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Epidemiology of Aeromonas species isolated from different samples in Al-Diwanyiah province

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Abstract

Approximately 1.3 million infants under five years old perished from gastroenteritis associated with Aeromonas species as of 2008. Because of ability into stay at water of chlorinated when it forms biofilms, as well as their resistance to antibiotics and virulence toxins, Aeromonas species must be continuously screened for in food and diarrheagenic feces. 50 samples of tap water, 50 samples of diarrheagenic feces, were randomly chosen from Diwaniyah city using standard protocol, and their Aeromonas species content was checked. " The reported Aeromonas species was identifie by a conventional method and then confirm at PCR uses a 16S rRNA primer. Only 80 samples was contain the bacteria and then antibiotic test was doing against 7 antibiotics and was some antibiotics have resistant to samples and other was sensitive to samples .the highest presence of isolates that belong to Aeromonas species was A hydrophilla in ratio (37.5%) while the lowest ratio was both species A trota (2.5%)and A jandaei (2.5%) the result of antibiotics test The results were as follows where high reistant percentage was of Gentamycin and Rifampin is 56.25% and 50% respectively ,whili the the high sensitive percentage of Erythromycin and meropenem was 53.75% and 42.5% respectively.whili the intermediate isolates of antibiotics was Streptomycin about 50%.this means that Erythromycin ,Meropenem and Chloramphenicol more effective against this bacteria and can use for treatment.

Keywords: Aeromonas, Epidemiology, Antibiotics resistance

1.Introduction

Gram-negative Bacteria, facultative an-aerobic, have rod-shape belonge into genus Aeromonas share morphological similarities with those of the Enterobacteriaceae family. The majority of the 14 species that have been identified have been linked to illnesses at humans (Igbinosa *et a*l.,2012; Ahmed at al., 2024) . A. *hydrophila*, A. caviae, and A. veronii is a most significant pathogen. In both fresh and salt water, organisms can be found everywhere. Gastroenteritis and wound infections (Bhowmick et al.,2018). with or without bacteremia, are two of the main disorders linked to hormones. While wound infections are caused by exposure to polluted water, gastroenteritis typically develops after consuming contaminated food or water. The worst kind of Aeromonas spp. Necrotizing fasciitis, which is typically fatal, can result from it. They are members of the Proteobacteria's Gamma subclass (Chauret et al., 2001). need antimicrobial therapy and maybe amputation (Qian et al., 2012). The specific functions of a few known putative virulence factors—such as endotoxins, hemolysins, enterotoxins, and adhesion factors—remain unclear (Bhaijee et al., 2015). The pH range that all Aeromonas can withstand is 4.5 into 5.5 (Isonhood and Drake, 2002). The Growth is best at concentrations between 0 to 4% of sodium chloride, and it cannot live at 6% (Harris et al., 1985). Aeromonad are present naturally at fresh vegetables, household animals, raw sewage water, processed drinking water, and food (Nishikawa and Kishi1 988). Numerous diseases in humans and animals are known to be caused by Aeromonas species, and certain motile of species are become very important foods and also waterborne pathogenes (Ansari et al., 2011). Chlorination has no effect on Aeromonas species' innate capacity to proliferate at water distributions systems, particularly in biofilms (Chauret et al., 2001). Some nations have adopte Aeromonad count such as a measure for quality of water as the presence for *Aeromonas* species at aquatics environment have identified as a possible healthly hazard (Borchardt *et al*., 2003). Numerous locations around a world, including California at 1988 and at college at Xingyi City, at 2012 China, had reported Aeromonas species outbreaks (Qian Zhang et al. 2012; Jam et al., 2018). There is a significant risk to the general public's health since Aeromonas species are quickly developing resistance to widely used medications (Overman and Janda (1999).

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Nonetheless, ongoing food monitoring and patient diarrhoea surveillance are necessary.

2. Experimental

2.1 Gathering of samples

100 samples were randomly selected for this study from tap water, and stool at different locations throughout the hospital. Patients who complained of diarrhea and were referred to Microbiology Laboratory had their stool samples taken in wide mouth screw-capped bottles. Three or more loose stools per day were considered diarrhea, and the samples were taken to. and processed one to two hours after being picked up. Additionally, cabbage was gathered from the five main markets that comprise the city of Al-Diwanyiah .

2.2 Media culture: Alkaline peptone water, Macchonkey agar, Aeromonas agar, and Blood agar are among the culture mediums utilized. Every piece of media was created in compliance with the guidelines provided by the manufacturer. 500 milliliters of distilled water were then added, and a mixture were homogenized in a waterbath to fully dissolve ingredients. The medium was autoclaved for 15 minutes at 121 degrees Celsius to disinfect it. After cooling to roughly 450C, it was poured to Petri dishe, left into set, then dry for 15 minutes at 370 degrees Celsius in the oven before use.

2.3. Aeromonas spp. isolation: *Aeromonas* agar was used, also blood agar media (Hussain et al., 2018), and Mac Conkey media (Merck) was inoculated with one grams/1 mL for each sample (tap water, and stool) that has been serial dilute at alkaline peptone water (PH 9.0) also incubated for 48 hours at 35ºC. Following a 24-hour incubation period, plates was checke of bacterial growth, and representatives' colonies—yellow colonies and green colonies and an opaque center—were chosen at random. To obtain pure cultures, isolates were repeatedly subcultured on nutrients agar and incubate in 37C of 24 h.

3.Results and discussion

3.1 Identification of *Aeromonas*: According to Michelim *et al*. (2005), the tween-calcium method was used to detect the presence of extracellular lipases. Each isolate was inoculated to Trypticase soy media and with 1% 20. lipase activity was Positive and verified by looking for development for a precipitate surrounding a colony after plates was incubated for 24 hours in 30ºC. The control was a plate that had been inoculated.

3.2 formation of protease and hemolytic activity. For this test, skimmed milk agar was utilized. It was made by mixing the proper agar with 1% (w/v) skim milk, as stated above (Harrigan and Mccance 1966). The media was autoclaved in a water bath for ten minutes at 110 degrees Celsius to disinfect it. They were cooled, then poured into sterilized Petri dishes and allowed to set.

The corresponding isolates were streaked across the plates to inoculate them. As a control, uninoculated plates were used. A clear area surrounding the streaking line in end for incubation periods showed that protease enzyme had hydrolyzed the casein.

Table 1. Show Aeromonas species

Aeromonas species	Percentage
A veronii	(18.75%) 15
A caviae	(22.5%)18
A hydrophilla	(37.5%) 30
A popoff	(12.5%) 10
A trota	(2.5%)2
A schubertii	(3.75%)3
A jandaei	(2.5%)2
Total	80

Table 2. the biochemical tests result of Aeromonas species

Biochemical test	Result
Gram stain	Negative
Motility	positive
Catalase	positive
Oxidase	positive
O/F	fermentative
H2S production	positive
Voges-Proskauer	positive
Esculin hydrolysis	positive
Growth in Vibriostatic agent	positive
Methyl red test	negative

Cultures cultivated by using peptone water with 1% D-glucose was used for this. After being suspended in

50% India ink, the bacteria were dry, fixed at methanol, also counterstaine for two minutes with use of carbol fuchsin. When viewed with an oil immersion lens, capsules were considered into be present if surrounding a clear zones bacterium was larger more than one-third for diameter for red-colore of bacteria (Lo et al., 2001). The plate approach was used to find hemolytic activity. After streaking each strain onto 10% sheep blood agar plates, they were cultured for 24 hours at 37°C. When a clear zone containing either beta or hemolysis type of alpha were present surround colony, hemolysis was deemed positives (Brenden and Janda 1987; Ghasemi et al., 2025). also were stain of flagella through by growing isolates on peptone water 1% D glucoses, from im-mature cultures creating a smear, and then flooding slide by Leifson stains after a shiny films stream for water wash appeare (Jabbour and Kanj, 2021). The slide was expose into 1% methylene blue, allowe into dry by air, and then examine under an oil immersion. The bacteria cell was blue in color, and the flagella were scarlet (Rabaan et al.,2001). table 2 show the result of biochemical test in the current study.

3.3 Profile of antibiotic susceptibility

After preparation, Mueller Hinton media was prepared. plates were inoculated with isolate, which had an initialy microbial loads for 6.0 x 105 c.f.u/ml, and 0.5 McFarlands standards suspension, and left for a short while into covery Mueller Hinton Agar's whole surfaces. After placing antibiotic discs on a agar surface, plates were incubated for 24 hours in 37^0C. CLSI breakpoints were used into determine and interpret zones for inhibition, that was classify as sensitive, intermediate sensitive, or resistant based on antibiotics used (CLSI 2024).

Table 3. Antibiotic sensitivity test of the *Aeromonas* species

Antibiotic type	Resistance	intermediate	Sensitive
chloramphenicol	30(37.5%)	17(21.25%)	33(41.25%)
Streptomycin	25(31.25%)	40(50%)	15(18.75%)
Erythromycin	15(18.75%)	22(27.5%)	43(53.75%)
Rifampin	40(50%)	20(25%)	20(25%)
meropenem	10(12.5%)	36(45%)	34(42.5%)
Cephradine	37(46.25%)	20(25%)	23(28.75%)
Gentamycin	45(56.25%)	18(22.5%)	17(21.25%)

From the table above table 3 show the result of antibiotics test the results were as follows where high reistant percentage was of Gentamycin and Rifampin is 56.25% and 50% respectively, whili the the highly sensitive percentage of Erythromycin and meropenem was 53.75% and 42.5% respectively.whili the intermediate isolates of antibiotics were Streptomycin about 50%. this means that Erythromycin, Meropenem and Chloramphenicol more effective against these bacteria and can use for treatment.

4. Conclusions

At the end of this work, we conclude that these bacteria may develop in the future and become more resistant to antibiotics, infect the human body's systems and organs, and cause serious diseases if not treated. Therefore, many studies must be conducted on these bacteria and the genes must be studied, especially antibiotic resistance genes and virulence genes.

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Conflict of interests

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