

Original Research Article

The anatomical site of perforation peritonitis and their microbiological profile: a cross-sectional study

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Received: 03 January 2020

Revised: 07 March 2020

Accepted: 12 March 2020

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ABSTRACT

Background: Initiation of early appropriate antibiotic therapy influences the outcome of perforation peritonitis, which otherwise is delayed till culture reports are available. The knowledge of microbial profile and sensitivity of peritoneal fluid culture with respect to the anatomical site of perforation peritonitis will help in initiation of early appropriate antibiotic therapy in the post-operative period.

Methods: A cross-sectional study conducted from January 2017 to December 2017 where intraoperative peritoneal fluid sample in patients of perforation peritonitis was subjected to culture (aerobic and anaerobic) and sensitivity and results analysed with respect to anatomical site of perforation.

Results: 50 patients were studied. The most common site of perforation was ileum (32%) followed by appendix (18%) and stomach (18%). In aerobic culture, the culture positivity rate was highest in colonic perforation (100%) and least in gastric perforation (44.4%). The most common organism isolated in all sites of perforation peritonitis was *E. coli* followed by *Klebsiella spp.* In anaerobic culture, although facultative anaerobes were isolated, no strict anaerobe was isolated. The most sensitive antibiotics covering all isolated organisms were gentamycin (p=0.006), colistin (p=0.018), piperacillin and tazobactam (p=0.022).

Conclusions: The predominant differential normal flora according to site of gastrointestinal tract was not reflected in the peritoneal fluid culture of patients with perforation peritonitis and *E. coli* was the most common organism isolated in all sites of perforation peritonitis. The antibiotic sensitivity profile showed the increasing resistance against third generation cephalosporins. Aminoglycosides, piperacillin and tazobactam, meropenem and colistin showed a significant antimicrobial activity against organisms isolated from cases of perforation peritonitis.

Keywords: Antibiotic sensitivity, Microbiological profile, Perforation peritonitis, Peritoneal fluid culture

INTRODUCTION

Intra-abdominal infections are one of the most common clinical problems in surgical practice and range from localized to generalized peritonitis.¹ Of the three types of peritonitis, secondary peritonitis is most common form originating from bowel pathologies such as perforation or ischaemia.² It is one of the most common surgical emergencies in the tertiary care centres in India with most of the patients presenting late in the course of disease. The mortality rates of intraabdominal infections

significantly depends on the anatomical site of perforation which in turn influences the source of the infection. Several studies have reported a mortality rate of 3-28% in gastroduodenal perforation, 20-38% for small bowel perforation and 20-45% in cases of large bowel perforation.³ The most accepted protocol of treatment for patients with secondary peritonitis due to hollow viscus perforation is resuscitation of the patient, removing the source of contamination as soon as possible along with the appropriate antimicrobial therapy.²

The knowledge of the microbial distribution according to anatomical site of perforation is essential, because understanding of the regional distribution and characteristics of bacteria will ensure an optimal empirical choice of antibiotic in these patients. It can be obtained by culture of peritoneal fluid obtained intraoperatively. Although some guidelines on empirical antibiotics for intraabdominal infections have been published, most studies on causative bacteria are quite old and were performed before the 2000s.

The purpose of this study is to evaluate the microbiological distribution and their sensitivity profile according to anatomical site of perforation, as identified from peritoneal fluid cultures in patients of perforation peritonitis.

METHODS

This descriptive cross-sectional study was conducted in the Department of Surgery and Microbiology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi. As it was pilot study and previous prevalence of perforation peritonitis in New Delhi was not known, sample size was not calculated. Previous year data of our institute was analysed. Average number of cases of perforation peritonitis treated by one surgical unit in previous year was found to be 50. So it was decided to study 50 cases. The study included patients presenting with perforation peritonitis from January 2017 to December 2017. Patients with diabetes mellitus and other immuno-compromised states, penetrating abdominal injury, multiple anatomical sites of perforation, gynaecological cause of perforation peritonitis, those managed with preoperative intrabdominal drain insertion and patients aged less than 18 years were excluded. Written informed and voluntary consent was obtained. After thorough history and general physical examination, patients suspected to have perforation peritonitis underwent imaging with X ray abdomen supine and chest posteroanterior erect film with both domes of diaphragm to confirm the diagnosis. CT abdomen was done as per the merit of the case. Routine laboratory investigations including hemogram, random blood sugar, renal function tests, arterial blood gas analysis etc. as per patient requirements were done. Pre-operatively broad spectrum antibiotic therapy (Amoxicillin+clavulanic acid and metronidazole, single dose, intravenous) was initiated and patients were taken up for emergency exploratory laparotomy through a vertical midline incision. At laparotomy, as soon as the peritoneum was opened, peritoneal fluid (10ml) was obtained for microbiological culture and sensitivity and intraoperative findings was noted in relation to site of perforation.

Collection and transport of specimen

A peritoneal fluid sample (10 ml) was collected with disposable needle and plastic syringe during surgery. For

isolation of strict anaerobes, 5ml of the fluid was introduced and transported in anaerobic Robertsons cooked meat (RCM) broth at room temperature. Rest of the 5 ml fluid was transported in the syringe for microscopy and isolation of aerobic micro-organisms.

Isolation and identification of microorganisms

Specimen was subjected to direct Gram's staining. For isolation of bacteria, specimen was inoculated on Blood agar and MacConkey agar and incubated overnight at 35 degree centigrade. For anerobic bacterial isolation additional set was incubated for 48 hours in anaerobic jar. RCM incubated anerobically, was observed for turbidity for 5 days. If found turbid, the broth was subjected to Gram's staining and twin set culture plates (aerobic and anaerobic) as described above.

The bacterial identification was carried out through conventional biochemical tests. Antimicrobial susceptibility testing was performed through disc diffusion method. The primary outcome of the study was to evaluate the microbiological profile in perforation peritonitis with respect to anatomical site of perforation. Secondary outcome was to determine the antibiotic sensitivity profile of microbes cultured from peritoneal fluid to commonly used antibiotics. Results was analysed using SPSS (version 17) software. For qualitative data, Chi-square test was used to observe the difference between two proportion for the paired values. For quantitative data, Student t - test was used, and data was expressed by the mean and SD of the difference between two means for paired observations.

RESULTS

50 patients were studied. The mean age of the patients in this study was 32.86±14.7 years and the median age was 30 years (range 18-65) (Table 1). The male:female ratio was 6.14:1 (Table 2).

Table 1: Age distribution.

Age groups (years)	Frequency	Percentage
18-27	20	40.0
28-37	15	30.0
38-47	7	14.0
48-57	2	4.0
58-67	6	12.0
Total	50	100.0
Mean±SD	32.86±14.17	
Median	30	
Min - Max	18 – 65	

The most common site of perforation was ileum (n=16) (32%) followed by appendix (n=9) (18%) and stomach (n=9) (18%) (Table 3).

Table 2: Sex distribution.

Sex	Frequency	Percentage
Female	7	14.0
Male	43	86.0
Total	50	100.0

Table 3: Anatomical site of perforation.

Anatomical site of perforation	Frequency	Percentage
Stomach	9	18.0
Duodenum	5	10.0
Jejunum	7	14.0
Ileum	16	32.0
Caecum	1	2.0
Appendix	9	18.0
Ascending colon	1	2.0
Rectum	1	2.0
Gallbladder	1	2.0
Total	50	100.0

Aerobic culture

Among 9 cases of stomach perforation, 5 cases (55.6%) were culture negative and 4 cases (44.4%) were culture positive. Out of culture positives, *E. coli* was isolated in 2 cases (50%), *Acinetobacter spp.* in 1 case (25%), *Klebsiella spp.* in 1 case (25%) (Table 4).

Table 4: Stomach perforation: aerobic culture.

Stomach perforation (n = 9)		
Aerobic culture	Number	Percentage
Culture negative	5	55.6
Culture positive	4	44.4
Organism		
<i>Acinetobacter spp.</i>	1	25.0
<i>E.coli</i>	2	50.0
<i>Klebsiella spp.</i>	1	25.0

Among 5 cases of duodenal perforation, 2 cases were culture negative (40%) and 3 cases were culture positive (60%). Out of culture positives, *E. coli* was isolated in 2 cases (66.7%) and *Klebsiella spp.* was isolated in 1 case (33.3%) (Table 5).

Among 7 cases of jejunal perforation, 1 case was culture negative (14.3%) and 6 cases were culture positive (85.7%). Out of culture positives, *E. coli* was isolated in 4 cases (66.7%). *Klebsiella spp.*, *Citrobacter spp.* and *Enterobacter spp.* were isolated in 1 case each (16.7%). One case was of polymicrobial infection with isolation of *E. coli* and *Klebsiella spp.* (Table 6).

Among 16 cases of ileal perforation, 6 cases were culture negative (37.5%) and 10 cases were culture positive (62.5%). Out of culture positives, *E. coli* was isolated in 9

cases (90%) and *Klebsiella spp.* was isolated in 2 cases (20%). One case was polymicrobial in which *E. coli* and *Klebsiella spp.* both were isolated (Table 7).

Table 5: Duodenal perforation: aerobic culture.

Duodenal perforation (n=5)		
Aerobic culture	Number	Percentage
Culture negative	2	40.0
Culture positive	3	60.0
Organism		
<i>E. coli</i>	2	66.7
<i>Klebsiella spp.</i>	1	33.3

Table 6: Jejunal perforation: aerobic culture.

Jejunal perforation (n= 7)		
Aerobic culture	Number	Percentage
Culture negative	1	14.3
Culture positive	6	85.7
Organism		
<i>Citrobacter spp.</i>	1	16.7
<i>Enterobacter spp.</i>	1	16.7
<i>E. coli</i>	4	66.7
<i>Klebsiella spp.</i>	1	16.70

Table 7: Ileal perforation: aerobic culture (n=16).

Aerobic culture	Number	Percentage
Culture negative	6	37.5
Culture positive	10	62.5
Organisms		
<i>E. coli</i>	9	90.0
<i>Klebsiella spp.</i>	2	20.0

Caecal perforation (n=1) was culture positive (100%) and *E. coli* was isolated. Among 9 cases of Appendicular perforation, 1 case was culture negative (11.1%) and 8 cases were culture positive (88.9%). Out of culture positives, *E. coli* was isolated in all cases (100%) (Table 8).

Table 8: Appendicular perforation: aerobic culture (n=9).

Aerobic culture	Number	Percentage
Culture negative	1	11.1
Culture positive	8	88.9
Organism		
<i>E.coli</i>	8	100.0

There was one case each of ascending colon and rectum perforation. Peritoneal fluid was culture positive (100%) and *E. coli* was isolated in both. Gallbladder perforation (n=1) was culture negative. Overall, most common organism isolated was *E. coli* (82.4%) (28 cases). *Klebsiella spp.* was isolated in 5 cases (14.7%). *Citrobacter spp.*, *Enterobacter spp.* and *Acinetobacter*

spp. was isolated in one case each (2.9%). 2 cases were polymicrobial with isolation of *E. coli* and *Klebsiella spp.* both in each case (Table 9).

Table 9: Overall aerobic culture.

Aerobic culture	Frequency	Percentage
Culture negative	16	30.8
Culture positive	34	69.2
Culture positive (34)		
<i>Citrobacter spp.</i>	1	2.9
<i>E.coli</i>	28	82.4
<i>Enterobacter spp.</i>	1	2.9
<i>Klebsiella spp.</i>	5	14.7
<i>Acinetobacter spp.</i>	1	2.9

Table 10: Aerobic culture: percentage antibiotic sensitivity.

Drugs	Aerobic culture					P value
	<i>Acinetobacter spp.</i>	<i>Citrobacter spp.</i>	<i>E. coli</i>	<i>Enterobacter spp.</i>	<i>Klebsiella spp.</i>	
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
Amoxyclav	0 (0.0)	0 (0.0)	3 (10.7)	0 (0.0)	1 (20.0)	0.773
Piperacillin and Tazobactam	1 (100)	1 (100)	11 (39.3)	0 (0.0)	2 (40.0)	0.022
Imipenem	0 (0.0)	0 (0.0)	10 (35.7)	0 (0.0)	3 (60.0)	0.056
Meropenem	1 (100)	0 (0.0)	12 (42.9)	0 (0.0)	2 (40.0)	0.031
Cephalexin	0 (0.0)	0 (0.0)	2 (7.1)	0 (0.0)	0 (0.0)	0.926
Cefotaxime	0 (0.0)	1 (100)	6 (21.4)	0 (0.0)	2 (40.0)	0.076
Ceftriaxone	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)	1 (20.0)	0.605
Ciprofloxacin	1 (100)	0 (0.0)	5 (17.9)	0 (0.0)	1 (20.0)	0.105
Gentamycin	0 (0.0)	1 (100)	15 (53.6)	0 (0.0)	3 (60.0)	0.006
Amikacin	1 (100)	1 (100)	10 (35.7)	0 (0.0)	2 (40.0)	0.027
Colistin	0 (0.0)	0 (0.0)	12 (42.9)	1 (100)	3 (60.0)	0.018

DISCUSSION

This study aimed to evaluate the microbiological profile of peritoneal fluid cultures in patients of perforation peritonitis with respect to anatomical site of perforation and to determine the antibiotic sensitivity profile of the microbes cultured from peritoneal fluid.

The mean age of the patients in this study was 32.86±14.7 years, similar to other studies which have been done in India.⁴⁻⁶ Perforation peritonitis is common in middle age group in India, probably due to smoking, alcoholism and higher incidence of abdominal tuberculosis and enteric perforation.⁷⁻¹²

The male:female ratio was 6.14:1, similar to that observed in other studies.^{3,4,13} In India, perforation peritonitis is common in males probably due to increased incidence of smoking, alcoholism. Male preponderance is also seen in abdominal tuberculosis enteric perforation

Anaerobic culture

Although facultative anaerobes were isolated, no strict anaerobic organism was isolated in any site of perforation peritonitis.

Antibiotic sensitivity profile

In aerobic culture, overall most sensitive antibiotic was gentamycin with p value of 0.006. Other antibiotics with significant sensitivity were colistin (p value - 0.018), piperacillin+tazobactam (p value - 0.022), amikacin (p value - 0.027), meropenem (p value -0.031) (Table 10).

which are the most common causes of perforation peritonitis in India.^{7,11,15,19,14}

The most common site of perforation in our study was Ileum similar to that observed by Yadav et al, a study from North India.⁴ This observation can be attributed to higher incidence of ileocaecal tuberculosis and typhoid, which are the more common causes of Ileal perforation in North India.^{4,16} The most common site of perforation in a study by Vishnu et al and Ravishankar et al was Gastroduodenal (51% and 94% respectively), both of which were studies from South India.^{3,13} Other studies also have shown that in South India, the most common site of perforation peritonitis is gastroduodenal while in North India, small bowel is the most common site of perforation peritonitis.^{16,17-22} This may be due to high incidence of peptic ulcer disease and lower incidence of abdominal tuberculosis and typhoid perforation in South India as compared to North India.

Aerobic culture

In gastric perforation, culture positivity was 44.4% (n=4) and *E. coli* was the most common organism isolated similar to that observed by Vishnu et al.³ The high percentage of culture negativity in gastric perforation can be attributed to high acidity of stomach due to which most microorganisms have survival difficulty.²³

In duodenal perforation, culture positivity was 60% (n=3) and the most common organism isolated was *E. coli*, similar to study by Punamiya et al.²⁴ The high percentage of culture negativity can be attributed to low microbial load (10^3 - 10^6) in duodenum compared to lower small bowel and colon.²⁵ The normal flora in duodenum is predominantly gram positive Cocci (*Enterococci*) and gram positive rods (*Lactobacilli*) but *E. coli* and *Klebsiella spp.* was isolated in Duodenal perforation similar to other studies.²⁶

In jejunal perforations, culture positivity was 85.7% (n=6) and *E. coli* was isolated as most common organism. In Ileal perforations, culture positivity was 62.5% (n=10) and *E. coli* was the most common organism isolated. In a study by Vishnu et al, culture negativity was 38% for small bowel perforations (jejunum and ileum combined). Out of culture positives, *E. coli* was major organism isolated. In our study, similar culture negativity rates was observed with respect to ileum. But jejunal perforations had much higher culture positivity rate. Normally the flora of jejunum and ileum is predominantly Gram negative bacilli (*Enterobacteriace*) and the same were isolated in cultures of jejunal and ileum perforation peritonitis with *E. coli* as the dominant isolate in both site of perforation peritonitis.²⁵

In appendicular perforation, culture positivity was 88.9% (n = 8) and *E. coli* was isolated in all cases (100%). In a study by Vishnu et al, 29% were culture negative and out of culture positives, *E. coli* was isolated in 47.2% and other species in the rest.³ In a study by Boueil et al, 26% were culture negative and out of culture positives, *E. coli* was isolated in 81% and others in rest. In our study, culture negativity was much lesser as compared to other studies.²⁷ Normal flora in appendix is of predominantly Gram negative bacilli in Appendix and the most common aerobic organism isolated in acute appendicitis is *E. coli*. This correlates with our study where *E. coli* was the only predominant isolate in the culture positives.^{25,28} In case of caecal, ascending colon and rectal perforation, culture positivity was 100% (n=1) and *E. coli* was isolated in all cases (100%), similar to study by Vishnu et al.³ This high rate of culture positivity can be attributed to higher load of microbial flora in large intestine.²⁵ Anaerobic organisms like bacteriodes predominate normally in flora of colon. Among aerobic flora, *E. coli* is the predominant organism.²⁹ This correlates with the results of our study where *E. coli* was predominantly isolated in culture of peritoneal fluid in colonic perforation.

Gall bladder perforation is mostly a complication of acute cholecystitis (calculous and acalculous) and the most common organism isolated in acute cholecystitis is *E. coli*.³⁰ We found only one case of gall bladder perforation and it was culture negative.

It has been observed that the bacterial flora of stomach is almost negligible due to low pH, the bacterial count in Duodenum is 10^3 - 10^6 per gram, in Jejunum and proximal Ileum is 10^5 - 10^8 per gram, in lower Ileum and Caecum is 10^8 - 10^{10} per gram, in colon is 10^{11} per gram.²⁵ This shows as we go from proximal to distal in gastrointestinal tract the load of microorganisms increase and it correlates with our study in which increase in culture positivity is noted as the level of perforation moves distally from stomach to rectum.

It has been seen that in duodenum and proximal ileum, *Enterococci* (Gram positive cocci) and *Lactobacillus spp.* (Gram positive bacilli) are the predominant organisms. In distal ileum and caecum, *Enterobacteriaceae* (Gram negative bacilli) predominate. In colon, anaerobes predominate (96-99%) of which *Bacteroides spp.* is most common.²⁵ But in our study, this differential predominant normal flora according to site of gastrointestinal tract was not reflected in the peritoneal fluid culture of patients with perforation peritonitis and *E. coli* was the most common organism isolated in all sites of perforation peritonitis. *E. coli*, gram negative bacilli was the predominant isolate from all sites of perforation, as observed in other mentioned studies.

Anaerobic culture

In our study, no strict anaerobic organism was isolated from any site of perforation peritonitis. In a study by Vishnu et al, no anaerobic organism was isolated in 18 cases of lower GI perforation tested for them. In a study by Jang et al, strict anaerobic organisms, *Bacteroides spp.* was isolated in 5.5% and *B. fragilis* was isolated in 1.4% cases.^{2,3} This low yield of strict anaerobes can be attributed to fastidious nature of anaerobic organisms, the strict conditions needed for anaerobic culture. *Bacteriodes spp.*, predominant part of anaerobic flora in Colon is a Gram negative, non sporing strict anaerobe and slow to grow on culture media unless there is adequate carbon dioxide tension in the anaerobic apparatus.³¹

Antibiotic sensitivity profile

In our study, overall most sensitive antibiotic was Gentamycin with p value of 0.006. Other sensitive antibiotics with p value less than 0.05 were colistin (p=0.018), piperacilin+tazobactum (p value=0.022), amikacin (p=0.027), meropenem (p value=0.031). In a study by Vishnu et al, *E. coli* isolates were mostly sensitive to amikacin (94%) followed by ceftazidime (91%). *Klebsiella spp.* species were sensitive to cephalosporins, aminoglycosides and ciprofloxacin.³

In a study by Ravishankar et al, *E. coli* showed sensitivity to ceftriaxone in about 87.5% followed by ciprofloxacin and amikacin of about 81.25%.¹³ In case of *Klebsiella* spp., the sensitivity to ceftriaxone is 91.07%, followed by amikacin which is about 78% and Ciprofloxacin 73.9%. Both *E. coli* and *Klebsiella* spp. showed high resistance to ampicillin and cotrimoxazole. Organisms were sensitive in most cases to ceftriaxone followed by ciprofloxacin and amikacin.

In a study by Punamiya et al, *E. coli* was most common organism isolated maximum sensitivity was found to piperacillin and tazobactam (51%) followed by cefotaxime (49%) and cefoperazone (48%) and ceftazidime (25%).²⁴

In our study, we noticed that there has been significant resistance to third generation cephalosporins compared to rest of the studies. It is probably because the rest of the studies are done before the year 2000 and there has been rampant use of third generation cephalosporins during that time leading to the development of resistance. But similar sensitivity pattern like other studies was observed to Aminoglycosides like gentamycin and amikacin.

Thus we can suggest the judicious use of piperacillin and tazobactam and aminoglycoside (gentamycin, amikacin) as the first line drugs empirically and change of antibiotic appropriately according to the culture report later in cases of perforation peritonitis.

CONCLUSION

The predominant differential normal flora according to site of gastrointestinal tract was not reflected in the peritoneal fluid culture of patients with perforation peritonitis and *E. coli* was the most common organism isolated in all sites of perforation peritonitis. The antibiotic sensitivity profile showed the increasing resistance against third generation cephalosporins, which have been commonly in use empirically. However Aminoglycosides still have a significant sensitivity profile. Piperacillin and tazobactam, meropenem and colistin also showed a significant antimicrobial activity against organisms isolated from cases of perforation peritonitis.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Lohith P, Jindal RK, Ghuliani D, Rajshekar P. The anatomical site of perforation peritonitis and their microbiological profile: a cross-sectional study. *Int Surg J* 2020;7:1251-7.