Original Article

Inducible Clindamycin Resistance among Staphylococci Isolated from Burn Patients

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ABSTRACT

Clindamycin has been used successfully to treat pneumonia and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*. However, inducible clindamycin resistance has been described as a cause of treatment failure of such infections. A total of 159 staphylococcal isolates from different clinical specimens from burn patients in Tripoli Burn Center were tested for inducible clindamycin resistance by the disk-diffusion induction test. Inducible clindamycin resistance was detected in 66.2% of 65 methicillin-resistant *S. aureus* isolates and in none of 55 methicillin-sensitive *S. aureus*, 10 methicillin-resistant coagulase negative staphylococci and 29 methicllin-sensitive coagulase negative staphylococci isolates. In our setting, clindamycin can be used for the treatment of infections due to staphylococci, but we recommend that staphylococci isolates, particularly methicillin-resistant *S. aureus*, are tested by the D-test before treatment.

INTRODUCTION

Burn patients are extremely susceptible to infections caused by Gram-positive organisms, particularly staphylococci [1]. *Staphylococcus aureus* has been recognized as one of the major causes of nosocomial infections worldwide, and its resistance to antimicrobials has complicated the treatment of infections due to these microorganisms. Clindamycin is frequently used to treat some staphylococcal infections, particularly skin and soft-tissue infections, and as an alternative in penicillin-allergic patients [2]. In addition, clindamycin has been shown to inhibit the production of *S. aureus* toxins, including Panton-Valentine Leukocidin toxin [3].

One of the major concerns regarding the use of clindamycin to treat staphylococcal infections is the possible presence of inducible resistance to clindamycin (ICR) [4,5]. In *S. aureus* and coagulase-negative staphylococci (CoNS), resistance to macrolides (*e.g.* erythromycin), lincosamides (e.g. clindmycin) and type B streptogramins (MLSB) can be the result of ribosomal target modification in which enzymes encoded by *erm* genes confer constitutive or inducible resistance to MLS drugs through methylation of the 23S rRNA [6]. Also, staphylococci can have an active efflux mechanism (encoded by *msrA* genes) that confers resistance to MSB only, but not to lincosamides [7,8]. Strains with constitutive resistance can be detected readily by standard susceptibility testing methods [9]. When tested by standard methods, clindamycin may appear active against staphylococci with IRC, and so this mode of resistance is identified by the disk-diffusion induction test (D-test) [6,7,10].

Frequencies of the different resistance patterns vary by geographic location, patient age, bacterial species, and bacterial susceptibility profile [11-17]. Because the incidence of ICR varies between hospitals [18], it is important to determine the prevalence of ICR in individual settings [9]. There are no data on the prevalence of ICR among staphylococci from clinical sources in North African countries, including Libya. The aim of the present work was to determine the prevalence of ICR among staphylococci isolated from infected sites of burn patients in Tripoli Burn Center.

MATERIALS AND METHODS

We examined 159 clinically significant, non-duplicate staphylococci isolated from different body sites of burn patients between January

and December 2007, at the Burn and Plastic Surgery Centre, Tripoli, Libya. Most (90%) were from swabs taken from skin burn wounds, 3% were from urine, 2% from blood, and 5% from other specimens. There were 65 MRSA, 55 methicillin-sensitive *S. aureus* (MSSA), 10 methicillin-resistant coagulase negative staphylococci (MRCoNS), and 29 methicllin-sensitive CoNS (MSCoNS). *S. aureus* and CoNS were identified by using standard bacteriological techniques [19]. Methicillin resistance was detected employing the cefoxitin disc diffusion test (Center for Disease Control and Prevention [http://www.cdc.gov/ncidod/dhqp/ar_lab_mrsa.html]) and confirmed by PBP2a agglutination test. The isolates were tested for susceptibility to clindamycin (2 µg) and erythromycin (15 µg) according to CLSI criteria [20]. Quality control was performed with *S. aureus* strain ATCC 25923 (American Type Culture Collection, Manassas, VA, USA). Isolates that were erythromycin-resistant (ER-R) and clindamycin-sensitive (CL-S) were tested for inducible resistance by the D-test. Erythromycin and clindamycin discs were placed 15 mm apart (edge to edge) on Mueller Hinton agar plate. Following incubation at 35°C for 17 hours, D-test positivity (ICR) was identified by flattening of the clindamycin zone between the erythromycin and clindamycin discs. The D-test was considered negative in the absence of flattening of the clindamycin zone. If the isolate was ER-R and CL-R, the isolate was considered to have a constitutive MLS_B (MLS_{Bc}) phenotype [2,18,21]. Unless stated otherwise, all materials used in the present work were obtained from Oxoid, Basingstoke, UK.

RESULT

Of the 159 staphylococci tested, 154 (96.9%) and 87 (54.7%) were susceptible to clindamycin and erythromycin, respectively. Susceptibility to both drugs was found in 87 (54.7%) of staphylococci examined (Table 1). On the other hand, resistance to both clindamycin and erythromycin (ER-R CL-R phenotype), which indicates MLS_{Bc}, was detected in only five isolates (3.2%); four were MRSA and one methicillin-resistant CNS (MRCNS). ICR was detected in 66.2% of the 65 MRSA isolates and in none of 55 MSSA, 10 MRCoNS and 29 MSCoNS isolates. Susceptibility to erythromycin and clindamycin among the 159 staphylococci isolates examined is shown in Table 1.

Table 1: Susceptibility to clindamycin and erythromycin among staphylococci isolated from burn patients.

Isolate*	Phenotype**			
	ER-R CL-R (constitutive) No. positive (%)	ER-S CL-S No. positive (%)	ER-R CL-S (inducible, D+) No. positive (%)	ER-R CL-S (susceptible, D-) No. positive (%)
MRSA (n=65)	4 (6.2)	13 (20)	43 (66.2)	5 (7.7)
MSSA (n=55)	0 (0.0)	50 (90.9)	0 (0.0)	5 (9.1)
MRCNS (n=10)	1 (10.0)	8 (80.0)	0 (0.0)	1 (10.0)
MSCNS (n=29)	0 (0.0)	16 (55.1)	0 (0.0)	13 (44.8)
Staphylococci (n=159)	5 (3.2)	87 (54.7)	43 (27.0)	24 (15.1)

*MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-susceptible *S. aureus*, MRCNS = methicillin-resistant coagulase negative staphylococci, MSCNS = methicillin-susceptible CNS.
**ER = erythromycin, CL = clindamycin, R = resistant, S = susceptible.

DISCUSSION

We examined 159 staphylococci isolates from burn patients for their susceptibility to clindamycin and erythromycin. The isolates were also tested for inducible and constitutive clindamycin resistance. More than 96% (154/159) and more than 54% (87/159) of isolates were susceptible to clindamycin and erythromycin, respectively. Azap et al. [9] from Turkey examined 408 staphylococci isolates from different clinical sources for susceptibility to clindamycin and erythromycin. They reported that 68% of their isolates were susceptible to clindamycin and 48.5% for erythromycin. They also found that 32% of the isolates were resistant to both clindamycin and erythromycin (MLS_{Bc} phenotype); these were mainly MRSA and MRCNS isolates. Similar to their findings, we found resistance to both drugs only among MRSA and MRCoNS.

However, the prevalence of resistance to both clindamycin and erythromycin among our staphylococci isolates was much lower (3%) than that reported by Azap et al [9]. Clindamycin is not frequently used at TBC for treatment of burn patients and this may explain the very high susceptibility rate (>96%) of staphylococci examined in the present study to this drug.

Recently, Farrell et al [22] examined 750 clinically significant *S. aureus* from five European countries. They reported ICR in 38% of community-acquired MRSA (CA-MRSA), in 6.7% of healthcare-associated MRSA (HA-MRSA), and in 63.6% of MSSA. A study from Saudi Arabia tested 291 clinical isolates of ER-R CL-S staphylococci [23]. It reported ICR in 43% of 81 MRSA, 70% of 70 MSSA, and 20.7% of 140 CNS. The study also found constitutive resistance (MLS_{Bc}) in 53%, 2.9% and 26% of MRSA, MSSA and CNS isolates, respectively. Here, we found MLS_{Bc} in 6.2% (4/65) of MRSA, 10% (1/10) of MRCoNS, and none of MSSA and MSCoNS. The difference between the prevalence of inducible and constitutive clindamycin resistance among our staphylococcal clinical isolates and those reported from the above-mentioned studies might be due to the type of patients studied in addition to the type of clinical specimens examined and differences in geographical location. Our staphylococci isolates were obtained from burn patients whereas the previously cited investigations were obtained from patients attending non-specialized general hospitals [22,23].

Failure of therapy with clindamycin in serious infections due to staphylococci with inducible MLS_B resistance is not uncommon. This led to questioning the safety of using clindamycin for any erythromycin-resistant staphylococci [2,10,24,25]. We detected ICR in 27% of staphylococci examined and found only in MRSA. Given that most burn infections are in the skin and soft-tissues, clindamycin is an attractive treatment for such infections because of its tolerability, low cost, oral adminsitration, and good tissue penetration [2,25]. Due to the restricted range of antibiotics available in Libya for the treatment of staphylococci infections, including MRSA, and the known limitation of vancomycin, clindamycin should be considered for the management of serious soft tissue infections in burn patients. However, to report clindamycin susceptibility accurately, staphylococci isolated from clinical specimens should first be subjected to the D-test to exclude isolates with ICR. Our findings indicate that erythromycin resistance is often caused by active efflux in Libya, especially in methicillin-susceptible isolates, which means that clindamycin can be used in these situations and ICR testing can be used to confirm susceptibility.

The D-test is simple, easy to perform and requires minimal resources. Therefore, we recommend that whenever clindamycin is intended for treatment of infections caused by staphylococci, particularly by MRSA, the isolated organism should be tested for ICR by the D-test before reporting clindamycin susceptibility. We hope that this policy will be adopted by the health authorities in Libyan hospitals and clinics. In the future, more studies from other hospitals are required to obtain a clearer picture of the prevalence of ICR among staphylococci in North Africa.

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