

Prevalence of vancomycin-resistant enterococci in hospitalized patients and those living in the community in the Czech Republic

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SUMMARY

Between July 1, 2002 and December 31, 2003, rectal swabs from both hospitalized patients and community subjects in the Czech Republic were taken to ascertain the prevalence of vancomycin-resistant enterococci (VRE). The swabs were used for isolating and identifying enterococci and their susceptibility to antibiotics. Vancomycin resistance phenotypes were verified by PCR detection of *vanA*, *vanB*, *vanC1* and *vanC2* genes. A molecular biology analysis was performed in *Enterococcus faecium* VanA strains.

During the observed period, 2,691 rectal swabs from the hospitalized patients and 6,529 rectal swabs from the subjects in community setting were examined. In total, 31 VRE of hospital origin and 13 community-population strains were isolated. The prevalence of VRE in the gastrointestinal tract was 1.9% in the hospitalized patients and 0.4% in the community subjects. The prevailing strains were *Enterococcus faecium* VanA (61.3%) in the VRE of hospital origin and *Enterococcus gallinarum* VanC (46.2%) in the community VRE. Mutual comparison between the hospital and community *Enterococcus faecium* VanA strains showed no similarity.

KEY WORDS: Vancomycin-resistant, enterococci, prevalence, Czech Republic

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INTRODUCTION

Enterococci are gram-positive bacteria commonly found as part of the natural microflora of the gastrointestinal tract in humans and animals. They are currently becoming increasingly more important as causative agents of nosocomial infections of the urinary and respiratory tracts, surgical lesions and bloodstream infections (Murdoch *et al.*, 2002, Cetinkaya *et al.*, 2000). Natural or acquired enterococcal resist-

ance to numerous antimicrobial agents represents a limiting factor in the treatment of enterococcal infections. Until recently, the effective antibiotics comprised glycopeptides (vancomycin and teicoplanin), but their medical use has been considerably restricted due to the occurrence of enterococci resistant to these antimicrobial drugs. Vancomycin-resistant enterococci (VRE) were first isolated in Europe in 1986 and a year later in the USA (Leclercq *et al.*, 1988, Utlley *et al.*, 1988, Kaplan *et al.*, 1988). At present, VRE are described in other countries from both hospitalized patients and subjects from the community population.

Although the main risk factor for VRE occurrence in the hospital setting is the excessive use of glycopeptides, their selection is also contributed to by third-generation cephalosporins, fluoro-

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quinolones and other antibiotics, particularly those acting upon anaerobic bacteria (Heath *et al.*, 1996). In the community setting, the source of VRE can be food of animal origin. According to Aarestrup, more than a half of the total world consumption of antimicrobial drugs is the use in livestock, not only to treat and prevent bacterial infections but also as growth promoters (Aarestrup 1999). In particular antimicrobial agents used for growth promotion and prophylaxis are considered the main cause of the development and spread of bacterial resistance. Despite the fact that the antibiotics administered to animals very often differ from those used in the human population, there is a very close relationship between them which is caused by cross-resistance. The examples are vancomycin and avoparcin, growth promoters whose administration in the past has probably led to the development and spread of VRE. According to several authors, the highest frequency of VRE was observed where avoparcin was administered to livestock and after its ban the frequency decreased not only in animals but also in humans (Bager *et al.*, 1997, Klare *et al.*, 1999).

The presented work aimed to determine the prevalence of VRE in rectal swabs from hospitalized patients as well as from subjects in the community of the Czech Republic.

MATERIAL AND METHODS

The rectal swabs were taken from subjects in the community setting of the District of Olomouc (Czech Republic) and from patients hospitalized in the Teaching Hospital in Olomouc (THO) between July 1, 2002 and December 31, 2003. Subsequently, these were simultaneously inoculated onto blood agar and diagnostic VRE agar (Oxoid) containing vancomycin (at a concentration of 6 mg/L) and meropenem (1 mg/L). After incubation for 18-24 hours at 37°C enterococci were identified according to the criteria established by Facklam and Collins and according to the biochemical properties using the En-coccus test (Pliva-Lachema) (Facklam and Collins 1989). In case of the VRE agar, all black growing colonies were identified. Only one isolate from each THO patient or a subject in the community setting was included in the study, in par-

ticular the strain isolated as the first one in case of repeated detection.

Resistance to vancomycin and teicoplanin was determined by a standardized dilution micromethod (NCCLS 2002). The limit concentrations for susceptible strains were set at 4 mg/L for vancomycin and 8 mg/L for teicoplanin. To determine the glycopeptide resistance phenotypes, vancomycin and teicoplanin were diluted to concentrations ranging from 2 to 1,024 mg/L. The dilution accuracy of both antibiotics was verified with *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 reference strains.

The vancomycin resistance phenotypes were verified by PCR detection of *vanA*, *vanB*, *vanC1* and *vanC2* genes (Dutka-Malen *et al.*, 1995).

The vancomycin-resistant *Enterococcus faecium* strains with the VanA phenotype were assessed using macrorestriction analysis by pulsed-field gel electrophoresis (PFGE). To determine the relationship of strains, fragment profiles of total chromosomal DNA digested with *SmaI* restriction endonuclease were used (Roche Diagnostics). DNA fragments were separated by PFGE (CHEF Mapper, Bio-Rad). Restriction profiles were statistically processed by cluster analysis (Gel Compar, Applied Maths) using methods applied by Kolar *et al.*, for molecular biology analysis of VRE of animal origin (Kolar *et al.*, 2002). PFGE profiles of individual strains were evaluated according to the criteria described by Goering (Goering 1998).

RESULTS

During the observed period, 2,691 rectal swabs from the hospitalized patients and 6,529 rectal swabs from the subjects in community setting were examined. Table 1 lists the number of identified enterococci including vancomycin-resistant strains. From the total of 31 hospital VRE, 29 strains (93.5%) were isolated from haematological patients. Only 2 strains of *Enterococcus gallinarum* VanC were isolated from patients with other diseases.

The species, phenotypes and genotypes of the identified VRE are given in Table 2. The results clearly show that the strains of hospital origin were dominated by *Enterococcus faecium* VanA

TABLE 1 - Prevalence of VRE in hospital and community settings.

	Total number of enterococci	Absolute number of VRE	Percentage of VRE
Hospitalized patients	1,595	31	1.9
Subjects in community setting	2,943	13	0.4
Total	4538	44	0.9

TABLE 2 - Identified VRE genotypes and phenotypes.

Species	Phenotype	Genotype	Number of strains	
			Hospitalized patients	Subjects in community setting
<i>Enterococcus faecium</i>	VanA	vanA	19	2
<i>Enterococcus faecium</i>	VanB	vanB	2	0
<i>Enterococcus faecalis</i>	VanA	vanA	2	0
<i>Enterococcus faecalis</i>	VanB	vanB	4	1
<i>Enterococcus gallinarum</i>	VanC	vanC1	3	6
<i>Enterococcus casseliflavus</i>	VanC	vanC2	1	4

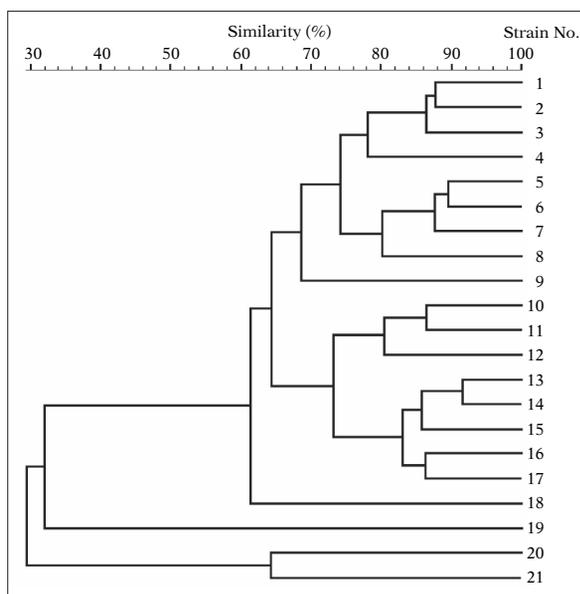


FIGURE 1 - Dendrogram showing *Sma*I macrorestriction pattern similarity of 21 *Enterococcus faecium* VanA isolates, constructed on the basis of Dice coefficients and UPGMA clustering.

Legend: 1 to 19 - hospital strains *Enterococcus faecium* VanA; 20 and 21 - community strains *Enterococcus faecium* VanA.

(61.3%) while *Enterococcus gallinarum* VanC (46.2%) was the prevailing strain in the community VRE.

Based on the macrorestriction analysis of total chromosomal DNA in 21 vancomycin-resistant *Enterococcus faecium* VanA strains, 21 unique restriction profiles were identified, with similarity ranging from 29 to 92%. Only two strain pairs (strains 5-6 and 13-14) exhibited DNA-profile similarity of at least 90%. In agreement with the above mentioned methods, these strains were isolated from 4 different patients hospitalized at the same THO ward over two months. Therefore these are assumed to belong to the same clonal type. Mutual comparison of hospital and community VRE showed no similarity. A dendrogram of similarity between all 21 analyzed strains is presented in Figure 1.

DISCUSSION

A complicating factor in the development of bacterial resistance is, among others, the development and spread of VRE. Due to difficult antibi-

otic treatment, they pose a potential risk in case of infections caused by these strains. This fact should be kept in mind especially in haemato-oncological patients who are in particular danger because of their compromised immunity. In our group, nearly 94% of hospital-setting VRE were isolated from patients with haemato-oncological diseases. Apart from the underlying disease itself, decreased function of the immune system and longer hospitalization, this could be explained by frequent administration of antibiotics to these patients. And it is the selection pressure of antimicrobial agents that can account for the significantly lower proportion of *Enterococcus gallinarum* and *Enterococcus casseliflavus* strains as compared to the community-setting VRE with the same strains representing 77%. On the other hand, the hospital-setting VRE were dominated by *Enterococcus faecium* VanA strains with the proportion exceeding 60%. The prevalence of VRE in the THO patients' rectal swabs reached 1.9%. The figure is comparable to those in other European studies assessing the prevalence of VRE in rectal swabs from hospitalized patients. According to Endtz *et al.*, in the Netherlands the prevalence of VRE was 2%; in the ICUs of selected French hospitals a 5 per cent prevalence was noted and in a Belgian study, the frequency of VRE in the gastrointestinal tract of hospitalized patients amounted to 4% (Endtz *et al.*, 1997, Boisivon *et al.*, 1997, Gordts *et al.*, 1995).

As seen from the molecular biology analyses in the present study, both endogenous and exogenous origin of *Enterococcus faecium* VanA strains can be assumed in the hospital conditions. The presence of only two clonal types of four strains was proved and the strains belonging to individual types exhibited DNA-profile similarity of at least 90%, suggesting that the strains are either identical or very closely related. So their clonal spread is highly probable, most likely by the hands of healthcare workers for the most part. Finding a relatively large number of unique genotypes of *Enterococcus faecium* VanA strains of hospital origin in the study may result from the fact that this strain set under study represented carrier isolates which may be community acquired. The hypothesis is supported by the confirmed presence of vancomycin-resistant *Enterococcus faecium* VanA strains in rectal

swabs from subjects in the community setting. However, identification of specific genetic linkage between highly variable community-acquired and epidemic hospital isolates would require additional particularized typing methods.

Carriage of VRE in the gastrointestinal tract of persons in the Czech Republic community was confirmed in 0.4%. The monitored District of Olomouc can be characterized as an agricultural region with an intensive livestock industry that utilized avoparcin in the past. In their work assessing the prevalence of rectal carriage in a French community area of a similar character, Gambarotto *et al.*, proved the prevalence of VRE in 12% of the population (Gambarotto *et al.*, 2000). In contrast, other European community-based studies documented a lower frequency, for example 2% in the Netherlands and the United Kingdom (Endtz *et al.*, 1997, Jordens *et al.*, 1994). The data show that community carriage of VRE in the Czech Republic is very low and does not exceed values stated for other European countries.

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