Benha Veterinary Medical Journal 45 (2023) 172-176

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Tracking the antibiotic resistance phenomenon in poultry meats under species and seasonal variations

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ARTICLE INFO

Keywords

Foodborne pathogens

Multidrug resistance

Seasonal variation

Received 08/09/2023
Accepted 19/09/2023
Available On-Line 01/10/2023

ABSTRACT

Antimicrobial-resistant bacteria in poultry meat pose a threat to public health. A total of ninety *E. coli* and *S. aureus* strains were isolated from 270 raw poultries (chicken, quail, and duck) drumstick samples purchased from local butchers in the Qalyubia governorate along winter, spring, and summer seasons of 2022 (30 isolates of each season). Isolates of each species per each season were examined for their antibiotic sensitivity using eight antibiotics represented by amoxicillin/clavulanic acid, ampicillin, ceftriaxone, ciprofloxacin, gentamicin, tetracycline, trimethoprim/sulfamethoxazole, and chloramphenicol by disc diffusion technique. Results revealed that the examined *E. coli* and *S. aureus* isolates had a variable sensitivity profile, where *S. aureus* showed higher resistance affinity than *E. coli*. Although the tested antibiotics showed variable sensitivity ratios, tetracycline showed the lowest sensitivity ratio either on *S. aureus* or *E. coli*. Isolates collected from duck samples showed higher resistance activity than those of chicken and quail, respectively. Moreover, the examined isolates detected in the summer season were more resistant to the tested antibiotics than those examined in spring and winter, respectively. In conclusion, fast-growing drug resistance of foodborne bacteria, especially, *S. aureus* and *E. coli*, of poultry meat origin appeared to be a critical health problem, especially during hot and humid weather of the summer season. So, close surveillance is required throughout the poultry production chain to prevent the spread of antimicrobial-resistant *bacterial* strains.

1. INTRODUCTION

Because of its flavor, nutritional content, and affordable price, poultry meat is one of the most sought-after and desired meats in the world (Bhaisare *et al.*, 2014). It can be served as whole carcasses or pieces, or as boneless meat. Despite being a wonderful source of high-quality protein, poultry meat can regrettably pick up a number of foodborne infections throughout various processing steps, which can lead to foodborne disease in humans (Bhandari *et al.*, 2013). Antibiotic resistance has been acknowledged as one of the main global health concerns, posing a threat to food security, human and animal health, and resulting in sizable economic losses, primarily as a result of the careless use of antibiotics in agriculture, the environment, animal, and human medicine (Manyi-Loh *et al.*, 2018).

Food and food production may be a source of human antibiotic-resistant bacteria and genes that have an effect on public health. Plasmids and transposons, which are mobile genetic elements, have the capacity to interact with or originate from the environment and foodborne bacteria to create hybrid elements. Many antibiotics, particularly those used as last-resort therapies for patients infected with multidrug-resistant bacteria, are susceptible to resistance encoded by these genetic structures. To control antibiotic-resistant foodborne bacteria, it's important to provide information, education, and training, as well as surveillance, monitoring, record-keeping, infection control, legislation, optimization, ~~and~~ reduced antibiotic use, and sustainable investment in alternatives (Caniça *et al.*, 2019).

Antibiotic resistance has reached a level that the World Health Organization believes poses a severe danger to world health (WHO, 2014). Already, antibiotic-resistant bacteria are responsible for more than 700,000 fatalities annually (Dadgostar, 2019). The rising pandemic of drug-resistant diseases is being fiercely fought by stakeholders from the governmental, international, academic, and food business sectors (Khan *et al.*, 2021).

Therefore, the current study focused on the antibiotic resistance behavior of *Escherichia coli* and *Staphylococcus aureus* of chicken, quail, and duck meat origin.

2. MATERIAL AND METHODS

2.1. Collection of the strains

Ninety *Escherichia coli* and *Staphylococcus aureus* strains were isolated from previously examined 270 random samples of raw chilled chicken, quail, and duck drumsticks along winter, spring, and summer seasons (30 of each product/each season) of 2023. Samples were collected from different butchers around Qalyubia governorate, Egypt. Samples were subjected to bacteriological detection of *E.*

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coli and *S. aureus* according to ISO (2001) and ISO (2003), respectively. Purified isolates were cultured on slopes for further examination.

2.2. Antibiotic sensitivity test

In vitro sensitivity test was done for each isolated *E. coli* and *S. aureus* strain of each season to study their sensitivity to different antibiotics (Tables 1&2) using the disc-diffusion method on Muller-Hinton agar and incubation for 24 hrs in 37 ℃ according to CLSI (2018).

2.3. Statistical analysis

Antibiotic sensitivity profiles were presented as a percentage (%) calculated in relation to the number of sensitive and resistant isolates in each season. The percentage of antibiotic-sensitive and resistant strains was calculated using the following equation:

$$\frac{No.of\ sensitive\ or\ resistant\ isolates}{Total\ No.of\ the\ isolates\ (30)} \times 100$$

Table 1 Reference guide for the used antibiotics against *S. aureus* isolates.

Antibiotic	Disc conc. (µ/g)	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm	
		Resistant	Sensitive
Ciprofloxacin (CIP)	5	≥ 15	≤ 21
Tetracycline (TE)	30	≥ 14	≤ 19
Cefotaxime (CTX)	30	≥ 21	≤ 22
Amoxicillin-Clavulanic acid (AMC)	30	susceptible staphylococci	
Gentamycin (GN)	10	≥ 12	≤ 15
Trimethoprim – sulfamethoxazole (SXT)	25	≥ 10	≤ 16
Ampicillin (AM)	10	susceptible staphylococci	

Table 2 Reference guide for the used antibiotics against *E. coli* isolates.

Antibiotic	Disc conc. (µ/g)	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm	
		Resistant	Sensitive
Amoxicillin/clavulanic acid (AMC)	30 µg	≥ 15	≤ 21
Ampicillin (AM)	10 µg	≥ 14	≤ 19
Ceftriaxone (CTX)	30 µg	≥ 21	≤ 22
Ciprofloxacin (CIP)	5 µg	≥ 15	≤ 21
Gentamicin (GN)	10 µg	≥ 12	≤ 15
Tetracycline (TE)	30 µg	≥ 10	≤ 16
Trimethoprim/ Sulfamethoxazole (SXT)	25 µg (1.25/23.75) mcg	≥ 10	≤ 16
Chloramphenicol (C)	30 µg	≥ 12	≤ 18

3. RESULTS

In vitro antibiotic sensitivity test was conducted on a total of ninety *S. aureus* isolates (30/each season per each species) as demonstrated in tables (3-5). Although isolates showed variable sensitivities against different used antibiotics, in general, they showed multi-drug resistance for about 87.5% (7/8) and 75.0% (6/8) of tested antibiotics on the isolates from chicken and quail samples, respectively. While *S. aureus* isolates from duck samples were totally resistant to all of the tested antibiotics in all seasons. Resistant isolates to more than three antibiotics was considered as a multi-drug resistant strain (MDR).

On the other hand, tables (6-8) recorded the antibiotic sensitivity profile of the examined *E. coli* isolates. Although they showed variable resistance to the tested antibiotics, in general, they showed higher sensitivity affinity to the tested antibiotics than the examined *S. aureus. Escherichia coli* showed sensitivity to 62.5% (5/8), 87.5% (7/8), and 50.0% (4/8) of the tested antibiotics in the examined isolates from chicken, quail, and duck samples, respectively. Results, also, showed higher resistance to the tested antibiotics in the examined isolates from duck samples, than those of chicken and quail, respectively.

Moreover, as a general view, either *S. aureus* or *E. coli* of summer isolates had higher resistance affinity to the tested antibiotics than in spring and summer, respectively.

Table 3 In-Vitro anti-microbial Sensitivity test for isolated *S. aureus* strains of chicken samples (n=30 / each season).

Antimicrobial agents	Sensitive						Resistant						AA
	Winter		Spring		Summer		Winter		Spring		Summer		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Amoxicillin/clavulanic acid (AMC)	10	33.3	7	23.3	4	13.3	20	66.7	23	76.7	26	86.7	R
Ampicillin (AM)	10	33.3	10	33.3	7	23.3	20	66.7	20	66.7	23	76.7	R
Ceftriaxone (CTX)	13	43.3	10	33.3	7	23.3	17	56.7	20	66.7	23	76.7	R
Ciprofloxacin (CIP)	17	56.7	15	50.0	10	33.3	13	43.3	15	50.0	20	66.7	R
Gentamicin (GN)	16	53.3	13	43.3	10	33.3	14	46.7	17	56.7	20	66.7	R
Tetracycline (TE)	10	33.3	7	23.3	0	0.0	20	66.7	23	76.7	30	100	R
Trimethoprim/ Sulfamethoxazole (SXT)	18	60.0	13	43.3	15	50.0	12	40.0	17	56.7	15	50.0	S
Chloramphenicol (C)	10	33.3	11	36.7	8	26.7	20	66.7	19	63.3	22	73.3	R

No.: Number of isolates, AA: Antibiogram activity, R: Resistant, S: Sensitive

Table 4 In-Vitro anti-microbial Sensitivity test for isolated *S. aureus* strains of quail samples (n=30 / each season).

Antimicrobial agents	Sensitive						Resistant						AA
	Winter		Spring		Summer		Winter		Spring		Summer		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Amoxicillin/clavulanic acid (AMC)	14	46.7	11	36.7	8	26.7	16	53.3	19	63.3	22	73.3	R
Ampicillin (AM)	14	46.7	13	43.3	10	33.3	16	53.3	16	53.3	19	63.3	R
Ceftriaxone (CTX)	17	56.7	14	46.7	11	36.7	13	43.3	16	53.3	19	63.3	R
Ciprofloxacin (CIP)	21	70.0	19	63.3	13	43.3	9	30.0	11	36.7	16	53.3	R
Gentamicin (GN)	20	66.7	17	56.7	14	46.7	10	33.3	13	43.3	16	53.3	S
Tetracycline (TE)	14	46.7	11	36.7	7	23.3	16	53.3	19	63.3	23	76.7	R
Trimethoprim/ Sulfamethoxazole (SXT)	22	73.3	17	56.7	19	63.3	8	26.7	13	43.3	11	36.7	S
Chloramphenicol (C)	14	46.7	15	50.0	12	40.0	16	53.3	15	50.0	18	60.0	R

No.: Number of isolates, AA: Antibiogram activity, R: Resistant, S: Sensitive

Table 5. In-vitro anti-microbial Sensitivity test for isolated *S. aureus* strains of duck samples (n=30 / each season).

Antimicrobial agents	Sensitive						Resistant						AA
	Winter		Spring		Summer		Winter		Spring		Summer		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Amoxicillin/clavulanic acid (AMC)	8	26.7	5	16.7	2	6.7	22	73.3	25	83.3	28	93.3	R
Ampicillin (AM)	8	26.7	8	26.7	6	20.0	22	73.3	22	73.3	24	80.0	R
Ceftriaxone (CTX)	11	36.7	8	26.7	5	16.7	19	63.3	22	73.3	25	83.3	R
Ciprofloxacin (CIP)	15	50.0	13	43.3	8	26.7	15	50.0	17	56.7	22	73.3	R
Gentamicin (GN)	14	46.7	11	36.7	8	26.7	16	53.3	19	63.3	22	73.3	R
Tetracycline (TE)	8	26.7	5	16.7	0	0	22	73.3	25	83.3	30	100	R
Trimethoprim/ Sulfamethoxazole (SXT)	16	53.3	11	36.7	13	43.3	14	46.7	19	63.3	17	56.7	R
Chloramphenicol (C)	8	26.7	9	30.0	6	20.0	22	73.3	21	70.0	24	80.0	R

No.: Number of isolates, AA: Antibiogram activity, R: Resistant, S: Sensitive

Table 6 In-vitro anti-microbial Sensitivity test for isolated *E. coli* strains of chicken samples (n=30 of each season).

Antimicrobial agents	Sensitive						Resistant						AA
	Winter		Spring		Summer		Winter		Spring		Summer		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Amoxicillin/clavulanic acid (AMC)	21	70	18	60	12	40	9	30	12	40	18	60	S
Ampicillin (AM)	15	50	15	50	9	30	15	50	15	50	21	70	R
Ceftriaxone (CTX)	12	40	15	50	12	40	18	60	15	50	18	60	R
Ciprofloxacin (CIP)	20	66.6	18	60	15	50	10	33.3	12	40	15	50	S
Gentamicin (GN)	18	60	18	60	15	50	12	40	12	40	15	50	S
Tetracycline (TE)	9	30	9	30	6	20	21	70	21	70	24	80	R
Trimethoprim/ Sulfamethoxazole (SXT)	18	60	15	50	15	50	12	40	15	50	15	50	S
Chloramphenicol (C)	22	73.3	21	70	17	56.6	8	26.7	9	30	13	43.4	S

No.: Number of isolates, AA: Antibiogram activity, R: Resistant, S: Sensitive

Table 7 In-vitro anti-microbial Sensitivity test for isolated *E. coli* strains of quail samples (n=30 of each season).

Antimicrobial agents	Sensitive						Resistant						AA
	Winter		Spring		Summer		Winter		Spring		Summer		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Amoxicillin/clavulanic acid (AMC)	25	83.3	22	73.3	15	50.0	5	16.7	8	26.7	15	50.0	S
Ampicillin (AM)	22	73.3	18	60.0	13	43.3	8	26.7	12	40.0	17	56.7	S
Ceftriaxone (CTX)	19	63.3	17	56.6	12	40.0	11	36.7	13	43.3	18	60.0	S
Ciprofloxacin (CIP)	20	66.6	18	60.0	12	40.0	10	33.3	12	40.0	18	60.0	S
Gentamicin (GN)	21	70.0	17	56.6	14	46.6	9	30.0	13	43.3	16	53.3	S
Tetracycline (TE)	12	40.0	10	33.3	8	26.6	18	60.0	20	66.6	12	40.0	R
Trimethoprim/ Sulfamethoxazole (SXT)	18	60.0	17	56.6	15	50.0	12	40.0	13	43.3	15	50.0	S
Chloramphenicol (C)	23	76.6	18	60.0	12	40.0	7	23.3	12	40.0	18	60.0	S

No.: Number of isolates, AA: Antibiogram activity, R: Resistant, S: Sensitive

Table 8 In-vitro anti-microbial Sensitivity test for isolated *E. coli* strains of duck samples (n=30 of each season).

Antimicrobial agents	Sensitive						Resistant						AA
	Winter		Spring		Summer		Winter		Spring		Summer		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Amoxicillin/clavulanic acid (AMC)	20	66.7	16	53.3	14	46.7	10	33.3	14	46.7	16	53.3	S
Ampicillin (AM)	12	40.0	14	46.7	10	33.3	18	60.0	16	53.3	20	66.7	R
Ceftriaxone (CTX)	13	43.3	15	50.0	10	33.3	17	56.7	15	50.0	20	66.7	R
Ciprofloxacin (CIP)	15	50.0	18	60.0	13	43.3	15	50.0	12	40.0	17	56.7	S
Gentamicin (GN)	14	46.7	12	40.0	12	40.0	16	53.3	18	60.0	18	60.0	S
Tetracycline (TE)	10	33.3	9	30.0	6	20.0	20	66.7	21	70.0	24	80.0	R
Trimethoprim/ Sulfamethoxazole (SXT)	12	40.0	12	40.0	10	33.3	18	60.0	18	60.0	20	66.7	R
Chloramphenicol (C)	20	66.7	18	60.0	17	56.7	10	33.3	12	40.0	13	43.3	S

No.: Number of isolates, AA: Antibiogram activity, R: Resistant, S: Sensitive

4. DISCUSSION

In small amounts, residues from drugs and other substances used frequently in livestock-raising techniques have been found in animal-derived foods. A small number of substances, mostly antibiotics, have been used to treat and safeguard the health of poultry. The presence of these substances residues in food products is a significant public health concern, particularly in light of the growing interest in, and awareness of the potential presence of drugs and their metabolites in the meat and meat products consumed by humans, as well as the emergence of antimicrobial resistance (AMR).

To avoid bacterial infections in food animals, antibiotics are often used prophylactically or sub-therapeutically. This, combined with the residue left behind, has led to the emergence of multidrug-resistant bacterial isolates, a serious public health concern. Several microorganisms have developed resistance to various antibiotics, which have triggered the expansion of novel antibiotics with a higher resistance level (Kimera *et al.*, 2021).

A major global public health issue is antimicrobial resistance (AMR). One of the main causes of AMR infection in humans is the overuse of antibiotics in poultry farming. More than 700,000 people die each year from antibiotic-resistant infections, which are predicted to surpass cancer mortality by 2050. This is because infections that are resistant to antibiotics are challenging to treat with current antibiotic regimens. Escherichia coli and Staphylococcus aureus are the bacteria most frequently involved in AMR-related fatalities *(Bole, 2022).*

Results of the antimicrobial sensitivity test for the detected *S. aureus* isolates as ~~were~~ summarized in tables (3-5) were somewhat agreed with those recorded by Wu *et al.* (2018) and Lika *et al.* (2021) who recorded that the *S. aureus* isolates were most resistant to tetracycline (88.24%) and chloramphenicol (61.77%); while lower resistance towards gentamycin (23.53%) and Trimethoprim/ Sulfamethoxazole (38.24%); however, all *S. aureus* isolates were resistant to amoxicillin and amoxicillin + clavulanic acid, and Igbinosa *et al.* (2023) who documented that their isolated *S. aureus* was resistant to tetracycline 64(58.2%), ciprofloxacin 71(64.6%), trimethoprim 58.2, 64.6 and 64.6%, respectively; while disagreed with those recorded by Owuna *et al.* (2015), who recorded more sensitivity of *S. aureus* isolates to gentamicin and ciprofloxacin. Most ~~of~~ *S. aureus* isolates were resistant to all β-lactams antibiotics, which is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP20) that has a lower affinity for binding β-lactams (penicillin, cephalosporins, and carbapenems). This allows resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections as mentioned by Amer *et al.* (2021).

Escherichia coli is normally a benign gut commensal, but certain strains include virulence characteristics that can lead to conditions including meningitis, hemorrhagic colitis, diarrhea, and urinary tract infections. *E. coli* strains are utilized as sentinel organisms for the surveillance of AMR with gram-negative resistance characteristics and as indicators of fecal contamination of food items since they are common in humans, animals, the environment, and foods (WHO, 2017).

Regarding with the obtained results of *E. coli* sensitivity test (Tables 6 to 8), ~~it~~ came in the same line with those recorded by Kim *et al.* (2020), who recorded a significant resistance of *E. coli* to ampicillin (69.1%) and tetracycline (64.0%); Mousavi *et al.* (2020) where their *E. coli* isolates exhibited resistance against ampicillin (95.45%), tetracycline (88.63%), gentamycin (84.09%) and Sulfa/trimethoprim (38.63%) antibiotics, while were sensitive to chloramphenicol (72.73%). Mensah *et al.* (2022) recorded that all of their examined *E. coli* isolates were sensitive to gentamicin and cefotaxime.

Variations between different authors may be attributed to differences in season and localities of collection, poultry species samples, and levels of hygienic quality.

Although AMR is a worldwide pandemic, it may be avoided. National governments have acknowledged that stopping the spread of AMR requires an understanding of its symptoms,

transmission, prevention, and treatment. All participants in the food supply chain must work together to ensure the safety of meat across the meat supply chain in order to stop the development of AMR. The role of consumers in the battle against AMR is crucial, and they must understand how important it is. Consumers can help to lessen the threat of AMR by taking precautions against AMR infections brought on by meat and animal products (Aljeldah, 2022).

The survival and reproduction of pathogenic bacteria, their vectors, and their animal reservoirs are directly influenced by climate conditions. Climate change has broadened the geographic distribution of several pathogenic bacteria as a result of persistently rising temperatures at higher latitudes. Extreme weather events linked to climate change occur more often, which fosters the growth of current pathogenic germs and the emergence of new illnesses (Coates and Norton, 2021).

Referring to the obtained results of the current study, *Staphylococcus aureus* and *Escherichia coli* isolates showed more resistance to the examined antibiotics in summer, while they were more sensitive in the winter season, which may be referred to that higher temperature and humidity ~~un~~ in relation to the global environmental climatic changes enhances microbial multiplication and infectivity as was mentioned by MacFadden *et al.* (2018).

Through a rise in temperature, climate change affects the emergence of antibiotic resistance. From 1980 to 2010, a study on the connections between temperature and antibiotic resistance has been carried out in the US. Resistance in *S. aureus* and *E. coli* was the main topic of discussion. According to this study, the percentage of resistant bacteria increased significantly for every 10 °C rise in minimum temperature, equaling 4.2% in *E. coli* and 2.7% in *S. aureus* (MacFadden *et al.*, 2018). Another study that looked at temperature's impact on antibiotic resistance in 28 European nations from 2000 to 2016 found a long-term relationship between ambient temperature and the pace of rise in antibiotic resistance. All drug classes for the pathogens showed faster gains in antibiotic resistance in European nations with 10 °C higher ambient temperatures during the course of the inquiry. The rate of antibiotic resistance rises ranged from 0.33 % year to 1.2%/year (McGough *et al.*, 2000).

5. CONCLUSIONS

Poultry meat appeared to be a source of multidrug-resistant foodborne pathogenic bacteria. *Staphylococcus aureus* and *Escherichia coli* of poultry meat origin appeared to be a critical health problem because of the emergence of AMR strains in the poultry food chain, especially during the hot and humid summer season. Moreover, bacterial isolates from duck samples showed more resistance affinity to the tested antibiotics than those of chicken and quail samples, respectively.

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