

Isolation and identification of hydatid cyst fluid – associated bacteria isolated from cattle and sheep livers in the southern parts of Iraq.

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Abstract

Hydatid cyst is a zoonotic disease caused by the larval stage of the worm *Echinococcus granulosus*. The study aimed to isolate and identification of bacteria associated the fluid of hydatid cysts isolated from the livers of cattle and sheep. 75 samples were collected from the liver of sheep and cattle infected with hydatid cysts during the period from February 2022 to March 2022 from South Iraq. The diagnosis was made using phenotypic and biochemical methods, where the percentage of infection with bacteria associated with hydatid cyst fluid isolated from sheep liver was 100%, while the percentage of infection with bacteria associated with hydatid cysts in cows liver was 88%, the results of biochemical tests were of bacteria *Escherichia coli*, *Shigella spp*, *Proteus spp*, *Providencia spp*, *Klebsiella spp*. To confirm the validity of the test, it was diagnosed using the Vitekll technique. Seven sample isolated from cattle liver were diagnosed, two of which were diagnosed for sheep liver and one for cow liver, and 4 isolates could not be identified, so the results of sheep isolates were *Staphylococcus lents*, *Aeromonas sobria*, Where it was inconsistent with the biochemical diagnosis, its biochemical diagnosis was *Shigella spp*, *E.coli*. As for the cow isolate, it was *Kocuria kristinae*, and the result was that there was no compatibility between it and the biochemical diagnosis. As for the antibiotic sensitivity test, where the isolates were more sensitive to antibiotics Vital Gentamicin , Ceftazidim , Cefepim Imipenem , Ciprofloxacin , Amikain , Trimethoprim / sulfamethoxazole , Piperacillin / Taz obactam, Ticarcillin/Clavulanic, Ticarcillin, Ticarcillin/clavulanic acid, Ticarcillin, Imipenem. Therefore, we conclude that the percentage of bacteria associated with sheep livers is higher than the percentage of bacteria in cows, and that negative bacteria are the most present for hydatid fluid bacteria.

Keywords: hydatid cysts, antibiotic, bacteria associated with hydatid cysts.

Introduction

Hydatid cysts is one of the important diseases common to humans and animals, especially in pastoral areas in the Middle East, Asia, Africa, Australia and South America (Santivaney *et al*, 2008). It is caused by *Echinococcus granulosus*, the worm. The adult on dogs, cats, foxes and other carnivores, as the adult worm is found in their small intestine and is called the hydatid cyst worm (Morar & Feldman, 2003). So far, it constitutes a public health problem in developing countries, especially in rural communities, where disease rates increase and the most important intermediate host for infection, including humans and animals (Abdulhameed *et al.*, 2019), and that there is a relationship between bacteria and parasites through increasing the pathological effects of bacteria and making them the host It is susceptible to bacterial infection, especially when the bacteria and parasite are present in the same organ and the other type of relationship. When the bacteria are transmitted to the host through the parasite, the stage of the parasite's invasion of the environment in which it resides occurs (Ziino *et al.*, 2009). Opportunistic bacteria are microorganisms isolated from a host suffering from stress due to parasitic infection (Al-Shemmari, 2017), while antibiotics are important biochemicals produced by living organisms and widely used in medicine (Al-Asoufi *et al.* 2017), where the aim of the study was to isolate and diagnose the bacteria associated with the hydatid cysts from the livers of sheep and cows and to know their sensitivity to many antibiotics.

Materials and methods

Sample collection

Between February and March 2022, 75 samples of sheep and cow livers were taken from the Nasiriyah slaughterhouse in Nasiriyah Governorate and Basra Governorate; 50 samples of sheep livers were taken from Old Basra and Al-Khadara in Basra Governorate, and 25 samples of cow livers were taken from the central Nasiriyah slaughterhouse. After being transported to the lab in the hours following their collection in a refrigerator, the scrubs were pulled by a medical syringe, taking into account the replacement of the syringe for each sample. The surface of the organ was sterilized by ethyl alcohol with a concentration of 70% and washed with distilled water.

Culturing and Identification

The fluids of the hydatid cysts of the isolated samples were cultivated by culturing them on different culture media, including solid communal media, to diagnose Gram-negative bacteria, as well as to distinguish between fermented and non-fermented bacteria for lactose, nutrient medium, and blood medium, by taking 50 microliters of hydatid cysts fluid and distributing it in the manner of publication on the culture media used, after which it incubator at a temperature of 37 ° for 24 hours (Quinn *et al.*, 2004). Then the microscopic examination was performed according to the recipe (Quinn *et al.*, 2004). The characteristics of the bacteria are determined for a gram stain dye using biochemical tests, including oxidase and cataleas test, and the IMViC test set for negative bacteria (Khleifal *et al.*, 2008).

Discussion and Results

During the period from February 2022 to March 2022, 75 hydatid cysts were collected. These cysts were isolated from the liver of cattls and sheep, and the fluids of the hydatid cysts were taken and cultivated on different culture media. We note the growth of bacterial colonies on the culture media, through which the percentage of bacteria associatids the hydatid cyst of isolated livers was found, as shown in Table No. (1).

Table (1) Percentage of hydatid cysts isolated isolated from cow and sheep livers

Host	Organsim	Number	bacteria associated with hydatids	percentage
Sheep	liver	50	50	100
Cow	liver	25	22	88

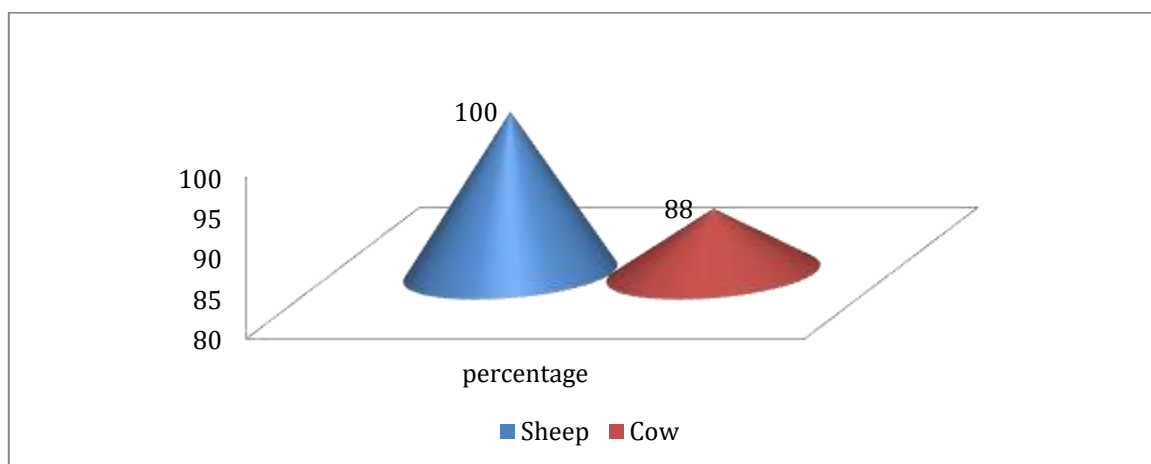


Figure 1 shows the percentage of bacteria associated with the fluid of the hydatid cyst isolated from cows and sheep, where cows constituted the lowest percentage.

The hydatid cysts in the organ 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, filled with water, and this is what happened with what was mentioned (Hammad, 2017). *Echinococcus granulosus* causing cystic *Echinococcosis* via the larval stage (Ahmed *et al.*, 2021) . Several previous studies have found a high incidence of bacterial infection at different rates in hydatid cysts isolated from cattle, and this is similar to the current study (Hadadi *et al.*, 2020; Ziino *et al.*, 2009). When these eggs arrive, hatch, and penetrate the

infected hexameroid embryo from the atmosphere into the mucous membrane, eventually leading to the formation of hydatid cysts in the liver. According to some opinions, the infection may have entered via the bile duct or enterohepatic circulation (Wani *et al.*, 2010). The liver is the organ most susceptible to hydatid cysts (Abdulhameed *et al.* 2018). This study focused on bacteria isolated in the livers of cattle animals, after culturing the samples, all of them showed growth colonies on the culture medium, through phenotypic identification, we note that the majority of isolates showed hemolysis on blood agar medium, while the other isolates that did not hemolysis were in the form of spherical white colonies, which grew All colonies were on MacConkey agar medium, but they gave bright pink colored colonies due to the inability to ferment the sugar lactose, and this is 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 gram-negative, with a percentage of 29% in sheep and 13% in cows, while the percentage of positive bacteria was 1% in sheep and 0% in cows from The total number of samples. (Levinson ,2016). After the results of the biochemical test showed that the most common and isolated bacteria, as shown in Table (2), are *E.coli*, *Shigella spp*, *Proteus spp*, *Providencia spp*, and *Klebsiella*, which indicates that Gram-negative bacteria are the most common bacterial invaders in hydatid cysts. Identification of negative bacteria from the hepatic hydatid cyst fluid, the results were similar to what was mentioned (Ziino, 2009: Abdullah *et al.*, 2021: Fallah *et al.*, 2014).

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>Proteus. spp</i>	<i>Providncia. spp</i>
Indol test	-	+	V	-	+
Methyl red test	-	+	+	+	+
Voges Prosker test	+	-	-	V	-
Citrate test	+	-	-	V	+
Oxidase test	-	-	-	-	-
Catalase test	-	+	-	-	-

+: the test result is positive
 heterogenous +/-

-: the test result is negative

v: the test result is

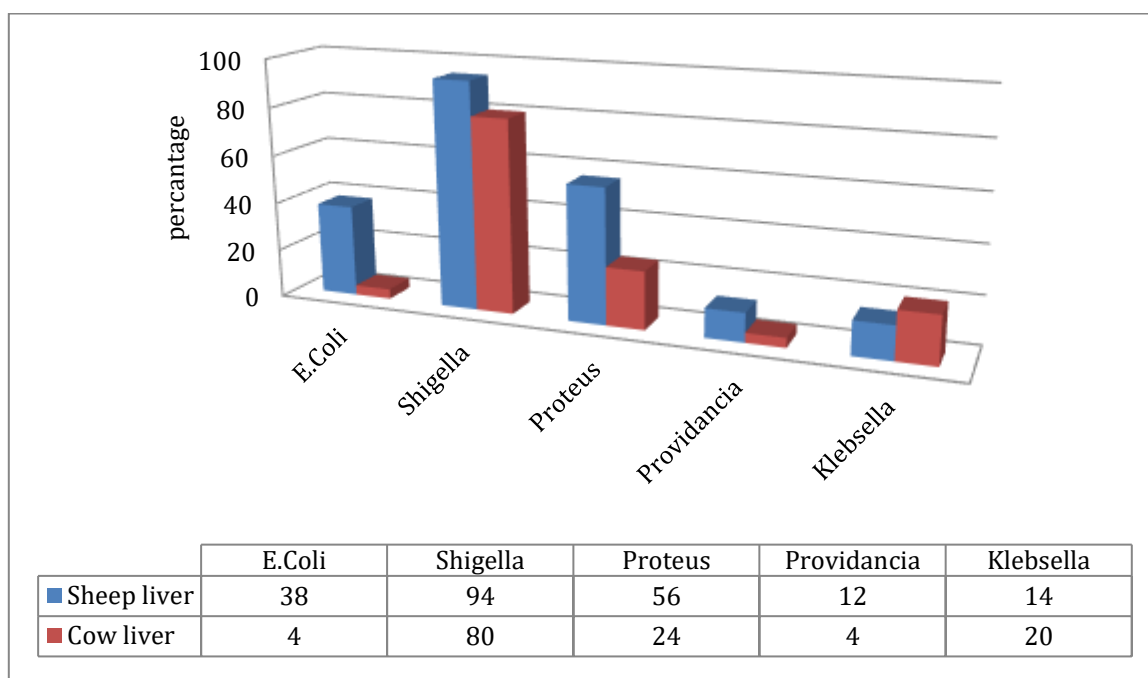


Figure 2 shows the percentage of bacteria species associated with the hydatid cyst fluid and isolated from the livers of both sheep and cows.

We noted that most of the isolates were negative for the oxidase test for all types of bacteria, catalase test. The results showed that the isolates possess the catalase enzyme that converts H_2O_2 into water and oxygen gas for *Providncia* and *Klebsiella* bacteria. The other isolates were tested negative. The results of the IMViC set of tests were positive for *E. Coli* isolates, *Providncia spp.*, for the indole test, as a result of the appearance of a red ring on the surface of the medium, and negative for the isolates of *Klebsiella*, *Proteus spp*, *Providncia spp*. This test is important for differentiating between *E. coli* and *Providncia. spp* and members of the other intestinal family are positive for the methyl red test, 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 used liquid medium, which is due to the bacteria not converting to glucose, except for the genus species *Klebsiella*. The result of the test was positive, and the color of the medium turned to purple. Positive for the citrate test for each of *Klebsiella spp*, *Providncia spp*, *Proteus spp*, 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 each of (Tille,2017). 7 isolates were identification, two belonging to sheep liver, one belonging to cow liver, and four that the Vitek was unable to identification, so the results for sheep isolates were *Staphylococcus lents*, *Aeromonas sobria*, as they were in violation of the biochemical identification, which was the identification of these isolates *Shigella spp*, *E. coli*, as for the bovine isolates, it belonged to *Kocuria kristinae*. As for the antibiotic sensitivity test, the isolates were more sensitive to the diagnosed antibiotics: Gentamicin, Ceftazidim, Ceftazidim, Cefepim Imipenem, Ciprofloxacin, Amikain, Trimethoprim / sulfamethoxazole, Piperacillin / Tazobactam, Ticarcillin / Clavulanic, Ticarcillin / Clavulanic. acid, Ticarcillin, and Imipenem, except for the genus *Proteus hauseri*, which has resistance to Ticarcillin, and this is similar to what was found (Max *et al.*, 2011).

Conclusion

The proportion of bacteria associated with the bacteria associated with the hydatid cysts was higher in sheep than in cows, as the proportion in sheep reached 100%, while in cows the proportion of bacteria accompanying the hydatid cysts was lowest, reaching 88%. The highest percentage, while positive bacteria had the lowest percentage in both organs.

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