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Common bacterial isolates of wound infections and their antimicrobial susceptibility, Gezira National Center for Pediatric Surgery, Sudan

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Abstract: Background *An increased antibiotic resistance of bacterial isolates from wound infections is a major therapeutic challenge. The aim of this study was to identify bacterial isolates associated with wound infection and to determine their current antimicrobial susceptibility profile.* ***Methods:*** *Descriptive cross-sectional study was followed, a total of 170 wound swabs were cultured aerobically at Gezira National Center for Pediatric Surgery, Wad Medani, Sudan in the period from December 2017 to march 2020. Swabs from different wound types were collected aseptically and analyzed using standard bacteriological procedures. Antimicrobial susceptibility testing was performed using disc diffusion technique as per the standard protocol. Demographic and bacteriological data were collected using a data extraction sheet. The data were cleaned, entered and analyzed using SPSS version 20.* ***Results:*** *Total positive bacterial growth revealed of 85.9% (146/170); of which 60% (102/170) were gram positive while 25.9% (44/170) were gram negative. Staphylococcus aureus was the predominant isolated organism 96 (56.5%), followed by Escherichia coli 34 (20%), Coaglase-negative staphylococci (CNS) 6 (3.5%), Klebsiella pneumoniae 6 (20%) and Pseudomonas aeruginosa 4 (2.4%). A 52.9% (90/170) of samples obtained from drainage surgical procedures, followed by 30% (51/170) incision, 11.1% (10/170) circumcision, 3.5% (6/170) appendectomy and 2.3% (4//170) laparotomy. Antimicrobial susceptibility for Staphylococcus aureus showed high level of drug resistance for methicillin with percentage of 96.9%, followed by cefotaxime 44.7% and cefixime 21.8%, while meropenine was the drug of choice with full sensitivity 100 % followed by vancomycin (90%), and gentamicin 86%.* ***Conclusions:*** *Staphylococcus aureus responsible for most cases of wound infections, and isolated gram negative rods were relatively more sensitive to selected antimicrobials.*

Introduction

The primary function of intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens (1). Exposure of subcutaneous tissue following a loss of skin integrity (i.e. wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Since wound colonization is most frequently poly-microbial, involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected (2). Infection in wound constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life as well as on the healing rate of the wound. Infected wounds are likely to be more painful, hypersensitive and odorous, resulting in increased discomfort and inconvenience for the patient (3). The prevalent organisms that have been associated with wound infection include *Staphylococcus aureus* which from various studies have been found to account for 20-40% and *Pseudomonas aeruginosa* 5-15% of the nosocomial infection, with infection mainly following surgery and burns. Other pathogens such as enterococci and members of the enterobacteriaceae have been implicated, especially in immune compromised patients and following abdominal surgery (4). Wound healing needs a good healthy environment so that the normal physiological process will result in a normal healing process with minimal scar formation. One of the most important strategies to keep the process of healing ongoing is to sterilize damaged tissue from any microbial infection (5). Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic resistant strains which in turn, has driven the continued search for new agents. Unfortunately, the increased costs of searching for effective antimicrobial agents and the decreased rate of new drug discovery has made the situation increasingly worrisome (6). The battle between bacteria and their susceptibility to drugs is yet problematic among public, researchers, clinicians, and drug companies who are looking for effective drugs. In addition, SSI by resistant bacteria worsens the condition and it has become serious problem in developing countries like Sudan owing to poor infection prevention program, crowding hospital environment, widespread uses of antibiotics, and irrational prescription of antimicrobial agents. Furthermore, recent studies assessing the bacterial isolates of SSIs and their susceptibility pattern in Sudan are scarce. Therefore, identification of a microbe and determining susceptibility pattern are beneficial to the patient and assist in selection of drug to avoid emergence of multidrug resistance organisms in hospital. It is also essential to take appropriate steps to control the spread of infection within the unit. Furthermore, the information gathered helps in planning antibiotic usage policy for SSI. Thus, the aim of this study was to assess bacterial pathogens and drug susceptibility pattern of surgical site infections at Gezira National Center for Pediatric Surgery in Wad Medani, Sudan.

Methods

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This descriptive study was conducted at Gezira National Center for Pediatric Surgery, Wad Medani, Sudan. Wad Medani is the capital of Gezira state, lies on the western bank of Blue Nile River about 85 Km from Khartoum, the capital of Sudan. Gezira National Center for Pediatric Surgery is one of the most important surgical centers in Africa for a period of 9 months from January to October 2018. A total of 170 pus samples were collected from patients suffering from surgical site infections. Samples were cultured on blood, chocolate and MacConkey's agar media, and incubated aerobically at 37°C for 24 hrs. Organisms were identified on the basis of colonial morphology, Gram staining and biochemical tests.

Inclusion and exclusion criteria's

No discrimination was made on the basis of age or gender, properly labeled samples of pediatric surgery patients with suspected surgical site infection were selected for study. Improperly labeled samples, samples collected from non-surgical sites, old stored samples and samples from Non-pediatric surgery patients were excluded.

Data and specimens collection

Wound swabs were taken from consented surgical site infected patients for microbiological analysis before wound dressing time to avoid skin flora contamination. The wound swab was collected by trained data collectors using sterile cotton swab on a separate sterile test tube or nutrient broth media. Patient specific socio-demographic characteristics and medical histories and all the other required information were collected using the structured questionnaire from the patient medical record and the responsible surgeon, when necessary.

Processing of specimens and identification

Pus specimens were transported immediately following collection by placing each swab in sterile nutrient broth media to the microbiology laboratory. All the specimens were inoculated onto blood agar, mannitol salt agar, and MacConkey's agar and chocolate agar within one hour of collection. The agar plates were incubated at 35–37°C aerobically examined for the presence of any growth after 24 hours. Those plates showed no growth were incubated for another 24 hours. The isolates were identified by colonial morphology, Gram's stain, and conventional biochemical tests such as catalase, coagulase, oxidase, and mannitol fermentation for Gram positive bacteria and urease, indole, citrate, and sugar utilization tests for Gram negative bacteria.

Antimicrobial susceptibility testing

Antibiotic susceptibility pattern of the isolates was studied by using Kirby-Bauer technique according to the criteria of the Clinical Laboratory Standards Institute (CLSI) by disc diffusion method. From a pure culture 3–5 pure colonies of bacteria were taken and transferred to a tube containing 5 mL sterile nutrient broth (Oxoid) and they were mixed gently until the turbidity of the suspension become adjusted to a 0.5 McFarland standard. Using sterile cotton swab, the bacteria were seeded evenly over the entire surface of Mueller-Hinton agar (pH 7.2–7.4) (Oxoid). The plates were left at room temperature to dry for 3–5 minutes and antibiotic discs (Oxoid) with the recommended concentrations were placed on the surface of a Muller-Hinton agar plate. Finally, the plates were incubated at 35–37°C for 18–24 hours. Diameters of growth inhibition around the discs were measured and interpreted as sensitive, intermediate, or resistant as per the standard protocol. The following antimicrobial agents were used with their respective concentration. Gentamicin (GEN, 10 µg), ciprofloxacin (CIP, 5 µg),vancomycin (VAN, 10 µg) , meropinem (MRP 10 µg) were used for both Gram positive and Gram negative bacteria. Methicillin (M, 5µg) for gram positive bacteria ceftriaxone (CTR, 30 µg), ceftazidime (CAZ, 30 µg), cefuroxime (CXM, 30 µg), cefixime (CFM, 5 µg), and cefotaxime (CTX, 30 µg) were used for Gram negative bacteria.

Quality control

The reliability of the study findings was guaranteed by implementing quality control (QC) measures throughout the whole processes of the laboratory works. All materials, equipment, and procedures were adequately controlled, and each procedures were aseptically performed. Culture media were tested for sterility and performance. To standardize the inoculums density of bacterial suspension for the susceptibility test, a barium sulfate ($BaSO_4$) turbidity standard, equivalent to a 0.5 McFarland standard, was used.

Statistical analysis

The Socio-demographic, clinical, and antimicrobial data were obtained from the patient's card and were extracted using a structured questionnaire. The patient's microbiology report was linked to the questionnaire with a code and was documented for each patient on the questionnaire. The information retrieved was used to analyze the rate of surgical site infection and the bacterial isolates and their susceptibility pattern. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Study findings were explained in words, percentage, and tables.

Ethical consideration

The study was approved after it was ethically reviewed by the research ethical review committee of Faculty of Medical Laboratory Sciences, University of Gezira and Ministry of Health, Gezira State, Sudan.

Results

A total of 170 specimens were collected from patients with clinical evidence of wound infection, Study subjects included 54.1% (92/170) males and 45.9% (78/170) females. The ages of the patients ranged from 5 to 16 years with mean of 34.68 ± 19.12 years, which divided in two group; less than 8 and 8 to 16 years. About 52.9% (90/170) of collected samples obtained from drainage surgical procedure, 51% (30/170) incision, 11.2% (19/170) circumcision, 3.5% (6/170) appendectomy and 2.4% (4/170) from laparotomy surgical procedure (Table 1). Superficial wound and deep tissue or/organ cases constituted 66.5% (113/170) and 33.5% (57/170) respectively. Most cases recorded as emergency with percentage of 84.1% (143/170). Preoperative post-operative antimicrobial therapy documented in 18.2 (31/170) and 22.4% (38/170) (Table 2).

Bacterial profile

Of the 170 swabs; 85.9% (146/170) gave significant bacterial growth and a total of 146 bacterial isolates were obtained; 102 (60%) were gram positive while 44 (25.9%) were gram negative. *Staphylococcus aureus* was the predominant organism isolated 65.8% (96/146), followed by *Escherichia coli* 23.3% (34/146), CNS 4.1% (6/146), *Klebsiella pneumoniae* 4.1% (6/146) and *Pseudomonas aeruginosa* 2.8% (4/146) (Figure 1) (Table 3).

Antimicrobial susceptibility pattern of bacterial isolates

Among gram positive bacteria *Staphylococcus aureus* showed resistance frequency to Methicillin 96.9%, cefotaxime (44.7%), cefixime (21.8%) cefepince (15%), ceftiraxone (15%), cefuroxime (12%) and gentamicin (8%), while sensitivity to meropenine and vancomycin revealed 100 % and 90% respectively. Gram negative rods isolated were considered to be higher sensitive to most of the antimicrobials tested (Table 4).

(Table 1). Socio-demographic characteristics of study subject No. 170

Characteristic		Frequency	Percentage
Gender	Male	92	54.1
	Female	78	45.9
Age (years)	Less than 8	103	60.6
	8 - 16	67	39.4
Surgical procedure	Drainage	90	52.9
	Incision	51	30.0
	Circumcision	19	11.2
	Appendectomy	6	3.5
	Laparotomy	4	2.4

(Table 2). Distribution of cases type and antimicrobial therapy

Type/Therapy	Frequency	Percentage

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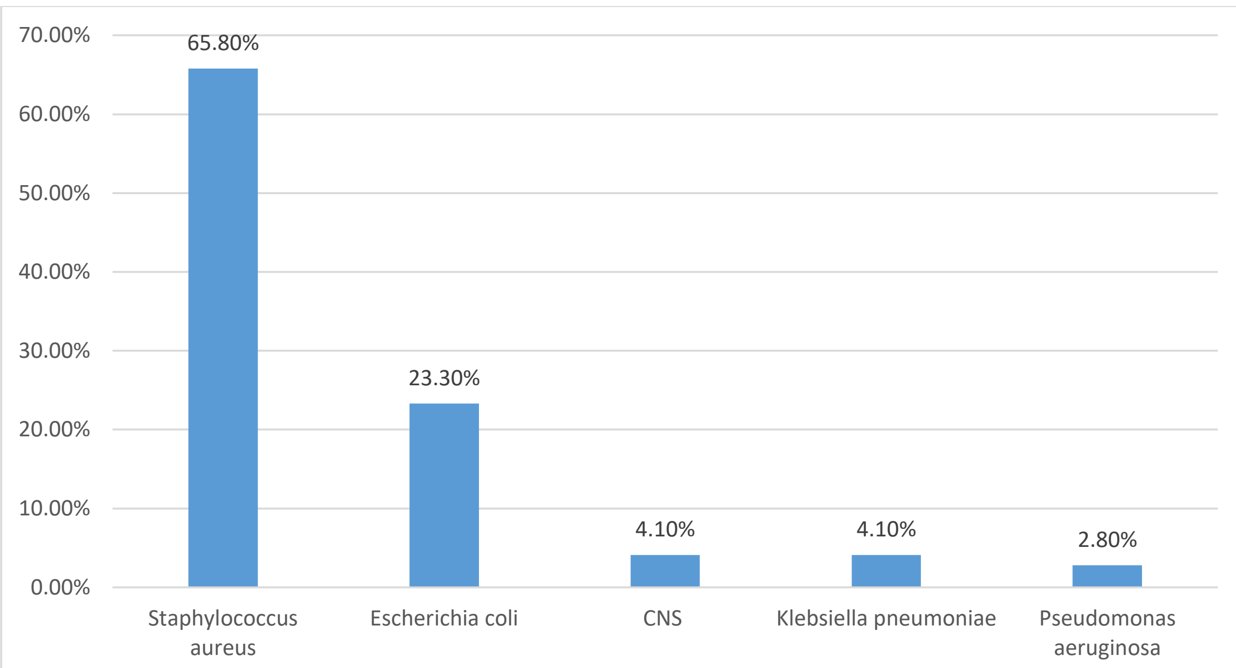
Case type	Emergency	143	84.1
	Elective	27	15.9
SSI class	Superficial	113	66.5
	Deep tissue or organ/space	57	33.5
Antimicrobial therapy	Preoperative	31	18.2
	Post-operative	38	22.4
	Not at all	101	59.4



(Figure 1). Frequency of common bacterial pathogens isolated from surgical-sites infections of study subject. No 146.

(Table 3). Distribution of isolated bacteria in different surgical procedures

Isolated bacteria	**Surgical procedure**					**Total**
	Drainage	**Incision**	**Circumcision**	**Appendectomy**	**Laparotomy**	
S.aureus	50	24	15	4	3	96
CNS	1	4	0	1	0	6
E.coli	15	15	2	1	1	34
K.pneumoniae	3	3	0	0	0	6
P.aeruginosa	3	1	0	0	0	4

Total	90	51	19	6	4	170

Table 4: Frequency of isolated bacteria in associated to different types of antibiotics

Antimicrobials		**Isolated bacteria**				
		S.aureus	**CNS**	***E.coli***	***K.pneumonia***	***P.aeruginosa***
CXM	**Sensitive**	76	5	28	3	0
	Resistance	12	1	5	2	4
	Intermediate	8	0	1	1	0
CTR	**Sensitive**	75	5	20	5	0
	Resistance	15	1	13	1	4
	Intermediate	6	0	1	0	0
CFM	**Sensitive**	69	4	22	4	0
	Resistance	21	1	7	0	3
	Intermediate	6	1	5	2	1
GEN	**Sensitive**	83	6	30	4	3
	Resistance	8	0	0	0	1
	Intermediate	5	0	4	2	0
CPM	**Sensitive**	77	5	25	5	1
	Resistance	15	1	8	0	2
	Intermediate	1	0	1	1	1
CIP	**Sensitive**	68	4	16	2	0
	Resistance	24	2	17	4	4
	Intermediate	4	0	1	0	0
	Sensitive	48	5	25	4	1
CTX	**Resistance**	43	0	8	1	3
	Intermediate	5	1	1	1	0
MRP	**Sensitive**	96	6	34	4	4
	Resistance	0	0	0	0	0
	Intermediate	0	0	0	2	0

VAN	**Sensitive**	87	4	0	0	0
	Resistance	5	0	0	6	0
	Intermediate	4	0	0	0	0
ME	**Sensitive**	3	0	NA	NA	NA
	Resistance	93	4	NA	NA	NA
	Intermediate	0	0	NA	NA	NA

NA: Not applicable

Discussion

The emergence of drug resistance among bacterial pathogens has been attributed to the high rates of morbidity and mortality associated with infection at surgical sites (SSIs) (7, 8). The current study conducted to identify possible bacterial pathogens and their drug susceptibility pattern in pediatric patients with SSI.

In this study most enrolled cases identified as drainage which is a widely used procedure in various care settings, including emergency departments. Possible contraindications are abscesses formation, promixity to structures of blood vessels and presence of foreign bodies (9). The data presented showed that *Staphylococcus aureus* (53%) and *Escherichia coli* (35 %) were the predominant organisms isolated from SSIs, this agreement with other previous studies in Ethiopia (10, 11, 12, 13). From this study, Gram-negative bacteria were more numerous than gram-positive bacteria, and this could be attributed to the large number of possible gram-negative species, such as those belonging to the family of enterobacteriaceae; this was agree to similar study done in Bangladesh (14), and Nepal (15, 16). In contrast, other findings from India recorded *Escherichia coli* as the most common pathogenic isolate followed by *Staphylococcus aureus* (17, 18).

There are several reasons for the dominance of *Staphylococcus aureus* as a cause of wound infection, including its spread in nature and carrying it in contaminated hands and noses, in addition to its transmission through contaminated surgical instruments and between patients (19). In addition to *Staphylococcus aureus* and *Escherichia coli*, other common bacterial isolates were CNS, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* which had been documented in different studies (20, 21, 22).

The current findings of *Staphylococcus aureus* in Sudan reported high resistance against tested antimicrobials which was indicated recently by Sanaa et al (23). Resistance to methicillin was very high at 96.9%, which is also similar to a study in Sudan in 2018 (24). Isolated *Staphylococcus aureus* expressed resistance against cefotaxime with percentage of 44.7%, in line, higher percentage was recorded in 2017 (25), the possible mechanism for resistance is the production of beta lactamase (26).

Conclusion

Staphylococcus aureus was the most common isolate from surgical-site infections infection followed by *Escherichia coli*. The observed resistance to treatment necessitates monitoring and conducting drug sensitivity tests routinely, which is not practiced in developing countries such as Sudan.

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