Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jul - Sep 2009 / v.11 / n.3 / 195 - 201

Detection and Transfer of Antimicrobial Resistance Gene Integron in *Salmonella Enteritidis* Derived from Avian Material

Author(s)

Okamoto AS[1]
Andreatti Filho RL[2]
Rocha TS[3]
Menconi A[4]
Marietto-Gonçalves GA[5]

[1] Doctoral Candidate, Department of Veterinary Clinics, FMVZ UNESP Campus Botucatu.
[2] Advisor responsible for the Ornithopathology service, FMVZ UNESP Campus Botucatu.
[3,4,5] Master Candidate, Department of Veterinary Clinics, FMVZ UNESP Campus Botucatu.

Mail Address

Adriano Sakai Okamoto
Avenida Vital Brasil, 630A
Vila São Lúcio
18.603-193, Botucatu, SP, Brazil

E-mail: sakai@fmvz.unesp.br

Keywords

Integron, gene transfer, resistance to antimicrobials, *Salmonella Enteritidis.*

Arrived: April/2009
Approved: August/2009

ABSTRACT

The expansion of global poultry production has increased the need to reduce or control the agents responsible for economic losses, including *Salmonella* spp. These bacteria are also of public health concern due to their potential to cause food poisoning, and, more recently, due to the antimicrobial resistance presented by these bacteria. Molecular biology is an important tool currently used in the diagnosis and research studies of main poultry diseases. The present studied analyzed 100 samples of *Salmonella* Enteritidis (SE) isolated from avian material aiming at detecting the class 1 integron gene, Integroninvolved in antimicrobial resistance, by means of polymerase chain reaction (PCR), and comparing it with plate inhibition test. Subsequently, *SE* samples were evaluated for their capacity to horizontally transfer this gene. There was no direct relationship between the presence of the class 1 *integron* gene and SE resistance to the 14 antimicrobials tested, as 80% of the studied samples were resistant to up to three antimicrobials, and did not present the aforementioned gene. However, horizontal transfer of this gene was accomplished in vitro (from *Escherichia coli* to *Salmonella* Enteritidis), demonstrating that capacity class 1 *integron* gene can be disseminated among enterobacteria.

INTRODUCTION

diseases, caused by one or more members of the genus *Salmonella*. These bacteria are Gram-negative, belong to the family Enterobacteriaceae, and most species are mobile. They are facultative anaerobic and aerobic bacteria, ferment sugars, producing gas and H2S, and possess a complex antigenic constitution (somatic "O", flagellar "H" and capsular "K", antigens) (Barrow, 2000).

According to Tood (1997), the high incidence of foodborne infections in humans is due to the ingestion of products of avian origin that are contaminated or unsuitably prepared, since the birds can be reservoirs of *Salmonella* spp. *Salmonella* can be introduced into poultry houses by means of contaminated feeds, particularly those that contain animal raw materials (Hofer *et al*., 1998), infection by transovarial route or contamination of the eggshell, by water, by direct contact with contaminated carriers or birds, and via vectors, such as insects, rodents, persons, and equipment (Gast *et al*., 1998).

The pathogenicity of *Salmonella* depends on a series of factors associated with the bacterium, with the bird, and with the conditions in which birds are raised. The association and penetration of the bacterium in the digestive mucosa is a prerequisite for systemic infection (Rychlik *et al*., 1998).

Microbial resistance is the loss of sensitivity by a microorganism to an antimicrobial to which it was originally susceptible. This resistance can be acquired by mutations in chromosomal DNA or by the acquisition of extra-chromosomal genetic material by means of plasmids and transposons (Vázquez *et al*., 2002).

The growing resistance of pathogenic bacteria to antimicrobials has raised the concern that the widespread use of antimicrobials in animal production may promote the development of resistant bacteria or resistance genes that can be transferred to bacteria that cause disease in humans (Wegener *et al*., 1997).

A major public health problem faced in recent decades has been the emergence and spread of antimicrobial resistance in bacterial populations, especially those of hospital origin. There is a significant increase in the frequency of isolates of bacteria that had once been sensitive to routine drugs, but are now resistant to all or nearly all drugs in the market (Nogueira *et al*., 1999).

Resistance to antimicrobial drugs was first reported in the studies published in 1907 by Paul Ehrlich, who recorded the emergence of trypanosomes resistant to rosaniline chemotherapy. The emergence of bacterial resistance was also recorded after sulfonamide and penicillin started to be used in veterinary and human medicine in the 1940s. There is a growing concern as to the proper use of antimicrobials. One negative aspect of the use of antimicrobials is the selection of multi-resistant microorganisms, limiting the therapeutic possibilities, and increasing not only the lethality rates, but also treatment costs (Nogueira *et al*., 2005).

The involvement of various *Salmonella* serotypes can be observed in cases of multi-resistance to diverse antimicrobials (Gutiérrez *et al*., 2000). In *S.* Typhimurium, *S.* Gallinarum, *S.* Enteritidis and *S.* Choleraesuis, the presence of plasmids of high molecular weight (50 to 100 kb) has been demonstrated, where genes encoding for toxins are found, as well as genes that confer multi-resistance to different antimicrobials (Vázquez *et al*., 2002).

The family *Enterobacteriaceae* includes genera that inhabit the intestinal tract of humans and other animals, as well as non-pathogenic (commensal) and pathogenic species (Howard *et al*., 1987). Many species belonging to this family that are present in the intestinal tract are frequently exposed to different antimicrobials, creating the potential to disseminate genes of resistance to antimicrobials (Goldstein *et al*., 2001).

The most widely known of the various identified genetic elements that participate in resistance gene transfer are plasmids (Amabile-Cuevas & Chicurel, 1992), transposons (Salyers *et al*., 1995), and bacteriophages (Stokes & Hall, 1989).

The transference of a resistance gene among different bacteria can occur via conjugation (Zatykaa & Thomasa, 1998), transduction (Thompson, 1994), or transformation (Cohens *et al*., 1972). In conjugation, resistance genes are transferred from one bacterium to another by means of a plasmid. In transduction, the resistance gene is integrated into a bacterium by means of a virus and, finally, in transformation, genes released into the external medium by dead bacteria are captured and assimilated (Vázquez *et al*., 2002).

Antimicrobial resistance is accomplished by Integronsintegrons that are present in plasmids or integrated within the chromosome of the bacterium (Goldstein *et al*., 2001). The function of plasmids and transposons is well known in bacterial multi-resistance to antimicrobials and in natural dissemination of resistance determinants (Amabile-Cuevas & Chicurel, 1992).

Integrons are genetic elements capable of integrating and expressing genes that induce resistance to antimicrobials, are identified in plasmids and transposons, and frequently contain one or more genes encoding resistance to antimicrobials (Stokes & Hall, 1989).

Integrons function as a system that captures genes that confer selective advantages to the bacterium. Due to their capacity to recognize a wide variety of recombination sequences, their exchange capacity and remote origin, integrons allow the bacterium to rapidly adapt to ecological changes, which most recent example undoubtedly is the adaptation to modern era chemotherapy (González *et al*., 2004). González *et al*. (1998) published the first evidence of the presence of integrons in Gram-negative bacilli isolated from biological residues in Chilean hospitals. The integrons are commonly associated with the family *Enterobacteriaceae*.

Four classes of integrons are known (1, 2, 3, and 4), with class 1 being predominant among the members of this family both in the normal and pathogenic microbiota of animals (Goldstein *et al*., 2001).

Based on the results found with different serotypes of the genus *Salmonella*, most isolates obtained from clinical cases are resistant to various antimicrobials and carry class 1 *integron*, involved in antimicrobial multi-resistance (Vázquez-Navarrete *et al*., 2005). The strains that present integrons are those that present the strongest resistance patterns (Munoz *et al*., 2000).

As consequence of the discovery and study of resistance gene transfer among bacteria, science has experienced an extraordinary advance culminating in the development of molecular techniques that allow the study and mapping of chromosomes and the subsequent manipulation of genes, applicable in eukaryotes as much as in prokaryotes (Nogueira *et al*., 1999).

Bacteria can acquire resistance to antimicrobials via horizontal transfer of the resistance gene (Top *et al*., 2000). Resistance genes are encoded by plasmids and these are frequently transferred among species, being common in *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter* spp., *Citrobacter freundii*, *Salmonella* spp., and *Serratia* spp., and their dissemination is apparently inevitable (De Champs *et al*., 1991).

The occurrence of horizontal transference of genes can be observed in experiments such as that of Moriarty (2009), which concluded that the group *Typhimurium* DT 104 of *Salmonella enterica* acquired resistance genes to multiple antibiotics derived from another bacterium through horizontal gene transference. There are evidences that its resistance to florphenicol originated from fish pathogens. As florphenicol, chloramphenicol, tetracyclines, fluorquinolones, and fish meal (which may contain high concentrations of bacteria) are used and transported all over the world, it possible that multi-resistance genes are horizontally propagated from aquatic bacteria to intestinal bacteria of terrestrial animals that ingested feed containing fish meal with bacteria with multiple-resistance genes (Moriarty, 2009).

The present study aimed at detecting class 1 integron gene associated to antimicrobial resistance present in *Salmonella* Enteritidis, by correlating the presence of this gene with multi-resistance to antimicrobials, as verified the plate inhibition test (antibiogram), and than to determine the possible horizontal transference of this gene between two enterobacteria.

MATERIAL AND METHODS

Bacterial samples in each type of sample

One hundred (100) samples of *Salmonella enterica* subspecies enterica serotype Enteritidis (SE) were obtained from chicken viscera (liver, cecum, yolk sac, or ovary), cloaca swabs, or carcasses. These bacteria were serotyped at Adolfo Lutz Institute in Sao Paulo, and then stocked in nutrient agar.

SE samples were reactivated in tubes containing 3mL brain-heart infusion (BHI) and incubated under aerobiosis at 41°C for 24 hours. They were then seeded onto Petri dishes containing brilliant green agar (BGA), were incubated as previously described, and maintained at 4°C until the moment of use.

The donor bacterium, *Escherichia coli*, was isolated from a water treatment plant in Porto, Portugal, and each sample was identified at the School of Biotechnology of the Portuguese Catholic University as positive for class 1 integron and as multi-resistant to all antimicrobials utilized in this experiment. The SE sample was used as receptor bacterium, and was obtained from the Ornithopathology Laboratory of the Department of Veterinary Clinics of FMVZ, UNESP at Botucatu, Brazil, and was shown to be sensitive to all the tested antimicrobials and negative for the presence of the antimicrobial resistance gene (class 1 *integron*).

Test of resistance to antimicrobials

The antibiogram was performed according to the methodology of Bauer and Kirby (1966), with incubation of five colonies from the sample in Mueller-Hinton (MH) broth at 37°C for two to eight hours, until achieving a turbidity equivalent to 0.5 on the MacFarland scale. After turbidity adjustment, a sterile swab was introduced, pressed against the tube wall in order to remove any excessive liquid, and then seeded on the surface of a Petri dish containing MH agar, rotating it at least twice. After the lid was placed, the dish was left at rest for five minutes to absorb any excessive humidity. Seven discs (Sensifar) impregnated with antimicrobials were placed at equal distances from each other on the surface of each dish. Subsequently, the dish was inverted and incubated at 37°C in aerobiosis. Dish readings were performed 18 hours after incubation and the diameter of inhibition halos was measured with the aid of a ruler. The results were analyzed using the table of Sensifar - Cefar*.

The 14 antimicrobials tested in the antibiogram were gentamyicin (10µg), enrofloxacin (5µg), chloramphenicol (30µg), neomycin (30µg), tetracycline (30µg), ampicillin (10µg), nalidixic acid (30µg), sulfonamides (300µg), trimethoprim (5µg), ceftiofur (30µg), amikacin (30µg), streptomycin (10µg), tobramycin (10µg), and ciprofloxacin (5µg).

Molecular Biology

Preparation of bacterial lysate

The bacterial lysate was obtained after culturing each sample for 24 hours at 41°C in BGA. The colonies

---

*Cefar Pharmaco Diagnostics Ltd. Clinical-Pharmaceutical Research and Diagnostic Products.

were transferred to a test tube containing sterile bi-distilled water until achieving a turbidity equivalent to 3 on the MacFarland scale, and then centrifuged at 15,000 rpm for 7 minutes. Next, the supernatant was discarded and 1mL of sterile bi-distilled water was added; the resulting suspension was submitted to 100°C for 10 minutes and then refrigerated (4°C), centrifuged at 15,000 rpm, and the supernatant was stored at -20°C until the moment of use (Álvarez *et al*., 2003)

Oligonucleotides

The oligonucleotides (primers) utilized for the gene that integrates into class 1 *integrons* (Álvarez *et al*., 2003; Goldstein *et al*., 2001) are specified in Table 1.

.

Table 1 - Oligonucleotides refer to class 1 integrons.

Gene	Oligonucleotides (5' → 3')	Base pair (bp)
Class 1 Integrons	GGCATCCAAGCAGCAA	900
	GAAGCAGACTTGACCTGA	

Amplification of bacterial DNA

After obtaining the lysate from SE samples, the presence of the gene was investigated by means of polymerase chain reaction (PCR).

Bacterial DNA was amplified in a sterile microtube, to which a buffer was added to PCR 1 X (100 mM Tris-HCl pH 8.8 at 25ºC, 50 mM KCl, 15 mM $MgCl_2$), 1mM of each oligonucleotide, 1 U of DNA Taq polymerase, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 13mL of ultrapure water and 15mL of lysate from each sample, totaling a final volume of 50mL (Silva *et al*., 2006).

Amplification consisted in the denaturation at 94°C for 10 minutes, followed by 35 cycles at 94°C for 1 minute, annealing at 54°C for 1 minute, and extension of DNA cassettes at 72°C for 2 minutes, with final extension at 72°C for 10 minutes (Silva *et al*., 2006).

Detection of the amplified product (electrophoresis in agarose gel)

To each 7mL of PCR product, 3mL of loading buffer were added (6X). After homogenization, 10mL was collected into each well with 1% agarose gel. The PCR products (molecular weight of 100 bp DNA ladder) were separated from the marker by electrophoresis in a 100 V current for 50 minutes. The gel was stained with ethidium bromide solution (10mg/mL) for 10 minutes and analyzed under ultraviolet light.

Horizontal gene transfer

In order to choose donor and receptor bacteria, the antibiogram test and PCR were performed prior to the beginning of the experiment to demonstrate not only their resistance or sensitivity, but also the presence or absence of genetic resistance to antimicrobials.

Adapting the technique of Hall and co-workers (1993), tetracycline, ampicillin, and trimetoprim were added at the concentration of 5mg/mL to nutrient agar broth, and at 100mg/mL to BGA. These antimicrobials were randomly chosen among those to which the SE sample (receptor) was found to be sensitive and the Escherichia coli sample (donor) to be resistant.

Transference was initiated by separately incubating the bacteria (donor and receptor) in nutrient broth at 37°C for 24 hours, which was subsequently diluted 100 times in nutrient broth preheated to 37°C, and incubated for another 4 hours. Cultures were then centrifuged at 7300 rpm, and the sediment from each bacterium (donor and receptor) was homogenized with 2 mL nutrient broth, seeded on nutrient agar, and incubated at 37°C for 24 hours. The dish was then washed with PBS and seeded on BGA, with the aid of a platinum loop.

SE and *E. coli* colonies were differentiated when the capacity of fermenting lactose was detected in the BGA medium. After identification, the bacteria were submitted to a new antibiogram and integron gene detection by PCR.

RESULTS AND DISCUSSION

The results of resistance and sensitivity of 100 *SE* samples isolated from avian material and challenged by antimicrobials are shown in Table 2.

Table 2 - Profile of antimicrobial resistance and sensitivity of 100 samples of *Salmonella* Enteritidis isolated from avian material.

	Number of samples		
Antimicrobials	Resistant	Intermediate	Sensitive
Sulfonamide	75	14	11
Nalidixic acid	57	16	27
Ampicillin	41	7	52
Tetracycline	18	4	78
Tobramycin	16	2	82
Enrofloxacin	14	30	56
Gentamicin	10	3	87
Neomycin	9	30	61
Ceftiofur	9	12	79
Streptomycin	7	10	83
Trimethoprim	5	9	86
Amikacin	4	1	95
Ciprofloxacin	1	5	94
Chloramphenicol	0	1	99

The most effective antimicrobial was chloramphenicol, to which 99% of the tested samples were sensitive. Similar results were observed by Cunha *et al*. (1981), who demonstrated 100% sensitivity to chloranfenicol of *S.* Typhi samples isolated from different Brazilian states. On the other hand, some other researchers have evidenced *SE* resistance to this antimicrobial since the 1960s in many countries, including Brazil (Asensi *et al*., 1994). The sensitivity to chloramphenicol is possibly due to the fact that the use of this antimicrobial in animal feeds has been prohibited since 2003 (Brasil, 2003), thereby reducing the exposure of bacteria to this drug.

The least effective antimicrobial (11%) among the samples was sulfonamide. The resistance of most of the *Salmonella* samples tested in this and other studies when challenged with sulfonamide may be due to the long-term and widespread use of this antimicrobial in both human and veterinary medicine (Nogueira *et al*., 2005).

Only eight (8%) of the *SE* samples presented sensitivity to all the antimicrobials tested. Seventy-two (72%) *SE* samples were resistant to one to three antimicrobials, while 15 (15%) samples showed resistance to four to six antimicrobials, and five (5%) were resistant to more than seven antimicrobials. One (1%) of these *SE* samples was resistant to as many as nine of the 14 antimicrobials tested (Figure 1).

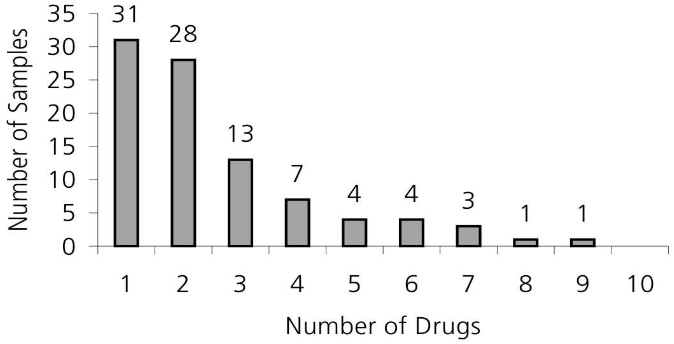

Figure 1 - Number of antimicrobial-resistant *Salmonella* Enteritidis samples isolated from avian material. * Gentamycin, enrofloxacin, chloramphenicol, neomycin, tetracycline, ampicillin, nalidixic acid, sulfonamides, trimethoprim, ceftiofur, amikacin, streptomycin, tobramycin and ciprofloxacin.

The gene for resistance to antimicrobials (class 1 integron) was not observed in any of the 100 *SE* samples analyzed by PCR, unlike other studies, such as that of Ebner (2004), where to presence of the gene was observed in 30.8% of the tested *SE* samples. Other genes or mechanisms may be involved in the multi-resistance to antimicrobials observed in the present *SE* isolates.

After applying the modified technique of in-vitro gene transfer proposed by Hall *et al*. (1993), *SE* and *Escherichia coli* colonies were submitted to a new antibiogram, when both showed resistance to tetracycline, ampicillin, and trimethoprim. The presence of the antimicrobial resistance gene (class 1 integron) was verified both in *E. coli* (donor) and *SE* (receptor), thus confirming transference of this gene between the two bacteria (Figure 2).

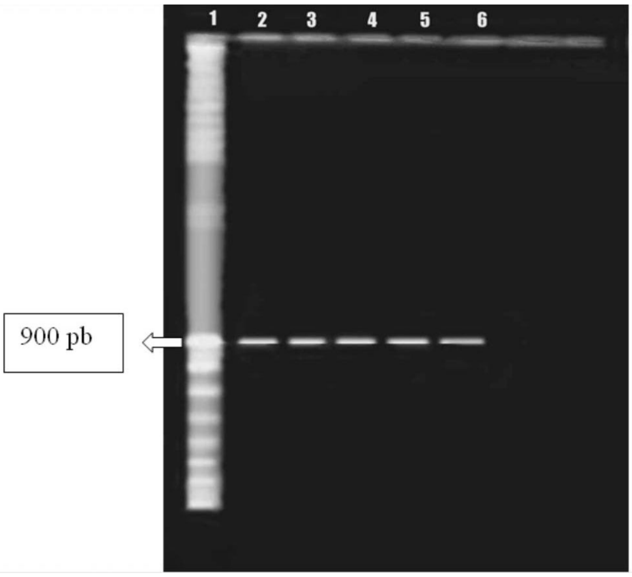

Figure 2 - Agarose gel showing bands of the antimicrobial resistance gene (class 1 integron). Band 1 shows the molecular weight marker, band 2 the positive control (*Escherichia coli* donor), and bands 3, 4, 5 and 6 positive receptors of *Salmonella* Enteritidis samples.

CONCLUSIONS

Although 80% of the *SE* samples isolated from avian material were shown to be resistant to at least three antimicrobials, this multi-resistance had no correlation with the presence of the antimicrobial resistance gene (class 1 *integron*) since none of the samples were positive for the presence of this gene.

Antimicrobial resistance gene (class 1integron) transfer can be performed *in vitro* by concomitant culture of *SE* (receptor) and *E. coli* (donor).

REFERENCES

Álvarez FM, Rodrígues ST, Brey FE, López MC, Piñeiro L. Asociación entre integrones de clase 1 com resistência a multiples antimicrobianos y plásmidos conjugativos en *Enterobacteriaceae*.

Revista Espanola de Quimioterapia 2003; 16(4):394-397.

Amabile-Cuevas CF, Chicurel M. Bacterial plasmids and gene flux. Cell 1992; 70:189-199.

Asensi MD, Hofer E. Serovars and multiple drug resistant *Salmonella* sp isolated from children in Rio de Janeiro - Brazil. Revista de Microbiologia de São Paulo 1994; 25:149-153.

Barrow PA. The paratyphoid salmonellae. Revue Scientifique et Technique Officef International des Epizooties 2000; 19:351-375.

Bauer AW, Kirby WMN. Antibiotic susceptibility testing by standartiezed simple disc method. American Journal Clinical Pathology 1996; 45:492-496.

Brasil. Ministério da Agricultura Pecuária e Abastecimento - Instrução Normativa nº9. Diário Oficial da União, Brasília, DF, 30 jun. 2003. Seção 1, p. 4.

Cohens N, Chang ACY, Hsu L. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proceedings of the National Academy of Sciences 1972; 69:2110-2114.

Cunha MAS, Suassuna IR, Suassuna I. Sensibilidade de Salmonella typhi a antimicrobianos em emprego clínico. Revista da Associação Médica Brasileira 1981; 27:267-268.

De Champs C, Sirot C, Chanal C, Poupart MC, Dumas MP, Dumas J. Concomitant dissemination of three extended-spectrum β-lactamase among different Enterobacteriaceae isolated in a French hospital. Journal Antimicrobial Chemotherapy 1991; 27:441-457.

Ebner P, Garner K, Mathew A. Class 1 *integrons* in various *Salmonella enterica* serovars isolated from animals and identification of genomic island SGI 1 in Salmonella enterica var. Meleagridis. Atlanta; 2004 [cited 2009 jul 11]. Available from: http://jac.oxfordjournals.org/cgi/reprint/dkh192v1.pdf

Gast RK, Mitchell BW, Holt PS. Airbone transmission of *Salmonella enteritidis* infection between groups of chicks in controlled-enviroment isolation cabinets. Avian Diseases 1009; 42(2):315-320.

Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ. Incidence of Class 1 and 2 Integrases in Clinical and Commensal Bacteria from Livestock, Companion Animals, and Exotics. Antimicrobial Agents and Chemotherapy 2001; 45(3):723-726.

González GR, Mella SM, Zemelman RZ, Bello HT, Domínguez MY. Integrones y cassettes genéticos de resistencia: estructura y rol frente a los antibacterianos.Revista Médica de Chile 2004; 132(5):619-626.

González GR, Sossa K, Mella SM, Zemelman RZ, Bello HT, Domínguez MY. Presence of integrons in isolates of different biotypes of *Acinetobacter baumannii* from Chilean hospitals. FEMS Microbiology Letter 1998; 161:125-128.

Gutiérrez CL, Montiel VE, Aguilera PP, González AMC. Serotipos de *Salmonella* identificados en los servicios de salud de México. Salud Pública de México 2000; 42:490-495.

Hall LMC, Livermore DM, Gur D, Akova M, Akalin HE. OXA-11, an extended-spectrun variant of oxa-10 (pse-2) β-lactamase from *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 1993; 37(8):1637-1644.

Hofer E, Silva Filho SJ, Reis EMF. Sorovares de *Salmonella* isolados de matérias primas e de ração para aves no Brasil. Pesquisa Veterinária Brasileira 1998; 18(1):21-27.

Holmes B, Gross RJ. Coliform bacteria: various other members of the Enterobacteriaceo. In: Parker MT, Collier LH, editors. Topley & Wilson´s principles of bacteriology, virology and immunity. 8th ed. London: Eduard Arnold; 1990. v.2, p.415-441.

Holt JG, Krieg NR, Sneeath PHA, Staley JT, Williams ST. Bergey's: manual of determinative bacteriology. 9th ed. Baltimore: Williams & Wilkins; 1994.

Howard BJ, Klass Ii J, Rubin SJ, Weissfeld AS, Tilton RC. Clinical and pathogenic microbiology. St Louis: The C. V. Mosby; 1987.

Moriarty DJW. Os perigos do uso de antibióticos na aquacultura. São Paulo [citado em 2009 jul 8]. Disponível em: http://www.aqualider.com.br/article.php?recid=88.

Muñoz J, González G, Bello H, Domínguez M, Mella S, Zemelman R. Prevalencia de integrones en cepas intrahospitalarias de Pseudomonas aeruginosa y *Acinetobacter* spp. y su relación con la resistencia a antimicrobianos aminoglicosidos. Anales Microbioles 2000; 3:72.

Nogueira IA, Brasil P, Conceição M, Martins IS. Recomendações para o uso adequado dos antimicrobianos [citado em 2006 dez 16]. Disponível em: http://www.saude.rj.gov.br/cecih/Antimicrobianos.doc

Nogueira MS, Nascimento AMA, Chartone-Souza E. Ação de produtos naturais na inibição do crescimento bacteriano e do fluxo gênico e na origem de mutantes resistentes. Genetics and Molecular Biology 1999; 22:431.

Poppe C. Epidemiology of *Salmonella enterica* serovar Enteritidis In: Saeed AM, Gast RK, Potter ME, Wall PG. *Salmonella enterica* serovar Enteritidis in humans and animals. Ames: Iowa State University Press; 1999. p. 3-18.

Rychlik I, Lovell MA, Barrow PA. The presence of genes homologous to the K88 genes faeH on the virulence plasmid of *Salmonella gallinarum*. Microbiology Letters 1998; 159:255-260.

Salyers AA, Shoemaker NB, Li LY, Stevens AM. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. Microbiology Review 1995; 59:579-590.

Silva MF, Vaz-Moreira I, Gonzalez-Pajuelo M, Nunes OC, Manaia CM. Antimicrobial resistance patterns in Enterobacteriaceae isolated from na urban wastewater treatment plant. FEMS Microbiology Ecology 2006; 1:1-11.

Stokes HW, Hall RM. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Molecular Microbiology 1989; 3:1669-1683.

Thompson B. Bacterial antibiotic resistence and evolution. Reason and Revelation 1994; 14:61-63.

Tood ECD. Epidemiology of the foodborne diseases: a Woldacide review. World Health Statistics 1997; 50:30-50.

Top EM, Moënne-Loccoz Y, Pembroke T, Thomas CM. Phenotypic traits conferred by plasmids. In: Thomas CM, editor. The horizontal gene pool. Reading: Harwood Academic; 2000. p. 249-285.

Vázquez NJ, López VY, Suárez GF, Eslava C, Verdugo RA. Caracterización y clonación de los genes que expresan una enterotoxina LT en Salmonella gallinarum. Anales del 27º Congreso Centroamericano y del Caribe de Avicultura; 20002; La Habana.

Vázquez-Navarrete J, Córdoba BC, López VY, Mancera MA. Identificacion Del gene da la integrasa Tipo I y perfil da resistência antimicrobiana em *Salmonella enteritidis*. Cuajimalpa [cited 2005 nov 24]. Available from: www.vet-uy.com/articulos/artic_micro/001/micro001.htm

Wegener HC, Bager F, Aarestrup FM. Vigilância da resistência aos antimicrobianos no homem, nos produtos alimentares e no gado na Dinamarca. Euro Surveillance 1997; 3(2):17-19.

Zatykaa M, Thomasa C M. Control of genes for conjugative transfer of plasmids and other mobile elements. FEMS Microbiology Reviews 1998; 21:291-319.