Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Physicochemical and Microbiological Quality of Poultry Drinking Water in Karak Governorate, Jordan

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Abstract

THE study aimed to investigate the microbiological and physicochemical quality of poultry drinking water in the Karak governorate, Jordan. Twenty-one water samples were collected from various poultry production locations, including broiler farms, layer farms, and local chicken butcher shops (Nattafat) in Karak. The susceptibility of the isolated gram-negative bacteria to commonly used antibiotics for poultry treatment, such as Cefotaxime, Amoxicillin-Clavulanate, Ciprofloxacin, and Gentamicin, were examined. The results revealed significant differences in the mean pH values and turbidity of water samples based on the source of collection. The values were 7.1 and 81.6 for broiler farms, 7.7 and 14.5 for layer farms, and 7.7 and 8.9 for butcher shops, respectively. Microbiological tests indicated that 66.7% of the total samples were contaminated with gram-negative bacteria, with the highest incidence of bacterial contamination found in broiler farms (85.7%). The isolated bacteria included *Escherichia coli, Serratia odorifera, Stenotrophomonas maltophilia, Klebsiella oxytoca, Raoultella ornithinolytica, Salmonella enterica ssp Arizona, Kluyvera spp, Citrobacter koseri,* and *Citrobacter amalonaticus*. Evaluation of the isolated bacteria's susceptibility to selected antibiotics showed that all bacterial isolates exhibited resistance against Amoxicillin-Clavulanate, Ciprofloxacin, and Gentamicin, except for E. coli, which showed partial sensitivity to gentamicin. All isolated bacteria, except *S. odorifera*, were susceptible to the cefotaxime antibiotic. In conclusion, the study emphasizes the importance of implementing a quality control system in poultry management to improve the quality of drinking water, reduce bacterial infections, and mitigate the emergence of multidrug-resistant (MDR) bacteria.

Keywords: Jordan, Poultry, Water Quality, Multidrug-Resistant (MDR), Physicochemical properties.

Introduction

Jordan is known for a shortage of water sources which directly impacts different agriculture sectors [1]. A shortage of water supply can affect the quality of drinking water available for animal consumption, particularly in poultry. Water quality, particularly good physiochemical and microbial properties, is crucial to the safe and healthy production of poultry, and consequently, to the general public's health [2,3]. Safe food, fewer antibiotic prescriptions, and a decline in the majority of diseases are partially dependent on clean drinking and cleaning water [4]. Water is usually stored in storage tanks that are exposed directly to sunlight and air which can directly influence its physiochemical and microbial status [5].

Water sources are among the most frequent environments in which bacteria are spread [6]. Water contamination with Gram-negative bacteria, particularly Enterobacteriaceae, is of interest due to their high pathogenicity, and resistance to a wide range of antibiotics [7]. For example, the Extended Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae can cleave the beta-lactam ring in the beta-lactam antibiotics, which are commonly used in poultry farms, mediating their resistance against this group of antibiotics [8]. Multiple antibiotic resistance (MAR) is a result of the widespread usage of antibiotics in poultry farms [9]. At the farm level, antibiotic administration is usually performed by dissolving medication in storage tanks. However, once storage tanks are refilled again with fresh water, filled tanks will certainly contain

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(Received 24 August 2024, accepted 17 October 2024)
DOI: 10.21608/EJVS.2024.315171.2336

residues of the antibiotics which contribute to the development of antibiotic-resistant bacteria which may infect animals as well as humans [8]. Thus, the value of good water quality is more pronounced to produce of free antibiotic animal products [10].

The primary aim of this study is to investigate the physicochemical and microbial properties of drinking water used in various poultry husbandry locations (broiler farms, layer farms, and local chicken butcher shops) across the Karak governorate in southern Jordan. Additionally, the study aims to assess the susceptibility of isolated Gram-negative bacteria in the drinking water to a group of antibiotics commonly used by poultry farms in Jordan.

Material and Methods

Samples Collection

Twenty-one water samples were collected at random from different types of poultry husbandry places (broiler farms (coded with B, n = 7), layer farms (coded with L, n = 7), and local chicken butcher shops (coded with S, n = 7), in December 2023. Five samples were collected from each water tank in the farm in sterile falcon tubes (25 ml/tube), and then samples from each farm were mixed and separated into two sterile and cleaned glass bottles. One bottle was used for the detection of physicochemical prosperities and the other was used for bacteria bacterial culture.

Physicochemical analysis of the water samples

Physical and chemical properties including water pH, Total dissolved Solids (TDS), and water turbidity were analysed. Water pH was determined using a pH test kit (CHECKIT® Comparator Test Kit pH, Lovibond, Germany) by following the manufacturer's instructions. TDS reading was taken by TDS meter as mg/L (ppm). Water turbidity was measured in terms of the Nephelometric Turbidity Unit (NTU) by a turbidity meter.

Isolation of gram-negative bacteria

Gram-negative bacteria were isolated by using Eosin methylene blue (EMB) media, which is a selective medium used for the isolation of most common gram-negative [10]. This was performed by inoculating 0.1 mL of diluted water sample on EMB agar and incubating the plates for 24h at 37C. The sample that exhibited bacterial growth, as indicated by the formation of the colonies, was considered contaminated. Colonies were classified based on morphological characteristics such as color, size, shape, margin, and others. Accordingly, each selected colony was subcultured on EMB agar to get pure isolates.

Identification of the Isolated *Enterobacteriaceae* and other non-fastidious Gram-Negative bacteria. A single colony of pure isolates was incubated for 24 hours in twenty wells included with the Analytical Profile Index (API) 20E, which is a biochemical panel for identification and differentiation of the family *Enterobacteriaceae* members and other non-fastidious gram-negative bacteria. The manufacturer's instructions were followed for conducting the 20 fundamental tests and interpreting the obtained results. Also, nitrate reduction and cytochrome-oxidase tests were used for further identification of *Enterobacteriaceae*, in which most of the family, with few exceptions, give positive nitrate reduction and negative oxidase results [11]. The *Salmonella* identified isolate was further confirmed by observing its growth on *Shigella-Salmonella* (SS) agar medium which appeared as a colorless colony with a black centre.

Antibiotic Susceptibility Tests

The disc diffusion technique, as described by Bauer [8], was used to examine bacterial susceptibility to commonly used antibiotics, including Cefotaxime (CXM, 30 mg), Amoxicillin-Clavulanate (AMX, 30 mg), Ciprofloxacin (CIP, 5 mg), and Gentamicin (CN, 10 mg). A single colony of bacteria was cultured in broth media and incubated at 37 °C for 24 hours. Subsequently, a bacterial suspension containing 1.5 x 10^8 CFU/mL (0.5 McFarland solution) was prepared. A volume of 100 µL of the bacterial suspension was spread on the surface of Muller Hinton Agar (MHA). Under aseptic conditions, a standard antibiotic disc was placed on the surface of the inoculated agar plate. The plates were then incubated for 24 hours at 37 °C, and the diameter of the inhibition zone formed around the disc was measured in millimetres. The resistance pattern of the bacteria was determined based on the size of the inhibition zone. [9].

Statistical analysis

One-way ANOVA was used to compare the means of pH, turbidity, and TDS values among different sources of water samples. Additionally, an independent t-test was conducted to analyse the differences in mean values of physicochemical measures (pH, turbidity, and TDS) between samples with and without Gram-negative bacteria growth. Significant differences between groups were determined when $P < 0.05$.

Results

Physicochemical Properties

physicochemical properties (pH, TDS, and turbidity) of water samples obtained from different locations are shown in Table 1. The mean pH value of samples collected from broiler farms was 7.07 ± 0.30, whereas the samples from layer farms were 7.72 ± 0.09, and the local chicken butcher shops samples were 7.71 ± 0.10. The statistical analysis revealed significant differences in pH values among the sample sources (P = 0.037). Similarly, the turbidity of the samples varied significantly (P = 0.033) among the three sources, with broiler farms samples showing the highest turbidity (81.61 ± 34.09), followed by layer farms samples (14.52 ± 4.00), and butcher shops samples (8.85 ± 2.31). However, there was no statistically significant variation (P = 0.700) in the total dissolved solids (TDS) measurements among the sample sources, with values of 847.14 ± 17.14 for broiler farm samples, 829.00 ± 36.41 for layer farms, and 860.00 ± 19.52 for butcher shops.

Isolation and identification of Enterobacteriaceae and other gram-negative bacteria

Bacterial isolation and identification revealed the presence of common poultry pathogenic gram-negative bacteria in the water samples. The biochemical test results identified the following bacteria: *Escherichia coli, Serratia odorifera, Stenotrophomonas maltophilia, Klebsiella oxytoca, Raoultella ornithinolytica, Salmonella enterica subsp. Arizona, Kluyvera spp., Citrobacter koseri,* and *Citrobacter amalonaticus* (Table 2).

In total, 14 out of the collected 21 samples (66.7 %) were found contaminated with Gram-negative bacteria. The highest incidence of bacterial contamination was found in broiler farms (85.7%), in which two samples were contaminated with *Serratia odorifera*, one sample was contaminated with *Stenotrophomonas maltophilia*, one sample was contaminated with Klebsiella oxytoca, one sample was contaminated with *Raoultella ornithinolytica*, and one sample was contaminated with *Salmonella enterica ssp* Arizona. On the other hand, 57.1% of samples collected from both layer farms and local chicken butcher shops were found contaminated with gram-negative bacteria. For layer farms sample, *kluyveraspp* was found in three samples, and Citrobacterkoseri\amalonaticus was found in one sample, while for local chicken butcher shops, two samples were contaminated with *Escherichia coli*, and two samples with *Serratia odorifera*.

Antibiotic Susceptibility of the Isolated Bacteria

As shown in Table 3, all isolated bacteria, except *Serratia odorifera* 1, were susceptible to the cefotaxime antibiotic, with the range of growth inhibition zones from 13 mm for *Stenotrophomonas maltophilia* to 33 mm for *Kluyvera* spp. However, all bacterial isolates showed resistance to Amoxicillin-Clavulanate, Ciprofloxacin, and Gentamicin, except for *E. coli*, which exhibited partial sensitivity to gentamicin with a 15 mm inhibition zone.

Effect of Gram-negative Bacteria Contamination on Physicochemical Quality of Water Samples

An independent t-test was conducted to examine the impact of Gram-negative bacteria contamination on the physicochemical quality of water samples. Table 4 displays the mean values of pH, turbidity, and TDS for samples with positive Gram-negative growth, which were 7.5, 38.6, and 857.4, respectively. In contrast, samples without Gram-negative bacteria had mean values of pH at 7.60, turbidity at 27.8, and TDS at 821.4. However, the statistical analysis revealed that there was no significant difference in the mean values of pH, turbidity, and TDS between Gram-negative contaminated samples and the non-contaminated group.

Discussion

Good water quality requires optimal physicochemical properties (pH, TDS, turbidity) and minimal microbial contamination, especially from pathogenic and antibiotic-resistant bacteria [4]. Providing high-quality drinking water to poultry farms is essential to ensure healthy and safe food production for human consumption [12]. Water sources in chicken farms are susceptible to bacterial contamination due to exposure to fecal matter, debris, dust, and other sources [10]. Water pollution in chicken farms often results from inadequate quality control measures for poultry drinking water [13]. Therefore, this study aimed to assess the quality of drinking water supplied to poultry farms in Karak governorate, Jordan, by evaluating physicochemical properties and microbial characteristics of water samples collected from broiler farms, layer farms, and local chicken butcher shops.

The pH of water can impact its taste, palatability, and absorption during digestion [2]. The recommended pH range for water is 6.5-8.5 [14, 15]. Water with a pH outside this range can be less palatable or affect absorption and performance [16]. In our study, the average pH value was 7.5, ranging from 5.5 to 8.0. Only one sample had a pH of 5.5, which is below the WHO preferred range. Statistical analysis revealed significant differences in pH values among different sample sources (P = 0.037). However, there was no significant difference in pH values based on the presence of Gram-negative bacteria (P = 0.513), suggesting no correlation between bacterial contamination and water pH.

TDS measured were within the recommended range of less than 1000 mg/L [17]. All our tested samples are within the range with approximately a mean of 845mg/l and without significant difference between means of samples collected from various sources. TDS affects dissolved vaccines and antibiotics in water and becomes unpleasant [16]. Turbidity was also determined to reflect the presence of non-dissolved and suspended matter such as salts, organic materials, and other microscopic organisms in water [16]. Turbidity is normally not above 5 NTU [17]. In this study, most of the samples (76.3 %) had higher turbidity values than the recommended level showing a high mean value = 35.0 NTU, with a minimum value equal to 1.7, while the maximum value was 247.6. however, the turbidity values were significantly varied among samples from various sources ($P = 0.033$), where broiler farm samples were the most turbid samples (81.6 NTU), followed by layer farms samples (14.5 NTU) and the least turbidity was from the butcher shop (8.9 NTU). This highly turbid water may affect the aesthetic value of poultry drinking water [18].

The current study found that the samples that exhibited positive growth for Gram-negative bacteria showed slightly lower pH, and slightly higher mean values of STD and turbidity levels than Gram-negative free samples. However, these differences in the physicochemical properties were statistically insignificant, indicating that Gram-negative bacteria contamination had no significant effect on the physicochemical quality of water samples. In contrast with our results, it was reported that acidic conditions are increased in the presence of bacterial growth as microbes release CO_2 which consequently decreases pH value [17]. Likewise, the presence of vast bacterial contamination could increase the turbidity of water samples [1]. The discrepancy between our findings with these reports may refer to the presence of other biological and chemical pollutants in water samples, which could affect the physicochemical quality more than Gram-negative bacteria growth. In addition, the statistically insignificant results could be attributed to the small sample size which is considered as a limitation of the present study.

The quality of drinking water is also affected by biological contaminants. Drinking water for humans and animals must be monitored and evaluated according to international standards. As part of the fecal-oral route, drinking water is considered a major source of spreading pathogenic microbes [19]. Poultry drinking water and water systems can act as infection sources and disease transporters [20]. Poultry drinking water is acceptable when the total bacterial count is less than 1000 CFU/mL, or the total coliform bacteria is less than 50 CFU/mL [21]. Another important criterion that must be critically considered to accept drinking water is the absence of all fecal *coliform* bacteria (zero CFU/mL) [21]. Fecal coliform bacteria, including *E. coli, Enterobacter sp, Klebsiella spp, Citrobacter spp,* and *Serratia spp.* are commonly used as a bacterial indicator of the sanitary quality of water [22].

In this study, broiler water samples showed the highest level of contamination, as indicated by the percentage of contaminated samples (40%) and the number of isolated bacteria (6 isolates). The percentages of contaminated samples for each layer farm and butcher shop were 26% and the isolates were 4. This might be due to the nature of the poultry house infrastructure, the number of birds, and the period for growing and caring for the birds [23]. Besides, all bacteria identified in this study are transmissible by fecal-oral route. Broiler, layer, and butcher water samples contain fecal coliform bacteria such as *E. coli, S. odorifera, K. oxytoca, S. enterica,* and *C. koseri*. Previous reports showed that *E. coli, Shigella, K. pneumonia, S. typhi, S. kentucky, and S. garoliare* the most common isolates from poultry drinking water [24-26].

Although the water sources can be evaluated, controlled, and pumped as high-quality water, they can be contaminated when it reaches the bird's houses or caches. Feces from the birds or spills from the birds' mouths are the major sources of contamination [27]. Also, most of the isolated bacteria from drinking water are opportunistic bacteria, such as those belonging to the *Citrobacter, Enterobacter,* and *Klebsiella genera* [28]. Thus, they may threaten human and poultry health [29].

Medication such as antibiotics is commonly given to poultry in drinking water. The use of drinking water as a route to deliver antibiotics has elevated the prevalence rate of antibiotic-resistant bacteria. The extensive use of the same water tanks and water system may emphasize the problem since it may increase the exposure time of the bacteria to the antibiotics, thus facilitating the emergence of antibiotic-resistant bacteria [16]. In addition, biofilm-forming bacteria are common in this water system. Biofilm-forming bacteria are 10 times more resistant to antibiotics [30, 31].

Previous reports found that poultry houses are infectious sources that contribute to the emergence and spreading of multidrug-resistant bacteria in poultry and humans [32]. In this study, the isolated bacteria appeared to be resistant to ciprofloxacin (100%), amoxicillin + clav (100%), and gentamycin (87.5%), while they were sensitive to cefotaxime. *Serratia odorifera* has been reported here as the most resistant bacteria. The species belonging to this genus have been characterized as beta-lactamase producers. *Serratia* spp. are intrinsically resistant to polymyxin, nitrofurantoin, cephalosporins, ampicillin, and ampicillin-sulbactam [33]. A high prevalence rate of ESBL in E. coli and other

Enterobacteriaceae members such as *Klebsiella spp, Salmonella spp,* and *Citrobacter spp* have been isolated from poultry farms water, and workers [3, 7]. Mustedanagic et al. [28] found that *Stenotrophomonas spp.* and *Citrobacter* spp isolated from drinking water samples are MDR.

Conclusion

The present study has concluded that the physicochemical quality of drinking water used in poultry production in Karak Governorate needs improvement, particularly in reducing high water turbidity, which could adversely affect the aesthetic value of poultry drinking water. Furthermore, several multidrug-resistant (MDR) pathogenic bacteria were isolated from poultry drinking water, highlighting the role of contaminated drinking water as a source of infections and the development of antibiotic resistance. Therefore, it is highly recommended to implement an efficient quality control system in poultry management to enhance the quality of drinking water, reduce the incidence of bacterial infections, and limit the occurrence of MDR bacteria. Further studies are needed to identify the management factors affecting the physicochemical and microbial quality of poultry drinking water, with a focus on those contributing to the presence of MDR bacteria in poultry farms and poultry products.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Statistical results of the physio-chemical analysis

Physio-chemical Character			
pH	Butcher shops	7.7 ± 0.1	0.037*
	Broiler	7.1 ± 0.3	
	Layer	7.7 ± 0.1	
Turbidity	Butcher shops	8.9 ± 2.3	0.033*
	Broiler	81.6 ± 34.1	
	Layer	14.5 ± 4.0	
TDS	Butcher shops	860.0 ± 19.5	0.700
	Broiler	847.1 ± 17.1	
	Layer	829.0 ± 36.4	

Data is presented as mean ± SE (n= 7). * significant difference among groups at P < 0.05.

TABLE 2. Identified Gram-negative bacteria in water samples collected from the poultry husbandry sources

Bacteria	Butcher shop	Broiler	Layer
Escherichia coli	2 (28.5%)		
Serratia odorifera 1	2 (28.5%)	2 (28.5%)	
Stenotrophomonasmaltophilia		1(14.3%)	
Klebsiella oxytoca		1(14.3%)	
Raoultellaornithinolytica		1(14.3%)	
Salmonella enterica ssp Arizona		1(14.3%)	
kluyveraspp			3 (42.9%)
Citrobacterkoseri\amalonaticus			1 (14.3%)
Total	4 (57.1%)	6 (85.7%)	4 (57.1%)

Data is presented as n (%)

TABLE 3. Susceptibility profile of the isolated Gram-negative bacteria to selected antibiotics

	Antibiotics			
Bacteria	**Cefotaxime**	**Ciprofloxacin**	**amoxicillin+clav**	**Gentamycin**
E.coli	26 mm	R	R	15 mm
Serratia odorifera 1	R	R	R	R
Stenotrophomonas maltophilia	13 mm	R	R	R
Klebsiella oxytoca	32 mm	R	R	R
Raoultellaornithinolytica	30 mm	R	R	R
Salmonella enterica ssp Arizona	31 mm	R	R	R
Kluyvera spp	33 mm	R	R	R
Citrobacter koseri\amalonaticus	21 mm	R	R	R

R: means resistant to the above antibiotics. Numbers indicate the mean of diameters (mm) of the growth inhibition zone for triplicates.

TABLE 4. Differences between mean values of physicochemical measures (pH, turbidity, and TDS) of samples with and without Gram-negative bacteria growth.

Physicochemical measures	**G-negative bacteria growth**	**N**	**Mean ± SE**	**Sig.**
pH	Positive	14	7.5 ± 0.2	0.703
	Negative	7	7.6 ± 0.2	
Turbidity	Positive	14	38.6 ± 17.6	0.726
	Negative	7	27.8 ± 19.2	
TDS	Positive	14	857.4 ± 12.5	0.114
	Negative	7	821.4± 35.5	

Data was expressed as mean ± SE. The statistical analysis was performed by Independent t-test. The difference between mean values was considered significant if $p < 0.05$. N: refers to the number of samples; Negative: refers to samples without Gram-negative bacteria growth; Positive: refers to Samples with Gram-negative bacteria growth.

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دراسة الجودة الفيزيائية والكيميائية والميكروبيولوجية لمياه شرب الدواجن في محافظة الكرك، الأردن

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الملخص

هدفت هذه الدراسة إلى التحقق من الجودة الميكروبيولوجية والفيزيائية والكيميائية لمياه شرب الدواجن في محافظة الكرك، الأردن. تم جمع 21 عينة مياه من مواقع مختلفة لإنتاج الدواجن مثل مزارع الدجاج اللحم، ومزارع الدجاج البياض، ومحلات جزارة الدجاج المحلية. تم تحديد الخواص الفيزيائية والكيميائية، مثل الرقم الهيدروجيني للمياه، والمواد الصلبة الذائبة الكلية (TDS)، وتعكر الماء. تم تقييم الجودة الميكروبية لمياه شرب الدواجن من خلال عزل وتحديد البكتيريا سالبة الجرام الموجودة في عينات المياه. بالإضافة إلى ذلك، تم فحص حساسية البكتيريا سالبة الجرام المعزولة للعديد من المضادات الحيوية المستخدمة بشكل متكرر لعلاج الدواجن، بما في ذلك سيفوتاكسيم، أموكسيسيلين-كلافولانيت، سيبروفلوكساسين، وجنتاميسين. أظهرت النتائج وجود فروق معنوية في متوسطات قيم الأس الهيدروجيني والعكارة لعينات المياه تبعاً لمصدر التجميع، حيث بلغت القيم 7.1 و81.6 لمزارع الدجاج اللحم، و7.7 و14.5 لمزارع البياض، و7.7 و8.9 لمحلات الجزارة، على التوالي. وأظهرت الاختبارات الميكروبيولوجية أن 66.7% من إجمالي العينات كانت ملوثة بالبكتيريا سالبة الجرام. أعلى نسبة تلوث بكتيري وجدت في مزارع الدجاج اللحم (85.7%). شملت البكتيريا المعزولة *Escherichia coli, Serratia odorifera, Stenotrophomonas maltophilia, Klebsiella oxytoca, Raoultella ornithinolytica, Salmonella enterica ssp Arizona, Kluyvera spp, Citrobacter koseri, and Citrobacter amalonaticus*. أظهر تقييم حساسية البكتيريا المعزولة للمضادات الحيوية المختارة أن جميع العزلات البكتيرية أظهرت مقاومة ضد الأموكسيسيلين-كلافولانيت، السيبروفلوكساسين، والجنتاميسين، باستثناء *E.coli* التي كانت حساسة جزئياً للجنتاميسين. كانت جميع البكتريا المعزولة باستثناء *S. odorifera* حساسة للمضاد الحيوي السيفوتاكسيم. علاوة على ذلك، أظهر التحليل الإحصائي أن التلوث بالبكتيريا سالبة الجرام لم يكن له أي آثار كبيرة على الخواص الفيزيائية والكيميائية لعينات المياه. وفي الختام، أبرزت الدراسة أهمية تطبيق نظام مراقبة الجودة في إدارة الدواجن لتحسين جودة مياه الشرب، والحد من حدوث الالتهابات البكتيرية، والبكتيريا المقاومة للأدوية المتعددة للحفاظ علي صحه الانسان.

الكلمات الدالة: الأردن، الدواجن، جودة المياه، مقاومة الأدوية المتعددة (MDR)، الخصائص الفيزيائية والكيميائية.