Meticillin-heteroresistant *Staphylococcus pasteurii* from an apheresis platelet product

About 1 in 1000–2000 apheresis platelet units is contaminated by bacteria. The platelet products are stored in oxygen-permeable bags, at 20–24 °C, for up to 5 days before being transfused, to preserve cell survival and function. Unfortunately, this may also permit growth of bacteria and transmission of these to recipients. Coagulase-negative staphylococci, *Staphylococcus aureus*, α -haemolytic streptococci, diphtheroids, *Propionibacterium acnes*, *Acinetobacter* species, *Escherichia coli*, *Serratia* species, *Klebsiella* species, *Enterobacter* species, *Providencia rettgeri* and *Yersinia enterocolitica* are known to contaminate platelet units (Riedel *et al.*, 2006; Savini *et al.*, 2008). Though contaminating staphylococci generally exert β -lactam susceptibility (as they are mostly donors' skin contaminants, not nosocomial isolates), meticillin resistance should always be excluded to avoid β -lactam failure when treating transfusion-related bacteraemias.

A *Staphylococcus pasteurii* strain, designated *Staphylococcus pasteurii* A, was isolated as a contaminant from an apheresis platelet unit from an adult first-time donor. Identification was obtained by rRNA sequencing. Given that the screened unit had not yet been transfused, an aliquot from the apheresis bag was newly examined to exclude the possibility that contamination had occurred while inoculating the BacT/Alert bottles (bioMérieux BacT/Alert is the instrument which is used in our department to screen platelet products for bacterial contamination) (Riedel *et al.*, 2006); interestingly, the same strain grew again. Antimicrobial susceptibilities were determined by bioMérieux Vitek2, and meticillin susceptibility was confirmed by a standard agar disc test (NCCLS, 2003). A 20 mm inhibition zone diameter was documented around the Oxoid meticillin disc, but two single colonies (*Staphylococcus pasteurii* B and C) were

observed within the inhibition area after 48 h incubation. These were both subcultured and showed the same phenotypic features as *Staphylococcus pasteurii* A (typical yellowish staphylococcal colonies), but exerted full meticillin resistance; an MIC ≥ 0.5 mg l⁻¹ was documented by Vitek2, whereas an 8 mm diameter meticillin inhibition zone was obtained by carrying out a disc test (NCCLS, 2003). Meticillin resistance thus appeared to be heterogeneously expressed within the population studied, and the isolate was finally considered β -lactam-resistant. The documented susceptibility profile was as follows: resistant to penicillin (MIC ≥ 0.5 mg l⁻¹); heteroresistant to meticillin/oxacillin (MIC ≥ 0.5 mg l⁻¹); susceptible to erythromycin (MIC ≤ 0.25 mg l⁻¹), clindamycin (MIC ≤ 0.5 mg l⁻¹), ciprofloxacin (MIC ≤ 0.5 mg l⁻¹), cotrimoxazole (MIC ≤ 10 mg l⁻¹), teicoplanin (MIC 1 mg l⁻¹) and vancomycin (MIC 1 mg l⁻¹).

Heteroresistance means the existence of a mixed population of drug-sensitive and drug-resistant organisms in a clinical isolate; in a most stringent sense, it is an exclusively phenotypic manifestation within a genotypically homogeneous strain. Heteroresistance may be undetected if only automated susceptibility tests are performed or only standard agar methods are carried out. This may lead to antimicrobial failure due to *in vivo* overgrowth of mutant drug-resistant clones. Meticillin and vancomycin heteroresistance has been found in *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus haemolyticus*, *Staphylococcus capitis*, *Staphylococcus auricularis* and *Staphylococcus warneri*. *Gardnerella vaginalis* metronidazole heteroresistance, *Acinetobacter baumannii* carbapenem heteroresistance and *Cryptococcus neoformans* fluconazole heteroresistance have also been reported in the literature. This phenomenon has also been described

in *Enterococcus faecium*, *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, but never in *Staphylococcus pasteurii*. The frequency of heteroresistance is known to be about one subclone in every 10⁵–10⁶ colonies, though this varies amongst different species. Heterogeneous resistance may represent a first step towards resistance, as it permits organisms to grow in the presence of an antibiotic, before the acquisition of resistance by the greater part of the microbial population (Falagas *et al.*, 2008; Pournaras *et al.*, 2005).

Heteroresistance is known to be difficult to detect, and there are few clinical data concerning this topic. Also, heteroresistance may not be evident before 48 h incubation even if agar methods are used. Hence we would suggest that laboratories carefully screen bacterial organisms for heterogeneous resistance to antimicrobials in daily clinical practice. Though clinical antimicrobial failures due to undetected heteroresistance have been reported, the therapeutic implications of this phenomenon have to be clarified and further clinical and experimental data are required (Falagas *et al.*, 2008).

Staphylococcus pasteurii is a coagulase-negative staphylococcus which contributes naturally to Italian sausage fermentation, and development of their typical colour and aroma (Iacumin *et al.*, 2006). Also, *Staphylococcus pasteurii* is one of the few micro-organisms which have been cultured from the stratosphere (Wainwright *et al.*, 2006). Concerning *Staphylococcus pasteurii* involvement in human disease, the organism has only recently been found to cause a proven bacteraemic episode in a leukaemia patient (Savini *et al.*, 2009), whereas the clinical significance of the isolates recovered by Chesneau *et al.* (1993) remained doubtful. The occurrence of platelet unit contamination by this species, however, may make it gain importance as an agent

of platelet-derived bacteraemias in the coming years. Also, widespread use of molecular methods for bacterial identification based on rRNA sequencing will probably lead to an increase in the number of *Staphylococcus pasteuri* isolates recovered from blood products and clinical specimens, given that automated methods which are commonly used in routine practice may fail to recognize this species on the basis of the biochemical profile. There are many unanswered questions about *Staphylococcus pasteuri*. We presently know very little about its ecological niches and its ability to cause disease in animals and man and to acquire resistance determinants. Nevertheless, its capacity to exert β -lactam resistance is of concern. To the best of our knowledge, this is the second isolation of *Staphylococcus pasteuri* from a blood product (Savini *et al.*, 2008). Our brief communication reports what we believe to be the first recovery of meticillin heteroresistance in the species discussed. This is thought to be due to the heterogeneous expression of the *mecA* gene within the bacterial population, and this could lead to *in vivo* selection of fully β -lactam-resistant mutant clones if a transfusion-related bacteraemia occurred.

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