Etiology of Peritonitis

Aldina Ahmetagic¹, Fatima Numanovic², Sead Ahmetagic³, Lejla Rakovac-Tupkovic¹, Humera Porobic-Jahic³
Department of Clinical Pharmacology, University Clinical Center Tuzla, Bosnia and Herzegovina¹
Polyclinic of Laboratory diagnostics, Department of Microbiology, University Clinical Center Tuzla, Bosnia and Herzegovina²
Clinic for Infectious Diseases, University Clinical Center Tuzla, Bosnia and Herzegovina³

Etiology of Peritonitis

1. INTRODUCTION
Peritonitis indicates diffuse peritonitis, or local, peritoneal inflammation due to any cause (1, 2).
The main signs that accompany peritonitis are abdominal pain, fever, tachypnea, tachycardia, hypotension, nausea, vomiting, and decreased bowel peristalsis. Clinical picture is changing with rising of symptoms of inflammation. With the APACHE II score (Acute Physiology and Chronic Health Evaluation, TISS-28 score (Simplified Therapeutic Intervention Scoring System), or Mamheim peritonitis index, which includes scoring system with prognostic value applied to patients with peritonitis, we could determine severity of the disease (3, 4).
Peritonitis generally occurs as a result of perforation of the gastrointestinal tract, and the sources of infection are microbials of the intestinal flora. In healthy adults they counts 400-600 different species of microorganisms, and 60 to 80% can be identified using existing microbiological techniques and procedures (5).
Precise etiology of abdominal infection depends of the perforation site. Stomach, duodenum and small intestine contain relatively few microorganisms. This situation is conditioned by the presence of gastric acid, which represents the first barrier to bacteria. In contrast to these organs, colon is colonized with 10¹¹ bacteria in gram of stool. Number of anaerobic bacteria increases in the distal parts of digestive system. Ileum contains an equal number of aerobic and anaerobic bacteria, while in the lowest parts of the digestive system, the proportion of the ratio aerobic – anaerobic bacterial infections is 1:10 000. Infections of proximal bowel and biliary system are often caused by gram-positive and gram-negative bacteria. Predominant isolates were Escherichia coli (60%), Klebsiella pneumoniae (26%), Proteus spp. 22%, Enterococcus spp. (17%), Enterobacter spp., Staphylococcus aureus (7%) and Streptococcus spp. (28%). In addition to these aerobic bacteria, in a significant number of these infections, anaerobic bacteria such as Bacteroides fragilis, Clostridium spp., Peptostreptococcus spp. and Prevotella spp. can be found. Regarding the etiology of perforated appendicitis and diverticulitis important role have some of the aerobic bacteria: Escherichia coli, Enterococci, Streptococcus viridans, Staphylococcus spp., Klebsiella spp. and Enterobacter spp. 80% of the isolated anaerobic bacteria were Bacteroides fragilis.

CONFLICT OF INTEREST: NONE DECLARED

ORIGINAL PAPER
Cause of the intraabdominal infections is significantly different for outpatient and in the hospital acquired infections. Escherichia coli and Bacteroides fragilis are the most commonly isolated bacteria in the outpatient acquired intraabdominal infections while in hospital acquired infections we isolated Staphylococcus epidermidis, Pseudomonas aeruginosa, Enterobacter spp., Entercoccus spp., Proteus spp. and Morganella spp. In patients who have taken antibiotic therapy before infection or were in the group of immunocompromised patients, often in addition to these bacterial isolates we found and Candida spp. (5, 6, 7, 8).

Knowing this diversity of intestinal flora is not surprising that peritonitis and intraabdominal infections almost always are polymicrobial. Yinnon and associates 1999th (9) reported that in 135 hemodialysed patients with peritonitis total of 481 microorganisms were isolated from 378 samples of peritoneal liquid.

Analyzing the results of microbiological materials from 150 patients with intraabdominal infections, Santos and colleagues in 2003 (10) in their study, conducted in Brazil, found that polymicrobial infection detected in 51.9% of cases, with two to nine organisms isolated per sample. In the same study, aerobic bacteria were found in 93.4% of cases, anaerobic bacteria in 30.2%, and fungi in 13.2% of cases. Leading isolated pathogens were Staphylococcus spp., Escherichia coli, Proteus spp. (10).

In a retrospective study (Switzerland), which covered the period from 1998 to 2000, in the material collected from 76 patients with secondary peritonitis, 156 different microorganisms were isolated (11). In the study of Solomkin and associates in 2003 (12), leading incidence of isolated bacteria in patients with intraabdominal infections was as follows: 1. facultative anaerobic and aerobic gram-negative bacteria (E. coli 71.3%, Klebsiella spp. 14.3%, 14.3% of Pseudomonas aeruginosa and other 12.3%); 2. anaerobic microorganisms: Bacteroides fragilis group–34.5%, Bacteroides spp., except B. fragilis–71%, Clostridium spp.–29.2%, Eubacterium spp.–16.5%, other anaerobic bacteria–19.4%; 3. Gram-positive microorganisms: Streptococcus spp.–38%, Enterococcus faecalis - 11.6%, Enterococcus spp. 7.8%, Staphylococcus aureus 3.5%.

Surgical infections require causal surgical treatment, in terms of sanitation or removal of septic debris, and control of infectious process. However, most infections of surgical origin also requires mandatory antibiotic therapy, due to the inability to surgery alone, because it completely eliminate the causes of infection, and prevent further propagation of causes of systemic infection (14, 15). Microbiological tests during and after surgical treatment are justified by the fact that they answer the following questions: Is this really the infection, whether it is of bacterial origin, what is the expected bacterial flora and what is the most effective antibiotic.

The aim of this study was to determine the etiology and sensitivity of commonly isolated bacteria to antibiotic therapy in patients with peritonitis in order to select appropriate antibiotics and possible changes of inadequate empirical therapy.

### 2. PATIENTS AND METHODS

#### 2.1. Patients

Study is performed at the Department of Clinical Pharmacology JZU-UKC Tuzla, Institute of Microbiology, Polyclinic for Laboratory Diagnostics, Surgical Clinic and Department of Anesthesiology and Reanimation JZU-UKC Tuzla, by prospective study, starting from June 1st 2009. to June 30th 2010. The study included 60 patients who underwent surgery for acute peritonitis at the Department of Surgery and Clinical Center Tuzla.

#### 2.2. Methods

1. We took the basic personal and anamnestic data from each patient through the questionnaire: name, last name and patient birth date, admission diagnosis, empirical antibiotic therapy given prior to surgery.
2. Setting the clinical diagnosis of peritonitis on the basis of the above mentioned clinical signs of disease and need for a surgical treatment, performed by surgeon at the admission to the Department of Surgery, JZU UKC Tuzla.
3. We sampled each patient for the microbiological examination, which was carried out by the Department of Microbiology, Polyclinic for Laboratory Diagnostics JZU-UKC Tuzla.; the content of the operative field, wound swab first, fourth and seventh postoperative day. Each sample was tested for the presence of aerobic, facultative anaerobic and anaerobic bacteria. Isolation and identification of bacteria was done using standard microbiologically accepted and established procedure. Besides the above methods, identification was performed using an automated system for identification of bacteria VITEK2 compact system. Each isolated strain was determined by susceptibility to antimicrobial agents using disc diffusion method and determination of minimum inhibitory concentration (MIC). The choice of antibacterial agents is dependent of the type of isolated bacteria. Identification of resistant types of bacteria (ESBL, ampC, MRSA) was performed according to CLSI recommendations.

4. Evaluation of data for each patient, obtained in accordance with the set objectives of the research, was done by clinical pharmacologist in the Department of Clinical Pharmacology JZU-UKC Tuzla and then notified the surgeon.

#### 2.3. Statistical Methods

The statistical analysis used the standard methods of descriptive statistics (Pearson’s correlation test). To test the statistical significance between groups were used for parametric and nonparametric tests, χ² and Student’s t-test. The results are presented graphically and in tabular form. Differences in values of $\chi^2 > 3.804$ we considered statistically significant.

### 3. RESULTS

In total sample were 60% men and 40% women, whereas in the group of patients with clinical signs of peritonitis caused by perforation of hollow organs were 55% men and 45% female, and in a group of patients with clinical signs of peritonitis caused by ileus were 57.2% of men and 42.8% women.

After microbiological processing of the content of the operative field, wound swabs and smears of drainage the most
isolated gram-positive bacteria were cocci – (coagulase-negative) Staphylococci in 11/58 (18.96%), followed by Staphylococcus aureus in 7/58 (12.6%) and Enterococcus faecalis/ faecium in 2/58 (3.44%) isolates. Regarding the gram-negative bacteria, in our study Escherichia coli were the most commonly isolated bacterium in 9/58 (15.51%), and Pseudomonas aeruginosa and Klebsiella pneumoniae were isolated in 6/58 (10.34%) of the cases. Distribution of other isolates is listed in Chart 1. Sensitivity of facultative anaerobic gram-negative bacilli to the antibiotics mentioned above was highest to the carbapenems and allosporins (25% -42%), while, regarding cephalosporins of the first generation (60%) was statistically higher than the sensitivity to the third-generation cephalosporins.

Sensitivity of Klebsiella pneumoniae to cephalosporins of the first generation (60%) was statistically higher than the sensitivity to the third-generation cephalosporins (25% -42%), while, regarding the Escherichia coli, there was no difference in sensitivity between these two generations of cephalosporins (75% -76.92%). Isolates of Pseudomonas aeruginosa were most sensitive to the third-generation cephalosporins and carbenem, and least sensitive to cephalosporins and penicillins. Sensitivity to other antibiotics is shown in Table 1.

The sensitivity of coagulase-negative Staphylococcus was the largest to vancomycin (100%), the lowest (10%) to Penicillin G; susceptibility of Staphylococcus aureus was the largest to vancomycin and amoxicillin/clavulanate in 100% cases and lowest to the Penicillin G in the 3.7% of the cases. The sensitivity of Enterococcus faecalis was the largest to the penicillins and vancomycin in 100%, and lowest to the tetracycline in 33.33% cases. Enterococcus faecium was susceptible to the vancomycin in 100% of the cases, to the quinolones in 20% of cases. Sensitivity to other antibiotics is shown in Table 2.

4. DISCUSSION

According to the research of Sartelli et al. (8, 9) and Solomkin and Edlund (5) infection of the proximal intestine and biliary system are commonly caused by aerobic and facultative anaerobic bacteria such as Escherichia coli (60%), Klebsiella pneumoniae (26%), Proteus spp. (22 %), Enterococcus spp. (17%), Enterobacter spp. and Staphylococcus aureus (7%) and streptococci (28%) and anaerobic bacteria from the genus Bacteroides (B. fragilis), Clostridium, Fusobacterium, and Prevotella. The causes of intra-abdominal infections are significantly different for outpatient and in hospital acquired infections. Escherichia coli and Bacteroides fragilis are the most commonly isolated bacteria in the outpatient acquired intra-abdominal infections while in hospital acquired infections most isolated were Staphylococcus epidermidis, Pseudomonas aeruginosa, Enterobacter spp. and Proteus spp., Enterococci, Morganella spp. In patients who were taken antibiotic therapy before infection or belong to the group of immunocompromised patients, often in addition to these bacterial isolates Candida spp. were also isolated (7, 8).

Solomkin and associates (12) in their study found that from patients with the highest incidence of intra-abdominal infections from facultative anaerobic and aerobic gram-negative bacteria were isolated Escherichia coli in 71.3%, Klebsiella spp. 14, 3%, Pseudomonas aeruginosa 14.3 % and other gram-negative bacteria; 12.3% of the anaerobic organisms were Bacteroides fragilis 34.5%, Bacteroides spp. (except B. fragilis)–71%, Clostridium spp.-- 29.2%, Streptococcus spp.--16.7%, compared to the results of Solomkins and associates in 2006 (5) we can see that we have same results regarding the coagulase-negative Staphylococcus as most commonly isolated gram-positive bacteria. Staphylococcus aureus was also isolated in 7/58 (12.6%) isolates, while Enterococcus faecalis / faecium in 2/58 (3.44%) isolates of the results according to the above authors. Since gram-negative bacteria in our study the most commonly isolated bacterium is Escherichia coli in 9/58 (15.51%), Pseudomonas aeruginosa and Klebsiella pneumoniae in 6/58 (10.34%), which is consistent with the results Solomkins and associates from 2006 (5) in which Escherichia coli was isolated in 71.3% of isolates be-
longing to the aerobic and facultative anaerobic bacteria.

Of anaerobic bacteria in our study we had matched the results with Solomkins and associates in 2003 (12) because we had the highest percentage of *Bacteroides species* isolated in 3/58 (5.17%) isolates and *Clostridium* sp. in a 1/58 (1.72%). In addition to these results it is important to emphasize that in our study even 20/78 (25.64%) isolates were not pathogenic bacteria.

According Solomkins and Edlund in 2006 (5) as a common cause of prolonged secondary peritonitis in immunocompromised patients, was the yeast *Candida albicans*, which in our study was isolated in 1/58 (1.72%) isolates.

Rossi and colleagues in 2006 (16) were in the Smart study on monitoring of antimicrobial resistance of isolates in patients with intraabdominal infections found that the resistance of *Enterobacteriaceae* to fluoroquinolones (ciprofloxacin) was 74.39%. The percentage of resistant *Escherichia coli* to ciprofloxacin was 17.3% and 28.9% of *Pseudomonas aeruginosa* isolates. In our study of *Escherichia coli* was resistant to ciprofloxacin in 41.7% of isolates, and *Pseudomonas aeruginosa* in 70% of isolates, probably due to the wide use of fluoroquinolones in both the primary and in secondary out hospital care.

Unlike the aforementioned studies Umgelter and associates from 2009 (17) in their studies of the application of first-line antibiotic therapy in patients with spontaneous bacterial peritonitis, found that the most common pathogens were *Escherichia coli* in 16.33% and the *Enterococcus faecalis* in 9.9% of respondents. *Escherichia coli* was resistant to cepotaxime in 33.3% of isolates, to amoxicillin/clavulanate in 38.6% of isolates, both isolates were resistant to ciprofloxacin in 45.2%.

In our study *Escherichia coli* was resistant to cepotaxime in 30.77% of the isolates, and amoxicillin/clavulanate in 43.48% of isolates. *Escherichia coli* and *Enterococcus faecalis* were resistant to ciprofloxacin in the range between 41.67 to 33.33% of the isolates.

Based on comparisons of our results with the results Umgelter and associates in 2009, it is seen that the resistance of our isolates were nearly similar to their results because the study was performed in a hospital, and research results of Rossi and colleagues refer to several meta-analysis of randomized trials. Microbiological isolates and their sensitivities were used to assess efficacy included empirical antibiotic therapy in surgical patients with peritonitis. Based on the results of microbiological findings in 50/60 (83.33%) of respondents empiric antibiotic therapy was appropriately involved, and in 50/60 (16.67%) was not adequately included, and it was changed on the basis of microbiological findings.

5. CONCLUSION
After microbiological processing of the content of the operative field, wound swabs and smears of drainage most commonly isolated are gram-positive cocci coagulase-negative *Staphylococci* in 18.92% (11/58), followed by *Staphylococcus aureus* in 7/58 (12.6%), and most gram-negative bacteria: *Escherichia coli* in 9/58 (15.51%), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in 6/58 (10.34%). Results of the sensitivity of isolated bacteria were included to facilitate justification of empirical antibiotic therapy and the change of inadequate empirical therapy in patients with peritonitis. On the basis of microbiological findings in 50/60 (83.33%) of respondents empiric antibiotic therapy was appropriately involved, and in 50/60 (16.67%) of respondents had not been adequately involved and changed due to results of microbiological findings.

REFERENCES