Inducible Clindamycin Resistance among Methicillin-Sensitive *Staphylococcus Aureus* Infections in Pediatric Patients

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ABSTRACT: **Background:** *Staphylococcus aureus* infections are a major cause of morbidity and mortality worldwide. Clindamycin is widely used in the treatment of staphylococcal infections; however, it is our impression that in the last few years, inducible clindamycin resistance (ICR) has become more prevalent.
Objective: To assess the prevalence of ICR in methicillin-sensitive *Staphylococcus aureus* (MSSA) infections among pediatric patients in Israel.
Methods: We reviewed the files of children diagnosed with MSSA infections during the period January 2006 to June 2007 for full antibiogram (including the D-test for ICR), phage typing and randomly amplified polymorphic DNA.
Results: Altogether, 240 MSSA isolates were recovered, mainly from wounds and abscesses. ICR was detected in 62 of 68 erythromycin-resistant/clindamycin-sensitive strains (91%); the ICR rate for the total number of isolates was 26% (62/240). Phage type analysis demonstrated that 38 of 61 ICR isolates (62%) were sensitive to group 2, compared to 42 of 172 isolates (24%) that did not express ICR ($P < 0.01$). On randomly amplified polymorphic DNA, phage type 2 isolates expressing ICR belonged to the same clone, which was different from ICR isolates sensitive to other phages and from isolates not expressing ICR.
Conclusions: Inducible clindamycin resistance is common among methicillin-sensitive *Staphylococcus aureus* in Israeli children. The D-test should be performed routinely in all MSSA isolates.

IMAJ 2011; 13: 605–608

KEY WORDS: *Staphylococcus aureus*, clindamycin resistance, methicillin-sensitive, pediatrics, antibiotic

Staphylococcus aureus infections are a major cause of morbidity and mortality worldwide. The growing interest in the resistance of *Staphylococcus aureus* to various antibiotics in the last two decades, especially methicillin, has led to the use of alternative agents such as clindamycin. Clindamycin has several advantages in the treatment of staphylococcal infections: It can be administered intravenously and orally with good bioavailability, it penetrates the skin and soft tissue easily, exerts an inhibitory action on toxin production, and is relatively inexpensive.

Clindamycin resistance among *Staphylococcus aureus* isolates is nearly always accompanied by macrolide resistance. Clindamycin resistance is mediated by the *erm* genes, which modify the drug-binding site that is shared by macrolides, lincosamides and group B streptogramin (MLSB) antibiotic groups. Some bacteria express these genes constitutively (MLSBc), and their concomitant erythromycin and clindamycin resistance is easily detected by standard susceptibility methods. However, other bacteria express these genes inducibly (MLSBi), that is, only when exposed to a strong methylase inducer such as erythromycin. These strains appear resistant to erythromycin and sensitive to clindamycin by routine susceptibility testing, but are found to be resistant to clindamycin when subjected to the agar disk-diffusion test (D-test) [1]. This phenomenon is called inducible clindamycin resistance.

Most studies have focused on the prevalence of ICR among methicillin-resistant *Staphylococcus aureus* isolates [2-5]. In Israel, the prevalence of MRSA in the community is very low, especially in children, with nasal colonization rates of less than 3% [6-7]. However, it is our impression that in the last few years, ICR has become more prevalent in methicillin-sensitive *Staphylococcus aureus* at our tertiary care pediatric hospital. Published data on ICR rates among *Staphylococcus aureus* isolates in Israel are lacking, and they could have important implications for treatment protocols against staphylococcal infections. The

ICR = inducible clindamycin resistance
MSSA = methicillin-sensitive *Staphylococcus aureus*

MRSA = methicillin-resistant *Staphylococcus aureus*

aim of the present study was to characterize the ICR phenomenon among MSSA isolates from infected children in Israel.

PATIENTS AND METHODS

Schneider Children's Medical Center of Israel is a tertiary care, university-affiliated hospital. We reviewed the medical records of all children aged 0–18 years diagnosed with MSSA infections from January 2006 to June 2007. Isolation sites included wounds, abscesses, blood, cerebrospinal fluid, lymph nodes, joint fluid, and central line tips. MSSA isolates from sputum or bronchial lavage were excluded, as were those regarded as colonization. When several serial MSSA isolates were grown from a single patient, only the first isolate was analyzed unless repeated isolates yielded a different antibiogram.

Staphylococcus aureus was identified in culture with the Slidex Staph-Kit (bioMerieux, Marey-Etoile, France) or the Pastorex Staph slide agglutination test (Sanofi Diagnostics Pasteur, Paris). Findings were confirmed by DNase (DNase test agar Hy-Laboratories, Rehovot, Israel) or the API-Staph test (ID 32 STAPH, bioMerieux). Susceptibility to trimethoprim/sulfamethoxazole, minocycline, chloramphenicol, rifampicin, gentamicin, ofloxacin and penicillin were tested by the disk diffusion method on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA), according to the procedures established by the Clinical and Laboratory Standards Institute [8]. Plates were incubated at 30^{0}C to define methicillin resistance (using oxacillin 1 µg/disk) and at 37^{0}C for other antibiotics, for 18 hours. The D-test was used to test for ICR, according to the recommendations of the Clinical and Laboratory Standards Institute [8]. The D-test was performed primarily for all isolates, and not only for those found to be resistant to erythromycin and susceptible to clindamycin by the regular susceptibility tests.

Phage typing was performed for most isolates using the technique of Blair and Williams [9], with phages issued by the International Reference Laboratory (Colindale, UK). The phages were obtained in two concentrations, routine test dilution and 100 routine test dilutions, as follows: lytic group 1: 29, 52, 52A, 79, 80; lytic group 2: 3A, 3C, 55, 71; lytic group 3: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85; lytic group 5: 94, 96; others: 81, 88, 89, 90, 92, D11. The lytic pattern was determined after 18 hours of incubation at 30^{0}C. In order to assess clonality, randomly amplified polymorphic DNA was performed, according to standardized published protocols [10]. The study was approved by the hospital's Helsinki's committee.

RESULTS

During the study period, 240 MSSA isolates were obtained from 232 patients treated at our center. The median age of the patients was 52.5 months. Isolation sites included suppurative wounds and abscesses in 206 patients (85.8%), blood in 20 (8.3%), tip of central lines in 7 (3%), lymph node aspirate in 6 (2.5%), and joint fluid in 1 (0.4%).

Table 1. Resistance of MSSA isolates to antibiotics other than clindamycin

Antibiotic	Number resistant (%)
Penicillin	233 (92.4)
Erythromycin	73 (30.4)
Gentamicin	16 (6.3)
Chloramphenicol	14 (5.6)
Ofloxacin	7 (2.8)
Trimethoprim-sulphamethoxazole	2 (0.8)
Fusidinic acid	2 (0.8)

No isolates were resistant to rifampin, minocycline or vancomycin

Table 1 illustrates the resistance rates of the isolates to different antimicrobial agents. As expected, most strains were resistant to penicillin (92%). Erythromycin resistance was also substantial (30%). Fewer than 5% were resistant to chloramphenicol and gentamicin, and an even lower percentage to trimethoprim-sulphamethoxazole, fusidinic acid and quinolones. No resistance was observed to vancomycin, rifampicin and minocycline. Five of the 73 isolates resistant to erythromycin were also resistant to clindamycin by regular susceptibility methods (MLSBc). Of the remaining 68 erythromycin-resistant/clindamycin-sensitive strains, 62 (91%) expressed ICR and only 6 (9%) were indeed clindamycin-sensitive. The ICR rate among all isolates was 25.8% (62/240).

Thereafter, phage group typing performed for most isolates revealed a statistically significant difference in the distribution of phage groups between the ICR and non-ICR groups [Figure 1]. Sixty-two percent of the ICR isolates reacted with phage group 2 compared to 24% of the non-ICR strains ($P < 0.01$). To assess whether these strains belonged to the same clone, we randomly selected 54 isolates for randomly amplified polymorphic DNA. All the ICR isolates that reacted with phage

Figure 1. Comparison of phage type groups between ICR and non-ICR isolates.

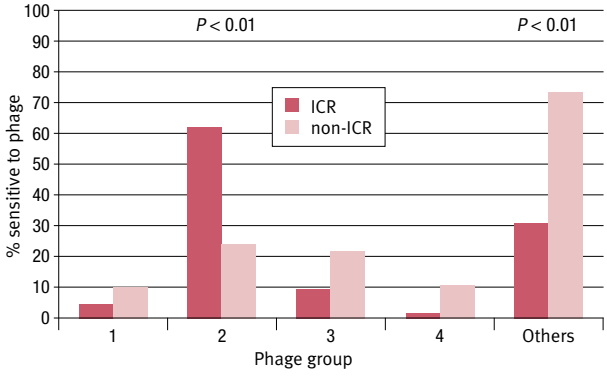
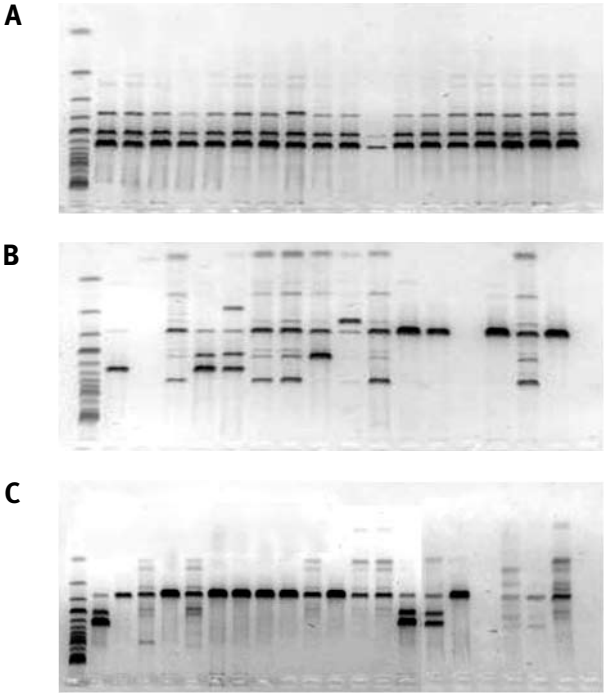


Figure 2. Randomly amplified polymorphic DNA of selected isolates: **[A]** positive ICR sensitive to phage 2 (n=18), **[B]** positive ICR sensitive to other phages (n=16), **[C]** negative ICR sensitive to phage 2 (n=20)



group 2 demonstrated the same fingerprint on randomly amplified polymorphic DNA [Figure 2A], indicating that they belonged to the same clone. By contrast, ICR isolates that reacted with other phage groups showed an additional three fingerprints that differed from the infecting clone [Figure 2B]. Isolates that reacted with phage group 2 but did not express ICR demonstrated two main clones [Figure 2C], which were also different from the clone causing most ICR infections.

DISCUSSION

The increasing prevalence of MRSA infections, especially with the spread of resistant strains in the community [11,12], poses a challenge to physicians in terms of the use of alternative antibiotic agents. Although clindamycin has been considered an acceptable option for patients with community-acquired MRSA infections, reports on high rates of clindamycin-resistant community-acquired MRSA strains are limiting its use [12]. Much less is known about ICR among MSSA strains. We focused on this question in the present study, because most infections due to MRSA in Israel are hospital-acquired, and the nasal colonization rate of MRSA strains in the community is very low (less than 1–3%) [6-7]. Recently, Nevet et al. in Israel [13] presented three children with community-acquired MRSA who were all sensitive to clindamycin [13].

Until this study, clindamycin resistance in our region was "believed to be low," possibly because the D-test is not performed routinely in most microbiological laboratories. This is the first study to assess ICR prevalence among MSSA isolates from infected children in Israel. We found that 91% of the erythromycin-resistant/clindamycin-sensitive strains expressed ICR, and the overall prevalence of clindamycin resistance among all MSSA isolates was 26%. These data are in line with other reports of ICR prevalence among MSSA isolates. In the United States and Europe, ICR was reported in 11–25% of isolates; in the Far East, rates were higher, reaching 30% [14-16].

ICR is an in vitro phenomenon, and its clinical significance is still uncertain. There are several reports of patients with infections caused by ICR MRSA infections in whom clinical deterioration was observed during treatment with clindamycin. Further molecular analysis demonstrated that the isolates had switched from a MLSBi to a MLSBc phenotype as a result of a mutation leading to constitutive resistance to MLSB antibiotic classes [14,17]. Study of the genetic profile of MLSBi strains may provide a clue to the understanding of this manifestation. MSLBi is mediated by *erm* genes. In *Staphylococcus aureus* infections, several main subsets of *erm* genes are involved, namely, erm (A), *erm* (B), and erm (C), and the expression of a combination of *erm* genes is possible [4]. The distribution of *erm* genes among MLSBi MSSA isolates differs worldwide [18-21]. Daurel and co-authors [22] reported that isolates expressing the *erm* (C) gene have a 14-fold higher mutation frequency than those expressing *erm* (A). This difference might be explained by the much more complex regulatory region of the *erm* (A) gene, leading to a lower probability of a spontaneous mutation causing constitutive expression [23].

It is also noteworthy, however, that some authors reported good outcomes in patients with ICR infections treated with clindamycin [24], so the implications of in vitro ICR remain controversial. Nevertheless, our data raise concerns regarding the reliability of clindamycin as a single empiric agent for the treatment of suspected staphylococcal infections. We believe that in our region, where 26% of MSSA isolates express ICR, clindamycin should not serve as monotherapy for serious MSSA infections until an antibiogram is obtained. However, in patients with minor skin infections or abscesses in which drainage itself has important therapeutic value [25], clindamycin can be administered pending culture results, as long as a good clinical follow-up is maintained.

The high rate of ICR in the present study indicates that the D-test should be performed routinely for all *Staphylococcus aureus* isolates when specimens are plated for culture. If the D-test is performed only after detection of an erythromycin-resistant/clindamycin-sensitive strain, the identification of ICR will take longer, extending the time the patient could be receiving ineffective treatment. By placing an erythromycin 15 µg disk and a clindamycin 2 µg disk adjacent to each other at

a distance of 15–26 mm apart on a Mueller-Hinton agar plate when susceptibility testing is being done, ICR can be detected the next day with the results of other antibiotic testing, thus avoiding the delay of an extra day for the D-test.

The MLSBi strains in our sample belonged to a total of four clones: one main clone reacting with phage group 2 and causing 62% of the ICR infections, and three other clones reacting with other phages. The predominance of the main clone is interesting, and prospective follow-up studies are needed to assess its spread. In the event of spread of the main clone, we may soon be witnessing an increase in the ICR rate. Fortunately, so far, the resistance in our region to other antibiotics, including oxacillin, trimethoprim-sulphamethoxazole and rifampicin, is very low.

Our study has several limitations: First, because of its retrospective design, we were unable to determine the relationship between previous antibiotic treatments, especially with macrolides, and the acquisition of ICR strains. A prospective study could address this issue, and additionally, evaluate the spread of ICR clones in our region. Second, we did not compare the severity of infection of ICR and non-ICR MSSA strains. Although it was our clinical impression that infections with ICR strains were more severe, this needs to be substantiated in a prospective study. Third, we included specimens obtained only at our hospital and not in primary care clinics in the community; therefore, our results might not reflect the true incidence of ICR in Israel. However, we provide unique data on ICR epidemiology in our region, which is characterized by low rates of community-acquired MRSA.

In conclusion, pediatric MSSA isolates in our region express relatively high rates of ICR, and this necessitates the routine performance of the D-test for all *Staphylococcus aureus* isolates. Our finding of a relatively high rate of ICR in MSSA infections should prompt physicians to be cautious with the use of clindamycin in the treatment of suspected staphylococcal infections pending culture results.

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