ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2, 231–236
www.aaem.pl

Prevalence and distribution of VRE (vancomycin resistant enterococci) and VSE (vancomycin susceptible enterococci) strains in the breeding environment

Krzysztof Skowron[1], Alicja Jeleńska[2], Zbigniew Paluszak[2], Beata Szala[2]

[1] *Department of Microbiology, Faculty of Pharmacy, Nicolaus Copernicus University in Toruń, Collegium Medicum of L. Rydygier in Bydgoszcz, Poland*
[2] *Department of Microbiology and Food Technology, University of Technology and Life Sciences, Bydgoszcz, Poland*

Skowron K, Jeleńska A, Paluszak Z, Szala B. Prevalence and distribution of VRE (vancomycin resistant enterococci) and VSE (vancomycin susceptible enterococci) strains in the breeding environment. Ann Agric Environ Med. 2016; 23(2): 231–236. doi: 10.5604/12321966.1203882

Abstract

Introduction. Intensive animal production causes numerous problems. Facilities connected with animal maintenance not only cause environmental pollution, but also pose a great sanitary and epidemiological threat. Long-term use of antibiotics in animal production lead animal-borne microorganisms to develop multiple resistance mechanisms, transferred to the typical environmental bacteria.

Objective. The aim of this study was assessment of *E. faecalis, E. faecium, E. durans* and *E. hirae* prevalence in samples gathered from swine production sectors, and determination of the contribution of VRE (vancomycin resistant enterococci) strains and their resistance. The degree of relationship between isolates of each species from genus *Enterococcus* was also determined.

Materials and method. 195 isolates were obtained, from which DNA was isolated. Genus identification was conducted with the primers specific to the 16S rRNA region, and identification of the species with primers specific to sequence of gene *sodA* in Multiplex PCR reaction. Resistance to vancomycin (6 μg×ml^{-1}) was tested using a screening method on Muller Hinton Agar. To assess resistance type Multiplex PCR, amplifying products corresponding to genes VanA, VanB and VanC, was conducted. Genotyping was conducted using the PCR-RAPD method.

Results. Among the 195 isolates, 133 (68%) belonged to *E. hirae*. The other species contributions were respectively: *E. faecalis* – 21%, *E. durans* – 8% and *E. faecium* – 3%. Only 2 isolates of *E. hirae,* being different strains, were resistant to vancomycin. Both were representing phenotype $VanC_1$. 60 genetically different strains were defined. The possible contamination paths involved animal feed and spreading of excrements by slaughtered individuals or on personnel's footwear.

Conclusions. The obtained results indicate a very low percentage of VRE strains in the tested piggery, resulting in a low health risk to piggery, slaughterhouse or abattoir employees

Key words

Enterococcus spp., VRE strains, breeding environment, swine production

INTRODUCTION

Intensive animal production causes numerous problems. The facilities connected with animal maintenance not only cause environmental pollution, but also pose a great sanitary and epidemiological threat. The excrements produced in huge amounts and containing a wide spectrum of microorganisms, including obligatory pathogens, are notably dangerous. Not only the virulence of these strains but also their resistance to antibiotics pose a great problem. Long-term use of antibiotics in animal production lead animal-borne microorganisms to develop various resistance mechanisms, which can be transferred to the typical environmental bacteria. Work with livestock may lead to severe infections, such as bronchitis, noted among 11.6% of piggery employees [1]. Due to the massive occurrence of microorganisms in the gastrointestinal tract of farm animals, they are also deposited in excrements and contaminate elements of livestock buildings. Among pathogens present in the breeding environment, species of genera *Salmonella, Leptospira, Treponema, Mycobacterium*, *Brucella*, *Bacillus* or *E. coli* can be noted [2].

Species of genus *Enterococcus* are important environmental microorganisms also present in animal production waste. They are human gastrointestinal tract commensals, accounting for 1% of intestinal microflora. *E. faecalis* accounts for 90–95% and *E. faecium* 5–10% of enterococci present in the gastrointestinal tract [3]. The main transmission route to the environment is fertilizing soil with slurry. Moreover, transmission and infection may occur due to work with the livestock or slurry; hence, the occupational groups predestined to infections with strains resistant to antibiotics are farmers, zootechnicians, vets, slaughterhouse and agricultural biogas plant employees [4].

Enterococci exhibit several resistance mechanisms against β lactams, aminoglycosydes and glycopeptides. HLPR strains (High-Level Penicilin Resistant) are resistant to high penicillin concentrations (MIC ≥ 256 μg×ml^{-1}) and HLAR (High-Level Aminoglycoside Resistance) to high aminoglycosydes concentrations (MIC ≥ 2000 μg×ml^{-1}).

Address for correspondence: Krzysztof Skowron, Department of Microbiology, Faculty of Pharmacy, Nicolaus Copernicus University in Toruń, Collegium Medicum of L. Rydygier in Bydgoszcz, 9 Marii Skłodowskiej-Curie, 85–094 Bydgoszcz, Poland
E-mail: skowron238@wp.pl

Received: 27 October 2013; accepted: 26 March January 2014

Due to the decreasing efficiency of aminoglycosydes and β lactams, glycopeptides, such as vancomycine, turned out to be solution in the treatment of severe enterococcal infections. Its function is to block the synthesis of cell wall peptidoglycans [5]. Frequent use of vancomycin caused enterococci to develop antibiotic resistance. A multitude of antibiotic resistance mechanisms acquisition led to the development of VRE (Vankomycin Resistant Enterococci), HLAR and MDR (Multiple Drug Resistant) strains. After few years of vancomycin use (1989–1993), the VRE contribution was 3.9% and exhibits an increasing tendency [6]. Five VRE phenotypes were identified, of these, 4 depend on the plasmid transfer (VanA, VanB, VanD, VanE), while VanC depends on the genome and is not transferred in conjugation. VRE resistance principle is modification of the antibiotic's aim, which are cell wall components [5].

The main sources of VRE strains are hospitals. Apart from the outbreak, such as patients involved in agriculture, other contamination routes are medical utensils and patients' transfer between medical facilities [7]. Other most significant infection sources are agriculture and municipal wastewater. VRE strains are isolated both from water treatment plants and agricultural or meat production waste, which means that VRE strains have already reached the food distribution chain [8].

Enterococci are etiological factors of numerous human and animal diseases. Synergistic occurrence is typical. The most frequent disease in swine production is diarrhea (*E. durans*) and endocarditis (*E. hirae*), in cattle myositis (*E. faecalis*) and cholangitis or pancreatitis (*E. hirae*). The cause of losses in poultry is sepsis (*E. hirae*, *E. durans* and *E. faecalis*) [9, 10].

Among clinical isolates, *E. faecalis* and *E. faecium* account for 90%. Other frequent species are *E. avium, E. gallinarum, E. casseliflavus, E. durans* and *E. raffinosus.* Diseases are connected with every system of the body, such as chronic and recurring urinary tract infection, mixed infection of the abdominal cavity, infections of soft tissues, burn wounds and inflammations of cerebral spinal fluid, bone marrow, gastric inflammation and endocarditis [3].

OBJECTIVE

The aim of this study was assessment of *E. faecalis, E. faecium, E. durans* and *E. hirae* prevalence in samples gathered from selected swine production sectors on an industrial farm, and determination of the contribution of VRE strains and their resistance. The degree of relationship between isolates of each species from genus *Enterococcus* collected from the breeding environment was also determined.

MATERIALS AND METHOD

The samples were collected in mid-July 2012 from an industrial piggery located in Kujawsko-Pomorskie province, Poland. Livestock was kept in separate group pens. Material were swabs from troughs, feeding passages, slurry channels (collected at 5 sites along the building axis), and fresh stool samples, from different production sectors, such as sector for suckling pigs, weaned pigs, piglets, porkers and the sexing sector. Samples were also collected from the loading ramp and platform. Swabs from pens were collected from each edge and in the centre. Only pens with healthy animals were tested. A total of 185 samples were gathered.

Decimal dilutions were made for each sample, up to 10^{-2}. Next, streaking was performed on the enterococcal selective medium Kanmycin Esculin Azide Agar (MERCK). Cultures were incubated for 48 hours at 37 °C. Enterococci grew as small grey colonies surrounded by an olive-black agar zone. Singular colonies were inoculated into nutrient broth (Nutrient Broth, MERCK). Cultures incubated for 18 hours at 37 °C were used to isolate DNA.

Vancomycin resistance testing. Vancomycin resistance was tested using screening on Mueller-Hinton Agar (OXOID) with vancomycin (6 $\mu g \times ml^{-1}$). Medium without antibiotic was used as control. Tested strains were suspended in 0.9% saline solution, so that their turbidity would be 0.5 on the McFarland scale. Next, medium with and without antibiotic was inoculated with suspensions. Cultures were incubated for 18 hours at 37 °C. Vancomycin susceptible strains grew on the control medium but not on the medium with antibiotic. VRE grew on both media [11].

Reference strains. Reference strains from the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, Poland, were used. The obtained strains were: *Enterococus durans* PCM 1857, *Enterococus faecium* PCM 1859, *Enterococcus faecalis* PCM 2673 and *Enterococus hirae* PCM 2559. Lyophilized strains were revived according to the distributor's procedure.

DNA isolation. DNA was isolated using *Genomic Mini AX Bacteria Spin* kit (A&A Biotechnology) according to the producer's procedure with own modification, involving double incubation with lysosyme (7 minutes each) at 37 °C, and double incubation with proteinase K (5 minutes each) at 50 °C, with breaks to vortex (TDB-120 Dry Block Termostat, BIOSAN; Multi-Vortex V-32, BIOSAN).

Identification of species using multiplex PCR method. Multiplex PCR was conducted, using isolated DNA, with starters specific to 16S rRNA sequence (identification of genus *Enterococcus*) and to gene *sodA* to identify species (Tab. 1). 3.0 µl of DNA and 22 µl of MasterMIX were used. MasterMIX components were: DreamTaq polymerase buffer– 2.5 µl (Fermentas), DreamTaq polymerase – 3.5u (Fermentas), dNTPs – 0.2 mM, $MgCl_2$ – 3.0 mM (Fermentas), primer Forward – 16 µM (Sigma-Aldrich), primer Rewers – 16 µM (Sigma-Aldrich), H_20 (DNA & RNA Free) (Sigma-Aldrich). Amplification conditions were: initial denaturation – 95 °C/4 min; main cycle (repeated 30 times): denaturation – 95 °C/30 sec; annealing – 55 °C/1 min; elongation – 72 °C/1 min; final amplification – 72 °C/7 min (MJ Mini Personal TermalCycler, BIO-RAD).

After the PCR, the 2-step electrophoresis (20V – 10 min and 85V – 110 min) was conducted on 2% agarose gel with nonspecific intercalating MidoriGreen dye (NIPPON Genetics Europe GmbH). Each sample had a common 440 bp product, which is proof of being a strain from the genus *Enterococus* [12].

Assessment of genetic relationship of strains from genus *Enterococcus*. In order to assess the degree of relationship between tested strains, the RAPD method was used with

9-nucleotyde starter 5'-ACGCGCCCT-3' [13]. 3.0 µl of DNA and 22 µl of MasterMIX were used. MasterMIX components: DreamTaq polymerase buffer– 2.5 µl (containing 1.5 mM $MgCl_2$), DreamTaq polymerase – 2.5u, dNTPs – 0.2 mM, primer 5 µM, H_20 (DNA & RNA Free). Preamplification consisted of 4 cycles: denaturation – 94 °C/45 sec; annealing – 30 °C/2 min; elongation – 72 °C/1 min. Main cycle (repeated 26 times): denaturation – 94 °C/5 sec; annealing – 36 °C/30 sec; elongation – 72 °C/30 sec; final amplification – 72 °C/10 min. Next, the 2-step electrophoresis was conducted on 1.5% agarose: 20V – 10 min, then 85V – 90 min.

Data matrixes documenting the RAPD products were created for each isolate. Next, the genetic relationship degree was determined between isolates of each species. Dendrograms were created using STATISTICA 10 PL (StatSoft), clustering according to Ward's method. The units were Euclidean distances.

Assessment of vancomycin resistance type of strains from genus *Enterococcus*. In order to determine vancomycin resistance, type multiplex PCR detecting VanA, VanB and VanC type was used (Tab. 1) [14]. 5.0 µl of DNA and 20 µl of MasterMIX were used. MasterMIX components: DreamTaq polymerase buffer– 2.5 µl, DreamTaq polymerase – 1.2u, primer Forward – 0.4 µM, primer Rewers – 0.4 µM, dNTPs – 0.2 mM, primer 5 µM, H_20 (DNA & RNA Free). Amplification conditions: initial denaturation – 95 °C/2 min; main cycle (repeated 25 times): denaturation – 94 °C/60 sec; annealing – 55 °C/60 sec; elongation – 72 °C/60 sec and final amplification – 72 °C/5 min. Next, the 2-step electrophoresis was conducted on 1.5% agarose: 20V – 10 min, then 85V – 90 min.

Table 1. Starters used in species identification and vancomycin resistance type assessment among genus *Enterococcus* [12, 14]

Starters specific to species from genus *Enterococcus*			
Species	Primer name	Primer sequence	Product size [bp]
Enterococcus spp.	16S1 16S2	5'-AACGCGAAGAACCTTAC-3' 5'-CGGTGTGTACAAGACCC-3'	440
E. durans	DU1 DU2	5'-CCTACTGATATTAAGACAGCG-3' 5'-TAATCCTAAGATAGGTGTTTG-3'	295
E. faecalis	FL1 FL2	5'-ACTTATGTGACTAACTTAACC-3' 5'-TAATGGTGAATCTTGGTTTGG-3'	360
E. faecium	FM1 FM2	5'-GAAAAAACAATAGAAGAATTAT-3' 5'-TGCTTTTTTGAATTCTTCTTTA-3'	215
E. hirae	HI1 HI2	5'-CTTTCTGATATGGATGCTGTC-3' 5'-TAAATTCTTCCTTAAATGTTG-3'	187
Starters specific to vancomycin resistance genes			
Resistance type	Primer name	Primer sequence	Product size [bp]
VanA	VanA1	5'-GGGAAAACGACAATTGC-3'	732
	VanA2	5'-GTACAATGCGGCCGTTA-3'	
VanB	VanB1	5'-ATGGGAAGCCGATAGTC-3'	635
	VanB2	5'- GATTTCGTTCCTCGACC-3'	
VanC	VanC1	5'- GGTATCAAGGAAACCTC-3'	$VanC_1$- 822 $VanC_2$/$VanC_3$-439
	VanC2	5'- CTTCCGCCATCATAGCT-3'	

RESULTS

Species identification of isolates and strains from genus *Enterococcus*. From the 185 environmental samples, 195 isolates from genus *Enterococcus* were obtained. *E. hirae* had the highest contribution to the enterococci population, accounting for 133 (68%) isolates. The other species contributions were, respectively: *E. faecalis* – 21% (40 isolates), *E. durans* – 8% (16 isolates) and *E. faecium* – 3% (6 isolates) (Tab. 2).

Table 2. Contribution of particular species of *Enterococcus* genus in the general population of isolates and strains obtained from different production sectors.

Production sector	Species Sampling place	*E. durans*		*E. faecalis*		*E. faecium*		*E. hirae*	
		isolates	strains	isolates	strains	isolates	strains	isolates	strains
Suckling pigs sector	Feeding passage	1	1	-	-	2	2	11	3
	Slurry channel	-	-	-	-	1	1	20	9
	Trough	-	-	2	1	1	1	9	3
	Faecal samples	-	-	-	-	-	-	5	3
Weaned pigs sector	Slurry channel	-	-	4	2	-	-	2	2
	Trough	-	-	8	1	-	-	1	1
	Faecal samples	-	-	-	-	-	-	2	1
Piglets sector	Feeding passage	-	-	2	2	-	-	7	4
	Trough	-	-	4	3	-	-	4	3
	a samples	-	-	-	-	-	-	7	3
Porkers sector	Feeding passage	2	1	-	-	-	-	8	4
	Trough	3	2	6	4	-	-	5	2
	Faecal samples	5	2	-	-	-	-	1	1
Sexing sector	Feeding passage	-	-	5	1	-	-	-	-
	Slurry channel	-	-	1	1	-	-	9	3
	Trough	-	-	3	2	2	2	12	5
	Faecal samples	-	-	3	3	-	-	20	3
Loading ramp	Ramp	1	1	2	2	-	-	6	3
	Platform	4	3	-	-	-	-	4	1
	Collectively within a species	16	7	40	13	6	4	133	36

60 genetically-different strains identified. Among ***E. hirae*** isolates they accounted for 36 (60.0%) strains and ***E. faecalis*** – 13 (21.7%) strains. ***E. durans*** and ***E. faecium*** had the lowest contributions to the general population of strains, which were 7 (11.7%) and 4 (6.6%), respectively (Tab. 2).

The presence of all 4 species was noted only in the suckling pigs sector. At other sampled sites, 2–3 species were present. Moreover, complete domination with ***E. hirae*** or ***E. faecalis*** in a sector was typical (Tab. 2).

In the suckling pigs sector, *E. hirae* was the most frequent species, accounting for 71% (15 strains) and *E. faecium* – 19% (4 strains). The lowest contributions belonged to *E. durans* and *E. faecalis*, both accounting for 5% (1 strain) (Tab. 2).

The weaned pigs sector was dominated with *E. hirae* (70% strains) and *E. faecalis* (30% strains). A similar situation was noted in the piglets sector, where *E. hirae* accounted for 67% strains, *E. faecalis* 33% strains (Tab. 2).

In the porkers' sector, *E. hirae, E. durans* and *E. faecalis* were noted. They accounted respectively for 44%, 31% and 25% strains. Same species were determined for the loading ramp, but *E. faecalis* (33% strains) dominated over *E. durans* (17% strains) at this site (Tab. 2).

In the sexing sector, *E. hirae* (55% strains) and *E. faecalis* (35% strains) dominated. *E. faecium* had the lowest contribution (10% strains) at this site (Tab. 2).

The platforms were colonized by *E. durans*, which accounted for 75% of the strains (Tab. 2).

Vancomycin resistance assessment among *Enterococcus* spp. Strains resistant to vancomycin at concentration 6 μg×ml^{-1} were determined only among *E. hirae*. Collectively, these isolates accounted for 1% (3.3% strains), and in the case of *E. hirae* species – 5.5% (Tab. 3). Both isolates were obtained from the sexing sector (slurry channel and trough samples). The multiplex PCR determined that both possessed VanC$_1$ phenotype (Tab. 3, Fig. 1).

Table 3. Contribution of VRE strains and isolates in each species

Species	Resistance to vancomycin (6 μg×ml^{-1})	No. of isolates [n (%)]	No. of strains [n (%)]
E. hirae	Resistant	2 (1.5%)	2 (5.5%)
	Susceptible	131 (98.5%)	34 (94.5%)
E. faecium	Resistant	0 (0%)	0 (0%)
	Susceptible	6 (100%)	4 (100%)
E. faecalis	Resistant	0 (0%)	0 (0%)
	Susceptible	40 (100%)	13 (100%)
E. durans	Resistant	0 (0%)	0 (0%)
	Susceptible	16 (100%)	7 (100%)

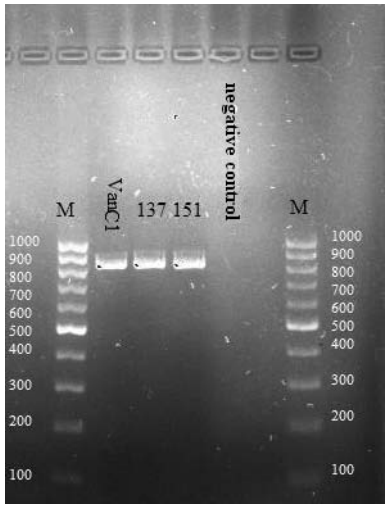

Figure 1. Electrophoresis from Multiplex PCR vancomycin resistance type assessment among *Enterococcus* spp.

Genetic relationship of strains from genus *Enterococcus*. Among 16 obtained *E. durans* isolates, 7 strains were differentiated. This research proved that *E. durans* isolates from troughs and feeding passage in the porkers sector formed one strain. Moreover, 7 isolates from faecal samples in the porkers sector, loading ramps and platform, formed one strain (Fig. 2).

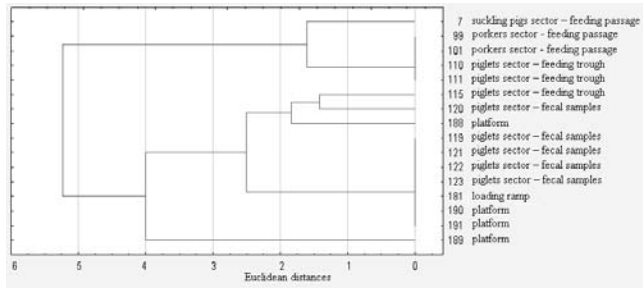

Figure 2. Genetic relationship of isolates isolates of *E. durans*

In the case of *E. faecalis,* 13 strains were noted. 5 of the isolates, forming one strain, originated in the suckling pigs, piglets and porkers sector. Furthermore, one strain consisting of 3 isolates was isolated from troughs and faecal samples in the sexing sector (Fig. 3).

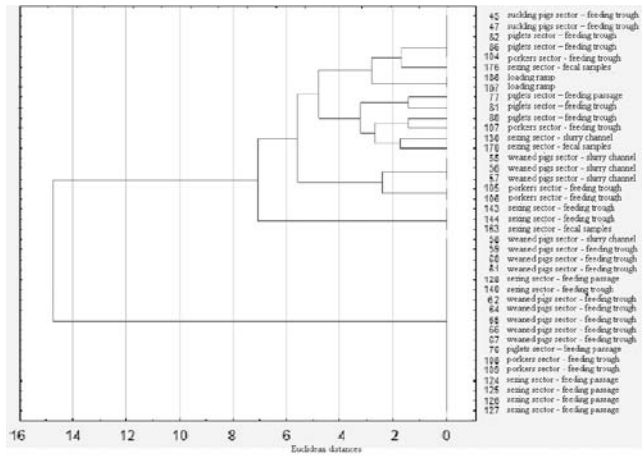

Figure 3. Genetic relationship of isolates of *E. faecalis*

There were 4 strains detected among *E. faecium* isolates, where one was formed by 3 genetically identical isolates. Each of them was isolated from a different environment: feeding passage and troughs in the suckling pigs sector, and troughs in sexing sector (Fig. 4).

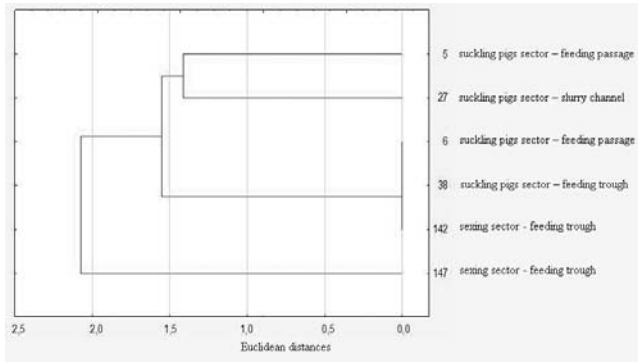

Figure 4. Genetic relationship of isolates of *E. faecium*

There were 36 strains obtained among *E. hirae*. In the slurry channel and feeding passage in suckling pigs sector, identical strains were determined. Another 7 identical isolates were noted from feeding passage, troughs and faecal samples from the piglets sector. In the slurry channel and troughs in sexing sector, another 8 identical isolates were detected (Fig. 5).

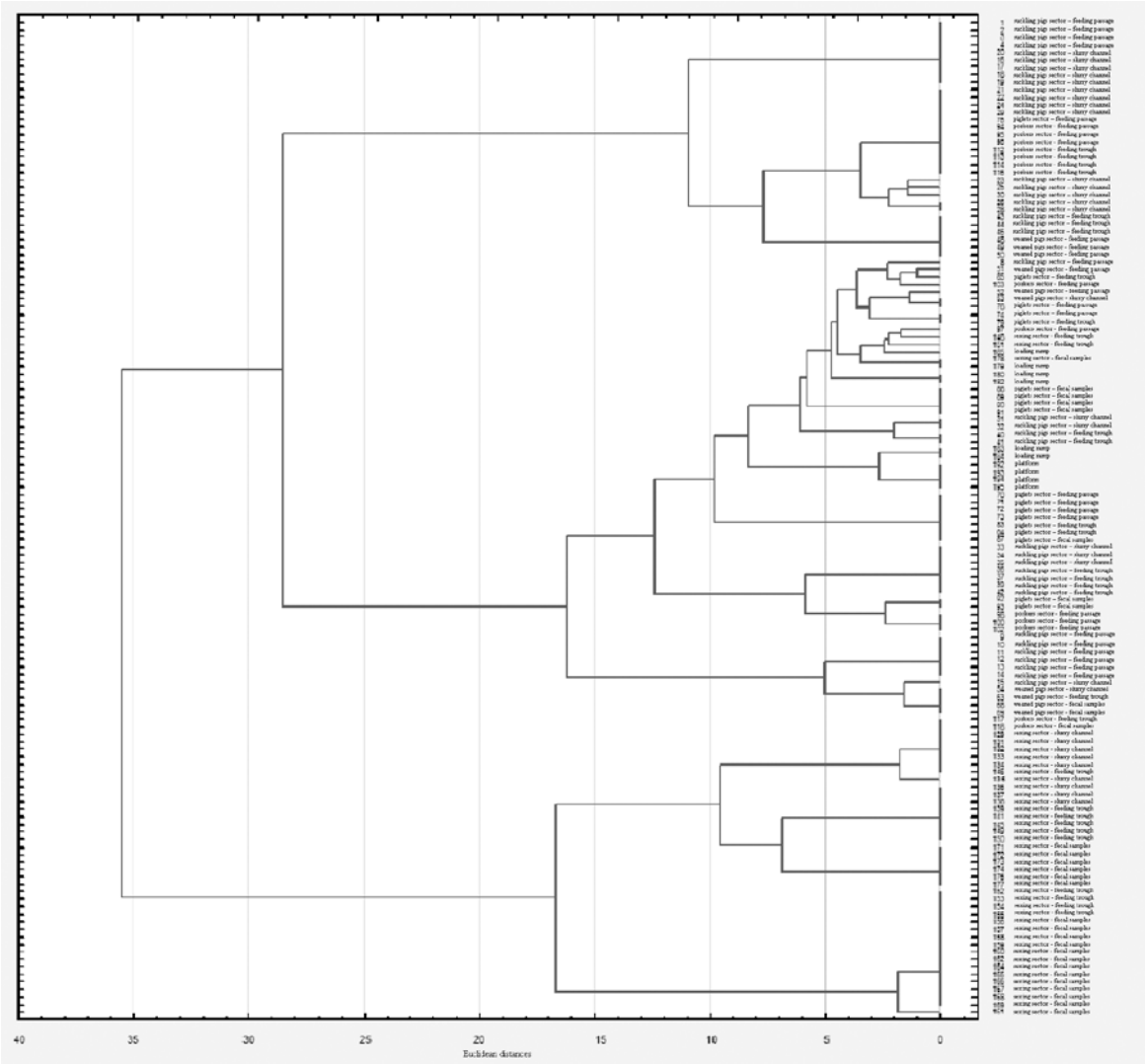

Figure 5. Genetic relationship of isolates of *E. hirae*

The results obtained from *E. durans* indicate that the main contamination routes were animal feed and transmission by slaughtered porkers driven onto the loading ramp, or on the footwear of the personnel (Fig. 2). A similar observation was made in the case of *E. faecium* and *E. hirae* (Fig. 4, Fig. 5). In the case of *E. faecalis* and *E. hirae,* contamination of troughs by excrements can be assumed (Fig. 3, Fig. 5).

DISCUSSION

A study by Kluczek (1998) proved a variety of species from genus *Enterococcus* in a piggery. Commonly isolated species were *E. faecalis* and *E. faecium*. Although the presented study also proved a variety of enterococci, their contribution, however, was different – *E. hirae* (Tab. 2) dominated [15].

According to Kluczek (1999), *E. cloace*, *E. agglomerans* and *E. faecium* were frequently isolated from pens of suckling pigs and sows. However, Kluczek did not indicate *E. hirae* in the suckling pigs sector, which was determined in the current research. Moreover, Kluczek (1999) isolated *E. casseliflavus* and *E. agglomerans* from the piglets sector, whereas in the current study *E. hirae* and *E. faecalis* were isolated [16].

It would be difficult to search for tendencies or analogies between the presented study and studies by other authors, because the species tested by the latter were not aim of the current research. Moreover, this study indicated sites for sample collecting, such as troughs, feeding passages, slurry channels and faecal samples, whereas other authors treated them collectively in every tested production sector.

According to Kühn (2000), 8% of isolates collected from production waste, slurry and swine faeces were resistant to vancomycin at a concentration of 8 $\mu g \times ml^{-1}$. That percentage was higher compared to the 1% at 6 $\mu g \times ml^{-1}$ in the presented study (Tab. 3) [17].

Garcia-Migura (2005) indicated that most of the VRE strains belonged to *E. faecium* or *E. faecalis*. The current study proved the lack of VRE strains among these species (Tab. 3). Research by Hershberger (2005) with vancomycin at concentration 16 $\mu g \times ml^{-1}$ was consistent in this matter (Tab. 3). Garcia-Migura's (2005) research concerning faecal *E. faecium* from 14 Welsh piggeries revealed 2–5% of VRE strains. Such strains were isolated both from ecological and industrial farms. In the case of samples from an ecological farm, VRE strains were discovered in environmental swabs, not in faecal samples. The presented study also proved a lack of VRE strains in faecal samples. All strains in Garcia-Migura's study had VanA phenotype with MIC > 64 $\mu g \times ml^{-1}$

against vancomycin. Genotyping with RFLP-PFGE (*SmaI)* proved the occurrence of horizontal gene transfer. In the current study, due to the $VanC_1$ resistance type, vertical gene transfer was expected [18, 19].

In contrast to the current study, most enterococcal antibiotic resistance studies focused on strains isolated from faecal samples. Other authors focused on the final by-product of animal breeding, such as the VRE contamination factor, without considering the breeding environment of livestock and meat as important sources of infections [18].

In Huycke's (1998) research, VRE strains in the case of *E. faecalis* accounted for 2% and *E. faecium* – 52%. Other clinical tests concerning *E. faecium* revealed VRE presence among isolates from catheters, wounds and blood. It is important to notice that the presented study proved the lack of VRE among these species (Tab. 3) [20, 21].

Research conducted on swine faeces in Lithuania by Ruzauskas (2009) detected 6% of VRE among genus *Enterococcus,* whereas only 3.3% of resistant strains were determined in the current study [22]. This means that only a small fraction of the strains present in the breeding environment pose an etiological factor of numerous infections. Moreover, these strains exhibited $VanC_1$ phenotype (Fig.1), which has a lower transmission ability compared to the dominant type VanA, predestined to horizontal gene transfer [5]. Hence, animal production is unlikely to be a source of infections. Epidemiologically, the evidence favours the tested piggery.

To-date, no tests concerning transmission of enterocicci in breeding environment have been conducted using the RAPD. Molla's (2010) research on *Salmonella* proved that among each strain there were isolates originating in animal feed and excrements, which confirms their mutual contamination in the production cycle [23]. Purwin's (2012) research proved the presence of enterococci as autochthonic microflora of vegetative parts of plants used as ensilage, which indicates animal feed as a contaminant of the breeding environment [24]. The presented research confirmed that contamination path in the case of *E. durans* (Fig. 2).

The methods used in the presented study obtained promising results, and it is worth noting that the study was a pilot one. However, the research is a premise for a more detailed assessment of the distribution of antibiotic resistant strains in the breeding environment, not only in the piggery environment, but also for chicken coops, cowsheds and biogas plants. Assessment of the presence of antibiotic resistant strains in the first element of the food distribution chain, such as piggeries, is reasonable and purposeful.

CONCLUSIONS

The variety of enterococci in tested piggery was determined. In most of the production sectors *E. hirae* was dominant, among which two different VRE strains resistant to vancomycin at concentration 6 $\mu g \times ml^{-1}$ were detected, both presenting $VanC_1$ phenotype. Both low percentage of VRE and resistance type are proof of reasonable prevention and treatment in tested piggery. Type $VanC_1$ is not subject to horizontal gene transfer, hence its ability to distribute in breeding environment is limited. Moreover, these strains are resistant to low vancomycin concentration compared to dominant, VanA type (MIC ≥ 64 $\mu g \times ml^{-1}$). This means low risk for piggery, slaughterhouse or abattoir employees having contact with animal products. Also transmission of the tested strains to the environment is limited, by means such as fertilizing with slurry produced in piggery.

REFERENCES

1. Cormier Y, Boulet LP, Bedard G, Tremblay G. Respiratory health of workers exposed to swine confinement buildings only or to both swine confinement buildings and dairy barns. Scand. J. Work Env Hea. 1991; 17(4): 269–75.
2. Kołacz R, Dobrzański Z. Higiena i dobrostan zwierząt gospodarskich. Wydawnictwo Akademii Rolniczej we Wrocławiu, 2006.
3. Gilmore MS. The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance. ASM Press, 2002.
4. Chang CW, Chung H, Huang CF, Su HJJ. Exposure of Workers to Airborne Microorganisms in Open-Air Swine Houses. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemoth. 1994; 34(4): 507–514.
5. Leclercq R, Courvalin P. Resistance to glycopeptides in enterococci. Clin Infect Dis. 1997; 24: 545–554.
6. Salem-Bekhit MM, Moussa IM, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian J Med Microbiol. 2012; 30(1): 44–51.
7. Shepard BD, Gilmore MS. Antibiotic-resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. Microbes Infect. 2002; 4: 215–240.
8. Bates J, Jordens JZ, Griffiths DT. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemoth. 1994; 34(4): 507–514.
9. Pérez-Rodríguez MT, Sopeña B, Longueira R, Lamas JL, Martínez-Vázquez C. Calf pyomyositis caused by Enterococcus faecalis, Calf pyomyositis caused by Enterococcus faecalis. Int J Med. 2010; 104 (6): 527.
10. Pattison M. Poultry Diseases. Saunders Elsevier, 2008.
11. Willey BM, Kreiswirth BN, Simor AE, Willaims G, Scriver SR, Phillips A, et al. Detection of Vancomycin Resistance in Enterococcus Species. J Clin Microbiol. 1992; 30 (7): 1621–1624.
12. Jackson CR, Fedorka-Cray PJ, Barrett JB. Use of a Genus- and Species-Specific Multiplex PCR for Identification of Enterococci. J Clin Microbiol. 2004; 42 (8): 3558–3565.
13. Hans-Jurg M, Quednau M, Samuelsson A, Isaksson B, Ahrne S, Jonasson J. Division of the genus Enterococcus into species groups using PCR-based molecular typing methods. Microbiology. 1998; 144(5): 1171–1179.
14. Perez-Hernandez X, Mendez-Alvarez S, Claverie-Martna F. A PCR assay for rapid detection of vancomycin-resistant enterococci. Diagn Micr Infec Dis. 2002; 42: 273–277.
15. Kluczek JP. Charakterystyka bakteriologiczna warchlakarni. Pr Kom Nauk Roln i Biol. 1998; 44: 63–68.
16. Kluczek Sz. Ogólna ocena flory bakteryjnej przemysłowej fermy trzody chlewnej. Pr Kom Nauk Roln i Biol. 1999; 46: 50–59.
17. Kühn I. Epidemiology and ecology of enterococci, with special reference to antibiotic resistant strains, in animals, humans and the environment. Int J Antimicrob Ag. 2000; 14: 337–342.
18. Garcia-Migura L, Pleydell E, Barnes S, Davies RH, Liebana E. Characterization of Vancomycin-Resistant Enterococcus faecium Isolates from Broiler Poultry and Pig Farms in England and Wales. J Clin Microbiol. 2005; 43 (7): 3283–9.
19. Hershberger E. Epidemiology of antimicrobial resistance in enterococci of animal origin. J Antimicrob Chemoth. 2005: 127–130.
20. Huycke MM, Sahm DF, Gilmore MS. Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. Emerg Infect Dis. 1998; 4(2): 239–249.
21. Dever LL, China C, Eng RHK, O'Donovan C, Johanson WG. Vancomycin-resistant Enterococcus faecium in a Veterans Affairs Medical Center: Association with antibiotic usage. 34th Interscience Conference on Antimicrobial Agents and Chemotherapy; Oct 4 – 7 1994; Orlando, Florida; Washington, American Society for Microbiology, 1995.
22. Ruzauskas M, Virgailis M, Šiugždinienė R, Sužiedėlienė E, Šeputienė V, Daugelavičius R, et al. Antimicrobial resistance of Enterococcus spp. isolated from livestock in Lithuania. Veterinarski Archiv. 2009; 79 (5): 439–449.
23. Molla B, Stermann A, Mathews J, Artuso-Ponte V, Abley M, Farmer W, et al. Salmonella enterica in Commercial Swine Feed and Subsequent Isolation of Phenotypically and Genotypically Related Strains from Fecal Samples. Appl Environ Microbiol. 2010; 76 (21): 7188–7193.
24. Purwin C, Lipiński K, Pysera B. Jakość higieniczna kiszonek, Życie Weterynaryjne. Życie Wet. 2012; 87(1): 37–40.