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A STUDY TO EVALUATE THE MICROBIOLOGICAL PROFILE IN PATIENTS WITH PERFORATION PERITONITIS WITH RESPECT TO ANATOMICAL SITE OF PERFORATION

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ABSTRACT

Aim: The aim of the present study was to evaluate the microbiological profile in patients with perforation peritonitis with respect to anatomical site of perforation.

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Material & methods: A prospective observational cross-sectional study conducted from 2021 to 2022 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: Out of total 100 patients, 49% were between 18-45 year of age group, 38% were between 46-65 year & 13 % were more than 65 years of age. Out of 100 patients, 76% were male and 24% were female. 43 patients had gastric perforation. 39 patients had ileal perforation. 6 patients had appendicular perforation. 6 patients had caecal perforation. 3 patients had jejunal perforation. 43 patients were positive for E.coli. 20 patients were positive for Klebsiella pneumonia. 8 patients were positive for Pseudomonas aeruginosa. 4 patients were positive for Candida albicans. 5 patients were positive for Acinetobacter spp.

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. Aminoglycosides, piperacillin and tazobactum, 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

1. INTRODUCTION

Intra-abdominal infections are one of the most common clinical problems in surgical practice and range from localized to generalized peritonitis. The peritoneum is the largest and the most complex serous membrane in the body which forms a closed sac (i.e. coelom). A parietal layer of the peritoneum reflects onto the abdominal visceral organs to form the visceral peritoneum.[1] Hence creating a potential space between the two layers otherwise known as peritoneal cavity. Peritonitis is defined as inflammation of the serosal membrane that lines the abdominal cavity and the organs contained therein.[2] Peritonitis is often caused by introduction of an infection into the sterile peritoneal environment through perforation of bowel, such as ruptured appendix or colonic diverticulum. Of the three types of peritonitis, secondary peritonitis is most common

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form originating from bowel pathologies such as perforation or ischaemia. The first clinical description of perforation peritonitis was made by Crisp in 1843. [3]

The commonly encountered sites of perforation peritonitis in Asian countries are Ileum (proximal), gastroduodenal, stomach, appendix, jejunum or gall bladder.[4] The male: female ratio of perforation peritonitis cases was 6.14:1 in India, probably due to increased incidence of smoking, alcoholism among males.[5] The various causes of perforation peritonitis includes Peptic ulcer perforation, Malignancy, Crohn's disease, Meckel diverticulum, intestinal tuberculosis, Incarcerated hernia, Ischemic bowel, Diverticulitis, Ulcerative colitis, Appendicitis, Colonic volvulus and Amoebic colitis. Most of perforation peritonitis cases presents as acute abdomen with tachycardia, abdominal guarding, rigidity, distension and absent bowel sounds. Patients usually are in septicemia with low blood pressure necessitating urgent resuscitation and immediate surgical intervention. Septicemia and septic shock leading to sudden cardiac arrest remains the most common cause of death among the perforation peritonitis patients.[6] The mortality rate because of perforation peritonitis significantly depends on the anatomical site of perforation which in turn influences the source of the infection. Smoking, trauma, peptic ulcer disease (PUD), abdominal tuberculosis, indiscriminate antibiotic and nonsteroidal anti-inflammatory (NSAID) drugs are important risk factors for perforation peritonitis.[7]

Diagnosis is made clinically and confirmed by the presence of pneumoperitoneum on radiographs. The knowledge of the microbial distribution according to anatomical site of perforation peritonitis is essential which can be obtained by culture of peritoneal fluid obtained intraoperatively. 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 / gram, in Jejunum and proximal Ileum is 10^5 -10^8 /gram, in lower Ileum and Caecum is 10^8-10^{10}/gram, in colon is 10^{11}/gram. This shows as we go from proximal to distal in gastrointestinal tract, the load of microorganisms increases.[8] E. coli was the most common organism isolated in stomach. In distal ileum and caecum, Enterobacteriaceae (Gram negative bacilli) predominate. In colon, anaerobes predominate (96-99%) of which Bacteroides spp. is most common.[9]

The aim of the present study was to evaluate the microbiological profile in patients with perforation peritonitis with respect to anatomical site of perforation.

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2. MATERIAL & METHODS

A Prospective observational cross-sectional study based on micro-organisms cultured from the peritoneal fluid and their sensitivity in 100 patients presenting in emergency room as case of perforation peritonitis. This study was done at the Department of General Surgery, Nehru Hospital B.R.D. Medical College, Gorakhpur U.P. during time period 2021-2022. After obtaining consent from the ethical committee and informed consent from the patients presenting to emergency room, patients with perforation peritonitis was clinically examined and emergency exploratory laparotomy was done, peritoneal fluid was taken intraoperatively and then sent to Microbiological Department for culture & drug sensitivity. Only those patients who have an identifiable perforation site in laparotomy were enrolled for the study.

Inclusion criteria

- Patients (>18 years of age) with clinical features including abdominal pain, generalized abdominal tenderness, guarding, rigidity, abdominal distension, decrease bowel sounds and presence of free gas under diaphragm on abdominal X-ray, on whom emergency exploratory laparotomy was performed.

Exclusion criteria

- Patients with primary peritonitis
- Patients with traumatic bowel perforation
- Peritonitis patients with no identifiable perforation site on laparotomy.

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. Preoperatively 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

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peritoneum was opened, peritoneal fluid (10ml) was obtained for microbiological culture and sensitivity and intraoperative findings were noted in relation to site of perforation.

Sample Collection

The procedure followed for collecting intra-operative samples are as follows. 10 ml of Peritoneal fluid was aspirated using a sterile syringe under strict aseptic precautions and well labelled with patient information, type of fluid and site of perforation. 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

After collection of samples, it was processed immediately or refrigerated at 4^0C-8^0C. Simultaneously, all the samples were kept in peptone broth 12-24 hours and tested for turbidity in broth. Samples were inoculated on blood agar, chocolate agar and Mac Conkey agar and incubated in aerobic condition in 370 C for 24-48 hours. Identification of isolated microorganisms was on the basis of colony characteristics, gram staining and standard biochemical tests [11] as per the standard protocol at species level. Bacterial concentrations (cfu/ml) were calculated. Microorganism with count >104 cfu/ml were submitted for identification & susceptibility test.[10,11]

Antibiotic susceptibility testing was done on Mueller-Hinton agar (Hi media, India) using standard disk diffusion (Kirby Bauer's) technique. This test and interpretation of result was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[12] (Clinical Laboratory Standard Institute, 2010). 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.

STATISTICAL ANALYSIS

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Data so collected was analysed by using Software Statistical Package for Social Sciences (SPSS) 16. The statistical testing was carried out by employing chi square test.

3. RESULTS

Table 1: Demographic details

AGE	**NO. OF PATIENTS**
18-45 YEARS	49
46-65 YEARS	38
>65 YEARS	13
SEX	NO. OF PATIENTS
MALE	76
FEMALE	24

Out of total 100 patients, 49% were between 18-45 year of age group, 38% were between 46-65 year & 13 % were more than 65 years of age. Out of 100 patients, 76% were male and 24% were female.

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Table 2: Distribution of patients according to site

SITE OF PERFORATION	TOTAL	CULTURE POSITIVE	CULTURE NEGATIVE
GASTRIC	**43**	27(62.7%)	16(37.2%)
ILEAL	**39**	37(94.8%)	2(5.12%)
CAECAL	**6**	6(100%)	0(0%)
APPENDICULAR	**6**	5(83.3%)	1(16.6%)
JEJUNAL	**3**	2(66.6%)	1(33.3%)
COLON	**1**	1(100%)	0(0%)
RECTAL	**1**	1(100%)	0(0%)
DUODENAL	**1**	1(100%)	0(0%)
TOTAL	**100**	80(80%)	20(20%)

43 patients had gastric perforation. 39 patients had ileal perforation. 6 patients had appendicular perforation. 6 patients had caecal perforation. 3 patients had jejunal perforation.

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Table 3: Distribution of patients according to organism cultured & site of perforation

SITE OF PERFORATION	E. COLI	KLEBSIELLA PNEUMONIAE	PSEUDOMONAS AERUGINOSA	CANDIDA ALBICANS	ACINETOBACTER SPP	TOTAL
GASTRIC	18(41.8%)	4(20%)	1(12.5%)	3(75%)	1(20%)	27(33.7%)
ILEAL	18(41.8%)	13(65%)	4(50%)	0(0%)	2(40%)	37(46.2%)
CAECAL	4(9.3%)	0(0%)	1(12.5%)	0(0%)	1(20%)	6(7.5%)
APPENDICULAR	3(6.9%)	0(0%)	0(0%)	1(25%)	1(20%)	5(6.25%)
JEJUNAL	0(0%)	2(10%)	0(0%)	0(0%)	0(0%)	2(2.5%)
COLON	0(0%)	0(0%)	1(12.5%)	0(0%)	0(0%)	1(1.25%)
RECTAL	0(0%)	0(0%)	1(12.5%)	0(0%)	0(0%)	1(1.25%)
DUODENAL	0(0%)	1(5%)	0(0%)	0(0%)	0(0%)	1(1.25%)
TOTAL	43	20	8	4	5	80

43 patients were positive for E.coli. 20 patients were positive for Klebsiella pneumonia. 8 patients were positive for Pseudomonas aeruginosa. 4 patients were positive for Candida albicans. 5 patients were positive for Acinetobacter spp.

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Table 4: Distribution of patients according to site of perforation & mortality

SITE OF PERFORATION	TOTAL CASE	MORTALITY	PERCENTAGE
GASTRIC	43	14	32.5%
ILEAL	39	12	30.7%
CAECAL	6	3	50%
JEJUNAL	3	1	33.3%
OTHERS	9	0	0%
TOTAL	100	30	30%

Out of total 43 patients of gastric perforation, mortality occurred in 14(32.5%) patients. Out of total 39 patients of ileal perforation, mortality occurred in 12(30.7%) patients. Out of total 6 patients of caecal perforation, mortality occurred in 3(50%) patients. Out of total 3 patients of jejunal perforation, mortality occurred in 1(33.3%) patient.

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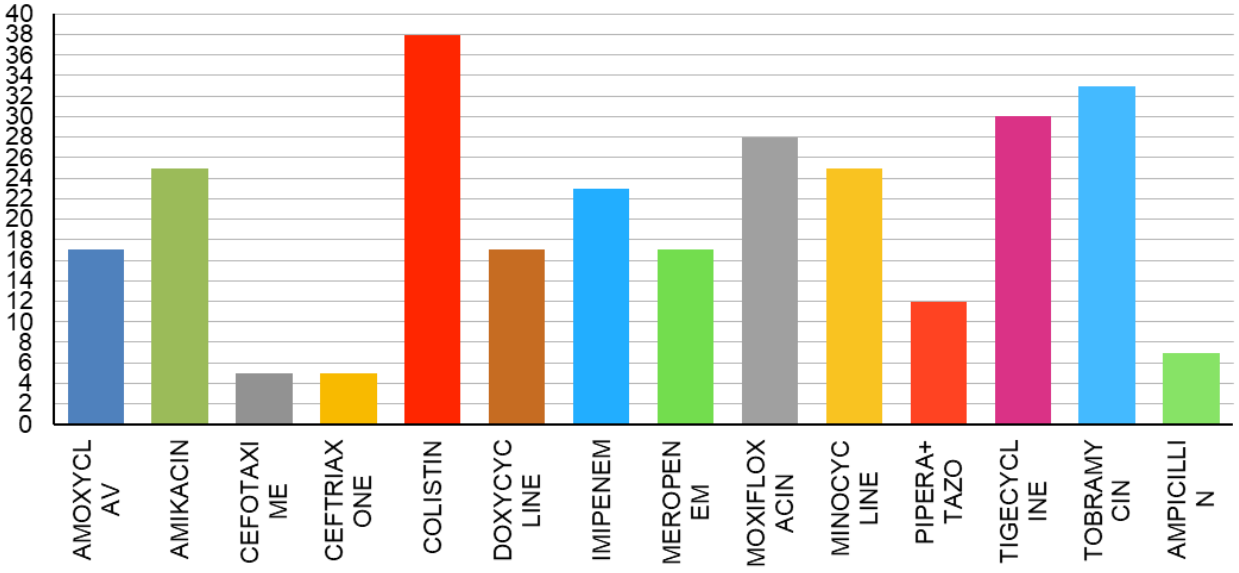

Fig. 1: Sensitivity of E. coli to various drugs

43 were positive for E.coli, amongst those 17(39.5%) were sensitive to Amoxyclav, 25(58.1%) were sensitive to Amikacin, 5(11.6%) were sensitive to Cefotaxime, 5(11.6%)were sensitive to Ceftriaxone, 38(88.3%) were sensitive to Colistin 17 (39.5%) were sensitive to Doxycycline, 23(53.4%) were sensitive to Imipenem, 17(39.5%) were sensitive to Meropenem, 28(65.1%) were sensitive to Moxifloxacin, 25(58.1%) were sensitive to Minocycline, 12(27.9%) were sensitive to Piperacillin+tazobactum, 30(69.7%) were sensitive to Tigecycline. 33(76.7%) were sensitive to Tobramycin 7(16.2%) were sensitive to Ampicillin.

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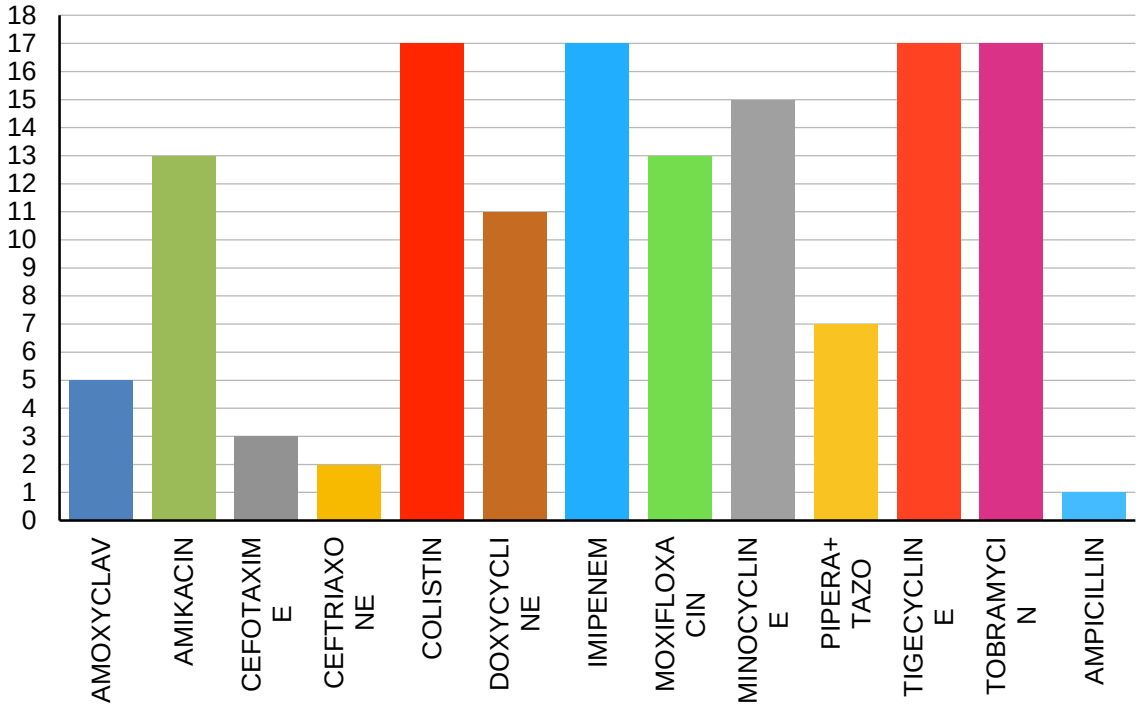

Fig. 2: Sensitivity of klebsiella pneumoniae to various drugs

20 were positive for Klebsiella pneumoniae, amongst those 5(25%) were sensitive to Amoxyclav, 13(65%) were sensitive to Amikacin, 3(15%) were sensitive to Cefotaxime, 2(10%) were sensitive to Ceftriaxone, 17(85%) were sensitive to Colistin, 11(55%) were sensitive to Doxycycline, 17(85%) were sensitive to Imipenem, 13(65%)were sensitive to Moxifloxacin, 15(75%) were sensitive to Minocycline, 7 (35%) were sensitive to Piperacillin+tazobactum, 17(85%) were sensitive to Tigecycline, 17(85%) were sensitive to Tobramycin, 1(5%) were sensitive to Ampicillin.

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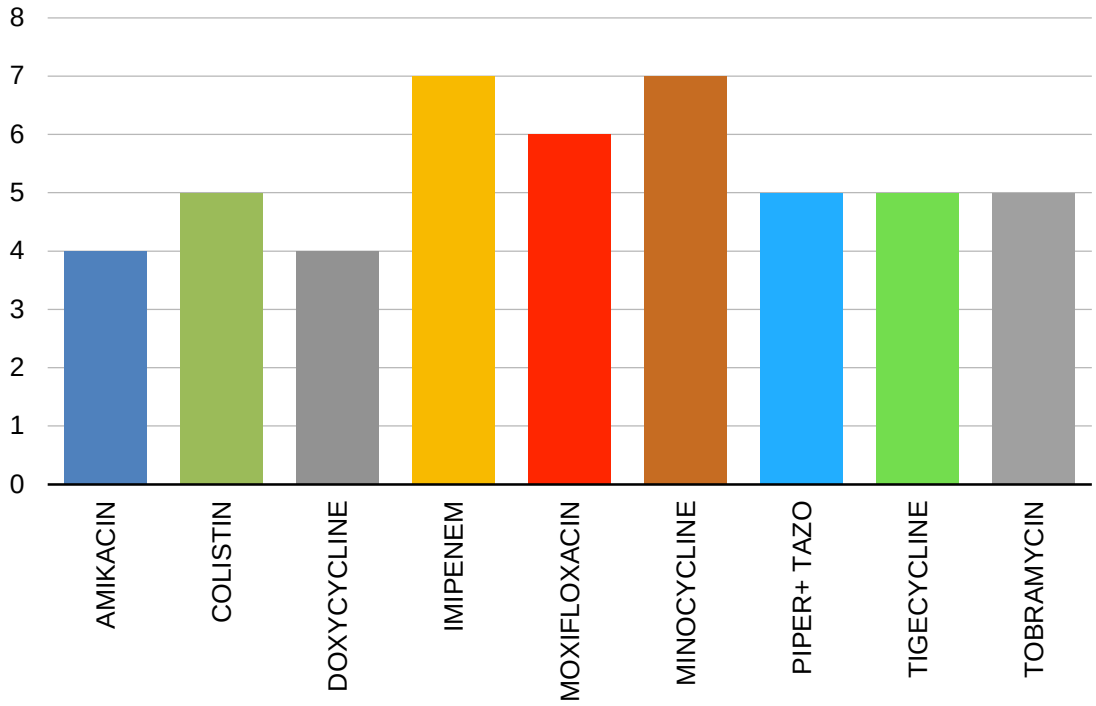

Fig. 3: Sensitivity of pseudomonas aeruginosa to various drugs

8 were positive for Pseudomonas aeruginosa, amongst those 4(50%) were sensitive to Amikacin, 5(62.5%) were sensitive to Colistin, 4(50%) were sensitive to Doxycycline, 7(87.5%) were sensitive to Imipenem, 6(75%) were sensitive to Moxifloxacin, 7(87.5%) were sensitive to Minocycline, 5(62.5%) were sensitive to Piperacillin+tazobactum, 5(62.5%) were sensitive to Tigecycline. 5(62.5%) were sensitive to Tobramycin.

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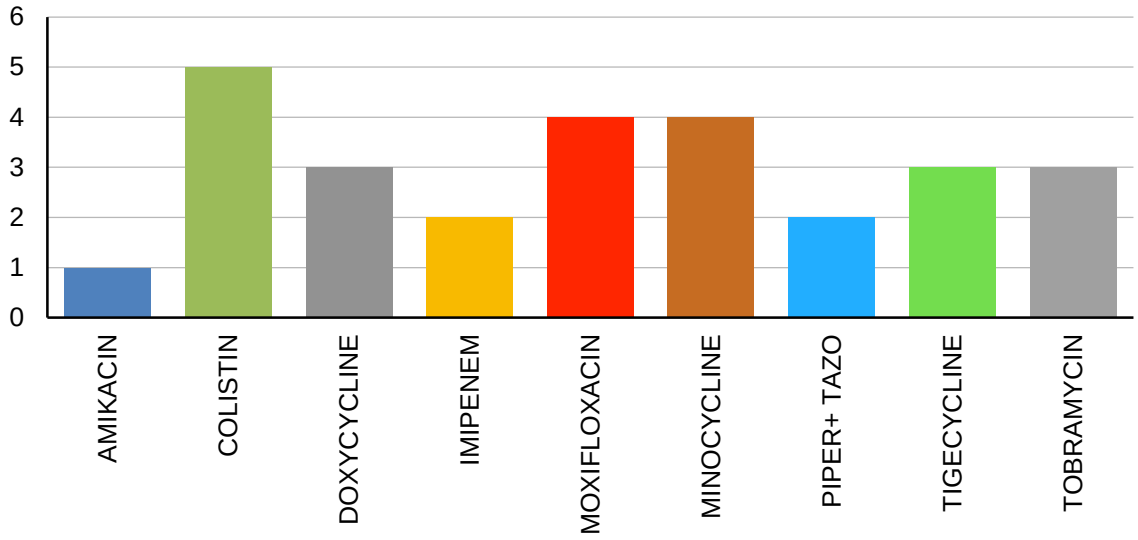

Fig. 4: Sensitivity of acinetobactor spp to various drugs

5 were positive for Acinetobactor, amongst those 1(20%) were sensitive to Amikacin, 5(100%) were sensitive to Colistin, 3(60%) were sensitive to Doxycycline, 2(40%) were sensitive to Imipenem, 4(80%) were sensitive to Moxifloxacin, 4(80%) were sensitive to Minocycline, 2(40%) were sensitive to Piperacillin+tazobactum, 3(60%) were sensitive to Tigecycline, 3(60%) were sensitive to Tobramycin.

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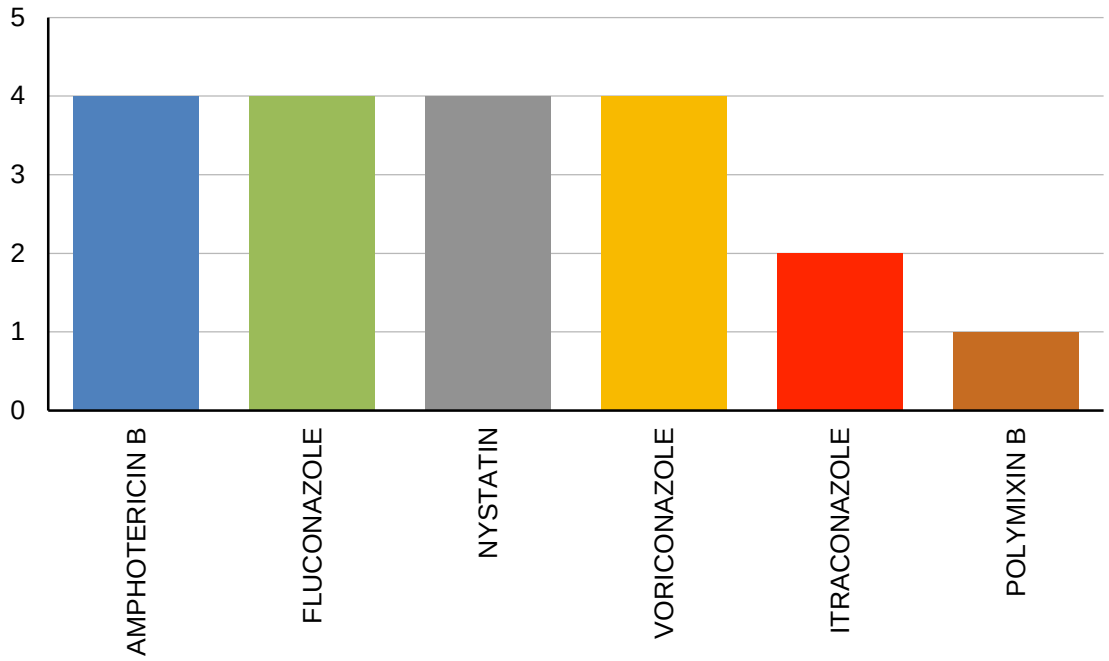

Fig. 5: Sensitivity of candida albicans to various drugs

4 were positive for Candida albicans, amongst those 4(100%) were sensitive to Amphotericin-B, 4(100%) were sensitive to Fluconazole, 4(100%) were sensitive to Nystatin, 4(100%) were sensitive to Voriconazole, 2(50%) were sensitive to Itraconazole, 1(25%) was sensitive to Polymixin-B.

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4. DISCUSSION

Intra-abdominal infections are one of the most common clinical problems in surgical practice and range from localized to generalized peritonitis.[12] Of the three types of peritonitis, secondary peritonitis is most common form originating from bowel pathologies such as perforation or ischaemia.[13] 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 depend 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.[14] Mean age of Perforation Peritonitis patient was 33.33 years. Most patients (49%) belonged to age group between 18-45 years which coincides with the age group where peptic ulcer disease is more prevalent. It was found similar to study done by Jhobta RS et al (2006)[15] in which it is most prevalent in males of age group of 30-40 years. In our study, it was found that males (76%) are 3 times more affected than females (24%). It was found in accordance with the studies done by Jhobta RS et al.[15]

In our study it was found that, gastric perforation (43%) is the commonest cause of perforation peritonitis followed by ileal perforation (39%) and then jejunal perforation (4%) which was found somewhat similar to study done by Yadav D et al (2013)[16], they found ileal perforation around 39%, jejunal perforation 4%. The cause may be Peptic Ulcer Disease, enteric perforation. E. coli was the most common organism isolated similar to that observed by Vishnu et al.[14] 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.[17] It was observed that maximum patients who were culture positive had duration of symptoms >2 days similar to study done by Chakma SM et al(2013)[18] which indicates earlier the presentation of patient, lesser is the chance of culture positivity from the peritoneal fluid because till the time being, the secondary infection has not set in. It was found that mortality amongst pseudomonas aeruginosa positive patients was highest ie., 50%, followed by E.coli ie., 30.2%, which was followed by Klebseilla pneumoniae and Candida albicans ie.,25% and least in acinatobacter patients ie., 20%. In a study by Ravishankar et al, E. coli showed sensitivity to ceftriaxone in about 87.5% followed by

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ciprofloxacin and amikacin of about 81.25%.[19] 20 were positive for Klebsiella pneumoniae, amongst those 5(25%) were sensitive to Amoxyclav, 13(65%) were sensitive to Amikacin, 3(15%) were sensitive to Cefotaxime, 2(10%) were sensitive to Ceftriaxone, 17(85%) were sensitive to Colistin, 11(55%) were sensitive to Doxycycline, 17(85%) were sensitive to Imipenem, 13(65%)were sensitive to Moxifloxacin, 15(75%) were sensitive to Minocycline, 7 (35%) were sensitive to Piperacillin+tazobactum, 17(85%) were sensitive to Tigecycline, 17(85%) were sensitive to Tobramycin, 1(5%) were sensitive to Ampicillin.

5. 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. Aminoglycosides, piperacillin and tazobactum, meropenem and colistin showed a significant antimicrobial activity against organisms isolated from cases of perforation peritonitis.

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