



ISOLATION AND IDENTIFICATION OF HYDATID CYST FLUID-ASSOCIATED BACTERIA ISOLATED FROM BUFFALO IN THE SOUTHERN OF IRAQ

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Abstract:

Echinococcus granulosus is known as the causative agent of hydatid disease. It belongs to the family Platyhelminthes within the family Taeniidae. Infected hydatid cysts are isolated from intermediate hosts. Fifty samples of liver and lung of buffaloes infected with hydatid cysts were collected during the period from February 2022 to March 2022 from southern Iraq. The aim of the study was to know the types of bacteria associated with hydatid cysts and isolated from the livers and lungs of buffaloes for the purpose of verifying this. The diagnosis was made by several tests, including phenotypic, biochemical and microscopic diagnosis, and then the antibiotic was tested To make sure of the tests, vitek test. The hydatid cyst fluid isolated from the lungs was 100%, and its percentage was in the liver, where it constituted 96%. The results of the biochemical diagnosis were *E.coli*, *Shigella spp*, *Proteus spp*, *Providencia*, and *Klebsiella*. For confirmation, two isolates isolated from the lungs were diagnosed using Vitek. It belonged to *Acinobacter lwoffii* and *Providencia rettgerii*, where the results were identical to the genus *Providencia* and contrary to the genus *Acinobacter* in the biochemical diagnosis, with the exception of the genus *Providencia spp*. As for the liver, four isolates were diagnosed and the results showed that they belong to *Mollerea wisconsensiss*, *Pseudomonas fluorescens*, *Shingomonas paucimobilis* and *E. coli*. The result was that there was no match between them and the biochemical diagnosis. Also, the result was that there was no match between them and the biochemical identification. As for the antibiotic sensitivity test, the most sensitive isolates to antibiotics were Gentamicin, Ceftazidim, Cefepim Imipenem, Ciprofloxacin, Amikain, Trimethoprim / sulfamethoxazole, Piperacillin / Tazobactam, Ticarcillin / Clavulanic, Ticarcillin / clavulanic acid, Ticarcillin, Imipenem.

Keywords: *Echinococcus granulosus*, hydatid cyst fluid- associated bacteria, antibiotic.

INTRODUCTION

Cystic echinococcosis caused by *Echinococcus granulosus* is one of the most important zoonotic parasitic diseases in the Middle East and other parts of the world (Eckert and Deplazes, 2004; Sadjjadi, 2006). It constitutes a public health problem and affects humans and types of livestock, especially in rural areas where buffaloes, cattle, goats, sheep, etc. are common, which act as the most important intermediate host in which infection occurs in the larval stage of hydatid cysts. However, dogs are represented by the final host of *Echinococcus* (Abdulhameed *et al.*, 2019). *Echinococcosis granlosis* becomes infected when eating infected hydatid cysts by animals, causing hepatitis and pneumonia that cause injuries in the body organs causing hydatid disease, and infection occurs in the liver, lungs and other organs of the body that causes economic losses in addition to the perpetuation of the affected organs (Jahed *et al.* 2013). With regard to the occurrence of bacteria in the hydatid fluid, *Echinococcus granulosus* exit eggs with the feces of infected carnivores, and ingestion of eggs by the intermediate host such as buffaloes leads to the formation of a layer of tumors that migrate through the intestinal mucosa and develop in the hydatid sacs inside the lungs or any other organ (Eckert and Deplazes, 2004). Direct contact between eggs and the external environment during the life cycle may lead to contamination of the hydatide fluid with bacteria (Ziino, 2009). The infection of the hydatid cysts with bacteria from the bile is usually due to rupture of the junction and emptying of the contents of the hydatid cyst into the bile duct or bronchioles (Fallah *et al.*, 2014). Given that the ways of infection of the liver hydatid cysts is entry from the bile passages, while the bloodstream is less dangerous and the rupture of the hydatid cyst is necessary for the entry of living organisms (Dew, 1928), so the study aimed to isolate and identification the bacteria

accompanying the fluid of the hydatid cyst and perform conventional tests and test the sensitivity of bacteria towards antibiotics vitality.

MATERIALS AND METHODS

Sample collection

In the months of February and March 2022, fifty samples of buffalo livers and lungs were obtained from the Nasiriyah slaughterhouse in the Nasiriyah governorate. Within hours of being collected, they were transported to the lab under refrigeration. The affected organ's surface was cleaned with distilled water after being sterilized with 70% ethyl alcohol. Sessile fluid extraction using a syringe for medical use, replacing the syringe after each sample.

Culturing and Identification

Blood agar, MacConky agar, Nutrint agar, Salmonella Shigella, and other culture media were used to culture 50 µl of hydatid fluid directly. The cultures were incubated at 37 °C for 24 hours (Quinn et al., 2004). The colonies produced were similar but distinct. Based on how this colony was described (Markey et al., 2013), the bacteria were also preserved by growing them on agar and glycerin 20%, incubating at 37 °C for 24 hours, storing them by freezing, and reculturing them monthly to maintain the activity and vitality of the bacteria. After that, the staining was performed according to the prescribed procedure (Quinn et al., 2004), and biochemical tests, including IMViC, oxidase and catalase tests, were performed according to the prescribed procedure (Quinn et al., 2004; Markey et al., 2013). The samples were sent to Al-Bayan laboratory for bacterial identification of the phenotypically identification isolates and their sensitivity to antibiotics using Vitek II.

DISCUSSION AND RESULTS

During the period from February 2022 to March 2022, 50 hydatid cysts were collected. The isolated hydatid cyst fluids were cultivated and after incubating them for 24 hours, we notice the emergence of bacterial colonies on the surface of the culture medium and some other media. The percentages of buffalo organs infected with bacteria accompanying the hydatid cyst fluids were found. These cysts were isolated from the livers and lungs of buffaloes, Table (1).

Table (1) Percentage of hydatid cysts isolated from buffalo livers and lungs

Host	Organsim	Number	number of infected bacteria	percentage
Buffallo	liver	25	25	100
	lung	25	24	96

Hydatid cysts in the organs were identified by the presence of a white or yellowish-white layer on the surface of the organ that resembles a bubble, especially in the liver and lung, as it is very clear, and this is consistent with what was mentioned (Hammad, 2017). This study focused on bacteria isolated from buffalo livers and lungs. After culturing the samples, all of them showed growth colonies on the culture medium, after which they were purified by planning on the culture medium after incubating them at 37 °C for 24 hours until a pure isolate was obtained. Through the phenotypic identification, we note that the majority of isolates showed hemolysis on blood agar medium, while the other isolates were non-hemolytic and were in the form of spherical white colonies, growth for all colonies on MacConkey agar medium, but it gave colored colonies due to the inability to The fermentation of lactose sugar was similar to other studies (Darweesh, 2021). Then a microscopic diagnosis was made, after using a gram stain to distinguish between positive and gram-negative bacteria, where most of the bacterial isolates were negative for the gram stain, as they constituted 4% in the liver and 10% in the lungs, while it was 1% for the lungs and 0% in the liver for the positive bacteria that formed The lowest percentage of the total number of isolated samples Levinson, (2016). Then the results of the biochemical tests to identify the bacteria, as in Table (2), showed that they are *E.coli*, *Shigella spp*, *Proteus spp*, *Providencia*, *Klebsiella*, and these results were similar to what was mentioned by (Ziino ., 2009), and Falah et al., where the relationship between Bacteria and parasite are clear even though the bile has become sterile due to bile flow and bacterial properties. Biliary tract obstruction and stasis may occur as a result of bacterial infection that gets access to the biliary system through the circulatory system, as in Table 2.

Table (2) shows the types of biochemical tests that were tested to identify bacterial species

Type of bacteria ,test	<i>klebsiella</i>	<i>E.Coli</i>	<i>Shigela. Spp</i>	<i>Serratia</i>	<i>Proteus. spp</i>	<i>Providncia. spp</i>
Indol test	-	+	V	-	-	+
Methyl red test	-	+	+	+	+	+
Voges Prosker test	+	-	-	+	V	-
Citrate test	+	-	-	+	V	+
Oxidase test	-	-	-	-	-	-
Catalase test	-	+	-	-	-	-
Hemolysis test	-	+	-	-	-	-

+: the test result is positive

-: the test result is negative

v: the test result is heterogeneous

+/-

Table (3) Percentages of bacteria species isolated from buffalo livers and lungs

Bacteria	No.lung	No. liver	Lung percentage	liver percentage
<i>Shigella spp</i>	10	14	40	56
<i>Proteus spp</i>	25	13	100	52
<i>E.coli</i>	3	3	12	12
<i>Serratia</i>	5	-	20	-
<i>Providancia spp</i>	1	-	4	-
<i>Klebsiella</i>	1	-	4	-

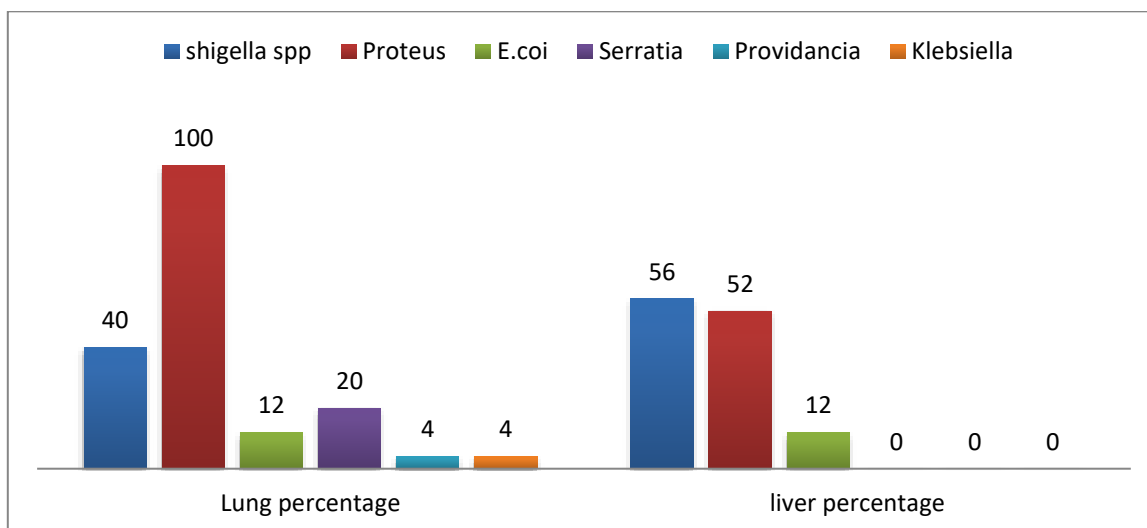


Figure (1) shows the percentage of bacteria numbers isolated from buffalo livers and lungs

Most of the isolates were negative for the oxidase test. The results showed that the isolates possess the enzyme catalase, which converts H₂O₂ into water and oxygen gas. The results of the IMViC group tests were positive for both the indole test, as a result of the appearance of a red ring on the surface of the medium. This test is important to differentiate between *Escherichia coli* and *Providencia spp* and members of the other intestinal family are positive for the methyl red test, as the color of the medium turns red. It is also negative for the Voges-Proskauer test, as a result of the appearance of a yellow to brown color in the liquid medium used, which is due to the bacteria not converting to glucose. Positive for the citrate test, which means the consumption and use of bacteria as the only source of carbon as a result of their possession of citrate permease, where the result was a change in the color of the medium to a bluish-green color, and this means the production of citric acid and the absence of a change in PH, and this is consistent with what was presented by (Tille, 2017). Five isolates were diagnosis with Vitek, two isolates were *Acinobacter Iwoffii*, *Providencia rettgeri*, while the other isolates could not be identification, as the results were identical to the genus *Providencia* and different to the genus *Acinobacter* in the biochemical identification, except for the genus *Providencia spp*. For the lungs, as for the liver, where 4 isolates were diagnosed and the results showed that they belong to *Mollerea wisconsensiss*, *Pseudomonas fluorescens*, *Shingomonas paucimobilis*, *E. coli* also, the result was that there was no match between them and the chemical diagnosis. As for the antibiotic sensitivity test, the isolates were more sensitive to the identification antibiotics, Gentamicin , Ceftazidim , Cefepim Imipenem , Ciprofloxacin , Amikain , Trimethoprim / sulfamethoxazole , Piperacillin / Tazobactam , Ticarcillin / Clavulanic , Ticarcillin / clavulanic acid , Ticarcillin , Imipenem except for the genus *Proteus hauseri* that has resistance to the anti-Ticarcillin antibiotic, and this is similar to what was found (Max *et al.* ,2011). The conclusion is that there are types of bacteria associated with the fluid of the hydatid cyst, where the negative bacteria are more common than the positive bacteria.

CONCLUSION

The proportion of bacteria associated with hydatid cysts in the lungs is higher than the percentage of bacteria associated with hydatid cysts in the liver, and negative bacteria are the most common types of bacteria from positive bacteria. The type of bacteria differed according to the organ, and most of the bacterial isolates were sensitive to various antibiotics.

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