INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Clinical Characteristics of Methicillin-resistant Coagulase-negative Staphylococcal Bacteremia in a Tertiary Hospital

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Abstract

Objective Coagulase-negative staphylococci are among the most frequently isolated microorganisms in blood cultures. The aim of this study was to assess [1] the clinical characteristics of methicillin-resistant, coagulase-negative staphylococci bacteremia and [2] the susceptibility of the isolated bacteria to glycopeptides.

Methods We retrospectively reviewed the medical records of 70 patients from whom methicillin-resistant coagulase-negative staphylococci had been isolated at Osaka City University Hospital between January 2010 and December 2013. We evaluated the patients' background, severity and prognosis of the disease, and the susceptibility of the isolated methicillin-resistant coagulase-negative staphylococci to glycopeptides.

Results Out of the 70 patients tested, 28 (40.0%) had leukemia, and 36 (51.4%) had been treated for febrile neutropenia. Infection with *Staphylococcus epidermidis* accounted for 78.6% of patients. Thirty-nine cases (55.7%) were related to intravascular catheters, and 39 (55.7%) were treated using teicoplanin as a first-line therapy. The 30-day mortality rate was 4.3%. Regarding susceptibility, 20% of all isolates were non-susceptible to teicoplanin. According to multivariate analyses, it was observed that premedication using glycopeptides was independently associated with teicoplanin non-susceptibility (p=0.03; hazard ratio = 5.64; 95% confidence interval, 1.16-26.76).

Conclusion Our results suggest that clinicians must use glycopeptides appropriately to prevent the development of further antibiotic resistance in methicillin-resistant coagulase-negative staphylococci.

Key words: methicillin-resistant coagulase-negative staphylococci, teicoplanin, catheter-related bloodstream infection, teicoplanin non-susceptibility

Introduction

Coagulase-negative staphylococci (CoNS) are common colonizers of human skin, and are among the most commonly isolated organisms in clinical microbiology laboratories. Furthermore, CoNS have become true pathogens, rather than simply culture contaminants (1-3), causing cardiovascular, joint, and bloodstream infections, among other conditions. In fact, CoNS are the most frequent cause of bloodstream infection, accounting for 36% of isolates (4). Patients with prosthetic devices, intravascular catheters, or other implanted foreign bodies are at particular risk of CoNS infection (5). However, even though CoNS are relatively common, they are associated with several unsolved clinical problems. For instance, it remains difficult for investigators to distinguish true CoNS infections from cases of culture contamination. This can lead to the erroneous use of antimicrobial therapies.

A high proportion of CoNS isolates are methicillin-

Table 1. Clinical Characteristics of Patients with MR-CoNS Bacteremia.

Sex (male/female)	38/32
Mean age (range)	53.5 (18-87)
Underlying disease	
Hematological malignancy	42 (60.0%)
Leukemia	28 (40.0%)
MDS	9 (12.9%)
Lymphoma	5 (7.1%)
Cardiovascular disease	9 (12.9%)
Solid tumor	8 (11.4%)
Diabetes mellitus	8 (11.4%)
Digestive disease	5 (7.1%)
Central nervous system disease	5 (7.1%)
Chronic kidney disease	4 (5.7%)
Others	8 (11.4%)
Comorbid condition	
Intravenous hyperalimentation catheter	62 (88.6%)
Use of antibiotics prior to isolation	49 (70.0%)
Febrile neutropenia	36 (51.4%)
Source of infection	
Intravascular device	39 (55.7%)
Unknown	31 (44.3%)
SOFA score	4.3 (0-16)
Mortality	3 (4.3%)

MDS: myelodysplastic syndrome, SOFA: sequential organ failure assessment

resistant (MR). For this reason (6), it is recommended to treat CoNS infections using glycopeptides (7). However, several groups have reported CoNS isolates that are less susceptible to glycopeptides (3, 8, 9), and it is important to diagnose and treat CoNS infections appropriately.

The purpose of the present study was to assess the clinical characteristics of patients with MR-CoNS bacteremia, as well as to identify the risk factors for glycopeptide resistance.

Materials and Methods

Study protocol

We retrospectively reviewed the medical records of 70 patients with MR-CoNS bacteremia who had been admitted to Osaka City University Hospital between January 2010 and December 2013. Osaka University Hospital had 969 beds in April 2013. We evaluated the patient background, severity, prognosis, and susceptibility to antibiotics. In cases where MR-CoNS had been isolated from the same patient on multiple occasions within a four-year period, we reviewed the first episode of MR-CoNS. The study was approved by the Ethics Committee of Osaka City University (number: 3310, date: 4/1/2016).

Identification of bacteria

All MR-CoNS isolates were identified using colony morphologic analyses, Gram staining, and catalase and coagulase testing. Isolates were identified using the MicroScan® WalkAway® system (Siemens, Munich, Germany) and recognized as MR-CoNS if the minimum inhibitory concentration (MIC) of oxacillin was ≥0.5 µg/mL. Furthermore, the MICs

of vancomycin (VCM), teicoplanin (TEIC), arbekacin (ABK), linezolid (LZD), and daptomycin (DAP) were determined using a dilution antimicrobial-susceptibility test in accordance with the manufacturer's instructions (Eiken Chemical, Japan). To test DAP specifically, we used Mueller-Hinton broth that was supplemented with 50 µg/mL of calcium. All plates were incubated at 35°C for 24 h.

Identification of infection

We diagnosed MR-CoNS bacteremia when a patient had two or more positive blood cultures on the same day and clinical signs of infection, such as a fever and chills, with or without local signs and symptoms. Catheter-related bloodstream infection (CRBSI) can only be definitively diagnosed when the same organism grows from both a catheter tip and at least one percutaneous blood culture, or when cultures from two blood samples (one from a catheter hub and the other from a peripheral vein) meet the CRBSI criteria for either quantitative blood cultures or for differential time to positivity (7).

Assessment of laboratory data

We recorded the white blood cell count and C-reactive protein levels on the day when the initial blood culture gave a positive result. Neutropenia was defined as a neutrophil count <500/µL. The severity of illness was assessed using the Sequential Organ Failure Assessment (SOFA) score (10).

Antimicrobial treatment

The attending physician selected the initial antimicrobial treatment. The first antibiotic used was only changed if the patient did not respond to treatment or showed side effects.

Statistical analysis

The patient characteristics and outcomes were compared using the SPSS 22.0 software program (IBM SPSS Statistics, NY, USA). Fisher's test was used for the univariate comparison of categorical data. Variables with a p value < 0.20 in the univariate analyses were included in a forward, stepwise, multivariate logistic regression to determine the risk factors for TEIC non-susceptible bacteremia. A p value <0.05 denoted a statistically significant difference.

Results

There were a total of 13,941 blood culture sets included in this study, and 314 sets were MR-CoNS-positive. The rate of patients with 2 blood culture sets during this study was 42%. There were 70 MR-CoNS bacteremia cases. Table 1 shows the clinical characteristics of the patients. MR-CoNS was isolated from 70 patients (38 men, 32 women) with a mean age of 53.5 years. Forty-two (60%) patients had a hematological malignancy; of these, 28 (70%) had leukemia. Thirty-six patients (51.4%) had been treated for febrile neutropenia. Thirty-nine (55.7%) cases were related to intravascular catheters. The mean SOFA score was 4.3.

Table 2. Antibiotics Used Prior to MR-CoNS Bacteremia.

Antibiotics	
Carbapenems	22 (31.4%)
Fourth-generation cephalosporins	19 (27.1%)
Tazobactam-piperacillin	12 (17.1%)
Glycopeptides	11 (15.7%)
Anti-fungal agents	10 (14.3%)
Quinolones	6 (8.6%)
Aminoglycosides	5 (7.1%)
Others	11 (15.7%)
No antibiotics	21 (30.0%)

Note: Oral quinolones were excluded

Table 3. Isolated Species of MR-CoNS.

Pathogen	
S. epidermidis	55 (78.6%)
S. haemolyticus	10 (14.3%)
S. capitis subsp. ureolyticus	2 (2.9%)
Others	3 (4.3%)

Note: *S. lugdunensis* was not isolated. Others: *S. hominis* subsp. *hominis* 1, *S. capitis* subsp. *capitis* 1, *S. hominis* subsp. *novobiosepticus* 1.

Table 4. The Minimum Inhibitory Concentrations (MICs) of MR-CoNS Strains.

Antibiotics	MIC range (µg/mL)	MIC_{50}	MIC_{90}	Non-susceptibility
VCM	≤ 0.5-2	1.5	2	0%
TEIC	≤ 0.5-32	8	16	20%
ABK	≤ 0.5-4	≤ 0.5	1.5	0%
LZD	≤ 0.5-2	0.75	1	0%
DAP	≤ 0.5-1	≤ 0.5	≤ 0.5	0%

VCM: vancomycin, TEIC: teicoplanin, ABK: arbekacin, LZD: linezolid, DAP: daptomycin

Twenty-two patients (31.4%) were treated prior to isolation using carbapenems, and 19 were treated using fourth-generation cephalosporins (Table 2). *Staphylococcus epidermidis* was the most frequent pathogen (55 cases; 78.6%), followed by *S. haemolyticus* (Table 3). Of the 70 patients, 39 (55.7%) were treated using TEIC as first-line therapy; 12 (17.1%) were treated using VCM, and 3 (4.2%) using LZD. None were treated using DAP. The MIC_{90} values of VCM, TEIC, ABK, LZD, and DAP were 2, 16, 1.5, 1, and 0.5, respectively. Fourteen patients (20%) showed no response to TEIC (Table 4). Among these, 8 (57.1%) had been treated using TEIC, but none died within 30 days.

Table 5 shows the results of a univariate analysis of the risk factors for TEIC non-susceptibility. Underlying diseases, comorbid conditions, severity, and prognosis did not differ markedly between the TEIC susceptible and non-susceptible groups. However, glycopeptide use prior to isolation was more frequent in the non-susceptible group than in the susceptible group (p=0.002). Teicoplanin was administered in nine cases within two months prior to the current blood stream infection. In the previous instance, the duration of therapy of teicoplanin was more than three days. The trough levels of this agent in the previous instance were 8.4-26.0 µg/mL (mean: 18.3 µg/mL). The trough levels of 7 cases (77.8%) were within 15-30 µg/mL, which is the target trough level. Furthermore, this factor was independently associated with TEIC non-susceptibility in the multivariate analyses (p=0.03; hazard ratio = 5.64; 95% confidence interval = 1.16-26.76; Table 6).

Discussion

The results of this study can be summarized as follows: [1] hematological malignancy and febrile neutropenia are common in patients with MR-CoNS infection, [2] the rates of catheter-related infection and TEIC therapy were high, [3] MR-CoNS bacteremia was associated with a low mortality rate, and [4] glycopeptide use prior to isolation was significantly associated with TEIC non-susceptibility.

In the present study, over half of the patients with MR-CoNS bacteremia had a hematological malignancy (60.0%), and around half (51.4%) had febrile neutropenia (Table 1). Similarly, Chen et al. found that CoNS was the most common Gram-positive pathogen among all patients with a hematological malignancy, and that 65% of these patients were neutropenic (11). In a prospective, multi-center study that involved 54 hospitals, patients with a hematological malignancy were stratified, and CoNS were found to be the leading pathogens, comprising 50.6% of the isolated strains (12). The increased risk of CoNS bacteremia in patients with a hematological malignancy may be related to chemotherapy-induced neutropenia, skin or mucosal disorders, or longer detainment of central venous catheters. In the present study, CoNS was associated with a high rate of catheter-related infection (55.7%; Table 1).

In general, VCM is recommended as a therapy for CRBSI and as a first-line therapy for MR-CoNS infection (7). However, in the present investigation, 55.7% of patients had been treated using TEIC as the first-line therapy. A prospective, randomized study involving VCM and TEIC therapy in 66 patients with neutropenia concluded that the drugs have equal efficacies (VCM: 44.7% vs. TEIC: 47.4%) and that red man syndrome occurs more frequently in patients treated with VCM (10.8%) (13). Another prospective, randomized study of VCM and TEIC therapy in febrile neutropenic patients reported that nephrotoxicity was more common in patients receiving VCM than among those receiving TEIC (14). It may be that many clinicians prefer to use TEIC therapy as a first regimen because the drug is safe for use with severe underlying diseases, such as hematological malignancy and febrile neutropenia.

The 30-day mortality rate was 4.3% in our study, which was not high. A prospective cohort study similarly reported that, in patients with cancer and febrile neutropenia, CoNS bacteremia resulted in lower 28-day mortality than bactere-

Table 5.   Univariate Analysis of Risk Factors Associated with TEIC Non-susceptibility.

Variables	Susceptible (n = 56)	Non-susceptible (n = 14)	p value
Age ≥ 70	10 (17.9%)	4 (26.6%)	0.37
Male sex	30 (53.7%)	8 (57.1%)	0.81
Leukemia	23 (41.1%)	5 (35.7%)	0.71
MDS	9 (16.1%)	0 (0%)	0.21
Lymphoma	3 (5.4%)	2 (14.3%)	0.25
Cardiovascular disease	8 (14.3%)	1 (7.1%)	0.48
Solid tumor	6 (10.7%)	2 (14.3%)	0.71
Diabetes mellitus	7 (12.5%)	1 (7.1%)	0.57
Digestive disease	3 (5.4%)	2 (14.3%)	0.25
Central nervous system disease	4 (7.1%)	1 (7.1%)	1.00
Chronic kidney disease	3 (5.4%)	1 (7.1%)	0.80
Intravenous hyperalimentation catheter	50 (89.3%)	12 (85.7%)	0.71
Febrile neutropenia	30 (53.6%)	6 (42.9%)	0.47
CRBSI	33 (58.9%)	6 (42.9%)	0.27
SOFA ≥ 5	16 (28.6%)	4 (28.6%)	1.00
CRP ≥ 5	15 (27.3%)	7 (50.0%)	0.07
S. epidermidis	12 (85.7%)	12 (76.8%)	0.47
Use of antibiotics prior to isolation			
Carbapenems	16 (28.6%)	7 (50.0%)	0.13
Fourth-generation cephalosporins	16 (28.6%)	3 (21.4%)	0.59
Tazobactam-piperacillin	9 (16.1%)	3 (21.4%)	0.63
Glycopeptides	5 (8.9%)	6 (42.9%)	0.002
Anti-fungal agents	7 (12.5%)	3 (21.4%)	0.39
Quinolones	6 (10.7%)	0 (0%)	0.39
Aminoglycosides	3 (53.6%)	2 (14.3%)	0.25
Mortality	2 (3.6%)	1 (7.1%)	0.56

MDS: myelodysplastic syndrome, CRBSI: catheter-related bloodstream infection, SOFA: sequential organ failure assessment, CRP: C-reactive protein

Table 6.   Multivariate Analysis of Risk Factors for TEIC Non-susceptibility.

Risk factor	OR (95% confidence interval)	p value
CRP ≥ 5	2.63 (0.70–9.91)	0.15
Premedication of carbapenems	1.69 (0.39–6.50)	0.50
Premedication of glycopeptide	5.64 (1.19–26.76)	0.03

CRP: C-reactive protein

mia caused by other pathogens (4.3% vs. 32.7%; log-rank p=0.009) (15). Furthermore, a meta-analysis reported a lower mortality in studies where CoNS caused greater than 30% of CRBSI (16). In our own previous report, the mortality rate from methicillin-resistant *S. aureus* (MRSA) bacteremia was 25.3% (17). *S. aureus* is associated with various virulence factors such as enterotoxin, toxic shock syndrome, and Panton-Valentine leucocidin. On the other hand, much less is known about the virulence mechanisms of CoNS, although various aspects of biofilm formation by *S. epidermidis* have been elucidated (18). It follows that the lower mortality from CoNS bacteremia may reflect the lower virulence of this organism.

In the current study, 20% of MR-CoNS bacteremia cases were non-susceptible to TEIC (Table 4), and premedication using glycopeptides was independently associated with TEIC non-susceptibility (Table 6). In the SENTRY Antimicrobial Surveillance Program study, 0.4% of all CNS isolates were TEIC resistant (19); in a United Kingdom study, 20.8% were non-susceptible to TEIC, but only 1 VCM-intermediate

CoNS isolated was found (20). Furthermore, in a 5-year retrospective study, Trueba et al. reported that 55% of MR-CoNS were resistant to TEIC (21). In contrast, TEIC resistance is very rare in MRSA (22, 23). Therefore, TEIC resistance is a serious problem in the treatment of MR-CoNS bacteremia, although there are regional differences in resistance rates. Furthermore, Boisson et al. reported a significant correlation between the incidence of bacteremia caused by CoNS with decreased susceptibility to TEIC and glycopeptide use (3). In addition, a 1-year, prospective, case-control study showed that previous exposure to β-lactams and glycopeptides, multiple hospitalizations in the previous year, and concomitant use were significantly associated with glycopeptide-resistant CoNS bacteremia (24). Thus, to prevent the spread of resistance to this agent, clinicians must use glycopeptides, especially TEIC, responsibly.

In the present study, the MICs of LZD and DAP were very low (Table 4). Therefore, these agents may be candidate treatments for MR-CoNS bacteremia. Indeed, a retrospective, multi-center, observational study reported that 94% of CoNS infections in neutropenic patients were responsive to DAP therapy (25), and the MR-CoNS eradication rates for LZD were high (85%) in microbiologically evaluable patients (26). In a recent, nationwide, prospective study involving 37 hospitals in France, LZD resistance occurred in 1.4% of isolates (27). Considering the low virulence and mortality of MR-CoNS infections, antibiotics other than anti-MRSA agents should be evaluated in the future.

Several limitations associated with the present study warrant mention. First, the rate of two blood culture sets from January 2010 to December 2013 was 42%. Therefore, our results may not reliably reflect the present situation regarding MR-CoNS bacteremia in our hospital. We need to improve the rate of two blood culture sets, as well as re-evaluate MR-CoNS bacteremia cases. Secondly, we did not perform a genetic analysis. Thus, a specific TEIC-resistant strain may have been endemic in the hematology unit. In the future, we will need to genetically characterize MR-CoNS bacteremia using pulse field gel electrophoresis and the staphylococcal cassette chromosome *mec*.

In conclusion, our results suggest that glycopeptides, especially TEIC, must be used appropriately to prevent antibiotic resistance in MR-CoNS. Furthermore, large prospective studies are required to evaluate alternate treatments for MR-CoNS bacteremia.

The authors state that they have no Conflict of Interest (COI).

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