J Clin Pathol 1988;**41**:1010–1012

Rapid conventional scheme for biochemical identification of antibiotic resistant enterobacteriaceae isolates from urine

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SUMMARY Six conventional biochemical tests were combined to produce an identification scheme. These tests included decarboxylation of lysine and ornithine, fermentation of glucose and cellobiose, indole production and urease production. Three hundred antibiotic resistant coliforms from urine specimens were tested by this scheme and also by the API 20E for comparison. Two hundred and seventy nine (93%) of organisms were correctly identified using the six tests, 17 (5·7%) were referred for further study, and four (1·3%) were misidentified. It is concluded that this combination of tests provides an inexpensive, accurate, and rapid tool for identification.

Apart from the practicality and clinical usefulness of routinely identifying bacterial isolates, another major consideration of any identification system is the cost.[1] In our department roughly half of all identifications performed on Gram negative organisms serve to biotype multiply resistant coliform organisms from urine specimens. These isolates are identified so that the spread of multiply resistant organisms can be monitored and hence controlled.

Both conventional tests[2] and commercial kits[3 4] have been used for the identification of these organisms. Commercial kits, however, are commonly designed to differentiate between at least 15 genera and over 60 species of enterobacteriaceae,[5] most of which are rarely, if ever, found in urine specimens. In view of this fact and because of the continuing desire to reduce costs in the hospital laboratory, we set out to design a simple and inexpensive scheme for identifying most enterobacteriaceae commonly found in urine specimens.

This system was called the Logic System (because of the tests entailed) and comprises six well known conventional tests: lysine decarboxylase, ornithine decarboxylase, glucose fermentation, indole production, cellobiose fermentation and urease production.

Material and methods

Three hundred antibiotic resistant urine isolates were tested by the Logic System and by API 20E (which had previously been used routinely for identifying these organisms in our laboratory). The two sets of identification results were then compared and the sensitivity and specificity of the Logic System was assessed using API 20E as a reference test. The cost-effectiveness of both schemes was also compared.

The following formulae were used in the preparation of conventional media.

Decarboxylase media were prepared from dehydrated Moeller decarboxylase base (Difco) according to the manufacturer's instructions. Following the addition of amino acid the pH was adjusted to 6·2.

Peptone water sugars were prepared containing 1% sugar—either glucose (BDH) or cellobiose (BDH), 1% proteose peptone (Oxoid), and 0·5% sodium chloride. Bromothymol blue was used as an indicator and the pH was adjusted to 7·6.

Tryptone water was prepared from dehydrated tryptone base (LAB M) according to the manufacturer's instructions.

Urea broth was prepared by Christensen's method,[6] which uses 2% urea (Oxoid).

Clinically important urine isolates colonially resembling members of the enterobacteriaceae were routinely subcultured on to diagnostic sensitivity test agar (DST) (Oxoid) for antibiotic sensitivity testing. Organisms which proved resistant to three or more "first line" antibiotics—that is, ampicillin, trimethoprim, cephalexin, cefuroxime, augmentin and gentamicin—were referred for further sensitivity testing and biochemical identification. Resistant isolates confirmed as being oxidase negative were identified

Accepted for publication 7 April 1988

simultaneously by the Logic System and API 20E.

Isolates were inoculated into the API 20E and results determined after 24 hours according to the manufacturer's instructions. Results of identification were determined from the API database and these were regarded as definitive for the purposes of this study. Occasionally extra tests suggested by the API database were undertaken to help resolve difficulties caused by profiles with low species discrimination.

The procedure for operation of the Logic System was carried out as follows: six plastic tubes were assembled, each containing eight drops of substrate (tube 1—lysine, tube 2—ornithine, tube 3—glucose, tube 4—tryptone water, tube 5—cellobiose and tube 6—urea solution).

The equivalent of four to five medium sized colonies was then removed from DST agar and emulsified in 0·4 ml physiological saline to produce a dense suspension. One drop of this suspension was added to each of the six tubes and sterile mineral oil added to tubes containing lysine, ornithine, glucose and urea. All tubes were incubated in a water bath at 37°C.

After four hours of incubation 4 drops of Kovac's reagent were added to the tube containing tryptone water to detect indole production. Any of the remaining tests showing a weak or doubtful colour change was reincubated for a maximum of one hour and then interpreted (table 1).

To interpret the identification of the isolates from results obtained by the Logic System, 18 acceptable profiles were agreed (table 2). Organisms producing a

Table 1 *Interpretation of logic test results*

Test	*Negative result*	*Positive result*
Lysine	Yellow	Grey/purple
Ornithine	Yellow	Grey/purple
Glucose	Blue/green	Yellow
Indole	Yellow/no change	Red (in reagent layer)
Cellobiose	Blue/green	Yellow
Urea	Yellow	Pink/red

Table 2 *List of acceptable Logic profiles*

Biochemical profile						*Identification*	*Additional requirement*
Lysine	*Ornithine*	*Glucose*	*Indole*	*Cellobiose*	*Urea*		
+	−	+	−	+	+ or −	*Klebsiella pneumoniae*	
+	−	+	+	+	+ or −	*Klebsiella oxytoca*	
+	+	+	−	+	−	*Enterobacter aerogenes*	
−	+	+	−	+	−	*Enterobacter cloacae*	
−	+	+	+	+	−	*Citrobacter diversus*	
−	−	+	−	−	−	*Citrobacter freundii*	Lactose positive
+	+ or −	+	+	−	−	*Escherichia coli*	
+ or −	+	+	+	−	−	*Escherichia coli*	
−	+	+	+	−	+	*Morganella morganii*	
−	+	+	−	−	+	*Proteus mirabilis*	
−	−	+	−	−	+	*Proteus penneri*	
−	−	+	+	−	+	*Proteus vulgaris*	
+	+	+	−	−	−	*Serratia* sp/*Salmonella* sp	Lactose negative
−	−	+	+	−	−	*Providencia* sp	Lactose negative

Table 3 *Comparative results of API 20E and Logic System*

API 20E result	*No of strains tested*	*No confirmed by logic*	*Isolates referred for further identification by logic*	*Isolates misidentified by logic*
Escherichia coli	90	88	1 (lactose +, indole −)	1 (identified as *Providencia* sp)
Enterobacter cloacae	41	41		
Enterobacter aerogenes	10	10		
Enterobacter agglomerans	1	0	1 (glucose +, lactose −)	
Klebsiella pneumoniae	32	31	1 (lysine −)	
Klebsiella oxytoca	28	28		
Morganella morganii	28	27		1 (identified as *Proteus mirabilis*)
Proteus mirabilis	15	15		
Proteus vulgaris	3	3		
Proteus penneri	3	3		
Providencia stuartii	4	4*		
Providencia alcalifaciens	1	1*		
Serratia marcescens	18	18*		
Citrobacter freundii	12	7	4 (2 lactose −, 1 ornithine +, 1 cellobiose +)	1 (identified as *Enterobacter cloacae*)
Citrobacter diversus	4	3		1 (identified as *E coli*)
Acinetobacter anitratum	7	0	7 (glucose −)	
Pseudomonas maltophilia	3	0	3 (glucose −)	
Total	300	279 (93%)	17 (5·7%)	4 (1·3%)

*Identified to genus level only by the Logic System.

profile which failed to match exactly with any of the 18 acceptable profiles were referred for further identification. This usually consisted of API 20E or 20NE, depending on the result of the glucose fermentation test.

To reduce the possibility of misidentification, organisms identified as *Citobacter freundii* must also be confirmed as lactose fermenting colonies on the original MacConkey isolation plate. Additionally, any isolates identified as *Providencia sp* or *Serratia sp* must be confirmed as lactose negative. This significantly reduces the risk of misidentifying indole negative *Escherichia coli* and decarboxylase negative *E coli*.

Organisms identified as *Serratia* sp were confirmed as being negative to poly-salmonella 'O' antisera to rule out the possibility of *Salmonella* sp. The probability of isolating a PSO negative, multiply resistant *Salmonella* sp in a urine specimen was considered to be negligible.

Results (table 3)

Discussion

The Logic System correctly identified 93% of isolates to genus or species level, referred 5·7% for further identification, and misidentified 1·3% of isolates.

The Logic System served as a first stage epidemiological tool for the monitoring of antibiotic resistant coliforms. Organisms which gave identical reactions in all six tests were listed and compared. Initially patterns of resistance or "antibiograms" were compared, including actual zone sizes, which increases the usefulness of the antibiogram as an epidemiological tool.[7]

If resistant coliform organisms are isolated from patients in close proximity to each other, and these isolates share identical biochemistry and a similar antibiogram, then the cross infection officer is informed and the need for action is discussed. A battery of supplementary biochemical tests (including API 20E) is then available if further biotyping of species is required.

It can be argued that the routine use of API 20E in preference to the Logic System would give a more valuable evaluation of the biotypes within a species, but we believe this argument is insubstantial for the following reasons. Firstly, the combined use of the Logic System with antibiogram was sufficient to differentiate between most isolates. Secondly, in many instances API 20E provided only a limited means of biotyping within a species—for example, 16 of 18 isolates of *Serratia marcescens* fell into two profiles and 25 of 28 isolates of *Morganella morganii* gave identical profiles but showed a range of sensitivity patterns.

As the main purpose of this project was to reduce costs a comparative costing scheme was carried out. The cost in the United Kingdom of an identification by the logic system in November 1987 was £0·09; the comparative cost of an API 20E was £1·39. These costs exclude the use of sterile saline, a pipette, and mineral oil as these are common to both systems. The labour time required is almost identical for both systems. All of the conventional media used are quick and easy to prepare and their high stability at 4°C means very little quality control is necessary. Overall, a substantial saving of about £1·20 for each isolate is estimated.

The inherent disadvantage of reducing the number of tests in an identification system is to increase the chance of misidentification, and rare organisms will occasionally cause a problem for any simple system. For example, using the logic system, a typical isolate of *Hafnia alvei* will be misidentified as *Serratia* sp but we have never encountered *Hafnia alvei* as a clinically important urine isolate.

From the results of this preliminary study, we believe the logic system to be an excellent tool for the identification of resistant organisms from urine specimens. The small increased risk of misidentification found here (1·3%) seems to be a small price to pay for an effective and much cheaper system. Rare isolates which may be misidentified are nevertheless biotyped and "matched up" which is, after all, the main reason for identifying them.

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