Daptomycin Nonsusceptible Enterococci: An Emerging Challenge for Clinicians

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Daptomycin is the only antibiotic with in vitro bactericidal activity against vancomycin-resistant Enterococcus (VRE) that is approved by the Food and Drug Administration (FDA). Data on the potential emergence of daptomycin nonsusceptibility among enterococci remain limited. We systematically reviewed the published literature for reports of isolates of enterococci that were daptomycin nonsusceptible and assessed the clinical significance and outcome of therapy. Based on susceptibility breakpoints approved by the Clinical Laboratory Standards Institute (CLSI), daptomycin has in vitro activity against >90% of enterococcal isolates. Less than 2% of enterococcal isolates were daptomycin nonsusceptible, with minimum inhibitory concentrations (MICs) >4 μg/mL. The prevalence of nonsusceptibility of VRE isolates to daptomycin may be overestimated due to the spread of clonally related isolates in health care settings. Clinicians should be aware of the possibility of the emergence of daptomycin nonsusceptibility and should closely monitor daptomycin MICs of enterococci isolated during treatment.

Antimicrobial drug resistance is a growing public health problem, and multidrug-resistant pathogens such as vancomycin-resistant enterococci (VRE) are increasing worldwide [1]. The limited therapeutic options currently available for the treatment of VRE infections emphasize the need for new antimicrobial agents with activity against these pathogens and for ongoing efforts to limit the transmission of VRE in health care settings [1].

Daptomycin, a cyclic lipopeptide approved for the treatment of complicated skin and soft-tissue infections and *Staphylococcus aureus* bloodstream infection, is the only antibiotic with in vitro bactericidal activity against VRE that is approved by the Food and Drug Administration (FDA). Data regarding the potential emergence of daptomycin nonsusceptibility among enterococci

remain limited. Here, we systematically review published literature for cases of daptomycin nonsusceptible enterococci (DNSE).

METHODS

We performed a literature search in PubMed and EMBASE (through April 2010) using the National Library of Medicine's medical subject headings (MeSH) terms "daptomycin," "enterococcus," "VRE," and "resistance," for articles that reported *Enterococcus* isolates nonsusceptible to daptomycin (microbiological failure). In addition, the references cited in these articles were examined to identify additional reports. *Enterococcus* isolates with daptomycin minimum inhibitory concentrations (MICs) >4 μg/mL, determined by broth dilution or Etest, or a zone of inhibition <11 mm by disk diffusion, were considered to be nonsusceptible, according to the Clinical Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards [NCCLS], 2003) [2]. According to FDA interpretative criteria, daptomycin nonsusceptibility breakpoint (4 mg/L) is available only

for vancomycin-susceptible *Enterococcus faecalis* [3]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has not issued interpretive criteria for enterococci and daptomycin.

RESULTS

From 2003 through 2010, we identified 23 studies reporting 150 DNSE (Supplementary Table 1) [4–27]. Of these 150 isolates, 140 (93.3%) were VRE [4–27], 9 (6.0%) were vancomycin-susceptible enterococci (VSE) [5, 14, 15], and in 1 (0.7%) case, vancomycin susceptibility was not reported [4]. One hundred thirty-two isolates (88%) were *Enterococcus faecium* [5, 7, 8, 10–15, 17–22, 24–27], 13 (8.7%) were *E faecalis* [6, 8–10, 14, 23, 26], 4 (2.7%) were other *Enterococcus* species [14, 16], and in 1 (0.7%) case, the species of *Enterococcus* was not reported [4]. The country of origin was documented for 144 (96%) of these 150 isolates, including 58 (40.3%) reported from Asia [10, 13, 19], 49 (34%) from Europe [12, 15, 21, 26], and 37 (25.7%) from North America [5–9, 14, 16–18, 22–24, 27]. The age and sex of the source patients were documented in only 8 cases; the mean age was 54.6 years, and 5 of 8 (62.5%) patients were female [7–9, 16, 17, 22–24]. The type of infection was documented in 27 cases [6–9, 11–13, 16, 17, 20, 22–24] and in all cases was bloodstream infection (BSI), including endocarditis in 4 (14.8%) of these cases [7–9]. In 2 large studies, the incidence of daptomycin nonsusceptibility was 0.1% (3 of 3258 *Enterococcus* isolates) [20] and 0.04% (2 of 4731 *Enterococcus* isolates) [21]. In one study in North America, 0.03% (1 of 2905) of *E faecalis*, 0.6% (11 of 1806) of *E faecium*, 0.02% (5 of 3336) of VSE species, and 0.6% (10 of 1560) of VRE were DNSE [14]. However, 2 studies in Asia [13, 19] and 1 in Europe [26] reported a much higher incidence of daptomycin nonsusceptibility among *E faecium* isolates (10%–10.2% and 19.1%, respectively). Some of these isolates were suspected to be clonally related, but this was not confirmed [13]. The prevalence of daptomycin nonsusceptibility was 0.2% (10 of 4051; range 0.1%–1.1%) for VSE isolates [5, 14, 15, 25], 1.0% (34/3445; range 0.0%–9.9%) for VRE isolates [5, 14, 15, 25], 2.6% (54 of 2092; range 0.6%–19.5%) for *E faecium* [5, 7, 11, 14, 15, 18, 20, 21, 25–27], and 0.1% (5/3398; range 0.0%–1.3%) for *E faecalis* isolates [6, 14, 26]. The overall prevalence of daptomycin nonsusceptibility for all *Enterococcus* isolates was 0.6% (111 of 17084; range 0.0%–19.1%).

The microbiological methods used to determine daptomycin susceptibility in *Enterococcus* isolates from 23 studies (Supplementary Table 2) included reference broth microdilution (BMD) (78.3%) [5, 7, 9, 11, 13–16, 18–27], agar dilution (4.3%) [10], Etest (30.4 %) [4, 7, 8, 12, 16, 17, 27], and disk diffusion (13%) [9, 22, 24]. Only 6 studies determined MICs by both diffusion and dilution methods (Supplementary Table 3) [7, 9, 16, 22, 24, 27]. In 2 cases, *E faecium* developed daptomycin nonsusceptibility de novo, without prior documented use of daptomycin [12, 17]. The duration of daptomycin administration before the isolation of DNSE was reported in only 8 cases and ranged from 14–69 days (mean, 32.3 days) [6–9, 16, 22–24]. The dosage of daptomycin was 6 mg/kg/day [7, 8, 22, 24], and 6 mg/kg every 48 h in 4 patients receiving hemodialysis [6, 9, 16, 23].

Treatment of DNSE isolates was reported in 6 cases. Linezolid was used in 4 cases [8, 16, 22, 24], ampicillin and gentamicin in 1 case [9], and linezolid and high-dose ampicillin in 1 case [23].

From a review of 31 daptomycin-nonsusceptible (DNS) *E faecium* isolates with available data on susceptibility to ampicillin [7, 12–15, 17, 18, 22, 24], 25 isolates (80.6%) were resistant to ampicillin in vitro [7, 12–15, 17, 18, 22, 24]. However, in one study, 6 of these DNSE isolates that were susceptible to ampicillin were clonally related [13]. If the duplicate clonal isolates are excluded, only 1 (3.8%) of 26 DNS *E faecium* isolates were susceptible to ampicillin in vitro. Available data on susceptibility to ampicillin was found in only 2 DNS *E faecalis* isolates, and both of these isolates were susceptible to ampicillin [9, 23] (Supplementary Table 1).

The clinical outcome of infection with DNSE was reported in only 8 cases [6–9, 16, 22–24] with clinical and microbiologic failure reported in all 8 cases, including 2 cases of endocarditis resulting in death despite treatment with alternative agents [8, 23].

DISCUSSION

Little is known about the frequency of the emergence of DNSE species. Although in vitro studies suggested that daptomycin resistance was unlikely to develop in vitro [2, 28–32], case reports of DNSE suggest this is an emerging clinical problem. Although clinical data, including site of infection and outcome, were not commonly reported, many infections associated with the isolation of DNSE infections were deep-seated—especially bloodstream infection and endocarditis, where there is a heavy bacterial load and penetration of the drug may be limited.

Among studies reporting DNSE, the overall prevalence of *Enterococcus* species with MIC >4 µg/mL was low. This prevalence likely overestimates the true prevalence of daptomycin nonsusceptibility among enterococci because of reporting bias and possible transmission of clonally related isolates in single institution reports [33]. However, there is a lack of data on daptomycin-nonsusceptible *Enterococcus* isolates from the World Health Organization Antimicrobial Resistance Information

Bank, and from international and national programs such as The Surveillance Network (TSN) database [34, 35].

In vitro studies have shown nearly uniform susceptibility of daptomycin against vancomycin-resistant *E faecium* and *E faecalis* strains with an MIC$_{90}$ of 2–4 µg/mL [14, 28]. Daptomycin MIC distributions for vancomycin-resistant *Enterococcus* strains are nearly identical to those of vancomycin-susceptible strains [5].

Mechanisms of Daptomycin Nonsusceptibility in *Enterococcus* Isolates

Daptomycin possesses a unique mechanism of action that targets the bacterial membrane in the presence of calcium. No cross-resistance with other classes of antimicrobial agents has been documented, making it an option for the treatment of infections caused by multidrug-resistant gram-positive organisms. The mechanisms of nonsusceptibility to daptomycin have not been characterized, but the risk of gene transfer of daptomycin resistance determinants has been raised as a potential threat [36]. In *S aureus*, daptomycin nonsusceptibility (MIC1 µg/mL) is associated with alterations in membrane phospholipid dynamics such that a relative positive charge is accumulated on the membrane outer leaflet [37–39]. This charge may then prevent permeabilization of the membrane by daptomycin-calcium complexes that result in membrane depolarization. This change in membrane phospholipid profile may also allow for increased resistance to cationic host defense peptides. However, these exact mechanisms do not appear to hold true for enterococci, as the genes identified in *S aureus* that are associated with daptomycin nonsusceptibility, *mprF*, *yycG*, and *rpoB*, are not altered in daptomycin-nonsusceptible *E faecium* isolates [40]. Although the resistance mechanism has yet to be determined in enterococci, reduced daptomycin diffusion into the bacterium due to the thickened cell walls of vancomycin-resistant isolates has been proposed [41–43]. Indeed, the emergence of heteroresistance to daptomycin following vancomycin exposure has been described for *S aureus* [44], but has been documented only rarely for enterococci [7].

Daptomycin has been shown to have a low spontaneous resistance rate [38]. Among enterococci, no spontaneously resistant mutants of *E faecium* have been isolated in vitro [38]. To our knowledge, there are only 2 reports of the development of spontaneous daptomycin nonsusceptibility in clinical *Enterococcus* isolates from patients with no documented prior exposure to the agent [12, 17]. Similarly, exposure to other classes of antibiotics has not been associated with the development of daptomycin nonsusceptibility. Resistance to vancomycin, teicoplanin, quinupristin/dalfopristin, or penicillin among the gram-positive isolates did not impact daptomycin activity [45].

Methods Used to Determine Nonsusceptibility of *Enterococcus* Isolates to Daptomycin

Although the majority of the included studies used the reference CLSI BMD method to determine susceptibility to daptomycin, 7 studies used Etest [4, 7, 8, 12, 16, 17, 27] and 3 studies used the disk diffusion method (in addition to BMD) (Supplementary Table 2) [9, 22, 24]. Discrepancies in daptomycin MIC results obtained by Etest and BMD have been documented for *S aureus* [46]. In a study at the Centers for Disease Control and Prevention (Atlanta, GA), MIC values by Etest tended to be 1 log$_2$ higher than MIC values by broth microdilution [46]. In one study comparing CLSI reference BMD and Etest for use of daptomycin in 124 *Enterococcus* spp strains, 69% of the Etest MIC values were found to be identical or within one doubling dilution of MICs determined with the BMD [4]. One study comparing BMD to Etest in 124 *Enterococci* strains found that 31% of the Etest and BMD MIC values differed by more than one dilution [4]. Of these 124 isolates, 32 (25.8%) had MIC values of 4 µg/mL by Etest compared to 8 of 124 (6.5%) isolates by BMD. Furthermore, 1 isolate (0.8%) had an MIC of 8 µg/mL by Etest, whereas no isolates were daptomycin nonsusceptible by BMD. Johnson et al. [47] found a higher overall agreement between Etest and BMD MIC values that may reflect the use of Iso-Sensitest agar. Several studies have shown that Ca^{2+} concentration can affect the results obtained by in vitro susceptibility testing, and strict adherence to CLSI (BMD) and BioMerieux (Etest) protocols is required [48, 49]. Calcium concentrations are known to fluctuate between production lots of Mueller Hinton agar, and this may account for the heightened MICs obtained by Etest. Furthermore, interpretation of daptomycin MICs by Etest is not trivial, and may be associated with higher reported MIC values. Generally, Etest tended to produce higher daptomycin MIC values than BMD [47]. It is recommended that a second method (BMD) be used to confirm Etest values >1 µg/mL. Of note, according to CLSI, disk diffusion is not considered reliable for testing of enterococci susceptibility to daptomycin [50].

To our knowledge, there are no large studies that investigate the performance of daptomycin susceptibility testing (in *Enterococcus* isolates) on the major commercial machines (eg, BioMerieux Vitek 2, BD Phoenix, or TREK Sensititre). A multicenter study compared CLSI reference agar and broth microdilution to Vitek 2 against 184 staphylococci at 3 centers, and found 98.2% overall agreement among Vitek 2, agar dilution, and broth dilution methods [51]. In a multisite evaluation study of 287 gram-positive isolates and 77 CDC challenge isolates, the performance of daptomycin on the Sensititre susceptibility system, using either the automated or manual reading method, was equivalent to its performance using the NCCLS microdilution reference method [52]. Further studies are needed

on comparison of the performance of daptomycin susceptibility testing on the major commercial machines versus Etest.

Clearly, the reproducibility of findings with the use of different microbiological methods has not been adequately evaluated.

Treatment Options for DNSE Isolates

The data presented here is suggestive that a daptomycin dose of 4 mg/kg should be used with caution to treat serious enterococcal infection because of the risk of daptomycin nonsusceptibility. This hypothesis is supported by previous studies showing the development of nonsusceptibility to daptomycin in high inoculum infections with gram-positive organisms that were treated with doses of daptomycin of 4 mg/kg [53]. One approach to decrease the development of daptomycin nonsusceptibility is to use doses greater than those currently approved for clinical use (ie, >4–6 mg/kg). The optimal dosing for enterococcal infection is not yet established; however, daily dosing at 6 mg/kg in the absence of renal insufficiency has been the most common dosing scheme. When daptomycin was tested against multidrug-resistant enterococcal isolates, in vitro pharmacodynamic models with simulated endocardial vegetations showed that 10 mg/kg/day resulted in more rapid and greater kill than 6 mg/kg/day, suggesting that higher doses may increase the efficacy of daptomycin in the treatment of enterococcal endocarditis [54, 55]. Increasing daptomycin doses to 10 mg/kg daily was shown to overcome resistance selection both in vitro and in animal models with S aureus or vancomycin-resistant enterococcal endocarditis [55, 56]. In humans, daptomycin doses up to 12 mg/kg for 14 days appear to be well tolerated [57, 58] and could potentially reduce the risk of the development of nonsusceptibility and treatment failure in deep-seated or complex infections. However, the safety profile with prolonged high-dose therapy is not well known.

Another potential approach for reducing the development of daptomycin-nonsusceptible enterococci is the use of combination therapy. Daptomycin has variable in vitro synergies with other antibiotics against methicillin-resistant S aureus (MRSA) [19, 59–62] and Enterococcus spp, including VRE [32, 59, 63, 64]. Synergies between daptomycin and other antibiotics, including β-lactams and aminoglycosides, have been reported, but data concerning the clinical efficacy of combinations with other antibiotics remain scarce (Supplementary Table 3) [29, 60, 63, 65]. There is increasing evidence that the combination of daptomycin and ampicillin is an important consideration for the treatment of cases of DNSE infections with persistent positive cultures [7, 66]. Sakoulas et al. [66] have recently demonstrated that ampicillin can alter the surface charge of daptomycin-nonsusceptible strains of VRE and apparently make the organism more likely to bind to daptomycin. Although in the current review we found that the majority of DNS E faecium isolates are resistant to ampicillin, this β-lactam may be tried in combination with daptomycin to treat DNS E faecium infections [7, 66], but further studies are needed to confirm these observations.

Further in vitro and in vivo studies of regimens containing daptomycin combined with other antibiotics are needed to improve our understanding of the mechanism of synergy and potential clinical use.

LIMITATIONS

This review has several limitations. Publication bias and other biases inherent in single-institution reports may underestimate or overestimate the prevalence of DNSE and its association with daptomycin therapy. Detailed clinical data were not available for most of the reported Enterococcus isolates that were nonsusceptible to daptomycin. Risk factors for and frequency of the emergence of daptomycin nonsusceptibility associated with therapy remain poorly characterized. In addition, the optimal management of serious DNSE infection is unknown. Case-control studies could better define the association between daptomycin dosage and the emergence and duration of DNSE. In addition, randomized prospective clinical studies are needed to define the optimal dosage of daptomycin for the treatment of serious VRE infections.

CONCLUSIONS

The mechanisms of nonsusceptibility to daptomycin have not been characterized and may not parallel those for S aureus. Clinicians should be aware of the possibility of the emergence of daptomycin nonsusceptible enterococcal strains, especially associated with the treatment of bloodstream infection with daptomycin, and should closely monitor the susceptibilities of sequential isolates recovered during treatment. The data presented herein is suggestive that a daptomycin dose of 4 mg/kg should be used with caution to treat serious enterococcal infection because of the risk of daptomycin nonsusceptibility. Since daptomycin may be one of the few microbiologically active agents against multidrug-resistant Enterococcus, further well-designed studies focused on the clinical effectiveness of daptomycin for infections caused by these pathogens, particularly for bloodstream infections, are required.

Supplementary Material

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/).

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