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IMPACT OF INFECTION BY SOME BACTERIAL ZOONOTIC PATHOGENS ON APPENDIX IN MAN

(With 4 Tables and 3 Figures)

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اثر الإصابة ببعض المسببات البكتيرية المشتركة على الذائدة الدودية فى الإنسان

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تعتبر بعض الميكروبات المعوية الممرضة من أهم الميكروبات التى تنتقل إلى الإنسان عن طريق الغذاء والماء الملوث والتى تؤدى إلى التهاب الذائدة الدودية والتى تنتشر بين الأطفال والبالغين. وقد أجريت هذه الدراسة لتحديد مدى تواجد كل من *E.coli* O157:H7، *Yersinia enterocolitica* و*Yersinia pseudotuberculosis* كمسببات لالتهاب الذائدة الدودية. كما تم تحديد نوع الالتهاب لهذه العينات وارتباطه بالميكروبات المعزولة. لذلك فقد تم جمع عدد ٨٠ عينة عشوائية من االذائدة الدودية والتى تم استئصالها من المرضى المصابين بأعراض التهاب الذائدة الدودية وذلك من مستشفى جامعة أسيوط فى الفترة من سبتمبر ٢٠٠٣ إلى يوليو ٢٠٠٤. وقد تم إجراء المسح البكتيرولوجى والهستوباثولوجى لهذه العينات. وقد كانت نسبة الإصابة بالالتهاب الحاد (٥٣,٧٥%) بينما كانت نسبة الإصابة بالالتهاب المزمن (٣٣,٧٥%). كما أظهرت النتائج أن (١٢,٥%) من العينات المفحوصة كانت غير مصابة. وقد تم عزل كل من *E.coli*O157:H7، *Yersinia enterocolitica* و *Yersinia pseudotuberculosis* بنسبة ١١,٢٥% ، ١٠% و٦,٢٥% ، من العينات المفحوصة على التوالى. وتمت مناقشة نتائج نوع الالتهاب لهذه العينات وارتباطه بالميكروبات المعزولة. وقد تمت دراسة مدى حساسية العترات المعزولة لعدد من المضادات الحيوية. وقد تبين أن بعض العترات المعزولة كانت مقاومة لبعض المضادات الحيوية. وقد تمت مناقشة الأهمية الصحية والوبائية لهذه الميكروبات ومصادر التلوث المختلفة، هذا بالإضافة إلى مناقشة التوصيات لكيفية الحد من انتشار هذه الميكروبات وكيفية الوقاية منها.

SUMMARY

Some zoonotic enteropathogenic microorganisms including *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are implicated as major bacterial causes of appendicitis. Appendiceal specimens were taken from 80 patients underwent appendectomy for presumed appendicitis at Assiut University Hospital between september

2003 to july 2004. All appendiceal specimens were screened with bacteriological and histopathological examination. This study was undertaken to investigate the incidence of *Escherichia coli* O157:H7; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* as a possible causes of appendicitis and to study their pathologic behavior. It has been estimated that 43 cases out of 80 (53.75%) showed acute inflammation including both catarrhal (12.5%) and suppurative (41.25%) inflammation. In addition, 33.75% of the examined appendices showed chronic inflammation where, 31.25% of the samples showed chronic inflammation only while, 2.5% of them had chronic inflammation mixed with suppuration. On the other hand, 12.5% of the examined appendices were normal. *E.coli* O157:H7 was recovered from 11.25% of the examined appendices, while *Y.enterocolitica* and *Y.pseudotuberculosis* were isolated from 10% and 6.25% of the examined appendices, respectively. Concerning the correlation between the isolated microorganisms and the different types of inflammation, *E.coli* O157:H7 has been isolated from 24.24% of the examined appendices with suppurative inflammation, while it was isolated from one patient with chronic inflammation with a rate of 4%. Moreover, *Y.enterocolitica* and *Y.pseudotuberculosis* were isolated from 18.18% and 15.15% of appendices with suppurative inflammation, respectively. It has been estimated that 6.25% of the examined cases of appendicitis were due to bilharzial infection, 3 (10%) of them were represented by chronic inflammation only while the remaining two cases were represented by chronic inflammation with suppuration. Interestingly, *Y. enterocolitica* was found in association with the two cases of specific inflammation (parasitic) both showed superadded suppuration. Antibiotic sensitivity pattern were studied and the results revealed that some of the recovered strains were resistant to several antibiotics. Suggestive measures to control transmission of foodborne pathogens were discussed.

Key words: *Appendicitis, E.coli* O157:H7, *Y.enterocolitica, Y.pseudotuberculosis, Antibiotic sensitivity pattern.*

INTRODUCTION

Foodborne diseases caused by microbial pathogens remain a significant international public health problem in the 21st century, so much that governments are intensifying their efforts to improve food safety (WHO, 2000). It has been estimated that the incidence of foodborne illness had been greatly increased recently due to increasing

numbers of working mothers and growing reliance on frozen meals, restaurant dining and take out foods (Ciestak *et al.*, 1997).

Many foodborne illness bacteria are excreted abundantly in the feces of infected animals or humans, which may contaminate food and water (Plaut, 2000). Bacterial foodborne zoonotic microorganisms are considered the most common causes of human illness (Thorns, 2000). Moreover, recent data indicated that antibiotic resistant strains of foodborne pathogens have emerged as an important public health problems in developed and developing countries all over the world (Schlundt, 2001; USDHHS, 2001 & Sayed and Abdel Hafez, 2005). Some zoonotic enteropathogenic microorganisms including *Escherichia coli* O157:H7; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are implicated as major bacterial causes of appendicitis (Cimolai *et al.*, 1990; Bennion *et al.*, 1991; Van Noyen *et al.*, 1991; Tarr *et al.*, 1992; Shorter *et al.*, 1998 ; Lamps *et al.*, 2001 and Sakellaris *et al.*, 2004).

Appendicitis is an inflammation of appendix; a finger like glandular piece of tissue that connects to the beginning of the large intestine, at the lower right side of the abdomen. Appendicitis is the most common acute surgical condition of the abdomen (Liu and Mcfadden, 1997). Approximately 7% of the population will have appendicitis in their life time with the peak incidence occurring between the ages of 10 and 30 years (Addis *et al.*, 1990 & Schwartz, 1994). However, acute appendicitis can occur at any time from infancy to very old age (Lamps, 2004). Delay in the diagnosis of appendicitis increases the risk of perforation, in turn increases the rate of postoperative complication, which is considered a challenge for physicians even with advent of improved diagnostic imaging techniques (Izbicki *et al.*, 1992; Velanovich and Satava, 1992; Calder and Gajraj, 1995 & Garcia Pena *et al.*, 2004).

This study was undertaken to investigate the incidence of *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* as a possible causes of appendicitis and to study their pathologic behavior.

MATERIALS and METHODS

Samples collection:

Eighty patients (40 males & 40 females) with an average age of 18.9yrs (range 8 – 46yrs) underwent appendectomy for presumed appendicitis at Assiut University Hospital between september 2003 to

july 2004. All appendiceal specimens were screened with bacteriological and histopathological examination.

Isolation and identification of *E.coli* O157:H7:

Enrichment Technique:

Bacteriological swabs were obtained from appendiceal specimens and enriched in modified Tryptic Soya Broth (mTSB) supplemented with novobiocin (20 mg/liter). The inoculated broth was incubated at 37°C for 24 hours. (De Boor and Heuvelink, 2000).

Isolation on Sorbitol MacConkey agar:

Loopful from the incubated broth was streaked onto Sorbitol MacConkey agar plates and incubated at 37°C for 24 hours (De Boor and Heuvelink, 2000).

Identification of *E.coli* O157:H7:

Non sorbitol fermenter colonies were identified morphologically by Gram's stain and biochemically as *E.coli* according to Varnam and Evans, (1991). A latex agglutination test (*E.coli* O157, Oxoid diagnostic reagents 620 M) was used for identification of *E.coli* serogroup O157 isolates. The Oxoid *E. coli* O157 latex was demonstrated by slide agglutination of *E.coli* strains possessing the O157 serogroup antigen according to Vernozy-Rozand, (1997). Bacto *E.coli* H7 antisera (Difco) was used to identify H7 strains according to manfacture's procedure using slide agglutination test.

Isolation and identification of *Yersinia* species:

Cold enrichment technique: swabs of appendiceal specimens were enriched in phosphate buffer saline pH 7.6 (PBS) supplemented with 2% peptone and incubated at 4°C for 14 days (Varnam and Evans, 1991).

Isolation on *Yersinia* selective agar:

Loopfuls from the enriched broth were streaked onto *Yersinia* selective agar plates supplemented with Cefsulodin-Irgasan-Novobiocin (CIN) and incubated at 28°C for 24 hours. Dark red, bull eye-like colonies were screened by biochemical reactions according to the technique described by Varnam and Evans (1991).

Antibiotic susceptibility test:

The antibiotic sensitivity patterns were determined for the recovered strains by using the disc diffusion method (Schroeder *et al.*, 2002). The following antibiotic discs were used: ampicillin 10µg, chloramphenicol (30µg), erythromycin (15µg), gentamycin (10µg) and tetracycline (30µg).

Histopathological examination:

All samples were observed grossly and were fixed in 10% formaline and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (H&E).

RESULTS

Table 1: Type of inflammation of the examined appendices

Type of inflammation	No./80	Total
Acute inflammation:		
Catarrhal	10 (12.5%)	43 (53.75%)
Suppurative	33 (41.25%)	
Chronic inflammation:		
Chronic	25(31.25%)	27 (33.75%)
Chronic +suppurative	2 (2.5%)	
Normal appendix	10 (12.5%)	10 (12.5%)

Table 2: Incidence of some zoonotic pathogens among the examined appendices

Sex	No.of samples	*E.coli* O157:H7	*Yersinia species*	
			Y. enterocolitica	*Y. pseudotuberculosis*
Female	40	6 (15%)	4 (10%)	3 (7.5%)
Male	40	3 (7.5%)	4(10%)	2 (5%)
Total	80	9 (11.25%)	8 (10%)	5 (6.25%)

Table 3: Correlation between type of inflammation and the isolated pathogens

Type of pathogens	Type of inflammation				Total
	Catarrhal	Suppurative	Chronic	Chronic+ Suppurative	
	No./10	No./33	No./25	No./2	No./80
E.coli O157:H7	-	8 (24.24%)	1 (4%)	-	9 (11.25%)
Y.enterocolitica	-	6 (18.18%)	-	*2(100%)	8 (10%)
Y.pseudotuberculosis	-	5 (15.15%)	-	-	5 (6.25%)
**Parasitic	-	-	3 (12%)	*2 (100%)	5 (6.25%)

* mixed infection (Y.enterocolitica and Parasitic infection).
** Bilharzial infection.

Table 4: Antibiotic sensitivty pattern of the isolated pathogens

Antimicrobial agents	*E.coli* O157:H7		*Y.enterocolitica*		*Y.pseudotuberculosis*	
	No./ 9		No./ 8		No./ 5	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	6(66.67%)	3(33.33%)	8(100%)	-	1(20%)	4 (80%)
Chloramphenicol	5(55.56%)	4(44.44%)	4 (50%)	4(50%)	1(20%)	4 (80%)
Erythromycin	7(77.78%)	2(22.22%)	7 (87.5%)	1 (12.5%)	2(40%)	3 (60%)
Garamycin	8(88.89%)	1(11.11%)	7 (87.5%)	1 (12.5%)	3 (60%)	2(40%)
Tetracycline	8(88.89%)	1(11.11%)	7 (87.5%)	1 (12.5%)	1(20%)	4 (80%)

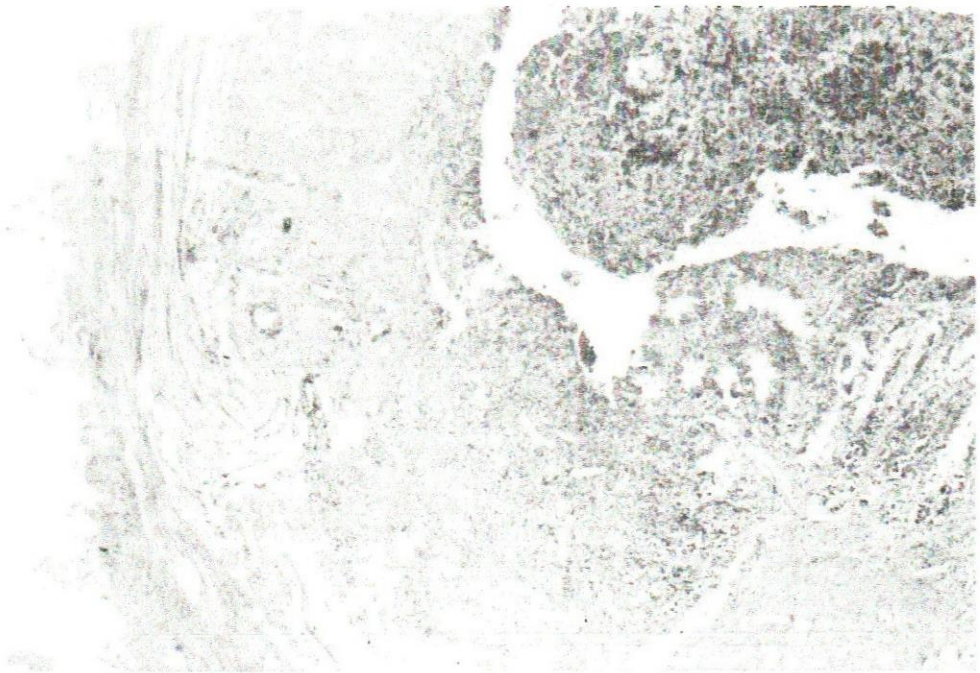

Figure 1: Acute suppurative appendicitis, showing ulceration of the mucosa with necrotic tissue within the lumen (H &E X40).

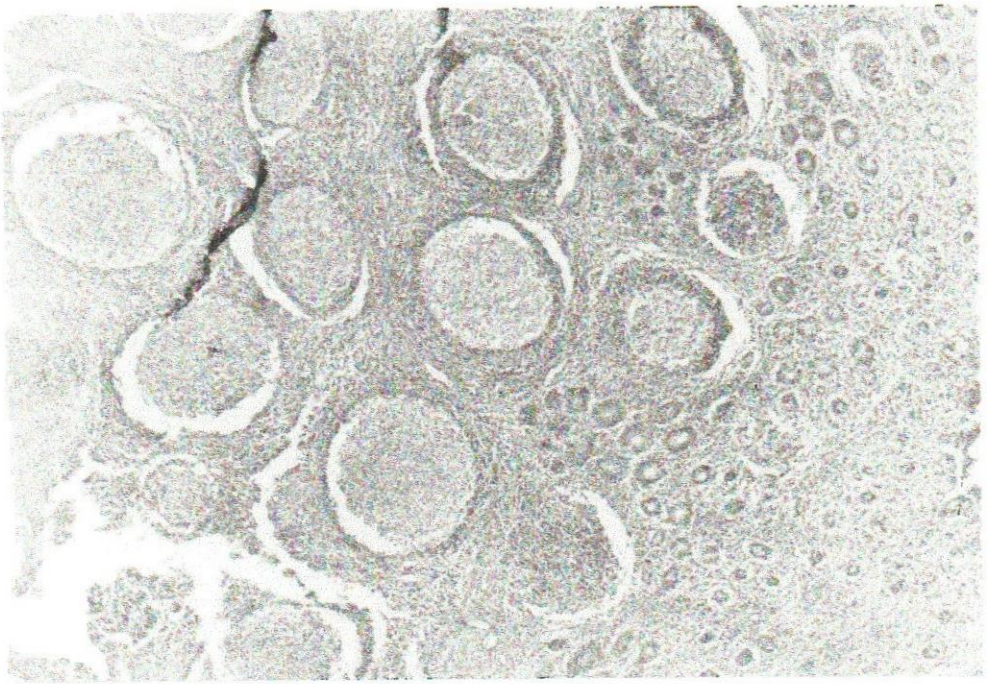

Figure 2: Chronic appendicitis with marked proliferation of the lymphoid follicles (H&E X40).

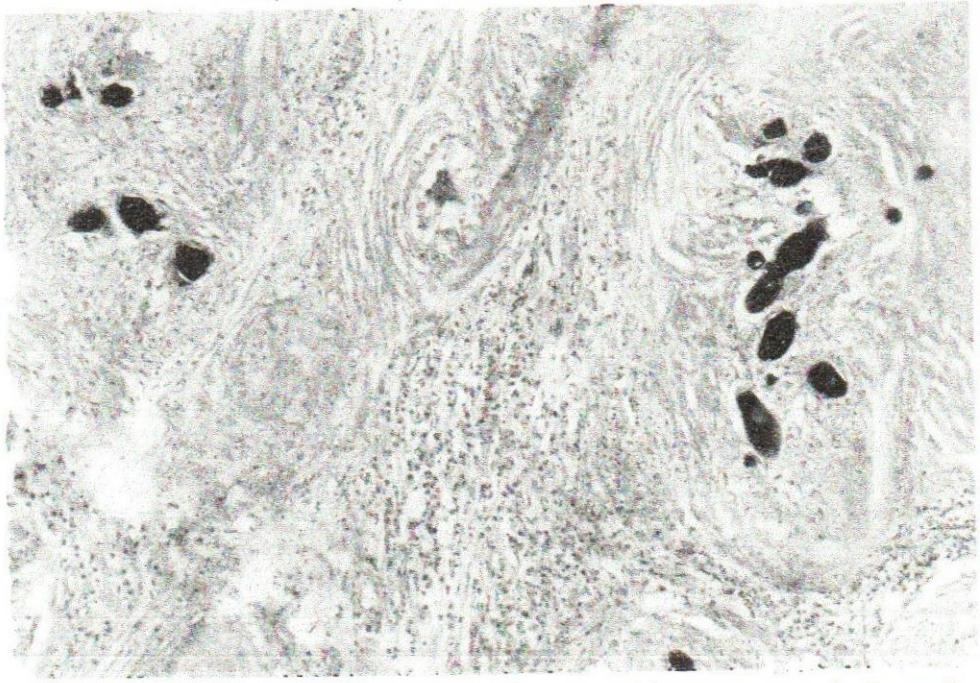

Figure 3: Chronic specific appendicitis showing schistosome eggs in the wall of the appendix with suppuration and mucosal ulceration (H&E X100)

DISCUSSION

The pathologic spectrum of the acutely inflammed appendix encompasses a wide range of infectious and non infectious entities. The appendix may suffer alone or may be involved through extention from other areas of the gastrointestinal tract. Appendicitis is usually precipitated by obstruction of the appendiceal lumen (Lamps, 2004).

The classical presentation of acute appendicitis begins with peri-umbilical pain that is colicky in nature of gradual onset, and increasing severity. Nausea, loss of appetite, vomiting and maliase may present as well as a low fever, within 6-18 hrs, then pain typically localizes to the right lower quadrant and becomes constant, usually with associated guarding and rebound tenderness (Williams and Myers, 1994).

Acute appendicitis is considered the most common presentation among patients presented for appendectomy (Esmer-Sanchez *et al.*, 2004). It is clear that some patients may suffer recurrent bouts of acute appendicitis, which develop to chronic appendicitis before appendiceal resection, moreover, patients with peri-appendiceal abscess or specific infections of the appendix may have chronic ongoing symptoms. However, most authorities agree that primary chronic appendicitis is not an entity that should be clinically or histologically recognized (Williams and Myers, 1994 & Carr, 2000).

In this study 43 out of 80 (53.75%) appendiceal samples showed acute inflammation as illustrated in (Table 1) including both catarrhal (12.5%) and suppurative (41.25%) inflammation. In addition, 33.75% of the patients had chronic inflammation where, 31.25% of the samples showed chronic inflammation only while, 2.5% of them had chronic inflammation mixed with suppuration (Figures 1, 2&3). Esmer-Sanchez *et al.*, (2004) reported that acute appendicitis was the most common presentation (79.1%). In addition, acute suppurative appendicitis were detected among 23.8%, 4.5% and 24% of the cases reported by Iul and Markov, (1990); Van-Noyen *et al.*, (1991) & Sakellaris *et al.*, (2005), respectively. However, It has been estimated in this study that 12.5% of the appendiceal specimens were normal as shown in (Table 1). It has been reported that when an appendix is removed for a clinical diagnosis of appendicitis; a certain percentage will be histologically normal (Lamps, 2004). Moreover, it has been reported that the rate of negative appendectomies was 12%, 20% and 20.3% recorded among patients underwent appendectomy for presumed appendicitis by Bennion *et al.*, (1991), Rao *et al.*, (1998) & Esmer-Sanchez *et al.*, (2004), respectively.

It has been explained that bacterial ileocecitis with clinical picture mimicking acute appendicitis, seems to be responsible for an appreciable number of unnecessary appendectomies. Some studies have investigated nonoperative management with parentral antibiotic treatment but 40% of these patients eventually required appendectomy (Schwartz, 1994). It has been recommended that it is better to differentiate infectious ileocecitis from appendicitis thus preventing an unnecessary laparotmy (Puylaret *et al.*, 1997).

A wide variety of pathogenic micro-organisms are incriminated as major causes of appendicitis (Bennion *et al.*, 1991 and Sakellaris *et al.*, 2004). The possible role of gut bacteria in both the development and the sequelae of acute appendicitis has also been the subject of great discussion (Jindal *et al.*, 1994 and Roberts, 1998).

Shiga toxin-producing *E.coli* (STEC) O157 has emerged as public health threat (Armstrong *et al.*, 1996). *E.coli* O157:H7 that is known to be associated with both sporadic and outbreak of human diseases ranging from uncomplicated diarrhea to haemorrhagic colitis and hameolytic uremic syndrome (Nelson *et al.*, 1998). More recently it was implicated as one of the most possible causes of acute appendicular syndrome (Cimolai *et al.*, 1990 & Tarr *et al.*, 1992). In this study *E.coli* O157:H7 had been isolated from 9 (11.25%) out of 80 samples of appendices as represented in (Table 2). However, Cimolai *et al.*, (1997) reported that *E.coli* O157:H7 was isolated from 20% of the examined appendices. Isolation of *E.coli* O157:H7 from cases of appendicitis expands the number of organisms that can cause bacterial ileocecitis (Tarr *et al.*, 1992).

Y.enterocolitica and Y.pseudotuberculosis are the two *Yersinia* species pertinent to human gastrointestinal disease. These Gram negative coccobacilli characteristicaly cause granulomatous appendicitis, which may or may not have associated enterocolitis and mesenteric adenitis (Naktin and Beavis, 1999 & Lamps *et al.*, 2001). Recent studies have concluded that there is significant overlap between the histological features of *Y.enterocolitica* and *Y.pseudotuberculosis* infection and that either species may show lymphoid hyperplasia, epithelioid granulomas with prominent lymphoid cuffing, transmural lymphoid aggregates giant cells, mucosal ulceration, cryptitis and concomitant lymph node involvement (Lamps *et al.*, 2001).

Y.enterocolitica usually affects children. The predominant symptom in small children is an acute enteritis which transform to bloody diarrhea. In older children and adolescents, the pseudoapp-

endicitis syndrome predominates with pain in the right illiac fossa (Butler, 1998). Results presented in (Table 2) declared that *Y.enterocolitica* was isolated from 10% of the examined patients. Higher incidence of isolation were reported by several authors (Puylaert *et al.*, 1989; Iul and Markov, 1990; & El Sherbeni, 1999 with a rate of 51.2%; 23.8%; and 17.1%, respectively. On the other hand, lower incidence rates (9.1% & 3.65%) were reported by Bennion *et al.*, (1991) & Van Noyen *et al.*, (1991), respectively. These differences in the rate of isolation of *Y.enterocolitica* could be attributed to several factors including seasonal variation (Delmas, 1983) as the frequency of isolation of the organism being much higher (25-50%) during winter than summer (0-17%) as stated by Toora *et al.*, (1989) as well as difference in the source of contamination in different geographical areas (Schiemann and Wauters, 1992).

Y.pseudotuberculosis mainly affects children, adolescents and young adults. The most common clinical forms is mesenteric adenitis or pseudoappendicitis with acute abdominal pain in the right iliac fossa, fever and vomiting (Acha and Szyfer, 1991). Infection by *Y. pseudotuberculosis* has become of increasing pathological importance. In this study *Y.pseudotuberculosis* had been isolated from 6.25% of the examined samples. Lower incidence had been isolated by Puylaert *et al.*, (1989) with a rate of 1.64%. The higher percentage of isolation of *Y.pseudotuberculosis* in this study confirm the belif that *Y.pseudotuberculosis* is common within the environment.

Concerning the isolated microorganisms from different types of inflammation (Table 3), *E.coli* O157:H7 has been isolated from 24.24% of the examined appendices with suppurative inflammation, while it was isolated from one patient with chronic inflammation of the appendix with a rate of 4%. Moreover, *Y.enterocolitica* and *Y.pseudotuberculosis* were isolated from 18.18% and 15.15% of appendices with suppurative inflammation, respectively.

Schistosomes are a rare cause of appendicitis even in nations where schistosomiasis is endemic. Histologically, appendices show transmural inflammation rich in eosinophils, with a granulomatous reaction to ova. Older granulomas may be fibrotic and hyalinized (Adebamowo *et al.*, 1991). However, it has been demonstrated at least in some cases that schistosomes do cause acute appendicitis, either by inducing granulomatous inflammation, or by producing such marked fibrosis that lumenal obstruction leads to signs and symptoms of acute appendicitis (Satti *et al.*, 1987).

It has been estimated in this study that 5 (6.25%) of the examined cases of appendicitis were due to bilharzial infection (Table 3), 3 (10%) of them were represented by chronic inflammation only while the remaning two cases were represented by chronic inflammation with suppuration as illustrated in (Figure 3). Interstingly, *Y.enterocolitica* was found in association with the two cases of specific inflammation (parasitic) with superadded suppuration indicating the possibility that suppuration is due to the presence of the organism.

The routine use of antimicrobials in animal production lead to emergence and dissemination of resistant pathogenic microorganisms which is considered as public health threat. (Witte, 1998). It was investigated that some of the recovered strains were resistant to several antibiotics as declared in (Table 4). The recovered *E.coli* O157:H7 was resistant to chloramphenicol (44.44%), followed by ampicillin (33.33%), then erythromycin (22.22%). Finally the strains were resistant to both garamycin and tetracycline with a rate of 11.11%. *Y.enterocolitica* was highly resistant to chloramphenicol (50%) followed by erythromycin, garamycin and tetracycline with a rate of 12.5% for each of them. While, *Yersinia pseudotuberculosis* were resistant to ampicillin, chloramphenicol and tetracycline with a rate of 80% followed by erythromycin with a rate of 60% and finally garamycin with a rate of 40%. The increasing rate of isolation of antimicrobial resistant microorganism in this study reflect the danger which makes clinical management of the cases difficult.

In conclusion, attempts to control foodborne illness by eliminating the pathogenic microorganisms from the animal host would be a monumental task. Control the use of antimicrobials in animal feed and monitor the recommended withdrawl periods for antibiotics when used. In addition human carriers may play an important role in transmission of foodborne infection so it is recommended to educate foodhandlers, who should be encouraged to behave responsibly in their work. Proper standered of hygiene should be observed in the handling and storage of cooked food.

Resturants and take away shops, must be hygienically constructed, clean, free from dirts and flies, moreover the staff should have clean hands, clean uniforms and medical certificate. Consumers should avoid eating raw or partially cooked foods of animal origin and take particular care with procedures which may lead to cross contamination from raw to cooked foods. Finally, control of foodborne illness request concentrated effort on the part of three principal partners

namely, governments, food industry and consumers. Differential diagnosis should be performed to differentiate both appendicitis and infectious ileocecitis to prevent unnecessary appendectomy.

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