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Association of Bacterial Species with Surgical Site Infections at Omdurman Teaching Hospital

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Abstract

Background: Wound infections are common complications of surgery and add significantly to the morbidity and mortality of patients and cost of treatment. Increase of infection is partly attributed to antimicrobial resistance of bacterial pathogens.

Objectives: To determine the association of bacterial species with surgical site infections at Omdurman Teaching Hospital

Materials and methods: This work was a comparative, longitudinal study conducted at Omdurman Teaching Hospital during the period from June 2015 to February 2016. It was which was run among surgical operation patients suffering of clean, clean contaminated, and contaminated wounds. These patients were followed up for a period of one month. Specimens were cultured, and Gram stain and biochemical tests were made for full identification. Antimicrobial susceptibility pattern of isolated pathogens was determined by Kirby-Bauer disc diffusion method.

Results: The study investigated 150 patients including 32.7% males and 67.3% females. Mean age ± SD was 38 (range 18-76 years). Infection was found in 6% clean, 8% clean contaminated, and 16% contaminated surgical wounds. Quarter of these wounds was superficially infected. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain was the most frequently isolated pathogenic organism from the infected postoperative wounds.

Conclusion: Meticulous follow up and routine culture techniques should be performed whenever surgical site infection (SSI) was suspected. The choice of antibiotics for treatment of SSIs should be guided by routine antimicrobial sensitivity testing, including MRSA and extended spectrum β-lactamase (ESBL) bacterial strains. No strong evidence was found to support the use of post-operative antibiotic prophylaxis in reducing post-operative surgical site infections.

Key words: Associated bacteria, Surgical site infections, Omdurman Teaching Hospital

Introduction:

Surgical site infection (SSI) is increasingly recognized as a measure of the quality of patient care by both healthcare providers and the public [(1)]. They are the second most common cause of hospital acquired infections.[(2)]

Other types of healthcare-associated infections mainly affecting surgical patients are postoperative respiratory and urinary tract infections, bacteraemia, and antibiotic related diarrheas [3].
Incisional infections are controlled easily; however deeper and more extensive infections may have devastating consequences [4]. The occurrence of such infections increases the length of hospital stay, admission to the intensive care unit, incidence of re-admission and risk of mortality [5].
Factors influencing the rate of infection can be categorized into those that arise from patient's health status, those related to the physical environment where surgical care is provided, and those resulting from clinical interventions that increase the patient inherent risk [6].
A prospective study was conducted among patients aged more than 18 years admitted during March 1st 2010 to 31th October 2010 in Khartoum Teaching Hospital. The frequency rate of wound infections in this study was 9% and the majority of the wound infections (120/96%) were superficial and only 5 (4%) were deep incisional wound infections [7].
A prevalence survey undertaken in the United Kingdom (2006) exhibited that approximately 8% of hospitalized patients had a health-care associated infection (HCAI). The American Association Insurance Services (AAIs) reported that 14% of these infections and nearly 5% of patients who had undergone a surgical procedure had developed an SSI [8].
However, prevalence studies tend to underestimate SSI since many of these infections occur after discharge of the patient from hospital. SSIs are associated with considerable morbidity; and it had been reported that over one third of postoperative deaths were associated, at least in part, with SSI [9]. However, it is important to understand that SSIs can range from a relatively trivial wound discharge without complications to a life-threatening condition. Other clinical outcomes of SSIs include: poor, cosmetically unacceptable scars, hypertrophic or keloid lesions, persistent pain and itching, restriction of movement (particularly over the joints), and a significant impact on emotional well-being [10].
Surgical site infections are an important target for the surveillance of health care-associated infections. In Sudan, there are insufficient data on SSIs as regard causes, risk factors, and types of bacteria related to clean, clean contaminated and contaminated wound infections.
Despite improvement in surgical procedures and administration of antibiotic prophylaxis prior to surgery, collection of specimens from patients with SSIs is recommended to improve the sensitivity and specificity of detection of bacteria. Still there is association with significant morbidity, prolonged hospitalization, and appreciable depletion of hospital resources [10]. This study aimed to collect proper data that may be used as a base for the surveillance of health care-associated and surgical site infections. It dealt with studying the prevalence of clean, clean contaminated and contaminated SSI wounds at Omdurman Teaching Hospital.
The study was also concerned with discussing the factors that were significantly associated with the types of bacterial species causing SSIs. Furthermore, the study had evaluated the role of prolonged postoperative antibiotic prophylaxis in decreasing the frequency rate of SSIs.

Materials and methods

This was a comparative, longitudinal study conducted at the Surgical Department of Omdurman Teaching Hospital (Sudan). Patients investigated were suffering from clean, clean contaminated, and contaminated surgical wounds. Inclusion criteria were patients referred from the surgical clinics with clean, clean contaminated and contaminated surgical wounds, aged 18 years and above. Exclusion criteria were severely ill patients and patients on antibiotics therapy at the time of investigation or two weeks before the time of surgery. Sampling technique was a random, non- probability purposive type. The sample frame was patients with clean, clean contaminated and contaminated surgical wounds. The sample strategy was a convenience type where patients were chosen on the basis of accessibility. The sample size was 50 patients with clean surgical wounds, 50 patients with clean-contaminated surgical wounds, and 50 patients with contaminated surgical wounds, i.e. 150 patients with surgical wounds.

Data were collected using medical records, laboratory tests, and interviewing of patients. The tools of data collection were sterile swabs, transport media, culture media, and sensitivity media, and antibiotic discs. In office check was done immediately after receiving the data to ensure accuracy, completeness and relevancy of data. The data was also checked, vetted and validated. The entered data were analyzed using the Software Statistical Package for Social Science (SPSS) version 19. Both dependent and independent variable were displayed as frequency tables. Appropriate statistical test (Chi square test) for association between culture results and independent variables was used to determine significance. The results were discussed and compared with expected frequency rates.

The study was expected to contribute in reducing morbidity and mortality by:

a) Raising awareness of the policy makers, health planners, professionals, and the public about SSIs. b) Raising the awareness of healthcare providers about SSIs and infection management. c) The results of our study will be providing the base line and putting a step forward for more researchers in this area.

Approval of the Sudan Medical Specialization Board of Clinical Microbiology was granted. Permission of Omdurman Teaching Hospital authorities to collect the specimens was granted. An informed consent was obtained from all patients investigated. The study facility area was revisited to discuss the results.

The study protocol started by collecting specimens under aseptic conditions and before an antiseptic dressing was applied. Two wound swabs were collected. Standard methods were followed to sustain the organism viability and immediately after specimen collection, swabs were inserted in the transport media. All samples were collected by sterile cotton wool swabs from the incisional site and inoculated in Robertson cooked meat media for anaerobic culture. Kanamycin blood agar with metronidazole disks was inoculated and incubated in an anaerobic jar with anaerobic gas generating kits overnight at 37°C. Blood agar and Mac Conkey were also inoculated and incubated aerobically at 37°C. The next day, the resultant colonies were examined macroscopically and microscopically. Gram stain was performed to check the morphology of the colonies. The isolates were identified using different, standard biochemical reactions. Oxidase test, Kligler iron agar test, citrate utilization test, urease test, and indole test were used for identification of Gram negative bacilli. While catalase test, DNase test, coagulase test, and novobiocin sensitivity test were used for identification of Gram positive organisms. Negative cultures were further incubated and reexamined after 48 hours. To achieve optimal conditions, specimens were processed according to the standard bacteriological techniques.

Antibiotic susceptibility tests were conducted using the agar diffusion technique. In this technique, 38g of Muller-Hinton agar were dissolved in one liter of distilled water, sterilized by autoclaving and poured in Petri dishes to give a depth of 3-4 mm. The plates were left to solidify and dried until there were no droplets of moisture on the agar surface. Inoculum was prepared by suspending one colony of the tested isolates in 3 ml of sterilized normal saline in a test tube. The plates containing Muller-Hinton agar were inoculated by immersing a swab in the inoculum and spreading it onto the entire surface of the medium. After drying, the third generation cephalosporins, i.e. ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg); were transferred with a forceps and pressed gently onto the surface of the medium to ensure even contact. The plates were then incubated overnight at 37°C. The diameters of inhibition zones were measured in mm, and read as per the guidelines of the Clinical Laboratory Standards Institute (CLSI). Inhibition zones were interpreted as susceptible, intermediate or resistant. If the organism was sensitive, it was considered as β-lactamase sensitive but not an extended-spectrum β-lactamase (ESBL) producer. On the other hand, if the organism was resistant, it was considered an ESBL producer.

To detect methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus* isolates were purified by picking up a discrete colony from the plate and streaked over slope of nutrient agar and incubated at 35-37° C for 18-24 hours. Detection of methicillin resistance was carried out by diluting five colonies of *S. aureus* grown overnight in 5 ml of distilled water to prepare a suspension equivalent in density to 0.5 Mc Farland barium sulfate standard unit. The entire surface of the Mueller-Hinton plate was covered with the required inoculum, and the plate was air dried for 15 min before the oxacillin disk was laid on the surface, and incubation was performed for 24 h at 37° C. All isolates showing no zone of inhibition with the oxacillin disc was considered MRSA strain.

All ESBL isolates were confirmed by the double-disk synergy test. To perform this test, an overnight culture of the test isolate was suspended in a McFarland broth (turbidity No. 0.5) and swabbed on a Mueller–Hinton agar plate. After drying, amoxicillin-clavulanic acid (amoxyclav) disc and the extended spectrum cephalosporin antibiotics (e.g. ceftazidime, cefotaxime, and ceftriaxone) were placed 20 mm apart. After overnight incubation, the presence of an enlarged inhibition zone indicates a positive synergy test. This enlargement was due to inhibition of the ESBL by the clavulanic acid disc, showing a key-hole phenomenon (Fig. 1).

Fig. (1): Double disk synergy test showing the key-hole phenomenon

Results

This study was conducted for a period of 8 months between June 2015 and February 2016. A total of 150 patients with clean, clean-contaminated, and contaminated surgical wounds were enrolled in the study.
Majority of the study population (101/67.3%) were females; and age distribution of patients was from 18 to 76 years (SD 38). Most of the patients studied (57/38%) were 16-30 years old (Table 1).

Table (1): Distribution of the patients investigated according to gender and age incidence

Age (years)	Frequency rate	Gender	Frequency rate
16-30	57 (38%)	Male	49 (32.7%)
31-45	40 (26.7%)	Female	101 (67.3%)
46-60	37 (24.7%)	Total	150 (100%)
61-75	15 (10%)		
76 and above	1(0.6%)		
Total	150 (100%)		

A total of 17 wound swabs were collected from patients with postoperative wound infections. Among these, 15 wound swabs yielded bacterial growth within 24hours incubation. 12 wound swabs yielded pure bacterial growth; while 3 wound swabs yielded mixed growth, and 2 wound swabs yielded no bacterial growth.
The 15 positive cultures yielded 14 aerobic bacterial isolates and one anaerobic isolate. Gram positive organisms were more prevalent than Gram negative bacteria accounting for 10 and 8 of isolates respectively. The commonly isolated bacterial species were 5 *Staphylococcus aureus*, 4 MRSA strains, 4 *Escherichia coli*, and 3 *Enterococcus spp*.
The total frequency rates of SSI wounds were 6% in clean, 8% in clean-contaminated and 16% in contaminated surgical wounds.
As shown in Table (2), clean surgical site incision wounds were found more associated (23/46%) with herniectomy surgical operations; while clean-contaminated wounds were more associated (28/56%) with appendicectomy operations, and contaminated wounds were more associated (36/72%) with laparotomy operations. This association was statistically significant ($p = 0.00$).
Surgical antibiotic postoperative prophylaxis was given to all 150 patients investigated. The antibiotics used were: metronidazole, cefuroxime, amoxyclav, meropenem, vancomycin, and gentamycin. Metronidazole and cefuroxime was the most common combination used in the surgical antibiotic postoperative prophylaxis (Table 3).

Table (2): Frequency rates of surgical site incision wounds according to type of surgical operation

Surgical operation type	Clean wounds	Clean-contaminated wounds	Contaminated wounds	Total
Lipomectomy	10 (20%)	0 (0%)	0 (0%)	10 (6.7%)
Thyroidectomy	13 (26%)	0 (0%)	0 (0%)	13 (8.7%)
Herniectomy	23 (46%)	0 (0%)	0 (0%)	23 (15.3%)
Fibroadenomectomy	4 (8%)	0 (0%)	0 (0%)	4 (2.7%)
Appendicectomy	0 (0%)	28 (56%)	0 (0%)	28 (18.7%)
Cholecystectomy	0 (0%)	22 (44%)	0 (0%)	22 (14.7%)
Anorectal surgery	0 (0%)	0 (0%)	14 (28%)	14 (9.3%)
Laparotomy	0 (0%)	0 (0%)	36 (72%)	36 (24%)
Total	50 (100%)	50 (100%)	50 (100%)	150 (100%)

p = 0.00

Table 3: Pattern of postoperative antibiotic used

Antibiotics	Frequency	Per cent
Metronidazole and cefuroxime	80	53.3
Metronidazole and amoxyclav	66	44.0
Meropenem and vancomycin	1	0.7
Gentamycin	3	2.0
Total	150	100

Also as shown in Table (4), 2 (20%) bacterial species were isolated from clean surgical site incision wounds, 4 (26.7%) bacterial species were isolated from clean-contaminated wounds, and 8 (53.3%) bacterial species were isolated from contaminated wounds. This association of bacterial isolates and SSI wounds was found statistically not significant (p = 0.48).

The risk factors detected among the patients studied were hypertension (4%) and diabetes mellitus (2%). Only 3 patients (2%) were cigarette smokers, and 147 patients (98%) were non-smokers. The commonest duration of injectable postoperative prophylaxis (75.3%) was 1-3 days; while the commonest duration of oral postoperative prophylaxis (80.7%) was 6-10 days (Table 5).

Table (4): Frequency rates of bacterial isolates according to type of surgical site incision wounds

Bacterial isolates	Clean wounds	Clean-Contaminated wounds	Contaminated wounds	Total
Enterococci	1(33.3%)	0 (0%)	0 (0%)	1 (6.7%)
MRSA	1(33.3%)	2 (50%)	0 (0%)	3 (20%)
MSSA	0 (0%)	0 (0%)	1 (12.5%)	1 (6.7%)
Escherichia coli	0 (0%)	1 (25%)	2 (25%)	3 (20.0%)
Enterococcus faecalis	0 (0%)	1 (25%)	1 (12.5%)	2 (13.3%)
Proteus vulgaris	0 (0%)	0 (0%)	1 (12.5%)	1(6.7%)
B. fragilis + *E. coli*	0 (0%)	0 (0%)	1 (12.5%)	1(6.7%)
MRSA + *K. pneumoniae*	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S. epidermidis and *K. pneumoniae*	0 (0%)	0 (0%)	1 (12.5%)	1(6.7%)
Streptococcus pyogenes	0 (0%)	0 (0%)	1 (12.5%)	1(6.7%)
Total	2 (100%)	4 (100%)	8 (100%)	14 (100%)

$p = 0.48$

Table (5): Frequency rates and duration of injectable and oral antibiotic postoperative regimens

Days	Injectable	Days	Oral
1 – 3	113 (75.3%)	1-5	28 (18.7%)
4 – 6	13 (8.7%)	6-10	121(80.7%)
7 and more	24 (16%)	11 and more	1(0.6%)
Total	150 (100%)	Total	150 (100%)

Discussion:

This study was among the few longitudinal studies of SSI in developing countries and patients were followed up almost one complete month. Majority of the study population (101/67.3%) were females; and age distribution of patients was from 18 to 76 years (SD 38). Most of the patients studied (57/38%) were 16-30 years old. Gender and age incidences were not significant predictors of SSI in our study.

In this study we assessed some associated risk factors such as diabetes mellitus, hypertension, and smoking. These risk factors were found not statistically significant.

The overall wound infections frequency rate in this study was found to increase progressively from clean (6%), to clean-contaminated (8%), to contaminated wounds (16%). Similar findings were reported by a study conducted at Khartoum Teaching Hospital where a frequency rate of 9% was detected in SSI [11]. Another study in Nigeria reported a high SSI frequency rate (27.3%) in contaminated wounds, a frequency rate (19.3%) in clean-contaminated wounds, and a (14.3%) frequency rate in clean wounds [12].
Also a study was performed at a University hospital in Brazil among general surgical patients reported a total SSI frequency rate of 16.9%, with a high frequency rate (17.8%) in clean-contaminated wounds and a (12.5%) frequency rate in contaminated wounds [13]. Another study done at Kilimanjaro Christian Medical Centre among general surgical patients reported the incidence rate of SSI as 19.4%, and the incidence rate as 50% in dirty surgery compared to the incidence rate of clean wounds (15.6%), the incidence rate of clean-contaminated wounds (17.7%), and the incidence rate of contaminated wounds (37%)[14].
Regarding the frequency rate of isolated organisms in clean, clean- contaminated and contaminated surgical wounds found in the present study, MRSA strains were the most common isolates in general surgery; while *Enterococcus* and *Escherichia coli* were the second most common isolates. The current study also documented a high frequency rate of methicillin resistance among *Staphylococcus aureus* isolated from SSIs. Even though, these results should be interpreted with great caution since confirmatory genotypic tests were not performed, and the data supports an increasing trend of MRSA infection in Sudan and other regional countries. Extended spectrum β-lactamase phenotype was detected in one strain of *Klebsiella pneumoniae*. In a study conducted in Tanzania among patients who underwent elective clean surgery, *S. aureus* was found the commonest isolate (36.1%), followed by *Klebsiella spp* (31.2%), and *Escherichia coli* (14.8%) [15]. Similar findings were reported among the general surgical patients in the northern part of Tanzania where *S. aureus* was reported to be the most commonly isolated micro-organism (27%), followed by *Escherichia coli* (14.8%), and *Klebsiella spp* (14.8%)[16].
Furthermore, another study carried out at a university hospital in Nigeria showed that the commonly isolated bacteria were *S. aureus* (25%), *Pseudomonas aeruginosa* (20%), *Escherichia coli* (15%), *Klebsiella oxytoca* (10%), and *Proteus mirabilis* (10%)[17]. Frequent isolation of *S. aureus* (28.8%) and *Escherichia coli* (27.1%) were reported among patients with abdominal surgical wounds in Ethiopia [18]. Studies conducted in East Africa among 63 surgical patients at university teaching hospital in Kenya reported that *S. aureus* was the most frequently isolated pathogen (54.7%); while *Proteus, Pseudomonas,* and *Escherichia coli* were 15.5%, 11.9%, and 2.3% respectively [19].
A similar finding was reported in a study at a referral hospital in Uganda where *S. aureus* was found the commonest isolate (45.1%), followed by *E. coli* (16.9%), and *Proteus mirabilis* (11.3%); and MRSA strains accounted for 25% of all *S. aureus* [20]. In United States of America, a study conducted at a small community hospital among SSI patients who underwent surgery reported that *S. aureus* was the commonest isolate (25.8%), followed by *Enterobacteriaceae species*,(12.4%), streptococci species (11.2%), enterococci species (7.9%), *Pseudomonas aeruginosa* (6.7%), and MRSA strains (4.5%) [21]. Another recent study in USA among postoperative hollow viscous injury patients documented *Escherichia coli* to be the most commonly isolated microorganism (64.7%), followed by enterococci species (41.2%), and *Bacteroides* (29.4%)[22].

On the other hand, a study conducted at a university hospital among surgical patients in Iran reported *S. aureus* to be the commonest bacterial pathogen isolated (43%), followed by *Escherichia coli* (21%), *Klebsiella spp* (13%), *Pseudomonas* (10%), and MRSA strains (78.9%) (23).

In the present study all patients investigated had received parental antibiotic prophylaxis on admission; and a 7days oral antibiotic prophylaxis after discharge. Also antibiotic prophylaxis was mainly performed by metronidazole, cefuroxime, and amoxyclav. The antibiotic prophylaxis was administered only to patients who really need it; so as to prevent the emergence of resistant pathogens and not to increase the cost of medical care.
Our study showed that there was no correlation between both the preoperative and postoperative antibiotic prophylaxis and development of SSIs. The mortality rate among the patients studied was 1.3%.
Conclusion: MRSA strains were the most common isolates among SSI patient.
There was no evidence that the use of preoperative and postoperative antibiotic prophylaxis can help in reducing postoperative infection in clean, clean-contaminated or contaminated SSI wounds.
From this study, it may be recommended that wound swabs culture, antimicrobial sensitivity testing, MRSA isolation, and ESBL detection must be applied to identify the aetiological agents and to determine the proper antibiotics for treatment. Since there is no evidence that the use of preoperative and postoperative antibiotic prophylaxis can help in reducing postoperative infection in SSIs, the use of antibiotic prophylaxis is not recommended. Hand hygiene technique should be adopted to maintain infection control and to reduce SSIs.

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