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Rapid Biochemical Screening for *Salmonella*, *Shigella*, *Yersinia*, and *Aeromonas* Isolates from Stool Specimens

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Four screens for the rapid (4 to 6 h) biochemical detection of pathogens from enteric isolation media are described. The *Salmonella* screen consisted of Kligler iron agar (KIA), motility-indole-urea-tryptophan-deamination semisolid medium (MIU-TDA), and the *o*-nitrophenyl-β-D-galactopyranoside (ONPG) test; the *Shigella* screen consisted of KIA, MIU-TDA, the ONPG test, and the lysine decarboxylation-indole test; the *Yersinia* screen consisted of a rhamnose broth; the *Aeromonas* screen consisted of a xylose agar plate. When tested on 2,102 fresh isolates and 71 stock strains, the screens correctly detected 212 enteric pathogens (sensitivity, 100%), with a specificity of 98.1%.

Various techniques for the rapid detection of enteropathogenic bacteria have been proposed, including same-day biochemical screening (1, 3, 5, 7, 8) and antigen detection (3, 6). In the study described here, we evaluated a system of four rapid (4 to 6 h) biochemical screens (RSs) for the presumptive identification of *Salmonella*, *Shigella*, *Yersinia*, and *Aeromonas* isolates from enteric isolation media and compared the results with those of a conventional two-tube 24-h screen.

Media and tests. The following media were prepared and, unless noted otherwise, were autoclaved for 15 min at 121°C. (i) Motility-indole-urea-tryptophan-deamination semisolid medium (MIU-TDA) (9) consisted of bio-Trypcase (bioMérieux, Marcy-l'Etoile, France), 30 g; KH_2PO_4 (Merck, Darmstadt, Germany), 2 g; NaCl (Merck), 5 g; agar (Merck), 3 g; 0.2% alcoholic solution of phenol red indicator (powder; Difco, Detroit, Mich.), 2 ml; L-tryptophan (Merck), 2 g; ammonium iron (III) citrate (Merck), 0.5 g; and distilled water, 900 ml; after autoclaving, the medium was cooled down to 50°C and 100 ml of a 20% sterile urea (crystalline; Merck) solution was added; 4 ml of the medium was aseptically dispensed per 13-by-75-mm capped tube [L-tryptophan is the substrate and ammonium iron (III) citrate is the indicator for the TDA reaction]. (ii) For the *o*-nitrophenyl-β-D-galactopyranoside (ONPG) test (β-galactosidase), Bacto Peptone (Difco), 1 g; NaCl (Merck), 0.5 g; and distilled water, 100 ml, were dispensed at 0.5 ml per tube; after inoculation, an ONPG disk (bioMérieux) was added aseptically. (iii) Rhamnose broth consisted of phenol red broth base (Difco), 1.6 g; rhamnose (Merck), 1 g; and distilled water, 100 ml; the medium was adjusted to pH 8.0; 0.5 ml of the medium was distributed to each tube, and the tubes were autoclaved for 15 min at 116°C. (iv) The xylose plate consisted of Phenol red broth base (Difco), 16 g; agar, 15 g; and distilled water, 1 liter; the medium was cooled down to 50°C and adjusted to pH 7.8, and xylose (Merck) was added aseptically to a final concentration of 0.4%; 15 ml of the medium was poured into each plate (diameter, 90 mm). (iv) Kligler iron agar (KIA) was provided by Merck. The following tests were performed according to the instructions of the manufacturer: lysine decarboxylation (LDC) and indole test (LDC-indole substrate tablet; Rosco, Taastrup, Denmark), in which indole production was detected by adding a drop of Kovács reagent (bioMérieux), and the cytochrome oxidase test (Bactident oxidase; Merck).

Methods. Fresh stool specimens were inoculated onto xylose-lysine-desoxycholate (XLD) agar with neutral red indicator (XLD I; Gibco, Paisly, Scotland), MacConkey agar (MC; MacConkey II agar; Becton Dickinson, Cockeysville, Md.), and cefsulodine-irgasan-novobiocin agar (CIN; Oxoid, Basingstoke, England) and into selenite enrichment broth (Gibco), which was subcultured after 18 to 24 h onto XLD agar (XLD II) (4). CIN was incubated at 29°C; the other media were incubated at 37°C. The conventional screen consisted of incubation in KIA and MIU-TDA overnight at 37°C; isolates from CIN plates, however, were incubated at 29°C. Suspected isolates were identified by the API 20E system (bioMérieux) and were serotyped if appropriate. A total of 2,102 fresh stool isolates and 71 stock strains were tested. All media used in the RSs were warmed at 37°C prior to use and incubated at 37°C, and the results were interpreted 4 to 6 h later.

RS for *Salmonella* spp. (i) From XLD I and XLD II, a nonacidified, H_2S-positive colony was subcultured, half into KIA and MIU-TDA and the other half in a test tube containing ONPG. (ii) From MC, a lactose-nonfermenting colony was subcultured onto KIA and MIU-TDA; after 4 h of incubation, H_2S-producing (traces on the lower side of the slant) and urease- and TDA-negative isolates were heavily inoculated from the KIA slant into an ONPG-containing test tube, the test tube was incubated at 37°C, and the results were read 1 h later. Suspected isolates in both cases were urease, TDA, and ONPG negative.

RS for *Shigella* spp. A non-acid-producing and oxidase-negative colony from XLD I or MC was subcultured into KIA, MIU-TDA, and ONPG-containing test tubes. A sweep of colonies of the same type was suspended in 0.25 ml of saline to obtain a turbidity of a 2 to 5 McFarland standard, an LDC-indole tablet was added, and the mixture was overlaid with three drops of sterile paraffin oil. The screen was interpreted by following the algorithm shown in Fig. 1. Isolates from MC showing traces of H_2S production were interpreted as an RS for *Salmonella* spp.

RS for *Yersinia* spp. The rhamnose broth was lightly inocu-

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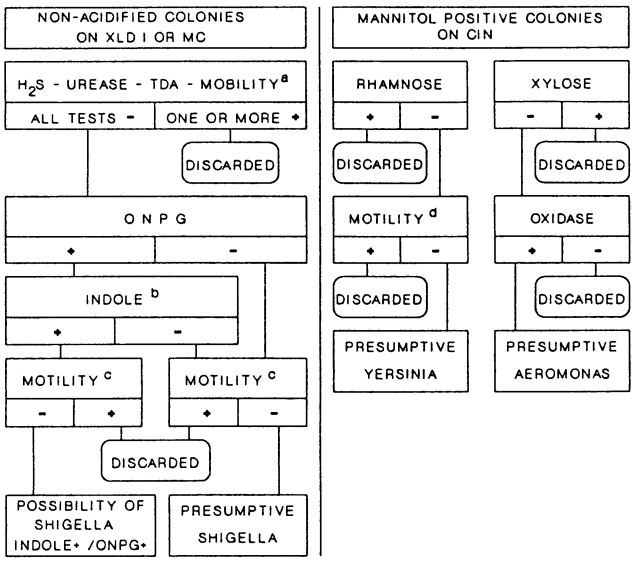

FIG. 1. Algorithms for presumptive identification of *Shigella*, *Yersinia*, and *Aeromonas* isolates. a, macroscopic motility test in MIU-TDA. b, reaction in LDC-indole test tube. c, microscopic examination of the ONPG test. d, microscopic examination of the rhamnose broth.

TABLE 1. Distribution of *Salmonella*, *Shigella*, *Yersinia*, and *Aeromonas* isolates among fresh stool isolates and stock strains

Species, serogroup, serotype, or biotype	No. tested	
	Stock strains	Fresh isolates
Salmonella spp.		
Serogroup B	0	54
Serogroup C	0	15
Serogroup D	0	37
Serogroup E	0	1
Serogroup G	0	1
Shigella dysenteriae	1	1
Shigella flexneri	14	4
Shigella boydii	6	1
Shigella sonnei	11	3
Shigella sp., nontypeable[a]	1	0
Yersinia enterocolitica		
Serotype O:3	17	12
Serotype O:9	1	0
Serotype O:5,27	1	1
Biotype 1A	0	1
Yersinia pseudotuberculosis	2	0
Aeromonas caviae	17	10

[a] Confirmed by the Centre National de Référence des Salmonella et Shigella, Institut Pasteur, Paris, France.

lated with a typical pinpoint mannitol-positive colony from CIN.

RS for *Aeromonas* spp. From a mannitol-positive colony from CIN, a thin streak of ±1 cm in length was made on a xylose plate; up to 16 isolates (separated by ±1 cm from each other) could be tested on the same plate. The *Yersinia* and *Aeromonas* screens were interpreted by following the algorithms shown in Fig. 1.

On 2,102 RSs performed, 794 were RSs for *Salmonella* spp., 415 were RSs for *Shigella* spp. 523 were RSs for *Yersinia* spp., and 370 were RSs for *Aeromonas* spp. Their specificities were 99.3, 95.1, 97.6, and 100%, respectively (5, 20, 12, and 0 false-positive results, respectively). The false-positive results of the RSs for *Salmonella* spp. included 1 *Edwardsiella tarda* isolate. All 212 strains of enteric pathogens, including 71 stock strains, were correctly detected by their respective RS (100% sensitivity) (Table 1).

Few limitations of the RS system were encountered. Indeed, except for the RS for *Shigella* spp., the RSs can be performed with a single colony from an isolation plate; the *Yersinia* and *Aeromonas* screens can both be inoculated with the same colony.

The *Shigella* screen requires sufficient growth from nonacidified colonies on MC or XLD I to obtain the dense suspension needed for the LDC-indole test. If this condition was not met, a conventional screen was inoculated, but this yielded no additional *Shigella* spp. If more colonies of each type need to be checked, one could dip an inoculating needle into a colony, inoculate a conventional screen, and then include the colony in the sweep for the LDC-indole test suspension. A rare biotype of *Shigella* spp. showing a simultaneous positivity in the ONPG and indole tests is described in some identification systems (the API 20E system). KIA and MIU-TDA of RSs with this biochemical profile (Fig. 1) were reincubated as a conventional screen, and acetate and Christensen citrate media were inoculated to exclude *Shigella* spp. (2). However, no ONPG- and indole-positive *Shigella* sp. was detected during the study. The LDC-indole test suspension is made from a sweep of colonies, and the presence of a mixture of colonies is possible; therefore, a positive reaction in one of these tests must be interpreted with caution: KIA and MIU-TDA of such isolates were reinterpreted after overnight incubation. In this regard, no anomalies were found.

E. tarda and *Yersinia pseudotuberculosis*, two rare enteric pathogens, were also detected by the RSs. Although the latter is rhamnose positive at 29°C, it is reported to be rhamnose negative at 37°C (2). KIA and MIU-TDA from the *Shigella* screen can also be reincubated to screen for other enteric pathogens such as plesiomonads, lactose-negative aeromonads, or H_2S-negative *Salmonella* spp. The RSs provide a pure culture for further identification and susceptibility testing, and growth on KIA of presumptive *Salmonella* isolates is usually sufficient for same-day serotyping.

The RSs fit easily into the routine work flow because they are simple to perform and the results are simple to interpret. This presumptive identification system, mostly based on negative biochemical reactions, reduced false-positive workup to 37 of 2,102 (1.8%) screens, thus saving time, effort, and material. In conclusion, the RSs reduce the delay in the final reporting of common enteric pathogens by 1 day and offer an efficient and cost-saving alternative to conventional screening systems.

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