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Short Communication

The rise and fall of a vancomycin-resistant clone of *Enterococcus faecium* among broilers in Sweden

Oskar Nilsson[a,*], Erik Alm[b], Christina Greko[a], Björn Bengtsson[a]

[a] Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), Uppsala, Sweden
[b] Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden

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ABSTRACT

Objectives: Historically, vancomycin-resistant enterococci (VRE) have been very common among farm animals in Europe, and they can still be readily isolated using media supplemented with vancomycin. An increase in the occurrence of VRE among broilers in Sweden was reported during 2000–2005. This was due to the spread of one clone of VRE in the apparent absence of selective pressure. The aims of this study were to estimate the current occurrence of VRE among Swedish broilers and to investigate if there had been any changes with regards to the dominating clone and antimicrobial resistance pattern.
Methods: Caecal samples (n = 100) collected at slaughter from healthy broilers were cultured on Slanetz-Bartley agar supplemented with vancomycin (16 mg/L). Presumptive VRE were identified to species using MALDI-TOF MS, susceptibility tested using broth micro-dilution, and whole genome sequenced to investigate the genetic relationship with previous isolates.
Results: Eleven (11%) of the samples were positive for VRE. This was a statistically significant decrease in the proportion of positive samples from 2010. The same clone as before still prevailed.
Conclusions: The occurrence of VRE among broilers in Sweden has decreased. However, just as the occurrence of VRE among Swedish broilers increased in the apparent absence of selective pressure, the reasons for the decrease is unknown.

1. Introduction

Vancomycin-resistant enterococci (VRE) are a significant cause of nosocomial infections, mainly in immunocompromised patients. In the early 1990s, many farm animals in Europe were colonised with VRE. This was associated with extensive use of the glycopeptide avoparcin as a growth promoter [1], a use that was discontinued in the European Union in 1997 (Commission Directive 97/6 EC). Following this, the occurrence of vancomycin resistance among randomly selected enterococci from farm animals decreased [2]. In Sweden, avoparcin was only used in the late 1970s and early 1980s [3,4], and all use of growth promoters was discontinued in 1986. In accordance, vancomycin resistance has been rare among enterococci from animals in Sweden [5–7].

While the occurrence of vancomycin resistance among randomly selected enterococci from farm animals has decreased in many countries, VRE can still be readily isolated using media supplemented with vancomycin [8,9]. Moreover, in Sweden, an increase in the proportion of VRE-positive samples from Swedish broilers from <1% in 2000 to >40% in 2005 was detected using such methods (Fig. 1) [10]. The increase was shown to be due to the spread of a single clone of *vanA*-carrying *Enterococcus faecium* in the apparent absence of selective pressure [10]. For more than 10 years (2000–2011) efforts were made to understand this phenomenon [11].

The aims of this study were to investigate (i) the current occurrence of VRE among Swedish broilers and (ii) if there had been any changes in the clonality and antimicrobial resistance pattern of VRE.

2. Materials and methods

2.1. Sampling

Caecal samples (n = 100) from healthy broilers were collected at six slaughterhouses representing over 95% of the total annual slaughter of broilers in Sweden. The samples were collected during March and April (n = 50) and September and October (n = 50), 2015.

* Corresponding author at: Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), SE-751 89, Uppsala, Sweden.
E-mail address: oskar.nilsson@sva.se (O. Nilsson).

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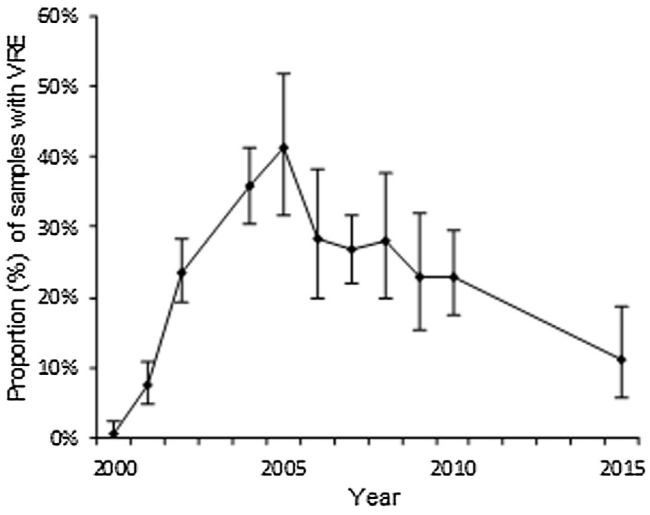

Fig. 1. Proportion of vancomycin-resistant enterococci-positive samples of intestinal content from broilers from 2000–2002, 2004–2010, and 2015; 95% confidence intervals are indicated. Number of samples cultured each year is between 99–351.

All samples were from unique broiler flocks but not from unique production sites.

2.2. Culturing and susceptibility testing

Sampling, culturing on Slanetz-Bartley agar (Oxoid, Basingstoke, UK) supplemented with vancomycin (16 mg/L, Sigma-Aldrich, Steinheim, Germany) and susceptibility testing using micro-dilution methods in VetMIC E-cocci panels (SVA, Uppsala, Sweden) were performed as previously described [10]. The antimicrobials that were tested are shown in Table 1. Resistance was determined as minimum inhibitory concentrations (MICs) above the epidemiological cut-off values suggested by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. For narasin, a cut-off > 2 mg/L was used instead of the recommended value of >4 mg/L.

2.3. Species confirmation and whole genome sequencing

Species identification was performed using MALDI-TOF MS (Bruker Daltonik GmbH). Whole genome sequencing was used to determine the gene responsible for the vancomycin resistance and to investigate the genetic relationship of the isolates from 2015. Briefly, DNA extracted from bacterial isolates (MagAttract® DNA Mini M48 Kit; Qiagen, Hilden, Germany) was sequenced using IonTorrent PGM technology (Thermo Fisher Scientific Inc., Waltham, MA, USA). Library and emulsion polymerase chain reaction (PCR) assays were performed according to the manufacturer's instructions. For comparison, two earlier isolates (2001-233 and 2007-70) previously determined as ST310 by multilocus sequence typing [10] were included in the whole genome sequencing.

The difference in occurrence of VRE between 2010 and 2015 was tested statistically using Pearson's χ^2 test (Stata software, release 13).

3. Results

3.1. Occurrence of VRE

Eleven (11%, Fig. 1) of the samples yielded growth of VRE and all of these were *Enterococcus faecium* carrying *vanA*. The decrease in the proportion of VRE-positive samples from 2010 (23%) to 2015 was statistically significant ($P = 0.001$).

Table 1
Multilocus sequence type (ST) and minimum inhibitory concentrations (MICs, mg/L) of vancomycin-resistant enterococci from healthy Swedish broilers (n = 11) from 2015.

Isolate	Sequence type	Antimicrobials tested (cut-off values, mg/L)										
		Van (> 4)	Nar (> 2)	Ery (> 4)	Bac (> 32)	Tet (> 4)	Amp (> 4)	Vir (> 4)	Str (> 128)	Gen (> 32)	Chl (> 32)	Lin (> 4)
2015-21	310	> 128	4	16	≤ 1	≤ 0.5	4	2	64	8	4	2
2015-24	310slv	> 128	8	8	2	1	4	2	64	8	8	2
2015-44	310	> 128	4	32	≤ 1	≤ 0.5	0.5	2	64	8	4	1
2015-54	310	> 128	8	16	≤ 1	1	≤ 0.25	2	64	8	8	2
2015-66	310	> 128	4	8	≤ 1	≤ 0.5	≤ 0.25	2	64	8	4	2
2015-71	310	> 128	8	16	≤ 1	≤ 0.5	0.5	2	64	16	4	2
2015-75	310	> 128	4	16	≤ 1	≤ 0.5	2	2	64	8	8	2
2015-76	310	> 128	8	16	≤ 1	≤ 0.5	2	2	64	8	8	2
2015-79	310	> 128	4	16	≤ 1	≤ 0.5	2	2	64	8	4	2
2015-80	310	> 128	8	8	≤ 1	≤ 0.5	≤ 0.25	2	64	16	8	2
2015-97	310	> 128	4	16	≤ 1	≤ 0.5	0.5	2	64	16	4	2

Tested antimicrobials: vancomycin (Van), narasin (Nar), erythromycin (Ery), bacitracin (Bac), tetracycline (Tet), ampicillin (Amp), virginiamycin (Vir), streptomycin (Str), gentamicin (Gen), chloramphenicol (Chl), and linezolid (Lin).
Shaded fields indicate resistance (i.e. MIC above the epidemiological cut-off values suggested by EUCAST [12] except for narasin where >2 mg/L was used instead of >4 mg/L.

3.2. Relationship of isolates

All but one of the isolates belonged to ST310, which was previously described to dominate among VRE from Swedish broilers [10]. The ST310 isolates clustered within 100 single nucleotide polymorphisms (SNPs) of each other, with the closest pair differing by six SNPs. The remaining isolate (2015-24) was a previously undescribed single locus variant of ST310 and only differed by a single base substitution in the PstS allele (A instead of a G at position 195). All the isolates had an antimicrobial susceptibility profile in accordance with the previously dominating VRE ST310 clone (Table 1) [10].

4. Discussion

Although efforts have been made to understand the epidemiology of VRE among Swedish broilers and how to decrease the occurrence [11], there have been no direct interventions aiming to reduce the occurrence of VRE, nor has there been any known general changes in for example routines for cleaning and disinfection between batches of birds over the last decade. Hence, just as the occurrence of VRE among Swedish broilers increased in the apparent absence of selective pressure, the reasons for the decrease is unknown.

In Sweden, the glycopeptide avoparcin was only used for some years in the late 1970s and early 1980s [3]. Furthermore, no growth promoters have been used since 1986, and therapeutic use of antimicrobials is now rare in Swedish broiler production [13]. However, the ionophore narasin is routinely used for prophylaxis of coccidiosis. The current group has previously, based on co-transfer of traits causing elevated MIC of vancomycin and narasin, hypothesised that use of narasin might play a role in persistence of vancomycin resistance among enterococci in Swedish broilers [14]. However, sequencing of the plasmids has shown that even though the mechanisms for the different traits are located on the same plasmids, they are not located next to each other [15]. Hence, it could be possible for the enterococci to preserve the genes causing elevated MIC of narasin but not the ones causing vancomycin resistance. Furthermore, the only change in the standard regimens regarding use of narasin in the Swedish broiler production is that the generally practiced withdrawal time has been shortened from 5 to 3 days since 2008. This would mean that the use of narasin has probably slightly increased concomitantly with the decreased occurrence of VRE among Swedish broilers described in this study. All of this taken together, the hypothesis that use of narasin plays a role in persistence of vancomycin resistance among enterococci in Swedish broilers is weakened.

Is perhaps the rise and fall of the VRE ST310 clone just a natural variation in which bacterial clones that constitute the bacterial population in the animals in a production system? It is also important to remember that even if, at times, a large proportion of Swedish broilers has been carrying VRE, the proportion of enterococci that has been vancomycin resistant has always been very low. This is indicated by the fact that over the years, only four out of 1176 randomly selected *Enterococcus faecium* from broilers in the Swedish surveillance programme Svarm have displayed vancomycin resistance [5–7].

In summary, selective culture changes in the occurrence of VRE among Swedish broilers for 15 years has been detected (from <2%, up to around 40%, and then down to 11%). It is unknown which factors have caused these changes, if there have been any real driving factors at all, or if the changes in occurrence are just a reflection of normal variation in the clones that constitute the bacterial population at any specific time point. These findings exemplify the complexity of the epidemiology of antimicrobial resistance.

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Competing interests

None.

Ethical approval

Not required.

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