Pathogens of spontaneous bacterial peritonitis change in northern China

Yan-Zi Gou, MD, PhD, Bo Liu, MS, MD, Lei Pan, MD, PhD, Hai-Tao Yu, MD, PhD, Jiu-Ping Wang, MD, PhD, Ding-Cheng Wang, MS, MD.

ABSTRACT

الأهداف: دراسة المكروبات وتحديد صفاتها بعد عزلها من المرضى المصابين بالتهاب الصفاق البكتيري التلقائي (spontaneous bacterial peritonitis).

الطريقة: لقد قمنا بمراجعة سجلات الحالات المصابة بالتهاب الصفاق البكتيري التلقائي وذلك خلال 14 عاماً من يناير 1996م إلى ديسمبر 2009م. شملت هذه الدراسة الاسترجاعية 780 مريضاً مُصاباً بالتهاب الصفاق البكتيري التلقائي والتليف الكبدي اللامُعاوض والاستسقاء ممن دخلوا إلى مستشفى تانغدو في مدينة أكسيان، مقاطعة شانكسي، الصين. تم تقسيم المرضى إلى مجموعتين وجُمعت البيانات السريرية ومن ثم تم عمل مقارنة فيما بينها. جُمعت سوائل الاستسقاء من المرضى، ثم زُرعت عينات البكتيريا المأخوذة من هذا السائل باستخدام نظام (MicroScan WalkAway 40 system).

النتائج: أظهرت الدراسة بأن هناك اختلافاً واضحاً بين نسبة المكروبات المعزولة من المجموعة الأولى (شملت 48 مريضاً من يناير 1996م إلى ديسمبر 2002م) ونسبة المكروبات المعزولة من المجموعة الثانية (شملت 50 مريضاً من يناير 2003م إلى ديسمبر 2009م) (χ^2=9.630, p=0.002). وقد اضطر المرضى الذين أظهرت نتائجهم إصابتهم بالبكتيريا بعد فحصها بصبغة غرام إلى تناول المضادات الحيوية لمدة 30 يوماً وذلك بصورة أكثر من هؤلاء الذين لم تظهر نتائجهم إصابتهم بالبكتيريا بعد فحصها بصبغة غرام (χ^2=12.285, p=0.000).

خاتمة: لقد تغير تركيب المكروبات المسببة للأمراض كثيراً في السنوات الأخيرة بشمال الصين، وفي البلدان الأخرى أيضاً، وقد أدى هذا التغير إلى زيادة مدة العلاج بالمضادات الحيوية وتكثيف طرق المعالجة، ولذلك يجب علينا البحث عن طريقة العلاج المثلى بسبب هذا التغير في تركيب المكروبات المسببة لمرض التهاب الصفاق البكتيري التلقائي.

Objective: To determine the identity of microorganisms isolated from patients diagnosed with spontaneous bacterial peritonitis (SBP).

Methods: We reviewed cases diagnosed with SBP over a 14-year period. The medical records of 780 SBP-diagnosed patients with decompensated cirrhosis and ascites admitted to Tangdu Hospital, Xian, Shaanxi Province, China were retrospectively reviewed between January 1996 and December 2009. The patients were placed into 2 groups, and the clinical data were compared between the 2 groups. Ascitic fluid was collected from these patients and cultured for bacteria using the MicroScan WalkAway 40 system.

Results: There was a significant difference in the ratio of pathogens between group A (48 patients, from January 1996 to December 2002) and group B (50 patients, from January 2003 to December 2009) (χ^2=9.630, p=0.002). The SBP patients with gram-positive bacteria needed significantly more antibiotics within 30 days compared to those with gram-negative bacteria (χ^2=12.285, p=0.000).

Conclusion: In recent years, the types of isolated pathogens have significantly changed in northern China. Such changes have also been observed in other countries and have been attributed to long-term antibiotic therapy and invasive procedures. Changes in the epidemiology of pathogens that cause SBP must be monitored for optimal treatment.

Saudi Med J 2010; Vol. 31 (10): 1152-1156

From the Center of Diagnosis and Treatment for Infectious Diseases (Gou, Liu, Pan, Yu, Wang JP), and the Clinical Laboratory Department (Wang DC), Tangdu Hospital, Fourth Military Medical University Xian, China.

Received 8th June 2010. Accepted 6th September 2010.

Address correspondence and reprint request to: Dr. Yan-Zi Gou, Center of Infectious Diseases, Tangdu Hospital, Fourth Military Medical University, No. 1 Xinsi Road, Ba Qiao District, Xian 710038, Shaanxi Province, China. Tel. +86 (29) 84777595 / +86 15309215488. Fax. +86 (29) 83537377. E-mail: gouyanzi@126.com

Disclosure. The authors have no conflicting interests, and this study was not supported or funded by any Drug Company.

Spontaneous bacterial peritonitis (SBP) is a frequent bacterial infection in patients with decompensated cirrhosis and it is associated with high mortality. Most pathogens causing SBP are derived from the intestinal microbial flora; mainly *Enterobacteriaceae* and *Escherichia coli (E. coli)* are the most frequently isolated pathogens. However, in recent years, the etiology of SBP has undergone some changes. The pathogens of SBP in cirrhosis have changed during recent years.[1-4] Gram-positive bacteria have emerged as the foremost cause of infection among patients. Gram-positive bacteria were the predominant pathogens associated with SBP in a study by Singh et al.[2] Although *Enterococcus faecalis* and *Viridans Streptococci* were the most common gram-positive pathogens, *Staphylococcus aureus (S. aureus)* accounted for 25% (5/20) of the gram-positive bacteria.[2] The emergence of gram-positive bacteria, including *S. aureus*, as significant pathogens in SBP in recent years has also been noted in other studies.[5] Different authors observed the increasing incidence of SBP caused by gram-positive bacteria in cirrhotic patients.[3] Angeloni et al[6] observed that an initial treatment with cefotaxime failed more frequently than expected. Their study supports the idea that the microbial etiology of SBP has changed in recent years.[6] Therefore, we performed a 14-year retrospective study to determine the change of microorganisms isolated from SBP cases at our hospital. Spontaneous bacterial peritonitis is a very serious complication in cirrhotic patients, empirical antibiotic therapy should be initiated before the results of ascitic fluid cultures are available and should cover the most commonly isolated microbial organisms. In recent years, quinolone prophylaxis in cirrhotic patients has been shown to decrease the incidence of SBP. Unfortunately, because of the increasing use of invasive procedures and quinolone prophylaxis, there have been studies indicating that the microbial etiology of SBP may have changed recently.[1] In particular, long-term administration of norfloxacin prophylaxis in cirrhotic patients is associated with the isolation of quinolone-resistant gram-negative bacteria from stool samples and the appearance of infections by these bacteria.[7] Moreover, invasive procedures and norfloxacin prophylaxis may promote carriage and bacterial infections of gram-positive bacteria.[1] Therefore, we studied the changes of bacteria isolated from our cirrhotic patients with SBP over a 14-year period.

Methods. All 780 SBP-diagnosed patients with decompensated cirrhosis and ascites admitted to Tangdu Hospital, Xian, Shaanxi Province, China between 1996 and 2009 were included in this retrospective study. We excluded patients with hepatocellular carcinoma, human immunodeficiency virus (HIV) infection, or heart failure. The SBP was diagnosed by an ascitic fluid polymorphonuclear (PMN) cell count of >250 cells/mm^3 and/or positive ascitic fluid cultures in the absence of clinical and laboratory evidence suggesting secondary peritonitis. Culture-positive SBP was defined as SBP with a positive ascitic fluid culture, meaning that a specific bacterium was isolated from at least 2 culture bottles. Finally, SBP was considered to be community-acquired when it was present at admission and considered nosocomial when it developed during hospitalization. A medical history, physical examination, laboratory tests, diagnostic paracentesis, and ascitic fluid cultures were performed according to our clinical practice in SBP patients. The data were collected in the hospital room, medical record library, and Clinical Laboratory Department. Medical records and laboratory data were reviewed. All pathogens were isolated from ascites of SBP patients in Tangdu Hospital, Xian, Shaanxi Province, China during the period January 1996 to December 2009. Ascitic fluid was collected by paracentesis, 10ml of ascitic fluid was inoculated into aerobic and anaerobic blood culture bottles for bacteriological examination at the patient's bedside using sterile techniques, and before the administration of antibiotics. Ascitic fluid cultures were performed using blood culture bottles (10ml, BD Company, New Jersey, USA). The samples were transported to the microbiology laboratory straight after. All ascitic fluid cultures were inoculated into blood plates at 35°C and were cultured for 18-24 hours; following this, the colonies (which were above +++) were Gram stained then pick single colony at last bacterial confirmatory was carried out by the Microscan WalkAway-40 system (American Dade Behring Company, American Dade Behring Company, New Castle,USA). The strains were determined according to the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1990).

Results were expressed as n (%) or mean values ± standard deviation. The statistical significance of the differences between the means of the experimental groups was tested by the 2-sample t-test. The differences in proportions were tested by the chi-square test. A difference was considered statistically significant when $p<0.05$. All data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) software version 12.0.

Results. Throughout the study, in 780 SBP-diagnosed patients with decompensated cirrhosis and ascites, SBP was diagnosed in 98 patients (male to female ratio was 3.1:1, patient's age was between 18-74 years) and ascitic fluid cultures were found to be positive for all of them. The patients were placed into 2 groups; group

A consisted of 48 cirrhotic patients hospitalized during the period from January 1996 through December 2002, and group B consisted of 50 patients hospitalized during the period from January 2003 through December 2009. No significant differences were found between the 2 groups regarding gender, age, or clinical situation. The clinical data were comparable between the groups. Gram-positive SBP was significantly more frequent in Group B than in Group A. Microorganisms isolated from the ascitic fluid of patients with SBP throughout the study (1996–2009) and from Groups A and B are shown in **Tables 1 & 2**. Clinical and laboratory data at the time of diagnosis were comparable for all groups. In Group A (48 patients), 73% of the SBP pathogens were gram-negative bacteria. The predominant bacteria were *E. coli* (46%), *Klebsiella oxytoca* (19%) and *Streptococcus* (14%) (**Table 1**).

In Group B (50 patients), 2 positive cultures (a strain of *Corynebacteria* and a strain of *Micrococcus luteus*) were considered contaminated. These 2 cases were excluded from the analysis. In the other 48 patients with cirrhosis, 53% of the pathogens were gram-positive bacteria, among which *Staphylococcus* was the major pathogen accounting for 37% of SBP cases, while *Streptococcus* accounted for 14% of the cases and methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated in 2% of cases (one isolate); 37% of the pathogens were gram-negative bacteria among which *Bacillus coli* accounted for 22% of the SBP cases. *Eumycetes* accounted for 6% of all pathogens, and combined infections accounted for 4% (**Table 2**). Of the 3 strains of *Eumycetes*, there was one strain of *Cryptococcus neoformans*, one strain of *Mycetes* and one strain of *Torulopsis glabrata*. Among 2 strains of combined infection, there was one strain of group D *Streptococcus* and one strain of *Enterococcus faecalis*. Gram-positive bacteria were significantly more frequently found to be the cause of SBP in patients from Group B than in those from Group A. There was a significant difference in the constituent ratio of pathogens between Group A and Group B (χ^2=9.630, p=0.002).

In Group B (during the period from January 2003 through December 2009), no significant differences in the age, gender, or Child-Pugh mean score were observed between patients with gram-positive bacteria and those with gram-negative bacteria. The SBP patients with gram-positive bacteria used significantly more antibiotics within 30 days compared with those with gram-negative bacteria (χ^2=12.285, p=0.000) (**Table 3**). In Group B, the occurrence of SBP was asymptomatic in 15 patients (31%). There was no statistically significant difference in flora between the symptomatic and asymptomatic patients. Compared to the 15 asymptomatic SBP patients, the 33 symptomatic SBP patients were found to have a higher Child-Pugh mean score (t=2.296, p=0.026). The in-hospital mortality for asymptomatic SBP was 13%, which is lower than the 21% for symptomatic SBP. Among patients with SBP, other clinical and laboratory data showed no significant difference between the symptomatic and asymptomatic groups.

Table 1 - Pathogens isolated from the ascitic fluid of cirrhotic patients with SBP from 1996-2002 (Group A).

Isolated pathogens	Number of strains	Constituent ratio (%)
Gram-negative bacteria		
All	35	73
Escherichia coli	22	46
Klebsiella oxytoca	9	19
Bacillus proteus	2	4
Other	2	4
Gram-positive bacteria		
All	13	27
Streptococci	7	14
Enterococci	4	8
Staphylococci	2	4
Eumycetes	0	0
Total	**48**	**100**

Table 2 - Pathogens isolated from the ascitic fluid of cirrhotic patients with SBP from 2003-2009 (Group B).

Isolated pathogens	Number of strains	Constituent ratio (%)
Gram-negative bacteria		
All	18	37
Bacillus coli	11	22
Klebsiella oxytoca	3	6
Other	4	8
Gram-positive bacteria		
All	26	53
Staphylococcus	18	37
Streptococcus	7	14
Bacillus cereus	1	2
Eumycetes	3	6
Combined infection	2	4
Total	**49**	**100**

Table 3 - Clinical characteristics of cirrhotic patients during 2003-2009 (Group B) with culture-positive spontaneous bacterial peritonitis (SBP); arranged by the microbial agent.

Patient characteristics	Gram-positive bacteria (N=26)	Gram-negative bacteria (N=18)	*P*-value
Mean age (years)	43.8±2.8	42.5±2.7	0.52
Gender, males (%)	22 (84)	14 (77)	0.56
Child-Pugh mean score	11.8±1.8	11.3±2.3	0.31
Prior antibiotic treatment within 30 days, n (%)	24 (92)	8 (44)	0.000
In-hospital mortality rate, n (%)	7 (26)	2 (11)	0.20

Discussion. Spontaneous bacterial peritonitis is a frequent complication of cirrhotic patients. Aerobic gram-negative bacteria that translocate from the intestinal lumen are considered responsible for most of SBP cases. Nevertheless, in recent years, the etiologies

for bacterial infections have undergone striking changes.[1] Cholongitas et al[3] reported their results from Athens, and found that cases of culture-positive SBP in cirrhotic patients have more frequently been caused by gram-positive bacteria in recent years. Moreover, the constituent ratio of pathogen greatly diversifies; this phenomenon is also confirmed by Campillo et al.[5] They observed that gram-positive pathogens were predominant among isolates from ascitic fluid cultures obtained from hospitalized cirrhotic patients with nosocomial SBP.[5] Our report also supports the view that gram-positive pathogens were predominant among ascites fluid samples from SBP patients.

Why did the constituent ratio of pathogens change greatly over the past 14 years in China? We propose 3 main possible reasons:

First, third-generation cephalosporins, especially cefotaxime was proposed as one of the 'gold standard' treatments. Current treatments use third-generation cephalosporins or oral quinolones.[8] Cefotaxime or other third-generation cephalosporins have been considered the first-choice empirical antibiotics in the treatment of cirrhotic patients with SBP.[9] In 1970, first-generation cephalosporins became available in China. First-generation cephalosporins were considered very effective in treating bacterial infections, especially against gram-positive bacteria.[10] However, these first-generation drugs were not effective against gram-negative bacteria.[10] Third-generation cephalosporins are very potent and have activity against gram-negative bacteria.[10] Of the antibiotics used for SBP treatment, cefotaxime is the best-studied and has excellent penetration into ascites without nephrotoxicity.[11] The incidence rate of SBP accompanied by gram-negative bacteria continues to decrease yearly. The clinical efficacy of third-generation cephalosporins against *Staphylococci* is considered to be worse than first- and second-generation.[10] *Staphylococcus* contributes to the majority of SBP cases caused by gram-positive bacteria. Indeed, the incidence rate of SBP with gram-positive bacteria has increased yearly. In our study, we found that SBP patients with gram-positive infections used significantly more antibiotics over a 30-day period than those patients with gram-negative infections. In short, the overuse of antibiotics over the past 20 years, especially the overuse of third-generation cephalosporins, may be causing the increasing frequency of gram-positive bacteria in SBP cases in China.

Second, broad-spectrum quinolones are currently used for oral treatment of uncomplicated SBP.[9] Norfloxacin is widely used to prevent SBP in cirrhosis in China. Norfloxacin is also generally used with cirrhotic patients to reduce the risk of gram-negative infections. Campillo et al[5] concluded that long-term administration of norfloxacin to cirrhotic patients reduces the risk of gram-negative infections, but increases the risk of severe hospital-acquired Staphylococcal infections. Our study confirms the validity of such an approach. Quinolone prophylaxis has been shown to reduce the recurrence of SBP and to improve the survival of these high-risk patients. However, there is a concern that the microbial causes of SBP may have changed in recent years with increasing involvement of quinolone-resistant gram-negative and gram-positive bacteria.[1] Such changes have also been observed in neutropenic cancer patients and have been attributed to long-term antibiotic therapy as primary or secondary prophylaxis to high-risk cirrhotic patients.[12]

Third, these epidemiological changes in the microbial causes of SBP have been associated with the increasing number of invasive procedures and hospitalization of cirrhotic patients in intensive care units, which promote the prevalence of gram-positive bacterial infections and increase the incidence of infections caused by these microbial strains (mainly MRSA).[13] Fernández et al[1] conducted a prospective study of all bacterial infections diagnosed in patients with cirrhosis between 1998 and 2000. They found that infections caused by gram-positive cocci had markedly increased in cirrhosis. This phenomenon may be related to the current high degree of instrumentation of cirrhotic patients. Ascitic fluid contamination, for example, from skin bacteria (mainly *Staphylococcus epidermidis [S. epidermidis]*), might be an alternative explanation. Nevertheless, all cultures were collected using a standard sterile technique, and strict criteria for positive cultures were used. In addition, *S. epidermidis*, as well as other potential gram-positive bacterial skin contaminants, were proportionally similar to gram-negative bacteria and those cases were excluded from the analysis. Moreover, SBP is often asymptomatic,[6] and no clinical criteria can be considered completely reliable for the diagnosis of SBP. Thus, SBP recognition requires ample use of diagnostic paracentesis. We think that it is advisable to carry out routine paracentesis in many cirrhotic patients with ascites.

Another result showed that the failure rate of cefotaxime therapy in the patients was very high (44%).[6] Song et al[13] proposed that ineffective initial therapies are responsible for the higher rate of treatment failure and mortality in SBP.[13] In these patients, because the isolated organisms were either intrinsically resistant to cefotaxime, capable of degrading expanded-spectrum cephalosporins or inherently resistant to cefotaxime, cefotaxime failed.

Although the use of antibiotics in the primary prophylaxis for SBP in patients with cirrhosis is controversial,[14] the extensive use of antibiotics in the management of cirrhotic patients still regularly occurs in China. The clinical efficacy of third-generation cephalosporins for gram-positive bacteria is worse

than first- and second-generation cephalosporins. The SBP patients with gram-positive bacteria had a higher mortality rate than those with gram-negative bacteria in our study. Campillo et al[5] observed that infections with *Staphylococci* were independently associated with a higher mortality rate.

Third-generation cephalosporins fail to resolve the infection in 7-17% of patients with SBP. In more than 40% of SBP patients cefotaxime failed.[6] *Enterococcus* infections are one cause of the failure of third-generation cephalosporin treatment of SBP because of their intrinsic resistance to cephalosporins. "The need to change this antibiotic treatment is higher than that reported in previous studies."[11,15]

In conclusion, our observations confirm that the frequency of gram-positive bacterial isolates from SBP patients has increased over the last 14 years. This phenomenon must be watched closely and taken into account when deciding on an appropriate treatment. When comparing our findings with other relevant studies, there has been an increase in the frequency of SBP caused by gram-positive bacteria in this study. The study has its limitations, the precise reason for this change needs further investigation. The recent change in its pathogens may have some important implications for the treatment of SBP, and a need for verifying the efficacy of current guidelines.[6] The changes of bacteria isolated from our cirrhotic patients should be taken into account. Before our results (which are studied from one geographical area) can be generalized, further investigation should be carried out in different areas.

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