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Phenotypic and genotypic detection of Aeromonas spp. isolated from drinking water

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***Abstract*---**Contamination of drinking-water is a significant concern for public health throughout the world. Microbial hazards make the largest contribution to waterborne disease in developed and developing countries. Biofilm-producing bacteria were identified by the Congo Red Agar (CRA) method we obtained isolated of *Aeromonas* spp. formed Biofilm. the antibiotic resistance screening test to a number of antibiotics on *Aeromonas spp.* isolates. The results revealed that isolates of *Aeromonas spp.* have high resistance (100%) to Amoxicillin , Vancomycin and Cefoxitin. Whereas some isolates of *Aeromonas spp.* observed moderate resistance to Imipenem and Erythromycin. PCR to detected presence of *fimH* and *papC* genes in *Aeromonas* spp. that isolated from drinking water contain *fimH* gene and contain *pap C. fimH* was an important factor in cell control and bacterial persistence under stress conditions, and it contributed to virulence through a variety of mechanisms.

***Keywords*---**Aeromonas spp., biofilm, drinking water.

Introduction

Aeromonas species are Gram-negative, non-spore forming, rod shaped, oxidase-positive, and facultative anaerobic bacteria. *Aeromonas* are divided into two groups namely the non-motile psychrophilic aeromonads and the motile mesophilic aeromonads. These bacteria are commonly found in freshwater reservoirs, soil and agricultural produce (Percival and Williams, 2014). The genus *Aeromonas* is regarded not only as an important disease-causing pathogen of fish or other cold-blooded species but also as the causative agent responsible for a variety of infectious complications in Immunocompromised persons (Janda and Abbott, 2010; Al-Hadraawy *et al.*, 2022). The genus *Aeromonas* comprises

flagellated widely distributed in freshwater, estuarine and marine environments. *Aeromonas* species may cause a variety of illnesses in humans, such as enterocolitis and septicemia, especially in warmer tropical or subtropical environments. *Aeromonas* septicemia is uncommon in temperate areas but can occur particularly in warm seasons. Immunocompromised conditions and recent ingestion of raw fish or shellfish are important characteristics of developing Aeromonas septicemia (Morinaga *et al* , 2011). *Aeromonas* strains have a wide range of virulence factors including adherence properties, lipopolysaccharides, toxins, siderophores , extracellular secretions, iron acquisition, colonization and quorum sensing mechanisms. Most of these virulence properties were reported in finfish (Beaz-Hidalgo and Figueras, 2013; Aljanaby et al., 2022). There are a number of virulence mechanisms possessed by *Aeromonas*, in particular the ability to be an avid biofilm former. In addition, many extracellular enzymes produced by *Aeromonas* are known to enhance its virulence and pathogenicity, including, lipases, cytolytic toxins, sulphatases, nucleases, lecithinase, staphylolysin, and amylase. Numerous epidemiological studies suggest that people become colonized by *Aeromonas* from contaminated drinking untreated water systems. (Percival and Williams, 2014; Alhasnawi and Aljanaby, 2022). *Aeromonas* strain was first identified in 1891. It was classified under genus Vibrionaceae in 1965 by the International Committee of Systematic Bacteriology. In 1986, it was categorized under a new family, namely Aeromonadacae based on the results of 16S rRNA and 5S Rrna gene sequence analysis and DNA hybridization studies - Under the family of Aeromonadacae, a total of 36 species were identified (Bravo and Figueras 2020).

The aim of this study: Molecular detection of some virulence genes in pathogenic bacteria isolated from different samples of drinking water in Al-Najaf Governorate.

1. Samples of drinking water were collected randomly from different areas of Najaf Governorate.
2. Determination of bacterial isolation using biochemical properties and vitek2 technique.
3. Identification of common bacteria isolated
4. Detection of some virulence factors for common isolate bacteria and the presence of genetic coding for some virulence factors using PCR technique.

Methods and materials

Sample

A total of 120 water sample were achieved during the period from October 2021 to March 2022. water sample collected from drinking water from a different region in Al-Najaf city. The samples were placed in separate sterile plastic bags before being immediately transported to a cool box filled with ice. All samples were transferred to the laboratory and cultured on MacConkey agar medium for 24 hours at 37° C. Isolates were purified several times until pure isolates were obtained, then subjected to microscopic and special biochemical tests before being transferred to VITEK 2 for identification.

Congo Red Agar (CRA)

It was made by mixing 52 grams of Brain heart infusion agar media with a liter of distilled water and autoclaving it for 15 minutes at 121°C/15 pressure. Congo red stain (0.8 g/L) was made as a concentrated aqueous solution and autoclaved for 15 minutes at 121°C, while sugar (50 g/L) was sterilized by filtering. After cooling to 55°C, both dye and sugar were added to the agar. After that, plates were infected and incubated aerobically at 37°C for 24 hours to detect biofilm formation (Freeman *et al.*,1989). Black colonies with a dry crystalline quality indicated a positive result. Weak slime producers stayed pink most of the time, however there was some darkening in the colonies' cores. An uncertain result was indicated by the darkening of the colonies in the absence of a dry crystalline colonial morphology.

Antibiotic Sensitivity Test : This test performed by Kirby-Bauer method

Extraction and Isolation of DNA

DNA of *Aeromonas spp.* isolates was prepared by boiling method. In brief, colonies were suspended in 100 microliters of sterile distilled water, boiled at 100C in a water bath for 15 minutes, then rapidly cooled at -20C for one hour, centrifuged, and the supernatant was saved for use in the amplification processes (Shah *et al.*, 2017). PCR amplification was used to identify the presence of genes. The primer used in this study was show in (Table 1).

Table 1
The sequence of Primer that were used in the present Study.

Primer `Type	Primer Sequence (5'-3')	Amplicon size (bp)	Preference
FimH	F:TGCAGAACGGATAAGCCGTGG R:GCAGTCACCTGCCCTCCGGTA	508	Johnson and Stell, (2000)
PapC	F:GTGGCAGTATGAGTAATGACCGTTA R:ATATCCTTTCTGCAGCGATGCAATA	200	Johnson and Stell.,(2000)

Each 25 µl of PCR reaction mixture for PCR contained 2.5µl of upstream primer, 2.5µl of downstream primer, 2.5µl of free nuclease water, 5 µl of DNA and 12.5µl of master mix thin walled PCR tube. The Thermal cycler conditions were as follow in (Table 2).

Table 2
PCR Program that Apply in the Thermo-Cycler

Gene	Temperature (C°) / Time			Cycles Number
	Initial	Condition of one cycle	Final	

	Denaturation	Denaturation	Annealing	Extension	Extension	
FimH	95/4 min.	95 \30 sec.	63\30 sec.	72\1min.	74\8min.	35
PapC	95\4min.	95\30sec.	63/30 sec.	72/1 min.	74\8min	35

Results and Discussion

Identification of Bacterial Species that Isolated From Drinking Water:

After collecting drinking water samples, they were cultured on differential media (blood agar, mannitol agar and MacConkey agar) to obtain pure isolates. Many bacterial isolates were identified using Gram stain test, bacterial culture, morphology and biochemical tests, where the bacteria were isolated and examined in the laboratory according to McFadden (2000). The most common bacteria was *Aeromonas spp.(A. hydrophila* , *A. salmonicida, A. sobria)* isolates Table (3). *Aeromonas* spp. grow well on common laboratory media including blood agar, MacConkey agar. On blood agar, *Aeromonas* forms circular colonies , 1-3 mm in diameter , large, round, raised, opaque; most colonies are beta-hemolytic.On MacConkey agar - typically non-lactose fermenting . *Aeromonas spp*. are oxidase and catalase positive.

Table 3
Detecting of Number and Percentage of Bacterial Isolates from Different Samples

NO.	Type of bacteria	Number of Bacterial Isolates	Percent (%)
1-	*Aeromonas hydrophila*	4	36.363
2-	*Aeromonas sobria*	4	36.363
3-	*Aeromonas salmonicida*	3	27.272
4-	Total	11	100

Biofilm Production by using Congo-Red Agar Method

Congo-Red Agar Method and Tube method was used to investigate the biofilm production , the results of this study showed that all bacterial isolate of *Aeromonas* spp. had ability to biofilm formation. This results were agreed with the result study of Almayali *et al.*, 2017 they showed that 100% of isolates were biofilm producers .while Oliveira and Cunha (2010) they showed that 82% of isolates were biofilm producers. In nature, bacteria can exist in planktonic and biofilm embedded state (Ghaima *et al.*,2017) . Biofilm growth is the most predominant made of growth for bacteria within the environment and is likely a survival mechanism. A biofilm is a bacterial population that is adherent to biological or non-biological surface and is enclosed by an extra-polymeric substance. Biofilm development is a sequential process initiated by the attachment of planktonic cells to a surface , then formation of micro colonies and biofilm maturation in which individual bacteria , as well as the entire community are embedded in a matrix composed of nucleic acid, protein and polysaccharides (Branda *et al.*,2005).

Antimicrobial Susceptibility Test

The susceptibility of bacterial isolates was tested against antibiotics that commonly used in the treatment of infections, based on the Kirby-Bauer disk diffusion method on Muller-Hinton agar. The results had been recorded based on measured the diameter of inhibition zones and then compared with (CLSI, 2021) , The results showed different susceptibility towards tested antibiotics Table (4) fig(1). many studies reported that the development of drug-resistance bacteria is due not only to the presence of drugs in the aquatic environment, but also to the density of resistance bacteria, antibiotic exposure time, and nutrient enriched environment . Long exposure time to sub therapeutic dose of antibiotics potentially leads to creating suitable conditions for resistance gene transfer. Furthermore, many studies documented the prevalence of amoxicillin and ciprofloxacin resistant bacteria in river water, waste water, and drinking water.

Table (4) shows the antibiotic resistance screening test to a number of antibiotics on *Aeromonas spp.* isolates. The results revealed that isolates of *Aeromonas spp* have high resistance (100%) to Amoxicillin , Vancomycin and Cefoxitin. Whereas some isolates of *Aeromonas spp.* observed moderate resistance to Imipenem and Erythromycin. A variety of antibiotics have been used to treat infection caused by *Aeromonas spp.* and have proved useful in many cases, but multiple antibiotic resistances are common among *Aeromonas spp.* (Kampfer *et al.,*1999 ; Janda and Abbott ,2010). Many strains of *Aeromonas spp.*are known to harbour mobile elements that encode antibiotic resistance and can be transferred among themselves or to other bacterial species' to establish multiple antibiotic resistances (Janda,2001). The widespread use of antibiotics has been identified as a major factor responsible for the increased incidence of antibiotics resistance (Andrea *et al.,*2006).

Holmberg and Farmer(1984) found that *Aeromonas spp* isolate obtained from patients were considered as multidrug resistant. These results confirmed data reported by other authors, indicating that bacteria are frequently and increasingly demonstrating multiple resistances to the antibiotics (Ko *et al.,*1996). The continuous use of even a single antibiotic over a period of weeks or months will select bacteria resistant to different kinds of antibiotics in addition to the one in use (Livermore., 2004). Transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons between and across different bacterial species with relative ease important factor that can contribute to the increase of multiresistant bacteria. Multiple antibiotics resistance can occur even in the absence of plasmid or transposon. The study published by Livermore (2000) shows that plasmid- and trasposon-free *E. coli* is resistant up to seven types of antibiotics.

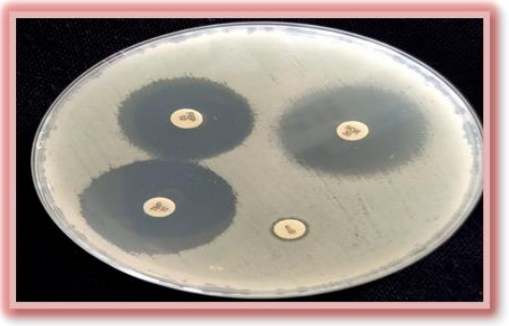
In agreement with Altwegg and Geiss,(1989) and Chandhury *et al.,*(1996) , this study demonstrates that all *Aeromonas spp* isolates are susceptible(100%) to these antibiotics(above antibiotic). Vila *et al.,*(2002) and Kannan *et al.,* (2011) who found that *Aeromonas spp* sensitive to Chloramphenicol, Ciprofloxacin and indicated to Chloramphenicol is the other antibiotic used in treatment and the isolates are susceptible to this antibiotic . The increase of antibiotics resistance in *Aeromonas spp* isolates is often related to the overdose and mistreatment of the

antibiotics prescribed. Heavy and widespread use of antibiotics in hospital doe s not only force the emergence of antibiotic resistance, but also promotes selection of drug-resistant organisms in the hospital environment (Janda and Abbott, 2010). Iraq is one of the developing countries where antibiotics sold over the country, an attitude that encourages self-medication. Furthermore, it is remarked that during period of time a group of antibiotics become to be more used than other antibiotics without susceptibility tests which may lead to variability in resistance to these antibiotics. The Feature in this study is the high prevalence of antibiotic by *Aeromonas spp.,*as the study shows that *Aeromonas spp.* bacteria had multiple resistance, and especially of many studies in world refer that plasmids are responsible for multiple resistance.

Table 4
Antibiotic Resistance of bacteria isolates from drinking water sample using disc diffusion method

NO	Type of Antibiotics	*Ae.hydrophila* n=4	*Ae.Sobria* n=4	*Ae.Salmonicida* n=3
1	Amoxicillin (AM)	4(100%)	3 (75%)	2(66.6%)
2	Amoxicillin/ clavulanic acid (AMC)	4(100%)	3 (75%)	1(33.3%)
3	Imipenem (IPM)	3 (75%)	3 (75%)	2(66.6%)
4	Amikacin (AK)	3 (75%)	4(100%)	3(100%)
5	Tobramycin (TOB)	0(0%)	2(50%)	0(0%)
6	Erythromycin (E)	2(50%)	2(50%)	3(100%)
7	Norfloxacin (NOR)	4(100%)	2(50%)	2(66.6%)
8	Levofloxacin (LEV)	3 (75%)	4(100%)	3(100%)
9	Trimethoprim (TMP)	3 (75%)	4(100%)	3(100%)
10	Cefepime (FEP)	3 (75%)	3 (75%)	0(0%)
11	Meropenem (MEM)	2(50%)	2(50%)	2(66.6%)
12	Cefoxitin (CTX)	4(100%)	4(100%)	3(100%)
13	Vancomycin (VAN)	4(100%)	4(100%)	3(100%)

Figure 1
Disk approximation test exhibiting positive on Muller Hinton agar surface after 24 hr. of incubation at 37°C**.**



Molecular Study of some Genes in isolates

Detection of *fimH* Gene

The production of PCR amplification between the extracted DNA and specific primers of *fimH* gene were detected by gel electrophoresis analysis using DNA marker and the products size was(508bp) figure (2) .The presence of the *fimH* gene was confirmed by PCR and the results indicated that the *fimH* gene was present in isolates. The results indicated that the *fimH* gene was presented in (*Aeromonas hydrophila* and *Aeromonas sobria*) as appeared in figure (2) .

Berry, *et al.*,2009 described the mechanism of action for *fimH* adhesin , this mechanism acts and interacts with urothelium, allowing *E.coli* to enter and form intracellular bacterial colonies (IBCs) after the first 6 hours of infection. IBCs are responsible for the recurrence, chronicity, and formation of bacterial reservoirs in the urothelium (Hanna *et al.*,2012). The mannose binding domain (LD) of the *fimH* is mainly responsible for adherence, which is the first step of infection (Merza, 2017). *fimH* adhesin is present in more than 80% of *E.coli* strains that cause UTIs. This adhesin is responsible for generating the adhesion of the bacteria to the urinary tissue, thus favoring colonization and subsequent invasion of the urothelium (Najafi *et al.*,2018) to the extent that the *fimH* gene was detected in more than(90%) of the *E.coli* strains. The high binding ability of *fimH* could result in increased bacterial binding to target cells and increased pathogenicity of pathogenic bacteria , thus, *fimH* can be used to design vaccine for prevention of bacterial infections by blocking the bacterial colonization and attachment . (Al-Mashhadi and Al-Fatlawi ,2021).

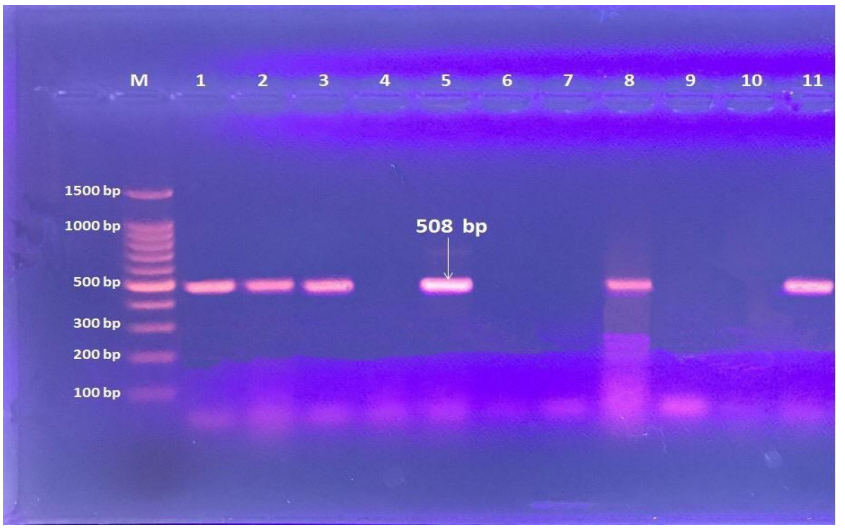


Figure 2. Ethidium Bromide-Stained Agarose Gel Electrophoresis of PCR Products from Extracted total DNA of bacterial isolates using Primer *fimH* Gene with Product (508)bp. The Electrophoresis was Performed at 70 volt for.90min. lane (L), DNA Molecular Size Marker (100-2000 bp ladder). Lanes (1 , 8) (*Aeromonas hydrophila and Aeromonas sobria* respectively) Show Positive Results with Gene *fimH* .

Detection of *Pap C* Gene

Polymerase chain reaction technique of the *Ae. salmonicida* isolates revealed that *papC* gene were gave positive result for this gene with product (200bp) as shown in figure (3). The results indicated that the *papC* adhesion gene was present in isolates. These results agree with(Abdul-Ghaffar and Abu-Risha,2017) .*PapC* gene is the second most common adhesins in *E.coli*. the results of a study by López-Banda *et al* .,(2014) indicated that more than 62% of *P. stutzeri* harbored *papC* gene. The high incidence of *papC* gene indicated that *E.coli* isolates have the ability to attach kidneys cell and cause pyelonephritis. while Johnson and Stell ,(2000) studied bacterial adhesins in patients with *E.coli* urosepsis and *papC* adhesin was (82%) .while *papC* in percentage 30% is the most prevalent in the *E.coli* strains of hemoculture in a study done by(Koga *et al.*,2014) how identified genetic features associated with virulence, and these results were compared with commensal isolates. the *papC* adhesion encoding operon, which shows a high prevalence among uropathogenic *E.coli* strains (75%–80% of pyelonephritis strains) (La Combe *et al.*,2019).

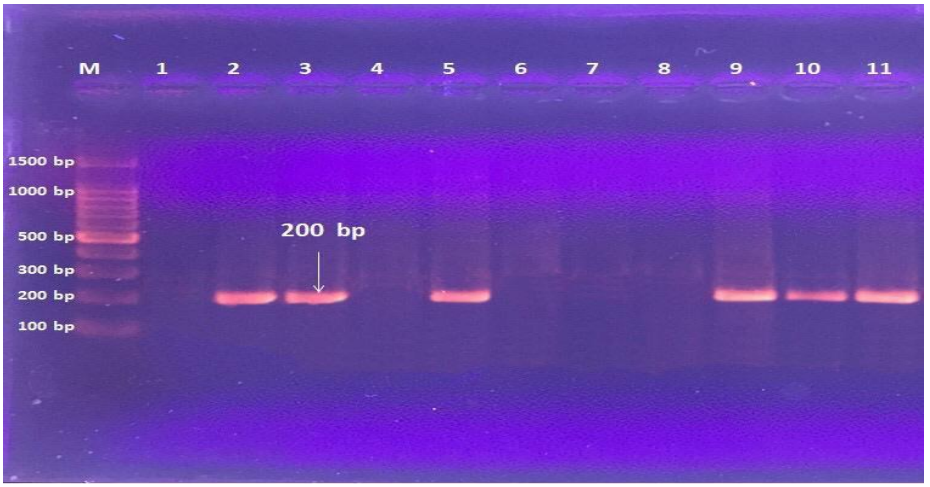


Figure 3. Ethidium Bromide-Stained Agarose Gel Electrophoresis of PCR products from Extracted Total DNA bacterial isolates under study Using Primer papC Gene with product(200bp).The Electrophoresis was Performed at 70 volt for 90min. lane (L), DNA molecular size marker (100 bp ladder). Lane (9,10) (*Aeromonas salmonicida* and *Acinetobacter lwoffii*) Show Positive Results with Gene *papC*

References

Abdul-Ghaffar, S. N. and Abu-Risha, R. A. (2017). Virulence Genes Profile of Escherichia coli Isolated from Urinary Catheterized and Non-Catheterized Patients. Iraqi Journal of Science, 58(2B), 820-835.

Al-Hadraawy, S. K., Alhadrawi, K. K., Aljanaby, I. A. J., Aljanaby, A. A. J., & Zabibah, R. S. (2022). Prevalence of pulmonary tuberculosis in Al-Najaf governate, Iraq. F1000Research, 11(675), 675.

Alhasnawi HMR, Aljanaby AAJ. Evaluation of Galectin-3 and CD19 in Helicobacter pylori patients infected with stomach cancer. Gene Reports 26 (2022) 101520. https://doi.org/10.1016/j.genrep.2022.101520.

Aljanaby, A.A.J., Al-Faham, Q.M.H., Aljanaby, I.A.J. and Hasan, T.H., 2022. Immunological role of cluster of differentiation 56 and cluster of differentiation 19 in patients infected with mycobacterium tuberculosis in Iraq. Gene Reports, p.101514. doi.org/10.1016/j.genrep.2022.101514

Al-Mashhadi K. M and Al-Fatlawi B. A. (2021) ; Molecular Detection of Some Virulance Gene in Pathogenic Gram Negative Bacteria Isolated from Different Clinical Cases in AL-Najaf Province. A Thesis , faculty of Science, University of Kufa

Almayali E. J. B. , Al-Muhanna A. S. and Al-Fatlawi B. A. (2017) . Molecular study to evaluate some virulence factors that associated with Bacterial Tonsillitis , *staphylococcus aureus* and *pseudomonas aeruginosa* . M.Sc. Thesis . Faculty of Science, University of Al-Kufa.

Altwegg, M., and Geiss, H. K. (1989). *Aeromonas* as a human pathogen. CRC Crit. Rev. Microbiol. 16:253–286.

Andréa. B; Cyrino,J.E.P.(2006). Antibiotic Resistance of Aeromonas hydrophila isolated from Piaractus mesopotamicus (Holmberg, 1887)and Oreochromis niloticus (Linnaeus, 1758). UFAM - Centro de Apoio Multidisciplinar - Divisão de Biotecnologia, Av. Rodrigo Otávio, 3000 - 69077-900 -Manaus, AM - Brasil.

Beaz-Hidalgo R. and Figueras, M.J. (2013) Aeromonas spp. whole genomes and virulence factors implicated in fish disease. J Fish Dis 36, 371–388.

Berry, R . ; Klummp , D . and Schaeffer , A.(2009). Urothelial cultures support Intracellular Bacterial Community Formation by uropathogenic Escherichia coli. Journal Infect Inmun. 77(7):2762-72.

Brandas .S.,Vlk A,.Frledmanl .,Kolter R.Bio -film :the matrix revisited .Trends Microbiol .13 ,20,2005.

Bravo F. A. and Figueras M.J. (2020) An update on the genus Aeromonas: taxonomy, epidemiology, and pathogenicity. Microorganisms 8, 129.

Chandhury ,A. ; Nath ,G. ; Shukla , B.N. and Sanyal , S.C.(1996). Biochemical, characterization , enteropathogenicity and antimicrobial resistance plasmids of clinical and environmental *Aeromonas* isolates J. of Medical Microbiology .44:434-437.

CLSI, CLSI M100-ED30: (2021) Performance Standards for Antimicrobial Susceptibility Testing, 31th Edition (2021).

Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. *J Clin Pathol.* 1989;42:872–4. https://doi.org/10.1136/jcp.42.8.872 PMid:2475530 PMCid: PMC1142068.

Ghaima, K. K.; Hamid, H. H. and Hasan, S. F. (2017). Biofilm formation, antibiotic resistance and detection of mannose-resistant Proteus-like (MR/P) fimbriae genes in Proteus mirabilis isolated from UTI. International Journal of ChemTech Research, 10(5), 964-971.

Hanna ,T. ; Totsika ,M .and Mansfield, K.(2012).Host-Pathogen Checkpoints and Population Bottlenecks in Persistent and Intracellular Uropathogenic E. coli Bladder Infection. FEMS Microbiol Rev.;36(3):616-648.

Holmberg,S . D.; Farmer . J. J . (1984). *Aeromonas hydrophila* and *Plesiomonas shigelloides* as causes of intestinal infections .Reviews of Infectious Diseases 6 : 633-639.

Janda, J. M.(2001). Chapter 59. *Aeromonas* and *Plesiomonas*, p. 1237–1270.*In* M. Sussman (ed.), Molecular medical microbiology, vol. 2. Academic Press, London, United Kingdom.

Janda, J.M. & Abbott, S.L. (2010). The genus *Aeromonas*, taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews* 23, pp. 35-73.

Johnson, J. R. and Stell, A. L. (2000). Extended Virulence Genotypes of detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic Escherichia coli Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. The Journal of Infectious Diseases. 181: 261.

Johnson, J. R. and Stell, A. L. (2000). Extended Virulence Genotypes of detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic Escherichia coli Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. The Journal of Infectious Diseases. 181: 261.

Kampfer, P.; Christmann, C.; Swings, J. and Huys,G. (1999). In vitro susceptibilities of *Aeromonas* genomic species to 69 antimicrobial agents. Syst. Appl. Microbial. 22:662–669.

Kannan, S.; Suresh, K. P.; Karkuzhali, K., Chattopadhyay, U. K& .Pal, D. (2001). Direct detection of diarrheagenic *Aeromonas* from faeces by polymerase chain reaction (PCR) targeting, (aerolysin toxin gene). Eur. Rev. Med. Pharmacol. Sci. 5(3),91-94.

Ko, W. C., *et al.* (1996). Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. Antimicrob. Agents Chemother. 40: 1260–1262.

Koga, V. L.; Tomazetto, G.; Cyoia, P. S.; Neves, M. S.; Vidotto, M.C.; Nakazato, G. and Kobayashi, R.K.T. (2014). Molecular Screening of Virulence Genes in Extraintestinal Pathogenic Escherichia coli Isolated from Human Blood Culture in Brazil. BioMed Research International. 9.

La Combe, B.; Clermont, O.; Messika, J.; Eveillard, M.; Kouatchet, A.; Lasocki, S. and Ricard, J. D. (2019). Pneumonia-specific Escherichia coli with distinct phylogenetic and virulence profiles, France, 2012–2014. Emerging infectious diseases, 25(4), 710.

Livermore,D.M.(2000). Quinupristin/dalfopristin and linezolid: where, when, which and whether to use? J Antimicrob Chemother; 46: 347–50.

Livermore,D.M.(2000). Quinupristin/dalfopristin and linezolid: where, when, which and whether to use? J Antimicrob Chemother; 46: 347–50.

López-Banda, D. A.; Carrillo-Casas, E. M.; Leyva-Leyva, M.; Orozco-Hoyuela, G.; Manjarrez-Hernández, Á. H.; Arroyo-Escalante, S. and Hernández-Castro, R. (2014). Identification of virulence factors genes in Escherichia coli isolates from women with urinary tract infection in Mexico. BioMed research international, 2014.

MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd edition. Lippincott Williams and Wilkins, USA.

Merza, N. S. (2017). Prevalence and Molecular Characterization of *Fim H* Gene in Escherichia Coli Isolates Recovered From Patients With Utis. Medical Journal of Babylon, 14(3), 470-477.

Morinaga Y., Yanagihara k., Araki N , Harada Y, Yamada K. , Akamatsu N., Matsuda Y, Nishino T, Hasegawa H., Izumikawa K. , Kakeya H. , Yamamoto Y., Yasuoka A., Kohno S., Kamihira S. .(2011) Clinical Characteristics of Seven Patients with Aeromonas Septicemia in a Japanese Hospital . The Tohoku Journal of Experime .../225 2011.

Najafi ,A.; Hasanpour. M.; Askary, A.;Aziemzadeh ,M. and Hashemi N.(2018) Distribution of pathogenicity island markers and virulence factors in new phylogenetic groups of 57 uropathogenic Escherichia coli isolates. Folia Microbiol (Praha). 63(3):335- 43.

Novoa, R. B. (2021). State of the art and future applications of digital health in Chile. *International Journal of Health & Medical Sciences*, *4*(3), 355-361. https://doi.org/10.31295/ijhms.v4n3.1772

Oliveira ,A .and cunha ,M .(2010) .Comparison of methods for the detection of biofilm production in coagulase-negtive staphylococci .BMC Res Notes l;3:260

Oliveira ,A .and cunha ,M .(2010) .Comparison of methods for the detection of biofilm production in coagulase-negtive staphylococci .BMC Res Notes l;3:260.

Percival S. L. and Williams D. W. (2014) Microbiology of Waterborne Diseases . (Second Edition) Chapter Three – *Aeromonas* . Microbiological Aspects and Risks 2014, Pages 49-64.

Shah R. K S 1, Zhao H Ni 1, Xiao Y S.1, Guo Q. W. 1, Fan L. (2017) ; The Determination and Correlation of Various Virulence Genes, ESBL, Serum Bactericidal Effect and Biofilm Formation of Clinical Isolated Classical Klebsiella pneumoniae and Hypervirulent Klebsiella pneumoniae from Respiratory Tract Infected Patients . Article , Pol J Microbiol . 2017 Dec 4;66(4):501-508. doi: 10.5604/01.3001.0010.7042.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864

Vila, J.;Marco, F.;Soler,L.;Chacon, M.and Figueras,M.J.(2002). In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonashydrophila*, and *Aeromonas veronii* biotype sobria. J. Antimicrob. Chemother. 49:697–702.