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CLINICAL & BASIC RESEARCH

Comparison of Short-Course and Conventional Antimicrobial Duration in Mild and Moderate Complicated Intra-Abdominal Infections

A randomised controlled trial

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abstract: ***Objectives:*** Studies have shown the feasibility of short-course antimicrobials in complicated intra-abdominal infection (CIAI) following source control procedure (SCP). This study aimed to compare postoperative complication rates in short-course (5 days) and conventional (7–10 days) duration groups after antimicrobial therapy. ***Methods:*** This was a single-centre, open-labelled, randomised controlled trial conducted in Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India, from July 2017 to December 2019 on patients with CIAI. Patients who were haemodynamically unstable, pregnant and had non-perforated, non-gangrenous appendicitis or cholecystitis were excluded. Primary endpoints were surgical site infection (SSI), recurrent intra-abdominal infection (IAI) and mortality. Secondary endpoints included time till occurrence of composite primary outcomes, duration of antimicrobial therapy, the length of hospital stays, antimicrobial-free interval, hospital-free days at 30 day intervals and the presence of extra-abdominal infections. ***Results:*** Overall, 140 patients were included whose demographic and clinico-pathological details were comparable in both groups. There was no difference in SSI (37% versus 35.6%) and recurrent IAI (5.7% versus 2.8%; $P = 0.76$); no mortality was observed in either groups. The composite primary outcome (37% versus 35.7%) was also similar in both groups. Secondary outcomes included the duration of antimicrobial therapy (5 versus 8 days; $P < 0.001$) and length of hospitalisation (5 versus 7 days; $P = 0.014$) were significant. Times till occurrence of SSI and recurrent IAI, incidence of extra-abdominal infection and resistant pathogens were comparable. ***Conclusion:*** Short-course antimicrobial therapy for 5 days following SCP for mild and moderate CIAI was comparable to conventional duration antimicrobial therapy, indicating similar efficacy.

Keywords: Abdominal Abscess; Antibiotic Prophylaxis; Antimicrobial Stewardship; Appendicitis, Perforated; Drug Resistance, Microbial; Intra-Abdominal Infection; Peritonitis; Surgical Wound Infection, India.

Advances in Knowledge

- *The use of short-course antimicrobials in complicated intra-abdominal infections is safe and efficacious.*
- *Short-course antimicrobial therapy leads to a reduced length of hospital stay.*
- *Short-course antimicrobial therapy has a comparable postoperative outcome to conventional antimicrobial therapy.*

Applications to Patient Care

- *Short-course antimicrobials help reduce the development of antibiotic resistances, which is considered a major concern across the globe.*
- *It also helps in early discharge of patients and for maintaining a better cost-benefit ratio in the hospitals.*

Complicated intra-abdominal infection (CIAI) is one of the most frequent cases encountered by a surgeon in an emergency scenario. CIAI is usually defined as abscess formation or peritonitis beyond the origin of the perforation of a hollow viscus in the peritoneal cavity, which requires an invasive procedure for source control.[1–3] The three pillars of management are fluid resuscitation, source control procedure (SCP) and the usage of antibiotics to eliminate residual organisms.[1–3] Patients undergoing major abdominal surgery, in which infective postoperative complications are anticipated, require perioperative antibiotic cover. This necessity is more pronounced in the subset of patients who undergo emergency abdominal surgery, especially when the patient has associated abdominal or generalised sepsis. Traditionally, in such cases, antibiotic coverage is stopped two days after the resolution of systemic inflammatory response syndrome as documented by the normalisation of total leukocyte counts and resolution of fever.[2]

Conventionally, the recommended duration of the use of antibiotics in these conditions is between 10–14 days.[3,4] However, recent evidence indicates that not all postoperative febrile episodes are due to active abdominal infection.[2–5] The Surviving Sepsis Campaign guidelines recommend that relevant cultures should be obtained and antibiotic therapy should be modified accordingly.[6] Recent reports imply that the duration of antibiotics can be shortened after a proper SCP

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is followed to control the sepsis and that there is no need for antibiotics to be continued for an extended period after an SCP.[3] Studies have shown the utility and efficacy of short-course antibiotic usage after SCP in complicated IAIs both in open and laparoscopic procedures.[2–5] However, despite this, many surgeons are still apprehensive in implementing the same. This trepidation is mostly attributed to the possibility of postoperative IAIs developing in patients, as well as multiple nosocomial infections that patients are exposed to in the hospital.[7] However, the decrease in the duration of antibiotics helps in shortening the length of hospital stay, and it has been shown to have comparable results in terms of postoperative complications.[6]

Management of a complicated IAI requires vigilant and timely intervention in order to contain the sepsis, which includes fluid and electrolyte correction, an effective SCP and judicious use of antimicrobials. All these three measures should be carried out expediently in order to achieve a good outcome. The duration of antibiotics is crucial as undertreatment and overtreatment can be detrimental to the patient. However, the optimum duration for the course of antibiotics is still debatable. Recent reports have shown that the use of a short course of antimicrobials after an effective SCP may be satisfactory for the control of infection, the rationale being that the SCP helps in eliminating a major portion of the sepsis, and thereafter, since the load of bacteria is expected to be largely reduced, the duration of antimicrobials can be safely truncated.[8]

Though studies have shown that short-course antimicrobial therapy is safe and effective compared with conventional long-course therapy, it is to be noted that a majority of these studies were carried out in Western countries, where the antibiotic usage is well regulated and antibiotic resistance is low. However, in the developing countries, with varying patient profile, poor nutritional status, delayed presentation, diverse aetiology of intra-abdominal infection, unrestricted antimicrobial usage with higher resistance pattern, the efficacy of short-course antimicrobial therapy needs to be studied to assess their effectiveness in these populations. Hence, this study aimed to compare the rates of postoperative complications in patients with complicated IAIs after conventional duration and short-course antimicrobial therapy.

Methods

This was a randomised controlled trial, which was single-centred, non-inferior and open-labelled, conducted in the Surgery Department in Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India, from July 2017 to December 2019. The study was registered at the Clinical Trials Registry in India (registration number: CTRI/2018/03/012682). This work has been reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Patients aged >18 years who presented to the emergency surgical unit and were diagnosed with complicated IAIs such as perforated/gangrenous appendicitis/cholecystitis, bowel gangrene/perforation and gastric/duodenal perforation with peritonitis were enrolled and assessed for eligibility. The diagnosis was confirmed by clinical examination and relevant laboratory and radiological investigations. Patients who were haemodynamically unstable, who were pregnant and who had non-perforated, non-gangrenous appendicitis or cholecystitis, infected necrotising pancreatitis, primary spontaneous bacterial peritonitis and infection associated with indwelling peritoneal dialysis catheter were excluded.

Patients who received antimicrobial therapy for either 5 days or of a conventional duration of 7–10 days were randomly assigned in a 1:1 ratio. A computer programme was used for block randomisation with block sizes of four and six selected randomly. The technique called 'serially numbered opaque sealed envelope' (SNOSE) was used for concealment during allocation. A person independent of the investigators had prepared these sealed envelopes. The nurse opened the envelope at the time of decision of surgery and group allocation was done.

Before the operation, all patients were stabilised by fluid resuscitation according to conventional guidelines and were started on intravenous (IV) empirical antibiotics.[9] Standard preoperative care was provided as per the routine protocol. Patients were admitted in the emergency surgical ward and laboratory investigations and imaging, including contrast enhanced computed tomography, were carried out for the diagnosis. Placement of a nasogastric tube at admission, urinary catheterisation and administration of crystalloids for fluid replacement were done. Patients received IV empirical antibiotic therapy with ceftriaxone and metronidazole or piperacillin–tazobactam depending on the possible grade of infection and IV acid reducing therapy with pantoprazole.[9,10]

All patients underwent open laparotomy and received standard SCP as per the primary diagnosis, which included omental patch closure for gastric or duodenal perforation, primary resection anastomosis or stoma for bowel gangrene, appendectomy and peritoneal lavage for gangrenous or perforated appendix, etc. Intraoperative fluid or specimen was sent for aerobic culture. Patients in the short-course group and in the conventional duration group received antimicrobial therapy for 5 days and for 7–10 days, respectively.[2] In both the groups, the antibiotics that were given were ceftriaxone with metronidazole or piperacillin–tazobactam based on a mild or moderate infection, which was diagnosed taking into account the total and differential leukocyte count, fever, respiratory rate and the possible organ involved based on the radiological investigation. These antibiotics were administered based on the antibiotic guidelines of the authors' institute, which is based not only on the sensitivity and resistance pattern of their hospital but also based on the standard guidelines of the international society.[3,10] In cases where the intraoperative pus/fluid culture showed a resistance pattern to the ongoing antibiotics, a sensitive antibiotic was given as per the culture and sensitivity report within 48 hours of starting the initial antibiotics. Subsequent occurrence of recurrent intra-abdominal infection and surgical site infection (SSI) were treated as per the standard protocol and with antibiotics based on the culture report.

Patients were monitored till the time of discharge for the presence of SSI, recurrent IAI or death due to any cause. In case of development of SSI or recurrent abdominal infection, a wound swab or percutaneously/surgically drained fluid was sent for culture and sensitivity. Patients were followed-up till the time of discharge and on days 15 and 30 postoperatively for occurrence of any of the primary outcomes and complications, for re-admission and for mortality.

The primary endpoints in the two groups were development of SSI, recurrent IAI and mortality. The primary outcome was assessed as a composite endpoint comprising any one, two or three of the primary endpoints. The secondary outcome included the time of the occurrence of composite primary outcomes, duration of antimicrobial therapy, the length of hospital stays, antimicrobial-free interval, hospital-free days at 30 days' interval and the presence of extra-abdominal infections.

The composite primary outcomes were used for power analysis. The sample size was calculated using nMaster software, Version 2.0 (Christian Medical College, Tamil Nadu, India). Assuming the proportion of composite primary outcome in the conventional duration group to be 30–40% and a non-inferiority margin of 10%, the sample size was calculated as 70 in each arm (total = 140), with a power of 80%, an alpha error of 5% and estimated loss to follow-up of 10%.[2]

Data were collected as per the specified proforma prepared by the investigators. Different demographic variables such as age, gender, address, organ of infection and SCP were collected and analysed.

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS), Version 20.0 (IBM Corporation, Armonk, New York, USA). Continuous variables such as time till the occurrence of composite primary outcome and duration of antimicrobial therapy were expressed as mean ± standard deviation or median (interquartile range) depending upon the normality of distribution. Categorical variables such as parameters of primary outcomes were expressed as proportions. The Chi-squared test was used to compare the proportions of

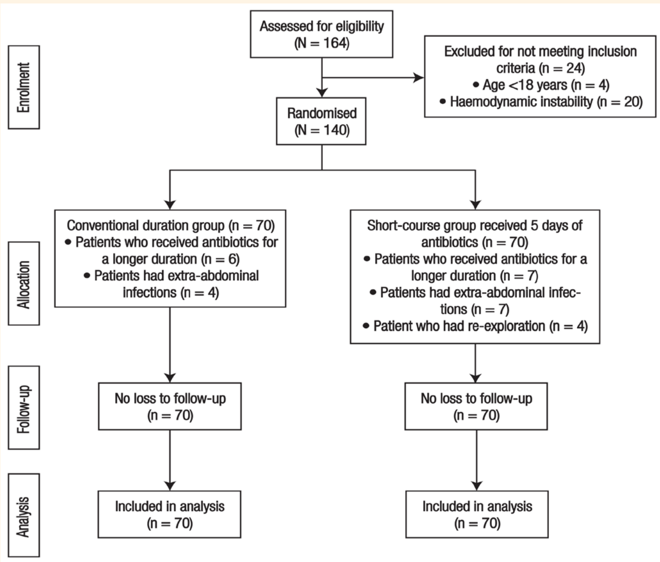

Figure 1: Flowchart for the study.

primary endpoints and composite primary outcome in the two groups. Secondary outcomes such as the time till the occurrence of primary endpoints and composite primary outcomes, duration of antimicrobial therapy, the length of hospital stay, antimicrobial-free interval and hospital-free days at 30 days' interval were compared using the Mann-Whitney U test. Fisher's test was used to compare the incidence of extra-abdominal infections and organisms of aerobic infection in the two groups. All results were interpreted as intention-to-treat analysis. A P value below 0.05 was considered as statistically significant.

The study was approved by the Institute Ethics Committee (JIP/IEC/2017/0239) and written informed consent was taken from all the participants.

Results

A total of 164 patients with complicated IAI were assessed for eligibility from July 2017 to May 2019. A total of 24 patients were excluded [Figure 1]. The remaining 140 patients were randomised, with 70 in the short-course group and 70 in the conventional duration group. There was no loss to follow-up.

Table 1: Baseline demographic and clinical characteristics in the short-course and conventional duration groups (N = 140)

Variables	n (%)		P value
	Short-course group (n = 70)	Conventional duration group (n = 70)	
Mean age in years ± SD	40 ± 15.5	43 ± 15.8	0.11
Male patients	54 (77.1)	51 (72.8)	0.55
Organ of origin			
Appendix	32 (45.7)	34 (48.5)	
Small bowel	23 (32.8)	27 (38.5)	
Stomach	11 (15.7)	6 (8.6)	0.465
Large bowel	3 (4.3)	2 (2.9)	
Gall bladder	1 (1.4)	1 (1.4)	
Source control procedure			
Appendectomy	32 (45.7)	34 (48.5)	
Omental patch closure	21 (30.0)	18 (25.7)	
Resection and anastomosis	8 (11.4)	7(10.0)	0.764
Resection and stoma	8 (11.4)	10(14.3)	
Pigtail	1 (1.4)	1 (1.4)	

SD = standard deviation; SCP = source control procedure.

The two groups were comparable in terms of all demographic and clinico-pathological characteristics [Table 1].

The incidence of superficial incisional SSI was 31.4% and 32.8%, deep incisional SSI was 2.8% and 1.4% and organ space infection was 2.8% and 1.4% in the short-course and the conventional duration antimicrobial therapy groups, respectively (P = 0.764) [Table 2]. Four and two patients developed recurrent IAI in the short-course group and in the conventional duration group, respectively, while two and one patients developed deep organ space infection in the short-course group and the conventional duration group, respectively. Except one patient in the short-course group who had postoperative leak after the initial adequate source control by omental patch closure for duodenal perforation (upper gastrointestinal tract), which resulted in recurrent IAI, the rest of the patients in both the groups had a small bowel or appendix (lower gastrointestinal tract) aetiology as a source of CIAI. The incidence of recurrent IAI was similar at 5.7% and 2.8% in the short-course and conventional duration antimicrobial therapy groups, respectively (P = 0.764). There was no mortality in both the groups. The composite primary outcome was 37% and 35.7% in the short-course and conventional duration groups, which was also similar (P = 0.726).

There was a significant reduction in the length of hospital stay by 2 days in the short-course and conventional duration antimicrobial therapy groups (P = 0.014) [Table 3]. There was a significant reduction in the duration of antimicrobial therapy by 3 days in the

Table 2: Comparison of primary endpoints and composite primary outcomes in the short-course and conventional duration groups (N = 140)

Surgical site infection	n (%)		P value*
	Short-course group (n = 70)	Conventional duration group (n = 70)	
Superficial incisional SSI	22 (31.4)	23 (32.8)	
Deep incisional SSI	2 (2.8)	1 (1.4)	0.764
Organ space infection	2 (2.8)	1 (1.4)	
Recurrent intra-abdominal infection	4 (5.7)	2 (2.8)	
Composite primary outcomes	27 (37)†	25 (35.7)†	

SSI = surgical site infection.
**Using Chi-squared test. †Patients with composite outcome (having any one of more than one type of SSI mentioned above).*

Table 3: Comparison of secondary outcomes in short-course and conventional duration groups (N = 140)

Secondary outcomes	Duration in days (range)		*P* value*
	Short-course group (n = 70)	Conventional duration group (n = 70)	
Antimicrobial therapy	5†	8 (7–10)	<0.001
Antimicrobial-free days at 30 days' interval	25†	22 (20–23)	<0.001
Hospitalisation after index procedure	5†	7 (7–10)	0.014
Hospital free days at 30 days' interval	25 (23–25)	23 (20–23)	0.012

*Using Mann-Whitney U test.
†The duration of antibiotics was fixed at five days in all the patients in short course group.

short-course and conventional duration antimicrobial therapy groups (*P* <0.001). The time till the occurrence of SSI was 3.8 ± 0.7 and 4.2 ± 1.2 days (*P* = 0.77) and recurrent IAI was 7 ± 1.8 and 5.3 ± 0.5 days (*P* = 0.195) in the short-duration and conventional duration groups, respectively. The time till the occurrence of composite primary outcome was 4.1 ± 1.6 and 4.5 ± 1.3 days in both the groups, which was similar (*P* = 0.256) [Table 4].

The incidence of extra-abdominal infections such as urinary tract infection (2.8% versus 2.8%), bloodstream infection (4.2% versus 2.8%), pulmonary infection (4.2% versus 5.6%) and vascular catheter-associated infection (1.4% versus 0.0%) were similar in the two groups (*P* = 0.582).

Escherichia coli (55.1% versus 49.7%) was the most common organism isolated from the culture specimen followed by *Enterococcus* (2.9% versus 7.6%), *Klebsiella* (2.9% versus 2.5%), *Pseudomonas* (2.9% versus 2.5%) and *Acinetobacter* (2.9% versus 2.5%) in the short-course and conventional duration groups, respectively [Table 5]. Nearly 33.3% of the short-course group and 35.2% of the conventional duration group showed poly-microbial growth.

Based on the intraoperative culture and sensitivity report, 9.2% of the study population required a change of antibiotics due to resistance of the primary antibiotics that were given perioperatively. Culture-sensitive antibiotics such as third-generation cephalosporins with metronidazole, piperacillin–tazobactam, meropenem or imipenem–cilastatin with metronidazole were used for SSI and recurrent intra-abdominal infection in the study population.

Table 4: Comparison of the time till occurrence of the primary endpoints and composite primary outcomes in short-course and conventional duration groups (N = 140)

Time to event	Duration in days		*P* value*
	Short-course group (n = 70)	Conventional duration group (n = 70)	
Surgical site infections	3.8 ± 0.7	4.2 ± 1.2	0.770
Recurrent intra-abdominal infection	7.0 ± 1.8	5.3 ± 0.5	0.195
Composite primary outcomes	4.1 ± 1.6	4.5 ± 1.3	0.256

*Using Mann-Whitney U test.

Table 5: Comparison of organisms of aerobic infection in short-course and conventional duration groups (N = 140)

Organisms of aerobic infection	n (%)		*P* value*
	Short-course group (n = 70)	Conventional duration group (n = 70)	
Aerobic infection	34 (48.5)	39 (55.7)	0.397
Anaerobic infection	2 (2.8)	2 (2.8)	0.97
E. coli	27 (38.5)	28 (40.0)	0.706
Enterococcus	10 (14.2)	10 (14.2)	
Klebsiella	5 (7.1)	8 (11.4)	
Pseudomonas	6 (8.5)	7 (10.0)	
Acinetobacter	1 (1.4)	3 (4.2)	
More than one organism	11 (15.7)	14 (20.0)	

*Using Fisher exact.

Discussion

This study showed that short-course antimicrobial therapy for 5 days following SCP for complicated IAI had similar outcomes to antimicrobial therapy for a conventional duration. A significant reduction in the length of hospitalisation in patients undergoing short-course antimicrobial therapy was also observed. The times till the occurrence of SSI and recurrence of IAI were comparable between the two groups. Also, the times till the occurrence of composite primary outcome were similar among the two groups. The incidences of extra-abdominal infections such as urinary tract infection, bloodstream infection, pulmonary infection and vascular catheter-associated infection were similar in the two groups. *E. coli* was the most common organism isolated from the culture specimens in both the groups, followed by *Enterococcus*, *Klebsiella*, *Pseudomonas* and *Acinetobacter*.

Only a few reports have been published on the use of antimicrobials for a shorter duration in complicated IAI.[2] Although recent guidelines and a few studies in this aspect have shown a similar outcome as short-course antimicrobial therapy, surgeons are still apprehensive in implementing the same in clinical practice, due to the life-threatening consequences of potential undertreatment.[3] In this study, the primary outcomes such as SSIs and recurrent IAIs were comparable in the short-course and conventional duration antimicrobial groups.

In this study, appendicular perforation was the commonest cause of complicated intra-abdominal infections. Though a significant number of patients had small and large bowel perforations, they could not be included in the study as they were haemodynamically unstable and required inotropes support at the time of presentation. This was consistent with the study conducted by Lopez *et al.*, in which appendicular perforation was the most common cause of peritonitis.[11] In this study, the most common organism isolated was *E. coli*, which was similar to a report from Italy.[1] In this report, after *E. coli*, *Enterococcus* was the next organism isolated in a very small number of cases while the cultures of many patients showed the growth of more than one aerobic organism. In a small subset of patients, anaerobic organisms such as *Bacteroides fragilis* were found growing in their culture. As per the Surgical Infection Society-Infectious Diseases Society of America guidelines, third-generation cephalosporins with metronidazole are recommended in patients with complicated IAIs who are at low risk, while in patients at high risk, piperacillin-tazobactam, meropenem or imipenem–cilastatin with metronidazole can be used.[12] Addition of metronidazole to the antibiotic therapy has been shown to reduce the postoperative complications as *B. fragilis* are obligate anaerobes in the distal small bowel and large bowel.[13] In this study, third-generation cephalosporins and metronidazole were given to most patients, while patients at high risk received piperacillin-tazobactam. Antimicrobials and its use in the complicated IAI are based on the susceptibility of the organisms.[14] The antibiotics in this study were used according to the hospital guidelines and policy.

Similar to this study, another report administered 4 ± 1 days of antibiotics in the short-course group and the conventional duration group received antibiotics till the fever resolved and white cell counts were normalised.[2] Schein *et al*. also reported the use of 3–5 days of antibiotics in patients with complicated IAI following SCP.[15] In this study, the short-course group and the conventional duration group received 5 and 7–10 days of antibiotics, respectively. In both the groups, a few patients received antibiotics for a longer time than the stipulated duration, when they had organ space infection, recurrent IAI and extra-abdominal infection. In a study from the United States and Canada, 10% of the patients had received antibiotics for a prolonged duration owing to the occurrence of wound infections and extra-abdominal infection.[2] In the short-course group of that study, four patients had re-exploration and two patients had organ space infection, which required a longer duration of antibiotics. Among the four patients, in one of the patients a postoperative leak occurred after the initial adequate source control by omental patch closure, which resulted in recurrent IAI. Two patients had anastomotic leak, which required re-exploration, and one other patient had an iatrogenic perforation, which was detected in the postoperative period. Though the majority of the studies advocate short-course antimicrobial therapy after adequate source control for mild to moderate IAI, a longer duration of therapy may be required for patients with severe IAI and showing features of severe sepsis. These patients may have an unpredictable clinical course that require a more complex and individualised approach for the diagnosis of ongoing sepsis, the reason for antimicrobial failure and continuous monitoring of inflammatory markers.[16] In that study, patients in the short-course and the conventional duration groups developed extra-abdominal infection at the rate of 12.6% and 11.2%, respectively. Pulmonary infection accounted for the majority of infections.

In the RCT by Sawyer *et al*., the recurrent intra-abdominal infection rate was considerably high with 36 (13.8%) in the control group versus 40 (15.6%) in the experimental group with (P = 0.67).[2] This was significantly lower in the current study with 4 (5.7%) in the short-course group versus 2 (2.8%) in the conventional duration group. Considering the lesser number of events in both the groups in the current study, the difference was not observed. In the study by Sawyer *et al*., the short-course group had 40.2% of infectious complications and the conventional duration group had 38.4% out of which SSIs accounted for a majority of cases, superficial incisional SSI being the most common. There was no mortality in both the groups. In another report, the rate of infectious complications were more than 20% in both the groups; however, the majority of the cases were recurrent IAIs.[2] There were two deaths in the study group and three deaths in the experimental group. Antibiotics such as ertapenem had been given for only 3 days to patients with mild to moderate IAIs with a successful outcome.[17]

The times taken till the occurrence of SSIs and recurrent IAIs were similar in the two groups. The time till the occurrence of the composite primary outcome was 4.1 days in the short-course group. This led to early detection and timely intervention, thus avoiding the need for readmissions.

In the current study, the antimicrobial-free days at the 30 day interval was 3 days less in the short-course group with comparable postoperative complications. The hospitalisation duration after the index procedure was 2 days less in the short-course group and was cost-effective.

There were certain limitations in this study. Firstly, in the majority of patients the source of IAI was from the appendix or the small bowel with mild to moderate severity. Severe IAI and colon as a source were found only in a limited number of patients. Hence, the results of this study are predominantly applicable to mild to moderate IAI. Secondly, the SSI rate in this study is high compared with the published literature from the Western population. As the authors' hospital is a public sector institute in a developing country, the patient population is usually from a low socioeconomic status, with poor knowledge of personal hygiene and self-care. The patients also mostly present late following the onset of symptoms, which could possibly lead to higher incidence of SSI. Previous published studies by the authors' institute on SSI have also shown a similar rate, indicating the possible role of patient population in the higher rate of SSI.[18,19]

Conclusion

This study showed that short-course antimicrobial therapy when compared with conventional duration therapy has comparable incidences of SSI and recurrent IAI in patients with mild and moderate complicated IAI. The time till the occurrence of composite primary outcomes and the presence of extra-abdominal infections were similar in both groups. There was a significant reduction in the duration of antimicrobial therapy and the length of hospital stays. Future studies are recommended to include critically ill patients to assess the efficacy of short-term antimicrobial therapy following SCP in severe CIAI.

Authors' Contribution

VK and SS conceptualised and BG contributed to the designed the study. PV collected the data and performed the investigation. PV, BG, TM and VK performed the data analysis. SS, TM and VK supervised the work. PV and BG drafted the manuscript. SS, BG, TM and VK reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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