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New Plate Medium for Screening and Presumptive Identification of Gram-Negative Urinary Tract Pathogens

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A new selective, differential plating medium to screen the common gram-negative urinary tract pathogens is described. The medium combines adonitol fermentation, phenylalanine deaminase, and β-glucuronidase tests and allows the indole and cytochrome oxidase tests to be performed directly from the plates. High-level agreement with individual conventional tests was recorded in comparative studies with 504 cultures of gram-negative rods. There was 100% agreement, except for the *Providencia* spp. indole spot test (61.6% agreement). Adonitol fermentation by *Providencia* species could not be determined. *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* were identified with a high efficiency (100, 85.7, 83.5, and 100% agreement, respectively) without further testing. There was 96% overall agreement for the 267 infected urine samples tested.

Members of the family *Enterobacteriaceae* are important causes of both community- and hospital-acquired infections (1, 2, 5); the most frequent of the community-acquired infections are those of the urinary tract due to *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

The present investigation was undertaken to evaluate a new medium (T-mod) for isolating and presumptively identifying members of the *Enterobacteriaceae* which commonly cause urinary tract infections.

(Some of the preliminary results of this study have been previously reported [M. C. Thaller, F. Berlutti, B. Dainelli, and R. Pezzi, Abstr. 14th Int. Congr. Microbiol., p. 77, 1986].)

A total of 504 gram-negative isolates were examined (see Table 2). These isolates included 493 from the collections of our institutes and 11 standard cultures from official collections, i.e., Collection de l'Institut Pasteur and American Type Culture Collection. In addition, 70 *Streptococcus faecalis*, 8 *Streptococcus faecium*, 48 *Staphylococcus aureus*, 12 *Staphylococcus epidermidis*, and 5 *Staphylococcus saprophyticus* isolates were used as controls for the inhibitory effect of the medium on the growth of gram-positive organisms (4). The identities of the strains were confirmed by the criteria of Farmer et al. (2), Palleroni (9), Kloos and Schleifer (6), and Mundt (8), following standard procedures (3, 10).

T-mod medium (Consiglio Nazionale delle Ricerche, patent 47512A/87, January 1987) contained the following components: Bacto-Peptone (Difco Laboratories, Detroit, Mich.), 20 g; adonitol, 10 g; L-tryptophan, 1.5 g; Teepol HB6 (catalog no. T9017; Sigma Chemical Co., St. Louis, Mo.), 2 ml; L-phenylalanine, 10 g; ferric ammonium citrate, 0.2 g; NaCl, 5 g; bromxylenol blue (Sigma), 0.03 g; agar, 15 g; deionized water, 1,000 ml. The medium was boiled until the components were completely dissolved, sterilized at 121°C for 15 min, and allowed to cool to 50 to 55°C. A 50-mg amount of 4-methyl-umbellifery l-β-D-glucuronide (catalog no. M9130; Sigma) was then added, and the melted medium was mixed, sterilized under flowing steam for 20 min, and poured into 100-mm-diameter culture plates (20 ml per plate). The final medium, which had a pH of 6.8 ± 0.1, appeared clear and green.

The test medium was inoculated with a single colony of a fresh isolate of a test strain. After overnight incubation at 37°C, readings were performed as follows. (i) The color of the colonies was recorded (blue, both adonitol fermentation and phenylalanine deaminase negative; yellow, adonitol fermentation positive; brown, phenylalanine deaminase positive). (ii) The colonies were tested for fluorescence under 366-nm light; the presence of blue fluorescence indicated a β-glucuronidase-positive test (11). (iii) The colonies were tested for cytochrome oxidase and indole with oxidase sticks (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and filter paper saturated with Kovacs reagent (12), respectively.

A total of 267 infected urine samples were streaked onto T-mod and MacConkey media and onto blood agar (control medium). After overnight incubation at 37°C, the gram-negative colonies were identified on the basis of T-mod reactions, and the identities of the isolates were confirmed by the criteria of Farmer et al. (2) and Palleroni (9), following standard procedures (3, 10).

The growth of *E. coli* CIP 548, *P. mirabilis* ATCC 29906, *K. pneumoniae* ATCC 4208, *Serratia marcescens* CIP 6755, *Citrobacter freundii* CIP 5732, and *Pseudomonas aeruginosa* (wild-type strain) was compared on MacConkey agar (Difco) and the test medium. The inhibition of gram-positive organisms was studied by streaking overnight brain heart infusion broth cultures with a calibrated loop on the test medium and 5% sheep blood agar.

Both the colony counts and sizes of the tested gram-negative strains showed no significative differences on T-mod and MacConkey media. The gram-positive organisms were all inhibited on T-mod medium after 48 h of incubation at 37°C.

In comparative trials all the gram-negative strains were examined for their responses both to T-mod medium and in individual adonitol, phenylalanine deaminase (3, 10), and β-glucuronidase (1) tests. The oxidase and indole tests were performed as spot tests directly on isolated colonies from the test medium and a companion blood agar plate, as described above. Agreement of 100% was obtained for the β-glucuro-

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nidase, adonitol, phenylalanine deaminase, indole, and cytochrome oxidase tests, with the following exceptions: (i) adonitol fermentation of *Proteus* and *Providencia* strains could not be recorded because of the presence of melaninlike pigments (7) and (ii) some *Providencia* isolates (all the *P. alcalifaciens* isolates and 18 of 24 *P. stuartii* isolates) yielded a false-negative indole spot test. The colony characteristics of the main gram-negative urinary tract pathogens on T-mod medium are given in Table 1; the results for the gram-negative strains in the β-glucuronidase, adonitol fermentation, phenylalanine deaminase, indole, and cytochrome oxidase tests are given in Table 2. The presumptive identifications, made on the basis of the results given in Tables 1 and 2, were 100% consistent with complete identification, except for *P. mirabilis* and *K. pneumoniae* (83.5 and 87.5%, respectively) isolates. The inconsistencies occurred with 30 organisms: 18 *P. stuartii* and 5 *P. alcalifaciens* isolates were misidentified as *P. mirabilis*, and 2 *S. marcescens* and 5 *Enterobacter cloacae* isolates were misidentified as *K. pneumoniae*.

Results obtained with the test medium for 267 infected urine samples led to the isolation and presumptive identification of 248 gram-negative strains. Of these, 232 were correctly identified, i.e., 217 isolates at the species level (*E. coli*, 150; *P. mirabilis*, 42; *K. pneumoniae*, 16; and *P. aeruginosa*, 16), 3 as *Providencia* spp. or *Morganella morganii*, 5 as *Klebsiella oxytoca* or *Citrobacter diversus*, and 6 as *C. freundii*, *S. marcescens*, or *E. cloacae*. Misidentifications occurred with 10 isolates: 1 *Proteus vulgaris* and 2 *P. stuartii* isolates were misidentified as *P. mirabilis*, 3 *S. marcescens* and 3 *E. cloacae* isolates were misidentified as *K. pneumoniae*, and 1 *Acinetobacter calcoaceticus* isolate was misidentified as *C. freundii*, *E. cloacae*, or *S. marcescens*. Total agreement with results of complete identification was 96%.

The test medium provides good presumptive identification of the gram-negative rods most frequently involved in urinary tract infections. It should be noted that the presence of brown melaninlike pigments (7) masks a positive adonitol reaction so that it is impossible to distinguish *P. alcalifaciens* and *Providencia rettgeri* from other members of the *Proteae* tribe. Also, *P. stuartii* and *P. alcalifaciens* strains might give a false-negative reaction in the indole spot test and could consequently be misidentified as *P. mirabilis*. These discrepancies do not affect the usefulness of the test medium because the *Providencia* species are less frequently recovered from infected urine than is *P. mirabilis*.

The efficiency and agreement percentages make T-mod medium a very useful tool in routine identification of urinary tract pathogens. The cost of a plate (20 ml) of T-mod medium made in-house was calculated by using the 1987 Difco and Sigma price lists for each specific compound at the maximum volume discount. The cost of a MacConkey medium plate (20 ml) was derived from the price of 1 lb of Difco dehydrated powder. Direct costs, including supplies and labor, and indirect costs were not considered. Although T-mod medium costs more than MacConkey medium ($0.43 and $0.06 per plate, respectively), it has several advantages over the latter: (i) it provides for high-efficiency presumptive diagnosis (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* are easily recognized), (ii) it is possible to recognize other species that infrequently cause urinary tract infections, and (iii) definitive identification of most commonly isolated gram-negative pathogens may be eliminated. Therefore, T-mod medium provides substantial savings in time and equipment and provides improved identification; thus, it may be preferred to MacConkey medium when urine samples must be examined.

TABLE 1. Colony characteristics of the main gram-negative urinary tract pathogens

Organism	Colony color
E. coli	Blue, rarely yellow
C. freundii	Blue
E. cloacae and *S. marcescens*	Blue, sometimes yellow
K. pneumoniae and *K. oxytoca*	Yellow
Providencia, *Morganella*, and *Proteus* spp.	Dark brown with dark brown halo
P. aeruginosa	Pale blue or light brown without brown halo

TABLE 2. Results for the main gram-negative pathogens of the urinary tract on T-mod medium in β-glucuronidase, adonitol, phenylalanine deaminase, indole, and cytochrome oxidase tests

Species (*n*)	Reaction (no. of isolates positive)[a]				
	β-Gluc	Ad	PAD	I	CO
E. coli (141)	+ (141)	− (0)	− (0)	+ (138)	− (0)
C. freundii (23)	− (0)	− (0)	− (0)	− (0)	− (0)
E. cloacae (15)	− (0)	V (5)	− (0)	− (0)	− (0)
K. pneumoniae (42)	− (0)	+ (42)	− (0)	− (0)	− (0)
K. oxytoca (7)	− (0)	+ (7)	− (0)	+ (7)	− (0)
S. marcescens (6)	− (0)	V (2)	− (0)	− (0)	− (0)
P. mirabilis (116)	− (0)	ND	+ (116)	− (0)	− (0)
M. morganii (38)	− (0)	ND	+ (38)	+ (38)	− (0)
P. rettgeri (31)	− (0)	ND	+ (31)	+ (31)	− (0)
P. stuartii (24)	− (0)	ND	+ (24)	V (6)	− (0)
P. alcalifaciens (5)	− (0)	ND	+ (5)	− (0)	− (0)
P. aeruginosa (56)	− (0)	− (0)	− (0)	− (0)	+ (56)

[a] β-Gluc, β-Glucuronidase; Ad, adonitol fermentation; PAD, phenylalanine deaminase; I, indole spot test; CO, cytochrome oxidase test; +, 95 to 100% positive; −, 0 to 5% positive; V, 6 to 94% positive; ND, not determined.

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