Differences in resistance and virulence between various sequence types of CC17 vancomycin-resistant Enterococcus faecium clinical isolates

Department of Microbiological Diagnostics and Infectious Immunology, Medical University of Białystok, Poland

Background:
Today, the expansion of hospital-adapted vancomycin resistant Enterococcus faecium (VRE) belonging to clonal complex (CC) 17 are a major problem in healthcare system [1]. The aim of this study was to compare the resistance and virulence between various sequence types of CC17 VRE clinical strains.

Materials/Methods:
Thirty VRE strains isolated from various materials from patients hospitalized in two University Hospitals in Białystok (Poland), since 12.2013 – 06.2015, were analysed. Identification and susceptibility testing were performed by VITEK2 system. Minimum Inhibitory Concentrations (MIC) of glycopeptides were determined with E-tests. The ability to hemolyze and to form biofilm were assessed by phenotypic methods: Columbia blood agar method and the tube/CRA method, respectively. Six virulence genes (esp - surface protein, hyl - hyaluronidase, acm - collagen adhesin, efa - endocarditis antigen, geE - gelatinase, cyl - cytolysin) were investigated by PCR followed by gel electrophoresis and DNA sequencing (Table 1). qPCR was used to establish the expression levels of vanA gene. Multilocus sequence typing (MLST) was done according to the scheme described in http://pubmlst.org/efaecium/ database. Sequence types (ST) were grouped into CCs by eBURST analysis (http://www.mlst.net).

Results:
MLST genotyping revealed five different STs: ST117 (n = 12), ST80 (n = 9), ST440 (n = 4), ST202 (n = 4) and ST18 (n = 1) belonging to CC17 (Figures 5 and 6). Comparative analysis (Figure 3) of these STs revealed that all ST80 and ST440, 33.3% of ST117, and 50% of ST202 strains were resistant to gentamicin (p<0.001). Resistance to streptomycin was found in all ST117, 33.3% of ST80, 25% of ST440, and 50% of ST202 strains (p<0.001). All isolates were resistant to ampicillin and imipenem, and were susceptible to linezolid and tigecycline. There were no differences in the levels of MIC of vancomycin (>256mg/L) and teicoplanin (>4mg/L) between various STs. All strains had VanA phenotype, whereas ST440 strains had VanA and VanB phenotype. The average vanA gene expression in ST117 was more than 5 times higher compared to other STs (Figure 2). The ability to α-hemolyze and to form biofilm occurred in >95% of tested isolates. All strains had acm and efa gene. esp gene was present in all ST117, 11.1% of ST80, 0% of ST440, and 100% of ST202 strains; hyl in 100%, 33.3%, 0%, and 75%, respectively (p<0.001) (Figure 4). None of tested isolates had geE and cyl genes.

Conclusions:
This study revealed that CC17 VRE strains show high levels of resistance and are well equipped with virulence traits. We found that isolates with various STs slightly differ from each other; statistically significant differences were found only in the case of resistance to aminoglycosides and in case of the prevalence of esp and hyl genes. Interestingly, despite that there were no differences in glycopeptide’s MICs between various STs, ST117 isolates exhibited the highest expression level of vanA gene. Further studies are needed to explain these observation.