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Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*

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A diverse collection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates resistant to tetracycline was screened by PCR for the presence of the resistance determinants *tetK*, *tetL*, *tetM* or *tetO*. Twenty-four of 66 isolates had *tetM* alone, 21 had *tetK* alone and 21 had both *tetK* and *tetM* (*tetKM*). All isolates were *tetL*- and *tetO*-negative. MICs of tetracycline, doxycycline and minocycline were evaluated for all isolates with or without preincubation in the presence of subinhibitory concentrations of tetracycline or minocycline. All isolates with one or more tetracycline resistance determinants were resistant to tetracycline 8 mg/L without induction of resistance. Some MRSA isolates of each of these three genotypes showed an unexpected lack of resistance to tetracyclines when the disc diffusion or agar dilution method was applied to uninduced cells. Resistance to tetracycline and doxycycline was greater (two- to four-fold) in *tetK* cells preincubated with tetracycline (*tetK* MRSA isolates were susceptible to minocycline ≤0.25 mg/L under all conditions tested). For isolates with *tetM* alone, preincubation with tetracycline or minocycline gave up to a four-fold increase in the level of resistance to doxycycline and minocycline. Induction of doxycycline and minocycline resistance was clearly observed for *tetKM* isolates when cells were preincubated with minocycline. This study suggests that, despite the results of susceptibility testing, all tetracycline-resistant *S. aureus* isolates should be treated as resistant to doxycycline, and all *tetM*-positive isolates should be treated as resistant to all tetracyclines. A double disc diffusion method has been developed to identify inducible resistance to minocycline and to distinguish between *tetK*, *tetM* and *tetKM* isolates.

Introduction

Tetracyclines are broad-spectrum antibiotics that have been widely used in human and veterinary medicine, as growth promoters in animal husbandry and even to treat bacterial infections in plants.[1,2] Not surprisingly, tetracycline resistance is prevalent in a diverse range of bacteria, and is encoded by a wide range of determinants.[3] Nevertheless, these relatively inexpensive antibiotics are, in some countries, still the second most frequently prescribed antimicrobial agents (after penicillins) for the treatment of a number of bacterial infections, including those caused by staphylococci.[2,4] During the last two decades one of the most important problems for the control of nosocomial infections has been the spread and persistence of epidemic methicillin-resistant *Staphylococcus aureus* (MRSA) strains, common nosocomial pathogens that are resistant to all β-lactam drugs.[5–11] MRSA are frequently resistant to multiple drugs, having acquired resistance to a variety of drugs such as tetracyclines, aminoglycosides, macrolides, lincosamides and others.[5,6] Recently, MRSA with reduced susceptibility to glycopeptides have been described.[12] Control of the spread of MRSA strains requires the implementation of efficient infection control programmes and appropriate antibiotic policies, for which the proper identification of known and emerging mechanisms of resistance is crucial.

A number of susceptibility testing guidelines recommend that *S. aureus* strains are routinely tested for resistance to

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tetracycline, but it is not clear how to interpret the results of such tests as breakpoints for susceptibility and resistance have not been agreed.[7–11,13]

Two mechanisms of resistance to tetracyclines have been identified in *Staphylococcus* spp.: (i) active efflux resulting from acquisition of the plasmid-located genes, *tetK*[14–16] and *tetL*, and (ii) ribosomal protection mediated by transposon-located or chromosomal *tetM* or *tetO* determinants.[17,18] *S. aureus* strains carrying *tetK* only have been described as resistant to tetracycline, but susceptible to minocycline.[19,20] The *tetM* gene is believed to confer resistance to all available drugs of the group, including tetracycline and minocycline.[19] Most *tetM*-positive isolates also carry the *tetK* gene and MRSA isolates are typically of *tetM* or *tetKM* genotype.[19] The *tetL* gene has been found only in *S. aureus* isolates already carrying the *tetM* gene.[19] There are no reports of *tetO*-positive *S. aureus* strains. Both drug efflux and ribosomal protection are inducible in *S. aureus in vitro*.[15,17]

In this study, 66 randomly selected tetracycline-resistant MRSA isolates were examined with regard to genotype and expression of resistance. The aim was to establish a method for the phenotypic identification of resistance to tetracyclines in *S. aureus*, particularly MRSA isolates.

Materials and methods

MRSA strains

Sixty-six clinical isolates were analysed, 52 of which were collected by the Sera and Vaccines Central Research Laboratory (SVCRL) between 1992 and 1997 from 16 hospitals in nine Polish towns. These isolates had already been typed by various methods, including macrorestriction analysis of *Sma*I-digested chromosomal DNA.[21–27] Of the remaining 14 isolates, three were from Turkey, three from the UK, two each from Bulgaria, Latvia and Slovenia, and one each from Hungary and Russia; these were collected between 1995 and 1997. Species identification was based on colony morphology, Gram's stain, cell morphology, presence of catalase, and the tube coagulase test with rabbit plasma (Biomed, Krakow, Poland). *S. aureus* 25923, *S. aureus* 29213, *Enterococcus faecalis* 29212 and *Escherichia coli* 25922, obtained from the American Type Culture Collection (ATCC), were used for quality control of susceptibility testing. All isolates were stored in tryptic-soy broth (Lab M, Bury, UK) frozen at –70°C with glycerol at concentrations of 10–30%.

Susceptibility testing

Resistance to methicillin was detected by screening on tryptic-soy agar (TSA) (Oxoid, Basingstoke, UK) supplemented with methicillin 25 mg/L as previously described,[28] and confirmed by PCR-based detection of the *mecA* gene.[29]

Resistance to tetracycline was detected by screening on Mueller–Hinton agar (Oxoid) supplemented with tetracycline 5 mg/L. A quarter of the screening plate was inoculated with cells from a single colony harvested from the TSA after overnight incubation. Strains were classified as resistant when any growth was observed after 20 h incubation.

For all isolates, MICs of tetracycline, doxycycline and minocycline were evaluated both with and without induction of resistance. For each strain, a single colony from overnight growth on TSA was used to inoculate three different Mueller–Hinton agar plates: (i) unsupplemented medium (lack of induction); (ii) Mueller–Hinton agar with tetracycline 5 mg/L (induction of resistance by tetracycline); and (iii) Mueller–Hinton agar with minocycline 0.5 mg/L (induction of resistance by minocycline). After overnight incubation, harvested cells were used to determine MICs using NCCLS guidelines.[9]

Resistance to tetracyclines was also determined by the disc diffusion method with tetracycline, doxycycline and minocycline discs, using NCCLS-recommended procedures.[13] The double disc test technique was developed for identification of inducible resistance to minocycline. This involved placing a 30 μg tetracycline disc, a 30 μg minocycline disc and a 5 μg tetracycline disc in a straight line, 8–9 mm apart, with the minocycline disc in the centre (Figure). The zone diameter around the minocycline disc was measured horizontally (in the plane of the surface of the agar) and vertically, perpendicular to the first measurement.

All cultures were incubated in ambient air. Except for methicillin resistance detection this was done at 35°C.

Cefotaxime, doxycycline, minocycline and oxacillin powders were obtained from Sigma–Aldrich (Poole, UK) tetracycline from NBL Gene Sciences (Cramlington, UK) and methicillin from SmithKline Beecham Pharmaceuticals (Betchworth, UK). Tetracycline (30 and 5 μg), doxycycline (30 μg) and minocycline (30 μg) discs were from Oxoid.

Statistical methods

Friedman's test[30] was used to determine the significance of changes in MICs after preincubation of cells of the same genotype with tetracycline and minocycline. The Kruskal–Wallis test[30] was used to compare differences between MICs for strains of different genotypes subjected to the same treatments. When these differences proved significant, Dunn's procedure[30] was used to make pairwise comparisons between treatments in the first case, and between genotypes in the second. A P value of <0.05 was considered significant.

Detection of tetK, tetL, tetM and tetO by PCR

DNA templates were prepared as follows. Tryptic soy broth supplemented with oxacillin and cefotaxime at 6 mg/L was inoculated with a single colony of an MRSA strain. After incubation with vigorous shaking for 2–4 days at 30°C,

500 μL of culture was centrifuged at 15 000**g** for 3 min. Pellets were resuspended in 400 μL of PBS, harvested by centrifugation, resuspended in 100 μL of 20 mM Tris–HCl (pH 8.4), 50 mM KCl and 3 mM $MgCl_2$, incubated for 10 min at 99°C in a Touchdown thermocycler (Hybaid, Teddington, UK), and then immediately centrifuged for 2 min at 15 000**g** at 4°C. Supernatants were used as DNA template for PCR. Each reaction was carried out in 50 μL of mix containing 20 mM Tris–HCl (pH 8.4) and 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.5 mM of each primer, 2.5 U of *Taq* DNA polymerase (Gibco BRL, Paisley, UK) and 2 μL of DNA template. The following primers were used: for *tetK* detection, tetK-up (5′-TATTTTGGCTTTGTATTCTTTCAT-3′) and tetK-rev (5′-GCTATACCTGTTCCCTCTGATAA-3′); for *tetL* detection, tetL-up (5′-ATAAATTGTTTCGGGTCGGTAAT-3′) and tetL-rev (5′-AACCAGCCAACTAATGACAATGAT-3′); for *tetM* detection, tetM-up (5′-AGTTTTAGCTCATGTTGATG-3′) and tetM-rev (5′-TCCGACTATTTAGACGACGG-3′) and for *tetO* detection, tetO-up (5′-AGCGTCAAAGGGGAATCACTATCC-3′) and tetO-rev (5′-CGGCGGGGTTGGCAAATA-3′). The PCR consisted of 35 cycles of 1 min at 95°C, 1 min at 50°C, 1 min 30 s at 72°C, followed by a final 5 min at 72°C, except for *tetO*, for which the annealing temperature was 55°C. Amplified products were run in 1.5% ultraPure agarose (Gibco BRL) with ethidium bromide and photographed under UV light. Detection of the 1862 bp fragment from positions 21–1882 of the published sequence of the *S. aureus tetM* gene[17] and a 1159 bp amplicon from positions 47–1205 of the published sequence of the *S. aureus tetK* gene[16] was taken as indicative of the presence of the *tetM* and *tetK* gene, respectively. The expected product for the *tetO* gene was 1723 bp (base pairs 146–1868 of the published sequence of the *Streptococcus mutans tetO* gene[31]) and that for the *tetL* gene was an amplicon of 1077 bp (base pairs 262–1338 of the published sequence of the *Enterococcus faecalis tetL* gene[32]). A 1 kb DNA ladder (Gibco BRL) was used as a molecular weight marker.

Results

The 66 tetracycline-resistant clinical isolates analysed all carried at least one of the *tet* genes; based on the results of the PCR, 24 isolates were classified as *tetM* genotype, 21 as *tetK* and 21 as *tetKM*. All MRSA analysed were *tetO*- and *tetL*-negative. Isolates of the *tetKM* genotype originated from Bulgaria, Latvia, Russia and Poland. The *tetM* genotype was identified in MRSA isolates from Hungary, Poland, Slovenia, Turkey and the UK. All isolates of the *tetK* genotype were from Poland.

MIC results for all 66 MRSA isolates are presented in Table I. In the absence of induction, the MICs of all three antibiotics varied among genotypes. However, pairwise comparisons between tetracycline MICs for isolates of *tetK* and *tetM* genotypes, and minocycline MICs for isolates of *tetM* and *tetKM* genotype, showed no significant differences ($P > 0.2$). In contrast, levels of tetracycline resistance in *tetKM* genotype isolates were significantly higher than those for both single-gene clusters ($P < 0.0001$), and the MIC geometric mean was over four-fold greater. MICs of doxycycline for *tetM* isolates were significantly higher than those for *tetK* isolates ($P < 0.05$). Isolates of the *tetKM* genotype again had significantly higher MICs than both of the single-gene isolates ($P < 0.0001$), and the geometric mean was also over four-fold greater. All MRSA of the *tetK* genotype were susceptible to minocycline ≤0.25 mg/L; they were significantly more susceptible than the other genotypes ($P < 0.0001$). The highest MICs of tetracyclines were observed in isolates of the *tetKM* genotype.

When comparing the three treatments (no preincubation, tetracycline preincubation and, for the *tetM* and *tetKM* isolates only, minocycline preincubation), the MICs were found to vary with treatment for all but one of the nine antibiotic–genotype combinations (Table I). The exception was for minocycline MICs for the *tetK* isolates, where preincubation with a subinhibitory concentration of tetracycline increased the geometric mean MIC by a factor of only 1.07. This treatment did, however, lead to increases in the MICs of tetracycline (2.4-fold increase compared with MICs for uninduced cells) and doxycycline (ratio 3.6) for the *tetK* isolates.

Comparison of MIC geometric means for isolates of the *tetM* genotype before and after induction showed that the greatest increase in MICs of minocycline occurred when cells were preincubated with tetracycline (ratio 4.4) and after induction by minocycline (ratio 3.9). The lowest increase was observed for MICs of tetracycline when induced by tetracycline (ratio 1.2) or minocycline (ratio 1.5). For doxycycline MICs, a 1.7-fold increase in the geometric mean after preincubation with tetracycline and a 2.5-fold increase after minocycline treatment were observed. All six of these increases were statistically significant ($P < 0.05$).

Induction of tetracycline resistance was weak in *tetKM* isolates; although there was significant variation between the three treatment groups ($P = 0.025$), neither of the pairwise comparisons between induced and uninduced cells showed significant differences on their own ($P > 0.2$ for tetracycline preincubation; $0.05 < P < 0.1$ for minocycline preincubation). Corresponding increases in the MIC geometric means were 1.1-fold and 1.2-fold, respectively. In MRSA of the same genotype, MICs of doxycycline increased 1.4- and 2.4-fold, and MICs of minocycline 1.6- and 2.8-fold after tetracycline and minocycline pretreatment, respectively. All but the first of these were significant at the 5% level.

No significant differences were found in MICs of tetracycline and minocycline between isolates induced with tetracycline and those induced with minocycline. However,

Table I. MICs of tetracyclines for MRSA isolates

Genotype (number of isolates)	Induction by: tetracycline 5 mg/L	Induction by: minocycline 0.5 mg/L	Antibiotic	MIC (mg/L): range	MIC$_{50}$	MIC$_{90}$	geometric mean	Pairwise comparison[a] of no induction versus induction
tet K-positive (21)	–	–	tetracycline	32–128	64	64	56.08	
	+	–		128–256	128	256	132.3	$P < 0.001$
	–	–	doxycycline	2–4	4	4	3.281	
	+	–		8–32	16	16	11.89	$P < 0.0001$
	–	–	minocycline	0.12–0.25	0.25	0.25	0.196	
	+	–		0.12–0.25	0.25	0.25	0.210	$P > 0.2$
tetM-positive (24)	–	–	tetracycline	16–128	64	64	50.8	
	+	–		32–128	64	64	65.88	$P < 0.05$
	–	+		16–128	64	128	73.94	$P < 0.01$
	–	–	doxycycline	4–8	4	8	5.496	
	+	–		8–32	8	16	9.514	$P < 0.01$
	–	+		8–16	16	16	13.85	$P < 0.0001$
	–	–	minocycline	1–8	2	4	2.448	
	+	–		2–16	8	16	10.68	$P < 0.0001$
	–	+		2–32	8	16	9.514	$P < 0.0001$
tetK- and *tetM*-positive (21)	–	–	tetracycline	128–256	256	256	231.9	
	+	–		256	256	256	256.0	$P > 0.2$
	–	+		256–512	256	256	273.5	$0.05 < P < 0.1$
	–	–	doxycycline	16–32	32	32	24.57	
	+	–		32–64	32	32	34.18	$0.1 < P < 0.2$
	–	+		4–128	64	64	57.97	$P < 0.0001$
	–	–	minocycline	0.5–16	8	8	4.565	
	+	–		0.5–32	8	16	7.246	$P < 0.05$
	–	+		2–32	16	32	12.70	$P < 0.0001$

All *tetM*-negative isolates were susceptible to minocycline at the concentration used to induce resistance (0.5 mg/L).

[a]For *tetM* and *tetKM* genotypes Friedman's test was performed first. In each case the null hypothesis that induction has no effect on MICs was rejected. For tetracycline MICs and *tetKM* isolates, $0.01 < P < 0.05$. In all other cases, $P < 0.0001$.

isolates induced with minocycline showed significantly higher doxycycline MICs than those induced with tetracycline for both *tetKM* and *tetM* isolates ($P < 0.01$ and $P < 0.05$, respectively).

In 12 out of 21 *tetKM* isolates, MICs of minocycline were ⩾8 mg/L, whereas only two *tetM* isolates had MICs as high as 8 mg/L. Both the MIC_{50} and MIC_{90} were 8 mg/L for *tetKM* isolates; for *tetM* isolates the corresponding values were 2 and 4 mg/L, respectively. For four of the *tetKM* isolates, MICs of minocycline were as low as ⩽1 mg/L for uninduced cells, and no more than one dilution higher when tetracycline was used as an inducer. In two of these, preincubation with minocycline clearly induced resistance, giving an eight-fold increase from 1 to 8 mg/L for the first, and a 64-fold increase from 0.5 to 32 mg/L for the second. For the other two isolates, induction by minocycline was weaker (leading to a two-fold increase in MIC).

Results of tetracycline susceptibility testing by the disc diffusion method are presented in Table II. Zone diameters around the 30 μg minocycline disc were ⩾27 mm for *tetK* isolates and ⩽25 mm for *tetM* isolates. Lack of inhibition around the 30 μg tetracycline disc was indicative of the *tetKM* genotype as a zone of inhibition was observed with all other genotypes. In the double disc test, an oval zone of inhibition around the minocycline disc was observed for all *tetM* isolates (Figure): horizontal zone diameters were 1–5 mm (mean 3.3 mm) smaller than vertical diameters. For isolates of the *tetK* and *tetKM* genotype, zones around the minocycline disc appeared circular. Stronger induction of resistance to minocycline was achieved using a 5 μg tetracycline disc than with a 30 μg disc (data not shown). Analysis of the results for all three tetracyclines generally showed good correlation between the agar dilution method using uninduced cells and the disc

Table II. Testing of MRSA strains for susceptibility to tetracyclines by disc diffusion

MRSA strains (number of isolates)	Zone diameter (mm)					
	tetracycline 30 μg		doxycycline 30 μg		minocycline 30 μg	
	range	mean	range	mean	range	mean
tetK-positive (21)	9–12	10.3	8–16	14.1	27–33	29.7
tetM-positive (24)	7–17	10.6	12–18	14.9	15–21	18.8
tetKM-positive (21)	6	6	8–12	9.1	13–25	16.3

[a]For minocycline 30 μg discs, the zone diameters were measured without induction of resistance (vertical measurement in double disc test).

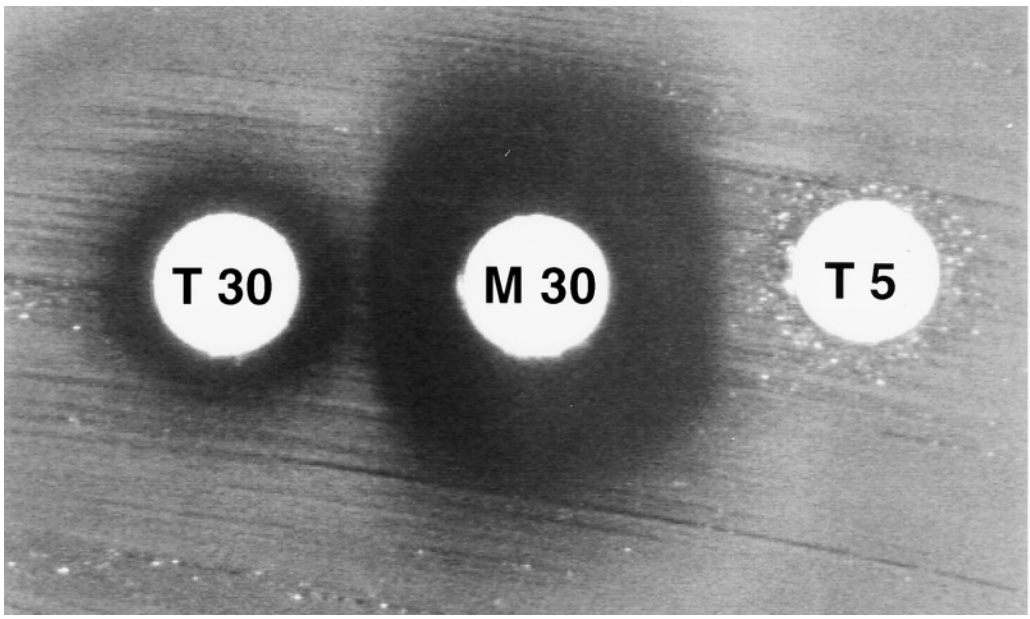

Figure. Positive results of double disc test in identification of the *tetM* phenotype in an MRSA strain. A 30 μg minocycline disc (M30) is in the centre, with 30 and 5 μg tetracycline discs (T30 and T5, respectively) about 8–9 mm away from it. Note the oval zone of inhibition around the minocycline disc.

diffusion method. An exception was doxycyline testing of isolates of the *tetK* genotype, for which zone diameters around the 30 μg doxycycline disc were smaller than would be expected from the doxycycline MICs.

Discussion

Tetracycline resistance is the second most common resistance phenotype in MRSA strains isolated in Poland. Epidemic MRSA in Poland can be divided into two types according to whether resistance to methicillin is expressed homogeneously or heterogeneously.[23,24] Isolates of the former type are resistant to multiple drugs, usually aminoglycosides, macrolides, lincosamides and other antistaphylococcal drugs. Strains of the latter type are generally susceptible to a wider range of drugs. However, resistance to tetracycline and doxycycline is common in both types.[21,24] The reported prevalence of tetracycline resistance among isolates of MRSA varies from *c*. 50% in centres where heterogeneous MRSA dominate,[22,23,27] to >95% in hospitals with homogeneous MRSA.[23–26] Tetracycline resistance is also prevalent in MRSA in Bulgaria and Turkey.[33,34] Resistance to tetracyclines was also common in MRSA in England and Wales before the mid-1990s;[6] since then, a decrease in resistance has been observed, probably connected with the nationwide spread of epidemic strains (EMRSA-15 and -16) susceptible to tetracycline.[35] No data on antibiotic resistance of MRSA from Hungary, Latvia, Russia and Slovenia are available.

PCR characterization of the *tet* determinants in the isolates selected for this study showed that they comprised three different genotypes. The *tetK* genotype was identified only in Polish isolates heterogeneously resistant to methicillin. This is the first report of a pure *tetK* genotype in MRSA. The other two genotypes identified, namely *tetM* alone and *tetKM*, were observed in Polish *S. aureus* isolates homogeneously resistant to methicillin and in isolates from other European countries.[19]

As reported previously,[19,20] *S. aureus* isolates of the *tetK* or *tetM* genotype were resistant to tetracycline both by breakpoint and agar dilution methods, and all isolates of the *tetK* genotype were susceptible to minocycline, irrespective of the breakpoints used. However, when French breakpoints were applied, the agar dilution method led us to identify *tetK*- and *tetM*-positive isolates as susceptible to doxycycline, and both agar dilution and diffusion methods indicated that some *tetM* and *tetKM* isolates were susceptible to minocycline.[11] Using NCCLS recommendations, some *tetM* isolates would be identified as intermediately susceptible to tetracycline by the disc diffusion method, and some *tetK* and *tetM* as intermediately susceptible to doxycycline by both methods.[9,13] In contrast, all 66 isolates would be identified as resistant to tetracycline and resistant or intermediately susceptible to doxycycline according to the Scandinavian breakpoints for both MICs and zone diameters.[9] There are no NCCLS or Scandinavian recommendations for minocycline breakpoints. Current BSAC guidelines do not mention testing staphylococci against tetracyclines.[8] However, according to MIC breakpoints previously recommended by the BSAC, all strains in this study would be identified as resistant to tetracycline.[36] All the breakpoints applied, for both agar dilution and disc diffusion, unanimously indicated full resistance to tetracycline and doxycycline only in the *tetKM* genotype.

It was confirmed that higher levels of resistance can be induced by subinhibitory concentrations of tetracyclines for both the resistance mechanisms described. After induction, all isolates were resistant to tetracycline and doxycycline according to all the guidelines applied, and an increase in resistance to minocycline was observed in *tetM* isolates. This is the first time that minocycline and tetracycline have been documented to 'cross-induce' resistance to each other in *tetM*-positive *S. aureus* isolates *in vitro*. An elevated level of tetracycline resistance in strains harbouring both genes has been described previously.[19,20] Here, higher levels of resistance for *tetKM* isolates were also observed for doxycycline and minocycline.

For the identification of tetracycline-resistant genotypes, the double disc test appears to be an alternative to molecular methods. The data suggest that lack of any zone of inhibition around a 30 μg tetracycline disc or a high level of resistance to tetracycline (MIC ⩾ 128 mg/L) and doxycycline (⩾16 mg/L) may predict the presence of *tetKM*. The presence of any zone of inhibition around a 30 μg tetracycline disc or low-level resistance to tetracycline (MICs ⩽ 64 mg/L) or doxycycline (MICs ⩽ 8 mg/L), together with a positive result in the double disc test (i.e. identification of inducible resistance to minocycline), predicts the *tetM* genotype. This result would also indicate resistance to all tetracyclines. In the case of low-level resistance to tetracycline and negative results in the double disc test (i.e. lack of induction), the *tetK* genotype and minocycline susceptibility might be predicted. MRSA strains in which resistance to tetracycline was identified were always resistant to doxycycline.

Double disc tests are already used for detecting other mechanisms of resistance (e.g. inducible-MLS_B in Gram-positive cocci,[37] chromosomally mediated β-lactamases[38] and extended-spectrum β-lactamases in Gram-negative bacilli[39]). The double disc test seems to be useful in the proper identification of the inducible *tetM* genotype, at least in MRSA. There is no need for the precise measurement of a zone of inhibition around a 5 μg tetracycline disc; using a 5 μg disc instead of a 30 μg one improves the expression of inducible resistance (Figure). If a 5 μg disc is not available, it could be replaced by a quarter of a 30 μg tetracycline one, though standardization of such a test might be difficult. The zones of inhibition around the doxycycline discs were relatively small considering the low doxycycline MICs for *tetK* isolates. This can be explained by induction of resistance by the drug as it diffuses into the agar.

Because MICs were determined using two-fold concentration increments, the statistical significance of the results was assessed using non-parametric tests. In eight of the nine genotype–antibiotic combinations, significant differences in the MICs between the three different treatments were found. In such cases pairwise comparisons between treatments can also be made. There is a strong case for using a higher overall level of significance than the traditional 5% when making such comparisons, since significant variation has already been found.[30] For this reason we report P values of <0.2 in Table I.

The data presented here indicate the importance of the proper recognition of tetracycline resistance mechanisms in *S. aureus* strains, and the necessity of revising existing breakpoints and interpretation guidelines. The consequences of failing to identify these mechanisms correctly, and of misclassifying strains likely to have inducible resistance as susceptible (as observed for minocycline in *tetM*-positive isolates[19]), need to be considered. It is possible that the prevalence of resistance to tetracyclines in *S. aureus* has been underestimated owing to the false identification of susceptibility. The importance of the induction of resistance for the outcome of infections when treated by drugs of this group should not be underestimated.

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