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Bacteriological Profile of Surgical Site Infection Following Gastrointestinal Surgery and Their Antibiogram

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ABSTRACT

Background: Surgical site infection is one of the common complication following abdominal surgery. It causes great morbidity and mortality, further increasing prevalence of multidrug resistant bacteria have made its management very challenging. The current study aims to identify causative agent responsible for surgical site infection and their antibiotic resistance patterns.

Methods: This study was conducted among patients developing surgical site infection following gastrointestinal surgery in Tribhuvan university teaching hospital over a period of one year. The samples were collected and processed according to standard methods. The bacterial pathogens with their antimicrobial susceptibility were determined and resistant pattern like methicillin resistant *Staphylococcus aureus* and extended spectrum beta lactamase were further detected.

Results: A total of 832 patients had under gone gastrointestinal surgery during the study period. Among them, 162 cases (19.5%) developed surgical site infection and 125 cases showed growth in culture. A total of 160 aerobic bacteria were isolated; *Escherichia coli* (29.9%) was the commonest organism with 40.8% being extended spectrum beta lactamase producer and 47.4% of *Staphylococcus aureus* were methicillin resistant. About 75.9% (85/112) of gram negative bacteria and 60.4% (29/48) gram positive bacteria were multi drug resistant.

Conclusions: The burden of multi drug resistant bacteria causing surgical site infection is high which needs to be addressed timely. Good surveillance of bacterial antibiogram and rational antimicrobial use is necessary to reduce emergence and spread of resistant bacteria.

Keywords: Extended Spectrum beta lactamase; gastrointestinal surgery; methicillin resistant *staphylococcus aureus*; multi drug resistance; surgical site infection

INTRODUCTION

Surgical site infection (SSI) refers to the infection that occur in the wound created by an invasive surgical procedure, majority of which become apparent within 30 days.[1,2] *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* are some of the common organisms responsible for SSI.[3-6] It is the third most common healthcare associated infection (HAI) accounting for 20% of all HAIs.[7,8] The rate of SSI can range from 2.5% to 41.9% depending on the type of operation and underlying patient status.[9,10] Also, the prevalence of MDR bacteria is in a rising trend, some study from Nepal show the prevalence being more than 60%.[11] However, the burden of MDR bacteria in SSI cases following gastrointestinal surgery have not been studied much. Thus, the aim of this study was to determine the burden of SSIs in GI surgery, identify the causative pathogen and their antibiotic susceptibility to commonly used antibiotics.

METHODS

This cross-sectional study was conducted at Tribhuvan University Teaching Hospital from October 2016 to September 2017; among patients developing SSI following gastrointestinal surgery. The study was conducted after approval from the Institutional Review Board (IRB) of Institute of Medicine, Tribhuvan University. The diagnosis of SSI was based on the criteria laid down by the Centers

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for Disease Control and Prevention (CDC).[12] All cases that had under gone GI surgery were followed up for clinical signs of SSI for 30 days. The stitches were usually removed on outpatient basis and follow up at 30 days was done through telephone conversation regarding the state of the wound. The patients were asked if they had discharge, swelling, pain or erythema at the incision site; if any of these symptoms were present, they were requested to come to the Out Patient Department (OPD) clinics.

Patient with implants and those not willing to give consent were excluded from the study. The wounds were classified as clean-contaminated (when an incision is made through alimentary tract under controlled conditions with no unusual contamination), contaminated (an operative wound in which there is major break in sterile technique or gross spillage from the gastrointestinal tract), or dirty wound (an incision undertaken when the viscera are perforated or when acute inflammation with pus is encountered during surgery).[2] As clean wound include incision in which no inflammation is encountered and alimentary tracts are not entered, this wound was excluded from our study.

Microbiological samples such as discharging pus, tissues, body fluid from the surgical site were collected aseptically and transported immediately to microbiology laboratory where it was further processed. The samples were inoculated into Blood agar, Chocolate agar and MacConkey agar. All plates were then incubated at 37°C for 24 to 48 hours; blood agar and chocolate agar plates were incubated in CO_2 incubator (10%). The isolates were identified using standard microbiological techniques like morphological appearance of the colonies, Gram's staining and various biochemical tests (catalase test, coagulase test, oxidase test, motility test, citrate utilization, urea hydrolysis, decarboxylase test, Hugh and Leifson's Oxidation fermentation test, MR/VP etc.)[13] were performed.

Antimicrobial susceptibility test of aerobic bacterial pathogen against different antibiotics were done by the standard modified Kirby Bauer Disc Diffusion method as recommended by CLSI using Mueller Hinton Agar (MHA) (Oxoid, UK).[14] The bacterial susceptibility towards different antimicrobial agents were then reported as 'sensitive', 'resistant' or 'intermediate' as recommend by CLSI guidelines. Isolates resistant to at least one agent in three or more antimicrobial categories were regarded as multi drug resistant (MDR) and bacterial isolates non-susceptible to at least one agent in all except only one or two categories were considered as Extensively drug-resistant (XDR).[15]

Screening of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant Coagulase negative *Staphylococcus* species (MRCoNS) was done by Cefoxitin (30 µg) disc diffusion method, organisms with the zone of inhibition ≤ 21 mm and ≤ 24mm for cefoxitin were considered MRSA and MRCoNS respectively. Also, Gram negative bacteria were further screened for ESBL production using ceftazidime (CAZ) (30 µg) and cefotaxime (CTX) (30 µg) disks. Isolates showing the zone of inhibition ≤22 mm for ceftazidime and ≤27 mm for cefotaxime was considered as a potential ESBL producer and confirmation was done by combination disk method where CAZ and CTX were tested alone and in combination with clavulanic acid (Ceftazidime-clavulanate 30/10µg and Cefotaxime-clavulanate 30/10 µg). Increase in ≥5mm zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone were confirmed as ESBL producer.[14]

RESULTS

The study was conducted among patients developing SSI following gastrointestinal surgery. A total of 832 gastrointestinal surgeries were performed during the study period, of them 162 (19.5%) patients developed clinical signs of surgical site infection. Majority (58%) of these cases had under gone emergency surgery. Clean contaminated wound (49%) was the commonest type of wound followed by contaminated wound (40%) and dirty wound (11%). Similarly, the most common type of SSI was superficial SSI (72.2%) followed by organ SSIs (24.1%) and deep SSI (3.7%). Among the total SSI cases maximum were male patient (57%) and most (52; 32.1%) belonged to age group 30-50 years.

Out of the total 162 SSI cases, the samples of 125 (77.1%) cases produced growth where 91 (72.8%) samples showed monomicrobial and 34 (27.2%) samples produced polymicrobial growth. A total of 164 organisms were isolated; among them 160 isolates were aerobic bacteria, gram negative bacteria constituted 70% (112/160) while 30% were gram positive bacteria. The most common isolate was *E. coli* (49; 29.9%) as depicted on Table 1.

Table1. Distribution of isolated organisms.

Organism isolated	Frequency	Percent
Escherichia coli	49	29.9
Staphylococcus aureus	19	11.6
Klebsiella pneumonia	15	9.2
Enterococcus species	14	8.6
Pseudomonas aeruginosa	14	8.6
Coagulase Negative *Staphylococci* species	13	7.9
Acinetobacter calcoaceticus baumannii complex	10	6.1
Citrobacter freundii	9	5.5
Enterobacter species	5	3.0
Klebsiella oxytoca	3	1.8
Citrobacter koseri	3	1.8
Proteus mirabilis	3	1.8
Streptococcus species	2	1.2
Acinetobacter lwoffii	1	0.6
Fungi		
Candida species	4	2.4
Total	164	100

In this study gram negative bacteria showed variable resistance to tested antibiotics as shown in Table-2. *E. coli*, was the most resistant of all Enterobacteriaceae; being resistant to many antibiotics like amoxycillin (95.9%), cefixime (91.9%), ofloxacin (89.8%), levofloxacin (65.3%), gentamycin (59.2%), piperacillin-tazobactam (55.1%) and imipenem (24.5%). Almost comparable rate of resistance was observed in isolates of *Citrobacter* spp. More than 50% of *Klebsiella* spp. were resistant to majority of antibiotics tested. Maximum (60%) *Enterobacter* spp. isolates were resistant to 3rd generation cephalosporin.

High (70-100%) percentage of *Acinetobacter* spp were resistant to cephalosporin, fluoroquinolones, aminoglycosides and even carbapenem. However, isolates of *Pseudomonas spp* were susceptible to majorities of antibiotics tested.

About 75.9% (85/112) of gram-negative isolates were multidrug resistant and 12.5% (14/112) were extensively drug resistant bacteria; further details are presented in Table- 3. Also, nearly 28.6% (32/112) of gram-negative isolates were ESBL producers; of them *E. coli* (40.8%) was the predominant ESBL producer.

Table 2. Antibiotic resistance pattern of Gram Negative Bacteria.

Antibiotics	Percentage of resistance among Gram negative bacteria					
	E. coli (N= 49)	*Klebsiella spp.* (N=18)	*P. aeruginosa* (N=14)	*Citrobacter spp.* (N=12)	*Acinetobacter spp.* (N=11)	*Enterobacter spp.* (N=5)
Ampicillin	95.9	-	-	91.6	-	100
Cefixime	91.9	66.7	-	83.3	-	60
Gentamycin	59.2	55.5	28.6	41.6	90.9	20
Amikacin	40.8	50	21.4	33.3	72.7	20
Ofloxacin	89.8	66.7	35.7	66.7	90.9	40
Levofloxacin	65.3	55.5	35.7	66.7	81.8	40
Cefepime	61.2	55.5	28.6	41,6	100	20
Ceftazidime	-	-	35.7	-	100	-
Piperacillin tazobactum	55.1	44.4	21.4	33.3	81.8	40
Ampicillin Sulbactam	-	-	-	-	81.8	-
Imipenem	24.5	27.7	21.4	33.3	81.8	0
Meropenem	24.5	27.7	21.4	33.3	81.8	0

Table 3. Burden of MDR and XDR among Gram negative bacteria.				
Bacteria	MDR		XDR	
	Number	%	Number	%
E. coli (N=49)	43	87.8	4	8.2
Klebsiella spp. (N=18)	12	66.7	1	6.7
P. aeruginosa (N=14)	6	42.9	2	14.3
Acinetobacter spp. (N=11)	11	100.0	5	40.0
Citrobacter spp. (N=12)	9	75	2	11.1
Enterobacter spp. (N=5)	3	60.0	0	0.0
P. mirabilis (N=3)	1	33.3	0	0.0
Total (N=112)	85	75.9	14	12.5

The antibiogram of gram positive bacteria is depicted in Table- 4. Most isolates of *Staphylococcus aureus* and CoNS showed susceptibility to doxycycline, clindamycin and amikacin, while all were sensitive to teicoplanin and linezolid. More than half isolated *Enterococcus* spp. were resistant to antibiotics like ampicillin, erythromycin and doxycycline while sensitive to chloramphenicol and vancomycin. However, 2 isolates were Vancomycin Resistant Enterococcus (VRE) and all these isolates were sensitive to linezolid.

Table 4. Antibiotic resistance pattern of Gram positive bacteria.			
Antibiotics	Percentage of resistance among Gram positive bacteria		
	S. aureus (N= 19)	CoNS (N=13)	*Enterococcus spp.* (N=14)
Ampicillin	84.2	92.3	71.4
Cotrimoxazole	57.9	61.5	-
Erythromycin	63.2	69.2	64.3
Clindamycin	31.6	23	-
Amikacin	10.5	15.4	-
Ofloxacin	52.6	46.2	-
Chloramphenicol	21	30	35.7
Doxycycline	26.3	30.7	50
Teicoplanin	0	0	-
Vancomycin	-	-	14.3
Linezolid	0	0	0

CoNS- Coagulase Negative Staphyloccocus species

About 60.4% (29/ 48) of gram-positive bacteria were MDR and 4.2% (2/48) were XDR their detail along with burden of other resistance patterns are depicted in Table 5.

Table 5. Burden of MDR, XDR and other resistances among Gram postive bacteria.												
Bacteria	MDR		XDR		MRSA		MRCoNS		iMLSB		VRE	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
S.aureus (N=19)	11	57.9	-	-	9	47.4	-	-	4	21.1	-	-
CoNS (N=13)	10	76.9	-	-	-	-	7	53.8	2	15.4	-	-
Enterococcus spp. (N=14)	8	57.1	2	18.2	-	-	-	-	-	-	2	14.3
Streptococcus spp. (N=2)	-	-	-	-	-	-	-	-	-	-	-	-
Total (N=48)	29	60.4	2	4.2	9	47.4	7	53.8	6	18.8	2	14.3

CoNS- Coagulase Negative *Staphyloccocus* species, MRCoNS- Methicillin Resistant Coagulase Negative *Staphyloccocus* species, iMLSB- Inducible Macrolide Lincosamide Streptogramin B , VRE- Vancomycin Resistant *Enterococci*

DISCUSSION

In this cross-sectional study, we assessed total 832 gastrointestinal surgeries cases, of them 162 (19.5%) cases developed clinical signs of surgical site infection. Out of total SSIs cases, 58% had under gone emergency surgery.

Several studies suggest rate of SSI can range from 2.5% to 41.9% depending on the type of operation.[9,10] In our study, as most of the surgeries were emergency cases involving gastrointestinal tract and clean wounds were not included hence the incidence might be slightly higher. Emergency surgeries are associated with higher risk of SSI, inadequate pre-operative preparation and the greater frequency of contaminated or dirty wounds could be the few reasons behind it.

We observed maximum isolated organisms (70%) were gram negative bacteria which is consistent with many other studies.[5,6] Studies which have also included anaerobic organism, suggest that polymicrobial pathogen are more commonly isolated in SSI cases.[16-18] As anaerobic organisms were not included in this study, majority cases (73%) showed monomicrobial pathogen which was in concordance with Negi et al study where only 5% polymicrobial growths were seen.[19]

The two most common pathogen isolated was *E. coli* (29.5%) and *Staphylococcus aureus* (11.5%) which is also seen in many other studies.[16,20] Earlier study by Banjara et al in 2003 showed *S. aurues* to be the predominant isolate but unlike this study it was not limited to GI surgeries.[21] Similarly, *Klebsiella species* and *Pseudomonas aeruginosa* were isolated in 9% and 8.4% of cases respectively which was consistent with other studies.[11,22] Also, organism like *Enterococcus* spp. contributed 8.4% and Coagulase negative *staphylococci* 7.8% which was similar to figures from Ethiopia and Uganda.[23] However, some study from Nepal show their prevalence in SSI to be extremely low.[11]

About 71.2% of total bacteria in this study were multidrug resistant which was extremely high compared to previous study done in 2003 from the same institute where only 47.2% MDR bacteria were reported. [21] Similar trend of rising resistance have been reported from different part of the world.[23-25] Also, gram negatives bacteria were found to be more resistant than the gram-positive bacteria as seen in many other studies.[10,26,27]

Third generation cephalosporins are commonly used antibiotics in our setting and the study shows rising concern about these drugs as majority (60-90%) of gram negative bacteria are resistant to them. One important reason could be the increasing prevalence of beta lactamase producing bacteria; beta-lactamases like ESBL are capable of conferring resistance to the penicillins, first, second, third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems). In a study conducted in National Cancer Institute of Mexico more than half (56.1%) of all SSIs occurring between 2008 and 2012 were caused by *E. coli*; of them 37.1% were ESBL producers.[28] Our study also showed a significant burden of ESBL producers (28.6%) among gram negative bacteria; especially in *E. coli* (40.8%, 20/49) and *Klebsiella* spp. (27.8%, 5/18). The outbreaks of infection with ESBL-producing organisms have been reported from virtually every part of the world.[29] There are very few drugs effective against ESBL producing bacteria one among them is carbapenem like imipenem and meropenem; however in this study carbapenem were not 100% susceptible, the most resistant of all was *Acinetobacter species*. Emergence of carbapenemases is a global treat as they confer resistance to all ß-lactams and also have ability to disseminate rapidly. [30]

About 60.4% of total gram-positive bacteria were multidrug resistant in this study. The burden of MRSA was 47.4%. which was much more compared to studies from India (10-15%).[19] However, Vancomycin resistance among these MRSA could not be assessed in this study which is its limitation. Similarly, more than half (57.1%) *Enterococcus* species were MDR and two among them were resistant to vancomycin which is in contrary to study from country like India and Japan, which were 100% susceptible.[16]

CONCLUSIONS

There is high rate of SSI among patients undergoing abdominal surgeries. *E. coli* is the most common organism responsible for SSI followed by *S. auerus*. The burden of MDR bacteria in SSI was too high, with gram negative bacteria being more than gram positive bacteria. The resistance pattern especially ESBL in gram negative bacteria and MRSA in gram positive bacteria is alarming. The most commonly prescribed antibiotics like amoxycillin, 3rd generation cephalosporin and fluoroquinolones were not much effective among gram negative isolates while aminoglycosides, pipericillin-tazobactam and carbapenems showed good effectiveness except for *Acinetobacter species*. There are limited treatment option against these resistant superbugs. Therefore, identifying the true pathogen, rational antimicrobial use and continuing surveillance of bacterial antimicrobial sensitivity tests at local level are necessary to reduce emergence and spread of resistant bacterial isolates.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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