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Two-Hospital Study of *Staphylococcus aureus* Susceptibility to Penicillin and Ampicillin by Autobac I

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A total of 322 clinical isolates of *Staphylococcus aureus* were tested with ampicillin and penicillin G by Autobac I. Of these, 74 non-beta-lactamase-producing strains were isolated, of which only 32 were initially reported susceptible to both antibiotics by Autobac I. Thirty-two strains were ampicillin susceptible but penicillin resistant by Autobac I, and nine others were "resistant" to both antibiotics but had light-scattering index values that neared the "susceptible" breakpoints established by Pfizer Diagnostics for ampicillin and penicillin. Two strains were intermediate by disk diffusion test. A method was devised by which accurate results for non-beta-lactamase-producing *S. aureus* could be determined by Autobac I. Substitution of the 0.22-μg ampicillin disk for the 0.2-U penicillin disk, coupled with extended incubation beyond the 3-h recommended interval, improved results. Alternative methods such as beta-lactamase enzyme assay or disk diffusion testing could be reserved for the occasional remaining cases of borderline light-scattering index-producing *S. aureus* (those nearing the susceptible break point, ≥0.60). Substitution of the ampicillin disk coupled with extended incubation time did not result in reporting of false susceptibility for beta-lactamase-producing strains, a clinically hazardous situation.

Automated susceptibility testing has been available since 1975 when Pfizer Diagnostics introduced the Autobac I. Extensive studies by the original collaborative group of Thornsberry et al. indicated a high overall correlation of test results for most organisms by Autobac I and the disk diffusion test (10). However, some organisms when tested with certain antimicrobial agents produced agreements of less than 90%, including *Staphylococcus aureus* tested with penicillin G (85.3%). Other workers noted similar disagreements with ampicillin and penicillin (4-6). Testing of the same strains on subsequent days results in some strains demonstrating a susceptible result one day and a resistant result the next (9).

We were aware early on of discrepancies with Autobac I when testing *S. aureus* with ampicillin and penicillin. Therefore we undertook a study to determine whether the use of the 0.22-μg ampicillin disk with the Autobac I would provide more consistent and reliable results than the use of the 0.2-U penicillin disk.

MATERIALS AND METHODS

Organisms. All antibiotic susceptibility tests were performed on fresh clinical isolates incubated at 35°C for 18 to 24 h on Trypticase soy agar supplemented with 5% sheep blood. Organisms which were selected for further testing were subcultured no more than twice before repeat studies were performed, or were frozen in 50% fetal calf serum-Trypticase soy broth medium and held at −70°C until used. Revitalization was accomplished by subculturing the frozen and thawed stock to Trypticase soy broth for 24 h of incubation and then to blood agar plates for an additional 24 h of incubation. The control organism was *S. aureus* ATCC 25923, provided in lyophilized form by Pfizer Diagnostics and maintained as directed.

Susceptibility test methods. The beta-lactamase test was performed according to the method of O'Callaghan et al. (8). Chromogenic cephalosporin was obtained from Glaxo Research Ltd., London. Also used was the penicilloic acid assay of Thornsberry and Kirvin (11). Penicillin powder was obtained from Squibb, Inc.

The standardized disk diffusion test recommended by the National Committee for Clinical Laboratory Standards (7) was used with the modification of Barry et al. (1), which allows direct standardization of a rapidly growing culture to a 0.5 McFarland turbidity standard with subsequent direct inoculation of a Mueller-Hinton plate. This allowed same-day testing of *S. aureus* strains by Autobac I and disk diffusion technique. Disks were supplied by BBL Microbiology Systems.

The Autobac I test was performed according to the manufacturer's directions, except where indicated. All disks and reagents were supplied by Pfizer Diagnostics.

Discrepant organisms. Organisms were considered discrepant if the results of the ampicillin and penicillin tests with Autobac I were dissimilar, i.e., the ampicillin result was "susceptible" and the penicillin result was "resistant" or vice versa. Organisms were

considered "possibly discrepant" if the light-scattering index (LSI) for ampicillin was ≥0.40 or ≤0.59 or the penicillin LSI was ≥0.50 or ≤0.90. Discrepant or possibly discrepant organisms were retested by an alternate method (standardized disk diffusion test or beta-lactamase assay or both). Precision trials and extended incubation trials were conducted with the control strain, *S. aureus* ATCC 25923, and with five randomly selected patient-derived *S. aureus* isolates which were known non-penicillinase producers. Except for extended incubation trials, all organisms were incubated for 3 h before initiation of readout, with the exception of an occasional strain which did not reach the required gate index of 0.90 or greater. These strains were reincubated for an additional 15-min interval, at which time automatic reading would be accomplished by Autobac I.

Mass assays. Two lots of 0.22-μg ampicillin disks and one lot of 0.2-U penicillin disks were tested for drug content. Cartridges were selected that had been properly stored since receipt in the laboratories and were unopened at the time of assay. All three lots were among those used in this study.

(i) Preparation of standards. Ampicillin and penicillin standards were prepared in 0.1 M phosphate buffer (pH 6.0) at 40× the final concentration desired; i.e., initial concentrations of 20, 10, and 5 μg or U/ml were used to prepare disks containing 0.5, 0.25, and 0.125 μg or U, respectively.

(ii) Method. The *Bacillus subtilis* assay method was used for mass assays. Briefly, 0.25-in. (ca. 0.63-cm) filter paper disks were placed onto an agar surface, and 0.025 ml of standard was dispensed onto each disk. Standards were done in triplicate on each plate. Autobac disks were placed directly onto the agar surface. Plates were incubated overnight, and inhibitory zones were read. Disk values were calculated from standards.

RESULTS

A total of 322 *S. aureus* strains were tested for susceptibility or resistance to penicillin and ampicillin with Autobac I and the disk diffusion test. By disk duffusion test, 248 (77%) were resistant to both antibiotics, 74 (23%) were susceptible to both, and 2 (<1%) were intermediate to both. These percentages approximate those which have been historically observed in our hospitals and other similar community general hospitals in the area.

Of the 74 susceptible isolates of *S. aureus* recovered at our hospitals, only 32 (43%) were susceptible to both ampicillin and penicillin by Autobac I. An additional 32 strains of susceptible *S. aureus* were determined to be resistant to penicillin but susceptible to ampicillin by Autobac I, a logically incongruous result. Only one organism was determined to be resistant to ampicillin but susceptible to penicillin by Autobac I, and this strain was later determined to be a non-penicillinase producer by beta-lactamase assay and disk diffusion test. Nine strains were resistant to both ampicillin and penicillin by Autoback I but had LSI values which indicated possible discrepancy. These also were further tested by beta-lactamase assay and disk diffusion test and were found to be uniformly susceptible to ampicillin and penicillin. Results of representative Autobac tests on the 40 discrepant or possibly discrepant strains are seen in Table 1.

Precision trials were performed with the control strain and five patient-derived strains randomly selected from the non-beta-lactamase-producing *S. aureus* isolated in the laboratories. Additional 0.5-h incubation periods after the initial 3-h reading resulted in increased LSI values for most disk wells (Table 2).

Scattered LSI values were observed with the laboratory control in spite of weekly changing of penicillin and ampicillin disks in the dispensers as recommended by Pfizer Diagnostics (Table 3).

The results of the disk mass assays are indicated in Table 4.

DISCUSSION

S. aureus demonstrated susceptibility to both ampicillin and penicillin by the disk diffusion test in our laboratories. Autobac I demonstrated significant numbers of discrepancies between the two antimicrobial agents when non-beta-lactamase-producing strains were tested. Beta-lactamase-producing strains produced consistent results with Autobac I testing of ampicillin and

TABLE 1. *Examples of variation in susceptibility with corresponding LSI values for beta-lactamase-negative S. aureus*

Culture no.[a]	Penicillin LSI[b]	Ampicillin LSI[b]
1456	0.61 (R)	0.64 (S)
1545	0.81 (R)	0.64 (S)
1620	0.71 (R)	0.53 (I)
1599	0.66 (R)	0.61 (S)
1686	0.67 (R)	0.57 (I)
1769	0.81 (R)	0.65 (S)
8458	0.74 (R)	0.64 (S)
6271	0.73 (R)	0.56 (I)
5988	0.81 (R)	0.65 (S)
6417	0.67 (R)	0.43 (R)
6330	0.60 (R)	0.50 (R)
9967	0.79 (R)	0.75 (S)
9868	0.89 (R)	0.99 (S)
9951	0.75 (R)	0.61 (S)
9132	1.00 (S)	0.56 (I)

[a] All strains demonstrated zones of ampicillin and penicillin inhibition of greater than 29 mm with the disk diffusion test.

[b] Parentheses indicate: R, resistant; I, intermediate; S, susceptible.

TABLE 2. *Effect of extended incubation on precision trials*

Incubation time (h)	Strain	Penicillin LSI		Ampicillin LSI		Discrepancies[a]	
		Range	Mean	Range	Mean	Penicillin	Ampicillin
3	ATCC 25923	0.68–0.87	0.77	0.55–0.69	0.62	0, 12, 0/12	0, 0, 4/12
	S 21	0.68–0.80	0.75	0.51–0.62	0.57	0, 12, 0/12	0, 0, 9/12
	S 22	0.66–0.82	0.76	0.51–0.73	0.63	0, 12, 0/12	0, 0, 3/12
	S 23	0.73–0.83	0.79	0.57–0.73	0.65	0, 12, 0/12	0, 0, 2/12
	S 24	0.75–0.88	0.79	0.64–0.76	0.70	0, 12, 0/12	0, 0, 0/12
	S 25	0.59–0.76	0.65	0.58–0.76	0.67	0, 12, 0/12	0, 0, 1/12
	S 26	0.64–0.75	0.70	0.58–0.67	0.63	0, 12, 0/12	0, 0, 1/12
3.5	ATCC 25923	0.77–0.91	0.83	0.64–0.75	0.70	0, 11, 0/12	0, 0, 0/12
	S 21	0.77–0.85	0.82	0.58–0.70	0.66	0, 12, 0/12	0, 0, 1/12
	S 22	0.72–0.88	0.81	0.55–0.76	0.68	0, 12, 0/12	0, 0, 3/12
	S 23	0.81–0.90	0.85	0.62–0.78	0.70	0, 12, 0/12	0, 0, 0/12
	S 24	0.77–0.91	0.83	0.64–0.78	0.71	0, 11, 0/12	0, 0, 0/12
	S 25	0.62–0.84	0.74	0.66–0.83	0.80	0, 12, 0/12	0, 0, 0/12
	S 26	0.70–0.83	0.77	0.66–0.73	0.70	0, 12, 0/12	0, 0, 0/12
4	ATCC 25923	0.84–0.94	0.89	0.75–0.83	0.79	0, 6, 0/12	0, 0, 0/12
	S 21	0.82–0.89	0.87	0.66–0.77	0.73	0, 12, 0/12	0, 0, 0/12
	S 22	0.77–0.91	0.86	0.59–0.80	0.73	0, 9, 0/12	0, 0, 0/12
	S 23	0.86–0.93	0.90	0.68–0.82	0.75	0, 3, 0/12	0, 0, 0/12
	S 24	0.75–0.88	0.81	0.65–0.81	0.71	0, 11, 0/12	0, 0, 0/12
	S 25	0.61–0.89	0.73	0.71–0.89	0.87	0, 12, 0/12	0, 0, 0/12
	S 26	0.78–0.89	0.84	0.75–0.81	0.78	0, 12, 0/12	0, 0, 0/12

[a] Numbers of very major, major, and minor discrepancies/total number of disk tests.

TABLE 3. *Effect of weekly changing of ampicillin and penicillin disks on LSI of control strain S. aureus ATCC 25923*

Date[a] (June)	Ampicillin LSI	Penicillin LSI
2	0.90	0.75
4	0.70	0.80
5[b]	0.85	0.80
6	1.00	0.75
7	0.50	0.70
9	0.85	0.95
10	0.75	0.85
11	0.60	0.75
12[b]	1.00	0.80
13	0.80	1.00
14	0.55	0.70
15	0.70	0.85
16	0.60	0.85
18	0.75	0.75
19[b]	0.65	0.80
20	0.75	0.85
21	0.60	0.70
22	0.80	0.70
23	0.85	0.90
24	0.75	0.90
25	0.90	0.75
27	0.60	0.95
30	0.65	0.50

[a] No tests were run on dates not listed (no patient tests, therefore no control tests).

[b] Disks were changed on these dates (fresh cartridges in use); disks were also changed on 26 June.

penicillin. Funnell and Guinness (4) reported 95% agreement with 110 strains of penicillinase-producing *S. aureus*, and suggested that results with penicillin could be improved for non-beta-lactamase producers by extending the incubation time and lowering the acceptable LSI range from ≥0.91–1.00 to ≥0.60–1.00 for penicillin. In our study, penicillin results did not provide accurate data for six non-beta-lactamase-producing strains even after extended incubation, using the presently recommended breakpoints of ≥0.91 to 1.00. However, substitution of the 0.22-μg ampicillin disk with extended incubation time to 4 h did produce acceptable LSI readings for an accurate interpretation of susceptible for all six non-beta-lactamase-producing strains. For those few strains which still might show a borderline LSI after 4 h of incubation, further extension of the incubation time for an additional period of time or use of a rapid alternate method for detection of beta-lactamase would be helpful. However, these recommendations should be substantiated by testing larger numbers of non-beta-lactamase-producing strains in other laboratories.

Waterworth (12) suggested that prolonged lag phase of some strains may slow division time with a resultant delay in the action of cell wall-active drugs. This may be the actual basis for the generally improved results observed after

TABLE 4. *Assay of some lots of in-use ampicillin and penicillin disks*[a]

Ampicillin lot 3865[b] (μg)	Ampicillin lot 3993[c] (μg)	Penicillin lot 3980[d] (U)
0.26	0.19	0.16
0.27	0.18	0.13
0.24	0.19	0.15
0.26	0.19	0.12
0.31	0.22	0.13
0.26	0.20	0.14
0.30	0.18	0.14
0.24	0.18	0.16
0.28	0.18	0.13
0.22	0.18	0.13
0.22	0.20	0.13
0.34	0.20	0.12
0.23	0.21	0.16
0.24	0.24	0.19
0.29	0.24	0.21
0.19	0.20	0.17
0.19	0.24	0.17
0.21	0.24	0.17
0.24	0.21	0.16
0.28	0.27	0.17
0.23	0.29	0.19
0.24	0.19	0.15
0.30	0.21	0.17
0.30	0.21	0.15
0.30	0.19	0.14
0.22	0.19	0.18
0.23	0.21	0.16
0.24	0.22	0.19
0.30	0.20	0.21
0.26	0.19	0.19
0.29	0.21	0.21
0.22	0.24	0.19
0.22	0.20	0.14
0.21	0.20	0.24
0.22	0.19	0.18
0.24	0.18	0.17
		0.16

[a] Stated nominal potency for ampicillin was 0.22 μg, and that for penicillin was 0.2 U.
[b] Mean, 0.25 μg.
[c] Mean, 0.21 μg.
[d] Mean, 0.16 U.

extended incubation. The allowed variation of 67% in disk mass (3) might be too great for these low-potency disks. The penicillin disks tested in our mass assay ranged from 60 to 120% of stated potency. The ampicillin disks ranged from 76 to 136% of stated potency for the first of two lots, and from 86 to 138% for the second of two lots. This disk-to-disk variation (Table 4) may also account for the LSI variation seen in the precision trials (Table 2).

We compared our overall discrepancy rate of 7.9% to the 14.7% of the original collaborative study of Thornsberry et al. (10). This verifies the overall accuracy of the Autobac I tests versus the disk diffusion test, since most strains encountered in the clinical laboratory are beta-lactamase producers.

We believe the use of the 0.22-μg ampicillin disk, with occasional extended incubation or other rapid-method back-up testing as directed by the test-reviewing technologist, can provide the most accurate results for *S. aureus* with the least reporting delay.

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