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ARE VANCOMYCIN-RESISTANT ENTEROCOCCI A PROBLEM IN POLISH HOSPITALS?

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ABSTRACT

Vancomycin-resistant enterococci are responsible for a significant percentage of hospital-acquired infections in the world. They can easily spread from patient to patient in the hospital environment, usually via the hands of medical staff. The infection most often develops in at-risk patients and poses an enormous epidemiological, as well as therapeutic, problem. In Poland, vancomycin-resistant enterococci are considered to be bacterial alert pathogens, currently viewed as particularly dangerous to public health. Until now, eight phenotypes of acquired vancomycin resistance have been described, but the most important clinically are *VanA* and *VanB* phenotypes because of their incidence and the speed of phenotype acquisition between enterococcal cells. VRE strains isolated in Poland belong to the clonal complex CC17, which is widespread in Europe and worldwide. First *VanA* VRE strains were described in 1996 in Gdansk. The first isolate of *VanB* VRE phenotype was isolated in 1999 from a patient undergoing long-term therapy with vancomycin and hospitalized in the intensive care unit of one of Warsaw's hospitals. The latest European Antimicrobial Resistance Surveillance Network report places Poland among countries with a proportion of resistant strains at 10 to <25%, along with Germany, Portugal, England, Greece, Romania and Latvia.

Key words: *vancomycin-resistant enterococci, VRE colonization, hospital-acquired infections*

OBJECTIVE

The objective of the present study is to evaluate the epidemiologicalsituation of the incidence of VRE strains in Polish hospitals based on a review of the available literature.

INTRODUCTION

Enterococci as normal flora. The natural habitat of enterococci in healthy people is the mucosa of the gastrointestinal tract, particularly colon, the oral cavity, and the urethra, and in women, also the vagina (1). Two main species of enterococci occur in the intestinal contents of healthy adults. *E. faecalis* (39–95%) is predominant, followed by *E. faecium* (3–47%) fulfilling a protective function against colonization of the mucosa by pathogenic bacteria (1). Enterococci also live in the gastrointestinal tract of animals and in the environment, water, sewage, as well as food (2,3).

First cases of VRE. For a very long time, enterococci were deemed relatively pathogenic microbes causing infection in humans only in rare cases (2,3). However, their significance as agents causing infections has been on the increase since the 1980s, when the first vancomycin-resistant (VRE – *Vancomycin-Resistant Enterococci*) strains were isolated from clinical material. In Europe, it was in England and France in 1988 and in the US in 1989 (4).

HOSPITAL-ACQUIRED INFECTIONS CAUSED BY ENTEROCOCCI

The genus *Enterococcus* is the etiologic agent of infections of, among others, the urinary tract, burn wounds, bedsores, cholangitis, endocarditis, sepsis, intra-abdom-

inal abscesses and peritonitis (4). Infections caused by enterococci develop in at-risk patients, including patients with proliferative disorders and concomitant neutropenia, patients with liver failure or chronic liver disease, and following organ transplants, as well as in patients treated in intensive care units (3,4). The biggest epidemiological challenge is posed by infections caused by enterococci that start in the hospital environment.

Enterococci display a high tolerance to abiotic components, e.g. the presence of disinfectants. They are capable of enduring extreme temperatures, high pH, and salt concentration (3). They can easily spread in the hospital environment from patient to patient, most frequently via the hands of medical staff (3).

ANTIBIOTIC RESISTANCE PHENOTYPES

Infections brought about by *Enterococcus spp.* also represent a significant therapeutic issue as these strains have a natural as well as acquired resistance to the majority of antibiotics commonly used in medicine. The genus *E. faecium* is naturally resistant to cephalosporins, low concentrations of aminoglycosides, clindamycin, trimethoprim/sulfamethoxazole, lincosamides and is characterized by decreased sensitivity to penicillin.

Enterococci also exhibit acquired resistance mechanisms, among which, currently, the greatest clinical and epidemiological significance is presented by resistance to high concentrations of aminoglycoside antibiotics (high-level aminoglicoside resistance; HLAR) and resistance to glycopeptides (vancomycin-resistant enterococci; VRE) (5). Eight phenotypes of acquired glycopeptide resistance have been described to date: *VanA*, *VanB* (variants *B1-B3*), *VanD*, *VanE*, *VanG*, *VanL*, *VanM*, and *VanN*. Also, one phenotype of natural resistance (*VanC*) is known which is unique to the species *E. gallinarum* and *E. casseliflavus* (6). The most clinically significant phenotypes are *VanA* and *VanB* due to their prevalence and the speed of acquisition of the phenotype between enterococcal cells using mobile genetic elements in the form of plasmids and transposons. The location of genes associated with phenotypes *VanA* and *VanB* in mobile genetic elements determines a high epidemic potential of these strains (3,5).

DEVELOPMENT OF VRE PHENOTYPE

The hospital environment is particularly accountable for selective pressure and has led to the development of multidrug-resistant enterococcal strains through the excessive application of many antibiotics and chemotherapeutics in inpatient, as well as outpatient, health care, including cephalosporins, aminoglycosides and fluoroquinolones, in the 1990s which led to the development of VRE strains (1). The development of glycopeptide resistance proceeded in two ways. In the US, VRE strains emerged due to the mass administration of vancomycin in the treatment of infections with methicillin resistant *S. aureus* (MRSA) strains, as well as *per os* for the treatment of antibiotic-associated diarrhea caused by *Clostridium difficile*. In the US, hospital reservoirs of VRE were not observed (3). While in Europe, the main reservoir of VRE strains was constituted by livestock animals (e.g. chickens) fed on fodder containing avoparcin (glycopeptide, banned in the EU in 1998). Food products of animal origin are considered to be one of the major vectors involved in the transmission of VRE strains from animals to humans. The second reservoir of VRE strains, also extramural, may be healthy people – asymptomatic carriers (7). Prior colonization with VRE strains may lead to VRE infection. However, in those with a properly functioning immune system, infections develop less frequently, because such a population of carriers is less vulnerable to infection. Rectal VRE carriage can persist for a very long time – from a few weeks to several months (8).

TREATMENT OPTIONS.

Therapeutic alternatives in the treatment of infections with multidrug-resistant enterococci, including those resistant to glycopeptides, are restricted to antibiotics introduced to hospital use relatively recently, such as quinupristin/dalfopristin, linezolid, tigecycline, daptomycin. However, these drugs are approved for the treatment of few conditions and antibiotic resistance to them has already been described in the literature (17).

VIRULENCE FACTORS

VRE isolates reported in Poland belong to the CC17 clonal complex, which is widespread in Europe and worldwide and was separated using multilocus sequence typing (MLST). *E. faecium* strains belonging to the CC17 clonal complex are characterized, apart from resistance to vancomycin, by resistance to ciprofloxacin and ampicillin and certain genetic traits (9). These involve the presence of potential virulence factors, among others, the extracellular surface protein Esp (encoded by the *esp* gene), hyaluronidase (encoded by the hyl_{Efm} gene) and the collagen-binding protein Acm (encoded by the *ace* gene). They facilitate enterococcal survival in the hospital environment. Enterococci that possess virulence determinants are potentially able to cause infection of a more severe course than strains deprived of them. Research is still in progress that will help to

understand better the differences in pathogenicity of pathogenic and nonpathogenic strains. (10,11,12).

VRE IN POLAND – LEGISLATION

Their unique properties and the role in hospital-acquired infections led to the classification of enterococci as alert pathogens. In Poland, since 1 January 2012, a Minister of Health regulation dated 23 December 2011 has been in force regarding the list of alert factors, records of hospital infections, and alert factors and reports on the current epidemiological situation of hospitals. Among bacterial alert pathogens, currently considered particularly dangerous to public health, enterococci (*Enterococcus* spp.) resistant to glycopeptides (VRE) or oxazolidinone (13) were listed in second place. This regulation is a continuation of the obligation to monitor hospital-acquired infections, which is regulated in Poland by the Act of 5 December 2008 on preventing and eliminating infections and infectious diseases in people. The Act also imposes an obligation to prevent and eliminate infections and infectious diseases in people, including the rules and procedures for identifying and monitoring the epidemiological situation, taking action against epidemics and preventative actions to inactivate the sources of infection, cutting the ways in which infections and infectious diseases spread and immunization of individuals susceptible to infection (14).

Disease outbreaks should be reported by Hospital Infection Control Teams to the regional branches of sanitary and epidemiological stations, and those, under the existing reporting system, should submit information to the Provincial Sanitary and Epidemiological Stations. Subsequent stages in the epidemiological surveillance system are: Chief Sanitary Inspector, the National Institute of Public Health – National Institute of Hygiene, the European Centre for Disease Control (ECDC) and the World Health Organization (WHO).

The most important element in the process of proper control of hospital-acquired infections is an appropriate system of registration. But suitable microbiological surveillance is also a crucial element of control. It consists in systematic collection, analysis and interpretation of data on etiological agents of infections and their sensitivity to antibiotics. This makes it possible to recognize the participation of individual agents in the selected clinical form of infection and the assessment of the changes in drug resistance (15). Sensitivity of the existing system of epidemiological surveillance is difficult to assess. It depends, among others, on diagnostic capabilities of medical personnel and the scope of research in microbiological laboratories, but also on the fact that doctors and heads of laboratories meet the requirement of reporting infections and alert pathogens.

EPIDEMIOLOGICAL SITUATION CONCERNING VRE IN POLAND

The first VRE isolates were reported in Poland in 1996. They were three *E. faecium* strains carrying the *vanA* operon, genetically unrelated, isolated from adult patients hospitalized in the Department of Hematology of the University Clinic in Gdańsk (16,17). The first VRE isolate with *vanB* phenotype, carrying the *vanB2* gene variant, was isolated in 1999 from a patient undergoing long-term vancomycin therapy hospitalized in the Intensive Care Unit (OIT) in one of Warsaw hospitals. The introduction of proper infection control procedures made it possible to prevent further spread of VRE in that hospital (18). In the period of 1999–2000, there was an outbreak (independent of the previous one) caused by enterococci with the *vanB* phenotype in another Warsaw hospital specializing in treating hematological patients (19). In 2005, there was a slightly different, mixed outbreak, caused by strains of the genus *E. faecium* and *E. raffinosus* carrying the *vanA* operon. The outbreak affected the Departments of: Hematology, Nephrology and Surgery of the University Hospital in Kraków (20).

However, the vast majority of outbreaks registered in Poland and caused by VRE was and is brought about by two species, *E. faecium* and *E. faecalis* (17). In 2009, there were two outbreaks with the VRE *vanA* phenotype, which occurred simultaneously at the Department of Oncology and the Department of Hematology and Transplantation in Warsaw (21). The literature also describes the occurrence of VRE strains originating from patients of University Hospital in Wrocław (years 2007–2009) as well as from patients after transplants of one of the hospitals in Warsaw (years 2010–2012) (10,16). In 2012 and 2013, there were 2 VRE outbreaks in one of the specialized hospitals in Kraków (unpublished author's data, 22,23).

Currently, disease outbreaks caused by VRE occur sporadically in Poland and are local in scope (Table I,17). In 2012, hospital outbreaks caused by *E. faecium* VRE constituted 1.3% of alert agents causing outbreaks compared to the total number of reported outbreaks. In 2013 it was 2% and in 2014, 1% (24,25). The clinical form of hospital-acquired infections, from which the *E. faecium* VRE etiologic agent was isolated (together with *K. pneumoniae* ESBL, *S. aureus* MRSA) were surgical site infections, which accounted for 6.3% in 2012, 6.9% in 2013, and 4.5% in 2014 among all clinical forms of hospital-acquired infections (21,22). The National Consultant for Medical Microbiology recognizes the fact that, in the upcoming years in intensive care units (ICU) in Poland, a bigger problem than infections with VRE will be the spread of infections with drug-resistant Gram-negative rods (KPC strains, carbapenem-resistant *A. baumannii* and multidrug-resistant *P. aeruginosa*) (26).

Table I. Notifiability of hospital outbreaks caused by VRE strains in annual reports of Provincial Sanitary--Epidemiological Stations in the years 2012–2014.

Province	Years		
	2012	2013	2014
Dolnośląskie	-	2 HLAR and VRE spp. (1 in nephrology unit, 1 in surgical unit)	-
Lubuskie	No data	-	-
Małopolskie	2 VREfm	2 VREfm	-
Mazowieckie	-	1 VREfm	No data
Opolskie	-	-	-
Podkarpackie	No data	No data	1 VREfm
Podlaskie	-	-	-
Śląskie	-	-	-
Świętokrzyskie	No data	No data	-
Wielkopolskie	-	No data	-
Zachodniopomorskie	-	-	-
Kujawsko-Pomorskie Lubelskie, Łódzkie, Pomorskie, Świętokrzyskie	*	*	*

Legend:
- there was no disease outbreak caused by VRE strains among the reported outbreaks
no data – no data concerning outbreaks caused by VRE in the report or lack of reports on Provincial Sanitary-Epidemiological Stations websites (on the basis of annual reports for the years 2012–2014 available on the websites belonging to provincial sanitary-epidemiological stations)
VREfm – *E. faecium* vancomycin-resistant strains
HLAR – resistance to high concentrations of aminoglycoside antibiotics

The occurrence of alert pathogens has become a major concern on a regional and global scale. In 1998, ECDC established the European Antimicrobial Resistance Surveillance Network (EARS-Net) collecting data concerning invasive infections with drug-resistant pathogens also from our country and collating them with data from other European countries. The data collected annually in EARS-Net (until 2009) placed Poland among countries in which VRE was not a significant epidemiological problem. The data for that period, however, should be analyzed carefully because the number of isolates tested derived from Poland throughout the year was limited, especially for *E. faecium* (17,28).

In Poland, as in other European countries (apart from Greece), *E. faecalis* VRE infections are not a major problem for healthcare facilities. This trend has persisted for several years and is true even today. While the situation concerning *E. faecium* VRE is different. According to EARS-Net data for 2009, Poland was included into the group of countries with a low level (<5%) of infections caused by *E. faecium* VRE strains. However, at the same time, the epidemiological situation of the neighboring countries (Germany and Slovakia: 5 to <10%; Lithuania: 10 to <25%) and countries where many Poles live and work (Ireland: 25 to <50%, England: 10 to <25%), as well as countries to which Poles like to travel (Greece: 25 to <50%, Portugal: 10 to <25%) was different and these were the countries with high and the highest proportions of infections caused by VRE in Europe. The latest EARS-Net report (data for 2013) places Poland among countries with the percentage of resistant strains at a level exceeding 10% but fewer than 25% together with Germany, Portugal, England, Greece, Romania and Latvia. The highest proportion of *E. faecium* VRE strains (>25%) still remains in Ireland (27). EARS-Net data are to be looked at with due attention and appropriately interpreted, because over the years notifiability of invasive infections caused by VRE in various European countries was significantly different on account of, among others, a variable number of laboratories which send their data over and due to the use of distinct guidelines according to which strains were classified as resistant (17,28). Perhaps the differences in the proportion of VRE strain occurrence in Europe are the result of disparate antibiotic policies in different countries.

CONCLUSION

Acquisition of resistance by bacteria of the genus *Enterococcus* is a result of plasticity of the genome of these cocci as well as continuous selective pressure which they are subjected to in the hospital environment. Heterogeneous strains and the ones more sensitive to antibiotics are being replaced by strains belonging to hyperepidemic clonal complexes of multidrug-resistant enterococci.

The majority of VRE outbreaks registered in Poland was and still is caused by the species *E. faecium*, which is the principal source of resistance to glycopeptides of phenotypes *vanA* and *vanB*. Epidemic outbreaks caused by VRE strains occur occasionally in Poland and are local in scope. VRE outbreaks described in the literature up to now have primarily occurred in facilities caring for oncology and oncology–hematology patients. The annual reports of the Provincial Sanitary–Epidemiological Stations indicate that in the past three years there has been no epidemic outbreak caused by VRE in the Opolskie, Podlaskie, Śląskie and Zachodniopomorskie provinces. Unfortunately, the data published on the website of the Provincial Sanitary–Epidemiological Station are of limited value because not every institution includes the data from the outbreak in its statements.

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