Changes in Etiologic and Antibiotic Resistance Profiles of Bacteria Causing Spontaneous Peritonitis in Egyptian Patients with Liver Cirrhosis

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ABSTRACT

To evaluate the recent changes in bacteria causing spontaneous bacterial peritonitis (SBP) in cirrhotic patients and its clinical significance on treatment, we compared the etiologic agents and their antibiotic resistance profiles over a four year period. In the prospective period of the study, 150 ascitic fluid (AF) samples obtained from SBP patients admitted consecutively to the Hepatology Department of Theodor Bilharz Research Institute during 2006-2007 were examined for polymorphonuclear leukocytes (PMLs) by blood cell counter and by a bedside dipstick test (Multistix SG10, Bayer). Blood and AF cultures were performed using BACTEC blood culture bottles and purified bacterial isolates were identified with a BBL Crystal Id System and software. Antibiotic sensitivity testing and extended-spectrum beta-lactamase (ESBL)- production were determined by disk diffusion and modified double disk synergy tests (MDDST) respectively. Genes encoding ESBLs were detected by PCR and typed by DNA sequencing. In the retrospective period of the study (2004-2005), the laboratory records of 140 episodes of SBP were examined for the culture positive rate, causes of SBP and their antibiotic resistance patterns recorded. Results showed that the overall culture positivity rate was significantly higher in prospective study period (32%) versus retrospective period (16.4 %) p<0.05 and the main bacterial isolates were E.coli, 47.8% and Klebsiella pneumoniae, 28.1% with no differences in the two study periods. Gram positive bacteria (GPB) were isolated more frequently in the prospective than retrospective period (25% versus 13%). Two opportunistic bacterial species (Staphylococcus haemolyticus and Pantoea agglomerans) were detected as a cause of SBP in the prospective period. Species identification of Pantoea isolate was confirmed by DNA extraction, sequencing of ribosomal RNA and phylogenetic analysis. ESBLs were detected among 17.6% of E. coli and Klebsiella isolates of the prospective period and all were of the CTX-M15 type. The rate of resistance to cefotaxime significantly increased from 45% to 72 % and to ciprofloxacin from 25% to 47% and treatment failure rate was 65% in recent years. No resistance was detected to imipenem over the entire study period. For the GPB, 50% were resistant to ampicillin/sulbactam, cefotaxime and gentamicin and one S. haemolyticus isolate was methicillin resistant. In conclusion the emergence of multidrug resistant opportunistic pathogens and ESBL-producing E. coli and Klebsiella as causal agents of SBP, together with an increase in resistance to antibiotics commonly used for the empiric treatment of bacterial peritonitis have serious implications on patient management in our region. Rapid diagnosis of SBP by a bedside dipstick test and identification of the causative organism by culture using blood culture bottles and direct sensitivity testing to establish an effective antibiotic therapy is recommended.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a frequent and serious complication that occurs in 10-30% of patients with cirrhosis and ascites (1). SBP is associated with high mortality rates (20-40%) that were previously attributed to the severity of cirrhosis (2). Recently several factors have been shown to contribute to the prognosis of the disease; these include associated bacteraemia, hepatocellular carcinoma and the microbiological characteristics of the causative agent of the infection (3, 4).

Bacterial overgrowth and translocation from the intestinal lumen to the mesenteric lymph nodes and ultimately to the blood are considered the key steps in the pathogenesis of SBP (5, 6). Our current understanding of the pathogenesis of SBP supports that classical SBP infection is mainly caused by enteric bacteria particularly Escherichia coli, the main facultative aerobe of the human intestinal flora (7).

It has been suggested that the microbiological causes of SBP and the susceptibility of the causative organisms to antibiotics are changing for several reasons. Since SBP is a serious complication in cirrhotic patients, empirical antibiotic therapy by third generation cephalosporins is usually initiated before the results of the ascitic fluid culture and sensitivity are available (8). However extended spectrum beta lactamase (ESBL)-producing strains have emerged among Enterobacteriaceae such as E. coli and Klebsiella spp., with an estimated prevalence of 25-35% among hospitalized patients in our institution (9, 10). It can be speculated that treatment failure with cephalosporins is a possibility in patients with SBP.

Moreover it has been demonstrated that the administration of oral quinolones for intestinal decontamination in high-risk group cirrhotic patients decreases the incidence, recurrence and mortality associated with recurrent episodes of SBP (11-13). However treatment with quinolones was associated with isolation of quinolone-resistant ...
resistant *E. coli* from stools (14, 15) and it may also promote carriage and SBP infections with multi-drug resistant Gram-positive bacteria at an increasing frequency (16). Changes in the microbial causes of SBP and bacterial antibiotic resistance patterns vary according to the location and time.

In this work we compared the changes in the profiles of causative agents and antibiotic resistance patterns over a four-year period in a two-year term manner in our research institute and we studied their influence on the clinical outcome on Egyptian patients with liver cirrhosis.

**PATIENTS AND METHODS**

**Retrospective Study Period (2004-2005)**

We retrospectively examined the medical records and the microbiology laboratory files of patients diagnosed clinically with SBP and who yielded positive results in ascitic fluid culture. The records were reviewed for the following data: age, sex, liver disease, Child–Pugh score, cause of cirrhosis, associated hepatocellular carcinoma, gastrointestinal bleeding, renal impairment, blood and ascitic fluid laboratory data and culture results, treatment and outcome of infection.

**Prospective Study Period (2006-2007):**

All patients with decompensated cirrhosis, ascites and clinical signs of SBP admitted to the Hepato-gastroenterology Department of Theodor Bilharz Research Institute between January 2006 and December 2007 were consecutively included in the study. Diagnosis of SBP was based on the presence of clinical signs of peritoneal infection (fever, increased confusion, diffuse abdominal pain and impaired renal function) or asymptomatic ascites with leucocytic count > 250 cells/mm². Patients with suspected secondary peritonitis or with non-neutrocytic ascites were excluded from the study. Detailed clinical history, complete physical examination, laboratory tests including complete blood count, prothrombin time, biochemical tests for liver and kidney functions and viral hepatitis markers were performed. Abdominal ultrasonography, upper endoscopy and diagnostic paracentesis were performed under aseptic conditions for all patients included in study. To assess the clinical outcome in those patients, the response to treatment was assessed 72h after antibiotic therapy and mortality rate within 30 days. The initial responses included resolution of fever, leucocytosis, and PML in ascitic fluid; and failure of response consisted of absence of improvement or clinical deterioration or death of patients.

**Leukocytic count**

Ascitic fluid samples were examined for polymorphnuclear leukocytes (PMLs) by two methods: Coulter automated cell counter and by a bedside dipstick test (Multistix SG10, Bayer Diagnostics, Bridgend, UK). The multisitix strip is a leucocyte esterase reagent strip that can rapidly detect leukocytes in biological fluids and is commonly used for testing urine samples. We assessed its utility as a rapid diagnostic test for diagnosis of SBP and its correlation with PML count and culture results.

**Bacterial cultures**

10 ml samples of blood and ascitic fluid were inoculated aseptically at the bedside of the patient into aerobic BACTEC blood culture bottles (Becton Dickinson, Sparks, MD, USA). All bottles were placed in the BACTEC 9050 system and incubated for 5 days. Bottles detected positive by the instrument were subjected to direct Gram staining and antibiogram testing. Aerobic subcultures were performed on MacConkey and blood agar plates. Cultured Gram-negative and Gram-positive bacteria were identified by the BD BBL CrystaT ID Identification systems for enteric Gram-negative and Gram-positive ID kit and software (Becton-Dickinson, Sparks, MD, USA). Extended-spectrum beta-lactamase (ESBL)-producing organisms were phenotypically determined by the modified double disk synergy test (MDDST) described by Pitout et al (17). *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively. Control strains were supplied by the Clinical Trials Program (CTP) located at Naval Medical Research Unit-3 (NAMRU-3), Cairo. Antimicrobial sensitivity testing was done according to the guidelines of the Clinical Laboratory Standard Institute (CLSI) disk diffusion methods. The following antibiotics were tested with Gram-negative isolates: ampicillin-sulbactam, amoxicillin-clavulanic acid, cefotaxime, cefoperazone, ciprofloxacin, amikacin, gentamicin and imipenem. Cefoxitin and vancomycin were included in the antibiogram of staphylococcal isolates for detection of methicillin and vancomycin resistance (MRS and VRS).

**Molecular identification of beta lactamase genes of ESBLs**

PCR was performed on isolates that were phenotypically identified as ESBL-producers. A previously described primer set (Chen et al (18), accession no. X92506) for detection of the *bla*<sub>CTX-M<sub>1</sub> was used. The primers were 5’- AAC CGT

200
DNA was extracted using a QIAamp DNA Mini Kit; (QIAGEN, Hilden, Germany). Amplification was performed with a Perkin Elmer 9600 thermocycler (Perkin Elmer, Cetus, Norwalk, CT, USA). PCR was performed as previously described by Chen et al (18) with a Tm of 57°C; expected amplicon size was 766 bp. PCR cleaning of amplified DNA product was performed using Exosap-IT kit (USB, Cleveland OH). Nucleotide sequences of the PCR products were determined by using the Big Dye terminator cycle sequencing kit version 3.1 and an ABI 3130XL automated genetic analyzer (Applied Biosystem, Foster City, CA, USA) according to the manufacturer’s instructions. The PCR fragments were sequenced in both directions with the same primers used for PCR amplification. A BLAST search for sequence identification was performed using the Blast algorithm at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi)

Statistical Analysis

Results were expressed as mean ± standard deviations (SD) or number (%). Numerical data were compared using Mann Whitney U test while categorical data were compared using Chi square test. Spearman rank correlation coefficient was used to determine significant correlations among different parameters. The data were considered significant if p values were equal to or less than 0.05.

RESULTS

Overall, 140 episodes of SBP were clinically recorded in the retrospective study period and 150 prospective cases were noted in prospective study period. The clinical characteristics of the patients showed that the main cause of cirrhosis was hepatitis C in both study periods but hepatitis B was significantly more common in patients of the first study period (p<0.05; Table1). Comparative analysis of the detection of leukocyte esterase using a rapid dipstick test (Multistix SG10) showed a significant correlation with AF leukocytic count and culture results (r= 0.873, 0.513 respectively p< 0.01; Figure 1). This indicates that the rapid dipstick test would be a good bedside test for diagnosis of SBP.

Ascitic fluid and/or blood culture-positive rate was higher in the prospective study (32 %) versus the retrospective study period (16.4%; p<0.05; Table 2). Gram-negative bacilli were the main organisms isolated during the two study periods (78.8%) and their rate of isolation was not significantly different. E. coli was the most common species (47.8%) recovered from patients; followed by K. pneumoniae (28.1%; Table 2). Gram- positive bacteria were found more frequently in SBP patients of the prospective as opposed to retrospective period (25% versus 13%) but the difference was not statistically significant. Twelve episodes were caused by GPB in the prospective period and were caused by, Staphylococcus haemolyticus (n=4), Staphylococcus aureus (n=2), Staphylococcus epidermidis (n=2), Enterococcus spp. (n=2) and Streptococcus viridans (n=2).

Among the GPB, 50% of isolates were resistant to the combination of ampicillin/sulbactam, cefotaxime and gentamicin while 33.3% were resistant to ciprofloxacin, clindamycin and doxycycline. A single S. haemolyticus isolate was methicillin resistant but vancomycin sensitive. Antibiotic resistance of isolated GNB over the study period (Table3), showed that 85.5% of the isolates were resistant to ampicillin-sulbactam and the rate of resistance was nearly equivocal between the 2 periods (85% and 86%). In contrast the resistance rate to cefotaxime and cefoperazone was significantly increased from 45% and 35% respectively in the retrospective study period to 72.2% and 66.6% respectively in the prospective study period (p <0.05). Furthermore, 70% (17 /24) of the cefotaxime-resistant isolates in prospective period were resistant to ciprofloxacin. However the resistance to amikacin was low in both study periods (5%, 5.5%) and none of the isolates were resistant to imipenem (Table 4).

The prevalence rate of ESBL-producing organisms among E. coli and K. pneumoniae isolated from ascitic fluid increased from 8.3% and 12.5% respectively in retrospective period to 18.2% and 16.7 % respectively in the prospective period, but the difference was not statistically significant (Table 4). ESBLs were recovered from 6 hospitalized patients; 3 were placed in the intensive care unit and all had history of previous treatment with cefotaxime within the prior 2 months. The antibiotic resistance pattern of the isolates indicated that they were only sensitive to amikacin, imipenem and meropenem; a single isolate was also sensitive to ciprofloxacin. PCR amplification and DNA sequencing of the ESBLs isolated from AF showed that they were all members of CTX-M1 group of ESBLs and that they all were genetically identical to the CTX-M 15 determinant.

Assessment of clinical response 72 hours after initial antimicrobial therapy in patients of prospective study period showed that treatment failure rate was 65% and mortality within 30 days was 19/97 patients (19.5%).
One isolate from ascitic fluid was identified biochemically as *Pantoea agglomerans* by two separate identification systems (BD BBL Crystal™ and API 20 E). The SBP episode caused by *P. agglomerans* occurred in a 65-year old female patient who had cellulitis in anterior abdominal wall, and showed clinical signs of peritonitis. Her blood culture result yielded a *S. haemolyticus* positive culture. The ascitic fluid leukocytic count for this patient was 250,000 cells/mm³. To confirm this result, DNA was extracted from the purified isolates and the small subunit (ssu) rRNA gene was sequenced as previously described (19). The assembled ssu rRNA gene was compared to available nucleotide sequences in the Genbank database and the closest identity was with an unknown species of *Enterobacteriacea* isolated from a human blood source (AY538694; Figure 2). Two *Pantoea* spp. were also very similar, a *Pantoea stewartii* subsp. *Stewartii* strain GSBP 2626 (AF373198) and a *Pantoea* spp. “Ward” (AY994301). This isolate was from a bacteraemic patient from a Korean pseudo-outbreak associated with contaminated cotton pledgets. An alignment and phylogenetic analysis of isolates from the *Pantoea* spp. and the clinical isolate supported this conclusion (Figure 2). The antibiogram of the isolate showed that it was resistant to all antibiotics tested except quinolones. Antibiotic therapy with ceftazidime and levofloxacin did not lead to a clinical improvement and the patient did not survive.

| Table 1: Clinical and Demographic Characteristics of Patients in the two study periods |
|----------------------------------|-------------------|-------------------|
| | Retrospective Period (n=140) | Prospective Period (n=150) |
| Age (mean±SD) | 48.5±10.9 | 50.8±12.8 |
| Sex (male/ female) | 80/ 60 | 95/ 55 |
| | 57.1/ 42.9% | 63.3/ 36.7% |
| Etiology of cirrhosis | | |
| *HCV* | 115 (82.15%) | 123 (82.0%) |
| *HBV* | 20 (14.28%) | 8 (5.33%) * |
| *HCV+HBV* | 4 (2.86%) | 7 (4.6%) |
| *Autoimmune* | 1(0.71%) | --- |
| Comorbidity | | |
| *Hepatocellular carcinoma* | 20 (14.28%) | 30 (20%) |
| *Gastrointestinal Bleeding* | 71 (50.71%) | 65 (43.33%) |
| *Renal impairment* | 62 (44.28%) | 77 (51.33%) |

*p < 0.05*
Table 2: Profiles of isolated microorganisms from patients with culture-positive spontaneous bacterial peritonitis

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Retrospective Period N of isolates =23 n (%)</th>
<th>Prospective Period N of isolates=48 n (%)</th>
<th>Total N of isolates =71 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>12 (52)</td>
<td>22 (46)</td>
<td>34 (47.8)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>8 (35)</td>
<td>12 (25)</td>
<td>20 (28.1)</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Pantoea agglomerans</em></td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total GNB n (%)</strong></td>
<td><strong>20 (87)</strong></td>
<td><strong>36 (75)</strong></td>
<td><strong>56 (78.8)</strong></td>
</tr>
<tr>
<td><em>Enterococci</em></td>
<td>3</td>
<td>2</td>
<td>5 (7)</td>
</tr>
<tr>
<td><em>Staph aureus</em></td>
<td>-</td>
<td>2</td>
<td>2 (2.8)</td>
</tr>
<tr>
<td><em>Staph haemolyticus</em></td>
<td>-</td>
<td>4</td>
<td>4 (5.6)</td>
</tr>
<tr>
<td><em>Staph epidermidis</em></td>
<td>-</td>
<td>2</td>
<td>2 (2.8)</td>
</tr>
<tr>
<td><em>Strept viridans</em></td>
<td>-</td>
<td>2</td>
<td>2 (2.8)</td>
</tr>
<tr>
<td><strong>Total GPB n (%)</strong></td>
<td><strong>3 (13)</strong></td>
<td><strong>12 (25)</strong></td>
<td><strong>15</strong></td>
</tr>
<tr>
<td><strong>Culture positivity (%)</strong></td>
<td><strong>16.4</strong></td>
<td><strong>32</strong></td>
<td><strong>23.1</strong></td>
</tr>
</tbody>
</table>

GNB: Gram-negative bacteria    GPB: Gram-positive bacteria   *p value<0.05

Table 3: Antibiotic resistance patterns of Gram negative isolates in the 2 study periods

<table>
<thead>
<tr>
<th></th>
<th>Retrospective Period (n=20) N (%)</th>
<th>Prospective Period (n=36) N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin/sulbactam</td>
<td>17(85)</td>
<td>31(86)</td>
<td>NS</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>9(45)</td>
<td>26(72.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>7 (35)</td>
<td>24 (66.6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5(25)</td>
<td>17(47.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1(5)</td>
<td>2(5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: non significant
Table 4: Proportion of ESBL –producing organisms among Escherichia coli and Klebsiella pneumoniae in the study periods

<table>
<thead>
<tr>
<th></th>
<th>Retrospective Period</th>
<th>Prospective Period</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>1/12 (8.3%)</td>
<td>4/22 (18.2%) NS</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1/8 (12.5%)</td>
<td>2/12 (16.7%) NS</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2/20 (10%)</td>
<td>6/34 (17.6%) NS</td>
</tr>
</tbody>
</table>

Figure 1: Correlation between multistix and both PMN count and ascitic fluid culture 
(r= 0.873; p< 0.01 & r= 0.513; p< 0.01, respectively).

Figure 2: Dendrogram based on the alignment of the small subunit rDNA of suspected Pantoea agglomerans isolate 474 (this study) and members of the Pantoea genus. In total, 1430bp of ssu-rDNA sequence was aligned using the Clustal X application in the BioEdit software package (Version 7.0.1). Dendrograms representing the relationship of the sequences from each strain were constructed using the program Mega4.0. Phylogenetic reconstructions were created using the neighbour-joining method with the Kimura 2-parameter substitution model and branches were evaluated using the bootstrapping method, with 2000 replications. Branch values below 70% were viewed as non-significant and are not shown in the dendrogram.
DISCUSSION

A substantial epidemiologic change in the etiology of SBP has been observed in recent years (7, 16, 20, 21). In the present study we report the emergence of two opportunistic bacterial species as causal agents of SBP in cirrhotic patients from Egypt. *Staphylococcus haemolyticus* was detected in blood and/or ascitic fluid samples of four patients and *Pantoea agglomerans* from the AF of one patient with SBP.

*S. haemolyticus* is a member of the *S. epidermidis* of the coagulase-negative staphylococci (CONS) that is frequently isolated from blood cultures and may cause septicemias, peritonitis, otitis and urinary tract infections particularly in patients with underlying disease (22). It is notorious for its multidrug resistance and early acquisition of resistance to methicillin and glycopeptides. This is due to the plasticity of its genome that allows frequent rearrangement and insertion of antibiotic resistance genes (23, 24). Our *S. haemolyticus* isolates causing SBP were multidrug resistant (R to three or more of the antibiotics tested) and one of them was methicillin resistant but vancomycin sensitive. Ascitic fluid and blood culture contamination from skin bacteria was excluded as all cultures were received by a standard sterile technique and isolation of the organism was confirmed from repeated blood culture bottles in case of detection of a CONS.

We also isolated a presumptive *Pantoea agglomerans* from the AF of a 65-year old female who suffered from advanced liver disease. She gave history of abdominal injury in a field and was being treated from abdominal cellulitis. The placement of the presumptive *Pantoea* isolate into the *Pantoea* genera was supported by nucleotide sequencing of amplified ssu rDNA and phylogenetic analysis. *P. agglomerans*, formerly known as *Enterobacter agglomerans*, is found in the gut of humans and animals and also in plants. It was recently reported as a plant pathogen causing human disease that was mostly associated with penetrating trauma by vegetative material and catheter-related bacteremia (25). Overall 56 cases of human infections with *Pantoea agglomerans* have been recorded worldwide including 2 peritonitis patients undergoing continuous ambulatory peritoneal dialysis (CAPD) (26,27). It is noteworthy to mention that the patient in the present study also suffered from bacteremia caused by a multidrug resistant strain of *S. haemolyticus*; denoting the impaired immune status of the patient. Infections in patients with end-stage liver disease by commensal bacteria is related to abnormalities in their natural defense mechanisms, alterations in the enteric flora and the growing utilization of invasive procedures that increase the risk of infections in these patients (28).

SBP is an infection of low microbial concentration (1 bacterium /ml of fluid) (29), so inoculation of ascitic fluid in blood culture bottles at bedside immediately post-paracentesis increases the yield of culture technique (8).

The culture positive rate in the second period of this study was significantly higher (32%) than the first study period (16%) p <0.05. This is because the former period corresponds to the introduction of the BACTEC system and blood culture bottles for culture of AF samples in our institution. Meanwhile in the previous period AF cultures were performed by the conventional method of inoculating centrifuged sediment on culture media. This culture-positive rate is comparable to that reported in previous studies (34-39%) (16, 30) but lower than that reported in others (41-59%) (20, 31).

As expected and as reported in previous studies (16, 20, 21), the main bacteria responsible for SBP cases were *E. coli* (47.8%) and *Klebsiella pneumoniae* (28.1%) with no differences between the 2 study periods. Despite the relatively low number of isolates recovered in the first study period, we observed an increase in the number of GPB isolated in the second period versus the first period (25% versus 13%). Staphylococcal isolates, particularly CONS, ranked in third position as causal agents of SBP and they were mostly multidrug resistant and one isolate was MR. These epidemiological changes may be attributed to the long term antibiotic therapy as primary or secondary prophylaxis in high-risk cirrhotic patients by quinolones to reduce recurrence and improve survival of SBP cases (11-13). Also the increase in invasive procedures and hospitalization of cirrhotic patients promotes the carriage of Gram-positive bacterial strains mainly the MRS and may ultimately lead to increase in infections by them (30).

The increase in antibiotic resistant bacteria has become a real threat to the effective treatment of SBP infections in recent years (28). In the present study, it was noticed that the rate of resistance to cefotaxime and ceftoperazone in GNB isolated from SBP patients increased significantly in the study period 2 (72% and 66% respectively). In parallel with the increase in resistance to cephalosporins, the emergence of ESBL-producers among *E.coli* and *Klebsiella* isolates causing SBP (17.6%), emphasize that broad-spectrum cephalosporins may no longer be effective in the empiric treatment of SBP cases or even when the organism are apparently susceptible to cephalosporin *in vitro*. This may explain the high treatment failure and mortality rate in patients of this study as they were all initially treated with...
cephalosporins. Similar results were reported in previous studies on the adverse clinical outcome of SBP infection with ESBL-producers (4, 20).

So AF culture and testing for presence of ESBLs are mandatory to ensure efficient antibiotic therapy in high-risk cirrhotic patients due to their severely impaired liver functions and immune systems. However cephalosporin is still the drug of choice for empiric therapy until culture results are available because SBP is a serious condition that has to be managed rapidly. A recent study suggested that cirrhotics who were on antibiotic prophylaxis were more likely to be infected with ESBL-producing organisms, and should be started empirically on second line antibiotics such as carbapenems upon diagnosis (31).

Quinolones are also known to be as effective as third generation cephalosporins in treatment of SBP (6, 33). In this study the resistance to ciprofloxacin increased from 25% to 47% in GNB and was 33% in GPB isolated from AF samples. Moreover 70% of the cefotaxime resistant strains were also resistant to ciprofloxacin. This may be explained by the increased use of the drug in treatment of endemic gastrointestinal infections and the administration of norfloxacin prophylaxis in high-risk SBP patients in our institution. This causes changes in antibiotic resistance of the bacterial flora due to antibiotic selection pressure. It has been reported that the outcome of norfloxacin prophylaxis is doubted in areas with high incidence of MDR bacteria as it can promote the selective growth of the resistant strains (29, 30). Restrictions of the use of prophylaxis to patients with greatest risk of SBP, or rotating antibiotics are possible alternatives (30, 33). Also the use of newer drugs as moxifloxacin and levofloxacin may have enhanced activity but their efficacy needs to be established in such cases. Although low resistance (5%) of the AF isolates to amikacin was recorded in the antibiogram sensitivity, its use is limited due to nephrotoxicity specially that hepatorenal syndrome is common with SBP.

Imipenem was the most effective drug on bacteria causing SBP in this study, so imipenem may be administered when culture results indicate resistance to cefotaxime and ciprofloxacin or when an ESBL-producing organism is detected or even if the patient’s condition is not improving 48h after initiation of empiric therapy. However imipenem-resistant strains are emerging and are known to spread via plasmids (34), so the drug has to be used only in justified cases to avoid serious clinical problems in the future.

In conclusion, bacteria causing SBP are recently showing changes in the etiologic profile including the emergence of multidrug resistant opportunistic pathogens and ESBL-producing E. coli and Klebsiella as causal agents of SBP, together with an increase in resistance to antibiotics commonly used for the empiric treatment of SBP. Third generation cephalosporins might not constitute the optimal therapeutic option for the treatment of SBP and this has serious implications on the therapy regime of SBP in our region. So rapid diagnosis of SBP by bedside dipstick test and AF culture on blood culture bottles and direct sensitivity testing is recommended to establish an effective antibiotic therapy.

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Long-term, prospective study on the incidence of Escherichia coli and Klebsiella pneumoniae
in the city of Cairo, Egypt.

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Background: The incidence of Escherichia coli and Klebsiella pneumoniae in the city of Cairo, Egypt, was investigated using a long-term, prospective study.

Methods: A total of 150 samples were collected from different sources over a period of 2 years. The samples were subjected to various biochemical and molecular tests, including MDDST and DNA sequencing.

Results: The incidence of Escherichia coli was found to be 16.3%, while Klebsiella pneumoniae was found to be 4.8%. Both organisms were found to be resistant to multiple antibiotics.

Conclusion: This study highlights the importance of monitoring the incidence of these organisms in urban areas and the need for developing effective treatment strategies.