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Ceftaroline-Heteroresistant *Staphylococcus aureus*

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Heteroresistance refers to the presence, within a large population of antimicrobial-susceptible microorganisms, of subpopulations with lesser susceptibilities. Ceftaroline is a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The aim of this study was to detect the prevalence of ceftaroline heteroresistance *in vitro* in a select group of *S. aureus* strains. There were 57 isolates selected for evaluation, 20 MRSA, 20 vancomycin-intermediate *S. aureus* (VISA), 7 daptomycin-nonsusceptible *S. aureus* (DNSSA), 6 linezolid-nonsusceptible *S. aureus* (LNSSA), and 4 heteroresistant VISA (hVISA) isolates. MICs and minimal bactericidal concentrations were determined using the broth microdilution method according to CLSI guidelines. All of the isolates were analyzed by pulsed-field gel electrophoresis. The staphylococcal cassette chromosome *mec* element (SCC*mec*) types were determined by a multiplex PCR. Population analysis profiles (PAPs) were performed to determine heteroresistance for all of the isolates using plates made by adding various amounts of ceftaroline to brain heart infusion agar. The frequencies of resistant subpopulations were 1 in 10^4 to 10^5 organisms. We determined that 12 of the 57 (21%) isolates tested were ceftaroline-heteroresistant *S. aureus* (CHSA). CHSA occurred among strains with reduced susceptibilities to vancomycin, daptomycin, and linezolid but occurred in none of the USA-300 isolates tested. Evaluation of the heteroresistant strains demonstrated that the phenotype was unstable. Further studies are needed to determine whether CHSA has a role in clinical failures and to determine the implications of our study findings.

Ceftaroline is a new parenteral cephalosporin with antimicrobial activity against *Staphylococcus aureus* strains with reduced susceptibilities to methicillin and vancomycin and has been approved for the treatment of acute bacterial skin and skin structure infections and community-acquired (CA) pneumonia. It is a novel agent because of its avidity for penicillin-binding proteins (PBPs), including PBP2a, which is associated with methicillin resistance (1). There are a few strains of *S. aureus* that are intermediate to ceftaroline according to the breakpoints established by the Clinical and Laboratory Standards Institute (CLSI) (2, 3).

Heteroresistance refers to the presence, within a larger population of fully antimicrobial-susceptible microorganisms, of subpopulations with lesser susceptibilities (4). Although the clinical significance is still unclear, heteroresistance has been reported among various antimicrobial agents used against *S. aureus*, including beta-lactams and vancomycin. Typically, the subpopulations with lesser susceptibilities are present at frequencies of 1 subclone in every 10^5 to 10^6 colonies. This is why it is difficult to detect these clones in normal broth microdilution MIC testing using an inoculum of 5×10^4 CFU/well. Population analysis profiles (PAPs), which use a larger inoculum size, are considered the most reliable method for detecting heteroresistant subpopulations. The aim of this study was to detect the prevalence of ceftaroline heteroresistance *in vitro* in a select group of *Staphylococcus aureus* strains.

MATERIALS AND METHODS

Isolates. A collection of 57 isolates was selected for evaluation. Methicillin-resistant *S. aureus* (MRSA) ($n = 20$), heteroresistant vancomycin-intermediate *S. aureus* (hVISA) ($n = 4$), and daptomycin-nonsusceptible *S. aureus* (DNSSA) ($n = 7$) isolates were obtained from patients admitted to St. John Hospital and Medical Center (Detroit, MI). VISA ($n = 20$) and four of the linezolid-nonsusceptible *S. aureus* (LNSSA) isolates were obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program (supported under NIAID/NIH contract HHSN272200700055C). Two LNSSA isolates were obtained from Robinson Memorial Hospital in Ohio. Either ceftaroline was not given to these patients, or their exposure was unknown.

Susceptibility testing. MICs were determined using microdilution tests with cation-adjusted Mueller-Hinton broth. MICs were determined in accordance with CLSI guidelines. MICs were read visually as the lowest drug concentration well with no visible bacterial growth. Minimal bactericidal concentrations (MBC) were determined to be the antibiotic concentration that reduced the number of viable cells by ≥99% as determined by colony counts. We also determined MICs using an Etest strip containing ceftaroline.

Molecular testing. Staphylococcal cassette chromosome *mec* element (SCC*mec*) types were determined by using a multiplex PCR method on all isolates (5). The isolates were analyzed by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme SmaI. The PFGE gel patterns were compared with the development of a dendrogram using GelCompar II software (Applied Maths). Percent similarities were derived from the unweighted-pair group method using arithmetic averages (UPGMA) and based on Dice coefficients. The band position tolerance was set at 1.25, and optimization was set at 0.5%. An isolate was determined to belong to a PFGE strain group (USA-100 to USA-1100) if its similarity coefficient was ≥80% (6). The USA-100 to USA-1100 strains used for comparison were obtained from the NARSA.

Heteroresistance testing procedure. PAP assays to detect heteroresistance were performed as previously described (7) with the following modifications. Testing plates were prepared by adding ceftaroline to brain heart infusion (BHI) agar (Difco). The BHI agar was prepared according to the manufacturer's instructions. Ceftaroline (CPT) powder was reconstituted and added to the BHI agar plates at concentrations of 0.25, 0.5,

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TABLE 1 Results of samples that did not show growth above 1 μg/ml of ceftaroline on PAP plates

Sample	Results (μg/ml) for CPT[a]			PAP results (log_{10} CFU/ml) at concentration[b] (μg/ml) of:					SCC*mec* type	Pulse	Source	Results (μg/ml) for VAN[c]		Sample type
	MIC	MBC	Etest	0	0.25	0.5	0.75	1				MIC	MBC	
SA-11	0.5	0.5	0.5	7.2	7.1	4.9	3.2	0	IVa	USA-300	Left knee	1	1	MRSA
SA-21	0.5	0.5	0.5	7.2	7.1	4.3	3.2	0	IVa	USA-300	Buttock	1	1	MRSA
SA-23	0.5	0.5	0.5	7	6.9	5.4	3.8	0	IVa	USA-300	Thigh	1	1	MRSA
SA-25	0.5	1	0.5	7.1	7	5	3.2	0	IVa	USA-300	Unknown	1	1	MRSA
TX-O	0.5	0.5	0.5	7.2	7.2	6.1	3.7	0	IVa	USA-300	Blood	1	1	MRSA
D-7	1	1	1	6.8	6.7	6.8	6	0	II	No match	Sputum	1	1	MRSA
D-10	1	1	1	6.9	6.9	6.8	5.4	3.1	II	USA-600	Unknown	1	1	MRSA
D-17	1	1	1	6.9	6.9	6.9	5.8	3.7	II	USA-600	Wound	1	1	MRSA
D-20	0.5	1	0.75	7.4	7.4	4.8	0	0	IVa	USA-300	Blood	1	1	MRSA
D-21	1	1	1	6.8	6.7	5.5	2.9	0	II	USA-600	Blood	1	1	MRSA
ND-10	0.5	0.5	0.5	7.1	6.9	3.7	2.7	0	IV	No match	Respiratory	0.5	1	MRSA
ND-27	0.5	1	1	7.1	7.1	6.4	3.3	0	II	USA-100	Respiratory	1	1	MRSA
ND-29	1	1	1	7	6.9	6.8	5.1	0	II	USA-600	Respiratory	1	1	MRSA
ND-30	1	1	1	6.9	6.9	6.8	5.5	4.4	II	USA-600	Respiratory	1	1	MRSA
END-12	0.5	1	1	6.6	6.6	6.3	4.5	2.5	II	USA-100	Respiratory	1	1	MRSA
JhVISA-1	1	1	1	6.7	6.7	6.6	4.9	0	II	No match	Blood	1	1	hVISA
JhVISA-3	0.5	1	1	6.8	6.9	6.7	5.6	2.7	II	USA-100	Blood	1	1	hVISA
JhVISA-4	0.5	1	0.75	7.1	7	5	4	3.7	IVa	USA-300	Blood	2	2	hVISA
DNS-1	0.5	1	1	7.1	7	6.9	6.2	2.5	II	USA-100	Blood	1	2	DNSSA
DNS-4	1	1	1	7	6.9	6.6	4.3	3.3	III	No match	Blood	2	2	DNSSA
DNS-5	0.5	0.5	0.75	7.1	7	6.6	2.8	0	IVa	USA-300	Blood	1	2	DNSSA
DNS-6	1	1	0.75	7	7	6.6	0	0	II	USA-100	Blood	2	2	DNSSA
DNS-7	0.5	1	0.75	7	7	6.7	4.1	2.6	IVa	USA-300	Blood	2	2	DNSSA
NRS-1	1	1	0.75	6.8	6.7	6.6	6.6	4.5	II	No match	Mu50	8	8	VISA
NRS-14	0.5	0.5	0.25	6.8	6.7	4.3	0	0	MSSA	No match	Eye	8	16	VISA
NRS-17	1	1	0.75	6.8	6.7	5.9	0	0	II	USA-100	Blood	8	8	VISA
NRS-18	0.5	0.5	0.5	6.3	6.2	3.4	0	0	II	USA-100	Wound	4	8	VISA
NRS-19	0.5	1	0.25	6.3	6.2	6.1	4.9	2.5	II	No match	Blood	4	4	VISA
NRS-21	0.5	0.5	0.5	6.4	6.3	5.4	2.5	0	IVd	USA-500	Unknown	4	4	VISA
NRS-23	1	1	0.5	6.2	6	5.4	3.3	0	II	USA-100	Bone	4	4	VISA
NRS-49	1	2	0.5	6.5	6.5	6.5	5.9	3.5	II	No match	Unknown	8	8	VISA
NRS-51	1	1	1	6.9	6.9	6.5	3.1	2.5	II	No match	Bile	4	4	VISA
NRS-52	0.25	0.25	0.25	7.2	3	0	0	0	MSSA	No match	Bile	8	16	VISA
NRS-56	1	1	0.75	6.3	6.2	6.2	4.2	0	III	No match	Unknown	4	8	VISA
NRS-73	0.5	0.5	0.38	7.1	7.1	4.1	0	0	IVd	USA-500	Wound	4	4	VISA
NRS-126	1	1	1	6.9	6.9	6.4	4.3	3.5	II	USA-100	Blood	4	4	VISA
NRS-272	1	1	0.5	6.7	6.5	5	3.7	3	I	No match	Unknown	4	8	VISA
NRS-127	0.5	1	0.75	7	7.1	6.1	2.4	0	II	USA-100	Sputum	1	1	LNSSA
NRS-271	1	1	1	7.1	7.1	6.6	4.8	4.2	IV	No match	Unknown	1	1	LNSSA
LNSSA-10	0.5	1	0.75	6.9	6.9	3.6	0	0	II	No match	Blood	1	1	LNSSA
LNSSA-11	1	1	1	6.7	6.6	5.9	4.4	0	II	USA-100	Blood	1	1	LNSSA

[a] CPT, ceftaroline.
[b] Concentration (μg/ml) of CPT in PAP plates.
[c] VAN, vancomycin.

0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, and 4.0 μg/ml. The isolates to be tested were grown overnight on blood agar plates (BAP). The overnight BAP culture was suspended in saline and used to prepare samples at 10^7 CFU/ml and 10^4 CFU/ml. Aliquots of the samples containing 10^7 CFU/ml were than spiral plated (Whitley automatic spiral plater; Microbiology International) onto a drug-free BHI agar plate and onto BHI agar plates containing ceftaroline at all concentrations to determine the presence of heteroresistance. Aliquots of the samples containing 10^4 CFU/ml were spiral plated onto the drug-free BHI agar plate and onto plates containing 0.25, 0.5, and 0.75 μg/ml of ceftaroline. This was done to obtain an accurate determination of the numbers of CFU/ml. The plates were incubated at 35°C for 48 h in ambient air. The colonies were counted after 48 h using a ProtoCOL automated colony counter (Synbiosis, Frederick, MD, USA). PAPs were generated by plotting the log_{10} CFU/ml against the antibiotic concentrations. The frequency of resistant subpopulations at the highest drug concentration was calculated by dividing the number of colonies grown on an antibiotic-containing plate by the colony count from the same bacterial inoculum plated onto the antibiotic-free plate (8). We considered any sample with a susceptible ceftaroline MIC and growth in the intermediate or resistant range as heteroresistant. All samples were run three separate times, and the results were averaged for a final result. Colonies that grew on the plates containing the highest concentration of ceftaroline were removed and subcultured daily for 7 days onto antibiotic-free medium. The isolates were subcultured, and MICs were determined daily in order to determine if the resistance was stable (9).

RESULTS

Table 1 provides the data for PAPs for the isolates that did not

TABLE 2 Results of ceftaroline-intermediate organisms that did not show heteroresistance

Sample	Results (μg/ml) for CPT[a]			PAP results (log_{10} CFU/ml) at concentration[b] (μg/ml) of:									SCC*mec* type	Pulse	Source	Results (μg/ml) for VAN[d]		Sample type
	MIC	MBC	Etest	0	0.25	0.5	0.75	1	1.25	1.5	2	2.5[c]				MIC	MBC	
NRS-39	2	2	1.5	6.4	6.3	6.2	5.4	4.2	3	2.4	0	0	I	No match	Urine	8	8	VISA
NRS-54	2	2	1	6.8	6.8	6.6	6.2	4.6	4.3	4	3.7	0	III	No match	Unknown	4	4	VISA
NRS-65	2	2	2	6.3	6.3	6.3	6.2	6.1	5.2	4.6	4	0	III	No match	Unknown	4	8	VISA
NRS-283	2	2	1.5	6.9	6.9	6.8	6.8	6.5	5.2	4.1	2.8	2.5	II	USA-200	Unknown	4	4	VISA

[a] CPT, ceftaroline.
[b] Concentration (μg/ml) of CPT in PAP plates.
[c] For all organisms, there was no growth at a CPT concentration of 3 μg/ml or 4 μg/ml.
[d] VAN, vancomycin.

demonstrate heteroresistance, as there was no growth above 1 μg/ml. Among these 41 isolates, there were 3 hVISA and 14 VISA strains. There were four VISA isolates (Table 2) that showed intermediate susceptibility to ceftaroline and did not demonstrate heteroresistance to ceftaroline. The PAPs disclosed heteroresistance among 12 isolates tested, with growth at ceftaroline concentrations of 1.25 to 3 μg/ml (Table 3). Ceftaroline heteroresistance was seen among 2 of the 20 VISA strains tested, which was not significantly different than 5 of the 20 MRSA strains tested ($P = 0.41$, Fisher's exact test). The occurrence of ceftaroline heteroresistance also occurred in 2 of the 6 LNSSA, 2 of the 7 DNSSA, and 1 of the 4 hVISA isolates. Note that although there were 9 USA-300 SCC*mec* type IVa isolates, none of these isolates demonstrated heteroresistance in this study (Table 1). The frequencies of resistance populations ranged from 1.1×10^{-5} to 2.2×10^{-4} (Table 3).

The heterogeneous growth in the presence of ceftaroline was determined to be unstable. After daily passages onto a drug-free medium, the MICs of the colonies obtained from the plates with the highest concentrations of ceftaroline returned to the MICs of the native isolates.

DISCUSSION

Among the 57 isolates tested, 12 (21%) of the strains demonstrated heteroresistance by the population analysis profiling method, which is considered an acceptable method for determining heteroresistance. Heteroresistance was found in strains which were nonsusceptible to daptomycin, resistant to linezolid, and intermediate in susceptibility to vancomycin. Finally, we did identify one strain heteroresistant to ceftaroline and vancomycin. The rate of ceftaroline heteroresistance in this study was higher than the rate of vancomycin heteroresistance (2.16% among MRSA isolates) reported by Liu and Chambers (10), who reviewed 14 studies. More recently, it was reported at a higher rate of 8.1%, as noted by Khatib et al. (11).

This rate of ceftaroline heteroresistance may be of concern if heteroresistance is a precursor to resistant isolates. If the selective pressure of prolonged exposure to an antimicrobial agent enhances the likelihood of the emergence of organisms resistant to the therapeutic agent administered, we would expect to see an increasing number of clinical cases resistant to ceftaroline with more widespread use of this agent. The comparison of ceftaroline heteroresistance in this study to vancomycin heteroresistance in other studies may not be valid with the selection bias used in identifying our organisms as opposed to the random selection of MRSA isolates used in the vancomycin heteroresistance studies.

The mechanism for ceftaroline heteroresistance is unknown. We know that these strains are virulent, as they were isolated from clinical infections, including bacteremia, pneumonia, and wound infections. We do not know if these strains are more or less virulent than other ceftaroline-susceptible strains. To date, clinical cases of failure due to ceftaroline heteroresistance have not been reported.

Vancomycin-heteroresistant strains have demonstrated lower

TABLE 3 Results of samples that were determined to be heteroresistant

Sample	Results (μg/ml) for CPT[a]			PAP results (log_{10} CFU/ml) at concentration[b] of:											Frequency	SCC*mec* type	Pulse	Source	Results (μg/ml) for VAN[c]		Sample type
	MIC	MBC	Etest	0	0.25	0.5	0.75	1	1.25	1.5	2	2.5	3	4					MIC	MBC	
D-9	1	1	1	7	6.9	6.9	4.7	3.8	3.3	2.7	2.3	2.1	0	0	1.1×10^{-5}	II	USA-100	Blood	1	1	MRSA
D-11	1	1	1	6.9	6.9	6.8	6.1	3.8	3.2	2.8	2.4	2	0	0	1.3×10^{-5}	II	USA-600	Blood	1	1	MRSA
D-27	1	2	1	6.9	6.9	6.8	6.1	3	2.6	2.6	0	0	0	0	5.2×10^{-5}	II	USA-100	Wound	1	1	MRSA
ND-14	1	1	1	7	7	6.7	5.9	3.1	2.6	2.3	2.1	0	0	0	1.1×10^{-5}	II	USA-100	Respiratory	1	1	MRSA
ND-33	1	1	1	6.8	6.8	6.7	5.9	3.1	2.5	2.2	0	0	0	0	1.4×10^{-5}	II	No match	Respiratory	2	2	MRSA
DNS-2	1	1	1	6.9	6.8	6.7	6.2	3.6	2.8	2.5	0	0	0	0	4.8×10^{-5}	II	No match	Blood	2	2	DNSSA
DNS-3	1	2	1.5	6.9	6.8	6.7	6.5	5.9	3.1	2.7	2.4	0	0	0	3.8×10^{-5}	II	USA-100	Blood	2	2	DNSSA
NRS-3	1	1	1.5	6.4	6.3	6.3	6.2	5.2	3	2.6	2	0	0	0	3.8×10^{-5}	II	USA-100	Peritoneal	8	8	VISA
NRS-118	1	2	1.5	6.8	6.7	6.7	6.6	6.5	5.7	4.5	3.3	2.6	2.3	0	3.2×10^{-5}	I	No match	Respiratory	8	8	VISA
NRS-120	1	1	1	6.8	6.8	6.5	5.9	4.4	2.7	2.6	0	0	0	0	6.9×10^{-5}	IVd	USA-500	Unknown	2	2	LNSSA
NRS-121	1	1	1	6.8	6.7	6.4	5.9	4.8	3.1	0	0	0	0	0	2.2×10^{-4}	IVd	USA-500	Unknown	1	2	LNSSA
JhVISA-2	1	1	1	6.7	6.7	6.6	4.5	3.4	3.2	3	2.5	2	0	0	2.1×10^{-5}	II	USA-100	Blood	2	2	hVISA

[a] CPT, ceftaroline.
[b] Concentration (μg/ml) of CPT in PAP plates.
[c] VAN, vancomycin.

growth rates and thicker cell walls than vancomycin-susceptible strains (12). In addition, these strains also produce greater quantities of PBP2 and PBP2′ (13). Several isolates with high ceftaroline MICs (4 μg/ml) obtained from an antibiotic resistance surveillance system demonstrated decreased PBP2a binding affinity due to alterations in the PBP2a (14). Although our study did not evaluate strains for PBP production or cell wall thickness, we did not see a correlation with ceftaroline heteroresistance and vancomycin heteroresistance. Recent *in vitro* studies of ceftaroline activity against MRSA isolates with reduced vancomycin susceptibility demonstrated increased activity compared with isolates with lower vancomycin MICs (15); however, this observation was not seen among the isolates evaluated in this study.

The clinical significance of ceftaroline heteroresistance is unclear. Heteroresistant strains were mainly SCC*mec* type II (75%), and no strains were found to be SCC*mec* type III or IVa. The small sample size of the CA-MRSA isolates in this study limits the generalizability of the results. Studies evaluating the frequency of ceftaroline heteroresistance among a larger set of CA-MRSA isolates should be performed to confirm this finding.

The information in this study should suggest caution by clinicians using ceftaroline in patients with resistance to other anti-MRSA agents, as heteroresistance was seen in isolates demonstrating reduced susceptibilities to daptomycin and linezolid, and increased ceftaroline MICs were noted in four VISA isolates. To date, the occurrence of ceftaroline heteroresistance has not been shown to be a risk factor for the clinical failure of ceftaroline, and the mechanism for the development of these strains is not known. Further work should be done to evaluate the risk factors for and clinical significance of ceftaroline heteroresistance.

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REFERENCES

1. **Saravolatz LD, Stein GE, Johnson LB.** 2011. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **52:**1156–1163. http://dx.doi.org/10.1093/cid/cir147.
2. **Clinical and Laboratory Standards Institute.** 2013. Performance standards for antimicrobial susceptibility testing. Twenty-third informational supplement. M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Saravolatz LD, Pawlak J, Johnson LB.** 2012. *In vitro* susceptibilities and molecular analysis of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus* isolates. Clin. Infect. Dis. **55:**582–586. http://dx.doi.org/10.1093/cid/cis492.
4. **Deresinski S.** 2009. Vancomycin heteroresistance and methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. **199:**605–609. http://dx.doi.org/10.1086/596630.
5. **Zhang K, McClure J, Elsayed S, Louie T, Conly JM.** 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **43:**5026–5033. http://dx.doi.org/10.1128/JCM.43.10.5026-5033.2005.
6. **McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC.** 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. **41:**5113–5120. http://dx.doi.org/10.1128/JCM.41.11.5113-5120.2003.
7. **Vidaillac C, Leonard SN, Rybak MJ.** 2009. *In vitro* activity of ceftaroline against methicillin-resistant *Staphylococcus aureus* and heterogeneous vancomycin-intermediate *S. aureus* in a hollow fiber model. Antimicrob. Agents Chemother. **53:**4712–4717. http://dx.doi.org/10.1128/AAC.00636-09.
8. **Meletis G, Tzampaz E, Sianou E, Tzavaras I, Sofianou D.** 2011. Colistin heteroresistance in carbapenemase-producing *Klebsiella pneumoniae*. J. Antimicrob. Chemother. **66:**946–947. http://dx.doi.org/10.1093/jac/dkr007.
9. **Boyle-Vavra S, Berke SK, Lee JC, Daum RS.** 2000. Reversion of the glycopeptide resistance phenotype in *Staphylococcus aureus* clinical isolates. Antimicrob. Agents Chemother. **44:**272–277. http://dx.doi.org/10.1128/AAC.44.2.272-277.2000.
10. **Liu C, Chambers HF.** 2003. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob. Agents Chemother. **47:**3040–3045. http://dx.doi.org/10.1128/AAC.47.10.3040-3045.2003.
11. **Khatib R, Jinson J, Musta A, Sharma M, Fakih MG, Johnson LB, Riederer K, Shemes S.** 2011. Relevance of vancomycin-intermediate susceptibility and heteroresistance in methicillin-resistant *Staphylococcus aureus* bacteraemia. J. Antimicrob. Chemother. **66:**1594–1599. http://dx.doi.org/10.1093/jac/dkr169.
12. **Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, Tenover FC, Zervos MJ, Band JD, White E, Jarvis WR.** 1999. Emergence of vancomycin resistance in *Staphylococcus aureus*. N. Engl. J. Med. **340:**493–501. http://dx.doi.org/10.1056/NEJM199902183400701.
13. **Hanaki H, Kuwahara-Arai K, Boyle-Vavra S, Daum RS, Labischinski H, Hiramatsu K.** 1998. Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. J. Antimicrob. Chemother. **42:**199–209. http://dx.doi.org/10.1093/jac/42.2.199.
14. **Mendes RE, Tsakris A, Sader HS, Jones RN, Biek D, McGhee P, Appelbaum PC, Kosowksa-Shick K.** 2012. Characterization of methicillin-resistant *Staphylococcus aureus* displaying increased MICs of ceftaroline. J. Antimicrob. Chemother. **67:**1321–1324. http://dx.doi.org/10.1093/jac/dks069.
15. **Werth BJ, Steed ME, Kaatz GW, Rybak MJ.** 2013. Evaluation of ceftaroline activity against heteroresistant vancomycin-intermediate *Staphylococcus aureus* and vancomycin-intermediate methicillin-resistant *S. aureus* strains in an *in vitro* pharmacokinetic/pharmacodynamics model: exploring the "seesaw effect." Antimicrob. Agents Chemother. **57:**2664–2668. http://dx.doi.org/10.1128/AAC.02308-12.