Egypt. J. Vet. Sci. **Vol. 54,** No. 3, pp. 479-490 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Association Between Antimicrobial Resistance and Biofilm Production of *Aeromonas hydrophila* Isolated from Beef and Mutton in Duhok Abattoir

Shereen A. Yousif [1], Nadhim S. Jakhsi [2] and Dhyaa M. Jwher [3]
[1]Department of Pathology and Microbiology, Collage of Veterinary Medicine, University of Duhok, Duhok, Iraq.
[2]Prof Dr.Vet.Public Health &Epidemiology, University of Zakho, Zakho, Iraq
[3]Dep. of Vet. Public Health, College of Vet. Med. University of Mosul, Mosul, Iraq

THE study aimed to investigate the presence of *A. hydrophila* in mutton and beef, and to determine the possible association between resistance to antibiotics and biofilm formation ability. A total of 91 meat samples; (60) mutton and (31) beef collected from local slaughterhouse in Duhok city. All samples were cultured and *A. hydrophila* strains were isolated using microbiological and biochemical assays then confirmed molecularly by amplifying 16S rRNA gene. The antimicrobials susceptibility test was implemented using Kirby-Bauer procedure and biofilm formation ability was quantified by micro-titer plate appliance. The bacterial identification revealed that 41(45.05%) isolates were belonging to *A. hydrophila* distributed as 28/60 (46.67%) for mutton and 13/31 (41.93%) for beef. Antibiotic susceptibility test results showed that the highest resistance was recorded for Cephalothin, Amoxicillin, and Tetracycline at a rate of 90.24%, 82.92%, and 85.36% respectively, while high sensitivity to Nitrofurantoin, Chloramphenicol, Cefixime, Trimethoprim, Ciprofloxacin, and Ceftriaxone was detected in 95.12%, 92.68%, 97.56%, 90.24%, 90.24%, and 97.56% of the isolates, respectively. The biofilm formation assay demonstrated that (90.23%) of obtained isolates were biofilm producers distributed among strong, intermediate and weak and there was a positive association between antibiotic resistance and biofilm formation. Our study's findings imply that the occurrence of *A. hydrophila* in food meats is a significant health hazard and may cause food-borne illnesses. Because Aeromonads can endure cold temperatures, and thrive in a variety of habitats, it is important to pay them more attention and strict sanitary practices should be used to limit bacterial contamination.

Keywords : *Aeromonas hydrophila*, Biofilm production, 16S rRNA, PCR, Duhok abattoir.

Introduction

Aeromonas hydrophila is a widely existing motile [1], aerobic, Gram negative, mesophilic bacteria, positive for oxidase- and catalase [2], belonging to *Aeromonadaceae* family [3]. These bacteria can grow and produce toxins at a wide range of temperatures (2-42) °C [4] indicating that storing food at refrigeration seems to be ineffective to control these pathogens [5] and due to their capability to grow in refrigerated food [6] they pose a serious risk to people safety and health [7, 8]. Aeromonas spp. incessantly isolated from many food products including seafood, fish and shellfish, raw meat, dairy and vegetables and have an ample host spectrum, including humans [9]. Recently, a lot of interest has been focused on this bacterium because it affects not only aquatic animals, causing the aquaculture sector

*Corresponding author: Shereen A. Yousif, E-mail: shereenomer65@yahoo.com, Tel.: 009647504715456
(Received 29/01/2023, accepted 26/02/2023)
DOI: 10.21608/EJVS.2023.188876.1432

to suffer tremendous financial losses [10], but causes infections in humans, such as septicemia, skin necrotizing fasciitis, gangrene, gastro-enteritis, and diarrhea among travelers due to unsuitable handling of meat and consumption of contaminated food [9].

The pathogenesis of Aeromonas spp. infection is convoluted and poorly understood [11]. These pathogens produce a diversified virulence factors; such as expression of cell membrane components, toxins, enzymes and other molecules such as lipopolysaccharides (LPS) present in the cell walls of Gram-negative bacteria [12]. The presence of such virulence elements helps these bacteria to stick, invade, destroy the host cells and defeat its immunological response [13]. Antibiotic resistance of *Aeromonas* is typically interposed by genes mapped to bacterial chromosomes or plasmids that give out resistance to generality of beta lactams [14]. *Aeromonas* spp. covers a variety of species that are broadly disseminated in aquatic environments like (sewage, oceanic, rivers and drinking water) as well as facilities used for water treatment [14], they may form biofilms in water pipes and are counted to be emerging pathogen by the Environmental Protection Agency (EPA) [15]. The development of biofilms is regarded as a component of many bacteria's harmful activity [16], Biofilms have emerged as major contributors to recurring infections and antimicrobial resistance [10]. Recently, many microorganisms have been identified molecularly. The sequences of several housekeeping genes such as *gyrB*, *rpoB* and *rpoD* were used as effective tools for the identification of Aeromonas species, also sequencing of *16S rRNA* is another sturdy molecular criteria for bacterial species identification [12]. Due to its stability and specificity, direct sequencing of *16S rRNA* is widely used as a unique marker for bacterial identification. Classification of bacteria according to genetic sequencing is very effective for phylogenetic identification at various levels [17]. Aeromonas' ability to grow combatively in cooling temperatures is a public health hazard [12]. The presence of *A. hydrophila* in animal products is a public health problem for humans, especially people who handle infected animals and their products [18].

Currently, there is limited data investigating *A. hydrophila* in raw meat in Duhok governorate/ Iraq. Therefore, the present work is designed and performed to investigate the prevalence of *A.hydrophila* in mutton and beef samples obtained from Duhok slaughterhouse, and to define the possible association between antibiotic resistance and production of biofilms by this species.

Materials and Methods

Sampling

The study was carried out in Duhok governorate/ Iraq. Meat samples were taken from Duhok slaughterhouse. A total of ninety-one raw meat samples were included in this study, 60 samples of sheep carcasses and 31 samples of beef carcasses were collected after final wash for the period between 15th September and 30th October 2022. Meat samples were collected aseptically using a polyethylene bag and placed in an ice box after that transported immediately to the "research center" in the College of Veterinary Medicine/ University of Duhok for diagnostic analysis.

Bacterial Isolation

Twenty-five grams from each meat sample were minced, peptone water added, homogenized and incubated overnight at 37°C as pre-enrichment step before being plated on blood agar (Accumix™/Belgium) with the addition of 5% blood of sheep then incubated as previous and on MacConkey agar (NEOGEN®/USA) followed by Gram staining [19].

Biochemical analysis using "VITEK 2" system

According to Elbehiry et al. [20], VITEK® 2 Gram-Negative Identification cards (bioMerieux Inc, France) were used to identify the isolates. The method was summarized by suspending 34-fresh colonies in 0.45% sterile normal saline. DensiChek™ (bioMerieux Inc, France) used to measure the optical density of the suspension and adjusted to 0.50-0.63 Mcfarland. The organism suspension was placed into the instrument and the desired organisms were matched with the strains used as references and stored in the system software.

Molecular investigation

DNA extraction

Thermal extraction (boiling method) is used for DNA extraction. According to Taha and Yassin [21] three morphologically similar colonies were selected and added to (1.5ml sterile tube) filled with 300 µl of sterile double distilled water, vortexed for (30s) and then heated at 95ºC for 10 min. The tubes were cooled immediately with ice and after that centrifuged for 10 min. About 150 µl supernatant was transferred to another sterile tube

and used as a template DNA for PCR. The pureness and concentricity of the DNA were measured using a Nano-Drop (Thermo Fisher Scientific™ USA). The extracted DNA samples were stored at -20 °C till used for further investigations by polymerase chain reaction (PCR).

Conventional PCR

For confirmation of the isolates as *A. hydrophila*, *16S rRNA* universal gene amplified and the primer set listed in (Table 1) were used.

The amount of PCR mixture was 25 µL which contained 1 µL forward primer, 1 µL reverse primer (Table 1), 12.5 µL AddStart Taq Master (ADDBIO INC, Korea), 5.5 µL sterilized double distilled water, and 5 µL DNA template. The mixture was put in PCR tubs (KIRGEN-China). The DNA samples were amplified using the thermo-cycler program (Applied Biosystems GeneAmp® PCR System ٩٧٠٠) which was adjusted to the following settings [22]: Initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 45 s then annealing at 52°C for 1min. followed by extension at 72°C for 1min., and a final extension at 72°C for 7 min. After completion of all cycles, the products were run on 1.5% agarose gel stained with safe dye (ADDBIO INC, Korea) and observed under UV Transilluminator (Vilber Lourmat Super Bright-France). To find the size of the PCR product the (100 bp) DNA ladder H3 RTU (GeneDireX, Taiwan) was used.

Sequencing identity analysis

For confirmation of the results, (10) PCR amplicons were chosen and sent to (Immunogene Center / North Korea) for DNA Sequencing. The outcomes were submitted to the similarity search using the BLAST search program of the "National Center for Biotechnology Information" (NCBI) [23].

Antibiotic Susceptibility

According to Jorgensen & Turnidge [24] Standard Kirby-Bauer disk diffusion technique and Mueller-Hinton agar were used to estimate the sensibility of *A. hydrophila* isolates against 12 different antibiotics (Bioanalyse®/Turkey): Tetracycline (TE 10µg), Cephalothin (KF 30µg), Amoxicillin (AX 10µg), Ciprofloxacin (CIP 10µg), Cefixime (CFM 5µg), Streptomycin (S 5µg), Ceftriaxone (CRO 10µg), Erythromycin (E 10µg), Gentamycin (CN 10µg), Trimethoprim (TMP 10µg), Nitrofurantoin (F 100µg) and Chloramphenicol (C 10µg). The suspension solution of the isolates was prepared equivalent to 0.5 McFarland opacity and transferred to Mueller-Hinton agars (Himedia®/India) . Discs saturated with antimicrobial agents were fixed on the agar then incubated at 37 °C for 24hrs. The inhibition zone was measured using a digital calibrator, and the test results were divided into three categories: resistant, intermediate and sensitive.

Biofilm detection

The 96 well micro-titer Plate method for biofilm detection described by Stepanovic et al. [25] was used to rate the potential of biofilm production of *A. hydrophila*. Trypticase Soy Broth (TSB) (Neogen®/USA) was used to reach an optical density (OD) of 0.8 at 630 nm of bacterial suspension by inoculation of the isolates and incubate for 24hrs, then 100 µL of the suspension piped in the wells (triple samples). The negative controls were the wells not inoculated with "TSB", the plate incubated for 96hrs at 28ºC. After that, the wells were washed with sterilized normal saline three times, (200 µL) methanol was added to each well for 15 min. after drying at room temperature. The drying step was repeated, then the plate was stained with 200 µL of 2% solution of Hucker's crystal violet (2g crystal violet, 0.8g ammonium oxalate, 20ml ethanol and 80ml distilled water). Five minutes later, the washing and drying steps were done again. After 15 min., 200µL of (ethanol-acetone) as discoloring solution was added. Finally, the intensity of absorption was measured using ELISA plate reader at 630 nm. The (ODs) of each sample were calculated by finding the mean of absorbance of three wells and comparing it to the mean absorbance of negative controls (ODnc), and classified to: non-biofilm production (ODnc<0.0600), weak biofilm

TABLE 1. Primers used for amplification of *16S rRNA* gene

Gene name	Primers	Sequences (5′-3′)	Amplicon size	Reference
16S rRNA	Forward	AGAGTTTGATCCTGGCTCAG	1498 bp	Abdulhasan et al. [22]
	Reverse	GGTTCACTTGTTACGACTT		

production (0.0600<ODs<0.0950), moderate (0.0950<ODs<0.1500), and strong production (ODs > 0.1500).

Results

The results of conventional cultural methods and gram staining revealed that out of 91 samples, 49 (53.84%) were positive for motile *Aeromonas* spp. distributed among 31/60 (51.66%) for mutton and 18/31 (58.06%) for beef (Table 2). These methods investigated (i) the hemolytic activity of the bacteria when after overnight incubation at 37°C, a zone of hemolysis around the colonies were observed on blood agar plates enriched with 5% of sheep blood and (ii) pale like colonies appeared on MacConkey agar indicating that the isolates are unable to ferment lactose sugar.

For identification of the isolates, the VITEK 2 system was applied and (41) isolates were identified to be *A. hydrophila* distributed between 28/60 (46.67%) for mutton and 13/31(41.93%) for beef with a high level of Discrimination for identifying of *A. hydrophila* up to 99%, (Fig. 1, Table 2).

The results of polymerase chain reaction (PCR) revealed that all the biochemically identified isolates were molecularly confirmed to be *A. hydrophila* (Fig. 2).

The sequences of *16S rRNA* gene compared with (NCBI) GenBank data through BLAST program. The following Accession Numbers (KR819398, KC252600 and MT279533) were matched with our study results and affirmed as *A. hydrophila* with 98-100% identity.

Antibiotic susceptibility test

The results of the antibiotic susceptibility test for 41 molecularly confirmed isolates showed that the highest resistance was recorded for Cephalothin, Amoxicillin, and Tetracycline at a rate of 90.24%, 82.92%, and 85.36% respectively, while the moderate resistance to Streptomycin, Erythromycin, and Gentamycin was reported in 80.48%, 75.61%, and 82.92% of the isolates respectively. High sensitivity to Nitrofurantoin, Chloramphenicol, Cefixime, Trimethoprim, Ciprofloxacin, and Ceftriaxone was detected in 95.12%, 92.68%, 97.56%, 90.24%, 90.24%, and 97.56% of the isolates respectively as shown in (Fig. 3).

Quantification of biofilm in micro-titer plates

The results of biofilm production for *A. hydrophila* isolates using micro-titer plate method showed that 37 (90.23%) of the isolates were biofilm producers at different levels (strong, intermediate and weak biofilm producers); 17 (41.46%) isolates were strong biofilm producer, 11 (26.82%) were moderate biofilm producer and

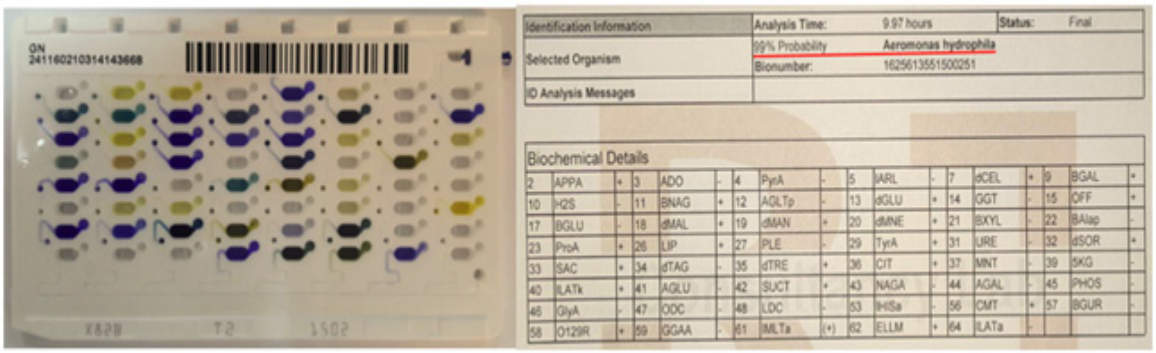

Fig. 1. Identification of bacterial species by VITEK 2 system.

TABLE 2. Isolation rates of *A. hydrophila* using cultural methods and VITEK 2 system.

Sample type	No. of Samples	Cultural methods		VITEK 2 system	
		No.	%	No.	%
Mutton	60	31	51.66	28	46.67
Beef	31	18	58.06	13	41.94
Total	91	49	**53.84**	41	**45.05**

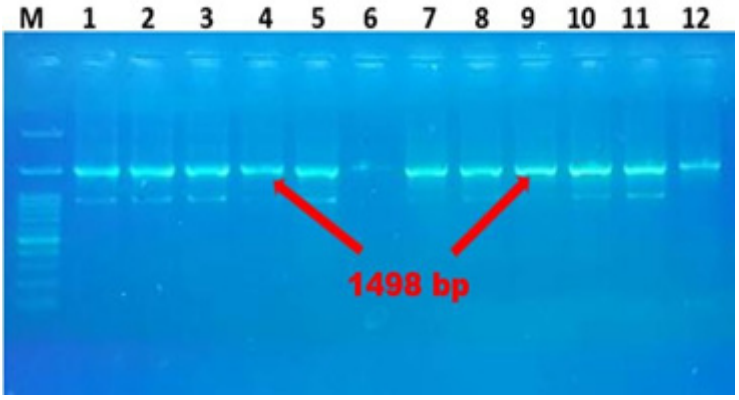

Fig. 2. Gel electrophoresis of PCR amplification of *16S rRNA* gene (1498 bp) of *A.hydrophila* isolates (Lane M; 100 bp Marker), Lane 1-12: bacterial isolates.

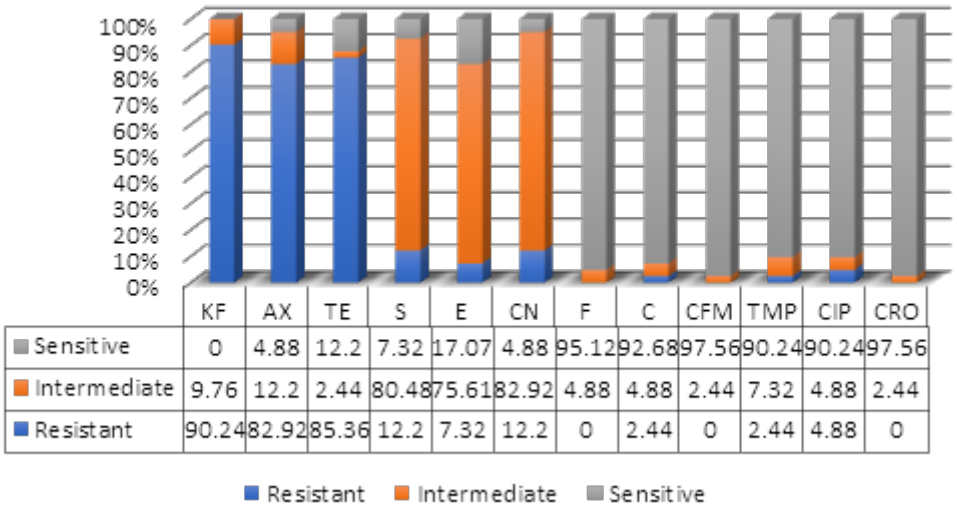

Fig. 3. Results of the Antibiogram of *A. hydrophila* isolated from the slaughterhouse in Duhok city.

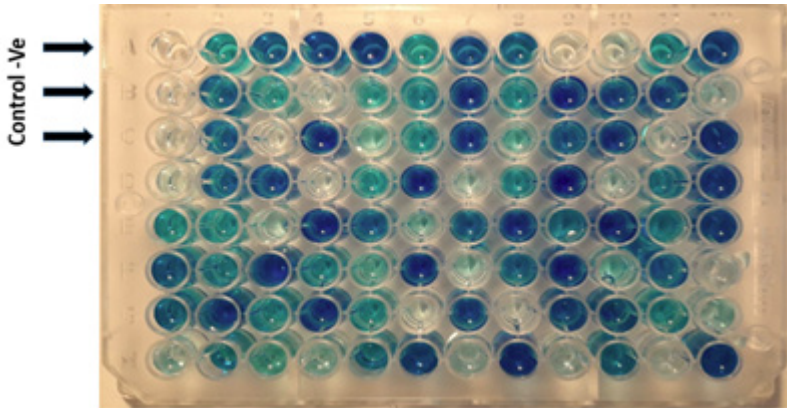

Fig. 4. Biofilm production Assay for *A. hydrophila* isolates using micro-titer Plate method

TABLE 3. Distribution of biofilm forming isolates of *A. hydrophila*.

Pattern of biofilm production	Range of ODs (nm)	Number of Isolates	%
Strong	0.1560 – 0.2230	17	**41.46**
Moderate	0.0950 - 0.1440	11	**26.82**
Weak	0.0650 - 0.0920	9	**21.95**
Total		**37**	**90.23**

9 (21.95%) were weak biofilm producer. And only 4 (9.77%) isolates were non-biofilm producers as shown in (Fig. 4) and (Table 3), the mean OD= 0.0600 was calculated for negative controls on the uncultured TSB.

Association between Biofilm producing Ability and Resistance to Antibiotics

The results of the antibiotic susceptibility test and biofilm production of *A. hydrophila* isolates revealed that there was a positive correlation between antibiotic resistance and biofilm production.

The isolates of higher resistance to the Cephalothin, Amoxicillin and Tetracycline were strong biofilm producers at a rate of 41.46%, while the ability of moderate biofilm production at a rate of 26.82% was for the isolates that showed intermediate resistance to Streptomycin, Erythromycin and Gentamycin also 21.95% of the isolates showed weak biofilm production which was sensitive to Nitrofurantoin, Chloramphenicol, Cefixime, Trimethoprim, and Ceftriaxone, except of Ciprofloxacin which was strong biofilm producer (Table 4).

Discussion

The study paid particular attention to evaluating the presence of pathogenic bacteria in meat, as this is a key point for ensuring consumer health and safety, because microbial contamination shortens food shelf life posing serious health risks [1, 26]. Aeromonas has received great attention because some strains of this microorganism tend to cause a significant intestinal disorders that are transmitted through food and have been linked to serious illnesses in human [12]. Existence of Aeromonads has been documented in food of animal origins like meat, poultry, dairy products and seafood, as well as in vegetables. Worldwide isolation of Aeromonas spp. from food revealed that the dominant spp. was A. hydrophila [27] and has been linked to 85% of gastroenteritis in human [28]. A. hydrophila strains are important emerging foodborne pathogens because they have the ability to grow at low temperatures as well as their exceptional ability to acclimate the extreme conditions in the environment [29].

Our findings in current study revealed that the level of contamination with A. hydrophila in total analyzed raw meat samples were 45.05%, the bacterial isolates were found in (46.67%) and (41.94%) of mutton and beef meat samples respectively. As traditional culturing and biochemical-based methods for bacterial identification are time-consuming and have some difficulties, Vitek-2 compact system and molecular assays were appreciably used for the identification of *A. hydrophila* from meat samples as simple, rapid and precise methods [30]. There were some diversities in the results obtained from

TABLE 4. Association between antibiotic susceptibility and biofilm production of A. *hydrophila* isolates.

Biofilm pattern	Antibiotics													Total
	KF		AX	TE	S	E	CN	F	C	CFM	TMP	CIP	CRO	
Strong	7	5	3	0	0	0	0	0	0	0	2	0		17
Moderate	0	0	0	3	5	3	0	0	0	0	0	0		11
Weak	0	0	0	0	0	0	2	2	2	1	0	2		9
Non	0	0	1	0	0	0	0	1	0	1	0	1		4
Total	7	5	4	3	5	3	2	3	2	2	2	3		**41**

previously mentioned methods. The problem with misidentification is due to two factors; The first reason is that these methods can identify strains at the genus level (Aeromonas) and not at the species level, the second one is misidentification with other strains such as Vibrio spp. [20]. Molecular assays particularly PCR is the most precise method for identification of microorganisms because of their stability and specificity, the *16S rRNA* gene is usually applied as a characteristic biomarker for bacterial identification via direct sequencing. [31, 32].

Since there is genetic heterogeneity among several *16S rRNA* genes in bacterial genomes, amplification of this gene alone is ineffective for identifying Aeromonas spp. unless it is followed by nucleotide sequencing [33, 34]. The identified bacteria were confirmed and the sequences were related to the GenBank database using "BLAST" program of the (NCBI) and more than 98% identity was found between the *16S rRNA* sequencing data from the current study and the *16S rRNA* gene sequences of *A.hydrophila* registered by other authors accessible at GenBank.

Our findings agreed with the results obtained by Kadry et al. [35] who found *A. hydrophila* in (40%) of beef meat samples and approximately similar to Elmanama et al. [36] who found that the occurrence of *A. hydrophila* in beef samples was (35%) but clearly different from ratio obtained by Rossi Júnior et al. [37] who isolated *A. hydrophila* from (3.3%) of beef samples. The isolation rate obtained in our study was greater than the rate registered in previous studies; Sharma and Kumar [38] and Ahmed et al. [39] founds that (13.13%) and (25%) of meat samples were contaminated with *A. hydrophila* respectively, also *A. hydrophila* registered in (12%) of goat meat and (7.69%) of beef samples in a study conducted by Kumar et al. [40], and lower rate obtained by Osman et al. [26] who found this pathogen in (6.5%) of mutton meat samples and in (0.0%) of beef raw meat samples. Higher incidence was reported by Ibrahim et al. [41] who found (60%) of beef and (58%) of mutton samples in Australia to be contaminated with this bacterium. The degree of variations in the results obtained by many authors can be due to the disparity of geographical locations, the methods and techniques adopted for isolation, samples collecting seasons [42], nutrition factors, livestock density, breeding systems, methods used in the slaughtering process, and the level of sanitation, which considered among the most important factors that affect the different levels of contamination with *A. hydrophila* in animal food samples [43]. The findings obtained in this study are concerning because different spp. of *Aeromonas* genus can be captured during various stages of the slaughtering process; via carcass surface, intestinal content, water, poorly cleaned equipment and handling. The broad use and misuse of antibiotics are important factors in the increased incidence of antibiotic-resistant microorganisms in food posing a risk to consumers [27]. The isolates obtained in our study showed strong resistance to Cephalothin, Amoxicillin, and Tetracycline while the intermediate resistance were to Streptomycin, Erythromycin and Gentamycin, the largest number of the isolates were sensitive to Nitrofurantoin, Chloramphenicol, Cefixime, Trimethoprim, Ciprofloxacin, and Ceftriaxone, these findings are nearly in line with the results reported by Moori-Bakhtiari et al. [48] who found that (100%) of *A. hydrophila* strains were susceptible to Ciprofloxacin and Trimethoprim, while Dias et al. [54] reported that (100%) of *A. hydrophila* isolates obtained from different animal samples were resistant to Ciprofloxacin, the variations in the findings could be attributed to the differences of the samples types and Aeromonas strains. A study conducted by Yang et al. [44] aimed to investigate the prevalence and antibiotic susceptibility of *A. hydrophilia* from grass carp in China, the authors found that most strains were resistant to Ampicillin (95.24%) and Tetracycline (88.89%), these results are strongly agreed with our findings, while we found opposite results regarding susceptibility to Streptomycin since they found that (79.37%) were resistance to Streptomycin. Another study conducted by Matyar et al. [45] revealed that (14.4%), (7.2%) and (11.3) of *A. hydrophila* isolates were resistance to Tetracycline, Gentamycin and Chloramphenicol respectively, on the other hand they found that a high proportion of *A. hydrophila* isolates were resistance to Cephalothin (86.6%), this is nearly similar to our finding in regards to Cephalothin (90%). Antibiotic resistance occurs due to different mechanisms; chromosomal mutation, change in drug target, formation of biofilms and etc. [46]. Numerous studies on food and animal products conducted in last few years revealed high detection rate of resistant bacteria as a result of antibiotic overuse and misuse in animal husbandry [47].

The results revealed that 90.23% of the examined isolates were capable to form biofilms and classified in terms of biofilm productivity as strong, intermediate and weak biofilm producers, this percentage is lower than results reported in previous studies by Moori-Bakhtiari et al. [48] and Mohamed et al. [49] who found that (100%) and (96%) of the isolates respectively are biofilm producers at different levels (strong, intermediate and weak). The biofilm formation ability of *A. hydrophila* and its relation to antibiotics susceptibility was evaluated, the results revealed that strong biofilm producer isolates were also resistant to 25% of the antibiotics used in the study (Cephalothin, Amoxicillin and Tetracycline). The isolates with strong biofilm production, which can act as a protective wall against antibiotics, may lead to easy transmission of resistant genes within this protective structure [50, 51]. The bacterial isolates that show moderate ability to produce biofilms exhibit intermediate resistance to several types of antibiotics (Gentamycin, Erythromycin and Streptomycin), while the isolates that classified as weak and non-biofilm producer were susceptible to 50% of the studied antibiotics (Nitrofurantoin, Chloramphenicol, Cefixime, Trimethoprim, Ciprofloxacin and Ceftriaxone). Biofilm production is a mutually beneficial behavior between microorganisms to enhance their survival. The possibility of gene transfers between microorganisms to develop antibiotic resistance and biofilm production cannot be ruled out. This fact confirms the great relationship between the two mechanisms [52]. Many studies have confirmed the existence of antibiotic-resistance bacterial isolates that have the ability to produce biofilm, while several bacterial isolates have the ability to form biofilm yet are not resistant to antibiotics. Additionally, there are some isolates who lack both abilities [53]. This was shown by the findings of our study when some isolates expressed high sensitivity to ciprofloxacin and strong biofilm production. Other studies are needed to investigate this association with other types of antibiotics and to provide evidence on the development of this resistance and its relationship with production of biofilms [48], bacteria›s ability to adhere to the surfaces of various materials and visceral organs may have clinical implications in terms of disease›s prolongation and emergence of antibiotic resistance [15].

Conclusions

Our findings showed that red meat produced in Duhok slaughterhouse is contaminated with *A. hydrophila* which may pose a risk to human health, and it is an indication of unhygienic procedures used in slaughterhouses. The study revealed multidrug resistance of *A.hydrophila* and its association with the production of biofilm that facilitate escape from antibiotics effect. Biofilm formation is a potent threat to public health and consequently the effectiveness of the antibiotics will decrease. It is essential that all meat processing and handling must take place under high standard of hygienic conditions, and the products must be as far away from all sources of contamination. To fully visualize the threats that these organisms bring to public health, more studies are needed to explore the abundance of *A. hydrophila* in clinical, food, and water samples.

Acknowledgement

The authors are grateful to Dr. Zanan Mohamed Taha, the director of Duhok Research Centre-College of Veterinary Medicine for his support during implementing this study.

Conflict of Interest

There are no competing interests.

Funding Statements

Self-funding.

References

1. Alhazmi, M. I., Isolation of Aeromonas spp. from Food Products: Emerging Aeromonas Infections and Their Significance in Public Health. *Journal of AOAC International*, **98** (4), 927–929 (2015).

2. Stratev, D., Vashin, I. and Rusev, V. Prevalence and survival of Aeromonas spp. in foods - a review. *Revue de Médecine Vétérinaire*, **163** (10), 486-494 (2012).

3. Das, A., Vinayasree, V., Santhosh, C. R. and Sree Hari, S. Surveillance of *Aeromonas sobria* and *Aeromonas hydrophila* from commercial food stuffs and environmental sources. *Journal of Experimental Sciences,* **3** (9), 36-42 (2012).

4. Martins, L. M., Marquez, R. F., and Yano, T. Incidence of toxic Aeromonas isolated from food and human infection. *FEMS Immunology and Medical Microbiology*, **32** (3), 237-242 (2002).

5. Sarker, B., Arif, M., Eashmen, N., Akter, M.R. and Kabir, S.M.L. Isolation, identification and antibiogram profile of *Aeromonas hydrophila* from broiler chickens in Mymensingh Sadar, Bangladesh. *Asian-Australasian J.Food Saf. Secur*., **4** (1), 22-30 (2020).

6. Praveen, P.K., Debnath, C., Shekhar, S., Dalai, N. and Ganguly, S. Incidence of Aeromonas spp. infection in fish and chicken meat and its related public health hazards: A review. *Veterinary World*, **9** (1), 6-11 (2016).

7. Stratev, D., Gurova, E., Vashin, I. and Daskalov, H. Multiplex PCR detection of heamolysin genes in β-heamolytic *Aeromonas hydrophila* strains isolated from fish and fish products. *Bulgarian. J. Agricul. Sci.*, **22** (2), 308-314 (2016).

8. Grave, I., Rudzate, A., Nagle, A., Miklasevics, E. and Gardovska, D. Prevalence of Aeromonas spp. Infection in Pediatric Patients Hospitalized with Gastroenteritis in Latvia between 2020 and 2021. *Children*, **9** (12), 1684 (2022).

9. Igbinosa, I. H., Igumbor, E. U., Aghdasi, F., Tom, M. and Okoh, A. I. Emerging Aeromonas species infections and their significance in public health. *Scientific World Journal*, **2012**: ID 625023 (2012).

10. Du, H., Pang, M., Dong, Y., Wu, Y., Wang, N., Liu, J., Awan, F., Lu, C. and Liu, Y. Identification and Characterization of an Aeromonas *hydrophila* Oligopeptidase Gene pepF Negatively Related to Biofilm Formation. *Frontiers in Microbiology*, **7**,1497 (2016).

11. Janda, J.M., and Abbott, S.L., The genus Aeromonas: taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev.*, **23** (1) 35-73 (2010).

12. Fernández-Bravo, Ana, and Maria José Figueras. An Update on the Genus *Aeromonas*: Taxonomy, Epidemiology, and Pathogenicity. *Microorganisms,* **8** (129) 3-6 (2020).

13. Gonçalves Pessoa, R.B., de Oliveira, W.F., Marques, D.S.C., dos Santos Correia, M.T., de Carvalho, E.V.M.M. and Coelho, L.C.B.B. The genus Aeromonas: A general approach. *Microb. Pathog.*, **130**, 81–94 (2019).

14. Aravena-Roman, M., Inglis, T. J., Henderson, B., Riley, T. V. and Chang, B. J. Antimicrobial Susceptibilities of Aeromonas strains isolated from clinical and environmental sources to 26 antimicrobial agents. *Antimicrobial Agents Chemotherapy*, **56** (2), 1110-1112 (2012).

15. Poole, T.L., Schlosser, W.D., Anderson, R.C., Norman, K.N., Beier, R.C. and Nisbet, D.J. Whole-Genome sequence of Aeromonas *hydrophila* CVM861isolated from diarrhetic neonatal swine. *Microorganisms*, **8** (11),164 (2020).

16. Sakimura, T., Kajiyama, S., Adachi, S., Chiba, K., Yonekura, A., Tomita, M., Koseki, H., Miyamoto, T., Tsurumoto, T., and Osaki, M. Biofilm-Forming Staphylococcus epidermidis Expressing Vancomycin Resistance Early after Adhesion to a Metal Surface. *BioMed. Research International*. **2015**, 1-8 (2015).

17. Sarkar, A., Saha, M. and Roy, P. Identification and typing of Aeromonas *hydrophila* through 16S rDNA-PCR fingerprinting. *Journal of Aquaculture Research and Development*, **3**(6), 1-4 (2012)

18. De Silva, B.C.J., Hossain, S., Wimalasena, S.H.M.P., Pathirana, H.N.K.S. and Heo, G.J. Putative virulence traits and antibiogram profile of Aeromonas spp. isolated from frozen white-leg shrimp (Litopenaeus vannamei) marketed in Korea. *J. Food Saf.*, **38** (4), e12470 (2018).

19. Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S. and Fitzpatrick, E. *Veterinary microbiology and microbial disease. John Wiley & Sons* (2011).

20. Elbehiry, A., Marzouk, E., Abdeen, E., Al-Dubaib, M., Alsayeqh, A., Ibrahem, M., Hamada, M., Alenzi, A., Moussa, I., and Hemeg, H. A. Proteomic characterization and discrimination of Aeromonas species recovered from meat and water samples with a spotlight on the antimicrobial resistance of *Aeromonas hydrophila*. *Microbiology Open*, **8** (11), e782 (2019).

21. Taha, Z.M. and Yassin, N.A. Prevalence of diarrheagenic Escherichia coli in animal products in Duhok province, Iraq. *Iran. J. Vet. Res.*, **20** (4), 255-262 (2019).

22. Abdulhasan, G. A., Alattar, N. S., and Jaddoa, N. T. M. Comparative Study of Some Virulence Factors and Analysis of Phylogenetic Tree by 16S rDNA Sequencing of Aeromonas hydrophila Isolated from Clinical and Environmental Samples. *Iraqi Journal of Science*, **60**(11), 2390–2397 (2019).

23. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., **28**(10), 2731-2739 (2011).

24. Jorgensen, J.H. and Turnidge, J.D. Susceptibility Test Methods: Dilution and Disk Diffusion Methods. *J. Clin. Microbiol*., **26** (1), 1253-1273 (2015).

25. Stepanovic, S., Vukovic, D., Dakic, I., Savic, B. and Svabic-Vlahovic, M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J. Microbiol. Methods*, **40** (2), 175-179 (2000).

26. Osman, K., Aly, M., Kheader, A. and Mabrok, K. Molecular detection of the Aeromonas virulence aerolysin gene in retail meats from different animal sources in Egypt. *World J. Microbiol. Biotechnol.*, **28** (5),1863-1870 (2012).

27. Stratev, D. and Odeyemi, O.A. Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review. *Journal of Infection and Public Health*, **9** (5), 535-544 (2016).

28. Duman, M., Saticioglu, I.B., Janda, J.M. and Altun, S. The determination of the infectious status and prevalence of motile Aeromonas species isolated from disease cases in rainbow trout (*Oncorhynchus mykiss*) and aquarium fish. *J. Fish Dis.*, **41**(12), 1843–1857 (2018)

29. Daskalov, H. The importance of *Aeromonas hydrophila* in food safety. *Food Control*, **17**(6), 474-483 (2006).

30. Ayeni, F.A., Gbarabon, T., Andersen, C. and Nørskov-Lauritsen, N. Comparison of identification and antimicrobial resistance pattern of Staphylococcus aureus isolated from Amassoma, Bayelsa state, Nigeria. *Afr. Health Sci.*, **15**(4), 1282-1288 (2015)

31. Janda, J. M., and Abbott, S. L. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology,* **45**, 2761-2764 (2007).

32. Hua, H. T., Bollet, C., Tercian, S., Drancourt, M. and Raoult, D. *Aeromonas popoffii* urinary tract infection. *Journal of Clinical Microbiology*, **42**, 5427-5428 (2004).

33. Alperi, A., Figueras, M.J., Inza, I., and Martínez-Murcia, A.J. Analysis of 16S rRNA gene mutations in a subset of *Aeromonas* strains and their impact in species delineation. *International Microbiology,* **11**(3),185-194 (2008).

34. Morandi, A., Zhaxybayeva, O., Gogarten, J. P. and Graf, J. Evolutionary and diagnostic implications of intragenomic heterogeneity in the 16S rRNA gene in *Aeromonas* strains. *J. Bacteriol*., **187**(18), 6561-6564 (2005).

35. Kadry, M. and Nader, S. M. Epidemiological Studies of *Aeromonas hydrophila* and its Public Health Importance, *International Journal of Microbiological Research,* **9** (3), 109-112 (2018).

36. Elmanama, A.A. and Ferwana, N. Yersinia enterocolitica and *Aeromonas hydrophila* in clinical, food and environmental samples in gaza strip. *J. Al Azhar Univ. Gaza*, **13**, 69-82 (2011).

37. Rossi Júnior, O.D., Amaral, L.A., Nader Filho, A. and Schocken-Iturrino, R.P. Bacteria of the genus Aeromonas in different locations throughout the process line of beef slaughtering. *RPCV*, **101**(557-558) 125-129 (2006).

38. Sharma, I. and Kumar, A. Occurrence of enterotoxigenic Aeromonas species in foods of animal origin in North East India. *Eur. Rev. Med. Pharmacol. Sci.*, **15**(8), 883-887 (2011).

39. Ahmed, R. M., Ismaiel, A. A., Abou Zeid, A., Ibrahim, R. A. and Enan, G. Biological characteristics of enteropathogenic Aeromonas species isolated from different Egyptian foods. *Biologia*., **76**(5), 1577-1586 (2021).

40. Kumar, A., Bachhil, V.N., Bhilegaonakar, K.N. and Agarwal, R.K. Occurrence of enterotoxigenic Aeromonas species in foods. *J. Comm. Dis*., **32**(3), 169–174 (2000).

41. Ibrahim, A. and Mac Rae, I.C. Incidence of Aeromonas and Listeria spp. in red meat and milk samples in Brisbane, Australia. *Int. J. Food Microbiol.*, **12**(2-3), 263–269 (1991).

42. Didugu, H., Thirtham, M., Nelapati, K., Reddy, K.K., Kumbhar, B.S., Poluru, A. and Pothanaboyina, G. A Study on the prevalence of Aeromonas spp. and its enterotoxin genes in samples of well water, tap water, and bottled water. *Veterinary World*, **8**(10),1237-1242 (2015).

43. Rather, M.A., Willayat, M.M., Wani, S.A., Munshi, Z.H. and Hussain, S.A. A multiplex PCR for detection of enterotoxin genes in Aeromonas species isolated from foods of animal origin and human diarrhoeal samples. *J. Appl. Microbiol*., **117**(6),1721-1729 (2014).

44. Yang, Y., Miao, P., Li, H., Tan, S., Yu, H. and Yu, H. Antibiotic susceptibility and molecular characterization of *Aeromonas hydrophila* from grass carp. *Journal of Food Safety*, **38**(1), e12393 (2017).

45. Matyar, F., Kaya, A. and Dinçer, S. Distribution and antibacterial drug resistance of Aeromonas spp. from fresh and brackish waters in Southern Turkey. *Annals of Microbiology*, **57**(3), 443-447 (2007).

46. Reygaert, W.C. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol.*, **4**(3), 482-501 (2018).

47. Yousif, S. A. and Jwher, D. Detection of multiple presence of antibiotic residues in slaughtered sheep at Duhok abattoir, Iraq. *Iraqi Journal of Veterinary Sciences*, **35**(1), 49–55 (2021).

48. Moori-Bakhtiari, N., Tulabi, Z. and Alishahi, M. Biofilm-Producing Ability and Antibiotic Resistance Pattern of Pathogenic Strains of *Aeromonas hydrophila*. *Jundishapur Journal of Microbiology*, **12**(12), e97640 (2020).

49. Mohamed, M., Ahmed, H., Rizk, M., Gharieb, R. and Abdel-Maksoud, S. *Aeromonas hydrophila* from Fish and Humans: Biofilm Formation and Genetic Relatedness. *Zagazig Veterinary Journal,* **47**(4), 340–351 (2019).

50. Tomas, J.M. The main Aeromonas pathogenic factors. *ISRN Microbiol*., **4**, 256-261 (2012).

51. Grilo, M. L., Pereira, A., Sousa-Santos, C., Robalo, J. I. and Oliveira, M. Climatic Alterations Influence Bacterial Growth, Biofilm Production and Antimicrobial Resistance Profiles in Aeromonas spp. *Antibiotics*, **10**(8), 1008 (2021).

52. Niederdorfer, R., Besemer, K., Battin, T.J. and Peter, H. Ecological strategies and metabolic trade-offs of complex environmental biofilms. *NPJ Biofilms and Microbiomes,* **3,** 1-6 (2017).

53. Nadell, C.D. and Bassler, B.L. A fitness trade-off between local competition and dispersal in *Vibrio cholerae* biofilms. *Proceedings of the National Academy of Sciences,* **108**(34), 14181-14185 (2011).

54. Dias, C., Borges, A., Saavedra, M.J. and Simoes, M. Biofilm formation and multidrug-resistant *Aeromonas* spp. from wild animals. *J. Glob. Antimicrob. Resist*., **12**,227-234 (2018).

مدى ارتباط مقاومة المضادات الحيوية وإنتاج الاغشية الحيوية لبكتريا الايروموناس هيدروفيلا المعزولة من لحوم الأغنام والابقار المجزورة في مسلخ مدينة دهوك

شيرين احمد يوسف[١] ، ناظم سليمان جقسي[٢] ضياء محمد طاهر جوهر[٣]
[١] قسم الامراض و البكتريولوجي - كلية الطب البيطري - جامعة دهوك - العراق.
[٢]رئيس جامعة زاخو - زاخو- العراق.
[٣] قسم الصحة العامة البيطرية – جامعة الموصل - العراق.

بكتريا الايروموناس هيدروفيلا هي أحدى أنواع جنس الايرموناس و قد تتواجد في الأغذية ذات المنشا الحيواني، ولها تأثيرات كبيرة على الصحة العامة. هدفت الدراسة الى التحري عن وجود هذه البكتريا في لحوم الأغنام والابقار المجزورة في مسلخ مدينة دهوك ودراسة العلاقة بين مقاومتها للمضادات الحيوية وإنتاجها للاغشية الحيوية. جمعت ٩١ عينة من اللحوم المجزورة في مسلخ دهوك، ٦٠ عينة من لحوم الاغنام و ٣١ عينة من لحوم الابقار. تم العزل باستخدام الطرق التقليدية والاختبارات الكيموحيوية ثم شخصت العزلات بالكشف الجزيئي عن الجين *16S rRNA* بتقنية تفاعل البلمرة المتسلسل. اجري فحص الحساسية للمضادات الحيوية بطريقة Kirby-Bauer واختبرت قدرة العزلات على انتاج الاغشية الحيوية باستخدام طريقة الصفيحة المعيارية. كشفت النتائج عن وجود ٤١ عزلة للايروموناس هيدروفيلا بنسبة ٤٥,٠٥٪، منها ٢٨ عزلة بنسبة ٤٦,٦٧٪ من لحوم الأغنام و ١٣عزلة بنسبة ٤١,٩٤٪ من لحوم الابقار. وأظهرت العزلات مقاومة عالية لكل من السيفالوثين والاموكسيسلين والتتراسايكلين بنسبة ٩٠,٢٤٪، ٨٢,٩٢٪ و٨٥,٣٦٪ على التوالي، في حين اظهرت حساسية عالية لكل من النيتروفيورانتين والكلورامفنكول والسفكسيم والترايميثبريم والسيبروفلوكساسين والسفراكسون بنسبة ٩٥,١٢٪ و٩٢,٦٨٪ و٩٧,٥٦٪ و٩٠,٢٤٪ و٩٠,٢٤٪ و٩٧,٥٦٪ على التوالي. واظهرت التنائج ان ٩٠,٢٣٪ من العزلات كانت منتجة للاغشية الحيوية وبدرجات مختلفة، وكانت هناك علاقة طردية بين المقاومة للمضادات الحيوية وانتاج الاغشية الحيوية بين العزلات. ان تواجد بكتريا الايروموناس هيدروفيلا في اللحوم يشكل خطرا على الصحة العامة. وبسبب قدرتها على تحمل درجات الحرارة المنخفضة والتعايش في مختلف البيئات، فمن الضروري تطبيق الإجراءات الصحية الصارمة لتقليل مستوى التلوث في اللحوم.

الكلمات الدالة: الايروموناس هيدروفيلا، الاغشية الحيوية،PCR، *16S rRNA*، مدينة دهوك.