Letters in Applied Microbiology 2005, **41**, 136–140 doi:10.1111/j.1472-765X.2005.01737.x

Comparison of bioMérieux API 20NE and Remel RapID NF Plus, identification systems of type strains of *Ralstonia pickettii*

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2004/1301: received 11 November 2004, revised and accepted 14 April 2005

ABSTRACT

C.C. ADLEY AND F.M. SAIEB. 2005.

Aims: Comparison of two commercial miniaturized rapid systems for the identification of *Ralstonia pickettii* strains.

Methods and Results: Varying identification results were encountered using the bioMérieux API NE system and the Remel IDS RapID NF Plus commercial systems for *R. pickettii*. To compare these two systems, eight strains of *R. pickettii* were purchased from different commercial culture collections. Additionally, 32 industrial and eight clinical isolates, initially identified using the Vitek Junior (bioMérieux) were tested. Total number of isolates tested was 48. The API 20NE identified 29 isolates, as *R. pickettii* but was unsuccessful with 19 isolates. The Remel IDS RapID NF Plus identified 46 isolates as *R. pickettii*. One clinical and one industrial isolates was identified as non-*R. pickettii* with both systems.

Conclusions: The above results indicate that the use of API 20NE system for examining the identification of *R. pickettii* strains is inconsistent.

Significance and Impact of the Study: This study demonstrated that the RapID NF Plus is more accurate as an inexpensive identification system for the identification of *R. pickettii*, a potential emerging organism of medically and industrial importance.

Keywords: bioMérieux API 20NE, identification, *Ralstonia pickettii*, Remel RapID NF Plus.

INTRODUCTION

Identification of Gram-negative nonfermenting rods by conventional methods is often difficult and time consuming, and commercial systems do not always provide reliable identification, especially for some genera or species (Wauters *et al.* 1995; Kiska *et al.* 1996; Van Pelt *et al.* 1999). Moreover, taxonomic studies have resulted in the description of an increasing number of new genera and species involved in nosocomial infections and requiring additional tests for identification. This was true, for instance, for the new genus *Ralstonia* (Vandamme *et al.* 1999; De Baere *et al.* 2001; Gilligan *et al.* 2003). The Rapid NF plus system utilizes conventional biochemical assay for the identification of medically important Gram-negative, nonfermentative bacteria and has been evaluated in several studies (Kitch *et al.* 1992; Kiska *et al.* 1996).

Ralstonia is a new genus that includes former members of *Burkholderia* species (*Burkholderia pickettii* and *Burkholderia solanacearum*). These organisms have been renamed as *Ralstonia pickettii*, *Ralstonia solanacearum* respectively. The genus was separated from *Burkholderia*, by phenotypic characteristics, cellular lipid and fatty acid analysis, rRNA–DNA hybridization, and phylogenetic analysis of 16S rDNA nucleotide sequences (Yabuuchi *et al.* 1995). *Ralstonia* spp. are aerobic Gram-negative, oxidase-positive, nonfermenta-

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tive rod, found in water and soil (Gilligan *et al.* 2003). Anderson *et al.* 1990 identified *R. pickettii* in biofilm formation in plastic water piping. Its potential to form biofilm is potentially because of its ability to produce homoserine lactone molecules (Adley and Saieb 2005). It has been identified in ultrapure water in industrial systems (Kulakov *et al.* 2002) and in the Space Shuttle water system (Koenig and Pierson 1997).

Ralstonia pickettii has also been identified as an opportunistic pathogen in nosocomial infections, especially among immunosuppressed patients (Lacey and Want 1991; Wertheim and Markovitz 1992). Nosocomial infection outbreaks with *R. pickettii* have been reported mainly in association with contamination of hospital supplies (Gardner and Shulman 1984; McNeil *et al.* 1985; Roberts *et al.* 1990; Lacey and Want 1991; Maki *et al.* 1991; Raveh *et al.* 1993; Labarca *et al.* 1999) and with contaminated chlorhexidine skin cleansing solutions (Kahan *et al.* 1983; Maroye *et al.* 2000). The emergence of *R. pickettii* in high-purity water systems necessitates re-looking at this organism.

In this context, it is important to evaluate commercial system for their abilities to detect the presence of *R. pickettii*. Here we evaluated two commercial systems, the bioMérieux API 20NE (bioMérieux, Marcy L'Etoile, France) and Remel RapID NF Plus (Remel, Lenexa, KA, USA), for the ability to identify isolates of *R. pickettii*.

We initially undertook these studies because we needed a relative inexpensive accurate method to identity *R. pickettii* isolates, as an ambiguity was observed in the identification of isolates in the laboratory using methods other than the bioMérieux Vitek Junior.

MATERIAL AND METHODS

Bacterial strains

Analysis was carried out on 48 strains of *R. pickettii* obtained from different sources. The type strains of *R. pickettii*, a clinical strain, were purchased from five different culture collections (see Table 1). One additional clinical strain, *R. pickettii* ATCC 49129 (Ralston *et al.* 1973; Yabuuchi *et al.* 1995), deposited as *Pseudomonas cepacia* (Burkholder) Palleroni and Holmes, American Type Culture Collection (Manassas, VA, USA) and a further soil strain from a rice field in Senegal (Garcia *et al.* 1977; Vandamme *et al.* 1999) of *R. pickettii* and deposited in two separate culture collection repositories, CCM 2846, Czech Collection of Microorganisms (Masaryk University, Brno, Czech Republic) and CCUG 18841 were analysed (Tables 1 and 2).

In addition, eight clinical strains were obtained from the collection of the microbiology laboratory of the Limerick Regional Hospital, isolated from the cystic fibrosis bench.

Table 1 Comparative identification of *Ralstonia pickettii* strains using bioMérieux API 20NE, Remel RapID NF Plus and the bioMérieux Vitek Junior systems

Strain no.	API 20NE (%)	RapID NF Plus (%)	Vitek Junior (%)
JCM 5969^{T}*	99·00	99·94	99·00
NCTC 11149^{T}*	95·10	99·94	99·00
DSM 6297^{T}*	95·10	99·94	99·00
ATCC 49129*†	92·40	99·99	99·00
CIP 73.23^{T}*	91·10	99·94	99·00
CCUG 3318^{T}*	91·10	99·94	99·00
CCUG 18841‡	0·00	99·71	99·00
CCM 2846‡	0·00	99·71	97·00

*JCM 5969^{T} Japan Collection of Microorganisms, the Institute of Physical and Chemical Research, Hirosawa, Wako-shi, Japan; NCTC 11149^{T} National Collection of Type Cultures, Central Public Health Laboratory, London, UK; DSM 6297^{T} Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; CIP 73.23 Collection Bactèrienne de l' Institut Pasteur, Paris, France, and CCUG 3318 Culture Collection University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden.
†Clinical isolate deposited as *Pseudomonas cepacia* (Burkholder) Palleroni and Holmes (Ralston *et al.* 1973).
‡Soil isolate from a rice field in Senegal deposited by Pichinoty (Garcia *et al.* 1977).
Superscript 'T' indicates type strain.

The remaining strains consisted of 32 isolates from a high-purity water system from an industrial setting.

Biotyping

Classical phenotypic tests were performed using the bioMérieux API 20NE system (http://www.biomerieux.com), consisting of eight enzymatic tests and 12 assimilation tests for the identification of nonfastidious Gram-negative rods. The Remel RapID NF Plus commercial system (http://www.remelinc.com) consists of 18 test scores based on microbial degradation of specific substrates. Both systems were used according to the protocol supplied by the manufacturers.

The analysis was carried out in triplicate for each test and using different batch lots. Among historic nonfermentative Gram-negative rods Tatum *et al.* (1974) devised two biovars, Va-1 and Va-2, according to their utilization of lactose and maltose, *R. pickettii* has been equated with biovar Va-2 (Pickett and Greenwood 1980). Lactose utilization was observed on MacConkey agar (Oxoid) at 35°C for 24 h. Lactose is broken down to give an acid product that turns the indicator deep red. Bacteria unable to ferment lactose utilize peptone, raising the pH of the medium, turning the indicator a buff colour (Tatum *et al.* 1974). Maltose utilization was recorded according to the API 20 NE system.

Table 2 Comparative identification of *Ralstonia pickettii* strains (eight clinical and 32 industrial) using bioMérieux API 20NE and Remel RapID NF Plus systems

Strain no.	API 20NE (%)	RapID NF Plus (%)
Industrial		
ULI 187	97·70	98·38
ULI 188	95·10	99·99
ULI 798	95·10	99·99
ULI 807	84·10	99·99
ULI 171	84·10	99·99
ULI 821	84·10	99·94
ULI 797	84·10	98·38
ULI 788	80·40	99·99
ULI 800	80·40	99·99
ULI 169	80·40	99·99
ULI 165	67·90	99·99
ULI 174	67·90	98·38
ULI 193	61·70	98·38
ULI 796	60·00	98·38
ULI 801	56·90	99·99
ULI 791	56·90	99·99
ULI 785	53·10	99·99
ULI 790	44·80	98·38
ULI 181	39·50	99·99
ULI 804	24·50	99·90
ULI 794	6·40	00·00
ULI 185	5·70	98·38
ULI 166	0·00	99·94
ULI 819	0·00	99·99
ULI 159	0·00	99·38
ULI 806	0·00	99·99
ULI 167	0·00	99·94
ULI 784	0·00	99·99
ULI 818	0·00	99·99
ULI 163	0·00	98·38
ULI 795	0·00	98·38
ULI 162	0·00	99·99
Clinical		
ULC 298	90·10	99·99
ULC 297	70·03	99·94
ULC 277	61·70	99·99
ULC 244	56·70	99·99
ULC 193	56·70	99·34
ULC 194	56·70	99·99
ULC 421	28·50	99·99
ULC 279	0·00	0·00

RESULTS AND DISCUSSION

The ID results obtained for the purchased strains are presented in Table 1, the clinical and industrial strains are presented in Table 2. All isolates were Gram-negative rods. Our results showed that these strains were biovar Va-2 (lactose and maltose negative).

In our studies, out of 40 strains (eight clinical and 32 industrial isolates) identified initially using the Vitek Junior, which is an expensive system and not all testing laboratories would have access to this system, the API 20NE misidentified 17 of 32 isolates as non-*R. pickettii*, cut-off points lower then 50%, whereas RapID NF Plus identify 38 isolates as *R. pickettii*. An important and unexpected result of this study was that the two instruments evaluated, differed in their rates of correct ID of *R. pickettii*, for which the Remel RapID NF system performed significantly better.

Eight purchased strains of *R. pickettii* were tested. The bioMérieux Vitek Junior identified all purchased strains as *R. pickettii*. The purchased strains were also tested using the API 20NE system, six strains of *R. pickettii* JCM 5969, NCTC 11149, DSM 6297, ATTC 49129, CIP 73.23 and CCUG 3318 were identified as *R. pickettii*, whereas CCM 2846 and CCUG 18841 were not (Table 1). The identification of these two identical purchased strains of *R. pickettii*, with the API 20NE system was inconsistent due to the Gelatine hydrolysis and assimilation of Mannitol biochemical tests that varied. *Ralstonia pickettii* strains were identified ('good identification', profile API 20NE 1041465 or 'low discrimination', profile API 20NE 0045457). Considering that all of these purchased isolates were catalogued as identical strains of *R. pickettii*, these results necessitated addressing this issue by the use of another method for confirming the identification of the strains.

In contrast, our results show that the Remel RapID NF system is more accurate in the identification of *R. pickettii*. It reconfirmed the identity of the eight purchased strains and the 38 of 40 industrial and clinical laboratory strains as *R. pickettii*. The Remel RapID NF system results were reproducible when compared with the bioMérieux API 20NE system prior to supplemental testing. Because the RapID NF Plus system is enzyme based, its ability to identify weakly oxidizing *R. pickettii* strains may be enhanced compared with those of the other commercial systems (Kiska *et al.* 1996). All strains were positive for glutmyl-β-naphthylamide, a key test for the identification of *R. pickettii* by this system. Using the RapID NF Plus system, the strains were identified as *R. pickettii* ('adequate identification', profile 400414). For the purposes of this study, RapID NF Plus identification of *R. pickettii* was taken as more accurate and repeatable.

In general, the study demonstrated insufficient accuracy of the API 20NE system. A study by Kiska *et al.* (1996) assessed the accuracy of the API Rapid (since replaced by the API 20NE) and the Vitek GNI systems, by studying 150 nonfermenting bacteria, including 58 isolates of *Burkholderis cepacia*. Their study included the RapID NF Plus and the Remel Uni-N/F Tek and N/F Screen. Again, the overall performances of these systems were relatively poor,

accuracies ranged from 57 to 80%, with the Rapid NF Plus being best for identifying nonfermenters in general.

In view of our results, it is observed that the use of API 20NE system for identification of *R. pickettii* was inconsistent. Overall, RapID NF Plus identified the *R. pickettii* strains in this investigation with greater reliability.

The RapID NF plus system was truly rapid, easy to use and interpret. Its use of carbon substrate assimilation enables it to provide more accurate identification of medically important bacteria than do other commercially available system. As no international gold standard for the routine laboratory identification of *R. pickettii* exists to date, the definition of a suitable identification system remains a topic for further investigation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance afforded by the personnel of the Microbiology Laboratory at the Mid-West Regional Hospital, Limerick, Ireland, and the local company that provided the industrial *R. pickettii* strains and Vitek Junior analysis.

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