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Distribution and Prevalence of Aeromonas hydrophila Isolates from Clinical Cases

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Abstract

The current research involved the investigation of the prevalence and laboratory patterns of Aeromonas hydrophila in human clinical samples. Two hundred clinical samples were examined of which 20 samples (10%) were positive to the A. hydrophila and 180 samples (90) were negative. The highest isolation rate was detected in stool samples of 15 positive isolates (15%), which showed gastrointestinal infections to have a strong association. The rates of lower isolation were found in urine samples (7%), and blood samples (6.7%). Wound/burn samples did not recover any isolates which implies that this pathogen does not play a major role in this type of infection among the population under study.

The first classification was carried out on morphological basis. On both MacConkey agar and blood agar, all isolates grew well as non-lactose-fermenting colonies and gave smooth and hemolytic colonies respectively. The capability of growing in enriched media was indicated by the growth on chocolate agar. The microscopic observation revealed all the isolates to be Gram-negative bacteria.

Anoxidase- and catalase-tests showed that all the isolates were positive, as expected of A. hydrophila. The system was identified by VITEK 2 Compact system with 100 percent consensus with the conventional methods. PCR showed the presence of the lipase gene in 90% and the protease gene in 100% of isolates, indicating the genetic presence of virulence-associated determinants. In general, the research confirms the clinical value of A. hydrophila, especially in gastrointestinal infections, and the importance of a combination of the conventional, automated, and molecular diagnostic methods.

Keywords: Aeromonas hydrophila; Clinical samples; Epidemiology; PCR; Virulence genes.

1. Introduction

Aeromonas hydrophila is a Gram-negative, non-obligatory anaerobic bacterium that has over the last few decades received an increasing amount of attention as an emerging opportunistic human pathogen [1]. Previously as an environmental microorganism, mostly related to freshwater and aquatic environments, A. hydrophila is currently considered a clinically important agent that can cause a broad range of clinical outcomes in human beings, including mild and self-limiting gastrointestinal diseases and severe and potentially fatal systemic diseases such as septicemia, necrotizing fasciitis and hepatobiliary infections [2,3]. It is believed that the growing clinical significance of this bacterium is interconnected with its ecological adaptability, potential virulence, and the growing antimicrobial resistance, and thus, it makes the study a significant topic of epidemiological and laboratory research across the globe [4].

A. hydrophila has the natural habitat in rivers, lakes, wastewater, swimming pools and aquaculture settings where it can survive under varied environmental conditions [5]. Exposure to human beings takes various forms such as consumption of contaminated water or food- especially seafood- direct contact with contaminated water in recreational or occupational exposures, and traumatic injuries in aquatic exposures [6,7]. The large spectrum of transmission pathways provides easy and close contact between humans and the organism particularly in areas that lack water sanitation facilities or are characterized by large scale aquaculture activities [8]. As a result, A. hydrophila-induced infections have been on the increase in both developing and developed countries, making it publically significant to the global health [9].

A. hydrophila is clinically related to varied manifestations of the disease. The most frequent manifestation is gastroenteritis, which occurs especially often in children and travelers; it is accompanied by diarrhea of watery or bloody nature, abdominal pain, fever, and vomiting [10,11]. In immunocompromised persons, such as patients with liver cirrhosis, malignancies, diabetes mellitus, or immunosuppressive therapy, organism may tend to invade extra intestinal sites resulting in wound infections, bacteremia, septic shock, and life-threatening soft tissue infections [12,13]. The majority of the studies have indicated high mortality rates with invasive A. hydrophila infections especially among patients with underlying chronic illnesses highlighting the severity of this pathogen clinically [14].

The virulence factors (complex) that allow colonization, tissue invasion, immune evasion and host cell destruction have been identified in the pathogenicity of A. hydrophila [15]. These are hemolysins, enterotoxins, proteases, lipases and secretion systems which together help in the organism to cause disease [16]. Molecular investigations have established that several clinical isolates contain numerous virulence linked genes, which could be the reason as to why the clinical results have been inconsistent among infected individuals [17]. The expression and the presence of these virulence determinants depend not only on the genetic factors but also environmental conditions which reiterates the significance of the integrated use of laboratory and epidemiological studies in the behavioral aspects of this pathogen [18].

Correct laboratory detection of A. hydrophila is still one of the most important elements of clinical diagnosis and epidemiological surveillance. Traditional diagnostics is based on the culture features and biochemical, such as oxidase positive and glucose fermentation, and hemolytic activity on blood agar diagnostics [19]. VITEK 2 automated identification systems have also enhanced the accuracy of diagnostic and decreased turnaround time in clinical microbiology laboratories [20]. However, Aeromonas species can also be misidentified by phenotypic similarity, which is why molecular methods can be used to perform a definitive diagnosis [21]. The real-time methods which include polymerase chain reaction (PCR)-based methods that focus on conserved genetic markers including the 16S rRNA gene have become indispensable in relation to reliable species-level identification and epidemiological investigations [22].

Epidemiologically, the prevalence and the distribution of A. hydrophila infection differ significantly at geographic level and are determined by environmental, climatic and socioeconomic factors [23]. During

the warmer seasons, infections are common especially in summer periods when water temperature is high and recreational water activities are high [24]. A. hydrophila poses a significant fish pathogen as well as a possible zoonosis in countries where the aquaculture sector is highly developed [25]. All these add up to the dynamically changing epidemiology of A. hydrophila that requires the study, in particular regions, to determine its clinical implication and its burden on human health.

In Iraq and other countries, there are little surveillance information available to A. hydrophila infections in human clinical samples, even though there are a number of risk factors including use of surface water, inconsistency in water treatment systems, and increasing aquaculture activities [26]. Inadequate local information prevents proper evaluation of the disease burden, transmission dynamics, and laboratory properties of the currently circulating strains. Hence, to fill this gap in knowledge, localized studies in epidemiology and laboratory are required to provide evidence-based data that would have an impact on the choice of diagnostic strategies, infection control, and the importance of future research [27].

Against these backgrounds, the current research paper is dedicated to the epidemiological and laboratory research of Aeromonas hydrophila found in human clinical samples with the aim of clarifying its distribution across various types of specimens, defining its laboratory classification characteristics, and deepening the knowledge on its clinical significance in the given local environment, as well as making a contribution to the global body of knowledge on this rapidly growing opportunistic pathogen [1-27].

2. Materials and Methods

Design of the study and sample collection.

This cross-sectional laboratory-based study was conducted to determine the prevalence and laboratory characteristics of Aeromonas hydrophila isolated from human clinical samples. The study was carried out in Al-Qadisiyah Governorate, Iraq, from September 15, 2025 to December 5, 2025.

A total of 200 clinical specimens were collected from patients attending local hospitals and diagnostic laboratories. The specimens included stool (n = 100), urine (n = 57), blood (n = 15), wound swabs (n = 15), and burn swabs (n = 13). All samples were collected using sterile containers or swabs and transported promptly to the microbiology laboratory for processing.

Isolation of Aeromonas hydrophila

Aeromonas hydrophila is a Gram-negative rod-shaped bacterium that was isolated on blood nutrient agar. Clinical samples were also cultured by virtue of routine bacteriological cultures. The samples of stool were placed on MacConkey agar and blood agar and chocolate agar as inoculums. The culturing of the urine samples was done with the help of the calibrated loops and blood samples were treated as per routine blood culture before subculture was performed in suitable media. One-to-one streak on the wound swabs and burn swabs was done on the same culture media. All inoculated plates were incubated at 37°C for 24 hours under aerobic conditions and examined for bacterial growth.

Morphological and Microscopic Identification.

The identification of Aeromonas hydrophila was done by presumptive identification by morphological characteristics and microscopic observation. The colonies which emerged as pale, non-lactose fermenting on the MacConkey agar and smooth colonies with or without hemolytic activity on blood agar and well-developed growth on chocolate agar were chosen to be analyzed further. Microscopic examination was done on Gram staining and the isolates that had Gram-negative bacilli were tested by biochemical test.

Biochemical Identification

Biochemical identification of the isolates was done to provide initial identification. Oxidase test was conducted with the help of oxidase reagent and a color change to purple within a few seconds was taken as a positive outcome. The catalase test was performed by placing the colonies of bacteria in the presence of hydrogen peroxide (H 2 O 2), whereby the appearance of visible bubbles represented a positive result. Isolates that were positive in both the oxidase and catalase tests were only treated as presumptive A. hydrophila and further confirmed.

Identification VITEK 2 Compact System.

To be certain enough all presumptive Aeromonas hydrophila isolates were tested with VITEK 2 Compact automated identification system. The pure colonies of bacteria were made as per the manufacturer guidelines and inserted in the system to analyze. Diagnostic accuracy was done by comparing identification outcomes obtained through the use of VITEK 2 Compact system with the traditional biochemical results.

Molecular Virulence Genes Detection.

Polymerase chain reaction (PCR) was used to molecularly detect the presence of different virulence-associated genes in Aeromonas hydrophila isolates. All confirmed isolates were extracted using the standard extraction methods, and stored at -20 C till further analysis.

PCR tests were conducted to identify only the presence of lipase gene and protease gene. The current local investigation was performed with the codes of specific primers designed against the genes using Primer3 software and synthesized commercially using conserved regions of the Aeromonas hydrophila sequences (Table 1).

Table (1): Primer used for Lipase and Protease Amplification

Genes	Primers	Product size
Lipase gene	Forward: 5’- ATGCCCTGAAAGCCTCTGAC-3	482 bp
	Reverse: 5’- CAGGTTCAGATACGGGGTCG-3’	
Protease gene	Forward: 5’- AAGAGCCCGAAATACGTGGG-3’	303 bp
	Reverse: 5’- AGAGAGTTGCATATCGACCCG-3’	

Each PCR reaction was performed in a total volume of 25 µL containing 12.5 µL of 2× PCR Master Mix (Thermo Scientific, USA), 1 µL of each primer (10 pmol/µL), 2 µL of template DNA, and nuclease-free water to complete the final volume. Amplification was performed in a thermal cycler under the following conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 7 minutes.

The PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized under UV transillumination. A 100 bp DNA ladder was used as a molecular size marker. Positive and negative controls were included in each PCR run to ensure assay validity.

Statistical Analysis

Data were analyzed using descriptive statistics (frequencies and percentages). The Chi-square test was applied to assess differences in isolation rates among different clinical sample categories. A P-value < 0.05 was considered statistically significant.

3. Results

Two hundred clinical samples were tested on the basis of Aeromonas hydrophila. Among them, 20 samples (10 percent) were positive in growth of A. hydrophila and 180 samples (90 percent) were negative.

The maximum level of isolation was registered on the stool samples where 15 out of 100 samples were positive (15%). Urine samples came next with 4 positive isolates of 57 samples (7.0%). This was compared to blood samples that had low isolation rates (and 1 positive isolate with 15 samples). None of the A. hydrophila isolates were found in the wound (0/15) or burn samples (0/13).

On the whole, the findings demonstrate that stool samples were the major source of the isolation of A. hydrophila, and the rates of isolation were significantly lower in other types of clinical samples or absent (Table 2).

Table (2): Distribution of *A. hydrophila* Isolates According to Clinical Samples

Clinical samples	Total samples	Positive isolates n (%)	Negative isolates n (%)
Stool	100	15 (15.0%)	85 (85.0%)
Urine	57	4 (7.0%)	53 (93.0%)
Blood	15	1 (6.7%)	14 (93.3%)
Wound	15	0 (0%)	15 (100%)
Burns	13	0 (0%)	13 (100%)
Total	200	20 (10.0%)	180 (90.0%)

Chi-square analysis showed no statistically significant difference in the distribution of *Aeromonas hydrophila* isolates among different clinical sample types ($\chi^2 = 6.64$, df = 4, P = 0.156).

Isolation and Identification of Aeromonas hydrophila

Morphological Identification

The Aeromonas hydrophila isolates were firstly discovered through the morphological features of the bacterial colonies after their growth on various culture media. Gram staining revealed that all the isolates were Gram-negative on microscopic examination as seen in Figure (1).

There was good growth of the isolates on the MacConkey agar, yielding pale colonies because of the failure to ferment lactose, as depicted in Figure (2). The cultivation on blood agar also showed smooth colonies, some of which had a hemolytic activity, which is regarded to be one of the virulence-associated features of this bacterial species (Figure 3). Moreover, the isolates exhibited obvious growth on chocolate agar, which means that they can grow on enriched media (Figure 4).

These morphological traits aided in initial assumption of isolates of the A. hydrophila which was later verified through microscopic observation, biochemical assays, VITEK system and molecular diagnosis.

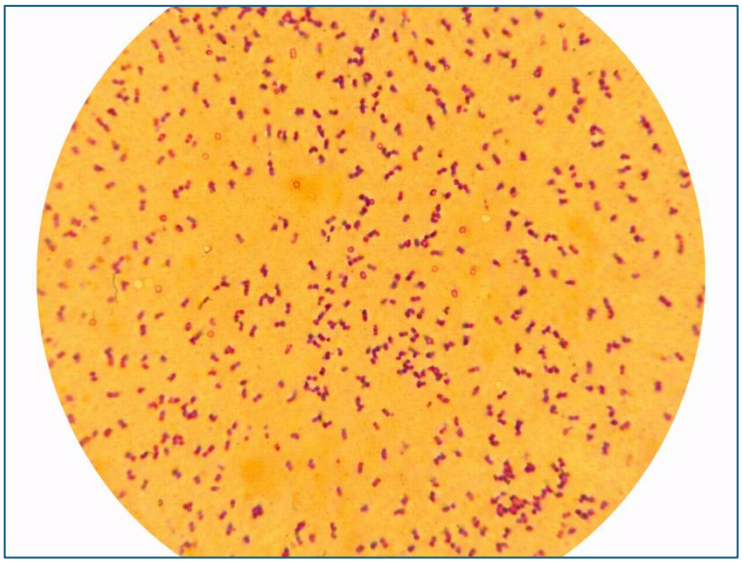

Figure (1): Microscopic appearance of *Aeromonas hydrophila* isolates showing Gram-negative reaction after Gram staining.

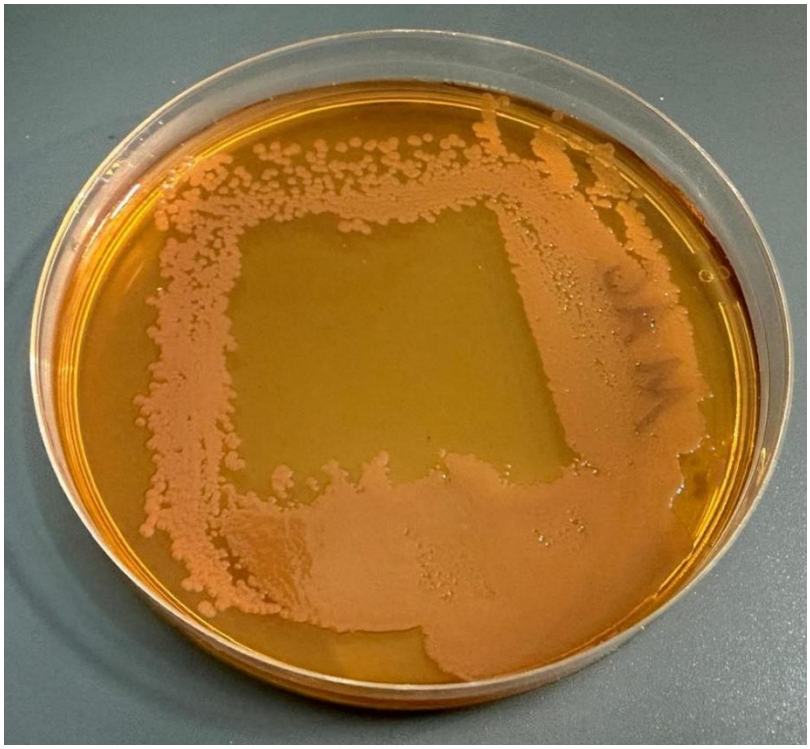

Figure (2): Colonial morphology of *Aeromonas hydrophila* on MacConkey agar.

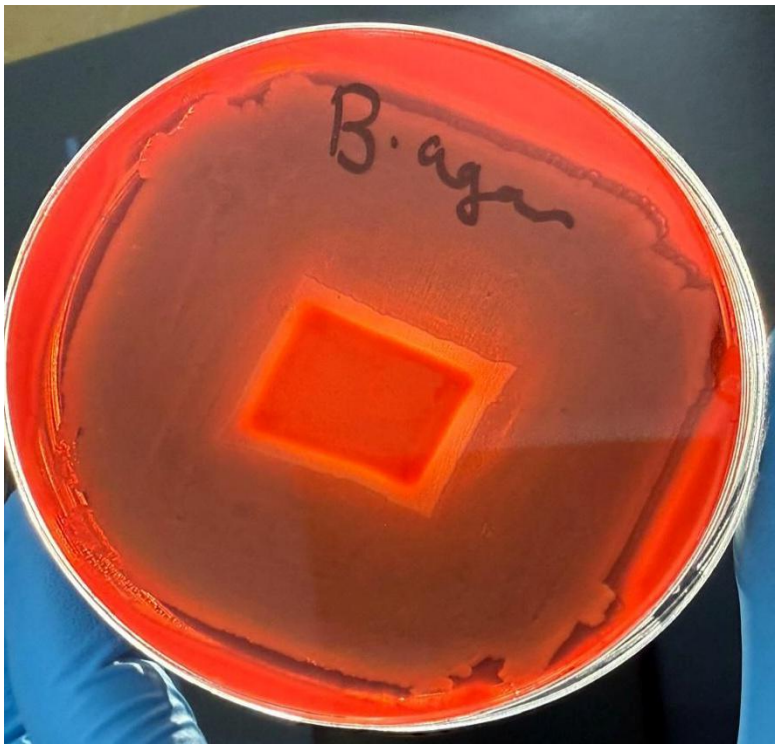

Figure (3): Hemolytic activity and colonial morphology of *Aeromonas hydrophila* on blood agar.

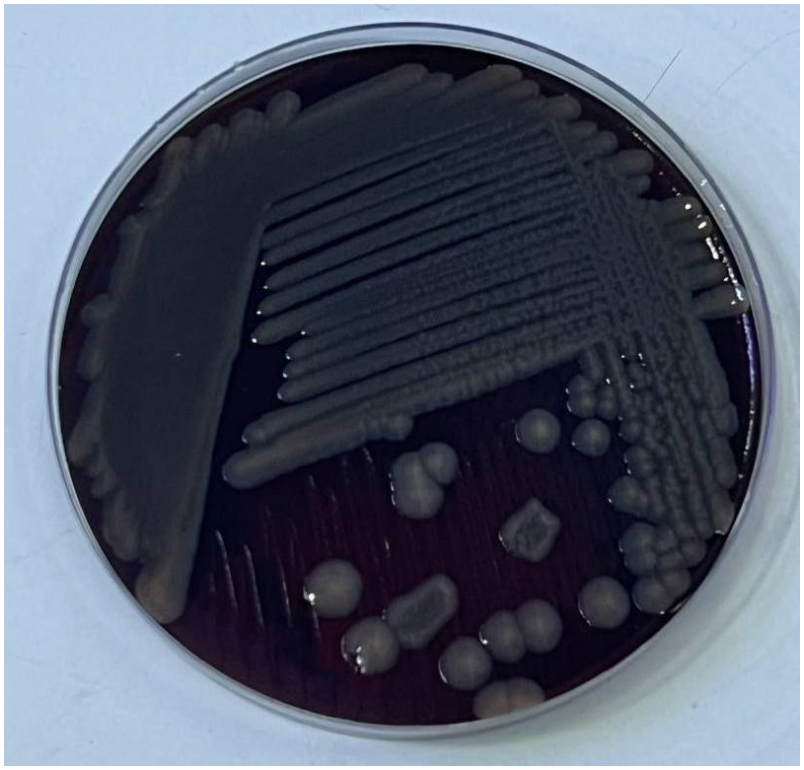

Figure (4): Growth characteristics of *Aeromonas hydrophila* on chocolate agar.

Biochemical Tests

As the preliminary diagnosis was supported and to ensure the identity of the bacteria, biochemical tests were done on the A. hydrophila isolates under study as follows:

A- Catalase Test

Even in the catalase test, all the isolates analyzed turned out to be able to produce catalase enzyme (3). The process of hydrogen peroxide (H_2O_2) decomposition and generation of water and oxygen led to the development of air bubbles on the glass slide surface which was covered with bacterial colony. The response is a significant biochemical trait of A. hydrophila.

B- Oxidase Test

Table (3) indicates that all the A. hydrophila isolates were positive to the oxidase test. The positive test was confirmed with the appearance of a purple color following the addition of oxidase reagent, which indicates the presence of cytochrome oxidase enzyme, which is a hydrogen acceptor in the transport chain and is a characteristic diagnostic test of A. hydrophila.

Table (3). Diagnostic biochemical tests for *A. hydrophila*

Test name	Result
Oxidase	+
Catalase	+

VITEK 2 Compact System Identification.

In order to guarantee the correctness of primary diagnosis and to ascertain successful isolation, more specific and sensitive diagnostic techniques were used. The final identification of the Aeromonas hydrophila isolates was done on the VITEK 2 Compact system. This system is regarded to be a modern and fast method of bacterial identification, which has the most accurate and reliable results without the chances of contamination which can disrupt the detection of the pathogens.

The outcome of the test performed with the help of the VITEK 2 Compact system was in full compliance with the biochemical test outcomes, which proved that all 20 isolates are A. hydrophila. The system was shown to be 100% accurate in identifying the isolates that were being investigated which shows the high level of efficiency and reliability of the system in giving the correct identity of this bacterial species.

The reason why this system was chosen among other systems of microbiological identification to identify bacterial species in the current study was due to its ease of use as well as the fact that it performs quickly and has high accuracy in the identification of bacterial species, which makes it a reliable diagnostic tool in microbiological investigations.

Identification of Lipase Gene in Aeromonas hydrophila Isolates.

The detection of Lipase gene using PCR showed that most of the isolates of Aeromonas hydrophila amplified successfully (Figure 5). The 18 out of 20 samples had a clear and specific DNA band and the size of the band was approximately 482 base pairs (bp) as compared to the 2 samples that had no amplification product (Isolates 1 and 15). The PCR concentration was specific, as the size of the amplicons in the positive samples was corresponding to the size of the expected Lipase gene.

The failure to amplify in the isolate 1 and 15 indicates that Lipase gene is absent or there is variation in the sequence which may prevent the binding of the primer in the isolate 1 and 15. Altogether, the fact that Lipase gene was found in most of the isolates suggests that it is widely spread among the considered population of Aeromonas hydrophila and it was one of the key virulence-related factors.

Based on this, 18 (90 percent) isolates were categorized into Lipase gene-positive and 2 (10 %) isolates were Lipase gene-negative indicating genetic variation among the isolates considered.

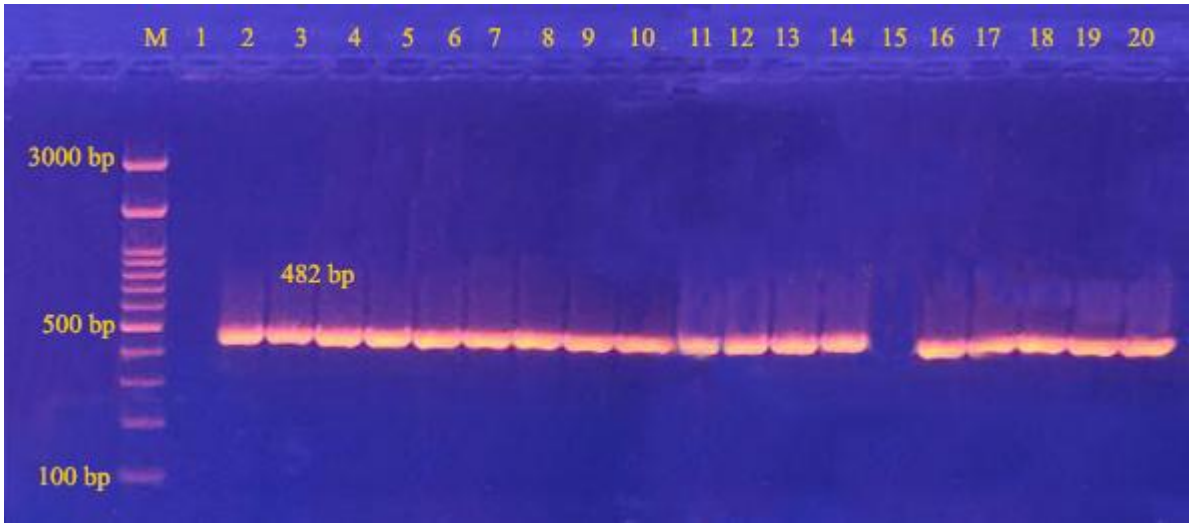

Figure (5): Detection of the Lipase gene (482 bp) in *Aeromonas hydrophila* isolates by PCR analysis. PCR amplification revealed a specific band at approximately 482 bp in 18 out of 20 examined samples. No amplification was observed in isolates 1 and 15, indicating the absence of the Lipase gene in these isolates.

Protease Gene of Aeromonas hydrophila Isolates Detection.

PCR was used to show that it was possible to amplify Protease gene in the isolates of Aeromonas hydrophila. The DNA band of particular size with a strong and specific pattern was seen in all the samples analyzed (n = 20). The observed amplicon size corresponded to the known size of the target Protease gene, which is why the primers were specific and the given PCR conditions used in this study were reliable (Figure 6).

A homogenous appearance of the amplification product at a common molecular weight in all the samples indicates that Protease gene is the one that is widely spread among the samples of Aeromonas hydrophila tested. Such observation indicates the genetic capability of these isolates to express protease enzyme, which is important in bacterial virulence as it is related to tissue disintegration, invasion in the host and disease pathogenesis.

Based on this, it was found that all Aeromonas hydrophila isolates used in the current study were found to be positive of the Protease gene which justifies the significance of the gene as a molecular virulence characterization marker in Aeromonas hydrophila.

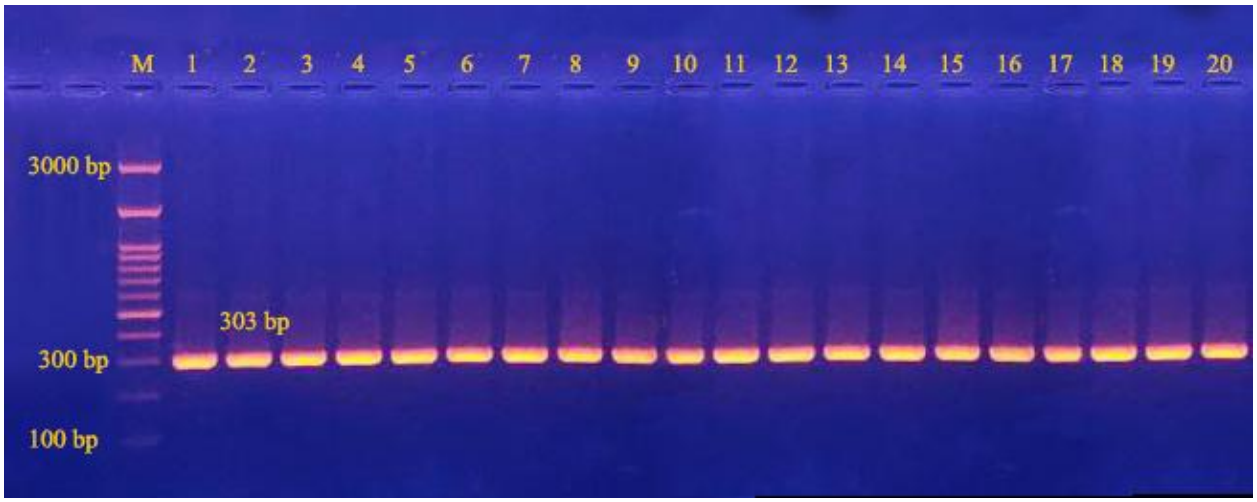

Figure (6): Detection of the Protease gene (303 bp) in *Aeromonas hydrophila* isolates by PCR analysis. A specific PCR amplification product of approximately 303 bp was detected in all tested samples (n = 20), indicating that all examined *Aeromonas hydrophila* isolates are positive for the Protease gene.

4. Discussion

The total isolation rate of Aeromonas hydrophila of the current local study was 10 percent meaning that this bacterium is an important clinically relevant opportunistic pathogen of the population under investigation. Similar rates of isolation have been observed in various hospital-based studies, in which A. hydrophila was identified on moderate frequencies in human clinical samples, mostly in gastrointestinal samples [28,29]. The variability in isolation rates may depend on the geographical location, exposure to the environment, and also, the difference in the methods of diagnosis.

The fact that most of the A. hydrophila (15%) are found in stool samples in this study confirms their pre-existing relationship with gastrointestinal infections. The same results have been reported in pediatric and adult diarrheal studies, in which Aeromonas species were often isolated in stool samples and associated with watery or dysenteric diarrhea [30,31]. In the current study, no isolates of wound and burn samples were found, whereas A. hydrophila is reported to cause skin and soft tissue infections after exposure to the aquatic environment [32]. The variation can be attributed to disparity in the exposure patterns, population of patients or excellent wound care practices within the local environment.

Its opportunistic nature and capacity to produce extraintestinal infections are indicated by the recovery of A. hydrophila in the urine and blood samples albeit at lower rates. In past investigations, it has been shown that A. hydrophila can cause infection of the urinary tract of the patient and bacteremia in immunocompromised patients or those with underlying chronic ailments [33,34]. A minimal percentage of isolation of blood samples is clinical, because invasive infections by this organism are usually identified as serious and leading to a higher number of deaths [35].

Morphological and cultural features of the isolates used in the study conformed with those previously reported in previous studies such as fermenting of non-lactose in MacConkey agar medium and haemolytic colonies in blood agar [36]. The hemolysis has been regarded as a significant sign of virulence, because it is an expression of toxin production that causes host cell damage [37]. The ability to grow on chocolate agar also indicates the ability of A. hydrophila to enriched media, through which it can survive in a variety of host environments.

Biochemical identification showed consistent positivity to oxidase and catalase test thus validity of these tests in preliminary identification of A. hydrophila. This is within the same lines of earlier reports which

pointed at the utility of traditional biochemical tests in the differentiation between Aeromonas and other Gram-negative bacteria [38]. Nevertheless, Aeromonas species can still be mistakenly identified based on their phenotypic similarity, which requires either automated or molecular tests [39].

VITEK 2 Compact system was shown to be fully concordant with the conventional identification techniques and all the isolates were confirmed as A. hydrophila. It has also been reported that similar high accuracy rates of automated identification systems have been observed in clinical microbiology laboratories, which confirms that they can be used in the rapid and dependable identification of bacteria [40]. Such use increases the confidence of the making of such diagnoses and shortens the turnaround time, which is instrumental in the proper clinical care.

Molecular evidence was used to determine the virulence related genes, where the lipase gene was found to be predominant (90 percent) and the protease gene was universal (100 percent). Past molecular investigations have revealed that lipase and protease enzymes play an important role in bacterial pathogenicity in promoting tissue invasion, nutrient acquisition, and avoidance of host immune immunity [41,42]. The high prevalence of these genes indicates that local isolates carry virulence-associated genetic determinants. However, gene presence alone does not confirm expression or clinical severity, and further functional studies are required.

The lack of lipase gene in a low percentage of isolates shows that there is genetic diversity in A. hydrophila strains. The comparable genetic heterogeneity has been described in other molecular epidemiological investigations that identified variations in virulence gene patterns in relation to differences in clinical presentation and severity of the disease [43]. These results bring into focus the relevance of molecular characterization in study of the pathogenic behavior of A. hydrophila and its effect on human health.

On the whole, the current local investigation shows that Aeromonas hydrophila is a clinically significant opportunistic pathogen, especially in gastrointestinal diseases. A combination of epidemiological information, traditional laboratory methods, automated identification, and molecular analysis offers a holistic solution to the accurate identification of this bacterium and description in clinical conditions.

Conclusions

The findings of the current local investigation suggest that Aeromonas hydrophila is an opportunistic pathogen of clinical interest, (All in all isolation rate is 10% in human clinical samples). Stool samples were the most important sources of isolation, which confirmed the presence of this bacteria in gastrointestinal infection, whereas low rates of isolation of urine and blood samples denote the fact that under some conditions, the bacteria can cause extraintestinal infections. The traditional morphological and biochemical procedures were good in identifying the initial identification of A. hydrophila, and the VITEK 2 Compact system has given quality and good verification on identification of bacteria. Molecular identification showed that virulence-associated genes in most cases and especially the protease gene, which was detected in all the isolates, and the lipase gene, which was detected in most of the isolates. These findings demonstrate the presence of virulence-associated genes among local isolates; however, additional studies are needed to evaluate their expression and clinical relevance. In general, the paper identifies the cruciality of the combination of epidemiological data with laboratory and molecular tools to properly identify and characterize A. hydrophila in clinical environments.

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