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Mosaic tetracycline resistance genes encoding ribosomal protection proteins

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First reported in 2003, mosaic tetracycline resistance genes are a subgroup of the genes encoding ribosomal protection proteins (RPPs). They are formed when two or more RPP-encoding genes recombine resulting in a functional chimera. To date, the majority of mosaic genes are derived from sections of three RPP genes, *tet*(O), *tet*(W) and *tet*(32), with others comprising *tet*(M) and *tet*(S). In this first review of mosaic genes, we report on their structure, diversity and prevalence, and suggest that these genes may be responsible for an under-reported contribution to tetracycline resistance in bacteria.

Introduction

Tetracyclines bind to the A-site on the bacterial ribosome, resulting in steric blocking of the aminoacyl-tRNA binding site, which prevents protein synthesis.[1] They are effective against both Gram-positive and Gram-negative bacteria and, due to the relative lack of major side effects and cheap cost, have been used extensively in the treatment of infections[2] as well as growth promoters in animal husbandry.[3]

Bacterial resistance to tetracycline is often mediated through the acquisition of DNA encoding proteins that confer resistance by one of three main mechanisms: ATP-dependent efflux, enzymatic inactivation of tetracycline, or ribosomal protection.[2] To date, a total of 47 different classes of tetracycline resistance gene, including [illegible] tetracycline resistance genes, have been reported. These include 21 predicted or proven to encode active efflux pumps, 12 encoding ribosomal protection proteins (RPPs), 2 encoding inactivating enzymes and 1 reported to confer resistance via an as yet undetermined mechanism, designated *tet*(U) (a full list is periodically updated by Roberts[4]). Although it has yet to be assigned a mechanistic class, *tet*(U) has been identified in *Enterococcus* and *Staphylococcus* isolates.[5,6] However, a study by Caryl *et al.*[7] reported that when *tet*(U) was cloned and expressed in *Escherichia coli*, the transformants were not resistant to tetracycline.

To be considered a new class of tetracycline resistance gene, it must encode a protein <80% identical to known tetracycline resistance proteins.[8] Determinants representing new classes were originally assigned a letter from the English alphabet.[9] However, as all letters are used, they are now assigned an Arabic numeral,[8] with new determinants referred to the Levy group (bonnie.marshall@tufts.edu) in order to obtain a designation prior to publication to avoid duplication and ensure taxonomic consistency.

RPPs

RPPs are a related group of proteins that, when bound to the ribosome, result in the release of tetracycline from the ribosome, enabling protein synthesis to proceed[10] (reviewed by Thaker *et al.*[11]). Of the 12 classes of RPP gene currently reported [*tet*(M), (O), (Q), (S), (T), (W), (32), (36), (44), *B*(P), *otr*(A) and *tet*], *tet*(M) is considered the most prevalent due to its association with the broad host range Tn*916*/Tn*1545* family of conjugative transposons.[12] However, a subgroup of RPP genes has been identified that consist of regions of different, already characterized RPP genes that appear to have undergone recombination forming a mosaic gene. It must be stressed here that the progenitors of mosaic genes are assumed based purely on the order in which they were discovered and we cannot be sure of the directionality of mosaic gene formation.

Mosaic RPP genes

In 2003, Stanton and Humphery[13] reported two RPP genes in *Megasphaera elsdenii* that encoded predicted proteins showing 89.1% and 91.9% identity to Tet(W) (accession number AJ222769) from *Butyrivibrio fibrisolvens*. As this was above the <80% cut-off, they did not qualify as a new resistance class

under the nomenclature system. However, further analysis of the amino acid sequence revealed variability in the percentage identity to Tet(W) across its length. The large central section in both sequences showed 98.1% identity to Tet(W), while small sections at the N- and C-terminal ends were found to have a lower amino acid sequence identity to Tet(W) [between 66.6% and 75.3%]. However, these same N- and C-terminal sections were shown to have between 99.3% and 100% amino acid identity to Tet(O) (accession number M18896), despite the central section showing identity to Tet(W). Given the evidence, this suggested recombination had occurred, creating a mosaic determinant with a central Tet(W) region flanked by two Tet(O) regions. Although never before observed between two different RPP classes, recombination resulting in functional genes has previously been reported between different phylotypes of *tet*(M)[14] as well as in other antibiotic resistance genes, such as *penA* and *pbp2x*, which confer resistance to penicillin.[15,16] Furthermore, *in vitro* experiments have successfully recombined *tet*(A) and *tet*(C) to create mosaics that confer resistance to tetracycline at levels comparable to the non-mosaic *tet*(C).[17]

The guideline for determining a new resistance gene class was established prior to the discovery of these mosaic RPP genes and none of the mosaic genes qualified as a new class when analysed as one single continuous sequence. It was clear, however, that these mosaic genes were different from their non-mosaic counterparts and that the current classification did not adequately reflect the true evolutionary background of these genes. Therefore, an expansion of the nomenclature system was suggested whereby the mosaic gene would receive a designation that reflected the structural order and class of the genes they comprised, better reflecting their variable nature.[18,19] For example, the two resistance genes reported in *M. elsdenii*, which comprised a central *tet*(W) region flanked by two *tet*(O) regions, were designated *tet*(O/W/O).[13]

Although Stanton and Humphrey[13] were the first to report mosaic RPP genes, Melville *et al.*[20] had unknowingly reported a mosaic gene 2 years previously. This resistance gene, found in *Clostridium saccharolyticum* K10, encoded a predicted protein that showed 76% amino acid identity to Tet(O) (accession number Y07780). As per the original nomenclature guidelines, it was given the new designation Tet(32). However, subsequent re-examination of the sequence found that only the central section showed <80% identity to known proteins, while the N- and C-terminal regions flanking the central section shared 100% and 97.7% identity, respectively, to Tet(O) (accession number M18896). The central region was still thought to represent a section of a new Tet(32) class and therefore the determinant was reclassified Tet(O/32/O).[18] Subsequently, the proposed full, non-mosaic sequences of Tet(32) have been reported in several isolates identified from the human oral cavity,[21,22] with the Tet(O/32/O) mosaic determinant now showing 89% amino acid identity to these.

Similarly, the previously reported *tet*(S) allele (accession number AY534326) on the conjugative transposon Tn*916S*[23] has subsequently been reclassified as a result of *in silico* analysis. The amino acid sequence shows identity to Tet(S) across 595 amino acids (1–595 inclusive), with the final 61 amino acids at the C-terminus end identical to Tet(M) (accession number U09422), resulting in it being reclassified as Tet(S/M).[24]

Mosaic gene diversity

To date, a total of 30 mosaic genes have been reported in the literature, of which 26 currently have sequences deposited in GenBank (Table 1). Some studies have reported multiple occurrences of known genes; however, many of these have been characterized by PCR amplification only. Structurally, these chimeric genes currently comprise either two [e.g. *tet*(O/W)], three [e.g. *tet*(O/W/O)], four [e.g. *tet*(O/W/32/O)] or six [e.g. *tet*(O/W/32/O/W/O)] different regions (Figure 1), with *tet*(O), *tet*(W) and *tet*(32) being the predominant RPP genes reported to form mosaic genes, comprising all but two of the reported variants, and *tet*(M) and *tet*(S) forming the remaining two.[24,25] Given the prevalence of *tet*(M) in certain samples, and the previous reports of self-recombination,[14,26] it is surprising that there are so few reports of mosaic genes containing *tet*(M). Furthermore, alignment of 12 representative RPP gene sequences shows *tet*(M) sharing 75% and 70% identity, respectively, to *tet*(O) and *tet*(44), which is higher than the percentage identity observed between the more commonly reported RPP mosaic genes comprising *tet*(O), (W) and (32) (Table 2). However, mosaic genes comprising *tet*(M) and any other gene, with the exception of *tet*(S), have yet to be reported. It is entirely possible that this may be due to a lack of investigation rather than an absence of recombination followed by fixation of the recombinant allele in the bacterial population. Alternatively, it is possible that there is little selective pressure for *tet*(M)-based mosaic genes if the resultant protein is no more efficient than Tet(M) itself and/or there is no indirect selective pressure for mosaicism. A similar situation may exist for other proteins, such as Tet(S). Stanton *et al.*[27] reported that the protein encoded by the *tet*(O/W/O) mosaic genes in *M. elsdenii* conferred a higher level of resistance to tetracycline than their non-mosaic counterparts, but similar investigations are still to be reported for other RPP genes. Therefore, the prevalence of certain mosaic gene variants could suggest that they are in some way more beneficial to the host than the non-mosaic genes they comprise.

PCR-based analysis

PCR-based assays have been developed to help researchers detect specific mosaic genes. Stanton and Humphrey[13] describe an assay that distinguished between the non-mosaic genes *tet*(O) and *tet*(W) and the mosaic *tet*(O/W/O) from *Megasphaera* strains, enabling them to detect *tet*(O/W/O) variants in six additional *M. elsdenii* strains. Patterson *et al.*[21] investigated the presence of mosaic genes using various specific oligonucleotide sets that either bound within the resistance genes or flanked them. Amplicons specific to *tet*(O/W), *tet*(O/32) and *tet*(W/32) were detected in faecal samples, with *tet*(O/32) being the most common of these mosaic amplicons; it was amplified in all 12 pig faecal samples and 6 of 7 human faecal samples tested. In contrast, the faecal samples from cows and sheep, as well as human saliva samples, failed to produce any amplicons for these mosaic genes, suggesting they were not present at detectable levels.

Chen *et al.*[28] also used an oligonucleotide primer set that annealed outside *tet*(O) to determine the presence of tetracycline resistance genes in two *Streptococcus suis* isolates. Although no amplicon was produced using internal, *tet*(O)-specific primers, the primers binding to flanking DNA yielded an amplicon, indicating the presence of mosaic genes [identified as *tet*(O/32/O) and

Table 1. A summary of the mosaic tetracycline genes reported to date

Gene	Organism	Source(s)	Accession number	Reference(s)
tet(O/W)	*Bifidobacterium thermophilum* B0219	environmental (pig slaughterhouse) sample	AM889118	32
tet(O/W)	*B. thermophilum* B0241	pig faeces	AM889119	32
tet(O/W)	*B. thermophilum* B0242	pig faeces	AM889120	32
tet(O/W)	*B. thermophilum* B0253	pig faeces	AM889121	32
tet(O/W)	*B. thermophilum* B0256	pig faeces	AM889122	32
tet(O/W)-2	*Megasphaera elsdenii* 25-51	swine faeces	AY485122	18,27
tet(O/W)-1 [$n=15^{a}$]	*M. elsdenii* 27-51	swine faeces	AY485126	27,33
tet(O/W/O)-4	uncultured bacterial clone	pig faeces	no accession number	21
tet(O/W/O)-3 [$n=9$]	uncultured bacterial clone	pig faeces	EF065524	21
tet(O/W/O)-2 [$n=28^{b}$]	*M. elsdenii* 14-14	swine caecum	AY196920	13,18,27,33
tet(O/W/O)-1 [$n=2$]	*M. elsdenii* 7-11	swine caecum	AY196921	13,18,27
tet(O/W/32/O) [$n=32$]	uncultured bacterial clone	pig faeces	EF065523	21
tet(O/W/32/O) [$n=7^{c}$]	*Streptococcus suis* Ss1303	pig (brain, lung and spleen) and human (CSF) samples	FM164392	34
tet(O/W/32/O)	*S. suis 32457*	diseased pig lung	FR823304	34,35
tet(O/W/32/O)	*Streptococcus gallolyticus* subsp. *gallolyticus* ATCC 2069 plasmid pSGG1	human blood	FR824044	36
tet(O/W/32/O)	*S. suis*	diseased pig (blood, brain, heart, joint and lung) samples	JQ740053	28
tet(O/W/32/O/W/O)	*Lactobacillus johnsonii* G41	human faeces	DQ525023	32
tet(O/W/32/O/W/O)	uncultured bacterial clone	pig faeces	DQ679926	21
tet(O/32/O)	*S. suis*	diseased pig (blood, brain, heart, joint and lung) samples	JQ740052	28
tet(O/32/O)	*Clostridium saccharolyticum* K10	human colon	AJ295238	18
tet(O/32/O)-2 [$n=3$]	uncultured bacterial clone	human and animal faecal samples	no accession number	21
tet(O/32/O)-3	uncultured bacterial clone	human and animal faecal samples	no accession number	21
tet(O/32/O)-4	uncultured bacterial clone	human and animal faecal samples	no accession number	21
tet(O/32/O)-5	uncultured bacterial clone	human and animal faecal samples	no accession number	21
tet(O/32/O)	*Dorea longicatena* AGR2136	rumen microbiome	NZ_AUJS01000017 (41 626–43 545 bp)	direct submission, analysed in this study
tet(O/32/O)	*Campylobacter coli* 202/04	human faeces	AINH01000038 (2361–4280 bp)	direct submission, analysed in this study
tet(O/32/O)	*C. coli* 317/04	human faeces	NZ_AINJ01000054 (2094–4013 bp)	direct submission, analysed in this study
tet(O/32/O)	*Campylobacter jejuni* subspecies *jejuni* 2008-894	human	AIOQ01000025 (14 515–16 434 bp)	direct submission, analysed in this study
tet(O/32/O)	*Roseburia intestinalis* XB6B4	human intestinal tract	FP929050 (2 873 814–2 875 733 bp)	direct submission, analysed in this study
tet(S/M)	*Streptococcus equinus* 1357	food	HM367711	25
tet(S/M)	*Streptococcus intermedius*	human isolate	AY534326	23,24

Continued

Table 1. *Continued*

Gene	Organism	Source(s)	Accession number	Reference(s)
tet(W/32/O)	*B. thermophilum* B0219	environmental (pig slaughterhouse sample	AM710601	32
tet(W/32/O)	*B. thermophilum* B0241	pig faeces	AM710602	32
tet(W/32/O)	*B. thermophilum* B0242	pig faeces	AM710603	32
tet(W/32/O)	*B. thermophilum* B0253	pig faeces	AM710604	32
tet(W/32/O)	*B. thermophilum* B0256	pig faeces	AM710605	32

The number given in square brackets indicates the instances of that mosaic gene variant reported, if more than one.
[a]Fourteen of the 15 *tet*(O/W)-1 variants were only determined by PCR analysis and so could be either *tet*(O/W)-1 or *tet*(O/W)-2.
[b]Eleven of the 28 *tet*(O/W/O)-2 variants were only determined by PCR analysis and so could be either *tet*(O/W/O)-2 or *tet*(O/W/O)-1.
[c]All *S. suis* isolates, but not the same strain.

tet(O/W/32/O)]. This full-length oligonucleotide primer set does aid the identification of mosaic genes; however, it is only specific for those with regions homologous to *tet*(O) flanking sequences. Since PCR strategies aimed at identifying resistance genes require knowledge of the sequence of the target, mosaic RPP genes are likely to be largely undetected and under-reported by PCR-based studies.

Reflecting the findings by Patterson *et al.*,[21] almost all the mosaic genes reported to date have originated from faecal samples, with the majority identified from a porcine origin and less commonly from humans (Table 1). The gut houses a complex and diverse bacterial community with potential for widespread horizontal gene transfer, and the mosaic genes found in faecal samples are likely to reflect the pool of non-mosaic genes present within the gut microbiota. Genes such as *tet*(W) and *tet*(O) are commonly reported from these types of samples,[29] but the prevalence of *tet*(32)-containing mosaic genes suggests that *tet*(32) may be more common than initially thought. In fact, *tet*(O/32/O) was found to be the most common mosaic gene in both the human and pig faecal samples tested and was present in almost as many samples tested as the non-mosaic *tet*(O) and *tet*(W) genes.[21] In contrast, mosaic genes have not yet been reported in faecal samples from bovine and ovine origin or in human saliva.[21] Why they are predominantly found in pigs while as yet unreported in other animals is not immediately clear, though the extensive use of tetracyclines in the swine industry[3,30,31] may have contributed to their selection.

Draft genome analysis

The advent of high-throughput genomic sequencing has led to an increase in the number of genomes being deposited in sequence databases. Many contain tetracycline resistance genes that are generically labelled simply as 'tetracycline resistance protein' or as '*tet*(M)-like', the designation of which may be a result of automated annotation pipelines. A preliminary search of the NCBI nucleotide database, using *tet*(O) (accession number Y07780) as the query, found that some of these generically labelled tetracycline resistance genes gave a partial match to *tet*(O). Further examination indicates that some are as yet uncharacterized and unreported mosaic genes, which have been further defined for this review using the nucleotide sequence to determine the crossover points. For example, the *tet*(M)-like gene (accession number NZ_AUJS01000017, location 41 626–43 545 bp) in the draft genome of *Dorea longicatena* AGR2136 from a human faecal sample appears to be a previously unreported variant of *tet*(O/32/O) (Figure 1).

Furthermore, the tetracycline resistance genes present in *Campylobacter jejuni* subspecies *jejuni* 2008-894, *Campylobacter coli* 202/04, *C. coli* 317/04 (accession numbers AIOQ01000025, AINH01000038 and NZ_AINJ01000054, respectively) and *Roseburia intestinalis* XB6B4 (accession number FP929050) are also structurally novel variants of *tet*(O/32/O) (Figure 1). The three mosaic genes present in the *Campylobacter* spp. are identical to each other, while that in *R. intestinalis* is different. Taking into account these newly defined genes, the total number of mosaic genes reported increases from 30 to 35 (not including those identified via PCR amplification only; Table 1) and suggests that other generically labelled tetracycline resistance genes

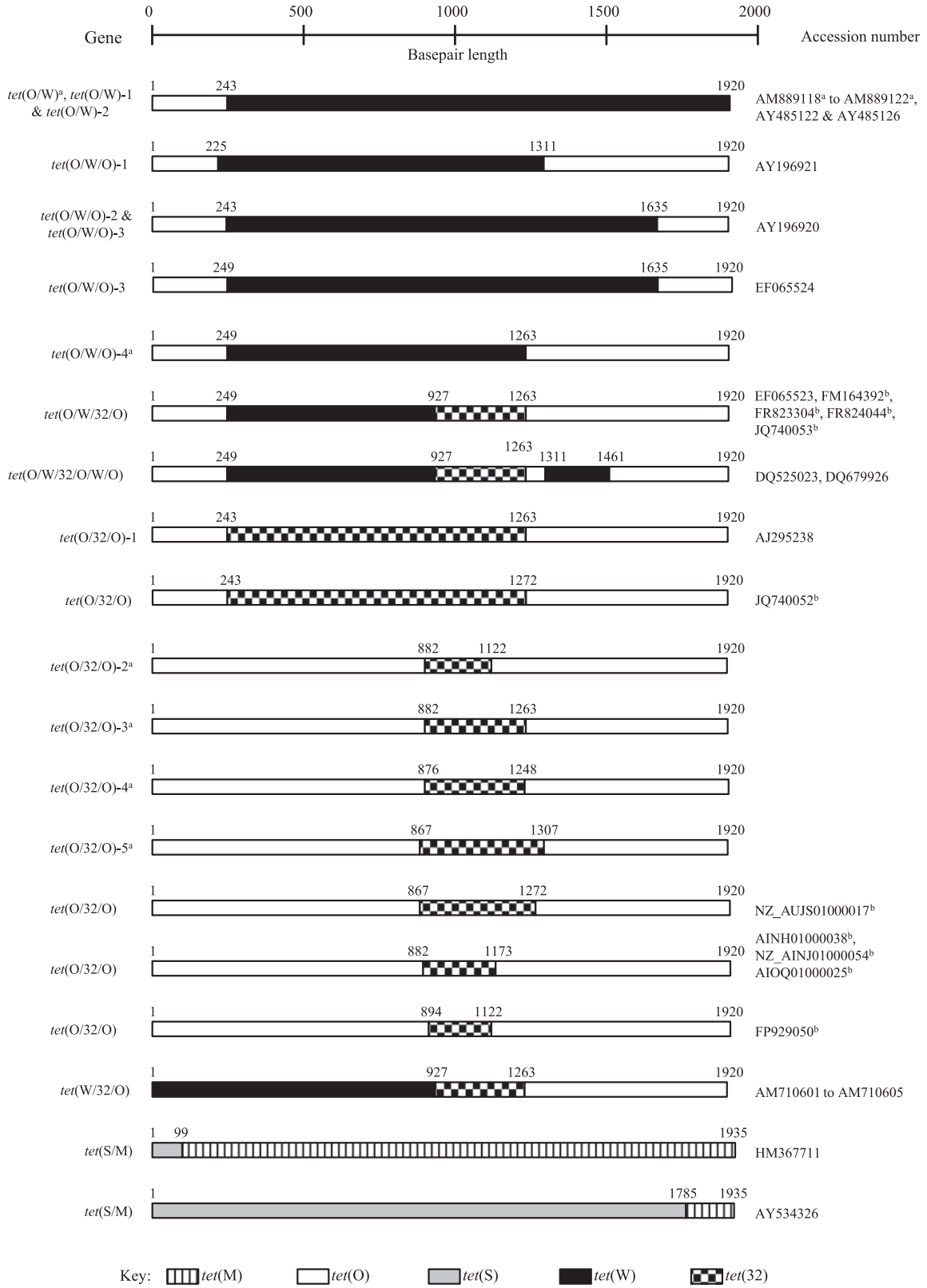

Figure 1. Schematic representation of reported mosaic tetracycline RPP genes. The coded bars indicate sequences of high identity to specific RPP genes: vertical line bars for *tet*(M), white bars for *tet*(O), grey bars for *tet*(S), black bars for *tet*(W) and checked bars for *tet*(32). The number above the bar indicates the reported crossover point. [a]Indicates those sequences that are incomplete or absent in GenBank, with the crossover points taken from the publication. [b]Indicates sequences that have been analysed in this study due to no specific crossover point(s) reported.

Table 2. Sequence identity matrix showing the percentage nucleotide identity between representatives of all 12 RPP gene classes, in descending order, compared with *tet*(M)

RPP gene	*tet*(M)	*tet*(S)	*tet*(O)	*tet*(44)	*tet*(32)	*tet*(W)	*tet*(T)	*tet*(36)	*tet*(Q)	*tetB*(P)	*otr*(A)	*tet*
tet(M)	100	78	75	70	69	64	57	49	46	23	11	11
tet(S)		100	70	69	67	62	56	56	48	11	11	10
tet(O)			100	69	69	65	56	49	48	15	12	11
tet(44)				100	71	64	50	58	46	15	11	10
tet(32)					100	67	55	49	47	11	12	10
tet(W)						100	12	45	15	5	14	12
tet(T)							100	57	56	18	8	2
tet(36)								100	64	9	11	11
tet(Q)									100	13	12	12
tetB(P)										100	1	1
otr(A)											100	63
tet												100

Accession numbers of representative genes included in the matrix: *tet*(M), U09422; *tet*(O), Y07780; *tetB*(P), AE001437; *tet*(Q), X58717; *tet*(S), X92946; *tet*(T), L42544; *tet*(W), AJ222769; *tet*(32), DQ647324; *tet*(36), AJ514254; *tet*(44), FN594949; *otr*(A), X53401; *tet*, AL939106.
Shaded boxes represent those genes currently reported to comprise mosaic genes.

present in the database [e.g. those labelled as *tet*(M)-like] could be further classified, helping to understand mosaic gene proliferation and diversity.

Conclusions

Our knowledge of the mosaic RPP gene group is steadily increasing since their discovery in 2003, with the majority derived from *tet*(O), *tet*(W) and *tet*(32) and others deriving from *tet*(M) and *tet*(S). It is clear that these genes are being under-reported both in terms of experimental detection and also within genomic data. Further work and increased attention on mosaic RPP genes is important if we are to understand the evolutionary selective pressures driving their fixation in bacterial populations and the subsequent effects on resistance and mobile genetic element evolution within their host.

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Transparency declarations

None to declare.

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