The first clinical isolate of *Enterococcus faecium* with the VanB phenotype in a teaching hospital in Greece

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Enterococci present a serious problem in hospitals, especially in the USA, where in intensive care and transplantation units as many as 15% of the strains are resistant to vancomycin. In Europe the situation with regard to vancomycin resistance in clinical isolates is better, usually with sporadic cases in intensive care and hematology units [1–3]. On the other hand, enterococci with various level of vancomycin resistance have been found colonizing the feces of healthy individuals in different parts of Europe [4,5].

In Greece, there have so far been very few reports of vancomycin-resistant enterococci (VRE), possibly because of the strict policy for vancomycin use that was established early enough in most hospitals by the infection control committees. We report a case of intra-abdominal infection with an *Enterococcus faecium* VanB isolate in a patient with Prader–Willy syndrome and acute pancreatitis.

A 14-year-old caucasian boy was admitted to the emergency room of our hospital for acute abdominal pain 6 h before, dyspnea, high fever and a low consciousness level. Physical examination revealed fever (40.5°C), acute abdominal pain and tenderness in the right part of the abdomen, which reflected to the right kidney area. Laboratory results showed a leukocyte count of 29 600/mm³ (80% neutrophils), hematocrit 48.2%, glucose 435 mg/dL (normal range 65–125), SGOT 760 IU/L (5–45), SGPT 410 IU/L (5–45), amylase 240 IU/L (0–220), and lactate dehydrogenase (LDH) 960 IU/L (0–450). Blood gases revealed metabolic acidosis (pO₂ 76, pCO₂ 27.5 and pH 7.45). Chest X-ray was normal, and abdominal ultrasound scanning, performed with great difficulty (due to excess weight), showed liquid in the peritoneal cavity.

The boy had had a known Prader–Willy syndrome since birth (mental deficiency, great obesity, increased appetite with ability to consume huge quantities of food, sexual infantilism and peripheral muscular atony). He also had a history of acute pancreatitis episodes. The boy was living in Bulgaria and the family had very recently moved to Greece as migrants.

The patient was admitted to the internal medicine department but was immediately transferred to the intensive care unit (ICU) because of septic shock with spasms and acute dyspnea. He was immediately started on antimicrobial treatment with amikacin and ceftriaxone; this was changed to meropenem, fluconazole and ornidazole after 5 days, and again to ceftazidime, fluconazole and ornidazole after 6 more days. His general condition remained the same, and high fever continued. A CT scan was performed with better preparation, and this time revealed a cyst in the proximity of the pancreatic body, fluid in the peritoneal cavity and fluid in the pleural space. Surgery revealed an abscess in the proximity of the head and the body of the pancreas; this was removed and the area was cleaned. The antimicrobial treatment was changed to co-trimoxazole, piperacillin+tazobactam and fluconazole. The cultures from the abscess were negative, as were all the blood cultures taken so far.

A small improvement in the patient's general condition was observed which lasted for 6 days. On the 28th hospital day, fever rose again and his general condition deteriorated. Treatment was changed again, to ciprofloxacin, vancomycin and fluconazole, with no results. A culture specimen was taken from the drainage on the 36th day, and revealed *Pseudomonas aeruginosa* and *Enterococcus* spp. The treatment was changed to imipenem and fluconazole. Another set of blood cultures was negative.

On the 40th hospitalization day, the patient was reoperated on but died during the surgical procedure as a result of oligemic shock.

The *Enterococcus* isolate was identified primarily by Gram stain, growth in the presence of bile and 6.5% NaCl and hydrolysis of esculin. A Kirby–Bauer disk
diffusion test was done, and species identification as well as the MIC measurement were performed by the PASCO MIC/ID semi-automatic system for Gram-positives (Difco Laboratories, Detroit, Mi, USA). The isolate was Enterococcus faecium and was resistant to ampicillin, ampicillin+sulbactam, chloramphenicol, ciprofloxacin, erythromycin, ofloxacin, rifampicin, cotrimoxazole, tobramycin and piperacillin+tazobactam. It had medium resistance to gentamicin (6 mg/L) and vancomycin (16 mg/L) and was susceptible to teicoplanin and to high levels of gentamicin (512 mg/L) and streptomycin (1024 mg/L). The strain did not produce β-lactamase (the test was performed using nitrocefin disks; Cefinase, BBL, Becton Dickinson Microbiology Systems, Lockeyville, MD, USA).

The PCR assay proposed by Dutka-Mallen et al [6] was performed to confirm species identification and determine the genotype of the isolate, which had a VanB phenotype (lane 4 in Figure 1). For quality control, the strains E. faecium BM4147 (lane 2 in Figure 1), which harbored the vanA gene, and E. faecalis V583 (lane 3 in Figure 1), with the vanB gene, were used.

The enterococci have naturally occurring resistance to various antimicrobial agents, including penicillins and cephalosporins. They can also develop plasmid-and transposon-mediated resistance to agents such as erythromycin, tetracycline, chloramphenicol and clindamycin.

Resistance to vancomycin was first described in 1988 [8]. Four major types of vancomycin resistance are known. Acquired inducible resistance to both vancomycin and teicoplanin characterizes the VanA type. The VanB type has various levels of acquired and inducible resistance to vancomycin but not to teicoplanin. The presence of VanC is an intrinsic property of certain strains such as E. gallinarum and E. casseliflavus and is characterized by low-level resistance to vancomycin. The VanD type has been very recently described and is still under investigation [7].

Because of the low level of vancomycin resistance of the VanB phenotype, it is sometimes difficult for clinical laboratories to detect it, especially those which use the Kirby–Bauer disk diffusion method for susceptibility testing. This difficulty was also shown by Q.C. Scheme, where hospitals performing the Kirby–Bauer method had difficulties in recognizing medium vancomycin resistance (A. Vatopoulos, personal communication). It is preferable to use a method of MIC measurement such as the Etest or the microdilution method, or, even better, to use a screening method of growth in brain–heart infusion agar supplemented with 6 mg/L vancomycin [9].

In Greece, up to now, there has been no significant vancomycin resistance. In both local and multicenter studies [10,11], the frequency of VRE clinical isolates was never higher than 1%, with the exception of one study [12] from northern Greece, which presented a rate of 7.6%. This rate changed to 0% 2 years later in the same institution [13]. In our hospital, surveillance using the WHONET software has never revealed, until now, a VRE strain [14], and the isolate under discussion was the first one with vancomycin resistance. To our knowledge, it is also the first fully characterized VanB E. faecium clinical isolate found in Greece.

The isolate was considered to be a colonizer rather than a true pathogen, since it was isolated from abdominal drainage only. Colonization was possibly the result of prolonged antibiotic use, especially vancomycin, or of VRE spread from patient to patient via the hands of personnel or various equipment. At the time of isolation, there was no screening program for VRE in patient fecal flora in the ICU, so we did not have the opportunity to screen the patient after the isolation because of his rapid deterioration and death. Immediately after isolation of the first VRE, a rectal culture surveillance program was started which is currently being carried out in all patients of the ICU and certain other departments of the hospital. The feces or perirectal swabs are cultured in bile–esculin–azide agar supplemented with 6 mg/L vancomycin [15]. Preliminary results of this study show a 33% rate of colonization with VRE among examined patients in the ICU. This high colonization rate may explain a possible patient-to-patient spread of the strain under discussion, via the hands of personnel.

There is only one very recent study in Greece on fecal carriage; this indicates a rate of VRE carriage (of the vanA genotype) of about 6% [16]. This study was

Figure 1 Agarose gel electrophoresis of the VanB PCR product. Lane 1 is the negative control.
performed in a primary and secondary healthcare center and it possibly indicates the colonization rate of the community.

This first VRE clinical isolate of the VanB phenotype is probably the tip of the vancomycin resistance ‘iceberg’ in our hospital. It indicates a possible need for adjustments in the testing of vancomycin resistance and in VRE carriage surveillance in all tertiary-care institutions, since the transfer of seriously ill patients from one hospital to another is common practice. Clinical laboratories must adopt a sensitivity testing method that is able to recognize the VanB phenotype, and should also apply a surveillance program for VRE based on the agar-screening test. They must also try to reinforce the infection control policies, to reduce the risk of nosocomial transmission of VRE.

References

The use of a genetic amplification technique (LCx MTB) to diagnose extrapulmonary tuberculosis

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The diagnosis of tuberculosis is well known as being a slow process, particularly in extrapulmonary cases. In these clinical presentations, the Zielh–Neelsen stain for acid-fast bacilli in smears tends to be negative, making