Biosis: Biological Systems (2020) 1(2): 85-90
https://doi.org/10.37819/biosis.001.02.0059

ORIGINAL RESEARCH

An Investigation of Antibacterial Resistance Patterns in Isolated Bacteria from Contaminated Water Samples in Poultry Slaughterhouses

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ABSTRACT

Nowadays, in the poultry industry, antibiotics are used to treat, prevent, and enhance poultry growth and production efficiency. Their irregular consumption has resulted in the spread of antibiotic-resistant bacteria in this industry. Antibiotic-resistant bacteria in contaminated waters can be transmitted into soil. The purpose of this study was to investigate the antibiotic resistance pattern of bacteria isolated from the water of chicken slaughterhouses around Hamadan (Iran) province. In this study, 20 water samples were collected from four slaughterhouses in Hamadan province (during spring and summer 2019). Initial isolation and identification of the bacteria were performed by pour plate culture and biochemical tests. The disc diffusion method was applied to investigate the resistance pattern. This study presents 109 screened isolates. Of these, 57.8% *E.coli*, 35.7% *Salmonella* spp., and 6.42% *Klebsiella* spp. were detected. Antibiograms of isolates showed that in *E.coli*, 23.09% were resistant to four types of the antibiotic tetracycline, amoxicillin, gentamicin, and chloramphenicol, 76.19% had only one type of antibiotic. Antibiotics for *Salmonella* spp. showed that 35.9% were resistant to tetracycline, gentamicin, and chloramphenicol, 64.10% to only one type of antibiotic. Also, in *Klebsiella* spp., 85.71% were sensitive to antibiotics, and only 14.28% were resistant to tetracycline. Conclusion: The results showed that the rate of multiple antibiotic resistance is relatively high, and contaminated water has a high potential for soil contamination. Therefore, resistant bacteria become more stable in the environment, and the health of the environment will be endangered. Therefore, it is necessary to study the antimicrobial resistance patterns of bacteria to study and maintain the health of the environment.

ARTICLE HISTORY

Received 24 May 2020
Revised 3 June 2020
Accepted 8 June 2020

KEYWORDS

Antibiogram
Resistant Bacteria
Slaughterhouse
Water

Introduction

Today, in human societies, poultry and poultry farming are considered an essential industry to provide the protein sources needed by the human population. In the poultry industry, antibiotics are used to treat, prevent, and increase the efficiency of poultry growth and production (use in the diet) (Donoghue, 2003). The use of antibiotics in this industry has caused increasing concern among consumers due to the creation of drug residues in food products (Ferguson et al., 2005). In recent decades, the rising use of antibiotics has led to the spread of resistant genes, resulting in increased antibiotic resistance of bacteria, which has led to a

reduction in the effectiveness of drugs (Johnson et al., 2005). Antibiotic-resistant bacteria have become widespread due to the overuse of antibiotics. One of the issues considered a severe threat to the industry is bacterial disease. Losses, growth reduction, production reduction, the removal of carcasses from the environment, and the high cost of treatment are among the significant damage to the poultry industry due to bacterial diseases (Joseph et al., 2001). The high rate of presence of some resistant bacteria such as *E. coli* in contaminated water and soil has been linked to this industry as a result of reduced antibiotic efficacy in resistant bacteria (Khan et al., 2014). Bacterial contaminations are spread in different ways, including through contaminated water in the environment (especially in the soil) and can contaminate both humans and poultry. It is estimated that in the USA, approximately 50% of antibiotics given to animals are without the presence of disease, and the damage caused by improper and inappropriate use of antibiotics is sometimes higher than the damage caused by the disease itself (Van den Bogaard et al., 2001).

Recent studies have reported the emergence of multiple resistance patterns and an increase in the number of resistant bacteria (Wajid et al., 2019). Among resistant bacteria, the *Enterobacteriaceae* family showed the highest rate of antibiotic resistance. *E. coli*, and *Salmonella* spp. have the highest frequency. *E. coli* often causes gastrointestinal infections in humans and mammals, and in domestic birds, it causes widespread or local non-gastrointestinal infections such as airway infection, Septicemia, and colibacillosis (Álvarez-Fernández et al., 2012).

Due to the prevalence of resistant bacteria in human societies, antibiotic resistance is of particular importance in terms of public health. This study aimed to investigate the antibiotic resistance patterns of contaminant bacteria of poultry slaughterhouses. Control and identification of the source of contamination in the spread of resistant bacteria is one way to deal with this problem. Due to the uncontrolled use of antibiotics in the poultry industry, and due to the recycling and use of poultry in the community, the transmission of resistant bacteria to the environment and humans is done. Slaughterhouses are one of the centers that easily allow this type of bacteria to enter the environment through drinking water. Water is one of the most critical pollutants due to its fluidity. Water easily contaminates soil and air and stabilizes pollution circulation in the ecosystem. Therefore, it is vital to study water pollution and prevent the spread of contamination with appropriate solutions. Thus, in this study, the antibiogram test is valuable to identify the patterns of antibiotic resistance and to prescribe and use antibiotics correctly.

Materials and Methods

Sample collection

To collect water samples, according to sampling standards, these were taken during spring and summer 2019 from the water of the consumption sections of four slaughterhouses in Hamadan Province (Iran). The physical and chemical specifications of the water sample are listed in Table 1. After sample collection, samples were transferred to the laboratory and glycerol stock of isolates stored at a 4ºC for further investigation.

Microbial culture and screening of bacterial isolates

To screen isolates, Nutrient Agar (Merk, Germany) culture medium was used. Thus, 20 ml of autoclaved culture medium, reduced to a temperature of 60ºC, was poured into sterile Petri dishes, and then 1 ml of each of the water samples was added to the culture medium. Bacterial cultures were cultured using Pour Plate and incubated at 37ºC for 24 h. The pure culture was provided from each of the existing colonies.

Biochemical and microbial identification of bacterial isolates

Macroscopic properties (shape and size of colonies) and microscopic properties (Gram staining) were used to identify isolates. Biochemical tests including MR-VP (Merk, Germany), TSI (Merk, Germany), SIM (Merk, Germany), Bailey Scoline (Merk, Germany), citrate, and culture on McConkey (Merk, Germany), EMB (Merk, Germany), *Salmonella-Shigella* (Merk, Germany), and Blood Agar, as well as nitrate reduction tests, were performed.

Antibacterial test by Kirby Bauer method

In order to determine the sensitivity and antibiotic resistance of screened isolates we used five high-consumption antibiotics common to Iran's poultry industry. The Disc diffusion test was performed on the Kirby Bauer method. In this study, was used tetracycline (30 micrograms), amoxicillin (10 micrograms), erythromycin (15 micrograms), gentamicin (10 micrograms) and chloramphenicol (30 micrograms). Antibiograms were performed according to the CLSI M100-S29 standard (CLSI, 2019). Briefly, bacteria inoculated in the of Muller-Hinton broth (0.5

McFarland 1.5×10^8 CFU) were cultured on the surface of Muller-Hinton Agar. Antibiotic discs were placed at regular intervals on the inoculated culture medium and incubated for 24 hours at 37ºC $A = \pi r^2$. After incubation, we used a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used. Finally, the resistance and sensitivity of each isolate were compared with the global CLSI standard (CLSI, 2019).

Table 1. The physical and chemical characteristics of the water samples (25ºC temperature).

Test type	Unit	Slaughterhouse 1	Slaughterhouse 2	Slaughterhouse 3	Slaughterhouse 4
pH		8.0	7.8	8.0	8.0
COD	mg/l	924	924	918	922
BOD	mg/l	451	452	455	451
DO	mg/l	2.1	2.0	2.2	2.1
Phosphate	mg/l	33.2	33.0	33.0	33.4
Nitrate	mg/l	83.4	83.0	82.8	83.2
Chloride	mg/l	439	430	417	433
Sulfate	mg/l	150	150	146	155
Ammonium	mg/l	60.2	59.8	60	60.7
TSS	mg/l	284	301	296	279
TDS	mg/l	2040	2040	2043	2036
Turbidity	NTU	397	397	397	~396
Sulfite	mg/l	0.9	0.89	0.75	0.92
Sulfide	mg/l	1.1	0.98	1.25	1.0

Statistical analysis

The obtained data were analyzed using the SPSS 26 version and Chi-square test at a significance level of $p \leq 0.05$.

Results and Discussion

In this study, 109 bacterial isolates were isolated. According to the present results, were reported that 63 cases (57.8%) of *E.coli*, 39 cases (35.7%) of *Salmonella* spp., and 7 cases (6.42%) of *Klebsiella* spp. Balakrishnan *et al*. (2018) studied the prevalence of *Salmonella* spp., in chicken meat and its slaughtering place from local markets in Orathanadu. A prevalence of 33.3% (5/15) and 60% (3/5) *Salmonella* was observed from chicken meat and water samples, respectively. (Balakrishnan et al., 2018).

Antibiogram test of isolates showed that in isolated *E. coli*, 76.19% (48 cases) resisted only one type of antibiotic and 23.09% (15 cases) resisted 2, 3, 4, or 5 antibiotics. The results of the discs diffusion test in *E. coli* showed in Tables 2 and 3, respectively. The chi-square test showed that the difference in the resistance and sensitivity of the isolates to the studied drugs was significant ($p\leq0.05$). The highest antibiotic resistance was observed against tetracycline. The highest and lowest frequency of the multi-antibiotic resistance of the isolates was 46.03% and 12.7%, respectively, which was significant statistically (Table 3). Seifi *et al*. (2015) examined antibiotic sensitivity and resistance of isolated *E.coli* in Mazandaran (Iran). They reported to the highest percentage of resistance to tetracycline (71.25%), erythromycin (65%), ampicillin (62.5%), fluoxetine (7.5%) and Florfenicol (7.5%), respectively. (Seifi et al., 2015).

Table 2. Resistance frequency of 63 isolated *E. coli* relative to only one type of antibiotic.

	Antibiotics	Resistant (%)	Sensitive (%)
1	Tetracycline	62 (98.41%)	1 (1.59%)
2	Amoxicillin	55 (87.30%)	8 (12.7%)
3	Erythromycin	59(93.65%)	4(6.35%)
4	Gentamicin	47(74.60%)	16(25.40%)
5	Chloramphenicol	46(73.01%)	17(26.99%)

Salmonella spp. Antibiogram test showed that 64.10% (25 cases) resisted only one type of antibiotic (Table 4), 35.9% (14 cases) resisted tetracycline, gentamicin, and chloramphenicol (Table 5). Among the 4 antibiotic resistance patterns, 25.64% of the isolates were the common pattern of number 1, 12.82% of the isolates were in the common pattern of number 2, 5.12% of the isolates were in the common pattern of number 3 and 56.41% of the isolates were in the common pattern of number 4. The highest and lowest frequencies of the multi-antibiotic resistance of the isolates were 56.41% and 5.12%, respectively, which was significant statistically ($p\leq0.05$). Shang *et al*. (2019) investigated the distribution, antimicrobial susceptibility patterns, serotypes, and genotypes of *Salmonella* spp. isolates from a slaughterhouse. Their results indicated that the highest antimicrobial resistance rate was against

nalidixic acid (92.7%), that 56.7% isolates were multidrug-resistant (MDR), and that the resistance profile of 20.7% isolates was relative to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (Shang et al., 2019).

Table 3. Multi-Antibiotic resistance patterns of 63 isolated *E. coli* relative to studied antibiotics.

Num. of patterns	Num. of Antibiotics	Resistance to antibiotics	Num. of isolated *E. coli*
1	2	TE- E	8 (12.7%)
2	3	TE- Gen- AMX	13 (20.63%)
3	4	TE- Chl- Gen- E	29 (46.03%)
4	5	TE- Chl- Gen- E- AMX	13 (20.63%)

Note: AMX: Amoxicillin; TE: Tetracycline; E: Erythromycin; Gen: Gentamicin; Chl: Chloramphenicol.

Also, in the case of *Klebsiella* spp., 85.71% were sensitive to amoxicillin, erythromycin, gentamicin, and chloramphenicol antibiotics, and only 14.28% were resistant to tetracycline. The results showed that among these isolates, also were observed cases with multiple resistances. Savin *et al*. (2020) reported that wastewater effluents from the poultry slaughterhouses exhibited clinically relevant bacteria (*E. coli* (39.4%), methicillin-resistant *S.aureus* (12.3%), *K. pneumonia* (10.8%), and species of the ACB (32.4%), which were widely distributed in their wastewater effluents (Savin et al., 2020).

The results showed that tetracycline resistance was present in screened isolates in this study. Tetracycline is one of the common antibiotics used as a dietary supplement and antibiotic in the poultry industry. Due to the excessive use of tetracycline, an increase in the appearance of resistant strains of this antibiotic is expected (Donoghue, 2003).

In this study, samples of water contaminated with *E. coli*, *salmonella* spp., and *Klebsiella* spp. have strains that show multiple antibiotic resistances, which pose a serious threat to the environment. Zhu *et al*. (2017) reported antimicrobial susceptibility profiles of The *Salmonella* isolates to 10 categories of antimicrobial agents using the Kirby–Bauer disc diffusion Method that were observed: nalidixic acid (99.5%), ampicillin (87.8%), tetracycline (51.9%), ciprofloxacin (48.7%), trimethoprim/ sulfamethoxazole (48.1%), and spectinomycin (34.4%). Antimicrobial resistance profiling showed that 60.8% of isolates were multidrug-resistant (MDR), and MDR strains increased from 44.7% to 78.6% along the slaughtering line (Zhu et al., 2017).

Table 4. Resistance frequency of 39 isolated *Salmonella* spp. relative to only one type of antibiotic.

	Antibiotics	Resistant (%)	Sensitive (%)
1	Tetracycline	31 (79.48%)	8 (20.52%)
2	Amoxicillin	17 (43.58%)	22 (56.42%)
3	Erythromycin	14(35.90%)	25(64.1%)
4	Gentamicin	9(23.07%)	30(76.93%)
5	Chloramphenicol	28(71.8%)	11(28.2%)

Table 5. Multi-Antibiotic resistance patterns of 39 isolated *Salmonella* spp. relative to studied antibiotics.

Num. of patterns	Num. of Antibiotics	Resistance to antibiotics	Num. of isolated *Salmonella* spp.
1	2	TE- Chl	10 (25.64%)
2	2	TE- Gen	5 (12.82%)
3	2	Chl- Gen	2 (5.12%)
4	3	TE- Chl- Gen	22 (56.41%)

Note: TE: Tetracycline; Gen: Gentamicin; Chl: Chloramphenicol.

Conclusions

Results show a high rate of multiple antibiotic resistance was observed in screened isolates. In slaughterhouses, because water is used to slaughter and wash carcasses, and the water is in direct contact with poultry, their pollution is high, and unfortunately, there is not enough management to control the outflow water, so the transfer of isolates to the environment outside the slaughterhouse occurs, and water can transmit these contaminants to the environment (especially soil) and make resistant bacteria more stable in the environment, thus endangering the health of the environment. Therefore, it is necessary to study the antibiotic resistance profiles of bacteria to monitor for antibacterial resistance and proper use of antibiotics in slaughterhouses, and it is also essential to preserve the health of the environment.

Acknowledgment

This project was funded by Malayer University, Malayer, Iran. The author thanks the biology department of Malayer University.

Conflict of interest

The author declares that she has no conflict of interest.

References

Álvarez-Fernández, E., Alonso-Calleja, C., García-Fernández, C., & Capita, R. (2012). Prevalence and antimicrobial resistance of Salmonella serotypes isolated from poultry in Spain: comparison between 1993 and 2006. *International Journal of Food Microbiology*, *153*(3), 281-287. https://doi.org/10.1016/j.ijfoodmicro.2011.11.011

Balakrishnan, S., Sangeetha, A., & Dhanalakshmi, M. (2018). Prevalence of Salmonella in chicken meat and its slaughtering place from local markets in Orathanadu, Thanjavur district, Tamil Nadu. *Journal of Entomology and Zoology Studies*, *6*(2), 2468-2247.

CLSI. (2019). *Performance standards for antimicrobial susceptibility testing; twenty-ninth informational supplement. CLSI document M100-S29*. Clinical and Laboratory Standards Institute, Wayne, PA.

Donoghue, D. J. (2003). Antibiotic residues in poultry tissues and eggs: human health concerns? *Poultry Science*, *82*(4), 618-621. https://doi.org/10.1093/ps/82.4.618

Ferguson, J., Baxter, A., Young, P., Kennedy, G., Elliott, C., Weigel, S., . . . Sharman, M. (2005). Detection of chloramphenicol and chloramphenicol glucuronide residues in poultry muscle, honey, prawn and milk using a surface plasmon resonance biosensor and Qflex® kit chloramphenicol. *Analytica Chimica Acta*, *529*(1-2), 109-113. https://doi.org/10.1016/j.aca.2004.11.042

Johnson, J. R., Kuskowski, M. A., Smith, K., O'Bryan, T. T., & Tatini, S. (2005). Antimicrobial-resistant and extraintestinal pathogenic Escherichia coli in retail foods. *The Journal of Infectious Diseases*, *191*(7), 1040-1049. https://doi.org/10.1086/428451

Joseph, S., Hayes, J., English, L., Carr, L., & Wagner, D. (2001). Implications of multiple antimicrobial-resistant enterococci associated with the poultry environment. *Food Additives & Contaminants*, *18*(12), 1118-1123. https://doi.org/10.1080/02652030110051275

Khan, M., Akhtar, N., Haque, M., Barua, A., Chowdhury, T., & Mullick, R. (2014). Isolation and identification of nonplasmid multidrug resistant E. coli from poultry wastes in Chittagong region. *Bangladesh. Journal of Bacteriology and Parasitology*, *5*(1), 1-7. http://dx.doi.org/10.4172/2155-9597.1000182

Savin, M., Bierbaum, G., Hammerl, J. A., Heinemann, C., Parcina, M., Sib, E., . . . Kreyenschmidt, J. (2020). ESKAPE bacteria and extended-spectrum-β-lactamase-producing Escherichia coli isolated from wastewater and process water from German poultry slaughterhouses. *Applied and Environmental Microbiology*, *86*(8). http://dx.doi.org/10.1128/AEM.02748-19

Seifi, S., Khoshbakht, R., & Atabak, A. (2015). Antibiotic susceptibility, serotyping and pathogenicity evaluation of avian Escherichia coli isolated from broilers in northern Iran. *Bulgarian Journal of Veterinary Medicine*, *18*(2). https://dx.doi.org/10.15547/bjvm.819

Shang, K., Wei, B., Jang, H.-K., & Kang, M. (2019). Phenotypic characteristics and genotypic correlation of antimicrobial resistant (AMR) Salmonella isolates from a poultry slaughterhouse and its downstream retail markets. *Food Control*, *100*, 35-45. https://doi.org/10.1016/j.foodcont.2018.12.046

van den Bogaard, A., London, N., Driessen, C., & Stobberingh, E. (2001). Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*, *47*(6), 763-771. https://doi.org/10.1093/jac/47.6.763

Wajid, M., Saleemi, M. K., Sarwar, Y., & Ali, A. (2019). Detection and characterization of multidrug-resistant Salmonella enterica serovar Infantis as an emerging threat in poultry farms of Faisalabad, Pakistan. *Journal of Applied Microbiology*, *127*(1), 248-261. https://dx.doi.org/10.1016/j.theriogenology.2019.08.034

Zhu, Y., Lai, H., Zou, L., Yin, S., Wang, C., Han, X., . . . Zhou, K. (2017). Antimicrobial resistance and resistance genes in Salmonella strains isolated from broiler chickens along the slaughtering process in China. *International Journal of Food Microbiology*, *259*, 43-51. https://doi.org/10.1016/j.ijfoodmicro.2017.07.023