Identification and Surveillance of Antimicrobial Resistance Dissemination in Animal Production

R. J. Bywater

Bywater Consultancy, Little Common House, Clungunford, Shropshire, SY7 0PL, United Kingdom

ABSTRACT Antimicrobial resistance is a growing problem in human medicine, and concern has been expressed that use of antimicrobials in animals may be a contributing factor. Although the majority of human pathogens showing antibiotic resistance have no link with animals, the issue of animal use of antimicrobials remains controversial, particularly with respect to antibiotic growth promoters (AGP). The European Union (EU) has withdrawn as AGP some compounds that remain in use in the United States. This difference in availability allows comparisons to be made of antimicrobial resistance outcomes with and without use of an AGP. Such comparisons so far show little apparent measurable benefit to human health resulting from the EU removal of AGP, and there is evidence of increased use of therapeutic antibiotics in animals to treat an apparent increased incidence of clinical disease. Microbial risk assessments are important in judging quantitatively or qualitatively whether the risk of using a particular AGP is acceptable in terms of potential hazard to human health. Resistance surveillance is an essential part of such microbial risk assessments, but such surveillance should be carefully planned to avoid confounding factors that could invalidate any conclusions.

(Key words: antibiotic resistance, surveillance, risk assessment, growth promoters)

INTRODUCTION

Antimicrobial resistance is an increasing problem in human medicine, and although it is recognized that this results mainly from antimicrobial use in human medicine, there has been concern that the use in animals may result in microbial resistance that could transfer to human pathogens. Antimicrobials are used in animals for treatment, disease prevention, and growth promotion, and the last of these has been subject to different approaches in Europe and the United States.

Antimicrobial growth promoters (AGP) are contentious, and the World Health Organisation has recommended (1997) that they should be phased out and replaced with alternatives. In the European Union (EU) this has begun to occur with a process of removal to be completed in 2006, but AGP continue to be used in the United States. Evidence that antibiotic use in animals is a major threat to human health remains sparse (Phillips et al., 2004), but there is a need for resistance surveillance in animals to explore the epidemiology of resistance dissemination. Application of microbial risk assessment (Snary et al., 2004) can determine whether or not there is real justification for concern with respect to a particular AGP.

©2005 Poultry Science Association, Inc.
Received for publication August 2, 2004.
Accepted for publication September 16, 2004.
1To whom correspondence should be addressed: rbywater@onetel.com.

ANTIMICROBIAL AGENTS IN ANIMAL PRODUCTION

What is the Contribution of Animal Sources to Resistance Among Human Pathogens?

One approach has been to direct a questionnaire to informed experts (Bywater and Casewell, 2000). This questionnaire was used to identify and prioritize the organisms most threatening to human health through antibiotic resistance and to estimate the perceived contribution from animal sources for each bacterial species. The experts involved were senior clinical medical microbiologists from the United States and Europe, and the results are shown in Figure 1. This survey suggests that the most important resistant pathogens such as methicillin-resistant Staphylococcus aureus were unrelated to animal sources, whereas those organisms for which there were possible animal sources of resistance were those viewed as less problematic. Organisms with a possible animal link were mainly zoonotic bacteria for which there is an obvious possibility that a resistant animal infection may be contracted by a human recipient. In total the perceived contribution from animals amounted to less than 4% of the overall human resistance problem.

Abbreviation Key: AGP = antibiotic growth promoter; EU = European Union; VRE = vancomycin-resistant enterococcus.
Antibiotic Use in Poultry

The poultry industry has been outstandingly successful in transforming an industry based originally on small individual producers into an industry based on large-scale, efficient units. Antimicrobials have been a factor in the success of the industry, whether for treatment of disease outbreaks, prevention of disease by group medication in the face of existing disease (metaphylaxis), or potential disease (prophylaxis). The final and most contentious use of antibiotics is for health maintenance or growth promotion, although the proportion used for this purpose (estimated as <10%; Animal Health Institute, 2003) is relatively small.

Availability of AGP in the EU and United States

Differences have arisen in the availability of AGP in the EU in comparison with the United States and are summarized in Table 1. The different usage patterns in the EU and the United States allow comparison of outcomes with respect to resistance and related matters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>United States</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine penicillin</td>
<td>Available</td>
<td>Withdrawn as AGP in several countries, 1972-1974</td>
</tr>
<tr>
<td>Tetracyclines (oxy- and chlor-)</td>
<td>Available</td>
<td>Withdrawn as AGP in several countries, 1972-1974</td>
</tr>
<tr>
<td>Zinc bacitracin</td>
<td>Available</td>
<td>Withdrawn in EU, 1999</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>Available</td>
<td>Withdrawn in EU, 1999</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>Never licensed</td>
<td>Withdrawn in EU, 1999</td>
</tr>
<tr>
<td>Tylosin</td>
<td>Available</td>
<td>Withdrawn in EU, 1999</td>
</tr>
<tr>
<td>Flavophospholipol</td>
<td>Available</td>
<td>To be withdrawn in EU, 2006</td>
</tr>
<tr>
<td>Monensin</td>
<td>Available</td>
<td>To be withdrawn in EU, 2006</td>
</tr>
<tr>
<td>Salinomycin</td>
<td>Available</td>
<td>To be withdrawn in EU, 2006</td>
</tr>
<tr>
<td>Avilamycin</td>
<td>Never licensed</td>
<td>To be withdrawn in EU, 2006</td>
</tr>
<tr>
<td>Avoparcin</td>
<td>Never licensed</td>
<td>Withdrawn in EU, 1997</td>
</tr>
</tbody>
</table>

Tetracyclines. The use of these compounds as AGP was ended in Europe in the 1970s, but they continue to be available for this purpose in the United States.

Background of the EU Ban

The United Kingdom has carried out surveillance of Salmonella incidence and resistance for many years. In the 1960s an alarming rise in multiresistant Salmonella typhimurium (strain DT 29) was observed. This led the to the Swann Report (1969), which recommended that antibiotics useful in therapy, (e.g., tetracyclines and procaine penicillin) should no longer be used as AGP. This restriction was enacted first in the United Kingdom and afterward in other European countries. Ironically, however, the original cause of concern (multiresistant Salmonella typhimurium DT 29) had peaked in 1965 and had largely disappeared before the ban was imposed. In the 1970s a different multiresistant strain (DT 204c) became prevalent, reached a peak, and eventually died, to be replaced in the 1990s by strain DT104, which again peaked and is at present declining (J. Threlfall, 2004, UK Food Standards Agency, personal communication).

The emergence and eventual decline of these organisms appears to be the result of clonal spread of dominant strains, which eventually lost their vigour and were replaced by other strains. The prevalence of such clones probably had little to do with availability of antibiotics, and much to do with clonal epidemiology (Hancock et al., 2000).

The ban on use of tetracycline as an AGP has not achieved its objective if judged by a comparison of the percentage tetracycline resistance in Salmonella spp. isolated from retail chicken in the United Kingdom (23%; Food Standards Agency, 2001) and the United States (19%; NARMS, 2000). This finding may be in part because tetracyclines are used in Europe for disease treatment, making it difficult to separate any selection pressure resulting from growth promotion as opposed to therapeutic use. As for resistance to tetracycline among bacteria within human intestines, a recent report showed that even in an isolated Bolivian community with virtually no exposure to antibiotics for human or animal use, (Bartelloni et al., 2004) tetracycline resistance of Escherichia coli was...
found to be 67%, suggesting that there is much yet to learn about the epidemiology of antibiotic resistance.

**Avoparcin.** Avoparcin is a glycopeptide related to vancomycin. Vancomycin is an important treatment for gram-positive infections (particularly due to *Staphylococcus* and *Enterococcus* species) in humans. Avoparcin was used as an AGP in Europe until 1997, but for commercial reasons was never licensed for any animal indication in the United States.

Vancomycin-resistant enterococcus (VRE) infection is a problem in human medicine, which has been suggested as originating from avoparcin use in animals (Witte, 1998). The contrasting history of avoparcin use in the United States and Europe invites comparison of outcomes. This shows paradoxically that the incidence of VRE among human isolates in the United States is high (CDC, 1999) despite the absence of animal use of avoparcin, whereas the VRE incidence in Europe is low (Schouten et al., 2000) despite the widespread use (before 1997) of avoparcin, with a resulting decline in resistance among animal isolates (DANMAP, 2003). After withdrawal of avoparcin, glycopeptide resistance in animals in Europe has declined to low levels (DANMAP, 2003), and clinical cases yielding VRE in hospitals has remained low. An exception was the United Kingdom, where VRE increased during the 1990s (Reacher et al., 2000), but the rising trend appeared to be unaffected by the avoparcin ban in 1997 and was probably related to hospital practices. Little connection appears, therefore, between clinical VRE cases and avoparcin use in animals. The explanation for the high prevalence of VRE in the United States lies in the greater human use of vancomycin in the United States compared with in Europe (Kirst et al., 1998), and no link with an animal source has been shown to be responsible.

**Virginiamycin.** This AGP is a streptogramin, which was widely used in Europe until its removal in 1999; it continues to be available in the United States. Virginiamycin has received attention after introduction of a related streptogramin, quinupristin/dalfopristin (Synercid), for human use in treatment of VRE. Because virginiamycin resistance can be found in *Enterococcus faecium* in treated animals, the concern is that there may be transmission to humans by colonization or by genetic transfer. Evidence of such transfer has been elusive, and reviews suggest that the risk to human health from virginiamycin use in animals is extremely low (Bafundo et al., 2003; Phillips et al., 2004). This was further confirmed by a recent report (Perri et al., 2004), which compared pulsed field gel electrophoresis (PGFE) patterns of enterococci from 24 farms with isolates from 10 hospitals in the same geographic area. There was, as expected, streptogramin resistance found among the animal isolates (37% of chicken enterococci), and some resistance (1%) was also found in human isolates. However PGFE comparison showed no evidence of spread from animals to humans, whereas in contrast there was evidence of spread between different hospitals. This is in keeping with the findings of a risk assessment suggesting that the chance of streptogramin resistance transfer to humans from animals treated with virginiamycin is very small (Cox and Popken, 2004).

**Effects of AGP Removals in Europe**

The removal of compounds as AGP in Europe, to be completed in 2006, has had some negative effects on animal health and welfare. This result could have been predicted by the Swedish experience after the ban on use of AGP in 1986), which was followed by an increase in disease (Swedish Ministry of Agriculture, 1997). The problems encountered in Sweden were necrotic enteritis in chickens and colitis in pigs, and similar effects were reported when Denmark terminated AGP use (WHO, 2002). The result in both cases was that although the removal of AGP resulted in lower overall use of antimicrobials, this was accompanied by an increase in the consumption of therapeutic antibiotics, as has been observed elsewhere in Europe where records are available, including the United Kingdom (VMD, 2002) and The Netherlands (MARAN, 2002). The increased need for therapy suggests that the effect of AGP had been, at least in part, to control disease.

**SURVEILLANCE OF ANTIBIOTIC RESISTANCE**

Adequate and comprehensive surveillance is essential for an understanding of the epidemiology of antimicrobial resistance in animals or in humans. Surveillance programs in the past have too often been ad hoc and sometimes flawed.

**Surveillance Program Pitfalls:**

**Source of Isolates**

**Clinical Isolates.** Clinical isolates form, by far, the most common source of sensitivity data from isolates from man or animals. Clinical laboratories examine isolates in an effort to determine the suitability of a current or future treatment. These samples, whether from a human or animal patient, are by definition from a sick individual, and such an isolate from a clinical case is likely to be biased toward a more resistant pattern due to previous treatment with selection of resistant bacteria as a result (Wray et al., 1993).

**Planned Surveillance Programs.** Increased interest in resistance issues and consequent funding has allowed the planning of organized studies. These should be ideal in that the source of the isolates can be chosen to eliminate bias and distortion. Thus far there have been relatively few studies in animals and even fewer that attempt international coverage.

The program being carried out in Denmark and reported annually since 1996 (DANMAP, 2003) is unusual in the breadth of sampling and the detailed reporting of trends. It also includes commensals and pathogens and attempts to correlate changes observed with consumption
of antibiotics. The program uses samples from slaughterhouses, from clinical cases and from human volunteers.

**Ad Hoc Surveillance Programs.** National surveillance of salmonella resistance has been carried out on isolates in several EU countries including The Netherlands, United Kingdom, and Germany. Annual reporting in the United Kingdom was instituted followed the Swann Report (1969) and has continued over 25 yr, thus satisfying the criterion of repeated observations over time. Testing is based on disc diffusion; sources of the isolates are largely clinical cases (see above), and there is no denominator information (the proportion of the population represented by the bacteria surveyed). Nevertheless, useful data emerge from such studies that illustrate changes in the prevalence of strains, which in turn is reflected in changes in susceptibility.

**Laboratory Methodology**

**Variations in Technique Among Laboratories.** If results are combined from more than one laboratory, quality control checks must ensure comparability of results. Many laboratories have established methods for susceptibility testing that have been in use for many years. They may each be perfectly defensible yet may differ sufficiently to prevent any comparison of data among laboratories.

**Variations Within Laboratories.** These variations are usually a reflection of poor quality control, which can result in many sources of error. Standard disks or solutions may be outdated, stored at the wrong temperature, or, in the case of discs, exposed to moisture (usually condensation), and so become inaccurate. Agar may be prepared incorrectly or poured at incorrect or inconsistent depth, and incubation conditions may be changed. All of these and other changes can affect results of disc diffusion or minimum inhibitory concentration determination.

**Breakpoints.** These are often a source of confusion. A clinical breakpoint should allow a prediction of clinical efficacy and be arrived at through a process that involves pharmacokinetic and clinical data. Breakpoints may thus be species specific; indeed sometimes they are disease specific. In practice there is a shortage of well-founded breakpoints for animals, and many are taken from human medicine with little thought as to whether this practice is appropriate. Moreover, there has been a lack of agreement among laboratories and countries as to what figure to take as a breakpoint (Baquero, 1990). However the US National Committee for Clinical Laboratory Standards (NCCLS) is becoming more widely accepted as an international and national standard.

**An International Surveillance Program.** A surveillance study of enteric bacteria (*E. coli*, *Salmonella* spp., and *Campylobacter* spp.) from chickens at time of slaughter (de Jong et al., 2003) was carried out in parallel in 4 EU countries but used a single testing laboratory, together with a common protocol. Thus the survey overcame the pitfalls identified above, and the results showed variations among countries, which could not be ascribed to differences in methodology. The differences might have reflected differences in antibiotic policies among countries, but the results also showed minimal resistance to modern antibiotics important to human health.

**Risk Assessment of Antibiotic Use in Animals.** Decisions on changes in antibiotic availability policy, especially with respect to use in animal production, should be on the basis of risk assessment. The approaches to microbiological risk assessment have recently been reviewed (Snary et al., 2004) and recently have been published for virginiamycin (Cox and Popken, 2004 ) and macrolides, including tylosin (Hurd et al., 2004). These findings suggest that the risk to human health is very small for the compounds assessed. As a basis for decision making, risk assessment appears preferable to the “precautionary principle,” sometimes viewed as an alternative, especially in the EU. The precautionary principle was introduced to take account of environmental chemical hazards that had not been subject to safety assessment and suggests that in the absence of definitive safety data, a pre-emptive removal of the threat should ensue. However, licensed animal antimicrobial products have, as part of the regulatory process, been assessed for safety (together with quality and efficacy). The licensing process for antimicrobials, including AGP, is thus itself precautionary, so arguably rendering the precautionary principle inappropriate and redundant.

**CONCLUSIONS**

Antimicrobial use during animal production remains controversial, but evidence of resulting harm to human health remains elusive. The EU removal of certain AGP has reduced antimicrobial resistance in animals in these countries but has been accompanied by increased need for antimicrobial therapy following disease problems in animals. Evidence of human health benefits attributable to AGP removals has been elusive. Qualitative and quantitative risk assessments for individual agents are needed to assess the degree of hazard. Such assessments need data from well-designed surveillance programs, which are essential to extend the present limited understanding of resistance epidemiology.

**REFERENCES**


DANMAP 2003. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. Danish Veterinary Laboratory Copenhagen, Denmark.


