Antimicrobial Resistance of F4+ Escherichia Coli Isolated from Swine in Italy

Antimicrobial resistance of F4+ *Escherichia coli* isolated from swine in Italy

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Abstract

Four hundred forty-two F4+ pathogenic *Escherichia coli* were isolated in a period of ten years (2002-2011), from pigs with diarrhea belonging to Italian herds of swine. The strains were analyzed for their susceptibility to 12 antimicrobials by the disk diffusion method. During the study period, a statistically significant proportion of isolates resistant to enrofloxacin (14.5% to 89.3%), marbofloxacin (5.4% to 60.7%), flumequine (49.1 to 92.9), danofloxacin (21.6 to 80%), aminosidine (45.4% to 71.4%), florfenicol (9.8% to 64.3%), thiamphenicol (50% to 92%) and cefquinome (3.8% to 44%) was recorded. An increase of resistance (not statistically significant) was also observed to gentamicin (63.6% to 85.7%), apramycin (61.8% to 82.1%), trimethoprim-sulphametoxazole (75% to 89.3%), tetracycline (97% to 100%) and erythromycin (92.4% to 100%). Based on antimicrobial multi-resistance, the strains were collected into three groups: I. resistant to 2-5 antimicrobials; II. resistant to 6-8 antimicrobials; III. resistant to 9-12 antimicrobials. The

number of isolates belonging to the first group showed a statistically significant decrease (*P<0.05*; R^2 0.896; r -0.9608), while the isolates belonging to the second and third groups showed a statistically significant increase (*P<0.05*; R^2 0.727; r 0.8701 and *P<0.05*; R^2 0.753; r 0.8890, respectively). The results of this study suggest the need for continued survey on the development of resistance.

Keywords: pig; F4+ *Escherichia coli*; antimicrobial resistance; disc diffusion.

1. Introduction

Escherichia coli is the causative agent of gastrointestinal diseases and septicemia in pigs and has been identified as a common foodborne pathogen for humans (Yasphal et al., 2011). Colibacillosis, caused by pathogenic *E.coli* is one of the most significant diseases in the pig industry that leads to various clinical symptoms. Diarrhea is the most common symptom of colibacillosis in pig livestock, and it is classified into two types: neonatal diarrhea, which occurs in suckling piglets, and post-weaning diarrhea, which occurs in pigs two weeks after weaning. The use of antimicrobials is the most effective measure for controlling pig colibacillosis outbreaks, and several publications have reported data about the in vitro activity of different antibiotics for treating this disease (Burch, 2005; Aarestrup et al., 2008).

E.coli is also a useful marker across all animal species, for showing where antimicrobial resistance lies. It is also an organism that readily gains resistance to many antimicrobials, so it could be considered a reasonable indicator of antimicrobial use (Burch, 2005). *E.coli* can serve as reservoir of antibiotic-resistance genes that can be transferred to bacterial pathogens of humans and animals (Van den Bogaard et al., 2001; Moubareck et al., 2003).

Even though commensal indicator organisms are targets for monitoring antimicrobial resistance, pathogenic types of *E.coli* can also give important information in antibiotic resistance surveys.

The development and spread of antibiotic resistance is a multifaceted problem based firstly on the assumption that any use of antibiotics increases the risk of resistance selection. For this reason, the restrictive and prudent use of existing antibiotics could effectively prevent "a resistance collapse". Each superfluous, indiscriminate or incomplete antibiotic therapy, for instance, promotes resistance selection and can increase the gene pool with antibiotic resistances in pathogens (Wallmann et al., 2006).

Increased antimicrobial resistance among pathogenic and commensal bacteria of animal origin is a growing concern in both veterinary and human medicine. For this reason, several countries are looking at different control options for the problem in Europe.

The aim of this study was to evaluate retrospectively, the trend in antimicrobial resistance and multi-resistance of *E.coli* F4+ strains isolated from swine in Italy.

2. Materials and methods

2.1. Bacterial isolates

A total of 442 F4+ *Escherichia coli* isolates from diseased pigs suffering from diarrhea were considered. The isolates were collected from 2002 to 2011 at the Diagnostic Sections of Reggio Emilia of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER). No more than two isolates of *E.coli* from the same herd per year were included in the study. Bacterial strains were isolated on blood agar plates at 37°C and routinely identified using Gram staining and according to biochemical standard procedures. All isolates were immediately sub-cultured on the same culture medium used

for the primary isolation and tested for antimicrobial susceptibility.

2.2. Antimicrobial susceptibility testing

E.coli isolates were tested for their susceptibility to a panel of antimicrobials by the disk diffusion method following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2008). The following antimicrobial agents were tested: apramycin (15 µg), cefquinome (30 µg), danofloxacin (5 µg), enrofloxacin (5 µg), erythromycin (15 µg), florfenicol (30 µg), thiamphenicol (30 µg), flumequine (30 µg), gentamicin (10 µg), marbofloxacin (5 µg), tetracycline (30 µg) and trimethoprim-sulphametoxazole (1,25/23,75 µg). The choice of antimicrobials to be tested over the ten-year period was based on the requests of submitting veterinarians, as well as on the basis of specific fields requirements. Regular quality assurance was performed among isolates processed using the American Type Culture Collection reference strain of *E.coli* (ATCC 25922, Oxoid, Milano, Italy) and *Staphylococcus aureus* (ATCC 25923, Oxoid, Milano, Italy). Isolates were classified as resistant, susceptible or intermediate to antimicrobials tested in accordance with the breakpoints proposed by the Comité de l'Antibiogramme de la Societé Française de Microbiologie (CASFM 2010). CLSI (2002; 2008) standards and criteria were applied for gentamicin, apramycin, florfenicol, erythromycin, trimethoprim-sulfametossazole and tetracyclines. Intermediate isolates were grouped with the resistant ones.

2.3. Data and statistical analyses

The antimicrobial resistance rate of *E.coli* was calculated on a yearly basis as the number of resistant isolates divided by the total number of tested isolates for a given antimicrobial.

On the basis of antimicrobial multi-resistance, the strains were attributed to three classes: I. resistant to 2-5 antimicrobials; II. resistant to 6-8 antimicrobials; III. resistant to 9-12 antimicrobials. The trend of resistance rate to the antibacterials and multi-resistance of *E.coli* strains was determined by the linear regression analysis. The percentage of resistance strains or the percentage of multi-resistance strains per groups was used as the dependent variable and the year of isolation (from 2002 to 2011) was used as the independent variable. Observations were weighted by the numbers of strain tested in each years.

A trend was considered statistically significant for $p < 0.05$. The regression coefficients were provided. Statistical analyses were performed using Intercooled Stata 7.0 software (Stata Corporation, College Station, TX, USA).

3. Results

The resistance rates and the trends in resistance of *E.coli* isolated from 2002 to 2011 to individual antimicrobials are shown in Table 1. Isolates showed a statistically significant increasing trend of resistance over the whole period to enrofloxacin (from 14.5% to 89.3%), marbofloxacin (from 5.4% to 60.7%), flumequine (from 49.1 to 92.9), danofloxacin (from 21.6 to 80%), florfenicol (from 9.8% to 64.3%), thiamphenicol (from 50% to 92%) and cefquinome (from 3.8% to 44%). An increasing resistance (not statistically significant) was also observed to gentamicin (from 63.6% to 85.7%), apramycin (from 61.8% to 82.1%), trimethoprim-sulphametoxazole (from 75% to 89.3%), tetracycline (from 97% to 100%) and erythromycin (from 92.4% to 100%).

All the strains resulted resistant to at least one antibiotic. Twelve strains (2.7%) were resistant to one antimicrobial only. Considering the distribution of the strains into the three

classes of multi-resistance, a shift was observed from the first to the second and third classes from 2002 to 2011. A statistically significant decrease of strains collected in the first class over the period of study (from 72.79% in 2002 to 6.5% in 2011) was observed. Conversely, the strains belonging to the second and third classes showed a statistically significant increasing trend, moving from 16.4% in 2002 to 54.8% in 2011 and from 0% in 2002 to 38.7% in 2011 (table 2 and figure 1).

4. Discussion

In the present study, the antimicrobial agents most commonly used for the treatment of pig diarrhea due to *E.coli* infection were taken into account in order to evaluate the antimicrobial resistance rates, the trends in antimicrobial resistance and the antimicrobial multi-resistance of *E.coli* F4+ strains isolated from swine in Italy from 2002 to 2011.

In accordance with Taylor et al. in 2009, a development of resistance to all fluoroquinolones tested was observed over the period of study, and the trend registered was statistically significant. In particular, our results, showing a high level of resistance of *E.coli* F4+ to enrofloxacin, are in agreement with previous studies performed in Austria (Mayrhofer et al., 2004) but disagree with the results obtained in Canada and Australia (Yasphal et al., 2011; Smith et al., 2010). Among the tested fluoroquinolones, resistance to marbofloxacin resulted lower than that observed for danofloxacin, enrofloxacin and flumequine. This reflects the low occurrence of cross-resistance between marbofloxacin and the other tested fluoroquinolones, suggesting a pattern of dichotomous fluoroquinolone resistance which has already been observed in other bacterial pathogens of both animal and human origin (Fitzgibbon et al., 1998; Vanni et al., 2011).

The in vitro resistance of *E.coli* F4+ isolates to all aminoglycosides tested showed an

increasing trend but resulted not statistically significant. In particular the levels of resistance to apramycin and gentamicin appeared very similar, and this is probably due to a cross resistance among aminoglycosides. It was reported that *E.coli* from pigs might have been an important reservoir for transfer of gentamicin resistance genes or bacteria to humans (Johnson et al., 1994). It was also reported that the occurrence of apramycin/gentamicin cross resistance in pigs was significantly correlated with the apramycin use (Jensen et al., 2006). Spread of gentamicin resistance in humans is of great concern, considering the importance of this antibiotic in human medicine (Zarrilli et al., 2005).

A statistically significant increasing trend of resistance to florfenicol and thiamphenicol has been demonstrated. In particular florfenicol has been licensed in Europe since 2000 for the therapy of bacterial infections in pigs, and the increased resistance to this relatively new molecule should be a reason of concern.

A statistically significant increasing trend of resistance to cefquinome was observed, even if this 4th generation cephalosporin resulted in one of the most effective antimicrobials in the therapy of *E.coli* diarrhea, showing the lowest percentage of resistance recorded in this study.

Trimethoprim-sulphametoxazole showed a reduction of the activity during the period considered, but this trend was not statistically significant. Erythromycin and tetracycline were found to be less active antimicrobials against *E.coli* F4+ tested in our study, as described in other studies performed in UK, Spain and Canada (Burch, 2005; Stannarius et al., 2000; Kozak et al., 2009). The high level of resistance to erythromycin observed was expected, because the antibiotic is considered to have moderate action on the member of the family *Enterobacteriaceae*. However, this level of resistance to erythromycin resulted

higher than these described by Medina et al., 2011 in *E.coli* isolated from calves, lambs and goats. The result obtained about tetracycline is probably due to the wide use of this antibiotic in the past for treating pig respiratory and enteric bacterial diseases as described in UK by Burch in 2005.

The high general level of resistance recorded in our study could be influenced by two main factors. The first is that all the intermediate isolates were classified as being resistant. The second is associated with the origin of *E.coli* strains sourcing from pigs with diarrhea—probably problematic from a therapeutic point of view.

Considering the test used for the antimicrobial resistance evaluation, it is widely known that the disc diffusion method may either over or underestimate the sensitivity of an organism to a certain antimicrobial. There are more accurate ways of assessing sensitivity, but for monitoring purposes, the disc diffusion remains a very useful method. The method used could have slightly influenced the percentages of resistance to antimicrobials but not the trend that can be considered as realistic.

The obtained results confirmed the antibiotic resistance of one of the most important problems in veterinary medicine. Antimicrobial agents have been extensively used in swine production for therapeutic, methaphylactic and prophylactic purposes, and the subsequent selective pressure has intensified the risk for the emergence of resistant bacteria (Jensen et al., 2006; Kozak et al., 2009). In particular, the development of resistance to fluoroquinolones tested and cefquinome is of great concern, considering the essential role of these antimicrobials for the treatment of infections in humans. It is not certain if the use of antimicrobials in pigs has a direct or indirect adverse effect on humans. However, the results obtained represent a problem of animal health compromising the control of pig diseases at herd level.

5. Conflict of interest

None of the Authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence the content of this paper.

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Table 1: Resistance to selected antimicrobials of 442 *E.coli* F4+ isolated from swine during 2002-2011 and statistical analysis of observed variations (R^2 and r = regression coefficients).

Antimicrobials	**Year of isolation – N° of strain tested - % of resistant isolates**										**Statistical analysis**		
	2002	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**P**	**R^2**	**r**
N° of strain tested	**55**	**53**	**63**	**45**	**68**	**39**	**44**	**18**	**29**	**28**			
Fluoroquinolones													
Enrofloxacin	14.5	25.4	11.1	42.2	39.7	46.1	65.9	50	72.4	89.3	<0.000	0.87	0.93
Marbofloxacin	5.4	16.7	7.9	31.1	38.2	38.5	50	38.9	62.1	60.7	<0.000	0.89	0.94
Flumequine	49.1	59.3	42.9	64.4	55.9	69.2	81.8	77.8	79.3	92.9	<0.01	0.74	0.90
Danofloxacin	21.6	22.2	12.7	44.4	39.7	35.1	66.7	50	79.3	80	<0.01	0.80	0.90
Aminoglycosides													
Gentamicin	63.6	65.4	74.6	66.7	83.6	51.3	59.1	77.8	75.9	85.7	>0.05	0.07	0.39
Apramycin	61.8	74.5	77.8	57.8	80.9	64.1	68.2	83.3	89.7	82.1	>0.05	0.22	0.58
Amphenicols													
Florfenicol	9.8	16.7	6.3	33.3	19.1	23.1	43.2	55.6	79.3	64.3	<0.001	0.77	0.90
Thiamphenicol	50	55	62	64	78	82	89	93	91	92	<0.000	0.93	0.97
Macrolides													
Erythromycin	92.4	100	92.1	100	100	94.9	93.2	100	100	100	>0.05	0.13	0.36
Cephalosporins													
Cefquinome	3.8	1.8	9.5	4.4	10.3	15.4	21.9	22.2	48.3	44	0.001	0.81	0.90
Others													
Trimethoprim-Sulphamethoxazole	75	63	84.1	95.6	83.8	82	70.4	94.4	58.6	89.3	>0.05	0.01	0.10
Tetracyclines	97.4	92.6	95.2	95.6	100	97.4	95.4	100	96.5	100	>0.05	0.20	0.46

Table 2: Percentage of resistant strains collected into three classes of multi-resistance.

		Multi-resistance classes		
Year		**2–5**	**6–8**	**9–12**
2002		72.7	16.4	0.0
2003		75.9	13.0	5.6
2004		79.0	17.7	1.6
2005		60.0	28.9	11.1
2006		57.4	20.6	22.1
2007		53.8	25.6	17.9
2008		29.4	25.0	8.8
2009		22.2	38.9	38.9
2010		10.3	37.9	51.7
2011		6.5	54.8	38.7
Statistical Analysis	**P**	$p<0.05$	$p<0.05$	$p<0.05$
	R^2	0.896	0.753	0.727
	r	-0.9608	0.8890	0.8701

Figure 1: Trends in percentage of *E.coli* F4+ resistant strains collected into three classes of multi-resistance from 2002 to 2011.