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Rationale for Eliminating *Staphylococcus* Breakpoints for β-Lactam Agents Other Than Penicillin, Oxacillin or Cefoxitin, and Ceftaroline

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Due to the ongoing concern about the reliability of *Staphylococcus* breakpoints (interpretive criteria) for other β-lactam agents, the Clinical and Laboratory Standards Institute recently approved the elimination of all breakpoints for antistaphylococcal β-lactams except for penicillin, oxacillin or cefoxitin, and ceftaroline. Routine testing of penicillin and oxacillin or cefoxitin should be used to infer susceptibility for all β-lactams with approved clinical indications for staphylococcal infections. It is critical for laboratories to reject requests for susceptibility testing of other β-lactams against staphylococci and to indicate that susceptibility to these agents can be predicted from the penicillin and oxacillin or cefoxitin results. This article reviews β-lactam resistance mechanisms in staphylococci, current antimicrobial susceptibility testing and reporting recommendations for β-lactams and staphylococci, and microbiologic data and clinical data supporting the elimination of staphylococcal breakpoints for other β-lactam agents.

Keywords. β-lactams and *Staphylococcus*; *Staphylococcus* susceptibility; CLSI breakpoints.

Staphylococci are ubiquitous colonizers of the skin and mucosa and are responsible for a variety of infections, including those involving the bloodstream, skin and soft tissue, lower respiratory tract, bone, and joints. Of the large number of species within the staphylococcal group, *Staphylococcus aureus* is considered to be the most virulent and is the leading cause of healthcare-associated infections [1]; however, coagulase-negative staphylococci (CoNS) are frequently associated with catheter and prosthetic device infections. Antimicrobial therapy is essential for most staphylococcal infections, and in vitro susceptibility testing plays a pivotal role in the selection of antimicrobial agents, as susceptibility of staphylococcal strains to first-line agents is not predictable [2]. For most staphylococcal isolates, susceptibility to penicillinase-stable penicillins (eg, oxacillin) is the most important result a laboratory can provide as this result will indicate whether or not a β-lactam agent (with the exception of ceftaroline, as discussed below) might be appropriate for treatment of an infection caused by the isolate. This paper discusses the rationale for recommending testing of only penicillin, oxacillin or cefoxitin, and ceftaroline to determine staphylococcal susceptibility to β-lactams. Susceptibility to these drugs allows inference of susceptibility to other antistaphylococcal β-lactams.

β-LACTAM RESISTANCE MECHANISMS IN STAPHYLOCOCCI AND THEIR DETECTION

Following its introduction in the 1940s, penicillin was used widely for treatment of *S. aureus* infections. However, penicillin resistance due to penicillinase production

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quickly emerged [3], and by the late 1960s, >80% of *S. aureus* isolates were resistant to penicillin [4]. Production of β-lactamase, which is conferred by *blaZ*, inactivates penicillin by hydrolyzing the β-lactam ring [5]. Four types of *blaZ* have been identified: types A, C, and D are plasmid-mediated, whereas B is typically chromosomal [6]. To circumvent the problem of penicillin hydrolysis by β-lactamase, researchers in 1959 synthesized methicillin, a related compound containing a β-lactam ring structure with added 2,6-dimethoxyphenyl side chains that protects the β-lactam ring from cleavage by penicillinase [7]. By 1961, within a year of the drug's introduction into clinical practice [8], methicillin-resistant *S. aureus* (MRSA) appeared in England, and by the 1980s MRSA had become widespread globally [9, 10].

The vast majority of methicillin resistance in *S. aureus* is mediated by *mecA*, which is carried on the mobile staphylococcal cassette chromosomal *mec* element (SCC*mec*) and encodes penicillin-binding protein (PBP) 2a. PBPs are essential for cell growth and survival in *Staphylococcus* species and have high affinity for most β-lactams; binding of β-lactams by native PBPs is lethal for staphylococcal cells [11–13]. PBP2a, an inducible transpeptidase, confers high-level resistance to methicillin and other β-lactams [14]. PBP2a has low affinity for β-lactams except ceftaroline and functions as a surrogate for the native high-affinity staphylococcal PBPs in the presence of high concentrations of β-lactams [11, 15–17].

In the 1980s, oxacillin, a semi-synthetic penicillinase-stable penicillin, was shown to be a reliable alternative to methicillin for detecting resistance to penicillinase-stable penicillins in staphylococci [18, 19]. In the 1990s, oxacillin replaced methicillin in clinical use in the United States and became the agent of choice for in vitro testing to represent penicillinase-stable penicillins when methicillin ceased to be commercially available. Other penicillinase-stable penicillins used clinically include nafcillin, dicloxacillin, cloxacillin, and flucloxacillin, all highly active antistaphylococcal antimicrobial agents [20–22]. Tests that target *mecA* or PBP2a are considered to be the most accurate methods of predicting resistance to oxacillin and other penicillinase-stable penicillins in staphylococci, and isolates that carry the *mecA* gene or produce PBP2a should be reported as oxacillin resistant [23].

Testing recommendations for detection of MRSA were further refined in the 2000s, when it was established that cefoxitin is more reliable than oxacillin for detection of *mecA*-mediated resistance in staphylococci [24]. Cefoxitin detects oxacillin heteroresistance better than oxacillin due to its strong induction of PBP2a [25, 26]. The Clinical and Laboratory Standards Institute (CLSI) now recommends cefoxitin disk diffusion (DD) or cefoxitin or oxacillin minimum inhibitory concentration (MIC) tests to test for *mecA*-mediated oxacillin resistance in *S. aureus* and *Staphylococcus lugdunensis*; for all other CoNS, cefoxitin DD is the preferred method [27–29].

Methicillin resistance in staphylococci can also occur by mechanisms other than *mecA*, although such mechanisms are believed to be rare. Other mechanisms of methicillin resistance include hyperproduction of β-lactamase (the borderline oxacillin-resistant *S. aureus* [BORSA] phenotype) [30], production of modified PBPs (MOD-SA) [31], and expression of a *mecA* homologue, termed *mecC* [32]. BORSA and MOD-SA typically demonstrate MICs near the oxacillin breakpoint, are not resistant to multiple agents, and are believed to have little clinical relevance. Resistance mediated by *mecC* can confer higher oxacillin MICs similar to *mecA*-mediated resistance, and has been documented in strains causing infection in both humans and animals [33–36]. Of note, the novel *mecA* homologue, *mecC*, cannot be detected by tests targeting *mecA* or PBP2a, instead requiring MIC-based cefoxitin or oxacillin susceptibility tests or tests directed at *mecC* [37, 38].

Previous versions of the CLSI M100 standard included staphylococcal MIC and DD breakpoints (interpretive criteria) for numerous additional antistaphylococcal β-lactams with a US Food and Drug Administration (FDA)–approved clinical indication for treating staphylococcal infections, including penicillins, β-lactam/β-lactamase inhibitor combinations, cephems, and carbapenems [39]. However, penicillin and oxacillin or cefoxitin were the only antimicrobial agents recommended for routine testing of staphylococci, and it was specified that results from these agents should be used to infer susceptibility to all other penicillins, β-lactam/β-lactamase inhibitor combinations, cephems, and carbapenems (Table 1). Additionally, it was noted that other β-lactams should never be reported as susceptible for methicillin-resistant staphylococci (MRS), even if tested as susceptible in vitro. Table 2 summarizes the β-lactam resistance mechanisms and testing methods for staphylococci.

ESTABLISHMENT OF VALIDATED β-LACTAM BREAKPOINTS

Most β-lactam breakpoints for staphylococci were established many years ago, prior to the development of the CLSI M23 [40] process currently used for establishing breakpoints. As such, there has been ongoing concern about the reliability of breakpoints other than those for oxacillin, cefoxitin and penicillin. The "inferred susceptibility" rule directing laboratories to infer results for other β-lactams from results of penicillin and oxacillin, and later cefoxitin, has been in place in the CLSI M100 standard since 1991, although *Staphylococcus* breakpoints for other β-lactam agents were also included.

At the June 2012 meeting of the CLSI Subcommittee on Antimicrobial Susceptibility Testing, it was decided to remove the DD and MIC breakpoints for all antistaphylococcal β-lactams. At the same time, DD and MIC breakpoints for ceftaroline, a new cephalosporin agent with activity against MRSA, were

Table 1. Inferred Susceptibility to β-Lactam Agents for Staphylococci Based on Testing of Penicillin and Oxacillin or Cefoxitin

Actual Susceptibility Result		
Penicillin	Oxacillin or Cefoxitin	Inferred Susceptibility Result
S	S	S to penicillins (penicillinase-labile[a] and stable[b]), β-lactam/β-lactamase inhibitor combinations[c], cephems[d], and carbapenems[e]
R	S	R to penicillinase-labile penicillins S to penicillinase-stable penicillins, β-lactam/β-lactamase inhibitor combinations, antistaphylococcal cephems, and carbapenems
R	R	R to penicillins, β-lactam/β-lactamase inhibitor combinations, cephems, and carbapenems except newer cephalosporins with anti-MRSA activity (when confirmed by standardized testing [eg, ceftaroline])

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; R, resistant; S, susceptible.

[a] Penicillinase-labile penicillins: amoxicillin, ampicillin, azlocillin, carbenicillin, mezlocillin, penicillin, piperacillin, ticarcillin.

[b] Penicillinase-stable penicillins: cloxacillin, dicloxacillin, flucloxacillin, methicillin, nafcillin oxacillin.

[c] β-Lactam/β-lactamase inhibitor combinations: amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid.

[d] Antistaphylococcal cephems include the oral cephems (cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, loracarbef) and the parenteral cephems (cefamandole, cefazolin, cefepime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, ceftizoxime, ceftriaxone, cefuroxime, cephalothin, ceftaroline moxalactam) for indications approved by the US Food and Drug Administration or other regulatory bodies in the country of use.

[e] Carbapenems: doripenem, ertapenem, imipenem, meropenem.

established for *S. aureus*, including MRSA. Susceptibility to ceftaroline can be inferred based on oxacillin or cefoxitin susceptibility, but because most but not all oxacillin- or cefoxitin-resistant *S. aureus* is ceftaroline susceptible, ceftaroline must be tested directly if it is to be reported for MRSA [27].

Now the CLSI unequivocally recommends that susceptibility to cephalosporins and other β-lactams with FDA-approved clinical indications for staphylococcal infections (Table 3) be deduced from the results of testing penicillin and oxacillin or cefoxitin (Table 1). Of note, ceftazidime is generally not thought to be a potent antistaphylococcal agent despite FDA-approved indications [41–43], and, in agreement with European Committee for Antimicrobial Susceptibility Testing (EUCAST), it has been recommended to exclude testing and reporting of staphylococcal susceptibility to this agent. Therefore, it is not included in the list of antistaphylococcal agents that can be inferred by testing penicillin and oxacillin or cefoxitin [44].

Testing and reporting recommendations for staphylococci are now similar for CLSI and the EUCAST (Table 2). Clinical breakpoints for antistaphylococcal β-lactams were never approved by EUCAST, which recommends that all antistaphylococcal cephalosporins, β-lactams/β-lactamase inhibitor combinations, and carbapenem results be inferred from cefoxitin susceptibility.

IN VITRO DATA SUPPORTING CLSI RECOMMENDATIONS

There is currently no strong evidence to support the categorization of an MRS strain as resistant to a β-lactam agent when in vitro susceptibility testing indicates that it is susceptible. However, due to the lack of appropriate clinical studies, including a small number of cases reporting clinical failure, it is postulated that all MRS isolates should be considered resistant to all antistaphylococcal cephalosporins, β-lactams/β-lactamase inhibitor combinations, and carbapenems, except for ceftaroline [27] and ceftobiprole [45], an agent recently approved for use in Europe for treatment of pneumonia. To our knowledge, there are no

Table 2. β-Lactam Resistance Mechanisms in Staphylococci, Detection Methods, and Reporting Recommendations[a]

Resistance Mechanism	Organism	Detection and Reporting: CLSI	Detection and Reporting: EUCAST
blaZ-mediated penicillinase (penicillin resistance)	All *Staphylococcus* species	Penicillin disk zone edge for *S. aureus* or induced β-lactamase test (Nitrocefin) for all CoNS	Penicillin disk zone edge for all *Staphylococcus* (notes that cephalosporin-based β-lactamase tests are unreliable for staphylococcal penicillinase)
mecA-mediated oxacillin resistance, PBP2a (oxacillin resistance)	*S. aureus*, *S. lugdunensis*	Cefoxitin disk diffusion or MIC, oxacillin MIC, *mecA* PCR, or PBP2a detection	Cefoxitin disk diffusion or MIC, oxacillin MIC, *mecA* PCR, or PBP2a detection
	CoNS	Cefoxitin disk diffusion or oxacillin MIC, *mecA* PCR, or PBP2a detection	Cefoxitin disk diffusion or oxacillin MIC, *mecA* PCR, or PBP2a detection
mecC-mediated oxacillin resistance	*S. aureus*, (1 report in CoNS)	Cefoxitin disk diffusion or MIC, oxacillin MIC, *mecC*, PCR	Cefoxitin disk diffusion or MIC, oxacillin MIC, *mecC* PCR

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; CoNS, coagulase-negative staphylococci; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction.

[a] Ceftaroline resistance for *Staphylococcus aureus* can be determined by performing disk diffusion or MIC susceptibility testing.

reports that indicate susceptibility results for other β-lactams have been useful for predicting clinical outcome once an isolate is known to be methicillin-susceptible staphylococci (MSS) or MRS. The occasional exception is a penicillin result for MSSA.

Several in vitro susceptibility studies have demonstrated that the vast majority of MSS test susceptible (based on previous CLSI interpretive criteria) to the cephalosporins and carbapenems clinically indicated to treat staphylococcal infections [46–48]. Some MSS isolates have been reported as resistant to the cephalosporins; however, detailed explanations of such observations are lacking. In a recent US survey of 4016 MSSA isolates collected between 2008 and 2010 from patients with a variety of infections, ceftriaxone MICs ranged from ≤0.06 to >8 μg/mL; 0.3% of isolates were considered resistant to ceftriaxone when using a combination of CLSI breakpoints (MIC ≥64 μg/mL) and FDA breakpoints (MIC ≥16 μg/mL). Of note, only 96% of the 4016 MSSA isolates were interpreted as susceptible to ceftriaxone, which may be attributed to the application of FDA breakpoints (MIC ≤4 μg/mL) rather than former CLSI breakpoints (MIC ≤8 μg/mL). The authors did not indicate if ceftriaxone MIC results for the 4% (n = 160) of nonsusceptible isolates were confirmed [47]. For CoNS, testing of 182 methicillin-susceptible isolates demonstrated 100% susceptibility to cefepime (MIC ≤8 μg/mL) and 98.3% susceptibility to ceftriaxone

Table 3. β-Lactam Agents With US Food and Drug Administration Indications for Treating Staphylococcal Infections[a]

Drug	Year Approved	Clinical Indications
Amoxicillin	1976	Ear, nose, throat, skin and skin structure, and lower respiratory tract infections
Amoxicillin-clavulanic acid	1984	Skin and skin structure infections
Ampicillin	1971	Respiratory tract infections, septicemia, and endocarditis
Ampicillin-sulbactam	1986	Skin and skin structure infections
Cefaclor	1979	Skin and skin structure infections
Cefamandole	1978	Lower respiratory tract, blood, skin and soft tissue, bone and joint infections
Cefazolin	1973	Respiratory tract, skin and skin structure, biliary tract, blood, bone and joint infections
Cefdinir	1997	Skin and skin structure infections
Cefepime	2010	Skin and skin structure infections
Cefmetazole	1989	Skin and soft tissue infections, urinary tract infections
Cefoperazone	1982	Respiratory tract, blood, skin and skin structure infections
Cefotaxime	2000	Lower respiratory tract, genitourinary, blood, skin and soft tissue, bone and joint infections
Cefotetan	1985	Lower respiratory tract, skin and skin structure, gynecologic, bone and joint infections
Cefpodoxime	1992	Skin and skin structure infections
Cefprozil	1991	Skin and skin structure infections
Ceftizoxime	1983	Blood, lower respiratory tract, urinary tract, intra-abdominal, skin and skin structure, bone and joint infections
Ceftriaxone	1984	Lower respiratory tract, blood, skin and soft tissue, bone and joint infections
Cefuroxime	1983	Lower respiratory tract, blood, skin and soft tissue, bone and joint infections
Cephalothin	1974	Skin and skin structure infections
Cloxacillin	1980	All infections caused by penicillinase-producing staphylococci that is methicillin susceptible
Dicloxacillin	1971	All infections caused by penicillinase-producing staphylococci that is methicillin susceptible
Ertapenem	2001	Skin and skin structure infections, osteomyelitis
Flucloxacillin	1971	Skin and soft tissue, respiratory tract, urinary tract, blood, and bone infections
Imipenem	1985	Lower respiratory tract, urinary tract, intra-abdominal, gynecologic, blood, skin and skin structure, bone and joint infections
Loracarbef	1991	Skin and skin structure infections
Meropenem	1996	Skin and skin structure infections
Methicillin	1961	All infections caused by penicillinase-producing staphylococci that is methicillin susceptible
Moxalactam	1980	Skin and soft tissue, bone and joint, respiratory tract infections
Nafcillin	1984	All infections caused by penicillinase-producing staphylococci that is methicillin susceptible
Oxacillin	1971	All infections caused by penicillinase-producing staphylococci that is methicillin susceptible
Penicillin	1964	Skin and soft tissue infection
Piperacillin-tazobactam	1993	Skin infections and nosocomial pneumonia
Ticarcillin-clavulanate	1985	Septicemia, lower respiratory tract, bone, joint, urinary tract, and gynecologic infections

[a] Despite US Food and Drug Administration–approved indications, it is the opinion of the authors that ceftazidime should not be used for staphylococcal infections.

(MIC ≤8 μg/mL). Ceftriaxone MICs ranged from ≤0.25 to >32 μg/mL, with 0.6% resistance (MIC >64 μg/mL). Confirmatory testing of the 1.7% (n = 3) of nonsusceptible isolates was not indicated in the study [48].

Conversely, although the majority of MRS isolates test resistant to the cephalosporins and carbapenems, it is not uncommon for some MRSA strains to test susceptible to various β-lactam agents [47, 49, 50]. In a study of 98 MRSA isolates, 16 exhibited cephalothin MICs of ≤2 μg/mL and 10 isolates had cefuroxime, cefotaxime, and/or cefepime MICs of ≤8 μg/mL, which would have been misinterpreted as susceptible. Another study reported a MIC range of ≤0.25 to >8 μg/mL to ceftriaxone in 4453 MRSA isolates, indicating susceptibility to ceftriaxone for some MRSA isolates when either FDA (MIC ≥16 μg/mL) or CLSI (MIC ≥64 μg/mL) breakpoints were used [47]. Although broth microdilution testing of 36 methicillin-susceptible CoNS strains demonstrated a correlation between susceptibility to methicillin (MIC ≤4 μg/mL) with susceptibility to cefradine, ceftriaxone, cephalothin, and cefamandole using former CLSI breakpoints (MIC ≤8 μg/mL), in vitro resistance to methicillin did not parallel resistance for 3 of the 4 agents tested against 26 methicillin-resistant CoNS isolates. The percentage of MRSA isolates that tested susceptible was 7.7% for ceftriaxone (MIC ≤8 μg/mL), 84.6% for cephalothin (MIC ≤8 μg/mL), 96.2% for cefamandole (MIC ≤8 μg/mL), and 0% for cefradine (MIC ≤8 μg/mL). Of note, selective testing of only highly methicillin-resistant subpopulations (MIC >128 μg/mL) of cells isolated from all 26 CoNS strains dramatically decreased percent of isolates susceptible to 0% for ceftriaxone, 3.8% for cephalothin, 46% for cefamandole and 0% for cefradine [50], demonstrating the presence of heteroresistant populations of MRS and potential for reporting falsely susceptible results when other β-lactams are tested in vitro [51, 52]. Table 4 summarizes published in vitro susceptibility studies for MSS and MRS.

CLINICAL DATA SUPPORTING CLSI RECOMMENDATIONS

Clinical data supporting CLSI recommendations were previously reported 26–44 years ago [54–64], and it is well accepted that numerous β-lactam agents are effective in treating infections caused by MSS but are ineffective for treating infections caused by MRS [56, 59, 61, 62, 64]. The efficacy of cefazolin in treating serious MSSA infections, including endocarditis and other deep-seated infections, is controversial. Some studies have reported cefazolin clinical failure in patients with serious MSSA infections due to the production of type A β-lactamase, instead reporting the superiority of nafcillin and oxacillin. These MSSA isolates are reported to have a significant rise in cefazolin MIC when the bacterial inoculum is increased, referred to as the inoculum effect [65–69]. However, clinical response to cefazolin, and probably other β-lactams, in patients with serious MSSA infections is a complex process dependent on multiple factors, including bacterial load, antibiotic penetration, host immune system, and surgical interventions, and the presence of a high-inoculum effect alone is unlikely to cause clinical failure [70]. In addition, contrasting studies, including a propensity-score-matched, case-control study, have reported clinical efficacy of cefazolin to be similar to nafcillin and cloxacillin for the treatment of MSSA bacteremia, including cases of endocarditis [20, 71]. Thus, future prospective studies are required to definitively determine the clinical efficacy of cefazolin, and other β-lactams, in the treatment of serious MSSA infections with high inoculum.

Despite the fact that MRS strains may test as susceptible to β-lactams using former CLSI breakpoints [55, 56, 59, 61], studies have indicated clinical failure when β-lactams were used to treat infections with *mecA*-positive staphylococci, regardless of the in vitro susceptibility results [54, 57, 58, 60, 63]. Clinical responses to cephalosporins (cephalothin, cephalexin, and cephaloridine) were evaluated in 31 patients with MRS septicemia, 7 of whom had endocarditis. All 31 strains had no zones of inhibition around methicillin (10 μg) and cephalexin (30 μg) disks, and 26 demonstrated reduced zones of inhibition for cephalothin (30 μg) and cephaloridine (30 μg) on trypticase soy agar containing 5% sodium chloride. When DD was performed on Mueller-Hinton agar, the same 26 strains demonstrated zones of 25–30 mm, which would have been interpreted as susceptible using former CLSI breakpoints, around the cephalothin and cephaloridine disks, confirming the ability of sodium chloride to improve the detection of β-lactam resistance [72] as well as the heterogeneous expression of resistance in these strains. MRS was recovered from blood culture after initiation of cephalosporin therapy in 21 of these patients, 20 of whom remained culture positive after day 3 of cephalosporin therapy. Importantly, in all 7 of the cases of endocarditis, cephalosporin therapy failed to produce negative blood cultures, whereas negative blood cultures were achieved in 75% of patients treated with non-β-lactam antistaphylococcal agents such as vancomycin and rifampin [54]. Overall, blood cultures from 17 of the patients remained positive until therapy was changed to a non-β-lactam agent, and 3 patients with endocarditis died. Multiple experimental models of endocarditis with methicillin-resistant strains of *S. aureus* and *S. epidermidis* have also demonstrated failure of therapy with β-lactams, including cephalothin, cefamandole, and imipenem [73–75].

Another study using macrobroth dilution and agar dilution methods demonstrated susceptibility (MIC range, 0.25–32 μg/mL) to cephalothin among 61 MRSA isolates recovered from various clinical sites from 23 patients, 16 of whom received a cephalosporin in the interim between admission and isolation of MRSA, and 10 of whom were confirmed to have definite

Table 4. Summary of In Vitro Susceptibility Studies for *Staphylococcus* Species and β-Lactams

Study	Isolates (No.)	Conclusions	Comments	Source
S. aureus				
1	MRSA (70) MSSA (24)	MSSA strains were highly susceptible (all MIC ≤4 μg/mL) to cephalothin, cefoperazone, and cefotaxime compared to MRSA strains. MIC_{50} and MIC_{90} of MSSA strains were 8- to-128-fold lower than MRSA isolates (MIC_{90} >32 for MRSA). MRSA stains had MIC range of 0.25–256 μg/mL. Strains with high MICs to methicillin (MIC ≥64 μg/mL) also had high MICs to cephalothin (MIC ≥32 μg/mL), cefoperazone (MIC ≥64 μg/mL), and cefotaxime (MIC ≥128 μg/mL).	Data support the deduction of cephalothin, cefoperazone, and cefotaxime results based on oxacillin or cefoxitin results.	[53]
2	MRSA (98)	MRSA isolates had high MIC_{50} and MIC_{90} values: cefuroxime (MIC_{50} >256, MIC_{90} >256) cefotaxime (MIC_{50} = 32, MIC_{90} >256), and cefepime (MIC_{50} = 48, MIC_{90} >256). Sixteen isolates exhibited MIC <2 μg/mL to cephalothin; 10 isolates were susceptible to cefuroxime, cefotaxime, or cefepime (MIC ≤8 μg/mL).	Majority of MRSA isolates have MICs >8 μg/mL to cefuroxime, cefotaxime, and cefepime, supporting the deduction of results for these agents based on oxacillin or cefoxitin results. Inclusion of breakpoints for β-lactams other than penicillin, oxacillin, and cefoxitin can lead to falsely susceptible results in MRS.	[49]
3	MSSA (1313)	MSSA isolates were 100% susceptible to cefepime (MIC ≤8 μg/mL), 99.8% susceptible to ceftriaxone (MIC ≤8 μg/mL), and 0% resistant to ceftriaxone (MIC ≥64 μg/mL) and cefepime (MIC ≥32 μg/mL).	Susceptibility of staphylococci to cefepime and ceftriaxone can be inferred from oxacillin or cefoxitin results.	[48]
4	MRSA (4453) MSSA (4016)	MSSA isolates had ceftriaxone MIC_{90} of 4 μg/mL, 96% of isolates had MICs to ceftriaxone <4 μg/mL, and 0.3% were considered resistant; 3.7% were not categorized as susceptible or resistant. 4% of MSSA isolates had ceftriaxone MICs >4 μg/mL and were considered ceftriaxone nonsusceptible using FDA breakpoints (MIC ≤4 μg/mL, susceptible). The actual MIC for these isolates was not reported. MSSA isolates demonstrated MIC_{90} of ≤0.12 μg/mL to meropenem. MRSA isolates were all (100%) resistant to ceftriaxone (MIC >64 μg/mL).	It is critical to know breakpoint criteria and methods used when evaluating reports in the literature. Authors specified that FDA breakpoints were applied when available but did not provide actual MIC values on those isolates categorized as resistant with MICs >4 μg/mL. This emphasizes that the inclusion of breakpoints for these ceftriaxone and meropenem can lead to falsely resistant results in MSSA. Results for ceftriaxone and meropenem can be inferred from oxacillin or cefoxitin results.	[47]
Coagulase-negative staphylococci				
5	MRCNS (26) MSCNS (36)	100% of MSCNS isolates were susceptible to cefradine, ceftriaxone, cephalothin, and cefamandole (MIC ≤8 μg/mL). Susceptible results for MRCNS isolates: 7.7% for ceftriaxone (MIC ≤8 μg/mL), 84.6% for cephalothin (MIC ≤8 μg/mL), 96.2% for cefamandole (MIC ≤8 μg/mL), and 0% for cefradine (MIC ≤8 μg/mL) Susceptible results for highly methicillin-resistant (MIC >128 μg/mL) subpopulation of CNS: 0% for ceftriaxone, 3.8% for cephalothin, 46% for cefamandole, and 0% for cefradine.	MSCNS can be considered susceptible to cefradine, ceftriaxone, cephalothin, and cefamandole. Presence of heteroresistant populations of MRS can lead to falsely susceptible results for cephalosporins. Inclusion of breakpoints for β-lactams other than the penicillin, oxacillin, and cefoxitin can lead to falsely susceptible results in MRCNS.	[50]
6	MSCNS (182)	100% of MSCNS isolates were susceptible to cefepime (MIC ≤8 μg/mL) and 98.3% were susceptible to ceftriaxone (MIC ≤8 μg/mL). The MIC_{90} for ceftriaxone was 4 μg/mL, and 0.6% (1 isolate) was considered resistant; 1.1% of isolates were not categorized as susceptible or resistant. 1.7% of MSCNS had ceftriaxone MICs >8 μg/mL and were considered ceftriaxone nonsusceptible using CLSI breakpoints (MIC ≤8 μg/mL, susceptible).	Susceptibility of staphylococci to cefepime and ceftriaxone can be inferred from oxacillin or cefoxitin results. This emphasizes that the inclusion of breakpoints for these cefepime and ceftriaxone can lead to falsely resistant results in MSCNS.	[48]

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; CNS, coagulase negative staphylococci; FDA, US Food and Drug Administration; MIC, minimum inhibitory concentration; MRS, methicillin-resistant staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*; MRCNS, methicillin-resistant coagulase negative staphylococci; MSCNS, methicillin-susceptible coagulase negative staphylococci; MSSA, methicillin-susceptible *Staphylococcus aureus*.

Table 5. Summary of Studies Demonstrating Clinical Response of *Staphylococcus* Species and β-Lactams

Study	Isolate	Infection	AST Result	Initial Antimicrobial Therapy	Outcome	Source
MSSA						
2	MSSA (294 pts)	Bacteremia	Not specified	β-lactams (267 pts) or vancomycin (27 pts)	Mortality rate was significantly higher in the vancomycin-treated group compared to the β-lactam–treated group	[76]
3	MSSA (123 pts)	Bacteremia	Susceptibility testing for cephems not performed	Cefazolin (46 pts) or vancomycin (77 pts)	Cure rate of 91.3% from cefazolin and 83.1% from vancomycin	[77]
MRSA						
1	(a) MRSA (17 pts) (b) MRSA (3 pts)	(a) Septicemia or endocarditis (b) Endocarditis	Susceptible to cephalothin, cephaloridine, and cephalexin by DD (25–30 mm zone) using Mueller-Hinton agar	(a) Cephalosporin (cephalothin, cephaloridine, cephalexin) ± aminoglycoside (b) Cephalosporin (cephalothin, cephaloridine, cephalexin)	(a) All blood cultures continue to be positive until therapy changed (b) All 3 patients died	[54]
2	MRSA (7 pts)	(a) Empyema (b) Empyema (c) Osteomyelitis (d) Bacteremia (e) Pneumonia (f) Wound infection (g) Bacteremia	All isolates tested had cephalothin MICs ranging from 0.25 to 32 μg/mL. All strains were considered susceptible to cephalothin. Actual MICs were not specified.	(a) Gentamicin, cefazolin (b) Cefazolin (c) Cefazolin (d) Cephalothin, gentamicin, vancomycin (e) Gentamicin, cefazolin (f) Cefazolin (g) Cefazolin	4/7 patients died	[57]
3	MRSA (10 pts)	Bacteremia	Resistant to cephalothin (MIC not indicated)	Cephalosporin (drug not specified)	8/10 patients died 1 of 2 patients who survived was also treated with vancomycin	[58]

Abbreviations: AST, antimicrobial susceptibility testing; DD, disk diffusion; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

MRSA infections. *Staphylococcus aureus* isolates were considered to be resistant to methicillin at MIC >12.5 μg/mL but breakpoint criteria for cephalothin were not specified by the authors. Despite in vitro susceptibility to cephalothin, neither cephalothin nor cefazolin alone or in combination with an aminoglycoside was successful in eradicating infections in 7 of 10 patients, 4 of whom died [57]. This clinical failure is consistent with another study of patients with MRSA bacteremia in which only 1 of 10 patients treated with a cephalosporin alone had a therapeutic response [58].

Regarding the importance of correctly identifying MSSA, one retrospective cohort and matched case-control study of 294 patients demonstrated that β-lactams are superior to vancomycin for treatment of MSSA bacteremia, with a 19% lower mortality rate with β-lactam therapy [76]. Overall, these clinical studies highlight the importance of avoiding β-lactams in cases of MRS infections, despite variable in vitro susceptibility results, and emphasize the efficacy of appropriate β-lactam treatment in cases of MSS infections (Table 5).

HURDLES FOR LABORATORY

With the elimination of most β-lactam breakpoints from the CLSI M100 standard, laboratories need only test penicillin and oxacillin or cefoxitin to routinely predict activities of other antistaphylococcal β-lactams. This recommendation has been in CLSI standards for >2 decades. However, if penicillins are not being considered for a specific staphylococcal infection, a laboratory may refrain from testing and reporting this agent. As noted previously, susceptibility to the new anti-MRSA cephalosporins (eg, ceftaroline) can be predicted by susceptibility to oxacillin or cefoxitin (ie, MSSA), but ceftaroline should be tested and reported if it is being considered for MRSA therapy [27].

Laboratories are also encouraged to include a comment with the report to emphasize that staphylococci that are resistant to oxacillin or cefoxitin must be considered resistant to all antistaphylococcal β-lactam drugs, except for the newer anti-MRSA cephalosporins, which must be specifically tested. A microbiology laboratory may report the interpretation for a specific antistaphylococcal β-lactam agent, but should specify that the result is inferred from penicillin and oxacillin or cefoxitin testing rather than testing of that agent. For example, if ceftriaxone is on the hospital formulary, a comment may be added to the report that MRSA strains are resistant to ceftriaxone.

CONCLUSIONS

The prevalence of MRSA remains high in the United States, with current rates of approximately 50% [47, 78]. Surveillance of antimicrobial resistance patterns for healthcare-associated infections reported in 2009–2010 to the National Healthcare Safety Network revealed MRSA rates of 43.7%–58.7%, depending on the type of healthcare-associated infection [79]. Although CLSI included breakpoints for β-lactams other than oxacillin, cefoxitin, penicillin, and ceftaroline in previous documents, sufficient evidence has now been accumulated to justify removal of these from the M100 standard. A consensus was reached by the CLSI Subcommittee on Antimicrobial Susceptibility Testing in June 2012 to remove all staphylococcal breakpoints for β-lactams except for the aforementioned agents, primarily based on the facts that (1) results from testing oxacillin or cefoxitin and penicillin can be used to deduce susceptibility for other antistaphylococcal β-lactams (for MRSA, ceftaroline must be tested separately); (2) the appropriateness of breakpoints for susceptibility testing of other β-lactams has not been rigorously examined; and (3) inclusion of other β-lactam breakpoints poses a risk for the reporting of MRS isolates as falsely susceptible and MSS as falsely resistant to these agents.

Notes

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Disclaimer. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Centers for Disease Control and Prevention (CDC). Use of trade names is for identification purposes only and does not constitute endorsement by the Department of Health and Human Services or the CDC.

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