ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1976, p. 804-809
Vol. 9, No. 5

Printed in U.S.A.

Comparison of Direct and Standardized Disk Diffusion Susceptibility Testing of Urine Cultures

GARY E. HOLLICK AND JOHN A. WASHINGTON II*
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 22 January 1976

A comparison between direct and standardized disk diffusion tests was made on a total of 300 urine specimens containing $\geq 10^5$ organisms/ml. Of these, 246 represented pure cultures and 54 represented mixed cultures. The number of major discrepancies per organism tested in pure culture was 18 (7.3%) and in mixed cultures it was 23 (42.6%). The percentage of major discrepancies per total number of antimicrobial drug comparisons made was 1.4%. Although this procedure may be of value in selected cases with pure cultures of organisms present in quantities $\geq 10^5$/ml, its use on a routine basis is not recommended.

It has recently been recommended by Kunin (7) that direct susceptibility tests be performed on urine specimens that contain bacteria in Gram-stained smears of the uncentrifuged specimen or in wet-mount preparations of centrifuged urinary sediments. Since two published reports (1, 9) of evaluations of direct susceptibility testing of urine have presented seemingly conflicting data about the procedure's reliability and accuracy, we performed a comparison between it and the standardized disk diffusion method in an attempt to reconcile these differences.

MATERIALS AND METHODS

Clean catch, midstream urine specimens collected by a urine collection service were used throughout the study. Initially, urine specimens were randomly selected for testing; however, due to their low rate of positivity, urine specimens were screened on receipt in the laboratory. Uncentrifuged, well-mixed urine was Gram stained and examined microscopically (×1,000). Only those specimens containing >2 organisms/field were selected for further study. Processing of urine specimens and identification procedures were performed as described by Washington (11).

Susceptibility test procedures. Direct susceptibility tests were performed on undiluted urine by swabbing the surface of Mueller-Hinton agar plates (BBL) with a sterile, cotton-tipped swab. Excess urine was expressed by pressing the swab against the side of the collection container. The plates were air dried for a maximum of 15 min, and high-content antimicrobial disks were distributed by means of a multidisk dispenser (BBL). Individual disks were pressed firmly on the agar surface with alcohol-flamed forceps. Plates were incubated at 37 C, and zone diameters were measured with calipers after 16 to 18 h of incubation.

The standard disk diffusion method, as described by Bauer et al. (3) and by the National Committee for Clinical Laboratory Standards (8), was performed on organisms isolated in pure culture and, in the case of mixed cultures, on those organisms present in concentrations exceeding 10^4 organisms/ml. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were included in each day's testing for quality control. Zone diameters, except those of carbenicillin, for both the direct and standard disk diffusion tests, were interpreted by guidelines set forth by the Food and Drug Administration (4, 5). Carbenicillin zone diameters were interpreted according to the manufacturer's specifications.

Zone diameter interpretations obtained with each method were compared, and discrepancies were defined as follows: very major discrepancies represented by resistance by the standard method and susceptibility by the direct method, major discrepancies represented by susceptibility by the standard method and resistance by the direct method, and minor discrepancies represented by intermediate susceptibility by one method and susceptibility or resistance by the other.

Ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), gentamicin (10 μg), kanamycin (30 μg), nitrofurantoin (300 μg), nalidixic acid (30 μg), and tetracycline (30 μg) disks were purchased commercially (BBL). Disk cartridges were received from the manufacturer sealed in plastic with desiccant. The cartridges were separated, wrapped in plastic, and frozen at −18 C with desiccant intact. A week's working supply was thawed as needed and, when not in use, refrigerated at 7 C in the multidisk dispenser, which contained calcium chloride. Disks were equilibrated to room temperature before use.

Mueller-Hinton agar plates purchased commercially (BBL) were checked for sterility, and the pH of each lot was determined before use. Plates were wrapped in plastic, stored at 7 C, and allowed to warm to room temperature before use.

Mixed culture acceptance criteria. If a mixed culture was obtained on the direct susceptibility test plate, a sufficiently large clear zone was taken to indicate susceptibility; one or more colonies within a

zone or growth to the disk was taken to indicate resistance. The direct susceptibility test was not interpreted if there was insufficient growth to permit zone resolution or the plate was overgrown with a combination of resistant organisms. Biochemically identical organisms with different colonial morphologies on primary isolation were considered to be the same organism irrespective of the susceptibility pattern obtained for each.

RESULTS

A total of 10 randomly selected urines and 290 urines with positive Gram-stained smears were found to be suitable for inclusion in the study. Pure cultures were obtained from 246 (82%) urine specimens, whereas 54 (18%) specimens yielded mixed cultures. Generally, specimens containing $\geq 10^5$ organisms/ml were required for accurate zone resolution; however, six specimens containing 10^4 to 10^5 organisms/ml gave zones adequate for analysis. When compared with the standard disk diffusion method, these zone diameters showed the greatest variability. In Table 1 are summarized the organisms isolated in pure culture. *E. coli* accounted for the majority of the isolates, followed by *Klebsiella* and *Proteus*.

Discrepant results observed with a single isolate. In Table 2 are indicated the number of organisms isolated in pure culture with at least one major and/or minor discrepancy in susceptibility. Very major discrepancies were noted in 2 (0.8%) instances, major discrepant results were observed in 16 (6.5%) instances, and minor discrepancies were observed in 53 (21.5%) instances. *E. coli, Klebsiella, Proteus,* and group D streptococci accounted for all of the very major and major discrepancies. All organisms except *Micrococcus* had at least one minor discrepancy. *E. coli,* being the organism most frequently isolated, also had the greatest number of minor discrepancies.

TABLE 1. *Distribution of organisms isolated in pure culture*[a]

Organism	% Isolated
Escherichia coli	72.4
Klebsiella	10.6
Proteus[b]	5.3
Streptococcus[c]	3.7
Pseudomonas aeruginosa	3.2
Staphylococcus epidermidis	2.8
Enterobacter[d]	0.8
Citrobacter diversus	0.8
Micrococcus	0.4

[a] Based on a total of 246 isolates.
[b] Includes 11 *P. mirabilis*, 1 *P. rettgeri*, and 1 *P. vulgaris*.
[c] Includes 8 group D streptococci and 1 group B streptococcus.
[d] Includes 1 *E. cloacae* and 1 *E. aerogenes*.

TABLE 2. *Number of organisms isolated in pure culture with at least one major and/or minor discrepant result*

Organism	Total no. isolated	No. (%) of discrepant results		
		Very major[a]	Major[b]	Minor[c]
E. coli	178	1 (0.6)	13 (7.3)	29 (16.3)
Klebsiella	26		2 (7.7)	11 (42.3)
Proteus	13	1 (7.7)		4 (30.8)
P. aeruginosa	8			2 (25.0)
S. epidermidis	7			1 (14.3)
Streptococci	9		1 (11.1)	4 (44.4)
C. diversus	2			1 (50.0)
Enterobacter	2			1 (50.0)
Micrococcus	1			

[a] Resistant by standard method and susceptible by direct method.
[b] Susceptible by standard method and resistant by direct method.
[c] Intermediately susceptible by one method and susceptible or resistant by the other.

Some organisms had more than one major or minor shift in susceptibility per test set (one set including eight antimicrobial drugs). This is reflected in Table 3, which shows the total number of major discrepancies observed between methods for each antimicrobial agent and each organism. Overall, 28 (1.4%) major discrepancies were observed, each antimicrobial agent having at least one discrepancy; no particular antimicrobial agent appeared to predominate. This is in contrast to that observed with minor discrepancies (Table 4). A total of 60 (3%) minor discrepancies were observed, of which 20 (33.3%) were with nitrofurantoin and 25 (41.7%) were with cephalothin. The remaining antimicrobial agents, by comparison, had relatively small numbers of minor discrepancies.

The overall correlation between the direct method and the standard method was found to be 95.5% (Table 5). The poorest correlations, 87.8 and 90.7%, were found to occur with cephalothin and nitrofurantoin, respectively. *E. coli, Klebsiella,* and gram-positive cocci gave the poorest correlation with cephalothin, whereas the *Klebsiella-Enterobacter* group gave the poorest correlation with nitrofurantoin. The highest correlation between methods was with gentamicin (99.6%).

Examination of the differences in zone diameters obtained with each method (Table 6) indicated that the majority (58.7%) of the differences were in the 0 to 1-mm range. A total of 10 (16.7%) minor discrepancies were noted to fall in this range, which might, in fact, reflect measurement error. One major discrepant re-

TABLE 3. *Total number of major[a] discrepancies observed between methods for each antimicrobial agent and microorganism*

Organism	Total no. isolated	Total no. of comparisons	No. of major discrepancies between methods for each antimicrobial agent[b]								
			Nitro	Tetra	Ampi	Ceph	N.A.	Kana	Carb	Gm.	Total
E. coli	178	1,424	1	5	5	4		3	4		22
Klebsiella	26	208	1			1	1				3
P. mirabilis	11	88	1	1							2
Streptococcus, group D	8	64								1	1
Total	246[c]	1,968[d]	3	6	5	5	1	3	4	1	28 (1.4%)

[a] Includes very major.

[b] Nitro, Nitrofurantoin; Tetra, tetracycline; Ampi, ampicillin; Ceph, cephalothin; N.A., nalidixic acid; Kana, kanamycin; Carb, carbenicillin; Gm., gentamicin.

[c] Includes those organisms in which no major discrepant result was observed.

[d] Includes all comparisons on organisms for which there was no major discrepancy.

TABLE 4. *Total number of minor discrepancies observed between methods for each antimicrobial agent and microorganism*

Organism	Total no. isolated	Total no. of comparisons	No. of minor discrepancies between methods for each antimicrobial agent[a]								
			Nitro	Tetra	Ampi	Ceph	N.A.	Kana	Carb	Gm.	Total
E. coli	178	1,424	10			19	2		2		33
Klebsiella	26	208	8		1	2	1	1	1		14
Proteus	13	104				1	1	1	1		4
P. aeruginosa	8	64		2							2
Enterobacter	2	16	1								1
Streptococcus, group D	8	64				2		1	1		4
S. epidermidis	7	56				1					1
C. diversus	2	16	1								1
Total	246[b]	1,968[b]	20	2	1	25	4	3	5		60(3%)

[a] Abbreviations are those used in Table 3.

[b] Includes those organisms where no minor discrepant result was observed.

TABLE 5. *Correlation between methods for each antimicrobial agent and microorganism*

Organism	Total no. isolated	Total no. of comparisons	% Correlation between methods for each antimicrobial agent[a]								
			Nitro	Tetra	Ampi	Ceph	N.A.	Kana	Carb	Gm.	Total
E. coli	178	1,424	93.8	97.2	97.2	87.1	98.9	98.3	96.6	100	96.1
Klebsiella	26	208	65.4	100	96.2	88.5	92.3	96.2	96.2	100	91.8
Proteus	13	104	92.3	92.3	100	92.3	92.3	92.3	92.3	100	94.2
P. aeruginosa	8	64	100	75.0	100	100	100	100	100	100	96.9
Enterobacter	2	16	50	100	100	100	100	100	100	100	93.7
Streptococcus	9	72	100	100	100	77.8	100	98.7	98.7	98.7	93.1
S. epidermidis	7	56	100	100	100	85.7	100	100	100	100	98.2
Micrococcus	1	8	100	100	100	100	100	100	100	100	100
C. diversus	2	16	50	100	100	100	100	100	100	100	93.7
Total	246	1,968	90.7	96.7	97.6	87.8	98.0	97.6	96.3	99.6	95.5

[a] Abbreviations are those shown in Table 3.

TABLE 6. *Differences in zone diameter observed between the direct and standard disk diffusion method*

Antimicrobial agent	Differences in zone diam (no.)				
	0-1 mm	1.1-2 mm	2.1-3 mm	3.1-4 mm	>4 mm
Nitrofurantoin	129	45	42	11	19
Tetracycline	182	34	12	6	12
Ampicillin	165	40	18	7	16
Cephalothin	129	51	33	14	19
Nalidixic acid	144	58	22	12	10
Kanamycin	145	58	16	9	18
Carbenicillin	130	52	30	12	22
Gentamicin	132	54	26	13	21
Percentage	58.7	19.9	10.1	4.3	7.0

sult in gentamicin also fell into this range. The majority of the minor discrepancies occurred when the difference was 1 to 4 mm. The remaining major discrepant results occurred when the zone diameter difference exceeded 4 mm.

Discrepant results observed with mixed isolates. A total of 54 urine specimens were found to contain more than one organism. By Gram stain alone, 30 (55.5%) of these urine specimens appeared to contain only gram-negative bacilli. In Table 7 are shown the total number of major and minor discrepancies that occurred with each type of mixed culture. At least one major discrepancy in susceptibility occurred in 23 (42.6%) of these combinations. *E. coli* was the most frequent organism isolated in mixed culture and accounted for the largest number of major and minor discrepant results. Group D streptococci isolated in conjunction with another organism were the single most frequent cause of major discrepancies in susceptibility. This was particularly striking with cephalothin, nalidixic acid, kanamycin, and gentamicin. Isolation of multiply resistant organisms in conjunction with susceptible organisms also led to many erroneous readings.

DISCUSSION

Because initiation of standardized susceptibility testing is contingent upon preparation of a broth culture of an isolated organism, such tests ordinarily require a minimum of 48 h after culture of specimen for their completion. It is tempting, therefore, in certain instances to try to shorten this process by applying antimicrobial disks directly onto an agar surface that has just been inoculated with the specimen.

Such "direct" methods for urine cultures have been evaluated by Barry et al. (1) and by Perez and Gillenwater (9) with differing interpretations of results. In the former study, there were 517 specimens containing more than 10^5 organisms/ml. Of these, 396 contained one organism, 100 contained two organisms, and 21 contained three organisms. In those specimens (396) with one organism, major discrepancies between the direct and standardized tests were encountered in 68 (17%) instances. For example, out of 232 instances in which *E. coli* was isolated in pure culture, there were 37 (16%) with major discrepancies between the two susceptibility testing methods. In the 100 specimens containing two organisms, there were 46 (46%) instances of major discrepancies. Finally, there were 10 (48%) major discrepancies observed in the 21 specimens containing three organisms.

TABLE 7. *Number of organisms isolated in mixed culture with at least one major and/or minor discrepant result*

Major organism isolated	No. of isolations	No. (%) of discrepant results	
		Major	Minor
E. coli and another[a]	37	15 (40.5)	10 (27.0)
Group D streptococci and another	3	1 (33.3)	2 (66.7)
Klebsiella and another	4	2 (50.0)	2 (50.0)
Proteus and another	2	1 (50.0)	1 (50.0)
S. aureus and another	2		1 (50.0)
P. aeruginosa and another	2	1 (50.0)	
S. epidermidis and another	2	1 (50.0)	
Citrobacter and another	1	1 (100)	
E. cloacae and another	1	1 (100)	1 (100)

[a] Other organisms isolated included 23 mixed flora, 15 streptococci, 4 *Proteus*, 4 *P. aeruginosa*, 3 staphylococci, 2 *E. coli*, 2 *Klebsiella*, and 1 *Lactobacillus*.

Although it is not specifically stated how many different antimicrobial agents were tested by Barry et al. (1), one might assume from other studies reported in their paper that as many as 10 different antimicrobial agents were tested. If this assumption is correct, one might interpret their data on a different basis. For example, assuming 10 antimicrobial agents tested against their 396 organisms in pure culture, there were 68 (1.7%) major discrepancies of 3,960 antimicrobial agent comparisons. This method of analysis of results was that used by Perez and Gillenwater (9).

Perez and Gillenwater (9) reported the overall correlation between direct and standardized testing to be 96.8% in 398 positive cultures. Very major and major discrepancies were observed in 0.5 and 2.7% of instances, respectively. On the other hand, if these data are analyzed in the manner used by Barry et al. (1), there were 137 "falsely negative" and "falsely positive" results (equivalent to the major discrepancies in the study by Barry et al. [1]) out of a total of 398 positive cultures. Since the authors stated that 97.9% (390) of their cultures contained one organism, major discrepancies occurred with approximately 35% of the organisms they tested, assuming only one major discrepancy per organism.

Basically, if we analyze our results according to the method used by Barry et al. (1), i.e., number or percentage of major discrepancies per number of organisms tested, we find them to be reasonably similar to theirs, although our percentage of very major and major discrepancies (7.3%) in testing pure cultures was somewhat lower than theirs (17%). If, on the other hand, we analyze our results by the method used by Perez and Gillenwater (9), i.e., number of very major and major discrepancies per total

number of antimicrobial agent comparisons made, we also find them to be similar to theirs, although our percentage of very major and major discrepancies (1.4%) was slightly lower than theirs (3.2%).

It should be stressed that all of the results analyzed in these two previous studies and nearly all of our results were derived from cultures containing organisms in quantities equal to or greater than 10^5/ml. Although the largest number of discrepancies encountered in these studies were minor, the vast majority of significant errors were major (resistant by direct testing and susceptible by standardized testing), rather than very major (susceptible by direct testing and resistant by standardized testing). Because it has been reported that from 68 to 86% of urine cultures from patients with true bacteriuria contained 10^6 or more colonies per ml (6) and because of the fact that no attempt is made in direct susceptibility testing to standardized the inoculum, it is reasonable to expect that smaller zone diameters may occur in direct testing than in the standardized method. In this regard, however, Bauer (2) reported in 1963 that even heavy inocula of susceptible organisms did not lead to results that would be inaccurately interpreted as representing resistance or intermediate susceptibility. By the same token, he found that testing light inocula of resistant organisms did not lead to results that would be inaccurately interpreted as signifying susceptibility.

The direct and standardized disk diffusion tests with pure cultures are essentially the same procedure used twice. The only variable is that in the direct method no attempt is made to standardize the inoculum. The variability observed in our study can validly be assessed only when it is compared with the reproducibility observed between replicates of the standard disk diffusion method. Thornsberry et al. (10) reported that, in a collaborative study of an automated susceptibility testing system, the interlaboratory estimated standard deviation for disk diffusion tests range from 1.3 to 2.0 mm for gram-negative organisms and enterococci. Furthermore, the intralaboratory estimated standard deviation was reported to range from 0.6 to 2.3 mm. From Table 6 it can be seen that approximately 78.6% of the differences observed between the direct and standardized method fell into the range of 0.6 to 2.3 mm. Thornsberry et al. (10) also found the intralaboratory reproducibility of interpretative results, based on tests performed on separate occasions, to be 91.7%, with major or very major shifts occurring in 1.2% and minor shifts in 7.1% of instances. Surprisingly, these values are very similar to the major and very major (1.4%) and min or (3%) discrepancies reported by us.

Whether or not direct susceptibility testing of urine cultures is warranted on a routine basis is debatable. First of all, the majority of urine specimens submitted to the clinical laboratory for culture are negative or contain fewer than 10^5 organisms/ml. Direct susceptibility testing of these types of specimens is both a waste of time and of antimicrobial drug disks. Second, not all laboratories routinely prepare a Gram-stained smear of urine specimens; however, such a smear is an important screening device both to detect significant bacteriuria ($\geq 10^5$ organisms/ml) and to assess the feasibility of direct susceptibility testing. Third, both our study and that of Barry et al. (1) demonstrated that, when two or more organisms were present, the instances of major discrepancies (percent per organisms tested) were greater than 40%. The direct susceptibility testing of mixtures is, therefore, clearly inadvisable. Furthermore, and unfortunately, the detection of mixtures by Gram-stained smears of well-mixed, uncentrifuged urine is unreliable. Fourth, it would appear to be necessary to confirm the susceptibility tests of organisms found to be resistant in direct testing, since most significant discrepancies were of the major variety, i.e., were resistant by direct testing and susceptible by standardized testing. Finally, the initial therapy of uncomplicated first or second episodes of urinary infections, in which *E. coli* is the most common organism and is usually susceptible to a variety of oral antimicrobial agents, is frequently empirically based. In this group of patients, therefore, the added costs of direct susceptibility may not be warranted unless the infection persists or recurs.

In conclusion, it is our opinion that the problems and costs associated with routine direct susceptibility tests outweigh their benefits. We do not, therefore, recommend the procedure on a routine basis. In selected cases, however, in which the urine collection technique is optimal and the specimen's transport to the laboratory is satisfactory, it may be worthwhile to consider direct susceptibility testing, subject to the conditions described above.

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