Appendectomy in pediatrics : the value of peritoneal Fluid Smear and its bacteriological Profile

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الخلاصة

أجريت دراسة استطلاعية على 54 طفل من اللذين يعانون من التهاب الزائدة الدودية واللذين أجريت لهم تداخلات جراحية لاستئصال الزائدة الدودية للفترة من الأول من حزيران 2007 م إلى نهاية مايس 2011 م في وحدة جراحة الأطفال/ مستشفى النسائية والأطفال التعليمي في الديوانية. تم اخذ مسحات من السائل البريتوني والإفرازات البريتونية ان وجدت مع عينات نسيجية من الزائدة الدودية بعد استئصالها وتم زرع عينات السائل البريتوني والإفرازات البريتونية والعينات النسيجية في الأوساط البكتيرية للكشف عن البكتريا الهوائية واللاهوائية. وجد بينهم 39 (72.2%) من الذكور و 15 (27.7%) من الاناث تراوحت أعمارهم بين (1،8-13) سنة ، بمتوسط عمر قدره 6.9 سنوات .اعتمادا على التحليل النسيجي ، وجد آن 34 من الأطفال مصابين بالتهاب الزائدة الدودية القيحي و 8 منهم مصابين بالتهاب الزائدة الدودية مع الغرغرينا، و6 منهم مصابين بمضاعفات التهاب الزائدة الدودية ألعفني و6 كانت عينة الزائدة الدودية غير مصابة. وجد أن من بين 108 من العينات التي أخذت لغرض الكشف البكتيري 80 (74.07%) ذات نتيجة موجبة . البكتريا اللاهوائية Bacteroides والهوائيةE.coli من اكثر الانواع التي تم عزلها . وكانت البكتيريا الهوائية ذات حساسية عالية لكل من الامبنيم وسيفترياكسون، والأميكاسين والكلندماسين، ولكنها كانت مقاومة تماما للأمبيسلين.اما البكتيريا اللاهوائية فكانت اعلى حساسية لكل من الكلورامفينيكول و كربنيسيلين وميترونيدازول واقلها للكليندامايسين.

Abstract

Acute appendicitis is the most common cause of abdominal pain requiring surgery in children. In most instances the infecting organisms are normal inhabitants of the lumen of appendix . Surgery allows easy microbiological sampling. Aspiration of fluid or pus in a syringe is preferred. Swabs are less suitable and only to be used when sampling with a syringe is not feasible. Antimicrobial susceptibility testing of the isolated bacteria and particularly of the anaerobes can be important to adjust therapy in case of the presence of multi resistant bacteria.

The aim of the study was to determine the bacteriological profile of acute appendicitis in children.

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Study design is a prospective descriptive study including children hospitalized for acute appendicitis. Tissue samples(a specimen of the appendix), peritoneal fluid swab from the appendicial fossa and the peritoneal exudates (if exists) obtained at surgery from 54 children with suspected acute appendicitis operated at the pediatric surgery unit at the Maternity and Child Teaching Hospital in Al-Diwaniyah province from the period 1st of June 2007 to the end of May 2011,   were examined histologically and by culture for aerobic and anaerobic bacteria.

 Out of these, 39 boys (72.2%) and 15 (27.7% girls). Their age ranged between (1.8-13) years, with a mean of 6.9 years. Of the all patients studied 34 presented with suppurative and phlegmnous appendicitis, 8 with gangrenous appendicitis, 6 with septic complications of appendicitis and 6 had normal appendices. Only 80 of the 108 swabs taken yielded a positive culture (74.07%). B*acteroides* species and *E.coli* were the predominant organisms in most normal and acutely inflamed appendices. Aerobic bacteria were highly sensible to imipenem, amikacin, ceftriaxone and clindamicyn, but it was absolutely resistance to ampicillin. For anaerobic bacteria, sensitivity rates from high to low were chloramphenicol, carbenicillin, metronidazole and clindamycin.

Although in our study no antibiotic regime was changed on the basis of a positive culture swab and the peritoneal culture swabs do not improve immediate postoperative therapy based on surgical impression and rapid histological reporting, however, the routine use of peritoneal culture swabs may be of value in identifying patients requiring outpatient follow-up.

Introduction

Acute appendicitis is the most common cause of abdominal pain requiring surgery in children. But it is an uncommon entity in young children and rare in infants. The classic description of appendicitis includes the onset of periumbilical pain followed by nausea, then migration of pain to the right lower quadrant (RLQ) and finally, vomiting and fever. However, this progression of symptoms is less common in children than adults [1]. Absence of

classic symptoms leads to a higher rate of appendiceal perforation in children. Pathogenesis of appendicitis is still uncertain, its significance in septic complications of appendicitis is well established [2,3].

The exact cause remains unclear, but luminal obstruction, diet, and familial factors have been suggested, and the etiology may be multifactorial in some cases [4,5]. Some bacteria and parasites were found in histopathological evaluations of the appendices [6]. Inflammation of the appendix ranges from minor, simple acute inflammation to suppurative necrosis and perforation, but in some appendectomized patients it could be histologically classified as normal appendices.

Most workers have found the qualitative flora of appendicitis does not differ appreciably from that of the normal appendix in adults and children [7]. Both *Streptococcus milleri* and *Bacteroides fragilis*, however, have been implicated in the pathogenesis of appendicitis by increased isolation rates in acute inflammation [8,9]. Less is known of the quantitative flora of the acutely inflamed and normal appendix. In adults anaerobic bacteria are more commonly found in counts of above 10^6 colony forming units/ml than aerobic bacteria in acute inflammation," and anaerobic bacteria, in particular bacteroides species, have been quantified in acutely inflamed and normal appendices. Cultures from inflamed appendices usually revealed that there is a hardly pyogenic organism. The most common organisms are a mixture of *Escherichia coli* (85%), *entercocci* (30%), *nonhaemolytic streptococci, anaerobic streptococci together with clostridium welchii* (30%) and *bacteroides* [10,11]. In most instances the infecting organisms are normal inhabitants of the lumen of appendix. Surgery allows easy microbiological sampling. Aspiration of fluid or pus in a syringe is preferred. Swabs are less suitable and only to be used when sampling with a syringe is not feasible. For anaerobic culture, special precautions should be taken to expel air bubbles after aspiration. Gram-staining and aerobic and anaerobic culture of pus is generally advocated. Blood cultures are also advised : generally two to three sets of an aerobic plus

anaerobic bottle are obtained with 10-30 min interval. Antimicrobial susceptibility testing of the isolated bacteria and particularly of the anaerobes can be important to adjust therapy in case of the presence of multi resistant bacteria [12].

The aim of the study was to determine the bacteriological profile of acute appendicitis in children and determining the influence of microbiological results on postoperative outcome in patients undergoing appendicectomy.

Materials and methods

Study design is a prospective descriptive study including children hospitalized for acute appendicitis. Tissue samples (a specimen of the appendix), peritoneal fluid swab from the appendicial fossa and the peritoneal exudates (if exists) obtained at surgery from 54 children with suspected acute appendicitis operated at the pediatric surgery unit at the Maternity and Child Teaching Hospital in Al- Diwaniyah province from the period 1st of June 2007 to the end of May 2011, were examined histologically and by culture for aerobic and anaerobic bacteria. After histology evaluation, patients were classified depending on the severity of inflammation into Suppurative and phlegmnous, Gangrenous, Septic and non-appendicitis (or normal histology) subgroups. All intra-operative microbiological swabs were taken using two microbiological swab supplemented with amieˏs and charcoal transport medium, and then immediately plated onto aerobic and anaerobic culture. For each specimen, the following anaerobic bacteriologic media were inoculated: brucella blood agar, *Bacteroides* bile esculin agar, phenylethyl alcohol blood agar, and sheep blood with kanamycin and vancomycin agar plates, and thioglycollate broth. Anaerobic plates were incubated in an incubator placed inside the anaerobic chamber (atmosphere,10% carbon dioxide) at 35°C. Aerobic media included trypticase soy sheep blood agar, Columbia colistin-nalidixic acid agar, and chocolate agar plates (incubated in 5% CO_2) and MacConkey agar plates (incubated in air). Fluid specimens were inoculated directly (0.05 mL per medium), and tissues were emulsified in 1.0 mL of

thioglycollate broth and inoculated to media (0.05 mL in to solid media and the remainder into the thioglycollate). Plates were streaked in a standardized quadrant format to facilitate enumeration of colonies [13]. Gram stains of all specimens were examined, and the plates were examined after I, 2, 5, and 10 days of incubation. The thioglycollate broths were subcultured, and isolates were identified only if no growth occurred on primary plates. All organisms were identified according to standard methods outlined in the *Wadsworth Anaerobic Bacteriology Manual* and *Bailey & Scott's Diagnostic Microbiology* [13,14]. Antibiotic susceptibilities of bacterial isolates were determined using the disk-diffusion method, according to the actual recommendations. The susceptibilities of aerobic and anaerobic bacteria were determined for antibiotics ciprofloxacin, ampicilin, trimethoprim/ Sulfamethoxazole, gentamicin, amikacin, cefotaxime, ceftriaxone, imipenem, clindamycin, vancomycin, carbenicillin, chloramphenicol, metronidazole, and tetracycline. Bacteria were enumerated semiquantitatively as follows: growth in the original inoculum area only, I+; growth over the first half of the plate, 2+; growth extending to the third quadrant, 3+; and growth extending throughout all areas of the plate, 4+. Significance of differences between groups was determined using Chi- square (x2) test analysis and two-tailed z-tests. *P*<0.05 was considered statistically significant, and all reported *P*-values were two-tailed [14].

Results

During the study period, 54 children were operated with clinical evidence of acute appendicitis .Out of these, 39 (72.2%) boys and 15 (27.7%)girls. Their age ranged between (1.8-13)years, with a mean of 6.9 years. Of the all patients studied 34 (60.71) presented with suppurative and phlegmnous appendicitis, 8 (14.28) with gangrenous appendicitis, 6 (10.71) with septic complications of appendicitis and 6 (10.71) had normal appendices. Overall, an intra-operative swab was taken in the patients, including those with normal appendices although swabs were taken more frequently in patients with 'complicated' appendicitis. Only

80 of the 108 swabs taken yielded a positive culture (74.07%). The proportion of patients with a positive culture, rang of types and semiquantitive counts of isolates increased with severity of histological diagnosis, table (1) and (2). The bacterial species isolated from appendices and appendix related infections, table (3). Anaerobes were more frequently isolated than aerobes and members of the *Bacteroide spp.* group were predominant. Polymicrobial infection was found in most of cases. Gram-negative bacilli were the most frequently isolated microorganisms among the aerobic bacteria with the predominant of *Escherichia coli* and *Klebsiella pneumoniue.* There were no significant differences in the proportions of different genera found among the four patient groups. The counts of the isolated organisms showed no significant differences within individual appendices (p>0.05). Between normal and inflamed appendices there was no significant difference in the counts of all isolates (0.05<p<0.1), We also observed differences in the number of organisms recovered between specimens of perforated appendices and those of gangrenous appendices: cultures of the peritoneal fluid were almost always positive for bacteria in cases of perforating appendicitis, whereas in cases of gangrenous appendicitis, fluid cultures were usually negative for pathogens but tissue cultures were positive. Table (3) shows that *bacteroides* species and *E.coli* were the predominant organisms in most normal and acutely inflamed appendices. Aerobic bacteria were highly sensible to imipenem, amikacin, ceftriaxone and clindamicyn, but it was absolutely resistance to ampicillin. For anaerobic bacteria, sensitivity rates from high to low were chloramphenicol, carbenicillin, metronidazole and clindamycin, table (4) .

There was no significant difference in the power of surgical or histological assessment of the appendicitis at predicting a positive peritoneal culture result. Complex appendicitis was more likely to be associated with a positive peritoneal culture (P < 0.0001).

Table (1):Frequency distribution of patients included in the study according to histopathology profile and positivity of culture.

Histopathology	Patient included in the study		Total smear (Appendiceal tissue and peritoneal fluid)	Positive culture	
	NO.	(%)		NO.	(%)
Suppurative and phlegmnous	34	60.71	68	56	82.35
Gangrenous	8	14.28	16	12	75
Septic	6	10.71	12	*11	91.66
Normal	6	10.71	12	1	8.33
Total	54	100	108	80	74.07

*p< 0.0001 {Complex(septic) vs normal}.

Table (2): Enumeration of types and semi quantitative counts of isolated organisms in relation to histopathology results were available for evaluation.

No. of patients with indicated type of appendicitis from whom both specimens were available	Appendiceal tissue	Peritoneal fluid	semi quantitative counts of isolates per specimen
Suppurative and phlegmnous			
10	Growth(one isolates per specimen)	No growth	1+
9	Growth(one isolates per specimen)	Growth(one isolates per specimen)	1+
6	Growth (one isolate per specimen)	Growth (1-2 isolates per specimen)	1+
4	Growth (2 isolates per specimen)	Growth (one isolates per specimen)	2+
4	Growth (1 isolates per specimen)	Growth (2 isolates per specimen)	2+
2	No growth	Growth (2 isolates per specimen)	1+
Gangrenous			
1	Growth (3 isolates per specimen)	Growth (3 isolates per specimen)	2+
1	Growth (2 isolates per specimen)	Growth (one isolates per specimen)	3+
6	Growth (2-3 isolate per specimen)	No growth	2+
Septic			
5	Growth (2-6 isolates per specimen)	Growth (3-5 isolates per specimen)	4+
1	No growth	Growth (2-6 isolates per specimen)	4+
Normal			
1	No growth	Growth (2 isolates per specimen)	1+
5	No growth	No growth	_

Table (3): Organisms isolated from   both Appendiceal tissue and peritoneal fluid specimens according to histological profile.

Bacterial species	Suppurative and phlegmnous (34)	Gangrenous (8)	Septic (6)	Normal (6)	Total (54)
Anaerobes					
Bacteroide fragilis	28	7	11	1	47
Bacteroide uniformis	0	1	1	0	2
Bacteroide vulgatus	1	0	1	0	2
Clostridium perfringens	1	2	1	0	4
Peptococcus spp.	0	0	1	0	1
Peptostreptococcus spp.	0	2	1	0	3
Total	30	12	16	1	59
Aerobes					
Escherichia coli	7	4	4	1	16
Klebsiella pneumoniae	4	2	4	0	10
Citrobacter freundi	0	1	2	0	3
Enterobacter cloacae	0	1	2	0	3
Pseudomonas aeruginosa	1	1	3	1	6
Streptococcus faecalis	0	1	1	0	2
Staphylococcus aureus	0	0	3	0	3
Total	12	10	19	2	43

Table(4):Antibiotic susceptibility pattern for common aerobic and anaerobic bacteria which were isolated from both Appendiceal tissue and peritoneal fluid specimens.

Organisms (% sensitivity) Drugs	*Bacteroide fragilis N=47*	*Escherichia coli N=16*	*Klebsiella pneumonia N=10*	*Pseudomonas aeruginosa N=6*
Ampicilin	-*	15	20	10
Ciprofloxacin	-	80	70	70
Amikacin	-	50	50	50
Cefotaxime	-	80	80	80
Ceftriaxone	-	80	80	80
Cefoxitin	-	80	80	80
Imipenem	-	100	100	100
Vancomycin	-	87	80	83
Trimethoprim/Sulfamethoxazole	-	75	70	66
Clindamycin	78	93	80	83
Carbenicillin	93	-	-	-
Chloramphenicol	100	-	-	-
Metronidazole	85	-	-	-
Tetracycline	32			

Disscision

Appendicitis is one of the most common and costly acute abdominal illnesses associated with inflammation [15] .In this study we investigated patients with acute appendicitis as well as patients with normal appendices and with septic complications of appendicitis. Both aerobic and anaerobic bacteria were isolated from all groups of patients, though in varied proportions. Since the microbial etiology of appendicitis was described in 1938, it has served as the prototype of mixed aerobic and anaerobic intra abdominal infections [16]. Whereas Altemeier's original investigations demonstrated mixed flora in patients with complicated appendicitis, subsequent studies have also documented mixed flora in patients with acute suppurative appendicitis [16 ,17]. However, investigators in these studies did not rigidly distinguish between acute and complicated appendicitis on the basis of pathology, a distinction which has made comparison of the bacteria encountered in each of these conditions difficult. In our

study, *E. coli* was the most frequently encountered aerobic bacteria, similar with previous reports of children with gangrenous and perforated appendicitis [18,19]. Perforated appendicitis was responsible for the majority of secondary bacterial peritonitis found in our study. Alexander noticed that between one third and three quarters of children present with Perforated appendicitis at the time of diagnosis depending on age [18]. There was little difference in the incidence of *Bacteroides* species in the various lesions except in patients with a perforated appendix where these organisms were found in most of the swabs. There are several issues regarding the importance of certain microorganisms isolated in secondary peritonitis. Although enterococci are frequently isolated as part of a polymicrobial intra-abdominal infection, their role as pathogens and the need for antibiotic coverage specifically toward this organism remains unclear. In a review of several trials [20]

Bacteroides are anaerobic Gram-negative bacilli which are relatively difficult to isolate in the routine laboratory without special precautions [21]. Care must be taken in the collection of the swab and it must be delivered to the laboratory in special anaerobic transport media. It is necessary to use special selective culture media to allow easy identification, and blood agar containing neomycin 0.01% is commonly used. It has been shown that using these precautions the rate of isolation can be significantly increased [22]. The shift from histologically normal toward gangrenous appendices was clearly associated with markedly elevated anaerobic bacterial counts in terms of species. *Bacteriod fragilis* is the most frequent anaerobic species found in acute appendicitis .Nonetheless *Bacteriod fragilis* is the most common isolate [23] . It is evident that some bacteria may pass the intact appendiceal wall prior to perforation ,while progressive infection and subsequent tissue damage with necrosis allows bacteria to move in to peritoneal cavity [23]. And here we must remember most of the bacteria that are implicated in intra-abdominal infections originate from the normal intestinal flora. With the exception of *Bacteroides* spp., most other anaerobes are never encountered in pathology but

they are our main barrier against colonization and infection by pathogens. This colonization barrier is particularly sensitive to different factors like antimicrobial treatments [12]. The longer the duration of peritonitis, the more anaerobes become present [24]. Although this study is too small to draw any definite conclusions, it is felt that *Bacteroides* should be considered an important pathogen in appendicitis and should be taken into account in the few ill patients where antibiotic treatment is contemplated. It was also noted that swabs taken from the surface of the appendix itself were more often positive than those from the peritoneal cavity, and this difference appears to be significant. It is important that the chemotherapy of post appendicectomy infections include an antibiotic active against *bacteroides.*

The debate remains open concerning the role of *Pseudomonas aeruginosa* in perforated appendicitis. *Pseudomonas aeruginosa* has been reported in previously healthy children in several studies [25-29] and even in 20% of young adults without co-morbidities in one study . In one pediatric study *P.aeruginosa* was isolated in up to 42% of peritoneal pus [29] . Two retrospective open (non-randomized) studies comparing different antibiotic regimens have found an increased complication rate in the group without antipseudomonal coverage. However, *P.aeruginosa* was often co-cultured with anaerobes [30] . In a retrospective study performed in a Belgian pediatric hospital, 23% of the children with peritonitis had *P. aeruginosa* in their peritoneal fluid. The presence of *P. aeruginosa* was correlated with an increased surgical complication rate, independently from the presence of anaerobes [31] . No prospective large trial has evaluated the role of *Pseudomonas aeruginosa* in patients (particularly children) with complicated appendicitis.

All studies questioning the use of intra-peritoneal swabs were open, non-randomized, and retrospective with incompletely matched control groups, non-standardized swab collection techniques, and consequently lacked power to inform surgical practice. They concluded that an appropriately powered randomized, blinded, prospective, controlled clinical trial is needed

to test for absolute efficacy in the use of peritoneal swabs in patient management. Until controlled trial data becomes available, it may be wise to continue peritoneal swabs at least in high-risk patients to decrease clinical and medico legal risk [32] .

Conclusion

Although in our study no antibiotic regime was changed on the basis of a positive culture swab and the peritoneal culture swabs do not improve immediate postoperative therapy based on surgical impression and rapid histological reporting, however, the routine use of peritoneal culture swabs may be of value in identifying patients requiring outpatient follow-up and Empiric antibiotic therapy in acute complicated appendicitis in children should be efficient against microorganisms were isolated.

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