SHORT ARTICLE

Identification of clinically isolated vancomycin-resistant enterococci: comparison of API and BBL Crystal systems

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Twenty-eight phenotypically separate strains of clinically isolated vancomycin-resistant enterococci have been investigated with two API identification kit systems (20 Strep and Rapid ID 32 Strep) and two BBL Crystal kits (Gram Positive and Rapid Gram Positive). All strains were identified as *Enterococcus faecium* by a reference laboratory. The Rapid ID 32 kit positively identified 15 of 28 strains (54%), but only two (7%) were identified correctly: 11 were identified as 'doubtful' or 'to genus level' and two gave 'unacceptable' profiles. The API 20 Strep kit identified 27 strains (96%), but only 16 (57%) were identified correctly as *E. faecium*. The Rapid ID 32 kit erred by either positively misidentifying vancomycin-resistant *E. faecium* as *E. casseliflavus* or *E. gallinarum*, or indicated that this was the most likely identification, while the API 20 Strep kit more commonly produced a misidentification as *E. casseliflavus*. The Crystal Gram Positive and Rapid Gram Positive kits correctly identified 26 (93%) and 27 (96%) of the strains, respectively.

Introduction

It is important to be able to identify vancomycin-resistant enterococci (VRE) accurately and rapidly so that their clinical significance can be ascertained [1, 2]. Two new systems, the BBL Crystal Rapid Gram Positive and Gram Positive kits (Becton Dickinson, Oxford), are now available that are capable of identifying enterococci. These kits incorporate 18 colorimetric and 11 fluorometric tests to generate a 10-digit code number interpreted by an electronic codebook. This report describes the performance of these new kits for the identification of vancomycin-resistant *Enterococcus faecium* strains, in comparison to API Strep 20 and API Rapid ID 32 kits (bioMérieux, Basingstoke).

Materials and methods

Bacterial strains

Twenty-eight VRE strains isolated from patients in this hospital were judged as phenotypically different on the basis of the combinations of differing biotypes determined by the two API kits, the two Crystal kits and the antibiotyping system described below.

Identification

All 28 strains had been identified as *E. faecium* by the Central Public Health Laboratory, 61 Colindale Avenue, London, by PCR. The numerical profiles generated in API Rapid ID 32 Strep, API 20 Strep, BBL Crystal Gram-positive and BBL Crystal Rapid Gram-Positive kits, were interpreted by databases v2.0, v6.0, v4.0 and v4.0, respectively.

Antibiogram determination

The 28 strains were tested by disk diffusion tests against the following discriminatory antibiotics: gentamicin (100 µg), rifampicin (5 µg), streptomycin (300 µg). Breakpoints were as described by the National Committee for Clinical Laboratory Standards [3].

Results

Strain identification

The API 32 kit produced 26 different numerical codes for the 28 strains. A total of 15 positive identifications (54%) was made (results being 'acceptable', 'good', 'very good' or 'excellent'): *E. casseliflavus* (eight), *E. gallinarum* (four), *E. faecium* 1 (two) and *E. durans* (one). Profiles from 11 strains were 'doubtful' or 'to genus only', with the highest probability identifications being: *E. gallinarum* (six), *E. faecium* 1 (three),

E. casseliflavus (two). The remaining two strains generated 'unacceptable' profiles.

The API 20 kit produced 10 different codes, two accounting for 18 strains. Twenty-seven strains (96%) could be identified: *E. faecium* (16), *E. casseliflavus* (10) and *E. durans* (one). The remaining strain was identified 'to genus only', *E. gallinarum* being the nearest species.

The two API kits agreed (positive or nearest identification) in 10 cases: *E. casseliflavus* (five), *E. faecium* (four) and *E. durans* (one).

The BBL Crystal Gram Positive kit generated eight different codes, one of which was found for 18 strains. Twenty-six strains were identified as *E. faecium*, one as *E. faecium* or *E. hirae* and one as *E. casseliflavus* or *E. gallinarum*. The Rapid Gram Positive kit also produced eight biotypes (19 strains were of the same biotype), that identified 27 strains as *E. faecium*, and gave an 'unacceptable' result for the remaining strain. The two Crystal kits agreed for 25 of the 28 strains.

Discussion

When the Reference Laboratory PCR speciation was taken as the 'gold standard', the API 32 kit was correct only twice (7%), misidentified 13 strains (46%) and produced inaccurate results for 13. The API 20 kit performed somewhat better, correctly identifying 16 strains (57%), but it misidentified 11 (39%) and was inaccurate for one. The Crystal kits were more accurate: the Gram Positive and Rapid Gram Positive correctly identified 26 (93%) and 27 (96%) strains respectively.

The discrepancies found with the API kits had not been reported previously for *E. faecium* [4, 5]. However, only relatively small numbers of *E. faecium* strains were examined in the earlier studies (e.g., 12 by Freney *et al.* [5]), and the accuracy of API kits for identifying vancomycin-resistant *E. faecium* had not been specifically investigated previously. *E. faecium* can be phenotypically variable [5–7], and the present study showed that certain biotypes were misidentified, particularly by the Rapid 32 kit. Perhaps it is these biotypes that are the most able to acquire resistance to vancomycin; alternatively, changes may occur in physiological properties when vancomycin resistance is acquired (i.e., the ability to utilise certain substrates is lost or gained [6]).

Previous information on the BBL Gram Positive kit [8, 9] reported good agreement with the API Strep system for 'enterococci', but neither the species examined nor the vancomycin resistance status was documented. An assessment of the Rapid Gram Positive kit has not been published previously.

It is concluded that the API kit systems are not suitable for the identification of VRE, and that the BBL Crystal kits performed well in this role. However, the API 32 kit may be useful for biotyping in the investigation of potential outbreaks [10]. Discriminatory antibiotyping as described here could provide an additional tool for epidemiological purposes, as has been found with methicillin-resistant *Staphylococcus aureus* [11].

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