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Letter

First detection of phenicol-oxazolidinone resistance gene *optrA* in *Aerococcus viridans* from bovine faeces, Italy

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Sir,

antibiotic resistance genes (ARGs) are now ubiquitous also in non-clinical environments. Specifically, livestock farms are considered major reservoirs of ARGs and pose a risk for human health since ARGs can be spread to human bacterial pathogens. Although many antimicrobials administered to animals are used exclusively in veterinary applications, most of them can drive co-selection of genes for the resistance to drugs critical in human medicine.

Oxazolidinones, including linezolid and tedizolid, are effective antimicrobial agents for the treatment of severe human infections due to MDR Gram-positive bacteria and they have never been approved for animal use. Nevertheless, acquired linezolid-resistance has arisen in bacteria of animal origin, as well as in human isolates, due to extensive use of phenicols in veterinary medicine that provide the selective pressure for a rapid dissemination of the *cfr*,[1] *optrA*[2] and *poxtA*[3] linezolid resistance genes on animal farms.[4] Analysis of the prevalence and distribution of linezolid-resistance genes in animal settings is important for evaluating the risk to public health and for development of control measures.

During a routine surveillance aimed at the detection of linezolid resistance genes in enterococci of animal origin, pooled faecal samples were collected from cattle farms where florfenicol was used previously. The samples were inoculated in buffered peptone water supplemented with florfenicol (10 mg/L) and incubated at 44°C for 48 hours. Then, 0.1 mL was inoculated in Slanetz Bartley agar plates supplemented with florfenicol (10 mg/L) and the enterococci grown on this medium were tested by PCR for the presence of *cfr*, *optr*A and *poxt*A genes.[5] Isolates carrying at least one linezolid resistance gene were identified by MALDI-TOF (Vitek-MS, bioMérieux). Interestingly, one *optrA*-positive isolate (called 1417-4A) belonged to the *Aerococcus viridans* species. Susceptibility testing, carried out by reference broth microdilution (www.clsi.org) and interpreted according to the EUCAST clinical breakpoints (version 10.0, www.eucast.org) revealed that MICs of linezolid, tedizolid, and florfenicol for *A. viridans* 1417-4A were 1 mg/L, 1 mg/L, and 16 mg/L, respectively. In filter mating experiments, using florfenicol (10 mg/L) for selection,[6] *optrA* gene was successfully transferred from *A. viridans* 1417-4A to *E. faecium* 64/3 recipient (transfer frequency, $1.7x10^{-5}$ per recipient). Two randomly selected transconjugants showed MICs of tedizolid and florfenicol identical to those for the donor, instead a linezolid MIC of 4 mg/L was recorded for both transconjugants. It is notable that a 4-fold increase in the linezolid MICs was detected in transconjugants compared to that of *E. faecium* 64/3

recipient (1 mg/L) suggesting that *optrA* of *A. viridans* could confer reduced linezolid susceptibility in enterococcal background.

In S1-PFGE and hybridization assays,[6] an *optrA*-specific probe hybridized with a ca. 30-kb plasmid both in donor and transconjugants.

In order to characterize the *optrA* genetic element, whole-genome sequence (WGS) of *A. viridans* 1417-4A was carried out by Illumina MiSeq platform (MicrobesNG, Birmingham, UK) using a 2 x 250 paired end approach. De novo assembly of WGS data was performed using the SPAdes software (http://bioinf.spbau.ru/spades).

Bioinformatics analysis revealed that *optrA* gene was located on a new 37,845-bp plasmid, named pAv-oprtA (G+C content, 32.0%) (accession no. MW364930). The map of the plasmid is shown in Figure 1 and the major characteristics of the ORFs are detailed in Table S1. Four relevant areas were detectable in pAv-*optrA*: (*i*) an antibiotic resistance region containing *optrA* (responsible for phenicols and oxazolidinone resistance) and *erm*(B) (responsible for macrolide, lincosamide and streptogramin B resistance) genes. The *optrA* gene was 98.0% identical to the DNA reference sequence (GenBank accession no. KP399637.1). Moreover, twenty-two amino acid changes were detected in the protein sequence compared to that of $OptrA_{E349}$ (96% identity, 98% similarity)(Figure S1);[2] (*ii*) a conjugation region spanning from *orf10* to *orf23*; (*iii*) a segment harbouring *repA* (*orf28*) and *parA* (*orf30*) genes responsible for plasmid replication and partitioning, respectively. The *repA* gene was not typeable by plasmid finder (https://cge.cbs.dtu.dk/services/PlasmidFinder/); (*iv*) a complete type 1 restriction-modification cassette (*orf35*, *orf1* and *orf2*) showing 96.0% DNA sequence identity with the corresponding region of *poxtA*-carrying pC25-1 and pC27-2 plasmids.[7] A post-segregational killing mechanism might be responsible for maintaining of pAV-optrA in *A. viridans* 1417-4A host.[8]

WGS analysis also ruled out the presence of *cfr*, *cfr*-like and *poxtA* genes. No mutations were detected in the genes encoding the 23S rRNA and ribosomal proteins.

Aerococci are widespread in the environment and frequently isolated from foods and animals and now are increasingly recognized as human pathogens.[9] To the best of our knowledge, this is the first report of the *optrA* gene in *Aerococcus* species, carried by a conjugative plasmid. *A. viridans* caused different human infection such as urinary tract infections, osteomyelitis, septic arthritis, septicaemia and endocarditis, therefore the presence of linezolid resistance *optrA* gene in this species and its ability to transfer to human pathogen *E. faecium*, are cause for concern.

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Transparency declarations

None to declare

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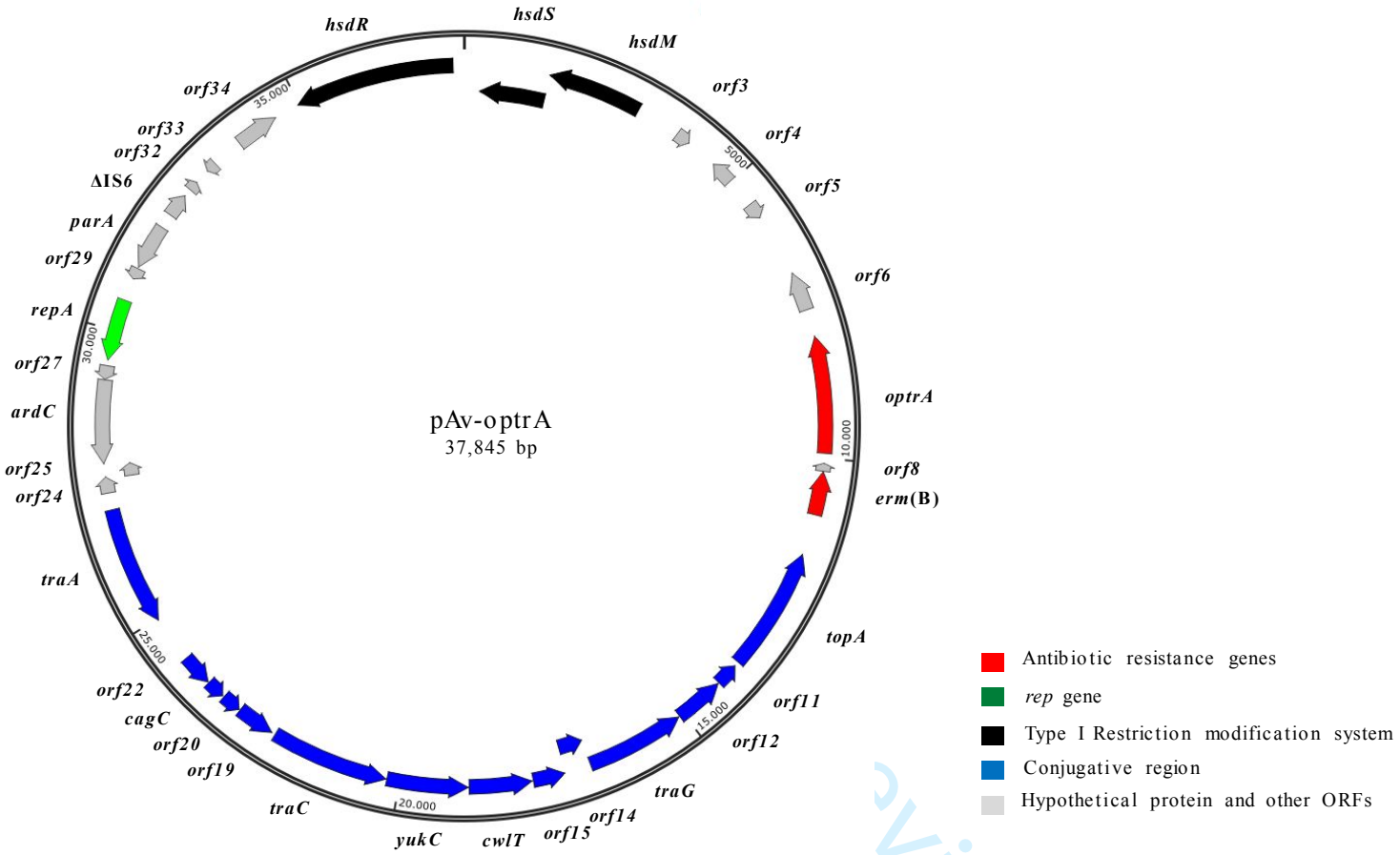
Figure

Figure 1. Schematic representation of the *optrA*-carrying pAv-*optrA* plasmid (16,500 bp) from *A. viridans* 1417-4A (accession no. MW364930).
Arrows indicate the position and direction of transcription of different genes.



Supplementary materials

Table S1. Amino acid sequence identities/similarities of putative proteins encoded by the pAv-optrA (GenBank accession no. MW364930).

ORF	Start (bp)	Stop (bp)	Size (amino acids)	Predicted function	BLASTP analysis[a]		
					Most significant database match	Accession no.	% Amino acid identity (% amino acid similarity)
orf1	1,449	283	388	Restriction endonuclease S subunit	Restriction endonuclease subunit S [*Aerococcus viridans*]	WP_111856479.1	65 (72)
orf2	3,038	1,449	528	Type I restriction-modification system DNA methylase subunit	Type I restriction-modification system subunit M [*A. viridans*]	WP_111856478.1	98 (99)
orf3	3,797	4,039	80	DNA-invertase hin	DNA-invertase hin [*A. viridans*]	SPT61043.1	100 (100)
orf4	4,979	4,581	132		Hypothetical protein [*Escherichia coli*]	WP_160516845.1	93 (95)
orf5	5,489	5,761	90		Hypothetical protein [*Aerococcus urinaeequi*]	HCT97980.1	43 (61)
orf6	7,486	6,833	217		DUF262 domain-containing protein [*Enterococcus faecalis*]	HAP3453445.1	100 (100)
orf7	9,915	7,948	655	ABC-F type ribosomal protection protein OptrA	ABC-F type ribosomal protection protein OptrA [*E. faecalis*]	HAP3438646.1	99 (99)
orf8	10,213	10,082	43		Hypothetical protein [Bacteria]	WP_000085855.1	100 (100)
orf9	10,955	10,218	245	Ribosomal RNA adenine methylase	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(B) [Firmicutes]	WP_001038790.1	100 (100)
orf10	13,753	11,648	701	DNA topoisomerase IA	Type IA DNA topoisomerase [*Enterococcus*]	WP_104846964.1	75 (85)
orf11	14,223	13,831	130		Hypothetical protein [*Tetragenococcus solitarius*]	WP_068710574.1	43 (64)
orf12	15,082	14,237	281		Unnamed protein product	WP_192982111.1	51 (72)
orf13	16,770	15,097	557	Type IV secretory system Conjugative DNA transfer	Type IV secretory system conjugative DNA transfer family protein [*Marinilactibacillus psychrotolerans*]	WP_138472019.1	86 (92)
orf14	17,207	16,770	145		Hypothetical protein [*Alkalibacterium gilvum*]	WP_091636415.1	54 (73)
orf15	17,764	17,222	180		Conjugal transfer protein [*Carnobacterium* sp. 1290_CSPC]	WP_157850759.1	46 (63)
orf16	18,835	17,783	350	CwlT-like N-terminal lysozyme domain	Lysozyme family protein [*Carnobacterium* sp. 1290_CSPC]	WP_053083829.1	62 (77)
orf17	20,214	18,850	454	Protein secretion system	Hypothetical protein [*Enterococcus faecium*]	EGP5098947.1	43 (68)
orf18	22,223	20,229	664	Type-IV secretion system protein TraC	DUF87 domain-containing protein [*Carnobacterium* sp. 1290_CSPC]	WP_053083830.1	77 (87)
orf19	22,941	22,291	216		Conjugal transfer protein [*Marinilactibacillus psychrotolerans*]	WP_138472028.1	64 (81)
orf20	23,289	22,957	110		Hypothetical protein [*M. psychrotolerans*]	WP_138472031.1	54 (77)

176	*orf21*	23,634	23,314	106	Cag pathogenicity island, type IV secretory system	Hypothetical protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729905.1	63 (78)
177								
178	*orf22*	24,188	23,646	180		Hypothetical protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729908.1	40 (66)
179	*orf23*	26,975	24,972	667	MobA/MobL family protein	MobA/MobL family protein [*Carnobacterium* sp. 1290_CSPC]	WP_053083831.1	52 (68)
180	*orf24*	27,266	27,532	88		Hypothetical protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729913.1	42 (71)
181	*orf25*	27,507	27,728	73		Hypothetical protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729916.1	62 (80)
182	*orf26*	29,159	27,756	467	Antirestriction protein ArdC	ImmA/IrrE family metallo-endopeptidase [*Carnobacterium* sp. 1290_CSPC]	WP_048729918.1	76 (83)
183								
184	*orf27*	29,398	29,165	77		Hypothetical protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729921.1	97 (97)
185	*orf28*	30,526	29,492	344	Replication initiator protein A (RepA)	Replication initiator protein A [*Carnobacterium* sp. 1290_CSPC]	WP_048729642.1	76 (85)
186	*orf29*	31,097	30,912	61		Hypothetical protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729641.1	92 (96)
187	*orf30*	31,901	31,128	257	Plasmid partitioning protein	ParA family protein [*Carnobacterium* sp. 1290_CSPC]	WP_048729640.1	89 (93)
188	*orf31*	32,141	32,548	135	DDE transposase	IS6 family transposase, partial [*E. faecium*]	WP_147239629.1	94 (95)
189	*orf32*	32,677	32,847	56		Hypothetical protein [Bacilli]	WP_164507104.1	100 (100)
190	*orf33*	33,279	33,106	57		Hypothetical protein FOB80_07155 [*A. viridans*]	QGS37267.1	100 (100)
191	*orf34*	33,808	34,545	245	Methyltransferase domain	Class I SAM-dependent methyltransferase [*Carnobacterium* sp. PL12RED10]	WP_149358818.1	99 (100)
192								
193	*orf35*	37,663	34,976	895	DEAD-like helicases superfamily	Type-1 restriction enzyme R protein [*A. viridans*]	SPT61052.1	99 (99)
194								

[a]For each ORF, only the most significant identity detected is listed.

Figure S1. Amino acid sequence alignment of the OptrA proteins of *A. viridans* 1417-4A (**Av**) and *E. faecalis* E349 (**Ef**). Mismatches are highlighted in yellow.

```
Av      1    LSEATFAIASTYVKEDMKMQYKIINGAVYYDGNMVLENIGIEINDNEKIAIVGRNGCGKT  60
             +S+ATFAIAST  KEDMKMQYKIINGAVYYDGNMVLENIGIEINDNEKIAIVGRNGCGKT
Ef      1    MSKATFAIASTNAKEDMKMQYKIINGAVYYDGNMVLENIGIEINDNEKIAIVGRNGCGKT  60

Query   61   TLLKAIIGEIELEEGTGESEFQVIKTGNPYISYLRQMPFEDESISMVDEVRTVFKTLIDM  120
             TLLKAIIGEIELEEGTGESEFQVIKTGNPYISYLRQMPFEDESISMVDEVRTVFKTLIDM
Sbjct   61   TLLKAIIGEIELEEGTGESEFQVIKTGNPYISYLRQMPFEDESISMVDEVRTVFKTLIDM  120

Query   121  EKKMKQLIDKMENQCDDKIINEYSDILERYMALGGLTYQKEYETMIRSMGFTEADDKKPI  180
             E KMKQLIDKMENQ DDKIINEYSDI ERYMALGGLTYQKEYETMIRSMGFTEAD KKPI
Sbjct   121  ENKMKQLIDKMENQYDDKIINEYSDISERYMALGGLTYQKEYETMIRSMGFTEADYKKPI  180

Query   181  SEFSGGQRTKIAFIKILLTKPDILLLDEPTNHLDIETIQWLESYLRSYKSTLVIISHDRM  240
             SEFSGGQRTKIAFIKILLTKPDILLLDEPTNHLDIETIQWLESYLRSYKSTLVIISHDRM
Sbjct   181  SEFSGGQRTKIAFIKILLTKPDILLLDEPTNHLDIETIQWLESYLRSYKSTLVIISHDRM  240

Query   241  FLNRIVDKVYEIEWGETKCYKGNYSAFEEQKRENHIKQQKDYDLQQIEIERITRLIERFR  300
             FLNRIVDKVYEIEWGETKCYKGNYSAFEEQKRENHIKQQKDYDLQQIEIERITRLIERFR
Sbjct   241  FLNRIVDKVYEIEWGETKCYKGNYSAFEEQKRENHIKQQKDYDLQQIEIERITRLIERFR  300

Query   301  YKPTKAKMVQSKIKLLQRMQILNAPDQYDTKTYMSKFQPRISSSRQVLSVSELVIGYDTP  360
             YKPTKAKMVQSKIKLLQRMQILNAPDQYDTKTYMSKFQPRISSSRQVLS SELVIGYDTP
Sbjct   301  YKPTKAKMVQSKIKLLQRMQILNAPDQYDTKTYMSKFQPRISSSRQVLSASELVIGYDTP  360

Query   361  LAKVNFNLERGQKLGIVGSNGIGKSTLLKTLMDGASALSGDFKFGYNVEISYFDQQLAQI  420
             LAKVNFNLERGQKLGIVGSNGIGKSTLLKTLM G +ALSGDFKFGYNVEISYFDQQLAQI
Sbjct   361  LAKVNFNLERGQKLGIVGSNGIGKSTLLKTLMGGVAALSGDFKFGYNVEISYFDQQLAQI  420

Query   421  SGDDTLFEIFQSEYPELNDTEVRTALGSFQFSGDDVFRPVSSLSGGEKVRLTLCKLLYKR  480
             SGDDTLFEIFQSEYPELNDTEVRTALGSFQFSGDDVFRPVSSLSGGEKVRLTLCKLLYKR
Sbjct   421  SGDDTLFEIFQSEYPELNDTEVRTALGSFQFSGDDVFRPVSSLSGGEKVRLTLCKLLYKR  480

Query   481  TNVLILDEPTNHMDIIGKENLENILCSYKGTIIFVSHDRYFTNKIADRLLVFDKDGVEFV  540
             TNVLILDEPTNHMDIIGKENLENILCSY+GTIIFVSHDRYFTNKIADRLLVFDKDGVEFV
Sbjct   481  TNVLILDEPTNHMDIIGKENLENILCSYQGTIIFVSHDRYFTNKIADRLLVFDKDGVEFV  540

Query   541  ESTYGEYEKKRLNSEKPFNYINVEKKVEKNNTVKGDRNSIEKEKVKKEKRIEKLEVLINQ  600
             +STYGEYEKKR+NSEKPFN I VE+KVEKNNTVKGDRNSIEKEKVKKEKRIEKLEVLINQ
Sbjct   541  QSTYGEYEKKRMNSEKPFNNIKVEQKVEKNNTVKGDRNSIEKEKVKKEKRIEKLEVLINQ  600

Query   601  YDEELERLNKIISQPNNSSDYIVLTELQKSIDEVKRCQGIYFNEWEQLMGELEVM  655
             YDEELERLNKIIS+PNNSSDYIVLTE+QKSID+VKRCQG YFNEWEQLM ELEVM
Sbjct   601  YDEELERLNKIISEPNNSSDYIVLTEIQKSIDDVKRCQGNYFNEWEQLMRELEVM  655
```