

Induction of clindamycin resistance in erythromycin-resistant, clindamycin susceptible and methicillin-resistant clinical Staphylococcal isolates

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ABSTRACT

Objectives: To demonstrate the in vitro ability of erythromycin to induce clindamycin in erythromycin resistant and clindamycin susceptible clinical isolates of *Staphylococci*.

Methods: We studied 291 clinical isolates of erythromycin-resistant (ER-R) clindamycin-susceptible *Staphylococci* (CL-S) at Almana General Hospitals, Al-Khobar, Dammam, Saudi Arabia during the period from June 2004 to May 2005. The isolates included 70 *Staphylococcus aureus*, 81 Methicillin Resistant *Staphylococcus aureus* (MRSA) and 140 coagulase-negative *Staphylococci* (CNS). We examined these isolates for inducible clindamycin resistance (ICR) by erythromycin induction test using double disc susceptibility test (D-test). Strains producing ICR show flattening of the clindamycin disc zone adjacent to the erythromycin disc.

Results: Of the 291 ER *Staphylococci* studied, 82 (28%) demonstrated constitutive clindamycin resistance [2 (2.9%) *S. aureus*, 43 (53%) MRSA and 37 (26%)

CNS]. Inducible clindamycin resistance was demonstrated in 113 (38.8%) of *Staphylococcal* isolates, including 84 (28.9%) from adult patients and 29 (10%) from pediatric patients. The incidence of ICR was 49 (70%) for *S. aureus*, 35 (43%) for MRSA and 29 (20.7%) for CNS. Overall, 96 (33%) of the isolates remained susceptible to clindamycin and were negative for clindamycin induction [19 (27%) *S. aureus*, 3 (3.7%) MRSA and 74 (52.8%) CNS].

Conclusion: We conclude that a significant number of ER-R CL-S staphylococcal isolates studied were positive for ICR. These isolates should be reported as clindamycin resistant. Given the high rate of inducible resistance to clindamycin in the staphylococcal isolates, we recommend that microbiology laboratories perform erythromycin induction test on all ER-R CL-S staphylococcal isolates prior to reporting clindamycin susceptibility.

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Macrolides (erythromycin), and lincosamides (clindamycin) antibiotics are frequently used for the treatment of staphylococcal skin and soft-tissue infections, particularly in penicillin-allergic patients. However, the increasing resistance of *Staphylococci* to macrolides has limited use. High

resistance to macrolides and lincosamides are increasingly being reported in *Methicillin Resistant Staphylococcus aureus* (MRSA).¹ Erythromycin resistance in *Staphylococci* may be due to an active efflux mechanism (encoded by *msrA*) causing resistance to macrolides and type B streptogramins

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(MSB) only or may be due to ribosomal target modification by erythromycin resistant methylases (*erm* genes) resulting in resistance to macrolides, lincosamide and type B streptogramins (MLSB).^{2,3} The MLSB resistance can be either constitutive or inducible. Strains with constitutive resistance show in vitro resistance to erythromycin and clindamycin, as active methylated mRNA is produced in the presence or absence of an inducer.⁴ The constitutive clindamycin resistance can normally be detected by standard Kirby-Bauer disc methods. Strains with inducible clindamycin resistance (ICR) demonstrate in vitro resistance to erythromycin, but appear susceptible to clindamycin.⁵ In these strains, erythromycin will effectively induce the *erm* gene and appear resistant while clindamycin is a poor inducer and therefore may appear sensitive. The ICR is not detected by standard susceptibility methods and requires the performance of an additional test.^{6,7} The ICR can be identified by erythromycin induction test commonly referred to as the D-test.⁸ In this test, strains with ICR will show flattening of the zone of inhibition around the clindamycin disc when an erythromycin disc is placed nearby forming a D-shape. In inducible strains, erythromycin induces the production of the methylase, which allows clindamycin resistance to be expressed; in non-inducible strains, clindamycin sensitive isolates remains susceptible to clindamycin. The prevalence of ICR among the erythromycin-resistant clindamycin susceptible (ER-R CL-S) clinical isolates of *Staphylococci* from our microbiology laboratory is more than 35%. As reporting clindamycin susceptibility for ER-R CL-S *Staphylococci* without checking for ICR may lead to clinical failure of clindamycin therapy,^{6,7,9} we carried out this study to demonstrate the incidence of ICR among the ER-R CL-S isolates of *Staphylococci* over a 12 month period.

Methods. Consecutive, non-repeated isolates of *Staphylococcus aureus* (*S. aureus*), MRSA and coagulase-negative *Staphylococci* (CNS) from patients at the Almana General Hospitals, Al-Khobar, Dammam, Saudi Arabia, were included in the study. The study period covers from June 2004 to May 2005. Identification of *S. aureus* and CNS were carried out by standard techniques.¹⁰ Methicillin resistance was detected according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations.¹¹ A total of 291 ER-R CL-S staphylococcal isolates were tested for ICR. Susceptibility to clindamycin (2µg) and erythromycin (15µg) was performed by the standard disc diffusion method as per NCCLS guidelines.¹¹ Demonstration of ICR was performed based on the standard NCCLS double disc diffusion test (D-test) on each isolate using Mueller-Hinton agar (Becton Dickinson) and standard 15µg erythromycin discs

and 2µg clindamycin discs (Mast Diagnostic).⁸ In the D-test, a 15µg erythromycin disc and 2µg clindamycin disc were placed on the plate in the area streaked for confluent growth, with a distance of 15 mm from disc edge to disc edge. Following incubation, ICR was identified by flattening of the clindamycin zone between the erythromycin and clindamycin discs (Figure 1). The ICR test was considered negative in the absence of flattening of clindamycin zone. Quality control of the erythromycin and clindamycin discs was performed with *S. aureus* ATCC 25923 according to the standard disc diffusion quality control procedure.¹¹

Results. A total of 291 erythromycin resistant clinical *Staphylococcal* isolates from adult (n=238) and pediatric (n=53) patients were tested for clindamycin resistance. The *Staphylococci* studied included 70 *S. aureus*, 81 MRSA and 140 CNS. Of the 291 isolates, 82 (28%) demonstrated constitutive resistance to clindamycin [2 (2.9%) *S. aureus*, 43 (53%) MRSA and 37 (26%) CNS]. Two different phenotypes were distinguishable for the ER-R CL-S *Staphylococci*: a novel phenotype that remained susceptible to clindamycin and strains positive for ICR. Of the 291 *Staphylococcal* isolates tested, 113 (38.8%) demonstrated ICR. The ICR was detected in 49 (70%) of *S. aureus*, 35 (43%) of MRSA and 29 (20.7%) of CNS. Eighty-four (28.9%) of the ICR strains were from adult patients [34 (72%) *S. aureus*, 29 (38.7%) MRSA and 21 (18%) CNS] and 29 (10%) from pediatric patients [15 (65%) *S. aureus*, 6 (100%) MRSA and 8 (33.3%) CNS] (Table 1). Overall, 96 (33%) of *Staphylococcal* isolates that exhibited the ER-R CL-S phenotype did not demonstrate ICR [19 (27%) *S. aureus*, 3 (3.7%) MRSA and 74 (52.8%) CNS] (Table 2).

Discussion. In this study, we used a simple method to detect ICR in the ER-R CL-S isolates of *S. aureus*, MRSA and CNS. We detected ICR in 38.8% of the *Staphylococcal* isolates. In a study from University of Iowa, 62% of the ER-R CL-S staphylococcal isolates demonstrated ICR.¹² Our isolates of *S. aureus* showed the lowest rate of constitutive resistance to clindamycin (2.9%) and highest inducible resistance (70%). The percentage of ICR in the *S. aureus* was close to that reported in an Indian study (76%).¹³ In our study, ICR in the *S. aureus* (70%) and MRSA (43%) isolates were higher than those reported in a study from 2 USA hospitals in which the incidences of ICR were 20 and 19% for *S. aureus*, 7 and 12% for MRSA.¹⁴ We demonstrated ICR in 20.7% of the erythromycin resistant CNS, which was lower than the 30% reported in a USA study.¹⁵ Although it is not clear to what extent the presence of ICR in vitro predicts clindamycin failure in vivo, a number of studies



Figure 1 - D-test showing a flattening of the clindamycin zone between the erythromycin (E) and clindamycin (DA) discs.

have reported clindamycin therapy failures in serious infections caused by *Staphylococci* expressing ICR.^{7,16,17} Variable clinical outcomes have been reported for clindamycin therapy of infections caused by *S. aureus* or MRSA exhibiting ICR.^{6,18-20} In a report by Drinkovic et al,⁶ one of 2 patients infected by MRSA responded to clindamycin therapy; a third patient with a foot infection due to *S. aureus* had a successful outcome with clindamycin therapy. In another report by Gopal Rao,¹⁹ a satisfactory clinical outcome was obtained in 2 patients treated with clindamycin for cellulitis caused by MRSA exhibiting ICR; a third patient with cellulitis and bacteremia failed clindamycin treatment. Many clinicians may be

Table 1 - Susceptibility of constitutive and inducible clindamycin resistance among the erythromycin resistant clindamycin susceptible staphylococcal isolates.

Bacteria	Number of isolates (%)			
	ER-R CL-R	ER-R CL-S	ER-R CL-SD⁺	ER-R CL-SD⁻
<i>Staphylococcus aureus</i>				
Adults (n=47)	2 (4)	45 (95.7)	34 (72)	11 (23)
Pediatrics (n=23)	0	23 (100)	15 (65)	8 (34.8)
<i>Methicillin resistant Staphylococcus aureus</i>				
Adults (n=75)	43 (57)	32 (42.6)	29 (38.7)	3 (4)
Pediatrics (n=6)	0	6 (100)	6 (100)	0
<i>Coagulase negative Staphylococci</i>				
Adults (n=116)	30 (25.9)	86 (74)	21 (18)	65 (56)
Pediatrics (n=24)	7 (29)	17 (70.8)	8 (33.3)	9 (37.5)
ER - erythromycin, CL - clindamycin, R - resistant, S - susceptible, D ⁺ - positive for inducible clindamycin resistance D ⁻ - negative for inducible clindamycin resistance				

Table 2 - Susceptibility to clindamycin among all *Staphylococci*.

Organism	Number of resistance (%)		
	Constitutive	Inducible	Susceptible D-
<i>Staphylococcus aureus</i> (n=70)	2 (2.9)	49 (70)	19 (27)
<i>Methicillin resistant Staphylococcus aureus</i> (n=81)	43 (53)	35 (43)	3 (3.7)
<i>Coagulase negative Staphylococci</i> (n=140)	37 (26)	29 (20.7)	74 (52.8)
Total (n=291)	82 (28)	113 (38.8)	96 (33)
D ⁻ - negative for inducible clindamycin resistance			

discouraged from using clindamycin for therapy of infection with MRSA expressing ICR because of concern over the possibility of emergence of clindamycin resistance during therapy. Clinicians should be cautious about the use of clindamycin in treating major infections caused by MRSA expressing ICR, as clinical failures may occur. Because clindamycin susceptible staphylococcal strains expressing ICR have the genetic potential to become resistant to clindamycin during therapy,²¹ there is a need for clinical evaluation of clindamycin efficacy in patients infected by these organisms. Erythromycin resistant *Staphylococci* should not be routinely considered clindamycin resistant without testing for inducible resistance, as doing so will deny a potentially effective therapy for patients infected by the truly susceptible strains. We found that 27% of isolates of *S. aureus* and 52.8% of CNS were susceptible to clindamycin and were non-inducers suggesting a possible role of clindamycin therapy for infections by such strains. Other reports have also demonstrated the existence of non-inducible clindamycin sensitive isolates that remained susceptible to clindamycin even after repeated passage in the presence of clindamycin.^{13,18} If inducible resistance to clindamycin is looked for on a routine basis, it can be used in those patients infected by truly clindamycin-susceptible strains. This testing is especially important in view of increasing resistance of *Staphylococci* to many antibiotics. The results of this study showed a high percentage of ICR in the clinical isolates of *Staphylococci* that were susceptible to clindamycin by the standard susceptibility test methods. As accurate susceptibility data are crucial for appropriate therapy decisions, we recommend that microbiology laboratories screen ER-R CL-S staphylococcal isolates for ICR before reporting clindamycin susceptibility.

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