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Evaluation of Cathra system for identifying Gram negative aerobic bacteria

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Abstract

The Cathra system is a commercial multipoint inoculation method for the identification of aerobic Gram negative bacteria. The system uses a replicator technique in which 21 different agar media can be inoculated simultaneously with 36 organisms. Identifications are made by use of a special computer database. The performance of this system was compared with that of the API 20E for the identification of 372 clinical isolates of Enterobacteriaceae and 133 miscellaneous Gram negative bacteria. For enterobacteria, the Cathra system was in 97% agreement with API 20E at species level and 98% at genus level. For miscellaneous Gram negative strains the two systems were in 59% agreement at species level and 77% at genus level.

The Cathra system is suitable for use in diagnostic laboratories, especially those with a heavy workload and a wish to use break-point sensitivity testing. The identification database for miscellaneous Gram negative organisms, however, needs to be expanded.

Several commercial systems for the identification of Gram negative aerobic organisms are available for use in clinical laboratories.[1-6] Most of these are based on conventional biochemical tests in dehydrated media which are rehydrated by addition of a liquid suspension of the test organism. Usually only one or a relatively small number of organisms can be tested at one time. The API 20E is the most widely used of these systems and is a reference for the evaluation of other kits.[7 8] The Cathra System (MCT Medical, Inc, Minnesota, USA) is a new bacterial identification system using agar replicator technology. Up to 36 organisms can be inoculated simultaneously on a variety of identification media in standard 15 x 10 cm Petri dishes. Twenty one different agar "repliplates" are available for the identification of Gram negative non-fastidious aerobic bacteria. The manufacturer can also provide antibiotic plates for sensitivity testing which can be inoculated at the same time as the biochemical media.

Results are entered into a "Replianalyzer" microcomputer which produces printouts of organism identities and suggestions for additional tests where necessary. Results can also be recorded on a score sheet to generate an octal code which is used to obtain identities from a profile register known as "Replidex".

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Methods

Five hundred and five recent clinical Gram negative bacterial isolates were examined: there were 372 Enterobacteriaceae and 133 miscellaneous Gram negative bacteria. Five reference strains were also tested: *Escherichia coli* NCTC 10418 and ATCC 25922; *Pseudomonas aeruginosa* NCTC 10662 and ATCC 27853; and *Salmonella typhimurium* 42R500.

The organisms were identified using the Cathra System (MCT Medical, Inc, Minnesota, USA), and the API 20E (API Systems, SA, Vercieu, France). The Cathra system provides 21, and the API 20E 20 biochemical tests; 13 tests are common to both systems (table 1) and indole production must be tested separately. Tests for bile tolerance, growth on colistin/nalidixic acid, cetrimide/kanamycin and colistin agars, hydrolysis of aesculin, utilisation of malonate, and acidification of lactose and cellobiose are available only in the Cathra system; detection of B galactosidase, urea hydrolysis, tryptophan deaminase, Voges-Prokauer reaction, gelatin liquefaction, and acidification of melibiose,

Table 1 Biochemical tests provided by Cathra and API 20E identification systems for Gram negative aerobic bacteria

	Cathra	*API 20E*
Tolerance to:		
Bile	+	—
Colistin/nalidixic acid	+	—
Cetrimide/kanamycin	+	—
Colistin	+	—
Fermentation of:		
Mannitol	+	+
Lactose	+	—
Cellobiose	+	—
Glucose	+	+
Arabinose	+	+
Sorbitol	+	+
Sucrose	+	+
Rhamnose	+	+
Inositol	+	+
Melibiose	—	+
Amygdalin	—	+
Possession of:		
Lysine decarboxylase	+	+
Ornithine decarboxylase	+	+
β-Galactosidase	—	+
Tryptophan deaminase	—	+
Utilisation of:		
Citrate	+	+
Malonate	+	—
Hydrolysis of:		
Aesculin	+	—
Arginine	+	+
Urea	—	+
Production of:		
Hydrogen sulphide	+	+
Indole	+	+
Acetoin (Voges-Proskauer reaction)	—	+
Liquefaction of gelatin	—	+

Table 2 Comparison of identifications between the Cathra system and API 20E

Enterobacteriaceae (n = 372)	
Agreement to species level	359 (96·5%)
to genus level	366 (98·4%)
Miscellaneous Gram negatives (n = 133)	
Agreement to species level	79 (59·4%)
to genus level	102 (76·7%)

amygdalin, and arabinose are provided only in the API 20E system.

A single well isolated colony, emulsified in 5 ml distilled water, was inoculated into the API 20E system according to the manufacturer's instructions, and then incubated at 37°C for 24 hours for enterobacteria and for 48 hours for organisms which did not ferment glucose and gave less than three positive reactions. The inoculum for the Cathra system was prepared by suspending one to three well isolated bacterial colonies in 2 ml sterile 1% tryptone broth and incubating until the turbidity matched that of a MacFarland 0·5 standard. These bacterial suspensions were added to wells of a seeding tray of which the first was filled with India ink to act as a marker for orientation. A maximum of 36 different bacterial cultures could be inoculated with a replicator device on to the Repliplates. After the inocula had absorbed into the media the plates were incubated at 35°C for 16–20 hours. The unused tryptone broth cultures were incubated for a further 14–18 hours at 35°C and the indole test was performed by adding Kovac's reagent.[9]

In this study the API 20E identities were assumed to be "correct", and the performance of the Cathra system was assessed against this standard.

Results

The number of identities for which the two systems were in agreement is shown in table 2. For enterobacteria there was 97% agreement at species level and 98% at genus level. One strain of *Enterobacter cloacae* was identified by Cathra as *Citrobacter freundii* and one strain of *E gergoviae* as *Klebsiella* sp. Among 22 strains of *Proteus vulgaris* tested, 17 were indole negative and were identified as *P penneri* by Cathra.

There was less agreement between the two systems in the identification of miscellaneous Gram negatives, with 59% agreement at species level and 77% at genus level. Thirty six out of 37 *Pseudomonas aeruginosa,* both *Plesiomonas shigelloides* isolates, and all three *Vibrio cholerae,* however, were correctly identified by the Cathra system. *Vibrio vulnificus* was not in the Cathra database so that the

Table 3 Comparison of results for identification of specific bacteria

	Cathra	
	Agreement with API 20E/Number tested	*"Incorrect" identification**
Enterobacteriaceae		
Escherichia coli	27/27	
Klebsiella pneumoniae	24/25	*Enterobacter agglomerans*
Klebsiella oxytoca	6/6	
Enterobacter aerogenes	7/8	*E cloacae*
Enterobacter cloacae	52/53	*Citrobacter freundii*
Enterobacter agglomerans	2/2	
Enterobacter gergoviae	0/1	*Klebsiella* sp
Enterobacter sakazakii	0/5	*E cloacae*
Citrobacter freundii	39/40	*Pseudomonas putida*
Citrobacter diversus	16/16	
Citrobacter amalonaticus	3/3	
Edwardsiella tarda	5/5	
Proteus mirabilis	27/27	
Proteus vulgaris	22/22	
Morganella morganii	32/33	*Proteus mirabilis*
Providencia stuartii	26/27	*Klebsiella oxytoca*
Providencia rettgeri	6/8	*Aeromonas* sp (1) *Providencia* sp (1)
Serratia marcescens	2/2	
Salmonella sp	28/28	
Shigella sp	33/33	
Yersinia enterocolitica	1/1	
Miscellaneous Gram negatives		
Acinetobacter anitratus	16/23	*Citrobacter freundii* (3) *Enterobacter* sp (2) *Aeromonas* sp (1) Non-fermenting organism (1)
Aeromonas hydrophila	0/16	*Aeromonas* sp (16)
Pseudomonas aeruginosa	36/37	*Xanthomonas maltophilia* (1)
Pseudomonas cepacia	5/8	*Acinetobacter anitratus* (2) *Serratia* sp (1)
Pseudomonas fluorescens	1/1	
Pseudomonas stutzeri	0/1	*Xanthomonas maltophilia* (1)
Xanthomonas maltophilia	12/17	*Pseudomonas cepacia* (1) *Pseudomonas fluorescens* (1) *Citrobacter freundii* (1) Non-fermenting organism (2)
Flavobacterium meningosepticum	0/12	Unidentified (10) *Xanthomonas maltophilia* (1) *Aeromonas* sp (1)
Flavobacterium sp	1/2	Unidentified (1)
Plesiomonas shigelloides	2/2	
Vibrio cholerae	3/3	
Vibrio parahaemolyticus	3/6	*Vibrio* sp (1) *Salmonella gallinarum* (2)
Vibrio alginolyticus	0/1	*Aeromonas* sp (1)
Vibrio vulnificus	0/3	*Vibrio parahaemolyticus* (3)
Pasteurella multocida	0/1	*Klebsiella oxytoca* (1)

*For the purpose of comparison, API 20E was assumed to be "correct".

three strains were identified as *V parahaemolyticus*. Cathra could identify most *Acinetobacter anitratus* and *Xanthomonas maltophilia*, but it was unsuccessful with *Flavobacterium*, and the database did not contain *F meningosepticum*. Neither did the database contain species of *Aeromonas*, so that all 16 *A hydrophila* strains were identified only to genus level.

Discussion

There have only been two previous evaluations[10 11] of the Cathra system. Brown and Washington compared the performance of Cathra with Enterotube and obtained similar results as ours for both Enterobacteriaceae and miscellaneous Gram negative organisms.[10] Bennett and Joynson, however, found only 65% and 57% correct enterobacterial identifications to the genus and species level, respectively, when Cathra was compared with API 20E.[11] The relatively poor results obtained in that study may be because they used the Replidex profile register[11] while Brown and Washington used the more extensive database of the Replianalyzer computer as we did.[10] The low percentages of correct identifications for miscellaneous Gram negative organisms in our study was mainly due to errors with *Flavobacterium* sp. The Cathra database did not contain *F meningosepticum*, but Cathra could correctly differentiate *P vulgaris* and *P penneri*,[12] while both organisms were identified as *P vulgaris* by API 20E.

As the formulation of some media in the Cathra system differs from conventional or the API 20E media, no attempt was made to compare individual reactions of the systems.[10] No test system can give a "correct" answer for every organism, but because the API 20E is well established and widely accepted,[5] we used it as a standard to evaluate the performance of Cathra. When disagreement occurred between the two systems, tests such as oxidase, motility, and oxidation/fermentation were performed as appropriate. These results always favoured the identifications provided by API 20E.

Discrepancies sometimes arose because of difficulties with the interpretation of colour reactions. Control strains were included in every test and reproducible results were always obtained. Where disagreement occurred between the two systems for the identification of test organisms, the strains were retested and in most cases the same results were obtained. The Cathra carbohydrate acidification plates (lactose, glucose, inositol and mannitol), malonate utilisation, and decarboxylation of ornithine, should be regarded as positive when a very pale pink colour is produced. For the detection of H_2S production, blackening of the colony, with or without diffusion of black pigment into the surrounding medium, should be considered positive. Scanty growth on colistin/nalidixic acid agar should be regarded as negative, because Gram negative organisms would not grow on this agar except for occasional strains of non-fermenters or *Proteus/Providencia* sp. Similarly, most enterobacteria would not grow on cetrimide/kanamycin agar, except for some *Proteus* sp and *Morganella* sp. Scanty growth on this medium should be regarded as negative. Doubtful results can be entered as questionable (?).

The Cathra system is simple and easy to use. All the Repliplates are prepared by the manufacturer and sealed in plastic bags to prevent desiccation and contamination during transport and storage. They are packed in four sets of five plates each, and each set is clearly labelled together with the date of expiration. Plates are therefore easily selected and there is little danger that plates will be missed or wrongly used. During our study less than 10 plates out of 300 were found to be contaminated or unusable, despite the subtropical climate in Southeast Asia and the need to ship media from Singapore to Hong Kong. The plate shelf life is usually more than three weeks and delivery by the manufacturer was reliable.

The system is convenient for use in clinical microbiology laboratories where many organisms are isolated each day. Throughput is much quicker with this multipoint inoculation system than with identification kits such as API 20E that need to be seeded individually. This methodology is especially convenient for laboratories that use break-point sensitivity testing, and Cathra can also supply the necessary antibiotic test plates.

The Replianalyzer microcomputer was a useful adjunct to the Cathra system. The programme enables the user to keep records of all the isolates tested and suggests additional tests when there is more than one probable identity for an organism. Supplementary test results can be entered manually into the computer, and the new identity of the organism is displayed. Individual result printouts can be issued as reports to the wards, while the laboratory can keep a summary report which includes the identifications and biochemical results.

In summary, the Cathra system is a useful and convenient method for the identification of clinical isolates of Enterobacteriaceae. It is less successful for the identification of the less common non-fermenters and the database needs to be extended for this group. The multipoint inoculation methodology is very suitable for busy laboratories and would be particularly useful for those using break-point sensitivity testing. The computed identification database and report generator provided with the system is helpful and easy to use, but inevitably increases the cost of the system.

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