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Prevalence and Antibiotic Susceptibility Pattern of Aerobic Bacteria Isolated from Iraqi Bed Sores Patients Admitted to Intensive Care Units

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Abstract: Bed sores (BSs), are injuries caused by ischaemia on the skin, adjacent tissues, and bones as a result of prolonged compression or shear forces.The National Pressure Ulcer Advisory Panel's (NPUAP) classification of pressure ulcer severity is one of the most widely accepted . The current study was determining prevalence and antimicrobial susceptibility pattern of aerobic bacteria that causes the BSs infection in Iraqi patients. Atotal of 82 BSs swabs specimens were collected from patients with bedsores infection due to prolonged bed lying in intensive care units (ICUs) from various hospitals in Iraq (AL-Yarmouk Teaching Hospital and and IBN –ALQUFF Hospital) during the period from October 2021 to February 2022, 43 men and 39 women, Samples were cultured on selective, enrichment, and special media and then incubated at 37 °C for 18-24 hours. Bacterial isolates were identified using various culture media, microscopic examination with Gram stain, antimicrobial susceptibility testing was performed using Viteck-2 system and Kirby- Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI) 2020. The prevalence of bedsores in hospitalized patients was 70/82 (85.4.7%) Both Gram positive cocci and Gram negative bacilli were isolated from 82 patients ;Among the Gram negative isolates, *Pseudomonas aeruginosa* had the highest frequency 21/40 (52.5%), followed by *Acinobacter baumanii* 4/40 (10%), *E.coli* 4/40(10%), *Klebsiella pneumoniae*3/40(7.5%), *Proteus mirabilis* 3/40(7.5%), *Pantoae spp* 2/40 (5*%), Aeromonas verronii1/40(2.5%), Kluyvera intermidia1/40(2.5%) and Sphingobacter thalophilum1/40(2.5%).* The gram positive cocci isolated was *Staphylococcus aureus, s.epidermis, Enterococcus faecalis, Kocuria Kristina*. The distribution of bedsores among the ages of the patients showed that age group within the range of >40 years recorded the highest incidence of bedsore infection. The most common site of bedsores was sacral region 34/70 (48.57%) followed by leg 11/70 (15.71%). All isolated *S. aureus* showed 94% resistance to Penicillin but showed variable susceptibility to other antibacterial used. *P. aeruginosa* was resistant to penicillin (100%) followed by Doxycycline (100%), amoxicillin-clavuanate, trimethoprime (90%). The results showed that all isolated bacteria considered multi-drug resistance organism (MDROs) of 67/70 (95.7%) except few strains of *P. aeruginosa* 2/21 (9.5%) and *S.aureus* 1/17 (5.8%).

Keywords: Bed sores, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, antibiotic susceptibility test.

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Introduction

Bed sores (BSs) are known as Pressure ulcers (PUs), a common clinical problem reported by patients with mobility limitations, sometimes, it can even be life threatening, its treatment imposes financial burdens on patient's family and society (1).

BSs are particularly common in elderly patients in public hospitals and home care settings, mainly in intensive care units where its incidence ranges

from (8 - 40) % (2). And recently years, increase morbidity and mortality due to infections with multidrug-resistant (MDR) ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) and it becomes a serious concern, globally (3).

Bed sores have high fatal rates also are difficult and expensive to treat.The Lack of treatment may lead to infection and sepsis with fatal results. BSs has been reported to develop in 15% of acute care patients and this rate increases up to 63%, compared to the earlier reports (4). The most common locations of BSs are between the buttocks (sacru), the bony protuberances in the sitting area (ischium) and the protuberances on the sides of the hip (trochanter). The occipital protuberance, the heel, and the lower side of the scapula are the other potential BSs areas in bedridden patients (1). BSs in hospitalized patients were major reservoir of multi-resistant GNB, also a high-risk population for the development of bacteremia with high mortality rates (5).

Moreover, BSs have been described as one the costliest and physically debilitating complication in the 20th century and represent the third most expensive disorder after cancer and cardiovascular diseases (7). *Pseudomonas aeruginosa* is one of the major causes of community-acquired infections, considered in 9-10% of nosocomial opportunistic infections (8) *P. aeruginosa* is well-known for being the leading cause of death from nosocomial infections, particularly in patients with severe BSs, causing sepsis in immunocompromised patients (9). The innate and adaptive resistance mechanisms of *P. aeruginosa* provide high resistance to several antibiotics. These mechanisms include: reducing the permeability of cell coatings, efflux pumps, obtaining resistance genes through plasmids and transposons, and changing the expression and function of chromosomally encoded mechanisms through mutation (10). The majority of the isolates from BSs are known to be resistant to ampicillin and amoxicillin. Large numbers of S. aureus are methicillin-resistant S. aureus (MRSA) and most bacteria isolated are sensitive to quinolones, aminoglycosides and monobactam (11). Antibiotic resistance is the hot topic of the 21st century as a result of the ever-increasing number of hospitalization due *to (MDR)* bacterial infections; Many scientists and medical professionals have emphasized the urgent need to prevent the emergence and spread of drug-resistant bacteria (12).

Materials and methods

Descriptive Cross-sectional study was conducted from October 2021 to February 2022. Eighty-two BSs swab specimens were collected from patients admitted to intensive care units of AL-yarmuk teaching Hospital and Ibn - alquff Hospital in Baghdad.

Macroscopic examination of bacterial isolates

All swab specimens were inoculated under aseptic technique in 5% Blood agar, MacConkey agar and Mannitol salt agar, Eosin methyl blue and prepared according to manufacturer's instruction. Inoculated culture media were incubated aerobically at 37°C overnight to study colonial phenotypes such as, colonial form, shape, color, size, and aroma (13).

Microscopic examination:

Gram staining was carried out initially to study morphological characteristics of clinical isolates (14).

Biochemical tests

All Gram positive coci identified by standard bacteriological test including; catalase test, coagulase test and 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 and by Api20E system used for identifing of gram negative isolates and API staph system (BioMerieux, France) and conformation using the compact VITEK 2 system.

Antibiotic susceptibility test (AST)

The AST for all isolates was performed by Kirby-Bauer disc diffusion method using Muller Hinton (*MH*) agar according to clinical laboratory standards institute (CLSI) guideline (CLSI, 2020) (14,15).

The antibiotics discs (Bioanalyse(USA)) used in this study were Penicillin (10 µg) , Azithromycin (15 mg) Doxycycline (30 µg) Chloramphenicol (30µg), Tetracycline (30µg), Ceftazidime (30µg), Meropenem (10µg), Gentamycin (10µg), Cefotaxime (30 mg), Ceftazidime(30mg), Azetreonam (30 µg), Imipenem (10 mg), Amoxacillin – Clavulanate(20-10mg), Levofloxacin (5 µg), Gentamicin(10mg), Amikacin (30mg), Trimethoprim (5mg), Chloramphenicol (30mg). Organisms that showed resistance to multiple types, classes or subclasses of antimicrobial agents were considered as (*MDR*)(16).

Results and discussion

Table (1) showed that 70/82 (85.36%) of specimens were positive for bacterial isolates includes 66/70 (94%) isolate with pure culture and 4/70 (6%) isolate with mixed culture, while 12/82 (14.63%)specimens were negative.

Table (1): Distribution of bed sores specimens according to bacterial isolates

Aerobic bacterial growth	No. (%) of specimens	Note
Positive	70(85%)	Pure culture 66 (94%) mixed culture 4 (6%)
Negative	12(15%)	---
Total No.	82(100%)	

The isolation rate was equals 35/70 (50%) in both of males and females in which its relationship with BSs incidence was non-significant differences (p-value 0.803). The highest overall infection rate was in the age group >40 years, while the lowest was in the age group <20 years, which were non-significant differences with p-value 0.813 and 0.892 respectively, as shown in Table (2).The most common infected sites was sacral region 34/70(48.57%), followed by leg 11/70 (15.71%),heel 10/50 (14.28%), neck 8/70 (11.42%), and buttock 7/70 (10%), which non-significant differences (p-value 0.766) for sacral as indicated in Table (2).

Table (2): Socio-demographic and clinical characteristics of bedsores infected patients

Characteristics	No. (%) of tested	No. (%) of culture positive	p-value
Gender			
Male	43/82 (52.44%)	35 (50%)	0.803 NS
Female	39/82 (47.56%)	35 (50%)	0.803 NS
Total	82 (100%)	70 (100%)	--
Age in year			
<20	12 (14.63%)	10 (14.28%)	0.892 NS
16-40	26 (31.71%)	20 (28.57%)	0.605 NS
>40	44 (53.66%)	40 (57.14%)	0.813 NS
Site of ulcer			
Sacral	40 (48.78%)	34 (48.57%)	0.766 NS
Neck	8 (9.75%)	8 (11.42%)	0.791 NS
Buttock	9 (10.9%)	7 (10%)	0.902 NS
Leg	13 (15.85%)	11(15.71%)	0.884 NS
Heel	12 (14.6%)	10 (14.28%)	0.892 NS
Total	82	70	

NS: Non-Significant.

According macroscopic and microscopic examination of bacterial isolates, the most common isolates in this study were Gram negative (G-ve) bacilli 40/70 (57.14%) with predominant isolate *Pseudomonas aeruginosa* (*P. aeruginosa*) 21/40 (30%) followed by other bacilli *Acinobacter baumanii* (*A.baumanii*) 4/40 (5.71%), 4/40 *Escherichia coli* (*E. coli*) (5.71%), *Klebsiella pneumonia (K. pneumonia*) and *Proteus mirabilis* (*P.mirabilis*) each one 3/40 (4.28%), *Pantoae spp* 2/40(2.85%), while *Aeromonas verronii(A. verronii), Kluyvera intermidia(K. intermidia)* and *Sphingobacter thalophilum*(*S. thalophilum*) each one 1/40(1.42. In the Gram positive(G+ve) bacteria the main organism identified was *Staphylococcus aureus* (*S.aureus*)17/70 (24.28%) followed by *Staphylococcus epidermis*(*S.epidermis*) 8/30(11.42%), *Enterococcus faecalis* (*E.faecalis*)3/30 (4.28%) and *Kocuria Kristina*(*K.kristina*) 2/30 (2.28%),. as shown in Table (3) and Figure(1).

Table (3): Distribution of positive bacterial isolates identified from study specimens

Type of isolates	No. of isolate	%
Gram positive isolates		
Staphylococcus aureus	17	24.28%
Staphylococcus epidermis	8	11.42%
Enterococcus faecalis	3	4.28%
Gram negative isolates		
Pseudomonas aeruginosa	21	30%
Klebsiella pneumoniae	3	4.28%
Acinetobacter baumannii	4	5.71%
Escherichia coli	4	5.71%
Proteus mirabilis	3	4.28%
Sphingobacter thalophilum	1	1.42%
Pantoae spp	2	2.85%
Aeromonas verronii	1	1.42%
Kluyvera intermidia	1	1.42%
Kocuria Kristina	2	2.85%
Total No.	70	100%

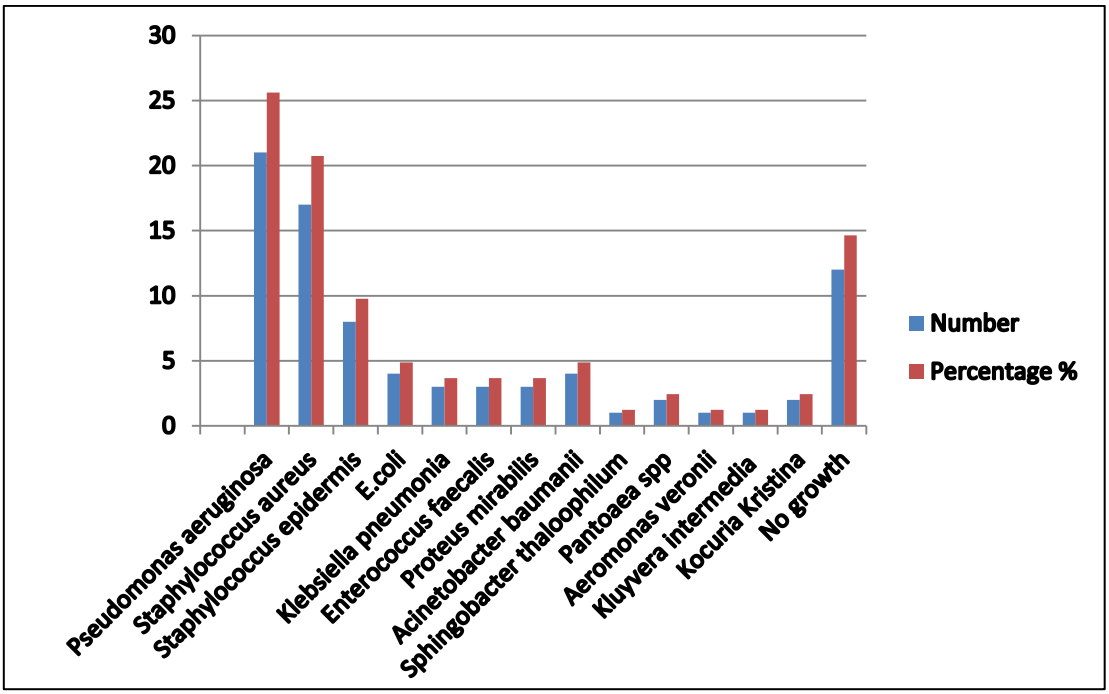

Figure (1): Distribution of bed sores samples according to the type of bacterial isolates.

The bacterial isolation and identification were confirmed by Vitek-2 system and by disc diffusion method were appeared that the most common species among the ESKAPASE for BSs was *P. aeruginosa* 21 (25.61%) and *S. aureus* 17(20.73%), followed by *S. epidermis* (9.76%), *A. baumanii* (4.88%), *E. coli* (4.8%), while *K. pneumonia, E. faecalis* and *P. mirabilis* (3.66%) each one, *Pantae spp.* and *K. kristina* (2.44%) each on, finally *S. thalophilum* , *A. veronii* and *K. intermedia* (1.22%) each on as shown in Figure (1).

The AST was conducted to all the isolates by using disc diffusion test towards 13 antimicrobial agents for *p.aeruginosa* Isolates showed a variable levels of resistance to Aminoglycoside group including gentamicin and amikacin 42.86% and 38.10% respectively, βeta-Lactam group including penicillin and augmentin 100% and 90.48% respectively, Cephalosporins 3rd generation class including Cefotaxime and ceftzidime 76.19% and 33.33% respectively. Carbapenem class including imipenem 52.32%, Tetracyclins represented by Doxycycline 100%, Fluroquinolone class including levofloxacin 47.62% and Polymyxins class including colistin 18%.

The results of AST as showed in figure (2) All *P. aeruginosa* isolates appeard resistance (100%) to penicillin and Doxycycline, followed by amoxicillin-clavuanate, trimethoprime, cefotaxime, chloramphenicol, imepenime, levofloxacin, gentamicin, amikacin, azetreonam, ceftazidime, aztronum and azithromycin reached to (90.48, 90.48, 76.19, 61.90, 52.38, 47.62, 42.86, 38.10, 33.33, 33.33 and 14.29) % respectively.

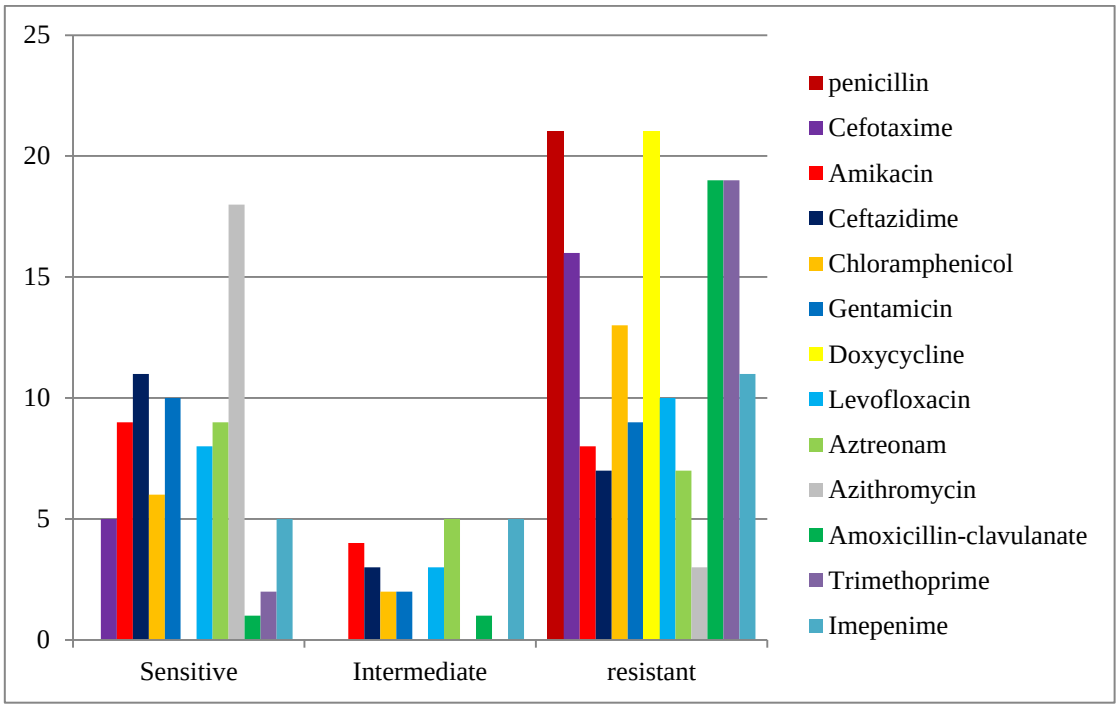

Figure (2): The susceptibility of *P.aeruginosa* isolates to antibiotics.

All *A.baumanii* isolates (100%) appeard resistant to Penicillin, cefotaxime, amikacin, cftazidime, chloramphenicol, gentamicin, levofloxacin, aztronam, amoxicillin-clavulanate, trimethoprime and imepenime followed by Doxycycline and azithromycin reached to (75 and 50)% respectively (Figure 3).

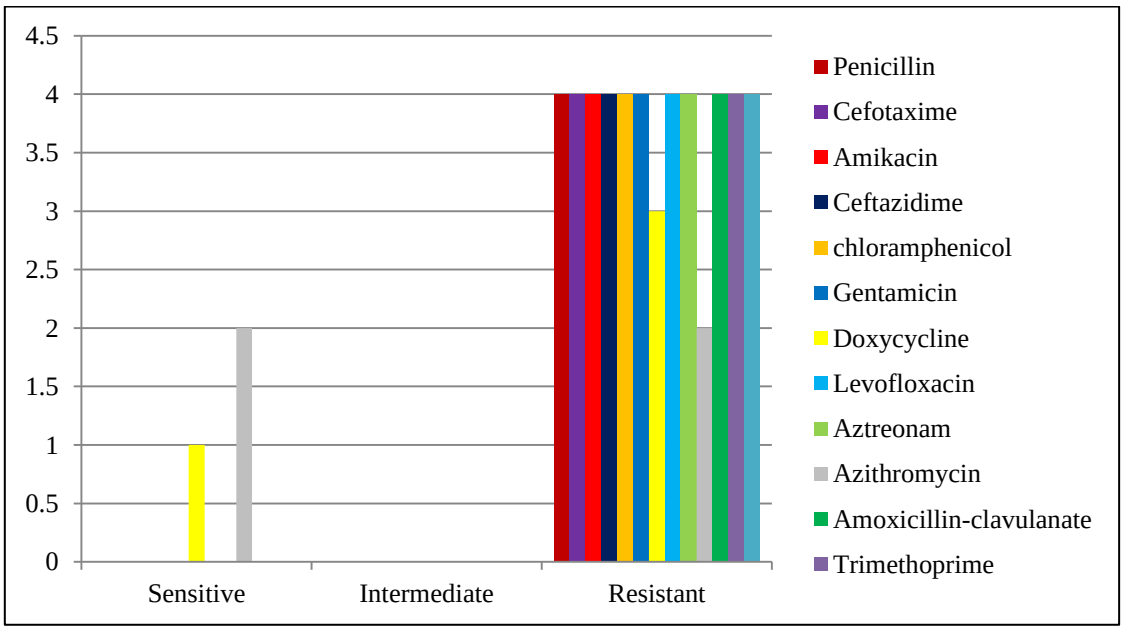

Figure (3): Sensitivity *A. baumanii* to antibiotic.

Table (4): Antimicrobial susceptibility pattern of Gram negative isolated from patients with bedsores.

Isolates	Number of strains resistant to																				
	PIN			CTX			AK			CAZ			C			GEN			DO		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Proteus mirabilis	0	0	3	0	0	3	2	0	1	2	0	1	0	1	2	1	1	1	1	0	2
Escherichia coli	0	0	4	0	0	4	0	0	4	1	1	2	0	2	2	2	0	2	2	0	2
Klebsiella pneumonia	0	0	3	0	0	3	1	0	2	2	0	1	0	1	2	1	0	2	1	0	2
Aeromonas veronii	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0
Pantae spp	0	0	2	0	0	2	2	0	0	2	0	0	0	0	2	0	2	0	0	0	2
Sphingobacter thalophilum	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0
Kluyvera intermedia	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1

Isolates	Number of strains resistant to																	
	LEV			Atm			Azm			Amc			TMT			IMP		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Proteus mirabilis	2	0	1	1	0	2	1	0	2	0	0	3	0	1	2	2	0	1
Escherichia coli	2	0	2	0	1	3	0	0	4	0	0	4	0	0	4	3	1	0
Klebsiella pneumonia	1	0	2	1	1	1	1	0	2	0	0	3	0	1	2	2	0	1
Aeromonas veronii	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0
Pantae spp	0	1	1	1	1	0	1	0	1	0	1	1	0	0	2	2	0	0
Sphingobacter thalophilum	0	1	0	0	0	1	1	0	0	0	0	1	0	0	1	0	1	0
Kluyvera intermedia	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1

Out of 70 isolates introduced to MDR organism criteria describes by clinical laboratory standards institute CLSI guidelines (resistant to three or more antimicrobial classes While XDR was defined as non-susceptibility to at least one agent in all, but two or fewer antimicrobial categories) (i.e, bacterial isolates remain susceptibility to only one or two categories) (16). All isolated bacteria considered as (MDROs) organism except few strains of and *S. aureus* and *P. aeruginosa* 3/17 (17.64%) , 2/21 (9.52%), respectively.

The BSs are also frequently colonized by several species of bacteria and surface cultures yield a polymicrobial flora of both G+ve and G-ve. The microorganisms most commonly isolated in chronic wounds are usually *S. aureus*, -hemolytic *Streptococci, Enterococcus spp*., aerobic GNB *Enterobacteriaceae* and *Pseudomonas spp*., and in particular, resistant bacteria such as methicillin-resistant *S. aureus* (MRSA), *Enterococcus spp*., ciprofloxacin resistant *Pseudomonas aeruginosa*, and extended-spectrum beta-lactamase *(E. coli*) (17).

The present study describes the distribution and antimicrobial susceptibility pattern of aerobic bacterial species isolated from bedsores infection. The rate of bacterial isolation was 70/82 (85 %), 35/70 (50%) from male and female respectively, in which its relationship with bedsores incidence was statistically insignificant. This result is consistent with other studies

done by Mostafa Shokati and Alzapir in which there was no statistical association between genders and bedsores incidence rate (18,1). Moreover, the result showed that bedsores infection more common among age group >40 (57%), which not agrees with the findings of work carried out by Khudair Al-Bedri1 and Alzapir who reported the bedsores were more common in men (19,1) in this study the result is equall between man and women.

The present study reported that the frequency of bedsores with respect to the location on the body was high at the sacral 34/70(48.57%) followed by leg 11/70(15.71%), heel 10/70(14.28%), neck8/70(11.42%), and buttock 7/70 (10%); These findings were quite different from those on another studies Shah (20). more frequent ulcers were found in the sacral region this is agree with Khudair Al-Bedri1(21) 46.9% and it's the same finding was reported in several recent studies by \Reihani *et al* (22) they showed that the most common sites were sacral regions (28.9%), (54%), (30%) and 72% respectively (22,19,1).

The present study revealed that G-ve bacteria were the prominent pathogens consisting 60% of the isolates with high frequency of *P. aeruginosa* followed by *A. baumannii*, *E. coli*, *K. pneumonia* , *P. mirabilis*, *P. spp* , *A. verronii* , *K. intermidia and S. thalophilum*. Which is matching to the result of a Khanafari (24) a who reported that *P. aeruginosa* (60%), *E. coli* (35%) and (5%) were dominant bacteria in all 20 bedsores samples. Also prospective study done by Dolati (25) was reported that the frequently of identified bacteria detected by aerobic culturing from the bedsores of all studied patients showed that *Pseudomonas* species 18/49 (36*%) S. aureus* 16/49 (32%) and *E. coli* 15/49 (30%). were the most abundant microorganisms isolated. However, *A. verronii1/40 (2.5%), K. intermidia1/40* (2.5%) *and S.thalophilum1/40 (2.5%)* were less frequency. In contrast study by Ghaly(23) have documented that *S. epidermidis* was the most prominent pathogen isolated from pressure sore (31.4%) followed by *P. vulgaris* (28.6%), *P. aeruginosa* (22.8%), *E. coli* (8.6%), *K. pneumoniae* (5.8%) and *S. aureus* (2.8%) .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 current study, *A. baumannii* showed resistance to all antimicrobial agents Figure (3). This result is corresponding with other studies. A study by Yang showed that77.8% of the patients were MDR (26). In addition, China's antimicrobial resistance monitoring program has widely identified extensive drug resistance to *A. baumannii* (XDRAB) and the study Jassim and Alash showed of resistance of *A. baumanii* to all antimicrobial agent (27).

The majority of the isolates in the present study were obtained from patients already on antimicrobial treatment and this could have led to the low recovery of antimicrobial susceptible pathogens. All isolates P. aeruginosa in present study were resistant to penicillin. Shown our study identified that Azithromycin was the most effective antibiotic against *P. aeruginosa*. On the other hand, most Gram-negative bacteria were highly resistance to Penicillin and Amoxicillin. Additionally Ciprofloxacin,as shown in Table(4) and Gentamycin are the most

antibiotics that were used for treating bedsores infection in Iraqi 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 pathogens or its pattern of sensitivity and this could be the cause of the prevalence of MDR bacteria.

Conclusion

The study findings indicate there are high prevalence of BSs among patients admitted to intensive care unit in IRAQ, with *P. aeruginosa* as the most prevalent isolate bacterium in the BSs patients Figure (2), with 85.71% sensitivity to Azithromycin, and 90% resistant to Amoxicillin-clavulante, Trimethoprime. All *S. aureus* isolates were resistant to Penicillin (94%). Additionally, all isolated bacteria considered MDROs organism except few strains of *P. aeruginosa* and *S. aureus*. The high isolation rate of aerobic bacteria from bedsores and increased resistance to drug that 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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