Prevalence and risk factors for surgical site infection after colorectal surgery: a multiple-center prospective study of 3,663 consecutive patients in China

Yu Zhang
Sichuan Academy of Medical Sciences and Sichuan People's Hospital
Hui-Quan Gan
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Jing-Fang Zhou
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Ya-Jie Gong
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Liu-Yi Li
Peking University First Hospital
Xin-Qiang Zhang
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Yue Meng
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Jie-Rong Chen
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Wei-Jiang Liu
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Long Ye
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Xiao-Xiao Wang
Guangdong Provincial People's Hospital & Guangdong Academy of Medical Sciences
Yun-Hu Zhao
Guangdong Provincial People's Hosptial & Guangdong Academy of Medical Sciences
Tie ying Hou (670293234@qq.com)
Guangdong Provincial People's hospital & Guangdong Academy of Medical Sciences
https://orcid.org/0000-0002-5290-5640

Research

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Abstract

Background: Surgical site infection (SSI) after colorectal surgery (CRS) remains a significant problem for its negative clinical outcomes. However, it is poorly understood in China . This study aims to investigate the prevalence, risk factors and microbiology of SSI after CRS.

Methods: A nationwide prospective multicenter design was applied. Patients in 19 Chinese hospitals from 2015 to 2018 were prospectively monitored for SSI after CRS. Demographic data, hospital characteristics, and potential perioperative risk factors were collected and analyzed, using univariate and multivariate logistic regression models.

Results: Among 3,663 study participants, 134(3.66%) episodes of SSI were identified. The prevalence rate of SSI decreased from 5.9 infections per 100 procedures in 2014 to 3.1 infections per 100 procedures in 2017 (prevalence rate ratio [PRR], 0.52; 95% CI, 0.28–0.94). The SSI rates were 1.88, 4.15, 6.27 and 11.58 per 100 operations for the National Nosocomial Infections Surveillance system (NNIS) risk index categories of 0, 1, and 2 or 3, respectively. Escherichia coli (54/134, 40.3%) and Klebsiella pneumoniae (10/134, 7.5%) were the most frequently isolated microorganisms. A high prevalence of antibiotic resistance were observed in our study, with rates of extended spectrum beta-lactamase-producing or carbapenem-resistant Escherichia coli and Klebsiella pneumonia of 50.0%(27/54) and 30.0%(3/10) respectively. Preoperative hospital stay $\geq$ 48h (OR=2.28, 95% CI: 1.03–5.02) and contaminated or dirty wound (OR=3.38, 95% CI: 1.88–6.06) were significantly associated with increasing risk of SSI after CRS.

Conclusion: A statistically significant but modest decrease in the prevalence rate of CRS SSI over the 4-year study period was observed in this study. Noticeably, the relatively high rates of multidrug-resistant pathogens causing SSI after CRS should be alert despite of the small number of isolates identified in our survey.

Introduction

Surgical site infection (SSI) is one of the most commonly reported hospital-acquired infections (HAIs), constituting up to 19.6% of all HAIs in Europe in 2011–2012[1]. The occurrence of an SSI following a surgery can double the risk of postoperative mortality, prolong hospital stay by 7 to 20 days and result in 5-times increased likelihood of readmission[2]. Aside from the deleterious impacts on patients, the development of SSIs also causes a substantial increase in the economic burden. An evaluation of a single SSI event has been reported at $12,000 - $35,000, and the annual nationwide hospital costs of SSI have been estimated ranging from $3 billion to $10 billion in the United States[3]. For both patient and economic reasons, the efforts of SSI reduction are recognized as a crucial quality-improvement priority for surgeons, institutions, and payers.

The SSI rate is particular high in those undergoing colorectal surgery (CRS) due to the clean-contaminated nature of the wound[4], ranging from 3 to 26% [5, 6]. Therefore, the prevention of SSIs after CRS has become a major target of quality improvement intiatives[7, 8]. Investigators in many countries have committed to develop infection control systems and a good example is the National Nosocomial Infection System (NNIS) of the US Centers for Disease Control and Prevention (CDC). The CDC'S NNIS system categorizes all CRS into the same "COLO" group, and the SSI rates within this group are stratified according to the NNIS risk index, which consists of scoring each operation by counting the number of risk factors present among the following: an American Society of Anesthesiologists (ASA) score of 3, 4, or 5; a wound classification of contaminated or dirty-infected; and duration of operation lasting more than 3 hours[9]. The NNIS risk index provided a valid comparison of SSI prevalence among different hospitals. However, additional risk factors including patient and surgical factors are also needed to be identified.

Systematic surveillance of SSIs is essential to accurately address the burden of these infections. Moreover, the surveillance with a feedback to institutions or surgeons has been reported to be effective in reducing the rates of SSIs[10, 11]. In developing countries, China particularly, only scarce data on SSIs after CRS have been reported to date, and previous evaluations of SSIs are hampered by single-hospital retrospective designs, small cohorts and single institutions [12]. Herein, we conducted a SSI surveillance program after CRS in a prospective multicenter design in China. The objectives of this study were to 1) evaluate the prevalence of SSI in colorectal surgery; 2) identify the risk factors for SSI for developing SSIs in these procedures; 3) determine the distribution of the pathogens isolated from post CRS SSIs.

Methods

Study design

A multicenter observational survey of prospective cohort of patients undergoing emerging or elective colorectal surgeries from 2015 to 2018 in 19 hospitals was performed. Patients were identified using an inpatient SSI Surveillance System database and hospital records. The outcome of interest was SSI, determined according to the Centers for Disease Control and Prevention (CDC) criteria[13] and divided into superficial incisional, deep incisional and organ-space SSI with a follow-up of 30 days. Post-discharge surveillance of CRS SSI was mandatory, including a review of electronic clinical records, checking readmissions and emergency visits, and reviewing microbiological and radiological information.

Inclusion and exclusion criteria

All patients who undergo elective or emerging colorectal operations via laparotomy were included in the study. Exclusion criteria for both types of procedures were 1) failure to provide information; 2) those who died during the procedure or immediately after the surgery; 3) Endoscopic operations.

Data collection

The data was collected using a standardized questionnaire. Data was recorded prospectively on each patient in the database, which was completed immediately postoperatively by the operating surgeon. The

Data obtained included patient, pre- /intra- and postoperative information such as demographic data, wound characteristics and laboratory investigations. Information collected included patient characteristics such as age, sex, height, weight; pre-operative variables such as operation class (elective or emergency), ASA grade (1, 2, 3, 4 or 5), wound classification (clean or I, clean/contaminated or II, contaminated or III, and dirty or IV), and preoperative hospital stay[14]; intra-operative data included duration of operation and multiple procedures; post-operative variables such as the diagnosis and classification of SSI (superficial incisional, deep incisional, or organ/space), date of SSI, date of hospital discharge, information of isolated pathogens and antimicrobial susceptibility.

Patient age was evaluated as a categorical variable ($<$60 years and $\geq$ 60 years). ASA score was evaluated as a categorical variable ($\leq$ 2 and $>$2). Duration of operation was evaluated as a categorical variable (first quartile, second quartile, third quartile and fourth quartile of the duration of operation). Also, for each case, the NNIS risk index was calculated and the risk index (0, 1, 2 and 3) was dichotomized for the analysis. Other variables were all categorical variables and are presented in the results.

Statistical analysis

Unadjusted log-binomial regression was applied to calculate the annual crude prevalence rates and prevalence rate ratios (PRRs) for all SSIs. And multivariate log-binomial regression model was constructed to further analyze SSI prevalence trends over time. The univariate relation between each independent factor and SSI was tested using the Student t test for the continuous variable and two-tailed Fisher exact test or chi-square test for categorical variables. Variables that were statistically significant or presented a trend ($P < .20$) were introduced in the multivariate logistic regression analysis for the study of the risk factors. P values $<$ 0.05 were considered to be statistically significant. In these cases, results were given as odds ratios (OR) and 95% confidence intervals (95% CI). All of the statistical analyses were performed using SPSS software (version 23.0, SPSS Inc, Chicago, IL).

Results

Prevalence of SSI and trends over time

A total of 134 SSIs occurred following 3,663 consecutive CRS procedures performed over the 4-year study period, giving an overall prevalence rate of 3.66 infections per 100 procedures. The prevalence rate of SSI decreased from 5.9 infections per 100 procedures in 2014 to 3.1 infections per 100 procedures in 2017 (prevalence rate ratio [PRR], 0.52; 95% CI, 0.28–0.94) (Table 1). The prevalence of different types of SSI after stratification by NNIS score is shown in Table 2. A total of 77(2.10%) patients had superficial incisional SSIs, 26(0.71%) had deep incisional SSIs and 31(0.85%) had organ/space SSIs. There was a considerable difference in SSI rate between each NNIS score group, and an increasing SSI rate with higher NNIS score was observed ($P_{trend} = 1.02 \cdot 10^{-10}$).

Table 1
Prevalence rates of surgical site infection (SSI) in 19 hospitals from 2015 through 2018

Year	No. of procedures	No. of SSIs	PR (95% CI)	PRR (95% CI)
2015	290	17	5.9(3.4–8.6)	1.00
2016	960	33	3.4(2.3–4.7)	0.57(0.31–1.04)
2017	1320	50	3.8(2.7–4.9)	0.63(0.36–1.11)
2018	1093	34	3.1(2.1–4.2)	0.52(0.28–0.94)
NOTE. Rates were calculated per 100 procedures. Prevalence rates (PRs), prevalence rate ratios (PRRs), and confidence intervals (CIs) were calculated with log-binomial regression models.				

Table 2
Prevalence of surgical site infection (SSI) after colorectal surgery by NNIS score

Type of SSI	SSI prevalence by NNIS score, % (no. of SSIs/no. of procedures)				
	0	1	2	3	Total
Superficial	1.15% (19/1,651)	2.32% (33/1,420)	3.61% (19/526)	6.32% (6/95)	2.10% (77/3663)
Deep	0.42% (7/1,651)	0.77% (11/1,420)	1.14% (6/526)	2.11% (2/95)	0.71% (26/3663)
Organ/space	0.12% (2/1,651)	0.35% (5/1,420)	0.76% (4/526)	2.11% (2/95)	0.85% (31/3663)
Total	1.88% (31/1,651)	4.15% (59/1,420)	6.27% (33/526)	11.58% (11/95)	3.66% (134/3663)
Note. NNIS: National Nosocomial Infections Surveillance system					

Risk factors for SSIs in colorectal surgery

The risk factors associated with SSI in CRS by univariate analysis were presented in Table 3. Emergent operation, a higher ASA score, contaminated or dirty wound and longer duration of operation were statistically associated with a higher prevalence of SSI. Older than 60 years, male sex, multiple procedures and preoperative hospital stay ≥ 48 h also exhibited a tendency to develop SSI, as their P values were less than 0.2.

Table 3

Univariate analysis of risk factors for surgical site infection in colon and rectal surgery

Variables	No. of SSI	No. of no SSI	Prevalence of SSI (%)	OR (95% CI)	P-value
Age, years					
<60	57	1115	4.86	1.00	
≥60	64	1684	3.66	0.74(0.516–1.07)	0.111
Sex					
Male	84	2005	4.02	1.00	
Female	50	1524	3.18	0.78(0.55–1.12)	0.179
BMI					
18.5 ~ 25	84	2236	3.62	1.00	
<18.5	16	345	4.43	1.24(0.72–2.13)	0.450
>25	27	587	4.40	1.22(0.79–1.91)	0.370
Emergent operation					
No	83	2796	2.88	1.00	
Yes	34	385	8.11	2.98(1.97–4.50)	$2.303\cdot10^{-7}$
ASA					
1 ~ 2	76	2454	3.00	1.00	
3 ~ 5	58	1071	5.14	1.75(1.423–2.48)	0.002
Multiple procedures					
No	103	2910	3.42	1.00	
Yes	14	272	4.90	1.45(0.82–2.58)	0.199
Preoperative hospital stay					

Note. *Senior doctor included deputy chief physician and chief physician. **Junior surgeon defined an intern doctor, junior physician and intermediate doctor.

Variables	No. of SSI	No. of no SSI	Prevalence of SSI (%)	OR (95% CI)	P-value
<48 h	17	331	4.89	1.00	
≥48 h	83	2576	3.12	0.63(0.37–1.07)	0.087
Wound class					
I or II	94	3153	2.89	1.00	
III or IV	40	367	9.83	3.66(2.149–5.38)	$4.36 \cdot 10^{-11}$
Duration of operation (min)					
≤120	21	867	2.36	1.00	
121–154	30	916	3.17	1.35(0.77–2.38)	0.296
155–200	30	870	3.33	1.42(0.81–2.51)	0.221
>200	53	876	5.71	2.50(1.49–4.18)	$4.82 \cdot 10^{-4}$
Type of surgeon					
Senior*	111	3215	3.34	1.00	
Junior**	22	269	7.56	2.37(1.48–3.81)	$3.63 \cdot 10^{-4}$

Note. *Senior doctor included deputy chief physician and chief physician. **Junior surgeon defined an intern doctor, junior physician and intermediate doctor.

The results of multivariate analysis were shown in Table 4. Independent risk factors for SSIs after colorectal surgery were preoperative hospital stay ≥ 48 h (OR 2.28, 95% CI 1.03–5.02) and contaminated or dirty wound (OR 3.38, 95% CI 1.88–6.06).

Table 4
Multivariate analysis of risk factors for surgical site infection in colon and rectal surgery

Variables	**OR**	**95% CI**	**P-value**
Age ≥ 60 years	0.62	0.38-1.00	0.050
Female sex	0.66	0.41–1.06	0.086
Emergent operation	1.78	0.82–3.86	0.146
ASA > 2	1.37	0.83–2.29	0.221
Multiple procedures	1.19	0.60–2.35	0.619
Preoperative hospital stay ≥ 48 h	2.28	1.03–5.02	0.042
III or IV wound class	3.38	1.88–6.06	$4.50 \cdot 10^{-5}$
Duration of operation > 75th	1.52	0.82–3.22	0.209
Junior surgeon	1.62	0.82–3.19	0.167

Microbiology cultures and antibiotic resistance

The microbiology results for isolates from SSI are shown in Table 5. Of the 134 CRS SSIs, 85 (63.43%) patients with 92 positive culture isolates were identified, and 6 patients (4.48%) had polymicrobial infections. Escherichia coli was the most common organism causing SSI (1.47 infections per 100 procedures) and was responsible for 54 (40.30%) colorectal SSIs. Besides, a preponderance of Klebsiella spp. (n = 10, 7.46%) in CRS SSI was observed, while Enterococcus and Pseudomonas aeruginosa were also frequent at rates of 6.72% (n = 9) and 5.97% (n = 8) respectively. Besides, cultures were either negative or not obtained for 36.57% (n = 49) of SSIs after CRS. Antibiotic resistance to one or more antibiotics occurred in 30 isolates (32.61%, 30/92), with rates of extended spectrum beta-lactamase-producing or carbapenem-resistant Escherichia coli (n = 27) and Klebsiella pneumonia (n = 3) of 50.0% and 30.0% respectively, carbapenem-resistant Pseudomonas aeruginosa (n = 2) and Acinetobacter baumanii (n = 1) of 25.0% and 25.0% respectively, but with low rates of typical multidrug-resistant microorganisms such as methicillin resistant Staphylococcus aureus (MRSA) (0.0%, n = 0).

Table 5
Organisms causing surgical site infections in colorectal surgery at 19 hospitals from 2015 through 2018

Isolated microorganisms	No.(%) (n = 134)	No. (%) of antibiotic resistant strains
Bacteria		
Escherichia coli	54 (40.3)	27 (50.0)
Klebsiella pneumoniae	10 (7.5)	3 (30.0)
Pseudomonas aeruginosa	8 (6.0)	2 (25.0)
Acinetobacter baumannii	4 (3.0)	1 (25.0)
Staphylococcus aureus	5(3.7)	0 (0)
Enerococcus	9 (6.7)	0 (0)
Other		
Candida spp.	2 (1.5)	—
Polymicrobial[a]	6 (4.5)	—
No pathogen identified[b]	49 (36.6)	—

[a]Polymicrobial infections were also included in individual SSI counts for each organism isolated.

[b]Negative cultures or no cultures taken.

Discussion

This series of postoperative SSIs in colorectal surgery, to date, is the largest multi-center prospective study in China. Overall, we identified SSIs in 134(3.66%) cases among 3663 patients with colorectal surgery. Historically, the prevalence of SSI in CRS has showed a wide range in prevalence mostly due to differences in the definition and the follow-up period[13, 15–17]. Recent reports[6, 18–20], however, continued to range widely in prevalence (2.4%-21.6%) despite the fact that the current definition of SSI issued by American CDC was globally accepted. Our SSI rate in colorectal surgery ranges in the lower field compared to other studies. Several factors may explain the discrepancy in SSI rates between our work and other surveys. First, the SSI rate of CRS varied across different hospitals; small hospital size (□250 beds) and community hospitals have been reported higher rates, while all member hospitals in our study are university-affiliated or tertiary-care hospitals which are generally thought to have more experience and high skills in colorectal operations. Also, the results from single-center and multicenter settings, benign and malignant neoplasms, and colon and rectal diseases may lead to the differences in CRS SSIs rates. Finally, our survey combines data from complex (deep incisional or organ-space) and superficial SSIs while some reports[19, 20] only reported complex SSI rates in CRS which, to some extent, explained the disparities.

The analysis revealed a statistically significant but modest decrease in the prevalence rate of CRS SSI over the 4-year study period. We hypothesized our surveillance program and improved SSI control practices were 2 of several factors that contributed to the decreased prevalence of SSI in CRS. It has been reported that SSI surveillance could help to reduce SSI[10, 13, 21]. Our surveillance program calculated the SSI rates according to stratification of NNIS index, determined procedure-specific risk factors, and periodical feedback of actual SSI rates to hospitals, which partly explained the reduction in SSI rates of CRS[13, 22]. Moreover, 'Guidelines for prevention and control of surgical site infection (Trial)' was issued by National Health Commission of the People's Republic of China (NHCPC; formerly the Chinese Ministry of Health) in 2010[23], and thoroughly described the pre-, intra- and post-operative preventive measures, such as surgical hand preparation, implement perioperative glycemic control, maintain perioperative normothermia, administration antibiotic prophylaxis within 120 minutes and no hair removal or clipping if needed, most of them were still recommended with high- or moderate-quality evidence by the latest guidelines released by American CDC in 2017[24] and WHO in 2016[25]. These measures had positive effect on reducing SSI which had be described through some previous studies[26, 27], however, the impact that these infection control measures had upon SSI rates in CRS could not be quantified in our study because we were not accessible to the hospitals involved.

Many factors, including patient- and procedure-related variables, influence surgical wound healing and affect a patient's risk of developing an SSI. NNIS risk index has been proved to be an useful tool to assess the risk of developing SSI and widely used to adjust the risk of SSI for the comparison among different institutions or surgeons[28–30]. In our survey, the risk of SSI after colorectal operations was found to increase as the NNIS risk index score increased like other studies[6, 19], and SSI rates stratified by NNIS index were similar to the corresponding U.S. NNIS rates[31]. Patient-dependent variables had little effect on the prevalence of SSI in our study. Age was reported to be a significant risk factor in few of the other operative procedures[32], but not in CRS[15, 16, 19, 33–35]. Interestingly, our present data indicated that patients older than 60 years showed a decreased risk tendency for SSI, even though the difference was not statistic significant. This result was mainly consistent with the data from NHSN in which age was not a risk factor in rectal surgery but showed an inverted relationship with risk of SSI in colon surgery[32]. We presumed that this inverse association could be in part explained by surgeons' additional consideration about older patients. They might be selected on the basis of indications for operation, and surgeons might also tend to avoid invasive techniques in treating older individuals. ASA score has been confirmed as an independent risk factor of SSI in a few studies[6, 16, 35], but this association could not be detected in some other surveys[5, 18, 19, 36]. The present data indicated the ASA score more than 2 was a predictive factor in SSI in CRS by the univariate analysis, whereas, not confirmed in the multivariate analysis. The controversy was also found for BMI index and sex. Higher BMI index and male sex were expected to be associated with the increasing risk of SSI higher BMI index [15, 18, 37], whereas, did not be confirmed in this study as well as some other surveys[5, 34, 35]. More researches are needed to clarify the effect of patient-dependent factors on the risk of SSI after CRS.

Most of the operation-related variables were detected to be associated with higher risk of developing SSI after CRS in the univariate analysis in this study, and 2 variables were finally confirmed in the multivariate analysis. Our results indicated that contaminated or dirty wound class significantly increased the risk of developing SSI after CRS, with 3.38 times higher risk compared to clean or clean-contaminated wound class. The National Research Council (1964) laid the foundation for a system of surgical wound classification[38] and higher bacterial contamination load was recognized as a greater risk for developing SSI that has been confirmed by numerous reports[9, 16, 18]. Preoperative hospital stay more than 48 h was also an independent risk factor in this survey, in keeping with the results of previous studies[34, 39–41]. It is difficult to explain why longer preoperative hospital stay increased risk of CRS SSI. One possible explanation derived from the finding that sicker or more elderly patients who need more diagnostic tests before surgery, as reflected in the American Society of Anesthesiologists classification. Besides, longer preoperative hospital stay may also increase patients' risks in developing SSIs by contacting with medical environment or personnel and colonization by microorganisms[34]. Some studies reported that emergent operation was associated with higher rate of SSI after CRS[6, 36], and longer surgery duration was an independent risk factor for development of SSI in CRS[16, 19, 36]. Nevertheless, neither of these factors were confirmed in our data, despite that increasing susceptibility of SSI was detected, whereas, not statistically significant. The impact of these 2 variables on developing SSI was remained questionable, because negative results were also found in some other reports[5, 34]. The association between SSI and the individual surgeon has been reported by some previous studies[10, 16, 42]. Our analysis showed surgeon with less experience in operation was a significant predictor in the univariate modeling analysis, although it did not remain so in the multiple regression model. Senior surgeons were generally thought to have more experience and high surgical technique in operation. A moderate quality of evidence showed that surgical procedures performed by high- or medium-volume surgeons had lower SSI rates compared to low volume ones in an unpublished systematic review conducted by WHO[43], however, there was controversial evidence when high- and medium-volume hospitals were compared, thus it remained unclear whether there was a linear relationship between procedure/surgeon volume and the SSI rate. Above all, more well-designed epidemiology study, including case-control study, cohort study and meta-analysis, or biological mechanism studies are needed to clarify the association of host- and surgery-dependent variables with SSI after CRS in the future.

Besides the host and operation factors, microorganism was also important in development of SSI. Physicians should be, particularly, cautious about the increases in high-virulence microorganisms, such as Staphylococcus aureus and Streptococcus pyogenes, and multidrug-resistant pathogens[44]. In our population, gram-negative microorganisms constituted more than 80% of the isolated strains, and Escherichia coli and Klebsiella pneumonia were the two most frequently isolated pathogens. Noticeably, high rates of multidrug-resistant were observed in Escherichia coli and Klebsiella pneumonia when compared with other series[36]. This discrepancy could be explained by the higher antibiotic pressure, use of broad-spectrum antibiotics, illness severity and prolonged treatment periods. Nevertheless, the results should be treated cautiously because of the relatively small number of isolated microorganism strains.

There were several limitations that should be acknowledged. First, we did not gather data from smaller hospitals in this study which may result in selection bias. However, the cases we studied were from 19 hospitals that were distributed nationwide. Besides, considerable patients in China preferred to perform colorectal surgeries in large healthcare settings such as university-affiliated or tertiary-care hospitals because of their better medical circumstances, more advanced armamentariums and higher qualified staffs. Thus, we believed that the results of this study in part reflected the situation in hospitals in China nationwide. Second, the retrospective analysis of prospectively collected data might lead to bias and the statistical correlations between the risk factors and SSI did not determine any “cause-and-effect” relationship between them. Third, certain risk factors that have been reported associated with SSI such as perioperative hypothermia, hyperglycaemia and blood transfusion were not collected. Last, nearly 37% of the patients who developed an SSI did not available for information of causative pathogens; besides, no anaerobic culture was done and lack of antibiotic policy may also have affected the SSI rate.

Despite of that, this study is to date the first time describing a prospective nationwide survey with a large number of patients undergoing colorectal surgery in China. Our survey provides an insight into the burden and microbiology of SSI after CRS in China. Moreover, physicians and administrators in medical institutions should be aware of the relative high antibiotic resistance in pathogens causing SSI. We also identifies that preoperative hospital stay ≥ 48 h and contaminated or dirty wound are both significantly associated with the increasing risk of SSI after CRS. However, more well-designed cohort with large population or meta-analysis and biological researches are needed to further clarify the relationship between risk factors with development of SSI after CRS, concerning that controversies are commonly observed among current surveys.

Conclusion

A statistically significant but modest decrease in the prevalence rate of CRS SSI over the 4-year study period was observed in this study. Noticeably, the relatively high rates of multidrug-resistant pathogens causing SSI after CRS should be alert despite of the small number of isolates identified in our survey.

Declarations

Ethics approval and consent to participate:

This study was approved by the Ethics Committee at Guangdong Provincial People’s Hospital & Guangdong Academy of Medical Sciences (reference: No. GDREC2019455H).

Consent for publication:

Not applicable.

Availability of data and material:

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Authors' contributions:

YZ and HQ G participated in the design of the study and drafted the manuscript; JF Z and YJ G performed the statistical analysis; LY L participated in its design; XQ Z, Y M, JR C, WJ L, LY, XX W and YH Z collected the data and participated in the statistical analysis; TY H conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

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