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Occurrence of Antibiotic Resistant Enterococci in Clinical Specimens from a Pediatric Hospital

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A b s t r a c t

Enterococcal infection has become a major clinical problem and *E. faecalis* and *E. faecium* are the most frequently isolated species. However, the isolation of other species (*E. casseliflavus*, *E. gallinarum*, *E. durans*) from clinical materials was reported recently. The aim of this study was to evaluate drug resistance of 97 species of enterococci isolated from clinical specimens of Upper Silesian Health Center of Child and Mother in Katowice. Each strain was tested for susceptibility to vancomycin, teicoplanin, aminoglycosides (gentamycin and streptomycin) and synercid (quinupristine/ dalfopristine) by the E-test method. Fifty three percent of studied enterococci demonstrated high level aminoglycoside resistance (HLAR) (MIC > 1024 μg/ml). Sixty three strains of *E. faecalis* were sensitive to vancomycin (MIC 1–4 μg/ml), but 5 strains demonstrated low sensitivity (3 strains with MIC = 6 μg/ml and 2 strains with MIC = 24 μg/ml). All studied enterococci were sensitive to teicoplanin (MIC < 2 μg/ml). A high percentage of *E. faecium* (70%) resistant to synercid was demonstrated (MIC = 2–24 μg/ml). Infection control and monitoring of antibiotic sensitivity among isolated hospital strains may prevent the transmission of resistant strains in a pediatric hospital.

K e y w o r d s: enterococci, HLAR, glycopeptides, synercid

The wide and uncontrolled use of antibiotics, especially wide spectrum antibiotics, has selected highly resistant bacterial strains. ESBL producing gram-negative *Enterobacteriaceae* or vancomycin resistant enterococci (VRE) are examples of such strains (Baier *et al.*, 1998; van der Braak *et al.*, 2002; Malik *et al.*, 1999; Perencevich *et al.,* 2004). According to recent publications (Schouten *et al.*, 1999; Silverman *et al.*, 1998) the number of infections caused by gram-positive bacteria: staphylococci (*S. aureus* and coagulase negative staphylococci – CNS) and enterococci has been rising in recent years, whereas the number of infections caused by gram-negative bacteria is falling.

Enterococci pose an important clinical problem despite the fact that they do not have any major virulence factors. Enterococci are naturally resistant to temperature and disinfectants which determines their pathogenic activity. They also have high natural resistance to many groups of antibiotics: cephalosporins, aminoglycosides and quinolones. Enterococci rapidly acquire resistance to antibiotics by means of mutations (high level resistance to aminoglycosides) or as a result of the transfer of genes located in plasmids/transposons or due to the incorporation of integrons (resistance to glycopeptides, mainly vancomycin – VRE) (Dzierżanowska *et al.*, 2004; Dzierżanowska, 2000; Facklam *et al.*, 1999).

Two mechanisms of resistance to aminoglycosides are typical for enterococci: medium level resistance to aminoglycosides which is a result of decreased permeability of bacterial cell wall membranes (MIC for streptomycin 62–500 mg/l) and high level resistance – HLAR due to mutation of a ribosomal gene locus which is a target for these antibiotics and also by production of specific enzymes that modify antibiotics (MIC for streptomycin >2000 mg/l).

Isolation of HLAR strains from clinical specimens does not allow the use of combined antibiotic therapy using antibiotics targeting bacterial cell wall components (penicillins or vancomycin) and aminoglycosides.

Resistance to vancomycin is a result of altered antibiotic binding target in the bacterial cell. Six phenotypes of resistance caused by the presence of *van A – van G* genes have been described. The most frequent is

phenotype Van A, responsible for resistance to vancomycin and teicoplanin, mainly in *E. faecalis* and *E. faecium* strains. This resistance appears often as a result of earlier antibiotic therapy. The gene responsible for this type of resistance is located in transposon Tn*1546* and can be transferred to other strains of enterococci as well as staphylococci. The frequency of such strains differs in Europe as well as around the world. The first vancomycin resistant strain was isolated in Poland in 1996 in a haematology unit (Bronk *et al.*, 1997; Hryniewicz *et al.*, 1998).

The recommended drug for treatment of infections caused by multiresistant enterococci and VRE is a streptogramin group representative-synercid (quinupristin/dalfopristin) (Dzierżanowska *et al.*, 2004; Hayes *et al.*, 2001; Hryniewicz *et al.*, 2003; Moellering *et al.*, 1999). Synercid is also used in cases of infections caused by methicillin resistant and vancomycin resistant staphylococci strains (MRSA, VRSA).

It has been proved that even after a few days in hospital, strains representing the physiological flora of the patient are replaced by hospital strains highly resistant to antibiotics (Dzierżanowska, 1999; Malik *et al.*, 1999; Neely *et al.*, 2001). The units with the highest risk of infection are: intensive care unit, neonatal unit, haematology, oncology and surgical units as well as burns units (Bronk *et al.*, 1997; Dzierżanowska, 1999; Malik *et al.*, 1999; Neely *et al.*, 2001; Ozorowski *et al.*, 2003).

The aim of this study was to evaluate the antibiotic resistance of enterococci isolated from clinical specimens of Upper Silesian Health Center of Child and Mother in Katowice.

Ninety seven strains of enterococci causing urinary tract infections in patients hospitalized in nephrology (65), pediatrics (9), intensive care, neonatal pathology (11), gastroenterology (12) units of the pediatric hospital – Health Center of Mother and Child in Katowice were studied.

The isolated strains of enterococci were identified by using routine laboratory diagnostic methods and Rapid ID 32 Strep, (BioMérieux, France) biochemical tests.

Antibiotic susceptibility of isolated strains was examined by: disc diffusion method for vancomycin, teicoplanin, gentamycin (120 μg), streptomycin (300 μg) and synercid with guidelines of Polish National Center of Bacterial Antibiotic Susceptibility and NCCLS and E-Test method (AB Biodisk, Sweden) (MICs for vancomycin, teicoplanin, gentamycin, streptomycin and synercid) (Table I).

Table I
MIC for selected antibiotics, inhibiting growth of enterococci, determined by E-test
(*Enterococcus faecalis*, *Enterococcus faecium* and others*)

MIC μg/ml	Vancomycin						Teicoplanin						Synercid			
	E. faecalis		*E. faecalis*		other*		*E. faecalis*		*E. faecalis*		other*		*E. faecalis*		other*	
	no	%	no	%	no	%	no	%	no	%	no	%	no	%	no	%
0.12	0	0	0	0	0	0	3	4.4	0	0	0	0	0	0	0	0
0.19	0	0	0	0	0	0	3	4.4	0	0	2	18.2	0	0	0	0
0.25	0	0	0	0	0	0	20	29.4	2	11.1	1	9.1	0	0	0	0
0,38	0	0	0	0	0	0	21	30.9	1	5.5	1	9.1	0	0	0	0
0.5	0	0	0	0	0	0	13	19.1	2	11.1	1	9.1	1	5.6	0	0
0.75	0	0	0	0	0	0	3	4.4	1	5.5	2	18.2	1	5.6	1	9.1
1	1	1.5	0	5.6	0	0	4	5.9	7	38.9	3	27.3	1	5.6	0	0
1.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	9.1
1.5	1	1.5	1	5.6	4	36.4	1	1.5	3	16.8	1	9.1	1	5.6	2	18.2
2	17	25	15	83.3	7	63.6	0	0	2	11.1	0	0	7	38.9	0	0
3	25	36.8	2	11.1	0	0	0	0	0	0	0	0	1	5.6	2	18.2
4	19	27.9	0	0	0	0	0	0	0	0	0	0	1	5.6	2	18.2
6	3	4.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	2	11.2	1	9.1
12	0	0	0	0	0	0	0	0	0	0	0	0	1	5.6	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	2	11.2	1	9.1
24	2	2.9	0	0	0	0	0	0	0	0	0	0	0	0	1	9.1
Total	68	100	18	100	11	100	68	100	18	100	11	100	18	100	11	100

* – *E. avium* (2), *E. casseliflavus* (3), *E. gallinarum* (3), *E. durans* (3)

We identified the following species among the examined enterococci: *E. faecalis* (68), *E. faecium* (18), *E. avium* (2), *E. casseliflavus* (3), *E. durans* (3), *E. gallinarum* (3). The most common species were *E. faecalis* (78%) and *E. faecium* (18%) which proves the results of other authors. The other species of enterococci were rarely isolated.

Fifty four percent of all enterococci (53) were characterized as HLAR strains. Seventeen strains (32%) presented high level resistance to both aminoglycosides, gentamicin and streptomycin. Twenty five (47%) strains presented high level resistance to streptomycin and were susceptible to high concentrations of gentamicin. The other eleven (21%) strains were resistant to high concentrations of gentamicin. High level resistance to aminoglycosides was confirmed by E-Test. MIC for aminoglycosides in E-Test was >1024 μg/ml for all HLAR strains, determined in disc-diffusion method. Similar results have been described also by other authors (Dzierżanowska *et al.*, 2004; Schouten *et al.*, 1999; Silverman *et al.*, 1998).

According to NCCLS, enterococci with MIC < 4 μg/ml for vancomycin are susceptible, with MIC 8–16 μg/ml are intermediate-susceptible and with MIC > 32 μg/ml are resistant (Dzierżanowska, 1999; Dzierżanowska, 2000; Hryniewicz *et al.*, 2003). Sixty three *E. faecalis* strains demonstrated MIC for vancomycin 1–4 μg/ml, five strains had decreased susceptibility (three strains with MIC = 6 μg/ml and two strains with MIC = 24 μg/ml), four of them were HLAR strains (MIC > 1024 μg/ml). All of *E. faecium* strains (18) demonstrated MIC for vancomycin <4 μg/ml, or were highly susceptible to vancomycin.

Other species of enterococci (*E. casseliflavus*, *E. gallinarum*, *etc.*) with MIC > 2 μg/ml show usually decreased susceptibility to vancomycin. Seven out of eleven strains of isolated *E. casseliflavus* and *E. gallinarum* presented MIC = 2 μg/ml for vancomycin.

Enterococci with MIC for teicoplanin <8 μg/ml are susceptible, with MIC = 16 μg/ml – are intermediate susceptible and with MIC > 32 μg/ml are resistant to teicoplanin, according to NCCLS. All our studied enterococci strains were susceptible to teicoplanin (MIC < 2 μg/ml). Enterococci with MIC for synercid <0,5 μg/ml are susceptible, with MIC = 1 μg/ml are intermediate susceptible, with MIC > 2 μg/ml – are resistant. Fourteen out of eighteen isolated *E. faecium* strains (70%) showed a MIC for synercid between 2–24 μg/ml, which means resistance to synercid. Among the other isolated species of enterococci (*E. casseliflavus*, *E. gallinarum*, *E. durans*) only seven strains were resistant to synercid (MIC > 2 μg/ml).

Our results showed that the majority of enterococci strains isolated from clinical specimens obtained from a pediatric hospital were HLAR strains (over 51% *E. faecalis*, 28% *E. faecium* and 21% other species) and most of them were resistant to streptomycin. Such results were also shown by other authors (Hryniewicz *et al.*, 2001; Randhava *et al.*, 2004; Schouten *et al.*, 1999; Silverman *et al.*, 1998).

We found five strains of *E. faecalis* with intermediate sensitivity to vancomycin and seven strains of *E. casseliflavus* and *E. gallinarum* with low level sensitivity to vancomycin. This is alarming news, because infections caused by these strains pose an important therapeutic problem. None of the isolated enterococci were resistant to teicoplanin.

Another alarming fact is also the high percentage of *E. faecium* (70%), *E. casseliflavus* and *E. gallinarum* (65%) resistant to synercid – a drug that has been begun to be used in hospitals in recent years.

Colonization of newborns in first days of life with antibiotic resistant hospital strains of enterococci, especially in intensive care units, creates additional therapeutic problems (van den Braak *et al.*, 2002; Malik *et al.*, 1999; Neely *et al.*, 2001; Ozorowski *et al.*, 2003).

It is possible to limit the spread of alarming pathogens in pediatric hospitals using a number of preventive methods, including appropriate antibiotic policy (Malik *et al.*, 1999; Neely *et al.*, 2001; Ozorowski *et al.*, 2003; Perencevich *et al.*, 2004). Infection control and monitoring of antibiotic susceptibility in isolated hospital strains may prevent further transmission of resistant strains in a pediatric hospital.

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