IDENTIFYING RESISTANCE™

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BIOMÉRIEUX

The macrolide antibiotic class has been used as first-line therapy to treat various bacterial infections including respiratory infections since the development of erythromycin in the 1950's.

Due to its frequent use and the development of other drugs in the macrolide class, macrolide resistance has increased rapidly throughout Europe and North America in recent years.

Resistance, that is now common in staphylococci and streptococci, is attributable to two mechanisms both of which are readily transmissible. As a result of the increased resistance in commonly treated respiratory pathogens, susceptibility testing is recommended in the case of treatment of serious illness such as community acquired pneumonia, particularly if the patient is allergic to β-lactams and quinolones.

It is an honor to have a column on this subject written by Prof. Roland Leclerq. Prof. Leclerq is one of the world's experts on the variety of ways that pathogens have invented to become resistant to this widely used antibiotic class.

Dee Shortridge, PhD

Director R&D Microbiology in Saint-Louis

State-of-the-Art

Macrolides and related antibiotics

Roland Leclercq

Roland Leclercq, MD, PhD, is professor of microbiology. After graduating at the University of Paris, France, he completed his PhD at the Institut Pasteur, Paris in the Laboratory of Professor Patrice Courvalin where he first characterized plasmid-mediated resistance to vancomycin in enterococci. He is currently head of the Microbiology department of the University-Hospital of Caen, France. He also serves on the French Committee on Antimicrobial Susceptibility Testing. His longstanding interest on mechanisms of antimicrobial resistance in Gram positive organisms, in particular resistance to glycopeptides and macrolides, lead to the publication of about 150 papers in peer reviewed scientific journals.

Macrolides and related antibiotics

The macrolides have been known for more than five decades and since the introduction in therapy of erythromycin, a number of these molecules have been developed for clinical use. Macrolides have a common structure formed by a large lactone ring. Erythromycin is a mixture of antibiotics that includes erythromycin A which is the active compound and has a fourteen-membered lactone ring with two sugars, L-cladinose and an amino sugar. Other commercially available macrolides derived from erythromycin A and include clarithromycin, dirithromycin, roxithromycin, and azithromycin which has an enlarged 15-membered ring resulting from a nitrogen insertion. The structural modifications of erythromycin A resulted in improved pharmacokinetic profiles and better tolerance but could not overcome cross-resistance between members of this class of antimicrobials. Certain 16-membered ring macrolides are also available in a few countries (spiramycin, josamycin,

Macrolides and related antibiotics

**Several antibiotics can inhibit *S.pneumoniae*.
And resistance exist for most of them.**

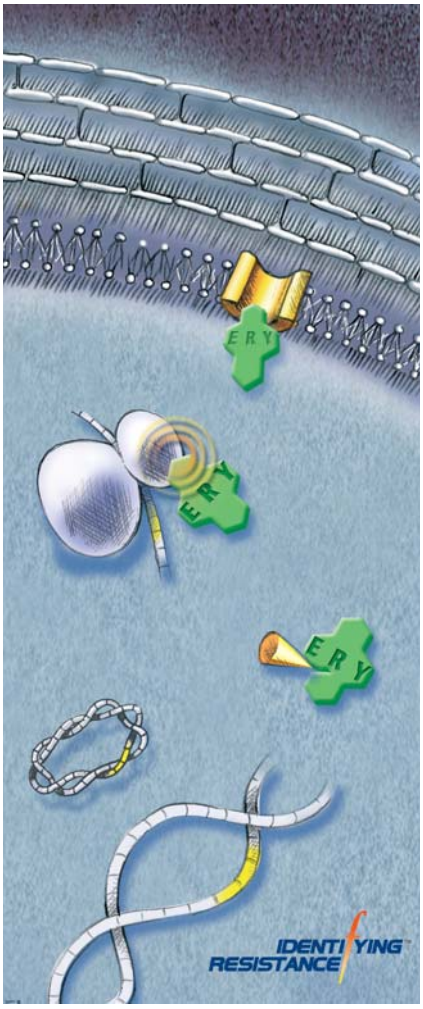

fig. 1

Staphyloccocci mainly resist macrolides through ribosomal methylation encoded by *erm*A and *arm*C genes. 14- and 15-membered macrolides are inducers. Mutations on *rrn* operon of the ribosomal RNA and for proteins L4 & L22 are rare. Resistance can also happen by efflux through ABC-transporters encoded by *msr* genes, or by drug modification by phosphotransferases (ery) and lincosamides nucleotidyltransferase (lin).

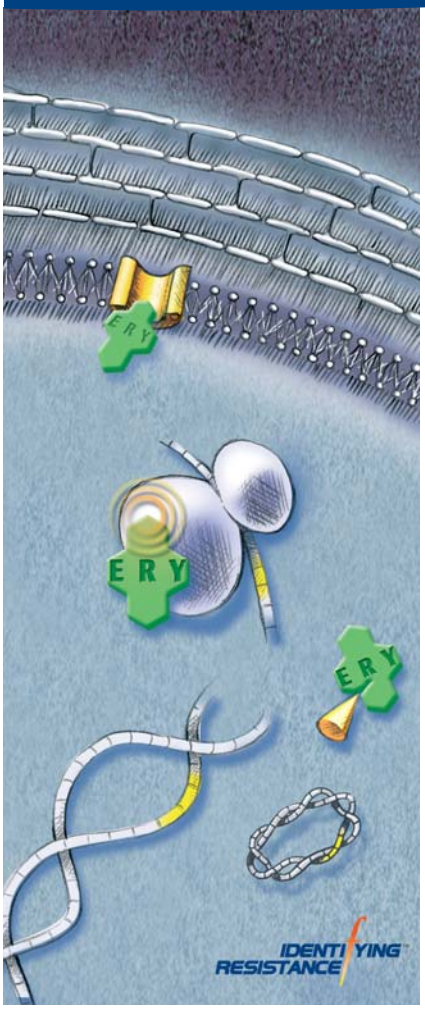

fig. 2

Streptococci mainly resist macrolides through ribosomal methylation encoded by *erm*B and *erm tr* genes. Erythromycin is inducer. Resistance can also happen by efflux through mef(A) gene (MFS class of pumps). Enterococci can also inactive macrolides by esterases.

midecamycin, and miocamycin) or for veterinary use (tylosin). The recently developed ketolide, telithromycin, is derived from clarithromycin and has two major modifications, replacement of L-cladinose by a keto-function and a 11-12-carbamate extension with an arylalkyl modification, which may explain increased intrinsic activity and activity against erythromycin-resistant strains.
Other antimicrobials although structurally different are related to macrolides because they share a similar mode of action and cross-resistance to a certain extent. Together with the macrolides, they form the MLS (macrolides-lincosamides-streptogramins) group of antibiotics. Lincosamide antibiotics (clindamycin and lincomycin) are devoid of a lactone ring. The streptogramins (quinupristin-dalfopristin, pristinamycin available in a few countries and virginiamycin for veterinary use) represent a strategy to overcome macrolide resistance. These antimicrobials are composed of two streptogramin factors, A and B, with a synergistic activity resulting from dual interaction with the ribosome. Due to this property, these molecules may retain activity against Gram-positive organisms displaying resistance to a single factor.
Activity of macrolides is related to protein synthesis inhibition. The bacterial ribosome is formed by a small 30S and a large 50S subunit. The latter is composed of 23S rRNA folded to form six domains numbered from I to VI and of a minimum of 30 proteins. The binding site of erythromycin is composed of domain V sequences nearby the peptidyltransferase center where the polypeptide chain is synthesised. Interactions with adenines at position 2058 and 2059 are particularly important. The binding site of erythromycin A is located within the tunnel that serves as a channel for the growing peptide. The surface of this tunnel is formed by 23S rRNA and several ribosomal proteins, including proteins L22 and L4.

Mechanisms of resistance to MLS antibiotics

1. Bacteria have several ways to resist macrolides

The spectrum of activity of macrolides is limited by the intrinsic resistance displayed by most Gram-negative bacilli to these compounds. Intrinsic resistance is related to the combined presence of an outer membrane which exhibits low permeability to macrolides and chromosomally-encoded proteins which pump the antibiotic out of the

cell. However, certain clinically important Gram-negative bacilli, such as *Bordetella pertussis*, *Campylobacter*, *Chlamydia*, *Helicobacter*, and *Legionella* are important exceptions.

Further, the Gram-positive microorganisms have collected mobile elements that help them evade the lethal effects of antibiotics. Bacteria have developed three ways of resistance against MLS antibiotics: 1) target site modification that prevents the binding of the antibiotic to its natural target, the ribosome, 2) efflux of the antibiotic which prevents the antimicrobial from reaching the ribosome and 3) inactivation of the antimicrobial molecule ***(figure 1)***.

2. Resistance by ribosomal modification

2.1. Ribosomal methylation : the MLS_B phenotype

Ribosomal modification by methylation was the first mechanism of resistance to erythromycin elucidated, remained unique for decades and is still highly prevalent. It is secondary to the acquisition of an *erm* gene (erythromycin ribosome methylase) usually carried by mobile elements. This gene encodes a ribosomal methylase which methylates 23S rRNA at a single site, adenine 2058. The modification markedly reduces the affinity of erythromycin for its target. Cross-resistance to macrolides, lincosamides, and streptogramins B which gave its name to the phenotype, MLS_B, is due to the overlapping binding sites of the drugs.

A wide range of microorganisms that are targets for macrolides and lincosamides express Erm methylases. Nearly forty *erm* genes have been reported so far. In pathogenic bacteria, these determinants are mostly borne by plasmids and transposons that are self-transferable and four major classes are detected, *erm*(A), *erm*(B), *erm*(C), and *erm*(F). *erm*(A) and *erm*(C) are typically staphylococcal gene classes while *erm*(B) class genes are mostly spread in streptococci and enterococci and *erm*(F) in *Bacteroides* and other anaerobic bacteria.

Expression of MLS_B resistance can be constitutive or inducible depending on a regulatory region which controls the expression of the *erm* gene. Constitutive expression leads to cross MLS_B resistance, irrespective of the *erm* gene. Inducible resistance leads to dissociated resistance to MLS_B antibiotics due to differences in inducing capacity of the antibiotics.

In staphylococci where the *erm*(A) and *erm*(C) determinants are predominant, the resistance phenotypes conferred by inducible expression of both determinants are similar and characterized by resistance to 14- and 15- membered ring macrolides which are inducers. By contrast, 16-membered ring macrolides, lincosamides, and streptogramins B which are not inducers remain active ***(Table 1)***. In disk-diffusion tests, a D-shaped zone due to induction of methylase production by erythromycin can be observed when a disk of erythromycin is placed nearby a disk of clindamycin. The use of clindamycin (or of a non inducer macrolide) for the treatment of an infection due to an inducibly resistant strain of *S. aureus* is not devoid of risk since constitutive mutants can be selected *in vitro* and *in vivo* at frequencies of approximately 10^{-7} in the presence of these antibiotics. Therefore, the NCCLS recommends to report the MLS inducibly resistant isolates as resistant to clindamycin.

The vast majority of resistance by ribosomal methylation in streptococci and enterococci is due to the spread of erm genes belonging to the *erm*(B) class and less frequently to the *erm*(TR) subset of the *erm*(A) class. Inducible expression of these genes gives rise to a variety of phenotypes differing from that of staphylococci. The MLS_B phenotype characterized by high-level cross resistance to macrolides and lincosamides, which is commonly detected in pneumococci, is frequently inducible. Some erythromycin-resistant strains may be apparently susceptible to clindamycin but express antagonism between erythromycin and clindamycin, and again, clindamycin should not be used. Interestingly, telithromycin is active against most erythromycin-resistant pneumococci since it is a poor inducer of methylase and has interaction with domain II of 23S rRNA which is not methylated by Erm methylases.

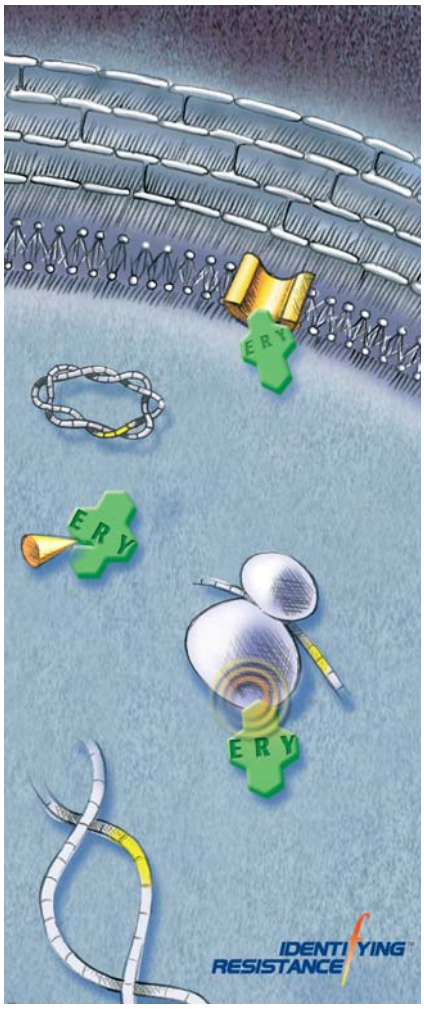

fig. 3

S.pneumoniae **mainly resist macrolides through ribosomal methylation encoded by *ermB* genes. Mutations can also happen for L4 and L22 proteins.**

2.2. Ribosomal mutation

The clinical importance of this mechanism was first recognised with identification of mutations at either A2058 or A2059 (*E. coli* numbering) in 23SrRNA of *Mycobacterium avium* and *Helicobacter pylori* resistant to clarithromycin. This mechanism is responsible for resistance in the vast majority, if not all, strains belonging to these species. These

Table 1 **Major phenotypes and genotypes of macrolide resistance in Gram-positive cocci due to ribosomal methylation, drug efflux, or drug inactivation.**

Species	*Mechanism*	*Gene class*	*Phenotype designation*	*Phenotype of resistance*			
				14-, 15-Md	Tel	16-Md	Cli
Staphylococcus sp.	Ribosomal methylation	*erm*(A), *erm*(C)	MLS_B inducible	R	s	s	s
			MLS_B constitutive	R	R	R	R
	Macrolide efflux	*msr*(A)	MS_B	R	S	S	S
Streptococcus and Enterococcus sp.	Ribosomal methylation	*erm*(B), *erm*(TR)	MLS_B inducible	R or I	S	R or I or s	R or I or s
			MLS_B constitutive	R	R	R	R
	Efflux	*mef*(A)	M	R	S	S	S

14/15/16-Md: 14/15/16-membered ring macrolides: read overleaf the list of antibiotics
Tel: telithromycin, Cli: clindamycin

R, resistant ; **S**, susceptible ; **I**, intermediate resistance
s, susceptible *in vitro* but risk of selection of constitutive mutants *in vivo*

bacteria possess one or two copies of the *rrn* operon encoding the ribosomal RNA. This mechanism has been reported only recently in pneumococci and staphylococci where it seems to be rare as compared to the traditional resistance conferred by mobile elements. Rarity of occurrence of this mechanism in these pathogens might be explained by the fact that their chromosome harbours several copies of the *rrn* gene at that high-level erythromycin resistance can only be achieved when at least half of the copies are mutated. In addition, mutations in ribosomal proteins L4 and L22 that confer erythromycin resistance have been identified in laboratory and clinical isolates of *S. pneumoniae* and *S. aureus*.

3. Resistance to macrolides by efflux

Acquired resistance to macrolides by active efflux has been detected in various bacterial species including streptococci and staphylococci.

The efflux proteins conferring acquired macrolide resistance usually found in *Staphylococccus* spp. are ABC-transporters encoded by plasmid-borne *msr* genes. ABC-transporters are pumps which require ATP to function and are usually formed by a channel composed of membrane domains and ATP-binding domains located at the cytosolic surface of the membrane. The *msr*(A) gene encodes a protein with two ATP-binding domains characteristic of ABC transporters. The nature of the transmembrane component of the MsrA pump remains unknown. The pump has specificity for 14- and 15-membered macrolides and type B streptogramins (the MS_B phenotype). The resistance is inducibly expressed. Erythromycin and other 14- and the 15-membered macrolides are inducers whereas streptogramins B are not. Therefore the strains are resistant to streptogramins B only after induction with erythromycin. Clindamycin is neither an inducer nor a substrate for the pump and thus the strains are fully susceptible to this antimicrobial ***(Table 1)***. This phenotype can be easily distinguished from the MLS_B inducible phenotype by the lack of interaction between erythromycin and clindamycin.

The *msr*(A) gene has not been found in streptococci. In streptococci, the genes responsible for efflux belong to the *mef*(A) gene class and are part of closely related large transposable elements. The Mef(A) pump belongs to the MFS class. It contains twelve transmembrane domains spanning the cytoplasmic membrane and efflux is driven by the protomotive force. The pump seems specific to erythromycin and its derivatives, including azithromycin. Resistance is expressed at moderate levels with erythromycin MICs comprised between 1 and 64 µg/ml (generally between 8 and 32 µg/ml). Because the 16-membered macrolides, the lincosamides, and the streptogramins B are not substrates of the pump, these antimicrobials remain active, even after induction with erythromycin. Resistance to erythromycin combined with susceptibility to clindamycin, whether the cells are induced or not with erythromycin, defines the M phenotype. Again, this phenotype can be easily distinguished from the MLS_B inducible phenotype by the lack of interaction between erythromycin and clindamycin.

4. Drug modification

Inherent to this mechanism of resistance, and unlike target modification, inactivation of antibiotics confers resistance to structurally related antibiotics only. A few staphylococcal isolates produce phosphotransferases which confer resistance to erythromycin and other 14- and 15-membered macrolides. Also, macrolide esterases and phosphotransferases have been reported in enterobacteria, although they are not targets for macrolides, Lincosamide nucleotidyltransferases found in staphylococci and *Enterococcus faecium*, respectively, inactivate lincosamides.

So far, these resistances have not been considered as of major clinical importance because of their rarity. However, an exception concerns the streptogramin class of antibiotics for which resistance is often explained by the combined production of enzymes inactivating the A factor (acetyltransferases encoded by the *vat* genes) and lyases (encoded by *vgb* genes). In addition, these genes are often combined with a *vga* gene putatively responsible for efflux of streptogramins A-type.

5. Incidence of macrolide resistance

It is rather difficult to provide a global figure on incidence of macrolide resistance. For instance, in *S. pneumoniae*, huge geographic differences resistance in resistance frequencies may be observed from very low to greater than 80%. In addition, considerable variations can be seen within a country depending on the source of the strains, patient age, sample origin, seasonal factors, and pneumococcal serotype. There is a need for physicians to be aware of local resistance patterns according to the patient age and the type of infection.

No noticeable difference between percentages of resistance to azithromycin, clarithromycin, and erythromycin can be observed, confirming cross resistance between these antimicrobials. By contrast, in several countries incidence of clindamycin resistance may be much lower than that of erythromycin due to the spread of erythromycin-resistant strains harbouring the efflux gene. For instance, this holds true for pneumococci in the United States in contrast to most European countries where *erm*(B)-containing strains are widespread. The reasons for these differences are unexplained. Remarkably, in intracellular pathogens, such as *Legionella*, *Chlamydia*, and in mycoplasma, resistance remains virtually unknown...

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To know more about….

Binding of macrolides to the ribosome

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Incidence of resistance

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VITEK 2

Evaluation of the VITEK 2 System for Identification and Antimicrobial Susceptibility Testing of Relevant Gram-Positive Cocci
M. Ligozzi, C. Bernini, M-G. Bonora, M. de Fatima, J. Zuliani, and R. Fontana
JCM, May 2002, 40, p. 1681-1686

The VITEK 2 system performed very well for erythromycin-resistant strains (S.pneumoniae), which are encountered in Italy more frequently than penicillin-resistant strains.

Evaluation of the New VITEK 2 System for Determination of the Susceptibility of Clinical Isolates of *Streptococcus pneumoniae*
M. Chavez, J-L. Garcia Lopez, J. Coronilla, A. Valverde, M-C. Serrano, R. Claro, E. Martin Mazuello
Chemotherapy, 2002, 48, p. 26-30

The best agreement was achieved with vancomycin (100%), erythromycin (95.8%) and tetracycline (95.8%).

Multicenter Evaluation of an Automated System Using Selected Bacteria That Harbour Challenging and Clinically Relevant Mechanisms of Resistance to Antibiotics
R. Leclercq, M-H. Nicolas-Chanoine, P. Nordmann, A. Philippon, P. Marchais, A. Buu-Hoi, H. Chardon, H. Dabernat, F. Doucet-Populaire, C. Grasmick, P. Legrand, C. Muller-Serieys, J. Nguyen, M-C. Ploy, M-E. Reverdy, M. Weber, R-J. Courcol
Eur J Clin Microbiology Infect Dis, 2001, 20, p. 626-635

For staphylococci,... the highest degree of agreement was 99.6% for erythromycin, fusidic acid, gentamicin and rifampin.

Hospital Saint-Joseph, Marseille, France

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The bioMérieux Forum

On November 29, bioMerieux UK held a joint symposium with the Health Protection Agency (HPA), in Sutton Coldfield. It was the 4th one, organised by our Dr Gill Webb.

Prof. Peter Borriello (HPA Centre for infections in Colindale, London) outlined the programme for the symposium and described how the political importance of the topic is now recognised.

Dr Michael Ford from Newcastle addressed the importance of antimicrobial resistance screening and recommended an ESBL screening similar to that for MRSA.

Dr David Livermore from HPA-ARMRL described the major shift that has been seen over the last five years in ESBL producers - from *Klebsiella* in patients in ICU to *E.coli* with CTX-M enzymes in complicated community cases.

Dr. Neil Woodford, from HPA-ARMRL, emphasised the fundamental need for AST and interpretive reading in our clinical microbiology laboratories, both to ensure appropriate patient management and to recognise local resistance issues.

Prof. Francis Drobniewski (HPA and University of London) reported that globally, there are 9 million new cases of Tuberculosis every year and 2 million deaths, most of which occur in the non-industrialised world, unlike those from MRSA.

Dr Derek Brown (BSAC, NEQAS, EUCAST) addressed the harmonisation of susceptibility breakpoints across Europe. He described how six active European committees and the American CLSI (former NCCLS) are involved in setting common breakpoints.

Dr. Mick Martin (Royal Bournemouth Hospital) addressed antibiotic abuse in our hospitals and how more appropriate prescribing can help control bacterial resistance.

Dr. Fleming from Birmingham linked the importance of patient management to bacterial resistance, and concluded by saying that investment in investigation routines is critical for the surveillance of bacterial resistance.

The take home message from the meeting was as expected - improved infection control in hospitals, effective surveillance, in both hospitals and the community, and the selective use of antimicrobials, are all vital to reduce the impact of antimicrobial resistance.

Identifying Resistance Symposia

Switzerland	**User's Meeting (no.1)** with Philippe Moreillon Bern, November 7-8
South Korea	**Identifying Resistance Symposium (no.4)** with Prof.Patrice Nordmann Seoul, November 17
U.K.	**Identifying & Controlling Resistance (no.4)** *read above* HPA & bioMérieux Joint Symposium Sutton Coldfield, November, 29
Germany	**Symposium "Wissen verbindet" (no.4)** Oberhausen, January 26-27, 2006

Did you know?

CLSI (nccls) recommandations

M100-S15 vol. 25 January 2005

Table 2C Staphylococcus spp.

Comment 26

Such isolates (that have inducible clindamycin resistance) should be reported as clindamycin resistant.

Table 2G *Streptococcus pneumoniae*

Comment 26

Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by using erythromycin.

Table 2H Streptococcus spp. other than *S.pneumoniae*

Comment 14

Such isolates (that have inducible clindamycin resistance) should be reported as clindamycin resistant.

Macrolides and related antibiotics

Macrolides		
14-membered ring macrolides		erythromycin, roxithromycin, clarithromycin, dirithromycin telithromycin (ketolide)
15-membered ring macrolides		azithromycin
16-membered ring macrolides		spiramycin, josamycin, myokamycin, midecamycin, tylosin
Lincosamides		
		clindamycin, lincomycin
Streptogramins		
	A-type	dalfopristin, pristinamycin IIA, virginiamycin M
	B-type	quinupristin, pristinamycin IA, virginiamycin S

VITEK2 Phenotypes

Staphylococci	macrolide resistant MLSb inducible macrolide resistant MLSb constitutive macrolide resistant to lincomycin macrolide resistant to streptogramins macrolide resistant MLSb+SA constitutive
enterococci	macrolide resistant MLSb
s. pneumoniae	macrolide resistant (MLSb) macrolide resistant (efflux) macrolide resistant to streptogramins
s. agalactiae	macrolide resistant (MLSb) macrolide MLSb inducible macrolide resistant (efflux) macrolide resistant to streptogramins

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Practical advice

What is the place of macrolides in therapy?

They are narrow spectrum drugs, only active on gram-positive bacteria and bacteriostatic agents. Thus they are second-line antibiotics either as an alternative to b-lactams or for specific indications.

What is new about them?

This is one of the rare antibiotic families with new drugs during the last years: quinupristin-dalfopristin, telithromycin.

Why perform antibiotic testing?

Because resistance to macrolides has been evolving during the last years.
After target alteration, efflux is increasing and even drug inactivation does occur.
An induction test is recommended to predict susceptibility of staphylococci to clindamycin.

IDENTIFYING™ RESISTANCE

INTERNATIONAL NEWSLETTER
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