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Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci

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Phenotypes of resistance to the macrolide-lincosamide-ketolide-streptogramin (MLKS) group of antibiotics have been determined in 540 clinical isolates of staphylococci (210 *Staphylococcus aureus* and 330 coagulase-negative species). Results of disc diffusion tests using erythromycin A, oleandomycin, rokitamycin, clindamycin, telithromycin, quinupristin and dalfopristin delineated four main groups corresponding to those defined classically using erythromycin and clindamycin only, but with sub-divisions. Resistance to erythromycin was more common in coagulase-negative strains (56%) than in *S. aureus* (16%); telithromycin, clindamycin, quinupristin–dalfopristin and rokitamycin were active against >97% of *S. aureus* strains and >88% of the coagulase-negative strains. The commonest resistance phenotype was 'inducible MLS_B' (12% in *S. aureus*, 31% in coagulase-negative strains); this group could be divided in terms of the different inducing abilities of erythromycin and oleandomycin. 'Constitutive MLS_B' and 'MS' phenotypes were more often found in coagulase-negative strains (11 and 13%, respectively) than in *S. aureus* (2 and 1%). Novel phenotypes were found during the isolation of constitutively resistant mutants from inducible strains, and of resistant mutants from 'MS' strains. This extended phenotyping scheme has revealed further complexities and evolutionary possibilities in patterns of resistance to this group of antibiotics.

Introduction

Interest in the macrolide antibiotics has increased greatly during the last decade. This was a consequence firstly of chemical modifications to the ring structure of erythromycin A resulting in newer macrolides such as azithromycin, clarithromycin and roxithromycin,[1,2] and secondly of the synthesis of the ketolides,[3] in which significant alterations have been made to the sugar side chains. The newer macrolides have remarkable pharmacokinetic properties, enlarging their spectrum compared with the archetypal macrolide erythromycin A, but all the resistance mechanisms that operate against the latter also apply to the former. Thus, there is complete cross-resistance. On the other hand, ketolides do not induce the enzyme responsible for the most common form of resistance to erythromycin, the so-called 'inducible MLS_B' phenotype.[4] Therefore ketolides, in contrast to the newer macrolides, remain active against many erythromycin-resistant strains.[5]

The purpose of the present study was to determine the incidence of various types of erythromycin resistance among a large number of unselected staphylococci isolated from patients in a university hospital, and to investigate how these resistance mechanisms affected susceptibility to antibiotics related to erythromycin A, namely another 14-membered macrolide (oleandomycin), a 16-membered macrolide (rokitamycin), a lincosamide (clindamycin), a ketolide (telithromycin) and representatives of the A and B components of the streptogramins (quinupristin and dalfopristin), alone and in combination.

Materials and methods

Bacterial strains

A total of 540 strains of individual staphylococci, comprising 210 *Staphylococcus aureus* and 330 coagulase-negative staphylococci (CNS), isolated in the Diagnostic Microbiology Laboratory of The Royal Free Hospital, London, UK, during June 1998, were identified by their colonial appearance, Gram's staining and production of catalase. *S. aureus* and CNS were differentiated using DNase and

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Staphaurex. CNS were identified where appropriate using API Staph kits.

The origin of each strain was checked by patient's name and hospital number, and possible duplicate strains excluded. For the methicillin-resistant strains, only one of each clonal type was included in the test population.

Antibiotics

Telithromycin (HMR 3647) was given by Hoechst Marion Roussel (Romainville, France); Synercid (RP 59500, seven parts quinupristin, three parts dalfopristin), dalfopristin (RP 54476) and quinupristin (RP 57669), each as the methane sulphonate, were given by Rhone-Poulenc Rorer (Collegeville, PA, USA); erythromycin BP free base was given by Lilly Industries (Basingstoke, UK); rokitamycin was given by ISF SpA (Milan, Italy); lincomycin hydrochloride and oleandomycin phosphate were purchased from Sigma (Poole, UK). Erythromycin was dissolved in ethanol, the other compounds in water.

Discs containing oleandomycin 15 μg were purchased from Mast Laboratories (Bootle, UK), and erythromycin 15 μg and clindamycin 2 μg from Unipath Laboratories (Basingstoke, UK). Discs containing Synercid 15 μg and telithromycin 15 μg were given by Rhone-Poulenc Rorer R-D (Antony, France) and Hoechst Marion Roussel, respectively. Discs containing 15 μg of either rokitamycin, dalfopristin or quinupristin were made as required by treating Whatman AA discs with the appropriate antibiotic solution.

Chemicals

These were obtained from Sigma (Poole, UK).

Media

Nutrient agar (NA), Mueller–Hinton agar (MHA) and broth (MHB) were from Unipath. 'Blood agar' was Columbia agar (Mast) + 5% whole horse blood.

Susceptibility testing

All 540 strains were tested by the breakpoint method against erythromycin (0.5 and 4 mg/L), lincomycin (1 and 2 mg/L) and Synercid (1 mg/L), on MHA inoculated with 10^4 cfu, incubated for 24 h in air.

The 215 strains resistant to erythromycin and the five sensitive to erythromycin but resistant to lincomycin were then screened by a disc diffusion method. An aqueous suspension of bacterial growth from blood agar was adjusted to McFarland 0.5, and inoculated by swab on MHA (60 μL in a plate of diameter 140 mm). Each plate was set with 13 discs (centres 2 cm apart) as shown in Figure 1. This arrangement allowed both the sensitivity pattern to individual compounds and interactions between the various antibiotics to be observed with a minimum amount of repetition.

Zone sizes and their shapes were read after overnight incubation, and then again after a further 24 h. If the nature of a specific interaction was not clear, the individual test was set up again in 90 mm plates, with the distances between discs being varied as appropriate.

Zones were interpreted according to their size and shape, as indicating sensitive or resistant, the latter being inducible if a 'D'-shaped zone was observed (the compound on the left being the inducer). Synergy was recorded if there was an extension of inhibition zone between two discs. The presence of satellite colonies within inhibition zones, and other evidence for the existence of sub-populations (e.g. some degree of 'target zone' formation) as well as unusual shapes of zones were noted.

Phenotypes were denoted by abbreviations of antibiotic class to which a strain was resistant: M, erythromycin; A, oleandomycin; L, clindamycin; S_A, dalfopristin; S_B, quinupristin; K, telithromycin; Mac, rokitamycin.

When resistance was inducible by erythromycin, i was prefixed. Thus, for example, the classical 'inducible MLS_B' phenotype is denoted here as M/i(LKS_BMac), and the classical 'constitutive MLS_B' phenotype is $MLKS_BMac$.

We also used the 13 disc method to determine the phenotypes of five strains (two MRSA, three *Staphylococcus haemolyticus*) that had previously shown anomalous susceptibilities to telithromycin.[5]

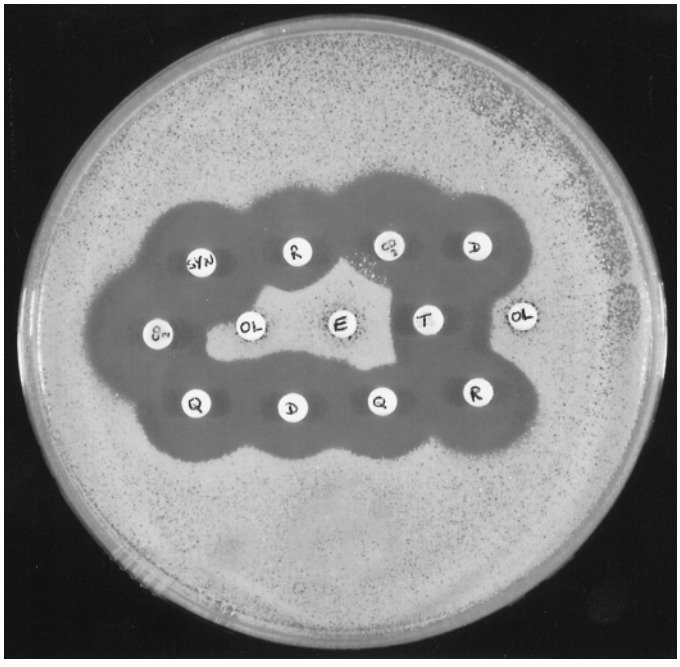

Figure 1. The 13 disc screening method for investigating activities of and interactions between MLKSMac antibiotics. Top row: Synercid (SYN), rokitamycin (R), clindamycin (CD2), dalfopristin (D). Middle row: clindamycin, oleandomycin (OL), erythromycin (E), telithromycin (T), oleandomycin. Bottom row: quinupristin (Q), dalfopristin, quinupristin, rokitamycin.

MIC determinations were made following the NCCLS agar dilution method.[6]

Selection of resistant mutants

Cultures in MHB were spun down and resuspended at 10× original concentration. A viable count was performed, and 0.1 mL (*c.* 10^9 cfu) was spread on MHA containing 1.2 mg/L telithromycin (i.e. at least 20 × MIC) and colonies counted after 48 h incubation. The proportion of cells able to grow was calculated, and their phenotypes determined by the 13 disc method described above.

Reversal of resistance

(i) Doubling dilutions of ethidium bromide in MHA were spot-inoculated with strains under test, and incubated overnight. For each strain, sub-culture was made from the 0.5 × MIC plate on to NA, and incubated overnight. Approximately 20 colonies from these plates were spot-inoculated on to MHA ± erythromycin (8 mg/L = *c.* 16 × MIC for sensitive strains). Those unable to grow in the presence of erythromycin had lost their resistance; they were also tested as in second screen above.
(ii) For strains in which an efflux mechanism was suspected, the MIC of erythromycin was determined alone and in the presence of (separately) dinitrophenol (20 mg/L), reserpine (20 mg/L) and carbonyl cyanide *m*-chlorophenol hydrazone (0.25 mg/L). These concentrations were chosen by solubility for the first two compounds and microbiological activity for the third. A four-fold diminution in MIC was taken as indicating inhibition of efflux.

Synergy/antagonism experiments

Interactions between oleandomycin and telithromycin were investigated and analysed by chequerboard titration on MHA using doubling dilutions, as described previously.[7] Results were interpreted as 'synergy', 'antagonism' or 'indifference'. The latter term was used in the sense originally defined by Jawetz & Gunnison,[8] meaning that each antibiotic in combination behaves as if the other were not there.

Assays of antibiotic destruction

Destruction of lincomycin and clindamycin was tested for by a modified Gots test[9] read after 48 h, and measured as described by Leclercq *et al.*[10] For the latter test, cells from overnight cultures were concentrated 60-fold in phosphate buffer containing 20 mg/L of the antibiotic and incubated at 37°C. A similar suspension of *S. aureus* Oxford was tested as a negative control. Antibiotic concentrations were determined at intervals by bioassay with *S. aureus* Oxford as indicator.

For technical reasons it was not possible to test for dalfopristin inactivation under these conditions.

Results

Sensitivity to individual agents

Sensitivity patterns are shown in Table I. CNS were significantly more often resistant to erythromycin, telithromycin, clindamycin, quinupristin or rokitamycin than were *S. aureus* strains ($P < 0.01$ by chi-squared test). Only five strains (two *Staphylococcus epidermidis*, two *Staphylococcus sciuri* and one *Staphylococcus simulans*) were resistant to dalfopristin (MIC 32 or 64 mg/L). All 540 strains tested were sensitive to Synercid, despite two (the *S. epidermidis* mentioned above, nos 152 and 538) being resistant to both components (Figure 2).

Type of resistance to erythromycin

The most common type of resistance to erythromycin was the 'inducible' variety (Table II); the 'constitutive' and 'MS' types were less common, especially among *S. aureus* strains. The incidence of all three types was higher among CNS than for *S. aureus* ($P < 0.01$).

Table I. Sensitivity of 540 staphylococcal strains to antibiotics of the MLKS[a] group

Antibiotic	Percentage of resistant strains		
	Staphylococcus aureus	CNS	all
Erythromycin	15.7	55	39.8
Oleandomycin	15.7	55	39.8
Telithromycin	2.4	10.6	7.5
Clindamycin	2.4	11.5	8
Quinupristin	2.4	10.6	7.4
Dalfopristin	0	1.5	0.9
Synercid	0	0	0
Rokitamycin	2.4	10.6	7.4

[a]MLKS, macrolide, lincosamide, ketolide, streptogramin.

Table II. Types of resistance to erythromycin in 540 staphylococcal strains

Resistance type	Incidence (%)	
	Staphylococcus aureus	CNS
Fully sensitive	84	44
Inducible	12	31
Constitutive	2	11
'MS'	1	13

Resistance phenotypes to MLKSMac antibiotics

Results obtained by analysing patterns of resistance deduced from the 13 disc screening test defined four groups, each of which could be divided into two.

Group A: destructive mechanism. Five of the 540 staphylococcal strains tested were resistant to lincomycin although sensitive to erythromycin. This pattern suggested the possible presence of a drug inactivation mechanism.[11] More detailed investigations of these strains showed this to be the case (Table III): the two *S. epidermidis* strains, phenotype L (group A_1), inactivated clindamycin rapidly and lincomycin more slowly, while the other three strains, phenotype LS_A, did not inactivate lincosamides.

The anomaly of strains rapidly inactivating clindamycin but being sensitive to the antibiotic has been reported previously.[10]

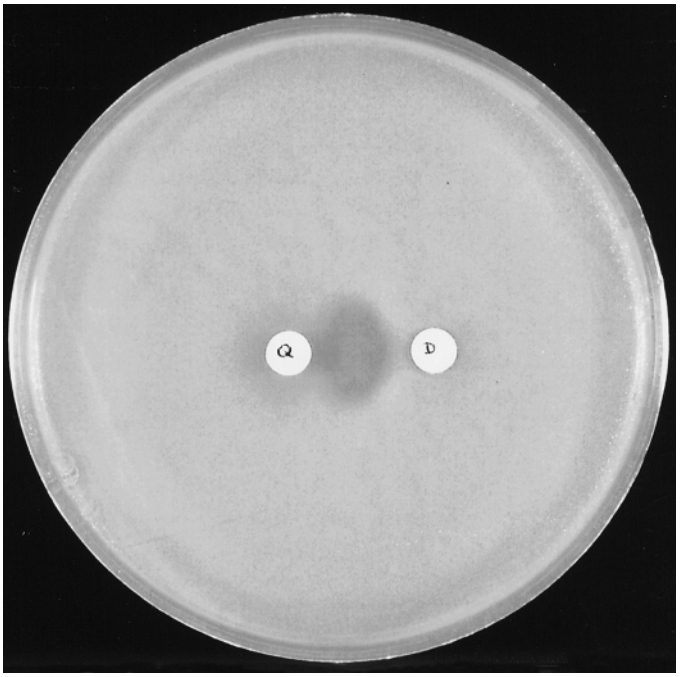

Figure 2. Synergy between dalfopristin (right) and quinupristin (left) against a strain resistant to both.

Group B: classical inducible 'MLS_B'. The 129 strains in this category (26 *S. aureus*, 103 CNS) were resistant to erythromycin and oleandomycin, and sensitive to clindamycin, telithromycin, quinupristin, dalfopristin and rokitamycin. For several strains, satellite colonies were observed in the truncated part of the zone between the erythromycin and clindamycin discs (e.g. as in Figure 1).

Strains could be divided into two groups on the basis of the inducing behaviour of erythromycin and oleandomycin. In organisms in group B_1 (15 *S. aureus*, 99 CNS), resistance to clindamycin, telithromycin, quinupristin and rokitamycin was induced by erythromycin and by oleandomycin.

The 15 strains in group B_2 (11 *S. aureus*, two *S. haemolyticus*, two *S. simulans*) were induced by erythromycin, but oleandomycin either did not induce (Figure 3) or had a variable effect, inducing resistance to some but not all of the agents depending on the strain. There was a disproportionate number of *S. aureus* strains in group B_2—73% compared with 20.1% in group B overall. All the group B strains had the phenotype M/i(LKS_BMac).

Group C: classical constitutive 'MLS_B'. Forty strains (five *S. aureus*, 35 CNS) with the phenotype $MLKS_BMac$ were classified as group C_1. Two other strains (*S. epidermidis* nos 152 and 538) were also resistant to dalfopristin (phenotype $MLKS_{AB}Mac$); they were classified as group C_2.

A zone of inhibition shaped like a shield (Figure 4) was seen when dalfopristin and quinupristin were tested side by side against group C_1 strains. The enhanced area of inhibition between the discs represents synergy between

Table III. Characteristics of five strains of coagulase-negative staphylococci resistant to lincomycin (phenotype A)

			Antibiotic sensitivity[a] and inactivation[b]		
Subgroup	Species	Strain no.	lincomycin	clindamycin	dalfopristin
A_1	*Staphylococcus epidermidis*	36	>16 (R) +	0.5 (S) ++	S
		384	>16 (R) +	0.25 (S) ++	S
A_2	*Staphylococcus sciuri*	406	16 (R) –	2 (I) –	64 (R)
		432	16 (R) –	1 (I) –	64 (R)
	Staphylococcus simulans	571	>16 (R) –	4 (R) –	32 (R)

[a]MIC in mg/L, susceptibility category (S, I, R) in parentheses.
[b]Degree of inactivation found indicated by symbols in brackets (see Materials and methods for experimental details).
++, complete inactivation within 24 h.
+, partial inactivation within 24 h.
–, no activation detected.

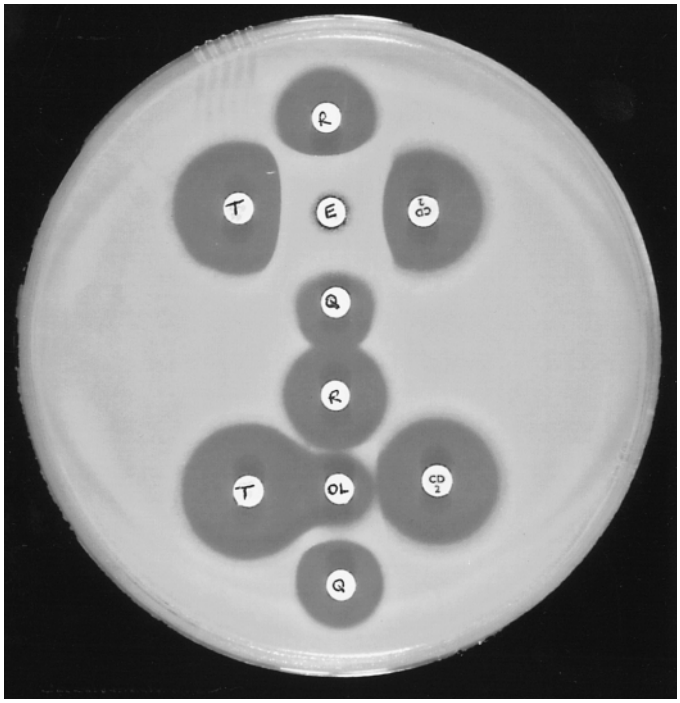

Figure 3. Differing behaviour of erythromycin and oleandomycin as inducing agents, illustrated using a strain from phenotypic group B_2. Top half: erythromycin (E) induces resistance to rokitamycin (R), clindamycin (CD2), quinupristin (Q) and telithromycin (T). Lower half: oleandomycin (OL) has little or no effect on activities of rokitamycin, clindamycin, quinupristin and telithromycin.

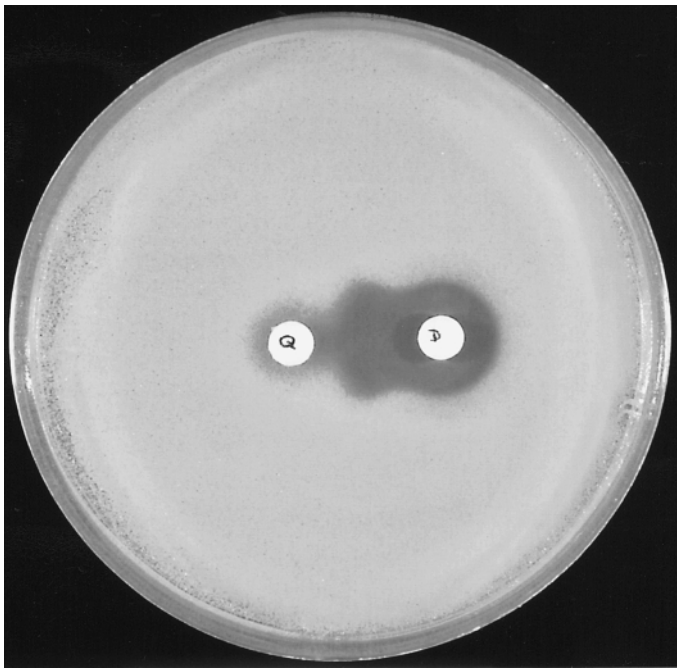

Figure 4. Shield-shaped inhibition zone between dalfopristin (right) and quinupristin (left) against a strain showing constitutive resistance (group C).

dalfopristin and quinupristin, despite the strains being resistant to the latter.

The five strains from our previous study that appeared constitutively resistant by conventional testing showed two further, novel, phenotypes. The two MRSA strains were ML (we have also seen this phenotype in diphtheroids, unpublished observations), and the three *S. haemolyticus* were $MLS_A/i(KS_B)$. We have called these phenotypes C_3 and C_4, respectively.

Group D: efflux mechanisms. Forty-six strains (two *S. aureus*, 44 CNS) were resistant to erythromycin and there was no induction of resistance to clindamycin. This pattern suggests 'active efflux'.[11] In the CNS strains, resistance to telithromycin and to quinupristin was induced by either erythromycin or oleandomycin, giving a phenotype of $M/i(KS_B)$; these were designated group D_1. In the *S. aureus* strains, however, no such induction occurred; these therefore had phenotype M, and were assigned to group D_2.

Further investigations on strains of different phenotypes

Inducibly resistant strains (group B). Twenty-one strains (13 *S. aureus*, eight CNS), made up of six B_1 and all 15 B_2 strains, were tested for the presence of mutants constitutively resistant to telithromycin. Ketolide-resistant colonies were isolated from 17 strains (81%), in greater numbers from B_1 strains (range 1 per 6×10^5–1 per 10^7, median 1 per 4×10^6) than in B_2 strains (range 1 per 10^6–1 per 10^9, median 1 per 10^8). The colonies isolated from 15 of these strains were C phenotype ($MLKS_BMac$) i.e. 'constitutively resistant'. Other novel phenotypes were found from colonies isolated during these experiments, including $MLKMac/iS_B$ (called C_5), from three *S. haemolyticus* strains.

Seventeen strains (nine *S. aureus*, eight CNS) were grown in the presence of ethidium bromide: clones sensitive to all MLKSMac antibiotics were isolated from one *S. aureus* and one *S. haemolyticus*.

The interactions between oleandomycin and telithromycin against B_2 strains were further investigated by the chequerboard method. For three strains (*S. simulans* nos 190 and 416, and *S. haemolyticus* no. 29), antagonism was found; for four (*S. aureus* nos 212, 342, 482 and 545) there was synergy; against the remaining eight strains each antibiotic behaved as if the other was not there ('indifference'). These results can be correlated with MICs of oleandomycin (Table IV): antagonism occurred for the highly resistant strains (MIC of oleandomycin $\geqslant 128$ mg/L), synergy for the least resistant strains (MICs 4–8 mg/L) and indifference in those strains for which MICs were intermediate (usually 8 or 16 mg/L).

B_2 strains were less resistant to oleandomycin than to erythromycin (Table IV), and several showed a small zone

of inhibition around the oleandomycin disc. The 'trailing endpoint' observed for erythromycin in the B_2 strains (Table IV) suggests some degree of heterogeneity. In contrast to the B_2 strains, fully sensitive strains were more sensitive to erythromycin than to oleandomycin, while B_1 strains grew in the presence of 128 mg/L of either macrolide.

Constitutively resistant strains (group C). Nine strains with constitutive resistance grown in the presence of ethidium bromide retained their phenotype.

Strains with efflux mechanisms. MICs were determined for 18 D_1 strains and the two D_2 strains (Table V). Six strains of the former sub-group and one of the latter (*S. aureus* no. 514) were plated on to agar containing 20 × MIC of telithromycin. Telithromycin-resistant colonies were found only from *S. aureus* no. 514; these were also resistant to quinupristin. Thus, a change of phenotype from M to MS_BK had occurred.

Compounds reported to be inhibitors of efflux pumps (reserpine, dinitrophenol and carbonylcyanide *m*-chlorophenyl hydrazone) had no effect on MIC of erythromycin against the 18 D_1 strains (results not shown). However, for the two D_2 strains *S. aureus* nos 321 and 514, dinitrophenol (but not the other compounds) reduced the MIC of erythromycin at least four-fold (to 32 and 2 mg/L, respectively).

Discussion

The availability of newer macrolides has resulted in greater use of this group of compounds, and this has, not surpris-

Table IV. Some characteristics of staphylococci of phenotype B_2

Species and strain no.	MIC (mg/L)		Telithromycin–oleandomycin interaction
	erythromycin[a]	oleandomycin	
Staphylococcus aureus 23	32/128	8	indifference
Staphylococcus haemolyticus 29	64/128	128	antagonism
MRSA 70	32/128	8	indifference
Staphylococcus simulans 190	64/128	128	antagonism
MRSA 203	32/128	16	indifference
S. aureus 206	16/128	8	indifference
S. aureus 212	2/4	4	synergy
S. haemolyticus 300	2/8	4	indifference
S. aureus 304	16/32	16	indifference
S. aureus 325	16/128	4	indifference
S. aureus 342	16/128	4	synergy
S. simulans 416	2/128	>128	antagonism
S. aureus 482	8/128	4	synergy
S. aureus 545	8/128	8	synergy
S. aureus 606	8/128	4	indifference

[a]Erythromycin showed a 'trailing end-point'. The first figure is the concentration at which heavy growth turns into light growth, the second figure where light growth stops.
MRSA, methicillin-resistant *Staphylococcus aureus.*

Table V. Activities of MSKMac antibiotics against staphylococci of phenotype D

Antibiotic	MIC (mg/L)				
	group D_1 (18 strains)			group D_2	
	range	mode	geometric mean	*Staphylococcus aureus* no. 321	*S. aureus* no. 514
Erythromycin	32–128	64	64	>128	8
Oleandomycin	16–128	64	44.7	>128	64
Rokitamycin	0.25–0.5	0.25	0.35	2	1
Quinupristin	1–4	1	1.4	8	2
Telithromycin	0.06–0.25	0.13	0.15	2	0.25

ingly, been followed by an increase in resistance to them. Although most attention has been paid in this respect to streptococci, it is clear that staphylococci have also been affected. In three recent studies[12–14] the incidences of resistance to erythromycin in *S. aureus* strains from Europe were 13–30%, and in the USA 20–50%. Most surveys[13,15–17] report that, as we found, CNS were more likely to be resistant than *S. aureus*. The situation in this hospital has changed since our last survey:[18] (i) the overall incidence of resistance to erythromycin in *S. aureus* has increased fivefold (from 3 to 15.7%), and the constitutive MLS_B phenotype has appeared in methicillin-sensitive *S. aureus*; (ii) in CNS, although the overall incidence of resistance is virtually unchanged (55% compared with 61%) and the MLS_B phenotype is still more frequently inducible than constitutive, the MS phenotype, absent previously, now makes up almost one-quarter of the resistant strains.

As expected, there was complete cross-resistance between the two 14-membered macrolides erythromycin and oleandomycin, while telithromycin, the 16-membered rokitamycin, clindamycin and quinupristin remained active against all but the strains with a constitutive MLS_B phenotype. This is because the latter four compounds do not induce the staphylococcal enzyme that confers resistance by ribosomal methylation.[19]

The experimental plan adopted in this investigation—testing the activities of and interactions between seven MLSK antibiotics including oleandomycin—has revealed novel phenotypes among clinical isolates and their laboratory derivatives. This further illustrates the considerable and apparently increasing complexity of resistance manifestations to this group of antibiotics,[11,19–23] as well as the shortcomings of conventional phenotyping in terms of susceptibility to erythromycin and clindamycin only (Table VI).

Phenotype A

Staphylococci of phenotype LS_A are unusual, and have previously been found mainly in *S. aureus*[24–26] and only where pristinamycin has been used clinically.[27] The three CNS strains found in the present study (two *S. sciuri* and one *S. simulans*) add to the six (four *S. sciuri*, two *S. haemolyticus*) reported previously.[27,28] Recent work[29] on *S. sciuri* suggests that this species is usually intrinsically resistant to dalfopristin, showing the LS_A phenotype.

Phenotype B

The behaviour of oleandomycin as an inducing agent enabled strains traditionally allotted to the 'inducible MLS_B' phenotype to be split into two groups. Despite having the same phenotype—M/i(LKS_BMac)—strains in group B_2 were less highly resistant to erythromycin than were group B_1 strains: none grew at 128 mg/L, and trailing end-points suggested heterogeneity. In contrast both to erythromycin-sensitive strains and B_1 strains, B_2 strains were more susceptible to oleandomycin than to erythromycin,

Table VI. Correlation of 'extended phenotype' classification with existing ('classical') scheme

Classical phenotype			Phenotype reported here		
erythromycin	clindamycin	epithet	group	resistances to MLKSMac	strains isolated
S	R	'LS_A'	A_1	L	2 *Staphylococcus epidermidis*
			A_2	LS_A	2 *Staphylococcus sciuri*
					1 *Staphylococcus simulans*
R	inducible	'inducible MLS_B'	B_1	M/i(LKS_BMac)[a]	15 *Staphylococcus aureus*, 99 CNS
			B_2	M/i(LKS_BMac)[b]	11 *S. aureus*, 2 *S. simulans*, 2 *Staphylococcus haemolyticus*
R	R	'constitutive MLS_B'	C_1	$MLKS_B$Mac	5 *S. aureus*, 35 CNS
			C_2	$MLKS_{AB}$Mac	2 *S. epidermidis*
			C_3	ML	2 MRSA[c]
			C_4	MLS_A/i(KS_B)	3 *S. haemolyticus*[c]
			C_5	MLKMac/iS_B	3 *S. haemolyticus*[d]
R	S	'MS'	D_1	M/i(KS_B)	44 CNS
			D_2	M	2 *S. aureus*
			D_3	MKS_B	1 *S. aureus*[d]

[a]Inducible by erythromycin and oleandomycin.
[b]Inducible by erythromycin only.
[c]Isolated in a previous study.[5]
[d]Selected by growth in the presence of telithromycin.

and often showed a small zone of inhibition around the oleandomycin disc. Whereas erythromycin induced resistance to clindamycin, telithromycin, quinupristin and rokitamycin in all group B strains, oleandomycin did so only in group B_1 strains: there was great variability in the interaction between oleandomycin and the other antibiotics for the group B_2 strains, and synergy was observed for some with telithromycin. Japanese workers[30,31] showed more than 30 years ago that oleandomycin and erythromycin may have different inducing abilities for certain staphylococci, but since then this phenomenon has been largely ignored.

Phenotype C

All the strains in this grouping would be classified as 'constitutively resistant' by conventional testing using only erythromycin and clindamycin. C_1 and C_2 strains had very similar phenotypes—$MLKS_BMac$ and $MLKS_{AB}Mac$, respectively. Another phenotype found during this investigation that would be classified loosely as 'constitutively resistant', but is in fact novel is that of the ketolide-resistant mutants selected from three *S. haemolyticus* strains from group B, whose phenotype was $MLKMac/iS_B$. It should be noted, however, that the phenotype found in the majority of phenotype B strains selected with telithromycin was the classical C_1 pattern.

Phenotype D

Strains that are resistant to erythromycin and sensitive to clindamycin (no induction) have been generally called 'MS' or 'PMS' in staphylococci,[32] and 'M phenotype' or novel resistance (NR) in streptococci.[33,34] The two *S. aureus* strains in this group (D_2) were resistant only to erythromycin and oleandomycin, in contrast to the CNS strains (D_1) in which resistance could be induced to quinupristin and telithromycin. Another difference was that the uncoupling agent dinitrophenol reduced the MICs of erythromycin for D_2 but not for D_1 strains.

Two additional novel phenotypes, MS_B/iK in *S. haemolyticus* and *Staphylococcus saprophyticus* (both D_1) and MS_BK in *S. aureus*, were produced by selection of these strains with telithromycin (Table VI).

Ketolides, the latest members of the macrolide group, show good activity against a wide range of Gram-positive species, including important respiratory pathogens. The survey reported above shows that telithromycin, which has the advantage over the quinupristin–dalfopristin combination of being orally bioavailable, is active against the great majority of staphylococci. Adding telithromycin to the battery of tests for resistance to the MLS antibiotic group has revealed some novel phenotypes, which may be of epidemiological interest.

From a clinical viewpoint, careful monitoring must be continued to determine the incidence of constitutive resistance, as such strains are insensitive to the $MLKS_B$ antibiotics. The relative ease of selection of constitutive mutants from inducible strains (group B→C conversion) also suggests that vigilance be exercised when novel members of this group are used to treat infections caused by inducible strains.

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