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Environmental Impact of Poultry Shop Solid Waste as a Reservoir of Pathogenic Bacteria in Samawah City, Iraq

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Abstract

Chicken farms and shops generate a lot of organic waste in the form of feathers, blood, intestines and other types of biological waste. If chicken waste is not disposed of correctly it could lead to severe environmental pollution and microbial contamination. Therefore, the objectives of the study were two fold: to determine the bacterial contamination found in the waste generated from feathers of commercial poultry; and to determine the antibiotic susceptibility profiles of the various bacteria isolated from poultry feathers. Microbiological techniques were used to isolate and identify bacteria present in samples taken from the feathers of broilers and layers produced in commercial facilities. Six genera of bacteria were identified as present in the analysed feather samples – Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp., Micrococcus spp., and Enterobacter spp. In addition to identifying the isolates, antimicrobial susceptibility testing was performed on each of the isolated bacterial species to determine their susceptibility to a variety of commonly used antibiotics. The results indicate that the isolated bacterial species exhibited a variety of responses when exposed to the different classes of antimicrobial agents. Some bacteria demonstrated susceptibility to specific antibiotics but had resistance against a variety of other antibiotics. The results show that there is a presence of potentially pathogenic or antibiotic-resistant bacteria in poultry waste and the importance of correct poultry waste disposal management and hygienic practices when disposing of poultry waste. Incorrectly disposed poultry waste may help spread infectious microorganisms and cause pollution of the environment. To summarize, these results provide further evidence that effective waste management systems, adequate hygiene measures, and control of antibiotic use in poultry production are critical to reducing environmental contamination and protecting human health from the potential microbial hazards associated with poultry waste.

Introduction

Feathers, blood residue, viscera, bones, and bedding material make up the solid waste produced by poultry stores, and this waste consists of a variety of organic substances. Poultry wastes are characterised by their high moisture content, rapid decomposition rates, and nutrient-rich components, including organic carbon, nitrogen and phosphorus. These characteristics make poultry waste attractive to agricultural and energy industries because of their potential value but also make them prone to microbial decomposition, which results in the production of toxic substances if they are not properly managed (Kelleher and Cantrell, 2002). Poultry retail operations in urban settings are often small in size and tend to be located in high-density areas. Waste generated from these operations is often dumped in open areas, along roadsides and streets, or in drainage ditches because of inadequate infrastructure and weak enforcement of regulations that govern waste disposal (Ribaudo and Gollehon, 2006). Improper waste disposal creates suitable conditions for the spread of microorganisms, insects and rodents, which serve as vectors for disease. Research shows that poultry litter is a source of many disease-causing organisms, including several species of Salmonella, E. coli, and other gastrointestinal bacteria which can have a detrimental effect on the health of humans (Heederik et al., 1991; Gržinić et al., 2023). Poultry litter also produces gases through decomposition, including ammonia (NH_3), hydrogen sulfide (H_2S), and methane (CH_4). The release of these gases has been proven to contribute to air pollution and can result in irritation to the respiratory system, headaches, and a variety of other health issues for those who live close to where poultry litter is dumped. Chronic exposure to these gases can lead to the exacerbation of chronic respiratory diseases and lower the overall air quality of urban neighbourhoods (Ritz et al., 2004; Parihar et al., 2019). Waste management systems in developing areas such as Iraq typically do not adequately address organic waste from small commercial outlets (e.g., chicken shops). The inadequate manner in which poultry waste is harvested, managed and treated has led to substantial environmental damage and increased risks to the health of the public (Al-Mohammed et al., 2021) . A lack of knowledge about proper disposal methods has also contributed to the improper disposal of poultry waste, creating a source of pollution for the environment and a threat to public health. Poultry shops in Samawah produce a large amount of poultry waste every day; much of this waste is disposed of improperly (e.g. thrown out into streets, next to the poultry shop where it was produced and at unfit locations). As a consequence, the improper disposal of poultry waste has created an unsanitary environment with unpleasant smells and has attracted a large number of insects and rodents, which pose a health risk to owners, employees and other people living or working around the shop. Lastly, with the continued expansion of the poultry industry coupled with improper disposal methods, there is growing concern about the effect of poultry waste on human health and the quality of the environment. Pollution of the air and contamination of surface and groundwater are just two of the many problems that poultry shops (Gržinić T., et al., 2023) are causing to the cities and communities where they exist. The growing demand from the community in the city of Samawah for poultry has led to many new poultry shops being established without any improvements to the waste management systems. The imbalance that exists between effectively managing poultry waste and providing sanitary products to the community warrants a better understanding of poultry waste, its effects on humans and the overall environment, and that the research study will contribute to disease-prevention efforts related to poultry waste, as well as provide experts-with the necessary information to create effective strategies against the transmission of the diseases associated with poultry waste, thereby improving poultry waste management in the future. Conduct an analysis of public health and environmental concerns related to solid waste discarded in Samawa City's poultry shops, including methods currently employed for handling and disposing of said waste; isolate/identify disease causing bacteria in discarded solid waste; and assess how much awareness exists amongst workers and residents who live near to the shops about disease transmission related to the solid waste generated by poultry shops.

Material and Methods

2.1 Chemicals and Biological Materials

The following chemical and biological materials were used in the experiments of our study:

Table 1. Chemical and biological materials were used in the experiments of the study

NO.	Materials	Company/ Origin
1	Oil Immersion	BDH (England)
2	Glycerol	BDH (England)
3	Urea Solution	Fluca (Switzerland)(
4	Physiological Normal Saline	Alfaiha (Jordon)
5	McFarland standard solution	Biomerieux (France)
6	Gram stains	SYRBIO (S.A.R.)
7	Ethanol (95%)	Origin, S.a .l. (Lebanon)

2.2 Equipment's and Instruments

Table 2. Equipment's and Instruments with their origin.

NO	Materials	Company / Origin
1	Petri Dishes	China
2	Slides	China
3	Cover Slides	China
4	Ependroff tubes	Germany
5	Test tubes	Jordon
6	Cylinders	China
7	Flasks	China
8	Pasture pipettes	China
9	Sterilized cotton swab	Jordon
10	Disposable syringe	China
11	Filter paper	China
12	Disposable surgical Gloves	Malaysia
13	Cotton	Jordon
14	Glass pencil	Germany
15	Centrifuge	Mlkro-200-Hettich , Germany
16	Hood	Lipshow, USA
17	Glass flasks and beakers	Marienfeld, Germany

2.3 Culture Media

Table 3. Culture media used in the present study.

NO	Materials	Company / Origin
1	Brain heart infusion broth	Oxoid / UK
2	Urinary tract infection Chromogenic Agar (UTIC)	Oxoid / UK
3	Muller Hinton agar	Oxoid / UK
4	Nutrient broth	Oxoid / UK
5	MacConkey agar	Oxoid / UK
6	Blood agar	Oxoid / UK

2.4 Preparation of Culture Media

The culture media included in this study were prepared according to the recommendations of the manufacturing company stated on the containers, and they were autoclaved at 121° C for 15 minutes, under 15 bar/in^2, and incubated at 37 $^{\circ}$ C for 24 hours to ensure their sterility and then kept at 4° C until they were used, these media.

2.5 Experimental Design

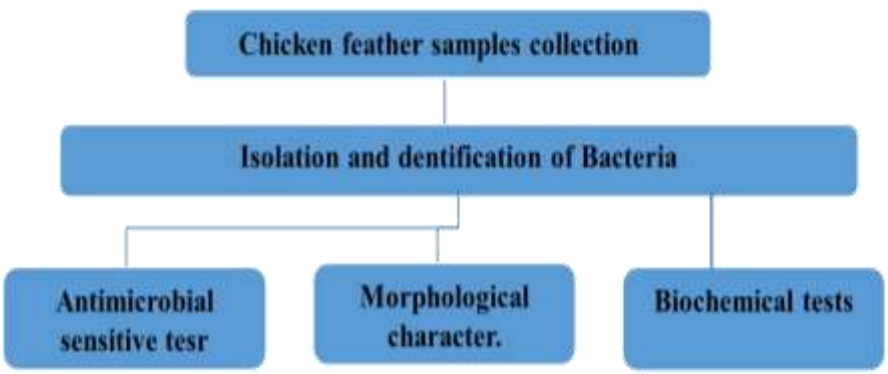


Figure 1. A schema of the experimental design

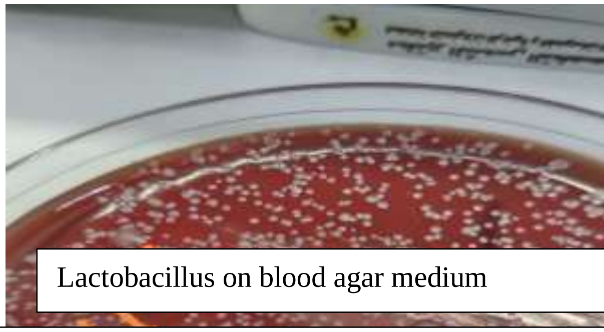
2.6 Sample Collection

A total of 20 chicken feather samples were collected from local poultry markets in Samawah city. These samples were placed in sterile containers and transported immediately to the laboratory for bacteriological analysis.

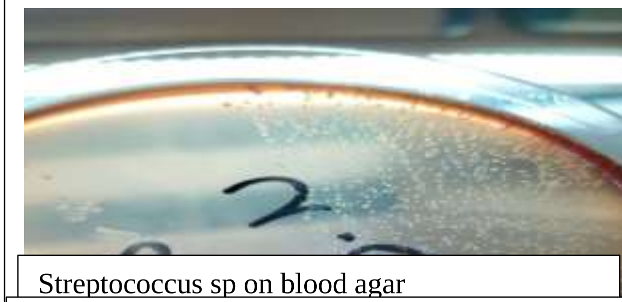
2.7 Isolation and identification of the bacteria

Culture plates containing MacConkey agar, blood agar, and UTIC agar were used to test each urine sample by spreading it directly onto a specifically labeled agar medium, followed by incubation for 24 hr. at 37°C.

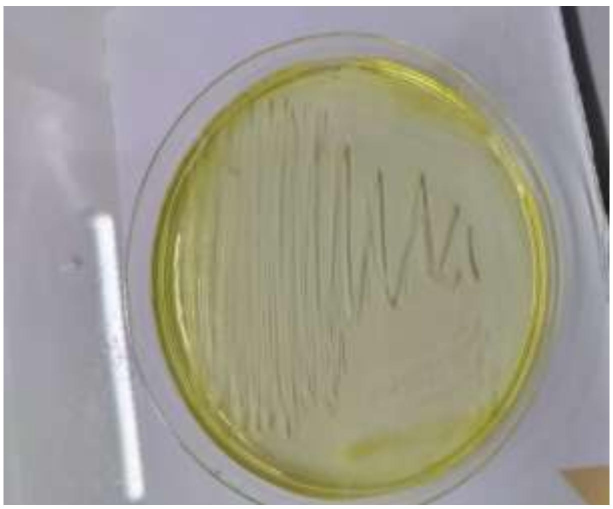
After incubation, the growth of bacteria was assessed on all culture plates (Senthilkumar et al., 2014), and bacteria isolated for identification per the UTIC media manual are listed in this section. **Figure 2.**

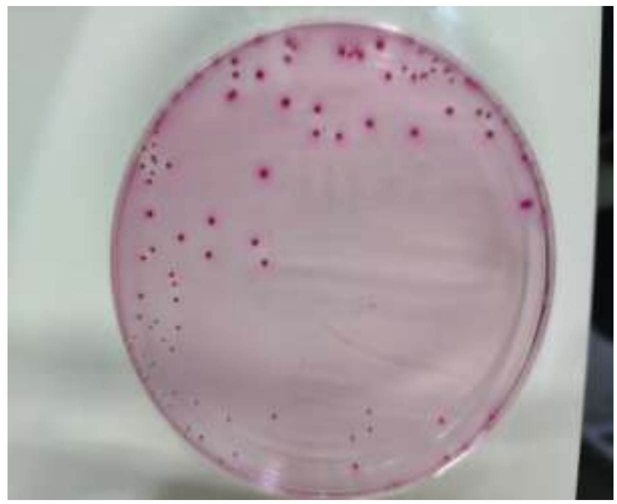


Lactobacillus on blood agar medium



Streptococcus sp on blood agar





Staphyloccus aureus on mannitol salt agar

lture N

E. coli in the middle of the MacConkey

2.8 Antibiotic susceptibility test

Using the Kirby-Bauer method, the antibiotic sensitivity of the pathogens was determined by testing for (AZM) Azithromycin; (TE) Tetracycline; (DOX) Doxycycline; (SXT) Trimethoprim-Sulfamethoxazole; (E) Erythromycin; (F) Nitrofurantoin; (VA) Vancomycin; (LZD) Linezolid; (LEV) Levofloxacin; (DA) Clindamycin; (RIF) Rifampin; (NOR) Norfloxacin, and the interpretation of results were done according to the CLSI guidelines. (Clinical and Laboratory Standards Institute, 2024).

Table 5. Antibiotics Used in the Antibiotic Susceptibility Test and Their Abbreviations

Antibatic	Symbol
Vancomycin	VA
Nitrofurantion	F
Chloramphenicol	C

Limpenem	lPM
Linezolid	LZ
Gentamicin	CN
Oflaxacin	OF
Levofloxacin	LE
Azithromycin	AZM
Clindamycin	DA
Teracycline	TE
Trimethoprim	TR
Erythromycin	E
Ciprofloxacin	CIP
Amikacin	AK
Norfloxacin	NOR
Tobramycin	TOB
Cefoxitin	FOX
Meropenem	MEM
Ampicillin	AMP
Amoxicillin/Clavylaniac acid	AMC
Aztreonan	ATM
Ceftriaxone	CRO
Nalidixic acid	NA
Netilmicin	NET

Results and Discussion

3.1 Results

Antibiotic susceptibility testing for E. coli isolates demonstrated 100% resistance to some antibiotics (tetracycline, cefoxitin, ampicillin, amoxicillin-clavulanic acid, ceftriaxone, and aztreonam). However, E. coli was found to be very susceptible to five others (ciprofloxacin, levofloxacin, meropenem, cefotaxime, and ofloxacin) and it is likely that patients who carry these strains have a much higher risk for acquiring multidrug-resistant E. coli..

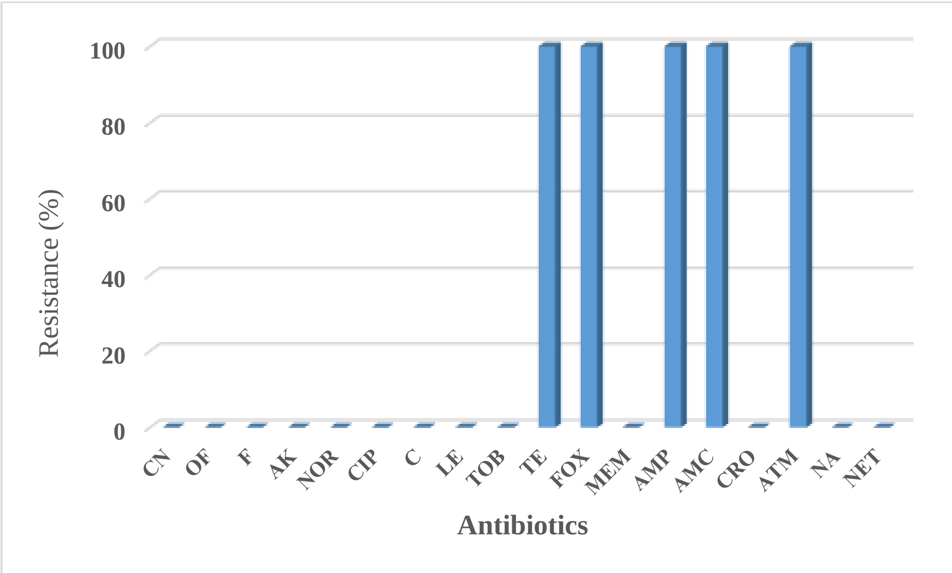


Figure 3. Antibiotic resistance pattern of E. coli isolates against different antimicrobial agents.

Figure 4 The Readable Toxicity Profile: Micrococcus spp. isolates' antibiotic susceptibility ln respect to different antibiotic classes have been completed. Altogether, the isolates from Micrococcus spp. were 100% resistant (all the isolates from Micrococcus spp. are 100 % resistant) to azithromycin (AZM) /clindamycin (DA). /trimethoprim (T R) and are therefore an excellent example of an antimicrobial agent which has not produced a resistance reaction with Micrococcus isolates Further, except for the three antibiotics mentioned above, all the isolates from Micrococcus spp. were sensitive (showing no or little resistance) against vancomycin (VA), erythromycin (E). ciprofloxacin (CIP) and tetracycline (TE). Our findings show Micrococcus spp. u isolates are still susceptible to most antibiotics except for some common antimicrobial agents.

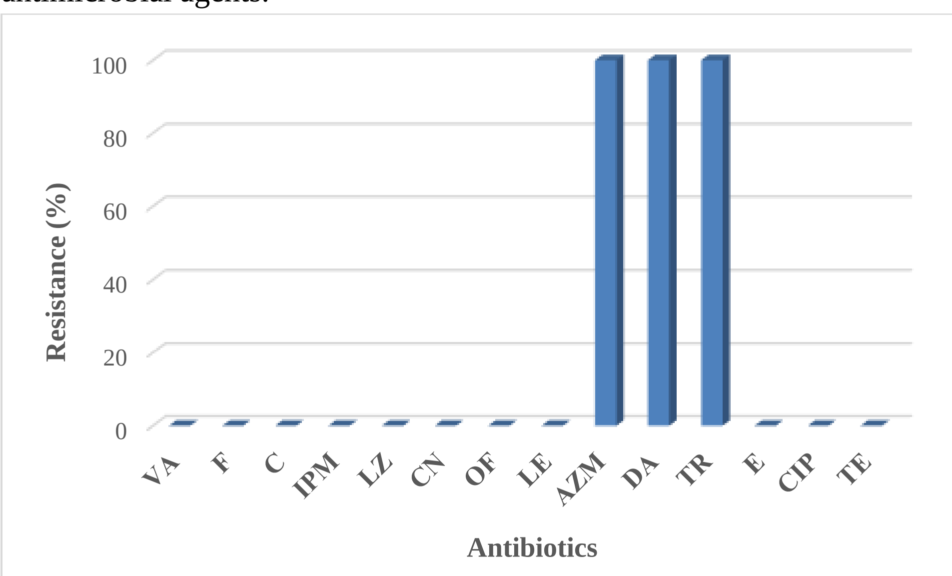


Figure 4. Antibiotic resistance pattern of Micrococcus spp. isolates against different antimicrobial agents.

Figure 5 presents Staphylococcus aureus (S.A.) strains' finished result indicated full (100%) resistance to the antibiotic Azithromycin (A), according to the A graph, and was the antibiotic with the largest vertical line. Conversely, the other antibiotics tested (VA, F, IPM, LZ, CN, OF, LE, D, TE, TR, E, CXT, C) showed little or no resistance against the isolates obtained from the antibiotic susceptibility pattern tests. This indicates that Azithromycin will not be a useful antibiotic when attempting to treat any S. aureus infections and that the other antibiotics would still be effective against these isolates.

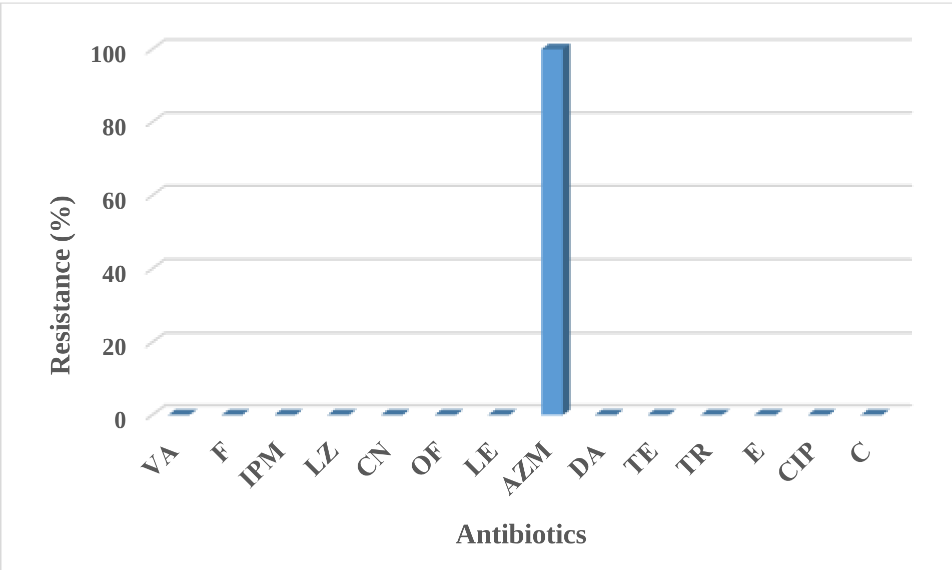


Figure 5. Antibiotic resistance pattern of Staphylococcus aureus isolates against different tested antibiotics, showing complete resistance to Azithromycin (AZM).

Figure 6 The pattern of antibiotic susceptibility for Staphylococcus epidermidis demonstrated by isolates was assessed against a range of antibiotics. The isolates were found to be 100% resistant to the antibiotics Azithromycin (AZM), Clindamycin (CL), and Tetracycline (TE), as indicated by the highest percentage resistance observed for these antibiotics in the chart. Isolates were also noted to be moderately resistant (approximately 50%) to Erythromycin (E) and Ciprofloxacin (CIP); however, all other antibiotics tested demonstrated to little or no resistance, including vancomycin (VA), fusidic acid (F), imipenem (IPM), linezolid (LZ), gentamicin (CN), ofloxacin (OF), levofloxacin (LE), daptomycin (D), trimethoprim/sulfamethoxazole (TR), and chloramphenicol (C). The results of this study indicated that S. epidermidis is multidrug resistant to the majority of the commonly used antibiotics tested, particularly AZM, CL, and TE.

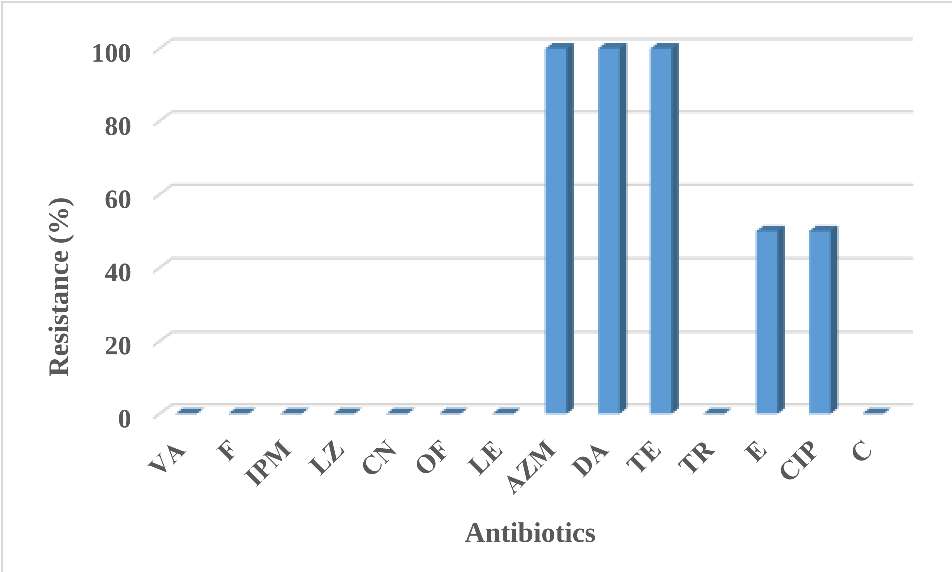


Figure 6. Antibiotic resistance pattern of Staphylococcus epidermidis isolates against different tested antibiotics, showing complete resistance to AZM, DA, and TE, and moderate resistance to E and CIP.

Figure 7 The antibiotic susceptibility profiles of various isolates of Streptococcus spp. were demonstrated through testing against a variety of antibiotics. The isolates were resistant (100% resistance) to azithromycin (AZM), clindamycin (DA) and erythromycin (E), with no effectiveness observed in these isolates. The isolates exhibited sensitivity to nearly all the other antibiotics tested (including vancomycin (VA), florfenicol (F), Imipenem (IPM), linezolid (LZ), ciprofloxacin (CIP), ceftazidime (C), P,) except for those listed above where there was some level of resistance. These results demonstrate that isolates of Streptococcus spp. have specific resistant properties toward certain antibiotics; specifically, macrolides and lincosamides.

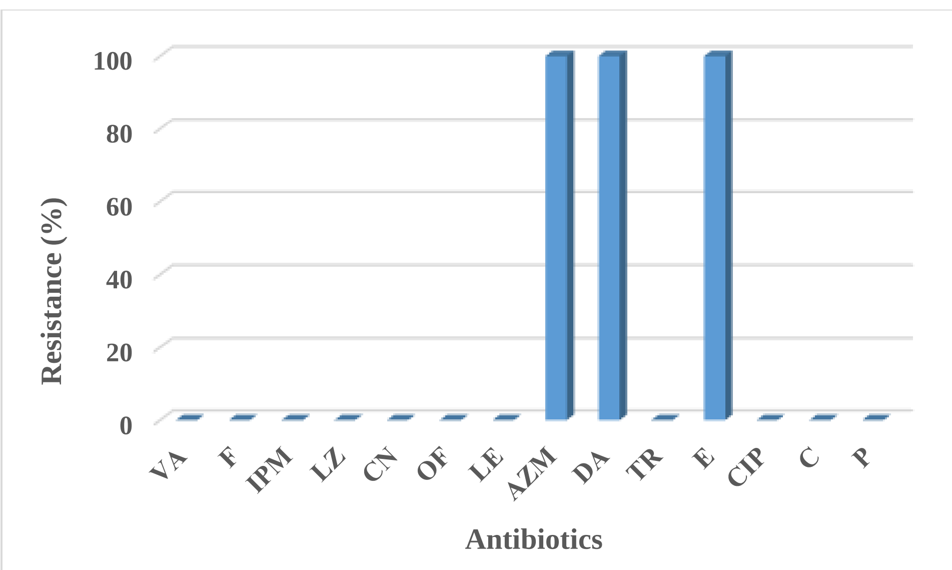


Figure 7. Antibiotic resistance pattern of Streptococcus spp. isolates against different tested antibiotics, showing complete resistance to AZM, DA, and E, while remaining sensitive to most other antibiotics.

Figure 8 The pattern of resistance to the various antimicrobials by Enterobacter spp. Isolates indicates that the isolates exhibited total anthracycline (100%) resistance to a number of the agents: F (nitrofurantoin), AK (amikacin), NOR (norfloxacin), CIP (ciprofloxacin), C (chloramphenicol), FOX (cefoxitin), AMP (ampicillin), AMC (amoxicillin/clavulanic acid), ATM (aztreonam), and NA (nalidixic acid). Conversely, the isolates had very little or no resistance to either CN (gentamicin), OF (ofloxacin), IP (imipenem), TOB (tobramycin), FF (fosfomycin), FTZ (fucitermin), MEM (meropenem), or netilmicin. These findings support the conclusion that the Enterobacter isolates are multi-drug-resistant, particularly against the following anti-infective classes: B-lactams, quinolones, and to a lesser extent some aminoglycosides. These data also suggest the need for the use of antimicrobial susceptibility testing prior to initiating therapy.

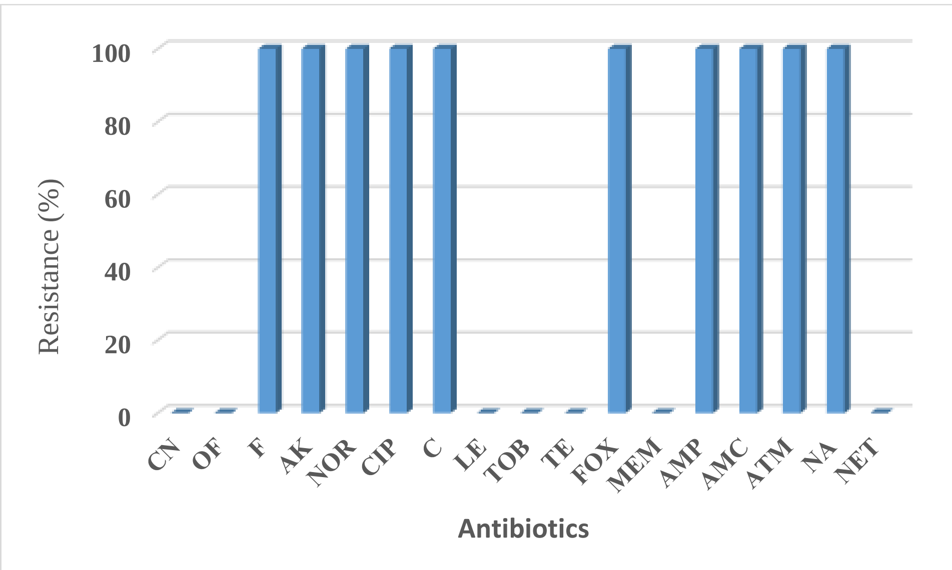


Figure 8. Antibiotic resistance profile of Enterobacter spp. isolates against commonly used antimicrobial agents, showing high resistance rates to multiple antibiotics including ampicillin, ciprofloxacin, norfloxacin, cefoxitin, and aztreonam.

Table 6 presents The isolation of Bacillus spp. and Lactobacillus spp. from the examined samples demonstrated their occurrence within the samples tested. Both of these organisms are expected to occur in the environment and are, therefore, indicative of either environmental contamination due to their ability to form spores (as Bacillus spp. do) or due to their natural population distributions in different environments (as can occur for Lactobacillus spp.). Proteus spp. and Pasteurella spp. were not recovered in any of the analysed samples; therefore, while these organisms may exist in the sampled environment, they did not exist at detectable concentrations under the laboratory conditions used during this study.

Table 6. Other isolated bacteria

Result	**Bacteria**
Isolated	Bacillus spp.
Isolated	Lactobacillus spp.

Not detected	Proteus spp.
Not detected	Pasteurella spp.

Discussion

In the city of Samawah, several chicken feather samples from various poultry markets were collected for the purpose of determining what bacteria are found in chicken feather waste and how dangerous these bacteria may be to public health. Laboratory methods used to isolate and identify bacterial species from poultry feather waste are discussed in Section 3. A total of 20 chicken feather samples were collected from different local poultry supply stores and brought to the laboratory using sterile techniques for microbiological examination. Different types of culture media were used to grow potential bacteria on the feather samples (MacConkey agars, blood agars, and UTIC agars) in order to isolate and identify pathogenic bacteria. After about 24 hours of incubation at a temperature of 37°C, all samples contained growth indicating contamination. The results from this research agree with prior research findings that have indicated that poultry wastes contain high levels of bacterial contamination as a result of their high organic matter content and their exposure to environmental conditions (Akinola et al., 2012; Williams and Barker, 2001). The results obtained on MacConkey agar where pink colonies (lactose fermenting) appeared, suggest the presence of Escherichia coli, a bacteria frequently identified from similar studies that have studied poultry waste (Tesfaye et al., 2017). Furthermore, a wide variety of colony characteristics (i.e., size, shape, colour, and texture) were present which demonstrates that there were multiple species of bacteria represented. Similar findings were noted in earlier studies where poultry waste samples exhibited heterogeneous colony morphologies due to the mixed nature of their microbial populations (Momoh et al., 2013).

It was found that the birds' feathers contained a significant amalgamation of both good bacteria (gram-positive) and harmful bacteria (gram-negative), which has been shown to be expected due to how much organic matter (i.e.: milk, blood, tissues) is found in the waste of poultry. It has already also been shown through several previous studies (Kabir, 2010) that birds tend to have a high concentration of diverse types of microbial populations, with some of each type being potential pathogens. The use of a combination of 3 tests for determining the different types of bacteria (morphological; staining; and biochemical) is both a commonly accepted and effective method within labs. The types of results that were found in this study are consistent with many previous studies about this type of testing (Cheesbrough, 2006). The reason that the feathers will support such a broad variety of different types of bacteria is attributed to the high levels of organic waste in the waste associated with chickens (e.g.: feathers, blood, and tissues). If proper management of the waste is not followed, the waste will act as a support media for fast growing microorganisms and therefore contaminate the environment. These observations are consistent with previous reports about how poultry waste contributes to the environmental contamination and spread of pathogens (Nahm, 2003). The results of the antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method demonstrated that bacterial isolates tested have different reactions to antibiotics commonly used in poultry production. Specifically, some of the isolates were susceptible while others were resistant. The findings of these studies confirm other reports of antibiotic-resistant bacteria in the poultry environment as a result of the widespread use of antibiotics in poultry production (Ritz et al., 2004; Kabir, 2010). The presence of antibiotic-resistant bacteria poses a serious health risk because it limits the ability of healthcare providers to effectively treat patients with bacterial pathogens. Additionally, other studies support concerns expressed in the present study regarding the role that poultry waste plays in being a potential source of antimicrobial-resistant pathogens that can transmit to humans through environmental exposure (Tesfaye et al., 2017). The study's overall conclusions are congruent with previously conducted studies and have shown that insulting egg farm operation practices and/or using poultry waste for anything other than manure has added to the spread and contaminating the environment with possibly harmful and/or antibiotic resistant bacteria. There is an immediate need to improve the hygienic practices of the handling of poultry products, to better manage poultry waste from their handling of animals, to limit the application of antibiotics used in producing poultry products, and therefore to protect both the local environment where the poultry is grown and the general health of people in that area. The presence of bacteria in feather waste found in the poultry industry underscores the need for effective waste management and hygiene practices within the production and distribution of poultry meat and eggs. Poultry workers who handle poultry on a daily basis and may, therefore, be at risk for exposure to potentially pathogenic microorganisms through either direct contact or through inhalation of dust from the surface of the work space may ultimately pose a risk to the environment (both human and other types of living being) by the improper disposal of poultry waste in a public area; the bacteria may ultimately make their way into the environment by way of insects or rodents, or into the water supply used by the public. The current findings concur with earlier studies indicating that poultry waste provides a vital source for the transmission of microbes that negatively impact the environment and human health (Akinola et al., 2012; Williams and Barker, 2001). Past research has shown that poultry feathers are capable of harboring microbial contaminants because they come into contact with animal tissues and blood as well as environmental surfaces during harvest and processing activities (Tesfaye et al., 2017). The buildup of poultry waste in urban settings without adequate systems for the disposal of the waste will lead to environmental degradation. The breakdown of organic poultry wastes can produce foul odors and release hazardous gases such as ammonia and hydrogen sulphide. This is consistent with previous studies that have demonstrated that emissions from poultry farms materially influence atmospheric conditions and contribute to the respiratory discomfort of individuals who live near to poultry markets (Nahm, 2003; Ritz et al., 2004). In conclusion, this study highlights the need for adequate sanitation practices and efficient waste management techniques in poultry markets located in Samawah City. To effectively reduce microbial contamination and preserve the health of the general public, the implementation of effective public awareness programs, appropriate waste disposal techniques, and improved sanitation practices among poultry shop employees will be essential.

Conclusion

This study assessed the environmental and health consequences of solid waste produced by poultry retailers in Samawah city, focusing mainly on chicken feathers produced as a by-product of the slaughtering/processing of birds. A variety of chicken feathers were gathered from the local poultry stores to gather information on the types of bacteria present and their susceptibility to different antibiotics through laboratory analysis. The findings indicate that the chicken feather samples contained significant amounts of microbial contamination, as several of the common types of bacteria associated with poultry waste were isolated from these samples. This study showed that the waste produced from poultry birds, specifically chicken feathers, creates conditions for the multiplication and growth of microorganisms. In addition, results indicated certain bacterial isolates displayed diverse levels of antibiotic resistance while also being susceptible to many of the antimicrobial agents examined here. The emergence of antibiotic resistance is now thought to be one of the major public health issues as they could indicate an overuse or misuse of antibiotics in poultry production. Furthermore, results support the notion that improper management or disposal of poultry waste at local markets may contribute to environmental contamination; such as air, soil, or water pollution. The accumulation of poultry waste can attract insects as well as rodents to carry disease(s). Therefore, poultry workers as well as surrounding residents are at an increased risk for health-related problems. This study also urged for further scientific studies to evaluate microbial contamination from poultry waste and the potential impact(s) on human health and environmental effects.

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