APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1996, p. 1885–1888 Vol. 62, No. 6
0099-2240/96/$04.00+0

Differential Susceptibility of Aeromonads and Coliforms to Cefsulodin

JOSE L. ALONSO,[1]* INMACULADA AMOROS,[1] AND MARIA A. ALONSO[2]

Instituto de Hidrología y Medio Natural, Universidad Politécnica, 46022 Valencia,[1] and Servicio de Análisis Clínicos, Hospital General del SVS de Alicante,[2] Spain

Received 16 January 1996/Accepted 25 March 1996

Cefsulodin was evaluated as a potential selective agent for aeromonads. Resistance of *Aeromonas* and coliform isolates was determined by using a standard disk diffusion technique. A total of 119 *Aeromonas* and 78 coliform strains were isolated. For 102 of 129 *Aeromonas* isolates (environmental and reference strains), the MIC of cefsulodin was <8 μg/ml. Results of MIC tests by the agar dilution method showed that a concentration of cefsulodin of 10 μg/ml or less inhibited the growth of 96% of isolates. In comparison, for 81 of 94 coliform isolates (environmental and reference strains), the MIC of cefsulodin was >32 μg/ml. Because cefsulodin suppresses growth of *Aeromonas* and other oxidase-positive organisms, total coliform (TC) and *Escherichia coli* counts on Chromocult Coliform agar (CC agar) without cefsulodin and on CC agar with 10 mg of cefsulodin per liter (CC-CFS) were compared. Variance analysis of data from 14 sewage-polluted irrigation water specimens did not demonstrate any statistically significant difference in the enumeration of *E. coli* with CC and CC-CFS media. On average, the CC agar recovered 2.46 times as many TCs as CC-CFS. However, *Aeromonas* colonies made up an average of 58.6% of the TC counts on CC agar. Because no *Aeromonas* spp. were recovered on CC-CFS, background interference was eliminated and the counts that were obtained reflected more accurately the number of TCs. Results of this study suggest that cefsulodin may be a useful selective agent against *Aeromonas* spp. which should be included in coliform chromogenic media when high levels of accompanying flora are expected.

Species of the genus *Aeromonas* are considered ubiquitous waterborne organisms (14). It is apparent that *Aeromonas* spp. interfere with coliform counts in drinking (15) and environmental (8) water samples. Lactose-fermenting aeromonads have been observed as false-positive colonies on coliform media (8, 9, 17, 24), elevating the total coliform (TC) count. The identification of TCs based on detection of β-galactosidase activity (18) is a significant departure from methods that utilize the bacterial end products of lactose fermentation (3). Recent studies have shown that chromogenic substrates of β-galactosidase can be used in rapid and simple methods that differentiate lactose-utilizing from non-lactose-utilizing members of the family *Enterobacteriaceae* (6, 23). However, most *Aeromonas* spp. are also β-galactosidase-positive (26). Ley et al. (16) found that 76% of β-galactosidase-positive bacteria isolated on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) were *Aeromonas* spp.

Most aeromonads are susceptible to the expanded-spectrum cephalosporins (13). Susceptibility of *Aeromonas* species to cephalosporins may help to distinguish these organisms from members of the family *Enterobacteriaceae*. Brenner et al. (5) found that cefsulodin added at 5 μg/ml to MI agar inhibited the growth of *Aeromonas* and *Flavobacterium* species.

In this study, cefsulodin was evaluated as a potential agent against *Aeromonas* species. We describe the evaluation of a new chromogenic medium, Chromocult Coliform agar (CC agar), developed by E. Merck AG (Darmstadt, Germany), containing two chromogenic glycosides for the detection of β-galactosidase and β-glucuronidase. The Salmon-GAL substrate causes a salmon to red color of the coliform colonies (Lac^+), and the substrate X-glucuronide is used for the identification of β-D-glucuronidase. *Escherichia coli* cleaves both Salmon-GAL and X-glucuronide, so that positive colonies take on a dark blue to violet color (Lac^+ Gus^+). To see whether we could eliminate *Aeromonas* interference from TC counts, we compared recoveries of TCs and *E. coli* from sewage-polluted irrigation waters on membrane filters by using CC agar without cefsulodin and CC agar with cefsulodin (CC-CFS).

MATERIALS AND METHODS

Bacterial strains. The reference strains used in this study are listed in Table 1. Two *Salmonella* strains were from our stock culture collection (isolated from freshwaters). A total of 119 *Aeromonas* strains were isolated from environmental waters on ampicillin-dextrin agar (ADA) (10), and 76 coliform strains were isolated from environmental waters on CC agar. All strains were grown and maintained on nutrient agar no. 2 (Oxoid, Basingstoke, United Kingdom). *Aeromonas* strains were incubated at 30°C, and all other strains were incubated at 35°C.

Biochemical identification. Identification of the *Aeromonas* strains to the genus level was done on the basis of the following tests: cytochrome oxidase activity, growth on TSI Agar (Difco Laboratories, Detroit, Mich.), and sensitivity to 10- and 150-μg disks (Oxoid) of the vibriostatic agent 0/129 on blood agar base (11). Conventional methods (esculin hydrolysis and production of gas from glucose) provided species identification (22). Incubation was carried out at 30°C. Isolates with one atypical reaction were classified as atypical strains. Identification of the coliform strains was done by growth and gas production in lauryl tryptose broth (LTB) (Merck). The tubes were incubated at 35°C for 48 h. All presumptive tubes, which were positive as defined in *Standard Methods* (3), were transferred into brilliant green bile broth (BGB) (Merck) and incubated at 35°C for 48 h. *E. coli* strains were identified by gas production in EC broth (Merck) and indole production in tryptone-water following incubation at 44.5°C for 24 h (3).

Susceptibility procedures. (i) Disk diffusion technique. Cefsulodin susceptibility tests were performed by a standard disk diffusion technique (4). Briefly, disks impregnated with 30 μg of cefsulodin (Diagnostics Pasteur, Marnes La Coquette, France) were manually applied to the surface of Mueller-Hinton agar (Merck) plates with sterile forceps. The plates were inverted and incubated at 30°C for *Aeromonas* strains and 35°C for all other strains. The following zone diameter breakpoints for cefsulodin were employed, as recommended by the manufacturers: >22 mm, sensitive; 14 to 22 mm, intermediate; and <14 mm, resistant.

(ii) Agar dilution method. MICs for *Aeromonas* and coliform strains that had intermediate resistance as determined by cefsulodin disk diffusion were determined by using a standard agar dilution method (25). A series of Mueller-Hinton

* Corresponding author. Mailing address: Instituto de Hidrología y Medio Natural, Universidad Politécnica, Camino de Vera 14, 46022 Valencia, Spain. Phone: 6-3877090. Fax: 6-3877009. Electronic mail address: jalonso@ihdr.upv.es.

TABLE 1. Reference strains used in cefsulodin susceptibility tests

Strain	Source[a]
Escherichia coli	
681	CECT
683	CECT
678	CECT
4177	CECT
R	NCTC
V	NCTC
Serratia odorifera 867	CECT
Hafnia alvei 157	CECT
Enterobacter aerogenes 684	CECT
Enterobacter cloacae 194	CECT
Edwarsiella tarda 849	CECT
Klebsiella pneumoniae subsp. *pneumoniae* 140	CECT
Klebsiella pneumoniae subsp. *ozaenae* 851	CECT
Klebsiella oxytoca 860	CECT
Citrobacter freundii 401	CECT
Citrobacter diversus 856	CECT
Vibrio cholerae 557	CECT
Pseudomonas aeruginosa 108	CECT
Aeromonas sobria 837	CECT
Aeromonas jandaei 4228	CECT
Aeromonas hydrophila	
398	CECT
839	CECT
Aeromonas caviae 838	CECT
Aeromonas media 4232	CECT
Aeromonas trota 4255	CECT
Aeromonas eucrenophila 4224	CECT
Aeromonas schubertii 4241	CECT
Aeromonas veronii 4257	CECT

[a] CECT, Coleccion Española de Cultivos Tipo; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

test plates containing 5, 10, and 15 μg of cefsulodin per ml (Sigma Chemical Co., St. Louis, Mo.) were prepared. The plates were inverted and incubated at 30°C for *Aeromonas* spp. and 35°C for all other strains. MICs were determined by placing plates on a dark background and observing for the lowest concentration that inhibited visible growth, which was recorded as the MIC. The MIC of cefsulodin was recorded in micrograms per milliliter.

Evaluation of selective media. (i) Sampling. A series of freshwater samples from three irrigation channels near Polytechnic University of Valencia were used to evaluate the ability of CC agar and CC-CFS to enumerate TCs and *E. coli*. All samples were collected in sterile glass bottles, refrigerated, and assayed within 1 h after collection.

(ii) Medium comparison. Dilutions of the water samples were made, and duplicates of each dilution were filtered through sterile 0.45-μm-pore-size membranes (Millipore Corp., Bedford, Mass.) by a standard membrane filtration technique (3). One membrane of each set of duplicates was placed on a preprepared layer of CC agar in a 47-mm-diameter petri dish. The second membrane of each duplicate pair was placed on CC-CFS (final concentration of cefsulodin, 10 mg/liter). One milliliter of a freshly prepared filter-sterilized solution of cefsulodin (10 mg/10 ml) was added to 100 ml of CC agar after the agar was tempered in a 50°C water bath. The membranes placed on CC agar and CC-CFS were incubated at 35°C for 24 h. All blue colonies (Lac^+ Gus^+) were counted as *E. coli*, and all salmon to red colonies (Lac^+) were counted as TCs. Salmon to red colonies were randomly selected from CC agar and CC-CFS and identified.

(iii) Accuracy of CC agar and CC-CFS. The accuracy of the enzymatic procedure was determined with two reference capsules of *E. coli* WR1 (prepared at the SVM, RIVM, Bilthoven, The Netherlands). The mean number of bacteria of reference capsules of the batch used was 41.0 CFU/100 ml. The content of each capsule was reconstituted in a tube with 10 ml of peptone saline (PS) solution consisting of 1 g of peptone per liter and 8.5 g of NaCl per liter, as recommended by the SVM. One milliliter of the resulting *E. coli* suspension was then added to 99 ml of PS solution and filtered through a 0.45-μm-pore-size membrane. The membranes were then layered onto CC agar and CC-CFS and incubated at 44.5°C for 24 h. Five replicates per capsule and agar were done.

Statistical analysis of the data. TC and *E. coli* colony counts from CC agar and CC-CFS were converted to $\log_{10}$ values for statistical analysis. A variance analysis was performed to determine whether *E. coli* counts from CC agar and CC-CFS differed significantly.

TABLE 2. Cefsulodin susceptibility of *Aeromonas* strains measured by disk agar diffusion method[a]

Bacterial strain	No. of isolates		
	R	I	S
A. caviae[b]	1	17	80
A. hydrophila	4	4	6
A. sobria	0	0	0
Aeromonas sp.	0	1	7
A. hydrophila 398[c]	0	0	1
A. hydrophila 839	0	0	1
A. sobria 837	0	0	1
A. caviae 838	0	1	0
A. media 4232	0	0	1
A. jandaei 4228	0	0	1
A. trota 4255	0	0	1
A. eucrenophila 4224	0	0	1
A. schubertii 4241	0	0	1
A. veronii bv. veronii 4257	0	0	1
Total	5	23	102

[a] Significance of zone diameters: resistant (R), >32 μg/ml; intermediate (I), 32 to 8 μg/ml; and sensitive (S), <8 μg/ml.
[b] Environmental strains.
[c] Reference strain.

RESULTS AND DISCUSSION

Aeromonas caviae was the mesophilic aeromonad most frequently isolated in our study. Geographical differences in the distribution of *Aeromonas* species from fecal specimens are evident (19). In environmental waters of Spain (1) and Japan (20), *A. caviae* is commonly found.

Cefsulodin susceptibility. The cefsulodin susceptibilities, as measured by disk agar diffusion, of *Aeromonas* spp. are presented in Table 2. Results for coliform strains are presented in Table 3. For 93 of 119 environmental *Aeromonas* isolates and for 9 of 10 reference *Aeromonas* isolates, the MIC of cefsulodin was <8 μg/ml. Altorfer et al. (2) tested 28 *Aeromonas* strains isolated from human specimens for cefsulodin suscep-

TABLE 3. Cefsulodin susceptibility of coliform strains measured by disk agar diffusion method[a]

Bacterial strain or group	No. of isolates		
	R	I	S
Coliform group[b]	22	4	0
Salmonella derby and *Salmonella anatum*	2	0	0
Escherichia coli	47	3	0
Klebsiella pneumoniae subsp. *ozaenae* 851[c]	0	1	0
Klebsiella pneumoniae subsp. *pneumoniae* 140	1	0	0
Klebsiella oxytoca 860	0	1	0
Serratia odorifera 867	1	0	0
Hafnia alvei 157	1	0	0
Enterobacter aerogenes 684	1	0	0
Edwarsiella tarda 849	0	1	0
Enterobacter cloacae 194	1	0	0
Citrobacter diversus 856	0	1	0
Citrobacter freundii 401	1	0	0
Escherichia coli	4[d]	2[e]	0
Total	81	13	0

[a] See Table 2, footnote *a*, for definitions of R, I, and S.
[b] Environmental strains. Coliform group: LTB^+ BGB^+.
[c] Reference strain.
[d] Strains CECT 681, CECT 678, NCTC R, and NCTC V.
[e] Strains CECT 683 and CECT 4177.

TABLE 4. Comparison of the numbers of TCs and *E. coli* detected on CC agar and CC-CFS

Sampling site	Mo/day (1995)	10^6 TCs/100 ml on[a]:			TC CC/CC-CFS ratio[d]	10^6 *E. coli* cells/100 ml on[a]:		*E. coli* CC/CC-CFS ratio[e]
		CC agar[b]	CC agar[c]	CC-CFS		CC agar	CC-CFS	
A	7/24	27	13	14	1.93	12	9	1.30
	7/25	71	34	28	2.53	20	19	1.05
	7/27	30	14	16	1.88	16	16	1.00
	9/6	52	24	20	2.60	14	15	0.93
Mean		45	21	20	2.25	16	15	1.07
B	7/25	29	12	13	2.23	11	12	0.91
	9/6	51	24	20	2.55	13	15	0.87
	9/7	65	27	26	2.50	23	24	0.96
	9/8	70	29	30	2.33	19	16	1.19
	11/15	25	15	10	2.50	7	6	1.17
Mean		48	20	20	2.42	15	15	1.02
C	9/8	32	11	15	2.13	6	7	0.86
	11/15	39	14	11	3.55	10	10	1.00
	11/17	44	15	16	2.75	7	7	1.00
	11/20	71	25	26	2.73	8	7	1.14
	11/21	42	15	18	2.33	8	10	0.80
Mean		46	16	17	2.70	8	8	0.96

[a] Values are the means of two replicates.
[b] Uncorrected counts.
[c] Corrected counts after subtraction of the proportionate number of lactose-positive *Aeromonas* spp. as determined for each sampling site.
[d] Ratios of the recoveries of TCs on CC (uncorrected TC counts) and CC-CFS media.
[e] Ratios of the recoveries of *E. coli* on CC and CC-CFS media.

tibility, and the MIC for 50% of the strains was 8 μg/ml. Fainstein et al. (7) reported that the MIC of cefsulodin for 50% of 16 clinical *Aeromonas hydrophila* strains tested was 12.5 μg/ml. Results of MIC tests by agar dilution showed that cefsulodin at 10 μg/ml or less inhibited the growth of 96% of environmental *Aeromonas* isolates. Moyer et al. (19) found that the recovery rate for *A. hydrophila* and *Aeromonas sobria* was half that of *A. caviae* on cefsulodin-irgasan-novobiocin agar (cefsulodin concentration, 4 μg/ml), indicating that *A. hydrophila* and *A. sobria* are more susceptible to cefsulodin than *A. caviae*. In contrast, we have found that *A. hydrophila* strains (for 4 of 14 strains, the MIC was >32 μg/ml) were more resistant to cefsulodin than either *A. caviae* or other *Aeromonas* sp. strains. No *A. sobria* strain was isolated from our samples.

For 81 of 94 coliform isolates (environmental and reference strains), the MIC of cefsulodin was >32 μg/ml, and for 13 of 21 coliform isolates the cefsulodin MIC, as determined by agar dilution, was found to be >15 μg/ml. For *Salmonella* isolates, the MIC of cefsulodin was found to be >32 μg/ml. It has been noted previously (21) that cefsulodin had poor activity against all members of the family *Enterobacteriaceae*. Neu et al. (21) reported that the MICs of cefsulodin for isolates of *E. coli*, *Citrobacter freundii*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella*, and *Proteus mirabilis* were in the range of 25 to 100 μg/ml. A lower concentration of cefsulodin (8 μg/ml) was required to inhibit *Pseudomonas aeruginosa* and *Vibrio cholerae* reference strains. It has been reported elsewhere (12) that cefsulodin inhibited 90% of clinical isolates of *P. aeruginosa.*

Evaluation of selective media. Because the purpose of using cefsulodin in CC agar is to suppress the *Aeromonas* flora, the growth levels of TCs and *E. coli* on CC agar and those on CC-CFS (10 mg of cefsulodin per liter) were compared. Comparative results for 14 sewage-polluted irrigation water samples are outlined in Table 4. Statistically, there was no significant difference between *E. coli* results obtained with the two media as determined by variance analysis. TC counts on CC media with and without cefsulodin are compared in Table 4. On average, the CC agar recovered 2.46 times as many TCs as CC-CFS. Data from the analysis of variance showed significant differences ($P = 0.01$) between TC counts on CC agar and CC-CFS. Species distributions of β-galactosidase-positive (Lac^+) bacteria isolated from each medium differed, as shown in Table 5. A slightly higher percentage of *Aeromonas* spp. was

TABLE 5. Verification of salmon to red (Lac^+) colonies observed on CC agar and CC-CFS

Sampling site	No. of isolates identified on:					
	CC agar			CC-CFS		
	CO^-[a]	CO^+[b]	Total	CO^-[a]	CO^+[b]	Total
A	22	27 (53.1)	49	46	0	46
B	16	23 (58.9)	39	8	0	8
C	14	26 (65.0)	40	27	0	27
Total	52[c]	76 (58.6)	128	81[d]	0	81

[a] CO^-, cytochrome oxidase activity negative.
[b] CO^+, cytochrome oxidase activity positive. All CO^+ isolates were identified as *Aeromonas* spp. The percentages of strains identified as *Aeromonas* spp. are indicated in parentheses.
[c] Number of isolates identified as LTB^+ BGB^+, 39 (75.0%); LTB^+ BGB^-, 5 (9.6%); LTB^- BGB^-, 8 (15.4%).
[d] Number of isolates identified as LTB^+ BGB^+, 59 (72.8%); LTB^+ BGB^-, 10 (12.3%); LTB^- BGB^-, 12 (14.8%).

recovered from site C (65.0%) than from sites A (53.1%) and B (58.9%). Because no *Aeromonas* spp. were recovered on CC-CFS (Table 5), background interference was eliminated, and the counts that were obtained reflected more accurately the number of TCs. Because of the large numbers of *Aeromonas* isolates recovered on CC agar, the TC counts were corrected to show the number of true coliforms that were recovered (Table 4). The overall analysis revealed no significant difference between the TC recoveries on CC agar (corrected counts) and those on CC-CFS. These findings indicate that the high counts observed in different sites are due mainly to the presence of *Aeromonas* spp. Several investigations have pointed out the presence of *Aeromonas* spp. in positive presumptive tests for coliforms, which thus elevates apparent coliform densities (false positive) (9, 15, 17). Ley et al. (16) found that *Aeromonas* spp. represented 76% of β-galactosidase-positive colonies isolated on X-Gal. Some similar problems were experienced by other workers (8). Brenner et al. (5) developed a membrane filter medium (MI agar) containing 5 mg of cefsulodin per liter. MI agar inhibited the growth of *Flavobacterium* and *Aeromonas* species (5).

The specificity of the media for TCs, which included all salmon to red colonies which were β-galactosidase positive and cytochrome oxidase negative, varied, depending on how TCs were defined. Coliforms are defined (3) as gram-negative nonspore-forming rods that ferment lactose with gas formation within 48 h at 35°C. However, all strains taxonomically assigned to the coliform group do not necessarily conform to the coliform definition stated in *Standard Methods* (3), because they may not ferment lactose, or if they do, they may not produce gas. We have verified 133 β-galactosidase-positive and cytochrome oxidase-negative strains in LTB and BGB. We encountered 98 strains (73.7%) positive for growth on LTB and BGB (LTB^+ BGB^+), 15 (11.3%) LTB^+ BGB^- strains, and 20 (15.0%) LTB^- BGB^- strains (Table 5). One LTB^- BGB^- strain was identified as *C. freundii* with the API 20E System (bioMérieux Ibérica, Madrid, Spain). In conventional medium, the target microbe must transport the substrate (e.g., lactose) through the cell membrane, transform the substrate to glucose, metabolize glucose through the glycolytic cycle to pyruvate, and then convert pyruvate to the desired end product, either acid or gas (6). Because conventional testing requires the microbe to go through many steps to yield a positive visible end point, a number of anomalous results may occur, such as false-negative gas production (6).

Recovery of the *E. coli* WR1 reference strain on CC and CC-CFS media was satisfactory. Averages of 103.6% on CC agar and 103.1% on CC-CFS were obtained.

The results of this study indicate the combination of CC agar with cefsulodin to be highly efficient for the inhibition of growth of *Aeromonas* and other oxidase-positive organisms (e.g., *Vibrio* spp.) from sewage-polluted water samples. We suggest the inclusion of cefsulodin at 10 mg/liter in chromogenic coliform media when high levels of aeromonads are present in the environmental waters.

ACKNOWLEDGMENT

The help extended for this project by E. Merck AG is gratefully acknowledged.

REFERENCES

1. **Alonso, J. L., M. S. Botella, and I. Amoros.** 1994. The occurrence of mesophilic aeromonads species in marine recreational waters of Valencia (Spain). J. Environ. Sci. Health **A29:**615–628.
2. **Altorfer, R., M. Altwegg, J. Zollinger-Iten, and A. von Graevenitz.** 1985. Growth of *Aeromonas* spp. on cefsulodin-irgasan-novobiocin agar selective for *Yersinia enterocolitica*. J. Clin. Microbiol. **22:**478–480.
3. **American Public Health Association.** 1989. Standard methods for the examination of water and wastewater, 17th ed. American Public Health Association, Washington, D.C.
4. **Barry, A. L., and C. Thornsberry.** 1991. Susceptibility tests: diffusion test procedures, p. 1117–1125. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
5. **Brenner, K. P., C. C. Rankin, Y. R. Roybal, G. N. Stelma, Jr., P. V. Scarpino, and A. P. Dufour.** 1993. New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. Appl. Environ. Microbiol. **59:**3534–3544.
6. **Edberg, S. C., M. J. Allen, D. B. Smith, and the National Collaborative Study.** 1988. National field evaluation of a defined-substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple-tube fermentation method. Appl. Environ. Microbiol. **54:**1595–1601.
7. **Fainstein, V., S. Weaver, and G. P. Body.** 1982. In vitro susceptibilities of *Aeromonas hydrophila* to new antibiotics. Antimicrob. Agents Chemother. **22:**513–514.
8. **Freier, T. A., and P. A. Hartman.** 1987. Improved membrane filtration media for enumeration of total coliforms and *Escherichia coli* from sewage and surface waters. Appl. Environ. Microbiol. **53:**1246–1250.
9. **Grabow, W. O. K., and M. du Preez.** 1979. Comparison of m-Endo LES, MacConkey, and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl. Environ. Microbiol. **38:**351–358.
10. **Havelaar, A. H., M. During, and J. F. Versteegh.** 1987. Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. J. Appl. Bacteriol. **62:**279–287.
11. **Hendrickson, D. A., and M. M. Kreunz.** 1991. Reagents and stains, p. 1289–1314. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
12. **Hussain, Q. S., O. Billington, and B. A. Cunha.** 1991. In vitro activity of cefsulodin against multiresistant isolates of *Pseudomonas aeruginosa*. Drugs Exp. Clin. Res. **17:**213–216.
13. **Janda, J. M., and P. S. Duffey.** 1988. Mesophilic aeromonads in human disease: current taxonomy, laboratory identification, and infectious disease spectrum. Rev. Infect. Dis. **10:**980–997.
14. **Kaper, J. B., H. Lockman, and R. R. Colwell.** 1981. *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. J. Appl. Bacteriol. **50:** 359–377.
15. **Leclerc, H., D. A. Mossel, P. A. Trinel, and F. Gavini.** 1977. Microbiological monitoring—a new test for fecal contamination, p. 23–36. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazard associated with water. American Society for Testing and Materials STP 635. American Society for Testing and Materials, Philadelphia.
16. **Ley, A., S. Barr, D. Fredenburgh, M. Taylor, and N. Walker.** 1993. Use of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside for the isolation of β-galactosidase-positive bacteria from municipal water supplies. Can. J. Microbiol. **39:**821–825.
17. **Mak, W. N.** 1977. Total coliform bacteria, p. 59–64. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazard associated with water. American Society for Testing and Materials STP 635. American Society for Testing and Materials, Philadelphia.
18. **Manafi, M., W. Kneifel, and S. Bascomb.** 1991. Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiol. Rev. **55:**335–348.
19. **Moyer, N. P., H. K. Geiss, M. Marinescu, A. Rigby, J. Robinson, and M. Altwegg.** 1991. Media and methods for isolation of aeromonads from fecal specimens. A multilaboratory study. Experientia **47:**409–412.
20. **Nakano, H., T. Kamuyana, K. Venkateswaran, H. Kawakami, and H. Hashimoto.** 1990. Distribution and characterization of hemolytic and enteropathogenic motile *Aeromonas* in aquatic environment. Microbiol. Immunol. **34:** 447–458.
21. **Neu, H. C., H. K. Geiss, M. Marinescu, A. Rigby, J. Robinson, and M. Altwegg.** 1991. Media and methods for isolation of aeromonads from fecal specimens. A multilaboratory study. Experientia **47:**409–412.
22. **Popoff, M.** 1984. Genus III. *Aeromonas* Kluyver and van Niel 1936, p. 545–548. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology. The Williams & Wilkins Co., Baltimore.
23. **Rambach, A.** 1990. New plate medium for facilitated differentiation of *Salmonella* spp. from *Proteus* spp. and other enteric bacteria. Appl. Environ. Microbiol. **56:**301–303.
24. **Ramteke, P. W., S. P. Pathak, and J. W. Bhattacherjee.** 1992. Incidence of *Aeromonas* in total coliform test. Indian J. Microbiol. **32:**193–196.
25. **Sahm, D. F., and J. A. Washington II.** 1991. Antibacterial susceptibility tests: dilution methods, p. 1105–1116. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
26. **Sakazaki, R., and A. Balows.** 1981. The genera *Vibrio*, *Plesiomonas* and *Aeromonas*, p. 1272–1301. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. A handbook of habitats, isolation and identification of bacteria. Springer-Verlag, New York.