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Molecular analysis of tetracycline resistant gene in gram-negative bacteria isolated from dairy farms

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Abstract

The present study was conducted from April 2019 to June 2019 in order to detect the tetracycline A resistant Gene in gram negative bacteria. A total of 40 buffalo's milk samples were collected randomly by aseptic technique, brought to laboratory. They were inoculated on Blood and MacConkey agars and then incubated at 37°C for 24 hours whereby growth of colonies were further confirmed with catalase test, Coagulase test, Oxidase test, Gram staining and API 20 E kit. Bacterial DNA was isolated using the boiling method. The Tet A gene (210 bp) was amplified in thermal cycler and run on 1.8-gram agarose gel with 50 kb ladder. The most predominant bacterial colonies observed were of Escherichia coli (10 (33.3%) followed by Klebsiella pneumonia 5 (16.7%), Klebsiella spp. 5 (16.7 %), Pseudomonas spp. 10 (33.3%) and prevalence of tetracycline A gene was 8 (26.7%).

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Introduction

Tetracycline is a group of broad-spectrum antibiotics that fights against infectious diseases caused by bacteria. Tetracycline helps to treat different bacterial infections of intestines, skin, respiratory tract, urinary tract, lymph nodes, genitals, and other body systems [1]. Tetracycline as a drug was introduced commercially in 1978 in World Health Organization List of Essential Medicines [2]. These suppress protein synthesis of bacteria by preventing the cooperation between aminoacyl RNA and bacterial ribosome [3]. Tetracycline antibiotics interact with target molecules to traversal the membrane system of susceptible organisms, e.g. gram-negative bacteria. Tetracyclines are broad-spectrum antibiotics, with efficiency against both; gram-positive and gram-negative bacteria. Generally, they work by inhibiting protein synthesis via restricting the function of ribosomal subunits [4]. Several tetracycline-resistant genes have been classified and characterized into different groups according to the mode of resistance that may include development of new ribosome protective genes in plasmids, or gene alterations that affect membrane porins, inactivate the drug molecule or 16S rRNA [5, 16]. Resistant microbes and resistive antibiotic genes exchange easily between ecosystems [6]. Antibiotic-resistant bacteria and disease-causing microbes act as pathogens and their property of being resistant creates a major health issue for human beings. In late 1970s, the main issue of disease-causing microbes was the identification of antibiotic-resistant pathogens [7]. Increase in the number of gram-negative bacteria as antibiotic-resistant organisms, is a major concern for the biologists today. This resistance towards different antibiotics can emerge due to antibiotic alterations, inactivation of enzyme by antibiotics that decrease the antibiotic uptake and loss of antibiotic activity due to frequent use and huge doses [8]. Approximately 350 bacterial genes isolated from livestock feces show Tetracycline resistance [9]. The main purpose of this study was to identify bacteria from bovine milk and determine the resistance for tetracycline A genes among them.

Materials and Methods

The study was conducted in the Molecular Biology laboratory, Virtual University of Pakistan during the period April 2019 to June 2019. A total of 40 buffalo's milk samples were collected randomly from dairy farms within different areas of district Rawalpindi Pakistan by aseptic technique. The samples were brought to laboratory and placed in a refrigerator at 4^0C until analyzed and then thawed at room temperature when needed. Milk samples were centrifuged separately, pellets were streaked directly on Blood agar and MacConkey agar and incubated at 37°C for 24 hours as done by Derebe and Solomon, 2017 [10]. After incubation colonies were identified by Catalase test, Coagulase test, Oxidase test, Gram staining and API 20 E kit (Table 1). DNA was extracted by boiling method as performed by Acharya *et al.* 2017 [11]. The TetA gene (210 bp) was amplified by using forward primer (5'-GCT ACA TCC TGC TTG CCT TC-3') and reverse primer (5'-CAT AGA TCG CCG TGA AGA GG-3') as previously used by Villedieu et al. 2003 [12]. The reaction mixture consisted of Taq polymerase (5 IU), dNTPs (0.2mM each), PCR buffer (100mM), 0.5mM forward and reverse primers. The steps of PCR included initial denaturation at 95 °C for 5 min and 35 cycles of amplification consisting of 30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C, with 10 min at 72 °C for the final extension. The PCR product was run on 1.8 % agarose gel with 50 kb ladder. The PCR product was observed using Gel Doc system.

Results and Discussion

Out of 40 samples, 30 (75%) showed bacterial growth while 10 (25%) did not show any growth of bacteria. Out of 30 samples, 8 (26.7%) were positive for tetracycline A (**Fig. 1**) and the most predominant bacteria were *Escherichia coli* 10 (33.3%), *Klebsiella pneumoniae* 5 (16.7%), *Klebsiella spp.* 5 (16.7 %), *Pseudomonas spp.* 10 (33.3%) (**Table 1**).

Table 1: Isolated microorganisms and tetracycline A percentage.

Microorganism Isolated	Number (%)	Tetracycline A (%)
Escherichia coli	10 (33.3 %)	4 (50%)
Klebsiella pneumonia	05 (16.7 %)	2 (25 %)
Klebsiella spp.	05 (16.7%)	1 (12.5 %)
Pseudomonas spp.	10 (33.3 %)	1 (12.5 %)
Total	30	8 (26.7 %)

In a study conducted in West Bengal, *tet (A)* gene resistance was observed in 5 samples (10%), which is in correspondence with the results of our study as it was 8 (26.7 %) in our samples [13]. Current study showed tetracycline A resistance in *E. coli* which correlates with the study conducted in 2015 on antimicrobial resistance by Skočková et al., 2015 [14]. Raw milk showed a high prevalence of tetracycline

resistant bacteria in the study of Chinese Tianjin University of Science and Technology [15]. The current study showed that tetracycline resistant A gene is common in gram negative bacteria isolated from milk of different dairy farms.

Table 2: Table indicating Biochemical tests performed on isolated colonies.

Biochemical tests	*E. coli*	*K. pneumonia*	*Klebsiella spp.*	*Pseudomonas spp.*
Indole test	Positive	Negative	Positive	Negative
Methyl red test	Positive	Negative	Positive	Negative
Voges Proskauer test	Negative	Positive	Positive	Negative
Citrate test	Negative	Positive	Positive	Positive
Oxidase Test	Negative	Negative	Negative	Positive
Catalase test	Positive	Positive	Positive	Positive
Coagulase test	Negative	Negative	Negative	Negative
H_2S test	Negative	Negative	Negative	Negative
Urease test	Negative	Positive	Negative	Negative
Glucose fermentation	Positive	Positive	Positive	Negative
Lactose fermentation	Positive	Positive	Positive	Negative

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Authors Contributions

Yasir Ali and Qurat-ul-Ain Ali Hira performed experiments, Ayesha Maqbool and Yasir Ali conceived and designed the study and wrote the manuscript. Tanveer Hussain reviewed the manuscript critically.

Conflict of interest

The authors declare no conflict of interest.

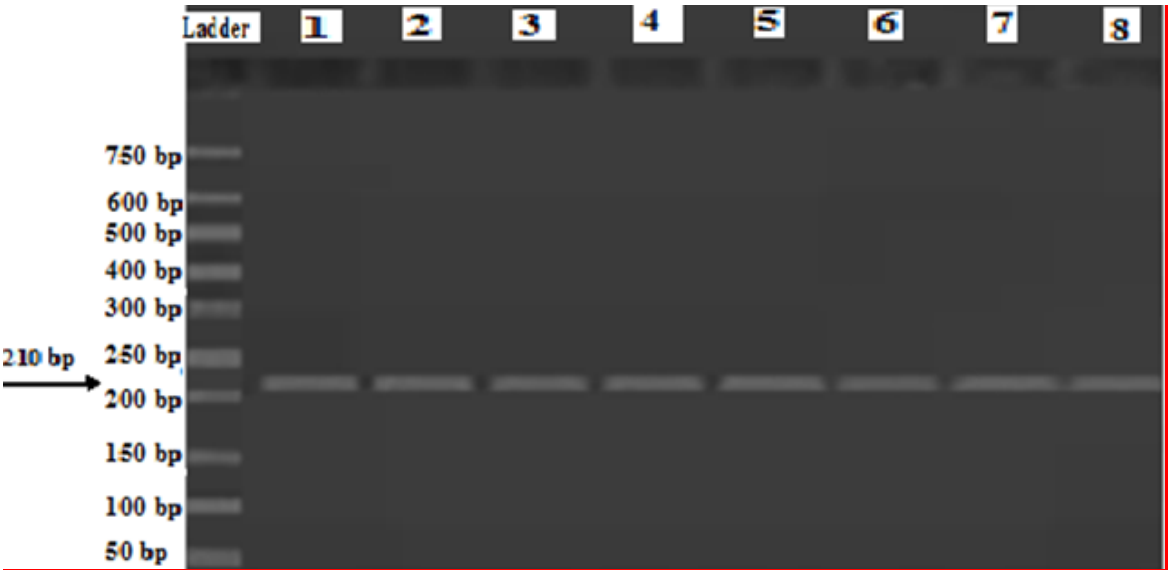

Fig. 1: Tetracycline A gene (210bp) with 50bp ladder indicated by agarose gel electrophoresis

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