

## INFLUENCE OF ANTIMICROBIAL TREATMENTS ON OCCURRENCE OF ANTIMICROBIAL RESISTANCE IN *Escherichia coli* FROM FAECAL FLORA OF PIGS

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### Introduction

Antibiotic use in food-producing animals is considered as a risk factor for emergence of resistant bacteria both in treated animals and in human beings. However, little is known about relation between therapeutic schemes and occurrence of resistance in herds where antibiotics are used.

The purpose of this investigation was to determine the effect of antimicrobial use on resistance in commercial swine facilities. *Escherichia coli* strains from faecal flora of pigs were used as indicator bacteria for evaluation of resistance.

### Material and Methods

Sixteen farrow-to-finish pig herds from west of France were included in the study. Depending on their antimicrobial use, they were classified as either low-users (LU), medium-users (MU) or high-users (HU) if number of collective treatments applied to animals during the study period was 2 or less, 3-4 or >4 respectively. Numbers of farms in different groups were 6 (LU), 4 (MU) and 6 (HU). In a second step, herds were classified as users (U) or non-users (NU) depending on whether a specific antimicrobial class was administered to pigs or not.

In each farm, 5 sows and 3 piglets from each sow were randomly selected for faecal sampling. Sows were sampled before farrowing and then 7, 30 and 60 days after farrowing and piglets at 7, 30, 60 and 150 days of age. Four indicator *Escherichia coli* strains were isolated from each faecal sample and tested for antimicrobial susceptibility by standard disk diffusion method. Isolates determined to be intermediate were classified together with resistant ones. For each sampling time and animal type, percentages of resistant strains were compared using chi square test in order to assess effect of antimicrobial use.

### Results

Among antimicrobials tested either no or few resistant strains were evidenced for the following: ceftiofur, cefquinome, florfenicol and colistin. Antimicrobials to which resistance was observed either transiently or continually during the study period were assessed by statistical analysis and results are illustrated in Tables 1 and 2.

Evolution of resistance with time was similar for all antimicrobials in pigs: percentage of resistant isolates increased during post-weaning period and decreased thereafter. Consequently lowest percentages were observed at the end of the fattening period. For sows, percentage of resistant strains were higher during lactating period (days 7 and 30). This evolution was more evident for amoxicillin and gentamicin than for trimethoprim-sulfamethoxazol and tetracycline. High variability of percentages of resistant strains was

observed for amoxicillin, gentamicin and trimethoprim-sulfamethoxazol depending both on herds and on sampling times. Results were more constant for tetracycline to which a high percentage of strains exhibited resistance.

**Table 1** Percentages of resistant *Escherichia coli* strains isolated from sows and pigs in herds exhibiting different levels of antimicrobial use

		N*	Amoxicillin	Gentamicin	Trimethoprim-sulfamethoxazol	Tétracycline
Sows	Day 0	LU 96	<b>16,7</b>	0	47,9	<b>87,5</b>
		MU 76	<b>34,2</b>	1,3	55,3	<b>73,7</b>
		HU 104	<b>26</b>	7,7	55,8	<b>86,5</b>
	Day 7	LU 92	<b>27,2</b>	<b>1,1</b>	51,1	<b>87</b>
		MU 73	<b>58,9</b>	<b>4,1</b>	65,8	<b>93,2</b>
		HU 104	<b>38,5</b>	<b>18,3</b>	57,7	<b>79,8</b>
	Day 30	LU 96	<b>27,1</b>	<b>0</b>	<b>39,6</b>	72,9
		MU 73	<b>50,7</b>	<b>1,4</b>	<b>74</b>	74
		HU 99	<b>50,5</b>	<b>23,2</b>	<b>57,6</b>	82,8
	Day 60	LU 92	<b>19,6</b>	3,3	<b>43,5</b>	<b>90,2</b>
		MU 46	<b>28,3</b>	0	<b>39,1</b>	<b>63</b>
		HU 96	<b>35,4</b>	8,3	<b>63,5</b>	<b>87,5</b>
Pigs	Day 7	LU 268	<b>23,5</b>	<b>0</b>	<b>36,9</b>	<b>67,2</b>
		MU 209	<b>54,1</b>	<b>1,4</b>	<b>58,4</b>	<b>76,1</b>
		HU 276	<b>44,9</b>	<b>19,9</b>	<b>57,2</b>	<b>83,4</b>
	Day 30	LU 263	<b>19,8</b>	<b>6,1</b>	<b>36,1</b>	<b>83,3</b>
		MU 204	<b>48,5</b>	<b>0,5</b>	<b>58,8</b>	<b>88,7</b>
		HU 239	<b>66,1</b>	<b>28</b>	<b>69,9</b>	<b>91,2</b>
	Day 60	LU 259	<b>26,3</b>	<b>7,3</b>	<b>34,7</b>	<b>84,6</b>
		MU 195	<b>70,3</b>	<b>21,5</b>	<b>86,4</b>	<b>99</b>
		HU 244	<b>54,5</b>	<b>31,6</b>	<b>82,4</b>	<b>93,9</b>
	Day 150	LU 202	<b>8,4</b>	1	<b>18,8</b>	<b>80,2</b>
		MU 94	<b>26,6</b>	0	<b>46,8</b>	<b>91,5</b>
		HU 203	<b>34</b>	0,5	<b>63,5</b>	<b>87,7</b>

\* number of *E coli* colonies tested

**Bold type indicate a significant effect of level of antimicrobial use ( $p < 0.05$ )**

Effect of level of antimicrobial use on percentage of resistant *E coli* was more noticeable in pigs than in sows. Indeed, for the first animal type, this effect was significant in all cases except for gentamicin at the end of the fattening period.

In sows, use of an antimicrobial class was not frequently associated with a significant increase of percentage of resistance to a member of this class except for aminoglycosides on gentamicin resistance. This association was observed in pigs for all antimicrobials except tetracycline.

**Table 2** Percentages of resistant *Escherichia coli* strains isolated from sows and pigs in herds using (U) or not using (NU) the class of tested antimicrobials

			Amoxicillin	Gentamicin	Trimethoprim-sulfamethoxazol	Tétracycline
Sows	Day 0	U	25.1	<b>7.6</b>	53.7	84.6
		NU	22.7	<b>0.4</b>	53.6	78.4
	Day 7	U	40.4	<b>18.2</b>	56.2	82.3
		NU	30.7	<b>1.9</b>	57.8	84.9
	Day 30	U	40.2	<b>23.2</b>	<b>64.5</b>	81
		NU	39.1	<b>0.4</b>	<b>51.7</b>	73.5
Day 60	U	<b>33.9</b>	<b>8.3</b>	56.5	<b>82.4</b>	
	NU	<b>18.5</b>	<b>2.3</b>	48.9	<b>84.5</b>	
Pigs	Day 7	U	<b>43.4</b>	<b>19.8</b>	54.5	<b>72.9</b>
		NU	<b>33.6</b>	<b>1.1</b>	49.1	<b>79.1</b>
	Day 30	U	<b>47.9</b>	<b>28</b>	<b>64.6</b>	87
		NU	<b>39.3</b>	<b>2.9</b>	<b>53.9</b>	86.2
	Day 60	U	<b>56.9</b>	<b>31.5</b>	<b>88.5</b>	91.9
		NU	<b>42.5</b>	<b>10.7</b>	<b>59.1</b>	92.2
Day 150	U	<b>29.8</b>	0.4	<b>69.8</b>	89.6	
	NU	<b>15.9</b>	0.4	<b>39.6</b>	84.2	

Bold type indicate a significant effect of level of antimicrobial use ( $p < 0.05$ )

Amoxicillin : U :  $\beta$ -lactam-user herds

Gentamicin : U : aminoglycoside-user herds

Trimethoprim-sulfa : U : trimethoprim-sulfamide-user herds

Tetracycline : U : tetracycline-user herds

## Discussion

Farms included in this study were selected for their use of antimicrobials similar to what is currently observed in french swine herds.

Percentages of resistant *E coli* strains exhibited variability depending on antimicrobial class with lowest levels of resistance observed for gentamicin. Gentamicin-resistant strains were less frequent here than observed in pigs in the USA where up to 92% of strains were resistant to this antimicrobial (4). On the opposite, percentages of resistance to tetracycline appeared to be very high. Both high prevalence and persistence of tetracycline-resistant strains have been previously demonstrated elsewhere (3,4).

Kinetic evolution of resistance was more or less noticeable depending on tested antimicrobial (decrease during fattening period was not very important for tetracycline) and has been previously reported with pathogenic bacterial strains (1). This variability in levels of resistance with time may be due to more frequent administration of antimicrobial treatments around farrowing for sows and during post-weaning period for pigs. However similar evolution has been observed in LU

herds without use of antimicrobials. Such a phenomenon could be linked to other factors that have been shown to interfere with resistance such as thermic stress or overcrowding (5).

Evaluation of impact of level of antimicrobial use on occurrence of resistant *E coli* strains in faecal flora suggested that the more antimicrobials were used in swine herds the more frequently resistant strains were selected. This effect was observed for antibiotics to which resistance in low-user herds was rather low whereas could not be evidenced for tetracycline. Moreover, relation between level of use and resistance was more noticeable in pigs than in sows, the later being less frequently treated than their progeny.

Effect of use of a given antibiotic on occurrence of resistance to this molecule and others from the same class was studied. A relation was observed between use of aminoglycosides and resistance to gentamicin both in sows and pigs although these antimicrobials were mainly administered to pigs in selected herds. Resistance to amoxicillin in pigs was associated with use of  $\beta$ -lactams, this class of antimicrobials being usually administered to piglets during either lactation or first part of post-weaning period. On the other hand, resistance to tetracycline was high and unrelated to the use of this class of antimicrobials.

## Conclusion

From this study level of use of antimicrobials was evidenced as a factor influencing prevalence of resistant *E coli* in faecal flora of pigs for some antimicrobials. However, further work is needed in order to describe more precisely effect of different antimicrobial treatments (molecule dose, administration route) on resistance.

Level of antimicrobial use has also to be more precisely studied using quantitative methods although measurement of drug consumption in veterinary medicine is difficult (2).

## Acknowledgements

This work was supported by funds from French Ministry of Agriculture.

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