Antimicrobial resistance, class 1 and 2 integrons and gene cassettes in avian Escherichia coli

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ABSTRACT

Seventy-four Escherichia coli isolates were collected from domestic, synanthropic free living birds as well as wild and exotic birds, all living in captivity. Antimicrobial susceptibility was tested against a panel of 9 antibiotics, and presence of integrons (Class 1 and Class 2) and gene cassettes was analysed by PCR and sequencing, respectively. Twenty-eight isolates proved positive for Class 1 integrons and 19 for Class 2. Gene cassette arrangements were determined in 23 integron-positive isolates, which harboured one (aadA1) two (dfrA1-aadA1) or three (dfrA7-dfrA1-aadA1, dfrA1-sat1-aadA1) cassettes in their variable region. E. coli multiresistance to antimicrobials was observed in all groups examined, in particular domestic and synanthropic birds showed resistance to at least 4 antibiotics. A large number of isolates from domestic and synanthropic birds proved to be Class 1 integron-positive, but unexpectedly, we observed many Class 2 integrons, usually considered less frequent.

Key words: Birds, E. coli, Antimicrobial resistance, Integrons.

RIASSUNTO

RESISTENZA ANTIMICROBICA, INTEGRONI DI CLASSE 1 E 2 E GENI CASSETTA IN CEPPI DI ESCHERICHIA COLI DI ORIGINE AVIARE

Sono stati eseguiti prelievi fecali da volatili domestici di allevamenti rurali, uccelli sinantropi catturati e specie selvatiche ed esotiche ospitate in un centro per selvatici. Sono stati esaminati 74 ceppi di Escherichia coli. Su tutti è stato eseguito l’antibiogramma e sono stati ricercati integroni di Classe 1 e Classe 2 (PCR) e geni cassetta (PCR e sequenziamento). Ventotto isolati sono risultati portatori di integroni di Classe 1 e 19 di Classe 2, in 23 di questi sono stati trovati geni cassetta. In tutti i gruppi esaminati è stata osservata multiresistenza antibiotica, particolarmente frequente nei pollame e nei sinantropi. È stato osservato un elevato numero di isolati portatori di integroni di Classe 1, ma inaspettatamente anche di ceppi con integroni di Classe 2, solitamente considerati poco frequenti.

Parole chiave: Uccelli, E. coli, Resistenza antimicrobica, Integroni.
Introduction

Antibiotic resistance (AR) is a major public health problem all over the world. Surveillance programmes concerning AR in bacteria from various animal species target overall commensal bacteria isolated from healthy domestic animals (indicator bacteria), while less attention has been paid to bacteria isolated from wild hosts. Little information is to be found regarding genetic resistance in bacteria of animal origin, particularly integron frequency. The latter are responsible for the transmission of the AR genes and seem to play a major role in the spread of antibiotic resistance in Gram negative bacteria. Infectious agents subsist in the environment even without selective pressure and internal AR genes even survive against discontinued drugs.

The aim of study was to investigate the occurrence of antimicrobial resistance, integrons (Class 1 and Class 2) and gene cassettes among E. coli isolated from domestic, synanthropic free-living birds as well as wild and exotic birds living in captivity.

Material and methods

During 2006-2007 cloacae swabs were collected from domestic birds (Gallus gallus var. domestica), synanthropic free-living birds (Corvus corone cornix), wild and exotic birds (Anas platyrhynchos, Alopochen aegyptiacus). Samples were obtained from two localities: a rural area in Piedmont (poultry and free-living animals) and a wildlife centre in Tuscany, where birds live in captivity.

Seventy-four E. coli isolates were cultured on MacConkey agar (Oxoid) and confirmation of species was performed using a commercial identification system (BBL, Becton Dickinson). Once identified, 1 strain of E. coli was taken from each sample. The NCCLS interpretative criteria was used by the disc diffusion method to test all isolates for susceptibility to the following antimicrobial agents: amoxycillin-clavulanic acid, streptomycin, gentamicin, amikacin, tetracycline, sulphamethoxazole, trimethoprim, enrofloxacin, and cefoperazone. For the purpose of our study, intermediate strains were considered as resistant.

Boiled suspensions of bacterial cells were used as templates in PCR reactions to screen integron-associated structures as follows: all isolates were tested for the presence of the integrase genes of Class 1 (IntI1) and Class 2 (IntI2) integrons by PCR (Kern et al., 2002; Orman et al., 2002). Strains containing the IntI1 or IntI2 genes underwent PCRs for amplification of the variable regions of Class 1 and Class 2 integrons (Lévesque et al., 1995; White et al., 2001).

Amplicons generated with primers for amplification of the variable regions were sequenced in order to determine the contents of inserted cassettes. The sequences obtained were analyzed with software CHROMAS 2.0 (Technelysium, Helensvale, Australia) and underwent BLAST analysis (BLAST®, NCBI, USA, Altschul et al., 1997).

Results and discussion

Phenotypic resistance frequencies for each bird group is given in Table 1. Resistance to one or more antibiotics was observed in 97.3% of isolates whereas 2 isolates from wild and exotic birds showed sensitivity at all (Table 2). E. coli multiresistance to antimicrobials was generally observed in all groups examined, in particular domestic and synanthropic birds showed resistance to at least 4 antibiotics. Streptomycin, detected in 64 (86.5%) isolates proved to be the prevailing type of resistance.

Forty-seven isolates (63.5%) carried integrase gene (Table 3), of which 13 (27.7%)
### Table 1. Percentages of resistant *E. coli* strains isolated from faecal swabs of birds.

| Antimicrobial agents       | Resistant strains (domestic birds) | Resistant strains (synanthropic birds) | Resistant strains (wild/exotic birds) |
|---------------------------|------------------------------------|----------------------------------------|--------------------------------------|
| Gentamicin                | 41.6                               | 85.7                                   | 44.8                                 |
| Cefoperazone              | 62.5                               | 90.5                                   | 20.7                                 |
| Amoxicillin-Clav. ac.     | 4.7                                | 23.8                                   | 27.6                                 |
| Tetracycline              | 79.2                               | 90.5                                   | 51.7                                 |
| Sulphmethoxazole          | 62.5                               | 57.1                                   | 13.8                                 |
| Enrofloxacin              | 25                                 | 95.2                                   | 13.8                                 |
| Streptomycin              | 95.8                               | 95.2                                   | 72.4                                 |
| Trimethoprim              | 62.5                               | 52.4                                   | 6.9                                  |
| Amikacin                  | 41.7                               | 76.2                                   | 62.1                                 |

### Table 2. *E. coli* in domestic, synanthropic and wild avian species: prevalence of Antibiotic Resistance (AR).

| N. AR | 24 strains from domestic birds | 21 strains from synanthropic birds | 29 strains from wild/exotic birds |
|-------|-------------------------------|-----------------------------------|----------------------------------|
| 0     | 0                             | 0                                 | 2 (6.9%)                         |
| 1     | 0                             | 0                                 | 3 (10.3%)                        |
| 2-3   | 1 (4.2%)                      | 0                                 | 16 (55.2%)                       |
| 4 or more | 23 (95.8%)               | 21 (100.0%)                       | 8 (27.6%)                        |

### Table 3. Characterization of integrons and gene cassettes in the integron-positive *E. coli* isolates.

| Integrons and gene cassettes | N. strains | Domestic birds N. (%) | Synanthropic birds N. (%) | Wild/exotic birds N. (%) |
|------------------------------|------------|------------------------|---------------------------|--------------------------|
| Class 1 integrons            | 28         | 15 (62.5)              | 12 (41.4)                 | 1 (4.8)                  |
| aadA1                        | 1          | -                      | 1 (4.8)                   | -                        |
| dfrA1-aadA1                  | 20         | 12 (50.0)              | 7 (33.3)                  | 1 (4.8)                  |
| dfrA7-dfrA1-aadA1            | 1          | -                      | 1 (4.8)                   | -                        |
| undetermined                 | 6          | 3 (12.5)               | 3 (14.3)                  | -                        |
| Class 2 integrons            | 19         | 4 (16.7)               | 14 (48.3)                 | 1 (4.8)                  |
| dfrA1-sat1-aadA1             | 1          | -                      | -                         | 1 (4.8)                  |
| undetermined                 | 18         | 4 (16.7)               | 14 (48.3)                 | -                        |
carried IntI1, 4 (8.5%) carried IntI2 and 15 (31.9%) carried both IntI1 and IntI2. Each variable region was sequenced and in Class 1 integrons, the sequencing revealed different gene cassettes, alone or in combination, which encoded resistance to trimethoprim (dfrA1 and dfrA7) and aminoglycosides (aadA1). Only a type of gene cassette, dfrA1-sat1-aadA1, was detected in one Class 2 integron. A large number of isolates from domestic and synantropic bird resulted carriers of Class 1 integrons. Unexpectedly we observed many Class 2 integrons, usually considered less frequent. Only 2 isolates from wild and exotic birds taken integrons (the one IntI1, the other IntI2) both carried of gene cassettes (Table 3).

The percentage of avian E. coli carrying integrons Class 1 is in line with literature regarding isolates of human and animal origin (Goldstein et al., 2001; White et al., 2001; Mathai et al., 2004). On the contrary we observed percentage of Class 2 integrons slightly higher than White et al. (2001) and Mathai et al. (2004), even if formerly Goldstein (2001) found more integrons Class 2 in birds and in cattle, than in other species.

Some integrons resulted undetermined, that is lacking in integrated gene cassettes. Their presence is very interesting because they are available for a future insertion of a gene cassette.

Most isolates carrying the variable region, possessed aadA gene alone or with other gene cassettes. Probably this gene, which encodes resistance to spectinomycin/streptomycin and trimethoprim, is one of the first cassettes to be acquired by the integrons, maybe for the selective pressure related to the use of these antibiotics.

**Conclusions**

Our results show that integrons and gene cassettes are frequent among all examined bird categories, even if with different percentages.

Among synanthropic birds, we investigated the presence of E. coli in crow (Corvus corone cornix), an omnivorous species that is among the most probable candidates for transmission of microbial agents at rural and urban areas. This animal probably reflect a very serious condition due to the contamination of his food and water sources with bacteria of animal and human origin. Although crows live in areas where the presence of antibiotics is seemingly rare they have an important role in maintaining E. coli multiresistant strains. Instead, the wild and exotic bird species examined are at times treated with antimicrobials if necessary, and not only do they show high sensitivity frequency but they also have fewer integrons; the latter being an index of a genetic “predisposition” to resistance.

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