Supplementary material to the manuscript: "The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview"

Antibiotic resistance in *E. coli* from poultry in Poland, United Kingdom, Germany, France and Spain

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POLAND

Poland began to implement a national monitoring programme for antibiotic resistance in commensal *E. coli* isolates from poultry in 2009 (Wasyl, et al., 2012). Figure 1 shows resistance to several antibiotics in the *E. coli* isolates from broilers at the slaughterhouse level from 2009 to 2014 (European Food Safety Authority and European Centre for Desease Prevention and Control, 2015, 2016; Wasyl, et al., 2013). The data in Figure 1 shows high resistance rates (from 70 to 90%) to ciprofloxacin, nalidixic acid and ampicillin, and 50 to 70% resistance against tetracycline, sulfamethoxazole and streptomycin. The reason for the 0% resistance rate to sulfamethoxazole in 2013 is not clear and may be explained with changes in the resistance detection values. Data from Wasyl, et al. (2013) correspond to data from the EU summary report on antibiotic resistance (European Food Safety Authority and European Centre for Desease Prevention and Control, 2014). Wasyl, et al. (2013) showed that the average resistance of multiresistant *E. coli* isolates from broilers in the period from 2009 to 2012 was 80.6%. The same study detected increasing trends of ampicillin and cefotaxime resistance in the observed *E. coli* isolates.

UNITED KINGDOM

AR data for *E. coli* isolated from broilers in the United Kingdom are available from two distinct AR-monitoring programmes: the EU monitoring and the clinical monitoring programmes (Veterinary Medicines Directorate, 2015). The EU monitoring programme isolated *E. coli* from healthy broilers across the United Kingdom. The clinical monitoring programme is passive monitoring. Its aim is the evaluation of AR to antibiotics of veterinary relevance in bacteria that are isolated from the clinical samples of diseased animals. Figure 2 presents the resistance rates of these tested *E. coli* isolates (Veterinary Medicines Directorate, 2015). Both monitoring programmes show high AR rates of *E. coli* to ampicillin and tetracycline. Bywater, et al. (2004) and Randall, et al. (2011) confirm the higher resistance rates to sulfonamides, ampicillin, and tetracycline in *E. coli* in UK. An analysis of the fluoroquinolone resistance in *E. coli* from faeces samples of 68 broiler farms in the UK detected resistance to ciprofloxacin at 50% of these farms (Taylor, et al., 2008).

GERMANY

The Federal Office of Consumer Protection and Food Safety reports on antibiotic resistance monitoring in Germany. Similar to the monitoring programmes of the UK, two different surveillance systems exist in Germany. The first one monitors healthy animals and the products thereof. The second system is the GERM Vet Report that contains data about the resistance of animal pathogens. Figure 3 shows results for *E. coli* isolates from intestinal samples of healthy broilers in Germany (European Food Safety Authority and European Centre for Desease Prevention and Control, 2011, 2016; German Federal Office of Consumer Protection and Food Safety, 2012a, 2013, 2015a, 2016a, Kaesbohrer, et al., 2012).

Germany monitors the AR in *E. coli* isolates from intestinal samples of broilers, chicken meat and diseased animals. As not all antibiotics are monitored to evaluate their resistance rates in diseased animals, it is only possible to compare the resistance rates of some antibiotics. All three systems show higher resistance rates to ampicillin and sulfamethoxazole than to other antibiotics (German Federal Office of Consumer Protection and Food Safety, 2012b, 2012c, 2014, 2015b, 2016b). The AR rates to ampicillin in *E. coli* from intestinal samples are approximately 60%, from meat samples approximately 68% and from diseased animals approximately 42%. The resistance rate to sulfamethoxazole is approximately 50% in *E. coli* from intestinal samples, 65% from meat samples and approximately 55% from diseased animals. For *E. coli* that were isolated from intestinal samples and retail meat, the resistance rates to ciprofloxacin, nalidixic acid, streptomycin, tetracycline and trimethoprim are between 40 and 60%. Except for trimethoprim, these antibiotics are not allowed for use in broilers in Germany. However, other representatives of the fluoroquinolones, aminoglycosides and tetracyclines may be used. The resistance rates of *E. coli* from diseased chickens to tetracycline and ciprofloxacin are approximately 30 and 7%, respectively. It seems that the resistance to ciprofloxacin is lower in diseased animals than in healthy animals, and resistance to cephalosporins is higher in diseased animals than in healthy animals. However, the resistance rates must be measured over longer periods of time to confirm these differences.

The plasmid-mediated colistin resistance gene MCR-1 that was detected by Lui et al. (2016) in China was also found in *E. coli* from broilers and chicken meat in Germany. The national monitoring programme of zoonotic agents for the years 2010-2014 demonstrates that 7% of the 1474 *E. coli* isolates tested from broilers were colistin-resistant and 93% of these colistin-resistant isolates harboured the MCR-1 gene (Irrgang, et al., 2016). The same study shows that 6% of the 580 *E. coli* isolates tested from chicken meat for the national monitoring of zoonotic agents in the years 2011-2014 showed colistin resistance.

FRANCE

France participates in the EU monitoring of AR in animals. Figure 4 shows the resistance rates of *E. coli* from broilers since 2005 (European Food Safety Authority and European Centre for Desease Prevention and Control, 2011; European Food Safety Authority and European Centre for Desease Prevention and Control, 2012; European Food Safety Authority and European Centre for Desease Prevention and Control, 2013; European Food Safety Authority and European Centre for Desease Prevention and Control, 2014; European Food Safety Authority and European Centre for Desease Prevention and Control, 2015; European Food Safety Authority and European Centre for Desease Prevention and Control, 2016).

The French Agency for Veterinary Medicinal Products (ANSES-ANMV) reports on national antibiotic resistance monitoring in France. The ANSES-ANMV provides reports on the French surveillance network for antibiotic resistance in pathogenic bacteria of animal origin (RESAPATH). The RESAPATH presents the results of the monitoring of AR in *E. coli* from diseased hens and broilers that are treated by veterinarians as part of their regular clinical services.

Tetracycline is approved for use in poultry in France. Due to the availability of the quantitative use of antibiotics in poultry, a comparison between antibiotic use and resistance is possible. According to this figure, more tetracyclines and polymyxins than other antibiotics are used in poultry. Consequently, the resistance rates to tetracyclines are also higher than those of other antibiotics. They are approximately 70% in all systems. However, there was a decrease in the use of tetracycline between 2007 and 2013, which was accompanied by a decrease of tetracycline-resistance rates in *E. coli* between 2011 and 2014. Thus, less use of an antibiotic may result in less resistance to that antibiotic. It may be that the lower antibiotic use in the country leads to lower resistance rates. This hypothesis needs to be confirmed in other countries, and the availability of quantitative data of antibiotic use allows this comparison.

Penicillins are quantitatively the third most frequently applied antibiotic class, after tetracyclines and polymyxins. The detected resistance rates for penicillins (50%) are lower than those of tetracyclines in France. There was an increase in penicillin use and resistance rates in *E. coli* between 2006 and 2014.

SPAIN

The Spanish antibiotic resistance surveillance network "Red de Vigilancia de Resistencias Antibioticas en Bacterias de Origen Veterinario" (VAV) was formed in 1996 (Moreno, 2000). The VAV reports present data on AR in *E. coli* that are taken from healthy broilers during the period from 1999 to 2005 (Ministry of Agriculture Fisheries and Food in Spain 2005, 2006). Additionally, AR data are available from the EU Summary reports. Figure 5 presents all available data.

The fluoroquinolones enrofloxacin and flumequine are allowed for use in poultry in Spain. The monitoring data show that AR to ciprofloxacin in *E. coli* increased from 17% in 2001 to 85% in 2014, and that of nalidixic acid increased from 60% in 2001 to 83% in 2014. Whether the increased use of the fluoroquinolones may have influenced this increase cannot be evaluated without the presence of quantitative AU data. Tetracycline and the penicillins are registered for use in Spain. The AR resistance rates to tetracycline and ampicillin are approximately 70%. A decrease of resistance to tetracycline was observed between 1999 and 2014. Neomycin and sulfamethoxazole are also approved for use in poultry. The resistance to neomycin is approximately 20%. However, the resistance rates to streptomycin, another aminoglycoside, and sulfamethoxazole are approximately 55%.

There is also a scientific study that detected resistance in *E. coli* to nalidixic acid, tetracycline, trimethoprim, gentamycin and ampicillin and showed 88, 75, 65, 40 and 38% resistance, respectively (Saenz, et al., 2001).

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FIGURE LEGENDS

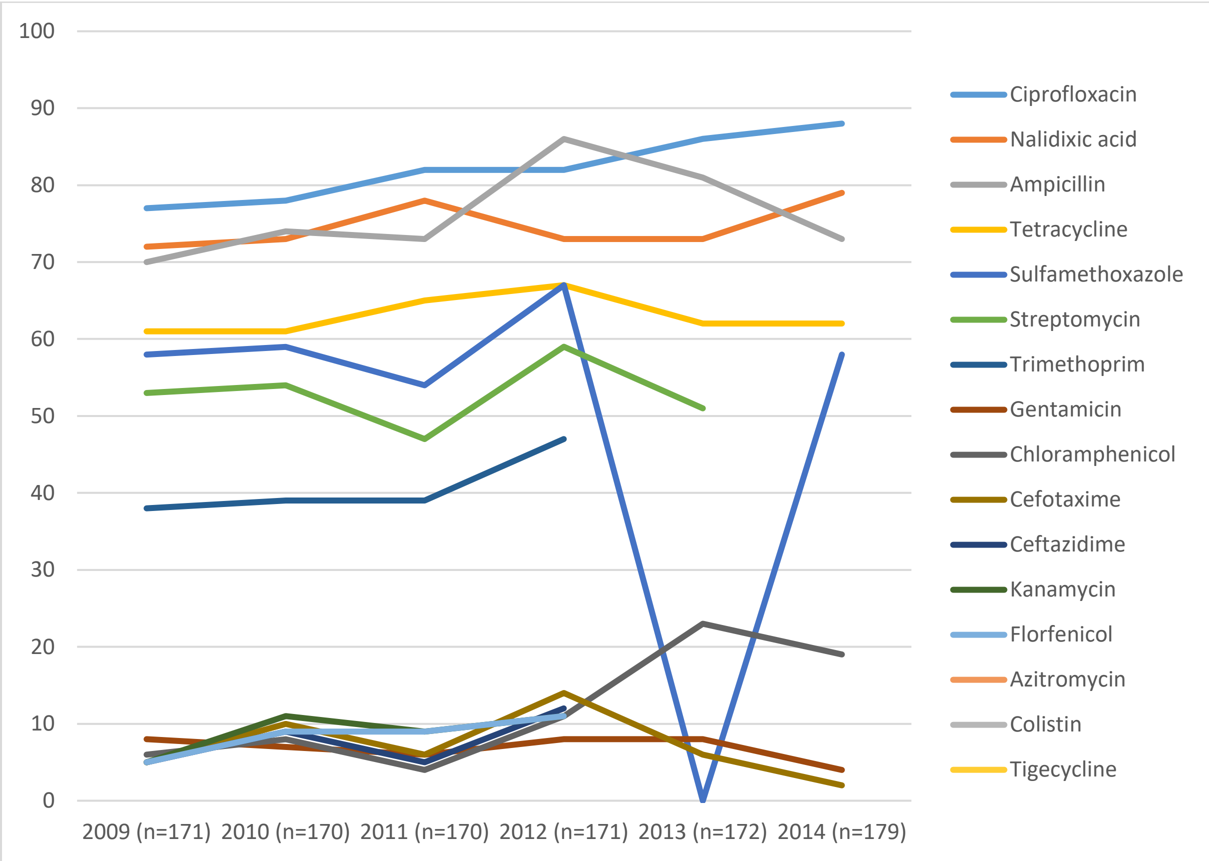

Figure 1. Percentages of antibiotic resistance in *E. coli* isolates from broilers at slaughterhouse level in the period from 2009 to 2014 in Poland, based on European Food Safety Authority and European Centre for Desease Prevention and Control 2015, 2016; Wasyl, et al., 2013).

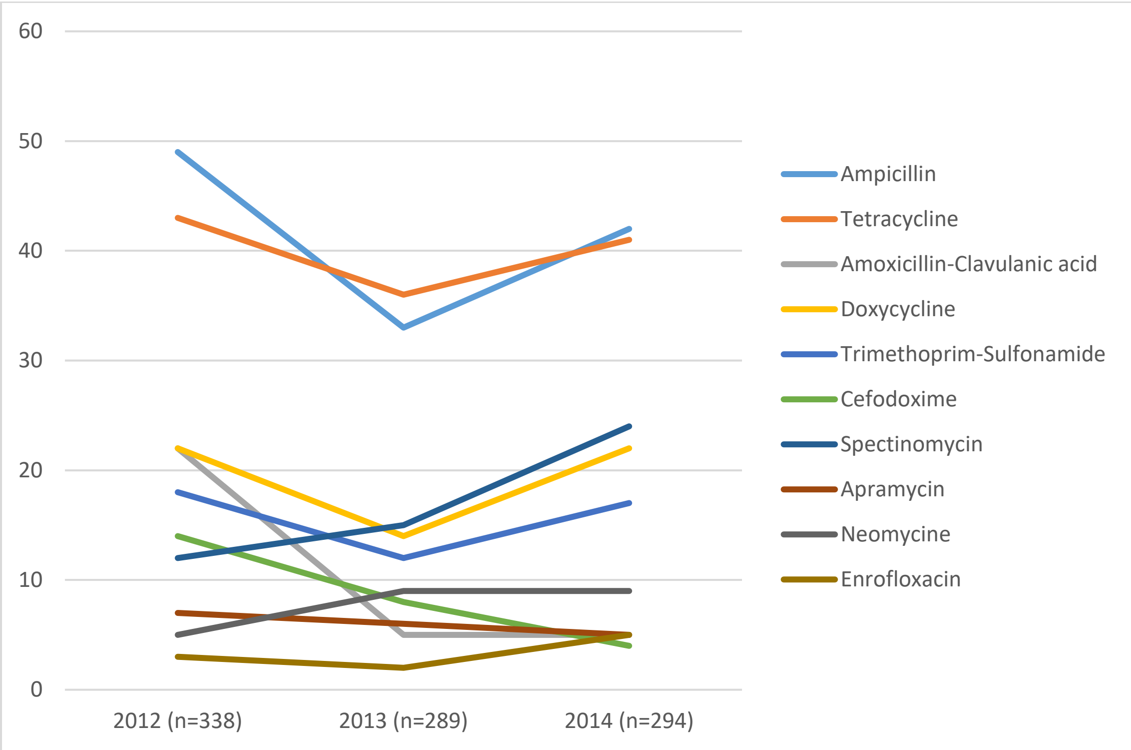

Figure 2. Percentages of antibiotic resistance in *E. coli* isolates from diseased broilers in the period from 2012 to 2014 in UK, based on Veterinary Medicines Directorate (2015).

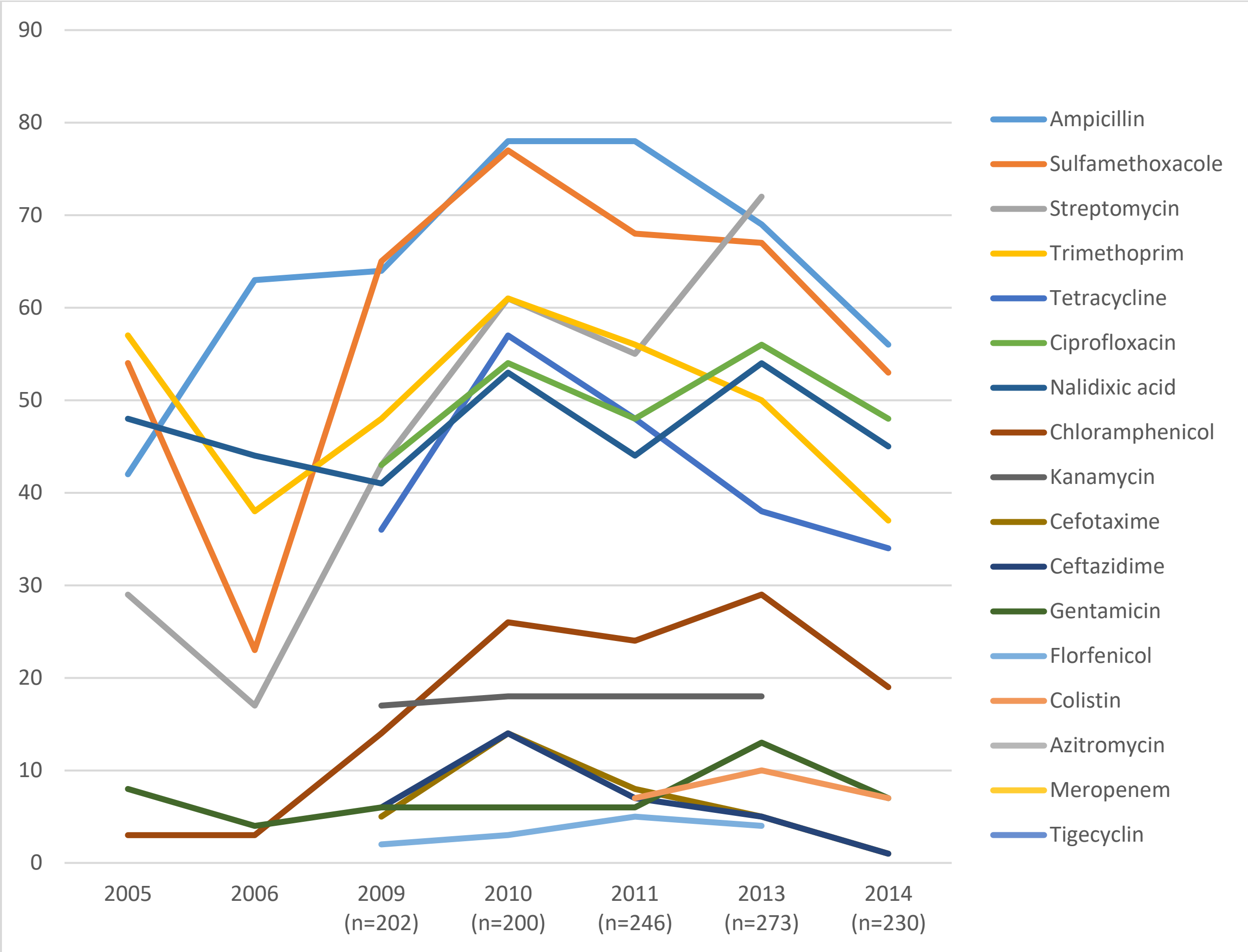

Figure 3. Percentages of antibiotic resistance in *E. coli* isolates from intestinal samples of healthy broilers in the period from 2005 to 2014 in Germany, based on European Food Safety Authority and European Centre for Desease Prevention and Control, 2011, 2016; German Federal Office of Consumer Protection and Food Safety, 2012a, 2013, 2015a, 2016a; Kaesbohrer, et al., 2012.

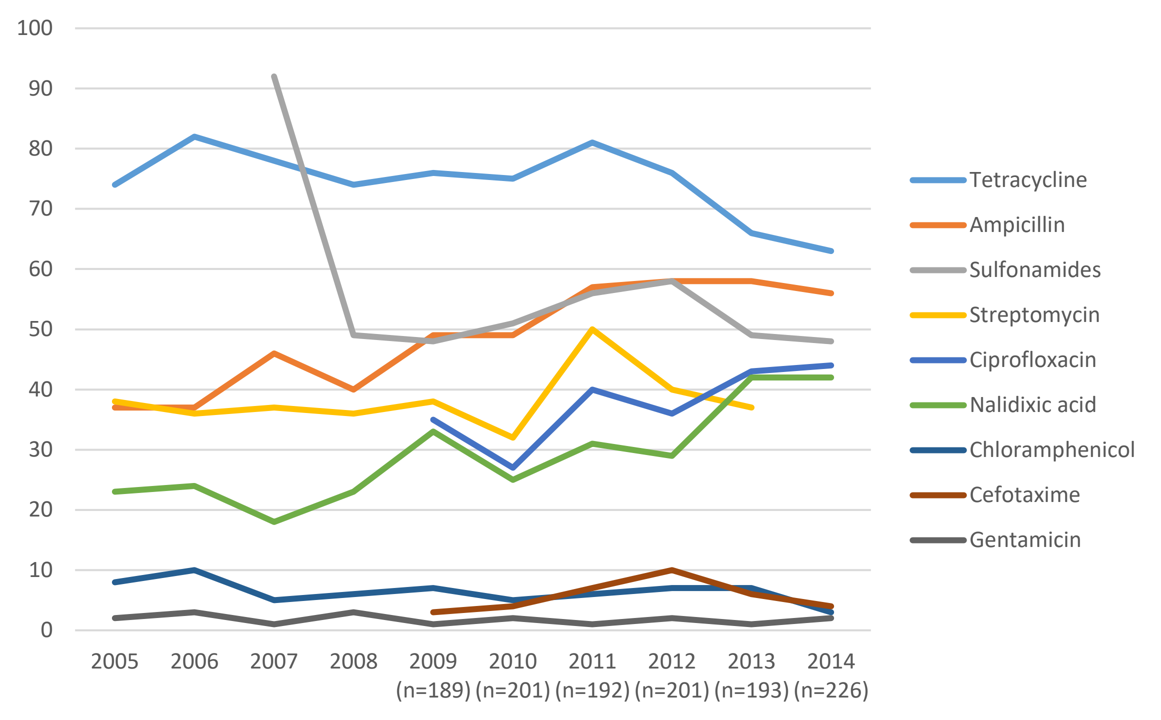

Figure 4. Percentages of antibiotic resistance in *E. coli* isolates from healthy broilers in the period from 2005 to 2014 in France, based on European Food Safety Authority and European Centre for Desease Prevention and Control 2011, 2012, 2013, 2014, 2015, 2016)

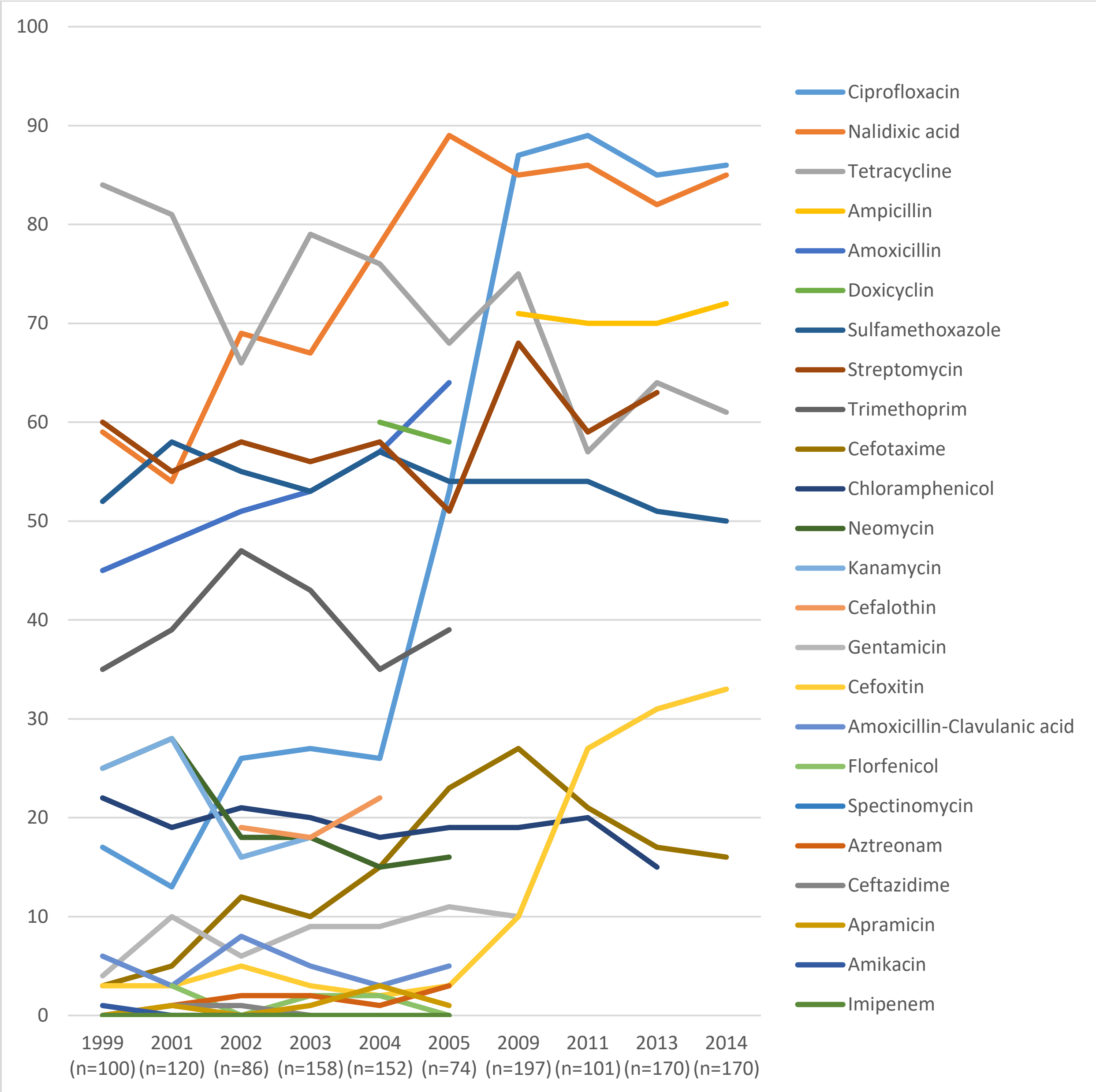

Figure 5. Percentages of antibiotic resistance in *E. coli* isolates from healthy broilers in the period from 1999 to 2014 in Spain, based on European Food Safety Authority and European Centre for Desease Prevention and Control, 2011, 2013, 2015, 2016; Ministry of Agriculture Fisheries and Food in Spain 2005, 2006)