Antimicrobial resistance prevalence of pathogenic and commensal *Escherichia coli* in food-producing animals in Belgium

Prevalentie van antimicrobiële resistentie van pathogene en commensale Escherichia coli *bij voedselproducerende dieren in België*

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ABSTRACT

In this article, detailed studies on antimicrobial resistance to commensal *E. coli* (in pigs, meat-producing bovines, broiler chickens and veal calves) and pathogenic *E. coli* (in pigs and bovines) in Belgium are presented for 2011. Broiler chicken and veal calf isolates of commensal *E. coli* demonstrated higher antimicrobial resistance prevalence than isolates from pigs and bovines. Fifty percent of *E. coli* isolates from broiler chickens were resistant to at least five antimicrobials, whereas sixty-one percent of bovine *E. coli* isolates were susceptible to all antimicrobials tested. On the other hand, bovine pathogenic *E. coli* isolates showed an extended resistance profile with more than half of the isolates being resistant to ten or more antimicrobials. The results are not significantly different from the results from previously published studies on commensal bacteria in pigs in Belgium, although different methodologies of sampling and susceptibility testing were used.

SAMENVATTING

In dit artikel worden gedetailleerde studies over antimicrobiële resistentie van commensale *E. coli* bij varkens, runderen, pluimvee en vleeskalveren en pathogene *E. coli* bij varkens en runderen in België beschreven voor 2011. Bij commensale *E. coli*-isolaten van pluimvee en vleeskalveren werd een hogere prevalentie van antimicrobiële resistentie vastgesteld dan bij isolaten van varkens en runderen. Vijftig procent van de *E. coli*-isolaten van vleeskuiken was resistent tegen ten minste vijf antimicrobiële middelen, terwijl 61% van de isolaten van runderen gevoelig was voor alle geteste antibiotica. Daarentegen vertoonden pathogene *E. coli*-isolaten van runderen een uitgebreid resistentieprofiel, met resistentie tegen tien of meer antimicrobiële middelen bij meer dan de helft van de isolaten. De resultaten zijn niet significant verschillend van de resultaten van eerder gepubliceerde studies over commensale bacteriën bij varkens en vleeskuikens in België, hoewel er verschillende methoden van bemonstering en gevoeligheidstesten werden gebruikt.

INTRODUCTION

E. coli is often used as an indicator bacterium for the presence of antimicrobial resistance of gram-negative bacteria because it is present in nearly all animal species. Murray et al. (1992) stated that resistance of commensal *E. coli* is an indication for the magnitude of the selective pressure from the use of antimicrobials in an animal population. Some *E. coli* strains are also major pathogens in several animal species. In pigs, several studies indicate that antimicrobial resistance is higher in pathogenic than in commensal *E. coli* strains (Boerlin et al., 2005, Hendriksen et al., 2008)

Transfer of antimicrobial resistance from food-producing animals to humans might happen via food, through environmental contamination such as recreational waters and by direct animal contact

(Wooldridge, 2012). Infections with bacteria, which are resistant to the antimicrobial used may result in treatment failures. Multi-resistance may necessitate the use of second-line antimicrobials for therapy, increasing the expenses as well as the chance of creating multi-resistant strains (Migliori et al. 2007). The World Health Organization (WHO) identifies antimicrobial drug resistance as a global concern and highlights the role of monitoring programs to provide sufficient data for the use in ongoing research focussing on combatting drug resistance. To stimulate the discussion and the research on antimicrobial resistance in veterinary medicine, McEwen et al. (2002) stated that although antimicrobial resistance is also of major concern for animal health, little is known about the magnitude of this problem.

Given the importance of antimicrobial resistance, the Belgian Federal Agency for the Safety of the Food Chain has established a monitoring program for antimicrobial resistance of indicator bacteria. Monitoring commensal *E. coli* complies with the guidelines set by European Food Safety Authority (EFSA). This monitoring program is the start of an annual returning program, which will allow to monitor the evolution of antimicrobial resistance, and to evaluate the effect of intervention measures taken. It also allows the comparison of national results with the results of other European countries (EFSA, 2012). Until recently, monitoring of the antimicrobial resistance of pathogenic *E. coli* in pigs and bovines has been performed at the Veterinary Agrochemical Research Centre (CODA-CERVA).

The aim of this study is to describe and summarize the results for the resistance of *E. coli* using the official monitoring program and to compare them with data from other point prevalence studies on *E. coli*. For all comparisons made between different studies, of which the raw data were available, the same interpretative criteria were applied (CLSI clinical breakpoints) to enhance comparability.

MATERIALS AND METHODS

Monitoring of antimicrobial resistance of commensal *E. coli* in poultry, pigs, meat-producing bovines and veal calves

All used sampling and analysis procedures are described in detail in the CODA-CERVA report on monitoring of antimicrobial resistance in *E. coli* in Belgium in 2011 (CODA CERVA, 2012). Briefly, fecal samples were collected by the inspectors of the FAVV-AFSCA from randomly selected, apparently healthy animals belonging to different categories (broiler chickens, n=420), pigs (>3 months old, n=157), meat-producing bovines (>7 months old, n=154) and veal calves (<7 months old, n=34) during 2011. The sampling ratio was one sample per farm. Isolates were identified as *E. coli* by Animal Health Care Flanders (DGZ) (inoculation on Kligler and indol medium) and the Walloon Regional Association for the Health and Identification of animals (ARSIA) (OPNG test, Ureum test and indol test), and were then sent to the CODA-CERVA reference laboratory for antimicrobial resistance testing, where antimicrobial susceptibility was tested using a microdilution broth method (Trek Diagnostics©). The minimum inhibitory concentration (MIC) was defined as the lowest concentration, by which no visible growth could be detected. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values were used to indicate breakpoints for resistant or susceptible isolates on fourteen antimicrobial agents, i. e. ampicillin, chloramphenicol, ciprofloxacin, colistin, florfenicol, cefotaxime, gentamicin, kanamycin, nalidixic acid, sulfomethoxazole, streptomycin, ceftazidime, tetracycline, trimethoprim. Multi-resistance median, i.e. the number of antimicrobials to which 50% of the strains are resistant, was calculated for each animal sector.

Monitoring of antimicrobial resistance of pathogenic *E. coli* in pigs and bovines

All used sampling and analysis procedures are described in the CODA-CERVA report on pathogenic agents in Belgium (CODA-CERVA, 2012). Briefly, strains originating from diseased pigs and bovines that showed symptoms compatible with an *E. coli* infection, e.g. neonatal diarrhea, were isolated and identified at the species level by the regional veterinary authorities, i.e. Dierengezondheidszorg Vlaanderen (DGZ) and by the Association Régionale de Santé et d'Identification Animales (ARSIA). Afterwards, the strains were sent to the CODA-CERVA. The bovine strains (all isolated from animals less than two weeks old) were selected on the basis of the presence of adhesion factors F17 and CS31A by agglutination (performed at ARSIA), and tellurite resistance was tested for potential Stx production and enterohemolysin production, ruling out most strains having no virulence factors. For both bovine and porcine strains, multiplex PCR tests were performed at CODA-CERVA for pathotyping (for the pigs: F4, F5, F6, F18, F41, Sta, Stb, LT, Stx2; for the bovines: CNF1, CNF2, eae, vt1, vt2, Sta, F5, F17, F41).

The antimicrobial susceptibility was measured using the Kirby-Bauer disk diffusion method (Neo-Sensitabs, Rosco© tablets, Taastrup, Denmark) and determined according to the Clinical and Laboratory Standards Institute (CLSI, 2008) guidelines and the CLSI clinical breakpoints. For the pigs, fourteen antimicrobials were used, while for the bovines twenty-four. Data were interpreted as susceptible, intermediate resistant and resistant. The intermediate resistant strains were reclassified as resistant.

In absence of good estimates of the characteristics of the used tests, the authors did not take into con-

sideration the sensitivity and specificity of the used tests. Therefore, the presented prevalence estimates are apparent prevalences.

Study comparisons: data preparation and considerations

Regarding broiler chickens, the results of the national monitoring data were compared with the data obtained by Persoons et al. (2010) who determined the susceptibility of commensal *E. coli* from fecal samples of healthy broilers. For isolation, the Kirby-Bauer disk diffusion method (NeoSensitabs, Rosco© tablets, Taastrup, Denmark) was used, and the determination of antimicrobial resistance was done according to the CLSI (2008) guidelines. In this study, fecal samples originating from 32 randomly selected broiler farms (30 samples per farm) were investigated between April 2007 and March 2008. All farms were visited twice (two sampling periods). In this comparison, the authors included the results from only one sampling (the second). From this sampling round, 912 strains were isolated. In order to make the comparisons valid, the raw data of the national monitoring of commensal *E. coli* in broilers were interpreted using the CLSI clinical breakpoints as used in the study by Persoons et al. (2010).

The results of the national monitoring of antimicrobial resistance of commensal *E. coli* in pigs were compared to the results on commensal *E. coli* in pigs by Callens et al. (2010), who used the Kirby-Bauer disk diffusion method (NeoSensitabs, Rosco© tablets, Taastrup, Denmark). The antimicrobial resistance prevalence was determined according to the CLSI (2008) guidelines. In this study, 824 strains originating from 45 Belgian randomly selected pig farms were tested (20 samples per farm). All animals were tested once. Similarly to the data on broilers, the raw data on the national monitoring of commensal *E. coli* in pigs were interpreted using the CLSI clinical breakpoints. The authors also compared the data on antimicrobial resistance of pathogenic *E. coli* with those of the national monitoring of commensal *E. coli* using the CLSI breakpoints.

Also for fluoroquinolones -a critically important class of antibiotics for human medicine-, there was a differentiation in the choice of antimicrobials between studies. In the studies of Persoons et al. (2010) and Callens et al. (2010), enrofloxacin was selected and used, whereas in the national monitoring report ciprofloxacin was used (CODA-CERVA, 2012). All fluoroquinolones have the same mechanism of action, i.e. inhibition of the topoisomerase genes leading to the inhibition of DNA replication (Hopkins et al., 2005). As there is full cross-resistance between fluoroquinolones, the authors directly compared the resistance prevalence to enrofloxacin and ciprofloxacin.

Data analysis

The antimicrobial resistance prevalence was measured for each animal sector. The exact 95% binomial confidence intervals were calculated.

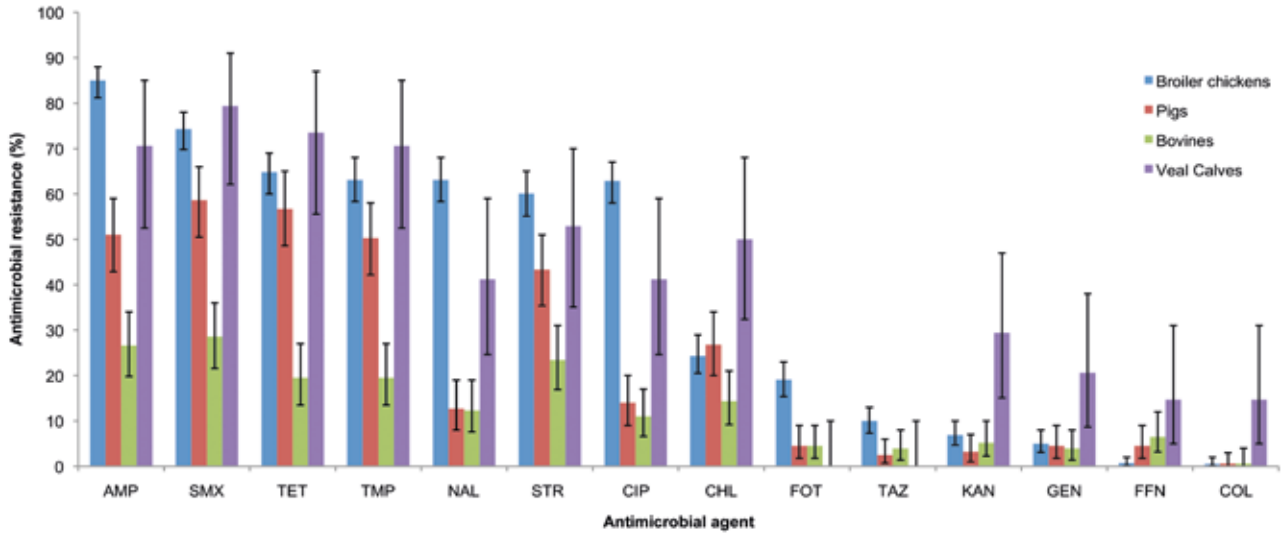
Data manipulations and analysis were performed in Microsoft Excel 2010 edition. IBM SPSS Statistics 21.0 was used for all statistical analysis. Differences in prevalence were studied using the Pearson's chi-square test or -if not suitable- the Fisher's exact test. The chi-squared test was not suitable only if the expected values in any of the cells of a contingency table was below 5 (usual rule of thumb). In those cases, the Fisher's exact test was preferred. Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.

RESULTS

National monitoring of antimicrobial resistance of commensal *E. coli*

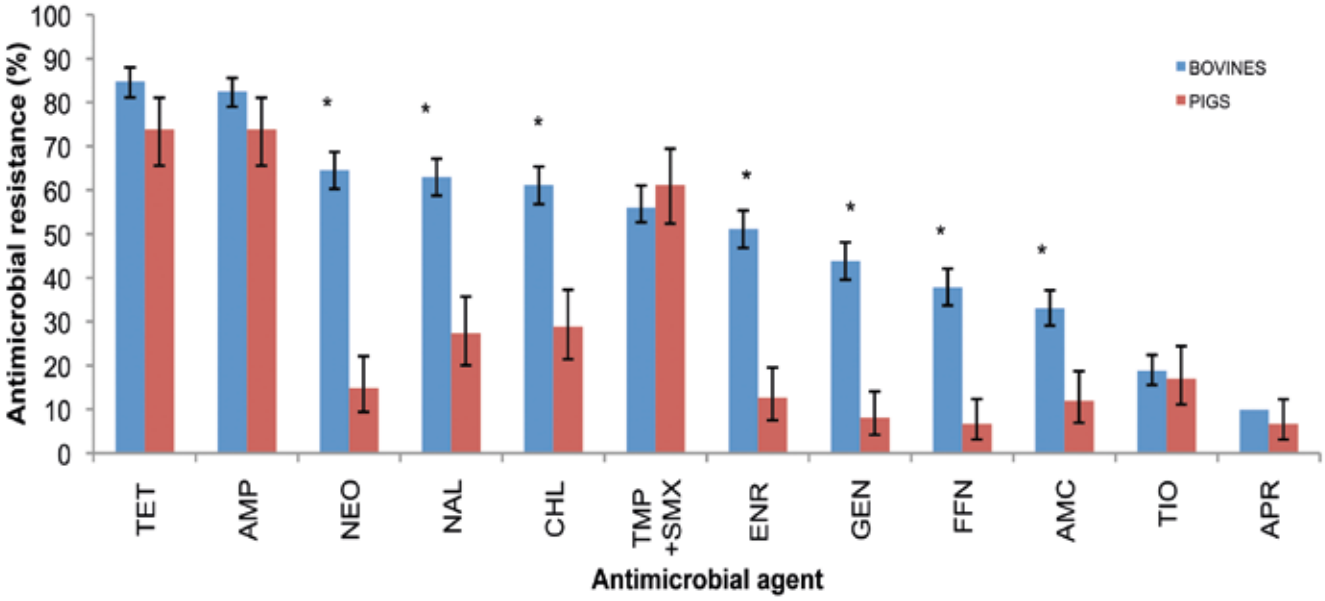
In this study, 420 isolates from broiler chickens, 157 from pigs, 154 from meat-producing cattle and 34 from veal calves were collected. The antimicrobial resistance results are presented for each animal sector in Figure 1. For broiler chickens, the antimicrobial resistance to ampicillin rose above 80%. For nalidixic acid, ciprofloxacin, sulphomethoxazole, tetracycline, streptomycin and trimethoprim, the antimicrobial resistance prevalence was higher than 60% but lower than 80%. Due to the importance of cephalosporins to human medicine, the 19% and the 10% antimicrobial resistance prevalences to the cephalosporins cefotaxime and ceftazidime, respectively, are noteworthy. For pigs, the resistance prevalence was above 50% to ampicillin, sulphomethoxazole, tetracycline, and trimethoprim. For bovines, the highest antimicrobial resistance prevalence was seen to sulphonamides and ampicillin, with a resistance prevalence of approximately 25%. Concerning veal calves, the resistance prevalence was higher than in pigs and bovines. More than 70% of the strains were resistant to ampicillin, sulphonamides, tetracycline and trimethoprim. As for veal calves, no cephalosporin resistance was found.

Multi-resistance median and strain susceptibility prevalences are presented in Table 1. For broiler chickens and veal calves, more than 50% (multi-resistance median) of the *E. coli* strains acquired resistance to at least five antimicrobials. For pigs, the multi-resistance median was three; for bovines, more than 50% of the strains were fully susceptible to all antimicrobials. Considering the isolates from broiler chickens in more detail, for ESBL suspected strains, the multi-resistance median was 6.5 antimicrobials and for AmpC suspected strains, the median was eight antimicrobials.



AMP: ampicillin, CHL: chloramphenicol, CIP: ciprofloxacin, COL: colistin, FFN: florfenicol, FOT: cefotaxime, GEN: gentamicin, KAN: kanamycin, NAL: nalidixic acid, SMX: sulfomethoxazole, STR: streptomycin, TAZ: ceftazidime, TET: tetracycline, TMP: trimethoprim

Figure 1. Indicator *Escherichia coli* isolates' resistance prevalence to fourteen antimicrobial agents, with exact 95% binomial confidence intervals. Four animal sectors were studied. The micro broth dilution method was used and epidemiological cut-off values were applied to determine the antimicrobial resistance prevalence, according to EUCAST standards. Significant differences (P<0.05) between studies are indicated with * (P-value was observed at 5%). Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.



AMP: ampicillin, AMO-CLA: amoxycillin- clavulanic acid, TET: tetracycline, TMP: Trimethoprim, SUL: sulfonamide, TIO: ceftiofur, NAL: nalidixic acid, ENR: enrofloxacin, APR: apramycin, NEO: neomycin, GEN: gentamycin, CHL: chloramphenicol, FFN: florfenicol

Figure 2. VAR report, pathogenic *E. coli* antimicrobial resistance prevalence in pigs and bovines in 2011. Additionally, the bars represent the exact 95% binomial confidence intervals of the prevalence results. The disk diffusion method was used and clinical breakpoints (CLSI standards) were implemented. Only antimicrobial agents that were commonly tested in both animal species are displayed in this figure. Significant differences between studies are indicated with * (P-value was observed at 5%). Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.

Table 1. Commensal *Escherichia coli* strain antimicrobial susceptibility prevalence, multi-resistance and main findings for each animal sector included

Animal sector	Strain susceptibility prevalence[a]	Multi-resistance median[b]
Broiler chickens	6.2 %	5 AMs[c] (6.5 for ESBL-suspected strains, 8 for AmpC-suspected strains)
Pigs	22.3 %	3 AMs
Bovines	61 %	0 AMs (5 for cephalosporin-resistant strains, 5 for FFN-resistant strains)
Veal calves	14.7 %	5 AMs (7 for colistin-resistant strains)

[a] : Percentage of the strains that remained fully susceptible to all antimicrobials
[b] : Number of antimicrobials, to which 50% of the strains were resistant
[c] : AMs = Antimicrobial agents; FFN=florfenicol

Table 2. Pathotypes found in pathogenic *E. coli* strains from pigs and bovines in 2011.

Pathotype	Number of strains (pigs)	Number of strains (bovines)
Colonizing strains	5	468
ETEC	35	11
VTEC	8	10
ETEC/VTEC	1	1
ETEC without attachment factor	8	-
NTEC	-	12
No virulence gene/ factor detected	76	35
No final conclusion possible[1]	-	5
Total number of strains	133	542

[1]: this applies only for bovine *E. coli* strains due to the different pathotyping technique used

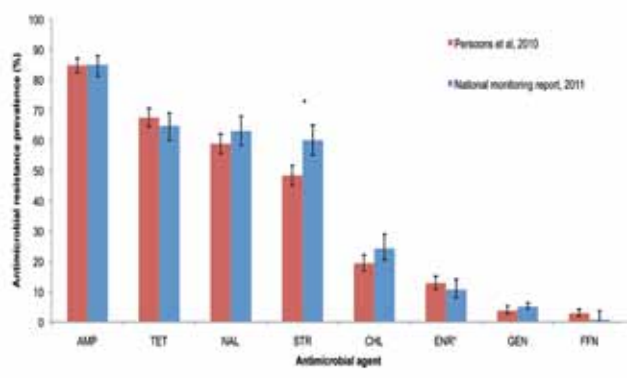
Monitoring of pathogenic *E. coli* from pigs and bovines

A total of 135 pig strains were retrieved, of which 133 were analyzed for virulence characteristics by PCR. In the majority of them (76/133), no virulence genes could be detected. The most prevalent pathotype was ETEC (Table 2). Few strains were positive for F41, F5 or F6 fimbriae. F4 was the most prevalent adhesion factor, followed by F18. Of the ETEC associated toxins, STb was the most prevalent. Hemolysis was seen in approximately 60% of the strains. Nearly 90% of the pathogenic strains were hemolytic. As for bovines, 545 strains were obtained, and the vast majority of them (468/565) were colonizing strains (Table 2). Regarding associations with adhesion factors, NTEC strains were mainly associated with F17 and CS31A. All but one STa strain were associated with F5 fimbriae. Of the 10 VTEC strains, six were associated with VT1, 3 with VT2 and one with both VT1 and VT2. EPEC strains were associated with F5 and/or F41. The majority of colonizing strains was CS31A or F17 positive.

Antimicrobial resistance prevalence for pigs and bovines are shown in Figure 2. The multi-resistance median for pigs was five out of 15 antimicrobials, and the strains that were fully susceptible were less than 8%. For bovines, the multi-resistance median was ten out of 24 antimicrobials, and the strains that remained fully susceptible to all antimicrobials were almost 4%.

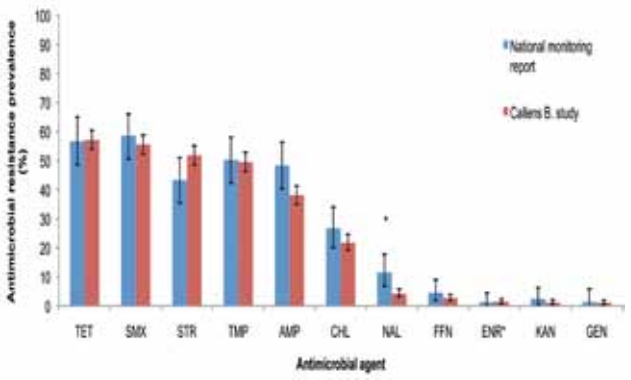
Study comparisons

Comparing the data of the national monitoring of the antimicrobial resistance of commensal *E. coli* from broilers with the data obtained from the study by Persoons et al. (2010), it was revealed that only the resistance prevalence to streptomycin was signifi-



AMP: ampicillin, NAL: nalidixic acid, TET: tetracycline, STR: streptomycin, CHL: chloramphenicol, ENR[1]: enrofloxacin (national monitoring report used ciprofloxacin), GEN: gentamycin, FFN: florfenicol

Figure 3. Indicator *E. coli* resistance in poultry. Comparison of the VAR report and the study of Persoons et al. (2010) is presented. Data harmonization with CLSI breakpoints for clinical resistance was applied to both datasets. The bars represent the exact 95% binomial confidence intervals of the prevalence results. Significant differences between both studies are indicated with * (P-value was observed at 5%). Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.



AMP: ampicillin, NAL: nalidixic acid, TET: tetracycline, STR: streptomycin, CHL: chloramphenicol, GEN: gentamycin, FFN: florfenicol, ENR[1]: enrofloxacin (national monitoring report used ciprofloxacin), SMX: sulfomethoxazole, TMP: trimethoprim, KAN: kanamycin

Figure 4. Indicator *E. coli* resistance in pigs. Comparison of the VAR report and the study of Callens et al. (2010) is presented. Data harmonization with CLSI breakpoints for clinical resistance was applied to both datasets.
The bars represent the exact 95% binomial confidence intervals of the prevalence results. Significant differences between both studies are indicated with * (P-value was observed at 5%). Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.

cantly different ($P < 0.05$) in the national monitoring report. Comparing the data of Callens et al. (2010) with the national monitoring of commensal *E. coli* from pigs, the only statistically significant difference found was for nalidixic acid ($P < 0.05$), which was higher in the national monitoring report (Figures 3 and 4). Finally, the authors compared the VAR commensal study and the pathogenic *E. coli* study on pigs and bovines (Figures 5 and 6). For pigs, significantly ($P < 0.05$) different antimicrobial resistance prevalences were shown in the pathogenic *E. coli* study to ampicillin, sulphamethoxazole, tetracycline and nalidixic acid. For bovines, significantly ($P < 0.05$) different prevalences in the pathogenic *E. coli* study were seen for ampicillin, sulphamethoxazole, tetracycline, nalidixic acid, trimethoprim, streptomycin, fluoroquinolones, chloramphenicol, kanamycin, gentamicin and florfenicol.

DISCUSSION

Commensal *E. coli*

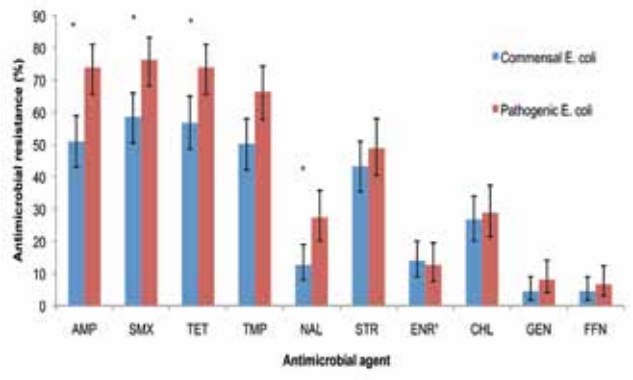
In the Belgian national monitoring program on antimicrobial resistance of commensal *E. coli*, samples were collected from broiler chickens, pigs, veal calves and bovines. Compared to other national monitoring programs conducted in European countries in 2010-2011, this was the only report- alongside with the MARAN report- that included data on the four major animal sectors, which complied with the EFSA guidelines (Chantziaras et al., 2013). In addition, the number of samples and the sampling protocol were -with the exception of veal calves- comparable with other national monitoring reports conducted in the same year, thus allowing a representative overview of the resistance situation (Bywater et al., 2004). However, a further increase of the number of samples will improve the power of the study regarding the analysing trends on antimicrobial resistance.

When comparing the results between animal species for commensal *E. coli*, veal calf isolates showed the highest antimicrobial resistance prevalence for eight antimicrobial agents. However, the low number of veal calf samples that were included, resulted in large confidence intervals. Hence, when compared to the other three animal sectors, the resistance prevalence results were not significantly different from those in broiler chickens or in pigs. Only when compared with bovines, and for all antimicrobials except cefotaxime and ceftazidime, the resistance prevalence was significantly higher for veal calves isolates. This difference is likely due to the very high use of antimicrobials in the veal production system (Pardon et al., 2013, Berge et al., 2010, Sato et al., 2005). Another possible explanation has been given by Walk et al. (2007). They suggest that the fitness cost of resistant bacteria becomes too large as the

host gastrointestinal tract matures and competition with other microbes increases.

Due to the importance for human medicine, the almost 15% antimicrobial resistance prevalence to colistin is to be noted. The colistin-resistant strains were highly multi-resistant, all of them being resistant to at least seven more antimicrobial agents. Gram-negative bacteria may develop resistance to colistin through chromosomal mutation or adaptation mechanisms rather than through a horizontal spread of mobile genetic elements (MGEs) carrying resistance genes (Falagas et al., 2005).

Broiler chicken isolates showed the highest antimicrobial resistance prevalence to the other six agents. The antimicrobial resistance prevalence to quinolones was higher than in the other animal species. Moreover, resistance to ceftazidime and cefotaxime was high, reaching a 20% prevalence for each of the cephalosporins. A closer look into multi-resistance patterns of the isolates provided valuable information. All ceftazidime-resistant strains were also resistant to cefotaxime. As shown in Table 1, there is a high multi-resistance pattern of the cephalosporin resistant strains. Due to the particular importance of cephalosporins for human health, molecular epidemiology analysis and further testing, e.g. the detection of plasmid-mediated genes, of the strains in such studies are warranted. After comparing the results of the national monitoring study for broilers with the results of the study of Persoons et al.(2010), no significant differences were found with the exception of streptomycin resistance, which was more prevalent in the results of the national monitoring report. This difference may be attributed to the accuracy of the disk diffusion method for streptomycin. Comparing the national monitoring study for pigs and the respective study of Callens (2010), only a small, yet significant difference was seen for nalidixic acid, of which the prevalence was higher in the national monitoring report (Figure 5). Contrary to the national monitoring study, all studies that used the disk diffusion method, did not test colistin as several publications have proved that the poor agar diffusion characteristics of colistin limit the accuracy of the disk diffusion test (Gales et al., 2001; Lo-Ten-Foe et al., 2007, Galani et al., 2008). In general, the monitoring of commensal *E. coli* did not show many statistically significant differences between the national monitoring and the two selected point prevalence studies, both confirming the high antimicrobial resistance prevalence in Belgium. This agreement suggests that both the studies and the monitoring program were capable of describing the general level of resistance in a representative manner. It may therefore be concluded that the described resistance levels of commensal *E. coli* in pigs and broilers are truly the current level of resistance, and are therefore a good reference point to check for evolutions in the coming years. Nevertheless, all of the comparisons also revealed that even when using different methods, a certain level of harmonization between the *E. coli* studies may be reached, acknowledging on beforehand the limitations that can be seen for some antimicrobials, e.g. colistin, streptomycin. Besides the methods used, special attention should also be drawn to the harmonization of sampling methods, age of animals and number of samples.



AMP: ampicillin, SMX: sulfomethoxazole, TET: tetracycline, NAL: nalidixic acid, STR: streptomycin, ENR[1]: enrofloxacin (national monitoring report used ciprofloxacin), CHL: chloramphenicol, GEN: gentamycin, FFN: florfenicol

Figure 5. Comparison of the VAR pathogenic *E. coli* study and the VAR commensal *E. coli* study. Data were collected from pig strains. Data harmonization with CLSI breakpoints for clinical resistance was applied to both datasets.
The bars represent the exact 95% binomial confidence intervals of the prevalence results. Significant differences between both studies are indicated with * (P-value was observed at 5%). Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.

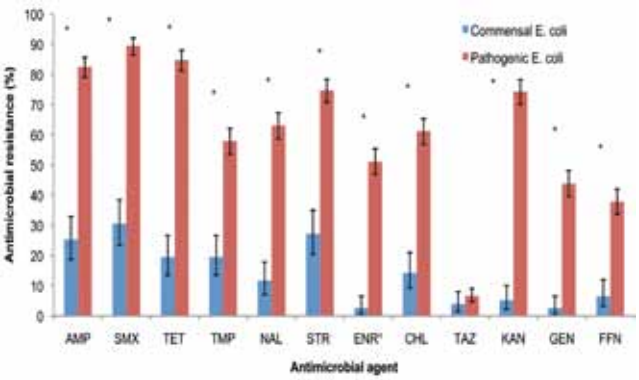


Figure 6. Comparison of the VAR pathogenic *E. coli* study and the VAR commensal *E. coli* study. Data were collected from bovine strains. Data harmonization with CLSI breakpoints for clinical resistance was applied to both datasets.
The bars represent the exact 95% binomial confidence intervals of the prevalence results. Significant differences between studies are indicated with * (P-value was observed at 5%). Multiple comparisons were dealt with using the Bonferroni correction method for each set of comparisons.

Pathogenic *E. coli* reports

Regarding the antimicrobial profile of the pathogenic *E. coli* isolates, pathogenic *E. coli* strains from bovines are more resistant to antimicrobials than those from pigs, as shown in Figure 2. Antimicrobial resistance to florfenicol was five times higher than in pigs, four times higher to neomycin and gentamicin and two times higher to chloramphenicol. No significant differences were seen for trimethoprim and its combination with sulphonamides, apramycin, ceftiofur and ampicillin. The use of the most recently introduced antimicrobials in veterinary medicine seems to have already been compromised in pathogenic *E. coli*, especially for strains isolated from bovines.

Strains resistant to cephalosporins and strains resistant to amoxicillin with clavulanic acid were clearly associated with multi-resistance. Co-resistance to ceftiofur was seen in 23 cases suggesting the presence of CMY encoding genes or other genes. Further testing is warranted, as these antimicrobials are critically important for human and veterinary medicine.

Several authors (Boerlin et al., 2005; Hendriksen et al., 2008) have shown that antimicrobial resistance more frequently occur in pathogenic than in commensal *E. coli* strains from pigs. When comparing the commensal and the pathogenic *E. coli* VAR studies, pathogenic strains were significantly more resistant to four out of ten antimicrobials (ampicillin, tetracycline, sulphonamides and nalidixic acid) in pigs (Figure 5). As for bovine strains, the differences between the antimicrobial resistance prevalence were more evident and more numerous (pathogenic strains were significantly more resistant to eleven out of twelve antimicrobials that were commonly tested) (Figure 6). It should be mentioned that for the pathogenic *E. coli* studies, isolates were collected from clinical cases. Age of the animals, genetic background of the *E. coli* isolates and possible previous administration of antimicrobials to the clinically ill animals could partially explain these differences. Hence, when seeing the differences in resistance prevalence between commensal and pathogenic *E. coli,* it appears that the results of one study cannot be used to predict the expected prevalence levels of the other and vice versa.

CONCLUSIONS

In 2011, a large scale national monitoring program on antimicrobial resistance of commensal and alongside with the pre-existing program concerning zoonotic bacteria was launched in Belgium. Antimicrobial resistance of commensal *E. coli* varied between animal species. Comparing the results from commensal *E. coli* point prevalence studies in research projects, using a different sampling and susceptibility testing methodology, the authors revealed that the results were highly comparable. Pathogenic *E. coli* strains both from bovines and pigs were more pathogenic than the respective commensal *E. coli* strains.

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Uit het verleden

HET SNIJDEN DER KOEIEN (UIT: “DE BOER”, 1902)

“De uitbreiding, welke de melknijverheid genomen heeft in ons land, heeft het belang groter gemaakt dat wij hechten aan alle middels welke kunnen helpen om de voortbrengst der grondstof, de melk, te vermeerderen. Om die reden willen wij een woord zeggen aangaande ene behandeling die in landen waar veel gekweekt wordt, sinds lang in voege is, maar bij ons om zo te zeggen nog onbekend is: het snijden der koeien.

Het snijden heeft bij het vrouwelijk dier dezelfde gevolgen als bij het mannelijke. Met het geslachtsleven uit te dooven, richt men al de levenskrachten van het lichaam naar het groeivermogen. In één woord, het snijden verzekert een betere benuttiging van het ingenomen voedsel, en als onmiddellijk gevolg, een vermeerdering van de opbrengst van het dier. Bij het slachten is de zuivere opbrengst 5 tot 6 % groter dan bij dieren die gevet worden in den staat der drachtigheid.

Wat de melkopbrengst betreft, geven gesneden koeien in het jaar dat volgt op deze behandeling,tenminste 1300 tot 1400 liters meer melk. Wat meer is: de melk ondergaat ene wijziging in haar hoedanigheid. Haar rijkdom in boterstoffen vermeerdert en haar samenstelling is bestendiger want ze ondergaat geen verandering meer onder invloed van tochtigheid, drachtigheid en kalftijd.

De voornaamste voorwaarde om te gelukken in het snijden van koeien is het tijdstip. Deze behandeling moet gebeuren zes weken tot twee maanden na het kalven terwijl de koe in volle melkgevigheid is. Tenzij in bijzondere omstandigheden, is de voordeligste ouderdom tussen 7 en 8 jaar. Tegenwoordig kan deze behandeling, dank aan de vooruitgang der ontsmettingsleer, gedaan worden zonder enig gevaar van verwikkeling.”

Uit: “100 jaar Boerenbond in Beeld”

Luc Devriese