, daptomycin resistance has been often associated with mutations in genes encoding two groups of proteins *i*) regulatory systems that orchestrate the cell envelope stress response (LiaFSR and YycFGH) and *ii*) enzymes involved in phospholipid metabolism. The isolate of our patient exhibited a novel substitution in the response regulator LiaR and also harboured a change in the active site of the cardiolipin synthase. Both

proteins have been previously shown to be important for the Daptomycin-non-susceptible phenotype and our results confirm previous observations.

In Spain, linezolid, daptomycin and tigecycline remain as therapeutic options against infections caused by multi-resistant enterococci¹⁹. However, there have been reports of increases in daptomycin MIC in isolates of methicillin-resistant *S. aureus* treated with this antibiotic, although values remain within the susceptibility range²⁰, and resistance to linezolid has been documented in *E. faecalis* and *E. faecium* in some Spanish hospitals²¹. To the best of our knowledge, this is the first case and characterization of emerging daptomycin resistance in *E. faecium* in a Spanish hospital in the absence of daptomycin exposure. Clinicians should be aware of the emergence of daptomycin non-susceptible enterococci in immunocompromised patients with or without a history of daptomycin exposure and check for daptomycin susceptibilities.

FUNDING

This work was supported only by the National Institute of Allergy and Infectious Diseases (National Institutes of Health [NIH] grant R01 AI093749 to C. A. A.).

TRANSPARENCY DECLARATIONS

All authors: None to declare.

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