

Antibiotic Resistance of Some Lactobacilli Isolated from the Digestive Tract of Broiler in Western Algeria

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Abstract: The lactobacilli of the digestive tract poultry are considered as a reservoir of genes for resistance to antibiotics. Lactobacilli colonizing the crop and intestines of broilers play an important role in maintaining the balance of the health of the animal. The current trend that aims to spread the use of lactobacilli as a probiotic in poultry is confronted by abuse as curative despite regulations and guidelines that prohibit the routine use of antibiotics for the treatment of microbial infections poultry. The study is conducted on the lactobacilli flora of broiler chickens in Western Algeria. Ten isolates from the crop and intestines broiler. Antimicrobial sensitivity test to ten different types of antibiotic were evaluated using the disc diffusion method. Inhibition zone diameter was measured of resistance or susceptibility.

Key words: Lactobacilli, antibiotics, broiler chickens, poultry infection, CMI, CMB, Algeria

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 (Mathur and Singh, 2005). Acquired antibiotic resistance, i.e., 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 via the food chain or within the gastrointestinal tract to pathogenic bacteria (Egervarn, 2009). The gastrointestinal tract microflora of poultry is a mixture of bacteria, fungi and protozoa but bacteria are the predominant microorganisms.

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 via down regulation of porin genes

(Tenover, 2006). Lactic acid bacteria may act as reservoirs of antibiotic resistance genes that can be transferred via the food chain or within the gastrointestinal tract to pathogenic bacteria (Egervarn, 2009).

The gastrointestinal tract microflora of poultry is a mixture of bacteria, fungi and protozoa but bacteria are the predominant microorganisms. Broiler chicken diets containing corn, sorghum, barley, oats or rye had various effects as corn- and sorghum-based diets increased numbers of *Enterococcus*, barley-based diet increased numbers of *Lactobacillus*, oats-based diet enhanced growth of *Escherichia* and *Lactococcus* and rye-based diet increased the number of *Streptococcus* in broiler chickens.

In poultry farming 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.

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). 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 (Zhou *et al.*, 2005).

MATERIALS AND METHODS

Bacterial cultures and growth conditions: The ten Lactic Acid Bacteria strains (LAB) in this study (Table 1) had been previously isolated from crop and intestine of broiler. The contents were suspended in MRS broth and incubated at 37°C for 18 h. After incubation, 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. Only the catalase negative colonies were picked up and cultured in MRS broth after incubation for 18 h at 37°C, cells were harvested by centrifugation (5000 g/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 (OD₆₀₀) of 0.1 (10⁸ cfu mL⁻¹).

Antibiotic susceptibility testing: Ten bacterial isolates were screened for antibiotic susceptibility. The Disc diffusion method was used according to the NCCLS instructions slightly modified and tested against ten antibiotics including: β -lactams group; penicillin G (6 μ g), ampicillin (10 μ g) and amoxycillin (30 μ g). Macrolide group: erythromycin (15 μ g), spiramycin (100 μ g), clindomycin (2 μ g), lincomycin (15 μ g). Aminosids group: gentamicin (10 μ g), naxilidic acid (30 μ g) fusidic acid (10 μ g). 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 discs were verified by *E. coli* ATCC 25922 reference strain as

quality control. Resistance and susceptibility were evaluated according to CASFM. In the 2nd part of the study, four antibiotics obtained from commercial sources, i.e., penicillin G, amoxicillin, ampicillin and gentamicin were determined using the reference Agar-dilution method recommended by the CA-SFM were used for the determination the MIC and MBC of some highly resistant strains. The final concentration ranges were 20, 40, 60, 80 and 100 μ g mL⁻¹.

The MICs were determined by microdilution method. The microtiter plates were incubated for 18 to 24 h at 37°C with a source of moisture to prevent dehydration. The MIC was determined by visual observation of the lowest concentration that yielded no visible growth. The MIC was defined as the concentration 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 AND DISCUSSION

The isolates were gram positive and all were homofermentaire, catalase negative, grew at 45°C, produce NH₃ from arginine, resist to 63°C for 30 min. The carbohydrate fermentation profiles of the isolates (Table 1) were affected according to Hammes and Hertel (2006). These results are similar with the results found by Abbas Hilmi *et al.* (2007). According this researcher, 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%), *Lactobacillus crispatus* (18.7%) and *Lactobacillus salivarius* (13.3%). In similar study, Lu *et al.* (2003) were found that the lactobacillus genera form 98% of the microbiota of ileum. The strain *Lactobacillus salivarius* LC1 is resistant to all antibiotics

Table 1: Carbohydrates fermented of isolated strains

Isolates	Carbohydrates										
	Galactose	Lactose	Maltose	Mannose	Mannitol	Raffinose	Salicin	Sucrose	Trehalose	Melibiose	Cellibiose
LbC1	+	+	+	-	+	+	-	+	+	+	-
LbC2	+	+	+	+	-	+	+	+	+	+	+
LbC3	+	+	+	+	-	+	+	+	-	+	+
LbC4	+	+	+	+	-	-	+	+	-	-	+
LbC5	-	-	+	+	-	+	+	+	+	-	+
LbI 1	+	+	+	+	-	-	+	+	-	-	+
LbI 2	+	+	+	-	+	+	-	+	+	+	-
LbI 3	+	+	+	+	-	+	+	+	+	+	+
LbI 4	+	+	+	+	-	+	+	+	-	+	+
LbI 5	+	+	+	+	-	-	+	+	-	-	+

LbC1: *Lactobacillus salivarius*, LbC2: *Lactobacillus johnsonii*, LbC3: *Lactobacillus gallinarum*, LbC4: *Lactobacillus crispatus*, LbC5: *Lactobacillus aviarus*, LbI1: *Lactobacillus crispatus*, LbI2: *Lactobacillus salivarius*, LbI3: *Lactobacillus acidophilus*, LbI4: *Lactobacillus gallinarum*, LbI5: *Lactobacillus crispatus*

tested but susceptible to amoxicillin, lincomycin and fusidic acid. *Lactobacillus jonshonii* LC2 is susceptible to penicillin, amoxicillin and clindomycin and resistant to the others antibiotics. *Lactobacillus gallinarum* LC3 is resistant to all antibiotics tested except Clindomycin and fusidic acid. *Lactobacillus crispatus* LC4 is susceptible both to amoxicillin and spiramycin. *Lactobacillus aviarus* LC5 is resistant to all antibiotics tested (Table 2 and 3).

The isolates from intestines were resistant to all antibiotics except *Lactobacillus gallinarum* LI4 was susceptible to ampicillin and *Lactobacillus crispatus* LI5 was susceptible to erythromycin. The isolates from intestines as indigenous microflora are most resistant than the isolates crop because their exposition and adaptation for long time to antibiotics. All the isolates were resistant to nalidixic 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. *Lactobacillus salivarius* LC1 and *Lactobacillus gallinarum* LC3 were showed susceptibility to fusidic acid. Zhou *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, metronidazole, nitrofurantoin, norfloxacin, streptomycin, sulphadiazine, teicoplanin and vancomycin.

All the isolates were resistant to gentamicin. Similary, Pulido *et al.* (2005) found that some isolates of lactobacilli were inhibited by gentamicin. Toomey were found that resistance among lactobacilli isolates appeared to vary between species.

Most of the observed resistances seemed to be intrinsic but some others could be compatible with transmissible determinants (Delgado *et al.*, 2005). Although, lactobacilli are generally susceptible to antibiotics that inhibit the synthesis of protein such as erythromycin and tetracycline (Ammor *et al.*, 2007; Essid *et al.*, 2009), we have found that all strains were resistant to erythromycin except *Lactobacillus crispatus* (LI5).

The determination of MICs shows that the all strains tested have an acquired resistance (Table 4). Knowledge of the distributions 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 (Egervarn, 2009). For ampicillin MICs of 20-80 $\mu\text{g mL}^{-1}$ for amoxicillin MICs of 40-60 $\mu\text{g mL}^{-1}$ for penicillin MICs of 20-40 $\mu\text{g mL}^{-1}$ were obtained (Table 4). This is in contrast to the common opinion of lactobacilli being susceptible to penicillins

Table 2: Measure of diameter inhibition zone (mm) of each isolate

Antibiotics	Strains									
	LbC1	LbC2	LbC3	LbC4	LbC5	LbI1	LbI2	LbI3	LbI4	LbI5
Erythromycin	11	10	12	0	0	10	10	9	0	25
Gentamicin	10	9	0	0	0	8	8	0	0	0
Penicillin	12	18	0	16	0	12	12	0	16	10
Amoxicillin	25	30	10	20	0	15	0	15	0	10
Lincomycin	20	8	0	0	0	0	8	0	10	10
Ampicillin	0	14	0	10	9	10	8	0	20	0
Spiramycin	10	10	0	20	0	0	12	0	17	9
Clindomycin	0	19	20	0	0	8	9	12	0	14
Nalidixic acid	0	0	0	0	7	0	0	0	0	0
Fusidic acid	21	12	30	0	0	0	12	0	17	9

Table 3: antibiotic susceptibility profiles of test strains

Antibiotics	Isolates from crops					Isolates from intestines				
	LbC1	LbC2	LbC3	LbC4	LbC5	LbI1	LbI2	LbI3	LbI4	LbI5
Erythromycin	R	R	R	R	R	R	R	R	R	S
Gentamicin	R	R	R	R	R	R	R	R	R	R
Penicillin	R	R	R	R	R	R	R	R	R	R
Amoxicillin	S	S	R	S	R	R	R	R	R	R
Lincomycin	S	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	S	R
Spiramycin	R	R	R	S	R	R	R	R	R	R
Clindomycin	R	S	S	R	R	R	R	R	R	R
Nalidixic acid	R	R	R	R	R	R	R	R	R	R
Fusidic acid	S	R	S	R	R	R	R	R	R	R

Table 4: Determination of CMI_s ($\mu\text{g mL}^{-1}$) and CMB_s ($\mu\text{g mL}^{-1}$) of some inherent isolates

Antibiotics	Isolates	CMI	CMB
Gentamicin	LbC3	40	80
	LbC4	40	80
	LbC5	60	100
	LbI 3	60	80
	LbI 4	40	80
Amoxicillin	LbI 5	40	60
	LbC5	60	100
	LbI2	40	80
	LbI4	40	60
	LbC1	60	80
Ampicillin	LbC3	60	80
	LbI3	20	40
	LbI5	80	100
Penicillin G	LbC3	20	60

in general (Danielsen and Wind, 2003; Hummel *et al.*, 2007). Finally, antibiotics resistance of *Lactobacillus* could also be regarded as a beneficial property. A resistant probiotic strain that is co-administered with an antibiotic may reduce the gastrointestinal side effects related to antibiotic treatment (Courvalin, 2006). By identifying strains with potentially non-transferable resistance genes, this field of application might gain wider acceptance and thus have a greater impact in the future.

CONCLUSION

All the isolates were resistant to Spiramycin and Gentamicin. Except one isolate is susceptible to Erythromycin; three are susceptible to Amoxicillin; one to Ampicillin; one to Penicillin G and one to Clindomycin. The Minimum Inhibitory Concentration (CMI) and the Concentration Minimum Bactericide (CMB) were evaluated for each strain resistant toward the most antibiotics employed for therapeutic purposes in broiler chickens in West Algeria.

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