



Multidrug Resistant Bacteria From Organic Chicken



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Abstract

Common bacteria associated with chicken and poultry include Salmonella, Enteritidis, *Staphylococcus aureus*, *Campylobacter jejuni* and *Listeria monocytogenes*¹. Poultry flocks are often raised under intensive care and large amounts of antimicrobials to promote growth and prevent the development of these bacteria. These conditions lead to selection of antibiotic resistant bacteria. However, if poultry are raised without the use of antibiotics it should not lead to the selection of antibiotic resistant bacteria. If antimicrobial resistant bacteria develop in chicken and other poultry, it may cause a threat to human health² From 1995 to 2016, consumption of chicken increased from 28.0 to 93 billion pounds per capita in America. The aim of this experiment was to explore whether a major store brand of organic chicken has antibiotic resistant bacteria on their whole chickens. To investigate the type of bacteria found in the whole chicken, we cultured bacteria under selective and non-selective media such as TSA, EMB and MSA plates. We then cultured the bacteria with twelve antibiotic discs to check for the presence of antibiotic resistant bacteria. Antibiotic resistance was measured based on the zones of inhibition on the Mueller-Hinton plates. To identify the bacteria found, we performed a gram stain, metabolic testing using Biolog plates, isolated the DNA and amplified the 16S rDNA before sequencing it.

Introduction

Poultry flocks are often raised under intensive care and large amounts of antimicrobials to promote growth and prevent the development of these bacteria. Conversely, if antimicrobial resistant poultry develop in chicken and other poultry, it results in treatment failure and a threat to human health. Exposure to these bacteria may lead to diarrhea and infection which may be fatal because of bacterial resistance to antibiotics. Many organic brands such as Natures Promise, Harvestland, Purdue, Coleman and Whole Foods claim that the poultry they provide to consumers contain no antibiotics, no animal byproducts in feed and no supplemental growth hormones. For this experiment, we've chosen the Natures Promise whole chicken³. Natures Promise is a brand that uses no antibiotics, no growth hormone, and no artificial ingredients in its feed. It is well established that antibiotic resistance is driven by the overuse and abuse of antibiotics. However, there are examples of antibiotic resistance arising not due to selective pressures. For example, some bacteria found in caves have shown antibiotic resistance. This study is meant to explore the presence of antibiotic resistant bacteria that have not been exposed to antibiotics.

Methods

Step 1: To investigate whether Natures Promise chicken has antibiotic resistant bacteria, we obtained their whole chicken.

Step 2: Next, we swabbed the chicken with a sterile cotton swab dipped in distilled water and spread the swab over three different selective and non selective media: TSA, MSA, EMB. Bacterial colonies were selected to streak on to Mueller Hinton plates. Each media contained three trails to ensure that the data collected was accurate.

Step 3: On the Mueller-Hinton plates, we used twelve different antibiotic filter discs such as penicillin, amoxicillin and colistin to test for antibiotic resistant bacteria.

Step 4: The zones of inhibition for each disc was measured to assess if the bacteria was resistant, somewhat resistant or sensitive.

Step 5: The bacteria that demonstrated multi-drug resistance was selected for further study. It was gram stained and tested using Biolog metabolic testing plates.

Step 6: Afterwards, DNA was isolated from the bacterial colonies that demonstrate multi-drug resistance, and the DNA was amplified through PCR using specific primers.

Step 7: Once the DNA has been amplified, gel electrophoresis was performed, the DNA was sequenced and DNA subway will be used to blast samples and see the data collected.

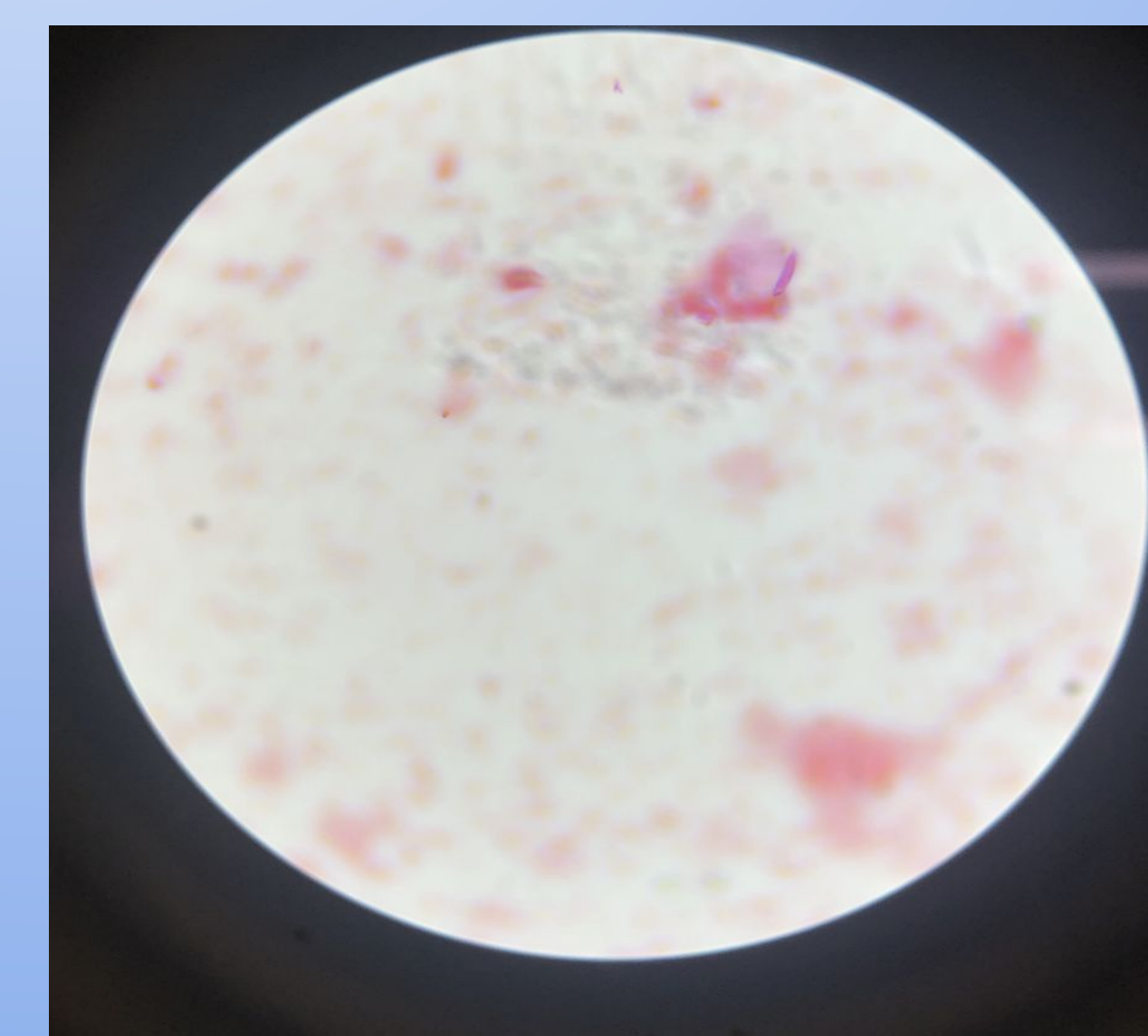
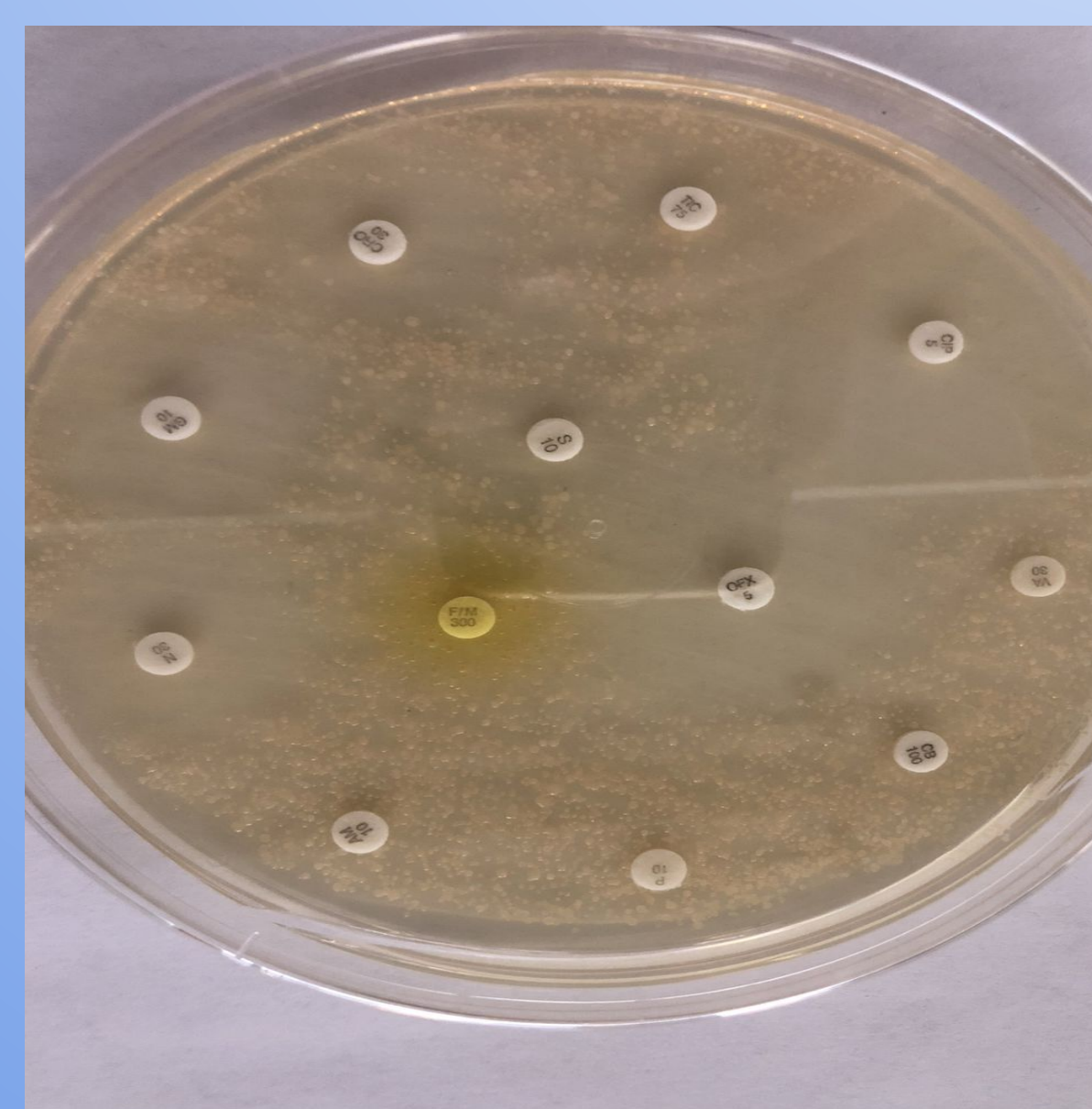
Methods (CONT.)

Step 7: We analyzed the biochemical traits of the bacteria and performed another round of PCR.

Step 8: Gel electrophoresis was performed and the DNA was also sequenced to determine the bacteria.

Data

Code	Zones of Inhibition			Name	Class of Antibiotic
CB 100	0	0	0	Carbenicillin 100mg	Penicillin
P 10	0	0	0	Penicillin 10mg	Penicillin
AM 10	0	0	0	Ampicillin 10mg	Penicillin
N 30	18mm	17mm	17mm	Neomycin 30mg	Aminoglycoside
GM 10	17mm	17mm	18mm	Gentamicin 10mg	Aminoglycoside
CRO 30	0	0	0	Ceftriaxone 30mg	Cephalosporin
TIC 75	0	0	0	Ticarcillin 75mg	Penicillin
CIP 5	27mm	27mm	25mm	Ciprofloxacin 5mg	Fluoroquinolone
VA 30	0	0	0	Vancomycin 30mg	Glycopeptide
OFX 5	21mm	22mm	25mm	Ofloxacin 5mg	Fluoroquinolone
F/M 300	0	0	0	Nitrofurantoin 300mg	Nitrofurantoin
S 10	0	0	0	Streptomycin 10mg	Aminoglycoside



Discussion

Based on the sizes of the zones of inhibition, the bacterium isolated was resistant to the antibiotics carbenicillin, penicillin, ampicillin, ceftriaxone, ticarcillin, vancomycin, nitrofurantoin, and streptomycin. This is significant because these antibiotics are classified into five different classes: penicillin, cephalosporin, glycopeptides, nitrofurantoin, and aminoglycosides. Blasting the 16S rDNA sequence resulting in high sequence similarity with *Yersinia entomophaga* and *Yersinia enterocolitica*. The Biolog identification was *Yersinia enterocolitica*. Because the results are not definitive because there are additional *Yersinia* species with significant similarities, further testing needs to be done. The resistance demonstrated by the bacteria on the whole chicken causes concerns because *Yersinia* species do cause human disease.

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