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Failure of Clindamycin To Eradicate Infection with Beta-Hemolytic Streptococci Inducibly Resistant to Clindamycin in an Animal Model and in Human Infections

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Inducible clindamycin resistance in beta-hemolytic streptococci remains an underrecognized phenomenon of unknown clinical significance. We performed an evaluation of inducible clindamycin resistance using an animal model as well as retrospectively reviewing the charts of patients treated with clindamycin monotherapy who were infected with beta-hemolytic streptococci inducibly resistant to clindamycin. The neutropenic mouse thigh model of infection was used to evaluate the *in vivo* activity of clindamycin against beta-hemolytic streptococci, including isolates susceptible, inducibly resistant, or constitutively resistant to clindamycin. The clinical microbiology laboratory information system and pharmacy databases were cross-referenced to identify patients with infections due to inducibly clindamycin-resistant beta-hemolytic streptococci who were treated with clindamycin monotherapy. Medical records of these patients were reviewed to evaluate microbiologic and clinical outcomes. Inducible clindamycin resistance resulted in impaired killing of beta-hemolytic streptococci in the animal model. Though suppressed initially, compared to those with constitutive resistance ($P = 0.0429$), by 48 h, colony counts of inducibly clindamycin-resistant organisms were similar to those of constitutively resistant isolates ($P = 0.1142$). In addition, we identified 8 patients infected with inducibly clindamycin-resistant beta-hemolytic streptococci who experienced clinical and microbiologic failure when treated with clindamycin monotherapy. These patients either improved initially and subsequently failed or never responded to clindamycin therapy. We have demonstrated in a murine model of infection and from human cases that inducible clindamycin resistance in beta-hemolytic streptococci is clinically significant. Routine testing and reporting by clinical laboratories should be encouraged and alternative antimicrobial agents considered when these organisms are encountered in clinical care.

Antimicrobial resistance in beta-hemolytic streptococci has increased over the past decade, most notably to the macrolide class of antibiotics (1, 2). β-Lactam antibiotics, including penicillinase-resistant semisynthetic penicillins or first-generation cephalosporins, remain frequently used agents in the care of skin and soft tissue infections (SSTI) and are predictably active against these organisms (3). However, the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has resulted in an increased use of non-β-lactam agents for empirical therapy of SSTI, many of which are less predictably active against beta-hemolytic streptococci. Clindamycin has been increasingly used as an agent capable of treating both CA-MRSA and beta-hemolytic streptococci. The appeal of clindamycin for SSTI includes its activity against many streptococci and *S. aureus* isolates, high oral bioavailability, extensive drug distribution, relatively low cost, and toxin inhibition properties.

Both constitutive and inducible clindamycin resistance is well recognized in *S. aureus*, and screening for inducible resistance is commonly performed in clinical microbiology laboratories using the "D-zone test" method described by the Clinical and Laboratory Standards Institute (CLSI) (4). Inducible clindamycin resistance is not well appreciated in beta-hemolytic streptococci, although mechanisms similar to those seen in *S. aureus* occur in streptococci (5). As in staphylococci, inducible clindamycin resistance in streptococci is not detected by standard broth microdilution testing, automated testing devices, standard disk diffusion testing, or E-test. The CLSI has described procedures for performance of the D-zone test in beta-hemolytic streptococci as well as in staphylococci, although evidence that such resistance is clinically important in the beta-hemolytic streptococci has until now been lacking. In this report, we describe results of a murine model of infection using group A *Streptococcus* (*Streptococcus pyogenes*; GAS) and group B *Streptococcus* (*Streptococcus agalactiae*; GBS) strains susceptible to clindamycin as well as those with constitutive or inducible resistance to clindamycin. We also describe 8 cases of SSTI in patients treated with clindamycin monotherapy that resulted in microbiologic and clinical failures due to the presence of inducible clindamycin resistance. We further discuss the mechanisms of clindamycin resistance in streptococci and review methods for the laboratory detection of inducible resistance in beta-hemolytic streptococci.

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TABLE 1 Strains of streptococci and MICs

Strain	MIC (μg/ml)	
	Clindamycin	Erythromycin
GAS 09-37	0.06 (inducible)	>32
GAS 08-06	>4 (constitutive)	>32
GBS 09-20	0.06 (noninducible)	1
GBS 09-62	0.06 (inducible)	1
GBS 09-59	>4 (constitutive)	4

MATERIALS AND METHODS

Bacterial strains. Clinical isolates of GAS and GBS retained by the University Health System San Antonio microbiology laboratory were used in this study. All isolates had been tested for susceptibility to erythromycin and clindamycin by the CLSI broth microdilution MIC method and by the disk diffusion method, including D-zone testing as well as PCR for the relevant resistance determinants (6). These included GAS 09-37 (inducible), GAS 08-06 (constitutive), GBS 09-20 (noninducible, clindamycin susceptible), GBS 09-62 (inducible), and GBS 09-59 (constitutive) (Table 1).

Antimicrobial agents. Clindamycin hydrochloride for the animal model studies was purchased from Sigma-Aldrich, St. Louis, MO.

Murine infection model. The neutropenic mouse thigh model of infection has previously been described in detail (7). Animals were maintained in accordance with criteria of the American Association for Accreditation of Laboratory Animal Care and investigation approved by the Animal Research Committee of the William S. Middleton Memorial Veterans Hospital. In each model, 6-week-old, specific-pathogen-free, female ICR/Swiss (CD1) mice weighing 23 to 27 g were used for the studies (Harlan Sprague-Dawley, Indianapolis, IN). Three mice were used at each sampling time point for each test group, including the control. Mice were rendered neutropenic (<100 neutrophils/mm^3) by injections of intraperitoneal cyclophosphamide (Mead Johnson, Evansville, IN) 4 days (150 mg/kg of body weight) and 1 day before thigh infection. Broth cultures were grown to logarithmic phase overnight to an absorbance of 0.3 at 580 nm. Cultures were diluted 1:10 with Mueller-Hinton broth.

Thigh infections with each of the strains were produced by the injection of 0.1 ml of inoculum into the thighs of halothane-anesthetized mice 2 h before they received therapy with 150 mg clindamycin/kg every 8 h. Bacterial counts at the start of therapy were ~10^7 CFU/thigh. Prior studies have shown that protein binding percentages of clindamycin in mouse and human sera are 82% and 77%, respectively (7). The clindamycin dosing strategy was designed to simulate the free-drug area under the curve (AUC) value obtained during intravenous administration in humans at a dose of 300 mg every 8 h. Saline control mice were sacrificed at 0, 6, and 24 h. Clindamycin-treated mice were sacrificed and sampled at 6, 12, 24, 48, and 72 h. Thighs were homogenized, serially diluted, and plated on tryptic soy agar (TSA) for CFU determinations. Statistical analysis and figures were created using GraphPad Prism version 6.02. Differences between $\log_{10}$ CFU/thigh were assessed using the unpaired *t* test. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

All five organisms grew well in control mice (i.e., 1.60 to 2.76 log CFU at 24 h; mean = 2.04 ± 0.38 log). All mice infected with susceptible or inducibly clindamycin-resistant strains of streptococci survived the entire 72-h period of the experiment. A few mice infected with the constitutively clindamycin-resistant strains had to be sacrificed before 72 h because of signs of severe infection. Colony counts were reduced significantly by clindamycin in the strain that was fully susceptible to clindamycin (Fig. 1). Streptococci with constitutive resistance to clindamycin produced growth similar to the saline-treated control mice (Fig. 1). Interestingly, the strains with inducible clindamycin resistance were initially suppressed by clindamycin therapy, and at 6 h the $\log_{10}$ CFU/thigh was significantly different compared to that of strains with constitutive resistance ($P = 0.0429$). These differences persisted at 12 and 24 h ($P = 0.0005$ and 0.002, respectively); by 48 h, regrowth occurred and differences between inducible and constitutively resistant strains were no longer significant ($P = 0.1142$) (Fig. 1).

Human cases. (i) Case 1. A female in her late 20s with a history of penicillin allergy presented to her OB physician with the complaint of worsening bilateral breast pain and tenderness during nursing. Diffuse erythema and pain were noted on physical examination with no evidence of abscess formation, and she was diag-

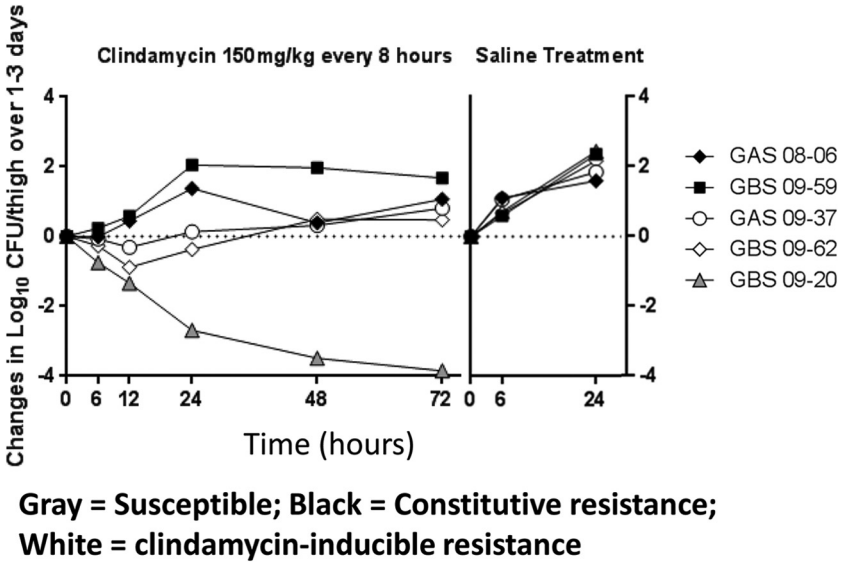

FIG 1 Activity of clindamycin versus GAS and GBS with differing clindamycin susceptibility in the neutropenic mouse thigh model. Gray, susceptible; black, constitutive resistance; white, clindamycin-inducible resistance.

nosed with bilateral mastitis. Oral clindamycin at 450 mg every 6 h was prescribed. Four days later, she again presented with no improvement of her initial symptoms, subjective fever, and no evidence of induration or fluctuance on repeat examination. She was admitted to the hospital for intravenous (i.v.) antibiotics. The patient's vital signs were significant for a fever of 103.5°F and a white blood cell count (WBC) of 12,800 cells/mm^3. All other laboratory values were within normal limits. Bilateral breast milk samples taken on admission grew GBS inducibly resistant to clindamycin. The patient was started on 1 g vancomycin every 12 h (q12h) and 80 mg gentamicin q8h, and the oral clindamycin was continued. Rapid improvement of symptoms occurred over the next 72 h, and she was discharged to complete 7 days of intravenous vancomycin therapy.

(ii) Case 2. A penicillin-allergic diabetic male in his mid-40s was admitted for treatment of an abscess and chronic infected ulcer near the 3rd digit of his left foot. He was started empirically on clindamycin at the time of admission. Wound cultures from the day of admission grew group G beta-hemolytic *Streptococcus* (GGS) inducibly resistant to clindamycin as well as mixed anaerobic bacteria. On hospital day 2, the patient was taken to surgery and had a malleolar amputation secondary to extensive infection and necrosis of the osseous tissue. Intraoperative cultures grew GGS with the aforementioned susceptibility profile and no anaerobes. The patient was continued on 600 mg clindamycin i.v. three times daily. On postoperative day 3 (hospital day 5), the wound showed minimal improvement, and the patient was taken back to surgery for further debridement. Intraoperative cultures again confirmed GGS with inducible clindamycin resistance, this time with methicillin-susceptible *S. aureus*. Gatifloxacin (400 mg i.v. daily) was added to his antibiotic regimen. Subsequent wound cultures after initiation of gatifloxacin no longer grew GGS.

(iii) Case 3. A female in her late 40s with no known drug allergies, hepatitis C, depression, and a history of recurrent cellulitis presented to her primary care provider with a 3-day history of worsening cellulitis and abscess formation of the right lower extremity. The patient denied fevers or chills and was afebrile; however, her WBC count was elevated at 13,400 cells/mm^3. The abscess was drained, and the material was not initially sent for culture. The patient was discharged on 10 days of 300 mg clindamycin orally every 6 h. Two days later, the patient returned to her primary care provider complaining of worsening swelling and pain, and her WBC count had decreased to 8,300 cells/mm^3. Fluid from the site of incision and drainage was cultured during her second office visit and grew GAS inducibly resistant to clindamycin. One double-strength tablet of trimethoprim-sulfamethoxazole twice daily was added to her antibiotic regimen, and she was sent home with wound care instructions that included warm compresses, elevation of the extremity, and washing the area with chlorhexidine soap. The patient was subsequently lost to follow-up.

(iv) Case 4. A diabetic male in his late 40s who had recently crushed his right ring finger in a car door presented to the emergency department complaining of increasing pain and swelling in the affected finger. The finger was found to have an abscess on the right volar aspect which was drained in the emergency department, and the patient was admitted for observation. Intravenous clindamycin, 600 mg every 8 h, was initiated. The Gram stain of the initial culture material revealed Gram-positive cocci in pairs, and the culture grew GBS inducibly resistant to clindamycin. On hospital day 3, the finger continued to appear infected with no improvement, and the patient was taken to the operating room (OR) for further exploration and debridement. Intraoperative cultures again grew GBS inducibly resistant to clindamycin. Culture results from hospital day 1 became available on hospital day 3, and the patient's therapy was changed to 2 g oxacillin every 6 h. The viability of the patient's finger continued to deteriorate, and he returned to the OR on hospital day 5 with subsequent amputation of the finger. No cultures were obtained. The patient improved on 2 g oxacillin every 6 h, and the wound was closed on hospital day 7.

(v) Case 5. A female in her late 40s with poorly controlled diabetes and a 1-month history of swelling and ulceration of her left 2nd toe presented to the emergency center with worsening pain and swelling. She had been prescribed 300 mg clindamycin every 8 h 7 days prior to admission. Despite the clindamycin, the symptoms continued to progress, and she presented to our emergency department. At the time of admission, she was in no acute distress and denied fevers, chills, and shortness of breath. Early on hospital day 2, podiatry performed an amputation of her left second digit, and the patient was started on piperacillin-tazobactam. Intraoperative cultures of the tissue as well as bone grew GBS with inducible resistance to clindamycin. The patient was converted to ampicillin-sulbactam after cultures returned and experienced an unremarkable remainder of her hospital stay.

(vi) Case 6. A male in his early to mid-30s with HIV/AIDS presented with increasing pain and cellulitis over his left anterior tibia for the past several days. The area was warm to the touch and slightly tender. The patient reported minor trauma to the area 2 weeks prior. He was in no acute distress but was febrile at 38.7°C. The patient received 600 mg of intravenous clindamycin as a one-time dose and was discharged to home with a prescription for 300 mg of oral clindamycin every 6 h. Three days after being seen in the clinic, 1 of the 2 blood cultures obtained during the clinic visit became positive for GGS with inducible clindamycin resistance. The patient was contacted at home and stated that the cellulitis had not improved. The patient was initiated on 1 g of amoxicillin three times daily and returned to the clinic 2 days later for further evaluation. By the time the patient returned to clinic, the cellulitis had begun to improve, and follow-up blood cultures were negative for the GGS. He completed 10 days of amoxicillin therapy without further complications.

(vii) Case 7. An obese female in her mid- to late 50s with uncontrolled diabetes and a history of liposarcoma 11 years prior presented to the emergency department with a 1-week history of cellulitis of her right lower extremity. The cellulitis and abscess were in the area where she had undergone resection and radiotherapy of the liposarcoma. She had been taking 300 mg clindamycin orally every 6 h for the past 7 days and reported that she had experienced an initial clinical response to the clindamycin but had subsequently worsened. In the emergency department, she was found to have a new ulceration of her right calf with surrounding cellulitis and abscess formation. The patient underwent surgical drainage of the abscess, and multiple intraoperative cultures were positive for GBS with inducible resistance to clindamycin. Postoperatively, the patient received vancomycin and piperacillin-tazobactam with rapid clinical response. She received 8 days of amoxicillin upon discharge and was seen in the clinic 1 week later with resolution of her infection.

(viii) Case 8. A poorly controlled diabetic patient in her early 40s presented to an outpatient clinic with labial cellulitis and pos-

sible abscess. She was given 600 mg of intramuscular clindamycin and a prescription for 300 mg clindamycin orally every 6 h. Two days later, the patient presented with worsening pain. She was admitted and received 600 mg clindamycin intravenously every 8 h and 1 g aztreonam every 8 h. On hospital day 3, she was taken to the OR for incision and drainage of the labial abscess. Cultures of the purulent material obtained during surgery revealed GBS with inducible clindamycin resistance. On hospital day 5, the patient was discharged to home with a prescription for trimethoprim-sulfamethoxazole, double strength, 1 tablet twice daily for 14 days. She was seen in an outpatient clinic for follow-up 10 days later with a normal noninfected postsurgical wound that was healing as expected.

DISCUSSION

Two primary mechanisms are responsible for resistance to macrolide antibiotics in beta-hemolytic streptococci. A specific efflux pump encoded by *mef* genes, namely, *mef*(A), extrudes 14- and 15-member macrolides such as erythromycin but not lincosamides such as clindamycin (3, 6, 8, 9). The second mechanism responsible for erythromycin resistance, which does result in lincosamide resistance, is encoded by the *erm* genes: *erm*(B) or *erm*(TR). These genes cause methylation of the 23S rRNA-binding site and subsequent resistance to erythromycin, clindamycin, and streptogramin B drugs (MLS_B phenotype) (8–12).

Phenotypically, resistance can be expressed constitutively ($cMLS_B$ phenotype) or inducibly ($iMLS_B$ phenotype) (12). Resistance is caused by the binding of a macrolide to an upstream translation attenuator sequence with resultant alteration of the mRNA secondary structure with exposure of the ribosomal binding site and translation of the *erm* methylase (11). $iMLS_B$ strains are resistant to 14-member macrolides (erythromycin, clarithromycin, and dirithromycin) and 15-member macrolides (azithromycin) and initially appear susceptible to the 16-member macrolides (josamycin and spiramycin), lincosamides (clindamycin), and group B streptogramins (13). However, in the presence of a macrolide inducer, such as erythromycin, lincosamide, and streptogramin B, resistance is observed.

Clinical implications. The CLSI has recommended a method for screening beta-hemolytic streptococci for $iMLS_B$ since 2005, although prior to this report no convincing clinical data have shown a lack of clindamycin efficacy in patients with $iMLS_B$ streptococci. Indeed, the data remain sparse even for $iMLS_B$ *S. aureus* infections, where this testing is more commonly performed. It had been our supposition that an inducibly clindamycin-resistant strain might undergo conversion to a constitutively resistant strain during high-inoculum infections with iMLSB strains during clindamycin therapy due to spontaneous mutation in the *erm* promoter gene. However, as noted in our eight patients, post-therapy strains from clinical failures continue to demonstrate the iMLSB phenotype rather than constitutive expression. Our animal model demonstrated impaired antibiotic killing of the iMLSB streptococcal strains by clindamycin. In some cases, colony counts in the mouse thighs with the iMLSB strains were similar to those with the constitutively resistant strains by 48 h of therapy. Thus, a lack of killing of the iMLSB streptococcal strains during clindamycin therapy may represent the real clinical significance of this genotype/phenotype. This pattern is similar to what was seen with clindamycin treatment in mice infected with inducibly clindamycin-resistant strains of *Staphylococcus aureus* at the same high starting inoculum (7, 14).

It is important to note that there are differences in the disk spacing for the D-zone test with streptococci and staphylococci. The CLSI recommendation for *S. aureus* specifies that a 15-μg erythromycin disk and a 2-μg clindamycin disk be placed 15 to 26 mm from the edge of one another on the surface of the inoculated agar test plate. However, because the inhibition zones are inherently smaller, the disks should be placed only 12 mm apart for beta-hemolytic streptococci (4). Other differences include the necessity of performing the testing of streptococci on sheep blood containing Mueller-Hinton agar incubated in 5% CO_2 (4).

Our eight cases and animal model data for the first time illustrate the risk of treatment with clindamycin when an $iMLS_B$ phenotype is present in beta-hemolytic streptococci. Interestingly, it required several years of screening and chart reviews to find these cases. The primary obstacle in locating these cases was the initial treatment of many patients with vancomycin, a β-lactam, or combination therapy of clindamycin with another agent possessing activity against these organisms. Another difficulty in identifying cases was that many patients with $iMLS_B$ beta-hemolytic streptococci were treated as outpatients and had minimal or no follow-up documentation in their medical record. Whether these patients were cured secondary to incision and drainage of an abscess and required no further follow-up or were subsequently seen in another health care facility remains an unanswered question.

Our eight cases and animal model data suggest that clinicians and clinical microbiologists need to be aware that strains with inducible clindamycin resistance are potentially problematic in SSTI attributed to beta-hemolytic streptococci and may contribute to clinical failure when clindamycin monotherapy is employed. Thus, the prior CLSI recommendation for screening beta-hemolytic streptococci for inducible resistance is affirmed by our findings. We encourage the routine detection and reporting of inducible clindamycin resistance in both *S. aureus* and beta-hemolytic streptococci, especially as clindamycin is increasingly used as an alternative to β-lactams for SSTI.

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