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Description of Leeds Acinetobacter Medium, a New Selective and Differential Medium for Isolation of Clinically Important *Acinetobacter* spp., and Comparison with Herellea Agar and Holton's Agar

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***Acinetobacter* spp. are responsible for an increasing number of opportunistic, nosocomial infections. They have been isolated from diverse inanimate objects in the hospital environment and are resistant to most of the commonly used antibiotics. Existing media for the isolation of *Acinetobacter* spp. are either nonselective, allowing the growth of unwanted bacteria, or too inhibitory, inhibiting the growth of many *Acinetobacter* strains. For the rapid isolation and effective control of *Acinetobacter* infection, a new selective and differential medium, Leeds Acinetobacter Medium (LAM), has been developed to isolate *Acinetobacter* spp. from clinical and environmental sources. The concentration of antibiotics and other ingredients in this medium have been determined according to the results of MIC and viable counts performed for these ingredients. LAM was compared with other selective and differential media for the isolation of *Acinetobacter* spp. from a local hospital environment and proved to be better in terms of recovery and selectivity.**

Acinetobacter spp. are ubiquitous bacteria that have been isolated from patients, the environment, soil, and water (1, 4). They have been responsible for frequent episodes of nosocomial infection and have been isolated from many diverse sources such as mechanical ventilators, peak flow meters, nebulizers, gloves, and dust (3, 6, 21). Carriage of the bacterium on the skin of the staff or patients has been suggested as the probable route of transmission in most outbreaks of nosocomial *Acinetobacter* spp. infections (4). Immunocompromised patients and those in intensive care units requiring assisted mechanical respiration are at particular risk of colonization or infection by *Acinetobacter* spp. (4, 6). The organism is often resistant to commonly used antibiotics, including most β-lactams and aminoglycosides, and may form a reservoir of antibiotic resistance genes in the hospital environment (24, 27). The genus *Acinetobacter* has been shown to comprise at least 17 DNA groups (genospecies). DNA groups 2, 3, and 13 are responsible for the nosocomial spread of infection (7).

Members of the genus *Acinetobacter* are strictly aerobic, nonmotile, non-spore-forming, gram-negative coccobacilli. They are catalase positive and oxidase negative (2). They all grow well on complex bacteriological media with a temperature optimum of 20 to 30°C without the need for extra growth factors (2, 14, 18).

Several selective and differential media are currently in use for the isolation of *Acinetobacter* spp. The first such medium used for the isolation of *Acinetobacter* spp. was reported by Mandel et al. (20) in 1964. This medium contained agar, soy peptone, pancreatic digest of casein, sodium chloride, lactose, maltose, bile salts, and bromocresol purple. The medium was modified to Herellea agar by substituting sucrose for maltose (19). Another selective and differential medium was described by Holton (16); that medium contained desiccated ox bile instead of the bile salts used by Mandel et al. (20) in the original formulation and the antibiotics ampicillin, cefsulodin, and vancomycin. Holton (16) excluded lactose and bromocresol purple from his medium and substituted instead fructose, mannitol, phenylalanine, and phenol red. Other selective media for the isolation of acinetobacters have been described but are not widely used (9, 13). We have observed that these existing media are either not sufficiently selective (because of the absence of appropriate inhibitory agents for other organisms) or too inhibitory (because of the presence of a high concentration of agents inhibitory for *Acinetobacter* spp., particularly ampicillin). We developed a new medium that can be used to isolate *Acinetobacter* spp. of DNA groups 1, 2, 3, and 13 from clinical and environmental sources. Ampicillin was excluded from the new medium, and the concentrations of the other antibiotics were adjusted after determining the MICs of a range of potentially useful antibiotics for a diverse collection of *Acinetobacter* spp. Leeds Acinetobacter Medium (LAM) was tested for inhibitory action by using a large collection of *Acinetobacter* spp. from environmental and clinical sources. Evaluation in use was carried out in two wards of a large tertiary-care referral hospital involving a semiquantitative comparison with existing media.

MATERIALS AND METHODS

Bacterial strains. A total of 134 strains of *Acinetobacter* spp. were used in the present work, of which 50 were clinical or environmental isolates from Leeds General Infirmary. These strains were identified as *Acinetobacter* spp. by using the API 20 NE system (Analytab Products, La Balme des Grottes, France) (26). Seventy-four strains were supplied by P. Gerner-Smidt (Department of Clinical Microbiology, Statens Seruminstitut, Copenhagen S, Denmark); these included nine American Type Culture Collection strains. Ten strains of *Acinetobacter* were supplied by K. J. Towner (Department of Microbiology and Public Health Laboratory Service, University Hospital, Nottingham, United Kingdom). Strains were grown on Iso-Sensitest agar at 30°C. For long-term storage, strains were kept in glycerol broth at −70°C. Growth of the following bacterial species was tested: *Serratia marcescens* (one strain), *Serratia*

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liquefaciens (two strains), *Klebsiella* spp. (four strains), *Escherichia coli* (four strains), *Providencia* spp. (three strains), *Stenotrophomonas* spp. (eight strains), *Citrobacter* spp. (four strains), *Pseudomonas aeruginosa* (four strains), *Burkholderia cepacia* (two strains), and one strain each of *Staphylococcus aureus*, *Staphylococcus xylosis*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, and *Staphylococcus capitis*.

Antimicrobial agents. Cephradine was obtained from Squibb (Hounslow, United Kingdom), vancomycin hydrochloride was from Eli Lilly (Basingstoke, England), cefsulodin was from Ciba (Horsham, England), and ampicillin was from Beecham (Brentford, England).

Susceptibility to selective antimicrobial agents and bile salts. The MICs of the various antibiotics used were determined by means of an agar incorporation technique (15). One milliliter of serial doubling dilutions of aqueous solutions of antibiotics was added to 19 ml of Iso-Sensitest agar (Oxoid CM 473; Unipath Ltd., Basingstoke, England) to a final concentration of 512 to 0.50 mg/liter. Strains were inoculated into Nutrient Broth (CM 1; Oxoid) and were incubated overnight on a shaker (100 rpm) at 30°C in air. Broths were diluted (1:100), and each plate was inoculated by using a multipoint inoculator (Mast Laboratories Ltd., Liverpool, United Kingdom) to give a final inoculum of approximately 10^4 CFU per spot. A total of 86 strains of *Acinetobacter* from four DNA groups were tested. *E. coli* NCTC 10418 (National Collection of Type Cultures, Colindale, United Kingdom) was used as a susceptible control strain.

The MIC of Bile Salts No. 3 (L 56; Oxoid) for 29 strains of *Acinetobacter* spp. was determined by using nutrient agar, Iso-Sensitest agar, and the bases of Herellea agar and Holton's agar. All other inhibitory agents, including antibiotics and indicators, were excluded from Herellea agar and Holton's agar. In one set of experiments sugars were also excluded from these media to exclude the possibility of any synergistic or other effect when used in combination with bile salts. The pHs of all of the media were adjusted to 7.0. Sterile solutions of different concentrations of Bile Salts No. 3 (0.25 to 3 g/liter) were added to the different media. The rest of the procedure described above for determining the MICs of the antibiotics was followed. Plates of the above-mentioned media without Bile Salts No. 3 were used as controls. Plates were incubated for 24 h in air at 30°C, and the growth on the different media was compared.

Preparation of LAM. LAM was prepared by adding the following ingredients to distilled water: Bacteriological Agar No. 1 (Oxoid L 11; Unipath Ltd.), 10 g/liter; acid casein hydrolysate (Oxoid L 41), 15 g/liter; neutralized soy peptone (Oxoid L 44), 5 g/liter; sodium chloride (Fisons Scientific Equipment, Loughborough, England), 5 g/liter; D-(−)-fructose (BDH Chemicals Ltd., Poole, England), 5 g/liter; sucrose (BDH), 5 g/liter; D-mannitol (Sigma Chemical Co. Ltd., Poole, England), 5 g/liter; L-phenylalanine (Sigma), 1 g/liter; ferric ammonium citrate (BDH), 0.4 g/liter; and phenol red (BDH), 0.02 g/liter. The pH of the medium was adjusted to 7.0, and the medium was steamed to dissolve the ingredients and then autoclaved for 15 min at 121°C and 15 lb/in^2. After cooling to 50 to 55°C, the following antibiotics were added: vancomycin at 10 mg/liter, cefsulodin at 15 mg/liter, and cephradine at 50 mg/liter. Freshly poured plates were orange in color. They were packed in plastic bags and stored at 4 ± 2°C in the dark. To determine the shelf-life of the medium, samples of plates were checked after 2 weeks by inoculation by using a spiral plater (Spiral System, Don Whitley Scientific Ltd., Shipley, United Kingdom) with strains of *Acinetobacter* spp. and other organisms. Herellea agar was prepared according to the manufacturer's (Difco Laboratories, Detroit, Mich.) instructions. Holton's medium was prepared according to the published formula (16), except that the concentration of pheylalanine was reduced to 1 g/liter instead of 10 g/liter, because the latter concentration was found to be too inhibitory.

Laboratory evaluation of LAM. The nutrient properties and selectivity of the media were assessed by using 134 strains of *Acinetobacter* spp. and 38 strains of the other bacterial genera detailed in Table 1. Three selective media, Herellea agar, Holton's agar, and LAM, were compared, with growth on Iso-Sensitest agar used as a control. Viable counts were determined by preparing serial dilutions of overnight Nutrient Broth (Oxoid CM 1) cultures and inoculating plates by using a spiral plater. Plates were incubated at 30°C in air and were examined after 24 and 48 h (Table 1). Colonies were counted according to the manufacturer's instructions, a viable count was determined by using the tables provided with the spiral plater, and the color change in the agar surrounding the colonies was noted. The productivity ratio (PR) was calculated by dividing the count on the test medium (Herellea agar, Holton's agar, LAM) by the count on the reference medium, Iso-Sensitest agar (Table 1). In order to assess the appearances of the bacterial strains when they were isolated, colonies were obtained by streaking out the different organisms and recording the growth.

In-use comparison of selective media for isolation of *Acinetobacter* spp. The new medium was used to isolate *Acinetobacter* spp. from a local hospital (Leeds General Infirmary); Herellea agar and Holton's agar were used at the same time, as was the nonselective Iso-Sensitest agar. The performance of each agar in terms of recovery and selectivity was assessed. One hundred five samples were taken from different inanimate objects in the Intensive Care Unit and one surgical ward. Each sample was collected on a single sterile swab soaked in sterile distilled water and was used to inoculate half of a plate containing each of the four media. The swab was rotated through 90 degrees between each plate. Each sample taken was streaked out onto each half plate to obtain the isolated colonies. These plates were incubated at 30°C in air and were examined after 24 and 48 h.

RESULTS

Susceptibilities to antimicrobial agents. The MIC data showed that ampicillin at 16 mg/liter inhibited the growth of approximately 40% of *Acinetobacter* strains (Table 2); in view of this the ampicillin in Holton's medium was replaced in LAM with cephradine at a concentration of 50 mg/liter. Piperacillin was tested as an alternative to ampicillin but was found to be unsuitable (Table 2). This concentration of cephradine inhibited the growth of only 5% of the *Acinetobacter* strains tested. Cefsulodin at 30 mg/liter inhibited the growth of 35% of the *Acinetobacter* strains tested, while cefsulodin at 15 mg/liter inhibited the growth of only 8% of the *Acinetobacter* strains tested. Consequently, the concentration of cefsulodin was reduced from 30 to 15 mg/liter in LAM. The concentration of L-phenylalanine was reduced to 1.0 g/liter after performing viable counts on Holton agar base without desiccated ox bile or antibiotics. The L and DL forms of phenylalanine were tested at 1.0 and 10 g/liter with two strains from different DNA groups of *Acinetobacter*. Fresh blood agar plates were used as noninhibitory controls for the comparison of growth. No growth was recorded with 10 g of the DL form of phenylalanine per liter, and the viable count was reduced to 14% of that observed on fresh blood agar with 1.0 g/liter. With 1 g of the L form of

TABLE 1. Productivity and selectivity of Herellea agar, Holton's agar, and LAM

Organism	No. of strains	Source[a]	Mean (±2SD) PR			Range of PR		
			Herellea agar	Holton's agar	LAM	Herellea agar	Holton's agar	LAM
Acinetobacter spp.								
DNA group 1	9	2[b], 7	0.24 (±0.65)	0	0.11 (±0.66)	0–1.0	0	0–1.0
DNA group 2	25	4[b]	0.55 (±0.85)	0.22 (±0.77)	0.56 (±0.98)	0.0013–1.0	0–1.0	0–1.0
DNA group 3	22	2[b], 7C	0.56 (±0.81)	0.19 (±0.12)	0.36 (±0.93)	0.014–1.0	0–0.28	0.0006–1.0
DNA group 8	4	3E, 1F	0.25 (±1.00)	0	0.25 (±1.00)	0.0005–1.0	0	0–1.0
DNA group 13	12	1[b]	0.76 (±0.83)	5.8×10^{-4} ($\pm 4.0 \times 10^{-3}$)	0.31 (±0.92)	0.0013–1.0	0–0.0007	0
Ungrouped	62	30C, 20E	0.68 (±0.78)	0.11 (±0.64)	0.61 (±0.94)	0–1.0	0–1.0	0–1.0
Stenotrophomonas maltophilia	8	5C, 3F	0.89 (±0.58)	2.5×10^{-4} ($\pm 1.4 \times 10^{-4}$)	9.6×10^{-4} ($\pm 3.6 \times 10^{-3}$)	0.18–1.0	0–0.002	$0–2 \times 10^{-4}$
Escherichia coli	4	3C, 1[b]	0.875 (±0.50)	0.59 (±0.987)	6×10^{-4} (±0.007)	0.50–1.0	0.37–1.0	$0.00–2.7 \times 10^{-3}$
Klebsiella pneumoniae	4	C	1.0 (±0.0)	0	0	1.0–1.0	1.0–1.0	0
Serratia marcescens	1	C	1.0 (±0.0)		1.0 (±0.00)	1.0–1.0	3×10^{-5}	1.0–1.0
Serratia liquefaciens	2	NCTC	1.0 (±0.0)	4.5×10^{-5} ($\pm 1.27 \times 10^{-4}$)	0.50 (±1.40)	1.0–1.0	$0–9 \times 10^{-5}$	0–1.0
Citrobacter spp.	4	C	1.0 (±0.0)	0.527 (±1.092)	0.75 (±1.00)	1.0–1.0	0.03–1.0	3×10^{-4}–1.0
Providencia spp.	2	L	1.0 (±0.0)	9.5×19^{-3} (±0.026)	0.59 (±1.15)	1.0–1.0	0–0.019	0.18–1.0
Providencia alcalifaciens	1	L	1.0 (±0.0)			1.0–1.0	8×10^{-4}	1×10^{-3}
Pseudomonas aeruginosa	4	L	1.0 (±0.0)	0	0	1.0–1.0	0	0
Burkholderia cepacia	2	E	0	0	0	0	0	0
Staphylococcus epidermidis	1	NCTC	0	0	0	0	0	0
Staphylococcus xylosis	1	NCTC	0	0	0	0	0	0
Staphylococcus hominis	1	NCTC	0	0	0	0	0	0
Staphylococcus capitis	1	NCTC	0	0	0	0	0	0
Staphylococcus haemolyticus	1	NCTC	0	0	0	0	0	0
Staphylococcus aureus	1	NCTC	0	0	0	0	0	0

[a] C, isolated from clinical material; F, isolated from food; E, isolated from environment; L, laboratory culture collection; NCTC, National Collection of Type Cultures.
[b] American Type Culture Collection strains.

TABLE 2. MICs of antibiotics for 86 strains of *Acinetobacter* spp.

Antimicrobial agent	MIC (mg/liter)[a]				Concn in LAM (mg/liter)
	Range	10%	50%	90%	
Ampicillin	2–512	8	32	>512	Nil
Piperacillin	2–512	8	16	128	Nil
Cefsulodin	8–512	32	64	256	15.0
Cephradine	8–512	128	256	>512	50.0

[a] 10%, 50%, and 90%, MICs for 10, 50, and 90% of strains tested, respectively.

phenylalanine per liter, 100% growth was recorded, while 7% growth was recorded with 10.0 g of the L form per liter.

Desiccated ox bile was excluded from LAM because this medium contains three antibiotics that proved to be sufficient for the inhibition of growth of most of the gram-positive organisms (Table 1).

In-use comparison of selective media for the isolation of *Acinetobacter* spp. Of 105 environmental samples collected from a local hospital (Leeds General Infirmary), 22 samples showed growth on LAM and 13 showed growth on Holton's medium, while 25 samples showed growth on Herellea agar. Oxidase-positive colonies were excluded from further identification, while colonies which were oxidase negative were tested by using the API 20 NE system. This identification system was chosen in preference to the API 20E system since it was designed for nonenteric gram-negative bacteria and it provides a better means of identification and discrimination of acinetobacters than the API 20E system. Ten isolates of *Acinetobacter* were obtained from LAM plates, while only four *Acinetobacter* isolates were obtained on Holton's agar or Herellea agar.

Genera such as *Providencia* which produce phenylpyruvic acid from the phenylalanine present in the medium turn the base of the agar to brown-black, so there is no need to flood the plates with 10% ferric chloride ions (5).

The base of the medium is turned mauve by the growth of *Acinetobacter* spp. This is due to the high alkalinity produced in the medium by the growth of the organisms, which changes the color of the phenol red indicator to mauve. This is due to the liberation of ammonium ions from complex nitrogenous materials present in the medium. The carbohydrates present in the medium are also extensively decomposed, but the alkalinity produced by aerobic organisms is greater than the acidity (17, 25).

Storage of the LAM plates for up to 2 weeks had no effect on the selective properties of the medium or the colony sizes and appearances of different organisms in comparison with those of organisms grown on fresh LAM plates.

Laboratory evaluation of LAM. The productivity and selectivity of Herellea agar, Holton's agar, and LAM for the growth of *Acinetobacter* spp. are shown in Table 1. Clinically important *Acinetobacter* strains belonging to DNA groups 2, 3, and 13 grow well on LAM in comparison with their growth on Holton's agar. Most of the ungrouped *Acinetobacter* strains were clinical isolates from a local hospital (Leeds General Infirmary); they also showed better growth on LAM. *Acinetobacter* strains from DNA groups 1 and 8 are mostly environmental and play very little role as human pathogens. Isolates of these environmental groups grow more poorly on all selective and differential media than other clinical isolates. *Klebsiella* spp., *Pseudomonas* spp., and gram-positive bacteria, including different species of staphylococci, do not grow on LAM.

One strain of *Acinetobacter haemolyticus* and one strain of *Acinetobacter johnsonii* were tested for their growth on LAM, but they failed to grow. However, those isolates also failed to grow on Holton's agar. The isolate of *A. johnsonii* grew on Herellea agar, but the *A. haemolyticus* isolate failed to grow on that medium.

Acinetobacter spp. usually produce circular, convex, smooth, opaque colonies with entire margins of 1 to 2 mm in diameter after 24 h of incubation at 30°C in air (Table 3).

DISCUSSION

Acinetobacter spp. are increasingly recognized as important causes of opportunistic, nosocomial infections. The pathogenic role of *Acinetobacter* spp. is limited to nosocomial infections, but a dramatic increase in the incidence of such infections has been noted in many reports during the last few years, particularly when compared with the other common bacterial causes of nosocomial infections. A seasonal variation has been reported by Retailliau et al. (22), with the rate of infection with *A. calcoaceticus* being twice as high in late summer as in the

TABLE 3. Comparison of morphologies of colonies of different nosocomial bacteria likely to be grown from Herellea agar, Holton's agar, and LAM

Bacteria (no. of strains)	Morphology after growth on:			Oxidase reaction
	Herellea agar	Holton's agar	LAM	
Acinetobacter spp. (134)	Pale lavender colonies with yellow background	Pink colonies with mauve background	Pink colonies with mauve background[a]	Negative
Stenotrophomonas maltophilia (8)[c]	Pale lavender colonies with yellow background	Pink colonies with mauve background	Pink colonies with mauve background[b]	Negative
Klebsiella pneumoniae (4)	Yellow colonies with yellow background	No growth	No growth	Negative
Burkholderia cepacia (2)	Yellow colonies with yellow background	Pink colonies with mauve background	Pink colonies with mauve background	Positive[d]
Citrobacter spp. (4)	Pale lavender colonies with yellow background	Yellow colonies with yellow background	Yellow colonies with yellow background	Negative
Providencia alcalifaciens (1)	Pale lavender colonies with yellow background	Pink colonies with mauve background	Brown colonies with brown-black background	Negative
Serratia marcescens (1)	Yellow colonies with yellow background	No growth	Pink colonies with yellow margins	Negative

[a] Colonies are circular, convex, smooth, and opaque with entire margins of 1 to 2 mm in diameter after 24 h at 30°C.
[b] Colonies are opaque and flat with rugose surfaces and crenated margins of 1 to 2 mm in diameter after 24 h at 30°C.
[c] Growth of *Stenotrophomonas maltophilia* is very poor on LAM, with a mean PR of 9.6×10^{-4} (Table 1).
[d] Ninety percent of strains were positive.

TABLE 4. Cumulative percent strains of *Acinetobacter* spp. inhibited by different concentrations of Bile Salts No. 3[a]

Concn (g/liter)	Cumulative % inhibition					
	Nutrient agar	Iso-Sensitest agar	Without sugars		With sugars	
			Herellea agar	Holton's agar	Herellea agar[b]	Holton's agar[c]
Without Bile Salts No. 3	0	0	0	0	0	0
0.25	0	6.8	3.4	6.8	3.4	3.4
0.50	3.4	6.8	6.8	6.8	6.8	3.4
0.75	3.4	6.8	13.7	10.3	6.8	6.8
1.0	6.8	6.8	17.2	13.7	6.8	13.7
1.25	6.8	10.3	17.2	17.2	10.3	13.7
1.50	6.8	13.7	17.2	17.2	13.7	13.7
2.0	6.8	17.2	20.6	20.6	17.2	20.6
2.50	6.8	17.2	44.8	51.7	17.2	20.6
3.0	6.8	17.2	58.6	55.1	17.2	20.6

[a] Bases of different selective and differential media were used without antibiotics and indicators. A total of 29 strains of *Acinetobacter* spp. from different DNA groups were used.
[b] Sugars used in Herellea agar: sucrose, 10 g/liter; lactose, 10 g/liter.
[c] Sugars used in Holton's agar: fructose, 5 g/liter; sucrose, 5 g/liter; mannitol, 5 g/liter.

early winter. In order to better understand the epidemiology of *Acinetobacter* infections, a rapid and effective technique is required for the isolation of *Acinetobacter* spp. from the hospital environment and clinical material. The media used in the past were either poorly selective, because of a suboptimal choice of antibiotics for the inhibition of growth of unwanted bacteria, or they were too inhibitory, because of the presence of antibiotics or bile salts which inhibited the growth of most *Acinetobacter* spp. when used at the recommended concentrations. The results presented in Table 4 show that the inhibitory effect on the growth of *Acinetobacter* spp. is less when sugars are added to the bases of different selective and differential media. *Acinetobacter lwoffii* and the strain of DNA group 1 (ATCC 23055) are very susceptible to even very low concentrations of Bile Salts No. 3, and this is why their growth is poor on each selective and differential medium.

The concentrations of antibiotics and the other ingredients in LAM are based on MIC and viable count data for a wide selection of both clinical and environmental strains representing all of the clinically important DNA groups. This new medium, when used in a comparative study for the isolation of *Acinetobacter* spp. from a local hospital environment, proved to be better than the existing selective and differential media. LAM recovered more *Acinetobacter* spp. isolates than the Herellea agar or Holton's agar. The differentiation of acinetobacters from other bacteria by colonial morphology on Herellea agar is difficult, because many other bacteria, including *Pseudomonas* spp., *Proteus* spp., and *Citrobacter* spp., also produce the same pale lavender colonies characteristic of *Acinetobacter* spp. (13). During a recent outbreak of nosocomial *Acinetobacter* infection in the Leeds General Infirmary, the organism was isolated from a fan, a tape recorder, a television, the beds of patients, a bedside tray, a window, an infusion pump, and the sinks and sluices of the affected ward. The infection was spread from the Intensive Care Unit to a surgical ward when the patients were transferred. *Acinetobacter* spp. were also isolated from the environment of the surgical ward. More than 90% of oxidase-negative organisms that grew on LAM were *Acinetobacter* spp., while oxidase-positive organisms were mostly *Burkholderia cepacia*. Oxidase-negative bacteria were mostly *Stenotrophomonas maltophilia*, which grew on LAM. The identification and differentiation of the different bacteria that grow on this medium are very easy because of the color change in the base of the medium and the morphologies of the colonies (Table 3). The API 20 NE system was used to confirm the identities of those organisms which were oxidase negative. *Acinetobacter* spp. isolated from clinical sources showed better growth than species of *Acinetobacter* isolated from environmental sources. *A. johnsonii* and *A. haemolyticus* failed to grow on LAM; however, these two species are only rarely encountered among clinical isolates (8, 10, 12, 23). Most of the environmental strains belong to DNA group 8 (*A. lwoffii*), which are generally more susceptible to Bile Salts No. 3 and β-lactam antibiotics (11). Consequently, environmental isolates grow poorly on selective media that contain Bile Salts No. 3 or β-lactam antibiotics. LAM is better than the existing Holton's agar and Herellea agar for the isolation of *Acinetobacter* spp. from clinical sources.

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