

Effect of some species of bacteria on viability of human hydatid cysts

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Abstract:

A total of 50 fertile human hydatid cases {33(66%) females and (34%) males}, obtained from Al-Ramadi public Hospital during the period from December 2003 to July 2004 were examined to study any bacterial infections. The specimens were obtained from hydatid fluid and then cultured on appropriate culture media to distinguish some species of bacteria which resulted in obtaining: *Staphylococcus aureus* (18%), *Pseudomonas aeruginosa*(12%), *Escherichia coli*(6%) and *Streptococcus pneumonia* (4%).

These bacteria were confirmed by isolation from interacyst fluid and blood culture technique. The possible routes of infection may be through blood, biliary ducts and bronchioles. The selectivity permeable of the cyst wall may be absent and that may allow some species of bacteria to enter inside the cyst.

Further, the percent viability decreased among cyst which yielded *S. aureus*, *P. aeruginosa* and other bacteria isolated after culturing compared with those of negative culture. Besides, the two types of protoscolex motilities (flame cell activity and constriction –relaxation movement) increased in cases of negative culture. This association holds true at three different temperatures (25°C, 37°C and 40°C).

Key words: Viability, Bacteria, Temperature, Hydatid cyst.

Introduction:

Hydatid disease was recognized as one of the world major zoonosis having a wide geographic distribution [1,2]. It still represents an important medical and sanitary problem in any regions and a challenge in common surgical practice world – wide [3].

It is well known that, hydatid cysts enter the body through the portal venous radicles and this haematogenous route is the primary route of entry and dissemination of hydatid disease [4].

It is a parasitic infection caused by the larval stage of *Echinococcus granulosus*, which is the causative agent of cystic hydatid disease or

cystic echinococcos that can involve various organs [5,6].

It is well accepted that bacterial infection of hydatid cysts could be considered as another hazardous risk factor, in addition to other complications usually associated with this serious parasitic infection in man [7,8].

[9] stated that bacterial infection of a cyst may occur, which can lead to a rapid increase in size, or to destruction of endocyst making it difficult to peel the cyst intact from its adventitia, perhaps causing a rupture of the hydatid cyst during the surgical intervention and dissemination of the

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parasite elements, thus may lead to secondary echinococcosis. In addition to that, [10], mentioned that hydatid cysts may become sterile due to action of secondary bacterial infection, or they may die and become calcified.

The aims of the study to investigate some of the microorganisms that may be present in the hydatid fluid and their relationship to the cyst viability .

Materials and methods :

A total of 50 fertile hydatid cysts were obtained from 33 females and 17 males from Ramdi general hospital during the period from December 2003 to July 2004. The specimens were taken from different ages and site of infection 40 liver, 5 lung, 3 kidney and 2 urinary bladder.

Hydatid materials which includes fluid and cysts whether intact or ruptured were transported as soon as possible to the microbiological laboratory inside closed sterile containers . On the other hand the age , sex and site of cyst for all patients were recorded .

Each specimen of hydatid materials was cultivated on nutrient agar, blood agar , Chocolate agar and MacConkey agar after sterilizing the area of intact cyst with 70% alcohol and aspiration by sterile disposable syringe . After 48 hrs of incubation positive cultured specimens were examined while negative cultured were furtherly incubated for other 24 hrs .

Gram stain was used according to [11,12] coagulase test affer [11], oxidase test affer and IMViC test [13] were used as confirmatory test. which include Indole test , Methyl red test, Vogas proskaeur test and Citrate utilization tests, in addition to aspiration the blood from patients for blood culture technique.

Statistical analyses were compute assisted using SPSS (Statistical Package for Social Sciences), while in

protoscoleces motilities the median used instead of mean to present the central tendency of data & non-parametric tests will be used to assess the statistical significance.

Results and discussion:

Table 1 indicates that 60% of the samples showed negative results in culture .The positive results obtained in remaining 40% of cases and they were as follows ;

Staphylococcus aureus (18%) ,
Pseudomonas aeruginosa (12%),
Escherichia coli (6%) and
Streptococcus pneumoniae (4%).

Two kinds of motility were seen, flame cell activity and constriction – relaxation activity (invagination-evagination movement) .One way of describing motility is noting the total absence of both types of movement in all examined protoscoleces.

The rate of cases with immotile protoscoleces was obviously higher (100%) among cases with clinical evidence of infection compared with those with no infection (68.1%). The evidence however failed to reach the level of statistical significance (Table 2).

The rate of cases with immotile protoscoleces was also higher (100%) among cases which receive medical treatment prior to surgery compared with those with no pretreatment (67.4%)

The isolated of *S. aureus* and *P. aeruginosa* increased the risk of having totally immotile protoscoleces by 6.1 and 3.8 times respectively compared to those with negative culture which failed also to reach the level of statistical significant . In these cases the presence of some bacterial species inside the hydatid cyst may indicate the absence of selectivity permeable of the cyst wall, this result was similar to [14].

Evidence of the absence of any sign of viability of the protoscolices after staining was documented to clear a dead cyst. The rate of cases with dead cyst was significantly higher (100%) among cases with clinical evidence compared with those with no infection (23.4%).

The rate of cases with dead cyst was also higher (75%) among cases which received medical treatment prior to surgery compared to those with no pretreatment (23.9%) (Table 3). The median percent of viability decreased from as high 46% among negative culture to 28%, 25% and 0% among cyst which yield *S. aureus*, *P. aeruginosa*, and other bacteria respectively after culturing. This negative association between the presence of bacteria and viability was statistically significant (Table 4).

The median percentage of protoscolices with flame activity or constriction-relaxation movement was significantly higher in the group with negative culture compared to those with *S. aureus*, *P. aeruginosa* and other bacteria isolated from culture media. This association holds true at 3 different temperatures (25°C, 37°C, and 40°C). It is worth noting that the median percentage of protoscolices with flame activity or constriction – relaxation movement increased steadily with rising temperature among the group with negative culture, while this positive trend was not observed among cysts in which bacteria were isolated from culture (Table 5).

In this study, the viability of human hydatid cyst showed high rate in high numbers of cases, except many cases such as, infected hydatid cyst which contained small numbers of dead and shrinkage protoscolices in addition to degeneration materials. These cases showed complete absence of any sign of viability (100%) due to presence of certain species of bacteria

such as *S. aureus*, *P. aeruginosa* & other species which were isolated. Therefore, these cases also showed absence of any sign of viability in (75%) due to the effect of treatment on hydatid materials with Tinidazole, compared to those with intact cyst.

Statistical analysis showed no significant difference in pretreatment cases with viability of hydatid cyst, probably because of small sample size.

Secondary bacterial infection of the cysts can result in liver abscesses, the organisms recovered from liver abscesses are varied and often reflect the origin of the infectious process. Mixed facultative and anaerobic species are isolates most frequently [15,16]. When the biliary tree is the source of infection, enteric gram negative aerobic bacilli and enterococci are common isolates. Anaerobes are not generally prominent in liver abscesses arising from biliary infections since anaerobes are not common constituents of the gall bladder unless previous surgery or stenting of the biliary tree has been performed. While with hematogenous spread of infection, a single organism is most commonly isolated including *S. aureus* or a streptococcal species [17,18].

Our results revealed, presence and isolates of some species of microorganisms from the cyst wall and fluid like; *S. aureus*, *P. aeruginosa*, *E. coli* and *S. pneumoniae* as confirmed isolation of bacteria from intracyst fluid and blood culture technique. This presence may be due to the absence of selective permeability of the cyst wall. The source of infection may be through blood or biliary ducts and bronchioles [1].

This study was supported by aspiration of blood from patients with hydatid disease that were suffering from bacteremia, and then cultured under sterile conditions. So, the large

numbers of cases which yielded a positive culture in appropriate culture media in vitro gave the same result after aspiration of the blood from patients with hydatid disease after surgical removal for blood culture .our result was similar to those obtained by [10].

In this study ,the presence and isolate of certain species of bacteria showed clearly effect of the percent of viability due to highly visible decrease of this percent .This negative association between the presence of bacteria and viability was statistically significant .In addition to the two types of motility like flame cell activity and constriction –relaxation it also showed more decrease in motility when compared to those with negative culture. This association holds true at 3 different temperatures (25°C, 37°C and 40°C).

Table 1: Frequency distribution of the study samples by results of culture.

Results of culture	N	%
Negative	30	60
<i>Staphylococcus aureus</i>	9	18
<i>Pseudomonas aeruginosa</i>	6	12
<i>Escherichia coli</i>	3	6
<i>Streptococcus pneumoniae</i>	2	4
Total	50	100

N= number of cases

Table 2: The risk of absence of any kind of motility of protoscolec by selected explanatory variables.

	Total	Absence of any kind of motility		P
	N*	N	%	
Clinical evidence of infected cyst				0.54 ^{[NS]**}
Negative	47	32	68.1	
Positive	3	3	100	
Pretreated cases				0.3 ^[NS]
Negative	46	31	67.4	
Positive	4	4	100	
Results of culture				
Negative	30	17	56.7	
<i>Staphylococcus aureus</i>	9	8	88.9	0.08 ^[NS]
<i>Pseudomonas aeruginosa</i>	6	5	83.3	0.22 ^[NS]
Others	5	5	100.0	0.08 ^[NS]
Isolation of bacteria in culture				0.01
Negative	30	17	56.7	
Positive	20	18	90.0	

* Number of cases.

** Non Significant.

Table 3: The risk of complete absence of any sign of viability of protoscolec by selected explanatory variables.

	Total	Complete absence of any sign of viability		
	N	N	%	P
Clinical evidence of infected cyst				0.02
Negative	47	11	23.4	
Positive	3	3	100.0	
Pretreated case				0.06 ^[NS]
Negative	46	11	23.9	
Positive	4	3	75.0	
Results of culture				
Negative	30	3	10.0	
<i>Staphylococcus aureus</i>	9	3	33.3	0.12 ^[NS]
<i>Pseudomonas aeruginosa</i>	6	3	50.0	0.04
Others	5	5	100	<0.001
Isolation of bacteria in culture				<0.001
Negative	30	3	10.0	
Positive	20	11	55.0	

Table 4: The difference in median viability (relative frequency of viable protoscolec) by result of culture.

	Percent viability		
	Range	Median	N
Negative	(0 - 72)	46	30
<i>Staphylococcus aureus</i>	(0 - 75)	28	9
<i>Pseudomonas aeruginosa</i>	(0 - 68)	25	6
Others	(0 - 0)	0	5
P (Kruskall-Wallis) = 0.001			

Table 5: The difference in median of Percent protoscolecemes with flame activity- and percent of those with constriction relaxation movement by result of culture and temperature.

	Percent protoscolecemes with flame activity-			Percent protoscolecemes with constriction-relaxation movement		
	25 °C	37 °C	40 °C	25 °C	37 °C	40 °C
Negative						
Range	(0 - 9.8)	(0 - 49.2)	(0 - 88.2)	(0 - 5.6)	(0 - 58.7)	(0 - 100)
Median	1.8	31.8	64.9	0.5	44.5	100
Number	30	30	30	30	30	30
<i>Staphylococcus aureus</i>						
Range	(0 - 1.3)	(0 - 41.2)	(0 - 19.8)	(0 - 1.1)	(0 - 41.8)	(0 - 100)
Median	0	0	0	0	0	0
Number	9	9	9	9	9	9
<i>Pseudomonas aeruginosa</i>						
Range	(0 - 1.9)	(0 - 42.3)	(0 - 18.2)	(0 - 2.1)	(0 - 36.3)	(0 - 100)
Median	0	0	0	0	0	0
Number	6	6	6	6	6	6
Others						
Range	(0 - 0)	(0 - 0)	(0 - 0)	(0 - 0)	(0 - 0)	(0 - 0)
Median	0	0	0	0	0	0
Number	5	5	5	5	5	5
P (Kruskall-Wallis)=	0.003	0.006	<0.001	0.002	0.001	0.006

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تأثير بعض الأنواع البكتيرية في حيوية الأكياس العدرية في الإنسان

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الخلاصة:

فحصت عينات الأكياس العدرية وضمت 50 كيسا مائيا بشريا خصبا 33 (66%) من الإناث و 17 (34%) من الذكور، من مستشفى الرمادي العام للمدة الممتدة من كانون الأول 2003 الى تموز 2004، بهدف دراسة الأصابات البكتيرية ، وأخذت هذه العينات من السائل العدري ثم زرعت في وسط زرع ملائم للكشف عن بعض الأجناس البكتيرية اذ تم الحصول على:

Staphylococcus aureus (18%) , *Escherichia coli* (6%) , *Pseudomonas aeruginosa* (12%) and *Streptococcus pneumoniae* (4%)

التي عزلت من داخل الأكياس فضلا عن تقنية زرع الدم . قد تكون هذه الأصابات دخلت عن طريق الدم ، القنوات الصفراوية والقصيبات ، او قد تكون النفاذية الأختيارية للكيس العدري مفقودة مما يسمح بدخول بعض الأجناس البكتيرية لداخل الكيس . قلت نسبة حيوية الرؤيسات الأولية في الأكياس التي كانت تحتوي على

S. aureus , *P. aeruginosa*

واجناس اخرى عزلت ، مقارنة بنتائج الزرع السلبية . الى جانب ذلك فإن حركتي الرؤيسات الأولية (الحركة اللهبية و حركة النقل - الأنيساط) ازدادت مع نتائج الزرع السلبية ، تزامنت هذه النتيجة مع درجات الحرارة الثلاث (25° م ، 37° م ، 40° م).