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Reproducibility of the MS-2 System for Identification of Members of the Family *Enterobacteriaceae*: a Collaborative Study with Blindly Assigned Reference Strains

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The reproducibility of identification and biochemical reactions for five different reference organisms of *Enterobacteriaceae*; *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*, and *Enterobacter cloacae*, were evaluated using the updated MS-2 system software (Abbott Laboratories, Diagnostic Division, Irving, Tex.) in a collaborative study involving 11 laboratories. When a total of 220 randomly coded test organisms were blindly examined, the MS-2 system correctly identified 92.7 and 86.8% for over 80 and 90% probability identification, respectively. Four organisms, *P. vulgaris*, *K. pneumoniae*, *E. coli*, and *S. marcescens*, were correctly identified in all laboratories with high probability, but 9 of 44 tests of *Enterobacter cloacae* resulted in misidentifications or low-likelihood (less than 80%) identifications. Accuracy was directly related to level of experience and familiarity with the MS-2 system in the individual laboratories. Biochemical reactions varied among the identification trials, especially in the identification of *S. marcescens* and *Enterobacter cloacae*. Among a total of 44 subcultures for each organism, 10 different biochemical patterns for *P. vulgaris*, 6 for *K. pneumoniae*, 9 for *E. coli*, 15 for *S. marcescens*, and 14 for *Enterobacter cloacae* were obtained. The results indicate that the MS-2 system performs with high accuracy and reproducibility in identifying *Enterobacteriaceae*, except for *Enterobacter cloacae*.

In recent years, considerable effort has been directed toward the development of automated microbiological diagnostic sytems, and several automated instruments are now available. Most automated systems perform antimicrobial susceptibility testing (4, 8, 14, 15), screen for urinary tract infections (1, 4, 5, 7, 9, 11–13), and identify bacteria (2, 3, 6, 10). It is well recognized that identification of gram-negative rods, especially *Enterobacteriaceae*, can be a major labor-intensive function in most clinical microbiology laboratories. The main advantages we can expect from automated bacterial identification are speed, precision, and accuracy, besides saving manual labor. Automated reading of biochemical reactions by instruments may assure higher reproducibility in reading biochemical reactions and in conclusive identification of organisms. Therefore, automation could contribute greatly to diagnostic bacteriology in clinical laboratories.

This paper presents the results of a collaborative study in 11 laboratories to evaluate the MS-2 system for identification of *Enterobacteriaceae*. The main purpose of this study was to evaluate the consistency of biochemical reactions and final identifications in the repeated tests of reference organisms randomly assigned to different laboratories for blind testing.

MATERIALS AND METHODS

Test bacteria. Three different reference organisms from the American Type Culture Collection (ATCC), Rockville, Md., *Proteus vulgaris* (ATCC 13315), *Klebsiella pneumoniae* (ATCC 13883), and *Escherichia coli* (ATCC 25922), were employed in this study. In addition, two bacteria from *Enterobacteriaceae*, *Serratia marcescens* (TU3SMA-30) and *Enterobacter cloacae* (TU3ECL-27), isolated at Tohoku University School of Medicine and used as laboratory reference strains, were included. The 44 subcultures of each organism were used and made up the total 220 randomly coded test organisms. The stock cultures were subcultured three times on blood agar plates before use. One fresh isolate of *E. coli*, *Proteus mirabilis*, *Citrobacter freundii*, or *Enterobacter aerogenes* was also included as a random coded addition for each day of testing.

Study profile. A total of 11 microbiology laboratories participated in this study. Six of the laboratories presently

TABLE 1. Summary of correct identification by MS-2 BID

Test organism	No. (%) of correct identifications (n = 44) at likelihood of[a]:	
	80%	90%
P. vulgaris	41 (93.2)	38 (86.4)
K. pneumoniae[b]	44 (100.0)	43 (97.7)
E. coli[b]	43 (97.7)	43 (97.7)
S. marcescens	41 (93.2)	40 (90.9)
Enterobacter cloacae[b]	35 (79.5)	27 (61.4)

[a] A total of 92.7% of the identifications were correct at >80% likelihood; 86.8% were correct at >90% likelihood.

[b] For *K. pneumoniae* and *E. coli*, the organisms with an acceptable identification were included; for *Enterobacter cloacae*, the organisms with identifications of both urease-positive and -negative were included.

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TABLE 2. MS-2 BID misidentifications and low-probability correct identifications (<80% likelihood)

Test organism	MS-2 identification	% Likelihood	Discrepant reaction(s)[b]
P. vulgaris	*Yersinia enterocolitica*	91.22	ARA(+), INO(+), MAN(+)
	Yersinia enterocolitica	50.95	MAN(+)
	Serratia rubidaea	70.71	ADO(+), ARA(+), INO(+), LAC(+), MAN(+)
E. coli	*Enterobacter agglomerans*	58.59	LYS(−), ORN(−), ADO(+), INO(+), SUC(+)
S. marcescens	*S. marcescens*	63.56	LAC(+), RHA(+)
	S. marcescens	34.13	GLU(−), ADO(−), LAC(+), RHA(+)
	Enterobacter aerogenes	87.09	LAC(+), RHA(+)
Enterobacter cloacae	*K. pneumoniae*	89.55	LYS(+), CIT(−)
	K. pneumoniae (4)[a]	80.24	LYS(+)
	Enterobacter agglomerans	94.64	ORN(−), CIT(−), URE(−)
	Enterobacter agglomerans	89.81	ORN(−), CIT(−), MAL(−), SUC(−), URE(−)
	Enterobacter aerogenes	88.91	LYS(+), URE(−)
	Serratia liquefaciens	94.84	MAL(−), URE(−), RHA(−), SUC(−)

[a] The same misidentifications were observed four times.

[b] Abbreviations: GLU, glucose; LYS, lysine; ORN, ornithine; CIT, citrate; MAL, malonate; ESC, esculin; URE, urease; ADO, adonitol; ARA, arabinose; INO, inositol; LAC, lactose; MAN, mannitol; RHA, rhamnose; SOR, sorbitol; SUC, sucrose; XYL, xylose; IND, indole. Sign in parentheses represents result obtained.

use the MS-2 biological identification (BID) in routine work and had at least 6 months of experience with the MS-2 BID. The remaining five laboratories had no previous experience with the MS-2 BID system. The six organisms chosen for identification were randomly coded at Tohoku University School of Medicine and included the five different reference strains and one fresh isolate previously described. All subcultures were randomly coded and tested blindly. These organisms were subcultured on heart infusion (HI) agar plates and then distributed to all participating laboratories. One day before BID testing, isolates were subcultured on blood agar plates at the respective laboratories. The MS-2 BID trials for all six strains were performed daily in every laboratory for 4 consecutive days from 19 to 22 April 1983.

MS-2 BID identification. For the MS-2 BID identification, MS-2 system operating procedures were carefully followed (10). Even when low probability of identifications or unexpected results were obtained, tests were not repeated. Resulting print-outs were sent directly to Tohoku University School of Medicine for detailed analysis. Further analysis of identification results and biochemical reactions was performed for the five reference strains. The latest MS-2 identification software (no. 01.03) was utilized in this collaborative study.

RESULTS

Accuracy of identification by MS-2 BID. Table 1 indicates the summary of correct identifications for each of the five reference strains with relatively high probability (over 80 and 90% likelihood). Of 220 identifications, 191 and 204 trials correctly identified the strains with over 90 and 80% likelihood, indicating 86.8 and 92.7% reproducibilities of genus-species identification, respectively. With four reference strains, *P. vulgaris* ATCC 13315, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, and *S. marcescens* TU3SMA-30, there were only a few misidentifications or low probability identifications. With *Enterobacter cloacae* (TU3ECL-27), misidentification occurred in 9 of 44 trials, and correct identification with probabilities between 80 to 90% were observed 8 times, even when we included both urease-negative and -positive results as correct identifications.

In Table 2, we have summarized the results of misidentifications and low-probability (less than 80% likelihood) identifications. Except for *Enterobacter cloacae*, the reaction(s) attributable to misidentifications were false-positive results in almost all the cases, although some false-negative reactions were observed, such as lysine and ornithine in *E. coli* and glucose and adonitol in *S. marcescens*. For *Entero-*

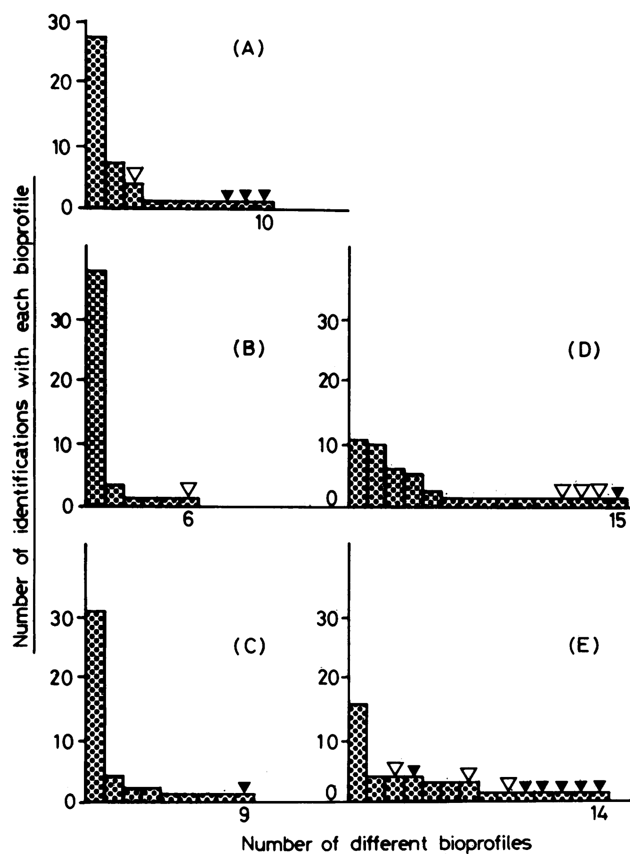

FIG. 1. The distribution of different biochemical patterns (bioprofile) for the identification of five reference *Enterobacteriaceae*: (A) *P. vulgaris*, (B) *K. pneumoniae*, (C) *E. coli*, (D) *S. marcescens* and (E) *Enterobacter cloacae*. The histogram indicates the number of identifications with the particular bioprofile for each bacterium. Open triangles indicate correct identifications with less than 90% likelihood, and closed triangles represent misidentifications.

TABLE 3. Positivity of biochemical reaction for five reference bacteria

Reaction	*P. vulgaris* Ref.[a]	*P. vulgaris* No. (%) positive	*K. pneumoniae* Ref.	*K. pneumoniae* No. (%) positive	*E. coli* Ref.	*E. coli* No. (%) positive	*S. marcescens* Ref.	*S. marcescens* No. (%) positive	*Enterobacter cloacae* Ref.	*Enterobacter cloacae* No. (%) positive
Glucose	+	43 (98)	+	44 (100)	+	44 (100)	+	43 (98)	+	43 (98)
Lysine	−	0 (0)	+	44 (100)	+	43 (98)	+	44 (100)	−	9 (20)
Ornithine	−	0 (0)	−	3 (7)	+	43 (98)	+	42 (95)	+	42 (95)
Citrate	−	0 (0)	+	43 (98)	−	0 (0)	+	44 (100)	+	36 (82)
Malonate	−	0 (0)	+	44 (100)	−	0 (0)	−	0 (0)	+	42 (95)
Esculin	−	0 (0)	+	44 (100)	−	0 (0)	−/+	16 (36)	+	44 (100)
Urease	+	44 (100)	+	43 (98)	−	0 (0)	−	0 (0)	+	33 (75)
Adonitol	−	1 (2)	+	44 (100)	−	3 (7)	+	41 (93)	−	0 (0)
Arabinose	−	3 (7)	+	44 (100)	+	44 (100)	−	0 (0)	+	44 (100)
Inositol	−	5 (11)	+	44 (100)	−	6 (14)	+	43 (98)	+	44 (100)
Lactose	−	1 (2)	+	42 (95)	+	44 (100)	−	5 (11)	+	30 (68)
Mannitol	−	3 (7)	+	44 (100)	+	44 (100)	+	44 (100)	+	44 (100)
Rhamnose	−	0 (0)	+	43 (98)	+	44 (100)	−	7 (16)	+	43 (98)
Sorbitol	−	1 (2)	+	43 (98)	+	42 (95)	+	44 (100)	+	44 (100)
Sucrose	+	43 (98)	+	44 (100)	−	8 (18)	+	44 (100)	+	42 (95)
Xylose	−/+	35 (80)	+	44 (100)	+	44 (100)	−/+	21 (48)	+	44 (100)
Indole	+	43 (98)	−	0 (0)	+	43 (98)	−	0 (0)	−	0 (0)

Test (n = 44) results for all organisms.

[a] Ref., reference reaction.

bacter cloacae, the reactions most frequently causing misidentifications were those of lysine, ornithine, and citrate.

Reproducibility of biochemical reactions. The 17 biochemical test reactions were recorded as a six-digit number (bioprofile) which indicated either positive or negative for each reaction. All the identification tests were classified according to the bioprofile number observed. Figure 1 shows the distribution of testings with different bioprofile numbers classified for each strain. Three reference organisms, *P. vulgaris*, *K. pneumoniae*, and *E. coli*, achieved relatively uniform biochemical patterns. There were 10 different bioprofiles from *P. vulgaris*, six for *K. pneumoniae*, and nine for *E. coli*. Among the total 44 testings, 27 cases (61.4%) for *P. vulgaris*, 37 cases (84.1%) for *K. pneumoniae*, and 31 cases (70.5%) for *E. coli* showed the identical BID bioprofiles. On the other hand, there were 15 different bioprofiles for *S. marcescens*, and 14 for *Enterobacter cloacae*. Also, the results obtained estimated that the reproducibility of the identical BID bioprofile numbers for these two bacteria were 11/44 (25.0%) and 16/44 (36.4%), respectively. In Table 3, we summarized the positivity for each biochemical reaction in each bacteria. As can be seen, the reactions for ATCC reference organisms, *P. vulgaris*, *K. pneumoniae*, and *E. coli* showed high reproducibility in both negative and positive reactions. The reproducibilities of almost all the reactions for these strains ranged over 93%, except for inositol (89%) and xylose (80%) for *P. vulgaris* and inositol (86%) and sucrose (82%) for *E. coli*. The biochemical reactions for *S. marcescens* identification, however, varied considerably. The reproducibilities for two reactions, esculin and xylose, were estimated to be 64 and 52%, respectively, and also several false positive reactions in lactose and rhamnose were observed. The most inconsistent results were obtained in identifying *Enterobacter cloacae*. With four reactions, the reproducibilities were estimated to be less than 90%, lysine (80%), citrate (82%), urease (75%) and lactose (68%). Also, one to two incidents of false-negative results for glucose, ornithine, malonate, rhamnose, and sucrose were observed.

Differences in biochemical reactions among laboratories. The occurrence of false-positive and false-negative reactions in identification of five test organisms by laboratories are summarized in Table 4. A total of 117 incorrect test interpretations were obtained, with 50 cases (43%) occurring in the identification of *Enterobacter cloacae*. For *P. vulgaris* 14 false-positive results occurred, with 13/14 (93%) coming from laboratories C, D, and H. These three laboratories contributed ca. 90% of the false results for *P. vulgaris*. For *E. coli*, 12 of the false positive interpretations of 17 came from laboratories C, G, and K. The trend of incorrect results clustered in certain laboratories was also demonstrated in the identification of *S. marcescens*. Namely, 12 false-positive results were noted, but of the 12 cases, 11 came from laboratories C, J, and K. When we excluded the results of *Enterobacter cloacae*, of the 46 false positive results recorded, nearly 60% came from laboratories C and K alone. Conversely, laboratories A, E, and F had no false-positive results throughout the replicate testings. Of the 21 false-negative results encountered, 62% came from laboratories G and J alone, and laboratories A, B, D, and E had no false-negatives. Eighty percent of all incorrect reagent reactions (both false-negative and false-positive) came from only four laboratories (C, G, J, and K), with the other seven laboratories contributing only 20% for four strains.

The results of *Enterobacter cloacae* were different from those of the former four bacteria. As can be seen in Table 4, the majority of incorrect reactions were false-negative, and the false-positive results were only observed with lysine in three laboratories (C, F, and K). Several false-negative results were observed in all laboratories participating in the study. Among 41 false-negative results, more than half of them were with three reagents, citrate (8), urease (11), and lactose (14).

The differences observed between laboratories were closely related to the experience and familiarity with the MS-2 BID system. Five laboratories (C, G, H, J, and K), which showed relatively higher incidences of false reactions, had only limited experience with the MS-2 BID system and had not used it for routine test identification. These five labora-

TABLE 4. Summary of false-positive and false-negative results by laboratory

Test organism	No. (% occurrence) of FP and FN by laboratory[a]:									
	A		B		C		D		E	
	FP	FN	FP	FN	FP	FN	FP	FN	FP	FN
P. vulgaris	0	0	1	0	7	0	3	0	0	0
K. pneumoniae	0	0	0	0	1	0	0	0	0	0
E. coli	0	0	1	0	4	0	0	0	0	0
S. marcescens	0	0	1	0	3	2	0	0	0	0
Subtotal	0 (0.0)	0 (0.0)	3 (6.5)	0 (0.0)	15 (32.6)	2 (9.5)	3 (6.5)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter cloacae	0	3	0	1	4	3	0	3	0	7
Total	0 (0.0)	3 (4.8)	3 (5.5)	1 (1.6)	19 (34.5)	5 (8.1)	3 (5.5)	3 (4.8)	0 (0.0)	7 (11.3)

[a] FP, False-positive results; FN, false-negative results.

tories subsequently contributed 75% (12 cases) of the total 16 misidentifications and low-likelihood (less than 80%) identifications observed with five bacteria tested (data not shown). The remaining six laboratories had at least 6 months of experience with the MS-2 BID system in routine work. The fact that the incubation time of BID cartridges at 35°C in identifying reference strains ranged from 294 to 334 min in the 11 laboratories, produced no significant effect on BID identifications (data not shown).

DISCUSSION

It was shown that the updated MS-2 BID system was capable of producing highly accurate results in identification of *Enterobacteriaceae*. In our study, it was demonstrated that the reproducibilities of genus-species identification with over 90 and 80% likelihoods, were 86.8% and 92.7%, respectively. The relatively low accuracy in identification was observed with *Enterobacter cloacae*, that is, six misidentifications and three low-probability (less than 80% likelihood) were observed of the total 44 tests. The reactions attributable to misidentifications were false-positive for lysine and false-negative for ornithine and citrate. Comparable results were also reported by McCracken et al. (10), who pointed out that an inherent weakness of the MS-2 BID system was in identifying various *Enterobacter* species.

On the other hand, the biochemical patterns obtained during identifications were not as uniform as we expected. In a total of forty-four tests for each strain, 10 different BID bioprofiles for *P. vulgaris*, 6 for *K. pneumoniae*, 9 for *E. coli*, 15 for *S. marcescens*, and 14 for *Enterobacter cloacae* were recorded. The results estimated that the reproducibility of the identical bioprofile numbers were 61.4, 84.1, and 70.5% for ATCC reference organisms *P. vulgaris*, *K. pneumoniae*, and *E. coli*, and 25.0 and 36.4% for in-house reference strains *S. marcescens* and *Enterobacter cloacae*, respectively. As for three organisms, *P. vulgaris*, *E. coli*, and *S. marcescens*, it was apparent that the most frequent discrepancies in biochemical reactions were false-positive results rather than false-negative results. In contrast, false-negative results were more frequently seen with *Enterobacter cloacae*. Although the reproducibility for each biochemical reaction ranged from 52% (xylose in *S. marcescens*) to 100%, almost all the reactions showed over 90% reproducibilities. In this sense, it is necessary to understand that the MS-2 BID system was designed to produce accurate identification results, unaffected by operator technical variables, rather than highly reproducible biotype of organisms. We are not sure what kind of variable factor(s) is responsible for this variability of biochemical reactions; however, it became apparent through our study that the accuracy and reproducibility of the results were clearly related to the individual testing laboratories involved, suggesting that technical manipulation of specimens and reagents can markedly influence the performance of the system. In our study, over 80% of all incorrect reagent reactions (both false-positive and false-negative) for four test organisms, excluding *Enterobacter cloacae*, came from only five laboratories, and also, 75% of misidentifications and low-probability (less than 80% likelihood) identifications with five organisms were observed in the same laboratories. In these laboratories, the technologists had no previous experience with the MS-2 BID system. Also, almost all of the false-positive results for lysine with *Enterobacter cloacae*, which were main cause for misidentifications, came from the same laboratories. The remaining six laboratories which had over 6 months of experience with the MS-2 system in routine work showed excellent accuracy and reproducibility in both identification results and biochemical reactions. The key variable factor when the automated identification instrument is used, is the manual preparation of cell suspensions. It is likely that our study profile somewhat differed from the routine work in this sense, because pure cultures were distributed in the study, however, the technologists usually use one well-isolated colony on the primary culture plate in which several different colonies coexist in routine work.

In conclusion, this collaborative study showed that, although the accuracy and reproducibility of the results were closely related to procedural manipulation in each laboratory, the updated MS-2 BID system was accurate and reproducible in genus-species identification of *Enterobacteriaceae*. Also, the ATCC reference strains were employed in the study should be useful for intralaboratory quality control studies for MS-2 BID.

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TABLE 4—*Continued*

No. (% occurrence) of FP and FN by laboratory:													
F		G		H		I		J		K		Total	
FP	FN	FP	FN	FP	FN	FP	FN	FP	FN	FP	FN	FP	FN
0	0	0	2	3	0	0	1	0	0	0	0	14	3
0	0	0	4	0	1	1	0	0	0	1	1	3	6
0	1	3	2	2	0	0	0	2	1	5	1	17	5
0	0	0	0	0	1	0	0	3	4	5	0	12	7
0 (0.0)	1 (4.7)	3 (6.5)	8 (38.1)	5 (10.9)	2 (9.5)	1 (2.2)	1 (4.7)	5 (10.9)	5 (23.8)	11 (23.9)	2 (9.5)	46 (100)	21 (100)
2	2	0	2	0	3	0	12	0	3	3	2	9	41
2 (3.6)	3 (4.8)	3 (5.5)	10 (16.1)	5 (9.1)	5 (8.1)	1 (1.8)	13 (21.0)	5 (9.1)	8 (12.9)	14 (25.5)	4 (6.5)	55 (100)	62 (100)

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