African Journal of Microbiology Research Vol. 5(13), pp. 1707-1709, 4 July, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.309
ISSN 1996-0808 ©2011 Academic Journals

*Short Communication*

# Antibiotic resistance of some lactobacilli isolated from the gut microflora of broiler

**Behira Belkacem* and Kihal Mebrouk**

Faculté des sciences, Laboratoire de microbiologie appliqué, Université d'Oran, Algérie.

Accepted 1 June, 2011

**To evaluate the impact of the antibiotic use in poultry and the development of the resistance in the gut microflora of broiler. Microbiological methods were used to investigate the susceptibility of ten lactobacilli stains isolated from digestive tract of broiler. The isolates were tested against the ten most used antibiotics in veterinary and human medicine in Algeria. The isolates exhibited high resistance to all antibiotics tested in the range 70 to 100%. The minimal inhibitory concentrations (MIC's) also were evaluated for each isolates. Lactobacilli of broiler's microflora are a reservoir of resistance genes able to dispread the antibioresistance phenomenon through the food chain and the environment. Further studies need to be performed to understand the mechanisms and the causes responsible for this phenomenon. This study shows the need to find alternatives and emergency measures to avoid repercussions on public health.**

**Key words:** Lactobacilli, antibiotics, resistance, broiler, microflora.

## INTRODUCTION

About 50 years ago, antibiotics were introduced for the treatment of microbial diseases. Since then, the greatest threat to the use of antimicrobial agents for therapy of bacterial infections has been the development of antimicrobial resistance in pathogenic bacteria (Shalini and Rameshwar, 2005). Acquired antibiotic resistance, that is, resistance genes located on conjugative or mobilizable plasmids and transposons can be found in species living in habitats (e.g. human and Lactic acid bacteria) may act as reservoirs of antibiotic resistance genes that can be transferred through the food chain or within the gastrointestinal tract to pathogenic bacteria (Egervärn, 2009). The gut micro flora of poultry is a mixture of bacteria, fungi, and protozoa, but bacteria are the predominant microorganisms (Gabriel et al., 2007). The microflora of the crop consists of large numbers of lactobacilli and smaller numbers of coliforms and streptococci. The lactobacilli remain dominant throughout the small intestine. It is only in the caeca where different nutritional conditions exist and residence time is longer that the strict anaerobes become the dominant components of the microflora (Fuller, 2001).

*Corresponding author. E-mail: behira58@gmail.com

Several mechanisms of antimicrobial resistance are readily spread to a variety of bacterial genera. First, the organism may acquire genes encoding enzymes, such as lactamases, that destroy the antibacterial agent before it can have an effect. Second, bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect. Third, bacteria may acquire several genes for a metabolic pathway which ultimately produces altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent, or bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site through down regulation of porin genes (Tenover, 2006). In poultry, as well as with other intensively reared animals, antibiotics may be administered though feed or drinking water to whole flocks rather than to individual animals. In the European community (EC), the water- or feed-based administration of antimicrobials to animals (at lower doses than those employed for therapeutic purposes) to enhance animal growth has been completely banned since January 2006. A key requirement for probiotic strains is that they should not carry transmissible antibiotic resistance genes. Ingestion of bacteria carrying such genes is undesirable as horizontal gene transfer to recipient bacteria in the gut could lead to the development of new antibiotic- resistant

pathogens (Zhoua et al., 2005).

## MATERIALS AND METHODS

### Bacterial cultures and growth conditions

Fifty broilers in the finishing phase obtained from different breeding region of western Algeria. After slaughter, the gastrointestinal tract is removed aseptically. The crops and intestines were separated. Contents were suspended in MRS broth and incubated at 37°C for 18 h. After isolation on solid MRS, ten colonies were selected randomly, the colonies were examined on the basis of different morphologies. All types of colonies were examined for catalase activity and were microscopically examined after Gram stain. Cells were harvested by centrifugation (5000 g) for 10 min and washed twice in sterile physiological water 0.9% NaCl. The washed cells were resuspended and diluted in physiological water 0.9% NaCl to form standard inoculum with an optical density at 600 nm ($OD_{600}$) of 0.1 ($10^8$ CFU $ml^{-1}$).

### Antibiotic susceptibility testing

Ten bacterial isolates were screened for antibiotic susceptibility. The disk diffusion method was used. The antibiotic disks were procured from Institute Pasteur Algeria. The isolates were tested against ten antibiotics including: Bêta-lactams group: penicillin G (6 µg), ampicillin (10 µg), amoxicillin (30 µg). Macrolide group: erytromycin (15 µg), spiramycin (100 µg), clindomycin (2 µg), lincomycin (15 µg). Aminosids group: gentamicin (10 µg), naxilidic acid (30 µg) fusidic acid (10 µg). The disks were placed on the agar surface, and the plates were incubated anaerobically for 18 h. Resistance was defined no zone of growth inhibition around the disk. All incubations were at 37°C. The disks were verified by *Escherichia coli* ATCC 25922 reference strain as quality control. Resistance and susceptibility were evaluated according to CA-SFM (2010). In the second part of this study, four antibiotics obtained from Rôche Laboratories (France), that is, penicillin G, amoxicillin, ampicillin and gentamicin were tested by using the reference agar-dilution method recommended by the CA-SFM for the determination the MIC and MBC of some highly resistant strains. The final concentrations ranges were 20, 40, 60, 80 and 100 µg $ml^{-1}$. The MICs were determined by microdilution method. The microtiter plates were incubated for 18 to 24 h at 37°C. The MIC was determined by visual observation of the lowest concentration that yielded no visible growth. The MIC was defined as the concen-tration of the antibiotic that elicited approximately 80% inhibition of growth. MBCs were determined by subculturing 0.1 ml aliquots from each tube onto MRS agar plates. Plates were incubated at 37°C for 48 h in anaerobiosis. The MBC was defined as the lowest antibiotic concentration yielding five or less visible colonies on agar.

## RESULTS

The isolates were Gram positive and all were homo-fermentaire, catalase negative, grew at 45°C, produce $NH_3$ from arginine, resist to 63°C for 30 min. The carbohydrate fermentation profiles of the isolates were affected according to Hammes and Hertel (2006). *Lactobacillus salivarius* LbC1 is resistant to erythromycin, gentamicin, penicillin, amoxicillin, lincomycin but susceptible to amoxicillin; lincomycin and fusidic acid. *Lactobacillus jonshonii* LbC2 is susceptible to penicillin, amoxicillin and clindomycin and resistant to the others antibiotics. *Lactobacillus gallinarum* LbC3 is resistant to all antibiotics tested except clindomycin and fusidic acid. *Lactobacillus crispatus* LbC4 is susceptible both to amoxicillin and spiramycin. *Lactobacillus aviarus* LC5 is resistant to all antibiotics tested. The isolates from intestines were resistant to all antibiotics except *L. gallinarum* LbI4 was susceptible to ampicillin and *L. crispatus* LbI5 was susceptible to erythromycin (Table 1).

## DISCUSSION

According to Hanan et al. (2007), nearly 98% of the sequences belonged to the genus *Lactobacillus* and the three most abundant *Lactobacillus* species detected in the crop samples were *Lactobacillus reuteri* (33%), *L. crispatus* (18.7%), and *L. salivarius* (13.3%). In similar study Jiangrang et al. (2003) were found that the *lactobacillus* genera form 98% of the microbiota of ileum. Our finding are similar of these results. All the isolates were resistant to naxilidic acid. Hummel et al. *(*2007) reported that lactobacilli seem to be intrinsically resistant to quinolones, e.g. ciprofloxacin and nalidixic acid, by a currently unknown resistance mechanism. *L. salivarius* LbC1 and *L. gallinarum* LC3 were showed susceptibility to fusidic acid but Zhoua et al. (2005) reported that many strains of lactobacilli were resistant to Gram-negative spectrum antibiotics (fusidic acid, nalidixic acid and polymyxin B) and aminoglycosides (gentamicin, kanamycin, neomycin, and streptomycin). All the isolates were resistant to penicillin. Generally lactobacilli seem to be sensitive to penicillins (Danielsen and Wind, 2003). Also Danielsen and Wind (2003) were reported that some lactobacilli have a high natural resistance to bacitracin, cefoxitin, ciprofloxacin, kanamycin, gentamicin, strepto-mycin and vancomycin. All the isolates were resistant to gentamicin. Similarly Reuben et al. (2005) found that some isolates of lactobacilli were inhibited by gentamicin. Most of the observed resistances seemed to be intrinsic, but some others could be compatible with transmissible determinants (Delgado et al., 2005). Al-though lactobacilli are generally susceptible to antibiotics that inhibit the synthesis of protein, such as erythromycin and tetra-cycline (Ammor et al., 2007; Essid et al., 2009), results shows that all strains were resistant to erythromycin except the strain *L. crispatus* LbI*5*. By contrast the determination of MICs shows that the all strains tested have an acquired resistance. Knowledge of the distribu-tions of antibiotic minimum inhibitory concentrations (MICs) for species is needed when using a phenotypic method to differentiate strains with acquired resistance from susceptible strains or strains with intrinsic resistance (Egervärn, 2009). For ampicillin MICs of 20 to 80 µg $ml^{-1}$, for amoxicillin MICs of 40 to 60 µg $ml^{-1}$, for penicillin MICs of 20 to 40 µg $ml^{-1}$ were obtained. Finally, antibiotics resistance of lactobacilli could also be regarded as a beneficial property. A resistant probiotic strain that is

**Table 1.** Antibiotic susceptibility profiles of strains.

| Antibiotic | Isolates of crops | | | | | Isolates of intestines | | | | | % of resistance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | LbC1 | LbC2 | LbC3 | LbC4 | LbC5 | LbI1 | LbI2 | LbI3 | LbI4 | LbI5 | |
| Erythromycin | R | R | R | R | R | R | R | R | R | S | 90 |
| Gentamicin | R | R | R | R | R | R | R | R | R | R | 100 |
| Penicillin | R | R | R | R | R | R | R | R | R | R | 100 |
| Amoxicillin | S | S | R | S | R | R | R | R | R | R | 70 |
| Lncomycin | S | R | R | R | R | R | R | R | R | R | 90 |
| Ampicillin | R | R | R | R | R | R | R | R | S | R | 90 |
| Spiramycin | R | R | R | S | R | R | R | R | R | R | 90 |
| Clindomicyn | R | S | S | R | R | R | R | R | R | R | 80 |
| Naxilidic acid | R | R | R | R | R | R | R | R | R | R | 100 |
| Fusidic acid | S | R | S | R | R | R | R | R | R | R | 80 |

R: Resistant, S: Senstive.

co-administered with an antibiotic may reduce the gastrointestinal side effects related to anti-biotic treatment but the risk due to transferable resistance genes to other commensalism bacteria is great.

## ACKNOWLEDGEMENTS

This work was supported by the Algerian Federation of Consumers.

### REFERENCES

Ammor MS, Bele ´n Flo ´rez A, Mayo B (2007). Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. Food Microbiol., 24: 559-570.

CA-SFM (2010). Comité de l'antibiogramme. Société Francaise de Microbiologie. www.sfm.asso.fr.

Danielsen M, Wind A (2003). Susceptibility of *Lactobacillus* spp. to antimicrobial agents. Int. J. Food Microbiol., 82: 1 –11.

Delgado S, Ana B, Baltasar M (2005). Antibiotic Susceptibility of *Lactobacillus* and *Bifidobacterium* species from the Human Gastrointestinal Tract. Curr. Microbiol., 50: 202–207.

Egervärn M (2009). Antibiotic resistance in *Lactobacillus reuteri* and *Lactobacillus plantarum*. Doctoral Thesis Swedish University of Agricultural Sciences Uppsala 2009.

Essid I, Medin M, Hassouna M (2009). Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat. Meat Sci., 81: 203-208.

Fuller R (2001). The Chicken Gut Microflora and Probiotic Supplements. J. Poult. Sci., 38: 189-196.

Gabriel I, Leconte M, Guillon J, Rideaud P, Moreau-Vauzelle C, Dupont C (2007). Individual variability of intestinal flora of the chicken Observed by Molecular Imprinting. Seventh Day of the Poultry Research, Tours(France), 28 and 29 March 2007.

Hammes WP, Hertel C (2006). The Genera *Lactobacillus* and *Carnobacterium*. Procaryotes, 4: 320-403.

Hanan T, Abbas H, Anu S, Juha A, Per E J (2007). Identification of the Most Abundant *Lactobacillus* species in the Crop of 1- and 5-Week-Old Broiler Chickens. Appl. Environ. Micrbiol., 73(24): 7867–7873.

Hummel AS, Hertel C, Holzapfel WH, Franz CM (2007). Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Appl. Environ. Microbiol., 73: 730-739.

Jiangrang L, Umelaalim I, Barry H, Charles H, John J, Maurer K, Margie D (2003). Diversity and Succession of the Intestinal Bacterial Community of the Maturing Broiler Chicken. Appl. Environ. Microbiol., 69: 6816–6824.

Reuben PD, Pulido T, Nabil BO, Rosario Lucas T, Hikmate A, Magdalena MC, Antonio G (2005). Resistance to antimicrobial agents in lactobacilli isolated from caper fermentations. Antonie van Leeuwenhoek, 88: 277–281.

Shalini M, Rameshwar SI (2005). Antibiotic resistance in food lactic acid bacteria—a review. Int. J. Food Microbiol., 105: 281– 295.

Tenover FC (2006). Mechanisms of Antimicrobial Resistance in Bacteria. Am. J. Med., 119: 3–10.

Zhoua JS, Pillidgec CJ, Gopal PK, Gill HS (2005). Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. Int. J. Food Microbiol., 98: 211 – 217.