

# Antimicrobial resistance in *Escherichia coli* strains isolated from Swiss weaned pigs and sows

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## Summary

Based on Directive (EC) No 99/2003, monitoring programs on the development of antimicrobial resistance in bacteria from livestock are implemented in many European countries. The aim of the present study was (i) to establish comprehensive baseline data on the antimicrobial resistance situation in *Escherichia coli* isolates obtained from healthy pigs (pooled fecal samples) originating from 60 Swiss pig-breeding farms, and (ii) to analyze differences in the resistance frequency between *Escherichia coli* isolates from weaned pigs and sows. Susceptibility testing (disc diffusion method) was performed on 429 isolates from weaned pigs and 431 isolates from sows. Overall, 17.7% of the isolates from weaned pigs and 22.5% of the *Escherichia coli* isolates from sows were susceptible to all antibiotics tested. Low resistance prevalence was found for amoxicillin, amoxicillin/clavulanic acid, ampicillin, cefquinome, ciprofloxacin, colistin, florfenicol, and gentamicin. The most frequently found resistances were against streptomycin (60.6% of the isolates from weaners and 64.3% of the isolates from sows), sulfonamide (51.5% and 26.9%), tetracycline (35.2% and 22.0%), and trimethoprim (27.5% and 11.1%). With exception of colistin, most resistances were found for those antibiotics commonly used on the farms. Except for ciprofloxacin and streptomycin, isolates from weaned pigs showed higher resistance prevalence than those from sows. This difference was significant for cefquinome, florfenicol, sulfonamide, tetracycline, and trimethoprim ( $p < 0.05$ ).

Keywords: weaned pigs, sows, fecal samples, *E. coli*, antimicrobial resistance

## Antibiotikaresistenzen bei *Escherichia coli*-Stämmen von Absetzferkeln und Galtsauen

Gemäss den Anforderungen der Richtlinie (EG) Nr. 99/2003 wurden in vielen europäischen Ländern Programme zur Überwachung von Antibiotika-Resistenzentwicklungen bei Bakterien von Nutztieren eingeführt. Das Ziel der vorliegenden Arbeit war es, (i) Baseline-Daten zum Status quo von Antibiotikaresistenzen bei *Escherichia coli* aus Sammelkotproben von gesunden Schweinen aus 60 Schweinezuchtbetrieben der Schweiz zu erheben und (ii) Unterschiede in der Häufigkeit von Resistenzen bei *Escherichia coli* von Absetzferkeln und Galtsauen zu analysieren. Mittels Agardiffusions-Methode wurde bei 429 Isolaten von Absetzferkeln und 431 Isolaten von Galtsauen eine Empfindlichkeitsprüfung durchgeführt. Von diesen Isolaten erwiesen sich insgesamt 17.7% (Absetzferkel) beziehungsweise 22.5% (Galtsauen) als empfindlich auf alle getesteten Antibiotika. Tiefe Resistenzraten wurden insbesondere für Amoxicillin, Amoxicillin/Clavulansäure, Ampicillin, Cefquinom, Ciprofloxacin, Colistin, Florfenicol und Gentamicin gefunden. Am häufigsten liessen sich Resistenzen gegen Streptomycin (60.6% der Isolate von Absetzferkeln und 64.3% der Isolate von Galtsauen), Sulfonamid (51.5% und 26.9%), Tetracyclin (35.2% und 22.0%), und Trimethoprim (27.5% und 11.1%) nachweisen. Mit Ausnahme von Colistin lagen die meisten Resistenzen gegen diejenigen Antibiotika vor, die auch häufig auf den Betrieben eingesetzt wurden. *Escherichia coli*-Isolate aus Absetzferkelkot wiesen mit Ausnahme von Ciprofloxacin und Streptomycin deutlich höhere Resistenzraten als Isolate aus Galtsauenkot auf. Für Cefquinom, Florfenicol, Sulfonamid, Tetracyclin und Trimethoprim erwiesen sich diese Unterschiede als signifikant ( $p < 0.05$ ).

Schlüsselwörter: Absetzferkel, Galtsauen, Kotproben, *E. coli*, Antibiotikaresistenz

## Introduction

Despite of the ban on the use of antimicrobial agents to promote growth in livestock animals in the European Union (EU) and Switzerland, antibiotics are still widely used in animal farming. The study of Arnold et al. (2004) provided for the first time an overview on the quantitative use of different antimicrobial agents in pig husbandry in Switzerland. Generally, sulfonamides, beta-lactams, and tetracyclines form the major part of antimicrobial drugs distributed for veterinary usage in Switzerland, followed by trimethoprim and aminoglycosides (Swissmedic, 2006). Associated with the use of antibiotics in animal farming and agriculture is the potential problem of resistance development in bacteria threatening public health (Witte, 1998; Aarestrup, 1999; Wegener et al., 1999; Barbosa and Levy, 2000; Sørensen et al., 2001; O'Brien, 2002). Worldwide, bacterial resistance has become an increasing problem in antimicrobial therapy (Tenover, 2001; Levy and Marshall, 2004).

Directive (EC) No 99/2003 on the monitoring of zoonoses and zoonotic agents requires the monitoring of antimicrobial resistances in the EU member states (Anonymous, 2003). This directive specifies that beside zoonotic agents the monitoring should cover also other agents. In particular, indicator organisms might be appropriate because such organisms constitute a reservoir of resistance genes, which can eventually be transferred to pathogenic bacteria (van den Bogaard and Stobberingh, 2000; Sørnum and L'Abée-Lund, 2002; Anonymous, 2003). Due to the equivalence agreement with the EU, the requirements of Directive (EC) No 99/2003 are also effective for Switzerland. In order to get information on the development of resistance in bacteria from livestock, various countries are about to establish or have established monitoring programs. The Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) has gained pioneer status by providing systematic, integrated, and continuous monitoring data of antimicrobial agent consumption and bacterial resistance in animals, food, and humans (Hammerum et al., 2007). Similar monitoring programs are now established e.g. in Norway (NORM-VET), Sweden (SVARM), or Canada (CIPARS). In Switzerland, a comparable comprehensive monitoring program is under way and in the meantime some data are available (Ledergerber et al., 2005; Ledergerber and di Labio, 2007). The Swiss program started with the monitoring of resistances in *Campylobacter* in poultry. In the year 2006, the bulk of data still originated from poultry, albeit pigs and cattle were also considered (Ledergerber and di Labio, 2007).

The aim of the present study was to establish comprehensive baseline data on the resistance situation in *Escherichia (E.) coli* isolates obtained from healthy pigs originating from 60 Swiss pig-breeding farms. Moreover, differences in the resistance frequency between *E. coli* isolates obtained from weaned pigs and sows were analyzed.

## Animals, Material and Methods

### Animals and sample collection

Pig farmers in eastern and central Switzerland were asked to participate in this study by veterinarians from the Swiss Swine Health Service during routine visits of the farms. Farms with 24 to 200 sows were considered. From October 2004 to April 2005, 60 Swiss pig-breeding farms recruited for this study were visited. In contrast to other established resistance monitoring programs (DANMAP; NORM-VET; SVARM), samples were taken on farms rather than at slaughter. Thereby, the resistance situation in the production facilities could be examined. Pooled fecal samples were collected from the floor of the pens containing apparently healthy weaned pigs or sows. Dunlop et al. (1999) recommended the use of pooled samples. Five samples were combined to make one pooled sample (Regula et al., 2003). The number of sows on each farm determined the quantity of samples. Two, three, and four samples per age group (weaned pigs, sows) were collected on farms with <50 sows, 50 to 150 sows, and >150 sows, respectively.

Weaned pigs considered for sampling had been weaned from the sows at least three weeks prior to sampling and had not been orally treated with antibiotics for at least 14 days. In total, 163 pooled fecal samples were obtained from weaner pens and 165 from sow pens. Data about the use of antibiotics during the last six months before sampling were obtained from the treatment records of the farms. If not available or adequate, data were supplemented by oral communications from the farmer.

### Strain isolation and susceptibility testing

From each pooled fecal sample, material was directly transferred by a moistened swab (0.85% NaCl) onto RAPID *E. coli* agar (BIORAD, Marnes La-Couquette, F). Presumptive colonies (violet onto RAPID *E. coli* agar due to the  $\beta$  galactosidase and  $\beta$ -D glucuronidase activity) were confirmed as *E. coli* by biochemical properties: lactose positive, o-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) positive, H<sub>2</sub>S negative, indole positive, and urease negative. Up to three *E. coli* isolates per plate were selected for resistance testing.

To test the susceptibility to antibiotics, the disc diffusion method was used. Therefore, *E. coli* isolates were first enriched in Trypticase Soy Broth (TSB, 211768, Becton Dickinson, Sparks, USA) and then plated on Müller-Hinton-Agar (CM 337, Oxoid Ltd., Basingstoke, Hampshire, UK) as described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (CLSI, 2002, 2004). The following antibiotic-impregnated discs were used: amoxicillin 25  $\mu$ g (bioMérieux SA, Marcy l'Etoile, F), amoxicillin/clavulanic acid 30  $\mu$ g (bioMérieux SA), ampicillin 10  $\mu$ g (bioMérieux SA), cefquinome 10  $\mu$ g

(Oxoid Ltd.), ciprofloxacin 5 µg (bioMérieux SA), colistin 10 µg (Becton Dickinson), florfenicol 30 µg (Mast Group Ltd., L20 1EA Bootle Merseyside, UK), gentamicin 10 µg (bioMérieux SA), streptomycin 10 µg (bioMérieux SA), sulfonamide 200 µg (bioMérieux SA), tetracycline 30 µg (bioMérieux SA), and trimethoprim 5 µg (Becton Dickinson). Zones of growth inhibition were evaluated in accordance with CLSI (formerly NCCLS) standards (CLSI, 2002, 2004) when adequate reference values were available. For amoxicillin, cefquinome, and sulfonamide standards recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM, 2008) were used. Since no established reference value was available for florfenicol, all *E. coli* isolates with inhibition zones <14 mm were regarded as possibly resistant (personal communication of V. Dehaas, Director EU Clinical Research, Schering-Plough Animal Health, 92307, Levallois-Perret Cedex, F). All isolates with intermediate growth were considered to be resistant. Statistical evaluation of data was performed using NCSS statistical software 2004 (NCSS, Kaysville, USA). To compare the respective resistance patterns of *E. coli* isolates obtained from weaned pigs and sows, contingency tables (Chi-square test) were used.

## Results

### Antimicrobial susceptibility testing of *E. coli*

Susceptibility testing was performed on 429 *E. coli* isolates from weaned pigs (58 farms) and 431 *E. coli* isolates from sows (59 farms). Overall, 17.7% of the isolates from weaned pigs and 22.5% of the isolates from sows were

susceptible to all antibiotics tested. Low resistance levels were found for amoxicillin, amoxicillin/clavulanic acid, ampicillin, cefquinome, ciprofloxacin, colistin, florfenicol, and gentamicin (Tab. 1). In both age groups, the most frequently found resistances were against streptomycin, sulfonamide, tetracycline and trimethoprim (Tab. 1). Except for ciprofloxacin and streptomycin, isolates from weaned pigs showed higher resistance prevalence than those from sows. For cefquinome, florfenicol, sulfonamide, tetracycline, and trimethoprim, differences between the two age groups (weaned pigs, sows) were significant ( $p < 0.05$ ). Single resistance was detected in 24.0% and 40.7% of the *E. coli* isolates from weaned pigs and sows, respectively. The proportion of multiple resistant *E. coli* was significantly higher ( $p < 0.05$ ) among isolates from weaned pigs (58.3%) than in those from sows (37.1%).

### Use of antibiotics on the farms

In general, the documentation of antibiotic use from the farmers proved to be insufficient to state accurately the quantity of antibiotics used. Therefore, the information is limited to the proportion of farms using the antibiotics considered in this study (Tab. 2 and 3). Colistin, sulfonamide, tetracycline, and trimethoprim were frequently used for oral group medication of weaned pigs (Tab. 2). None of the farmers had treated groups of sows orally during the last six months before sampling. For treatment of individual animals, the combination of dihydrostreptomycin and penicillin G and the combination of trimethoprim and sulfonamide were most commonly used (Tab. 3). None of the farmers had utilized Nuflor® (Schering-Plough Animal Health, Branchburg, USA), the only florfenicol preparation registered for use in pigs in Switzerland.

Table 1: Prevalence of antibiotic resistance in *E. coli* strains from weaned pigs and sows from 60 Swiss pig breeding farms (C.I., confidence interval).

| Antibiotic agent            | Weaned pigs (n=429) |             | Sows (n=431)       |             |
|-----------------------------|---------------------|-------------|--------------------|-------------|
|                             | Resistant isolates  | 95% C.I.    | Resistant isolates | 95% C.I.    |
| Amoxicillin                 | 8.9%                | 6.3 – 12.0  | 7.4%               | 5.1 – 10.3  |
| Amoxicillin/clavulanic acid | 2.6%                | 1.3 – 4.5   | 1.4%               | 0.5 – 3.0   |
| Ampicillin                  | 8.6%                | 6.1 – 11.7  | 5.8%               | 3.8 – 8.4   |
| Cefquinome                  | 4.0%                | 2.2 – 6.3   | 0.5%               | 0.0 – 1.7   |
| Ciprofloxacin               | 0.5%                | 0.1 – 1.7   | 1.4%               | 0.5 – 3.0   |
| Colistin                    | 3.7%                | 2.1 – 6.0   | 3.5%               | 2.0 – 5.7   |
| Florfenicol                 | 5.6%                | 3.6 – 8.2   | 0.5%               | 0.1 – 1.7   |
| Gentamicin                  | 2.1%                | 1.0 – 3.9   | 0.7%               | 0.1 – 2.0   |
| Streptomycin                | 60.6%               | 55.8 – 65.3 | 64.3%              | 59.5 – 68.8 |
| Sulfonamide                 | 51.5%               | 46.7 – 56.3 | 26.9%              | 22.8 – 31.4 |
| Tetracycline                | 35.2%               | 30.7 – 39.9 | 22.0%              | 18.2 – 26.3 |
| Trimethoprim                | 27.5%               | 23.3 – 32.0 | 11.1%              | 8.3 – 14.5  |

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Table 2: Oral group treatment of weaned pigs on the 60 farms with antibiotics tested in this study within six months before sampling.

| Antibiotic agent | % of farms using antibiotics for oral group treatment |                                      |       |
|------------------|---|--------------------------------------|-------|
|                  | Only if indicated <sup>a</sup>                        | Metaphylactic treatment <sup>b</sup> | Total |
| Colistin         | 26.7  | 25.0                                 | 51.7  |
| Sulfonamide      | 15.0  | 6.7                                  | 21.7  |
| Tetracycline     | 10.0  | 3.3                                  | 13.3  |
| Trimethoprim     | 10.0  | 3.3                                  | 13.3  |
| Amoxicillin      | 1.7   | 0.0                                  | 1.7   |
| No medication    | –   | –                                    | 31.7  |

<sup>a</sup> generally no treatment was necessary, only single litters with symptoms of disease have been treated after weaning

<sup>b</sup> diagnosis of a herd problem is on hand; therefore all litters were treated after weaning for a certain period of time

## Discussion

The results of the present study on the occurrence of antimicrobial resistances (AR) in *E. coli* from apparently healthy pigs (weaned pigs, sows) showed that about 20% of the pig isolates were susceptible to all antibiotics tested. A comparable study was recently performed in Switzerland to evaluate the level of AR in bacteria from healthy calves at slaughter (di Labio et al., 2007). Thereby, the overall resistance prevalence in *E. coli* isolates from veal calves was 69%. Other AR prevalence data from broilers, pigs, and cattle are available from the Swiss zoonoses report 2006 (Ledergerber and di Labio, 2007). In the EU, the annual community summary report of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) provides an overview on the resistance situation in bacteria from livestock including *E. coli* from pigs (Anonymous, 2007).

In the present study, high resistance rates were detected for streptomycin, sulfonamide, tetracycline, and trimethoprim (Tab. 1). Although the quantity of antibiotics used in the different farms could not be assessed exactly, higher resistance rates were generally found for those antibiotics, which were used more frequently on the farms (Tab. 2 and 3). Concurrent resistance of *E. coli* to streptomycin, sulfonamide, and tetracycline is often observed, suggesting frequent application of these antibiotics and a genetic linkage of resistance determinants (Dewulf et al., 2007). In the EU, high AR levels in *E. coli* from pigs were reported for tetracycline (overall average 68.1%), streptomycin (overall average 36.4%), and ampicillin (overall average 27.8%), but the reported levels varied considerably among the member states (Anonymous, 2007). Isolates from different member states may originate from different categories of animals. This presents a source of variation in the results, because prevalence of AR in indicator bacteria can differ markedly in different ages or classes of animals. In Denmark, DANMAP 2005 provides a set of data for AR in *E. coli* from fattening pigs. These isolates also showed little resistance against amoxicillin, amoxicillin/clavulanic acid, gentamicin, florfenicol, ciprofloxacin, and colistin. But in contrast to our results, high prevalence of ampicillin resistance was present in the Danish pigs.

Table 3: Treatment of individual animals on the 60 farms with antibiotics tested in this study within six months before sampling.

| Antibiotic agent                     | % of farms using antibiotics for individual animal treatment |
|--------------------------------------|--|
| Dihydrostreptomycin and penicillin G | 55.0   |
| Trimethoprim and sulfonamide         | 40.0   |
| Enrofloxacin                         | 36.7   |
| Gentamicin                           | 30.0   |
| Tetracycline                         | 13.3   |
| Cephalosporins (cefquinome)          | 11.7   |
| Colistin                             | 11.7   |
| Amoxicillin                          | 6.7  |
| Amoxicillin/clavulanic acid          | 3.3  |
| Ampicillin                           | 1.7  |

Although more than 50% of the farmers had used colistin in weaned pigs within six months before sampling in our study (Tab. 2), less than 4% of the evaluated *E. coli* isolates were resistant to this antibiotic. In this context it must be considered that differing colistin susceptibility results were reported for the disc diffusion and the broth microdilution method (Gales et al., 2001). Tested on the same strain collection, the disc diffusion method tended to yield more false-susceptible results. Therefore, the low prevalence of colistin resistance in pigs from Swiss breeding farms might be an underestimation. Otherwise, it may be assumed that colistin does not belong to the group of antibiotics against which high resistance rates evolve. To obtain more reliable data, minimal inhibitory concentration (MIC) data for colistin are required in future studies. For the generally higher resistance prevalence in the *E. coli* isolates from weaned pigs than from sows there are two possible explanations. First, oral administration of antibiotics was almost restricted to groups of weaned pigs and individual suckling piglets. A major part of the antimicrobial treatments in pigs is administered during the suckling, weaning, and early fattening period (Timmerman et al., 2006). By adding antibiotics to the feedstuff, subtherapeutic doses, which are considered to promote resistance development, are more likely to occur because equal dosage is difficult to guarantee for all animals. On

the other hand, sows were generally treated less often than weaned pigs and adult pigs were not treated orally in any of the investigated farms. In sows, antibiotics were mostly applied to single animals by intramuscular injection. Second, the incomplete colonization barrier in the gut flora of weaned pigs may also contribute to the different AR rates between the two age groups. Whereas the gastrointestinal tract of adult mammals is colonized by a bacterial population, which is rather stable in size and over time, the intestinal flora of young animals is still under development and can be influenced easier by extrinsic factors like changes in feed composition and selective pressure of antibiotics (Mackie et al., 1999).

The present study shows that healthy pigs serve as reservoir for resistant *E. coli*. The resistance situation in Switzerland is comparable with other European countries. As a veterinary public health issue, the surveillance of the

AR situation in bacteria from livestock is of great importance because resistant bacteria may be transferred from the animals to humans by direct contact or by food of animal origin. The monitoring of AR in indicator bacteria enables the evaluation of trends in the occurrence of AR in animals, even when zoonotic pathogens are scarce or absent. Our results on the occurrence of AR in *E. coli* from apparently healthy pigs (weaned pigs, sows) constitute baseline data on the actual situation and are valuable in the implementation and maintenance of the national monitoring program for AR in Swiss pig-breeding farms.

## Acknowledgement

This work was partly funded by the Swiss Federal Veterinary Office.

### Antibiorésistance des souches d'*Escherichia coli* chez les porcelets au sevrage et les truies non allaitantes

Conformément aux exigences de la directive (UE) no 99/2003, des programmes de surveillance du développement de résistances aux bactéries chez les animaux de rentes ont été introduits dans plusieurs pays européens. Le but du présent travail était : (1) de relever des données de bases quant à la situation d'antibiorésistance chez *Escherichia coli* provenant de pools d'échantillons de selles de 60 élevages suisses et (2) d'analyser les différences dans la fréquence de ces résistances entre les porcelets au sevrage et les truies. Un test de sensibilité a été effectué au moyen d'une diffusion sur gel d'agar sur 429 isolats de porcelets et 431 de truies non allaitantes. 17.7% (porcelets) respectivement 22.5% (truies) de ces isolats se sont montrés sensibles à tous les antibiotiques testés. Les taux de résistances les plus bas ont été trouvés en particulier face à l'amoxicilline, l'amoxicilline/acide clavulanique, l'ampicilline, le cefquinone, la ciprofloxacine, la colistine, le florfenicol, et la gentamycine. Les résistances les plus fréquentes concernaient la streptomycine (60.6% des isolats de porcelets et 64.3% des isolats de truies) les sulfamidés (51.5% et 26.9%) la tetracycline (35.2% et 22%) et le triméthoprime (27.5 et 11.1%). À l'exception de la colistine, la plupart des résistances concernaient les antibiotiques qui étaient le plus souvent utilisés dans les exploitations. Les isolats de porcelets présentaient, sauf pour la ciprofloxacine et la streptomycine, des taux de résistances nettement plus élevés que ceux provenant des truies. Pour le cefquinone, le florfenicol les sulfamidés, la tetracycline et le triméthoprime, ces différences étaient significatives ( $p < 0.05$ ).

### Resistenza agli antibiotici dei ceppi di *Escherichia coli* nei suinetti e nei giovani suini

Sulla base delle esigenze delle direttive (EU) Nr. 99/2003, sono state introdotti in molti Paesi europei dei programmi per sorvegliare le apparizioni di resistenze agli antibiotici nei batteri negli animali da reddito. Scopo di questo studio era di (i) ottenere dati di base, provenienti da campioni di escrementi raccolti in suini sani in 60 aziende di allevamento in Svizzera, sullo stato della resistenza antibiotica dell'*Escherichia coli* e (ii) analizzare le differenze nella frequenza delle resistenze all'*Escherichia coli* nei suinetti e nei giovani suini. È stato eseguito un esame della sensibilità tramite il metodo della agar diffusione su 429 isolati di suinetti e 431 isolati di giovani suini. Da questi isolati sono risultati come sensibili a tutti gli antibiotici testati in totale 17.7% (suinetti) rel. 22.5% (giovani suini). Basate percentuali di resistenza sono state trovate in particolare per l'amoxicillina, amoxicillina/acido clavulanico, ampicillina, cefquinone, ciprofloxacina, colistina, florfenicol e gentamicina. Di frequente si sono rilevate resistenze verso streptomycina (60.6% degli isolati di suinetti e 64.3% di isolati di giovani suini), sulfonamide (51.5% e 26.9%), tetraciclina (35.2% e 22.0%), e trimetoprima (27.5% e 11.1%). Ad eccezione della colistina la maggior parte delle resistenze si presentavano verso quegli antibiotici che vengono utilizzati maggiormente nelle aziende. Gli isolati di *Escherichia coli* provenienti da escrementi di suinetti mostravano con eccezione della ciprofloxacina e della streptomycina delle chiare e alte percentuali di resistenza per rapporto agli isolati di escrementi dei piccoli suini. Per quel che riguarda cefquinone, florfenicol, sulfonamide, tetraciclina e trimetoprima queste differenze sono risultate significative ( $p < 0.05$ ).

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Received: 19 July 2007

Accepted: 15 August 2008