EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

OPINION OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON AVILAMYCIN

(adopted on 28 April 2000)

Background

(1) On 1st January 1999, Sweden applied the safeguard clause for certain antibiotics authorised as feed additives, including avilamycin. This measure was transmitted to the European Commission accompanied by scientific justifications for the action taken.

The company responsible for marketing the antibiotic feed additive avilamycin Eli Lilly and Company Ltd, submitted dossiers in response to the Swedish action. Member States also submitted comments on the Swedish decision.

(2) The European Commission initiated a multi-disciplinary exercise on the cross sectorial subject of antimicrobial resistance. The Scientific Steering Committee carried out this review and adopted its opinion on 28 May 1999.

As far as antibiotics used as growth promoters are concerned, the Scientific Steering Committee recommended that *"the use of agents from classes which are or may be used in human or veterinary medicine (i.e. where there is a risk of selecting cross-resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and ultimately abolished."*

(3) It has been brought to the attention of the Commission that everninomicin, an antibiotic belonging to the same class as avilamycin, is under development for use in human medicine.

Terms of reference

On the basis of the available scientific information (including data submitted by Sweden, other Member States and Eli Lilly and Company Ltd), and taking into consideration the opinion of the Scientific Steering Committee, the Scientific Committee on Animal Nutrition is requested to answer the following questions:

(1) What is the prevalence of resistance to avilamycin in enterococci isolated from livestock?

(2) Is there an association

(a) between avilamycin use and resistance to avilamycin in bacteria of animal origin

(b) between a decrease in the use of avilamycin and a decrease in the prevalence of resistance to avilamycin in bacteria of animal origin?

(3) Does resistance to avilamycin in enterococci reduce their susceptibility to everninomicin?

(4) Is there any information on the prevalence of resistance to avilamycin /everninomicin in bacteria isolated from food of animal origin and amongst the human population (e.g. community and hospitals)?

(5) Is the transfer of avilamycin resistant enterococci and/or of the resistance gene(s) to avilamycin to humans possible? What are the direct or indirect factors that favour or exclude such a possibility?

Opinion of the Committee

1. INTRODUCTION

Avilamycin is a mixture of oligosaccharides of the orthosomycin group produced by *Streptomyces viridochromogenes.* Avilamycin is mainly active against Gram-positive bacteria and has been used for about ten years for growth promotion in swine and poultry. It has never been used for therapeutic purposes in either human or veterinary medicine but a related compound, everninomicin is under development for approval for use in human medicine.

2. WHAT IS THE PREVALENCE OF RESISTANCE TO AVILAMYCIN IN ENTEROCOCCI ISOLATED FROM LIVESTOCK?

Normally the Minimal Inhibitory Concentration (MIC) of avilamycin for enterococci varies between 0.12 and 4 mg/l. Because avilamycin is not intended for therapeutic use, no recommended breakpoints according to the NCCLS or others exist to separate clinically resistant and sensitive strains. However, according to the bimodal distribution of the MICs, enterococcal strains with an MIC of more than 8 mg/l are clearly separated from the normal population and may therefore, from a

microbiological point of view, be considered as resistant. This is the definition of resistance adopted in this report.

Data on the prevalence of avilamycin resistance among enterococci are limited. In Denmark avilamycin has been used almost exclusively in poultry since 1990 and information on the prevalence of resistance is available from the Danish monitoring programme started at the end of 1995 (DANMAP 1997; 1998). In broiler isolates of *Enterococcus faecium*, the prevalence of resistance was 63, 79, 65 and 28% in 1995, 1996, 1997 and 1998, respectively. In pigs and cattle the prevalence of resistance was 0% in 1998. Among *Enterococcus faecalis* isolates from pigs and broilers, the prevalence of resistance was very low at 1 and 0% respectively.

In Belgium, among 199 strains of *Enterococcus faecium* and 154 strains of *E. faecalis* of animal origin isolated in 1996, 1997, and 1998, only two strains of *E. faecium* were resistant to avilamycin (MIC> 32 mg/l). The MICs of the other strains of *E. faecium* and of all the strains of *E. faecalis* varied between 0.12 and 2 mg/l (Butaye *et al.*, 1999).

Conclusion: On the basis of the limited epidemiological information, it appears that the prevalence of resistance to avilamycin is high amongst *E. faecium* strains isolated from animal species. A similar pattern of resistance is not seen amongst *E. faecalis* isolates.

3. THE ASSOCIATION BETWEEN AVILAMYCIN USE AND RESISTANCE IN BACTERIA OF ANIMAL ORIGIN

3.1. Is there an association between use of avilamycin and resistance to avilamycin in bacteria of animal origin?

No data have been published on the prevalence of resistance among enterococci isolated from animals before the introduction of avilamycin as feed additive.

In Denmark, the consumption of avilamycin increased between 1990 and 1996 from 10 to 2740 kg/annum most of which for use in broilers.

In a recent Danish study, the prevalence of avilamycin resistance in *E. faecium* isolated from birds reared in farms exposed to avilamycin was compared with the resistance prevalence among isolates from poultry farms not exposed (Aarestrup *et al.*, submitted for publication). The percentage of isolates from exposed farms resistant to avilamycin was 72% (64 out of 88), with values for individual farms ranging from 25 to 100%. For non-exposed farms, the corresponding value for resistant isolates was 23% (24 out of 104) with a range of 0 to 90%. A significantly lower prevalence of avilamycin-resistant strains was found in enterococci isolated from the farms not exposed at the time of studies to avilamycin.

Avilamycin has not been used as feed additive for pigs from Denmark and no avilamycin resistant *E. faecium* strains have been found among Danish pig isolates.

In a 56-day trial it was shown that the incorporation of avilamycin at 30 ppm (normal dose: 2.5 to 10 ppm) in the diet of chickens resulted in selection of resistant staphylococci. Most of these avilamycin-resistant isolates also became resistant to erythromycin (dossier of the company, November 1998, section 19).

Recently the company provided three other studies, two with broilers and one with pig carcasses suggesting that the use of avilamycin as a feed additive did not increase the incidence of resistance among enterococci. However:

- for broilers, these studies are isolated short term experiments or based on a small number of strains tested and the results can hardly be extrapolated to animal husbandry under field conditions

- for pigs, enterococci isolated from carcasses in slaughterhouses were not shown to be representative for enterococci isolated from live animals.

Thus, these studies can not be taken as evidence for a non-existing association between use of avilamycin and development of avilamycin resistance.

Conclusion: There is an association between use of avilamycin and prevalence of resistance in bacteria of animals exposed to avilamycin.

3.2. Is there an association between the decrease in use of avilamycin and the decrease in the prevalence of resistance to avilamycin in bacteria of animal origin?

The use of avilamycin as feed additive was discontinued in Denmark in February 1998 on a voluntary basis and only 7 kg was used during the year. Less than a year later, monitoring studies showed that the resistance in *E. faecium* isolates from broilers fell from 65% seen in 1997 to 28% in 1998. These results suggest that this decrease in avilamycin resistance is caused by the substantially reduced use of avilamycin in chicken feed.

Conclusion: There is an association between the decrease in use of avilamycin and the reduction in the prevalence of resistance to avilamycin in bacteria isolated from chickens.

4. DOES RESISTANCE TO AVILAMYCIN IN ENTEROCOCCI REDUCE THEIR SUSCEPTIBILITY TO EVERNINOMICIN?

Avilamycin acts on the bacterial ribosome and interferes with the attachment of tRNA by binding to the 30S subunit (Wolf, 1973). Recent studies seem to confirm that the binding target site for both avilamycin and everninomicin is the L16 ribosomal protein and that modifications in the protein induce a reduced susceptibility of bacteria to each product (Aarestrup and Jensen, submitted; Adrian and Klugman., 1998).

No data has been published on mechanisms of resistance, but it seems logical to assume that structural changes in the 30S subunit which confer resistance to

avilamycin will also do so for everninomicin as the two compounds are structurally very similar.

One publication suggests that a proportion of avilamycin resistant enterococci are also less susceptible to everninomicin (Aarestrup, 1998). Based on 86 *E. faecium* strains isolated from birds, a bimodal distribution of the MIC of avilamycin is observed. Avilamycin susceptible enterococci (<8 mg/l) had MIC for everninomicin ranging from 0.064 to 0.75 mg/l and avilamycin resistant enterococci (> 8mg/l) had MIC of everninomicin ranging from 1.5 to 16 mg/l. The bimodal distribution of the MICs of avilamycin was in parallel with that of everninomicin. Different techniques were used to determine the MIC of avilamycin (plating on twofold dilution series in Müller-Hinton agar) and everninomicin (E-test). The same method preferably should have been used for both substances, but this does not invalidate the conclusions.

If we accept the provisional breakpoints recommended for everninomicin by the NCCLS, only one strain (MIC = 64 mg/l) would be considered as clinically resistant to everninomicin.

Conclusion: There are both theoretical and practical data to suggest that resistance to avilamycin in enterococci also reduces their susceptibility to everninomicin

5. IS THERE ANY INFORMATION ON THE PREVALENCE OF RESISTANCE TO AVILAMYCIN/EVERNINOMICIN IN BACTERIA FROM FOOD OF ANIMAL ORIGIN AND IN THE HUMAN POPULATION (E.G. COMMUNITY AND HOSPITALS)?

Although avilamycin was used in Denmark only in poultry, *E. faecium* resistant to avilamycin were isolated from a variety of different food categories including beef (13%), broiler meat (12%), other poultry meat (4%) and vegetables (7%). No avilamycin resistant *E. faecium* were isolated from pork. For *E. faecalis*, the prevalence was: pork meat 5%, beef 6%, broiler meat 12%, other poultry meat 25% and vegetables 8%. (DANMAP 1998).

No other surveys have been made.

Published information on prevalence of resistance to everninomicin in the human population in the community or hospitals is scarce.

In Denmark, a total of 15 *E. faecium* and 49 *E. faecalis* strains were isolated from pig producers and abattoir workers. All were susceptible to avilamycin (DANMAP 97).

Using non-selective media no resistance was found (MIC 90 = 1 mg/l) in a large number of hospital isolates of *E. faecium* and *E. faecalis* collected from 27 European countries (an average of 155 isolates per country) in 1997 (Schouten *et al.*, 1999).

A study conducted recently in Europe and North America demonstrated that among 959 enterococci strains isolated from humans, no strain resistant to everninomicin (MIC 90 = 1 mg/l) was found (Jones *et al.*, submitted).

Information on the prevalence of resistance in the human population outside hospitals is not available.

Conclusion: Enterococci resistant to avilamycin have been isolated from food of various animal, but also plant origin in Denmark. No avilamycin/everninomicin-resistant enterococci have been isolated from patients in European hospitals. No information is available on the prevalence of avilamycin/everninomicin-resistant enterococci in the human population outside hospitals.

6. IS THE TRANSFER OF AVILAMYCIN RESISTANT ENTEROCOCCI AND/OR THE GENE(S) CONFERING RESISTANCE TO AVILAMYCIN TO HUMANS POSSIBLE? WHAT ARE THE DIRECT OR INDIRECT FACTORS THAT FAVOUR OR EXCLUDE SUCH A POSSIBILITY?

Only limited information is available on the genetic basis for the decreased susceptibility to avilamycin/everninomicin in enterococci or other bacteria. Since susceptibility to avilamycin rapidly decreases in *in vitro* and *in vivo* experiments, it may be assumed that the « resistance » is caused by mutations in the chromosome. This assumption is further supported by new data (Aarestrup and Jensen, submitted; McNicholas *et al.,* 1999). This means that the resistance would not be easily transferred to other bacteria by means of plasmids and transposons. However, while the horizontal transfer of avilamycin resistance genes between enterococcal strains or enterococci and other bacteria may be very limited (or even non-existent), the possibility of horizontal transfer of avilamycin resistance by plasmids and/or transposons can not be completely excluded.

Human ingestion of avilamycin-resistant enterococci of animal origin will occur via the food chain. There is evidence that enterococci from animals can colonise humans, at least transiently (Stobbering, 1999). The chances of avilamycin resistant enterococci entering the human food chain depend on the prevalence of resistant strains among the animals and the degree of contamination of products of animal origin. The probability of humans in hospitals being colonised with antibiotic-resistant enterococci is likely to be influenced by the condition of the patient. Immuno-compromised patients treated with antibiotics are more liable to be colonised than healthy people. The main elements influencing the likelihood of patients in hospitals being infected with avilamycin-resistant enterococci of animal origin is the selective pressure exerted on enterococci from use of avilamycin in farmed animals and the selective pressure exerted on enterococci from use of everninomicin in hospitals.

Conclusion: Transfer of avilamycin resistant enterococci from animals to humans is possible *e.g.* via the food chain. The likelihood of such transfer or colonisation is influenced by the prevalence of resistant enterococci in the animal population, the degree of contamination of food products of animal origin and the selective pressure exerted on human patients from everninomicin use in hospitals. There is presently no evidence that transfer of avilamycin resistance genes between enterococci or other bacteria occur, although the possibility of such a transfer cannot be completely excluded.

7. CONCLUSIONS

Notwithstanding the limited information available, it has been shown that:

- the use of avilamycin as feed additive is associated with development of resistance in enterococci and staphylococci among exposed animals.
- the decreased use of avilamycin is associated with decreased prevalence of resistance among enterococci of animal origin.
- reduced susceptibility to avilamycin in enterococci is correlated with reduced susceptibility to everninomicin.

In addition,

- Transfer of avilamycin/everninomicin resistant enterococci from animals to humans will occur mostly via the food chain but its extent is impossible to estimate.
- Transfer of avilamycin-resistance genes between animal and human bacteria has not been demonstrated but cannot be completely excluded.

Finally,

- The likelihood of avilamycin resistant bacteria from farm animals colonising humans will depend on the prevalence of resistant bacteria in animals and food of animal origin and on the selective pressure exerted on humans' microflora from the use of everninomicin in hospitals.

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Wolf, H., 1973. Avilamycin, an inhibitor of the 30S ribosomal subunits function. *FEBS-Lett.*, **36 :** 181-186.

ADDITIONAL MATERIAL CONSULTED BY SCAN DURING THE PREPARATION OF THIS OPINION

(1) Data provided by the Swedish Government in support of its invoking of the safeguard clause, which included:
- The medical impact of the use of antibiotics in food animals. Report from a WHO meeting. Berlin, October 13-17 1997
- Opinion of the Economic and Social Committee of the European Communities on resistance to antibiotics as a threat to public health. 1998 - 38 pages. N° ESC-98-016-E
- Report from the Invitational EU Conference on the Microbial Threat. Copenhagen, Denmark, 9-10 September 1998.
- Report of the Health Council of the Netherlands: Committee on Antimicrobial Growth Promoters. Risjvijk, 1998. N° 1998/15E

(2) Dossiers provided by the Eli Lilly and Company Limited:
- Tylosin and avilamycin as growth promoters in food animals, November 1998;
- Response to Council Regulation 2821/98/EC, February 1999 and supplementary dossier May 1999.

(3) Comments of Member States on the above dossiers and data

(4) Response of Eli Lilly and Company Limited to comments

(5) Other papers:
- Antimicrobial resistant bacteria in food (Danish Veterinary and Food Administration);
- DANMAP 98 - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans;