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Etiology of spontaneous bacterial peritonitis and determination of their antibiotic susceptibility patterns in Iran

Mirmejad R¹, Jeddi F², Kiani J³, Khoobdel M^{4*}¹Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran²Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran³Department of Virology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran⁴Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

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ABSTRACT

Objective: To determine the causative agents of spontaneous bacterial peritonitis (SBP) in patients with liver disease and ascites who referred to the pediatrics ward of Tehran Imam Khomeini Hospital in Iran during January to December 2008. **Methods:** In this study, from 85 patients with liver disease and ascites, ascite samples were taken and the causative bacterial agents were determined by direct microscopy, culture and biochemical tests. Subsequently, antibiotic susceptibility tests by disk diffusion method (Kirby–Bauer test) were performed on each bacterial isolate. **Results:** Among 85 examined samples, 32 bacterial and 2 yeast agents were isolated. Among bacterial cases, *Escherichia coli* (31.3%) and coagulase negative *Staphylococci* (18.8%) were the most predominant and *Streptococci* and *Enterobacteriaceae* were the next common agents, respectively. Antibiogram tests revealed that most of isolated coagulase negative *Staphylococci* were resistant to ampicillin, penicillin, cotrimoxazole and cephalosporin (first generation); and most of the gram negative isolates were resistant to amikacin, gentamicin and vancomycin. **Conclusions:** In total agreement with similar studies performed previously in other parts of the world, the present survey indicates that, *Escherichia coli* and coagulase negative *Staphylococci* are the most common causes of SBP in children and a third generation of cephalosporin such as ceftriaxone and cefoxitin can be a suitable antibiotic for empirical therapy of children with SBP.

1. Introduction

Since 1970, when spontaneous bacterial peritonitis (SBP) was first described and up to present, the mortality rate has been steadily decreasing from 80% to 30%. This has been mainly due to prompt diagnosis and early initiation of adequate therapy^[1]. Actually, SBP is the infection of the ascitic fluid that occurs in the absence of a visceral perforation and in the absence of an intraabdominal inflammatory focus such as abscess, acute pancreatitis or cholecystitis^[2]. SBP is defined as an ascitic fluid infection without a demonstrable intraabdominal pain. Although in recent studies, it was shown that SBP can be diagnosed by careful evaluation of intraabdominal pain^[3,4]. Because SBP is in most cases, a monomicrobial infection, the presence of more microorganisms in the culture (>1), must

raise the suspicion of secondary peritonitis. These kinds of infections are identified by evaluating the number of polymorphonuclear leucocytes (PMN) and bacteriological cultures^[4,5]. The diagnosis of secondary bacterial peritonitis must be made early in the course of illness, because without the adequate surgical treatment, the evolution is very severe. Most of the time, multimicrobial peritonitis is because of intestinal rupture during operation induced by operation scars, post operational adhesiveness, or by intestinal blockage^[6,7].

Regarding the etiology, over 60% of the SBP episodes are produced by gram-negative *Enteric bacilli*. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the most frequently isolated microorganisms but there may be different patterns in children^[8]. It has been ascertained that certain *E. coli* strains can translocate the intestinal mucosa more often probably because of a higher capacity to adhere to it and because of a higher virulence that determines a higher resistance to the defense mechanisms of the host^[9]. In about 25% of the cases, gram-positive cocci are involved: *Streptococci* (frequently *Pneumococcus*) and *Enterococci* spp. Although the bowel floras are predominantly anaerobic, SBP

*Corresponding author: Dr. Khoobdel M, Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, P.O. Box: 19945/581.
Tel: +98-2188600062
E-mail: khoobdel@yahoo.com

is very seldom produced by anaerobic microorganisms due to their incapacity to translocate the intestinal mucosa and due to the high volume of oxygen in the intestinal wall and the tissues that surround it^[10].

Because etiologies factors of peritonitis in children and adults are very variable, therefore, identification of the causative factors is essential for proper therapy and prevention of sequels^[11,12]. In each country, with reference to the antibiotic usage their pattern of consumption, there are great differences in antibiotic sensitivity and resistance. Since antibiotic therapy in our country is based on other countries treatment program or published books and reports, it may cause unfavorable consequences^[13,14]. The aim of the present study was to determine the causative agents of SBP in children with liver disease and ascites who referred to a children hospital and determination of their antibiotic susceptibility patterns *in vitro* method.

2. Materials and methods

In this cross-sectional study, patient sampling was performed by an easy non-random method. The study population was children who referred to Imam Khomeini Hospital during January to December 2008. 85 children with liver diseases and ascetics with symptoms like fever, abdominal pain, diarrhea, blood pressure drop and tachycardia were selected for this survey. Samples of ascitic fluid were taken by a medical specialist with special needles. Then samples were sent to microbiology laboratory to determine the etiologic agents and pattern of antibiotic resistance. Specimens were cultured on chocolate agar, blood agar, EMB agar, MacConkey agar, and nutrient agar. After 24–48 hours of incubation, any growing bacteria were identified by tests such as catalase, oxidase, biochemical characteristics determination (sugar and amino acid fermentation and sensitive to optochin and bile or sodium

deoxycholate) and complementary tests such as coagulase, culture on special media, *e.g.* SS agar and Manitol salt agar^[10,13]. Following determination of the etiologic agents, the isolated bacteria were subjected to antibiogram test by disk diffusion method (Kirby–Bauer Test) (NCCLS, 2000). Pattern of antibiotic resistance for each isolated agent was determined by 15 types of different antibiotics, including ampicillin, cephalothin, amikacin, cefoxitin, ciprofloxacin, penicillin G, erythromycin, *etc* (NCCLS, 2001). Data were analyzed by means of statistical software. We used descriptive analysis to estimate the frequencies.

3. Results

From a total of 85 ascitic fluid samples submitted to the laboratory, for direct microscopic examination and culture, 32 bacterial strain cases and 2 yeasts samples were isolated. The bacterial isolates included *E. coli* (31.2%), coagulase negative *Staphylococci* (18.8%), *Streptococcus pneumoniae* (*S. pneumoniae*) (15.6%), *Pseudomonas* spp.(12.5%), *Enterobacter* spp. (3.1%), *K. pneumoniae* (3.1%), *Proteus* spp. (3.1%) and *Serratia* spp. (3.1%).

In this study, different species of bacteria have different level of sensitivity against various antibiotics demonstrated. *E. coli*, being the most prevalent causative agent of peritonitis, was most sensitive to amikacin, cefoxitin, vancomycin, ciprofloxacin, ceftriaxone, *etc*, whereas only 10%–20% of the *E. coli* isolates were sensitive to sulfamethoxazole and ampicillin. The bacteria were not sensitive to cotrimoxazole and penicillin G. Most other gram negative bacilli showed sensitivity to cefoxitin, vancomycin, ciprofloxacin and ceftriaxone (50%–100%). The gram positive cocci isolates including coagulase negative *Staphylococci* and *S. pneumoniae* were predominantly sensitive to vancomycin, ampicillin, gentamicin and cephalothin (Table 1).

Table 1
Susceptibility percent of bacteria isolated from ascitic fluid in patients who referred to Imam Khomeini Hospital in Iran during 2008.

Bacteria	Antibiotic Ampicillin	Sulfamethoxazole	Cephalothin	Gentamicin	Penicillin G	Chloramphenicol	Cotrimoxazole	Amikacin	Erythromycin	Cefoxitin	Ceftriaxone	Clavulanic acid	Vancomycin	Ciprofloxacin	Carbenicillin
<i>E. coli</i>	10	20	40	50	0	20	0	80	0	70	70	70	70	70	40
Coagulase negative <i>Staphylococci</i>	50	66	83	83	33	50	16	83	33	50	50	50	50	0	0
<i>Pneumococcus</i>	80	60	60	60	40	60	0	20	40	60	60	80	80	0	0
<i>Pseudomonas</i> spp.	0	0	0	60	0	0	0	60	0	0	0	0	25	50	0
<i>K. pneumoniae</i>	0	0	0	0	0	0	0	0	0	100	0	100	0	100	0
<i>Enterobacter</i> spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Proteus</i> spp.	0	0	0	0	0	0	0	100	0	100	100	100	100	100	0

4. Discussion

Since the first descriptions of SBP by Kerr *et al* in 1963 and Conn and Fessel in 1964^[10], the clinical presentation, treatment and prognosis of this disease have been well established. However, other aspects are still under investigation such as its pathogenesis, diagnosis and prevention^[10].

Although most episodes of SBP occur in patients with advanced cirrhosis with ascites to symptoms and signs like fever (69%), abdominal pain (59%), signs of hepatic encephalopathy, abdominal tenderness (very rare), diarrhea, illus, shock and hypothermia, occasionally it has been observed in non-cirrhotic patients such as fulminate hepatic failure, nephritic syndrome and congestive heart failure^[3].

In a study which was performed by Haghghat *et al* in Shiraz, Iran, children with SBP had fever (92.3%), abdominal

pain (92.3%), abdominal tenderness (92.3%), change in level of consciousness (38.5%) and decreased bowel sounds (23%)^[14].

In the present study, most of the children had hepatic failure and ascites with clinical manifestations such as fever, abdominal tenderness, diarrhea, hypothermia and tachycardia. Because SBP is a monomicrobial infection in most cases, the presence of more microorganisms in the culture (>1), must raise the suspicion of secondary peritonitis.

Peritonitis is a severe infection in individual who have ascetics^[2]. Most gram-negative *Enteric bacilli* especially *E. coli* can translocate the intestinal mucosa and is the most prevalent cause of bacterial peritonitis, but recent studies show that gram positive bacteria are becoming increasingly associated with bacterial peritonitis^[14,15]. The results of the present study indicated that gram negative bacteria especially *E. coli* are among the primary causes of bacterial peritonitis, whereas gram positive bacteria (*Staphylococcus* and *Streptococcus*) are the second most probable cause. It

is possible that individuals who have peritonitis can get contaminated with environmental microorganism unrelated to hospital nosocomial infection^[15,16].

In a study conducted in Greece, *E. coli* (14 of 42 cases) was the most prevalent bacteria isolated from ascitic fluid with *Klebsiella* spp, and *Enterobacter* spp being the next common isolates^[15]. In comparison, the results of the present study shows that *E. coli* is the main etiological agent (10 of 82 cases) and coagulase negative *Staphylococci* is the next. This difference shows that the etiologic patterns peritonitis may vary in different geographic regions.

One particular study indicated that all of the isolated bacteria from individuals who had SBP, except *Enterococci*, were sensitive to ciprofloxacin and ceftriaxone^[14]. Other studies show that fluoroquinolones and third generation of cephalosporin such as ceftriaxone or cefotaxime can be a suitable antibiotic for empirical therapy of SBP^[17,18].

Our study showed that most of the isolated bacteria are sensitive to third generation cephalosporins and fluoroquinolones. The *E. coli* strains isolated in this study were 80% sensitivity to amikacin and showed sensitivity 70% to cephoxitin, ceftriaxon, ciprofloxacin and vancomycin. However, coagulase negative *Staphylococci* showed 83% sensitivity to amikacin, cephalothin and gentamicin and 50% sensitivity to vancomycin and ceftriaxone. Other isolated *Enterobacteriaceae* (*Proteus*, *Kelbseilla* and *Serratia*) from peritonitis samples were sensitive to vancomycin, ciprofloxacin, cefoxitin and ceftriaxone. They did not show any sensitivity to gentamicin, cotrimoxazole, cephalothin and ampicillin.

At last, it should be mentioned that in the present study, we used clinical symptoms and bacteriological cultures for diagnosis of SBP, whereas, in most other studies involving SBP diagnosis, investigators have used PMN counting (in both ascetic fluids and blood samples) in addition to bacteriological culture.

The results of this investigation indicated that when facing patients with peritonitis, physician should consider the followings. Firstly, SBP in children who have liver disease and ascites with one or several causal factors (gram negative and gram positive bacteria) may occur with clinical symptoms like fever, abdominal pain, hepatic encephalopathy, diarrhea, shock and hypothermia. Secondly, most of the isolated bacteria from the patients are resistant to most antibiotics except third generation of cephalosporin and fluoroquinolones. Therefore, physicians may consider beginning initial treatment with third generation cephalosporins and/or fluoroquinolones, and then send the sample to the laboratory to determine the causative agents and their sensitivities. In addition, to prevent the increment of antibiotic resistance, antibiotic administration should not be performed without definite diagnosis and necessity of treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Strauss E, Caly WR. Spontaneous bacterial peritonitis: a therapeutic update. *Expert Rev Anti Infect Ther* 2006; **4**: 249–260.
- [2] Câruntu FA, Benea L. Spontaneous bacterial peritonitis: pathogenesis, diagnosis, and treatment. *J Gastrointest Liver Dis* 2006; **15**: 51–56.
- [3] Koenig M, Cathébras P. Abdominal tenderness in ascites patients does not indicate spontaneous bacterial peritonitis. *Eur J Int Med* 2007; **18**: 448.
- [4] Wallerstedt S, Olsson R, Simrén M, Broomé U, Wahlin S, Löoff L, et al. Abdominal tenderness in ascites patients indicates spontaneous bacterial peritonitis. *Eur J Int Med* 2007; **18**: 44–47.
- [5] Sheer TA, Runyon BA. Spontaneous bacterial peritonitis. *Dig Dis* 2005; **23**: 39–46.
- [6] Amadon MN, Arroyo V. Ascites and spontaneous bacterial peritonitis. In: Schiff ER, Sorrell MF, Maddrey WC. (eds.) *Schiff's diseases of the liver*. Philadelphia: Lippincott Williams Wilkins; 2003, p. 559–594.
- [7] Ozmen S, Dursun M, Yilmaz S. Spontaneous bacterial peritonitis: pathogenesis, diagnosis, and management. *Acta Gastroenterol Belg* 2006; **69**: 276–282.
- [8] Levison ME, Bush LM. Peritonitis and intraperitoneal abscesses. In: Mandell GL, Bennett JE, Dolin R. (eds.) *Principles and practice of infectious diseases*. 6th ed. Philadelphia: Elsevier, Churchill Livingstone; 2005, p. 927–951.
- [9] Alaniz C, Regal RE. Spontaneous bacterial peritonitis: A review of treatment options. *P T* 2009; **34**: 204–210.
- [10] Filik L, Unal S. Clinical and laboratory features of spontaneous bacterial peritonitis. *East Afr Med J* 2004; **81**: 474–479.
- [11] Vinnitskaia EV, Osipov AG, Drozdov VN, Petrakov AV, Lazebnik LB. New approaches to diagnostics of spontaneous bacterial peritonitis in liver cirrhosis. *Eksp Klin Gastroenterol* 2008; **2**: 32–39.
- [12] Fernández J, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, et al. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterol* 2007; **133**: 818–824.
- [13] Zhang JM, Weng XH. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis. *Zhonghua Gan Zang Bing Za Zhi* 2005; **13**: 459–460.
- [14] Haghghat M, Dehghani SM, Alborzi A, Imanieh MH, Pourabbas B, Kalani M. Organisms causing spontaneous bacterial peritonitis in children with liver disease and ascites in Southern Iran. *World J Gastroenterol* 2006; **12**(36): 5890–5892.
- [15] Cholongitas E, Papatheodoridis GV, Lahanas A, Xanthaki A, Kontou-Kastellanou H, Archimandritis AJ. Increasing frequency of Gram-positive bacteria in spontaneous bacterial peritonitis. *Liver Int* 2005; **25**: 57–61.
- [16] Parsi MA, Atreja A, Zein NN. Spontaneous bacterial peritonitis: recent data on incidence and treatment. *Cleve Clin J Med* 2004; **71**: 569–565.
- [17] Bauer TM, Follo A, Navasa M, Vila J, Planas R, Clemente G, et al. Daily norfloxacin is more effective than weekly rifloxacin in prevention of spontaneous bacterial peritonitis recurrence. *Dig Dis Sci* 2002; **47**: 1356–1361.
- [18] Solomkin JS, Mazuski JE, Baron EJ. Guidelines for the selection of anti-infective agents for complicated intraabdominal infections. *Clin Infect Dis* 2003; **37**: 997–1005.