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Antibiotic Susceptibility Pattern of Bacterial Isolates from Wound Infections among Patients Attending a Tertiary Care Hospital in Oman

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Wound infections are considered as a major cause of morbidity and mortality around the world and it is associated with long hospital stays and increased costs. This study aims to determine the prevalence of bacterial pathogens, associated risk factors and the antibiotic susceptibility patterns of wound infections among patients attending a tertiary care hospital in Oman. One hundred and sixty wound swabs were collected using clean, sterile swabs from patients attending Sultan Qaboos University Hospital(SQUH), as a tertiary care hospital in Oman. These wound swabs were inoculated into appropriate culture media. A microscopical examination was carried out in order to have a preliminary idea of the causative microorganism until the culture reports were available. Bacterial growth was identified by morphological aspects of colonies, followed by different biochemical tests. BD Phoenix™ system was used to confirm microbial identification and to determine the antibiotic sensitivities. Out of 160 wound swabs, 93(58.1%) were positive for wound infections. *Staphylococcus aureus* was the most prevalent microorganism. Elderly patients above 70 years had more wound infections compared to other age groups. Most of the isolated Gram-positive bacteria were sensitive to vancomycin except *Enterococcus gallinarum*. Isolated Gram-negative bacteria were 100% resistant to ampicillin except for *Proteus mirabilis*. Multi-drug resistant (MDR) organisms had quit high prevalence in wound infections among Omani patients, therefore there is a need for adequate intervention to limit the spread and evolution of further resistance.

Keywords: Wound infections, Antibiotic resistance, Oman.

A wound is defined as “a breakdown in the protective function of the skin, the loss of continuity of epithelium, with or without loss of underlying connective tissue”[1]. These wounds range from minor cuts and burns to major surgical wounds and body ulcers[1,2].

Pathogen infecting wounds can originate either from the external environment or from the patient’s endogenous flora such as the patient’s skin, mucous membranes, or gastrointestinal tract[3].

Wounds are classified into acute and chronic wounds. An acute wound is usually caused by external damage to the skin which is the case in surgical wounds, burns, bites and minor cuts. While chronic wounds are usually caused by disturbance of the dermal and epidermal tissue by an endogenous mechanism due to a predisposing condition such as diabetic foot ulcers and pressure sores [4].

Wound infections usually occur when the virulence factors of the pathogen overcome the host immune system [5, 6].

The causative agents of wound infections may vary with the geographical location, from hospital to hospital and with different surgical procedures performed[7]. The most common causative agents of wound infections are *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*[7].

Many studies around the world have been conducted to identify the bacterial species isolated from wound infections.A study carried out in Italy, showed that the most common bacterial species isolated from different types of wounds were *Staphylococcus aureus* (37%), followed by *Pseudomonas aeruginosa* (17%), *Proteus mirabilis* (10%), *Escherichia coli* (6%) and *Corynebacterium* spp. (5%) [8].

A previously, published study carried out in south-west Ethiopia showed the predominance of *Staphylococcus aureus* (32.4%) followed by *Escherichia coli* (20%), *Proteus* species (16%), Coagulase negative *Staphylococci* (14.5%), *Klebsiella pneumoniae* (10%) and *Pseudomonas aeruginosa* (8%) [9].

Another study carried outin Egypt showed that *Pseudomonas aeruginosa* was the most frequent isolated microorganism from burn infections in cancer patients. While *Staphylococcus aureus* was the most frequent isolated microorganism from wound infections in cancer patients [10].

A previously published study from Saudi Arabia showed that only 23 patients out of 131 patients admitted to surgical wards and surgical intensive care unit in King Khalid University Hospital had bacteria isolated from their surgical sites[11]. In addition, emergency operations showed a higher rate of infections in comparison to elective operations. The most common bacterial isolates were *Escherichia coli* followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*[11].

Bacterial wound infections are treated with different types of antibiotics. The selection of the suitable antibiotic depends on a number of factors including the causative agent, the site and severity of the infection [3]. The chosen antibiotic should be able to eliminate the microorganism completely and at the same time, cause the least adverse effect by reducing the possibilities of the microorganism to have a tendency to develop antibiotic resistance [3].

Unfortunately, bacteria have developed several mechanisms through which it can resist the action of antibiotics. This includes mutations in genes encoding the target site of the antibiotic, over-expression of efflux pumps that extrude the drug from the cell, and protection of the antibiotic target site by specific proteins [12, 13]. Sydnor and Perl(2011)showed that multi drug resistant bacteria had shown a higher mortality rate compared to those caused by susceptible ones[14].

In a study conducted in Italy, Gram positive bacterial isolates from wound swabs were susceptible to vancomycin and linezolid, while Gram negative bacterial isolates showed quite high resistance to most of the tested antibiotics, where amikacin was the most effective against them[8].

In a study carried out in Nigeria, all wound isolated microorganisms were resistant to cloxacillin [15]. *Pseudomonas aeruginosa* had a very high resistance to tested antibiotics, with the lowest resistance to ofloxacin, while *Klebsiella pneumonia* was relatively susceptible to nitrofurantoin[15]. Multiple-antibiotic resistant strains including *Pseudomonas aeruginosa*, coliforms and *Staphylococcus aureus* were resistant to all tested antibiotics[15].

This study and for the first time was carried out to firstly identify the causative bacterial pathogens of wound infections among patients attending a tertiary care hospital in Oman and to determine the antibiotic susceptibility patterns of the isolated bacterial pathogens and finally, to investigate the risk factors contributing to wound infections.

MATERIALS AND METHODS

Specimens

One hundred and sixty wound swabs were collected using clean, sterile swabs from patients attending SQUH followed by adequate labelling of the sample with necessary data (patient's name, medical record number, episode number, age, gender and date of collection). All patients with suspected wound infections and attended Sultan Qaboos University Hospital in the period from August until November 2018 were included in this present study. Patients on antibiotic therapy 2 weeks before the study was excluded.

To avoid contamination of wound swab samples, all the swabbing was carried out following the hospital guidelines by a well-trained and qualified Medical officer at the Sultan Qaboos University Hospital.

All swabs were sent in Amies transport media immediately after collection to the Microbiology Laboratory at the Hospital for Microbiological analysis. Once samples arrived at the laboratory, they were updated on the LabTrack system and request forms including all patients' information were printed out.

Ethical approval

The ethical approval for this research was obtained from the Research Ethics Committee College of Medicine and Health Science, Sultan Qaboos University, Muscat, Oman. (MREC#1689).

Microscopical examination of wound swabs

Wound swabs were smeared on a glass slide, heat fixed on a hot plate and stained with gram stain. A microscopical examination was carried out in order to have a preliminary idea of the causative microorganism until the culture reports were available.

Detection of pathogenic bacteria in wound swabs using routine culture media

Detection of the causative microorganism of wound infection was done by culturing the wound swabs into blood agar, CLED agar, neomycin blood agar and Sabouraud agar. Inoculation of wound swabs into Sabouraud agar was only performed

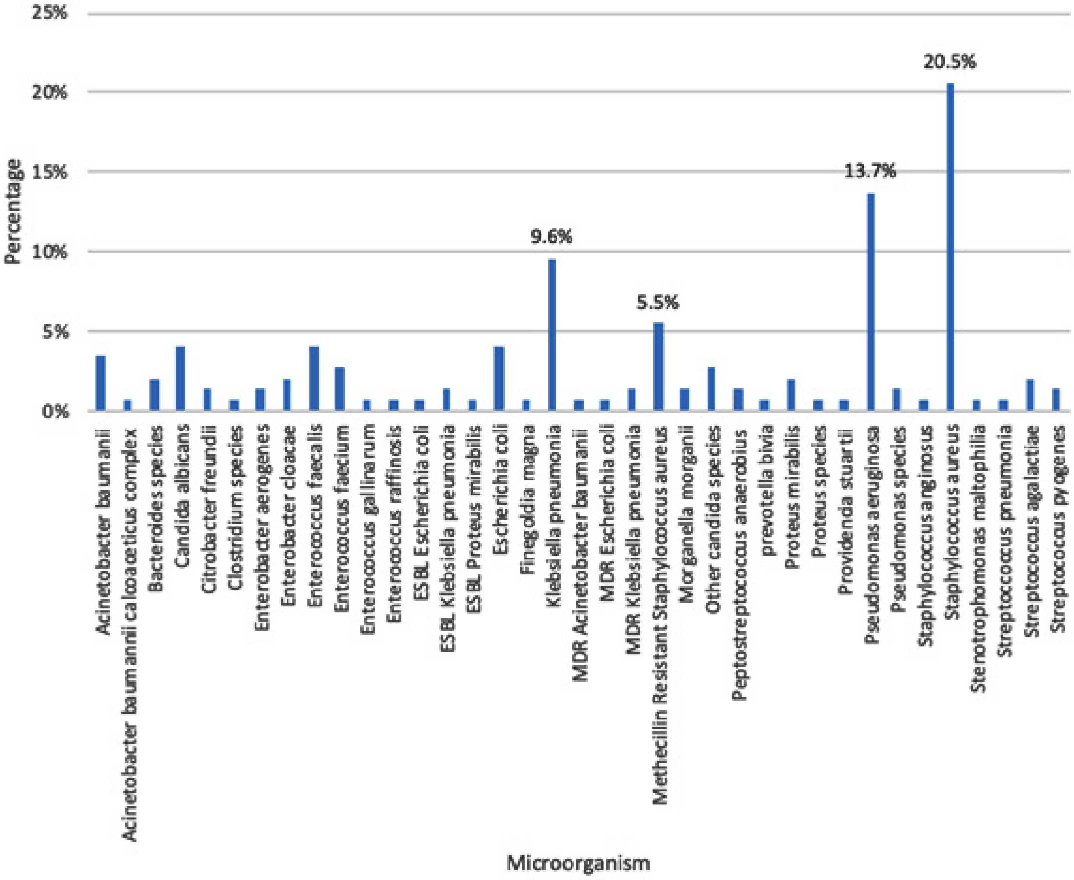

Fig. 1. Prevalence of pathogens detected in wound swabs obtained from Omani patients attending SQUH

if yeasts were seen during the microscopical examination. All plates except neomycin agar were incubated in aerobic conditions for 24 hours at 37°C, while neomycin agar was incubated in 7% CO_2 at 37°C for 48 hours after the addition of 5 mg metronidazole (MTZ) disk to the plate. After incubation, the bacterial growth was identified by morphological aspects of the colonies, followed by different biochemical tests such as coagulase, catalase and oxidase tests. The identification of the pathogens was confirmed using automated BD Phoenix^{TM} system.

Identification and antibiotic susceptibility testing of bacterial pathogens in wound swabs using BD Phoenix system

The BD Phoenix^{TM} automated identification and susceptibility system provides accurate, rapid and reliable identification of known and newly emerging antimicrobial resistance. Bacterial colonies from culture plates were added to ID broth to prepare a 0.5 McFarland suspension with the aid of BD Phoenix^{TM} nephelometer. 25ml of prepared ID broth was added to AST broth in addition to one drop of AST indicator. ID broth and AST broth were inoculated into the panel wells and purity plates were prepared from the inoculum fluid for purity check. Finally, panels were loaded into BD Phoenix^{TM} automated system and reports were printed out when the processing was completed. Manual antibiotic susceptibility testing using disk diffusion method was done for some microorganisms if there was no suitable panel for the identified microorganism. Interpretation of results was done according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations.

Data analysis

Patients' information was recorded in the Microsoft Excel program. Statistical analysis was conducted using both Microsoft Excel program and the Statistical Package for Social Science (SPSS)software in which the mean, median and frequencies for categorized variables were analyzed.

RESULTS

Clinical features

During the data collection period from August until November 2018, a total of 160 patients were recruited. Patients were aged between 0-90 years with a median age of 46 years. Samples were collected from Sultan Qaboos University Hospital (SQUH), which is a referral hospital from all regions of Oman. Out of the 160 patients,87 were males and 73 were females. A wound swab was collected from each patient. The majority of the cases (20%) were 70 years old and above. The sociodemographic characteristics of patients are summarized in Table 1.

Occurrence of pathogens

Out of 160 wound swabs, 93 (58.1%) were positive for wound infections. Out of the 93 positive wound swabs obtained from Omani patients, 47(50.5%) were male and 46 (49.46%) were female. The most abundant isolated pathogenic microorganisms were *Staphylococcus aureus*(20.5%), followed by *Pseudomonas aeruginosa*(13.7%), *Klebsiella pneumoniae*(9.6%) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) (5.5%).The prevalence of pathogens detected in wound swabs obtained from Omani patients attending SQUH is summarized in Fig. 1.

In about 52 cases (55.9%), the infection was caused by a single microorganism, while in 41 cases (44.1%) the infection was polymicrobial. 75.6 % of the polymicrobial infections were caused by two microorganisms, while 24.4% only were caused by three microorganisms.

The prevalence of bacterial pathogens in male patients

A total of 47 wound swabs obtained from male patients had positive results for bacterial cultures. *Staphylococcus aureus* was the most common bacterial pathogen isolated from males with 22.2% followed by *Klebsiella pneumoniae* (11.1%), *Pseudomonas aeruginosa* (8.3%) and Methicillin Resistant *Staphylococcus aureus* (MRSA) (5.6%). The type and the percentage of bacterial pathogens detected in wound swabs obtained from male patients are summarized in Figure 2.

The prevalence of bacterial pathogens in female patients

A total of 46wound swabs obtained from female patients had positive results for bacterial cultures. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common bacterial pathogens isolated from females with (19.4% each), followed by *Klebsiella pneumoniae*

Table 1. The sociodemographic characteristics of involved patients

Variables	Number of cases	Percentage
Gender		
Male	87	54.4%
Female	73	45.6%
Age (median-range)	46 (0-90)	
Age group(years)		
0-9	27	16.6%
10-19	5	3.1%
20-29	15	9.4%
30-39	22	13.8%
40-49	17	10.6%
50-59	23	14.4%
60-69	19	11.9%
≥70	32	20.0%

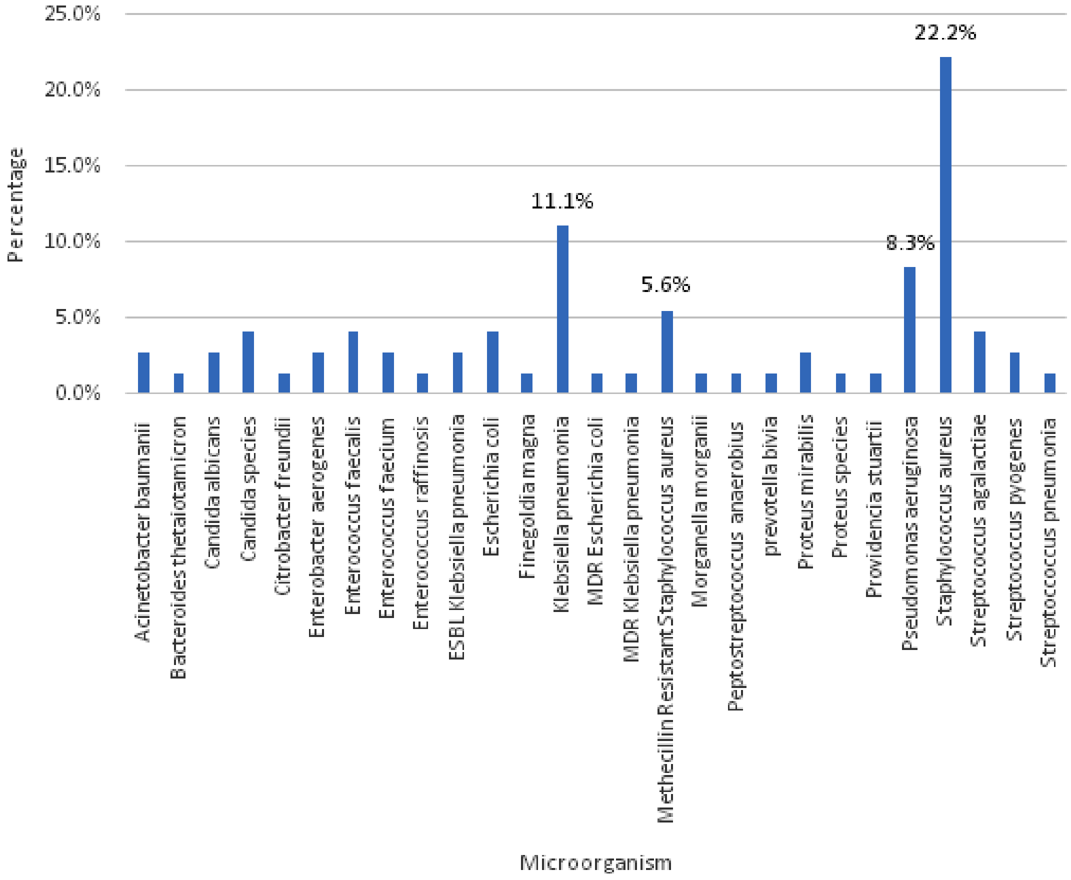

Fig. 2. The prevalence of bacterial pathogens obtained from wound swabs in male

(8.3%) and *Candida albicans* (5.6 %).The type and the percentage of microbial pathogens detected in wound swabs obtained from female patients are summarized in Figure 3.

Association of wound infections with age

Staphylococcus aureus was the most common bacterial species affecting most of the age groups and it was more associated with patients from (0-9)years old. Whereas *Pseudomonas aeruginosa* was more associated with patients who were 70 years old and above. Elderly patients above 70 years had more wound infections compared to other age groups. Table 2 shows the distribution of wound infections in association with age groups.

Association of wound infections with the anatomical site

Twenty-six-point three percent (26.3%) of wound infections were located on the abdomen,

Table 2. The distribution of wound infections in association with age groups

Microorganism	0-9	10-19	20-29	30-39	40-49	50-59	60-69	>=70
Acinetobacter baumanii						2	1	2
Acinetobacter baumannii calcoaceticus complex						1		0
Bacteroides species						1		2
Candida albicans	2	1						3
Candida species				1		1		2
Citrobacter freundii						1		1
Clostridium species								1
Enterobacter aerogenes							1	1
Enterobacter cloacae				1			2	0
Enterococcus faecalis				1		2	1	2
Enterococcus faecium			1			2		1
Enterococcus gallinarum								1
Enterococcus raffinosis				1				0
ESBL Escherichia coli						1		0
ESBL Klebsiella pneumonia				1			1	0
ESBL Proteus mirabilis						1		0
Escherichia coli			2	1		2		1
Finegoldia magna				1				0
Klebsiella pneumonia	2	1		3	2	1	3	2
MDR Acinetobacter baumanii							1	0
MDR Escherichia coli						1		0
MDR Klebsiella pneumonia							2	0
Methecillin Resistant Staphylococcus aureus	2	1	1	2	1	1		0
Morganella morganii					1			1
Peptostreptococcusanaerobius				1			1	0
prevotellabivia				1				0
Proteus mirabilis	1			1		1		0
Proteus species			1					0
Providencia stuartii								1
Pseudomonas aeruginosa	2		1	1		5	2	9
Pseudomonas species				2			1	0
Staphylococcus anginosus						1		0
Staphylococcus aureus	10		2	5	3	6	1	3
Stenotrophomonas maltophilia	1							0
Streptococcus agalactiae					1			2
Streptococcus pyogenes	2							0
Streptoccoccus pneumonia	1							0
Total	23	3	8	23	8	30	17	35

Table 3. The distribution of wound infections in association with the anatomical site

Microorganism	Abdomen	Arm	Back	Breast / Chest	Foot	Genitalia	Head and Neck	Leg	unpecified
Acinetobacter baumanii	2		1				1		1
Acinetobacter baumannii calcoaceticus complex					1				
Bacteroides species	1		1						1
Candida albicans	1	1	3						1
Candida species	2		1						1
Citrobacter freundii	1							1	
Clostridium species			1						
Enterobacter aerogenes				2					
Enterobacter cloacae	2						1		
Enterococcus faecalis	2				1		1	1	1
Enterococcus faecium	1		1			1		1	
Enterococcus gallinarum									1
Enterococcus raffinosis								1	
ESBL Escherichia coli	1								
ESBL Klebsiella pneumonia	2								
ESBL Proteus mirabilis									1
Escherichia coli	1			1	1	1		2	
Finegoldia magna				1					
Klebsiella pneumonia	3		2	4	1	2	2		
MDR Acinetobacter baumanii									1
MDR Escherichia coli								1	
MDR Klebsiella pneumonia									2
Methecillin Resistant Staphylococcus aureus	3	1	1		1			2	
Morganella morganii							1	1	
Peptostreptococcusanaerobius				1		1			
prevotellabivia						1			
Proteus mirabilis	1			1	1				
Proteus species									1
Providencia stuartii					1				
Pseudomonas aeruginosa	3		2	3	1	2		2	6
Pseudomonas species	1		1						
Staphylococcus anginosus				1					
Staphylococcus aureus	7		3	5	3	3	2	5	2
Stenotrophomonas maltophilia									1
Streptococcus agalactiae					1			1	1
Streptococcus pyogenes					1			1	
Streptoccoccus pneumonia				1					
total	34	2	17	20	13	11	8	19	21

Table 4. Antibiotic resistance pattern of Gram-positive bacteria to different antibiotics

	CL	D	ER	G	L	P	TS	TC	T	V
Staphylococcus aureus	3.2%	0%	9.7%	0%	0%	96%	6.9%	20.7%	0%	0%
Enterococcus faecalis	100%	0%	66.7%	100%	0%		100%	66%%	0%	0%
Enterococcus faecium	100%		66.7%	100%	0%		100%	33.3%	0%	0%
Enterococcus gallinarum	100%	0%	0%	100%	0%		100%		0%	100%
Enterococcus raffinosis							100%			0%
MRSA	22.2%	0%	33.3%	22.2%	0%	100%	25%	11.1%	0%	0%
Streptococcus agalactiae	0%	0%	0%		0%	0%		50%		
Streptococcus anginosus	0%					0%	0%			
Streptococcus pneumoniae	0%		0%			0%	100%	0%		0%
Streptococcus pyogenes	0%		0%		0%	0%	50%	0%		0%
Providencia stuartii	100%			100%			100%			

CL:Clindamycin, D:Daptomycin, ER:Erythromycin, G:Gentamycin, L:Linezolid, P: Penicillin, TS:Trimethoprime/ Sulfamethoxazole, TC:Tetracyclin, T:Teicoplanin, V:Vancomycin followed by breast/chest (18.8%), legs (16.3%), back (12.5%), foot(10.0%),genitalia (8.8%), head and neck (5.0%) and arm (2.5%). Most *Staphylococcus aureus* caused wound infections were found on the abdomen, whereas most of *Pseudomonas aeruginosa* caused infections were found on the abdomen and breast/chest area. The distribution of wound infections in association with the anatomical site is summarized in Table 3.

Association of wound infections with the wound type

Wound infections were more associated with surgical wounds (17), followed by abscesses (7) and bedsores (7), traumatic wounds (4), burns (3), diabetic ulcers (3), ulcers (1) and scars (1).

Antibiotic resistance pattern

Most of the isolated Gram-positive bacteria were sensitive to vancomycin except *Enterococcus gallinarum* which exhibited complete resistance to vancomycin. All isolated Gram-negative bacteria were 100% resistant to ampicillin except *Proteus mirabilis* which was sensitive to it.

Staphylococcus aureus, MRSA and *Enterococcus* species were completely sensitive to Teicoplanin. *Klebsiella pneumonia* was completely sensitive to Amikacin, Gentamycinand Trimethoprim/Sulfamethoxazole.

Antibiotic resistance of all isolated microorganisms from wound infections are summarized in Table 4 and Table 5.

DISCUSSION

In the present study, 93 (58.1%) of the 160 wound swabs collected from patients who attended a tertiary care hospital in Oman for the

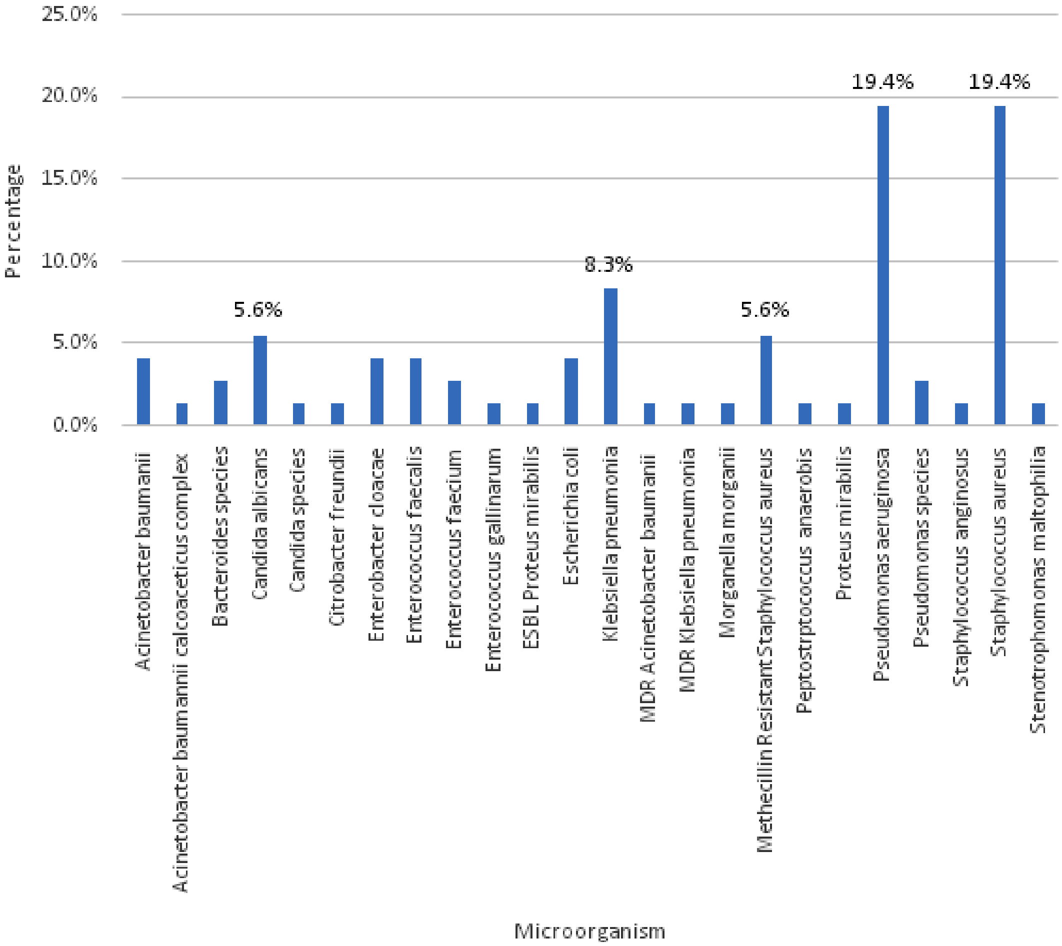

Fig. 3. The prevalence of microbial pathogens obtained from wound swabs in female

Table 5. Antibiotic resistance pattern of Gram-negative bacteria to different antibiotics

	A	AC	AK	CZ	CP	CX	N	G	M	TS	PB
Acinetobacter baumannii	100%	100%	0%	0%	33.3%	100%	100%	0%	0%	0%	0%
Citrobacter freundii	100%	100%	0%	0%	0%	100%	50%	0%	0%	0%	0%
Enterobacter aerogenes	100%	100%	0%	0%	0%	100%	0%	0%	0%	0%	0%
Enterobacter cloacae	100%	100%	0%	0%	0%	100%	100%	66.7%	0%	33.3%	0%
ESBL Escherichia coli	100%	100%	0%	100%	66.7%	0%	0%	0%	0%	33.3%	0%
ESBL Klebsiella pneumonia	100%	100%	0%	100%	50%	0%	0%	50%	0%	100%	0%
ESBL Proteus mirabilis	100%	0%	100%	100%	100%	0%	100%	100%	0%	100%	0%
Escherichia coli	100%	66.7%	0%	66.7%	33.3%	33.3%	0%	0%	0%	33.3%	0%
Klebsiella pneumonia	100%	25%	0%	12.5%	12.5%	12.5%	12.5%	0%	14.3%	0%	12.5%
MDR Acinetobacter baumannii	100%	100%	100%	100%	100%	100%	100%	66.7%	100%	33.3%	100%
MDR Escherichia coli	100%	100%	0%	100%	100%	0%	0%	0%	0%	100%	100%
MDR Klebsiella pneumonia	100%	88.9%	77.8%	100%	100%	88.9%	88.9%	100%	44.4%	100%	88.9%
Morganella morganii	100%	100%	0%	0%	0%	0%	100%	0%	0%	0%	0%
	A	AC	AK	CZ	CP	CX	N	G	M	TS	PB
Proteus mirabilis	0%	0%	0%	0%	0%	0%	100%	0%	0%	0%	0%
Pseudomonas aeroginosa	100%	100%	0%	14.3%	0%	100%	100%	4.5%	22.7%	100%	13.6%
Pseudomonas luteola			0%	0%	0%			0%	0%	0%	0%
Stenotrophomonas maltophilia	100%	100%	100%	0%		100%	100%	100%	100%	0%	100%
Pseudomonas species			0%	0%	0%			0%	0%		0%

A:Ampicillin, AC:Amoxicillin/Clavulanate, AK:Amikacin, CZ:Ceftazidime, CP:Ciproflaxin,CX:Cifoxitin,N:Nitrofurantoin, G:Gentamycin, M:Meropenem, TS:Trimethoprime/Sulfamethoxazole, PB:Piperacillin/Tazobactam period from August until November 2018 were positive for wound infections. This finding was to some degree comparable with a study conducted in Italy, where 217 (69.5%) out of 312 wound swabs were positive for wound infections[8].

In our study, the number of infected wounds associated with females 46 (49.5%) was almost the same as males 47(50.5%). These results were consistent with a study conducted in Nigeria[15].

In the present study,*Staphylococcus aureus* was the most predominant bacterial species isolated from wound infections. This result was consistent with the studies conducted in Italy, South-west Ethiopia and Egypt[8–10]. However, the results of this study were inconsistent with the result of another study carried out in Nigeria[15], where *Pseudomonas aeruginosa* was the most commonly detected pathogen[15]. This could be due to different economic and environmental factors in the two regions.

Pseudomonas aeruginosa was the second most isolated microorganism in the present study. In many studies [8–11,15]*P.aeroginosa* was found to be either the first or second most isolated microorganism from wound infections. Rossi *et al.*, (2015), reported that *S.aureus* is usually isolated from the superficial layers of wounds, while *P.aeroginosa* lay in the deepest region of wounds[16].

In the present study, most of the wound infections were monomicrobial (55.9%),while (44.1%) of wound infections were polymicrobial. This result was consistent with a study conducted in Italy, where the monomicrobial infections (72.8%) were more frequent than polymicrobial infections(27.2%)[8]. Our results were also consistent with a study carried out in south-west Ethiopia, where 91.6% of wound infections were monomicrobial, while only 8.4% were polymicrobial infections [9].

It is well known that *S.aureus* and *P.aeroginosa* produce many virulence factors that worsen infections and delay healing[16]. The co-infection of wound with both *S.aureus* and *P.aeroginosa*is found to be more virulent than a single infection of each microorganism separately[16,17].

In the present study, elderly patients who were 70 years old and above had more wound infections compared to other age groups. A similar finding was seen in a previous study carried out in Nigeria, where patients aged between 21-30 years were more prevalent to wound infections[15]

The present study showed that the highest number of wound infections were located on the abdomen (26.3%). This result was inconsistent with the results of a previous carried study carried out in south-west Ethiopia, where about 30% of wound infections were located on legs[9].

Our results showed that the highest numbers of infections were detected in surgical wounds. This result was inconsistent with studies carried out in Nigeria and South-west Ethiopia, where non-traumatic and traumatic wounds were the highest types of wounds associated with wound infections, respectively [9,15]. This could be due to the contamination of surgical instruments with biofilms and inappropriate disinfection of surgical sites[18,19,20].

In the present study, we found that all Gram negative bacteria exhibited complete resistance to ampicillin except *Proteus mirabilis* which exhibited no resistance. Mama *et al.* (2014) found that the isolated Gram-negative bacteria were 100% resistant to ampicillin except for *Proteus* species, where 9% were sensitive to ampicillin[9].

In the present study, Methicillin-Resistant *Staphylococcus aureus* (MRSA) was the fourth (5.5%) most common pathogen isolated from infected wound. This finding is inconsistent with a previously published work where a higher rate of MRSA was detected [21].

In conclusion, our data suggested that the major pathogen that was associated with wound infections among patients attending Sultan Qaboos University Hospital was *Staphylococcus aureus*. The majority of wound infection cases were diagnosed in elderly patients whose ages were 70 years and above. Polymicrobial infections were recognized in 41 cases (44.1%) of the wound infection cases. For antimicrobial resistance, all isolated Gram-positive bacteria were sensitive to vancomycin except *Enterococcus gallinarum* which interestingly exhibited complete resistant to vancomycin. All isolated Gram-negative bacteria were 100% resistant to ampicillin except *Proteus mirabilis*. In the present study, multi-drug resistant

(MDR) organisms had quiet high prevalence in wound infections among patients attending a tertiary care hospital in Oman;therefore, there is a need for effective intervention to limit the spread and evolution of further antibiotic resistant bacterial pathogens among this unique a group of patients.

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