Diagnostic Microbiology and Infectious Disease 76 (2013) 497–501

Contents lists available at SciVerse ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Susceptibility of enterococci to daptomycin is dependent upon testing methodology☆,☆☆,★

Kendall A. Bryant[b], Amity L. Roberts[b], Mark E. Rupp[a], James R. Anderson[c], Elizabeth R. Lyden[c], Paul D. Fey[a,b], Trevor C. Van Schooneveld[a,*]

[a] Division of Infectious Disease, Department of Internal Medicine, College of Medicine, University of Nebraska Medical Center, 984031 Nebraska Medical Center, Omaha, NE 68198-4031, USA
[b] Department of Pathology and Microbiology, College of Medicine, University of Nebraska Medical Center, 984031 Nebraska Medical Center, Omaha, NE 68198-4031, USA
[c] College of Public Health, University of Nebraska Medical Center, 984031 Nebraska Medical Center, Omaha, NE 68198-4031, USA

ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form 17 April 2013
Accepted 21 April 2013
Available online 27 May 2013

Keywords:
Daptomycin
Susceptibility testing
Enterococcus

ABSTRACT

An increase in daptomycin nonsusceptible enterococci (DNSE) was noted in our institution (8.3% 2008 to 34.5% 2011) using MicroScan methods which may overestimate DNSE prevalence. DNSE ($N = 150$) from the clinical laboratory (2008–2011) underwent susceptibility testing using broth microdilution (BMD), Etest, Sensititire, MicroScan prompt (MSP), and MicroScan turbidity (MST) with only 20% of isolates confirmed as nonsusceptible. Categorical and essential agreement were highest with MSP and MST, but both missed the majority of resistant isolates (70% and 87% missed). Etest MIC values were statistically higher, more likely to be nonsusceptible, had the lowest very major error rate (37%), and the highest falsely nonsusceptible rate (22%). Sensititre MIC values were not statistically different from BMD, but missed 57% of DNSE. PFGE analysis did not define a clonal outbreak. These findings suggest that MicroScan methods overestimate nonsusceptibility, and the lack of correlation between methods raises questions regarding which method is most effective at confirming nonsusceptibility.

1. Introduction

Enterococci, including both *Enterococcus faecalis* and *Enterococcus faecium*, are important clinical pathogens and cause a significant number of healthcare-associated infections (Hidron et al., 2008). Resistance to antimicrobials is common among enterococci, particularly *E. faecium*. Fortunately, agents with activity against these resistant strains have been developed including linezolid, quinupristin/dalfopristin, tigecycline, and daptomycin. The use of these newer agents with increased activity against resistant enterococci, particularly vancomycin-resistant enterococci (VRE), has not been clearly associated with improved outcomes (Erlandson et al., 2008). Daptomycin is a cyclic lipopeptide which is rapidly bactericidal against a broad spectrum of Gram-positive cocci including enterococci (Rybak et al., 2000). Daptomycin's unique mechanism of action allows activity against isolates harboring resistance to commonly used antimicrobials such as beta-lactams and vancomycin. The widespread emergence of *E. faecium* resistant to vancomycin coupled with the toxicity associated with alternatives such as linezolid and quinupristin-dalfopristin has increased daptomycin use in treatment of enterococcal infections (Canton et al., 2010; Kullar et al., 2011; Mohr et al., 2009).

Enterococcal isolates with daptomycin minimal inhibitory concentration (MIC) results of ≤4 μg/mL are considered susceptible, while those >4 μg/mL are reported as nonsusceptible (CLSI, 2013). Daptomycin susceptibility rates for enterococci are high; in a large survey of US clinical isolates from 2005 to 2010, less than 0.4% of *E. faecalis* or *E. faecium* isolates were classified as nonsusceptible to daptomycin (Sader et al., 2011). Another survey evaluated daptomycin susceptibility in both vancomycin-susceptible and vancomycin-resistant enterococci and noted that 99.9% and 99.4% of strains were susceptible, respectively (Pfaller et al., 2007). Recently, reports of enterococci not susceptible to daptomycin have begun to emerge (Eliopoulous et al., 2011; Kelesidis et al., 2011, 2012a, b). These reports of daptomycin nonsusceptible enterococci (DNSE) have not shown a consistent association with daptomycin use, nor have they generally been clonal in nature (Eliopoulous et al., 2011; Kelesidis et al., 2012a; Storm et al., 2012).

☆ Presented in part at the 112th General Meeting American Society of Microbiology, San Francisco, CA, June 16–19, 2012.
☆☆ Support: This work was supported by a grant from Cubist Pharmaceuticals.
★ Disclosure: T.C.V. has received research funding from ViroPharma and has served as a consultant for Cubist Pharmaceuticals. M.E.R. has received research funding from 3M, served as a consultant for Molynlcke, and served on an advisory board for 3M and Semprus Biosciences. All other authors report no conflicts of interest.
* Corresponding author. Tel.: +402-559-8376; fax:+402-559-8730.
E-mail address: tvanscho@unmc.edu (T.C. Van Schooneveld).

0732-8893/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.diagmicrobio.2013.04.019

At our institution, a progressive decline in daptomycin susceptibility in *E. faecium* over the years 2008 to 2010 was noted. Based upon our institutional antibiogram, daptomycin susceptibility declined from 91.7% in 2008 to 81.6% in 2009, 65.5% in 2010, and 76.2% in 2011. Vancomycin-resistant strains of *E. faecium* predominated during this time, although vancomycin resistance rates remained relatively stable (2008, 63.1%; 2009, 73%; 2010, 69.2%; and 2011, 78% resistance). These data suggested a significant increase in DNSE at our institution to levels not previously reported. One explanation for this may have been the emergence and spread of a clonal isolate with daptomycin resistance. The fact that the rate of colonization/infection with VRE had declined at our institution from 1.44 cases per 1000 patient days in 2008 to 0.86 in 2010 made this less likely (unpublished observation). Also, use of daptomycin was relatively infrequent (averaged 8.3 days of therapy [DOT]/1000 patient days [PD]), although use did increase during the time period 2009–2011 (2009, 6.4 DOT/1000 PD; 2010, 7.10 DOT/1000 PD; and 2011, 11.4 DOT/1000 PD).

A second possibility was that the susceptibility testing method used in our clinical microbiology laboratory (MicroScan) may have been "overcalling" daptomycin nonsusceptibility. A recent technical service bulletin highlighted this issue and recommended that isolates of *E. faecium* with daptomycin MIC of >4 μg/mL be confirmed with a second method (Siemens Healthcare Diagnostics I, 2011). There have been a number of reports highlighting variability in daptomycin MIC results between testing methods in *Staphylococcus aureus* (Fuchs et al., 2001; Keel et al., 2010; Kruzel et al., 2011; Sader et al., 2009). Broth microdilution (BMD) is considered the reference standard by the Clinical Laboratory Standards Institute (CLSI), but other methods have been used to evaluate daptomycin susceptibility in enterococci including Etest, disk diffusion (not considered a valid method), Vitek 2, and Sensititre with varying levels of agreement (CLSI, 2013; Eliopoulous et al., 2011; Fuchs et al., 2001; Jorgensen and Crawford, 2006; Rathe et al., 2010). Based upon these findings, we sought to determine whether there was a true increase in DNSE and whether this increase was clonal in nature or whether discrepancies in susceptibility results were responsible for the increases in DNSE. We also desired to determine which susceptibility method was most effective at differentiating daptomycin-susceptible from nonsusceptible isolates.

2. Materials and methods

2.1. Bacterial isolates

Enterococcal isolates from The Nebraska Medical Center clinical microbiology laboratory previously defined as daptomycin nonsusceptible (MIC>4) by the MicroScan Walkaway 90 SI using the prompt method to inoculate PC33 panels (Siemens, Tarrytown, NY, USA) were selected by convenience and retrieved from storage in a −80 °C freezer. Not all DNSE isolates from each year were utilized. The anatomic source of the isolates was not recorded. The study consisted of 150 enterococcal isolates: *E. faecium* ($n = 147$), *E. faecalis* ($n = 2$), and *E. gallinarum* ($n = 1$) collected from a 4-year period: 2008 ($n = 67$), 2009 ($n = 7$), 2010 ($n = 8$), and 2011 ($n = 68$).

2.2. Susceptibility testing

Daptomycin susceptibility testing was performed in triplicate for each isolate using 5 methods: BMD, Etest, Sensititre, MicroScan prompt method (MSP), and MicroScan turbidity method (MST). BMD microtiter plates were prepared in-house following standard procedures (Garcia et al., 2010) using a bacterial inoculum of 5×10^5 CFU/mL and Difco cation-adjusted Mueller-Hinton broth (Becton Dickinson, Sparks, MD, USA) containing 50 mg/L of calcium. The BMD plates contained wells from 0.125 to 64 μg/mL in doubling dilutions of daptomycin (Cubist Pharmaceuticals, Lexington, MA, USA). The MicroScan Turbidity Meter (Siemens, Tarrytown, NY, USA) was used to prepare an inoculum equivalent to a 0.5 McFarland turbidity standard (1.5×10^8) (Remel, Lenexa, KS, USA); this inoculum, which was confirmed using standard dilution plating, was used to inoculate the MicroScan panel PC33 (Siemens; MIC wells, to 4 μg/mL) using standard methodologies and read on a MicroScan autoSCAN-4 system (Siemens). For the MSP method, 3 isolated colonies were selected using the prompt inoculation system, and the inoculation fluid was used for MicroScan analysis using the same panels and analysis methods as described above for the MST method. Etest (bioMérieux, Marcy l'Etoile, France; MIC values, 0.016 to 256 μg/mL) was performed using cation-adjusted Mueller-Hinton agar according to the manufacturer's recommendations. Susceptibility testing using Sensititre panels was performed according to the manufacturer's recommendations using panel GPN3F (TREK Diagnostic Systems, Cleveland, OH, USA; MIC wells, 0.25 to 8 μg/mL). All method results were assessed by a single trained reviewer. All susceptibility controls and interpretations were performed as described by the CLSI M100-S22 document; *E. faecalis* 29212 was used as a quality control strain for all susceptibility testing methods (CLSI, 2013).

2.3. Molecular methods

Pulsed-field gel electrophoresis (PFGE) using the protocol described by Turabelidze et al. (2000) was performed on DNSE isolates as defined by BMD. PFGE gels were analyzed and relatedness calculated using the Bionumerics software (Applied Maths, Austin, TX, USA).

2.4. Data analysis

As all isolates were tested in triplicate, modal MIC was used for analysis, and when not available median values were used. DNSE were defined as having a BMD MIC >4 μg/mL. Etest results that did not occur as a doubling dilution were adjusted to the next highest dilution (i.e., Etest MIC = 6 μg/mL adjusted to 8) for analysis unless otherwise noted. When the MIC was greater than the upper or lower limit of the testing method, the next higher or lower dilution was considered to be the MIC (i.e., MIC >4 μg/mL was considered to be 8 μg/mL for MIC analysis). Essential agreement (within ±1 dilution) and categorical agreement (same interpretation) were calculated along with discrepancy error rates. Very major errors were defined as isolates falsely determined to be susceptible and rates calculated by dividing the number of errors by the total number of resistant isolates. Major errors were defined as isolates falsely determined to be resistant and rates calculated by dividing the number of major errors by the number of susceptible organisms. Minor errors did not occur as no intermediate category exists for daptomycin. Weighted kappa statistics were calculated for each method using BMD as the gold standard and geometric means were also calculated. Statistical modeling was performed on the data using all tested replicates. MIC was modeled in 2 ways. In the first model, MIC was dichotomized as susceptible (≤4) or nonsusceptible (>4), and a logistic regression model was used to evaluate the probability that a sample will be classified as nonsusceptible as a function of year of isolation or of testing method, accounting for the variability across testing method and replicate. In the second model, the log base 2 of MIC was taken and a general linear regression model was fit to assess the change in MIC values over time and to compare each of the 4 antimicrobial susceptibility tests with the gold standard BMD. All analyses were done using