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Evaluation of the Role of Culture, PCR, and Sequencing in the Diagnosis of Spontaneous Bacterial Peritonitis in Patients with Ascites

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A B S T R A C T

Spontaneous bacterial peritonitis (SBP) means the development of peritonitis (infection in the abdominal cavity) despite the absence of an obvious source of infection. It occurs almost exclusively in people with portal hypertension, usually as a result of liver cirrhosis, our aim of this study was detection of bacterial infections in SBP by conventional method and molecular biology. (19.5%) ascitic fluid (AF) samples were culture positive. PCR results show that bactDNA was detected in 56 samples (28%), 18 samples were sequenced for molecular identification of the amplified bactDNA fragments. 10 out of 18 were undetermined, while 6 were *E. coli* and 2 were *Streptococci*. Our conclusion is that the detection of bacterial DNA in the ascitic fluid provides an important role in diagnosis of SBP.

Introduction

Definition of Spontaneous bacterial peritonitis (SBP) is the development of peritonitis regardless of the absence of a source of infection that occurs mostly in patients with portal hypertension, as a result of liver cirrhosis (Lata *et al.*, 2009). Spontaneous bacterial peritonitis is defined by the peritoneal inflammatory response and ascitic fluid neutrophil count ≥250/mm3 is currently considered the cutoff point for SBP diagnosis (Runyon, 2009).

Ascitic fluid infection can classify into five types according to polymorphonuclear cell count, ascitic fluid culture results and clinical circumstances: classic culture positive SBP, culture negative SBP, also known as culture negative neutrocytic ascetic (CNNA), monomicrobial and polymicrobial buster-associates, and secondary peritonitis (Rimola *et al.*, 2000 and Runyon, 2009).

SBP is the most frequent bacterial infection in cirrhosis and represented as 10–30% of all recorded bacterial infections in hospitalized patients (Fernández *et al.*, 2003).

The incidence of SBP is 12% in hospitalized patients (Cadranel *et al.*, 2013 and Singal *et al.*, 2014), while 3.5% in outpatient (Evans *et al.*, 2003). In spite the great efforts have done for treatment of these patients, the morbidity

and mortality remain high (Runyon, 2009 and Saab *et al.,* 2009)

Bacterial translocation (BT) is one of a most common cause of SBP. However, particularly in hospital acquired SBP, other sources, as transient bacteremia due to invasive procedures, can cause SBP (Wiest, and Garcia- Tsao, 2005). Gram-negative *Entero-bacteriaceae* combines the major causative organisms of SBP (Runyon, 2009 and Liver, 2010).

The risks factors that lead to change the bacterial spectrum and resistance pattern of causative organisms that lead to an increased incidence of SBP due to Gram positive and third-generation cephalosporin TGC-resistant bacteria includes hospitalization, increasing invasive procedures and antibiotic prophylaxis in cirrhotic patients (Runyon, 2009 and Biecker, 2011)

Firstly, the mortality exceeded 90% but it can reduce to 20% with accurate diagnosis and treatment (Tandon, and Garcia-Tsao, 2008, Khan *et al.,* 2009 and Saud and Baltodano, 2010). Although, the elevation of PMN count and the cultures of the ascitic fluid are con-sidered the golden standard for the diagnosis of SBP, it has some disadvantages. First, the results of the ascitic fluid culture are not rapidly available so delaying the diagnosis and treatment of infection (Runyon *et al.,* 2013).

Second, the most frequent variants of ascitic fluid infection is culture-negative neutrocytic ascites, which occurs in approximately 30% to 50% of patients because the ascitic fluid culture is not positive in all patients with ascitic fluid neutrophil count $\geq 250/mm^3$ (Runyon, 2009) and also called 'culture-negative SBP' in which bacteria are present in a low concentration and therefore could not diagnosed by microbiological culture methods (Rimola *et al.,* 2000).

This fact was supported by (Moore and Aithal 2006), the authors were found that bedside inoculation of about 20 mL of ascitic fluid into blood culture bottles increases culture positivity to approximately 70–90% while conventional cultures were 30–40%.

So, the growth of bacteria in the ascites culture does not establish the diagnosis of SBP, since bacteria are detected only in about 40% of SBP cases (Gines *et al.,* 2010 and Tandon *et al.,* 2013).

So great efforts were established for improvement the sensitivity of microbiological ascites analysis.

Overall, detection of bacteria in the ascites by PCR-based methods failed to improve test accuracy (Soriano *et al.,* 2011 and Jalan *et al.,* 2013).

Detection of bacterial DNA (bactDNA) using various approaches has been proposed in the ascitic fluid of patients with cirrhosis (Francés *et al.,* 2008 and Bruns *et al.,* 2009).

The advantage of such a system would be helping the immediate detection of the causative bacteria, so allow more rapid antibiotic treatment. BactDNA is found in the ascitic fluid of about 40% of patients with cirrhosis and mainly were Gram negative bacteria (Bruns *et al.,* 2009). But, detection of bact DNA in ascites or serum was not associated with an enhanced incidence of SBP and has no role in prediction of the development of bacterial infections (Zapater *et al.,* 2008).

The aim of our study is to detect the bacterial infection in the ascetic fluid samples by conventional method and detection of bacterial DNA by PCR and sequencing.

Materials and Methods

Samples

A cross-sectional study was carried out in the Microbiology and Immunology Department, Faculty of Medicine, Minia University, during the period from June 2014 to January 2016,

200 ascitic fluid samples were collected from patients admitted to the Tropical Medicine Department, Minia University Hospital with ascites on top of liver cirrhosis diagnosed both clinically and ultrasonographic.

Exclusion criteria included the presence of neoplastic ascites, any other cause of increased ascitic fluid neutrophil count (i.e. bloody ascites, pancreatitis, and tuberculosis), and antibiotic intake within the preceding 2 weeks, including selective intestinal decontamination with norfloxacin. Written informed consent was obtained from all patients.

Each patient history was recorded with the help of the patient himself and the resident doctors, including; age, gender, residence, total bilirubin, direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), albumin, urea, creatinine, blood total leukocytic count (TLC), and polymorphonuclear leukocytes in ascetic fluid (PMN).

Paracentesis was carried out for all patients at bedside in sterile conditions, Blood culture bottle were each inoculated with 5 ml of ascitic fluid at the patient's bedside aseptically using standard precautions of infection control to ensure that a sterile sample was collected before delivery to the microbiology laboratory, incubated at 37º C for 7 days, and examined daily for turbidity. Subcultures, both aerobically and anaerobically, were done on blood agar and MacConkey's agar plates. Positive cultures were identified by Gram's staining and microscopic examination then performing standard biochemical reactions.

An ascitic fluid aliquot was stored at -80º C until DNA extraction was performed.

Detection of 16s rRNA gene by PCR

DNA was extracted using Zymo-research Quick-gDNA MiniPrep (D3006) DNA extraction kits (Zymo research, USA) according to manufacturer's instructions.

PCR conditions

The primers used for amplification of the targeted gene:

Forward primer sequence: 5' AGAGTTT GATCATGGCTCAG-3' Reverse primer sequence: 3' ACCGCGACTGCTGCTGGC AC-5'

The primers located at positions 7-27 531-514 (*Escherichia coli* numbering).

PCR conditions for the *16s rRNA* gene comprised 35-cycle PCR was run using the following the amplification protocol profile:

95 °C for 5 min followed by 40 cycles composed of 94°C for 45 seconds, 55 °C for 45 seconds and 72 °C for 60 seconds and a final extension step at 72 °C for 10 min.

Agarose gel electrophoresis

Samples (10µL) were loaded in 2% agarose gel, stained with ethidium bromide. In addition, one lane was used for a DNA size marker (10 µL). The gel was then electrophoresed for 30 min at 100 volts. The DNA was then visualized under a UV transilluminator (Biometra Goettingen, Germany) and an image was recorded.

DNA sequencing

PCR products (for the samples that were culture negative); were sequenced in both directions using the universal 27f and 519r primers by Macrogen Inc. (Seoul, Korea). Each sequence was compared with GenBank sequences by using the basic local alignment scratch tool (BLAST) algorithm.

Statistical analysis

Statistical analysis was performed by the statistical Package for the Social Sciences software (SPSS) version 11.0. Comparisons between groups were done using Chi-square test for qualitative variables and independent sample t-test for normally distributed quantitative variables, while non-parametrical Mann–Whitney test was used for quantitative variables with no normal distribution.

Results and Discussion

200 patients with ascites on top of liver cirrhosis were included in the study; Cirrhosis was diagnosed based on clinical and ultrasonographic findings. 39 (19.5%) ascitic fluid (AF) samples were culture positive with a single organism, 36 of them were classical spontaneous bacterial peritonitis (c SBP) as the PMN count in the AF was more than 250 cell/mm^3, and the remaining three were mono-bacterascites (MNBA) as the PMN count was less than 250 cell/mm^3, 161 (80.5%) were culture negative, with 8 were culture negative neutrocytic ascites (CNNA) as the PMN count in the AF was more than 250 cell/mm^3, the others were culture negative non-neutrocytic ascites (CNNNA) as the PMN count was less than 250 cell/mm^3.

Table 1 shows the difference in the characteristics of the patients in the 2 groups (culture positive and culture negative) as shown in table 1.

The bacteriological profile for the bacteria in the culture positive group was as following as shown in table 2 and in figure 1.

Gram negative bacteria in 20 (51.3%) ascitic fluid samples, mainly *E coli* (30.8%), then *Klebiella* (12.8%), *Enterobacter* (5.1%), and *Citrobacter* (2.6%) while the Gram positive bacteria was found in 19 (48.7%) ascitic fluid samples, mainly *Enterococcus* (20.5%), followed by *Staph. epidermidis* (17.9%), then *Staph. aureus* (5.1%), and *Strept. pneumoniae* (5.1%).

PCR results show that bactDNA was detected in 56 samples (28%), regarding the culture positive samples, bactDNA was detected in 38 out of 39 AF samples as one AF sample was culture positive (*Staph. epidermidis*) but also was repetitively negative for PCR (it was repeated twice) as shown in figure 2. 18 AF samples were culture negative but bactDNA could be detected by the PCR. Those 18 samples were sequenced for molecular identification of the amplified bactDNA fragments.

Results of sequencing as in table 3, 10 out of 18 were undetermined, while 6 were *E. coli* and 2 were *Streptococci*.

Sensitivity, specificity, PPV, NPV and total accuracy of ascitic fluid culture considering PCR of bactDNA in the ascitic fluid as the gold standard shown in table 4 and figure 3.

In the present study, the prevalence of SBP is found to be 23.5 %. In Sagar *et al.*, prevalence of Spontaneous Bacterial Peritonitis was found to be 18% (Sagar, 2016). In Sheikhbahaei *et al.*, (2014), SBP was found in 24.33% of samples, in an Iranian study by Saqib *et al.*, SBP prevalence was 31% (Saqib *et al.*, 2012), our results also correlate with El-Bendary *et al.*, as the frequency of SBP was 25%.

Table.1 The characteristics of the patients of positive and negative culture

	Culture		P value
	-Ve (n=161)	**+Ve (n=39)**	
Age Range Mean ± SD	 (42-78) 58.2±8.6	 (34-79) 55.1±10	0.052
Gender Male Female	 103(64%) 58(36%)	 24(61.5%) 15(38.5%)	0.777
Residence Rural Urban	 121(75.2%) 40(24.8%)	 25(64.1%) 14(35.9%)	0.163
Diabetic -Ve +Ve	 89(61%) 57(39%)	 23(60.5%) 15(39.5%)	0.961
HB Range Mean ± SD	 (6.3-15) 9.5±1.9	 (6-12) 8.7±1.5	**0.017***
WBC ($x10^3$) Range Mean ± SD	 (3.2-56) 17.3±7.9	 (3.8-44.3) 18.5±9	0.415
Platelets ($x10^3$) Range Mean ± SD	 (16-173) 84.2±34.1	 (31-354) 105.5±56.8	**0.003***
($)Total bilirubin Range Mean ± SD	 (0.6-10.3) 3.1±2	 (0.4-23.5) 3.9±5	0.921
($)Direct bilirubin Range Mean ± SD	 (0.2-7.6) 1.8±1.4	 (0.2-19.5) 2.4±4.1	0.792
Albumin Range Mean ± SD	 (0.8-28) 2.3±0.5	 (0.9-28) 2.4±0.5	0.432
($)ALT Range Mean ± SD	 (12-496.4) 83.8±72.3	 (16-324) 77.9±73.6	0.349
($)AST Range Mean ± SD	 (1.2-1554) 148.2±192.6	 (23.3-700) 139.9±129.3	0.155
($)Urea Range Mean ± SD	 (3-225) 75±53.4	 (26-244) 99.7±62.1	**0.016***
($)Creatinine Range Mean ± SD	 (0.3-7.6) 1.6±1.3	 (0.5-6) 1.7±1.3	0.283

- Independent sample t test for parametric quantitative data between the two groups
- ($)Mann Whitney test for non-parametric quantitative data between the two groups
- *: Significant difference at p value < 0.05

Table.2 The bacteriological profile in culture positive AF samples

	Descriptive statistics (n=39)
Culture	
E coli	12(30.8%)
Klebsiella	5(12.8%)
Citrobacter	1(2.6%)
Enterobacter	2(5.1%)
Enterococcus	8(20.5%)
Staph. epidermidis	7(17.9%)
Staph. aureus	2(5.1%)
Strept. pneumoniae	2(5.1%)

Table.3 The results of Ascitic fluid culture, PCR and sequencing

No. of cases (200)	culture	PCR	Sequencing
38	**positive**	**Positive**	**Not done**
1	**positive**	**negative**	**Not done**
10	**negative**	**Positive**	**Undetermined**
6	**negative**	**Positive**	***E coli***
2	**negative**	**Positive**	***Streptococcus***
143	**Negative**	**negative**	**Not done**

The phi coefficient of correlation between ascitic fluid culture and PCR was 0.491, i.e. moderate correlation (p-value =0.001).

Table.4 The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of culture (PCR as a gold standard)

	Sensitivity	Specificity	PPV	NPV	Accuracy
Culture	**66.67**	**99.3**	**97.4**	**88.2**	**90**

	AUC	Std. error	P value	95% CI
Culture	**0.83**	**0.03**	**<0.001***	**0.77 - 0.88**

Fig.1 Graph shows the bacteriological profile

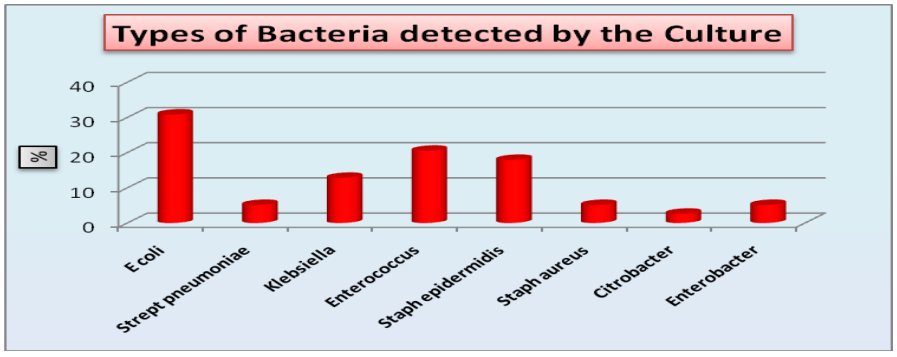
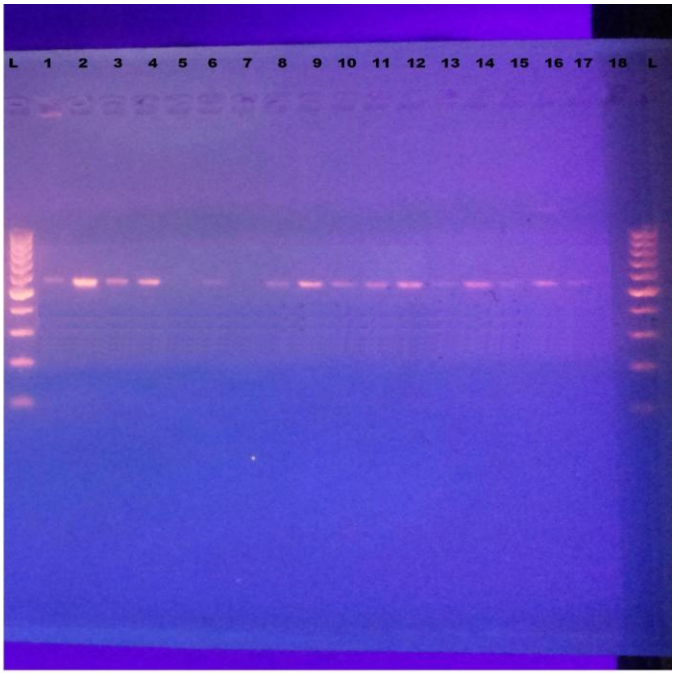
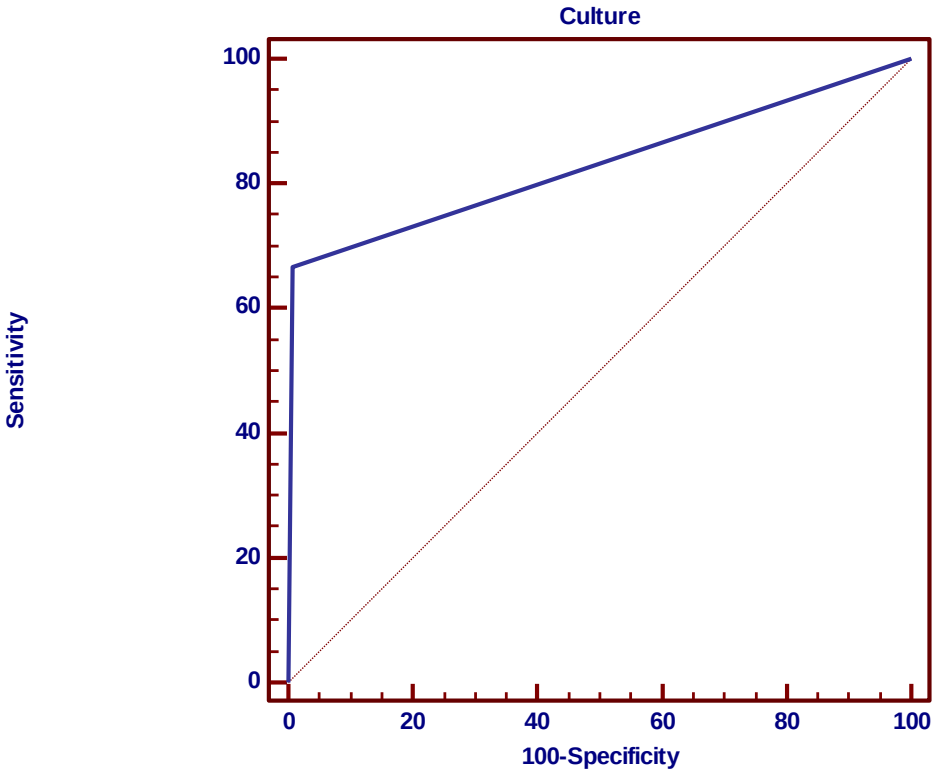


Fig.2 PCR products on the gel



All samples were positive except no. (5, 7, 18) that were negative.

Fig.3 ROC curve analysis of culture (PCR as a gold standard)



In this study, the SBP cases (47 patients) were divided to three main variants: 76.9% were classic SBP, 8 (17%) were CNNA and 2 (6.1%) were MNBA. These result was agreed with Doddamani *et al.,* that observed classic SBP cases were 66.67%, CNNA 25% and MNBA 8.3% of cases (Doddamani *et al.,* 2010).while in Bibi *et al.,* it was reported that CNNA was the most common variant (52.6%) in meanwhile the classic SBP was21% and MNBA was 26.3%. this difference may be due to having different populations with different severity of the disease and host factors (Bibi *et al.,* 2015).

Bacterial translocation is one of the main causes of SBP development so Gram negative bacteria from the *Enterobacteriaceae* family were the predominant causative agents (Conn, 2000). In spite of intestinal bacteria had been suggested to translocate into the mesenteric lymph nodes, more than 70 different microbial species have been isolated in ascitic fluid from patients with SBP (Bibi *et al.,* 2015).

In the present study, all positive ascitic fluid cultures revealed the growth of a single organism with the slight predominance of Gram negative over Gram positive bacteria (51.3% vs. 48.7%, respectively). in Bibi *et al.,*(2015), the Gram negative bacilli were isolated from 85% of AF samples, in Sheikhbahaei *et al.,* Gram negative bacteria were detected in 62.9% of the AF samples while the Gram positive were only in 28.8% of the samples. In Oladimeji *et al.,* (2013) Gram negative bacteria were responsible for 66.7% of the cases and Gram positive in 33.3%. In El-Bendary *et al.,* (2013), gram negative bacteria were detected in 58.2% of culture positive samples while Gram positive were detected in 41.8%.

Molecular techniques can increase the rates and velocity of bacterial identification in comparison with microbiological cultures (Andrade *et al.,* 2008). In the present study, bactDNA was detected in ascitic fluid in 56 samples out of 200 (28%) from patients with liver cirrhosis and ascites, 38 of them only had culture positive ascitic fluid, BactDNA was detected in 18 culture negative ascitic fluid samples.

BactDNA was not detected in one culture positive ascitic fluid sample. Possible explanations considered: (a) low concentration of the bactDNA in the AF sample that could not be detected by PCR, (b) contamination of the culture after collecting the sample or (c) Technical difficulties in disrupting the cell wall of these organisms may result in failure in DNA extraction (Vieira *et al.,* 2007 and Soriano *et al.,* 2011).

Sequencing of culture negative samples with PCR positive for 16s rRNA gene could detect the organism in 8 out of 18 AF sample (45%) and the results was undetermined in 10 out of 18 (55%) samples, the possible explanation was the following possibilities: (a) suboptimal sequencing reaction due to a low initial DNA concentration, or (b) a mix of amplification products corresponding to different bacterial species as a consequence of previous polybacterial translocations leading to the presence of polybacterial DNA (with or without viable bacteria) in ascitic fluid (Soriano *et al.,* 2011).

By considering that PCR of bactDNA in ascitic fluid as the gold standard, the culture of AF samples using the blood culture technique shows sensitivity 66.67 and specificity 99.3 with total accuracy 90%. In Esparcia *et al.,* culture technique shows sensitivity 80.6 and specificity 100 with total accuracy 89% (Esparcia *et al.,* 2001).

The using of 16S rRNA gene-based methods in detection of bactDNA suggested an

important role of molecular evidence of bacterial translocation (BT) in patients with CNNNA (Fam N *et al.,* 2008).

In the present study bactDNA was detected in 38 out of 39 culture positive samples (97.5%), and in 18 out of 161 culture negative AF samples (11.2%). These results come in agree with Vieira *et al.,* (2007).

Different methods result in a broad variability in detected AF bactDNA ranging from around 10% (Fam *et al.,* 2008) with single-plex or multiplex PCR, 30-40 % with 16s rRNA PCR (Zapater *et al.,* 2008), up to 60% with real-time TaqMan PCR (26), which is well comparable to the prevalence of AF bactDNA in our study cohort. Although the results obtained by some techniques support the traditional concept of monomicrobial BT present in serum and AF (Vieira *et al.,* 2007 and Tandon *et al.,* 2013), more recent studies using 16S rRNA-based fingerprinting analyses rather suggest polymicrobial peritoneal diversity questioning this canonical concept (Zapater *et al.,* 2008).

From this study, it can be concluded that ascetic fluid infection is caused by a variety of Gram negative and Gram positive organisms. Detection of bacterial DNA in the ascitic fluid provides a molecular evidence of bacterial translocation in patients with liver cirrhosis and ascites, as reported by Such *et al.,* (2002). Moreover, bacterial DNA could be present in a significant number of patients with culture negative non-neutrocytic ascites. So the early detection of bacterial DNA in those cases and early initiation of prophylactic intestinal decontamination might be helpful in overall patients' survival.

Ethical statement

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and obtaining consent from all patients enrolled in this study

References

Andrade SS, Bispo PJM and Gales AC. 2008. Advances in the microbiological diagnosis of sepsis. Shock 30(Suppl. 1): 41–6.

Badawy, A. A., Zaher, T. I., Sharaf, S. M., Emara, M. H., Shaheen, N. E., and Aly, T. F. 2013.Effect of alternative antibiotics in treatment of cefotaxime resistant spontaneous bacterial peritonitis. World Journal of Gastroenterology: WJG, 19(8), 1271.

Bibi S, Ahmed W and Arif A, 2015. Clinical, Laboratory and Bacterial Profile of spontaneous bacterial peritonitis in cirrhotic liver disease patients. Journal of the College of Physicians and Surgeons Pakistan; 25(2): 95-99.

Bruns, T., Sachse, S., Straube, E., Assefa, S., Herrmann, A., Hagel, S., Lehmann, M. and Stallmach, A. 2009. Identification of bacterial DNA in neutrocytic and non- neutrocytic cirrhotic ascites by means of a multiplex polymerase chain reaction. Liver International. 29, 1206-1214.

Cadranel J.-F., Nousbaum, J.-B., Bessaguet, C., Nahon, P., Nguyen-Khac, E., Moreau, R., Thévenot, T., Silvain, C., Bureau, C. and Nouel, O. 2013. Low incidence of spontaneous bacterial peritonitis in asymptomatic cirrhotic outpatients. World journal of hepatology. 104-105.

Conn HO. Unusual presentations of BSP. In: Rodes J, Navasa M, editors. 2000. Spontaneous bacterial peritonitis: the disease, pathogenesis and treatment. London: Informa Healthcare; Pp. 47–74.

Doddamani, G. B., Pujar, S., and Kora, S. A. 2010. Spontaneous Bacterial Peritonitis in Ascites: A prospective study in a tertiary care hospital. J Clin Diagn Res, 4, 2737-41.

Escudero, A. 2008. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis.

Esparcia, O., Montemayor, M., Ginovart, G., Pomar, V., Soriano, G., Pericas, R., and Coll, P. 2011. Diagnostic accuracy of a 16S ribosomal DNA gene-based molecular technique (RT-PCR, microarray, and sequencing) for bacterial meningitis, early-onset neonatal sepsis, and spontaneous bacterial peritonitis. Diagnostic microbiology and infectious disease 69(2), 153-160.

Evans, L. T., Kim, W. R., Poterucha, J. J. and Kamath, P. S. 2003. Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. Hepatology 37, 897-901.

Fam N, Ghali A and Shemis M, 2008. Bacterial DNA in Egyptian patients with cirrhosis and culture-negative non-neutrocytic ascites: a marker of bacterial translocation and a prognostic indicator. J Appl Sci Res, 4(6): 607–12.

Fernández, J., Navasa, M., Gómez, J., Colmenero, J., Vila, J., Arroyo, V. and Rodés, J. 2002. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. Hepatology 35, 140-108.

Francés, R., Zapater, P., González Navajas, J. M., Muñoz, C., Caño, R., Moreu, R., Pascual, S., Bellot, P., Pérez- Mateo, M. and Such, J. 2008. Bacterial DNA in patients with cirrhosis and noninfected ascites mimics the soluble immune response established in patients with spontaneous bacterial peritonitis. Hepatology 47, 978-985.

Gines, P., Angeli, P., Lenz, K., Moller, S., Moore, K. and Moreu, R. 2010. European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol, 53.

Jalan, R., Fernandez, J., Wiest, R., Schnabl, B., Moreau, R., Angeli, P., Stadlbauer, V., Gustot, T., Bernardi, M. and Canton, R. 2014. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. Journal of hepatology. 60, 1310-1324.

Khan, J., Pikkarainen, P., Karvonen, A.-L., Mäkelä, T., Peräaho, M., Pehkonen, E. and Collin, P. 2009. Ascites: aetiology, mortality and the prevalence of spontaneous bacterial peritonitis. Scandinavian journal of gastroenterology 44, 970-974.

Lata, J., Stiburek, O. and Kopacova. M. 2009. Spontaneous bacterial peritonitis: a severe complication of liver cirrhosis. World journal of gastroenterology: WJG 15: 5505.

Liver, E. a. F. T. S. O. T. 2010. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. Journal of Hepatology, 53, 397-417.

Moore, C. M. and Van Thiel, D. H. 2013. Cirrhotic ascites review: Pathophysiology, diagnosis and management. World journal of hepatology, 5, 251.

Moore, K. P. and Aithal G. P. 2006. Guidelines on the management of ascites in cirrhosis. Gut, 55, vi1-vi12.

Oladimeji, A. A., Temi, A. P., Adekunle, A. E., Taiwo, R. H., and Ayokunle, D. S. 2013. Prevalence of spontaneous bacterial peritonitis in liver cirrhosis with ascites. Pan African Medical Journal, 15(1).

Rimola A., García-Tsao, G., Navasa, M., Piddock, L. J., Planas, R., Bernard, B., Inadomi, J. M. and Club, I. A. 2000. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. Journal of hepatology 32, 142-53.

Runyon, B. 2004a. Early events in spontaneous bacterial peritonitis. Gut 53, 782-784.

Runyon, B. A. 2009. Management of adult patients with ascites due to cirrhosis: an update. Hepatology,49, 2087-107.

Saab, S., Hernandez, J. C., Chi, A. C. and Tong, M. J. 2009. Oral antibiotic prophylaxis reduces spontaneous bacterial peritonitis occurrence and improves short-term survival in cirrhosis: a meta-analysis. The American journal of gastroenterology 104, 993-1001.

Sagar KV, Reddy PR and Chandrasekhar S. 2016. A clinical study of spontaneous bacterial peritonitis in cirrhosis of liver with ascites in tertiary care hospital. J Evid Based Med Health. 3(1), 36-41.

Saqib, A., Khan, R. R., Masood, Z., and Ul Haque, I. 2012. Frequency of Spontaneous Bacterial Peritonitis (SBP) in Cirrhotic Patients with Ascites due to Hepatitis B and C. JUMDC, 3, 22-26.

Saud, B. and Baltodano, J. 2010. Ascites. Decision Making in Medicine (Third Edition). An Algorithmic Approach.192-193.

Sheikhbahaei, S., Abdollahi, A., Hafezi-Nejad, N., and Zare, E. 2014. Patterns of antimicrobial resistance in the causative organisms of spontaneous bacterial peritonitis: a single centre, six-year experience of 1981 samples. International Journal of Hepatology.

Singal, A., Salameh, H. and Kamath, P. 2014. Prevalence and in- hospital mortality trends of infections among patients with cirrhosis: a nationwide study of hospitalised patients in the United States. Alimentary pharmacology & therapeutics 40, 105-12.

Soriano, G., Esparcia, O., Montemayor, M., Guarner Argente, C., Pericas, R., Torras, X., and Coll, P. 2011. Bacterial DNA in the diagnosis of spontaneous bacterial peritonitis. Alimentary pharmacology & therapeutics 33(2), 275-284.

Such J, Frances R and Munoz C, 2002. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, nonneutrocytic ascites. Hepatology; 36(1):135–41.

Tandon, P. and Garcia-Tsao, G. 2008. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Seminars in liver disease, GEORG THIEME VERLAG, 26.

Tandon, P., Kumar, D., Seo, Y. S., Chang, H.-J., Chaulk, J., Carbonneau, M., Qamar, H., Keough, A., Mansoor, N. and Ma, M. 2013. The 22/11 risk prediction model: a validated model for predicting 30-day mortality in patients with cirrhosis and spontaneous bacterial peritonitis. The American journal of gastroenterology. 108, 1473-9.

Vieira SM, Da Silveira TR and Matte U. 2007. Amplification of bacterial DNA does not distinguish patients with ascitic fluid infection from those colonized by bacteria. J Pediatr Gastroenterol Nutr, 44(5):603–607.

Wiest, R. and Garcia-Tsao, G. 2005. Bacterial translocation (BT) in cirrhosis. Hepatology, 41, 422-33.

Zapater, P., Francés, R., González-Navajas, J. M., De La Hoz, M. A., Moreu, R., Pascual, S., Monfort, D., Montoliu, S., Vila, C. and Escudero, A. 2008. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis.

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