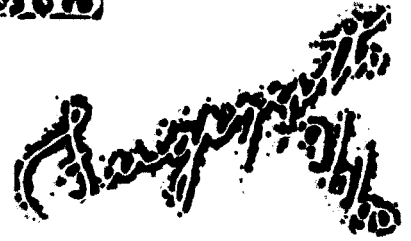


Urinary Tract Infection: A Method for Revealing Hidden Pathogens

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After the report by Kass¹ on asymptomatic bacteriuria, laboratory reports based on the colony count in urine culture became the point of departure for practically all studies on the clinical aspects and diagnosis of urinary tract infection (UTI). There have been reports challenging the value of the 100,000 count, and some even indicating that counts as low as 150-200/ml are significant.^{2,3,4} Yet medical laboratories continue to lean heavily on the concept of high "significant numbers" as the basis on which to report as etiologic agents those organisms found to emerge as such.

As for laboratory procedure, the convenient practice of direct agar plate culture (certainly applicable to the study of well-adapted stock organisms) does not take into account that the organisms in cases of deep-seated infection such as UTI do not behave as "classroom" stock cultures. They are often in a hydrophilic state (a little-known concept), and do not emerge at all on the direct plating of a specimen on agar—a finding that was the thrust of a 4 year procedural study* involving more than 6,500 urines, and challenging the universally adopted bacteriologic protocol for the diagnosis of UTI. This 4-year study of the bacteriology of UTI presented evidence

emphasizing the need for more sophisticated culture procedures in order to attain a reliable appraisal of the bacterial status of patients with UTI.

A few salient features of this study are summarized in Table 1. It can be seen that by extending incubation periods beyond 48 hours, the number of patients having so-called "significant colony counts" of 100,000/ml or more was doubled, and the number of agar-plate cultures with appreciable growth was increased from 19.7% to 41.2%. Furthermore, the addition of a primary broth culture to the routine test procedure resulted in the recovery of more than twice as many beta-hemolytic streptococci, and tripled the total number of catheterized kidney urines in which significant organisms were found. In other words, the recovery of organisms on culture increased as measures were taken to

provide more favorable conditions for bacterial growth.

An additional feature in the laboratory diagnosis of UTI, observed in a continuation of the foregoing study,⁵ is that urine specimens often contain antibacterial (growth-suppressant) components, some of which are considered to derive from antibiotic treatment of the patient. In tests on 612 specimens obtained from a local cooperating hospital laboratory, 170 (or 27.8%) were found, by a special test procedure, to contain antibiotics (mostly broad-spectrum). On the basis of this finding it was postulated that the presence of these substances in a urine specimen cannot possibly fail to influence the culture results reported to the clinician and the consequent evaluation of the patient's microbial status.

Since it is well known that bacterial growth is suppressed in the presence of the appropriate antibiotics, it seemed logical to question what one should expect of a specimen if those suppressants were not present. Therefore, exploratory studies were directed toward devising a procedure for removing these growth-suppressing components from the culture environment, thereby presumably liberating the suppressed bacteria to grow normally.

After considerable preliminary trial, a method was developed whereby aliquots of urine specimens were "washed," thereby rinsing their bacteria clean of inhibitory components and removing them so

Table 1. Comparison of Percentage Recoveries Between Standard (24 Hour) Culture and the Extended (48+ Hour) Research Procedure

Effect of increasing incubation time from:	24 hr.	to	48+ hr.	Outcomes
Agar plate culture growth	19.7%		41.2%	•
Colony counts of 100,000/ml	6.4%		12.4%	•
Recovery from culture on:	agar	vs.	agar+broth	
Beta hemolytic streptococci	1.9%		4.7%	•
Catheterized bladder urine	61.4%		77.3%	•
Catheterized kidney urine	24.3%		78.1%	•
• = 6,562 specimens	1,864		4,698	
• = 625 specimens	226		397	

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they could not interfere with bacterial metabolism in culture. The results of this were so dramatic that this "washing" manipulation was adopted routinely in processing all subsequent (more than 2,500) specimens.

Procedure

Although the goal of the washing procedure was to enhance the emergence of any microbial species present in bladder urine, and this was accomplished by primary culture in liquid media, after eliminating urine-chemical components from the culture—as noted above—need for comparison dictated a routine consisting of processing two broth cultures in parallel (with and without washing). Consequently, for each specimen, five drops (by micropipette) of well-mixed urine were transferred to a 13- by 100-mm tube containing 1.5 ml of trypticase broth. This represented a direct broth culture of the urine. For the washing procedure, 10 drops of the peptic urine sample to be "ashed" were aseptically added to a screw-cap tube (13 by 100 mm) containing 4.5 ml of sterile salt solution. The salt solution consisted of 5 g NaCl plus 0.05 g proteose peptone (Difco) as a buffer, per 100 ml distilled water.

The screw-cap tube was then capped securely, held in an upright position, and passed by smooth up-and-down excursions over an arc of about 1 foot, two times per second, 10 seconds (20 complete cycles). Mode of agitation devised only after many preliminary tests, after which it was centrifuged at 2,000 rpm for 20 minutes, the supernatant poured off (presumably leaving the "ashed" bacteria in the bottom), 1.5 ml of trypticase soy broth (as in the unwashed culture) added to the residue, and the tube cap replaced.

Both broth tubes were then again shaken briefly and incubated at 35°C until growth appeared usually in 3 days or for as long as 6 days if negative in 3 days.

For the purposes of purification and identification of the organisms recovered in each tube, and for sensi-

tivity testing of these organisms, each broth culture was subcultured in due time, to appropriate agars, using standard isolation techniques. To select the best agar media for subculture, each broth was examined by wet mount under 430X magnification, to ascertain the nature and morphology of the organisms present. Generally, for preliminary separation and identification according to the organisms seen, ~~the~~ eosin methylene blue, ~~and~~ inoculated for (Gram-negative) bacillary forms; ~~blood agar for anaerobes~~; and selective phenylethyl alcohol agar for retrieving Gram-positive organisms (cocci)—suppressing Gram negative bacilli if any were present. Although transfers of the broth cultures can be made to ~~the~~ the agars mentioned here without the prior examination of wet mounts, cocci in the presence of other organisms may be missed if one is not aware of their presence in the broth and fails to make special efforts to isolate and identify them.

Both screw-cap tubes were then subcultured as above, in order to identify and compare the bacterial species recovered from each.

Besides these steps, all urines were also transferred directly to blood agar in approximately 0.025-ml amounts, (which is about 25 times the amount used in "standard plate count" or 0.001-ml loop pro-

cedures), to ascertain whether there was growth or no growth in comparison to broth culture. The colony count was not included in this procedure because (as will be seen later) such a manipulation serves no purpose in UTI bacteriology, where the etiologic agents mentioned above may well be in the hydrophilic state or must be freed of suppressant urine components in order to emerge at all.

Results

Besides the data shown in Table 1, the importance of subjecting broth cultures to the washing procedure is brought out by Table 2, which summarizes the data derived from two groups of tests in which whole direct-agar cultures of urine (0.025 ml) were compared to cultures in broth. The third and fourth columns from the left indicate that a substantial number (16% and 17.3%, respectively) of the urines did yield growth in broth, but not on agar. These results confirm the finding (Table 1) that culture on agar alone is insufficient for the complete bacteriologic appraisal of urine specimens.

Another series of results to be considered is represented in Table 3. This relates to a special group of 69 specimens (found to be negative on agar culture) for which, in the first 24 hours of incubation, there was disagreement in terms of growth

Table 2. Culture Results on Primary Inoculation of Whole Urine to Agar vs. Broth

Agar	NG	Grth	NG	NG	Grth**	Total
Broth	NG	Grth	Grth	Grth*	NG	
Group I						
No.	438	498	109	70	3	1,118
%	39.2	44.9	9.7	6.3	0.27	
			16.0			
Group II						
No.	121	169	39	23	1	352
%	34.4	48.0	11.1	6.2	0.28	
			17.3			

*Colonies: no growth in 24 hours, but growth in 48 hours.
 **Colonies: 2-4 colonies (questionable significance).
 Grth = Growth
 NG = no growth
 Group I specimens furnished by four clinical laboratories (Pecos, Nevada; Group II by one facility (Rapid City, South Dakota).

versus no growth between broth cultures of the whole urine and broth cultures of the respective "washed" residues. In 40 of these 69 specimens, the washed residues yielded bacterial growth, as compared to only 1 of the whole urine cultures that produced growth. The retrieval differences are also shown for incubations of 48 and 72 hours. Ultimately the washing procedure resulted in growth from all 69 urines, while 32 (46.8%) of the respective whole-urine broth cultures remained negative, attesting to the importance of the washing procedure for the recovery of suppressed organisms.

More surprising still are the last two rows of figures in Table 3, showing that while 25 of the 69 urines did contain detectable antibiotics (inhibitors), 44 (63.8%) did not. This would indicate that urines may contain bacteria-suppressing components other than antibiotics, and that these too are removed by the washing procedure, freeing the suppressed bacteria to emerge on culture. Also noteworthy is that the medical laboratory from which these specimens were obtained reported its results of the standard agar plate culture procedure as 46 = "no growth" and 16 = "normal flora" (no data were received on the remaining 7 specimens).

Moving on to Table 4, which organizes the results from 108 agar-culture-negative specimens from among 800 consecutive urines, the left-hand column gives the findings resulting from direct broth culture of the urine samples. Group A = no growth; Group B = Gram positive organisms; Group C = Gram negative organisms; and Group D = others. The columns to the right in Table 4 list the additional isolates arising from broth culture of the respective washed aliquots. The totals in the far right column give the numbers of isolates that did not emerge on direct broth culture, especially the 38 in Group A. Also, noteworthy are the 46 isolates found only in the washed aliquots (*Streptococcus* column). 16 of

which were beta hemolytic, and the 28 mixed members of the *Staphylococcus-Gallia* group in Column 3—together constituting 74 significant Gram-positive isolates of the 108 missed by the clinical laboratory. These organisms cannot be ignored merely because they are not found by a "standard" procedure."

Discussion

It should first be noted that "growth" in the studies described here is not meant to imply that a definitive identification was made of these organisms as etiologic

agents. Since there seems to be no specifically unique UTI pathogen, as there is for gonorrhea or salmonellosis, the laboratory cannot declare any organism that it isolates to be an etiologic agent, although it is becoming obvious that Gram-positive cocci must be accepted as such under these test conditions. The designation must instead be a cooperative decision between the laboratory and the physician, with close observation of the patient—an ideal arrangement that is almost impossible to achieve).

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Table 4. Isolates Obtained from Washed Urine Aliquots as Compared to Those Obtained from Direct Culture of Respective Urines

Group	From Direct Broth Culture		Additional Isolates from Culture of Respective Washed Aliquots				Total
			Strep	Staph-Gallia	Gram neg.	Other	
A	No growth	(18)	8	15	9	6	38
B	Gram positive	(17)	-	6	8	-	12
	Strep* Staph-Gallia		19		6		25
C	Gram negative	(23)					
	<i>Coliform</i> (**)		11	2	3	1	17
	<i>Klebsiella</i> <i>Proteus</i>		2	-	1	-	4
D	Others	(10)					
	Yeast Lactobacilli		4 1	4 1	- -	- -	8 2
Totals			44***	28	27	7	108

*Three of these were beta strep.

**Includes *proteus* and other enterobacteriaceae.

***Sixteen of these were beta strep.

Table 3. Results From 69 Specimens Yielding No Growth on Agar but with Disagreement Between Broth Culture of Whole Urine (Whole U) and of the Respective Washed Aliquots (WSH U).

Specimen	Result	Incubation Time			Total No. Spec's
		24 Hr.	48 Hr.	72 Hr.	
Whole U	NG	(48)	(39)	(32)	32
Whole U	Grth	1	29	7	37
WSH U	NG	(29)	(4)	(0)	0
WSH U	Grth	40	23	6	69
Ant-B*	Yes	16	8	1	25
	No	23	14	3	44

*Detectable Antibiotics

Grth = growth

NG = no growth