Original article

Spontaneous bacterial peritonitis: risk factors and causative organisms

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Abstract

Objective: To determine the prevalent pathogens responsible for spontaneous bacterial peritonitis (SBP) and their sensitivity pattern, to test the efficiency of different culture techniques in microbial isolation, and to study the diagnostic predictors of such cases. **Methods**: One hundred eight SBP episodes from 92 adult patients were compared to 88 cirrhotic ascites patients cross-matched with age and sex without SBP. Ascitic fluid was subjected to cytological, biochemical examination and culture on both conventional and blood culture bottles at the bedside for bacterial identification and antimicrobial susceptibility testing. **Results**: The prevalence of SBP was 25.02 %. Logistic regression analysis revealed that; previous SBP episode, low ascitic fluid protein levels, high serum creatinine and low serum albumin levels were the independent significant predictors of SBP. About forty-five per cent of SBP episodes were detected by conventional culture compared to 73.15 % by modified technique with a significant difference. Gram-negative bacteria were the cause of SBP in 46 (58.23 %) culture positive episodes. *Escherichia coli* and *Staphylococcus aureus* were the most commonly detected organisms. Resistance to different antibiotics was high. **Conclusion**: Culture of ascitic fluid in blood culture bottles at bedside increases the sensitivity of SBP detection. There is a recent increase in Gram-positive pathogen with emergence of multidrug resistance. These recent changes may have an impact on guidelines for management and treatment of SBP in our locality.

Keywords: Spontaneous bacterial peritonitis; Paracentesis; Bedside inoculation; Blood bottle culture

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a serious and potentially life-threatening complication of cirrhotic ascites[1]. Today, even with intensive treatment, the in-hospital mortality is still between 10 % and 30 %. In patients who recovered from an episode of SBP, recurrence of SBP is common, estimated to be 43 % at 6 months and 69 % at one year[2-4].

SBP may be asymptomatic or present with insidious, non specific symptoms; in addition, by using conventional culture techniques, the ascetic fluid culture outcome is negative in up to 60 % of patients. As a rapid diagnosis and an early treatment have a crucial role, the empirical antibiotic treatment cannot therefore be delayed to the moment when the laboratory results are available[5]. Moreover, recent data have been suggested that the causative agents for SBP and antibiotic resistance rate vary according to the time and regions[6-8]. This study aims to determine the prevalent pathogens responsible for SBP in our locality and their sensitivity pattern, to test the

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efficiency of bedside inoculation culture in blood bottles technique versus conventional procedure in microbial isolation, and to study the predictive value of different clinical and laboratory variables in diagnosis of such patients.

MATERIALS AND METHODS

Population and methods

This study was conducted at Tropical Medicine Unit, Mansoura University Hospital, from August, 2006 to August, 2008, 432 diagnostic paracentesis were performed prospectively in 215 cirrhotic patients with ascites consecutively admitted to the hospital. One hundred—eight SBP episodes from 92 adult patients were compared to 88 cirrhotic ascites patients cross—matched with age and sex without SBP.

Diagnosis of cirrhosis was based on clinical, biochemical, radiological and/or histo—pathological data. Only subjects with serum—ascites albumin gradient (SAAG) > 1.1 g/dL were enrolled. A detailed medical history was taken from each patient and complete physical examination was performed. Exclusion criteria were recent previous antibiotic therapy, evidence of intra—abdominal source of infection, hepatocellular carcinoma, intra—abdominal malignancy, presence of urinary tract infection or data of organic renal failure (ultrasonographic evidence of obstructive uropathy or parenchymal renal disease or hematuria and/or proteinuria).

Routine investigations carried out in every case including haemogram, serum bilirubin, Alanine transaminase (ALT), Aspartate transaminase (AST), alkaline phosphatase, serum proteins with Albumin/Globulin ratio, serum creatinine, prothrombin time and urine analysis. Abdominal ultrasonography and chest X—ray were done to all cases.

Diagnostic paracentesis was performed at admission and during the hospital stay for treatment of SBP. Ascetic fluid was drown under all aseptic precautions; naked eye inspection of color and transparency (as first evidence of infection) of the aspirated samples inside the syringe were done (trying to read the mark on the syringe through the aspirated ascetic fluid) then 15 mL were sent to the laboratory for cytological and biochemical examination, 15 mL were cultured by conventional method, and another 15 mL was inoculated into blood culture bottles at the bedside in both aerobic and anaerobic media for bacterial identification and antimicrobial susceptibility testing. Diagnosis of classic SBP and its variants (mono—microbial non— neutrocytic bacter—ascitis (MNB) and culture—negative neutrocytic ascites (CNNA) was made depending on the polymorphnuclear count $\geqslant$ 250 cell/ mm^3, and/or monomicrobial growth in ascitic fluid culture without evidence of an intra—abdominal surgically treatable source of infection, and no recent use of antibiotics.

Bedside inoculation culture was done using BACTEC Standard/10 Aerobic and anaerobic blood culture bottles (Becton, Dickinson and Company Maryland, USA). Before inoculating, the bottle septum was swabed with alcohol. Fifteen mL of specimen was aseptically injected directly per vial. Inoculated vials were placed in the BACTEC fluorescent series instrument as soon as possible for incubation and monitoring. Vials entered into the instrument were automatically tested every ten minutes for the duration of the testing protocol period. Positive vials were determined by the BACTEC fluorescent series instrument and identified as such. Positive vials were subcultured and a Gram—stained slide prepared. Subcultures to selective media and direct antimicrobial susceptibility test were prepared from fluid in the BACTEC vials.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 11. Categorical variables were presented as number and percent. Chi squire or Fisher's exact test was used for comparison between groups, as appropriate. Quantitative variables were presented as mean and standard deviation and t—test was used for comparison between groups. Significant predictors in bivariate analysis were entered into logistic regression analysis model using forward Wald technique to detect the independent predictors of the outcome. $P \leqslant 0.05$ was considered to be statistically significant.

RESULTS

During the study period, a total of 431 diagnostic paracentesis were performed in 215 cirrhotic patients with ascites. One hundred—eight SBP episodes occurred in ninety—two adult cirrhotic were compared to 88 non SBP (NSBP) ascetic patients with ascites cross—matched for age and sex (103 men and 77 women) their age ranged from 38 to 59 years with mean of 48.9 $\pm$ 4.5 years hospitalized in tropical medicine unit,

Mansoura University Hospital. Demographic and clinical characteristics of these patients are shown in Table 1.

Table 1 Demographic, clinical, and biochemical characteristics of the patients.

	Ascetic (NSBP) (88)	Ascetic (SBP) patients(92) episode(108)	Test of significance
Sex M F	51(57.95%) 37(42.05%)	52(56.52%) 40(43.48%)	$X^2=0.97$ $P=0.3$
Previous episodes	9(10.23%)	36(33.33%)	$\chi^2=14.6$ $P=0.000$
Prev. Paracentesis	19(21.59%)	47(43.51%)	$\chi^2=10.4$ $P=0.001$
Abdominal cellulitis	4(4.55%)	18(16.67%)	$\chi^2=7.2$ $P=0.010$
Ascitic flu. echoes	5(5.68%)	18(16.67%)	$\chi^2=4.6$ $P=0.031$
Turbid ascites	3(3.41%)	14(12.96)	$\chi^2=4.5$ $P=0.035$
Ascitic flu. protien	479±181.9	406±197.7	$t=2.6$ $P=0.009$
S. creatinine	1.5±0.4	1.7±0.8	$t=3.0$ $P=0.003$
S. albumin	2.2±0.3	2.3±0.4	$t=2.2$ $P=0.030$

At time of admission SBP was asymptomatic in 20 out of 108 episodes (18.52 %), fever was present in 28 cases (25.93 %), chills in 8 (7.41 %), abdominal wall redness and edema mainly in the infra-umbilical region in 18 (16.67 %) Figure 1. Past history of the presence of risk factors for SBP was detected in 47 episodes (43.52 %) as history of paracentesis, history of previous SBP episodes in 36 (33.33 %), history of rupture umbilical hernia in 11 (10.19 %), and previous attacks of haematemesis and/or melena or even endoscopic band ligation or injection sclerotherapy in 16 (14.81%). There was a significant difference between NSBP and SBP groups as regard abdominal wall edema and redness (cellulites), ($P=0.01$), as well as history of previous paracentesis and history of previous SBP episodes ($P=0.001$). Ultrasonographic examination of the patients revealed that, 18 out of 108 SBP episodes (16.67 %) had a characteristic marked ascites with numerous fine hyper-echoic internal particulates Figure 2.

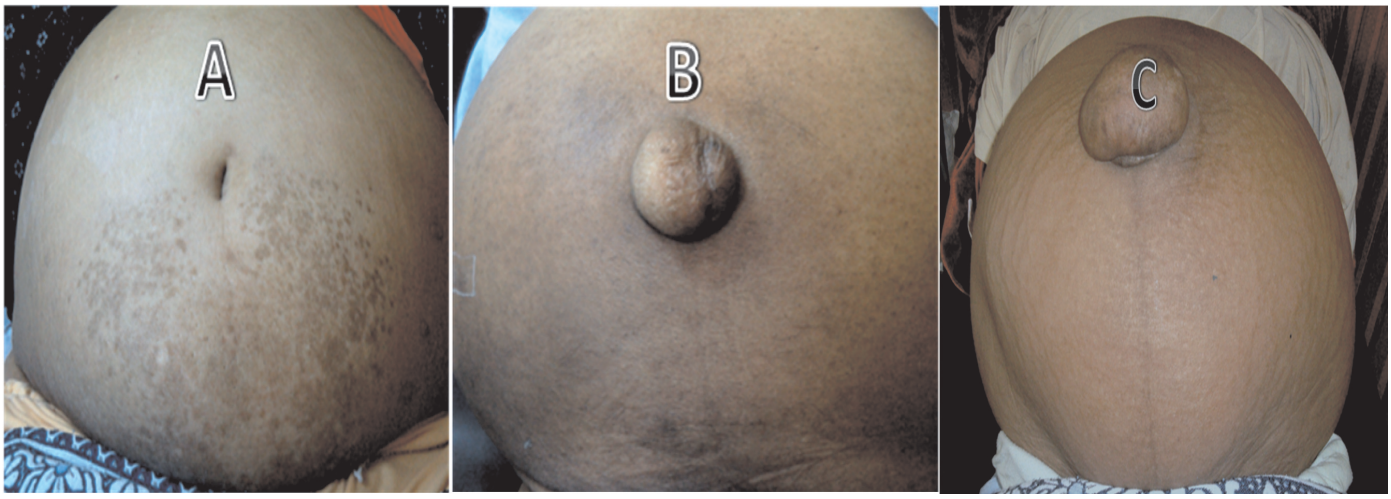

Figure 1 Abdominal wall redness and edema in patients with SBP. A: Female patient with recent onset ascites; B: Male patient with history of paracentesis; C: Female patient with history of perforated ulcer on top of umbilical hernia 2 months ago.

Inspection of color and transparency of the aspirated ascetic fluid revealed that 14 out of 108 SBP episodes (12.96 %) had slightly turbid ascites when inspected inside the syringe by naked eye (trying to read the mark on the syringe through the aspirated ascetic fluid).

Comparing the biochemical parameters between the NSBP group and SBP, there were a significant difference as regard ascitic fluid protein levels ($P=0.009$), serum albumin levels ($P=0.03$) and serum creatinine levels ($P=0.003$) (Table 1). There was no significant difference between the two groups regarding total leukocytic count, heamoglobin level, ascitic fluid glucose

levels, serum bilirubin or prothrombin time. Using logistic regression analysis; previous SBP episode, low ascetic fluid protein levels, high serum creatinine, and low serum albumin levels, all had a significant prediction of SBP ($P=0.000$) (Table 2).

Table 2 Logistic regression analysis of significant predictors of SBP ascetic patients and SBP positive culture.

		β	*P*	OR (95% CI)
SBP ascetic patients	Previous SBP episode	1.2	0.007	3.2 (1.4−7.6)
	Ascitic fluid protein	−0.002	0.030	0.93 (0.9−0.99)
	Creatinine	0.8	0.010	2.1 (1.2−3.8)
	Serum albumin	1.7	0.001	5.3 (2.0−13.9)
	Constant	−4.1		
	Model χ^2	35.9	$P=0.000$	
	Percent predicted	67.3		
SBP positive culture	Ascitic fluid protein	−0.004	0.001	0.996(0.994−0.998)
	Constant	5.5		
	Model χ^2	35.2	$P=0.000$	
	Percent predicted	79.6		

r=reference group, OR = odds ratio, CI = confidence interval

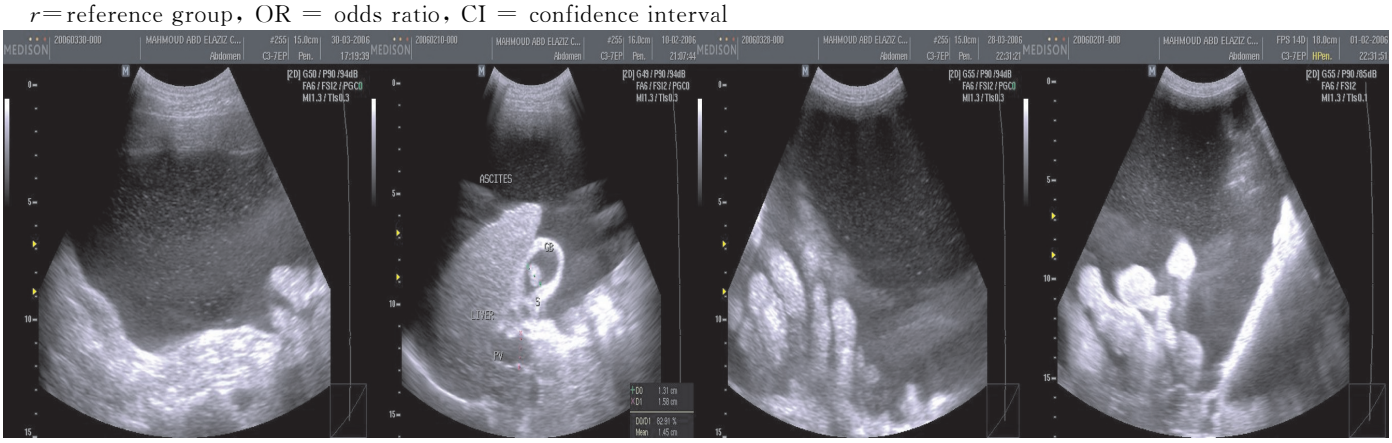

Figure 2 Abdominal ultrasonography showing ascetic fluid with numerous fine hyper-echoic internal particulates in patients with SBP

Forty-nine (45.37 %) episodes of SBP were detected by the conventional culture compared to 79 (73.15 %) by modified technique with significant difference ($\chi^2=17.259$, $P<0.001$). Twenty-nine (26.85 %) cases were culture negative neutrocytic ascites (CNNA) according to bedside blood culture result compared to fifty-nine (54.63 %) dected by conventional cuture with significant difference($\chi^2=17.259$, $P<0.001$). Thirty-nine (36.11 %) of them were culture positive with ascitic fluid total leukocytic count ⩾ 250 cells/ mm^3 (classic SBP), 40 cases (37.04%) were monomicrobial non-neutrocytic bacterascites (MNB), and 29 cases (26.85%) were culture negative neutrocytic ascites. Comparing the clinical and biochemical data of both culture positive and culture negative patients, there was no significant difference except for low ascetic fluid protein levels and high serum creatinine in patients with positive culture. Low ascetic fluid protein levels was the only significant predictor (more than 5 fold increase risk to develop positive culture SBP; $P=0.000$) (Table 2).

Gram-negative bacteria were the cause of SBP in 46 (58.23 %) episodes while Gram-positive bacteria were the isolated organisms in 33 cases (41.77 %). *Escherichia coli* and *Staphylococcus aureus* were the most commonly detected organisms in 40 (50.63 %) and 26 (32.91 %) cases respectively (Table 3). However there was no significant difference regarding clinical or biochemical data between SBP episode due to Gram-negative or Gram-positive micro-organisms.

The present study revealed that, 31.65 % of cultures were sensitive to levofloxacin, 29.11 % were sensitive to Cefotaxime, 20.25 % were sensitive to amoxicillin-clavulanic acid, 18.99 % were sensitive to Meropenem, 17.72 % were

sensitive to ciprofloxacin, and 15.19 % were sensitive to ceftazidime. On the other hand, antibiotic resistant rates to ciprofloxacin were 25.32 %, 24.05 % to ceftazidime and 21.52 % to cefotaxime.

Table 3 Frequency of isolated organisms in ascetic fluid cultures of 79 specimens with positive culture.

Category	Organism	Number of positive cultures	Percent (%)
Gram-ve organisms (46, 58.23%)	*E. coli*	40	50.63
	K. pneumonia	4	5.06
	K. oxytocca	1	1.27
	P. mirabilis	1	1.27
Gram +ve organisms (33, 41.77%)	*Staph aureus*	26	32.91
	Diplococci	3	3.80

DISCUSSION

Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication of cirrhotic patients with ascites, and continues to be an important source of morbidity and mortality[9]. The prevalence of SBP in unselected, hospitalized, cirrhotic patients with ascites has been reported to range between 10 % and 30 %[10]. This figure decrease to only 3.6 % in patients with uncomplicated ascites (patients admitted only for treatment of ascites with paracentesis) [11]. In this study, the prevalence of SBP was 25.06 %. This high prevalence may be due to presence of many risk factors for developing of SBP as 33.33 % of patients had a history of previous episode. Previous studies revealed that, the cumulative recurrence rate of SBP at 6 month is 43 % and within one year of follow up is approximately 70 % and even those recovering from an SBP episode should be considered potential candidates for liver transplantation as the survival expectancy is very poor[11,12].

Another 58.33 % had history of invasive maneuvers; paracentesis in 43.52 % , rapture umbilical hernia in 10.19 % and gastrointestinal bleeding with injection sclerotherapy or banding in 14.81 %. Cirrhotic patients with gastrointestinal bleeding are at higher risk for SBP. This has been attributed to temporary impairment in reticulo-endothelial system function, to breach of the mucous membranes that usually function as barriers to bacterial entrance to the body, and also to endoscopic procedures in bleeding patients[13,14]. This risk increase if associated with hemorrhagic shock due to change in mucosal permeability.

Symptoms and signs of SBP may be insidious or non specific. In addition, clinical presentation of SBP depends on the stage at which the infection is diagnosed. In the early stages, most patients are asymptomatic. As the disease progresses, patients show signs and symptoms of peritoneal infection[2].

In this study, fever and abdominal pain present in 25.93 %, 30.56 % of SBP patients. There was no significant difference between them and those without SBP. On the other hand, the frequency of abdominal wall cellulites was significantly higher in SBP patients ($P = 0.01$). Infection is frequent in cirrhotic patients, and this may be attributed to qualitative neutrophile abnormalities, low complement serum levels and reticulo-endothelial system suppression[15], moreover, 35 % of our patients had refractory ascites and anterior wall edema which facilitate abdominal wall infections.

Ultrasonographic examination of the patients revealed that, 16.67 % of SBP patients had a characteristic marked ascites with numerous fine hyper-echoic internal particulates. Komatsuda et al[16], reported that, the presence or absence of echo spots within the ascites helped differentiate transudate from exudate ascites.

A low concentration of ascetic fluid protein is associated with an increased risk of SBP in cirrhotic patients with ascites[17]. Many studies of cirrhotic patients with ascites found variables associated with an increased risk of SBP, but only an ascetic fluid protein concentration less than 1 g/dL showed an independent predictive value[2], and this agree with our finding as there was significant decrease in total ascetic protein levels in SBP patients than that of non SBP ($P=0.003$). However, this association may be attributed to decreased the endogenous antimicrobial activity (opsonic activity) of human ascetic fluid that has been shown to correlate directly with the protein concentration of the ascetic fluid and patients with deficient opsonic activity in the ascetic fluid have been shown to be predisposed to SBP[18,19].

Seventy five percent of SBP occurs in Child-Pugh class C, and this may explains the significant lower serum albumin levels in SBP patients of this study than non SBP one ($P=0.03$), and supports the view that SBP is more common in patients with advanced liver disease[17].

On admission, serum creatinine levels in SBP

patients were significantly higher than non SBP group. However, patients with SBP develop severe impairment of circulatory function leading to multi-organ failure[11], and can trigger a number of complication such as hepatic encephalopathy and renal failure, and the latter is probably the strongest independent predictor of death in a study by Follo et al[20], on 252 consecutive episodes of SBP, the mortality rate was 100 % when associated with progressive renal impairment, 31 % when associated with steady renal impairment, and only 7 % in those without renal impairment.

Fourty-nine (45.37 %) episodes of SBP were detected by the conventional culture compared to 79 (73.15 %) by modified technique with a significant P value <0.001. These results are near to that reported by[21] who had got 93 % positivity by the modified technique versus 57 % by the conventional technique. Bobadilla et al[22], had reported a culture positivity of 81 % by the modified technique compared to 52 % by the conventional technique, while Agarwal et al[23], reported 35.7 % cases of SBP detected by the conventional culture compared to 78.5 % by the modified technique of culturing ascetic fluid in blood culture bottles. In patients with SBP, studies based on quantitative cultures of ascetic fluid have shown a median bacterial concentration of one organism (one bacterium) per milliliter of ascetic fluid[24]. This low concentration explains the low sensitivity of conventional culture techniques for detection of the responsible microorganism, and it explains the low sensitivity of Gram-staining for SBP (approximately 10 %), while the modified technique has a greater probability of detecting microorganisms as it treats ascetic fluid as if it was blood, and hence, can detect a very low concentration of microorganisms[23].

Bacteria isolated from the ascetic fluid in patients with SBP are usually those of the normal intestinal flora with aerobic Gram-negative bacilli being responsible for more than 60 % of all cases. *Escherichia coli* accounts for nearly half of these cases, followed by *Klebsiella* species and other gram negative bacteria[15,25]. In early study by Wilcox and Dismukes[26], *Escherichia coli* was responsible for 45% of cases and *Staphylococcus aureus* for 12 % of cases while Runyon[27] demonstrated *Escherichia coli* in 27.3 % of cases of SBP and *Staphylococcus aureus* in 6.8 %. Increased frequency of the SBP episodes due to Gram-positive bacteria has been ascertained by many recent studies[6,8,28,29]. This study confirms the results of these studies in our locality as Gram-negative bacteria were the cause of SBP in 46 (58.23 %) episodes while Gram-positive bacteria were the isolated organisms in 33 cases (41.77%). *Escherichia coli* and *Staphylococcus aureus* were the most commonly detected organisms in 40 (50.63%) and 26 (32.91 %) cases respectively.

In addition to these data, the higher incidence of *Staphylococcus aureus* in our study may be attributed to the past history of many invasive maneuvers; injection scelero-therapy, band ligation and paracentesis which favor infection with Gram positive organisms. However, patients with low ascetic fluid protein levels had more than 5 fold increased risk to develop SBP episodes with positive cultures on regression analysis.

The high rates of microbial resistance are in accordance with many studies that document an increase in the frequency of multiple antibiotic resistant bacteria. Singh et al[7], detected increased frequency to multiple antibiotic resistant from 8.3 % to 38.5 % , while Park and his colleague[8] observed increasing antibiotic resistance rates to cefotaxime from 5.4 % to 14.8 % , and to ciprofloxacin from 4.3 % to 28.4 %, with isolation of methicillin-resistant *staphylococcus* in 28 % and vancomycin-resistant *Enterococci* in 31 % of resistant cases. This may be due to the extensive use of quinilones, and in particular, to the employment of norfloxacin for SBP prophylaxis and the routine use of cefotaxime as an initial empirical therapy. This empirical wide use of cefotaxime leads to 44 % treatment failure rates of the initial therapy in a study by Angeloni et al[30] and his colleagues who isolated a resistant stains capable of degrading the expanded-spectrum cephalosporins as extended spectrum beta lactamase producing *Escherichia coli* (ESPL-positive *E coli*), *Enterobacter* and *enterococcus*.

In conclusion, SBP was and still a frequent serious complication of cirrhotic ascites. Previous SBP episode, low ascitic fluid protein levels, high serum creatinine and low serum albumin levels are significant predictors of SBP. Beside cytological and biochemical examination, culture of ascitic fluid in blood culture bottles at bedside increases the sensitivity of SBP detection and must be a routine in every hospitalized patient with cirrhotic ascites. Gram-negative organisms still the prevalent microorganisms causing SBP

but there is a significant recent increase in Gram-positive pathogen with emergence of multidrug resistance especially for ciprofloxacin, Ceftazidime and cefotaxime. These recent changes may have an impact on guidelines for management and treatment of SBP in our locality.

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