Brief Report

Aerobic bacteriological profile and antibiotic sensitivity pattern of peritoneal fluid isolates in patients with intra-abdominal infections due to perforated intra-abdominal viscus in two Tertiary Care Hospitals in Colombo, Sri Lanka

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Abstract

This study aimed to determine the aerobic bacteriological profile and antibiotic sensitivity pattern of peritoneal fluid isolates in patients presenting with intra-abdominal infections due to perforated abdominal viscus /viscera to guide empiric antibiotic therapy. A descriptive study was carried out involving in-ward patients with evidence of intra-abdominal infections due to perforated abdominal viscus following surgery in Colombo South Teaching Hospital (CSTH) and National Hospital of Sri Lanka (NHSL) from 21/11/2022 to 20/03/2023. Forty-nine patients, who had undergone laparotomy were recruited. The majority (91.9%) of the peritoneal fluid isolates were Gram negatives: *Escherichia coli (*33.9%) and *Klebsiella pneumoniae (*19.4%). Meropenem, amikacin and piperacillin-tazobactam demonstrated a sensitivity of 89.5%, 68.4%, 89.5% for coliforms respectively in appendicular perforations and 100%, 80%,75% in small intestinal perforations, highlighting their role as the most reliable therapeutic option. Based on in vitro susceptibility data, higher resistance rates were observed for ampicillin (88%), amoxicillin-clavulanate (64%), and cefuroxime (56%). Study data indicate that these agents are unsuitable as monotherapy options.

Keywords: intra-abdominal infections, peritoneal fluid, antibiotic, intestinal perforations

Introduction

Intra-abdominal infections are a major cause of sepsis.[1] These infections range widely from mild appendicitis to faecal peritonitis.[2] Primary peritonitis develops without a macroscopic gastrointestinal lesion by translocation and haematogenous dissemination. Often, it is monomicrobial.[3] Secondary peritonitis on the other hand results from a defect in the digestive system and a direct leak into the peritoneal cavity. Perforated intra-abdominal viscera leads to secondary peritonitis. According to Grotelueschen et al.(2019)[4], major causes for secondary peritonitis are hollow organ perforation or anastomotic leakage. A study by Sartelli et al.*(*2014) showed *Enterobacteriaceae* were the most frequently isolated bacteria in intra-abdominal infections with *E. coli* and *Klebsiella* spp.as the most prevalent. *Streptococcus* spp. and anaerobes

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were the primary pathogens isolated in community acquired intraabdominal infections, apart from *Enterobacteriaceae*. There was no difference in the isolation rate of community and hospital-acquired infections for *Pseudomonas aeruginosa*, which was low.[2]

It is known that both the cause of peritonitis and antimicrobial therapy affects the severity of peritonitis. The death rate increases when empiric antibiotics are delayed.[5] There is a scarcity of information regarding antibiotic sensitivity test (AST) patterns of isolated organisms in patients presenting with perforated abdominal viscera due to the low numbers of peritoneal fluid cultures. Global data suggest that the most prevalent organism isolated from cultures of peritoneal fluid is *E.coli.*[6,7,8] Isolated bacteria have been reported to be sensitive to meropenem and amikacin.[7] The most effective antibiotic regimens against *Enterobacteriaceae* were piperacillin/tazobactam with gentamicin, cefotaxime, cefotaxime with gentamicin, meropenem, tigecycline plus gentamicin or tigecycline plus ciprofloxacin.[4] In regional studies, the most common perforation site was the gastro duodenal region[9,10] whereas global studies showed colon as the commonest site of perforation.[11,12] According to Nwobodo et al.(2022), antibiotic resistance may have worsened during the COVID-19 pandemic due to the overuse of antibiotics in humans, continued misuse in agriculture, and limited development of new antimicrobials.[13]

There is limited data in Sri Lanka on the microbiological spectrum and resistance trends in intra-abdominal infections. Of 80 surgical events, 15% were sterile in an Indian study[7] with positive cultures in 85%. However, no such study has been conducted in Sri Lanka. This study aims to fill this gap and guide clinicians to start effective antibiotic treatment early.

Methods

A hospital based descriptive study was carried out in the surgical units of CSTH, NHSL and microbiology laboratory of CSTH from 21st November 2022 to 20th March 2023.

Forty-nine inward patients with evidence of intra-abdominal infections due to a perforated abdominal viscus and undergone surgery were included in this study. To calculate the sample size, the formula $N= Z^2P(1-P) / d^2$ was used. To calculate the sample size, proportion was considered as 85%. Z=1.64 (90% confidence interval), P=0.85 (85%) proportion, prevalence of positive samples, d=precision=0.1 as the prevalence is >60 %.[14]

The 3 criteria for inclusion in the study were a) all patients with surgical or radiological evidence of intraabdominal infections b) perforated abdominal viscus/viscera confirmed at laparotomy c) a sample of peritoneal fluid/pus collected during surgery for microbiological investigations. Full blood count including white cell count (WBC) and C-reactive protein (CRP) were tested. Patients with perforated abdominal viscus/viscera in whom a surgical procedure was not performed or sample not collected were excluded.

Peritoneal fluid samples were transported to the microbiology laboratory without delay. Peritoneal fluid samples were processed according to the Standard Operating Procedures of the Laboratory Manual in Microbiology 2011.[15] Lactose fermenters were identified by automated identification (Vitek®) and other isolates by conventional biochemical methods. AST was performed according to the disc diffusion test recommendations of the Clinical Laboratory Standards Institute (CLSI 2022).[16] Bacteriological culture media were quality controlled according to manufacturer's instructions.

All data was analysed using 2022 SPSS software.

Peritoneal fluid samples were classified according to the perforated site (appendix, small intestine, colon). Number of species from a site/ total number of the same species isolated from all samples is expressed as a percentage. Sensitivity of isolates obtained from each site to each tested antibiotic is expressed as a percentage of the total number of isolates tested from the same site. Age and sex of patients were also analysed similarly (e.g., number of males per site/total number of males)

Results

Of the 49 eligible patients, 67% were males and 33% were females. Of the 49 patients, 13 (26.5%) belonged to the 21-40 age group and only 1 (2.1%) was in the 81-100 age group. The mean age of the patients was 42 ± 22 years. Sixty two peritoneal samples from the 49 patients were sent for culture.

Table 1: Aetiological agents isolated from positive peritoneal fluid cultures

Gram-negatives	*Escherichia coli*	21	33.9
	Klebsiella pneumoniae	12	19.4
	Enterobacter aerogenes	4	6.5
	Proteus spp	3	4.8
	Pseudomonas aeruginosa	7	11.3
	Other non-lactose fermenters	10	16.1
	Total	57	91.9
Gram-positives	*Streptococcus* spp	5	8.1
	Total	62	100

As depicted in Table 1, of the 62 isolates, 57 (91.9%) were Gram negatives with distribution of species as shown and 5 (8.1%) were Gram positive organisms

Among the 49 patients, the most common perforation site was the appendix (n=23;46.9%) followed by small intestine (n=15; 30.6%). The large intestine was the third common site for perforation (n=3; 6.1%). The most common reason for perforation was acute appendicitis (43.5%) followed by intestinal obstruction (17.7%) and iatrogenic (16.1%). Other reasons were trauma (8.1%), malignancy (8.1%), acute cholecystitis (4.8%) and peptic ulcer disease (1.6%).

Table 2: Antibiotic sensitivity patterns of Gram-negative organisms isolated from peritoneal fluid cultures

Antibiotic	**Total no tested**	**Sensitive**		**Intermediate**		**Resistant**		
		n	**%**	**n**	**%**	**n**	**%**	
Ceftazidime	6	6	100	-	-	-	-	tested only for *Pseudomonas*
Meropenem	57	49	86	-	-	8	14	
Piperacillin-tazobactam	57	46	80.7	1	1.8	10	17.5	
Amikacin	57	43	75.4	4	7.1	10	17.5	
Ticarcillin-clavulanate	57	34	59.6	8	14	15	26.4	
Ciprofloxacin	57	32	56.4	3	5	22	38.6	
Gentamicin	57	32	56.1	11	19.3	14	24.6	
Cotrimoxazole	50	28	56	-	-	22	44	7 isolates not tested for *Pseudomonas* spp
Cefotaxime	50	26	52	1	2	23	46	
Cefuroxime	50	20	40	2	4	28	56	
Amoxicillin-clavulanate	50	16	32	2	4	32	64	
Ampicillin	50	6	12	-	-	44	88	

Among antibiotic sensitivity patterns of Gram-negative organisms, meropenem (86%), amikacin (75.4%) and piperacillin-tazobactam (80.7%) had the highest sensitivity whereas ampicillin(12%), amoxicillin-clavulanate(32%) and cefuroxime (40%) had the lowest sensitivity (Table 2).

Coliforms isolated from peritoneal fluid samples following perforation of the appendix had the highest sensitivity of 89.5%, 89.5% and 68.4% to meropenem, amikacin and piperacillin-tazobactam respectively. The lowest sensitivity rates of 42.1 %, 26.3% and 13.6% were noted for cefuroxime, co-amoxiclav, and ampicillin respectively. All tested *Pseudomonas* sp, were sensitive to meropenem, amikacin, piperacillin tazobactam and ceftazidime.

Coliforms isolated from peritoneal fluid samples following perforation of the small intestine had the highest sensitivity of 39.6%, 39.4%, 36.7% and 34.9% to amikacin, ciprofloxacin, meropenem and piperacillin-tazobactam respectively. All tested *Pseudomonas* spp. from these samples were sensitive to meropenem, amikacin, piperacillin-tazobactam and ceftazidime.

A greater percentage of coliforms isolated from peritoneal fluid samples following appendicular (52,6%) and small intestinal perforations (60%) were sensitive to cefotaxime than to co-amoxiclav, cefuroxime, ampicillin, and cefotaxime.

Discussion

The purpose of this study was to identify the microorganisms that cause secondary peritonitis and to determine their susceptibility to antibiotics commonly used in empiric treatment, in order to determine the most suitable antibiotic or combination for empiric therapy.

According to international guidelines, selecting an agent as an empirical antibiotic must be based upon resistance patterns in the local community. Commonly used empirical antibiotics are either cefuroxime or co-amoxiclav in combination with metronidazole.[17]

Apart from a single patient who sustained an injury during biliary tract surgery, all cases including the two bladder perforations during cystoscopy originated from the community.

Carbapenem (meropenem) resistant rates in our peritoneal fluid isolates of 9.5% in *E. coli* and 41.6% in *K. pneumonia* are similar to meropenem resistance rates of blood culture isolates of *E. coli* (10.6%) and *K. pneumoniae* (47.5%) as submitted to data in Global Antimicrobial Resistance and Use Surveillance System (GLASS).[18]

The WHO AWaRe classification categorises antibiotics into Access (first/second choice, narrow spectrum), Watch (higher resistance potential, requires monitoring), and Reserve (last-resort, multi-drug resistant pathogens) groups to support antibiotic stewardship.[19] AST patterns reflect the antimicrobial resistance in the community. According to our study, though carbapenem (a Watch group antibiotic) resistance is low, Access group antibiotics (ampicillin, cefuroxime, amoxicillin-clavulanate) show high levels of resistance, emphasising the need for a strong antibiotic stewardship programme.

The AST results in the current study show high levels of resistance to these agents leading to a therapeutic dilemma. Watch group antibiotics – aminoglycosides such as amikacin (75.4%) and gentamicin (56.1%), along with ciprofloxacin (56.4%) and ticarcillin–clavulanate (59.6%) – demonstrated comparatively higher sensitivity rates than Access group antibiotics such as

cefuroxime (40%) and co-amoxiclav (32%). According to the AST results in this study, Watch group antibiotics (meropenem and piperacillin- tazobactam) were the most appropriate empiric agents for monotherapy. The national guidelines -'Empirical and prophylactic use of antimicrobials' recommends piperacillin-tazobactam or carbapenems as empirical antibiotics for patients who present with community-acquired intra-abdominal infections.[19]

The results of this study suggest that the use of cefuroxime, cefotaxime and co-amoxiclav as monotherapy as empirical treatment for patients presenting with intra-abdominal organ perforations cannot be recommended.

Limitations: The results of this study carried out in only two centres cannot be generalised to the whole population. In addition, the true pathogen profile is not reflected as anaerobic bacterial pathogens were not included in the study. Due to the non-availability of resources, final identification was done only for lactose fermenting organisms and could not be done for other organisms. Though the study met the required sample number, another study with a larger sample size may change our findings. Among the 62 isolates, 11 were not analysed for susceptibility patterns in relation to perforation site as the numbers were too small in each perforation site.

Future directions: This study could be used as a pilot study and a study designed with a larger sample size and involving several institutions would be appropriate to generate more accurate data. Strong antibiotic stewardship programme for the community is needed.

Conclusion

Appendicitis, which mostly affects young males, was the most common reason for perforation. Perforation could have been avoided by seeking of medical care early. The use of cefuroxime and co-amoxiclav as single agents for treatment of secondary peritonitis cannot be advocated but combining with an aminoglycoside will improve coverage and add a synergistic effect for aerobic flora.

Declaration

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Author contributions:
HHKTD: contributed in developing the research proposal, sorting permission and ethical clearance from relevant bodies, data collection, sample processing, data entry, analysis, research writing and presentation.
NSC: contributed in developing the research proposal, sorting permission and ethical clearance from relevant bodies, sample processing in the Colombo South Teaching Hospital, guiding on report writing and presentation.
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