مجلة جامعة بابل / العلوم الصرفة والتطبيقية / العدد (4) / المجلد (17) : 2009

Isolation of Bacteria from Appendicitis and Determination the Colicin Production of *Escherichia coli*

Zainab Hammod Al-Saadi Lena Fadhil Al-Gibouri Abeer Thaher Al-Hassnawi
University of Babylon, College of medicine

Abstract

This study was carried out for isolation and identification the bacteria causing appendicitis for 80 patients; 62 (77.5%) were males and 18 (22.5%) were females, who had undergoing appendicectomy in Hilla Teaching Hospital. Their ages ranged from (1-40) years.

In the present study, *E.coli* was isolated in higher ratio (76.2%). Another types of bacteria were isolated such as *Staphylococcus epidermidis*, non haemolytic *Streptococcus* and *Pseudomonas aeruginosa* about (10%), (8.7%) and (5%) respectively.

E.coli represents the high percentage of bacteria in this study, therefore, we investigated the bacteriocin production properties of *E. coli* strains. 40 (65.5%) of *E.coli* strains produced bacteriocin while 21 (34.4%) of *E.coli* strains didn't produce bacteriocin.

الخلاصة

أجريت هذه الدراسة لغرض عزل وتشخيص البكتريا المسببة لالتهاب الزائدة الدودية ل 80 مريض: 62(77,5%) من الذكور و18(22,5%) من الإناث تم استئصال الزائدة الدودية لهم في مستشفى الحلة التعليمي للفترة من أيلول/2007 إلى تشرين الثاني/2007 وكان معدل أعمارهم يتراوح ما بين (1−40) سنة.

في هذه الدراسة تم عزل *E.coli* بأعلى نسبة حوالي (76,2%) كما وتم عزل أنواع أخرى من البكتريا مثل: *Staphylococcus epidermidis* ، *non-haemolytic Streptococcus* و *Pseudomonas aeruginosa* بنسب (10%) ,(8,7%) و (5%)على التوالي.

سجلت *E.coli* أعلى نسبة في هذه الدراسة لذا فقد تم دراسة خواص إنتاج البكتريوسين لعتر إل *E.coli* وقد أظهرت النتائج بان 40 (65.5%) من العتر منتجة للبكتريوسين بينما 21 (34.4%) من العتر كانت غير منتجة للبكتريوسين.

Introduction

Appendicitis is an inflammation of the appendix. The cause of appendicitis relates to blockage of the inside of the appendix, known as the lumen. The blockage leads to increased pressure, impaired blood flow and inflammation (NDDIC, 2004). Appendicitis occurs at any age, it accounts for approximately 1% of all surgical operations. Although, it is rare in infants, appendicitis becomes increasingly common throughout childhood, reaching its maximal frequency between the ages of 10 and 30 years (Gorbach, 1998).

Bacteria play an important role in appendicitis (Forbes,1961; Rickett and Jackson,1969). Rains and David 1984 found that the most common bacteria present in appendix are a mixture of *E.coli* (found in 85% of cases), *enterococci* (30%) and non-haemolytic *Streptococci* (30%). *E.coli* isolated in high percentage because it is known to produce two types of bacteriocins, the first is colicin and the second is microcins (Braun, *et al*.,2002). Colicin was first discovered by Gratia in 1925 as a highly specific antibiotic produced by strains of *E.coli* and active against other strains in the same species (Gratia, 1925). Colicins are large molecular weight proteins with antimicrobial properties. They are produced by strains of Enterobacteriaceae carrying specific plasmids called colicinogenic factors (Morlon, *et al*., 1983). Bacteriocins of Gram negative bacteria generally exert their action by adsorption to their specific receptors on the sensitive cells are translocated to their targets within the cells (Koniski, 1982; Riley and Gordon,1996).

Colicin recognizes specific receptor on the surface of the sensitive cells, and destroys them by different mechanisms (Chung, 2003). Despite their different mode of action, most colicins have the same domain structures with N-terminal, central, and C-terminal regions. N-terminal region of colicins is important for uptake of colicins into sensitive cells. A central

region is involved in binding to the receptors in outer membrane and C-terminal region is essential for exerting its activity (Tilby, *et al.*,1978). When colicin derivatives, mutated in the uptake domain (N-terminal) and the receptor binding domain (central region), were translocated into cells by induced permeabilization using osmotic shock, the derivatives showed killing activity in vitro or in vivo (Tilby, *et al*., 1978; Braun, *et al*., 1980).

Material and Methods

Eighty patients (62 were males and 18 were females) undergoing appendicectomy at the Hilla Teaching Hospital were included in this study. Swabs were taken from the appendix fossa of all patients after sterilization the external surface of the appendix. The swabs were cultured directly on blood agar, MacConkey and Nutrient agar. The agar plates were incubated aerobically and examined after 24 hours. The bacterial isolates were diagnosed microscopically and biochemically. The biochemical tests were included: catalase test, oxidase test, simmon's citrate, mannitol fermentation, methyl red, voges-proskauer, motility and indole production(Collee, *et al*.,1996).

Within the results *E.coli* was reported in high ratio. The production of *E.coli* bacteriocin were investigated. To demonstrate their production: Stab inoculate multiple strains on separate multiple brain heart infusion agar Petri-dishes, incubate at 37ºc for 24 h. After removing the cell growth with a sterile glass slide, residual cells on the agar surface are killed by exposure to chloroform vapor for 30 minutes. Overlay each plate with one of the strains, incubate again at 30ºc for 24 h . After this process, the presence of bacteriocins can be inferred if there are zones of growth inhibition around stabs(Chemie,2008).

Results

Between September/ 2007 and November/ 2007 appendicectomy was carried out on (80) patients; (62)were males and (18) were females, the mean age was 6 (7.5%) years, ranging between (31-40) years, the peak age group affected was (11-20) years comprising 36(45%) of cases (table 1).

Bacteria were isolated from 80 (100%) patients (table 2). The commonest bacteria that isolated in the present study was *E.coli* 61(76.2%). The other Gram-negative bacilli isolated was *Pseudomonas aeruginosa*. Gram-positive cocci were found less commonly and were usually *Staphylococcus epidermidis* and non haemolytic *Streptococcus*. Out of 61 appendicitis *E.coli* strains, 40(65.5%) produced colicin and 21 (34.4%) non produced colicin (table 3).

Table (1). The Incidence of Appendicitis According to the Age and Sex.

Age groups(years)	Female	Male	Total
1 -10	2	7	9
11 – 20	9	27	36
21 – 30	6	23	29
31 – 40	1	5	6
Total	18	62	80

Table (2). Bacterial Isolates rate of Appendicitis.

Type of Bacteria	No. & %
Escherichia coli	61(76.2)
Staphylococcus epidermidis	8 (10)
Non haemolytic *Streptococcus*	7 (8.7)

Pseudomonas aeruginosa	4 (5)
Total	80 (100)

Table (3). Bacteriocin Production from *E.coli* Isolated from Patients of Appendicitis.

Production of bacteriocin	No. & %
produced	40 (65.5)
Non produced	21 (34.4)
Total	61 (100)

Discussion

The present study revealed that the peak incidence of appendicitis is in the second and third decades of life. Males and females are equally affected, except between puberty and age 25, when males predominant in a 3:2 ratio. Although appendicitis rarely occurs in infants, it increases in frequency between 2 and 4 years of age, reaches a peak between 10 and 20 years of age, and decreases after that. About of 80% of cases occur before 45 years of age. The results of present study agree with the results of (Dale and Daniel, 2003; Kasper, 2005).

There are few reports of the bacteriology of appendicitis and the organisms have usually been referred to the normal enteric flora. Gilmore and Martin(1974) found *E.coli* to be the commonest organism isolated from the appendix fossa. Only 43 of the cultures 48% taken during the operation were bacteria grown, and these were mainly *E.coli*. Furthermore, bacteriologic analysis revealed that *E.coli* was the predominant species isolated (Juric, *et al*.,2001). The results of present study agree with the result of (Juric, *et al*.,2001; Vankemmelbeke, *et al.*,2005).

In this study, the colicin production was detected in 61 appendicitis *E.coli* strains. Forten isolates 65.5% produced colicin. Previous studies performed in Lzmir with uropathogenic *E.coli* strains, the rate of colicin production were found to be 23% and 22% (Gratia,1925; Koniski, 1982).

McGeahie(1965) reported that 534 of *E.coli* strains isolated from the urine samples of patients, 36.3% produced colicins. Davies, *et al*.,(1981) found that the colicinogenity samples of hospitalized patients was 41%. Nowicki (1983) studied 400 uropathogenic *E.coli* strains and found that 42.7% were colicinogenic. Waleh (1981) determined colicin production to be 15% in 653 uropathogenic *E.coli* strains.

The colicin production percentages reported for uropathogenic *E.coli* strains in those studies were lower than the results of the present study 65.5%. The difference in the reported frequencies of colicin producing strains may be due to differences in the characteristics of the *E.coli* strains found in different countries during various periods (Morlon, *et al*.,1983).

Conclusions

1. Males are more affected with appendicitis than females.
2. The age group (11-20) is the most susceptible for appendicitis.
3. *E.coli* is the commonest bacterial isolates of appendicitis.
4. *E.coli* strains are considered good producer for bacteriocin that acts as spreader factor.

References

Braun, V.; Frenz, J.; Hantke, K and Schaller,K.(1980). Penetration of Colicin M into Cells of *E.coli*. J. Bacteriol. 142, 162-168.

Braun,V.; Patzer,S.I. and Hantke, K. (2002). Ton- dependent colicins and microcins: modular design and evolution. *Biochimie* 84, 365-380. (Medline).

Chemie.De. (2008). Bacteriocin: encyclopedia of chemistry, analytics & pharmaceutics with 64,557 entries.(Internet).

Chung, H.J.(2003). Control of food borne pathogens by bacteriocin-like substances from lactobacillus spp. In combination with high pressure processing. Ph. D. the Ohio State University, (Internet).

Collee, J.G.; Marmion, B.P; Fraser, A.G. and Simmons, A. (1996). Practical Medical Microbiology. 14th ed. Churchill Livingstone. P 131-149.

Dale,D.C. and Daniel, D.F.(2003). Scientific American Medicine. Vol. 1 web. MD. Inc.P.758-760.

Davies, D.L.; Falkiner, F.R.; and Hardy, K.G.(1981). Colicin V production by clinical isolates of *E.coli*. Infect Immun 31: 2: 574-579.

Forbes, G.B.(1961). Staphylococcal infection of operation wounds with special reference to topical antibiotic prophylaxis. *Lancet*, 2, 505-509.

Gilmore, O.J.A., and Martin, T.D.M.(1974). Aetiology and prevention of wound infection in appendicectomy. *Brit J. Surg*., 61, 281-287.

Gorbach,S.L. (1998). Infectious Diseases. W.B.SAUNDERS company.2nd ed. Vol. 1. P.822.

Gratia, A. (1925). Sur un remarquable example d'antagoisme entre deux douches de colibacille. C.R.Soc. Biol. 93, 1040-1041.

Juric, I; Primorac, D; Zagar,Z.; and Biocic,M. (2001). Frequency of portal and systemic bacteremia in acute appendicitis.43(2): 152-6.

Kasper,D.L. (2005). Harrison's principles of internal medicine. 16thed. Vol. 11.MaGraw-Hill Medical publishing Division. 1805-1806.

Koniski, J.1982. Colicins and other bacteriocins with established mode of action, Ann. Rev. Microbiol. 36, 125-144.

McGeachie, J.(1965) Bacteriocin typing in urinary infection. Zentrablatt W. B.L Bakt 1 Abt(Orig) 196: 377-384.

Morlon, J.; lioubes, R.; Varenne, S.; Chartier, M. and Lazunski, C. (1983). Complete nucleotide sequence of the structural gene for colicin A, a gene translated of non-uniform rate. J. Mol. Biol. 170, 271-285.

National Digestive Diseases Information Clearinghouse(NDDIC).(2004). Bacteriocin. p 1, (Internet).

Nowicki,B. (1983). Colicin production and colicin sensivity typing of Nephropathogenic Serotyes of *E. coli*. Zbi Blatt Hyg I .Abt (Orig A) 254: 397-402.

Rains, A.J.H and David, H. R.(1984). Bailey and lore's. Short Practice of Surgery. 19 th.p.1010.

Rickett, J.W.S., and Jackson, B.T. (1969). Topical ampicillin in the appendicectomy wound: report of a double-blind trial. *Brit. Med. J.,* 4, 206-207.

Riley, M.A. and Gordon,D.M. (1996). The ecology and evolution of bacteriocins. J. Indust. Microbiol. 17, 151-158.

Tilby, M.; Hindenach, I. and Henning, U. (1978). Bypass of receptor-mediated resistance to colicin E3 in E.coli K12. J. Bacteriol. 136, 1189-1191.

Vankemmelbeke,M.; Healy,B. and James,R. (2005). Rapid detection of colicin E9-Induced DNA damage using *E.coli* cells carrying SOS promoter-Lux fusions. J. of bacteriology. P4900-4907. Vol.187, No.14.

Waleh, N.S. (1981). Distribution of Group E colicin types in Shiraz-Iran. (1968). Jour Gen Microbiol 127: 219-222.