THE EVALUATION OF THE PATHOGENIC ROLE AND ANTIMICROBIAL RESISTANCE OF ENTEROCOCCUS SPECIES

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Abstract: The aim of the study consisted in the evaluation of the pathogenic role and resistance to antibiotics of enterococcal strains isolated in a 3 years period (2013-2015) from hospitalized patients. The spectrum of enterococcal infections in hospitalized patients was large and relative constant in the studied period, being dominated by urinary tract infections (38.8%), wound infections (30.1%) and intraabdominal and pelvic abscesses (10.9%). The highest level of resistance were recorded to macrolides and fluoroquinolones. The Enterococcus strains had high sensitivity to glycopeptides.

Key words: Enterococcus, infections, antimicrobial resistance.

1. Introduction

Enterococcus genre includes gram positive catalase negative cocci, natural residents of human intestine. Due to the disruption of the commensal relationship with the host, Enterococcus can be involved in urinary and biliary tract infections, endocarditis, intra-abdominal and pelvic infections, wounds infections, rarely meningitis. [4], [7], [9]

Enterococcus represent an important cause of nosocomial infections and can determine sporadic outbreaks in some hospital wards. In the hospitals, enterococci are considered the second most common cause of urinary tract and wounds infections and the third most common cause of bacteriemia. [4], [7]

Urinary infections are the most common location in hospitalized patients (cystitis, pyelonephritis, prostatitis, renal abscesses) and are frequently associated with structural abnormalities or local catheterization. [4], [7]

Enterococcus sp. are intrinsically resistant to cephalosporins. The sensitivity to other antibiotics varies widely. The acquisition of high-level resistance to aminoglycosides and the emergence of vancomycin resistant strains are the most concerning problem in our days.

The resistance to β-lactams is due to β-lactamases or structural changes of the PBP (penicillin binding protein). [3], [5], [7], [9]

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Enterococcus genre shows a low level of natural resistance to aminoglycosides but with gentamicin being active. At the wild strains of Enterococcus the synergy with β-lactams and glycopeptides is present. Due to chromosomal mutations, HLAR (High Level Aminoglycosides Resistant) strains are being selected case in which the synergy with β-lactams is lost.

The enterococci resistance to glycopeptides is associated with changes in the bacterial wall (due to the synthesis of a new precursor of the peptidoglycan). [3], [5], [7], [9]

2. Material and Methods

The study was retrospective, descriptive and was conducted in the Clinical County Emergency Hospital of Brașov in the period 1.01.2013-31.12.2015. The aim of the study was to evaluate the pathogenic role of these germs and to determine the level of antibiotic resistance of the enterococci isolated from the hospitalized patients. The objectives of the study have consisted in the evaluation of the etiologic spectrum of enterococcal infections and the study of the resistance patterns of the isolates strains in order to optimize the prescription of antibiotics.

The processing of the biological samples was performed in the Clinical laboratory of this medical unit. During the study there were considered as having ethiopathogenic significance the enterococci isolated from the samples with inflammatory characteristics (purulent, with polymorphonuclear neutrophil leukocytes on the gram smear).

For the isolation of enterococci there were used Columbia Agar with 5% sheep blood, Mac Conkey Agar and Brilliance UTI Agar, incubated aerobically at 37° C for 24 hours.

The genre identification of the isolates was based on morphological, cultural and biochemical characteristics (Aesculin Bile Agar), being confirmed in certain clinical situations by VITEK 2 Compact system.

The routine antibiotic susceptibility testing of the Enterococcus sp. was performed by Kirby-Bauer diffusion method, interpreted according to the C.L.S.I. guideline (Clinical and Laboratory Standards Institute) from 2013, 2014, respectively 2015.

The antimicrobials that have been tested for Enterococcus strains were ampicillin (A), ciprofloxacin (Cip), cloramphenicol (C), erythromycin (E), linezolid (Lzd), levo-floxacin (Lev), norfloxacin (Nor), penicillin (P), rihampicin (R), teicoplanin (Tec) and vancomycin (Va).

For detection of the HLAR strains 120 µg gentamicin disks have been used.

3. Results and Discussions

The first objective of the study was to assess the involvement in pathology of the enterococci isolated from the hospitalized patients.

Figure 1 shows the dynamics of the number of enterococci in the studied period.
Figure 2 shows the spectrum of infections caused by Enterococcus sp. during the study.

![Figure 2. The spectrum of infections with Enterococcus sp. in the studied period](image)

The analyzed serous fluids were peritoneal (52), pleural (3), pericardic (3) and sinovial fluids (1). Other biological samples from which Enterococcus sp. has been isolated were blood, bile, genital secretions and catheters fragments.

We have also analyzed the spectrum of the infections with Enterococcus sp. in each year of the study to see if there have been significant differences, as shown in Figures 3-5.

![Fig. 3. Enterococcus infections in 2013](image)

![Fig. 4. Enterococcus infections in 2014](image)

![Fig. 5. Enterococcus infections in 2015](image)
From the results it can be observed that the spectrum of infections with Enterococcus was relatively constant during the study.

The second objective of the retrospective study was to analyze the level of antibiotic resistance of Enterococcus sp. The results of the antibiograms during the studied period are illustrated in Figure 6.

In Figures 7-9 are presented the results obtained for the antibiotic susceptibility tests for each year of study.

During the study, higher level of resistance were recorded to macrolides (erythromycin - 56.1%), fluoroquinolones (ciprofloxacin – 46.6%, levofloxacin – 46.3%, norfloxacin – 39.9%) and rifampicin (43.3%). The isolates strains had high sensitivity to glycopeptides (vancomycin – 99.2%; teicoplanin – 99.2%). There were also observed various levels of resistance to the other tested antibiotics.
During the study it was also analyzed the dynamics of the number of HLAR strains, knowing that these strains represent a major infections control challenge and an important cause of nosocomial infections. The results are shown in Figure 10. The pathogenic role of HLAR strains for the entire studied period are illustrated in Figure 11.
Fig. 10. The dynamics of the number of HLAR strains in the studied period

Fig. 11. The spectrum of infections with HLAR strains in the studied period

The pathogenic role of HLAR strains for each year of the studied period are illustrated in Figures 12-14.

Fig. 12. Infections with HLAR strains in 2013

Fig. 13. Infections with HLAR strains in 2014

Fig. 14. Infections with HLAR strains in 2015
It was also analyzed the resistance to antibiotics of the HLAR strains, which is an important aspect in the evaluation of the therapeutical options for these infections. The results for the entire period and for each year is illustrated in Figures 15-18.

During the study it was recorded an increase of the number of Enterococcus strains, especially in the last year.

The results regarding the pathogenic role of Enterococcus sp. were similar to those obtained in previous studies in the same medical unit, these germs being most commonly involved in the urinary tract infections and wound infections of the hospitalized patients. [1], [2], [6]

As in other studies conducted in the same hospital, the isolated strains have presented resistance to all tested antibiotics, especially to fluoroquinolones.

Also these germs have retained their sensitivity to glycopeptides. [1], [2], [6]

The levels of resistance to antibiotics of Enterococcus species resulting from this study falls within the data published by the European EARS-Net surveillance system in 2014. The HLAR strains share (39.85%) was lower than the percentage for Romania (76.5% - E. faecalis; 84.2% - E. faecium) but greater than the percentage for Europe (28.8%) in 2014. The resistance to vancomycin was very low (0.8%) compared to the national percentage (3.9% - E. faecalis, 25% - E. faecium) [8], [9]
4. Conclusions

- The spectrum of enterococcal infections in hospitalized patients was large and relatively constant in the studied period, being dominated by urinary tract infections (38.8%), wound infections (30.1%) and intraabdominal and pelvic abscesses (10.9%).
- During the studied period, higher level of resistance was recorded to macrolides and fluoroquinolones. The Enterococcus sp. had high sensitivity to glycopeptides. There were obtained various levels of resistance to the other tested antibiotics.
- The share of HLAR strains was relatively high (39.85%) and constant during the study.
- The results of the study sustain the importance of the identification of the species and of the resistance phenotypes in case of these germs in order to optimize the etiological therapy.

References