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Influence of Housing Systems on Microbial Load and Antimicrobial Resistance Patterns of *Escherichia coli* Isolates from Eggs Produced for Human Consumption

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ABSTRACT

Microbial counts (aerobic bacteria, psychrotrophs, *Enterobacteriaceae,* coliforms, *Pseudomonas* spp., *Enterococcus* spp., *Staphylococcus* spp., and molds and yeasts) were obtained for the shells of 240 table eggs in northwestern Spain. Eggs from six sources (40 samples in each) were analyzed: chicken eggs from five different housing systems (conventional battery cages, barn, free range, organic, and domestic breeding) and quail eggs (cages). A total of 120 *Escherichia coli* strains (20 from each source) were tested by the disk diffusion method for resistance to 12 antimicrobial drugs of veterinary and human health significance. Aerobic plate counts ranged from 1.96 $\pm$ 1.0 (barn) to 3.69 $\pm$ 0.7 (domestic) log CFU/cm^2. Counts for most microbial groups differed significantly between sources. Eggs from domestic production had the highest contamination loads ($P < 0.05$) for aerobic bacteria, *Enterococcus* spp., and molds and yeasts and the highest prevalence of *E. coli*. Twenty-three *E. coli* isolates (19.17%) were susceptible to all antimicrobials tested, and 80.83% were resistant to one (22.50%) or more (58.33%) antimicrobials. The housing system had a significant influence ($P < 0.05$) on the average resistance per strain, with the highest resistance in conventional cage (2.85) and barn (3.10) systems followed by free range (1.55) and quail (1.95). Eggs from organic (1.00) and domestic (0.75) production systems had the lowest resistance per strain. The highest prevalence of resistance was observed for the groups of antimicrobials more frequently used on poultry farms. Our results suggest that a relationship exists between the prevalence of antimicrobial resistance in *E. coli* strains and the more frequent use of antimicrobials in conventional (cage, barn, and free range) than in domestic and organic chicken housing systems. Education covering good sanitary practices for handling eggs to avoid cross-contamination or inadequate cooking is needed.

Escherichia coli is one of the common microorganisms of the gastrointestinal tract of animals and human beings. Although most isolates are nonpathogenic and are considered merely indicators of fecal contamination in food, 10 to 15% of *E. coli* strains are opportunistic and pathogenic *(7)*. *E. coli* and *Enterococcus* spp. also are considered indicators of antibiotic resistance *(37)*.

Serious concerns about bacterial resistance to drugs have been increasing at both national and international levels *(9)*. Antimicrobial resistance has been defined as a global pandemic *(18),* one of the major global public health threats, and one of the major health challenges of the 21st century *(40)*. The widespread use (especially overuse or misuse) of antimicrobials in humans and animals often is involved in the emergence, selection, and dissemination of multidrug-resistant bacterial strains. A link between the agricultural use of antibiotics and the emergence of drug-resistant bacterial strains causing human infections has been suggested, and many resistant bacteria have been isolated from food samples in recent years *(8)*. Antibiotic resistance among *Salmonella, Campylobacter, E. coli,* and *Enterococcus* isolates from animals and foodstuffs is monitored in the European Union *(20)*. However, few published studies have included information on antimicrobial resistance in bacteria, particularly *E. coli,* recovered from eggshells *(31)*.

The European Union is the second highest producer of table eggs, following China, with more than 6.5 million tons and an average consumption of 235 eggs per capita *(36)*. In recent years, several different methods and systems for producing eggs have evolved and have been addressed by European Council Directive 1999/74/EC *(22),* Council Regulation (EEC) No 2092/91 *(21),* Council Regulation (EC) No 1804/1999 *(23),* and Commission Regulation (EC) No 2295/2003 *(19)*. These systems differ in how the birds are housed, fed, and managed. Hens can be confined in battery cages, which are small enclosures with welded wire mesh sloping floors. In barn systems, the layers are kept on litter, and the birds have freedom to move around within the poultry house, whereas in free-range systems the layers also have access to an outdoor run. In organic (ecological) production facilities, hens must be free range and must be ranged on organic land. In contrast to conventional production of poultry, where antimicrobial agents are

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TABLE 1. *Culture media and techniques, incubation times, and temperatures used for microbiological analysis*

Microorganism	Culture medium	Culture technique	Incubation	
			Temp (°C)	Time (h)
Aerobic bacteria	Plate count agar	Spread plate (0.1 ml)	30	72
Psychrotrophs	Plate count agar	Pour plate (1 ml)	7	240
Enterobacteriaceae	Violet red bile glucose agar	Pour plate (1 ml) (plates were overlaid)	35	24
Fecal coliforms	Violet red bile agar	Pour plate (1 ml) (plates were overlaid)	44	24
Pseudomonas spp.	*Pseudomonas* agar with cephaloridine, fucidin, and cetrimide	Spread plate (0.1 ml)	25	24
Enterococcus spp.	Kanamycin aesculin azide agar	Pour plate (1 ml)	42	24
Staphylococcus spp.	Baird-Parker agar	Spread plate (0.1 ml)	35	48
Yeasts and molds	Oxytetracycline glucose yeast extract agar	Spread plate (0.1 ml)	25	120

widely used for treatment, control, and prevention of diseases, organic production practice involves restricted use of antimicrobials. In addition to being subjected to strict rules regarding the use of antimicrobials, organic birds must be given only organically produced feed and supplements.

Consumption in the European Union by type of eggs is 75% from conventional cages, 14% from barns, 9% from free range, and 2% from organic production *(36)*. Domestically produced eggs from small family holdings frequently are sold in Spanish traditional local markets. Thus, consumers face a broad range of products at very different prices but without any real information about specific qualities. European Council Directive 1999/74/EC *(22)* imposes a full ban that took effect on 1 January 2012 on the housing of laying birds in conventional battery cages, which are considered poor for poultry welfare.

Because high numbers of microorganisms on the eggshell can increase the risk of microbial penetration of the eggshell, contamination of the egg content, and of cross-contamination of other eggs, the microbial load on eggshells is an issue of concern *(28)*. To date, information on the microbiological contamination of the shells of eggs in northwestern Spain has been unavailable, and no investigations have been undertaken to determine the level of resistance to commonly used antimicrobial agents by bacteria present on table eggs. This study was therefore conducted to determine the microbial loads on the shells of chicken and quail eggs collected at retail outlets in northwestern Spain and to gather information about the antimicrobial resistance of *E. coli* isolates from eggshells. The influence of housing systems (conventional cage, barn, free range, organic, and domestic breeding) on microbial counts and bacterial drug resistance was assessed for chicken eggs.

MATERIALS AND METHODS

Sample collection. Fifty samples of commercial grade A chicken eggs (size L *(19)*) and 10 samples of quail eggs were collected from October 2008 to September 2009 in different supermarkets in the Province of León in northwestern Spain 5 days before their expiration dates. Each sample consisted of 12 eggs from the same batch (one boxed dozen). Quail eggs were from conventional cage systems, and the chicken eggs came from five different housing systems: conventional battery cages, barn, free range, organic, and domestic production. Eggs were collected based on the code numbers stamped on the packages (0 for organic production, 1 for free range, 2 for barn, and 3 for cage systems). Eggs from small family holdings were purchased in traditional local markets within the week in which the eggs were laid. Five brands (two samples for each brand) of quail eggs and chicken eggs were tested for all housing systems. Four eggs were randomly selected and tested individually in each sample. Thus, a total of 240 eggs were studied. On arrival in the laboratory, the eggs were kept at 4 $\pm$ 1°C and analyzed within 24 h of purchase.

Microbial counts. To recover bacteria, individual eggshells (after removal of egg content) were placed in a mortar of sterile porcelain and crushed for 1 min. The contents of the mortar were placed in a plastic bag with 66 ml (chicken eggs) or 22 ml (quail eggs) of buffered peptone water (Oxoid Ltd., Hampshire, UK), and homogenized (Masticator IUL, Barcelona, Spain) for 2 min. Previous experiments revealed that the average surface areas of chicken and quail eggs were 66 and 22 cm^2, respectively. Details of the culture media (all from Oxoid) and incubation parameters used are shown in Table 1. Duplicate culture plates were incubated under aerobic conditions. Eggshell dirt (e.g., feces, dust, egg yolk, egg white, feathers, and blood) was evaluated visually. To detect cracked eggs, eggs were examined visually using candle light.

Isolation and identification of *E. coli*. Once the bacterial counts were determined, one to four colonies on violet red bile agar (VRBA) were selected for each egg sample, transferred onto tryptone soy agar (Oxoid), and incubated under the same time and temperature conditions as used for isolation, to obtain pure cultures. The pure cultures were evaluated preliminarily for their colony and cell morphology, Gram staining, and oxidase and catalase activities. Presumptive *E. coli* strains were confirmed on the basis of the presence of beta-glucuronidase (ability to hydrolyze 4-methylumbelliferyl-beta-D-glucuronide), beta-galactosidase (ability to hydrolyze ortho-nitrophenyl-beta-D-galactopyranoside), and tryptophanase (ability to produce indole from tryptophan) using the *E. coli* test (Liofilchem s.r.l., Teramo, Italy).

Determination of the antimicrobial sensitivity of *E. coli* isolates. A total of 120 isolates (20 from each housing system) were used for antimicrobial susceptibility testing. Isolates were screened for susceptibility to a panel of 12 antibiotics on Mueller-Hinton agar (Oxoid) by a disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) *(13)*. The following disks (Liofilchem) were used: gentamicin (CN; 10 μg), ampicillin-sulbactam (AMS; 20 μg), amoxicillin–clavulanic acid (AUG; 30 μg), piperacillin-tazobactam (TZP; 110 μg), cefotaxime (CTX; 30 μg), sulfamethoxazole-trimethoprim (SXT; 25 μg), chloramphenicol (C; 30 μg), ciprofloxacin (CIP; 5 μg), nalidixic

acid (NA; 30 μg), tetracycline (TE; 30 μg), nitrofurantoin (F; 300 μg), and phosphomycin (FOS; 200 μg). The inhibition zones were measured and scored as sensitive, intermediate, and resistant according to the CLSI guidelines. Isolates of intermediate susceptibility were counted together with the isolates that were resistant sensu stricto *(11)*. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as reference strains for antibiotic disk controls. An isolate was considered multidrug resistant when it was resistant or intermediately resistant to two or more antimicrobials *(10)*.

Statistical analysis. Microbial counts were converted to log CFU per square centimeter. Means and standard deviations were calculated. Data were evaluated with an analysis of variance (ANOVA) and Duncan's multiple range test. An ANOVA was performed for the two variables of microbial group and housing system and their interactions. ANOVAs for all microbial groups also were performed. Hypothesis tests were conducted to determine whether means differed significantly between housing systems. The prevalence of resistant strains and the multiresistance patterns were compared with the two-tailed Fisher's exact and chi-square tests. Student's *t* test was performed to compare the average number of antibiotics to which the strains were resistant. Significance was set at $P < 0.05$. The tests were carried out using the Statistica 6.0 software package (Statsoft Ltd., Tulsa, OK).

RESULTS

Microbial counts. ANOVA of the two variables, microbial group and housing system, revealed a significant influence ($P < 0.01$) of both variables and their interaction. The aerobic plate counts (APCs) were similar for all chicken and quail eggshells ($P > 0.05$) at 2.49 ± 1.1 and 2.59 ± 1.6 log CFU/cm^2, respectively. No difference was found between chicken and quail eggshells for *Enterococcus* spp. (0.49 ± 1.0 and 0.65 ± 0.9 log CFU/cm^2, respectively) and *Staphylococcus* spp. (1.85 ± 1.0 and 1.85 ± 0.7 log CFU/cm^2, respectively). In contrast, chicken eggshells had higher counts ($P < 0.05$) than did quail eggs for psychrotrophs (1.79 ± 0.8 and 1.19 ± 0.8 log CFU/cm^2, respectively), *Enterobacteriaceae* (0.81 ± 1.0 and 0.19 ± 0.5 log CFU/cm^2, respectively), fecal coliforms (0.65 ± 0.9 and 0.16 ± 0.4 log CFU/cm^2, respectively), and *Pseudomonas* spp. (1.89 ± 1.1 and 1.27 ± 0.6 log CFU/cm^2, respectively). Counts for molds and yeasts were higher ($P < 0.05$) on quail eggshells (2.44 ± 1.6 log CFU/cm^2) than chicken eggshells (1.52 ± 1.2 log CFU/cm^2).

The influence of the housing system on microbial counts on chicken eggs is shown in Table 2. APCs, *Enterococcus* spp. counts, and yeast and mold counts were highest ($P < 0.05$) on eggshells from domestic production. The highest microbial counts ($P < 0.05$) for psychrotrophs were found in free-range and domestic eggs, for *Enterobacteriaceae* were found in cage, barn, organic, and domestic eggs, for coliforms were found in barn and domestic eggs, for *Pseudomonas* spp. were found in free-range eggs, and for *S. aureus* were found in cage, barn, and domestic eggs. The percentages of eggs on which each microbial group was detected were 80 to 100% for psychrotrophs, 20 to 75% for *Enterobacteriaceae,* 10 to 75% for fecal coliforms, 25 to 80% for *Enterococcus* spp., and 70 to 100% for yeasts and molds. All eggs were

TABLE 2. *Microbial counts on shells of eggs collected from retail outlets in northwestern Spain*[a]

Microorganism	Chicken eggs					Quail eggs (conventional cage)
	Conventional cage	Barn	Free range	Organic	Domestic production	
Aerobic plate counts	2.34 ± 1.2 AB a (100%)	1.96 ± 1.0 A a (100%)	2.18 ± 0.9 AB a (100%)	2.25 ± 1.1 AB a (100%)	3.69 ± 0.7 C a (100%)	2.59 ± 1.6 B a (100%)
Psychrotrophs	1.54 ± 1.2 AB bc (80%)	1.71 ± 1.1 A ab (100%)	2.19 ± 0.5 C a (100%)	1.41 ± 0.5 AB b (100%)	2.11 ± 0.4 C bc (100%)	1.19 ± 0.8 B b (100%)
Enterobacteriaceae	0.91 ± 1.2 A d (45%)	0.89 ± 0.6 A c (75%)	0.26 ± 0.8 B b (20%)	0.90 ± 1.3 A c (50%)	1.10 ± 0.9 A d (65%)	0.19 ± 0.5 B c (20%)
Fecal coliforms	0.10 ± 0.2 A e (15%)	1.35 ± 1.0 B bc (70%)	0.19 ± 0.8 A b (10%)	0.25 ± 0.5 A d (25%)	1.35 ± 0.9 B de (75%)	0.16 ± 0.5 A c (15%)
Pseudomonas spp.	1.94 ± 1.3 ABC ab (100%)	1.56 ± 0.8 AB ac (100%)	2.39 ± 1.1 C a (100%)	1.49 ± 1.02 AB b (100%)	2.08 ± 1.2 AC bf (100%)	1.27 ± 0.6 B bd (100%)
Enterococcus spp.	0.13 ± 0.4 A e (25%)	0.13 ± 0.3 A d (25%)	0.10 ± 0.4 A b (25%)	0.27 ± 0.4 A d (55%)	1.84 ± 1.4 B be (80%)	0.65 ± 0.9 C bc (60%)
Staphylococcus spp.	2.14 ± 1.0 AB a (100%)	1.94 ± 1.0 AC a (100%)	1.30 ± 0.7 D c (100%)	1.36 ± 0.4 CD b (100%)	2.49 ± 1.2 A cfg (100%)	1.85 ± 0.7 BCD de (100%)
Molds and yeasts	1.02 ± 1.1 A cd (75%)	1.11 ± 1.0 A c (70%)	1.22 ± 0.8 A c (100%)	1.30 ± 0.9 A bc (80%)	2.97 ± 1.0 B g (100%)	2.44 ± 1.7 C ae (80%)

[a] Values are log CFU per square centimeter. Within a row, means with no uppercase letters in common are significantly different ($P < 0.05$). Within a column, means with no lowercase letters in common are significantly different ($P < 0.05$). Values in parentheses are the percentage of eggs contaminated with each microbial group.

TABLE 3. *Sensitive, resistant, and multiresistant patterns in* Escherichia coli *strains isolated from the shells of eggs collected from retail outlets in northwestern Spain*[a]

No. of antimicrobials to which eggs were resistant	Chicken eggs					Quail eggs (conventional cage) ($n = 20$)	Mean ($n = 120$)
	Conventional cage ($n = 20$)	Barn ($n = 20$)	Free range ($n = 20$)	Organic ($n = 20$)	Domestic production ($n = 20$)		
0	1 (5) A a	1 (5) A a	1 (5) A a	6 (30) B a	14 (70) C a	0 (0) A a	23 (19.17) a
1	0 (0) A a	0 (0) A a	9 (45) B b	8 (40) B a	2 (10) AC b	8 (40) B b	27 (22.50) a
≥2	19 (95) A b	19 (95) A b	10 (50) B b	6 (30) BC a	4 (20) C b	12 (60) B b	70 (58.33) b

[a] Values are the number (percentage) of *E. coli* isolates. Within a row, means with no uppercase letters in common are significantly different ($P < 0.05$). Within a column, means with no lowercase letters in common are significantly different ($P < 0.05$).

contaminated with aerobic bacteria, *Pseudomonas* spp., and *S. aureus* (Table 2).

Of the 240 samples tested, 45% were positive for *E. coli,* with a range of 20% (conventional cages) to 85% (domestic production). Barn, free-range, and organic chicken eggs and quail eggs had *E. coli* prevalences of 80, 25, 35, and 25%, respectively.

A total of 42 (17.5%) of 240 eggs analyzed had dirt on the shell. None of the conventional cage, free-range, or organic eggs had dirt, whereas 10, 15, and 80% of barn, quail, and domestic eggs, respectively, had dirt. Intact shells (without cracks) were confirmed for all eggs sampled.

Antimicrobial resistance. A total of 120 *E. coli* strains (20 from each type of housing system) were analyzed. Twenty-three (19.17%) of these isolates were susceptible to all antimicrobials tested, 27 (22.50%) were resistant to one antimicrobial, and 70 (58.33%) were multiresistant (resistant to two or more antimicrobials) (Table 3). Resistance to two (21 strains; 17.50%), three (43 strains; 35.83%), four (4 strains; 3.33%), and five (2 strains; 1.67%) antimicrobials was observed among multiresistant strains. The highest frequency ($P < 0.05$) of multiresistance (95% strains) was observed in isolates from conventional cage and barn systems. The highest frequency ($P < 0.05$) of sensitive strains was detected in eggs from domestic (70%) and organic (30%) production.

The mean number of antimicrobials to which each strain type was resistant differed among housing systems, with the highest ($P < 0.001$) resistance observed in conventional cage (2.85 antimicrobials) and barn (3.10 antimicrobials) systems and the lowest in free-range (1.55 antimicrobials), organic (1.00 antimicrobials), and domestic (0.75 antimicrobials) production. Chicken eggs (all production types) and quail eggs had a similar ($P > 0.05$) mean number of antimicrobials to which each strain type was resistant: 1.85 and 1.95 antimicrobials, respectively.

The highest prevalence of resistance was observed for TE (60.83% of strains tested) and AUG (51.67%), followed by SXT (27.50%), and F and FOS (17.50%). Low prevalence of resistance (from 0.83 to 2.50% of strains) was detected for the rest of antimicrobials tested (Table 4).

TABLE 4. *Frequency of resistance to antimicrobial agents among* Escherichia coli *isolates from the shells of eggs collected from retail outlets in northwestern Spain*[a]

Drug[b]	Chicken eggs					Quail eggs (conventional cage) ($n = 20$)	Mean ($n = 120$)
	Conventional cage ($n = 20$)	Barn ($n = 20$)	Free range ($n = 20$)	Organic ($n = 20$)	Domestic production ($n = 20$)		
CN	0 (0) A a	2 (10) A a	0 (0) A a	0 (0) A a	0 (0) A a	0 (0) A a	2 (1.67) a
AMS	0 (0) A a	0 (0) A a	0 (0) A a	3 (15) A a	0 (0) A a	0 (0) A a	3 (2.50) a
AUG	18 (90) A b	18 (90) A b	7 (35) BC b	4 (20) B a	4 (20) B a	11 (55) AC b	62 (51.67) b
TZP	0 (0) A a	2 (10) A a	0 (0) A a	0 (0) A a	0 (0) A a	0 (0) A a	2 (1.67) a
CTX	0 (0) A a	1 (5) A a	0 (0) A a	0 (0) A a	0 (0) A a	0 (0) A a	1 (0.83) a
SXT	17 (85) A b	10 (50) B c	1 (5) C a	3 (15) C a	2 (10) C a	0 (0) C a	33 (27.50) c
C	0 (0) A a	3 (15) A a	0 (0) A a	0 (0) A a	0 (0) A a	0 (0) A a	3 (2.50) a
CIP	0 (0) A a	0 (0) A a	0 (0) A a	0 (0) A a	1 (5) A a	0 (0) Aa	1 (0.83) a
NA	0 (0) A a	1 (5) A a	0 (0) A a	0 (0) A a	1 (5) A a	0 (0) A a	2 (1.67) a
TE	19 (95) A b	18 (90) AB b	14 (70) B c	0 (0) C a	4 (20) C a	18 (90) AB d	73 (60.83) b
F	3 (15) A a	4 (20) AB a	9 (45) B bc	0 (0) A a	1 (5) A a	4 (20) AB c	21 (17.5) d
FOS	0 (0) A a	3 (15) A a	0 (0) A a	10 (50) B b	2 (10) A a	6 (30) AB bc	21 (17.50) d

[a] Values are the number (percentage) of *E. coli* isolates resistant. Within a row, means with no uppercase letters in common are significantly different ($P < 0.05$). Within a column, means with no lowercase letters in common are significantly different ($P < 0.05$).

[b] Drugs tested were gentamicin (CN; 10 μg), ampicillin-sulbactam (AMS; 20 μg), amoxicillin–clavulanic acid (AUG; 30 μg), piperacillin-tazobactam (TZP; 110 μg), cefotaxime (CTX; 30 μg), sulfamethoxazole-trimethoprim (SXT; 25 μg), chloramphenicol (C; 30 μg), ciprofloxacin (CIP; 5 μg), nalidixic acid (NA; 30 μg), tetracycline (TE; 30 μg), nitrofurantoin (F; 300 μg), and phosphomycin (FOS; 200 μg).

TABLE 5. *Resistance patterns among resistant and multiresistant* Escherichia coli *isolates from eggshells*[a]

Resistance pattern	No. of strains
CN, AUG, TZP, CTX, FOS	1
AUG, SXT, CIP, NA, TE	1
AUG, C, TE, FOS	2
AUG, TZP, NA, TE	1
AUG, TE, F, FOS	1
CN, AUG, TE	1
AUG, SXT, TE	30
AUG, TE, FOS	12
AUG, SXT	1
AUG, TE	8
TE, F	10
TE, FOS	2
AMS	3
AUG	4
SXT	1
C	1
TE	5
F	10
FOS	3

[a] Drugs tested were gentamicin (CN; 10 μg), ampicillin-sulbactam (AMS; 20 μg), amoxicillin–clavulanic acid (AUG; 30 μg), piperacillin-tazobactam (TZP; 110 μg), cefotaxime (CTX; 30 μg), sulfamethoxazole-trimethoprim (SXT; 25 μg), chloramphenicol (C; 30 μg), ciprofloxacin (CIP; 5 μg), nalidixic acid (NA; 30 μg), tetracycline (TE; 30 μg), nitrofurantoin (F; 300 μg), and phosphomycin (FOS; 200 μg).

Table 5 shows the antimicrobial resistance patterns among *E. coli* isolates. The most common patterns were AUG, SXT, TE (30 strains; 25%), AUG, TE, FOS (12 strains; 10%), TE, F (10 strains; 8.33%), and F (10 strains; 8.33%). The remaining 35 resistant or multiresistant strains had 16 different patterns.

DISCUSSION

Microbial counts. With the exception of eggs from domestic production (3.69 ± 0.7 log CFU/cm^2), APCs in the present study were lower than reported values of 2.61 to 5 log CFU per eggshell *(6, 16, 27, 39)*. However, most reports refer to initial eggshell contamination, whereas the samples in the present study were analyzed after several days of retail display. Storage of table eggs, whether temporarily refrigerated or not, resulted in a significant decrease in bacterial eggshell contamination *(15)*. The method used for the recovery of bacteria from the eggshell could also be partially responsible for the lower counts obtained in the present study. De Reu et al. *(14)* found that washing of intact eggs in buffered peptone water or phosphate buffer saline by rubbing resulted in statistically higher counts than obtained when the shell was crushed in buffered peptone water. The APCs for eggshells in this study were below 5 log CFU per eggshell, a limit considered as acceptable as an indicator of hygienic quality *(15)*.

Other researches have found differences in bacterial contamination of eggshells depending on housing systems. Eggs from hens kept in floor systems were more contaminated with aerobic bacteria than were eggs from cage systems *(15, 16)*. Differences in farm construction or management also could influence bacterial eggshell contamination *(15),* which may explain certain discrepancies between the findings in the present study and those in previous reports. The handling of eggs in the food supply chain also may explain some of the differences.

The higher microbial counts on eggs from domestic production compared with eggs from other housing systems could be associated with the higher prevalence of dirty eggs. However, Wall et al. *(39)* suggested that the correlation between visual shell contamination and bacterial contamination is poor. Thus, rating bacterial contamination of eggshells based on visual examination may not be highly reliable. The fact that eggs produced in the conventional cage system were cleaner than those from barn production is congruent with the findings of Djukić-Stojčić et al. *(17),* who reported that cleanliness of eggs from hens kept in floor systems depends on the conditions of the environment, the percentage of eggs taken out of the nest, and the organization of the work on the farm (e.g., how often eggs are collected and the cleanliness of the nests).

The high average percentage of eggshells contaminated with *E. coli* strains (45%) was expected because freshly laid eggs are readily contaminated with feces during laying and in their environment *(3)*. Thus, the high prevalence of *E. coli* on domestic eggs could be related to the frequent presence of dirt on these samples. Similar to our results, Ali Akond et al. *(5)* reported in Bangladesh that 42% of eggshells were contaminated with *E. coli*.

Antimicrobial resistance. The high prevalence of resistant or multiresistant *E. coli* strains on table eggs in the present study (80.83%) is comparable to the figures reported by other authors *(1, 4, 5, 32)*. This high prevalence of resistance, which may have important therapeutic implications, may be due to the overuse of antimicrobial agents on birds laying eggs *(9)*. This overuse of antimicrobials favors the emergence, selection, and dissemination of antimicrobial resistance among bacterial pathogens and birds' endogenous fecal microbiota *(29, 30)*. Because eggshells become contaminated during laying, resistant fecal *E. coli* strains from poultry can infect humans via the food chain. Bacteria can acquire antimicrobial resistance from environmental exposure, and multidrug-resistant bacteria have been detected in poultry and eggs from farms that did not report antibiotic use *(29)*. Resistant bacteria from food may colonize the human intestinal tract and pass on resistance genes horizontally to endogenous human bacteria. Drug resistance in the avian intestinal tract may persist for a long time even in the absence of antibiotics *(12)*. Thus, in several countries chickens (both their meat and their eggs) have been described as a source of antibiotic resistant bacteria in humans *(38)*.

Numerous strains in the present study (58.33%) were resistant to two or more antimicrobial agents. An outcome of the proliferation of these multidrug-resistant strains is a reduction in the number and types of antimicrobials that are effective in clinical practice. The high prevalence of

multidrug-resistant *E. coli* isolates in conventional chicken housing systems (cage, barn, and free range), where only a limited number of antimicrobials are frequently used, highlights the fact that resistance mechanisms may be linked *(9)*. The detection of strains resistant to nutrofurantoin, an antimicrobial agent banned in the middle 1990s for veterinary use in the European Union, provides additional support for the hypothesis that drug application may select for resistance not merely to the applied drug but to multiple drugs.

Similar to findings by other authors *(33, 35),* the higher prevalence of antimicrobial resistance in the current study was observed in *E. coli* strains isolated from conventional cage, barn, and free-range housing compared with organic and domestic production systems, in which antimicrobial use was assumed to be less, suggesting that these alternative housing systems may limit the development and spread of antimicrobial resistance among foodborne bacteria. In addition to antibiotic use, crowding and poor sanitation, two factors typically associated with intensive poultry farming (e.g., cage systems), also are major forces selecting for antimicrobial resistance. A prevalent belief on the part of consumers is that organic and domestic production eggs are healthier and safer than conventional eggs *(26)*. Results found in the present study justify in part this consumer perception. However, compared with conventional cage systems outdoor (barn, free-range, domestic, or organic) systems are inherently less controllable from a hygienic point of view and can be affected by pollutants that have not been an issue for intensive farms, resulting in increased food safety risk from microbial infections or environmental contamination *(24)*.

The high percentage of *E. coli* strains resistant to AUG, SXT, and TE in the present study is consistent with findings by other authors *(25, 31, 32, 38)*. These agents are among the main drugs used against infectious diseases in chicken flocks in numerous countries worldwide, including Spain. Hence, some level of resistance is expected to have emerged over time *(2, 29)*. The small percentage of strains resistant to fluoroquinolones in the current study (0 to 5%) is surprising because these drugs are among those most frequently used in poultry farms in Spain *(10)*. Previous studies in Spain revealed a high prevalence of resistance to fluoroquinolones in *E. coli* strains from both chicken and human populations *(34)*.

The microbiological contamination of eggshells differed substantially between housing systems. Domestically produced eggs had the highest percentage of dirty samples, the highest counts for most microbial groups, and the highest prevalence of *E. coli*. The high prevalence on eggshells of *E. coli* strains resistant or multiresistant to antimicrobial agents, some of which are used in treating human diseases, poses a potential health hazard to consumers. The results obtained in this study suggest a relationship between antibiotic resistance and housing system. The prevalence of antimicrobial resistance was significantly higher in conventional egg production systems than in organic and domestic systems. These findings could reflect the less prevalent use of antibiotics in these housing systems and highlight the need for more prudent use of antimicrobials in all food-producing animals. Because resistant bacteria that survive antimicrobial treatment survive longer than do the drug residues in foods, the monitoring of antimicrobial-resistant bacteria on eggshells could be one method for detecting antibiotic use in laying hens and uncovering fraudulent use of antimicrobial treatments in organic breeding systems.

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