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Transferring of Lactobacillus antibiotic resistant genes to Salmonella

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Abstract

Antibiotic resistance is a worldwide issue and becoming more problematic due to extensive misuse of antibiotics. The present study was aimed to analyze role of *Lactobacillus* in transmission of antibiotic resistance genes (*tet*M, *erm*B, *sul*2) to *Salmonella* and verification of these genes by real time polymerase chain reaction. A total of thirty fecal samples (15 were indigenous and 15 were broilers) were collected and analyzed by real time polymerase chain reaction. The results indicated that there was high expression of antibiotic resistance genes in *Lactobacillus* in case of broiler chicken than indigenous ones indicating *Lactobacillus* as a reservoir of antibiotic resistance genes but found to be non-significant in transferring these genes to *Salmonella*. In conclusion, the excessive use of animal growth promoters in poultry assists in acquisition of antibiotic resistance genes by normal micro-biota.

Key words: Broiler, Non-significant, Antibiotic resistance, Real time polymerase chain

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1. INTRODUCTION

Salmonella enterica is a zoonotic pathogen and transfers from animal to humans with the consumption of animal products, contaminated meat, or other contaminated food products[1]. It has been reported that about 1,335 foodborne outbreaks and 36,490 food related illnesses occur due to *Salmonella enteric*[2]. Tetracycline resistance in *Salmonella* is attributed due the efflux pump that expels tetracycline out from the cell[3]. Whereas resistance against sulfonamide in *Salmonella* has been recognized due to the presence of *sul* genes which states an insensitive type of dihydrofolate synthetase[4].

Antibiotic resistance is a global issue and because of this, bacteria are becoming more resistant against antibiotics by developing such tools of antibiotic resistance such as: enzymatic degradation, target modification and target substitution[5]. This could either due to mutation or acquirement of a particular antibiotic resistance (AR) gene by horizontal transfer[6]. The accessibility of antibiotics to be used for treatment of infectious diseases has considerably improved the human health and animal welfare. But massive misuse of antibiotics causes the development of antimicrobial resistance in commensals and pathogenic bacteria[7].

Lactobacillus species isolated from meat and fermented milk products have offered resistance to tetracycline, vancomycin and erythromycin[8]. In pathogenic or commensal strains, AR genes produce an indirect risk for animals and humans and as well as the enlargement of gene pool for different other pathogenic and exogenous bacteria to grab them to advance antibiotic resistance. There are multiple

pathways of antibiotic resistance genes transfer including conjugation and transformation as well as transduction. Horizontal gene transfer (HGT) of antimicrobial resistance genesis significantly enhanced by plasmids, transposons and integrons[9]. The AR genes frequently exist on mobile elements like integrons, plasmids and transposons[10].

It has been reported that possible increase of resistance happened because of excessive use of antibiotics[11]. The incidence of AR genes; *erm*(B)and *tet*(M) for erythromycin and tetracycline respectively in *Lactobacilli* represents the most pervasive determinants of resistance, Moreover, in genetic linkage of *Lactobacillus paracasei,* both these genes were frequently stated[12]. The current study is intended for the evaluation of *Lactobacillus* role in transmitting the AR genes to *Salmonella*.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The following research was conducted in Institute of Microbiology University of Agriculture, Faisalabad. A total of fifteen samples were taken from three indigenous poultry birds (5 samples from each chicken) and fifteen samples from three broiler birds (5 samples from each chicken). Total thirty fecal samples were taken from the colon region of birds for the isolation of *Lactobacillus* and *Salmonella*. Bead containing collection tubes were used for the collection of all the samples. FavorPrep Stool DNA Isolation Mini Kit was used for gDNA isolation. With the help of Nano drop, exact DNA concentration was assessed using 1000 spectrophotometer Thermo scientific®. According to the requirement of PCR master mix, upto 50 ng/µl DNA was utilized in master mix as a DNA template[13].

2.2PCR Amplification and Gel electrophoresis

Lactobacillus and *Salmonellae* were detected by PCR amplification of DNA samples using primers

(**LAA;** F: CATCCAGTGCAAACCTAAGAG, R:GATCCGCTTGCCTTCGCA & **Inv-A;** F: CGGTGGTTTTAAGCGTACTCTT, R:CGAATATGCTCCACAAGGTTA) by a micro processed controlled swift Maxi thermal cycler block (Esco technologies Inc. France). The amplified products were then visualized on 1% agarose gel[14].

2.3 Real Time PCR (q-RT-PCR):

This was done for analysis of three antibiotic resistance genes named as *Tet*M, *erm*B and *Sul*2 using primers Table1 and protocol followed by Haarman and Knol[15]. The steps followed were: For q-RT-PCR, dNTPs up to 0.5µL were mixed with F101 buffer up to 2.5µL. 0.5µL of forward primer and 0.5µL reverse primer as well as amplified gDNA in a100ng⁄µl concentration was used. After addition of 0.25µL Taq polymerase and 25µL ddH_2O, following conditions were provided to run PCR. PCR cycling parameters were as follows: Initial denaturation for 1 min 1 cycle at 95°C, denaturation for 30 sec 28 cycles at 95°C.

Table 1. Primers for Erythromycin (*ermB*), Sulfonamide (*sul2*), Tetracycline *tet*(M), *Lactobacillus* (LAA), *Salmonella* invasion protein (Inv-A) and for Hyper-variable region (V2).

Genes	Primers Sequences (5'-3')
ermB	F: TGGTATTCCAAATGCGTAATG R: CTGTGGTATGGCGGGTAAGT
sul2	F: GCAGGCGCGTAAGCTGA R: GGCTCGTGTGTGCGGATG
***tet* M**	F: CGAACAAGAGGAAAGCATAAG R: CAATACAATAGGAGCAAGC

Annealing for 20 sec 28 cycles at 58°C, extension for 1 min 28 cycle at 72°C, elongation for 5 min 1 cycle at 72°C and finally kept on at 4°C. Materials used for q-RT PCR were placed under the UV light for 30 min. The gDNA up to 20ng/µL was required for each reaction and primers were used in dilution at a ratio of 1:20

from a solution of 5µM. For each reaction, master mix of 5µL Dynamo Flash and ROX up to 0.25µL was added into each valve. After that, 10 µL of HPLC water was added, the mixed reaction mixed was used for q-RT-PCR. For q-RT-PCR, 20ng/µL gDNA was needed for each reaction and primers at a dilution of 1:20 from a 5 µM solution.

For each reaction ROX up to 0.25 µL and master mix of Dynamo Flash up to 5µL were put into each valve. Then, HPLC water up to 10µLwas put into the reaction mixture and used for q-RT-PCR. Initial denaturation was done for 10 min at 95°C (1 cycle) and again denaturation for 10 second at 95°C (40 cycles). Annealing for 20 sec at 60°C (40 cycles), extension for 2 min 35 sec at 72°C (40 cycle), melting curve for 15 sec at 95°C, melting curve at 60°C + 0.3°Cup to 95°C.The q-RT-PCR analysis was based on the mean CT values.

3. RESULTS AND DISCUSSIONS

This study was aimed to observe the presence of antibiotic resistance genes in the colon region of indigenous and broiler chicken and to assess the role of *Lactobacillus* in transmission of these genes to *Salmonella.*

3.1 PCR analysis of *Lactobacillus*

The detection of *Lactobacillus* was made confirm by presence of LAA gene consisting of 250 bp Fig. 1. While appearance of 796bp band yielded from Inv-A primer amplification confirmed the presence of *Salmonella* Fig.2.

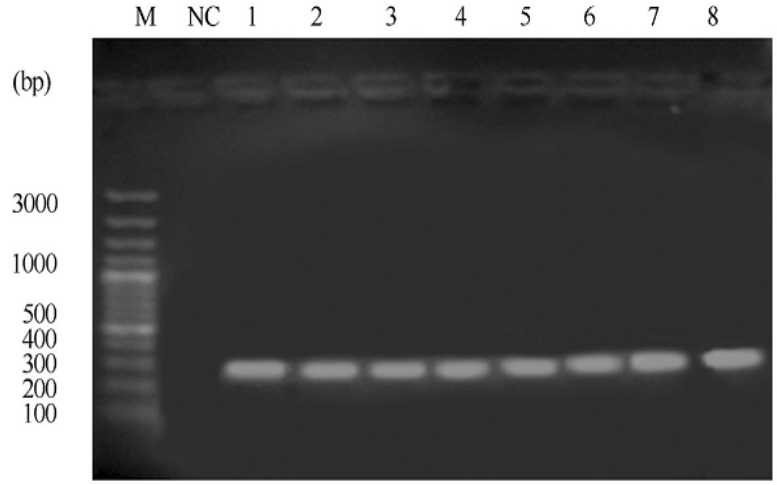

Fig. 1. PCR analysis of *Lactobacillus*. Lane M: marker of 3000 bp, Lane NC: negative control, other lanes indicate the bands for LAA gene (250 bp) of *Lactobacillus*.

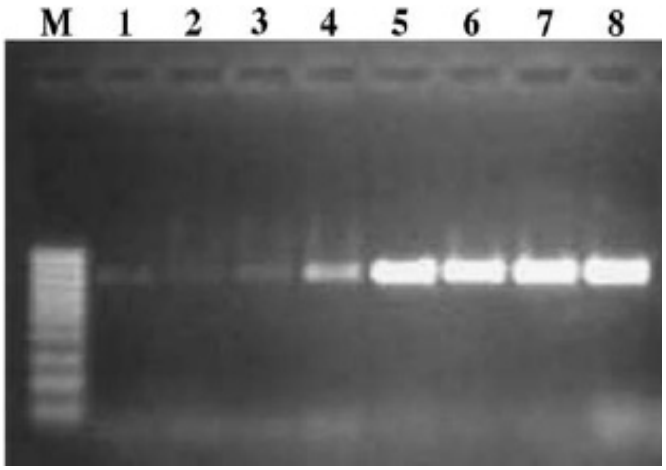

Fig. 2. PCR analysis. Lane M: marker of 1kb, Lane 1: negative control, other lanes indicate the bands for InvA gene (796 bp) of *Salmonella*.

3.2 *Tet* M gene expression level

The q-RT-PCR expression of *tet*M demonstrated that the expression of *tet*M in *Lactobacillus* in case of broiler chicken sample was higher as compared to their expression in *Salmonella* and indigenous chicken representing the role of *Lactobacillus* as a reservoir of *tet*M gene but the results were non-significant to explain the transfer of *tet*M gene from *Lactobacillus* to *Salmonella*. The q-RT-PCR results are also indicating the higher expression of *tet*M gene in broiler as compared to indigenous chickens which may be due to the excessive use of antibiotic growth promoters in broiler chickens Fig.3 and Table2.

Table 2. CT values of *tet* M gene in *Lactobacillus*, *Salmonella*, Indigenous and Broiler.

	Indigenous	**Broiler**
Lactobacillus	0.0315	5.16
Salmonella	0.00385	0.0039

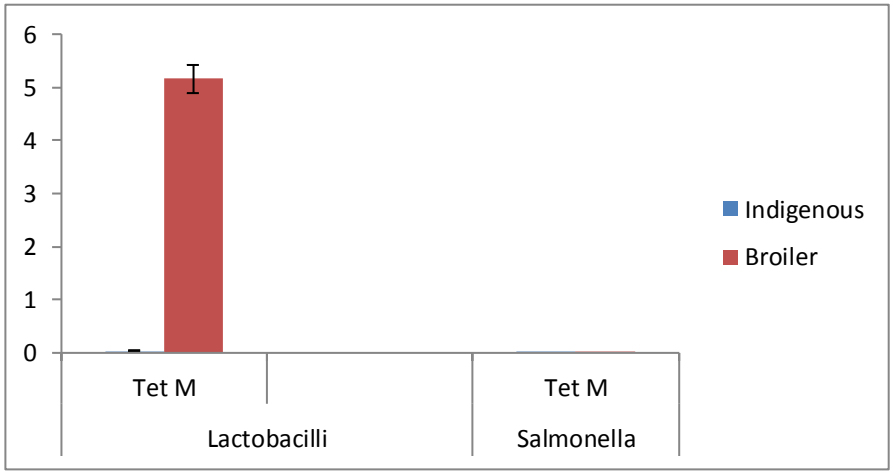

Fig. 3. *Tet* M gene expression level in indigenous and broiler samples.

3.3 *erm* B gene expression level

The q-RT-PCR results of *erm*B gene showed the expression of this gene in *Lactobacillus* and *Salmonella* in case of indigenous and broiler chickens indicating *Lactobacillus* as a reservoir for *erm*B gene. The transfer of these genes to *Salmonella* has been non-signified due to low *erm*B gene expression in *Salmonella* in case of broiler and indigenous chicken Fig.4 and Table3.

Table 3. CT values of *erm* B gene in *Lactobacillus*, *Salmonella*, Indigenous and Broiler.

	Indigenous	**Broiler**
Lactobacillus	0.0155	4.18
Salmonella	0.00495	0.0083

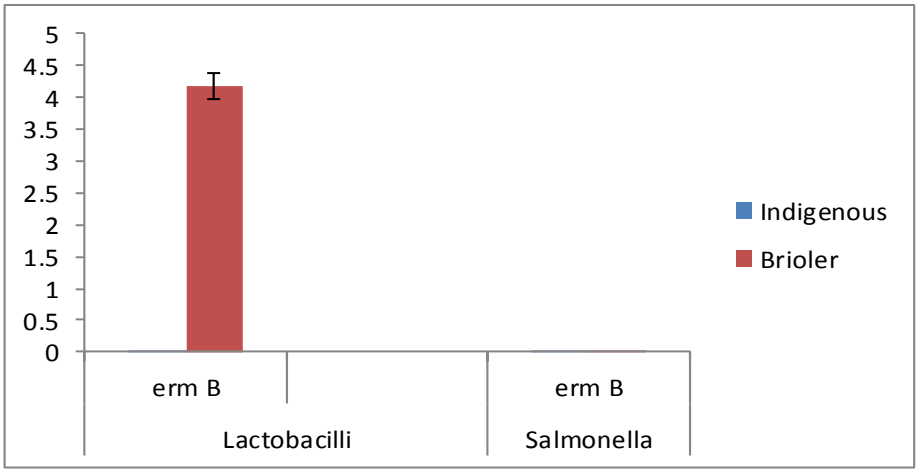

Fig. 4. *erm* B gene expression level in indigenous and broiler samples.

3.4 *Sul* 2 gene expression level

The *sul*2 results revealed its expression in *Lactobacillus* and *salmonella* in indigenous as well as in broiler chickens. The higher expression of *sul*2 gene in *Lactobacillus* of broiler chickens characterizes that the *lactobacillus* in broiler chickens serves as a reservoir for *sul 2* gene Fig.5 andTable4.

Table 4. CT values of *sul* 2 gene in *Lactobacillus*, *Salmonella*, Indigenous and Broiler.

	Indigenous	**Broiler**
Lactobacillus	0.0265	3.06
Salmonella	0.00155	0.006

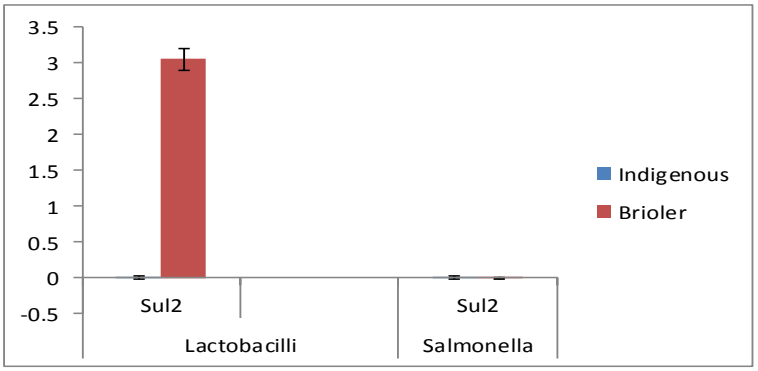

Fig. 5. *Sul* 2 gene expression level in indigenous and broiler samples.

3.5 CT values of AR genes

The analysis of CT values for different AR genes in q-RT-PCR represents that the *tet*M gene is showing the highest expression among the three antibiotic resistance genes Fig.6. It was found that the most frequent antibiotic resistance gene was *tet*M followed by *erm*B and *sul*2. The q-RT-PCR results of present study exhibited that *Lactobacillus* serves as a reservoir of antibiotic resistance genes due to presence and higher expression level of AR genes (*tet*M, *sul*2 and *erm*B) in *Lactobacillus* in broiler chicken.

The transmission of antibiotic resistance genes to *Salmonella* was proved to be non-significant because of the very lower CT values and expression of antibiotic resistance genes in *Lactobacillus* in case of indigenous and in *Salmonella* in case of indigenous and broiler chicken specimens. Similar work was performed by[16] who detected tetracycline genes (*tet*M) from *Lactobacillus* isolates. Detection of antibiotic resistance

pattern mediated by *tet*M and *tet*L genes in different *Enterococcus* bacteria was also demonstrated[17]. The susceptibility of *Lactobacillus paracasei* to tetracycline and erythromycin and found that this bacterium may act as reservoir for transferring antibiotic resistance genes to other pathogenic bacteria[12].

In a previous study detection of *erm*B and *tet*M genes from different lactic acid bacteria isolates of fermented foods was determined[18]. The present study is closely related to the previous study in which role of *Lactobacillus* species in transferring *tet*M and *erm*B genes to other *Enterococcus* species was investigated[19]. The results of the present research is also closely related to the previous research in which the role of antibiotic growth promoters in the acquisition of antibiotic resistance genes in commensals and pathogenic bacteria to develop antibiotic resistance was described[5].

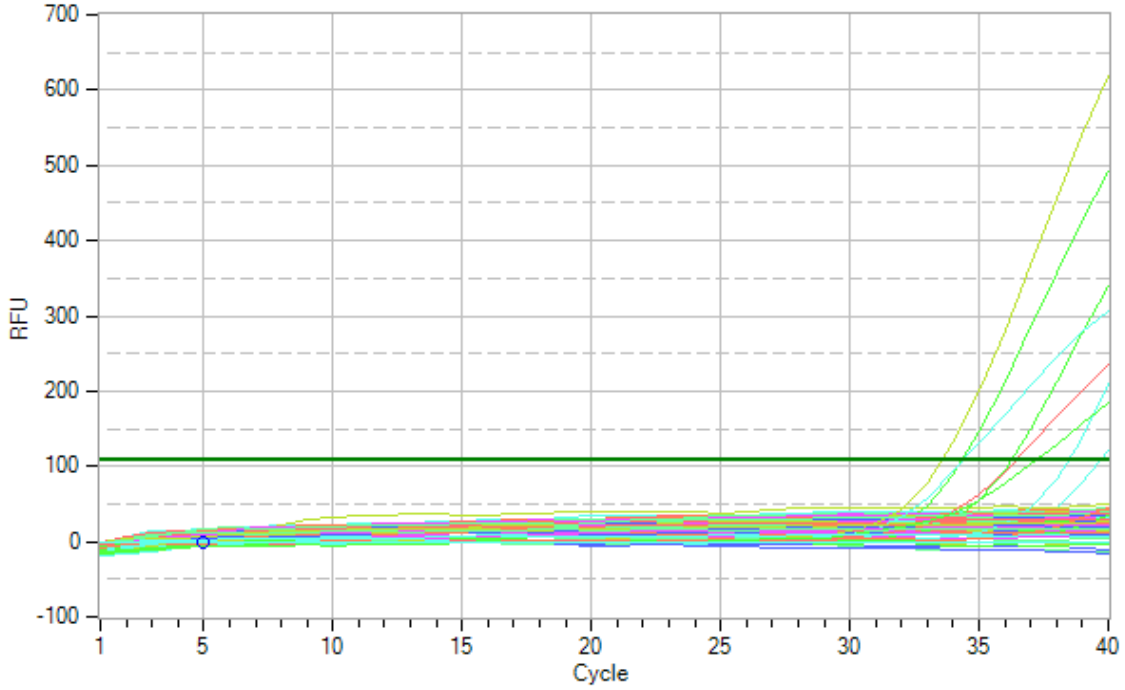

Fig. 6. CT values of AR genes and their expression level in indigenous and broiler samples.

4. CONCLUSIONS

It is concluded that the excessive use of animal growth promoters in poultry assists in acquisition of antibiotic resistance genes by normal micro-biota and hence, development of antibiotic resistance in these strains. The study may assist to offer the future aspects to discover the potential role of horizontally transmission of antibiotic resistance genes from *Lactobacillus* to other pathogenic bacteria.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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