Class 1 and class 2 integrons in avian pathogenic *Escherichia coli* from poultry in Italy

Lara Cavicchio,[*] Giorgia Dotto,[*] Martina Giacomelli,[*] Davide Giovanardi,[†] Guido Grilli,[‡] Maria Pia Franciosini,[§] Angela Trocino,[*] and Alessandra Piccirillo[*,1]

[*]*Department of Comparative Biomedicine and Food Science, University of Padova, 35020 Legnaro (PD), Italy;* [†]*Laboratorio Tre Valli, 37132 San Michele Extra (VR), Italy;* [‡]*Department of Veterinary Science and Public Health, University of Milan, 20133 Milano, Italy; and* [§]*Department of Biopathologic Science and Hygiene of Food and Animal Production, University of Perugia, 06126 Perugia, Italy*

ABSTRACT The aim of this study was to investigate the occurrence of class 1 and 2 integrons in avian pathogenic *Escherichia coli* (APEC) from poultry in northern Italy. Strains were tested for phenotypic resistance to aminoglycosides and sulphonamides, and the association between the presence of integrons and the resistance to these antimicrobials was evaluated. A total of 299 isolates (158 from turkeys, 110 from broilers, and 31 from layer hens) were collected from 200 industrial farms. Antimicrobial susceptibility test by the disk diffusion method was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. All strains were screened for the presence of class 1 and 2 integrons by PCR and sequencing. About 55% of APEC contained integrons (class 1, 49.8%; class 2, 10.4%). Different variants of the *aadA* (5 variants) and the *dfrA* (4 variants) genes, encoding for streptomycin and trimethoprim resistance respectively, were detected in integron-positive isolates. Less common gene cassettes, such as *sat*, *estX*, and *orfF*, were also identified. Fifteen and 4 gene cassette arrays were found among class 1 and 2 integrons, respectively. High levels of resistance were observed for triple sulphonamides (79.3%), streptomycin (67.2%), and sulfamethoxazole combined with trimethoprim (62.2%), whereas resistance against gentamycin (16.7%), kanamycin (14.7%), and apramycin 3.0%) was low. Integron positivity was significantly higher in isolates phenotypically resistant to aminoglycosides (63.6% vs. 37.8%, $P < 0.001$) and sulfonamides (64.1% vs. 21.1%, $P < 0.001$) than in susceptible ones. Integron-borne aminoglycoside and sulfonamide resistance in APEC represents a concern for the poultry industry in Italy, since they are among the most commonly used antimicrobials in poultry therapy.

Key words: avian pathogenic *Escherichia coli*, poultry, antimicrobial resistance, integron, aminoglycoside, sulfonamide

2015 Poultry Science 94:1202–1208
http://dx.doi.org/10.3382/ps/pev095

INTRODUCTION

Escherichia coli is a commensal bacterium of the normal intestinal flora of humans and animals. However, pathogenic strains able to cause intestinal and extraintestinal infections, including gastroenteritis, urinary tract infections, meningitis, peritonitis, and septicaemia, have been recognized in humans (Kaper et al., 2004). In poultry, pathogenic strains of *E. coli,* known as avian pathogenic *Escherichia coli* (APEC), can cause localized or systemic infections, such as acute fatal septicemia or subacute pericarditis and airsacculitis (Nolan et al., 2013). Avian colibacillosis can be secondary to other respiratory infections, inappropriate husbandry practices, or environmental predisposing factors (Nolan et al., 2013) and can cause great economic losses to the poultry industry due to mortality and slaughter condemnation (Lutful Kabir, 2010).

In recent years, antimicrobial resistance of bacteria has become one of the most serious public health threats. Several authors (Hammerum and Heuer, 2009; Landers et al., 2012) suggest that the selective pressure consequent to the use of antimicrobials in humans and animals may promote resistance in both commensal and pathogenic bacteria, including *E. coli*. Indeed, in poultry, commensal and pathogenic *E. coli* have increasingly become resistant to many antimicrobials, including aminoglycosides and sulfonamides (Gyles, 2008). The antimicrobial resistance of APEC may pose serious therapeutic problems when treating sick birds. Furthermore, the resistance of poultry bacteria may represent a public health threat due to the potential transmission to humans of resistant bacteria (Hammerum and Heuer, 2009).

Received November 24, 2014.
Accepted January 31, 2015.
[1]Corresponding author: alessandra.piccirillo@unipd.it

Several mechanisms of antimicrobial resistance have been described in *E. coli* from humans and animals, and integrons seem to play a key role in the emergence and diffusion of multidrug resistant *E. coli* (Saenz et al., 2004). Integrons are genetic structures able to capture, integrate, and express genes contained in gene cassettes, which are discrete mobile units comprising genes usually encoding for antimicrobial resistance, and a recombination site recognized by an integrase (Hall, 2012). Based on differences in the integrase gene, integrons are classified in several families and those belonging to class 1 and 2 are the most frequently detected in *Enterobacteriaceae* (Partridge et al., 2009). These genetic elements have been described in commensal and pathogenic *E. coli* from humans and several animal species (Partridge et al., 2009; Hall, 2012).

A number of studies have documented the presence of integrons in commensal and pathogenic *E. coli* isolated from poultry farms (Yang et al., 2004; Nogrady et al., 2006; Kim et al., 2007; Ahmed et al., 2013; Dessie et al., 2013; Oosterik et al., 2014) and poultry meat and meat products (Trobos et al., 2008; Altalhi et al., 2010; Soufi et al., 2011; Dessie et al., 2013). However, very few papers investigating on the presence of integrons in commensal (Nebbia et al., 2008) and pathogenic *E. coli* (Piccirillo et al., 2014) from Italian poultry have been published to date. Therefore, we carried out a study aimed at assessing the occurrence of class 1 and 2 integrons in APEC isolates from commercial poultry farms in northern Italy. The gene cassette content of integron-positive isolates was characterized, as well as the phenotypic resistance to aminoglycosides and sulphonamides.

MATERIALS AND METHODS

Bacterial Isolates

Between 2008 and 2012, avian pathogenic *E. coli* strains were recovered from commercial poultry affected by colibacillosis in 200 farms throughout northern Italy. In detail, 158 strains were isolated from meat turkeys, 110 from broilers and 31 from laying hens for a total of 299 isolates. Samples from tissues and viscera showing lesions consistent with colisepticemia (e.g., pericarditis, perihepatitis, airsacculitis) were plated on eosin-methylene blue (EMB) agar (OXOID, Basingstoke, UK) and incubated for 18 h to 24 h at 37°C. From each sample, a single colony of *E. coli* was identified using RapID E20 (bioMérieux Vitek, Hazelwood, MO, USA).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was performed using the disk diffusion method on Mueller-Hinton Agar (OXOID), according to the procedure recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008). All isolates were tested for susceptibility to apramycin (APR, 15 μg), gentamicin (GM, 10 μg), streptomycin (S, 10 μg), kanamycin (K, 30 μg), sulfamethoxazole + trimethoprim (SXT, 25 μg), and triple sulphonamides (S3, 300 μg). All disks were obtained from OXOID. *Escherichia coli* ATCC 25922 was used as quality control strain.

Integron Detection and Characterization

Total DNA was extracted by boiling for 20 min a suspension of bacterial cells in 200 μl of sterile RNase/DNase free water (Sigma-Aldrich, Milan, Italy). The real-time PCR assay described by Piccirillo et al. (2013) was used to screen isolates for the presence of class 1 and class 2 integrons. Integron-positive strains were further processed by PCR and sequencing to identify gene cassettes. Primers described by Lévesque et al. (1995) and White et al. (2001) were used to amplify the variable region of class 1 and 2 integrons, respectively. Amplicons were sequenced on both strands using the BigDye Terminator Cycle Sequencing Kit v3.1 in the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Editing of chromatograms and assembly of nucleotide sequences were performed by the software ChromasPro v. 1.42 (Technelysium Pty Ltd., Tewantin, Australia). Gene cassettes were identified by the BLASTN software available online (http://blast.ncbi.nlm.nih.gov/).

Statistical Analysis

Differences in the occurrence of integrons in resistant *vs* susceptible and intermediate isolates to the 6 antimicrobials were tested for turkeys, broilers, and laying hens by the Chi-square (χ^2) test and using the SAS software (Institute Inc., Cary, NC, USA). The same procedure was followed for assessing statistical differences in the occurrence of *aadA*, *dfrA,* and *sat* genes in the same isolates. Differences among means with $P < 0.05$ were accepted as statistically significant.

RESULTS AND DISCUSSION

Out of 299 APEC isolates examined in this study, 167 (55.9%) were found positive for integrons. Of these, 149 (49.8%) and 31 (10.4%) isolates harbored class 1 and class 2 integrons, respectively (Table 1). Thirteen strains (4.3%) were positive for both classes. Positivity to class 1 integrons was significantly different among poultry species (41.8%, 61.8%, and 48.4% in isolates from turkeys, chickens, and layer hens, respectively; $P < 0.01$) (Table 1), whereas no significant difference was observed for class 2 integrons (12.0%, 9.1%, and 6.5% in isolates from turkeys, broilers, and layer hens, respectively; $P = 0.56$). These percentages of integron positivity, as well as the lower frequency of class 2 compared to class 1 integrons and the concurrent presence of both classes of integrons, are

Table 1. Integron positivity and gene cassette content of APEC isolates from poultry.

Integrons/resistance genes	Turkeys Positive no. (%)	Broilers Positive no. (%)	Layer hens Positive no. (%)	Total Positive no. (%)
Class 1 integrons	66 (41.8)	68 (61.8)	15 (48.4)	149 (49.8)
none	18 (27.3)	24 (35.3)	4 (26.7)	46 (30.9)
aadA	3 (4.5)	0 (0.0)	0 (0.0)	3 (2.0)
aadA1	16 (24.2)	14 (20.6)	2 (13.3)	32 (21.5)
aadA2	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.7)
aadA5	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.7)
aadA13	1 (1.5)	0 (0.0)	0 (0.0)	1 (0.7)
dfrVII	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.7)
sat	0 (0.0)	4 (5.9)	0 (0.0)	4 (2.7)
estX	0 (0.0)	0 (0.0)	8 (53.3)	8 (5.4)
aadA1-dfrA1	17 (25.8)	10 (14.7)	0 (0.0)	27 (18.1)
aadA2-dfrA12	2 (3.0)	0 (0.0)	0 (0.0)	2 (1.3)
aadA5-dfrA17	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.7)
dfrA1-aadA1	8 (12.1)	9 (13.2)	1 (6.7)	18 (12.1)
sat2-aadA1	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.7)
estX-aadA1	1 (1.5)	1 (1.5)	0 (0.0)	2 (1.3)
dfrA12-orfF-aadA2	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.7)
Class 2 integrons	19 (12.0)	10 (9.1)	2 (6.5)	31 (10.4)
none	5 (26.3)	3 (30.0)	2 (100.0)	10 (32.3)
aadA1-dfrA1	2 (10.5)	2 (20.0)	0 (0.0)	4 (12.9)
dfrA1-aadA1	3 (15.8)	4 (40.0)	0 (0.0)	7 (22.6)
sat2-aadA1	1 (5.3)	0 (0.0)	0 (0.0)	1 (3.2)
dfrA1-sat2-aadA1	8 (42.1)	0 (0.0)	0 (0.0)	8 (25.8)

consistent with previous studies. In 2001, Goldstein et al. detected 66%, 14%, and 11% of positive isolates for class 1, class 2, and both integrons, respectively, in 100 avian pathogenic *E. coli* from poultry. More recently, Ahmed et al. (2013) found class 1 and class 2 integrons in 46.6% (34/73 isolates) and 9.6% (7/73 isolates) of APEC isolated from broilers, respectively, whereas Povilonis et al. (2010) detected a lower positivity, i.e., 18.5% (5/27 isolates) and 3.7% (1/27 isolates) for class 1 and class 2 integrons, respectively. In laying hens, class 1 but not class 2 integrons were found in 21.6% of isolates (21/97) by Oosterik et al. (2014). In a previous study carried out in Italy, Piccirillo et al. (2014) detected the presence of *intI1* and *intI2* genes in 6 (12.5%) and 9 (18.7%) isolates, respectively, among 48 APEC from commercial turkeys, with 1 isolate carrying both classes of integrons. Other studies investigating only on the presence of class 1 integrons have been carried out in the USA (Bass et al., 1999), Hungary (Nogrady et al., 2006), Korea (Kim et al., 2007), and China (Yang et al., 2004), where positivity of 63% (63/100 isolates), 41% (11/27 isolates), 39.6% (40/101 isolates), and 59% (42/71 isolates) of isolates, respectively, was documented.

The most common gene cassettes identified in class 1 integron-positive isolates belonged to the *aadA* (90/149 isolates, 60.4%) and the *dfrA* (50/149 isolates, 33.6%) gene families, with 5 and 4 different variants, respectively (Table 1). Similarly, most of the class 2 integron-positive isolates carried the *aadA* (21/31 isolates, 67.7%) and the *dfrA* (20/31 isolates, 64.5%) genes, both with 1 variant (*aadA1*). The *sat* (5/149 isolates, 3.4%), the *estX* (10/149 isolates, 6.7%) and the *orfF* (1/149 isolate, 0.7%) genes were also found in *intI*1-positive isolates, and the *sat* gene in *intI*2-positive isolates (10/31 isolates, 32.3%). Many gene cassettes able to confer resistance to several antimicrobial classes have been described in class 1 and class 2 integrons, but those more frequently found are *dfrA*, *aadA,* and, for class 2 integrons, *sat* (Partridge et al., 2009; Hall, 2012). The reason for the presence of a limited number and type of gene cassettes in integrons is unclear; however, it could be due to the reduced ability of integrase to promote their insertion in the recombination site (Van Essen-Zandbergen et al., 2007), to the frequent location of integrons in mobile genetic elements, such as transposons and/or plasmids, promoting their dissemination in the environment (Vinué et al., 2008), or to the exchange of gene cassettes under conditions of antimicrobial selective pressure (Kim et al., 2007). In the present study, 30.9% and 32.3% of class 1 and class 2 integron-positive isolates to the real-time PCR screening, respectively, could not be amplified using endpoint PCR to sequence the variable region. This finding may be due to an empty variable region or to variations in the conserved region targeted by the primers used to amplify the gene cassettes, as previously documented (Goldstein et al., 2001; Povilonis et al., 2010; Dessie et al., 2013).

Fifteen and 4 different gene cassette arrangements were identified among class 1 and 2 integrons, respectively (Table 1). In class 1 integrons, the most common arrays comprised the *aadA1* gene alone (21.5%), followed by the combinations *aadA1-dfrA1* (18.1%) and *dfrA1-aadA1* (12.1%). In class 2 integrons, the combinations *dfrA1-sat2-aadA1* (25.8%) and *dfrA1-aadA1* (22.6%) were the most frequently identified. The distribution of gene cassettes among turkey, chicken, and layer hen isolates was similar (Table 1), except for class 1 integron-positive strains from laying hens, which

contained predominantly the *estX* gene (53.3%). The genes identified in class 1 and class 2 integrons in this study are known to encode for an adenyltransferase conferring resistance to streptomycin and spectinomycin (*aadA*) and for a dihydrofolate reductase conferring resistance to trimethoprim (*dfr*) (Partridge et al., 2009). The *estX* gene encodes for a hypothetical esterase or hydrolase and some authors (Partridge, 2005) suggest a similarity with the *sat* gene, responsible for resistance to streptothricin; the *orfF* gene encodes for a protein whose function is still unknown (Partridge et al., 2009). In this study we describe the presence of the *estX* gene in class 1 integrons, which is usually reported in class 2 integrons (Partridge, 2005; Partridge et al., 2009; Hall, 2012). The number and the type of gene cassettes found in both class 1 and class 2 integrons in the present study are in agreement with that reported previously in APEC isolates from poultry. Class 1 integrons harboring only the *aadA1* gene have been reported by Bass et al. (1999) and Nogrady et al. (2006), as well as the *aadA1* gene combined with the *dfrA1* (Cocchi et al., 2007). A high variability (up to 6 distinct arrays) among gene cassettes contained in class 1 integron positive APEC from chickens has been documented by Yang et al. (2004) and Kim et al. (2007), with a predominance of the *dhfrI-aadA1* and the *aadA1* arrangements. Ahmed et al. (2013) identified 10 and 3 different gene cassette arrays in class 1 and class 2 integrons, respectively, in 34 APEC strains isolated from broilers, with several variants of the *aadA* and the *dfrA* genes. Besides the *aadA1* and the *dfrA1* gene cassettes in both class 1 and class 2 integrons, Povilonis et al. (2010) described an atypical gene cassette array, the *aacA4-catB3-dfrA1-orfX*, in class 1 integrons. In a previous study (Piccirillo et al., 2014), three different cassette arrays (*i.e. dfrA1-aadA1*, *dfrA1-sat2-aadA1*, *estX-aadA1*) were identified in class 1 and class 2 integron-positive APEC from turkeys.

All APEC were tested for resistance against some members of the aminoglycoside and sulphonamide classes (Table 2). High levels of resistance were observed for triple sulphonamides (79.3%), streptomycin (67.2%), and sulfamethoxazole combined with trimethoprim (62.2%). In contrast, a low number of strains showed resistance to gentamycin (16.7%), kanamycin (14.7%), and apramycin (3.0%). Resistance phenotypes of turkey, broiler, and layer hen isolates are detailed in Table 2. The percentages of isolates resistant to apramycin (2.5%, 4.5%, and 0.0%; $P = 0.37$), kanamycin (11.4%, 20.0%, and 12.9%; $P = 0.14$) and triple sulphonamides (80.4%, 76.4%, and 83.9%; $P = 0.58$) were similar among turkeys, broilers, and layer hens, respectively. For streptomycin (75.3%, 62.7%, and 41.9%; $P < 0.001$), gentamycin (18.4%, 18.2%, and 3.2%; $P < 0.10$) and sulfamethoxazole combined with trimethoprim (67.1%, 63.6%, and 32.3%; $P < 0.01$), however, significant differences were detected among isolates from turkeys, broilers, and layer hens, respectively. A possible association between

Table 2. Resistance against aminoglycosides and potentiated sulphonamides of APEC isolates from poultry.

Antimicrobial	Turkeys			Broilers			Layer hens			Total		
	Percentage of resistance (no.)			Percentage of resistance (no.)			Percentage of resistance (no.)			Percentage of resistance (no.)		
	S	I	R	S	I	R	S	I	R	S	I	R
Apramycin	96.2 (152)	1.3 (2)	2.5 (4)	95.5 (105)	0.0 (0)	4.5 (5)	100.0 (31)	0.0 (0)	0.0 (0)	96.3 (288)	0.7 (2)	3.0 (9)
Gentamycin	80.4 (127)	1.3 (2)	18.4 (29)	73.6 (81)	8.2 (9)	18.2 (20)	93.5 (29)	3.2 (1)	3.2 (1)	237 (79.3)	4.0 (12)	16.7 (50)
Streptomycin	12.7 (20)	12.0 (19)	75.3 (119)	14.5 (16)	22.7 (25)	62.7 (69)	32.3 (10)	25.8 (8)	41.9 (13)	15.7 (46)	17.4 (52)	67.2 (201)
Kanamycin	50.6 (80)	38.0 (60)	11.4 (18)	43.6 (48)	36.4 (40)	20.0 (22)	58.1 (18)	29.0 (9)	12.9 (4)	48.8 (146)	36.5 (109)	14.7 (44)
Triple sulphonamides	14.6 (23)	5.1 (8)	80.4 (127)	22.7 (25)	0.9 (1)	76.4 (84)	16.1 (5)	0.0 (0)	83.9 (26)	17.7 (53)	3.0 (9)	79.3 (237)
Sulfamethoxazole + trimethoprim	31.6 (50)	1.3 (2)	67.1 (106)	36.4 (40)	0.0 (0)	63.6 (70)	67.7 (21)	0.0 (0)	32.3 (10)	37.1 (111)	0.7 (2)	62.2 (186)

S = sensitive; I = intermediate; R = Resistant.

resistance to these antimicrobials and the integron presence (Table 3) as well as the presence of the *aadA*, *dfrA,* and *sat* genes (Table 4) was evaluated. In turkeys, the occurrence of integrons was significantly higher in isolates resistant to aminoglycosides (57.4% vs. 30.6%, $P < 0.001$), particularly in those resistant to gentamycin (75.9 vs. 45.7%, $P < 0.01$) and streptomycin (57.1% vs. 33.3%, $P < 0.01$). In broilers, the higher occurrence of integrons in isolates resistant to aminoglycosides compared to susceptible ones (76.4% vs. 39.5%, $P < 0.001$) depended on the differences between resistant and susceptible isolates to streptomycin (78.3% vs. 39.0%, $P < 0.001$). In layer hens, no significant difference in the occurrence of integrons in isolates resistant and susceptible to aminoglycosides was noticed. In turkeys and broilers, the occurrence of integrons was significantly higher in isolates resistant to sulphonamides, including triple sulphonamides and sulfamethoxazole combined with trimethoprim, than in those susceptible (sulphonamides: 58.0% vs. 18.5%, $P < 0.01$ in turkeys; 76.5% vs. 20.0%, $P < 0.001$ in broilers). Considering aminoglycosides and streptomycin, the occurrence of the gene *aadA* was significantly higher in resistant isolates rather than in susceptible ones, both in turkeys (aminoglycosides: 43.4% vs. 16.7%, $P < 0.01$; streptomycin: 43.7% vs. 18%, $P < 0.01$) and broilers (aminoglycosides: 47.2% vs. 26.3%, $P = 0.03$; streptomycin: 47.8% vs. 26.8%, $P = 0.03$) (Table 4). The occurrence of the *sat* gene was higher in isolates resistant to aminoglycosides and streptomycin compared to susceptible ones only when turkey, broiler, and layer hen isolates were considered together (aminoglycosides: 6.7% vs. 1.1%; $P = 0.04$; streptomycin: 7.0% vs. 1.0%, $P = 0.03$). Considering sulphonamides and sulfamethoxazole combined with trimethoprim, the occurrence of the *dfrA* gene was significantly higher in resistant isolates rather than in susceptible ones, both in turkeys (sulphonamides: 29.8% vs. 3.7%, $P < 0.01$; sulfamethoxazole + trimethoprim: 36.8% vs. 1.9%, $P < 0.001$) and in broilers (sulphonamides: 31.8% vs. 0.0%, $P = 0.001$; sulfamethoxazole + trimethoprim: 37.1% vs. 2.5%, $P < 0.001$) (Table 4). No significant difference in the occurrence of the *aadA*, *dfrA,* and *sat* genes in resistant and susceptible APEC from layer hens was detected (Table 4). In this case, an increase in the dataset size would be advisable.

Results of our study suggest that integron-borne aminoglycoside and sulphonamide resistance is an important mechanism underlying the resistance to these antimicrobials. Nevertheless, some isolates not harboring integrons showed to be resistant to aminoglycosides and sulphonamides, as well as some isolates susceptible to these antimicrobials harbored integrons. This finding may be explained by other molecular resistance mechanisms and by the lack of expression of gene cassettes contained in integrons, as described by other authors (Bass et al., 1999; Lanz et al., 2003; Nogrady et al., 2006; Kim et al., 2007). However, further work will be carried out to support this observation, as well as to

Table 3. Occurrence of class 1 and 2 integrons in resistant and susceptible APEC isolates from poultry.

Antimicrobial	Turkeys			Broilers			Layer hens			Total		
	Resistant no. (%)	Susceptible no. (%)	Prob.	Resistant no. (%)	Susceptible no. (%)	Prob.	Resistant no. (%)	Susceptible no. (%)	Prob.	Resistant no. (%)	Susceptible no. (%)	Prob.
Aminoglycosides	70/122 (57.4)	11/36 (30.6)	<0.01	55/72 (76.4)	15/38 (39.5)	<0.001	8/15 (53.3)	8/16 (50.0)	0.85	133/209 (63.6)	34/90 (37.8)	<0.001
Apramycin	2/4 (50.0)	79/154 (51.3)	0.96	3/5 (60)	67/105 (63.8)	0.86	0/0 (0.0)	16/31 (51.6)	n.e.*	5/9 (55.6)	162/290 (55.9)	0.99
Gentamycin	22/29 (75.9)	59/129 (45.7)	<0.01	15/20 (75.0)	55/90 (61.1)	0.24	0/1 (0.0)	16/30 (53.3)	0.29	37/50 (74.0)	130/249 (52.2)	<0.05
Streptomycin	68/119 (57.1)	13/39 (33.3)	<0.01	54/69 (78.3)	16/41 (39.0)	<0.001	7/13 (53.9)	9/18 (50.0)	0.83	129/201 (64.2)	38/98 (38.8)	<0.001
Kanamycin	9/18 (50.0)	72/140 (51.4)	0.91	17/22 (77.3%)	53/88 (60.2)	0.14	2/4 (50.0)	14 (51.9)	0.94	28/44 (63.6)	139/255 (54.5)	0.26
Sulfonamides	76/131 (58.0)	5/27 (18.5)	<0.001	65/85 (76.5)	5/25 (20.0)	<0.001	14/26 (53.9)	2/5 (40.0)	0.57	155/242 (64.1)	12/57 (21.1)	<0.001
Triple sulfonamides	76/127 (59.8)	5/31 (16.1)	<0.001	64/84 (76.2)	6/26 (23.1)	<0.001	14/26 (53.9)	2/5 (40.0)	0.57	154/237 (65.0)	13/62 (21.0)	<0.001
Sulfamethoxazole + Trimethoprim	67/106 (63.2)	14/52 (26.9)	<0.001	59/70 (84.3)	11/40 (27.5)	<0.001	6/10 (60.0)	10/21 (47.6)	0.52	132/186 (71.0)	35/113 (31.0)	<0.001

*n.e. = not estimable.

Table 4. Occurrence of *aadA*, *dfrA*, and *sat* genes in resistant and susceptible APEC isolates from poultry.

	Turkeys			Broilers			Layer hens			Total		
Antimicrobial	Resistant no. (%)	Susceptible no. (%)	Prob.	Resistant no. (%)	Susceptible no. (%)	Prob.	Resistant no. (%)	Susceptible no. (%)	Prob.	Resistant no. (%)	Susceptible no. (%)	Prob.
Aminoglycosides												
aadA	53/122 (43.4)	6/36 (16.7)	<0.01	34/72 (47.2)	10/38 (26.3)	0.03	1/15 (6.7)	2/16 (12.5)	0.58	88/209 (42.1)	18/90 (20.0)	<0.001
sat	9/122 (7.4)	0/36 (0.0)	0.09	5/72 (6.9)	1/38 (2.6)	0.34	0/15 (0.0)	0/16 (0.0)	n.e.*	14/209 (6.7)	1/90 (1.1)	0.04
Streptomycin												
aadA	52/119 (43.7)	7/39 (18.0)	<0.01	33/69 (47.8)	11/41 (26.8)	0.03	1/13 (7.7)	2/18 (11.1)	0.75	86/201 (42.8)	20/98 (20.4)	<0.001
sat	9/119 (7.6)	0/39 (0.0)	0.08	5/69 (7.3)	1/41 (2.4)	0.28	0/13 (0.0)	0/18 (0.0)	n.e.*	14/201 (7.0)	1/98 (1.0)	0.03
Sulfonamides												
dfrA	39/131 (29.8)	1/27 (3.7)	<0.01	27/85 (31.8)	0/25 (0.0)	0.001	1/26 (3.9)	0/5 (0.0)	0.66	67/242 (27.7)	1/57 (1.8)	<0.001
Sulfamethoxazole + Trimethoprim												
dfrA	39/106 (36.8)	1/52 (1.9)	<0.001	26/70 (37.1)	1/40 (2.5)	<0.001	1/10 (10.0)	0/21 (0.0)	0.14	66/186 (35.5)	2/113 (1.8)	<0.001

*n.e. = not estimable.

establish a chromosomal or plasmidic location of integrons.

In conclusion, studies focusing on the occurrence of integrons, involved in the transfer of resistance genes, can provide useful information not only for a better understanding of the resistance mechanisms, but also for the development of policies for use of antimicrobials. A widespread dissemination of class 1 and class 2 integrons and a significant association between the presence of integrons and resistance against aminoglycosides and sulphonamides were found, suggesting that integron-borne aminoglycoside and sulfonamide resistance may represent a serious concern in APEC from poultry in Italy. These antimicrobials are among the most commonly used in the poultry industry and they should be used parsimoniously in order to preserve the efficacy of the therapeutic intervention. Monitoring the antimicrobial resistance and the underlying genetic mechanisms in poultry bacteria is needed both for animal and human health.

ACKNOWLEDGMENTS

The present study was supported by grants from the University of Padua (CPDA095771/09). The authors wish to thank Dr Marianne Sunde (National Veterinary Institute, Norway), Dr Stefan Schwarz (Friedrich-Loeffler Institut, Germany), Dr Beatriz Guerra-Roman (Federal Institute for Risk Assessment, Germany), and Dr Dik Mevius (Centraal Veterinair Instituut, The Netherlands) who kindly provided integron positive controls used in this study.

REFERENCES

Ahmed, A. M., T. Shimamoto, and T. Shimamoto. 2013. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int. J. Med. Microbiol. 303:475–483.

Altalhi, A. D., Y. A. Gherbawy, and S. A. Hassan. 2010. Antibiotic resistance in *Escherichia coli* isolated from retail raw chicken meat in Taif, Saudi Arabia. Foodborne Pathog. Dis. 7:281–285.

Bass, L., C. A. Liebert, M. D. Lee, A. O. Summers, D. G. White, S. G. Thayer, and J. J. Maurer. 1999. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. Antimicrob. Agents Chemother. 43:2925–2929.

Clinical and Laboratory Standards Institute. 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard M31-A3. CLSI, Wayne, PA.

Cocchi, S., E. Grasselli, M. Gutacker, C. Benagli, M. Convert, and J. C. Piffaretti. 2007. Distribution and characterization of integrons in *Escherichia coli* strains of animal and human origin. FEMS Immunol. Med. Microbiol. 50:126–132.

Dessie, H. K., D. H. Bae, and Y. J. Lee. 2013. Characterization of integrons and their cassettes in *Escherichia coli* and *Salmonella* isolates from poultry in Korea. Poult Sci. 92:3036–3043.

Gyles, C. L. 2008. Antimicrobial resistance in selected bacteria from poultry. Anim. Health Res. Rev. 9:149–158.

Goldstein, C., M. D. Lee, S. Sanchez, C. Hudson, B. Phillips, B. Register, M. Grady, C. Liebert, A. O. Summers, D. G. White, and J. J. Maurer. 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob. Agents Chemother. 45:723–726.

Hall, R. M. 2012. Integrons and gene cassettes: Hotspots of diversity in bacterial genomes. Ann. N. Y. Acad. Sci. 1267:71–78.

Hammerum, A. M., and O. E. Heuer. 2009. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. Clin. Infect. Dis. 48:916–921.

Kaper, J. B., J. P. Nataro, and H. L. Mobley. 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. 2:123–140.

Kim, T. E., Y. W. Jeong, S. H. Cho, S. J. Kim, and H. J. Kwon. 2007. Chronological study of antibiotic resistances and their relevant genes in Korean avian pathogenic *Escherichia coli* isolates. J. Clin. Microbiol. 45:3309–3315.

Landers, T. F., B. Cohen, T. E. Wittum, and E. L. Larson. 2012. A review of antibiotic use in food animals: Perspective, policy, and potential. Public Health Rep. 127:4–22.

Lanz, R., P. Kuhnert, and P. Boerlin. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91:73–84.

Lévesque, C., L. Piché, C. Larose, and P. H. Roy. 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. 39:185–191.

Lutful Kabir, S. M. 2010. Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. J. Environ. Res. Public Health 7:89–114.

Nebbia, P., C. Tramuta, M. Giammarino, C. Ambrogi, S. Salvarani, A. Marotta, and P. Robino. 2008. Antimicrobial resistance, class 1 and 2 integrons and gene cassettes in avian *Escherichia coli*. Ital. J. Anim. Sci. 7:391–395.

Nogrady, N., J. P. Paszti, H. Piko, and B. Nagy. 2006. Class 1 integrons and their conjugal transfer with and without virulence-associated genes in extra-intestinal and intestinal *Escherichia coli* of poultry. Avian Pathol. 35:349–356.

Nolan, L. K., H. J. Barnes, J. P. Vaillancourt, T. Abdul-Aziz, and C. M. Logue. 2013. Colibacillosis. Pages 751–805 in Diseases of Poultry. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, and V. Nair, eds. Iowa State Press, Ames, IA, USA.

Oosterik, L. H., L. Peeters, I. Mutuku, B. M. Goddeeris, and P. Butaye. 2014. Susceptibility of avian pathogenic *Escherichia coli* from laying hens in Belgium to antibiotics and disinfectants and integron prevalence. Avian Dis. 58:271–278.

Partridge, S. R. 2005. Correctly identifying the streptothricin resistance gene cassette. J. Clin. Microbiol. 43:4298–4300.

Partridge, S. R., G. Tsafnat, E. Coiera, and J. R. Iredell. 2009. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol. Rev. 33:757–784.

Piccirillo, A., G. Dotto, C. Salata, and M. Giacomelli. 2013. Absence of class 1 and class 2 integrons among *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in Italy. J. Antimicrob. Chemother. 68:2683–2685.

Piccirillo, A., D. Giovanardi, G. Dotto, G. Grilli, C. Montesissa, C. Boldrin, C. Salata, and M. Giacomelli. 2014. Antimicrobial resistance and class 1 and 2 integrons in *Escherichia coli* from meat turkeys in Northern Italy. Avian Pathol. 14: 1–10.

Povilonis, J., V. Seputiene, M. Ruzauskas, R. Siugzdiniene, M. Virgailis, A. Pavilonis, and E. Suziedeliene. 2010. Transferable class 1 and 2 integrons in *Escherichia coli* and *Salmonella enterica* isolates of human and animal origin in Lithuania. Foodborne Pathog. Dis. 7: 1185–1192.

Saenz, Y., L. Brinãs, E. Domínguez, J. Ruiz, M. Zarazaga, J. Vila, and C. Torres. 2004. Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. Antimicrob. Agents Chemother. 48: 3996–4001.

Soufi, L., Y. Saenz, L. Vinuè, M. S. Abbassi, E. Ruiz, M. Zaragaza, A. B. Hassen, S. Hammami, and C. Torres. 2011. *Escherichia coli* of poultry food origin as reservoir of sulphonamide resistance genes and integrons. Int. J. Food Microbiol. 144: 497–502.

Trobos, M., L. Jacobsen, K. E. Olsen, N. Frimondt-Moller, A. M. Hammerum, K. Pedersen, Y. Agerso, L. J. Porsbo, and J. E. Olsen. 2008. Prevalence of sulphonamide resistance and class 1 integron genes in *Escherichia coli* isolates obtained from broilers, broiler meat, healthy humans and urinary infections in Denmark. Int. J. Antimicrob. Agents 32:367–369.

Van Essen-Zandbergen, A., H. Smith, K. Veldman, and D. Mevius. 2007. Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. J. Antimicrob. Chemother. 59:746–750.

Vinué, L., Y. Sáenz, S. Somalo, E. Escudero, M. A. Moreno, F. Ruiz-Larrea, and C. Torres. 2008. Prevalence and diversity of integrons and associated resistance genes in faecal *Escherichia coli* isolates of healthy humans in Spain. J. Antimicrob. Chemother. 62:934–937.

White, P. A., C. J. McIver, and W. D. Rawlinson. 2001. Integrons and gene cassettes in the *Enterobacteriaceae*. Antimicrob. Agents Chemother. 45:2658–2661.

Yang, H., S. Chen, D. G. White, S. Zhao, P. McDermott, R. Walker, and J. Meng. 2004. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J. Clin. Microbiol. 42:3483–3489.