The International Innovations Journal of Applied Science (IIJAS) Vol. 2, No.2, 15-09-2025
https://doi.org/10.61856/drt2nx79

Scientific Events Gate

The International Innovations Journal of Applied Science

Journal homepage: https://iijas.eventsgate.org/iijas

ISSN: 3009-1853 (Online)

Isolation and Characterization of Multidrug Resistant *E.coli* and *S.aureus* From Selected Poultry Farms in Palestine

Arwa Mujahed
Department of Applied Chemistry and Biology, College of Applied Science, Palestine Polytechnic University, Palestine.

ARTICLE INFO

Article history:
Received xxxx
Revised xxxx,
Accepted xxxx,
Available online xxxx

Keywords:
MDR bacteria,
Poultry
Antibiotics
Antibiotic resistant bacteria
Drinking water
Resistant
Sensitivity

ABSTRACT

Poultry is one of the world's fastest-growing sources of meat. As a result, antibiotics are increasingly being used to treat diseased hens and even to prevent infectious bacterial diseases, as well as growth promoters in diets at sub-therapeutic levels. This Inappropriate and indiscriminate usage of antibiotics results in the development of antibiotic-resistant bacteria. As a result, there is increasing public and government interest in reducing the inappropriate use of antibiotics in animal farming due to rising global concerns that antibiotic-resistant bacteria can be transmitted from animals to humans. The objectives of this study were to isolate and characterize multidrug-resistant bacteria (MDR) from poultry litter and water source samples from selected farms in Hebron/Palestine. Several antibiotic-resistant bacteria were isolated from poultry litter and water sources such as *Klebsiella, Shigella, E.coli, Staphylococcus aureus and epidermidis, Morexella, Neisseria, Clostridium, Salmonella, Brucella, Enterobacter, and Bordetella*. However, this study was focused on MDR *E.coli* and *S. aureus* which were tested using different identification techniques and exposed to several widely used antibiotics via disc diffusion method. The results showed that the overall isolation rate of *E.coli* and *S.aureus* in all samples isolated from the four farms (poultry litter and water source) was 100%, and the antibiotic sensitivity test for these bacteria indicated resistant percentage range from 80% to 100%. In conclusion, the best antibiotics recommended for usage against the growth of these bacteria were Ceftriaxone، Sulfamethoxazole and Ceftazidime because they produce synergism effect when were used together.

1. Introduction

The poultry sector is among the fastest growing agro-based industries worldwide due to increasing demand for egg and meat products, accounting for approximately a quarter of all meat produced in the year 2000 (Bolan et al.,2010).

The poultry industry plays an important role in Palestinian agricultural economy. It contributes 40% to 50% of the income of the animal production sector (12% to 15% of the agricultural income). The recent statistics showed that the total population of layer hens and broilers to be 3.6 and 71 million respectively (Palestinian Central Bureau of Statistics Website, 2020/2021).

The great challenge of poultry production is the potential outbreaks of infectious diseases (Bolan et al.,2010). Numerous common microbial pathogens are responsible for diseases in poultry and can be found in fresh poultry litter. These include *Salmonella* sp., *Campylobacter* spp., and *Escherichia coli (E. coli)* (Ejeh et al., 2017) (Ngogang et al., 2021). The lack of research in this area and inadequate management of these pathogens in poultry litter have contributed to several food-borne disease outbreaks in countries like Palestine, Bangladesh, Nigeria, and Cameroon (Ejeh et

* Corresponding author.
E-mail address: arwam@ppu.edu

al., 2017) (Ngogang et al., 2021) (Khan et al., 2014).

The use of antibiotics in poultry and livestock production is a key practice for treating and preventing infectious bacterial diseases, as well as for promoting growth at sub-therapeutic levels in feeds. However, this practice is believed to have contributed to the growing issue of bacterial antibiotic resistance in recent years (Apata et al., 2009). The indiscriminate use of antibiotics can lead to resistance not only in pathogenic bacteria but also in the natural bacterial flora of both affected animals and humans (Lie et al., 2019). This occurs in part because some poultry farmers utilize antibiotics as growth promoters, viewing them as a cost-effective management strategy (William et al., 2012). On the other hand, some farmers use antibiotics as a preventive strategy to address the prevalent unsanitary conditions and insufficient biosecurity. As a result, residues of antibiotics may be present in the litter, which exposes bacteria continuously and poses a considerable risk of developing resistance.

Antimicrobials have been used in animal production since 1910, when workers across America staged protests and riots due to a lack of meat products. Sweden is recognized as the first country to ban the use of antibiotics for non-therapeutic purposes, such as prophylaxis. Following Sweden's lead, Denmark, the Netherlands, the United Kingdom, and several other European Union nations implemented similar bans during the 1980s and 1990s. Additionally, various countries have prohibited certain classes of antibiotics or created regulations to limit the use of specific antibiotics in animal farming. Despite these measures, it is estimated that livestock production, including poultry, accounts for over 60% of all antibiotics produced. The use of antibiotics in poultry and livestock farming provides advantages for farmers and contributes to the economy. The likely spread of antibiotic resistant pathogenic and non-pathogenic organisms could have serious public health consequences. Despite these advancements, it is estimated that livestock production accounts for more than 60% of all antibiotics produced (Christian et al., 2018).

Factors that have contributed to the growing resistance problem include extensive use of antibiotics in poultry as growth promoters and most importantly for the control and treatment of diseases, and improper prescribing of antimicrobial therapy. There can be cases of improper antibiotic prescribing, like when a broad-spectrum drug is initially prescribed even though it isn't needed or is later determined to be ineffective against the pathogens responsible for the infection (Yu VL., 2011). Recent studies have identified incomplete human metabolism and the incorrect disposal of antibiotics into sewage treatment plants as significant contributors to the release of antibiotics into the environment (Rizzo et al., 2013). This allows bacteria to have enough time to protect themselves by modifying their DNA and biological systems, allowing them to thrive and reproduce more easily (Galvin et al.,2010). These antibiotic resistance genes have the potential to infect wildlife in the natural environment when the treated water is released.

Flies found around broiler chicken facilities may play a role in spreading drug-resistant bacteria from these sites, which could heighten the risk of human infections. This transfer likely happens when flies feed on waste and decaying carcasses, leading to the ingestion of bacteria or contamination of their feet, legs, proboscis, and wings. These flies can then mechanically transmit microbes through physical contact, or they might defecate or regurgitate bacteria from their digestive system onto food or other surfaces (Nichols, 2005). The rise of multidrug resistance could significantly affect the treatment and management of infectious diseases in both animals and humans (Mamza et al., 2010). Therefore, the objectives of this study were to isolate and characterize multidrug-resistant bacteria from poultry litter and water source samples from selected farms in Hebron/Palestine.

2. Methodology

2.1 Sample collection

A total of 8 poultry litter samples and 8 (100ml) water samples were collected from four selected broilers and local chickens' farms in Hebron/Palestine. The litter samples taken from each of these poultry farms consisted of dry feces gathered from the open fields near the poultry cages. Using sterile gloves, litter was mixed and samples were collected in sterile wide mouthed-containers. But the water samples were collected in a sterilized bottle with led from water sources of each farm. After that, the samples were properly labelled and stored in sterile plastic containers, then placed in a cooler with ice packs before being transferred to the laboratory of the Department of Applied Biology and Chemistry at Palestine Polytechnic University for further analysis.

2.2 Isolation and Identification of Pathogens for poultry litter samples

A pre-enrichment suspension was prepared by adding 25 mg of poultry litter to 225 ml of buffered peptone water, which was then incubated at 37°C for 24 hours. Bacterial species were isolated by plating the pre-enrichment suspension on MacConkey agar, mannitol salt agar, and XLD agar, followed by incubation as described by Ngogang et al. (2021). The isolation and identification of bacteria were performed using standard bacteriological methods. MacConkey agar, EMB, mannitol salt agar, and nutrient agar were utilized for culturing the specimens and for primary identification. For further characterization and accurate identification, bacterial colonies were examined using specific biochemical and microbiological tests, including the oxidase test, catalase test, and Gram staining (Gyles, 2008).

2.3 Isolation and Identification of Pathogens for water sources samples

The total sample volume of 100 ml was thoroughly mixed and filtrated using membrane filtration method through a cellulose nitrate. Each filter was placed on M-endo ager and LB agar media. With two plates per water source sample, each one was then incubated at 37°C for 24 h. The bacterial colonies on filter were isolated and cultured on EMB, macconkey, and mannitol salt agar, also further identification was done using specific biochemical and microbiological tests, the same that was done for poultry litter samples.

2.4 Antibiotic susceptibility testing

Antibiotic susceptibility testing was done for both poultry litter samples and water sources samples using the disc diffusion method as described in (Miles et al.,2006). Bacterial isolates were grown in nutrient broth for 24 hours and 0.5 McFarland standard was prepared to compare the turbidity. Freshly prepared Mueller-Hinton agar was inoculated with the standardized inoculum using sterile cotton swabs. The plates were covered and allowed to dry.Commercially available antibiotic-impregnated filter paper discs were placed on the surface of the agar, and the plates were incubated at 37°C for 24 hours. The inhibition zones were measured to the nearest millimeter, indicated by the absence of microbial growth due to the inhibitory concentrations of the antibiotics. The inhibitions were read using a Vernier caliper. CLSI standards (2015) were used to classify susceptibility of the isolates as susceptible (S), intermediate (I) or resistant (R). Explaining research chronological, including research design, research procedure (in the form of algorithms, Pseudocode or other), how to test and data acquisition (Snyder, 2019).

3. Results and discussion

3.1 Isolation and Antimicrobial susceptibility of the Escherichia coli isolates to antibiotics

Escherichia coli is a gram-negative commensal bacterium found in the intestines of both humans and animals. It typically produces pink colonies (lactose positive) with a surrounding pink area on Macconkey agar, is usually motile, does not produce H_2S, and is non-spore forming.

The overall isolation rate of *Escherichia coli* in all samples isolated from the four farms

https://doi.org/10.61856/drt2nx79

(poultry litter and water source) was 100% (Table 1).

Table 1: Isolation of *Escherichia coli* from poultry litter and water source of the 4 selected farms

S/No	Sample type	Number of processed samples	Number of positive samples
1	Poultry litter	8 (50)	8 (100)
2	Water source	8 (50)	8 (100)
	Total	16 (100)	16 (100)

The standard disc diffusion method outlined by Miles et al. (2006) was employed to assess the sensitivity to various antimicrobial gents in vitro. A total of ten antibiotics were selected, as detailed in Table 2. *E. coli* isolates of poultry litter samples were highly resistant 100% to Ceftazedime, Trimethoprim+sulfamethoxazole, Nalidixic acid, Streptomycin,Vancomycin and Cefotaxime respectively, and 75% resistant to Erythromycin, Azithromycin, Ceftriaxone, and Amoxicillin.

E. coli isolates of water source samples (Table 3) were highly resistant to Ceftriaxone, Ceftazedime, Erythromycin, Amoxicillin, Trimethoprim+sulfamethoxazole, Nalidixic acid, Streptomycin, Vancomycin, and Cefotaxime (100%) followed by Azithromycin (75%). Ceftriaxone، Sulfamethoxazole and Ceftazidime are observed to producesynergism when used together.

Table 2: Resistance pattern of *Escherichia coli* isolates from poultry litter

Isolate	Antibiotic profile	R (%)	I (%)	S (%)	MAR	MDR
L1-1	CRO, CAZ, AM, SXT,NA,S, VA,CTX	8 (80)	0 (0)	2(20)	0.8	+
L1-2	CRO, CAZ, AM, SXT,NA,S, VA,CTX	8 (80)	0 (0)	2(20)	0.8	+
L2-1	CAZ,E, SXT, NA, S, AZM, VA, CTX	8 (80)	0 (0)	2(20)	0.8	+
L2-2	CAZ,E, SXT, NA, S, AZM, VA, CTX	8 (80)	0 (0)	2(20)	0.8	+
L3-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
L3-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
L4-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
L4-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+

KEY: CRO = Ceftriaxone CAZ = Ceftazedime E = Erythromycin

AM = Amoxicillin SXT = Trimethoprim+sulfamethoxazole NA = Nalidixic acid

S = Streptomycin AZM = Azithromycin VA = Vancomycin CTX = Cefotaxime

R= Resistant I = Intermediate S = Susceptible

MAR= Multi antibiotic resistance

MDR= Multi-drug resistance (when the isolate is resistant to more than 3 antibiotics)

L1-1 = *E. coli* isolates from poultry litter farm 1 sample 1

L1-2 = *E. coli* isolates from poultry litter farm 1 sample 2

L2-1 = *E. coli* isolates from poultry litter farm 2 sample 1

L2-2 = *E. coli* isolates from poultry litter farm 2 sample 2

Key: W1-1 = *E. coli* isolates from water source farm 1 sample 1

W1-2 = *E. coli* isolates from water source farm 1 sample 2

W2-1 = *E. coli* isolates from water source farm 2 sample 1

W2-2 = *E. coli* isolates from water source farm 2 sample

Table 3: Resistance pattern of *Escherichia coli* isolates from water source

Isolate	Antibiotic profile	R (%)	I (%)	S (%)	MAR	MDR
W1-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
W1-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
W2-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
W2-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
W3-1	CRO, CAZ, E, AM, SXT, NA, S, VA, CTX	9 (90)	0 (0)	1 (10)	0.9	+
W3-2	CRO, CAZ, E, AM, SXT, NA, S, VA, CTX	9 (90)	0 (0)	1 (10)	0.9	+
W4-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
W4-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+

In this study, it was found that over 75% of the *E. coli* isolates showed resistance to more than three antibiotics. This is consistent with the study by (Moustafa & Mourad.,2015) which provided direct evidence that antimicrobial use in animals selects for antimicrobial-resistant bacteria that may be transferred to humans through food or direct

contact with animals. Multidrug resistance to more than two antimicrobial agents was detected in 6 of the isolates. This study found that multiple antibiotic resistance was prevalent among E. coli. These findings align with earlier research conducted in Nigeria (Olonitola et al., 2015). Salihu et al. (2014) further noted that the widespread use of antibiotics in poultry is due to their easy availability and low cost. The resistance seen in E. coli isolated from local chickens is believed to have arisen from the transfer of resistance gene(s) from other hosts within the same production environment (Salihu et al., 2014). It was

In our study, high isolation rate 100.0% of *E.coli* from poultry litter was observed. A possible explanation for this, may be due to the increased use of antibiotics for treatment and as growth promoter in broiler chickens (Ejeh et al.,2017). Similar MAR index from both poultry litter (0.8- 1.0) and water source (0.9-1.0) were recorded in this study which may imply transfer of *E. coli* from water source to the poultry by drinking, especially in farm 4 that has the same antibiotic profile and MAR for both poultry litter and water source. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used (Miranda et al.,2008). In addition, MAR index values greater than 0.2 indicate existence of isolate from high risk contaminated source with frequency use of antibiotics while values less than or equal to 0.2 show bacteria from source with less antibiotics usage (Zinnahet al.,2008). Higher MAR indices as shown in the results of this work a great efforts need for surveillance and remedial measures which is public health concern as litter is used as a source of manure and solving water source contamination problem, that affects the human and animal health. High level of antibiotic resistance of the *E. coli* isolates (100%) to Ceftazedime, Trimethoprim+sulfamethoxazole, Nalidixic acid, Streptomycin, Vancomycin and Cefotaxime has been identified and this is because heavy metals as well as antibiotics used in animal farming might promote the spread of antibiotics resistance via co-selection (Abdel-Tawab et al.,2015), and resistance to antibiotics can be conferred by chromosomal or mobile genetic elements (e.g. plasmid). These findings are in agreement with that of (Romanus & Amobietal., 2012), with high prevalence of *E. coli* strains that are resistance to commonly prescribed antibiotics. This was consistent with findings in this study in which it was observed that more than 50% of the E. coli isolates showed a MDR pattern, with the highest resistance profile being associated with streptomycin and amoxicillin. These findings were also consistent with those in previous study, in which it was also noticed that E. coli isolates from cattle had high resistance against streptomycin, amoxicillin, sulfamethoxazole+trimethoprim, ciprofloxacin, and ampicillin (Zinnah et al.,2008).

Further research has shown that the use of antimicrobials in veterinary medicine, both as therapeutic and preventive measures, as well as their role as growth promoters, significantly affects the prevalence of resistance in bacteria found in animals. This situation raises concerns about the potential for antibiotic resistance to develop in human pathogens. Additionally, it was noted that bacterial isolates resistant to two or more antibiotics may have come from high-risk contamination sources, such as commercial poultry farms, where the use of antibiotics is prevalent (Moustafa & Mourad, 2015).

3.1 Isolation and Antimicrobial susceptibility of the Staphylococcus aureus isolates to antibiotics

Staphylococcus aureus is a Gram-positive cocci and a facultative anaerobe that appears in clusters (grape-like clusters), ferments many carbohydrates (e.g. mannitol) with the production of lactic acid but no gas, Non-motile and Non-spore forming bacteria (Christian et al.,2018) .

The overall isolation rate of *Staphylococcus aureus* (*S.aureus*) in all samples isolated from the four farms (poultry litter and water source) was 100% (Table 4).

https://doi.org/10.61856/drt2nx79

Table 4: Isolation of *Staphylococcus aureus* from poultry litter and water source of the 4 selected farms

S/No	Sample type	Number of processed samples	Number of positive samples
1	Poultry litter	8 (50)	8 (100)
2	Water source	8 (50)	8 (100)
	Total	16 (100)	16 (100)

The standard disc diffusion method as described in (Miles et al.,2006), was used for the in vitro determination of the sensitivity to the antimicrobial agents. Ten antibiotics were chosen as shown in Table 5. The results of antibiotic resistance profile have been shown in Table 4. *S.aureus* isolates of poultry litter samples were highly resistant (100%) to Ceftriaxone, Ceftazedime, Erythromycin, Trimethoprim+sulfamethoxazole, Nalidixic acid, Streptomycin, Azithromycin, Vancomycin, and Amoxicillin, and 75% resistant to Ceftriaxone which give intermediate (I) in S4-1 and S4-2 *S.aureus* isolates.

Table 5: Resistance pattern of *Staphylococcus aureus* isolates from poultry litter

Isolate	Antibiotic profile	R (%)	I (%)	S (%)	MAR	MDR
S1-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
S1-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
S2-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
S2-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
S3-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
S3-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
S4-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA,	9 (90)	1 (10)	0 (0)	0.9	+
S4-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA,	9 (90)	1 (10)	0 (0)	0.9	+

On the other hand, *S.aureus* isolates of water source samples (Table 6) were highly resistant to Ceftriaxone, Erythromycin, Trimethoprim+sulfamethoxazole, Nalidixic acid, Azithromycin, Vancomycin, Amoxicillin, and Cefotaxime (100%) followed by (75%) for Streptomycin (which was Intermediate in T3-1 and T3-2 isolates) and Ceftazidime (which was Susceptible in T3-1 and T3-2 isolates).

In our study, high isolation rate 100.0% of *S. aureus* from poultry litter was observed. One possible explanation for this could be the increased use of antibiotics for treatment and as growth promoters in broiler chickens (Ejeh et al., 2017). Similar MAR index from both poultry litter (0.9- 1.0) and water source (0.8-1.0) were recorded in this study which may imply transfer of *S.aureus* from water source to the poultry by drinking, especially in farm no. 1 and farm no.2 that has the same antibiotic profile and MAR for both poultry litter and water source. MAR index values greater than

Key: S1-1 = *Staphylococcus aureus* isolates from poultry litter farm 1 sample 1

S1-2 = *Staphylococcus aureus* isolates from poultry litter farm 1 sample 2

S2-1 = *Staphylococcus aureus* isolates from poultry litter farm 2 sample 1

S2-2 = *Staphylococcus aureus* isolates from poultry litter farm 2 sample 2

Table 6: Resistance pattern of *Staphylococcus aureus* isolates from water source

Isolate	Antibiotic profile	R (%)	I (%)	S (%)	MAR	MDR
T1-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
T1-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
T2-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
T2-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
T3-1	CRO, E, AM, SXT, NA, AZM,VA, CTX	8 (80)	1 (10)	1 (10)	0.8	+
T3-2	CRO, E, AM, SXT, NA, AZM,VA, CTX	8 (80)	1 (10)	1 (10)	0.8	+
T4-1	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+
T4-2	CRO, CAZ, E, AM, SXT, NA, S, AZM,VA, CTX	10 (100)	0 (0)	0 (0)	1	+

Key: T1-1 = *Staphylococcus aureus* isolates from water source farm 1 sample 1

T1-2 = *Staphylococcus aureus* isolates from water source farm 1 sample 2

T2-1 = *Staphylococcus aureus* isolates from water source farm 2 sample 1

T2-2 = *Staphylococcus aureus* isolates from water source farm 2 sample 2

The 0.2 indicate existence of isolate from high risk contaminated source with frequency use of antibiotics while values less than or equal to 0.2 show bacteria from source with less antibiotics usage (Zinnah et al.,2008). Higher MAR indices as shown in the results of this work a great efforts need for surveillance and remedial measures which is public health concern as litter is used as a source of manure and solving water source contamination problem, that affects the human and animal health. The inappropriate use of growth-promoting antibiotics, along with the selective pressure exerted by antimicrobials, is a major factor contributing to the rise of antibiotic resistance (Manie et al., 1998). In animal husbandry, the use of antibiotics in animal feed is becoming more common to help prevent disease outbreaks (Khachatourians, 1998). This study found that Ceftriaxone, Ceftazidime, Erythromycin, Trimethoprim+sulfamethoxazole, Nalidixic acid, Streptomycin, Azithromycin, Vancomycin, and Amoxicillin were resistant to all strains isolated from poultry litter. Resistance can develop through various mechanisms, such as chromosomal mutations or plasmid transfer. Staphylococcus aureus strains from clinical samples were also found to be resistant to these antibiotics (Naseer B.S. & Jayaraj Y., 2010). However, there have been no documented cases of vancomycin resistance in poultry. Although vancomycin is not used in poultry, resistance may arise from the transfer of resistance genes from older antibiotics to newer ones available on the market (Summers, 2002).

In a previous report, erythromycin was shown to be an effective antibiotic against Staphylococcus aureus (Hassam et al., 1978). However, in our study, all strains exhibited resistance to erythromycin, which will complicate treatment.

In our study, cefotaxime demonstrated antimicrobial susceptibility to *S. aureus,* aligning with research conducted in 2002 that reported a 97.8% sensitivity of *S. aureus* to cefotaxime (Zafar et al., 2012).

Beta-lactamase producing S. aureus has been identified in humans, animals, and various organs or tissues in chickens. Staphylococcal beta-lactamase is located on plasmids and can be either non-inducible or inducible when antibiotics are present (Maddux M.S., 1991). Beta-lactamase activity in S. aureus and E. coli isolated from chicken were found to be 8.8% and 11%, respectively (Mamza et al.,2010). Reports showed that S. aureus from various samples had beta-lactamase production (Salimnia H. & Brown W.,2005), and as well showed multiple drug resistance that also was observed in water samples that may be acquired from resistance genes that occur in water, horizontal gene transfer, or from the antibiotics that accidently found in water from sewerage, or pharmaceutical factories waste, or from the electrolytes that is found in water (Mamza et al., 2010).

Staphylococcus aureus mainly affects chickens and turkeys. β-lactams were previously considered the primary treatment for staphylococcal infections. However, with the rise of high resistance levels to these and other medications, there are now only a limited number of treatments available for these infections. MRSA, or methicillin-resistant *Staphylococcus aureus*, is a superbug that resists nearly all drugs used to treat *S. aureus* (Christian et al., 2018).

4. Conclusions

The isolation and characterization of MDR *E. coli* and *S. aureus* from poultry litter and water source are concerning. The best antibiotics that are recommended for usage which provide the best results in inhibition of the growth of bacteria were Ceftriaxone، Sulfamethoxazole and Ceftazidime because they produce synergism when used together.

The potential for these MDR bacteria to enter the food chain poses significant health risks to both humans and animals. To combat the rise of bacterial resistance in poultry farms in Palestine and globally, it is essential to implement bacterial surveillance programs. This effort should be a collaborative initiative.

Acknowledgement

We appreciate the farm owners for allowing us to access samples throughout the study especially Dr. Hazem Tamimi the Chairman of Al- Tamimi Farms. This research received no external funding.

References

Abdel-Tawab, A. A., Ammar, A. M., Nasef, S. A., & Reda, R. M. (2015). Prevalence of E. coli in diseased chickens with its antibiogram pattern. Benha Veterinary Medical Journal, 28(2), 224–230. https://doi.org/10.21608/bvmj.2015.32507

Apata, D. F. (2009). Antibiotic resistance in poultry. International Journal of Poultry Science, 8(4), 404–408. https://doi.org/10.3923/ijps.2009.404.408

Bolan, N. S., Stogy, A. A., Chuasavathil, T., Seshadri, B., Rothrock, M. J., & Panneerselvam, P. (2010). Uses and management of poultry litter. World's Poultry Science Journal, 66(4), 673–698. https://doi.org/10.1017/S0043933910000656

Christian, A., Vivian, E., Boamah, C., Ngofi, Z., & Frank Boateng, O. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. In Y. Kumar (Ed.), Antimicrobial Resistance – A Global Threat. IntechOpen. https://doi.org/10.5772/intechopen.79371

Clinical and Laboratory Standards Institute (CLSI). (2015). Performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement (CLSI document M100-S25). Wayne, PA: CLSI.

Ejeh, F. E., Lawan, F. A., Abdulsalam, H., Mamman, P. H., & Kwanashie, C. N. (2017). Multiple antimicrobial resistance of Escherichia coli and Salmonella species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria. Sokoto Journal of Veterinary Sciences, 15(3), 45–53. https://doi.org/10.4314/sokjvs.v15i3.7

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris, D., & Cormican, M. (2010). Enumeration and characterization of antimicrobial resistant Escherichia coli bacteria in effluent from municipal, hospital, and secondary treatment facility sources. Applied and Environmental Microbiology, 76(14), 4772–4779. https://doi.org/10.1128/AEM.02898-09

Gyles, C. L. (2008). Antimicrobial resistance in selected bacteria from poultry. Animal Health Research Reviews, 9(2), 149–158. https://doi.org/10.1017/S1466252308001552

Hassam, Z. A., Shaw, E. J., Shooter, R., & Caro, D. (1978). Changes in antibiotic sensitivity in strains of Staphylococcus aureus, 1952–78. British Medical Journal, 2(6136), 536–537. https://doi.org/10.1136/bmj.2.6136.536

Khachatourians, G. G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association Journal, 159(9), 1129–1136.

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

Lie, X., Payne, J. B., Santos, F. B., Levine, J. B., Anderson, K. E., & Shaleon, B. W. (2019). Salmonella populations and prevalence in layer feces from commercial high rise houses and characterization of the Salmonella isolates. United States Department of Agriculture.

Maddux, M. S. (1991). Effects of β-lactamase-mediated antimicrobial resistance: The role of β-lactamase inhibitors. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 11(2P2), 40S–50S.

Mamza, S. A., Egwu, G. O., & Mshelia, G. D. (2010). Antibiotic susceptibility patterns of beta-lactamase-producing Escherichia coli and Staphylococcus aureus isolated from chickens in Maiduguri (Arid zone), Nigeria. Veterinarski Arhiv, 80(2), 283–297.

Manie, T., Khan, S., Brözel, V., Veith, W., & Gouws, P. (1998). Antimicrobial resistance of bacteria isolated from slaughtered and retail chickens in South Africa. Letters in Applied Microbiology, 26(4), 253–258. https://doi.org/10.1046/j.1472-765X.1998.00312.x

Miles, T. D., McLaughlin, W., & Brown, P. D. (2006). Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. BMC Veterinary Research, 2(1), 7. https://doi.org/10.1186/1746-6148-2-7

Miranda, J. M., Vazquez, B. I., Fente, C. A., Barros-Velázquez, J., Cepeda, A., & Franco, C. M. (2008). Evolution of resistance in poultry intestinal Escherichia coli during three commonly used antimicrobial therapeutic treatments in poultry. Poultry Science, 87(8), 1643–1648. https://doi.org/10.3382/ps.2007-00485

Moustafa, S., & Mourad, D. (2015). Resistance to 3rd generation cephalosporin of Escherichia coli isolated from the feces of healthy broilers chickens in Algeria. Journal of Veterinary Medicine and Animal Health, 7(8), 290–295. https://doi.org/10.5897/JVMAH2015.0396

Naseer, B. S., & Jayaraj, Y. (2010). Identification of multiresistant Staphylococcus aureus in clinical specimens. Research Journal of Medical Sciences, 4(2), 204–207. https://doi.org/10.3923/rjmsci.2010.204.207

Ngogang, M. P., Ernest, T., Kariuki, J., MouliomMouiche, M. M., Ngogang, J., Wade, A., & van der Sande, M. A. B. (2021). Microbial contamination of chicken litter manure and antimicrobial resistance threat in an urban area setting in Cameroon. Antibiotics, 10(1), 20. https://doi.org/10.3390/antibiotics10010020

Nichols, G. L. (2005). Fly transmission of Campylobacter. Emerging Infectious Diseases, 11(3), 361–364. https://doi.org/10.3201/eid1103.040460

Olonitola, O. S., Fahrenfeld, N., & Pruden, A. (2015). Antibiotic resistance profiles among mesophilic aerobic bacteria in Nigerian chicken litter and associated antibiotic resistance genes. Poultry Science, 94(5), 867–874. https://doi.org/10.3382/ps/pev069

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of the Total Environment, 447, 345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032

Romanus, I. I., Chinyere, O. E., & Amobi, N. E. (2012). Antimicrobial resistance of Escherichia coli isolated from animal and human clinical samples. Global Research Journal of Microbiology, 2(1), 85–89.

Salihu, A. E., Onwuliri, F. C., & Mawak, J. O. (2014). Antimicrobial resistance profiles of Salmonella gallinarum isolates from free range chickens in Nasarawa State, Nigeria. International Journal of Bacteriology Research, 2(1), 19–27.

Salimnia, H., & Brown, W. (2005). Detection of oxacillin resistance in Staphylococcus aureus: Comparison of phoenix oxacillin and cefoxitin MICs, microscan oxacillin MIC, oxacillin and cefoxitin disk diffusion, and mecA gene detection. ICAAC, 12–20.

Snyder, H. (2019). Literature review as a research methodology: An overview and guidelines. Journal of Business Research, 104, 333–339. https://doi.org/10.1016/j.jbusres.2019.07.039

Summers, A. O. (2002). Generally overlooked fundamentals of bacterial genetics and ecology. Clinical Infectious Diseases, 34(Suppl. 3), S85–S92. https://doi.org/10.1086/340245

William, U. D., Hang'ombe, B., Victor, Z. C., & King, N. S. (2012). Distribution of virulence genes and antimicrobial susceptibility of Salmonella isolated from dogs and chickens in Zambia.

Yu, V. L. (2011). Guidelines for hospital-acquired pneumonia and health-care-associated pneumonia: A vulnerability, a pitfall, and a fatal flaw. The Lancet Infectious Diseases, 11(3), 248–252. https://doi.org/10.1016/S1473-3099(11)70005-6

Zafar, A. I., Ejaz, H. A., Ahmed, J. M., & Javed, A. (2012). Frequency and antimicrobial susceptibility of Staphylococcus aureus isolated from pus samples of paediatric patients. Pakistan Journal of Medical and Health Sciences, 6(1), 32–35.

Zinnah, M. H., Haque, M. T., & Islam, M. T. (2008). Drug sensitivity pattern of Escherichia coli isolated from samples of different biological and environmental sources. Bangladesh Journal of Veterinary Medicine, 6(1), 13–18. https://doi.org/10.3329/bjvm.v6i1.1332