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ABSTRACT

***Objectives*: To assess the performance of the API20E bacteria identification system at a teaching hospital in Kenya.**
***Design*: Retrospective study.**
***Setting*: The microbiology laboratoryoratory of the Aga Khan University teaching Hospital.**
***Subjects*: One thousand six hundred and fifty eight API20E records.**
***Main outcome measures*: The accuracy in identifying the bacteria species.**
***Results*: One thousand four hundred and forty two (87.6%) isolates had the exact identity, 199 (12%) nearest identity, and seven (0.4%) no identity. The performance varied among the species; *Acinetobacter baumanii* had 140 (99.3%) isolates with the exact identity and only one (0.7%) with the nearest identity compared with *Aeromonas hydrophila* which had five (17.2%) with exact and 24 (82.8%) with nearest.**
***Conclusions*: The API20E system is a robust bacteria identification method which can serve small and medium clinical microbiology laboratoryoratories that may not afford automated systems. Adhering to the manufacturer's instructions and good laboratoryoratory practice can improve the performance of this method.**

INTRODUCTION

Bacteria identification to the genus and species levels in a clinical laboratoryoratory is crucial in guiding the choice of antimicrobial therapy to report. Delineation of the biochemical activities of a microbial isolate is the most convenient way to narrow the search path towards the identity of an unknown strain.

Among the most commonly utilised microbial biochemical activities are fermentation of sugars (carbohydrates), utilisation of certain carbon sources, production of certain unique fermentation products, possession of specific enzymes, etc.

The API20E system (API; bioMérieux, France) is a plastic strip with microtubes containing dehydrated substrates, originally designed for the identification of Enterobacteriaceae so that identification of fermenters with the system would be straightforward. The API20E system was extended to include non-fermenters by the addition of six supplementary tests and prolonged incubation of the API20E tests.

A seven-digit bionumber calculated from the observed reactions is compared with the Profile Index to get the bacteria identity.

A number of authors evaluated the API20E system for the identification of Enterobacteriaceae and have reported a high level of agreement with conventional methods in both biochemical reactions and identifications (1, 2).

The performance of the API20E system in the laboratoryoratory depends largely on following the manufacturer's instructions namely; use of colonies from a pure culture, making a homogeneous bacterial suspension, proper inoculation into the cupules and incubating in the recommended environment and duration. Additional tests are done when the seven digit profile is not discriminatory enough.

The Aga Khan Hospital microbiology laboratoryoratory started using the API20E system in the early 90's.

It was supplementary to the routine biochemical identification tests by tube methods and was in most cases performed to confirm a presumptive identity and/or resolve inconclusive results by the primary tests.

API20E remained the major identification system

until the acquisition of the automated Vitek 2 (Vitek; bioMérieux, France) system in the middle of 2010.

MATERIALS AND METHODS

API20E records from the year 2006 to 2010 were reviewed, and a total of 1658 entries in which biodata were availaboratoryle formed the study.

Bacteria identification was categorised into three analytic profiles; exact identity, nearest identity and no identity.

An exact profile matched an entry in the profile index; a nearest profile category differed by one or two minor biochemical reactions while no profile meant an isolate could not be identified based on the biochemical reactions of the entries in the index.

RESULTS

Analytic profiles: One thousand four hundred and fifty two (87.6%) isolates had exact identity, 199 (12%) nearest identity, and 7 (0.4%) no identity. Only 11 repeats were documented.

The performance varied among the species; *Acinetobacter baumanii* had140 (99.3%) isolates with the exact identity and only one (0.7%) with a nearest identity whereas *Aeromonas hydrophila* had five (17.2%) with exact and 24 (82.8%) with nearest. *Citrobacter freundii* had almost equal proportions in both profiles (Table1)

Table 1
Analytic profiles and spectrum of bacteria identified by API20E

	Exact identity (%)	Analytic profile Nearest identity (%)	Total
Acinetobacter baumanii	140 (99.3)	1 (0.7)	141
Aeromonas hydrophila	5 (17.2)	24 (82.8)	29
Burkholderia cepacia	4 (100)	0	4
Chryseomonas leutola	1	0	1
Citrobacter braakii	0	1	1
Citrobacter freundii	13 (52)	12 (48)	25
Citrobacter koseri	22 (84.6)	4 (15.4)	26
Citrobacter youngae	1(100)	0	1
Enterobacter aerogenes	7(87.5)	1(22.5)	8
Enterobacter asburiae	3 (75)	1(25)	4
Enterobacter cloacae	77 (91.7)	7 (8.3)	84
Enterobacter jurgusonii	0	1	1
Enterobacter sakazakii	4 (50)	4 (50)	8
Enterobacter vulneris	1	0	1
Escherichia coli	721 (95)	38 (5)	759
Escherichia fergusonii	1 (33.3)	2 (66.6)	3
Flavibacterium oryzihabitans	4 (100)	0	4
Flavimonas oryzihabitans	3 (100)	0	3
Hafnia alvei	1	0	1
Klebsiella ornithinolytica	2 (66.7)	1 (33.3)	3
Klebsiella oxytoca	29 (85.3)	5 (4.7)	34
Klebsiella pneumoniae	211 (93.4)	15 (6.6)	226
Klebsiella terrigena	36 (97.3)	1(2.7)	37
Kluyvera species	16 (21.9)	57 (78.1)	73
Morganella morganii	26 (96.3)	1(3.7)	27

Ochrobactrum anthropi	2	0	2
Pantoea species	10 (76.9)	3 (20.1)	13
Pasteurella haemolytica	0	1	1
Proteus mirabilis	33 (86.8)	5 (13.2)	38
Proteus penneri	1	0	1
Proteus rettgeri	1	0	1
Proteus vulgaris	13(92.8)	1(7.2)	14
Providencia rettgeri	4 (100)	0	4
Pseudomonas aeruginosa	8 (61.5)	5 (38.5)	13
Pseudomonas oryzihabitans	2 (66.7)	1 (33.3)	3
Pseudomonas putida	1	1	2
Raoultella ornithinolytica	3 (100)	0	3
Raoultella planticola	1	0	1
Raoultella terrigena	16 (100)	0	16
Salmonella species	5 (100)	0	5
Salmonella typhi	3 (100)	0	3
Serratia fecaria	1	0	1
Serratia liquefaciens	1	0	1
Serratia marcescens	7 (100)	0	7
Serratia odonfera	1	0	1
Serratia plymuthica	1	1	2
Stenotrophomonas maltophilia	10 (66.7)	5 (33.3)	15
Unidentified	_	_	7
Total	1452	199	1658

Escherichia coli was the most isolated species at 46%. The majority of the bacteria identified were lactose fermenters (81.7%) and the non-lactose fermenters constituted 18.3%.

DISCUSSION

We reviewed the performance of the API20E system in our microbiology laboratory based on the analytic profiles obtained for over a thousand isolates.

It is evident from the above results that API20E did perform reasonably well in our laboratory giving an exact profile in 87.5% of the isolates tested, a nearest profile in 12%, and <0.5% having no profile.

Several causes could lead to not obtaining an exact profile; failure to follow the manufacturer's instructions on the period of incubation or the performance of supplementary tests, or both (3).

Going through our records it was not clearly stated whether supplementary tests were performed for nearest and no profiles. The omission of supplementary testing may explain why identification of some bacteria was less satisfactory than others. A good example is of *Aeromonas hydrophila* and Kluyvera species which had less "exact profile" and more of "nearest profile" than *Acinetobacter baumanii*.

Other possibilities were failure to have a pure culture (mixed organisms) or to perform a Gram stain to identify aerobic spore bacilli leading to un-interpretable results. The purity of cultures that returned nearest profiles was not always documented. Interestingly when we switched to the automated system there were no more *Klyuvera spp.* isolated raising the question of which between the API20E system and Vitek 2 correctly identifies this isolate.

The API20E system still retains an important role in bacterial identification in settings where the automated identification instruments are out of reach. But even in established laboratories, the API20E system is still useful for identifying certain organisms that may not have a panel in the automated system. Rapid and accurate identification of bacterial pathogens is a fundamental goal of clinical microbiology. In order to guide therapy effectively, it is important to follow the manufacturer's instructions and the approved standard operating procedures so as to minimise misidentifications and repeat testing.

There are studies that have compared the accuracy of the API20E system with the newer identification platforms. In a study by O'hara *et al* after

the initial incubation, 194 (77.0%), 213 (84.5%), and 198 (78.6%) strains were correct to the genus and species levels with the API, Vitek, and MicroScan Walk/Away (W/A; Baxter Diagnostics, USA) respectively. After additional biochemical tests were performed, as directed by each manufacturer's protocol, the numbers of strains correctly identified to the genus and species levels were 241 (95.6%), 234 (92.8%), and 243 (96.4%)with the three systems, respectively (4) . Anne Robinson *et al* demonstrated that of the 381 isolates from the family *Enterobacteriaceae*, API20E and Crystal correctly identified 90.3 and 91.6% by 18 to 24 hours without supplemental testing, respectively, and Vitek identified 92.4 and 96.1% following 10 and 18 hours of incubation, respectively (5).

From these and other studies it is apparent that the API20E system performance was comparable to the automated systems in use then. However, this is not to say there were no drawbacks in its application in our microbiology laboratoryoratory. The challenges we faced while using this system were; the manual reading of the results (colour change) was at times subjective when the change was not clear cut. Others included difficulties in maintaining the in-use API20E packs airtight, and the disposal of the waste strips which were bulky and had potentially infectious isolates in open cupules. The long time taken to manually process the isolates in this system was at times overwhelming as the workload increased. Archiving and retrieval of the information on the API20E score sheets was tedious.

Unfortunately for the isolates that could not be identified at our laboratory there were no arrangements to have them sent to a reference centre.

There are no reports within our region of laboratoryoratories' experience with the API20E system making it difficult to compare our performance with others in a similar environment. This is likely to remain so, as more centres move to the automated systems.

In conclusion, the API20E system is a robust bacteria identification method which can serve small and medium clinical microbiology laboratories that may not afford automated systems. Adhering to the manufacturer's instructions and good laboratoryoratory practice improves the performance of this method.

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