

Trends in antimicrobial resistance of *E. coli* isolated from pigs at slaughter

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Abstract

Countrywide, harmonized microbiological resistance monitoring of indicator *Escherichia coli* isolated from healthy pigs at slaughter was run in 2009 and 2010. Resistance to each of the 13 tested antimicrobials was found, reaching a maximum value of 40% in the case of streptomycin and tetracycline. Cephalosporin resistance was sparse and less than 10% of strains possessed quinolone resistance. Numerous resistance profiles comprised up to 11 compounds from 7 antimicrobial classes. Year-to-year trends in resistance were not significant, with the exception of the decrease of the number of non-resistant strains ($P < 0,05$). The most frequent resistances correlate with the consumption of antimicrobials used for swine treatment. The results justified the need for continuous resistance monitoring followed by study on genetic background of the resistance. The attention is also drawn to the public health impact including possible therapeutic failures with drugs critically important for human medicine.

Introduction

Public health concerns have led numerous countries to monitoring of antimicrobial resistance in indicator bacteria. The results provide a baseline needed for the assessment of public health threats due to possible therapeutic failures and control of emerging resistances and resistance mechanisms. Furthermore, they are a measurement of antibiotic consumption in animal production leading also to the issue of residues that might compromise consumers' health. European Food Safety Authority recommended a harmonized antimicrobial resistance monitoring scheme of *Escherichia (E.) coli* aiming at comprehensive and comparable data at the EU level. Pigs are one of the target populations that should be covered by randomized sampling at slaughter to obtain a reasonable number of 170 isolates guaranteeing a predefined sensitivity of the testing. *E. coli* isolation and identification procedures, resistance testing and reporting are also addressed in the guidelines [1].

During the study period 36,9 mln of pigs were slaughtered at approximately 700 abattoirs in Poland (Tab. 1). The aim of the study was to assess the level of microbiological resistance in indicator *E. coli* strains isolated from random, healthy pigs at slaughter and the identify resistance trends in 2009 – 2010.

Material and Methods

The abattoirs contributing to 70% of annual pig slaughter capacity were chosen to perform randomised sampling by veterinary officers (Tab. 1). The randomisation of sampling was validated using index of diversity (D) [2] on the following variables: farm of origin of sampled animal (farm ID), geographical location of farm (county area code), and sampling date (Tab. 2.). Mapping of farm locations was used to visualise overall coverage of the country territory (data not shown). Each sampling contained rectal swabs collected from 3 consecutive animals from slaughter line. The samples were referred to the laboratory in transportation medium in ambient conditions by a courier service. They were directly streaked on MacConkey agar the first isolate showing typical *E. coli* morphology was used for biochemical confirmation followed by antimicrobial resistance testing with microbroth dilution method (Sensititre, TREK D.S.). MIC values of 13 antimicrobials tested (Tab. 1) were interpreted according to EUCAST epidemiological criteria to split bacterial population into wild-type (WT) and non-wild type (NWT) strains. The latter group was considered as microbiological resistant due to the possible presence of resistance mechanism against given antimicrobial.

Results

A sufficient number of samples was collected evenly over the study period from pigs originated from a number of farms located all over the country. The D index close to the maximum value of 1 confirmed satisfactory randomisation of sampling (Tab. 2).

Tab. 1. Annual pigslaughter data

	2009	2010
Number of slaughtered pigs (Veterinary Service data)	17420288	19488804
Number of involved slaughterhouses (Veterinary Service data)	706	694
Number (%) of slaughterhouses selected for sampling	109 (15%)	94 (14%)
Contribution of selected slaughterhouses to overall annual slaughter	70%	70%

Tab. 2. Randomization of sampling

	2009	2010
Number of collected samples	181	175
Number of sampled farms (D)	108 (0,988)*	92 (0,985)*
Number of counties – geographical location of farms (D)	70 (0,974)*	90 (0,993)*
Number of sampling days (D)	65 (0,980)*	92 (0,990)*
Number of tested strains (<i>E. coli</i> recovery rate)	178 (98,3%)	170 (97,1%)

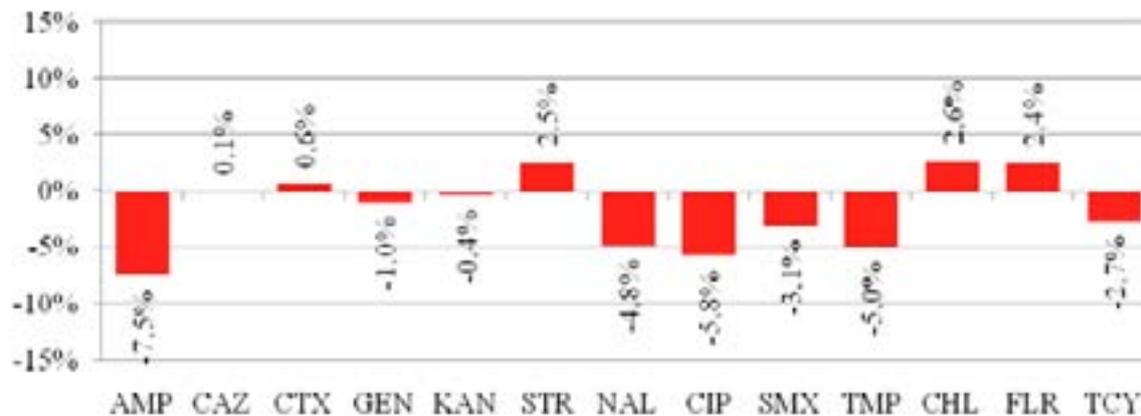
* data were not available for all sample sets

The frequency of resistance in tested *E. coli* strains were shown in Tab. 3. Microbiological resistance to each of compounds tested was found and in the case of streptomycin and tetracycline it was reaching 40%. Cephalosporin resistance was sparse in both study periods whereas up to 10% of strains possessed quinolone resistance. Year-to-year changes in resistance level were not significant (Fig. 1). They tend to increase in cephalosporins, phenicols, and streptomycin but decline in other compounds, reaching with maximum decrease in ampicillin (7,5%) and ciprofloxacin (5,8%).

Tab. 3. Microbiological resistance in *E. coli*

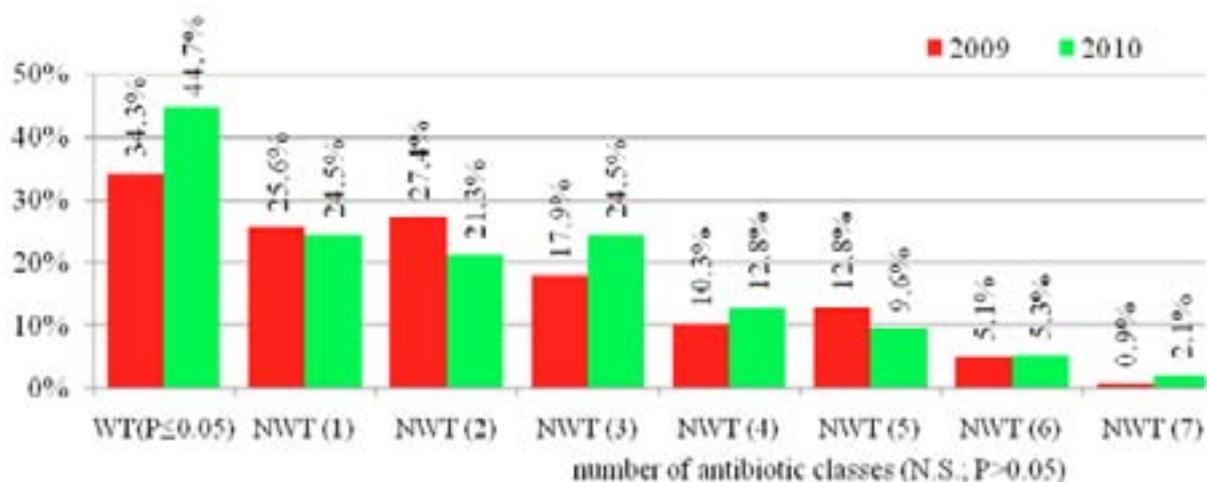
antimicrobial	NWT > mg/L	Microbiological resistance			
		2009 (N=178)		2010 (N=170)	
		N	%	N	%
Ampicillin (AMP)	8	49	27,5%	34	20,0%
Ceftazidime (CAZ)	0,25	2	1,1%	2	1,2%
Cefotaxime (CTX)	0,5	1	0,6%	2	1,2%
Gentamicin (GEN)	2	6	3,4%	4	2,4%
Kanamycin (KAN)	8	9	5,1%	8	4,7%
Streptomycin (STR)	16	71	39,9%	72	42,4%
Nalidixic acid (NAL)	16	18	10,1%	9	5,3%
Ciprofloxacin (CIP)	0,032	24	13,5%	13	7,6%
Sulfamethoxazole (SMX)	256*	60	33,7%	52	30,6%
Trimethoprim (TMP)	2	34	19,1%	24	14,1%
Chloramphenicol (CHL)	16	11	6,2%	15	8,8%
Florfenicol (FLR)	16	2	1,1%	6	3,5%
Tetracycline (TCY)	8	74	41,6%	66	38,8%

Figure 1. Trends in microbiological resistance 2009-2010 (N.S.; P>0.05)



The percentage of WT strains with no resistance mechanisms increased from 34,3% to 44,7% (P 0.05). Fifty-five resistance profiles were observed in 2009 compared to 45 in the next year. The most complex profile comprised 11 antimicrobials from 7 antimicrobial classes (AmpNalCipChlFlrTcyStrGenKanSmxTmp). The shift in the number of strains resistant to one to seven antimicrobial classes was not significant, although occurrence of resistance to 2 classes decreased by 6.1% from 27,4% in 2009. The opposite trend (6,5%) was noted in strains resistant to 3 classes reaching the value of 24,5% in 2010.

Figure 2. Trends in resistance profiles 2009-2010



Discussion

Human infections with antibiotic resistant bacteria are often traced back to animal reservoirs of the pathogen or resistance mechanisms [3, 4]. We believe the present study is one of the first reports of the harmonised resistance monitoring as recommended in the EU [1]. Tetracycline, penicillins, sulphonamides and aminoglycosides constitute 83,7% of the compounds used in swine treatment in Poland [5] and the resistances observed in our study demonstrate the correlation with antimicrobial consumption resulting in the selection pressure [6, 7]. The observed resistance levels are in concordance with the other studies [7].

An association between *E. coli* resistance from animal, human, and environmental sources has been reported [3, 7], but resistance mechanisms may significantly differ [8]. Besides the compounds widely used in pig production, our results draw the attention to the antimicrobials showing less frequent resistances, but considered "critically important" for human medicine, such as cephalosporins and fluoroquinolones [9]. Our previous studies demonstrated that pigs are often colonised with *E. coli* resistant to extended spectrum cephalosporinases (ESC), mostly CTX-M1 and CMY-2 [8, 10]. The

higher number of ciprofloxacin compared to nalidixic acid resistant strains proved the presence of plasmid mediated quinolone resistance (PMQR). A subset of those strains were included in the international collaborative study in several European countries and carried *qnrS1* and *qnrB1.5* genes [11]. Both PMQR and ESC resistance mechanism may spread horizontally compromising public health safety [3, 4, 6].

Conclusion

We conclude that countrywide, randomized, and harmonized monitoring introduced in pigs at slaughter revealed comparable data on the most crucial microbiological resistances in indicator *E. coli*, that are correlated with antibiotics used for pig treatment. Some variations were observed over two years, but only a decrease in number of resistant isolates was statistically significant. The trends in *E. coli* resistance and the complex resistance profiles justify the need for continuous monitoring followed by the identification of genetic backgrounds of the resistance mechanisms to reduce public health risk due to the transmission of resistant pathogens of animal origin.

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