

Relation between antimicrobial use and resistance in Belgian pig herds

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Abstract

The aim of this study was to determine the link between the characteristics of antimicrobial therapy and occurrence of antimicrobial resistance in *Escherichia coli* of clinically healthy pigs exposed to antimicrobial treatments.

A total of 918 *Escherichia coli* isolates were obtained from faecal samples, collected from 50 pig herds at the end of the fattening period and susceptibility was tested towards 15 different antimicrobial agents, using the disk diffusion method. The Antimicrobial Resistance Index (ARI) of each isolate was calculated, as the number of antimicrobials to which resistance was found divided by the number of drugs tested. The antimicrobial resistance percentage per class (ARclass) was defined as the percentage of *E. coli* strains clinically resistant to that specific class. Data on group level antimicrobial use in the sampled herds was collected and quantified as treatment incidences (TI) based on the used daily dose pig (UDDpig) and the animal daily dose pig (ADDpig) (number of pigs treated with one ADDpig or UDDpig/1000 pigs at risk/day). The UDDpig/ADDpig ratio gives an indication of the correctness of dosing.

The TIADDpig for group level use was 235.7 per 1000 pigs at risk per day, whereas the TIUDDpig equaled 200.7. This means that in reality, fewer pigs were treated with the same amount of antimicrobials than theoretically expected and thus antimicrobials were generally overdosed. Generalized linear regression analysis showed a significant relation between the TIADDpig and the ARI ($p < 0.01$), whereas there were no significant links for the TIUDDpig ($p > 0.05$). Analysis of the antimicrobial resistance for β -lactam antimicrobials and tetracyclines suggests that the effect of correct or incorrect dosing on resistance development was different for the different antimicrobial classes tested. Besides the amount of administered antimicrobial agents, the frequency of drug administration may play a role in the selection of antimicrobial resistance in commensal *E. coli*.

Introduction

The emergence of resistant bacteria is a well documented threat to the effectiveness of therapy in both human and veterinary medicine (Schwarz et al., 2001; Catry et al., 2003). Besides several bacterial-associated factors, the characteristics of an antimicrobial therapy are believed to contribute to the rate and the extent of antimicrobial resistance development (Schwarz et al., 2001; Catry et al., 2003). The selection pressure exerted by the use of antimicrobial agents might be influenced by several features such as dose, duration of treatment and frequency of therapy, but few data are available to support these hypotheses. The goal of antimicrobial therapy is to help an animal in its cure from disease by a reduction or elimination of pathogenic bacteria. Yet, selection pressure will also be exerted on the commensal bacterial flora during metaphylactic and prophylactic antimicrobial use. So a high selection pressure will be exerted mainly on the commensal flora. The aim of this study is to investigate the link between the characteristics of antimicrobial therapy and occurrence of antimicrobial resistance in *Escherichia coli* exposed to antimicrobial treatments.

Material and Methods

Study design, data and sample collection

Fifty closed or semi-closed pig fattening herds were randomly selected from the Belgian farm-animal identification and registration database (SANITEL, 2010) and visited between January and October 2010. The herds held at least 150 sows and 600 fattening pigs. On each herd 20 randomly selected fattening pigs were sampled within 3 weeks before slaughter. Faecal samples were taken after rectal stimulation and were put in a sterile recipient. The samples were inoculated the same day of collection.

All data on antimicrobial group level treatments applied between birth and time of sampling of the selected animals were

collected retrospectively. A group treatment was defined as each prophylactic or metaphylactic administration of antimicrobials to all the animals of the same production group. For each group treatment, following data were gathered: product name, duration of therapy, dose applied, administration route, age of the treated animals and body weight at time of treatment (estimated by means of a standard growth table).

Data on the external and internal biosecurity level on the herds were obtained by means of the biocheck biosecurity quantification system as described in detail in Laanen et al. (2011).

Bacteriological analysis and susceptibility testing

The isolation, identification and susceptibility testing of *Escherichia coli* was performed as previously described in Persoons et al. (2010).

Following antimicrobial groups were tested (agents): β -lactam antimicrobials (ampicillin, amoxicillin-clavulanic acid and ceftiofur), phenicols (chloramphenicol and florfenicol), tetracycline, aminoglycosides (gentamicin, streptomycin, apramycin, neomycin and kanamycin), quinolones (enrofloxacin, nalidixic acid), sulfonamides (sulfadiazine), pyrimidines (trimethoprim).

Data were dichotomized into resistant or susceptible by allocating the intermediately susceptible isolates to the susceptible group.

Quantification of drug consumption

Antimicrobial drug consumption was quantified as treatment incidences (TI) based on the animal daily dose pig (ADDpig) and the used daily dose pig (UDDpig). TIADDpig and TIUDDpig (number of pigs treated with one ADDpig or UDDpig/1000 pigs at risk per day or number of days during which a pig is administered antimicrobials in a theoretical live of 1000 days) were calculated based on the acquired data, according to the method described by Timmerman et al. (2006). The UDDpig/ADDpig ratio of each antimicrobial treatment gives an idea of the correctness of dosing. A variation of 0.2 under or above 1 (range 0.8-1.2) was considered as correct dosing. The 'frequency of therapy' is a count for every new start of an antimicrobial therapy administered during a pigs' lifetime.

Data analysis

The Antimicrobial Resistance Index (ARI) for each isolate was calculated as the number of antimicrobials to which resistance was found divided by the number of drugs tested. The average ARI per herd was used in further analysis.

Antimicrobial resistance percentage per antimicrobial class (ARclass) was defined as the percentage of *E. coli* strains resistant to that specific class. For those antimicrobial classes where more than one antimicrobial was tested, a strain was determined "resistant" if it was resistant against at least one antimicrobial of that class.

A generalized linear regression model was developed to study the possible relationship between the TI based on ADDpig or UDDpig, the UDDpig/ADDpig ratio, the frequency of therapy and the external and internal biosecurity scores on the one hand and ARI on the other hand. In this analysis a combination of all antimicrobial treatments was used as a measure of the total consumption on the herd.

For the seven different antimicrobial classes a logistic regression model with antimicrobial resistance per class (ARclass) as a binomial outcome variable and the potential risk factors listed above as covariates was performed. The antimicrobial treatments tested in this analysis were restricted to the ones corresponding to the class of the dependent variable.

All statistical analyses were performed in SPSS 19.0 (SPSS inc., Chicago Illinois, USA).

Results

An average isolation success of 18.4 isolates per 20 sampled pigs per herd was achieved, resulting in a total of 918 *Escherichia coli* isolates.

The average TIADDpig for group level use was 235.7 per 1000 pigs at risk per day (SD = 222.7, Min = 0, Max = 1322.1), whereas the average TIUDDpig equaled 200.7 (SD = 136, Min = 0, Max = 699). This means that on average, fewer pigs were treated with the same amount of antimicrobials than theoretically possible which suggests that the antimicrobials are on average overdosed. A significant positive relation between the TIADDpig and the ARI ($p < 0.01$) was seen, whereas a link could be found between the TIUDDpig and the ARI ($p = 0.085$). Likewise higher TI showed higher ARclass for both the β -lactam antimicrobial agents as for the tetracyclines. Analysis of other antimicrobial classes included in the susceptibility testing, could not be performed since no sufficient data on antimicrobial use of other classes were available.

Lower values for ARI after correct dosing compared to under- or overdosing (ARI equals 0.16, 0.18 and 0.22 respectively) were seen. Yet these differences are borderline non-significant ($p = 0.07$).

Table 1. Results of the logistic regression model for correctness of dosing for the β -lactam antimicrobial class and the tetracyclines (OR = Odds Ratio; AR_{class} = Antimicrobial Resistance per class of antimicrobials)

Antimicrobial class	Correctness of dosing	AR_{class}	OR	p-value
β -lactams	Under	42,8%	Ref.	
	Correct	42,2%	0.98	>0.05
	Over	40,9%	0.93	>0.05
Tetracyclines	Under	62.1%	Ref.	
	Correct	31.6%	0.28	<0.05
	Over	79.2%	2.32	<0.01

Ref.: reference category

Table 2: results of the logistic regression model for frequency of treatment for the β -lactam antimicrobial class and the tetracyclines (OR = Odds Ratio; AR_{class} = Antimicrobial Resistance per class of antimicrobials)

Antimicrobial Class	Frequency of antimicrobial therapy	AR_{class}	OR	p-value
β -lactams	0	23.2%	Ref.	
	1	37.0%	1.94	<0.01
	2	47.9%	3.04	<0.01
	3	36.2%	1.88	<0.05
	4	39.7%	2.17	<0.05
	5	26.3	1.18	>0.05
	6	47.4	2.97	<0.05
Tetracyclines	0	54.7%	Ref.	
	1	68.4%	1.79	<0.01
	2	57.9%	1.14	>0.05

The correlation between the ARI and the external ($r = -0.16$, $p = 0.12$) and internal ($r = -0.18$, $p = 0.07$) biosecurity was slightly negative.

Discussion

Moderate to high resistance percentages were seen for respectively β -lactam antimicrobials and tetracyclines even when these antimicrobials had not been used (0 level of frequency of therapy). High resistance to tetracycline in commensal *E. coli* from pigs has been reported several times (Hendriksen et al., 2008). The use of tetracyclines in swine production has been considerable since their discovery (Chopra and Roberts, 2001; Schwarz et al., 2001). As such the high antimicrobial resistance prevalence, even without tetracycline treatment, is not surprising.

The observed relation between TI and ARI indicates that the amount and number of days during which a pig is administered antimicrobials seems to be of importance for the presence of antimicrobial resistance. A TI_{UDDpig} lower than the TI_{ADDpig} reflects an average overdosing of antimicrobial treatments. A miscalculation of the bodyweight at moment of administration (Timmerman et al., 2006), intentional overdosing to aim at less disease or lack of precision could also be possible causes. The stronger link between TI_{ADDpig} (quantifying the duration of selection pressure assuming a correct dosing) in comparison to the TI_{UDDpig} (quantifying the true duration of selection pressure) and ARI could be an indication that the total amount of antimicrobials given is more influential than the actual duration of the selection pressure. Moreover, also the frequency of treatment seems to play a role in the degree of antimicrobial resistance both for β -lactam antimicrobials as for tetracyclines.

Although under discussion, antimicrobial concentrations lower than prescribed concentrations are thought to create a favourable opportunity for less sensitive bacterial populations, resulting in an exponential increase of the number of resistant bacteria after treatment (Catry et al., 2003). This could explain the higher AR_{class} for the tetracyclines after underdosing tetracyclines compared to correct dosing. Yet, a higher AR_{class} for the tetracyclines was also found after overdosing tetracyclines. These findings require more detailed research to ascertain the biological relevance. In this study, no effect

of correct or incorrect dosing on the AR_{class} was seen for the β -lactam antimicrobial class. Different ways of spread of resistance and mechanisms of acquiring antimicrobial resistance between tetracyclines and β -lactam antimicrobials of the tested *E. coli* isolates could possibly explain these observed differences.

Higher correlations were found for internal biosecurity than for external biosecurity and ARI. Most likely this is due to the lower antimicrobial use in herds with a higher internal biosecurity (Laanen et al., 2011), consequently leading to a lower antimicrobial resistance of *E. coli*.

Conclusion

The use of antimicrobials selects for the acquisition and spread of antimicrobial resistance. The hypothesis that incorrect dosing is a risk factor for antimicrobial resistance selection is probably drug dependant whereas it seems that the frequency of drug administration may play a role in the selection of resistance to β -lactam antimicrobials and tetracyclines. A higher biosecurity score is related to less antimicrobial resistance.

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