Journal of Global Antimicrobial Resistance 36 (2024) 112–115

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Short Communication

The first tigecycline resistant *Enterococcus faecium* in Norway was related to tigecycline exposure

Kristin Hegstad[a,b,*], Anna K. Pöntinen[a,c], Jørgen V. Bjørnholt[d,e], Else Quist-Paulsen[d], Arnfinn Sundsfjord[a,b]

[a] *Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway*
[b] *Research group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway*
[c] *Department of Biostatistics, Faculty of Medicine, University of Oslo, Oslo, Norway*
[d] *Department of Clinical Microbiology, Oslo University Hospital, Oslo, Norway*
[e] *Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway*

ARTICLE INFO

Article history:
Received 25 September 2023
Revised 30 November 2023
Accepted 8 December 2023
Available online 19 December 2023

Editor: Dr Yen-Hsu Chen

Keywords:
Enterococcus
Tigecycline resistance
Mechanism
Deletions
Ribosomal protein S10
tet(M) leader peptide

ABSTRACT

Objectives: We describe the first tigecycline resistant enterococcal isolate in Norway and the mechanisms involved.
Material and methods: The Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res). received in 2022 an *Enterococcus faecium* blood culture isolate with decreased susceptibility to tigecycline from a hospitalized patient in the South-Eastern Norway Health region for confirmatory testing. K-res verified a tigecycline-resistant *E. faecium* (TigR) with broth microdilution MIC of 0.5 mg/L. The patient had received treatment with tigecycline because of an infection with a linezolid- and vancomycin-resistant but tigecycline susceptible *E. faecium* (TigS) 47 days prior to the detection of the corresponding tigecycline-resistant isolate. Whole-genome comparisons, cgMLST and SNP analyses revealed that the two ST117 strains were closely related.
Results: The TigR isolate showed a novel deletion of 2 amino acids (K57Y58) in a polymorphic region of ribosomal protein S10 previously associated with tigecycline resistance and a deletion of the *tet(M)* leader peptide previously related to increased expression of *tet(M)* and tigecycline resistance in enterococci.
Conclusions: Genomic and epidemiological analyses confirm that the two *E. faecium* (TigR and TigS) are closely related isolates of the same strain and that the two deletions (in *rpsJ* and of *tet(M)* leader peptide) account for the tigecycline resistance in TigR.

1. Introduction

The enterococci have since the introduction of antimicrobial agents undergone a genomic transition from harmless gut commensals to be leading causes of multidrug resistant hospital infections [1]. Particularly *Enterococcus faecium* is very adept at acquiring resistance to a wide spectrum of antibiotics and represent an emerging health concern [2].

Tigecycline is one of the last resort antibiotics that is increasingly used because of the rising prevalence of vancomycin-resistant enterococci. The occurrence of tigecycline resistance in clinical isolates of enterococci has been low (<1%) but is increasing worldwide. The overall tigecycline resistance in *E. faecium* (1%) is higher than in *Enterococcus faecalis* (0.3%), and the tigecycline-resistant *E. faecium* prevalence is higher in Europe (3.5%) than in Asia (1.3%) and America (0.3%) [3]. Acquired tigecycline resistance is most often conveyed via mutations in inherent genes selected for by tigecycline exposure. Mutations in a specific region of ribosomal protein S10 (RpsJ) and mutations that contribute to increased expression of the ribosomal protection protein Tet(M) or efflux pump Tet(L) have been shown to contribute to tigecycline resistance in enterococci [4–7].

In this study, we describe the finding of the first confirmed tigecycline resistant enterococcal isolate in Norway and the mechanisms involved.

* Corresponding author. Mailing address: UiT The Arctic University of Norway, PO Box 6050 Langnes, N-9037 Tromsø, Norway
E-mail address: kristin.hegstad@uit.no (K. Hegstad).

https://doi.org/10.1016/j.jgar.2023.12.002

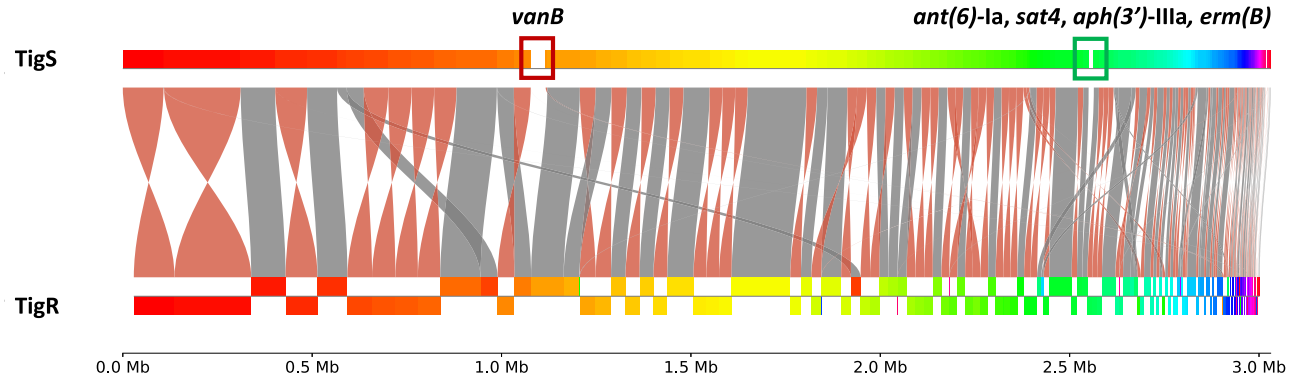

Fig. 1. Alignment of TigS and TigR genomes. Main differences between their genomes are due to additional mobile genetic regions in TigS indicated by red rectangle (region containing Tn*1549* harbouring *vanB*) and green rectangle (region containing *ant(6)*-Ia, *sat4*, *aph(3')*-III, *erm(B)*).

Table 1
Relevant characteristics of the isolates.

Isolate	Isolation date	ST	CT	Amp MIC mg/L	Cip MIC mg/L	Gen MIC mg/L	Lin MIC mg/L	D-Q MIC mg/L	Tei MIC mg/L	Tig MIC mg/L	Van MIC mg/L	Str MIC mg/L	*tet* genes (coverage/ identity)[a]	Differences in AMR genes and AMR mutations
TigS	02.02.22	117	6485	>32	>16	<32	>8	1	<0.5	0.12	4	>1024	*tet(M)* (100/100)	*ant(6)*-Ia, *sat4*, *aph(3')*-IIIa, *erm(B)*, *vanB* cluster, 23S rDNA G2576T
TigR	21.03.22	117	6485	>32	>16	<32	2	<0.25	<0.5	0.5	1	<512	*tet(M)* (100/100)	

Amp, ampicillin; AMR, antimicrobial resistance; Cip, ciprofloxacin; D-Q, Dalfopristin-Quinupristin; Gen, gentamicin; Lin, linezolid; Str, streptomycin; Tei, teicoplanin; Tig, tigecycline; Van, vancomycin.
[a] *tet(M)* reference GenBank Accession No AM990992

2. Material and methods

2.1. Case description and bacterial isolates

In spring 2022, the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res) received an *E. faecium* (TigR) (Table 1), isolated from blood culture, with decreased susceptibility to tigecycline from a patient hospitalised in the South-Eastern Norway Health region. The patient had been treated with tigecycline because of an infection with a linezolid- and vancomycin-resistant *E. faecium* (TigS) 47 days prior to the tigecycline-resistant isolate. Both *E. faecium* isolates (TigR and TigS) were included for genomic comparisons to investigate relatedness and potential resistance mechanisms.

2.2. Phenotypic analyses

Species identification was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany). Primary laboratory revealed tigecycline resistance by disc diffusion (MAST GROUP). K-res confirmed the antimicrobial susceptibility testing (AST) observation using disc diffusion (Oxoid, Thermo Fisher Scientific, Waltham, USA), gradient test (Liofilchem, Roseto degli Abruzzi, Italy) and broth microdilution (BMD; EUENCF Sensititre plate, Thermo Fisher Scientific). The European Committee on AST (EUCAST) clinical breakpoints for resistance were used for interpretation; minimum inhibitory concentration (MIC) > 0.25 mg/L and/or disc diffusion zone diameter < 22 mm for *E. faecium* [8].

2.3. Whole genome sequencing, assembly, and detection of resistance genes

Bacterial genomic DNA was isolated with the Qiagen MagAttract HMW DNA isolation kit (Qiagen, Hilden, Germany) and sequenced by NextSeq500 paired-end platform using Nextera XT DNA library preparation kit and Mid Output 300 cycles cell according to standard protocols (Illumina, San Diego, USA). The reads were trimmed using Trimmomatic v.0.39, with contigs shorter than 200 bp and with lower than 2x coverage removed by default. Assembly of genome sequences was performed using Spades v.3.12.0. Presence of antimicrobial resistance genes and mutations was screened from assemblies using AMRFinderPlus v.3.10.11 with the –plus option and minimum coverage and identity both of 90%.

2.4. Genome comparisons

Multi-locus sequence types (MLST) were retrieved using mlst v.2.19.0 by comparing the sequence data to the MLST database (https://pubmlst.org/organisms/enterococcus-faecium). SeqSphere+ software V6.0.2 (Ridom GmbH, Münster, Germany) was used to determine core genome MLST based on a scheme with 1423 core genes of *E. faecium* [9]. To determine core SNP differences between the TigR and TigS isolates, two approaches were used: 1. Nullarbor v.2.0.20191013 pipeline [10] using strain E1 (CP018065.1) as a reference. 2. Selecting the closest isolate (E8414) among the hybrid assemblies from an extensive *E. faecium* collection [11] as a reference, based on core distances using k-mers and core threshold of 0.007 by PopPUNK v.2.4.0 [12]. Subsequently, sequence reads were mapped against the reference chromosome using smalt v.0.7.6, and snp-sites v.2.5.1 was run on the alignment to retrieve SNP positions. Genomic alignment and visualisation were performed using pgv-pmauve v.0.3.2 of the pyGenomeViz python package.

3. Results and discussion

3.1. Detection of tigecycline resistance

A blood culture isolate of *E. faecium* with tigecycline inhibition zone of 21 mm (disc diffusion) was recovered from a hospitalized

A.

Reference	Sample	Tig MIC	Alignment (42 47 52 57 62 67 72)
	Efm WT DO/Aus0004	0.125	LPTERSLYTI*IRATHKYKD*SREQFEMRTHKR
Cattoir *et al.*:	Efm AusTig/HMtig1+2	0.25	LPTERSLYTI*IRATHKYK**Y**SREQFEMRTHKR
	Efm EF16	0.5	LPTERSLYTI*IRATH**E**YKD*SREQFEMRTHKR
Niebel *et al.*:	Efm 3I	0.5	LPTERSLYTI*IR**E**T**R**KYKD*SREQFEMRTHKR
	Efm 2R	8	LPTERSLYTI*-----KYKD*SREQFEMRTHKR
	Efm 1R	8	LPTERSLYTI*-RATHK**H**KD*SREQFEMRTHKR
Beabout *et al*:	Efs S613	0.5	*----**Q**YKD*
	Efm 105 mutants	≥0.18	*R**EA**HKYKD*
	Efm 105 mutants	≥0.18	*RAT**RN**YKD*
	Efm 105 mutants	≥0.18	*RATH**E**YKD*
	Efm 105 mutants	≥0.18	*RATH**RD**KD*
	Efm 105 mutants	≥0.18	*RATHKYK**Y***
	Efm 105 mutants	≥0.18	*RATHK**S**KD*
	Efm R499 mutants	≥0.18	*RATHKYK**Y***
	Efm R499 mutants	≥0.18	*----**Q**YKD*
	Efm R499 mutants	≥0.18	*RATH**E**YKD*
	Efm R499 mutants	≥0.18	*RATH**RD**KD*
	Efm R499 mutants	≥0.18	*RATHK**D**KD*
	Efm R499 mutants	≥0.18	*RATHK**S**KD*
Dabul *et al*:	Efs ST103 isolates	1-2	LPTERSLYT**I***IRAT----D*SREQFEMRTHKR
Bender *et al.*:	Efm UW16540 + n=10	>2	LPTERSLYTI*I----KYKD*SREQFEMRTHKR
Boukthir *et al*:	Efm 17-477	8	LPTERSLYTI***M**--THKYKD*SREQFEMRTHKR
	Efm 18-481/18-626	1	LPTERSLYTI*----**N**KYKD*SREQFEMRTHKR
	Efm 18-785	2	LPTERSLYTI*IRATH**R**YKD*SREQFEMRTHKR
	Efm 18-394.1	0.5	LPTERSLYTI*IRATHKYK**Y***SREQFEMRTHKR
Bai et al:	Efs	16	LPTERSLYT**I***-----**Q**YKD*SREQFEMRTHKR
K-res:	**TigR**	0.5	LPTERSLYTI*IRATH--KD*SREQFEMRTHKR
	TigS	0.12	LPTERSLYTI*IRATHKYKD*SREQFEMRTHKR

B.

```
TigR AAATAATTGGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCAC-T
TigS AAATAATTGGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCATGT

TigR G---------------GGA--------------TTTTTATGCCTATGGTTATGCATAAAAATCCCAGTGAT
TigS GATTCTAAAGTATCCGGAGAATATCTGTATGCTTTGTATGCCTATGGTTATGCATAAAAATCCCAGTGAT
                   RBS          leader peptide
TigR AAAAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGTTTTTGAATGGAGGAAAATCACATGAAAAATT
TigS AAAAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGTTTTTGAATGGAGGAAAATCACATGAAAAATT
     transcriptional terminator                        RBS      tet(M) start
```

Fig. 2. Genomic differences associated with tigecycline resistance in isolate TigR compared with TigS. A. Alignment of the region of S10 showing amino acid changes potentially implicated in tigecycline resistance identified through mutations in the *rpsJ* gene. Wildtype sequence from reference Efau004_00094 (GenBank Accession No AFC62180.1). Amino acids are numbered according to the reference protein. Red italic letters indicate polymorphic region and mutations/deletions are highlighted by bold text. Tigecycline MIC > 0.25 mg/L which are defined as resistance according to EUCAST are highlighted in red. The TigR isolate had a deletion in the polymorphic region (red) while the TigS isolate showed identical amino acid sequence to the wildtype. B. Alignment of *tet(M)* region of TigR against TigS shows that TigR have a deletion in the tetracycline resistance leader peptide. Their *tet(M)* gene is identical (Table 1). Mutations/deletions as well as ribosomal binding sites (RBS) and start of leader peptide and *tet(M)* are highlighted by bold text. Leader peptide is highlighted by red italics and transcriptional terminator by underlining.

patient that had received treatment with tigecycline. K-res confirmed the findings of a tigecycline-resistant *E. faecium* with disc diffusion (20 mm), gradient test (MIC = 1 mg/L) and BMD (MIC= 0.5 mg/L). The isolate was sent to the EUCAST Development Laboratory (EDL) which also confirmed a tigecycline-resistant *E. faecium* by equivalent methods. This is the first confirmed tigecycline resistant enterococcal isolate in Norway.

3.2. Close relatedness of E. faecium *isolates with different resistance profiles from the same patient*

Genomic comparison of the TigR isolate with the corresponding TigS *E. faecium* isolate retrieved from the same patient 47 days prior to TigR revealed that they show the same sequence and cluster type, ST117-CT6485. There was only one allelic difference

between TigR and TigS according to core genome MLST analyses in SeqSphere+ and 1 SNP difference in their core genome according to Nullarbor (data not shown), indicating that the isolates are closely related.

Further core SNP analyses using reference isolate E8414 [11] identified a SNP difference in two genes as compared with the reference isolate: the *rpsJ* 30S ribosomal protein S10 and the *ghrB* glyoxylate/hydroxypyruvate reductase B. An additional difference in an intergenic region between two genes encoding hypothetical proteins was also observed (data not shown).

The differences in their accessory genome were due the presence of mobile genetic regions with 1) Tn*1549* harbouring *vanB* and 2) *ant(6)*-Ia, *sat4*, *aph(3')*-III and *erm(B)* in the TigS isolate that was not present in the TigR isolate (Figure 1). The TigS isolates further showed a G2576T mutation in 23S rDNA accounting for the linezolid resistance of this isolate. Both isolates shared the same mutations in Pbp5 (V24A, S27G, R34Q, G66E, A68T, E85D, E100Q, K144Q, T172A, L177I, D204G, A216S, T324A, M485A, N496K, A499T, E525D, E629V, P667S) and ParC (S80I)/GyrA (S83Y), which are involved in ampicillin and quinolone resistance, respectively.

3.3. Identification of the tigecycline resistance determinants

Genomic comparison of the TigR isolate with the closely related TigS *E. faecium* isolate revealed a 2 amino acids (K57Y58) deletion in the TigR isolate in a specific polymorphic region of ribosomal protein S10, previously associated with tigecycline resistance in enterococci [4,6,13–17], while the TigS isolate showed identical amino acid sequence to the expected wild type. This specific deletion has previously not been described to be associated with tigecycline resistance (Figure 2A). Additionally, both isolates had an identical *tet(M)* gene, but the TigR isolate showed a deletion of the *tet(M)* leader peptide that was not present in TigS (Figure 2B). This deletion of the *tet(M)* regulator has previously been confirmed to contribute to increased expression of *tet(M)* and tigecycline resistance [4,6].

K-res has after this first case received other tigecycline resistant *E. faecium* isolates which have shown different mutations in the specific region of ribosomal protein S10 (RpsJ) that may contribute to tigecycline resistance in enterococci. However, without having a susceptible closely related isolate to compare with like in this case it is difficult to prove that a novel mutation is the reason for resistance since there might be other changes in the genome that are not accounted for.

4. Conclusion

Genome comparison, phylogenetic inference, and SNP analyses, in concert with the temporal relatedness and shared host, confirm that the two *E. faecium* genomes (TigR and TigS) are closely related isolates of the same strain. It is most likely the identified changes (*rpsJ* mutations and deletion of *tet(M)* leader peptide) that account for the tigecycline resistance in the TigR isolate. It is important to identify and report novel genetic variants leading to resistance so these can be added to the public databases/tools and thus help others confirming the genetic basis for resistance.

Funding: This work received no specific grant from any funding agency.

Competing interests: None declared.

Ethical approval: Since this study contain only limited anonymized patient data, the study was approved by the Data Protection Officer at Oslo University Hospital.

Sequence information: Genome sequence data generated in this study are published under Accession numbers ERS15990991 (TigS named KresVRE0143) and ERR11677588 (TigR named KresTRE0001).

Acknowledgments: We thank Bjørg C. Haldorsen and Siv Heidi Barkhald at K-res for technical assistance with identification of isolates and initial AST testing and Erika Matuschek and Jenny Åhman at EDL for confirming our AST data.

References

[1] Gilmore MS, Lebreton F, van Schaik W. Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. Curr Opin Microbiol 2013;16:10–16. doi:10.1016/j.mib.2013.01.006.
[2] Cattoir V. The multifaceted lifestyle of enterococci: genetic diversity, ecology and risks for public health. Curr Opin Microbiol 2022;65:73–80. doi:10.1016/j.mib.2021.10.013.
[3] Dadashi M, Sharifian P, Bostanshirin N, Hajikhani B, Bostanghadiri N, Khosravi-Dehaghi N, et al. The global prevalence of daptomycin, tigecycline, and linezolid-resistant *Enterococcus faecium* strains from human clinical samples: a systematic review and meta-analysis. Front Med 2021;8:720647. doi:10.3389/fmed.2021.720647.
[4] Beabout K, Hammerstrom TG, Wang TT, Bhatty M, Christie PJ, Saxer G, et al. Rampant parasexuality evolves in a hospital pathogen during antibiotic selection. Mol Biol Evol 2015;32:2585–97. doi:10.1093/molbev/msv133.
[5] Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, et al. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug Resist Updat 2018;40:25–39. doi:10.1016/j.drup.2018.10.002.
[6] Bender JK, Klare K, Fleige C, Werner G. A nosocomial cluster of tigecycline- and vancomycin-resistant *Enterococcus faecium* isolates and the impact of *rpsJ* and *tet*(M) mutations on tigecycline resistance. Microb Drug Resist 2020;6:576–82. doi:10.1089/mdr.2019.0346.
[7] Bai B, Chen C, Zhao Y, Xu G, Yu Z, Tam VH, et al. In vitro activity of tigecycline and proteomic analysis of tigecycline adaptation strategies in clinical *Enterococcus faecalis* isolates from China. J Glob Antimicrob Resist 2022;30:66–74. doi:10.1016/j.jgar.2022.04.022.
[8] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.0, 2023. http://www.eucast.org
[9] de Been M, Pinholt M, Top J, Bletz S, Mellmann A, van Schaik W, et al. Core genome multilocus sequence typing scheme for high-resolution typing of *Enterococcus faecium*. J Clin Microbiol 2015;53:3788–97 10.1128/JCM.01946–15.
[10] Seemann T, Goncalves da Silva A, Bulach DM, Schultz MB, Kwong JC, Howden BP. Nullarbor . Github. Available from: https://github.com/tseemann/nullarbor [Accessed 03.06.22].
[11] Arredondo-Alonso S, Top J, McNally A, Puranen S, Pesonen M, Pensar J, et al. Plasmids shaped the recent emergence of the major nosocomial pathogen *Enterococcus faecium*. mBio 2020;11:e03284 e03219. doi:10.1128/mBio.03284-19.
[12] Lees JA, Harris SR, Tonkin-Hill G, Gladstone RA, Lo SW, Weiser JN, et al. Fast and flexible bacterial genomic epidemiology with PopPUNK. Genome Res 2019;29:304–16. doi:10.1101/gr.241455.118.
[13] Cattoir V, Isnard C, Cisquer T, Odhiambo A, Bucquet F, Guerin F, et al. Genomic analysis of reduced susceptibility to tigecycline in *Enterococcus faecium*. Antimicrob Agents Chemother 2015;59:239–44. doi:10.1128/AAC.04174-14.
[14] Niebel M, Quick J, Prieto AMG, Hill LRL, Pike R, Huber D, et al. Deletions in a ribosomal protein-coding gene are associated with tigecycline resistance in *Enterococcus faecium*. Int J Antimicrob Agents 2015;46:572–5. doi:10.1016/j.ijantimicag.2015.07.009.
[15] Beabout K, Hammerstrom TG, Perez AM, Magalhaes BF, Prater AG, Clements TP, et al. The ribosomal S10 protein is a general target for decreased tigecycline susceptibility. Antimicrob Agents Chemother 2015;59:5561–6. doi:10.1128/AAC.00547-15.
[16] Dabul ANG, Avaca-Crusca JS, Navais RB, Merlo TP, Van Tyne D, Gilmore MS, et al. Molecular basis for the emergence of a new hospital endemic tigecycline resistant *Enterococcus faecalis* ST103 lineage. Infect Genet Evol 2019;67:23–32. doi:10.1016/j.meegid.2018.10.018.
[17] Boukthir S, Dejoies L, Zouari A, Collet A, Potrel S, Auger G, et al. In vitro activity of ervacycline and mechanisms of resistance in enterococci. Int J Antimicrob Agents 2020;56:106215. doi:10.1016/j.ijantimicag.2020.106215.