Original Research Article

Study of bacterial flora and their antibiotic sensitivity in peritonitis of various causes

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ABSTRACT

Background: Peritonitis is a common emergency encountered by surgeons the world over. Despite a better understanding of pathophysiology, advances in diagnosis, surgery, antimicrobial therapy and intensive care support, peritonitis remains a potentially fatal affliction. Intra-abdominal sepsis is an important cause of mortality and morbidity. The treatment is based on rapid fluid resuscitation, initiation of antibiotic therapy and surgical intervention. The antibiotic chosen must cover the most frequently expected bacterial species depending upon the site of perforation. Objectives of the study were to identify the type of organism present in bowel perforation and their sensitivity pattern to different antibiotics. A guideline will be framed for advising antibiotics to be used for different kinds of perforation.

Methods: This was a prospective study of one year on 50 patients of secondary peritonitis due to bowel perforation, conducted in Amaltas Institute of Medical Sciences, Dewas.

Results: This study included 50 patients with an average age of 36 years (range: 3 days-75 years). There were 40 males and 10 females. The mean duration of hospitalization was 10.6 days (range: 3-25 days) with predominant site of perforation was ileum. E. coli emerged as main pathogenic microbe even in site specific culture, was closely followed by Klebsiella. A combination of third generation cephalosporins with sulbactam and metronidazole has been the most promising therapy to treat secondary bacterial peritonitis due to bowel perforation. It needs to be emphasized that although the sensitivity studies reveal an edge for meropenem over ceftazidime sulbactam, yet the preference of cephalosporin with sulbactam over meropenem is justified, considering the economic constraints and with a suitable foresight, to keep meropenem as a reserve drug because trends indicate that our microbes are fast becoming resistant to the promising combination of third generation cephalosporin with sulbactam and metronidazole.

Conclusions: This study suggests that the current recommended empirical antibiotics need to be reassessed.

Keywords: Antibiotic sensitivity, Bacterial flora, Intra-abdominal sepsis, Peritonitis

INTRODUCTION

On the basis of source and nature of the microbial contamination peritonitis can be classified as primary, secondary and tertiary. Primary peritonitis is infection, often monomicrobial, of the peritoneal fluid without visceral perforation. Secondary peritonitis arises subsequent to loss of integrity of a hollow viscus and is
SBP is defined as a monomicrobial infection of the ascitic fluid, which is not accompanied by a definite evidence of a surgically treatable origin.\textsuperscript{3,6} The infection occurs following a translocation or hematogenous dissemination of the intestinal flora. Intestinal bacterial overgrowth can also exacerbate the condition.\textsuperscript{3,5} Studies have indicated that gram-negative Enterobacteriaceae such as \textit{Escherichia coli} (\textit{E. coli}) was the most common isolated organisms in SBP.\textsuperscript{3,5,7} Diagnosis of SBP is established by an elevated ascitic fluid polymorphonuclear leukocyte (PMNL) count (\geq 250 cells/mm\textsuperscript{3}).\textsuperscript{3,6} Some studies suggest that the type and the etiology of SBP have been changing in the recent years. Involvement with gram-positive bacteria and increased frequency of multiple antibiotic resistant bacteria are evidences that support this viewpoint.\textsuperscript{3-10} Because of anatomy of the peritoneal cavity, there is rapid absorption of bacterial endotoxin and inflammatory mediator into the systemic circulation hence in generalized peritonitis can lead to a cascade of infection, sepsis, multisystem organ failure (MSOF) and death, if not treated in a timely manner. Portal hypertension, splanchnic vasodilatation and activation of the renin-angiotensin cascade leads to sodium and water retention and fluid overflow into the peritoneal cavity.\textsuperscript{1}

The treatment is based on rapid fluid resuscitation, initiation of antibiotic therapy and surgical intervention. The antibiotic chosen must cover the most frequently expected bacterial species depending upon the site of perforation. Initially a broad-spectrum antibiotic is chosen while the microbiological results are pending. But despite of the diagnostic, therapeutic and surgical progress and availability of large variety of antibiotics, intra-abdominal sepsis remains an important cause of morbidity and mortality.

Particularly in patients who have multiple pre-morbid conditions present to the hospital after a significant delay or those who are immune compromised. Various studies including Misdell et al found that in appropriate initial antibiotic coverage was highly associated with persistent infection.\textsuperscript{11} Hence, this study was done to study various organism found in cases of perforation peritonitis of difference sites of gastrointestinal tract and their sensitivity pattern, so as to form a recommendation for most effective empirical antibiotic regimen, so as to reduce morbidity and mortality. The study was done to identify the type of organism present in bowel perforation and their sensitivity pattern to different antibiotics. A guideline will be framed for advising antibiotics to be used for different kinds of perforation.

**METHODS**

This study was a prospective study in 50 patients with peritonitis due to bowel perforations, completed in one-year duration at a tertiary care teaching hospital, Indore, Madhya Pradesh. This study was included the patients of all age groups. All patients with stomach, small bowel, large bowel, and appendicular perforations of various etiologies like peptic ulcer, UC, enteric perforation and perforation due to blunt injury abdomen.

**Inclusion criteria**

Patients with co-morbid condition like diabetes mellitus, chronic obstructive pulmonary disease, ischemic heart disease, hypertension, immunosuppressed had also been included in the study.

**Exclusion criteria**

Exclusion criteria were patients with penetrating injuries or patients with biliary leak, perforation may be due to bowel infarction or gangrene.

All patients in the present study were diagnosed as primary peritonitis, because there was no contamination with bowel flora. After documentation of perforation on the basis of history, physical examination, X-ray, USG, CT, or as an intra-operative finding were captured. Patient confirmed with diagnosis of perforation peritonitis were resuscitated with intravenous fluid and stabilizing the patient vitals were planned for emergency laparotomy and taken up for surgery after getting consent from the patient and his/her attenders. Peritoneal aspirate was collected in sterile container at the time of laparotomy and was immediately sent for the culture and sensitivity report to microbiology department. Antibiotic was changed according to sensitivity report obtained.

**Intraoperative procedure**

Emergency laparotomy done using midline incision and peritoneal fluid was obtained from confirmed non-traumatic cases and sent for aerobic microbiological culture. Following which perforation closure is done using vicryl with live omental patch and abdomen is closed after keeping abdominal drains.

**Post-operative case**

Following surgery patient were given routine postoperative care with intravenous fluids and antibiotics.
Peritoneal fluid culture reports were followed up and the isolated organisms were tested for antimicrobial sensitivity by Kirby-Bauer disc diffusion method using ampicillin, amikacin, ciprofloxacin, ceftriaxone and cotrimoxazole and the culture reports were obtained. Antibiotics were changed according to the sensitivity pattern of organism grown in the culture.

**Antibiotic susceptibility testing**

Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. Antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, UK). The organisms were tested against erythromycin (10µg), penicillin G, gentamicin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), ceftriaxone (30 µg). Amoxicillin/clavulanic acid (30 µg) and amikacin (30 µg). Growth inhibition zone diameters were measured in millilitres and results interpreted as recommended by the Clinical laboratory standards institute (CLSI) guidelines 2013.

**Minimal inhibitory concentration (MIC) determination**

Broth microdilution technique was performed using nutrient broth to determine MIC. For the interpretation of MIC, breakpoints were referred from CLSI guidelines 2013.

**RESULTS**

This study included 50 patients with average 36 years, range (3 days-75 years). There were 40 males and 10 females. The mean duration of hospitalization was 10.6 days (3-25 days) Table 1 with predominant site of perforation was ileum (Table 2).

**Table 1: Demographic data and clinical characteristics of the study participants.**

| Average age | 36 years |
|-------------|----------|
| Range       | (3 days-75 years) |
| Sex M/F     | 40/10 (4:1) |
| Mean duration of hospitalization | 10.6 days (Range 3-25 days) |

In this study E. coli was the most common organism isolated followed by Klebsiella (Table 3). Only 50 patients (100%) had cultures taken intra/postoperatively. Eight of these were reported as no growth (16%), and none of the culture specimens were "lost" before culture. The most common bacteria identified were E. coli (50%), Klebsiella (24%) and Streptococci Group D (12%) Table 3.

Among the 50 peritonitis cases, mainly 10 (20%) gastric, 10 (20%) duodenal, ileal 14 (28%) and 05 (10%) perforation related cases Table 2. An additional 06 patients (12%) had perforated colonic lesions from either diverticular disease or other colonic pathology (e.g., perforated colon carcinoma, cecal perforation from distention, etc.) Table 2.

**Table 2: Anatomical site-specific distribution of perforation (n=50).**

| Site of perforation | No. of patients | Percentage |
|---------------------|----------------|------------|
| Gastric             | 10             | 20         |
| Duodenal            | 10             | 20         |
| Jejunal             | 05             | 10         |
| Ileal               | 14             | 28         |
| Appendicular        | 05             | 10         |
| Caecum              | 03             | 06         |
| Colon               | 03             | 06         |
| Total               | 50             | 100        |

Among the "other cases identified in Table 2 were perforations of the small intestine, perforated pseudocysts, perforated necrotic intestine, (thought to be from other than vascular occlusion), perforated duodenum, perforated gastric ulcer cases and delayed recognition of two stab wounds involving the intestine.

**Table 3: Peritoneal isolates at laparotomy.**

| Organism                  | Number of patients | %  |
|---------------------------|--------------------|----|
| **Gram negative organism**|                    |    |
| E. coli                   | 25                 | 50 |
| Klebsiella                | 12                 | 24 |
| Pseudomonas               | -                  | -  |
| Proteus                   | -                  | -  |
| Citrabacter               | 2                  | 4  |
| Flavobacter               | 1                  | 2  |
| **Gram positive organism**|                    |    |
| Streptococci Group D      | 6                  | 12 |
| Staphylococcus aureus     | 4                  | 8  |
| Yeast                     |                    |    |
| Candida                   | 5                  | 10 |
| Sterile                   | 8                  | 16 |

In few cases there was fungal growth or contamination (10%). The identified bacterial species were consistent with other reports of peritonitis cultures in the literature.

E coli and Klebsiella isolated in almost all cases have been uniformly sensitive to meropenem and almost 90% of them have been sensitive to third generation cephalosporin with sulbactam.

Klebsiella isolates have been more sensitive to piperacillin with tazobactam and quinolones than E coli, while their sensitivity to other drugs have been less than 50% Table 5.

E coli and Klebsiella isolated in almost all cases have been uniformly sensitive to meropenem and almost 90%
of them have been sensitive to third generation cephalosporin with sulbactam.

Table 4: Site specific distribution of single organism.

| Site of perforation | Organism          | E. coli | Klebsiella | Citrobacter | Staph aureus | Streptococcidi D | Favobacter | Candida | Sterile | Multiple organism |
|---------------------|-------------------|---------|------------|-------------|---------------|------------------|------------|---------|---------|------------------|
| Gastric (10)        |                   | 1       | -          | -           | -             | -                | 4          | 4       | 1       |                  |
| Duodenum (10)       |                   | -       | 1          | 2           | 2             | 1                | 1          | 2       | 1       |                  |
| Jejunal (5)         |                   | -       | -          | 1           | -             | -                | -          |         | -       | 3                |
| Ileal (14)          |                   | 6       | 1          | 1           | -             | -                | -          | -       | 6       |                  |
| Appendicular (5)    |                   | 3       | -          | -           | -             | -                | -          | 2       | -       |                  |
| Ceccal (3)          |                   | 2       | -          | -           | -             | -                | -          |         | -       | 1                |
| Colonic (3)         |                   | 1       | 1          | -           | -             | -                | -          | -       | -       | 1                |
| Gastric (10)        |                   | 1       | -          | -           | -             | -                | 4          | 4       | 1       |                  |

Table 5: Antibiotic sensitivity pattern of isolated organisms.

| Isolated organism | Mox-clav | Cefazidine | Ceftrioxone | Cefaparzone + Sulbactam | Piperacillin + Tazobactam | Ticarcillin + Clavulanic acid | Meropenem | Quinolone | Amikacin |
|-------------------|----------|------------|-------------|-------------------------|---------------------------|-------------------------------|-----------|-----------|----------|
| E. coli           | 2        | 6          | 6           | 20                      | 12                        | 2                             | 23        | 5         | 5        |
| Klbesiella        | -        | 4          | 4           | 9                       | 9                         | -                             | 12        | 5         | 2        |
| Strept-D          | 1        | 1          | -           | 2                       | -                         | -                             | 3         | -         | 1        |
| Staph aureus      | 1        | 1          | -           | 3                       | -                         | -                             | 3         | 1         | -        |
| Citrobacter       | -        | 1          | 1           | 2                       | 1                         | -                             | 2         | 2         | -        |
| Flavobacter       | -        | -          | -           | 1                       | 1                         | 1                             | 1         | 1         | -        |
| Candida           | -        | -          | -           | -                       | -                         | -                             | -         | -         | -        |

Klebsiella isolates have been more sensitive to piperacillin with tazobactam and quinolones than E coli, while their sensitivity to other drugs have been less than 50%. Group A received a combination of third generation cephalosporins + sulbactam and metronidazole. Group B patients were given a combination of a quinolone and metronidazole. Group C received a combination of carbapenem or piperacillin and tazobactam along with metronidazole and comprised of patients with significant comorbid factors and compromised immune status. Group D included other patients where aminoglycosides, β-lactams, azeonam, amoxicillin, clavulanic acid and metronidazole were prescribed in various combinations.

Table 6: Comparison of outcome variables among patients receiving Group A, Group B, Group C and Group D antibiotics.

| Variable                   | Group A (n=34) | Group B (n=6) | Group C (n=4) | Group D (n=6) |
|----------------------------|---------------|---------------|---------------|--------------|
| Duration of hospitalization| 10.6          | 8             | 14.5          | 12.5         |
| Wound infection            | 3 (8.82%)     | 1 (16.67%)    | 3 (75%)       | 1 (16.67%)   |
| Wound dehiscence           | 2 (5.88%)     | -             | 1 (25%)       | -            |
| Deaths                     | 5 (14.4%)     | -             | 1 (25%)       | 1 (16.67%)   |

Group A: Third generation cephalosporin + sulbactam + metronidazole. Group B: Quinolone, Metronidazole. Group C: Carbapenem or piperacillin + Tazobactam + Metronidazole. Group D: Miscellaneous Third generation cephalosporins+ sulbactam, amikacin, metronidazole. Third generation cephalosporin + sulbactam, azeonam, metronidazole Mox Clav, Metronidazol.

Morbidity and mortality were studied in these groups. Wound infection was maximum (75%) in Group C while only 8.82% in Group A. The patients in Group B and Group D had wound infection rates of approximately 16% each. Mortality figures and incidence of wound dehiscence have also been the highest among patients of Group C, 25% each while patients in Group A and Group B each had about 15% mortality. Patients in Group A had nearly 6% incidence of wound dehiscence while there was...
The present study was done to evaluate 50 cases of secondary bacterial peritonitis. The prevalence of site of perforation, the most common organisms isolated and their sensitivity to antibiotics were studied. In the present study ileal perforation was the commonest (28%), followed by gastric (20%), duodenal (20%), jejunal (10%) and appendicular (10%). Colonic (6%) and caecal perforations (6%) were found in the least number of cases (Table 2). Sanjay Gupta and Robin Kaushik were analyzed for the site and cause of perforation and the mortality in secondary peritonitis, and observed that duodenal ulcer was the most commonly encountered perforation, followed by small bowel and appendicular perforations. Colonic perforations were uncommon. While Mosdell et al found appendicular perforation as most common cause for secondary peritonitis, also been confirmed by D Genne et al in their study.2,11,16

The results of culture and sensitivity of the peritoneal fluid at laparotomy revealed that organisms isolated were E. coli (50%) followed by Klebsiella (24%), other organisms found were streptococci group D (12%), staphylococcus aureus (8%), Citrobacter (4%), and Candida in 10% patients and 16% patients revealed sterile culture. None of the 50 patients of peritonitis showed anaerobic isolation in present study due to non-inclusion of suitable culture media for anaerobes and that seems to have become the major limitation of the present study. On the other hand, Solomkin et al and Mosdell et al11 reported the incidence of B. fragilis 22.8% and 44.5% respectively and clostridia isolation rates were 17.9% and 5.8% respectively. However, D Genne et al have confirmed E coli to be the most common microbe isolated from the peritoneal fluid cultures, in 26% cases followed closely by anaerobes in 25% of cases.14,16-18

E coli and Klebsiella isolated in almost all cases have been uniformly sensitive to meropenem and almost 90% of them have been sensitive to third generation cephalosporin with sulbactam. Klebsiella isolates have been more sensitive to piperacillin with tazobactam and quinolones than E coli, while their sensitivity to other drugs have been less than 50%. Group A received a
combination of third generation cephalosporins + sulbactam and metronidazole. Group B patients were given a combination of a quinolone and metronidazole. Group C received a combination of carbapenem or piperacillin and tazobactam along with metronidazole and comprised of patients with significant comorbid factors and compromised immune status. Group D included other patients where aminoglycosides, β-lactams, adenoma, amoxicillin, clavulanic acid and metronidazole were prescribed in various combinations. In that way, metronidazole as an ant anaerobic drug was used in all patients. No antifungal therapy was included in the regimens because existing literature suggests that Candida normally does not appear to be pathogenic and does not require systemic therapy.19

Krobot et al., in a multicenter study of 162 patients with perforated appendicitis, found that appropriateness of initial parenteral antibiotic therapy was a predictor of clinical success and length of stay.20 Similarly, they demonstrated a high risk of post-operative infections in patients with inadequate empirical treatment.

Morbidity and mortality were studied in these groups. Wound infection was maximum (75%) in Group C while only 8.82% in Group A. The patients in Group B and Group D had wound infection rates of approximately 16% each. Mortality figures and incidence of wound dehiscence have also been the highest among patients of Group C, 25% each while patients in Group A and Group B each had about 15% mortality. Patients in Group A had nearly 6% incidence of wound dehiscence while there was no recorded wound dehiscence in patients in Group B and D.

The differences in mortality and morbidity rates and incidence of wound dehiscence among the different groups were not found to be statistically significant, probably because the study included less number of patients. However, the wound infection rate was higher in patients belonging to Group C and was found statistically significant (p≤0.01). Mean duration of hospital stay was analyzed in the various groups and was found to be highest in Group C and lowest in Group B and the difference was found to be clinically significant (p≤0.05).

The present study finally revealed that a combination of third generation cephalosporins with sulbactam and metronidazole has been the most promising therapy to treat secondary bacterial peritonitis due to bowel perforation. It needs to be emphasized that although the sensitivity studies reveal an edge for meropenem over cefaperazone sulbactam, yet the preference of cephalosporin with sulbactam over meropenem is justified, considering the economic constraints and with a suitable foresight, to keep meropenem as a reserve drug because trends indicate that our microbes are fast becoming resistant to the promising combination of third generation cephalosporin with sulbactam and metronidazole.

CONCLUSION

The present study demonstrates that half of the patients included in the study showed a preponderance of E. coli as the main pathogenic microbe, closely followed by Klebsiella in half of the remaining 50% cases. E. coli has also emerged as the predominant organism implicated in the pathogenesis even if we consider a site-specific culture. Colonic perforations were uncommon. E coli and Klebsiella isolated in almost all cases have been uniformly sensitive to meropenem and almost 90% of them have been sensitive to third generation cephalosporin with sulbactam. This study suggests that the current recommended empirical antibiotics need to be reassessed. The empirical treatment of SBP should be adapted to the local epidemiological pattern of antibiotic susceptibility, in order to decrease the morbidity and mortality associated with SBP.

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