SHORT COMMUNICATION

Cluster of linezolid resistant *Enterococcus faecium* ST117 in Norwegian hospitals

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Abstract

A linezolid resistant, vancomycin susceptible *E. faecium* strain was isolated from three patients who had not received linezolid. The first patient was hospitalised in the same hospitals and wards as the two following patients. The *E. faecium* isolates were resistant to linezolid (MIC 8-32 mg/L), ampicillin and high levels of gentamicin. Resistance to linezolid was associated with a G2576T mutation in 23S rDNA. The *cfr* linezolid resistance gene was not detected. The three isolates showed identical DNA fingerprints by PFGE, belonged to ST117 and harboured virulence genes *esp*, *hyl*, *acm*, *efaAfm*, *srgA*, *ecbA*, *scm*, *pilA*, *pilB* and *pstD* typically associated with high-risk *E. faecium* genotypes. The linezolid resistant *E. faecium* high-risk clone caused bacteraemia in the first two cancer patients and survived in the hospital environment for more than a year before appearing in the urethral catheter of the third patient.

Introduction

The oxazolidinone antibiotic linezolid has been available since 2000 as a therapeutic alternative against antibiotic resistant Gram-positive cocci. It inhibits bacterial protein synthesis through binding in the A site pocket at the peptidyltransferase centre, domain V of the 23S ribosomal RNA of the 50S subunit [1]. Recently the first human isolate of *Enterococcus faecalis* with transferable linezolid resistance encoded by the *cfr* (chloramphenicol-florfenicol resistance) gene was recovered from a patient in Thailand [2]. The *cfr* gene encodes a methyltransferase which has previously been reported to methylate nucleotide A2503 in the 23S rRNA of staphylococci, thereby causing resistance to several antimicrobial compounds including linezolid. However, in enterococci linezolid resistance has mainly been caused by point mutations in 23S rDNA with a G2576U transition in the central loop of domain V as the most common [3-7]. *Enterococcus faecium* has 6 alleles of 23S rRNA genes. The level of linezolid resistance expressed correlates with the number of mutated 23S rRNA genes [8].

Linezolid resistance rates (< 1 %) have remained low for staphylococci, enterococci and streptococci monitored in medicals centres across Europe, Canada, Latin America, the US and the Asia-Pacific region [3-7]. Linezolid resistant enterococci have only been reported twice in Scandinavia [3, 9]. Here we report the first cluster of linezolid resistant *Enterococcus* with identical DNA fingerprints identified in Scandinavian hospitals.

Material and methods

Bacterial isolates

During the period from July 2012 to October 2013, three linezolid resistant *E. faecium* isolates were recovered from 3 patients. *E. faecium* strains UW3698, UW3695, UW3936 and

UW3939 containing the point mutation G2576U in 23S rRNA [10] as well as a *cfr* positive *Staphylococcus epidermidis* strain were used as positive controls.

Bacterial identification and susceptibility testing

Identification of the *E. faecium* isolates was performed according to standard bacteriological procedures. The isolates were confirmed to be *E. faecium* by *ddl* specific PCR [11] and Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) using Bruker Microflex with Biotyper 3.0 software (Bruker Daltonik GmbH, Bremen, Germany).

Susceptibility testing was performed by the EUCAST disk diffusion method [12] for ampicillin and gentamicin and MIC gradient tests for linezolid, vancomycin and teicoplanin (Etest, bioMérieux , Marcy l'Etoile, France or MIC Test strip, Liofilchem, Roseto degli Abruzzi, Italy) using EUCAST clinical breakpoints [13].

Detection of linezolid resistance mechanism and virulence genes

The linezolid resistance gene *cfr* gene was searched for by PCR analysis [14]. Amplification of the 23S rDNA encoding domain V and subsequent *Nhe*I digestion [15] was used to reveal the G2576T mutation showing one 746-bp band corresponding to the wild type undigested amplification product, and two bands of 557 and 189 bp representing *Nhe*I digested mutant alleles.

The selected virulence genes searched for by PCRs (A. Sivertsen, H. Billström, Ö. Melefors, B. Olsson Liljequist, K. Tegmark Wisell, M. Ullberg, V. Özenci, A. Sundsfjord, and K. Hegstad, submitted for publication)[16] are associated with high-risk genotypes of *E. faecium* and encode proteins involved in biofilm formation (*esp*), hyaluronidase production (*hyl*), host

tissue attachment (*acm, efaAfm, srgA, ecbA, scm*), pili formation (*pilA/B*) and intestinal colonization during antibiotic treatment (*pstD*).

Pulsed-field gel electrophoresis (PFGE) and Multi Locus Sequence Typing (MLST)

PFGE after *Sma*I digestion was performed as described by Saeedi *et al.* [17]. The bands were separated with switch time 1 to 35 for 29 hours at 6V/cm with 120° angle, 12°C, 1.2% agarose and 0.5xTBE buffer [18]. MLST was performed using the primers adk1n, adk2n, atp1n, atp2n, ddl1, ddl2, gdh1, gdh2, gyd1, gyd2, pstS1n, pstS2n, purK1n and purK2n [19].

Results

Patient characteristics

A 44-y-old man (patient 1) with kidney cancer had previously had a nephrectomy and surgical removal of metastatic brain and lung lesions at several hospitals over a 3 year time period. He was admitted July 2012 to ward A at hospital 1 and diagnosed with peritonitis from perforated colon. He was initially treated with cefotaxime and metronidazole and from day 9 with meropenem. Blood cultures taken on the same day were negative. The next day he was moved to ward B for further medical treatment, but deteriorated one week later due to persistent peritonitis. Blood cultures revealed growth of linezolid resistant, vancomycin susceptible *E. faecium* (LR-VSEfm) (isolate 1), and he was treated with vancomycin. On hospital day 24 laparoscopic drainage of the peritoneum was performed and two days later he was moved to ward C at his local hospital 2. The patient died some months later from his cancer.

A 61-y-old woman (patient 2) with inoperable metastatic cancer of the pancreas and carcinomatosis was first admitted to ward B at hospital 1 to be evaluated for cytostatic

106 treatment. After a few days she was moved to ward A because of increasing cholestasis. She
107 received external bile drainage and started treatment with cefotaxime and metronidazole for
108 cholangitis. The next day she was moved back to ward B and received piperacillin-
109 tazobactam followed by meropenem and vancomycin due to increasing general malaise, fever
110 and chills. Blood cultures revealed growth of LR-VSEfm (isolate 2). Some days later she was
111 moved to her local hospital 3 for further antibiotic treatment and supportive care. She was
112 readmitted to hospital 3 after a few weeks because of cholangitis. *Klebsiella* sp. and linezolid
113 susceptible *E. faecium* grew in her blood cultures. She died a few months later from her
114 cancer.

115 An 80-y-old paraplegic man (patient 3) with a permanent urethral catheter, decubital
116 ulcer and heart failure was admitted to ward C at his local hospital 2 with general malaise and
117 fever. Blood cultures revealed growth of *Staphylococcus aureus*. He was treated with
118 ciprofloxacin, penicillin, metronidazol, then piperacillin-tazobactam and finally meropenem
119 for suspected chronic osteomyelitis and a prostatic abscess. LR-VSEfm (isolate 3) was
120 recovered from his urethral catheter on day 15. There were no indications of catheter-
121 associated urinary tract infection and the patient did not receive specific treatment. He was
122 treated for a total of 6 weeks with meropenem until resolution of symptoms.
123

124 *Context of the cases*

125 Patient 1 was admitted to ward A at hospital 1 just 3 days before admission of patient 2 to the
126 same ward. The LR-VSEfm strain from patient 1 was revealed while staying at ward B at the
127 same hospital where patient 2 was admitted 2 days later. The two patients stayed there
128 simultaneously for 10 days before patient 2 had growth of the strain in blood culture. Patient 1
129 was subsequently transferred to ward C at hospital 2. Patient 3 was admitted to ward C at
130 hospital 2 one year later and eventually harboured the strain in a urethral catheter.

None of the patients had received linezolid before detection of the linezolid resistant strain. We have no information of any infection control measures conducted at the different departments after detection of this strain.

Isolate characteristics

The three *E. faecium* isolates were resistant to linezolid (MIC 8-32 mg/L), ampicillin and high levels of gentamicin, but susceptible to vancomycin and teicoplanin. The isolates did not contain the *cfr* gene mediating transferable linezolid resistance but rather showed heterozygosis for the G2576T mutation of 23S rDNA previously found to be involved in linezolid resistance. Furthermore, the *Sma*I PFGE patterns (Figure 1) were identical for the three isolates and they all belonged to ST117 and were positive for all tested virulence genes.

Discussion

23S rDNA mutational resistance often occurs after therapy with oxazolidinone [20, 21]. Previous exposure to linezolid was not recorded for any of these three patients, but they all had at least one known risk factor for the development of mutation based linezolid resistance in *Enterococcus* such as immunosuppression, prior surgery and previous exposure to β–lactam antibiotics [22].

The three LR-VSEfm isolates belonged to ST117, a single locus variant of ST17, and thus represent one of the well-known hospital adapted high-risk clonal lineages of *E. faecium* [23]. ST17 is associated with hospital outbreaks and, like the LR-VSEfm isolates described here, typically contains many antimicrobial resistance and virulence properties [23, 24]. The identical PFGE patterns as well as hospitalisation in the same wards may indicate nosocomial spread of this LR-VSEfm ST117 strain, although it should be noted that the third isolate

appeared more than a year after the first two. Nosocomial spread of linezolid resistant enterococci to patients not previously treated with linezolid has been documented before [25] and suggests that linezolid resistant enterococci may remain relatively fit despite of their heterozygous resistance to linezolid. An LR-VSEfm ST117 strain was recently reported to persist for 41 days in the intestine of a patient with hematologic malignancy after linezolid treatment was discontinued [26]. Furthermore, environmental survival of *E. faecium* has been documented up to about 1400 days [27]. The long time span between cases 2 and 3 confirms the ability of *E. faecium* strains to survive in the hospital environment for long periods of time.

Recent European surveys have documented a pronounced increase (19.3% per year) in bacteraemia caused by multidrug resistant *E. faecium* clonal lineages [28]. Moreover, a significant increase in bloodstream infection due to vancomycin susceptible *E. faecium* has been observed in cancer patients in Barcelona where ST117 isolates have predominated since 2009 [29]. In line with these reports, the ST117 high-risk clone described in the present study was apparently able to cause bacteraemia in the first two cancer patients and then survived in the hospital environment for more than a year before being isolated from the urethral catheter of the third patient.

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Figure 1. PFGE illustrating identical DNA fingerprints of the three LR-VSEfm isolates. 1, 2 and 3 indicate lanes with *Sma*I digested total DNA from isolates 1, 2 and 3, respectively. L indicates low range marker (New England BioLabs).