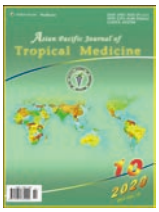

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Misidentification of multidrug resistant *Enterococcus faecium* using a commercial identification method

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Enterococcus (*E.*) *faecium* is recognized as a leading cause of nosocomial infections worldwide. Infection with the organism is often difficult to treat due to its inherent ability to acquire glycopeptide resistance genes and other virulence genes[1]. Laboratory identification of this organism in healthcare settings tends to rely on commercially available standardized biochemical tests such as the API 20 Strep[2]. Incorrect identification of the enterococci isolates could lead to improper antimicrobial therapy and infection management strategies[2]. A retrospective study was undertaken to speciate and characterize the archived enterococci isolates previously identified using the API 20 Strep during routine microbiological cultures at the University Malaya Medical Center diagnostic laboratory. Special emphasis was given to enterococci isolates that gave poor species identification using the API 20 Strep.

Archived bacteria isolates stored in the specimen repository at the Tropical Infectious Diseases Research & Education Centre, University of Malaya were subjected to Gram staining, microscopy, biochemical and API 20 Strep tests. A total of seven enterococci isolates (*E. gallinarum*, *n*=4; *E. durans*, *n*=3 and *Leuconostoc* spp., *n*=1) isolated in 2011 were selected for the study. The enterococci isolates were among those recorded as having inadequate species identification (66.0%-68.4% identity) determined using the API 20 Strep (Table 1). Additionally, the recorded *Leuconostoc* isolate was also unsatisfactory using the API 20 Strep (49.6% identity) (Table 1), which was found positive for the pyrrolidonyl arylamidase test, raising suspicion that it was previously misidentified. All bacteria isolates were maintained on Columbia agar with 5% sheep blood at 37 ℃ under aerobic condition. Genomic DNA was extracted from the bacteria isolates using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) and the 16S rDNA gene was amplified using overlapping primers[3]. The amplified partial 16S rDNA sequences were submitted for BLASTn search, resulting in *E. faecium* (>98.0% identity) for all the eight selected bacteria isolates (Table 1).

Multilocus sequence typing performed according to the protocols by Homan *et al*.[4] found three *E. faecium* isolates with sequence types (ST) 78 and ST80, respectively and one with ST17 and ST203, respectively. Amplification of glycopeptide resistance genes[5] found that all the *E. faecium* isolates carried the *vanA*, with two isolates also carrying the $vanC_1$. Examination for the presence of virulence genes[6] revealed that all the *E. faecium* isolates possessed the extracellular surface protein gene, *esp*. Furthermore, all eight isolates possessed at least one of these genes; the *asa1* (aggregation substance), *hyl* (hyaluronidase) and *cylA* (cytolysin), with UM-127 carrying three virulence genes (*esp*, *hyl* and *cylA*). Disk diffusion tests performed strictly according to the guidelines by the Clinical and Laboratory Standards Institute demonstrated that all the *E. faecium* isolates were resistant to ampicillin, penicillin, erythromycin, ciprofloxacin and vancomycin. Three out of eight *E. faecium* isolates were found resistant to teicoplanin.

As determined by multilocus sequence typing, all *E. faecium* isolates in this study belonged to the high risk clonal complex 17 (CC17)[1]. Isolates from CC17 are colonizers of the healthcare facilities found in many continents and are currently also found among animals and the environment[1]. All the STs (ST17, ST78, ST80 and ST203) found in this study had previously been reported

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Table 1. Genotypic and phenotypic features of *Enterococcus faecium* isolates in this study.

Isolate name	UM-1A	UM-124	UM-125	UM-127	UM-128	UM-129	UM-134	UM-138
Identification *via* 16S rDNA sequencing	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*
Identification *via* API 20 Strep*	*Enterococcus durans* (68.4%)	*Enterococcus gallinarum* (66.0%)	*Enterococcus gallinarum* (66.0%)	*Enterococcus gallinarum* (66.0%)	*Enterococcus durans* (68.4%)	*Enterococcus gallinarum* (66.0%)	*Enterococcus durans* (68.4%)	*Leuconostoc* spp. (49.6%)
Sequence type	78	78	78	80	80	203	17	80
Antimicrobial resistance phenotype#	AMP, ERY, PCN, CIP, VAN	AMP, ERY, PCN, CIP, VAN	AMP, ERY, PCN, CIP, VAN	AMP, ERY, PCN, CIP, VAN	AMP, ERY, PCN, CIP, TEC, VAN	AMP, ERY, PCN, CIP, VAN	AMP, ERY, PCN, CIP, TEC, VAN	AMP, ERY, PCN, CIP, TEC, VAN
Glycopeptide resistance gene	*vanA*	*vanA*	*vanA*	*vanA*, $vanC_1$	*vanA*	*vanA*	*vanA*, $vanC_1$	*vanA*
Virulence gene	*esp*, *asa1*	*esp*, *hyl*	*esp*, *asa1*	*esp*, *hyl*, cylA	*esp*, *asa1*	*esp*, *asa1*	*esp*, *hyl*	*esp*, *asa1*

#AMP: ampicillin; ERY: erythromycin; PCN: penicillin; CIP: ciprofloxacin; VAN: vancomycin; TEC: teicoplanin. *The identification percentage was calculated by algorithms of the manufacturer, based on biochemical reactions of the respective *Enterococcus faecalis* isolates.

in clinical cases in Malaysia[7], suggesting the CC17 isolates had already established themselves in the local hospital environment. Accordingly, accurate bacteria species identification is crucial to determine the appropriate antimicrobial therapy and for determining whether the bacteria is a risk for other hospital personnel, patients and the public[3]. The misidentification of the Enterococci species using the API 20 Strep possibly contributed to the maintenance and persistence of the CC17 in the University Malaya Medical Center since 2011. Besides, infection with *E. durans* and *E. gallinarum* are commonly associated with a lower risk of mortality[8,9], undermining the gravity and impact of *E. faecium* infections on the patients. *Leuconostoc* spp. are associated to the food industry for its use in food and beverage fermentation[10] and as such will most likely be dismissed as an environmental contaminant. It was quite possible that the patients infected by the *E. faecium* isolates in this study did not receive optimum antimicrobial treatment as a result of the misidentification of bacteria by API 20 Strep.

Furthermore, persistence and continuous survival of *E. faecium* in the hospital environment most likely facilitated the acquisition and also the horizontal transfer of antimicrobial resistance and virulence genes. Hence, it was not unexpected to find all the *E. faecium* isolates harboring the *vanA*, as well as expressing resistance not only to the glycopeptide, but also to the macrolide, penicillin and quinolone antimicrobials. Persistence may also be due to the function of the extracellular surface protein, *esp* and the aggregation substance, *asa1*, which mediate initial attachment of *E. faecium* to host cell surfaces[1]. These virulence genes work in tandem with the hyaluronidase, *hyl* and the cytolysin, *cylA*, to hydrolyze host cells, triggering the inflammatory process and subsequently causing disease[1]. Detection of $vanC_1$ in UM-127 and UM-134 could possibly be explained by gene acquisition from *E. gallinarum* or other enterococci, as *E. faecalis* harboring the $vanC_1$ has been reported in Malaysia before[5].

In essence, accurate bacteria species identification is pivotal for epidemiology investigations with the aim of curbing the spread of multidrug resistant enterococcal infections. Our findings suggest that the current commercial diagnostic platform needs improvement in the ability to identify and differentiate against the newer multidrug resistant bacteria. In contrast, 16S rDNA sequencing was shown to be highly reliable for the identification of enterococci down to the species level and should be considered in addition to the API 20 Strep in the clinical laboratory diagnostic settings.

Ethics statement

This study received approval from the University Malaya Medical Center Medical Ethics Committee (MECID. No. 20149-575).

Conflict of interest statement

The authors declare that there is no competing interest.

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Authors' contributions

S.K.L., N.A.A.C.M.S. and N.H.M. performed the experiments. S.K.L. wrote the manuscript together with S.A., who obtained funding for the study.

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