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Case-Control Study Comparing *De Novo* and Daptomycin-Exposed Daptomycin-Nonsusceptible *Enterococcus* Infections

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Understanding factors associated with *de novo* daptomycin-nonsusceptible *Enterococcus* (DNSE) infections will aid in better understanding the mechanisms of daptomycin nonsusceptibility. We conducted a case-control study to compare patients with DNSE infections who were daptomycin treatment naïve ($n = 9$) and those with DNSE infections who had exposure to daptomycin ($n = 13$). Less frequent exposure to antimicrobials, increased susceptibility to nitrofurantoin and gentamicin, and shorter duration of hospitalization were associated with *de novo* DNSE infection, suggesting a potential community reservoir.

There have been several reports of daptomycin-nonsusceptible *Enterococcus* (DNSE) infections developing during daptomycin therapy (11). DNSE infections in which there was no prior use of daptomycin therapy have also been reported (8, 9). Little is known about how these *de novo* DNSE infections develop or about the risk factors associated with them. We conducted a case-control study to compare demographic, epidemiological, clinical, and microbiological features between patients with *de novo* DNSE infections and patients with DNSE infections that developed after exposure to daptomycin. Determining risk factors associated with *de novo* DNSE infections will aid in understanding the mechanisms of daptomycin nonsusceptibility.

The UCLA clinical microbiology laboratory routinely tests all clinically significant Gram-positive organisms for daptomycin susceptibility by the CLSI reference broth microdilution (BMD) method (2). DNSE species isolated at the UCLA Health System from 1 January 2007 to 31 March 2011 were identified by querying the laboratory electronic database for *Enterococcus* species with a daptomycin MIC of >4 μg/ml (3, 11). Case patients were patients with at least one positive DNSE clinical culture with no known history of daptomycin exposure. Control patients were those with at least one positive DNSE clinical culture who had received daptomycin within 3 months prior to the first isolation of a DNSE strain. For patients with DNSE species isolated from multiple specimens, we only examined exposures before the first isolation of a DNSE species.

We reviewed medical records and recorded patient demographics, comorbidities, antibiotic exposures and durations within the prior 3 months, hospitalizations and surgeries within the prior 3 months, and antimicrobial susceptibilities. All patients and family members were interviewed by at least one infectious diseases physician. This study was approved by the UCLA Institutional Review Board.

Enterococcus isolates were identified by the Vitek Legacy (bioMérieux, Durham, NC). Enterococcal antimicrobial MIC susceptibility testing was performed by the CLSI reference BMD method (2). Screening for high-level aminoglycoside resistance was performed in brain heart infusion broth supplemented with gentamicin (500 μg/ml) or streptomycin (1,000 μg/ml) (2). All MICs were interpreted using CLSI interpretive criteria (4). Daptomycin MICs were confirmed by repeat BMD testing at the time of isolation and were later verified for 14 available DNSE isolates by Etest (bioMérieux, Durham, NC) on Mueller-Hinton agar (BBL, Sparks, MD). The genetic relatedness of these 14 available DNSE isolates was determined by repetitive-element PCR (rep-PCR) using a semiautomated system (DiversiLab, bioMérieux, Durham, NC) and a DiversiLab *Enterococcus* kit (bioMérieux, Durham, NC).

We determined clinical (if the patient completed at least 5 days of therapy and had resolution of signs and symptoms of DNSE infection) and microbiological (lack of positive cultures at least 7 days after cessation of antienterococcal antibiotics) responses to treatment of the DNSE infection episode when possible.

Univariate and multivariate models were constructed (SAS, version 9.2). The odds ratios (ORs) and 95% confidence limits (CLs) of demographic, epidemiological, clinical, and microbiological factors were calculated. In addition, an unpaired Student *t* test was used for comparison of continuous variables. A two-tailed *P* value of <0.05 was considered statistically significant.

During the study period, a total of 22 patients with 23 DNSE isolates were identified. Nine (41%) of the patients with DNSE infections had no history of daptomycin exposure (case patients), whereas 13 had received daptomycin treatment in the preceding 3 months (control patients). Of the 23 episodes of DNSE infection, DNSE species were isolated from blood ($n = 10$ [43.5%]), urine ($n = 5$ [21.7%]), an abdominal source ($n = 5$ [21.7%]), and wounds ($n = 3$ [13.0%]). Of the 23 DNSE isolates, 18 (78.3%) were *Enterococcus faecium*, including 6 (66.6%) of the 9 case isolates and 12 (85.7%) of the 14 control isolates ($P > 0.05$). Three isolates (13.0%) were *Enterococcus faecalis*, one (4.3%) was *Entero-*

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TABLE 1 Association of prior recent antibiotic exposure, duration of hospitalization, and antimicrobial sensitivities with *de novo* DNSE infection[a]

Factor	Adjusted OR[b]	95% CLs	*P* value
Absence of recent exposure to expanded-spectrum cephalosporins	27.62	1.42, 535.82	0.028
Hospitalization of <30 days	14.76	1.28, 169.92	0.031
Presence of nitrofurantoin activity	18.99	1.49, 242.71	0.024
Absence of gentamicin resistance	16.29	1.29, 205.82	0.031

[a] Significant factors only.
[b] Adjusted for age, gender, and immunosuppression.

coccus gallinarum, and one isolate was not identified to the species level. All 18 *E. faecium* isolates were resistant to ampicillin, and 15 (65.2%) of the 23 DNSE isolates were resistant to vancomycin. Although *E. faecium* isolates from case patients were more likely to be vancomycin resistant than those from control patients (5/5 [100%] versus 10/13 [76.9%]), this difference was not statistically significant ($P > 0.05$).

Case patients and control patients were similar in age, gender, immunosuppression status, frequency of prior surgery, receipt of or duration of vancomycin therapy, presence of a nidus of DNSE infection (such as a central venous catheter or abscess), clinical and microbiologic response, and 30-day mortality after the first DNSE infection (data not shown).

Fourteen DNSE isolates were available for typing by rep-PCR, including five isolates from case patients and nine from control patients. Four DNSE isolates were unique. Among 10 *E. faecium* isolates tested, six Diversilab patterns (clones) were identified. Only one *E. faecium* isolate from a case patient was identical to an *E. faecium* isolate obtained from a control patient; interestingly, the case patient was hospitalized 1 year before the control patient. Similarly, all three *E. faecalis* isolates tested were identical by rep-PCR. Of these, two were from case patients and the third was from a patient who had received 90 days of daptomycin treatment before the isolation of a DNSE strain. These three patients were hospitalized in different wards at different times over a 12-month period, their hospitalizations did not overlap, and no epidemiological link was identified between them.

All of the daptomycin-exposed control patients had a history of prior hospitalization within 3 months, compared to only two-thirds of the case patients that had a history of prior hospitalization within 3 months ($P = 0.029$). After adjusting for age, gender, and immune status, compared to control patients, case patients without prior daptomycin exposure had a significantly shorter duration of hospitalization preceding the first isolation of DNSE and significantly less exposure to antibiotics associated with the development of vancomycin-resistant enterococci (VRE), including expanded-spectrum cephalosporins (Table 1). Of note, recent antianaerobic therapy was common among both case and control patients (6/9 versus 13/13, respectively; $P = 0.029$). VRE commonly cause colonic colonization (6, 12), and glycopeptide-resistant (1) and daptomycin-nonsusceptible (5) bowel anaerobes have been reported. Recently, we suggested that the interplay between anaerobes and enterococci may have a possible role in the dissemination of daptomycin resistance (10). The roles of horizontal gene transfer between DNS bacteria and enterococci in the gut and prior antimicrobial exposure in promoting the emergence of DNSE infection remain speculative, especially for *de novo* DNSE infections.

In this study, DNSE isolates from case and control patients were typically susceptible to linezolid and tigecycline, whereas nitrofurantoin and gentamicin were significantly more active against *de novo* DNSE isolates than control patient isolates after adjusting for age, gender, and immune status (Table 1). The geometric means of daptomycin MICs obtained by Etest were 28 μg/ml for the control patients and 5.5 μg/ml for the case patients; this difference was not statistically significant ($P = 0.06$), but only 5 case patient isolates were available for this analysis. The lower frequency of prior antimicrobial exposure among case patients and the increased susceptibility of their DNSE isolates suggest a possible community reservoir of DNSE strains or, alternatively, an increased risk of transmission of these strains within our facility.

The possibility of a community reservoir of DNSE strains (8) is further supported by our identification of a clonally related *E. faecalis* isolate shared among two daptomycin-unexposed patients and one daptomycin-exposed patient. It is unclear if other risk factors, such as exposure to animal products and horizontal gene transfer of daptomycin resistance determinants, may promote the development and transmission of DNSE strains in the community (T. Kelesidis, R. Humphries, D. Z. Uslan, and D. Pegues, unpublished observation).

The limitations of our study include the retrospective study design and the small number of cases identified. Because we did not obtain complete medical records from outside referring facilities, we may have underestimated antimicrobial exposures. In addition, while all daptomycin MICs for the DNSE isolates in this study were confirmed by repeat testing, 9 isolates were not available for supplemental testing by Etest. Of those isolates tested by Etest, 3 (13%) tested with an MIC of 4 μg/ml, which, by the CLSI interpretive criteria, is considered susceptible, and 3 were less than a dilution above the CLSI susceptible breakpoint by Etest. However, it has been documented by others that Etest MICs are generally lower than those obtained by the reference broth microdilution method (7). Nonetheless, this study describes the largest series of *de novo* DNSE infections and is the first attempt to define factors associated with DNSE infection in patients with no prior exposure to daptomycin. Case-control studies focused on potential community exposures outside those associated with health care may better define risk factors associated with the emergence of *de novo* DNSE resistance.

The acquisition and emergence of daptomycin resistance among enterococci pose both treatment and infection control challenges. Clinicians should be vigilant not only for the emergence of DNSE infection associated with prolonged daptomycin administration for treatment of serious enterococcal infection but also for DNSE infection arising *de novo*.

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