JOURNAL OF CLINICAL MICROBIOLOGY, June 1995, p. 1665–1667 Vol. 33, No. 6
0095-1137/95/$04.00+0


# Multicenter Clinical Laboratory Evaluation of a β-Lactamase Disk Assay Employing a Novel Chromogenic Cephalosporin, S1

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Received 30 November 1994/Returned for modification 10 February 1995/Accepted 15 March 1995

**S1, a new chromogenic cephalosporin (International BioClinical, Inc., Portland, Oreg.), was used to detect β-lactamase production among a variety of commonly encountered bacteria in a four-center collaborative study. Results of an S1 disk assay were compared with those obtained by a nitrocefin-based disk procedure (Cefinase; Becton-Dickinson Microbiology Systems, Cockeysville, Md.), with repetitive testing of five quality control organisms and with individual tests of recent clinical isolates of *Neisseria gonorrhoeae* (162 strains), *Haemophilus influenzae* (162 strains), *Moraxella catarrhalis* (155 strains), *Staphylococcus aureus* (161 strains), and *Bacteroides fragilis* (164 strains). The performances of the two β-lactamase disk assays were comparable for the first three species cited above. However, the S1 assay appeared to be a more sensitive procedure than the Cefinase assay when applied to *S. aureus* and *B. fragilis*, with respect to both total numbers of positive results and length of time to a definitive positive endpoint.**

Determination of β-lactamase production in the clinical microbiology laboratory has served as a useful means for predicting the activity of the penicillins against certain bacteria, in particular *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Moraxella catarrhalis*, and *Staphylococcus aureus* (16, 19, 23). A variety of β-lactamase detection techniques have been developed, including acidometric (2, 6, 10, 18, 20, 22, 24), iodometric (4, 12, 21), and chromogenic cephalosporin-based assays. Among the last, at least three reagents have been described: PADAC (1, 3, 8, 11), CENTA (9), and nitrocefin (13, 17), the last of which has become most commonly utilized. Reasons for the popularity of this test system are its commercial availability (e.g., Cefinase disks [Becton-Dickinson Microbiology Systems, Cockeysville, Md.] and Dry-Slide [Difco, Inc., Detroit, Mich.]), logistical simplicity, availability of results within seconds to minutes, reliability, and the fact that the nitrocefin disk test has proven to be the only accurate means for detecting β-lactamase production by *M. catarrhalis*. False-negative results are often obtained for this bacterium when tested with acidimetric or iodometric assays or procedures based on the other chromogenic cephalosporins (5, 7). Unfortunately, even nitrocefin-based β-lactamase assays have not proven useful in detecting β-lactamase production by organisms in the *Bacteroides fragilis* group, organisms commonly found to be penicillin resistant by virtue of production of a β-lactamase. For this reason, development of new, chromogenic cephalosporin β-lactamase reagents is highly desirable.

Recently, Sutton and colleagues described a novel chromogenic cephalosporin, called S1 or cefesone (23). This compound, 3-(2,4-dinitrostyryl)-(6*R*,7*R*)-7-phenylacetamido-ceph-3-em-4-carboxylate, has been shown in a single-center study to be a potentially useful substrate for detecting β-lactamase production among a variety of commonly encountered bacteria. The intent of the current study was to systematically evaluate a disk assay for β-lactamase production based on the S1 chromogenic substrate (International BioClinical, Inc., Portland, Oreg.) in four clinical microbiology laboratories using recent isolates of *H. influenzae*, *Streptococcus pneumoniae*, *M. catarrhalis*, *S. aureus*, and the *B. fragilis* group. S1 β-lactamase results were compared with those obtained with the Cefinase disk procedure.

Each of the four participating laboratories examined ca. 40 recent clinical isolates of each of the following organisms: *N. gonorrhoeae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, and the *B. fragilis* group. Colonies (16 to 24 h) on enriched chocolate agar (i.e., *N. gonorrhoeae* and *H. influenzae*), 5% sheep blood agar (i.e., *M. catarrhalis* and *S. aureus*), or supplemented brucella anaerobic agar plus 5% sheep blood (i.e., *B. fragilis*) were used for β-lactamase assays. On each day of testing, the following five quality control strains were also each examined once: *Escherichia coli* ATCC 35218, *B. fragilis* ATCC 25285, *S. aureus* ATCC 29213, *H. influenzae* ATCC 10211, and *S. aureus* ATCC 25923. The expected β-lactamase assay results for these control organisms were positive, positive, positive, negative, and negative, respectively.

A nitrocefin β-lactamase assay using Cefinase disks was performed explicitly according to the manufacturer's instructions. Briefly, 6-mm-diameter filter paper disks impregnated with nitrocefin were moistened with 0.85% NaCl, and several well-isolated colonies of test organisms were transferred to the disk with a wooden applicator stick or wire loop. The investigational β-lactamase assay using disks impregnated with the S1 chromogenic cephalosporin was performed in exactly the same manner. Disks were examined for the appearance of a pink-red coloration for up to 60 min with isolates of *S. aureus*, up to 30 min with *B. fragilis* group isolates, and up to 10 min with the other three species. The length of time to the appearance of a definitive positive reaction was noted.

Methicillin MICs for selected isolates of staphylococci were determined by using a broth microdilution method as described by the National Committee for Clinical Laboratory

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TABLE 1. Concordance of results of S1 and Cefinase disk β-lactamase assays applied to clinical isolates of selected bacteria in four laboratories[a]

| Organism (no. tested)[b] | S1 β-lactamase test result | No. of strains yielding the indicated Cefinase β-lactamase test result | |
|---|---|---|---|
| | | Positive | Negative |
| *N. gonorrhoeae* (162) | Positive | 72 | 0 |
| | Negative | 0 | 90 |
| *H. influenzae* (162) | Positive | 79 | 0 |
| | Negative | 0 | 83 |
| *M. catarrhalis* (155) | Positive | 129 | 0 |
| | Negative | 0 | 26 |
| *S. aureus* (161) | Positive | 128 | 2[c] |
| | Negative | 0 | 31 |

[a] Combined results of all four laboratories.
[b] The numbers of clinical isolates of each of the study organisms were as follows for laboratories A through D: *N. gonorrhoeae*, 40, 42, 40, and 40, respectively; *H. influenzae*, 40, 42, 40, and 40, respectively; *M. catarrhalis*, 40, 34, 41, and 40, respectively; and *S. aureus*, 40, 41, 40, and 40, respectively.
[c] Additional results obtained with these two isolates of *S. aureus* are described in the text.

TABLE 2. Concordance of results of S1 and Cefinase disk β-lactamase assays with clinical isolates of *B. fragilis* in four laboratories

| Laboratory | S1 β-lactamase test result | No. of strains yielding the indicated Cefinase β-lactamase test result | |
|---|---|---|---|
| | | Positive | Negative |
| A | Positive | 13 | 23 |
| | Negative | 0 | 4 |
| B | Positive | 6 | 0 |
| | Negative | 0 | 37 |
| C | Positive | 39 | 0 |
| | Negative | 0 | 2 |
| D | Positive | 11 | 26 |
| | Negative | 0 | 3 |

Standards (15). The ampicillin-sulbactam (2:1) and penicillin MICs for selected *B. fragilis* group isolates were determined by using a microdilution procedure in brain heart infusion broth as described by the National Committee for Clinical Laboratory Standards (14).

Intra- and interlaboratory reproducibility was assessed by repetitive testing of the five quality control strains noted above. Each quality control strain was tested 21 times: 5 times in laboratory A, 8 times in laboratory B, 3 times in laboratory C, and 5 times in laboratory D. Complete concordance between the results of the S1 and Cefinase β-lactamase assays was observed with four of the five quality control strains. Only *B. fragilis* ATCC 25285 yielded discordant results, with 10 of 21 determinations falsely negative by the nitrocefin assay. Among these 10 discrepancies, 4 were obtained in laboratory A, 1 was obtained in laboratory C, and 5 were obtained in laboratory D. The mean lengths of time to positive results with *E. coli* ATCC 35218 and *S. aureus* ATCC 29213 varied between 1.4 and 1.8 min within each assay. With *B. fragilis* ATCC 25285, positive results were obtained with each assay at 3.6 to 4.2 min.

As noted in Table 1, exact concordance between the results of the S1 and Cefinase β-lactamase assays was observed for clinical isolates of *N. gonorrhoeae* (162 isolates), *H. influenzae* (162 isolates), *M. catarrhalis* (155 isolates), and all but 2 of 161 isolates of *S. aureus*. The two discordant results obtained with *S. aureus* were both from laboratory A and appeared to be false-negative Cefinase test results, since the MICs of penicillin for both isolates, following induction with 0.05 μg of methicillin per ml, were found to be >1.0 μg/ml.

The overall mean lengths of time to a definitive positive test result were very similar with the two assays for *H. influenzae*, *N. gonorrhoeae*, and *M. catarrhalis*, i.e., 36 to 48 s. With clinical isolates of *S. aureus*, the overall mean lengths of time to a positive test result were consistently longer with both β-lactamase assays (i.e., 7.0 min with the S1 assay and 10.6 min with the Cefinase test). The S1 assay, however, clearly yielded definitive positive results more quickly than the Cefinase procedure. For *S. aureus*, in general, laboratories A and C were similar with respect to mean lengths of time to a positive test result and faster than laboratories B and D which, in turn, were comparable.

The results of tests with clinical isolates of *B. fragilis* are depicted in Table 2. Because conspicuous interlaboratory variability was noted with this organism group, results are indexed by individual laboratory. On the assumption that the large majority of *B. fragilis* isolates produced β-lactamase, large numbers of apparent false-negative results were obtained with the Cefinase assay in laboratories A and D. In laboratory B, both β-lactamase assays yielded numerous false-negative results. Only in laboratory C were most isolates of *B. fragilis* (i.e., 39 of 41) found to be positive with both the S1 and the Cefinase assays. The mean lengths of time to a strong positive test result also varied among laboratories (i.e., 3.5 to 8.0 min with the S1 assay and 3.7 to 5.8 min with the Cefinase procedure). In general, however, positive S1 results were observed 1 to 2 min sooner than were positive results with the Cefinase procedure.

In an attempt to clarify the apparent interlaboratory variability noted with the two β-lactamase assays for isolates of the *B. fragilis* group, 10 isolates were obtained from each of the four study centers and examined in a single laboratory (laboratory A). The MICs of ampicillin-sulbactam and penicillin were determined, and both β-lactamase assays were repeated. Thirty-one of these 40 isolates were judged as clearly producing β-lactamase on the basis of the disparity between ampicillin-sulbactam MICs (≤1.0 μg/ml) and penicillin MICs (≥16 μg/ml). All 31 isolates yielded positive results with the S1 assay; 20 (64.5%) were positive with the Cefinase procedure. Six of the 40 *B. fragilis* group isolates appeared to be β-lactamase negative, since the MICs of penicillin for these isolates were ≤4.0 μg/ml. One of these six isolates produced weakly positive results after 30 min with the S1 assay; all were negative with the Cefinase disk procedure. The one isolate which yielded an apparent weakly positive S1 result was negative upon retesting. Interestingly, the MICs of penicillin and ampicillin-sulbactam for the remaining three isolates in this collection of 40 *B. fragilis* group organisms were high (≥32 μg/ml). All three isolates were positive with the S1 assay; two of the three were Cefinase positive.

S1, a novel new chromogenic cephalosporin, proved to be an effective substrate for detection of β-lactamase production by several commonly encountered bacteria when tested in a disk assay format. Comparison of results obtained by the S1 disk assay with those obtained by the Cefinase disk procedure revealed complete agreement for numerous clinical isolates of *H. influenzae*, *N. gonorrhoeae*, and *M. catarrhalis* in terms of numbers of positive and negative results and length of time to a positive test result. The results obtained with the S1 reagent with *M. catarrhalis* are particularly important, as nitrocefin has heretofore been the only effective reagent for detecting β-lactamase production by this organism (5, 7).

With clinical isolates of *S. aureus*, the S1 disk test was found to be slightly more sensitive than the nitrocefin procedure, both in terms of total numbers of true-positive test results and in terms of length of time to a definitive positive test result. Enhanced sensitivity of the S1 assay was even more conspicuous with the *B. fragilis* group, for which, among a total of 164 clinical isolates, 49 (29.9%) were positive in the S1 assay but negative with the nitrocefin procedure. Furthermore, when comparisons were restricted to isolates that were positive by both assays, the length of time to a positive test result was consistently shorter with the S1 assay (mean, 2.3 min faster).

The results obtained with *B. fragilis* group isolates deserve further mention. In terms of percentages of strains found to yield specific patterns of reactivity with the two assays, laboratories fell into one of three groups. Laboratory C found 39 of 41 strains (95.1%) to be positive with both assays; 2 of 41 strains were negative by both procedures. By contrast, laboratories A and D both found roughly two-thirds of test strains to yield apparent true-positive results with the S1 assay but false-negative results with Cefinase disks, i.e., 23 of 40 strains (57.5%) in laboratory A and 26 of 40 strains (65.0%) in laboratory D. These discordant results were judged as representing false-negative nitrocefin findings at least in laboratory A, since for the 23 isolates in this laboratory with positive S1 but negative nitrocefin results, in all cases, the penicillin MIC was noted to be >8 μg/ml on the basis of a broth microdilution procedure which used supplemented brain heart infusion broth as a growth medium. Laboratory B, on the other hand, observed absolute concordance between the two assays with *B. fragilis* isolates; however, 37 of 43 strains (86.0%) yielded unexpected negative results with both assays. It is presumed that these represented false-negative results, since the vast majority of *B. fragilis* group isolates would be expected to be resistant to penicillin because of production of β-lactamase. Penicillin MICs were not available for the test isolates of *B. fragilis* in laboratory B.

There is no obvious explanation for the interlaboratory variation observed with the nitrocefin disk test with *B. fragilis* or the conspicuously lower sensitivity observed in laboratory B with the S1 procedure for this organism group. However, among the four participating laboratories, lengths of time to a positive test result were generally longest in laboratory B for all five organism groups. This was perhaps due to observer bias, i.e., higher thresholds for calling results positive, or possible use of smaller inocula for β-lactamase tests in laboratory B. The results obtained with the control organisms seem to support the former assumption (data not given).

In conclusion, use of the S1 chromogenic cephalosporin in a disk assay was found to be at least comparable to the Cefinase disk procedure as a means of detecting β-lactamase production by *N. gonorrhoeae*, *H. influenzae*, and *M. catarrhalis*. The S1 assay was superior for identifying β-lactamase-producing isolates of *S. aureus*. Results obtained with *B. fragilis* were variable and require further study; however, the S1 reagent may provide the first useful means for detecting β-lactamase activity with this organism group as well.

We thank Debbie McQuaid for excellent secretarial assistance.

This investigation was jointly funded by grants from International BioClinical, Inc., and the University of Iowa College of Medicine, Iowa City.

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