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Intrinsic and Unusual Resistance to Macrolide, Lincosamide, and Streptogramin Antibiotics in Bacteria

ROLAND LECLERCQ[1] AND PATRICE COURVALIN[2]†*

Service de Bactériologie-Virologie-Hygiène, Hôpital Henri Mondor, Université Paris XII, 94010 Créteil, France,[1] and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093[2]

INTRODUCTION

In the accompanying review (21), we considered bacterial resistance to macrolide, lincosamide, and streptogramin (MLS) antibiotics secondary to target modification, the most common mechanism in nature. In this paper, we will focus on intrinsic resistance and on resistance acquired by modification or active efflux of the antibiotics. We will also examine the clinical implications and the consequences for in vitro detection of MLS resistance of these various mechanisms.

INTRINSIC RESISTANCE

MLS resistance of gram-negative bacilli, in particular, members of the family *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp., is probably due to the relative impermeability of the cellular outer membrane to these hydrophobic compounds, as indicated by the erythromycin sensitivity of *Escherichia coli* ribosomes in cellular systems (24). MLS antibiotics cannot therefore be used in the treatment of systemic infections caused by these microorganisms. However, oral erythromycin is locally active in the intestinal tract, in which the concentrations achieved are far higher than the MICs (2 to 256 μg/ml) against most strains of enterobacteria (3). This observation supports the use of this drug for intestinal decontamination in the prevention of traveler's diarrhea and of septicemia caused by gram-negative bacilli in neutropenic patients (3).

Lincosamide antibiotics display reduced activity against certain bacterial species, in addition to members of the family *Enterobacteriaceae*, *Pseudomonas* spp., and other gram-negative bacilli. The lincomycin resistance of *Enterococcus* spp. (except for certain strains of *Enterococcus durans* and *Enterococcus faecium*), *Staphylococcus cohnii*, *Staphylococcus xylosus*, *Staphylococcus sciuri* (18), *Haemophilus* spp., and *Neisseria* spp. is helpful in bacterial identification. Enterococci are, in addition, resistant to streptogramin A-type antibiotics.

ACQUIRED RESISTANCE

Three mechanisms account for acquired resistance to MLS antibiotics: modification of the target of the antibiotics, inactivation of the antibiotics, and active efflux of the antibiotics. In the first type of resistance, a single alteration in 23S rRNA confers broad cross-resistance to macrolides, lincosamides, and streptogramin B-type antibiotics (the so-called MLS phenotype) (21); the other two types confer resistance to structurally related antibiotics only.

* Corresponding author.
† Present address: Unité des Agents Antibactériens, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France.

ANTIBIOTIC MODIFICATION

Various inactivating enzymes are responsible for resistance acquired by antibiotic modification (Table 1).

Macrolides. Macrolide-modifying enzymes have been detected in *Lactobacillus* strains of animal origin (11). Neither the mechanism of drug inactivation nor the corresponding gene(s) has been characterized. Recently, members of the family *Enterobacteriaceae* highly resistant to erythromycin because of inactivation were reported (2, 4, 27). Most of the strains were isolated from feces or blood cultures during selective digestive tract decontamination with erythromycin in neutropenic patients. The strains inactivate the lactone ring of 14-membered macrolides by producing erythromycin esterases (8) or a macrolide 2′-phosphotransferase (27). The 16-membered macrolides are not efficiently utilized as substrates by these enzymes. Two types of esterases, I (349 amino acids) and II (419 amino acids), encoded by the *ereA* (28) and *ereB* (5) (erythromycin resistance esterase) genes, respectively, have been found. The G+C content of *ereB* (36%), as opposed to that of *ereA* (50%), is significantly different from the base composition of the *E. coli* chromosome (50% G+C content). The difference is mostly due to a specific codon usage in *ereB* which is different from that of *E. coli*. This observation strongly suggests that *ereB* is of exogenous origin and has been acquired from a phylogenetically remote bacterium, possibly a gram-positive coccus (5, 6).

ereB is frequently associated with *ermB* (encoding an rRNA methylase) in enterobacteria highly resistant to erythromycin. The two genes contribute synergistically to the high degree of erythromycin resistance of these strains (7).

Lincosamides. *Staphylococcus aureus* and *Lactobacillus* strains of animal origin resistant to lincosamides because of inactivation have been reported but not investigated (10, 11). Human clinical isolates *Staphylococcus haemolyticus* BM4610 and *S. aureus* BM4611 are resistant to high levels of lincomycin and are apparently susceptible to clindamycin (20) (Fig. 1). However, the MBCs of clindamycin against these isolates are greatly increased (20) (Table 2). The isolates produce a 3-lincomycin 4-clindamycin *O*-nucleotidyltransferase [LNT(3,4)] specified by small, nonconjugative plasmids (9). The nucleotide sequences of the genes, designated *linA* and *linA′* (lincosamide nucleotidylation), were determined and found to be closely related (9). The genes encode two isozymes of 161 amino acids with a high degree of similarity (91%). The distribution of these determinants in clinical isolates of staphylococci was analyzed by DNA-DNA hybridization with intragenic probes. The *linA* gene was detected in *S. haemolyticus*, *Staphylococcus epidermidis*, *S. cohnii*, *Staphylococcus hominis*, and *S. aureus*, and the *linA′* gene was detected in *S. epidermidis*, *S. cohnii*, and *S. aureus* (20). Lincomycin-resistant isolates that did not harbor *linA* or *linA′* were found to contain sequences ("*linA*-

TABLE 1. Resistance to MLS due to inactivation in clinically important bacterial species

Host	Phenotype[a]	Enzyme	Localization	Gene	Reference
Staphylococcus aureus	S_A	Streptogramin A *O*-acetyltransferase	pIP524	*saa*	23
	S_B	Streptogramin B hydrolase	pIP524	*sbh*	22
	L	ND[b]	ND		10
	L	[LNT(3,4)]	pIP856	*linA'*	9
S. haemolyticus	L	[LNT(3,4)]	pIP855	*linA*	9, 20
Lactobacillus spp.	M				11
	S_A				11
	ML	ND	ND		11
	MS				11
	MLS				11
Clostridium perfringens	S	ND	ND		12
Pseudomonas spp.	M	ND	ND		16
Escherichia coli	M	Erythromycin esterase type I	pIP1100	*ereA*	2, 8, 28
	M	Erythromycin esterase type II	pIP1527	*ereB*	5
	M	Macrolide 2'-phosphotransferase	ND		27

[a] L, lincosamides; M, macrolides; S, streptogramin antibiotics; S_A, streptogramin A-type antibiotics; S_B, streptogramin B-type antibiotics.
[b] ND, not determined.

like'' genes) distinct from but related to the two genes. Inactivation of lincosamides in staphylococci appears therefore due to a family of closely related sequences that form a continuum of genes.

Streptogramin antibiotics. Resistance to streptogramin antibiotics because of modification of both factors was first described in 1975 in *S. aureus* (22, 23). The resistant strains harbor a large plasmid containing genes *saa* (streptogramin A acetyltransferase) and *sbh* (streptogramin B hydrolase) encoding streptogramin A *O*-acetyltransferase (23) and streptogramin B hydrolase, respectively (22). Most of the strains are also resistant to low levels of lincosamides, despite the fact that these antibiotics are not inactivated (Fig. 1 and Table 2).

Recently, resistance to factor A of streptogramin antibiotics and to lincosamides was described in *S. aureus* (14) (Fig. 1 and Table 2). Some of the strains inactivate streptogramin A-type antibiotics. The corresponding determinant, *lsa* (lincosamides and streptogramin A), is chromosomal, and the resistance mechanism has not yet been elucidated.

ACTIVE EFFLUX

Two types of resistance due to active efflux were recently reported in staphylococci (Table 2). In the first one, a strain of *S. epidermidis* was resistant to 14- and 15-membered macrolides (19). This low-level resistance was expressed constitutively, and the absence of antagonism between 14-membered molecules and other macrolides differentiated this phenotype from classical inducible MLS resistance (Fig. 1). The uptake of erythromycin by resistant cells was decreased, and resistance was due to active efflux of 14-membered macrolides (17). The resistance gene, *erpA* (erythromycin resistance permeability), is borne by a 26.5-kb plasmid and codes for a 60-kDa protein present only in the membrane fractions of resistant cells. Other strains of *S. epidermidis* were inducibly resistant to 14-membered macrolides and streptogramin B-type antibiotics. Analysis of the nucleotide sequence of the *msrA* (macrolide streptogramin resistance) gene responsible for this resistance sug-

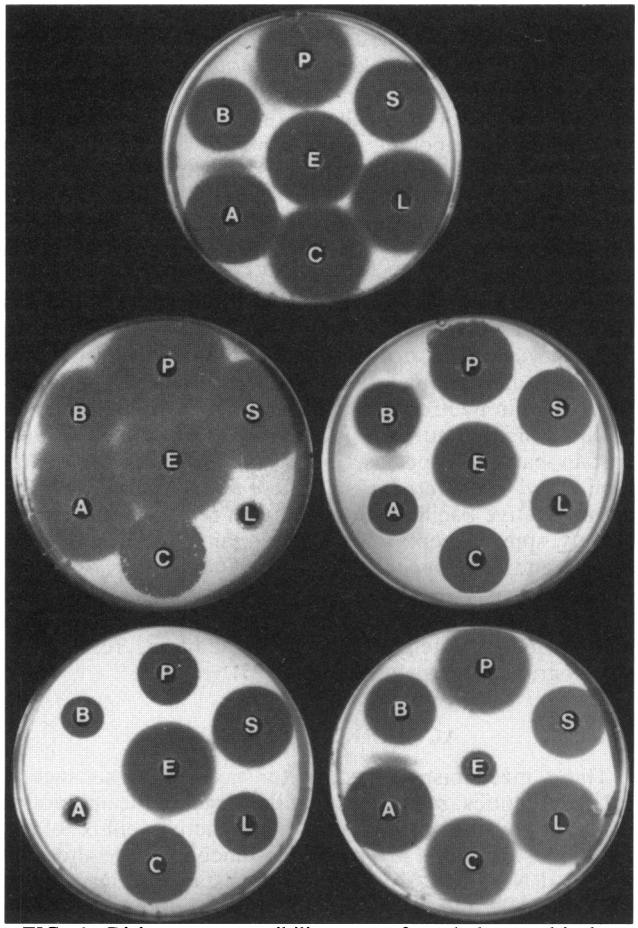

FIG. 1. Disk agar susceptibility tests of staphylococcal isolates. Top, susceptible strain; middle left, strain harboring the *linA* gene; middle right, strain harboring the *lsa* gene; bottom left, strain harboring the *saa* and *sbh* genes; bottom right, strain resistant because of active efflux. C, clindamycin (2 U); E, erythromycin (15 U); L, lincomycin (15 μg); S, spiramycin (100 μg); A, pristinamycin II (20 μg); B, pristinamycin I (40 μg); P, pristinamycin (15 μg).

TABLE 2. Types of MLS resistance in staphylococci

Mechanism	Genotype	Type of resistance to[a]:							
		Ery	Ole	Mac	Lin	Cli	SgB	SgA	Sg
Target modification	*erm*[b]	R	S/R	S	S	S	S	S	S
	erm[c]	R	R	R	R	R	R	S	s
Drug inactivation	*linA*	S	S	S	R	s	S	S	S
	lsa	S	S	S	I	I	S	R	I
	saa-sbh	S	S	S	S/I	S/I	R	R	R
Active efflux	*erpA*[d]	R	R	S	S	S	S	S	S
	msrA	R	R	S	S	S	R[e]	S	ND

[a] I, intermediate resistance; R, resistance; S, susceptibility; s, diminished susceptibility to bacteriostatic and/or bactericidal activity; ND, not determined. Cli, clindamycin; Ery, erythromycin; Lin, lincomycin; Mac, 16-membered macrolides; Ole, oleandomycin; SgA, streptogramin A-type antibiotics (pristinamycin factor II, virginiamycin factor M); SgB, streptogramin B-type antibiotics (pristinamycin factor I, virginiamycin factor S); Sg, streptogramin antibiotics.
[b] Inducible.
[c] Constitutive.
[d] Detected in coagulase-negative staphylococci only.
[e] After induction by erythromycin.

gested that it encodes an ATP-binding protein that functions as a drug efflux pump (30).

CLINICAL RELEVANCE

The multiplicity and complexity of MLS resistance phenotypes of bacteria are largely due to the recent detection of new mechanisms of resistance, mainly enzymatic drug inactivation. However, from a practical point of view, these new mechanisms currently have a limited importance because of their low incidence. Inactivation of lincosamides is present in 4 to 8% of coagulase-negative staphylococci but in only 0.2% of *S. aureus* strains (20). Less than 5% of *S. aureus* clinical isolates modify the streptogramin antibiotics (13). In contrast, the MLS resistance phenotype accounts for nearly all of the resistant strains isolated in clinical practice. In staphylococci, the prevalence of this phenotype in hospital settings is between 15 and 45%, but generalization is difficult because of important local variations (13). Nevertheless, erythromycin resistance in methicillin-resistant strains is higher than 90% in numerous countries (25).

Less than 5% of beta-hemolytic streptococci are resistant to MLS antibiotics (13) but, again, local variations can be observed. For instance, an increase in resistance from 12% in 1972 to 83% in 1977 followed by a decrease to 35.4% in 1978 was reported in Japan (26). This evolution was possibly due to the epidemic spread of certain strains and to a high consumption of antibiotics from 1972 to 1977.

Pneumococci are often susceptible to MLS antibiotics. However, evolution toward resistance has been observed in France. The first strains resistant to erythromycin appeared in 1976, and their incidence progressively increased to 20 to 30% in 1986 (1). This evolution may have been due to the epidemic spread of strains of serotypes 6, 14, 19, and 23 and to a rapid dissemination of Tn*1545*-related transposons in pneumococci.

ANTIBIOTICS TO BE TESTED IN VITRO

Staphylococci. For staphylococci, testing of erythromycin and lincomycin is sufficient to allow the identification of inducible and constitutive MLS resistance. The results for erythromycin, susceptible or resistant, also apply to other 14-membered macrolides and to the 15-membered macrolides. The lincomycin response is valid for lincosamides and 16-membered macrolides in the case of MLS resistance. Lincomycin, rather than clindamycin, should be tested routinely, since it allows better detection of lincosamide resistance (20). The addition of a 16-membered macrolide is advisable for the detection of combined phenotypes (for instance, inducible MLS resistance associated with lincomycin resistance due to inactivation). In countries in which streptogramin antibiotics are used, a streptogramin must be tested. Factor A alone should also be included to detect resistance to this component, which leads to diminished susceptibility to the streptogramin complex (Table 2).

Streptococci. Since, whether inducible or constitutive, resistance is crossed among macrolides, lincosamides, and streptogramin B-type antibiotics, testing of erythromycin appears sufficient for streptococci. However, the recent detection of a pneumococcal strain susceptible to erythromycin and resistant to 16-membered macrolides and streptogramin antibiotics (15) indicates that the latter drugs should be tested in countries in which they are used. Lincomycin is useful for the identification of *Enterococcus faecalis*.

Anaerobic bacteria. For anaerobic bacteria, clindamycin must be tested. However, MLS resistance of *Bacteroides* spp. inducible by this antibiotic has been described and is often not detected by the disk agar diffusion method after 48 h of incubation (29). These strains are resistant to high levels of erythromycin, and we therefore recommend that this antibiotic be tested in addition to clindamycin to detect this unusual inducible resistance.

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