MINIREVIEW

Update on macrolide–lincosamide–streptogramin, ketolide, and oxazolidinone resistance genes

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Abstract

This Minireview summarizes the changes in the field of bacterial resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone (MLSKO) antibiotics since the nomenclature review in 1999. A total of 66 genes conferring resistance to this group of antibiotics has now been identified and includes 13 new rRNA methylase genes, four ATP-binding transporter genes coding for efflux proteins, and five new inactivating enzymes. During this same time period, 73 new genera carrying known rRNA methylase genes and 87 new genera carrying known efflux and/or inactivating genes have been recognized. The number of bacteria with mutations in the genes for 23S rRNA, L4 and L22 ribosomal proteins, resulting in reduced susceptibility to some members of the group of MLSKO antibiotics has also increased and now includes nine different Gram-positive and 10 different Gram-negative genera. New conjugative transposons carrying different MLSKO genes along with an increased number of antibiotics and/or heavy metal resistance genes have been identified. These mobile elements may play a role in the continued spread of the MLSKO resistance genes into new species, genera, and ecosystems.

Introduction

The first macrolide, erythromycin, was discovered in 1952 and since then macrolides have had an important role in treating infectious diseases (Kirst, 2002). Erythromycin has a moderate spectrum of activity which has been enhanced with the production of the newer semi-synthetic derivatives such as azithromycin, clarithromycin, and more recently, the ketolides (Kirst, 2002). Macrolides are used to treat acute upper and lower respiratory tract infections due to a variety of Gram-positive bacteria, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae, Legionella pneumophila, Haemophilus influenzae*, *Moraxella catarrhalis*, skin and soft tissue infections, sexually transmitted diseases caused by *Chlamydia trachomatis*, *Treponema pallidum* or *Ureaplasma urealyticum*, chronic pulmonary infections in cystic fibrosis patients, and diseases caused by *Bordetella pertusis*, *Borrelia burgdorferi*, *Bartonella henselae*, *Campylobacter* spp., *Listeria monocytogenes*, *Rickettsia* spp., *Rhodococcus equi*, as well as *Mycobacterium avium-intracellulare* and other atypical mycobacterium. Macrolides have also been used to treat diseases due to single cell eukaryotes such as *Babesia microti*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Pneumocystis carinii*, *Plasmodium* species, and *Toxoplasma gondii* (Iacoviello & Zinner, 2002). Macrolides influence bacterial virulence factors and cause reduction of adherence by *Moraxella catarrhalis* to ciliated epithelial cells, decreased adherence of *Neisseria meningitidis*, decreased binding of fibronectin to *Staphylococcus aureus*, and a variety of effects on *Pseudomonas aeruginosa* infecting cystic fibrosis patients. Macrolides also have anti-inflammatory characteristics which provide improvement in severe steroid-dependent asthma, diffuse pan-bronchiolitis, atheroma, cancer, and arthritis (Iacoviello & Zinner, 2002). A detailed review on macrolides as potent immunomodulators is now available (Giamarellos-Bourboulis, 2008).

Macrolides, lincosamides, streptogramins, ketolides (semi-synthetic derivatives of erythromycin A), and oxazolidinones (MLSKO), though chemically distinct, are usually considered together (Vester & Douthwaite, 2001; Zhanel *et al*., 2001; Sutcliffe & Leclercq, 2003). The MLSK antibiotics share overlapping binding sites on the 50S subunit of

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the ribosome, while linezolid binds to the 50S ribosomal subunit but is not affected by methylation of the *erm* genes (Franceschi *et al*., 2004; Poehlsgaard & Douthwaite, 2005; Tu *et al*., 2005). MLSKO antibiotics inhibit protein synthesis by binding to the 50S ribosomal subunit and blocking peptide bond formation and/or translation. More details on the interaction between these antibiotics and the ribosome can be found in a number of recent papers some of which are referenced in this review (Vester & Douthwaite, 2001; Lui & Douthwaite, 2002; Douthwaite *et al*., 2005; Poehlsgaard & Douthwaite, 2005; Tu *et al*., 2005). Shortly after the introduction of erythromycin in the 1950s into clinical practice, bacterial resistance to erythromycin was reported in *Staphylococci* (Zhanel *et al*., 2001). Since then a large number of bacteria have been identified that are resistant to macrolides due to the presence of a number of different genes which included 13 identical or nearly identical genes (98–100% amino acid identity) which coded for the *erm*(B) rRNA methylase gene but had different names because there was no standardized nomenclature or criteria used (Roberts *et al*., 1999). Much of this information including individual GenBank numbers, DNA, and amino acid homology within the gene class and association with particular plasmids or transposons can be found at http://faculty.washington.edu/marilynr/. To remedy this situation, colleagues and I wrote a review standardizing the nomenclature for genes which conferred resistance to macrolide, lincosamide, and streptogramin antibiotics which required that the new gene must be functional and have $< 80\%$ identity at the amino acid level with all previously characterized MLSKO genes (Roberts *et al*., 1999; http://faculty.washington.edu/marilynr/). In addition, a nomenclature center was established that provides a central place to assign names to new genes and a website was developed for the dissemination of information on resistance genes which encode for rRNA methylases, efflux and inactivating enzymes that confer resistance to MLSKO antibiotics. The site is modified twice a year or as needed, to reflect the ongoing changes in the field (http://faculty.washington.edu/marilynr/). The aim of this Minireview will be to focus on information available after the 1999 review was published (Roberts *et al*., 1999). In the current review I have also included a section on mutations in the genes coding for 23S rRNA domain, L4 and L22 proteins since the majority of isolates with resistance to ketolide and/or oxazolidinones have been shown to carry mutations in one or more of these three genes. In addition, there is a section on new transposons and linkages between MLSKO resistance genes and other antibiotic and/or heavy metal resistance genes and their association with integrons or particular elements. The literature since 1999 is extensive and cannot be adequately referenced in this current Minireview. A more complete list of MLSKO references can be found with the following reviews and papers (Roberts, 1997, 2007; Vester & Douthwaite, 2001; Zhanel *et al*., 2001; Wang *et al*., 2003; Webber & Piddock, 2003; Sutcliffe & Leclercq, 2003; Roberts & Sutcliffe, 2005) and with updates at http://faculty.washington.edu/marilynr/.

New resistance genes

There have been 22 new MLSKO-resistance genes identified since the 1999 publication which include 13 new genes coding for rRNA methylases, four genes coding for efflux proteins, four found coding for transferases, and one coding for a phosphorylase (Table 1).

Table 1. Mechanisms of MLS resistance genes

	rRNA methylase	Efflux	Inactivating enzymes $n = 19$			
Time	$n = 33$	$n = 14$	Esterases $n = 2$	Lysases $n = 2$	Transferases $n = 11$	Phosphorylases $n = 4$
Listed in 1999*	$n = 20$	$n = 10$	$n = 2$	$n = 2$	$n = 7$	$n = 3$
	erm(A), *erm*(B), *erm*(C), *erm* (D), *erm*(E)	*car*(A), *msr*(A)	*ere*(A)	*vgb*(A)	*lnu*(A), *lnu*(B)	*mph*(A)
	erm(F), *erm*(G), *erm*(H), *erm*(I), *erm*(N)	*ole*(B), *ole*(C)	*ere*(B)	*vgb*(B)	*vat*(A), *vat*(B)	*mph*(B)
	erm(O), *erm*(Q), *erm*(R), *erm*(S), *erm*(T)	*srm*(B), *tlr*(C), *vga*(A)[†], *vga*(B)			*vat*(C), *vat*(D)	*mph*(c)
	erm(U), *erm*(V), *erm*(W), *erm* (X), *erm*(Y)	*lmr*(A), *mef*(A)			*vat*(E)	
Not listed 1999	$n = 13$	$n = 4$	$n = 0$	$n = 0$	$n = 4$	$n = 1$
	erm(Z), *erm*(30), *erm*(31), *erm*(32), *erm*(33)	*msr*(C), *msr*(D)			*lnu*(C), *lnu*(D)	*mph*(D)
	erm(34), *erm*(35), *erm*(36), *erm*(37), *erm*(38)	*lsa*(A), *lsa*(B)			*lnu*(F), *vat*(F)	
	erm(39), *erm*(40), *erm*(41)					

Data taken from http://faculty.washington.edu/marilynr/ and other publications (Heir *et al*., 2004; Roberts & Sutcliffe, 2005; Nash *et al*., 2006; Novotna & Janata, 2006; Roberts, 2007).

*Roberts *et al*. (1999).

[†]*vga*(A)$_{lc}$ is a variant of *vga*(A) but it is worth separating in Table 2 because it is the only recognized variant because of its' extended range in conferring resistance to streptogramin A and lincomycin (Novotna & Janata, 2006).

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rRNA methylases

Currently there are 66 MLSKO resistance genes and 33 are *erm* genes that code for rRNA methylases which add one or two methyl groups to a single adenine (A2058 in *Escherichia coli*) in the 23S rRNA moiety and generally conferred resistance to macrolides, lincosamides, and streptogramin B antibiotics (MLS_B) (Table 1) (Poehlsgaard & Douthwaite, 2005). Out of the 33 *erm* genes, 13 are new including four which have been renamed; *erm*(Z) formerly *pikR1*, *erm*(31) formerly *pik2*, *erm*(32) formerly *tlr*(B), and *erm*(30) all from *Streptomyces*, and five innate methylase genes [*erm*(37), *erm*(38), *erm*(39), *erm*(40), and *erm*(41)] identified from different species of *Mycobacterium* which confer resistance to macrolides and lincosamides but not to streptogramin B (Nash *et al.*, 2006). The *erm*(33) gene, from *Staphylococcus* has 62% DNA and 58% amino acid homology to both an *erm*(A) and an *erm*(C) gene, respectively. The DNA sequence of *erm*(33) indicates that it was likely created by a recombination event between these two genes (Schwarz *et al.*, 2002). The remaining three genes, *erm*(34) from *Bacillus*, *erm*(35) from *Bacteriodes*, and *erm*(36) from *Micrococcus* may be found in other genera but currently have not been used extensively in screening resistant bacteria (Tables 1 and 2).

Efflux proteins

There are now 14 different genes that code for either ATP-transporters or Major Facilitator Transporters and the genes produce proteins that pump one or more of the MLSKO antibiotics out of the cell (Table 1). Of the four new genes identified since 1999, one is an innate enterococcal gene [*msr*(C)], while the *msr*(D) gene is always found linked to the newly described efflux gene *mef*(A) and two genes, *lsa*(A) and *lsa*(B), coding for lincosamide efflux proteins (Tables 1 and 2) (Singh *et al.*, 2002; Kehrenberg *et al.*, 2004; Reynolds & Cove, 2005; Roberts & Sutcliffe, 2005). In addition, a variant $vga(A)_{lc}$ of the previously described *vga*(A) gene is listed in Tables 1 and 2 because unlike the *vga*(A) gene which confers resistance to streptogramin A, the $vga(A)_{lc}$ variant confers resistance to streptogramin A and lincomycin and thus merits a variant status (Novotna & Janata, 2006).

Inactivation enzymes

Currently there are 19 inactivating enzymes including two esterases, two lyases, 11 transferases, and four phosphorylases. Of these 19, four genes coding for transferases and one gene coding for a phosphorylase were not in the 1999 review (Table 1) (Roberts *et al.*, 1999). Unfortunately the *mph*(D) phosphorylase gene had not been fully sequenced.

Additional enzyme

Another 23S rRNA methyltransferase, encoded by *cfr* gene, and first identified on staphylococcal plasmids from animals should also be mentioned (Long *et al.*, 2006). This gene conferred resistance to lincosamides, oxazolidinones, streptogramin A, phenicols and pleuromutilins, but not macrolides, and thus differs from *erm* rRNA methylase genes and has not been included in Tables 1 and 2.

Mutations

Over the past 10 years, an increasing number of isolates that are resistant to MLSKO antibiotics have been identified which contain mutations in the V domain of the 23S rRNA genes, and/or the genes coding the ribosomal proteins L4 and L22 (Franceschi *et al.*, 2004). The majority of telithromycin (ketolide) and/or linezolid (oxazolidinone) resistant bacteria carry mutations in one of these three genes (Garza-Ramos *et al.*, 2001; Jones *et al.*, 2002; Farrell *et al.*, 2004; Douthwaite *et al.*, 2005; Roberts & Sutcliffe, 2005; Toh *et al.*, 2007). These mutational changes have been described in both Gram-positive and Gram-negative bacteria (Table 3) and alter the function of the 23S rRNA and/or proteins resulting in moderately decreased susceptibility to one or more of the MLSKO antibiotics (Garza-Ramos *et al.*, 2001; Vester & Douthwaite, 2001; Jones *et al.*, 2002; Ng *et al.*, 2002; Sutcliffe & Leclercq, 2003; Farrell *et al.*, 2004; Lukehart *et al.*, 2004; Douthwaite *et al.*, 2005; Morozumi *et al.*, 2005; Tu *et al.*, 2005; Luthje & Schwarz, 2006; Binet & Maurelli, 2007; Florez *et al.*, 2007; Hong *et al.*, 2007; Mayrhofer *et al.*, 2007). These changes are normally passed to daughter cells during replication and generally not passed between strains or between different genera. *In vitro* selection experiments often obtained the same mutants as found in clinical isolates, while clinical strains carrying both mutations and one or more acquired MLSKO resistance genes has also been identified. Isolates with both mutations and acquired genes are often labeled as having acquired MLSKO resistance genes but not mutations which may lead to an under estimation of the number of resistant mutations in some studies.

Mutations 23S rRNA

Mutations in the 23S rRNA can result in increased resistance to macrolides, lincosamides, streptogramin B, telithromycin and/or linezolid in both Gram-positive and Gram-negative bacteria (Sutcliffe & Leclercq, 2003; Poehlsgaard & Douthwaite, 2005). Eight Gram-positive and nine Gram-negative genera, including the intracellular pathogen *C. trachomatis* and two *Treponema* species, have been identified with 23S rRNA mutations (Table 3). In addition, laboratory derived mutants have been selected in other species and genera (Pereyre *et al.*, 2007). Various mutations

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Table 2. Distribution of MLS genes in new bacterial genera

Gene	Total #	Number of new genera since 1999[†]	GenBank	Genera
rRNA Methylases				
Confers resistance to macrolides, lincosamides, streptogramin B				
erm(A)	7	4		*Bacteriodes, Helcococcus, Peptostreptococcus, Prevotella*
erm(B)	33	23		*Acinetobacter, Aerococcus, Arcanobacterium, Bacillus, Bacteriodes, Citrobacter, Corynebacterium, Enterobacter, Eubacterium, Fusobacterium, Gemella, Haemophilus Lactobacillus, Micrococcus, Pantoeae Peptostreptococcus, Porphyromonas, Proteus, Pseudomonas, Ruminococcus, Rothia, Serratia, Treponema*
erm(C)	16	8		*Actinomyces, Bacteriodes, Corynebacterium, Enterococcus, Haemophilus, Micrococcus, Prevotella, Peptostreptococcus*
erm(D)	2	1		*Salmonella*
erm(E)	6	5		*Bacteroides, Eubacterium, Fusobacterium, Ruminococcus, Shigella*
erm(F)	24	8		*Corynebacterium, Enterococcus, Lactobacillus, Mobiluncus, Peptostreptococcus, Ruminococcus, Shigella, Staphylococcus*
erm(G)	7	5		*Catenibacterium, Lactobacillus, Prevotella Porphyromonas, Staphylococcus*
erm(H)*	1	0		
erm(I)*	1	0		
erm(N)*	1	0		
erm(O)*	1	0		
erm(Q)	6	2	L42817	*Bacteroides, Staphylococcus*
erm(R)*	1	0		
erm(S)*	1	0		
erm(T)	2	1	AY894138	*Streptococcus*
erm(U)*	1	0		
erm(V)	3	2		*Eubacterium, Fusobacterium*
erm(W)*	1	0		
erm(X)	4	3	NC_005206	*Arcanobacterium, Bifidobacterium Propionibacterium*
erm(Y)*	1	0		
erm(Z)*	1	0	AM709783[§]	*Streptomyces*
erm(30)*	1	1	AF079138	*Streptomyces*
erm(31)*	1	1	AF079138	*Streptomyces*
erm(32)*	1	0	AJ009971	*Streptomyces*
erm(33)[‡]	1	1	AJ313523	*Staphylococcus*
erm(34)	1	1	AY234334	*Bacillus*
erm(35)	1	1	F319779	*Bacteriodes*
erm(36)	1	1	AF462611	*Micrococcus*
erm(37)	1	1	Z74025	*Mycobacterium*
erm(38)	1	1	AY154657	*Mycobacterium*
erm(39)	1	1	AY487229	*Mycobacterium*
erm(40)	1	1	AY570506	*Mycobacterium*
erm(41)	1	1	EU177504	*Mycobacterium*
Efflux genes				
Confers resistance to lincomycin, erythromycin or erythromycin + streptogramin B, olenadomycin, spiramycin, tylosin streptogramin A, or lincomycin + streptogramin A				
ATP-binding Transporters				
car(A)*	1	0		
msr(A)	7	6		*Corynebacterium, Enterobacter, Enterococcus, Gemella, Pseudomonas, Streptococcus*
msr(C)	1	1	AY004350, AF313494 AJ243209	*Enterococcus*
msr(D)[¶]	20	20	AF227521, SA318993, AF274302	*Acinetobacter, Bacteroides*[§]*, Citrobacter, Corynebacterium, Enterobacter, Enterococcus, Escherichia, Fusobacterium, Gemella, Klebsiella, Morganella, Neisseria, Proteus, Providencia, Pseudomonas, Ralstonia, Serratia, Staphylococcus, Streptococcus, Stenotrophomonas,*
lsa(A)	1	1	AY4225127	*Enterococcus*
lsa(B)	1	1	AJ579365	*Staphylococcus*
ole(A)*	1	0		

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Table 2. Continued.

Gene	Total #	Number of new genera since 1999[†]	GenBank	Genera
ole(B)*	1	0		
ole(C)*	1	0		
srm(B)*	1	0		
tlr(C)	1	0		
vga(A)	1	0		
vga(A)$_{lc}$[‖]	1	1	DQ823382	*Streptococccus*
vga(B)	2	1		*Enterococcus*
Major facilitators				
lmr(A)*	1	1	X59926	*Streptomyces*
mef(A)	24	19		*Acinetobacter*, *Bacteroides*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Fusobacterium*, *Gemella*, *Klebsiella*, *Lactobacillus*, *Morganella*, *Neisseria*, *Pantoeae*, *Providencia*, *Proteus*, *Ralstonia*, *Pseudomonas*, *Salmonella*, *Serratia*, *Stenotrophomona*
Inactivating genes				
Esterases				
Confers resistance (usually high level) to erythromycin				
ere(A)	11	7		*Pantoeae*, *Providencia*, *Pseudomonas*, *Serratia*, *Staphylococcus*, *Stenotrophomonas*, *Vibrio*
ere(B)	8	5		*Acinetobacter*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Staphylococcus*
Lyases				
Confers resistance to streptogramin B, currently only in Gram-positive genera				
vgb(A)	2	0		
vgb(B)	1	0		
Transferases				
Confers resistance to streptogramin A				
lnu(A)	2	1		*Clostridium*
lnu(B)	3	2	AJ238249	*Staphylococcus*, *Clostridium*
lnu(C)	1	1	AY928180	*Streptococcus*
lnu(D)	1	1	EF452177	*Streptococcus*
lnu(F)	2	2	AJ561197	*Escherichia*, *Salmonella*
vat(A)	1	0		
vat(B)	2	1		*Enterococcus*
vat(C)	1	0		
vat(D)	1	0		
vat(E)	2	1	AJ488494, NC_004566	*Lactobacillus*
vat(F)	1	1	AF170730	*Yersinia*
Phosphorylases				
Confers resistance to macrolides				
mph(A)	10	9		*Aeromonas*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Pantoeae*, *Pseudomonas*, *Proteus, Serratia*, *Stenotrophomonas*
mph(B)	4	3		*Enterobacter, Pseudomonas, Proteus*
mph(C)	2	1		*Stenotrophomonas*
mph(D)	6	6	AB048591 only partially sequenced	*Escherichia*, *Klebsiella*, *Pantoeae*, *Proteus*, *Pseudomonas*, *Stenotrophomonas*

Data taken from http://faculty.washington.edu/marilynr/ and other publications (Cousin *et al*., 2003; Sutcliffe & Leclercq, 2003; Wang *et al*., 2003; Heir *et al*., 2004; Szczepanowski *et al*., 2004; Roberts & Sutcliffe, 2005; Novotna & Janata, 2006; Nash *et al*., 2006; Ghosh & LaPara, 2007; Mayrhofer *et al*., 2007; Roberts, 2007).

*Genes that have not been examined in surveillance studies.

†Roberts *et al*., 1999.

‡Hybrid between *erm*(A) and *erm*(C) $<80\%$ aa identity with either gene.

§Recently sequenced.

¶The *msr*(D) may not be functional in the *Bacteroides* isolate described (Wang *et al*., 2003).

‖Variant of *vga*(A) which confers resistance to lincomycin and streptogramin A rather than only streptogramin A, given variant status because of the change in resistance pattern.

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Table 3. Mutations in 23S rRNA, L4, and/or L4 gene which increase MLSKO resistance

Bacteria	23S rRNA	L4	L22
Gram-positive and related genera			
Arcanobacterium pyogenes	Yes		
Bacillus subtilis		Yes	Yes
Enterococcus faecalis	Yes*	Yes	Yes
Lactobacillus rhamonosus	Yes		
Mycobacterium spp.†	Yes		
Mycoplasma spp.‡	Yes		
Propionibacterium species§	Yes		
Staphylococcus aureus	Yes		Yes
Streptococcus pneumoniae	Yes	Yes¶	Yes
Streptococcus pyogenes	Yes	Yes	Yes
Turicella	Yes		
Gram-negative			
Brachyspira hyodysenteriae	Yes		
Bordetella pertusis	Yes		
Campylobacter jejuni	Yes	Yes‖	Yes‖
Campylobacter coli	Yes	Yes‖	Yes‖
Chlamydia trachomatis	Yes	Yes**	
Escherichia coli	Yes	Yes	Yes
Haemophilus influenzae	Yes	Yes	Yes
Helicobacter pylori	Yes		
Neisseria gonorrhoeae	Yes		
Rickettsia spp.			Yes
Treponema palladium	Yes		
Treponema denticola	Yes		

Data taken from various publications (Boumghar *et al*., 2008; Jensen *et al*., 2000; Haroche *et al*., 2002; Ng *et al*., 2002; Gfeller *et al*., 2003; Heir *et al*., 2004; Morozumi *et al*., 2005; Roberts & Sutcliffe, 2005; Garcia-Migura *et al*., 2007). Most of the missing information above is due to lack of data on the genes coding for L4, L22 or rarely 23S rRNA and does not represent that no mutations have been identified. *In vitro* selected mutans with increased resistance to macrolides such as; *Ureaplasma parvum* (Pereyre *et al*., 2007); *Chlamydia psittaci* have also been identified (Binet & Maurelli, 2007).

*Decreased linezolid resistance also found.

†Includes *M. avium, M. chelonae, M. intracellulare, M. abscessusm M. smegmatis, M. kansassi*.

‡Includes *M. hominis, M. gallisepticum*, and *M. pneumoniae*.

§Include *P. acnes*, *P. avidum*, *P. granulosum*.

¶6 bp deletion decreases linezolid, macrolide and chloramphenicol susceptibility (Wolter *et al*., 2005).

‖Not clear if associated with reduced susceptibility (Sutcliffe & Leclercq, 2003).

***In vitro* selected.

have been identified but the most common are in domain V with mutations at positions A2058 or A2059 the most frequently identified (*E. coli* numbering) (Vester & Douthwaite, 2001). Mutations in other positions within the domain V of the 23S rRNA have been associated with increased linezolid resistance. The first bacteria identified with mutations in their 23S rRNA had one or two copies of these genes such as *Mycobacterium* or *Helicobacter*. However, soon it was clear that other bacteria carrying more than two gene copies were also acquiring mutations and there was a correlation between the number of *rrl* genes that carried mutations and level of resistance to MLSKO antibiotics. Detailed reviews on specific mutations have been published (Vester & Douthwaite, 2001; Sutcliffe & Leclercq, 2003; Franceschi *et al*., 2004; Roberts & Sutcliffe, 2005). It is very likely that more species and genera carry mutations in the 23S rRNA than given in Table 3.

Mutations in ribosomal proteins L4 and/or L22

There have been a number of mutations identified in the L4 and L22 ribosomal proteins and include single amino acid changes, insertion and/or deletion which add or delete one or more amino acids to these proteins (Table 3). Three Gram-positive and five Gram-negative genera with mutations in the L4 gene and four Gram-positive and five Gram-negative genera with mutations in the L22 gene have been described. Details of various mutations that confer macrolide resistance in *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *H. influenzae* can be found in Franceschi *et al*. (2004). It is very likely that Table 3 underestimates the number of species and genera which carry mutations in the L4 and/or L22.

Mutations in intrinsic genes

Virtually all bacteria have multiple efflux pumps which import and export a variety of different compounds. As the understanding of intrinsic efflux pumps has increased it is clear that some of these pumps have roles in mediating low-moderate levels of antibiotic resistances in Gram-positive and Gram-negative bacteria (Webber & Piddock, 2003; Li & Nikaido, 2004). These efflux pumps use the proton motive force or ATP as energy sources for transporting multiple antibiotics, dyes, detergents, and disinfectants across the cell membrane. Exposure to any number of chemicals may lead to mutational changes that result in increased resistance to a variety of antibiotics. One example is triclosan, this antibacterial compound can be found in soap, toothpaste, and other products and can select for bacteria which have mutations in their efflux pumps and/or in genes that regulate expression of the genes coding for the efflux proteins. The *mtrR* gene encodes for a regulator protein that regulates transcription of the efflux proteins. Missense, deletions, and/or insertions within the *mtrR* gene, of *Neisseria gonorrhoeae*, increases resistance to multiple antibiotic resistant classes including macrolides (Zarantonelli *et al*., 2001). Upstream of the *mtrR* a one base pair deletion of an adenine results in the loss of expression of the *mtrR* and leads to a four-fold increase in resistance to erythromycin, penicillin, and tetracycline (Zarantonelli *et al*., 2001; Cousin *et al*., 2003). This resistance is clinically relevant and can lead to treatment failures. In the 2005 surveillance, coordinated

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by the Centers for Disease Control, 5.7% of the isolates had acquired antibiotic-resistance genes while 13.9% had chromosomal mediated mutational resistance in the United States (http://www.cdc.gov/std/Gisp2005/GISPSurvSupp2005short.pdf). In contrast, acquired resistance is much more prevalent in Asia (Tapsall, 2005). Thus illustrating that on occasion, mutational resistance may be more common for some bacteria in particular geographical areas than acquired resistance.

Spread of acquired MLSKO genes and/or mutations

Currently 66 genes are listed in Table 1, 22 of which have been described in the past 9 years (Table 1). During this same time period, 73 new genera carrying known rRNA methylase genes and 87 new genera carrying known efflux and/or inactivating genes have been identified (Table 2). Twenty-three (35%) of the genes are now in multiple genera (2–33 genera) with 18 of the genes found in both Gram-positive and Gram-negative bacteria (Table 2). It is not clear why only some of the genes are found in multiple species and others are not. For example, the *Mycobacterium* and *Streptomycetes erm* genes have a high $>70\%$ G+C content, while the rest of the *erm* genes $<40\%$ G+C content. It is possible that some of the *Streptomycetes* genes are in *Mycobacterium* and/or *Norcardia* spp. similar to the tetracycline resistance *Streptomycetes otr*(A), *otr*(B), *otr*(C) genes or vice versa, however, it is unlikely that the high % G+C *erm* genes will be found in other genera though the experiments have not been done (Chopra & Roberts, 2001). In other cases the genes have not been used in surveillance studies and may be found in more species than listed in Table 2. These genes are marked with an asterisk in Table 2.

rRNA methylases

The rRNA methylases are the largest group of acquired MLSKO genes and include innate genes found in *Streptomycetes*, where they provide a self-protective mechanism for these macrolide producers, and innate genes in naturally macrolide resistant *Mycobacterium* species (Nash *et al.*, 2006). Each species of *Mycobacterium* carries a unique *erm* gene and together with the *Streptomycetes* genes have a $>70\%$ G+C content, while the rest of the *erm* genes $<40\%$ G+C content (Chopra & Roberts, 2001).

Ten *erm* genes have been identified in multiple genera with the *erm*(B) gene found in the widest host range, the most genera and in both Gram-positive and Gram-negative bacteria, aerobic, and anaerobic genera and in most ecosystems that have been examined (Table 2). This may be due to *erm*(B) genes association with mobile elements and linkage to a number of different genes (Table 4). The *erm*(B) gene

Table 4. MLS antibiotic resistance genes linked to other genes or elements

Gene	Linkage	Phenotype/element
rRNA methylases		
erm(B)	*tet*(M)	Tetracycline
	tet(Q)	Tetracycline
	erm(G)	MLS_B
	mef(A)	Erythromycin
	msr(D)	Erythromycin
	cfr	Lincosamide, linezolid, Streptogramin A Chloramphenicol, florfenicol
	aadE	Streptomycin
	aphA-3	Aminoglycoside
	lnu(A)	Lincosamide
	vat(A)	Streptogramin A
	vat(B)	Streptogramin A
	vat(D)	Streptogramin A
	tcrB	Copper
	merA	Mercury reductase
	merX	Mercury reductase
	Tn*1545*, Tn*5384**	Transposon
	mef(A), Tn*2009*, Tn*2010*	Macrolide
	vanA	Vancomycin, teicoplanin
erm(F)	*tet*(Q)	Tetracycline
	tet(X)	Tetracycline
	rteA, rteB	Regulatory genes
	orf1	Unknown
erm(G)	*tet*(Q)	Tetracycline
	mef(A)	Erythromycin
	msr(D)	Erythromycin
erm(Y)	*msr*(A)	Erythromycin
	mph(C)	Erythromycin
erm(33)	*cfr*	Lincosamide, linezolid, Streptogramin A Chloramphenicol, florfenicol
	lsa(B)	Lincomycin
ATP-binding Transporters		
msr(A)	*mph*(C)	Erythromycin
	erm(Y)	MLS_B
msr(D)	*mef*(A)	Erythromycin
	erm(B)	MLS_B
	orf6	Unknown
	tet(M)	Tetracycline
	tet(O)	Tetracycline
	catQ	Chloramphenicol
	Tn*2009*, Tn*2010*, Tn*1207.1*, Tn*1207.3*, MEGA[†]	Transposons
lsa(B)	*erm*(33)	MLS_B
	cfr	Lincosamide, linezolid, Streptogramin A Chloramphenicol, florfenicol
Major Facilitators		
mef(A)	*msr*(D)	Erythromycin
	erm(B)	MLS_B
	tet(M)	Tetracycline

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Table 4. Continued.

Gene	Linkage	Phenotype/element
	tet(O)	Tetracycline
	orf3	Unknown
	Tn*2009*, Tn*2010*, Tn*1207.1*, Tn*1207.3*, MEGA[†]	Transposons
	catQ	Chloramphenicol
Esterases		
ere(A)	Class 1 integron[‡]	
	Class 2 integron[‡]	
	sat	Streptothricin
	aadA1	Aminoglycoside adenyltransferase
	orfX	Unknown function
	dfrA5	Trimethoprim
Lyases		
vgb(B)	*vat*(C)	Streptogramin A
	vat(B)	Streptogramin A
Transferases		
vat(A)	*vgb*(A)	Streptogramin B
vat(B)	*vga*(B)	Streptogramin A
	vat(D)	Streptogramin A
	vat(E)	Streptogramin A
vat(C)	*vgb*(B)	Streptogramin B
vat(D)	*vgb*(A)	Streptogramin B
	erm(B)	MLS_B
	erm(T)	MLS_B
lnu(A)	*erm*(B)	MLS_B
	mph(C)	Macrolides
lnu(F)	Class 1 integron[‡]	
Phosphorylases		
mph(A)	*mrx*	Hydrophobic protein Unknown function
	mphR(A)	Regulator protein
	IS26, IS*6100*	Insertion sequences
	Class 1 integron[‡]	
mph(C)	*cadD*/*cadA*	Cadmium efflux
	mrs(A)	Erythromycin
	erm(Y)	MLS_B

Data are from a number of publications (Gfeller *et al*., 2003; Jensen *et al*., 2000; Haroche *et al*., 2002; Matsuoka *et al*., 2003; Wang *et al*., 2003; Brenciani *et al*., 2004; Heir *et al*., 2004; Hasman *et al*., 2006; Poole *et al*., 2006; De Graef *et al*., 2007; Del Grosso *et al*., 2007; Kehrenberg *et al*., 2007; Mingoia *et al*., 2007; Rice, 2007; Roberts, 2007; Jackson *et al*., 2008).

*Found in a number of Tn*916*-Tn*1545*-like transposons;

[†]found in a number of different transposons, these are just a few,

[‡]integron codes for an integrase (*intI1, intI2*) genes that code for proteins which mediate recombination between a recombination site (*attI*) and a target recombination sequence (*attC*: 59-base element).

was first identified in *Enterococcus* spp. from the 1950s and from *S. pneumoniae* in 1967 and has been identified in 23 new genera over the last 8 years (Roberts & Sutcliffe, 2005; http://faculty.washington.edu/marilynr/). The *erm*(B) gene is found in 33 different genera (Table 2) and has the widest host range of any of the acquired resistance genes listed in Table 1. The *erm*(B) gene is associated with conjugative transposons located in chromosomes as well as on plasmids. The *erm*(B) gene has also been found on nonconjugative transposons such as Tn*917* and Tn*551*. The next most common gene is *erm*(F) found in a total of 24 different genera many of which are anaerobes, including eight new genera. Interestingly, both the *erm*(B) and *erm*(F) genes have been identified in a 1955 *N. gonorrhoeae* isolate (Cousin *et al*., 2003). The *erm*(C) was identified in eight new genera and in a total of 16 different genera, the *erm*(G) gene in seven genera including five new genera, the *erm*(A) gene has been found in seven genera of which four are new, the *erm*(E) gene has been found in six genera including five new genera, the *erm*(X) gene has been found in four genera including three new genera, the *erm*(Q) gene in six genera including two new genera, the *erm*(V) gene in three genera including two new genera, while the *erm*(T) gene is in two genera with one new (Table 2).

In the last 8 years, telithromycin resistant *S. pyogenes*, *S. aureus* and *S. epidemidis* have been reported (Farrell *et al*., 2004). Each of these strains carried a constitutively expressed *erm*(A) or *erm*(B) gene. Douthwaite *et al*. (2005) found that there was variation in how well the *erm*(A) and *erm*(B) enzymes dimethylated the rRNA molecules within the host bacteria. The enzymes which had the greatest proportion of the rRNA molecules modified conferred the highest levels of telithromycin resistance with a range of MICs from 4 to $> 64\,\mu g\,mL^{-1}$, which explains the association between constitutively expressed *erm*(A) or *erm*(B) genes and ketolide resistance.

Efflux genes

Among the efflux new genes the most prevalent was the *msr*(D) gene, which has been identified in 19 different genera, however, this gene always is downstream of the *mef*(A) gene and has been identified in 24 genera, of which 19 are new (Ojo *et al*., 2006a). This suggests that every strain which carries a *mef*(A) gene should also carry the *msr*(D) gene and the presence of the *msr*(D) gene should be checked in all *mef*(A) positive Gram-positive and Gram-negative bacteria (Table 2). Among previously described efflux genes six new genera including Gram-positive and Gram-negative have been identified carrying *msr*(A) (Ojo *et al*., 2006b).

Inactivating genes

The new lincosamide transferases genes, *lnu*(C) and *lnu*(D) have both been characterized from *Streptococcus* spp. while *lnu*(F) gene has been found in *Escherichia* and *Salmonella* (Achard *et al*., 2005; Petinaki *et al*., 2008). The innate *vat*(F) gene codes for a streptogramin A acetyltransferase found in *Yersinia*, while the other *vat* genes are found

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in Gram-positive bacteria and normally associated with plasmids (Seoane & Lobo, 2000). The new macrolide phosphorylase gene, *mph*(D) and is the only gene listed in Table 1 which has not been completely sequenced. The two erythromycin esterases, *ere*(A) and *ere*(B) genes, have been identified in seven Gram-positive and five new Gram-negative genera. Nine new Gram-negative genera carrying the macrolide phosphotransferases *mph*(A) gene, and three new Gram-negative genera carrying the *mph*(B) gene have been found (Table 2). The *mph*(C) gene has recently been identified in Gram-negative *Stenotrophomonas* and this is the only macrolide phosphotransferases found in both Gram-positive and Gram-negative bacteria. It would therefore be of interest to determine if the other *mph* genes are also found in Gram-negative genera. The transferase coded by the *vat*(E) gene has recently been found in *Lactobacillus fermentum* linked to an *erm*(T) gene and represents the first species other than *Streptococcus* spp. and *Enterococcus* spp. to carry a *vat* gene (Gfeller *et al*., 2003).

Other enzyme

The first bacterial isolate with a *cfr* gene was a bovine *Staphylococcus sciuri* isolated from a florfenicol surveillance study of animals (Schwarz *et al*., 2000). More recently a methicillin and linezolid resistant *S. aureus* was isolated in 2005 from Columbia, South America from a patient. In this isolate the *cfr* gene was located downstream of an *erm*(B) gene and the two genes were co-transcribed from the *erm*(B) promoter (Toh *et al*., 2007). This gene cluster was found in the chromosome flanked by an IS*21-558* element, which has been implicated in the mobility of the *cfr* gene (Kehrenberg *et al*., 2007). This *S. aureus* is also the first human linezolid resistant isolate characterized which is resistant due to the acquisition of an acquired resistance gene rather than to mutations. The identification of this human isolate suggests that more human strains with the *cfr* gene should be anticipated in the future.

Mutations

The current mutations to the 23S rRNA ribosomal proteins L4 and L22 which confer increased resistance to MLSKO antibiotics are listed in Table 3. Both Gram-positive and Gram-negative species have been identified with mutations, however, only some of the bacteria listed have mutations in all three genes. This is most likely due to lack of available data rather than the actual lack of mutations in the bacteria listed. Some of these mutations also confer resistance to ketolides and oxazolidinone (Garza-Ramos *et al*., 2001; Jones *et al*., 2002; Liu & Douthwaite, 2002; Franceschi *et al*., 2004; Farrell *et al*., 2004; Douthwaite *et al*., 2005; Hong *et al*., 2007).

Mobile elements and new linkages

Lateral gene exchange was demonstrated *c*. 50 years ago and it is hypothesized that gene exchange is the primary way most bacteria acquire and transfer antibiotic resistance genes between species, genera, and ecosystems (Watanabe, 1963). Mobile elements found in bacteria include plasmids, transposons, conjugative transposons, and integrons. All may carry one or more different genes which confer antibiotic resistances, resistance to one or more heavy metals, genes coding for enzymes which allow the host to utilize alternative energy sources, genes coding for the production of toxins, bacteriocins, and/or virulence factors, genes for self-replication, conjugative and/or mobilizable genes, and in some cases genes which confer susceptibility to specific bacteriophages. Some of the earliest plasmids were identified in Japanese *Shigella* spp. in the 1950s and conferred resistance to three or four different class of antibiotics. Plasmids differ in their bacterial host range with some such as those found in *Haemophilus* spp. having a very restrictive range that allows transfer between strains of a closely related species, to broad host range plasmids which are able to be transferred and survive in a variety of unrelated genera. In contrast, transposons and conjugative transposons tend to have broader host ranges, and less host restriction than plasmids (Roberts, 1989; Rice, 2007).

Conjugative transposons (CTns) are found in chromosomes and plasmids of a variety of bacterial species and in many ecosystems. They may carry multiple antibiotic resistance genes, multiple heavy metal resistance genes, and are widely disseminated in bacterial populations (Rice, 2007). The first CTn was Tn*916*, an 18 kb element that codes for tetracycline and minocycline resistance due to the *tet*(M) gene, and the first member of the Tn*916*-family of transposons. This and other CTns carry genes needed for their own conjugal transfer with integration into the new host relatively nonselectively in some species and highly site-specific in others (Rice, 2007). At about the same time a second transposon designated Tn*1545* was described which carried multiple different antibiotic resistance genes including the rRNA methylase *erm*(B) gene and an *aphA-3* gene coding for kanamycin resistance (Courvalin & Carlier, 1987). Both of these genes were integrated downstream of the *tet*(M). The Tn*916*-family of CTns has been identified in 27 different Gram-positive and 22 different Gram-negative genera. Many more genera have acquired the Tn*916*-like elements *in vitro*. More recently, composite transposons have been described where one or more transposons have been inserted within other transposons creating new multidrug resistant elements such as the composite element Tn*5385*. Tn*5385* codes for tetracycline/minocycline, erythromycin, gentamicin, penicillin, streptomycin, and mercury resistance and shares genes with both staphylococcal and

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enterococcal elements. An interesting feature of the composite elements is their ability to transfer as a single unit to a recipient or to transfer the inserted transposon separately thus allowing for different combinations of genes to transfer depending on which element was received (Rice, 2007).

New CTns and plasmids have been described in the last few years which identify linkages between a variety of MLSKO and other genes. Some of the other genes and elements associated with acquired MLSKO genes are illustrated in Table 4. These include elements which link the *tet*(M), *mef*(A)-*msr*(D) genes(Figueiredo *et al*., 2006), the tetracycline resistant gene *tet*(O) linked upstream of the *mef*(A) and *msr*(D) genes (Brenciani *et al*., 2004), the linkage between the *vat*(E) and *erm*(B) genes (Jensen *et al*., 2000) and *erm*(B) and copper resistances genes (Hasman *et al*., 2006).

CTns may also play a role in maintaining specific genes in the bacterial population without selective pressure or without functioning in the particular bacteria. A good example can be found in the anaerobic *Bacteroides* spp. which often carry the *erm*(F) gene which confers resistance to the bacterial host and linked to a *tet*(X) gene, which codes for an enzyme that breaks down tetracycline in the presence of oxygen, but is nonfunctional in its anaerobic host (Chopra & Roberts, 2001). The hypothesis has been that the *erm*(F)-*tet*(X) genes which are now part of the CTnDOT element originated in an aerobic bacteria and was transferred as a unit to *Bacteroides* where the *tet*(X) gene product is nonfunctional. Recent data on a tetracycline resistant *Sphingobacterium* which carries the *tet*(X) gene, indicates that the genes flanking this *tet*(X) are the same genes as found flanking the *tet*(X) gene on the *Bacteroides* CTnDOT element except the *erm*(F) gene is missing in the *Sphingobacterium* isolate (Ghosh & LaPara, 2007; S. Ghosh, pers. Commun.). Thus, linkage between genes may be one way to keep non-expressed genes or genes which are currently not under selective pressure in bacterial populations.

Mobile elements and MLSKO genes

Sixteen of the MLSKO resistance genes have been linked to other antibiotic or heavy metal resistance genes, class 1 or 2 integrons and/or specific transposons (Table 4). Linkage of various antibiotic resistance genes in one mobile element allows the recipient to be converted from a susceptible isolate to a multiple resistant one with the integration of mobile elements carrying multiple antibiotic resistance genes. Selection for transfer of these elements can be done using multiple different antibiotics or chemicals as was observed with the replacement of avoparcin with tylosin without changes in the level of avoparcin resistant enterococci. It was then determined that the same plasmid which carried the *vanA* gene cluster for avoparcin resistance also carried an *erm*(B) gene which made the isolates resistant to tylosin. Therefore, when tylosin replaced avoparcin for animal use, the *vanA* gene remained because of its linkage with the *erm*(B) gene (Garcia-Migura *et al*., 2007). However, some caution does need to be taken when thinking about co-selection and resulting carriage of antibiotic resistant bacteria as our study on children treated for caries illustrates (Roberts *et al*., accepted). In this study, 75 children were treated with amalgam fillings and 75 were treated with composite fillings. Oral and urine cultures were taken before treatment, after treatment, and for 7 years of follow-up and then analyzed to determine whether the amalgam treated group had increased levels of antibiotic and/or mercury resistant commensal flora over baseline and higher levels than the control group treated with composite material. We found that there was no cultural evidence that children with amalgam fillings had higher levels of antibiotic or mercury resistant commensal oral or urinary bacteria as compared with the children treated with composite material.

Future

Erythromycin was discovered over 60 years ago, but its use has diminished over time due to increased bacterial resistance. However, with the addition of the newer macrolide derivatives in the 1980s and more recently the ketolides and oxazolidinones, this group of antibiotics remains an important class of drugs for the treatment of a variety of community and hospital infectious diseases caused by Gram-positive and Gram-negative bacteria, and *Mycobacterium* spp. Dr Cohen (1992), The Centers for Diseases Control in the USA, coined the phrase 'Post-Antimicrobial Era' over 10 years ago to mean a time when community and hospital acquired infections will primarily be due to multidrug resistant pathogens where few or no viable antibiotic therapies exist for treating infections that were once treatable. Disease due to multidrug resistant pathogens increases the morbidity, mortality, and cost associated with treatment of these infections and the number of these infections increases yearly. To illustrate this point a recent study on methicillin resistant *S. aureus* (MRSA) infections from 2005 in the United States estimated that $>90\,000$ MRSA infections occurred with $>18\,000$ associated deaths (Klevens *et al*., 2007). This coupled with the low number of novel antibiotic classes currently in development, suggests that the 'post-antimicrobial era' is almost here for the industrialized world for some multidrug resistant pathogens and for practical purposes has already been reached in much of the developing world. In tune with this concern is the increased numbers of ketolide resistant streptococci, oxazolidinone resistant enterococci, and staphylococci which have been identified suggesting that these current new antibiotics will also lose their effectiveness as more bacteria become

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resistant. Multiple strategies are needed to preserve the use of the currently effective MLSKO antibiotics. These strategies include the development of newer MLSKO derivatives, development of new classes of antibiotics and nonantibiotic therapies. Changes in infection control practices, personal hygiene, sanitation improvements, in agricultural and animal husbandry practices are also needed. In addition, a much better understanding is needed on how antibiotic resistance develops in different bacterial populations, why some of these genes spread quickly, and why others stay more localized. However, most importantly more resources are needed to monitor changes in antibiotic resistance patterns and the development of ways to extend the life of our current antibiotics such as using two different antibiotics for therapy instead of one antibiotic.

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