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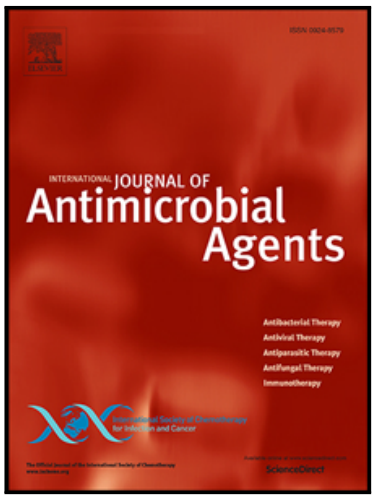

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Highlights

- First report of vancomycin-resistant *E. faecium* in Danish chicken meat since 2010
- Persistence of a pVEF4-like plasmid in VREfm from poultry for more than 18 years
- *ω/ε/ζ* toxin-antitoxin system likely responsible for long term persistence
- Co-transfer of the non-conjugative plasmid pVEF4 with another larger plasmid

Vancomycin resistance in *Enterococcus faecium* isolated from Danish chicken meat is located on a pVEF4-like plasmid persisting in poultry for 18 years

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VRE, pVEF4, *vanA*, *Enterococcus faecium*, retail chicken meat, ST32 (CT1068)

Abstract

The occurrence of vancomycin-resistant *Enterococcus faecium* (VREfm) in food has public health relevance since foodborne VREfm may colonize the gut of consumers and transfer vancomycin resistance genes to the indigenous gut microbiota. Therefore, we determined occurrence and elucidated genetic traits of VREfm in Danish retail chicken meat. Three out of 40 samples (7.5%) from two slaughterhouses yielded VREfm (vancomycin MIC > 32mg/L). This is the first report of VREfm in Danish retail poultry meat since 2010 (DANMAP). All three VREfm belonged to the sequence type ST32, cluster type CT1068. Using whole genome sequencing, we detected transposon Tn*1546* harbouring the *vanA* operon encoding vancomycin resistance. The *vanA* operon was located on a 43.4kb plasmid highly similar (99.9% identity across 97.5% of the sequence) to pVEF4 which was observed in VREfm in Norwegian poultry in 1998 as well as in Danish poultry in 2010. The remarkable persistence of a pVEF4-like plasmid in enterococcal populations may be explained by the presence of two independent plasmid stability systems namely the *ω/ε/ζ* toxin-antitoxin system and the *prgOPN* gene cluster. Filter mating experiments showed that the pVEF4-like plasmid could transfer between *E. faecium* strains *in vitro* and that transfer occurred concomitantly with a larger, co-residing plasmid. The data presented here indicates that poultry meat constitutes a reservoir of VREfm and further investigations are needed to assess the risk of foodborne transmission to humans.

Highlights

- First report of vancomycin-resistant *E. faecium* in Danish chicken meat since 2010
- Persistence of a pVEF4-like plasmid in VREfm from poultry for more than 18 years
- Two independent plasmid stability systems might contribute to long-term persistence
- Co-transfer of the non-conjugative pVEF4-like plasmid with another larger plasmid

Abbreviations:

- VREfm: vancomycin-resistant *Enterococcus faecium*
- VRE: vancomycin-resistant enterococci
- ST: sequence type
- CT: cluster type
- CC: clonal complex
- MLST: multi-locus sequence typing
- cgMLST: core genome MLST

1. Introduction

Enterococci are natural members of the intestinal microbiota of animals and humans but also range among the pathogens that frequently cause nosocomial infections around the globe, such as urinary tract infections, bacteraemia, endocarditis and wound infections [1]. The number of cases of infections with vancomycin-resistant enterococci (VRE) has increased dramatically in recent years with vancomycin-resistant *Enterococcus faecium* (VREfm) being the most prevalent species [2]. Vancomycin is a cell-wall active glycopeptide that is used for treatment of infections by Gram-positive bacteria. Nine variants (*vanABCDEGLMN*) of glycopeptide resistance determinants have been reported in enterococci worldwide, of which the *vanA* type is most prevalent [3]. *vanA* is normally carried on plasmids as part of transposon Tn*1546*, which can transfer between enterococci. This indicates that VRE of animal or food origin may represent a reservoir of resistance genes that are able to spread to human-associated strains [4].

In the 1990s, VREfm carrying Tn*1546* were commonly found in the intestinal flora of farm animals in Europe. After the withdrawal of the growth promoter and vancomycin-analogue avoparcin in the mid to late 1990s, the prevalence of VREfm in farm animals in Europe rapidly decreased [5]. Despite this, several VREfm clones can still be isolated globally from poultry meat [6]. In Denmark, information on the prevalence of VRE in poultry and poultry meat is limited to non-selective screening within the frame of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). The last reports of VREfm isolates from poultry and poultry meat in Denmark published as part of DANMAP were in 2008 (2%) and 2010 (1%), respectively [7,8]. Nonetheless, another Danish study carried out in 2010 using vancomycin for selective enrichment, found high prevalence (ca. 50%) of VREfm at the poultry flock level [9]. Thus, it appears that VREfm circulate in the Danish poultry population albeit at low prevalence, with unknown consequences for meat contamination and public health. The aim of this study was to screen Danish retail chicken meat for VREfm using a selective enrichment method and to genetically characterize the strains and plasmids obtained.

2. Materials and Methods

2.1 Sample collection

In total, 40 chicken meat samples were purchased in nine Danish supermarkets on three days from March to May 2016. All samples were within the expiration date and kept refrigerated at 5 °C for a maximum of 24

hours until analysis. The meat derived from chickens conventionally raised and slaughtered in two different slaughterhouses in Denmark.

2.2 Isolation and identification

One-hundred grams of each sample were aseptically cut, collected in a stomacher bag and rinsed with 250 mL buffered peptone water (BPW) (Oxoid, United Kingdom). For a first enrichment, 10 mL of this sample broth was added to 90 mL tryptic soy broth (TSB) (Oxoid, United Kingdom) with 6.5% NaCl and incubated at 37 °C overnight. Further, isolation was carried out as described previously [9], with minor changes: 1 mL of the overnight culture was added to 5 mL VRE broth (Oxoid, United Kingdom) containing 16 µg/mL vancomycin. After shaking incubation at 42 °C overnight, one 10 µL-loop was spread on vancomycin-containing (16 µg/mL) Slanetz and Bartley agar (3% agar), which was incubated at 42 °C for 48 h. Two colonies per plate with typical enterococci morphology were transferred to Blood Agar Base plates (Oxoid, United Kingdom) (3% agar) and incubated at 37 °C overnight. Species identification was performed by MALDI-TOF MS. Enterococci strains were stored in 50% glycerol at -80 °C. Additional characterization was carried out with only one isolate per meat sample.

2.3 Antimicrobial susceptibility testing

The VREfm isolates were tested for antimicrobial susceptibility by MIC determination using commercial Sensititre™ plates (COMPGP1F, Thermo Scientific) and disc diffusion test for linezolid. CLSI instructions were followed for inoculum preparation and MIC value interpretation [10].

2.4 Whole genome sequencing (WGS) and bioinformatics analysis

Genomic DNA was extracted using lysozyme lysis (20 mg/mL; incubation at 37 °C for 30 min), proteinase K treatment (56 °C for 30 min) and isolation of genomic DNA using the DNeasy Blood and Tissue Kit (Qiagen, Germany). Fragment libraries were constructed using the Nextera Kit (Illumina, United Kingdom) and sequenced by 251-bp paired-end sequencing (MiSeq, Illumina) according to the manufacturer's instructions. The paired-end Illumina data was assembled using the *de novo* assembly algorithm integrated in CLCbio's Genomics Workbench 10.1.1 (Qiagen, Denmark).

ResFinder (version 2.1) and MLST (version 1.7 at http://www.genomicepidemiology.org) were used to identify acquired antimicrobial resistance genes and sequence types (ST), respectively [11,12]. For

antimicrobial resistance genes, a threshold of 90% identity was used. As previously described by de Been et al. [13], core genome MLST (cgMLST) was performed on the draft assemblies using the SeqSphere software v4.1.7 (Ridom) to further assign cluster types (CT).

Complete sequences of plasmids from isolate VRE2881 were obtained by assembly of MinION long-reads and polishing with MiSeq short-read data as follows. Plasmid DNA was isolated using a Qiagen tip-100 Plasmid Midi kit according to the manufacturer's instructions, with volume of buffer P1, P2 and P3 increased to 10 mL each as recommended for 100 mL starting culture. DNA was eluted in Tris-Cl pH 8.5. A nanopore MinION library was prepared using the Native Barcoding Ligation Kit, SQK-NSK007 + EXP-NDB002. It was sequenced on a FLO-MIN106 R9.4 Spot-ON flow cell in a MinION Mk1B according to the manufacturer's instructions (Oxford Nanopore Technologies) with the following modifications: 2 µg input DNA; G-Tube at 5 krpm; 0.4 vol AMPure XP beads. Fast5-format read files were base-called with a two-direction (2D) workflow using ONT's Metrichor software and yielded 3098 passed 2D reads amounting to 20 megabases. Poretools (v0.6.0) was used to extract fastq-files. Adaptor sequences were removed from fastq-files using Porechop (v0.2.2). Unicycler (v0.4.0) was used for hybrid assembly. Using CLCbio's Genomics Workbench 10.1.1 MiSeq paired-end reads were aligned to the circular contigs to error-correct individual positions that diverged between the assembly and the read data.

2.5 Plasmid conjugation

To investigate the transferability of the *vanA*-harbouring plasmid between the VREfm donors and the vancomycin-susceptible, fusidic acid- and rifampicin-resistant *E. faecium* recipient strain 64/3, *in vitro* mating experiments were performed according to the method by Clewell *et al*. [14] with few modifications. Briefly, freshly diluted overnight cultures (1:10) of donor and recipient were incubated in a 37 °C shaker until late exponential phase (OD600 = 0.8-1.0). One hundred µL of the donor culture were mixed with 900 µL of recipient culture, and 100 µL of this solution were dispensed onto a sterile filter (0.45µm; Pall Corporation, USA) placed on a 5% BHI sheep blood agar plate and allowed to dry. After incubation at 37 °C for 24 h, the filter was rinsed in 400 µL saline by vortex mixing, and 150 µL of the rinsed suspension were spread on BHI agar plates supplemented with vancomycin (32 µg/mL), fusidic acid (32 µg/ml) and rifampicin (32 µg/ml). As a control, the donor and recipient were plated separately on the same type of plates. The plates were incubated at 37 °C for 24 h. Putative transconjugants were screened for *vanA* by PCR (fwd. primer: 5'- AACAACTTACGCGGCACT-'3, rev. primer: 5'- AAAGTGCGAAAAACCTTG-'3) and amplicons were sequenced (Macrogen, The Netherlands). Furthermore, one representative from each of the three conjugation experiments was subjected to Illumina paired end sequencing as described above to

confirm transfer of the *vanA* cluster to the recipient. All *vanA*-positive transconjugants were stored in 50% glycerol solution at -80 °C.

3. Results and Discussion

By selective enrichment for vancomycin resistance, VREfm were detected in three out of 40 meat samples from conventionally raised chickens obtained from Danish supermarkets corresponding to a prevalence of 7.5% (table 1). All VREfm isolates were resistant to vancomycin (MIC > 32 µg/mL) and rifampicin (MIC > 4 µg/mL). This is the first report of VREfm in Danish chicken meat since 2010. Within the national surveillance program DANMAP, the last VREfm were detected at low prevalence in poultry (2%) in 2008 and in chicken meat (1%) in 2010, respectively [7,8]. However, DANMAP does not apply selective enrichment for vancomycin resistance and therefore is less sensitive in detecting VREfm.

WGS analysis showed that all three VREfm strains belong to ST32, cluster type CT1068, sharing 1422 out of 1423 alleles of the *E. faecium* cgMLST scheme suggesting they are epidemiologically related although the strains originate from meat slaughtered at two different slaughterhouses. Since poultry production in Europe is highly centralized, the presence of highly similar VREfm isolates in different flocks could be a result of VREfm colonization in birds at the top of the production pyramid. ST32 belongs to CC22, which comprises strains of various origins. To our knowledge this is the first report of VREfm ST32 of poultry origin as all the previously reported animal-related strains demonstrated susceptibility towards vancomycin.

Due to the close similarity between the three VREfm isolates, only one of these (named VRE2881) was selected for full plasmid sequencing. Sequence analysis showed that VRE2881 harbours a 43.4 kb plasmid (GC-content 35.8%, 51 CDS) with a full-length *repUS1* replicase (and a truncated *rep18* replicase) as well as a larger plasmid named pHLSA of 162.7 kb (GC-content 35.7%, 188 CDS, GenBank accession no. MG674581) with a full-length *repUS15* replicase and a truncated *repE* (pAMbeta1) replicase. WGS and BLAST alignment of Illumina sequencing data from the two other VREfm isolates strongly indicated that both plasmids are also present in these isolates. The 43.4 kb plasmid harbours the full *vanA* gene cluster embedded in transposon Tn*1546* (10.851 bp, GenBank accession no. M97297.1). It is closely related (99.9% identity across 97.5% of the sequence) to a previously published linear DNA fragment of 44.4 kb, constituting the almost complete sequence of plasmid pVEF4 that was isolated from VREfm present in Norwegian poultry in 1998 (GenBank accession no. FN424376.1) [15]. Therefore, the 43.4 kb plasmid in VRE2881 was named

pVEF4_A; GenBank accession no. AMG674582. The pVEF4_A sequence was circularized by combining MinION and MiSeq techniques (Fig. 1).

Comparison of the sequence of pVEF4_A with approximately 400 draft and complete genome sequences of *E. faecium* from the NCBI RefSeq database resulted in 10 plasmids with high similarity. Eight of those were from *E. faecium* isolates obtained from chicken (six from Denmark, one from Belgium, one from Sweden), one from a Dutch patient and the source of the last is unknown. The six isolates from Danish chicken carrying pVEF4_A like plasmids belonged to ST22, ST38, ST417 and ST784 and all showed transferrable vancomycin resistance [9]. These findings correlate with the poultry origin of plasmid pVEF4_A and raise the question how this plasmid, or highly similar variants of it, has persisted in poultry production for such a long time without known selective pressure. pVEF4 and pVEF4_A both carry the *ω/ε/ζ* toxin-antitoxin system (Fig. 1) with 95% to 99% similarity to the *ω/ε/ζ* toxin-antitoxin system in pVEF1 and pVEF2 plasmids [16]. This three-component toxin-antitoxin system might contribute to the long-term persistence of the plasmid. Post-segregational killing systems are able to confer plasmid stability, especially for low copy number plasmids, and have been previously described for pVEF-type plasmids [17]. It was also proposed that the *prgOPN* gene cluster, which has been partially detected on plasmid pVEF4_A (Fig. 1) plays a role in the stabilization of certain plasmids, including pVEF4, by a toxin-antitoxin-independent mechanism [18].

Filter mating experiments showed the successful conjugation of plasmid pVEF4_A from all three VREfm isolates to the vancomycin-susceptible *E. faecium* 64/3. For all three isolates tested, > 10^3 CFU/mL of transconjugants were obtained. PCR and WGS-based MLST analysis confirmed the presence of *vanA* in the transconjugants (ST21 as *E. faecium* 64/3) and further showed that in all cases the 162.7 kb plasmid pHLSA had co-transferred to the recipient strain. In contrast to pVEF4_A the plasmid pHLSA encodes conjugation proteins of the TraG/TraD family, belonging to the Type IV Secretion System [19] suggesting that it mediates the transfer of pVEF4_A. In this experiment the recipient strain *E. faecium* strain 64/3 is of clinical origin, demonstrating that transfer of vancomycin resistance can take place between strains of animal and human origin *in vitro* and has previously been demonstrated *in vivo* [20].

4. Conclusion

Chicken meat constitutes a reservoir for VREfm and the risk of transmission during food handling or consumption cannot be excluded. The plasmids encoding vancomycin resistance are surprisingly conserved over time and place and they may be transferrable. Additional data is needed concerning the prevalence of

VREfm in meat and to address the risk of transmission of *vanA*-containing plasmids to other bacterial species present on meat and in the human gut.

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Declarations

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Competing Interests: The authors declare that they have no competing or conflicts interests related to this study.

Ethical Approval: Not required

References

[1] Van Tyne D, Gilmore MS. Friend Turned Foe: Evolution of Enterococcal Virulence and Antibiotic Resistance. Annu Rev Microbiol 2014;68:337–56. doi:10.1146/annurev-micro-091213-113003.

[2] Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro Surveill Bull Eur Sur Les Mal Transm = Eur Commun Dis Bull 2008;13. doi:19046 [pii].

[3] Cattoir V, Leclercq R. Twenty-five years of shared life with vancomycin-resistant enterococci: Is it time to divorce. J Antimicrob Chemother 2013;68:731–42. doi:10.1093/jac/dks469.

[4] Hammerum a M. Enterococci of animal origin and their significance for public health. Clin Microbiol Infect 2012;18:619–25. doi:10.1111/j.1469-0691.2012.03829.x.

[5] Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob

Agents Chemother 2001;45:2054–9. doi:10.1128/AAC.45.7.2054-2059.2001.

[6] Bortolaia V, Espinosa-Gongora C, Guardabassi L. Human health risks associated with antimicrobial-resistant enterococci and Staphylococcus aureus on poultry meat. Clin Microbiol Infect 2016;22:130–40. doi:10.1016/j.cmi.2015.12.003.

[7] DANMAP. DANMAP 2010 - Use of antimicrobial agents and occurence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2011.

[8] DANMAP. Danmap 2008 - Use of antimicrobial agents and occurence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2008.

[9] Bortolaia V, Mander M, Jensen LB, Olsen JE, Guardabassi L. Persistence of Vancomycin Resistance in Multiple Clones of Enterococcus faecium Isolated from Danish Broilers 15 Years after the Ban of Avoparcin. Antimicrob Agents Chemother 2015;59:2926–9. doi:10.1128/AAC.05072-14.

[10] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25. 2015.

[11] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4. doi:10.1093/jac/dks261.

[12] Larsen M V., Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 2012;50:1355–61. doi:10.1128/JCM.06094-11.

[13] De Been M, Van Schaik W, Cheng L, Corander J, Willems RJ. Recent recombination events in the core genome are associated with adaptive evolution in Enterococcus faecium. Genome Biol Evol 2013;5:1524–35. doi:10.1093/gbe/evt111.

[14] Clewell DB, An FY, White BA, Gawron-Burke C. Streptococcus faecalis sex pheromone (cAM373) also produced by Staphylococcus aureus and identification of a conjugative transposon (Tn918). J Bacteriol 1985;162:1212–20.

[15] Sletvold H, Johnsen PJ, Wikmark O-G, Simonsen GS, Sundsfjord A, Nielsen KM. Tn1546 is part of a larger plasmid-encoded genetic unit horizontally disseminated among clonal Enterococcus faecium lineages. J Antimicrob Chemother 2010;65:1894–906. doi:10.1093/jac/dkq219.

[16] Sletvold H, Johnsen PJ, Simonsen GS, Aasnæs B, Sundsfjord A, Nielsen KM. Comparative DNA analysis of two vanA plasmids from Enterococcus faecium strains isolated from poultry and a poultry farmer in Norway. Antimicrob Agents Chemother 2007;51:736–9. doi:10.1128/AAC.00557-06.

[17] Sletvold H, Johnsen PJ, Hamre I, Simonsen GS, Sundsfjord A, Nielsen KM. Complete sequence of Enterococcus faecium pVEF3 and the detection of an ω-ε-ζ toxin-antitoxin module and an ABC transporter. Plasmid 2008;60:75–85. doi:10.1016/j.plasmid.2008.04.002.

[18] Li X, Alvarez V, Harper WJ, Wang HH. Persistent, toxin-antitoxin system-independent, tetracycline resistance-encoding plasmid from a dairy Enterococcus faecium Isolate. Appl Environ Microbiol 2011;77:7096–103. doi:10.1128/AEM.05168-11.

[19] Arends K, Celik E-K, Probst I, Goessweiner-Mohr N, Fercher C, Grumet L, et al. TraG Encoded by the pIP501 Type IV Secretion System Is a Two-Domain Peptidoglycan-Degrading Enzyme Essential for Conjugative Transfer. J Bacteriol 2013;195:4436–44. doi:10.1128/JB.02263-12.

[20] Lester CH, Frimodt-Møller N, Sørensen TL, Monnet DL, Hammerum AM. In vivo transfer of the vanA resistance gene from an Enterococcus faecium isolate of animal origin to an E. faecium isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother 2006;50:596–9. doi:10.1128/AAC.50.2.596-599.2006.

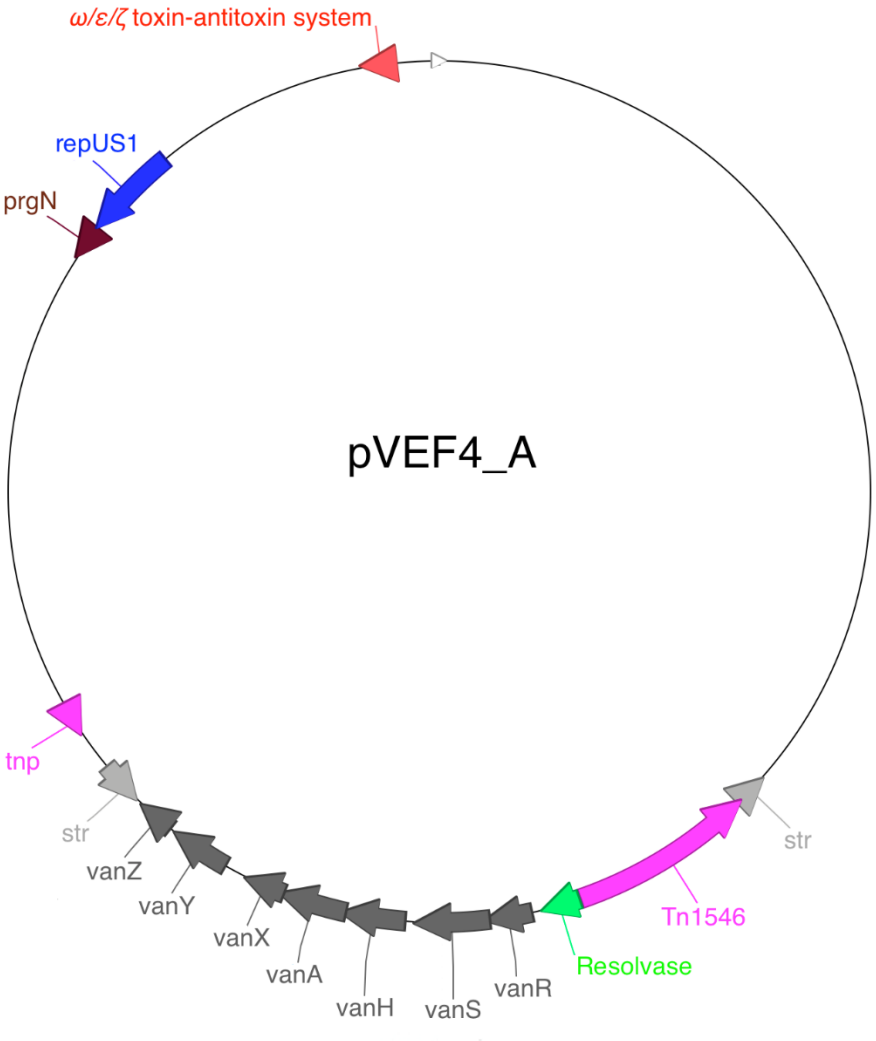

Fig. 1. Closed map of pVEF4_A. Indicated on the pVEF4_A map are the *ω/ε/ζ* toxin-antitoxin system, a *repUS1* replicase, *prgN* being a part of the *prgOPN* gene cluster, the streptomycin resistance gene *str*, transposon Tn*1546* and the *vanA* operon. This figure was generated by ApE A plasmid Editor (v2.0.53) http://biologylabs.utah.edu/jorgensen/wayned/ape/.

Table 1: Information on the three vancomycin-resistant E. faecium isolates. Resistance towards streptomycin was not tested.

Isolate no.	Origin	Production date	Sequence type	Cluster type	Genome size (bp)	Plasmids	Resistance profile	Resistance genes
VRE2881	chicken leg Company A, slaughter house B	13.04.2016	ST32	CT1068	2651941	*vanA* carrying pVEF4_A (43.4kb), pHLSA (162.7kb)	vancomycin, rifampicin	*str*, *vanA*
VRE2882	chicken breast Company B, slaughter house A	25.04.2016	ST32	CT1068	2660381	*vanA* carrying pVEF4_A (43.4kb), pHLSA (162.7kb)	vancomycin, rifampicin	*str*, *vanA*
VRE2883	chicken breast Company C, slaughter house B	11.05.2016	ST32	CT1068	2661125	*vanA* carrying pVEF4_A (43.4kb), pHLSA (162.7kb)	vancomycin, rifampicin	*str*, *vanA*