

Primary Culture Media for Routine Urine Processing

JOAN C. FUNG,^{1*} BARBARA LUCIA,¹ EDNA CLARK,¹ MARJORIE BERMAN,¹ JACK GOLDSTEIN,²
AND RICHARD F. D'AMATO³

North Shore University Hospital, Manhasset, New York 11030,¹ Analytab Products, Plainview, New York 11803,² and The Catholic Medical Center of Brooklyn and Queens, Jamaica, New York 11432³

Received 17 March 1982/Accepted 24 June 1982

It has been recommended that routine microbiological processing of urine specimens include quantitative plating onto blood agar medium along with a selective and differential agar such as MacConkey agar for gram-negative organisms. Few data have been published to justify this combination. To evaluate the validity of this recommendation 2,553 midstream, clean-voided urine samples were quantitatively plated onto blood agar, MacConkey agar, and colistin-nalidixic acid agar, which is a selective medium for gram-positive organisms. The amounts of growth on each of the three media were compared. Results indicated that the best medium combination was colistin-nalidixic acid agar and MacConkey agar. The use of colistin-nalidixic acid agar instead of blood agar increased the detection of significant growth of enterococci, lactobacilli, and *Torulopsis glabrata*.

Urine samples comprise a large majority of the specimens submitted to a hospital microbiology laboratory. Plating media recommended for clean-voided, midstream urines include the use of blood agar (BA) with a selective and differential agar for gram-negative organisms such as eosin methylene blue or MacConkey agar (McC) (1). A recent survey (Clin. Microbiol. Newsletter 1979, 1(22):1-5) showed that in 234 laboratories over 90% used BA, over 90% used eosin methylene blue or McC, and approximately 30% used either colistin-nalidixic acid agar (CNA) or phenylethyl alcohol agar (PEA) as the primary plating media for urine specimens.

The selection of the best combination of media to use for primary plating is based, of course, on the ability of the chosen media to support growth and isolation of the suspected causative agent(s). However, the inclusion of many different media in primary isolation may be excessive and not cost effective. The purpose of the present study was to determine which combination of media is the best for the routine primary plating of urine specimens.

MATERIALS AND METHODS

Urine specimens. A total of 2,553 clean-voided, midstream urine specimens were submitted during a 7-week period of April and May 1981 to the Bacteriology Section of North Shore University Hospital Microbiology Laboratory. Urine specimens not processed within 30 min subsequent to collection were refrigerated. All specimens were processed within 12 h after collection.

Urine culture procedure. Each urine specimen was

plated onto commercially prepared BA (Trypticase soy agar with 5% sheep blood), McC, and Columbia CNA (all from BBL Microbiology Systems, Cockeysville, Md.) (7). Each lot of medium was quality controlled, as recommended by the manufacturer, before use. Urine specimens were plated, using a 0.001-ml calibrated loop. Plates were incubated at 35°C aerobically and examined at 18 to 24 h and at 48 h of incubation. Cultures were quantitated, and those microorganisms isolated in the range of $\geq 10^4$ colony-forming units (CFU) per ml were identified. Specimens containing three or more isolates in counts of $\geq 10^4$ CFU/ml but with no single isolate in the $\geq 10^5$ range were reported as "contaminated," and a repeat specimen was requested. "Significant" bacteruria is defined as a clean-voided, midstream specimen containing a bacterial count of $\geq 10^5$ CFU/ml, and a "significant" funguria was defined as a fungal count of $\geq 10^4$ CFU/ml.

Organism identification. Bacterial identifications were performed with the API 20E system (Analytab Products, Plainview, N.Y.) or by conventional procedures (15). Beta-hemolytic streptococci were presumptively identified as group B *Streptococcus* isolates, using the standard CAMP test (15). Yeast isolated from urine specimens requested only for routine and not for fungal culture were identified, using the germ tube test or Tween 80-oxgall-cafeic acid-Dairs (TOC) agar (8). Yeast cells with a positive germ tube test or producing pseudomycelium and chlamydospores on TOC were reported as *Candida albicans*. Those yeasts which produced no pseudomycelium and no browning on TOC and grew as tiny, white, raised nonhemolytic colonies on BA were reported as yeast-cells resembling *Torulopsis glabrata* (16); such isolates have subsequently been confirmed to be *T. glabrata*, using the Uni-Yeast-Tek system (Flow Laboratories, Inc., McLean, Va.). Yeasts producing only

TABLE 1. Microorganisms isolated from urine specimens

Organism	Monomicrobial					Polymicrobial
	Total	Inpatient	Outpatient	Female	Male	Total
Gram-negative						
<i>Escherichia coli</i>	159	103	56	125	34	21
<i>Klebsiella pneumoniae</i>	17	13	4	10	7	8
<i>Proteus mirabilis</i>	16	9	7	12	4	7
<i>Pseudomonas aeruginosa</i>	18	18	0	9	9	7
<i>Enterobacter cloacae</i>	5	5	0	4	1	3
<i>Serratia marcescens</i>	4	4	0	1	3	0
Others	6	6	0	4	2	3
Gram-positive						
<i>Enterococcus</i> species	26	25	1	17	9	29
Group B streptococci	15	14	1	15	0	4
Alpha streptococci	4	2	2	4	0	1
<i>Staphylococcus aureus</i>	2	1	1	0	2	0
Coagulase-negative staphylococci	14	11	3	6	8	4
<i>Lactobacillus</i> species	16	11	5	16	0	7
<i>Corynebacterium</i> species	12	11	1	6	6	6
Yeast						
<i>Candida albicans</i>	27	24	3	12	15	6
<i>Torulopsis glabrata</i>	24	24	0	21	3	5
Others	7	7	0	7	0	2

pseudomycelium and blastospores on TOC with no chlamydo spores or browning of the TOC medium were reported as "yeast cells not *Candida albicans*."

Simulated urine specimens. Simulated urine specimens were made with Mueller-Hinton broth. Suspensions containing 10^7 , 10^5 , and 10^3 CFU of gram-negative bacteria per ml mixed with 10^7 , 10^5 , and 10^3 CFU of enterococci per ml were plated onto BA, McC, CNA, and PEA (BBL), using a 0.001-ml loop. Quantitation of growth was recorded after 18 to 24 h and at 48 h of incubation at 35°C. Likewise, broth suspensions containing approximately 2×10^6 CFU of *T. glabrata* per ml were plated onto BA, PEA, and CNA, and the plates were examined for growth after 48 h of incubation at 35°C.

Yeast strains. Yeast isolates consisted of *Candida albicans* (24 strains), *Candida tropicalis* (10), *Candida krusei* (10), *Candida paratropicalis* (10), *Candida guilliermondii* (9), *Candida parapsilosis* (7), *Candida lusitanae* (10), *Torulopsis candida* (9), *T. glabrata* (30), *Cryptococcus neoformans* (10), *Cryptococcus albidus* subsp. *albidus* and *Cryptococcus albidus* subsp. *diffuens* (10), *Saccharomyces cerevisiae* (9), and *Saccharomyces rosei* (4) obtained from a variety of clinical sources and sent to API Diagnostic Laboratories from various clinical laboratories throughout the United States for confirmation of identification. Organisms were identified, using the API 20C system (Analytab Products) and conventional Wickerham carbohydrate assimilations (15).

RESULTS

Of the 2,553 clean-voided midstream urine specimens processed, 744 (29%) were from male patients, 1,809 (71%) from female patients, 463

(18%) from outpatients and 2,090 (82%) from inpatients. A total of 463 specimens (18%) contained $\geq 10^5$ bacterial CFU/ml or $\geq 10^4$ yeast CFU/ml (or both), of which 34 (7.3%) were reported as contaminated.

Of the 463 specimens with significant growth, 372 (80%) had only a single organism. The distribution of the microorganisms isolated from monomicrobial urine specimens was 225 (60%) gram-negative rods, 89 (24%) gram-positive organisms, and 48 (16%) yeasts (Table 1). The predominant organism causing monomicrobial urinary tract infections (UTI) was *Escherichia coli* (43%). *E. coli* was involved in 67% of the outpatient and only 36% of the inpatient monomicrobial UTI. Yeasts, gram-positive organisms, and non-*E. coli* gram-negative rods played major roles in UTI of hospitalized individuals. Group B *Streptococcus* isolates, lactobacilli, and *T. glabrata* were isolated more frequently from urine specimens of female patients.

Fifty-seven specimens were polymicrobial. Of these, 49 had only two organisms in the significant range, whereas 8 others were obtained from patients whose urine samples repeatedly contained significant growth of three or more organisms. Polymicrobial cultures were encountered from urine specimens of 34 females and 33 males. Of these, only four were from outpatients. Enterococci were observed in approximately half of the specimens which contained two or more organisms in the significant range; this far exceeded their isolation in only 7% of

TABLE 2. Discrepancy in colony counts on microbiological media used for urine cultures

Organism	Discrepant medium combination ^a	No. of discrepancies	Type of bacteriuria	
			Monomicrobial	Polymicrobial
Enterococci	CNA+/BA-	13	0	13
<i>Lactobacillus</i> species	CNA+/BA-	10	4	6
<i>Torulopsis glabrata</i>	CNA+/BA-	22	14	8
Group B streptococci	CNA+/BA-	3	0	3
<i>Corynebacterium</i> species	CNA+/BA-	1	1	0
	CNA-/BA+	2	2	0
Coagulase-negative staphylococci	CNA+/BA-	1	1	0
	CNA-/BA+	3	1	2
<i>Staphylococcus aureus</i>	CNA+/BA-	1	0	1
Yeasts not <i>Candida albicans</i>	CNA+/BA-	2	1	1
<i>Escherichia coli</i>	McC+/BA-	1	1	0

^a CNA+/BA- indicates that a higher colony count was observed on CNA than on BA plates (at least a 10-fold increase in colony-forming units). CNA-/BA+ and McC+/BA- indicate that higher colony counts were observed on BA and McC than on CNA and BA, respectively.

monomicrobial UTI (Table 1).

For each urine specimen processed, the amounts of growth on each of the three media, BA, McC, and CNA, were quantitated. A 10-fold difference in the number of colony-forming units per milliliter was considered to be a discrepant result. Discrepancies in colony counts were observed in 59 urine specimens. Of these, 55 would have resulted in a difference of laboratory interpretation of the culture. Discrepancies in colony counts were observed most frequently between the CNA and BA plates (Table 2). Of the 59 discrepancies, 55 were the result of more growth on CNA than on BA. The discrepancies occurred with equal frequency for both monomicrobial and polymicrobial specimens.

The discrepancies in colony counts between CNA and BA were notable for enterococci (Table 2). Often, enterococci were missed or underestimated when one or more gram-negative rods were present in significant numbers. Simulated urine suspensions containing 10^7 , 10^5 , and 10^3 CFU of *E. coli* per ml and 10^7 , 10^5 , and 10^3 CFU of enterococci per ml were plated onto CNA, McC, and BA media, using the 0.001-ml loop. A discrepancy in colony counts was observed for the suspension containing 10^7 CFU of *E. coli* per ml and 10^5 CFU of enterococci per ml; 75 colonies of enterococci were observed on the CNA plate, whereas only 4 colonies grew on the BA plate. In a separate set of experiments, similar discrepancies were observed in broth suspensions containing (colony-forming units per milliliter) 10^5 *E. coli*/ 10^4 enterococci, 10^5 *Klebsiella pneumoniae*/ 10^4 enterococci, 10^7 *K. pneumoniae*/ 10^5 enterococci, 10^7 *E. coli*/ 10^5 *Streptococcus bovis*, 10^7 *K. pneumoniae*/ 10^5 *Streptococcus bovis*, 10^5 *E. coli*/ 10^4 group B streptococci, 10^5 *K. pneumoniae*/ 10^4 group B streptococci, 10^5 *E. coli*/ 10^4 lactobacilli, and 10^5

K. pneumoniae/ 10^4 lactobacilli. Likewise, discrepancies were observed between BA and PEA, with suspensions (colony-forming units per milliliter) of 10^5 *E. coli*/ 10^4 enterococci and 10^5 *K. pneumoniae*/ 10^4 enterococci. No discrepancy was observed in broth suspensions containing 10^6 , 10^4 , and 10^2 CFU of *Candida albicans* per ml and 10^7 , 10^5 , and 10^3 CFU of enterococci per ml or with yeast mixed with 10^6 , 10^4 , and 10^2 CFU of *Streptococcus bovis* per ml.

In addition to enterococci, 19 lactobacilli and 22 *T. glabrata* strains were isolated from urines in much higher numbers on CNA than on BA plates. To determine whether this difference can be attributed to the difference in the ability of CNA and BA to support the growth of *T. glabrata*, 10 *T. glabrata* strains, suspended in saline from 1×10^6 to 3×10^6 CFU/ml, were quantitatively plated onto CNA and BA plates, using a 0.001-ml loop. All 10 isolates were recovered on CNA in the range of 100 to 300 colonies per plate. However, only 4 of the 10 strains grew on BA. These four strains were isolated in the same quantities on BA as on CNA, but the colony size on BA was noticeably

TABLE 3. Growth of *Torulopsis glabrata* on CNA and BA media

No. of isolates	Colony size	
	CNA	BA
12	+ ^a	-
2	++	-
14	++	(+)
1	+++	(+)
1	+	+

^a Colony sizes were graded in decreasing size from +++ to +. The (+) indicates barely visible colonies, and a minus indicates no growth.

smaller than on CNA. Thirty strains of *T. glabrata* were examined for their growth characteristics on BA and CNA (Table 3). All but one of these *T. glabrata* strains grew only on CNA plates or as larger colonies on CNA than on BA; the one strain grew equally well on both media. Eight *T. glabrata* strains were tested for their ability to grow on PEA; only one grew. This isolate grew very poorly on PEA but as large colonies on CNA medium.

To examine the growth characteristics of other yeasts on CNA and BA, various species of yeasts, including those most frequently encountered in clinical specimens, were examined. The better growth of *T. glabrata* on CNA than on BA was observed also for eight of the nine *Saccharomyces cervisiae* tested. Eight of ten *Cryptococcus neoformans* grew better on BA than on CNA. *Cryptococcus albidus* and *T. candida* grew poorly on both media. Essentially, all *Candida* species and *Saccharomyces rosei* examined grew well on both media.

Only 1 of the 18 coagulase-negative strains isolated in the significant range was identified as *Staphylococcus saprophyticus*. This strain was isolated only on BA and not on CNA and was from a female outpatient. The other three coagulase-negative staphylococci isolated which showed a discrepancy in growth between CNA and BA were identified as *Staphylococcus epidermidis*.

DISCUSSION

Recommended media for microbiological examination of urine samples are BA and either McC or eosin methylene blue (1), however, data accumulated in the present investigation indicate that CNA with McC may be a better plating combination. CNA is superior to BA in the quantitative recovery of enterococci, lactobacilli, and *T. glabrata*. The underestimation of enterococci appears to be due to the presence of significant gram-negative bacterial growth on BA medium. Of the 21 polymicrobial urines, 13 contained both enterococci and a gram-negative organism in a significant amount and had fewer colony counts on BA than on CNA. Gram-negative rods suppressing or obscuring the growth of enterococci on BA plates included *Pseudomonas aeruginosa*, *K. pneumoniae*, *E. coli*, *Proteus mirabilis*, and *Citrobacter freundii*. None of the eight urine specimens containing enterococci with either a significant number of yeast cells or a second gram-positive bacterial organism had a discrepant result between BA and CNA quantitation. These observations, using clinical specimens, were supported by simulated urine suspensions containing different concentrations of coliforms mixed with various gram-positive organisms. In suspensions con-

taining $\geq 10^5$ CFU of coliforms per ml and 10^4 to 10^5 CFU of gram-positive organisms per ml, the presence of the gram-positive organism on BA was underestimated. It may be that the mucoid colonies of gram-negative rods obscure the presence of underlying smaller enterococcal colonies.

Of the 29 *T. glabrata* isolated, 22 grew only on CNA plates or grew in higher quantities on CNA plates than on BA plates. The component present in CNA medium which supports and enhances the growth of *T. glabrata* is not known. It may be that the polypeptone peptone, a constituent of CNA and of Sabouraud dextrose agar but not of BA, is the important factor. Of the 23 isolates of *Lactobacillus*, 10 grew only, or in increased amounts, on the CNA versus the BA plates. It may be that CNA contains certain vitamins (20) required for *Lactobacillus* growth which are absent in BA medium.

The clinical significance of lactobacilli and *T. glabrata* in urine specimens is unclear since both are members of the normal flora of the urogenital tract (2, 10, 12, 17, 19). *T. glabrata* infrequently may cause UTI (12, 18) and was isolated on repeat urine specimens in $\geq 10^5$ cfu/ml from two patients in this study, suggesting its possible significance.

Enterococci may also colonize the urogenital tract and have frequently been isolated from polymicrobial urines (6, 14) and in complicated cases of UTI (1, 11), as was found in this study. However, the quantitative amounts of enterococci may be underestimated in the presence of significant growths of gram-negative rods when BA and not CNA medium is used. It may be that if a CNA-McC plating combination is used instead of the recommended BA-McC combination, higher incidences of enterococcus-involving polymicrobial bacteriuria will be observed.

Aside from those for enterococci, lactobacilli, and *T. glabrata*, the discrepancies in the amount of growth observed for other organisms were minor. Discrepancies between the colony counts on BA and McC were essentially nil. Thus, the omission of the BA plate and its replacement with CNA should have no, or an insignificant, effect on the isolation rate of gram-negative rods. The only instance in which there was increased growth on McC relative to BA was with one *E. coli* isolate; the colony count on BA was 60 versus 7 on McC. PEA may be used in place of CNA as the gram-positive selective plate for routine urine cultures. However, PEA is unable to support the growth of *T. glabrata*.

Fastidious organisms such as anaerobes (4, 9), mycobacteria (5), and *Haemophilus* species (21) have been implicated in UTI. The incidence of these organisms causing UTI is, however, very low (3, 13); thus, including media and incubation

conditions for their growth in routine urine processing is unnecessary and uneconomical. When UTI is still suspected but there is a negative routine urine culture, a Gram stain and additional media should be included in the culture of a follow-up urine specimen.

In summary, a higher isolation rate was observed when CNA and McC were used as primary culture media for routine urine processing. This CNA-McC combination proved to be a better combination of medium than the presently recommended BA-McC combination.

ACKNOWLEDGMENTS

We thank the staff of the Division of Microbiology at North Shore University Hospital for their cooperation in this study, Henry Isenberg, Erwin Neter, and Syed Qadri for their helpful suggestions, and Phyllis Vogel for the typing of this manuscript.

LITERATURE CITED

1. Barry, A. L., P. B. Smith, and M. Turck. 1975. Cumitech 2. Laboratory diagnosis of urinary tract infections. Coordinating ed., T. L. Gavan. American Society for Microbiology, Washington, D.C.
2. Bartlett, J. G., A. B. Onderdonk, E. Drude, C. Goldstein, M. Anderka, S. Alpert, and W. M. McCormack. 1977. Quantitative bacteriology of the vaginal flora. *J. Infect. Dis.* **136**:271-277.
3. Boyd, D. E., D. J. Flournoy, and S. M. Hussain Qadri. 1980. An evaluation of the gram stain and chocolate agar culture as a part of a routine urine culture set-up. *Med. Microbiol. Immunol.* **169**:63-66.
4. Brook, J. 1980. Urinary tract infection caused by anaerobic bacteria in children. *Urology* **16**:596-598.
5. Brooker, W. J., and A. C. Aufder heide. 1980. Genitourinary tract infections due to atypical Mycobacteria. *J. Urol.* **124**:242-244.
6. Bump, C. M. 1977. Bacteriuria in a pediatric population. *Am. J. Med. Technol.* **43**:851-855.
7. Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi. 1966. A new culture medium for medical bacteriology. *Am. J. Clin. Pathol.* **36**:502-504.
8. Fleming, W. H. III, J. M. Hopkins, and G. A. Land. 1977. New culture medium for the presumptive identification of *Candida albicans* and *Cryptococcus neoformans*. *J. Clin. Microbiol.* **5**:236-243.
9. Gargan, R. A., W. Brumfitt, and J. M. Hamilton-Miller. 1980. Do anaerobes cause urinary infection? *Lancet* **i**:37.
10. Grimley, P. M., L. D. Wright, and A. E. Jennings. 1965. *Torulopsis glabrata* infection in man. *Am. J. Clin. Pathol.* **43**:216-223.
11. Gross, P. A., M. Flower, and G. Barden. 1976. Polymicrobial bacteriuria: significant association with bacteremia. *J. Clin. Microbiol.* **3**:246-250.
12. Guze, L. B., and L. D. Haley. 1958. Fungus infections of the urinary tract. *Yale J. Biol. Med.* **30**:292-305.
13. Hole, R. 1980. Fastidious organisms as urinary pathogens. *Lancet.* **i**:546.
14. Laurell, G., and G. Lindbom. 1960. Urinary tract infections. I. Drugs and bugs in urinary tract infection from the bacteriological point of view. *Acta Paediatr.* **49**:189-196.
15. Lennette, E. H., A. Balow, W. J. Hausler, Jr., and J. P. Truant (ed.). 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
16. Marks, M. J., and E. O'Toole. 1970. Laboratory identification of *Torulopsis glabrata*: typical appearance on routine bacteriological media. *Appl. Microbiol.* **19**:184-185.
17. Marrie, T. J., G. K. M. Harding, and A. R. Ronald. 1978. Anaerobic aerobic urethral flora in healthy females. *J. Clin. Microbiol.* **8**:67-72.
18. Newman, D. M., and J. M. Hoog. 1969. *Torulopsis glabrata* pyelonephritis. *J. Urol.* **102**:547-548.
19. Pfau, A., and T. Sacks. 1977. The bacterial flora of the vaginal vestibule, urethra and vagina in the normal premenopausal woman. *J. Urol.* **118**:292-295.
20. Rogosa, M. J. G. Franklin, and K. D. Perry. 1961. Correlation of the vitamin requirements with cultural and biochemical characters of *Lactobacillus* ssp. *J. Gen. Microbiol.* **25**:473-482.
21. Schuit, K. E. 1979. Isolation of *Haemophilus* in urine cultures from children. *J. Pediatr.* **95**:565-566.