

Original Research Article

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Prevalence and Antimicrobial Susceptibility Pattern of Aerobic Bacteria Isolated from Patients with Bedsores Admitted to Intensive Care Units in Khartoum State

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ABSTRACT

Bedsores remain a significant healthcare concern, it is one of the most dominant problems reported by patients with mobility limitations, sometimes, it can even be life threatening, its treatment imposes financial burdens on patient's family and society. The aim of this study was to determine prevalence and antimicrobial susceptibility pattern of aerobic bacteria that causes the bedsores infection in Sudanese patients. Methods: A total of 57 bedsores swabs specimens were collected from patients with bedsores infection due to prolonged bed lying in ICUs from various hospitals in Khartoum State during the period from December 2019 to February 2020. Isolation and identification of culture isolates was done using standard bacteriological techniques and antimicrobial susceptibility testing was performed using Kirby- Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Out of 57 patients enrolled in the study, the prevalence of bedsores in ICUs patients was 50/57 (87.7%) Both Gram positive cocci and Gram negative bacilli were isolated from 50 patients out of 57 patients. Among the Gram negative isolates, *Pseudomonas aeruginosa* had the highest frequency 15/41 (36.5%), followed by *Klebsiella spp* 12/41 (29.2%) and *Escherichia coli* 4/41 (9.7%). The frequencies of *Citrobacter freundii*, *Proteus spp* and *Salmonella spp*, *Acinetobacter baumannii* were 3/41 (7.3%) and 2/41 (4.8%) respectively. The only gram positive cocci isolated was *Staphylococcus aureus*. The distribution of bedsores among the ages of the ICUs patients showed that age group within the range of 66-80 years recorded the highest incidence of bed sore infection. The most common site of bedsores was sacral region 36/50 (72%) followed by neck 5/50 (10%). All isolated *Staphylococcus aureus* showed 100% resistance to Penicillin and Oxacillin but showed variable susceptibility to other antibacterial used. *Pseudomonas aeruginosa* was resistant to Co-trimoxazole 13/15 (86.7%), Ciprofloxacin, and Ceftazidime 12/15 (80%). The results showed that all isolated bacteria considered multi-drug resistance organism (MDROs) except few strains of *Pseudomonas aeruginosa* 2/15 (13.3%) and *Citrobacter freundii* 1/3 (33.3%). The overall prevalence of bedsores in this study was relatively high with *Pseudomonas aeruginosa* as the major causative agents. Multi-drug resistance of 50/53 (94.3%) was observed among the isolated bacteria.

Keywords

Bedsores,
Intensive Care
units, *Pseudomonas
aeruginosa*,
Pressure ulcer,
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Introduction

Bedsore defined as a painful often reddened area of degenerating ulcerated skin caused by pressure and lack of movement, and worsened by exposure to urine or other irritating substance on skin (Yarkony, 1994). Bedsore also known as pressure ulcer or decubitus ulcer. It is one of the most dominant problems reported by patients with mobility limitation, sometimes it can even be life threatening and its treatment imposes financial burdens on patients family and society (Repić *et al.*, 2014; Agrawal *et al.*, 2012). Moreover bedsore have been described as one the most costly and physically debilitating complication in the 20th century and represent the third most expensive disorder after cancer and cardiovascular diseases (Burdette-Taylor *et al.*, 2002). The microorganisms like Enterobacteriaceae (*Escherichia coli* and *Klebsiella pneumoniae*) and non-fermenting GNB mainly *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently associated with bedsore infection and some of this isolated bacteria exhibit multi resistant to different antibiotics including; *Pseudomonas aeruginosa*, *Proteus spp*, *Klebsiella spp*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Acinetobacter spp*, *Escherichia coli* and *Enterobacter spp* (Braga *et al.*, 2017). The World Health Organization (WHO) uses the incidence and prevalence of pressure ulcers as an indicator of the quality of patient care services and treatment principles (Ghodela *et al.*, 2018). A good prevention and treatment is obligatory to avoid bedsore that can easily infect and lead to more morbidity. Curative dressings can help wound healing and avoid further problems (Chhugani *et al.*, 2017). All pus related infections including bed sore are tricky to manage now a days due to multi-drug resistant (MDRs) bacteria (due to widespread use of prophylactic and empiric antibiotics), increased severity of illness and greater

numbers of immune-compromised patients undergoing surgical procedures (Sarin *et al.*, 2013). The main purpose of the present study was to assess the prevalence of aerobic bacteria isolated from bedsore infection from patients admitted to intensive care unit in Khartoum state and determine their antimicrobial susceptibility patterns.

Materials and Methods

Descriptive Cross sectional study was conducted from December 2019 to February 2020. Fifty seven bedsore swabs specimens were collected from patients admitted to intensive care unit at Soba University Hospital, Royal care International Hospital, Military Hospital and Omdurman Teaching Hospital. All swabs specimens were inoculated under aseptic technique in 5% Blood agar, MacConkey agar and Mannitol salt agar prepared according to manufacturer's instruction. The inoculated culture media were incubated aerobically at 37C° overnight. Gram staining was carried out initially to study morphological characteristics of clinical isolates, all Gram positive cocci identified by standard bacteriological test including; catalase test, DNase test, coagulase test and Novobiocin sensitivity test was done to identify *Staphylococcus spp*. Gram negative bacilli isolates were identified by standard conventional biochemical tests including; motility test, oxidase test, Kligler iron agar, Citrate utilization test, Urease hydrolysis test, Methyl Red test and Indole production test. Antimicrobial susceptibility test for all isolates was performed by Kirby-Bauer disc diffusion method using Muller Hinton agar according to clinical laboratory standards institute (CLSI) guideline using Muller-Hinton agar (MH) (CLSI, 2015). The antibiotics discs (oxid limited, England) used in this study were Amoxicillin (10µg), Chloramphenicol (30µg), Tetracycline (30µg), Cefotaxime (30µg), Meropenem (10µg), Gentamycin (10µg),

Ciprofloxacin (5µg), Vancomycin (30µg), Erythromycin (15µg), Oxacillin (1µg), Co-trimoxazole (15µg), Doxycillin (10µg) Penicillin (10µg). *Staphylococcus aureus* ATCC 25923 strain was used as control organism for gram positive cocci and *Escherichia coli* ATCC 25922 strain for gram negative bacilli respectively. Organisms that showed resistance to multiple types, classes or subclasses of antimicrobial agents were considered as multi-drugs resistant (Magiorakos *et al.*, 2012).

Statistical analysis

Data were statistically analysed using Statistical Packaged for Social Science (SPSS) software version 20, frequency and percentages were calculated for categorical and ordinal variables. Chi-square test was performed and p-value less than 0.05 considered statically significant.

Results and Discussion

In the present study total number of 53 clinical microorganism were isolated from 57 bedsores swabs collected from Soba University Hospital, Royal care International Hospital, Military Hospital and Omdurman Teaching Hospital during the period from December 2019 to February 2020. Out of 57 swab specimens examined 50/57 (87.7%) were positive for bacterial growth consist of 47/50 (94%) swabs with pure bacterial isolates and 3/50 (6%) swabs with mixed pathogens and 7/57 (12.3%) swabs were negative for bacterial growth. The isolation rate was higher in males 36/50 (72%) compared to females 14/50 (28%) in which its relationship with bedsores incidence was insignificant statistically p-value (0.778). The highest overall infection rate was in the age group 66 - 80 years, while the lowest was in the age group 80 - 95 years, which were statically insignificant with p-value (0.679). The most

common infected sites was sacral region 36/50 (72%) followed by neck 5/50 (10%), buttock 4/50 (8%), leg 3/50 (6%) and heel 2/50 (4%), which statically insignificant p-value (0.569) as indicated in Table (1).

The most common isolates in this study were Gram negative bacilli 41/53 (77.3%) with predominant isolate *Pseudomonas aeruginosa* 15/41 (36.5%) followed by other bacilli like *Klebsiella spp* 12/41 (29.2%) 4/41 *Escherichia coli* (9.7%), *Citrobacter freundii* 3/41 (7.3%), *Proteus spp* 3/41 (7.3%), *Salmonella spp* 2/41 (4.8%) and *Acinetobacter baumannii* 2/41 (4.8%). In the Gram positive bacteria the main organism identified was *Staphylococcus aureus* 12/53 (22.6%) as shown in Table (2). The result showed insignificant relationship between isolated bacteria and underline disease and period of hospitalization p-value (0.181), (0.488) respectively.

The antimicrobial susceptibility patterns of isolated bacteria from bedsores revealed that all isolated *Staphylococcus aureus* were resistant to Penicillin and Oxacillin (100%) as shown in table (3). Meropenem is relatively effective formost isolated *Pseudomonas aeruginosa* and Gram negative bacilli except; *Proteus spp*, *Salmonella spp*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* which were found to be (100%) resistant as indicated in table (4). *Pseudomonas aeruginosa* was resistant to Co-trimoxazole 13/15 (86.7%), Amoxicillin, Ciprofloxacin, and Ceftazidime (80%), as shown in table (5). Out of 53 bacterial isolates introduced to multi-drug resistant organism criteria describes by clinical laboratory standards institute CLSI guidelines (resistant to three or more antimicrobial classes (Livesley *et al.*, 2002). All isolated bacteria considered as (MDROs) organism except few strain of *Pseudomonas aeruginosa* and *Citrobacter freundii* 2/15 (13.3%), 1/3 (33.3%) respectively shown in table (6).

Table.1 Socio-demographic and clinical characteristics of bedsores infected patients (December 2019 to February 2020)

Characteristics	Number (%) of tested	Number (%) of culture positive	p-value
Gender			
Male	43/57 (75.4%)	36 (72%)	.778
Female	14/57 (24.5%)	14 (28%)	
Total	57 (100%)	50 (100%)	
Age in year			
20 – 35	12 (21%)	9 (18%)	.679
36 – 50	10 (17.6%)	9 (18%)	
51 – 65	10 (17.6%)	9 (18%)	
66 – 80	21 (36.8%)	20 (40%)	
81 – 95	4 (7%)	3 (6%)	
Site of ulcer			
Sacral	38 (66.7%)	36 (72%)	.569
Neck	7 (12.2%)	5 (10%)	
Buttock	6 (10.5%)	4 (8%)	
Leg	4 (7%)	3 (6%)	
Heel	2 (3.5%)	2 (4%)	
Total	57 (100%)	50 (100%)	

Table.2 Distribution of positive bacterial isolates identified from bedsores swab specimens

Type of isolates	Number of isolate	Percentage
Gram positive isolates		
<i>Staphylococcus aureus</i>	12	22.6%
Gram negative isolates		
<i>Pseudomonas aeruginosa</i>	15	28.3%
<i>Proteus vulgaris</i>	2	3.7%
<i>Klebsiella ozaenae</i>	4	7.5%
<i>Escherichia coli</i>	4	7.5%
<i>Proteus mirabilis</i>	1	1.8%
<i>Citrobacter freundii</i>	3	5.8%
<i>Klebsiella pneumoniae</i>	3	5.8%
<i>Acinetobacter baumannii</i>	2	3.7%
<i>Klebsiella oxytoca</i>	5	9.5%
<i>Salmonella spp</i>	2	3.7%
Total	53	100%

Table.3 Antimicrobial Susceptibility Pattern of *Staphylococcus aureus*

<i>Staphylococcus aureus</i>	Antimicrobial agent								
	COT	GEN	TE	CIP	AMX	ERY	PEN	VAN	OXA
Sensitive	4	3	5	3	4	2	0	3	0
Resistant	8	9	7	9	8	10	12	9	12

PEN = Penicillin; COT= Co-trimexazole; GEN = Gentamicin; TE = Tetracycline; ERY= Erythromycin OXA = Oxacillin; VAN = Vancomycin; AMX = Amoxicillin; CIP = Ciprofloxacin

Table.4 Antimicrobial Susceptibility Pattern of *Enterobacteriaceae* Isolated from Bedsores (December 2019 to February 2020)

Isolates	Number of strains resistant to													
	CIP		COT		AMX		TE		GEN		CAM		MEM	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>Proteus vulgaris</i>	1	1	0	2	1	1	0	2	1	1	0	2	0	2
<i>Klebsiella ozaenae</i>	1	3	0	4	0	4	0	4	2	2	2	2	1	3
<i>Escherichia coli</i>	1	3	0	4	0	4	0	4	3	1	1	3	4	0
<i>Proteus mirabilis</i>	0	1	0	1	1	0	0	1	1	0	0	1	0	1
<i>Citrobacter freundii</i>	2	1	1	2	1	2	2	1	1	2	1	2	1	2
<i>Klebsiella pneumoniae</i>	0	3	1	2	1	2	0	3	2	1	0	3	0	3
<i>Klebsiella oxytoca</i>	2	3	2	3	0	5	1	4	2	3	2	3	2	3
<i>Salmonella spp</i>	1	1	0	2	0	2	0	2	1	1	1	1	0	2

CAM = Chloramphenicol; COT = Co-trimexazole; GEN = Gentamicin; TE = Tetracycline; AMX= Amoxicillin CIP = Ciprofloxacin

Table.5 Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Isolates	Number of strains resistant to													
	CIP		COT		MEM		DO		GEN		AMX		CAZ	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>Pseudomonas aeruginosa</i>	3	12	2	13	6	9	5	10	6	9	3	12	3	12
<i>Acinetobacter baumannii</i>	1	1	0	2	0	2	0	2	0	2	0	2	0	2

CAM = Chloramphenicol; COT = Co-trimexazole; GEN = Gentamicin; CAZ= Ceftazidime; AMX= Amoxicillin CIP = Ciprofloxacin; DO = Doxycycline

Table.6 Antimicrobial Susceptibility Pattern for MDR- Bedsore Isolates

Isolated bacteria	Sensitive	Resistant (MDROS)	Total
<i>Pseudomonas aeruginosa</i>	2 (13.3%)	13 (86.6%)	15
<i>Proteus vulgaris</i>	0	2 (100%)	2
<i>Klebsiella ozaenae</i>	0	4 (100%)	4
<i>Escherichia coli</i>	0	4 (100%)	4
<i>Citrobacter freundii</i>	1 (33.3%)	2 (66.9%)	3
<i>Acinetobacter baumannii</i>	0	2 (100%)	2
<i>Salmonella spp</i>	0	2 (100%)	2
<i>Proteus mirabilis</i>	0	1 (100%)	1
<i>Staphylococcus aureus</i>	0	12 (100%)	12
<i>Klebsiella pneumoniae</i>	0	3 (100%)	3
<i>Klebsiella oxytoca</i>	0	5 (100%)	5

MDROS = Multi-Drugs Resistant Micro-organisms

Bedsore have important consequences both for patients and for the health care system. It can lead to severe or intolerable pain, are prone to infection and are associated with high mortality rates. They also inflict a considerable economic burden on the health care system (Reihani *et al.*, 2007). The present study describes the distribution and antimicrobial susceptibility pattern of aerobic bacterial species isolated from bedsore infection. The rate of bacterial isolation was 36/50 (72 %), 4/50 (28%) from male and female respectively, in which its relationship with bedsore incidence was statistically insignificant. This result is consistent with other studies done by (Reihani *et al.*, 2007), (Gallagher *et al.*, 2008) and (Mostafa Shokati Ahmadabad, 2015) in which there was no statistical association between genders and bedsore incidence rate. Moreover, the result showed that bedsore infection more common among age group 66-80 (40%). Which agrees with the findings of earlier work carried out by (Abbott *et al.*, 2002) who reported the bedsore were more common in men and in patients over 60 years of age. The present study reported that the frequency of bedsore with respect to the location on the body was

high at the sacral 36/50(72%) followed by neck 5/50(10%), buttock 4/50 (8%), leg 3/50(6%) and heel 2/50(4%). The same finding was reported in several recent studies by (Reihani *et al.*, 2007; Mostafa Shokati Ahmadabad, 2015) they showed that the most common sites were sacral regions (28.9%), (54%), (30%) respectively. The present study revealed that gram-negative bacteria were the prominent pathogens consisting 77.3% of the isolates with high frequency of *Pseudomonas aeruginosa* 15/41 (36.5%) followed by *Klebsiella spp* 12/41 (29.2%) 4/41 *Escherichia coli* (9.7%), *Citrobacter freundii* 3/41 (7.3%), *Proteus spp*3/41 (7.3%), *Salmonella spp* 2/41 (4.8%) and *Acinetobacter baumannii* 2/41 (4.8%). Similar to study by (Khanafari *et al.*, 2013) who repeated that *Pseudomonas aeruginosa* (60%), *Escherichia coli* (35%) and *Staphylococcus aureus* (5%) were dominant bacteria in all 20 bedsore samples. Also prospective study done by (Dolati *et al.*, 2017) was reported that the frequently of identified bacteria detected by aerobic culturing from the bedsore of all studied patients showed that *Pseudomonas species* 18/49 (36%) *Staphylococcus aureus* 16/49 (32%) and *Escherichia coli* 15/49 (30%) were the most

abundant microorganisms isolated. However, *Proteus spp* 4/49 (8%) and *Klebsiella spp* 5/49 (10%) were less frequency. In contrast study by Ghaly have documented that *Staphylococcus epidermidis* was the most prominent pathogen isolated from pressure sore (31.4%) followed by *Proteus vulgaris* (28.6%), *Pseudomonas aeruginosa* (22.8%), *Escherichia coli* (8.6%), *Klebsiella pneumoniae* (5.8%) and *Staphylococcus aureus* (2.8%) (Ghaly *et al.*, 2010). The possible reason for variation in these studies could be attributed to differences in the populations investigated; diversity of bedsores sites, as well as timing of specimen collections. In the present study the majority of the isolates were obtained from patients already on antimicrobial treatment and this could have led to the low recovery of antimicrobial susceptible pathogens. According to achieved results from present study, it was found that 87.7% of patients hospitalized in ICU affected by bedsores (pressure ulcer). While in a study conducted in four European countries was found that the PU prevalence in ICU was 14% in Italy 4% in Denmark, 38% in Netherlands and 49% in Germany (Weststrate *et al.*, 2001). A different studies reported that the incidence rate of PU was 16% in Spain (Manzano *et al.*, 2010), 15.5% in Turkey (Karayurt *et al.*, 2016), 39.3% in Saudi Arabia (Tayyib *et al.*, 2016), 13.6% in Brazil (Becker *et al.*, 2017). In addition, a prevalence study in the Dutch found that the PU prevalence was 28.7%. (Bours *et al.*, 2001). The differences in the prevalence could be due to different infection control programs and general hygiene levels of the country. All isolates *Staphylococcus aureus* in present study were resistant to penicillin and Oxacillin. Furthermore, our study identified that Meropenem and Doxacillin was the most effective antibiotic against *Pseudomonas aeruginosa*. On the other hand, most Gram negative bacteria were highly resistance to Co- trimoxazole and

Amoxicillin. Additionally Ciprofloxacin, Co-trimoxazole and Gentamycin are the most antibiotics that were used for treating bedsores infection in Khartoum state hospitals. However the drugs were given immediately upon admission either combined or alternatively depending on the severity of infection, but not on the types of pathogen or its pattern of sensitivity and this could be the cause of the prevalence of Multi-drugs resistant bacteria (94.3%) from the total isolates.

The present study findings indicate there are high prevalence of bedsores among patients admitted to intensive care unit in Khartoum state, with *Pseudomonas aeruginosa* as the most prevalent isolate bacterium in the bedsores patients, with 40% sensitivity to Meropenem, Gentamicin and 80% resistant to Amoxicillin, Ciprofloxacin and Ceftazidime. All *Staphylococcus aureus* isolates were resistant to Penicillin and Oxacillin (100%). Additionally all isolated bacteria considered MDROs organism except few strain of *Pseudomonas aeruginosa* and *Citrobacter freundii*. The high isolation rate of aerobic bacteria from bedsores and increased drug resistance to the commonly used antibiotics warrants the need for immediate measures ensuring effective infection prevention and rational use of antimicrobial agents leading to minimize infection rate and emergence of drug resistance also alarm for physicians to change their treatment pattern depending on antimicrobial susceptibility results.

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