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Comparison of Reagent-impregnated Paper Strips and Conventional Tests for Distinguishing *Escherichia* from *Aerobacter*: Correlation with Colonial Morphology

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The means for distinguishing *Escherichia* from *Aerobacter* (*Enterobacter*) differ in laboratories and range from complete dependence on colonial reactions on typical gram-negative media to reliance on one or more of the classical indole, methyl red, Voges-Proskauer, citrate (IMViC) parameters. Three colonial types (one prejudged as *Escherichia*) of lactose-positive rods were catalogued on each of the most commonly used selective media, MacConkey Agar, Endo Agar, and E M B Agar. Each cultural type was presumptively diagnosed and then compared with the expected outcome of individual IMViC tests. The distribution of preliminary identifications was similar from growth patterns on MacConkey Agar and E M B Agar, but it differed markedly from Endo Agar. When organisms initially diagnosed by cultural methods were compared by single IMViC tests, it was found that for each colonial type one of the biochemical parameters was best suited. Thus, for those types initially considered *Escherichia*, the methyl red or Voges-Proskauer test results agreed most consistently; for other types, the citrate reaction was most satisfactory. In addition, when newly formulated reagent-impregnated paper strip methods for indole, Voges-Proskauer, and citrate were evaluated and compared to the standard methods, agreement was 97% for indole, 90% for Voges-Proskauer, and 95% for Simmons' citrate.

The necessity for distinguishing *Escherichia* from *Aerobacter* (*Enterobacter*) is most relevant to two of the purposes outlined by Nungester (6) for the identification of microorganisms. The pertinent aims as stated by Steel (9) are (i) "to aid epidemiologists in tracing sources of infections, and (ii) to accumulate data of interest to those studying infectious diseases."

Clearly, the primary goal of the clinical microbiologist is rapid identification and not classification. Thus, we believe that the fundamental diagnostic tests selected are crucial for a prompt diagnosis of unknown infective agents. If one were to employ the esoteric device of numerical taxonomy regardless of whether principles of Adansonian or "cluster analysis" were used, it seems that the gram-negative bacteria display several clusters of genera. One cluster corresponds to the entire family of *Enterobacteriaceae*, yet it includes some of the *Pasteurella*, *Vibrio*, *Proteus*, and *Aeromonas* species that may be intermediate with the other families (7). Moreover, enlisting the aid of deoxyribonucleic acid base composition analysis for the purpose of differentiating *Aerobacter* from *Escherichia* is of little consequence (3).

We are aware that the means for discriminating *Escherichia* from *Aerobacter* differ in laboratories and range from complete dependence on gross morphology exhibited on typical gram-negative media to reliance on one or more of the classical indole, methyl red, Voges-Proskauer, citrate (IMViC) parameters. Hence, we undertook this study to compare IMViC criteria with colonial characteristics of lactose-positive rods on three widely used media, namely, MacConkey Agar (MAC; Albimi Laboratories, Inc., Flushing, N.Y.), Endo Agar, and E M B Agar (Difco). In addition, newly formulated paper strip methods for three of the IMViC tests [PathoTec-I (indole), PathoTec-VP (Voges-Proskauer), and PathoTec-C (citrate)], supplied by General Diagnostics Division, Warner-Chilcott Laboratories, Morris

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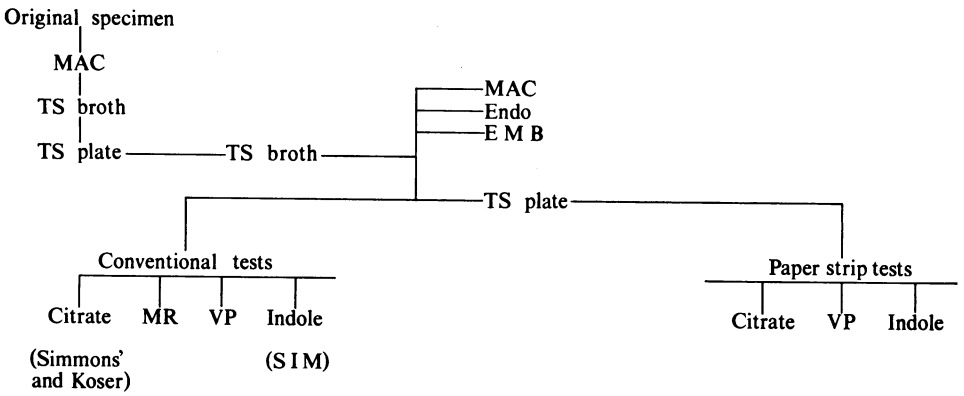

FIG. 1. *Flow chart indicates handling of clinical isolates for observation of colonial morphology and performance of biochemical procedures.*

Plains, N.J., were evaluated and compared with conventional IMViC tests.

MATERIALS AND METHODS

Gram-negative lactose-positive organisms obtained from various routine pathological specimens received in this laboratory formed the material for this study and were handled as shown in Fig. 1. At the outset they were not identified, but subsequent to their selection, strains were identified to insure that only prompt lactose-fermenting gram-negative rods were studied.

Initially, each specimen was cultured according to laboratory routine which included inoculation of a MAC plate. Individual colonies were fished from MAC, transferred to a tryptic soy (TS) broth tube, incubated 4 hr, and streaked out on a TS plate. To insure purity, bacteria collected from the TS plate were subcultured to TS broth, incubated 18 hr, and lastly plated out on MAC, Endo, E M B, and TS plates. The final TS plate served as the source of inoculum for both conventional and experimental paper strip tests.

MAC Agar, Endo Agar, and E M B Agar were purchased as the dehydrated product and prepared according to the directions.

After initial familiarization with the media, the colonial types arising on the three gram-negative media were catalogued. On MAC we recognized: (i) a pink to red, bile-salt precipitated colony (presumptively *Escherichia*); (ii) a yellow colony; and (iii) a pink colony. On Endo we noted: (i) a metallic colony (presumptively *Escherichia*); (ii) a dark pink colony; and (iii) a light pink colony. On E M B we recognized: (i) a metallic colony (presumptively *Escherichia*); (ii) a pink colony with a black center; and (iii) a gray colony (Table 1).

Conventional IMViC tests were essentially those described previously (8). Koser's and Simmons' formulations were used for the citrate test, and the presence of an alkaline blue slant on the latter was interpreted as a positive reaction. Peptone broth (Albimi Laboratories, Inc.) was inoculated and incubated for 48 hr for the methyl red (MR) and Voges-Proskauer (VP) tests. The O'Meara modified reagent was utilized to detect acetoin production. Indole formation was assayed in S I M (Difco) medium utilizing Kovac's reagent.

RESULTS

The various morphological types exhibited on the lactose media during the 48-hr incubation period were catalogued (Table 1). According to gross morphology, there were 38 colonies presumptively identified as *Escherichia* (A-type) on MAC, 12 on Endo, and 35 on E M B after 24 hr of incubation. The results with MAC and E M B were consistent regarding the preliminary estimation of *Escherichia* as opposed to Endo, which demonstrated about one-third the number of this supposedly identified group. After 48 hr of incubation, reactions on Endo and E M B remained essentially the same; however, 12 less than the 38 colonies conditionally diagnosed *Escherichia* were exhibited on MAC. For non-*Escherichia* types (B and C), there were 62 colonies on MAC, 88 on

TABLE 1. *Colonial morphological types exhibited by 100 gram-negative, lactose-positive clinical isolates on MAC, Endo, and E M B Agars*

Medium	Colony types[a]					
	A		B		C	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
MAC....	38	26	1	64	61	10
Endo....	12	10	64	47	24	43 (13)[b]
E M B...	35	34	65	60	0	6

[a] On MAC, A = pink to red, bile-salt precipitated, B = yellow, and C = pink. On Endo, A = metallic, B = dark pink, and C = light pink. On EMB, A = metallic, B = pink with black center, and C = gray.

[b] Light pink colonies at 24 hr which turned lighter at 48 hr.

Endo, and 65 on E M B following 24 hr of incubation. Again, these results showed good accord between MAC and E M B. Within this group, an additional 24 hr of incubation produced 74 B and C (*Aerobacter*) colonies on MAC, 90 on Endo, and 66 on E M B; MAC and E M B in this group exhibited much greater disparity.

Individual IMViC results are compared with colonial characteristics on the three selective media (Tables 2 to 4). Comparisons of single IMViC tests can be made because their results imply that an *Aerobacter* cannot have the same result as an *Escherichia*. Yet, it is realized that biochemical characteristics of bacterial strains overlap, as will be demonstrated by our data.

The best conformity with any one criterion for an A-type colony grown on MAC for 24 hr was with VP (Table 2). Positive test results were recorded; however, VP results for A-type colonies should be negative. Thus, the percentage agreement would be 73.7%. In close proximity was MR test data with 71%, and the indole reaction which was intermediate between MR and citrate. Only one B-type colony grew after 24 hr of incubation precluding any valid conclusions. For 48-hr-old B-type colonies, the only adequate comparison obtained was with citrate; indole was medial among the parameters. For 24-hr-old C-type colonies, citrate yielded superior results, followed by indole and MR in that order. For C-type (48-hr-old) colonies, agreements were markedly diminished and ranged from 20 to 40%.

Table 3 compares Endo colony types with corresponding IMViC reactions. VP and MR results correlated with A-type colonies of the order of 90%. Agreement was better with Koser's citrate than with Simmons' citrate for B- and C-type colonies (24 hr); C-type colonies yielded perfect

TABLE 2. *Comparison of colonial morphological types on MAC Agar with positive IMViC parameters*

Colony type[a]	Time of incubation	No.	MR		VP		Indole		Citrate Koser				Citrate Simmons			
									24 hr		48 hr		Growth		ABS[b]	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	hr															
A	24	38	27	71.0	10	26.3	26	68.4	15	39.4	20	52.6	28	73.6	15	39.4
	48	26	22	84.6	6	23.0	21	80.7	8	30.7	14	53.8	18	69.2	7	26.9
B	24	1	1	100	0	0	1	100	1	100	1	100	1	100	1	100
	48	64	22	34.3	46	71.8	14	21.8	57	89.0	62	96.8	64	100	51	79.6
C	24	61	25	40.9	41	67.2	17	27.8	53	86.8	56	91.8	58	95.0	46	75.4
	48	10	8	80.0	2	20.0	8	80.0	3	30.0	3	30.0	5	50.0	4	40.0

[a] A = pink to red, bile-salt precipitated. B = yellow. C = pink.
[b] ABS, alkaline blue slant.

TABLE 3. *Comparison of colonial morphological types on Endo Agar with positive IMViC parameters*

Colony type[a]	Time of incubation	No.	MR		VP		Indole		Citrate Koser				Citrate Simmons			
									24 hr		48 hr		Growth		ABS[b]	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	hr															
A	24	12	11	91.6	1	8.3	8	66.6	3	25.0	5	41.6	8	66.6	4	33.3
	48	10	8	80.0	1	10.0	5	50.0	3	30.0	4	40.0	8	80.0	4	40.0
B	24	64	32	50.0	37	57.8	31	48.4	42	65.6	49	76.5	56	87.5	37	57.8
	48	47	29	61.7	21	44.6	27	57.4	27	57.4	31	65.9	36	76.5	23	48.9
C	24	24	9	37.5	17	70.8	4	16.6	22	91.6	24	100	24	100	22	91.6
	48	43	16	37.2	31	72.0	9	20.9	37	86.0	42	97.6	43	100	34	79.0

[a] A = metallic. B = dark pink. C = light pink.
[b] ABS, alkaline blue slant.

TABLE 4. *Comparison of colonial morphological types on E M B Agar with positive IMViC parameters*

Colony types[a]	Time of incubation	No.	MR		VP		Indole		Citrate Koser				Citrate Simmons			
									24 hr		48 hr		Growth		ABS[b]	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	hr															
A	24	35	28	80.0	8	22.8	26	74.2	10	28.5	16	45.7	23	65.7	11	31.4
	48	34	29	85.2	7	20.5	27	79.4	9	26.4	15	44.1	22	64.7	10	29.4
B	24	65	24	36.9	47	72.3	16	24.6	57	87.6	61	93.8	64	98.4	51	78.4
	48	60	23	38.3	41	68.3	14	23.3	52	86.6	56	93.3	59	98.3	46	76.6
C	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	6	1	16.6	6	100	0	0	6	100	6	100	6	100	6	100

[a] A = metallic. B = pink, with black centers. C = gray.
[b] ABS, alkaline blue slant.

TABLE 5. *Agreement between conventional biochemical tests and reagent-impregnated paper strip method*

Test	Conventional tests		Paper strip tests		Frequency of agreement between conventional and paper strip tests		Agreement (%)
	No. positive	No. negative	No. positive	No. negative	Positive	Negative	
Indole	42	58	43	57	41	56	97
VP	55	45	61	39	53	37	90
Citrate, Koser	77	23	74	26	69	18	87
Citrate, Simmons	57	43	61	39	56	39	95

correlation with Koser's formulation. In general, C-type colonies compared very favorably with all parameters. For comparisons of Endo, 24- and 48-hr data revealed better consistency with the younger colonies.

IMViC reactions were also related to colonial types cultivated on E M B plates (Table 4). In this case, the best correlation between any one IMViC criterion and an A-type colony either 24 or 48 hr old was with the MR test; VP followed with agreement percentages of about 70 to 80%. For all B-type colonies, the best conformity was with the citrates; indole was next in order. C-type colonies were absent after 24 hr of incubation, but there was perfect correlation between 48-hr C-type colonies and IMViC tests; however, it should be noted that there were only six colonies with which to make the comparison.

Tabel 5 depicts the agreement between conventional IMViC tests and newly formulated reagent-impregnated strip techniques. The number of positives was recorded, and the conventional method was employed as a standard for comparative purposes. Of a total of 100 strains, 55 were positive according to the conventional VP method, and 61 were positive with the strip technique. Of the 61 positive strips, 8 were "falsely" positive and 2 were "falsely" negative, which resulted in 90% agreement. For indole, agreement was 97% with 2 "false" positives and 1 "false" negative. Conformity between conventional and newly formulated tests for the citrate reaction was 95 and 87% for Simmons' and Koser's citrate, respectively.

DISCUSSION

Taylor et al. (Bacteriol. Proc., p. 89, 1967) surveyed hospitals of 250- to 400-bed capacity and found that at least 50% of them relied solely on gross morphology for their indentification of lactose-positive gram-negative rods. Our own experience supports this conclusion. Moreover, we are concerned about the results of performance tests conducted by qualified supervisory agencies of clinical microbiology laboratories. Are incidents of incorrect diagnoses due to lack of knowledge or experience or rather misidentification because of the criteria used to codify them?

The conclusion of the comparative studies reported in this paper with IMViC biochemical parameters and colonial morphology is that the best single criterion between an IMViC parameter and a non-*Escherichia*-type colony is a citrate reaction, whereas the best single correlation between an IMViC test and an *Escherichia*-type colony is either an MR or VP reaction. We

realize that the pragmatic aspects of clinical microbiology preclude the use of all IMViC tests on each individual colony isolated on gram-negative medium. Hence, the above conclusions may be valid as a substantive aid to gross colonial morphological reactions.

It is not our onus in this paper to validate the standard IMViC reference techniques. However, it should be noted that they may not possess any real taxonomic value for medical microbiology (9). As with any biochemical tests, best results can be achieved only when the limitations of each method and possible sources of technical error are realized and minimized. For each of the orthodox procedures, the temperature of incubation, length of incubation, and reagents employed for the detection of end product or condition represent variables and hence possible sources of error (1, 2, 4, 5, 8, 10). The number of variables has been reduced in the impregnated-paper strip tests, and thus the probable error has diminished.

Experimental IMViC strips which were studied included tests for VP, indole, and citrate. A color code chart for interpretation of reactions, which has now been included in the marketed product, was not available at the time this study was initiated, and our judgments of color were made from written descriptions. Correlation between the strips and conventional tests was of the order of 90% or better (except for Koser's citrate medium).

In general, we found that these strips served as a practical and dependable facilitation of rather complex biochemical methods that required 2 to 5 days for final evaluation. The longest procedure with the reagent-impregnated strips represents a time span of 4.5 hr; thus, their greatest advantage lies in the fact that an indole, VP, and citrate test can be completed within the working day.

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