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Daptomycin (DAP) Susceptibility Testing of *Staphylococcus aureus* Isolates by Broth Microdilution and Etest

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REVISED ABSTRACT

Background: In the face of increasing antibiotic resistance among isolates of *Staphylococcus aureus*, it is imperative that accurate reliable susceptibility results are obtained against the newer anti-staphylococcal agents. Daptomycin, a new lipopeptide antibiotic, has a functional requirement for calcium. Therefore, unless controlled, susceptibility test results may be inaccurate. Such has been the case with the disk diffusion assay which was found not to be reliable when testing this agent.
Methods: In this investigation, two Sensititre microdilution tests and Etest for DAP were evaluated. The challenge set: 7 baseline strains of *S. aureus*, and 7 related non-susceptible strains that were isolated while the patient was receiving daptomycin were tested by the CLSI broth microdilution method, Sensititre JustOne strips, Sensititre GPN3F panels and Etest. An additional 30 isolates of *S. aureus* with MICs to vancomycin of 2 µg/ml were tested with Sensititre JustOne strips and Etest to determine the activity of daptomycin against these isolates.
Results: Of the fourteen isolates of *S. aureus*, 7 baseline and 7 with elevated MICs which developed on therapy were tested by the broth microdilution method and the aforementioned assays. There were 3 major errors (false non-susceptible) seen with the Sensititre panels and two each with the JustOne strips and with Etest. One of the isolates tested consistently non-susceptible by all three methods but tested susceptible by the reference method. The 30 isolates of *S. aureus* with elevated MICs to vancomycin all tested susceptible to DAP. The DAP range and MIC_{90} with the JustOne Strips and Etest were 0.06-1.0 µg/ml, 1.0 µg/ml and 0.19-0.75 µg/ml, 0.75 µg/ml respectively.
Conclusion: Isolates of *S. aureus* with elevated MICs to vancomycin were susceptible to daptomycin. Isolates testing non-susceptible to daptomycin should be confirmed.

INTRODUCTION

Reports of isolates of *Staphylococcus aureus* that exhibit non-susceptibility to vancomycin are of growing concern. In addition, we have observed an increase in the number of *S. aureus* isolates that have MICs to vancomycin of 2 µg/ml. Although this value is within the susceptible range, in prior years MIC values this high were not observed. An elevation in vancomycin MICs have been associated with poorer clinical outcomes (1,2).
Daptomycin is a lipopeptide antibiotic with activity against *S. aureus* and other gram-positive microorganisms. This antibiotic requires calcium to be active which can pose a problem when performing in vitro susceptibility testing. Due to this requirement disk diffusion cannot be utilized to perform in vitro susceptibility tests. In this investigation, broth microdilution methods were compared to Etest for susceptibility testing of *S. aureus* isolates that have elevated MICs to vancomycin. In addition, 7 pairs of *S. aureus* isolates that were obtained pre and post daptomycin therapy, were also tested by these methods.

METHODS

Isolates:

S. aureus isolates with vancomycin MIC values of ≥ 2 µg/ml as determined by testing in the Vitek 2 (bioMerieux, Durham, NC) were re-tested using Sensititre (TREK Diagnostic Systems, Cleveland OH) Gram positive microdilution panels. Those isolates confirmed to have elevated MICs to vancomycin were included in the study. Seven pairs of *S. aureus* isolates obtained from patients who received daptomycin were obtained from Cubist Pharmaceuticals. These post therapy isolate of each pair was reported to have a MIC to daptomycin in the non-susceptible range.

METHODS

Susceptibility Testing:

Daptomycin MICs of clinical isolates of *S. aureus* with MICs to vancomycin of ≥ 2 µg/ml were tested by a microdilution method, Sensititre JustOne strips (Trek Diagnostics Systems) and by Etest (AB Biodisk, Piscataway, NJ). The JustOne strip consists of a single row of microdilution wells containing doubling dilutions of an antibiotic (Fig.1). A 0.5 McFarland suspension was made from cultures grown overnight on Trypticase Soy Blood Agar plates to be used in each assay according to the manufacturers' recommendation.

Daptomycin MICs for the pre and post therapy *S. aureus* isolates were obtained by using Sensititre JustOne strips, Sensititre Gram positive microtiter panels (GPN3F) and Etest strips. The results generated by these assays were compared to those previously run using a reference microdilution method. The reference method was performed in the laboratories of Cubist Pharmaceuticals. Isolates yielding discrepant results were retested by all four methods in an attempt to resolve these discrepancies.

RESULTS

A total of 30 clinical isolates were recovered which demonstrated MICs to vancomycin of 2 µg/ml. The MIC range, MIC_{50} and MIC_{90} for daptomycin as determined using the Sensititre JustOne strips and Etest are shown in Table 1. All of the *S. aureus* isolates were susceptible to daptomycin with a MIC of ≤ 1µg/ml. There was excellent agreement between the two methods with 90% of the isolates exhibiting either the same MIC value or a MIC within 1 two-fold dilution. Two isolates exhibited a two-fold difference and one a three-fold difference in the MIC with the value being higher with the Etest in all three instances.

Table 1. MIC Results for Daptomycin with *S. aureus* Clinical Isolates

	JustOne	Etest
Range	0.06-1.0 µg/ml	0.19-0.75 µg/ml
MIC_{50}	0.5 µg/ml	0.5 µg/ml
MIC_{90}	1.0 µg/ml	0.75 µg/ml

RESULTS cont.

There were seven pairs of *S. aureus* isolates that were obtained from patients whose infections did not resolve despite receiving daptomycin therapy. Both the parent and break through isolates were tested by a reference method, Sensititre Gram-positive panels, Sensititre JustOne strips and Etest. These results are shown in Table 2. Compared to the reference microdilution method there were three isolates that yielded major errors (false resistance) with the Sensititre panels, and two isolates each which gave major errors with the JustOne strips and Etest. Isolate 1663 consistently yielded non-susceptible results with the three test systems and a susceptible result with the reference methods. Isolate CB-184 initially was reported to have a MIC of 4 µg/ml when first isolated. Subsequent subcultures could only recover a susceptible strain which was confirmed by all four methods.

Table 2. Comparison of a reference microdilution method with three test methods on isolates of *S. aureus* obtained prior to, and subsequent to, daptomycin therapy

	Daptomycin MIC (µg/ml)			
Isolate	Reference MIC	Sensititre Panel	Etest	JustOne Strip
CB-182	0.5	0.5	0.38	0.25
CB-183	4	4	2	2
CB-1482	0.5	0.5	2	2
CB-184	0.5	≤0.25	0.125	0.25
CB-1483	0.25	4	0.5	0.5
CB-185	4	8	4	4
CB-1627	0.5	0.5	0.5	0.25
CB-1630	4	8	2	2
CB-1631	0.5	2	0.5	0.25
CB-1634	4	4	2	2
CB-1635	1	0.5	1	1
CB-1638	4	>8	8	16
CB-1663	0.5	4	2	2
CB-1664	4	8	4	4

DISCUSSION

The isolation of *S. aureus* with increased MICs to vancomycin undoubtedly will result in the use of other agents for the treatment of staphylococcal infections. Daptomycin, with its' unique mechanism of action and cidal activity, makes it a viable therapeutic option for treating these infections. This antibiotic has a functional requirement for calcium that can be problematic when performing susceptibility tests. False resistant susceptibility results will occur in the absence of adequate calcium levels. Additionally the disk diffusion assay has been deemed to be unreliable and is not recommended.

In this study, clinical isolates of *S. aureus* with MIC values to vancomycin of 2 µg/ml were tested by two methods to determine the in vitro activity of daptomycin. These isolates were all susceptible to daptomycin when tested by individual microdilution MIC strips and Etest. In both instances the amount of calcium contained in the test system can be controlled by the manufacturer.

DISCUSSION cont.

Results comparing a reference microdilution method to three commercial test systems with pre and post therapy isolates of *S. aureus* showed some inconsistencies. Each assay method recorded some false resistant results. The Sensititre panel results yielded three major errors but otherwise was within 1 two-fold dilution of the reference method with 10/14 isolates. The JustOne and Etest results each yielded two false resistant results but otherwise was in close agreement with the reference method. Isolate CB1663 reproducibly yielded discrepant results with the three commercial systems and the reference method despite repeated testing. There were no very major errors (false susceptible results) with any of the three commercial systems. Of interest was the fact that the resistance noted initially in isolate CB-184 was not stable and was lost upon subculture.

The dependency of daptomycin on calcium can be a challenge when performing susceptibility tests. The calcium concentration must be controlled when determining MIC values. In this investigation the three commercial test systems employed yielded results that were reliable in the susceptible range. Any isolate yielding a result in the resistant range should be re-tested, preferably by a different methodology.

CONCLUSIONS

1. Isolates of *S. aureus* with MICs to vancomycin of 2 µg/ml were susceptible to daptomycin as determined by both broth microdilution and Etest.

2. In this study there were no false susceptible results using the three commercial test systems with daptomycin.

3. Any isolate of *S. aureus* yielding a MIC in the resistant range should be confirmed.

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