International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Isolation of Multi-drug Resistant Bacteria from Patients Admitted to Aljala Trauma Hospital in Benghazi, Libya

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Abstract

Background: The high prevalence of multidrug-resistant (MDR) bacteria is a worldwide problem. The overuse and misuse of antimicrobial agents favored the emergence of MDR bacteria and rendered infectious agents difficult to treat. The aim of this study was to isolate bacteria responsible for wound infections in inpatients and outpatients treated at Aljala Trauma Hospital, Benghazi, Libya.

Methods: A cross-sectional study was conducted at Al-Jala Trauma Hospital from January to July 2019. Wound swab samples were collected from 230 patients and cultured on different media. An antibiogram of isolates was determined. We were using the disk diffusion technique (Kirby-Bauer technique).

Results: Three hundred fifty-five isolates were able to grow on culture media. A single etiological agent was identified in 149 patients and multiple agents were found in 81 patients. The predominant pathogens isolated were *Pseudomonas aeruginosa* and *Staphylococcus aureus* (21%), *E. coli* (15%) *Klebsiella* (14%), *Acinetobacter* (7%), *CoNS* and *Enterobacter* (6%), while *Enterococcus* sp. (4%), *proteus* sp. (3%) and *S. pyogens*(%1)

The overall MDR among Gram-positive and Gram-negative bacterial isolates were (32.7%) and (67.3%) respectively. The percentage of MRSA was 16%, surprisingly the prevalence of MDR among *Pseudomonas aeruginosa, Acinetobacter* sp., *Proteus* sp., and *S. pyogens* were very high (100%) and most of the other bacteria isolated were resistant to most of the antibiotics used. Moreover, Co-negative isolates appeared highly resistant to Oxacillin (89%), *Enterococcus* sp. (90%), and *Staph aureus* sp. (79%)

Conclusion: The prevalence of multi-drug resistance among Gram-positive and Gram-negative bacteria is high and this is considered a serious problem. It is necessary to follow the bio gram of bacteria causing wound infections in the hospital as this would be beneficial to patients as well as help physicians in the selection of appropriate treatment. Moreover, aseptic techniques, hand hygiene, and wearing personal protective equipment will improve patient outcomes and help reduce the spread of pathogens among patients and in the hospital.

Keywords: Antimicrobial resistance, antibiotics, wound infections

Introduction:s

Antibiotic resistance is a global health problem facing modern medicine, in which bacteria have adapted their resistance to antimicrobial agents. The rapid spread of resistant bacteria along with deceleration in

the invention of new antibacterial agents is recognized all over the world, as a serious threat to the treatment of life-threatening infections.

The term multidrug-resistance (MDR) applies to a bacterium that is resistant to a number of antimicrobial drugs (Magiorakos *et al.*, 2013), several scientific articles have been devoted to the evolution of multidrug-resistant bacteria in different countries (Kateryna, 2015; John *et al.*, 2014*;* Hazim, *et al*., 2019*).*

In Libya, the problem of antibiotic resistance is very serious, as drug resistance to frequently administered antibiotics is extremely widespread. Over-counter antibiotics are considered one factor that might cause antibiotic resistance to spread. A study carried out on self-medication of antibiotics found that the total of interviewed individuals with self-medication percentage was 50% (Scicluna *et al*., 2009). Furthermore, this is may be attributed to the war in Libya, which led to casualties among the military and the civilian population. Several studies found a high level of resistance to the antibiotics tested especially among *Acinetobacter* spp., followed by coagulase-negative *Staphylococci*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp isolated from wound infections associated with war injuries. (Benedikt *et al*., 2018; Franka *et al.*, 2012; Dau *et al.*, 2013*)*. Failure to apply the necessary procedures to enhance the prevention of infection within the hospitals, poor infection prevention and control, water, sanitation, and hygiene initiatives would promote the spread of drug-resistant bacteria (Ahmed et al., 2022; Morgan *et al.*, 2011).

High prevalence rates (68%) of methicillin-resistant *Staphylococcus aureus* (MRSA) were reported in the last decade among *S. aureus* isolated from patients with burns and surgical wound infections. Besides, high resistance rates were observed among enteric bacteria against commonly used drugs (Sifaw, *et al.*,2013).

Other researchers have shown that the etiologic structure and antibiotic sensitivity of bacterial spp. differs in various countries. So, there is a need to examine regional data. The present study was undertaken to know the bacteriological profile and antibiotic susceptibility patterns of pathogens causing pyogenic infections in our hospital, especially after the war in order to help clinicians formulate an empirical treatment for the patients

Materials and methods.

Study design and population

A cross-sectional study was conducted from January to July 2019. at Al-Jala Hospital. The hospital has 200 beds offering different specialized services and it is a teaching hospital that was opened in 1968 as a surgical and accident hospital covering the eastern region of Libya.

Wound sample collection and processing

Wound swab samples were collected under an aseptic technique from 230 patients and inoculated into appropriate media. The hospital followed standard operating procedures to process and identify microorganisms up to their genus and/or species level. Bacterial isolates were identified based on their morphological characteristics, Gram stain, and confirmatory biochemical tests. The plates were incubated aerobically at 370C for 18-24 hours. For Gram-positive bacteria, identification was done through catalase reaction, coagulase test, and testing for hemolytic activity on blood agar. Gram-negative bacterial strains were characterized based on morphological appearances on MacConkey agar and blood agar, followed by biochemical experiments such as the Citrate test, Urease test, Oxidase test, Indole and Motility test, and Triple sugar iron test. Mueller-Hinton agar medium has been used for the determination of antibiotic susceptibility tests.

Antibiotic sensitivity testing

Antibiotic sensitivity testing (AST) was performed against different classes of antimicrobials and antibiotic susceptibility tests were performed using the disk diffusion technique (Kirby-Bauer technique). Clinical and Laboratory Standards Institute (CLSI) guidelines were used to determine the results. The tested antimicrobial discs were routinely supplied from Oxoid and Bioanalyse.

Multiple Drug Resistance (MDR)

The MDR isolates were identified based on the guidelines provided by the European Centre for Disease Prevention and Control (ECDC) and the CDC. An MDR isolate is an isolate that exhibits resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Data analysis

The total number of bacterial isolates were analyzed using Microsoft Excel 2016, and the total number of isolates tested for antibiotic susceptibility were calculated.

Results:

Out of 230 patients, the gender distribution was found 140 (61%) males and 90 (39%) females as shown in (Table 1). The largest percentage of patient's positive cultures were from age group 21-40 years 103 (45%) followed by 41-60 years 72 (31%) then, >60 years 32(14%) and the least ≤ 20 years 23(10%) (Table 2).

Table 1: Distribution of positive wound swab cultures according to sex (n=230)

Sex	Patients with positive cultures and a percentage
Male	140 (61%)
Female	90 (39%)

Table 2: Distribution of positive wound swab cultures and percentage-wise

Patient's age group	No. of positive samples
≤ 20 years	23 (10%)
21-40 years	103 (45%)
41-60 years	72 (31%)
>60 years	32 (14%)

Bacterial pathogens were isolated from 230 patients, a single etiological agent was identified in 149 patients, and multiple agents were found in 81 patients. There were 355 etiological agents isolated, 239 were gram-negative bacteria, in which *pseudomonas aerogenosa* was the predominate pathogen, followed by *E. coli*, *Klebseilla* spp., *Acinetobacter* spp., *Enterobacter* spp., *Proteus* spp., and their percentage were (21 %, 15%, 14%, 7%, 6%, 3%, 2.0%) respectively, and others include *Morgenella morgani*, *Provednsia* spp., and *Serratia spp.*. Moreover, 116 were Gram-positive bacteria, in which *S. aureus* was the predominant organism (21 %) followed by Coagulase-negative *Staphylococcus* (CoNS) (6%), then *Enterococcus* spp. (4%), and *Streptococcus pyogene*s (1%) Table 3.

Table 3: Prevalence of microorganisms isolated from positive wound swab culture

Organisms isolated	Male	Female	Total	Percentage (%)
Pseudomonas aeruginosa	46	28	74	21%
Escherichia coli	38	14	52	15%

Klebsiella pneumoniae	29	20	49	14%
Acinetobacter spp.	15	10	25	7%
Enterobacter spp.	12	8	20	6%
Proteus mirabilis	6	5	11	3%
Others	3	5	8	2%
Staphylococcus aureus	35	38	73	21%
CoNS	13	10	23	6%
Enterococcus spp.	8	7	15	4%
Streptococcus spp.	4	1	5	1%
Total	209(59%)	146(41%)	355	100%

Others include *Morgenella morgani, Provednsia* spp., *and Serratia* spp.

In our study, *Pseudomonas aeruginosa* was found highly resistant to Ampicillin-sulbactam (98%), Septrin (95%), ceftriaxone (93%), Doxycyclin (85%). And more susceptible to Colistin (91%) and Aztronam (75%), whereas, the rate of *E. coli* resistance to trimethoprim/Sulphamethoxazole was (80%) and no resistance to colistin was found. However, *Klebsiella* spp. was highly resistant to Tobramycin (87%), Ampicillin-sulbactam (83%), and Amikacin (71%). Though *Acinetobacter* spp. was the highest-resistant microorganism in our study, it was resistant to Ceftazidime and Tobramycin (100%), While *Enterobacte*r spp. sensitive to Amikacin (95%) and Colistin (100%), and the rate of resistance to Aztreonam was (99%), Ampicillin/sulbactam (95%), and Azithromycin (93%). Among *Proteus* spp. it showed 100% susceptibility to Imipenem, and tobramycin (Table 4).

Bacterial isolates		CT	CAZ	AK	CIP	LEVO	DO	SXT	AZTH	GN	TO	IMP	AZT	CEF	AM/SUL
Pseudomonas (n=74)	**S**	**40** 91%	**16** 36%	**27 42 %**	**29 42 %**	**24 41 %**	**8 15 %**	**3 5 %**	**15 42 %**	**17 30 %**	**7 22 %**	**26 45 %**	**47 75 %**	**4 7 %**	**1 2%**
	R	**4** 9%	**28** 64 %	**37 58 %**	**40 58 %**	**34 59 %**	**47 85 %**	**53 95 %**	**21 58 %**	**40 70 %**	**25 78 %**	**32 55 %**	**16 25 %**	**54 93 %**	**53 98 %**
E.coli (n=52)	**S**	**35 100 %**	**24 67 %**	**37 46 %**	**26 58 %**	**14 40 %**	**22 54 %**	**7 20 %**	**13 68 %**	**24 53 %**	**23 88 %**	**38 97 %**	**25 51 %**	**35 74 %**	**12 33 %**
	R	**- 0.0 %**	**12 33 %**	**44 54 %**	**19 42 %**	**21 60 %**	**19 46 %**	**28 80 %**	**6 32 %**	**21 47 %**	**3 12 %**	**1 3%**	**24 49 %**	**12 26 %**	**24 67 %**
	S	**25**	**16**	**27**	**23**	**22**	**16**	**13**	**10**	**12**	**3**	**27**	**17**	**13**	**5**

Klebsiella (n=49)		**66 %**	**55 %**	**64 %**	**61 %**	**56 %**	**52 %**	**36 %**	**50 %**	**39 %**	**13 %**	**90 %**	**45 %**	**33 %**	**17 %**
	R	**13 34 %**	**13 45 %**	**15 36 %**	**15 39 %**	**17 44 %**	**15 48 %**	**23 64 %**	**10 50 %**	**19 61 %**	**21 87**	**3 10 %**	**21 55 %**	**27 67 %**	**25 83 %**
Acinetobacter (n=24)	**S**	**8 73 %**	**- 0.0 %**	**6 30 %**	**3 13 %**	**4 21 %**	**10 59 %**	**2 11 %**	**2 13 %**	**1 6 %**	**- 0.0 %**	**4 20 %**	**3 19 %**	**1 6 %**	**1 6%**
	R	3 27%	13 100 %	14 70 %	20 87 %	15 79%	7 41 %	17 89 %	13 87%	16 94 %	6 100 %	16 80 %	13 81 %	16 94 %	17 94 %
Enterobacter (n=20)	**S**	12 100 %	7 47 %	21 95 %	15 71 %	17 85%	16 84 %	9 47 %	1 7%	11 55 %	2 20 %	17 89 %	15 71 %	7 35 %	1 5%
	R	- 0.0 %	8 53 %	1 5%	6 29 %	3 15%	3 16 %	10 53 %	13 93%	9 45 %	8 80 %	2 11 %	6 29 %	13 65 %	18 95 %
Proteus (n=14)	**S**	1 14%	6 50 %	7 70 %	8 80 %	7 64%	1 10 %	2 20 %	2 50%	5 45 %	- 0.0 %	5 100 %	6 60 %	5 45 %	3 30 %
	R	6 86%	6 50 %	3 30 %	2 20 %	4 36%	9 90 %	8 80 %	2 50%	2 71 %	2 100 %	- 0.0 %	4 40 %	6 55 %	7 70 %

Table 4: Antibiotic sensitivity pattern of Gram-negative bacteria isolated from wound swab

CT: Colistin; CAZ:Ceftazidime; AK: Amikacin; CIP:Ciprofloxacin; LEV:Levofloxacin; DO:Doxycycline; SXT: Trimethoprim/Sulphamethoxazole; AZTH:Azithromycin; GN:Gentamycin TO:Tobramycin; IMP:Imipenem; AZT=Aztreonam; CEF : Ceftriaxone; AM+SUL:Ampicillin/sulbactam. Note: not all the antibiotics were tested for all 355 isolates.

Table 5: Antibiotic sensitivity pattern of Gram-positive bacteria

Bacterial isolates		**Ox**	CAZ	**CD**	AK	AZ	SXT	FA	AMX	**CRO**	**GN**	**DO**	**AM**	AM	IMP	AZTH	**E**	Lev	**VA**
S.aureus (n=73)	S	28 41 %	28 61 %	38 88 %	16 10 0 %	5 38 %	39 74 %	13 25 %	15 48 %	17 40 %	13 43 %	15 39 %	3 18 %	36 64 %	26 84 %	10 59 %	57 80 %	53 87 %	-
	R	40	18 39 %	5 12 %	-	8 62 %	14 26 %	39	16 52 %	26 60 %	17 57 %	23 61 %	14	20	5 16 %	7 41 %	14	8	-

		59%			0.0%			75%					82%	36%			20%	13%	
S.pyogen (n=5)	S	125%	-0.0%	1100%	5100%	120%	375%	120%	480%	240%	-0.0%	-	267%	480%	5100%	360%	240%	480%	5100%
	R	375%	-0.0%	-0.0%	-0.0%	480%	125%	480%	120%	360%	2100%	-	133%	120%	-0.0%	240%	360%	120%	-0.0%
CoNS (n=23)	S	211%	675%	1376%	17100%	457%	850%	18%	325%	417%	225%	550%	444%	1376%	15100%	646%	1447%	18778%	1890%
	R	1689%	225%	424%	-0.0%	342%	850%	1292%	975%	1983%	675%	550%	556%	424%	-0.0%	754%	1653%	522%	210%
Enterococcus spp. (n=15)	S	110%	-0.0%	225%	113%	-0.0%	18%	117%	250%	117%	225%	320%	125%	982%	5100%	267%	215%	1275%	-
	R	990%	8100%	675&	787%	4100%	1192%	583%	250%	583%	675%	1280%	375%	218%	-	133%	1185%	425%	-

OX: Oxacillin; CAZ: Ceftazidime; CD: Clindamycin; AK= Amikacin; AZ: Aztreonam; SXT: Trimethoprim/Sulphamethoxazole; FA: Fucidin; AMX: Amoxicillin, CRO: Ceftriaxone, GN: Gentamycin,DO: Doxycycline, AM: Ampicillin, IMP: Imipenem; AZTH: Azithromycin; AM/SUL: Ampicillin/sulbactam; E: Erythromycin; LEV: Levofloxacin; VA: Vancomycin,

The MDR rate seen in this study was extremely high in both Gram-negative and Gram-positive bacteria, the percentage of MDR in *Pseudomonas*, *Proteus* and *Acinetobacter*, *S. hemolyticus* were (100%), however the MDR rate for other bacteria ranged from (80% - 88.5%) such as *E. coli*, *Klebsiella*, and *Enterobacter* in Gram-negative bacteria and *Enterococcus* in Gram positive bacteria. Among coagulase-negative *Staphylococci*, the resistance rate was calculated as 87%, while *S. aureus* showed (62%) as shown in table5

Table: 5 Classes of antimicrobial resisted to No

Bacteria	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	Average (%)
Psedumonase	1	6	7	8	7	5	1	7	10	9	11	2	74(100%)
E. coli	2	2	10	5	11	6	6	1	2	1	-	-	46(88.5%)
Klebsiella	8	-	3	5	3	5	4	2	8	3	2	-	43(88%)
Acinetobacter	1	-	-	-	-		3	2	7	3	4	5	25(100%)
Enterobacter	2	2	3	2	-	2	4	1	-	-	-	-	16(80%)
Proteus	3	1	2	1	-	-	3	-	3	1	-	-	14(100%)
S. aureus	6	7	10	6	3	6	2	2	2	1	1	-	46(63%)
CoNS	1	4	-	3	6	1	2	2	1	-	-	-	20(87%)
Enterococcus	2	1	-	1	2	1	2	1	2	1	1	-	14(93%)
S. hemolyticus	1	-	-	1	1	1	-	-	1	-	-	-	5 (100%)

Discussion

Open wounds are susceptible to colonization by bacteria, leading to poly-microbial growth. The presence of bacterial communities, whether single or multiple, is determined by various factors such as wound condition, microbial load, previous treatment, skin moisture, and nutrient availability.(Ibrahim Shebl, 2019)(Kaur Gill and Sharma, 2019).

In the present study, we found that a single etiological agent was more prevalent (65%) than multi-etiological agents (35%). The results were consistent with previous studies by (Bessa *et al.*, 2015), (Kaur Gill and Sharma, 2019) which reported a similar predominance of single etiological agents over multi-etiological agents. However, these findings disagree with the study by Hassan in Egypt who reported a higher prevalence of single etiological agent isolates at 60% and 40% of mixed bacterial species (Hassan *et al.*, 2022).

Our study revealed that the rate of wound infection was more common in males (61%) than females (39%), this finding was consistent with a study in India by (Kaur Gill and Sharma, 2019). and disagreed with the findings of Ibrahim in Egypt and Mulu in Ethiopia who reported that the ratio of male to female was 1:2.1(Mulu *et al.*, 2012) (Ibrahim Shebl, 2019).

Antibiotic resistance (AR) has become a major concern in clinical settings globally, with its impact on rising healthcare costs, patient morbidity and mortality due to infectious diseases. This problem is particularly severe in developing countries where there is limited information available on the susceptibility patterns of bacterial isolates to antibiotics ((Hart and Kariuki, 1998). It is crucial to understand that the excessive and improper usage of antibiotics are leading causes of the increase in antibiotic resistance. This is mainly due to incorrect diagnoses and unwarranted prescriptions, which make up almost half of all antibiotic prescriptions for patients(Chamoun *et al.*, 2016) (Hart and Kariuki, 1998).

The study demonstrated that the predominant bacteria were *pseudomonas aeruginosa* and *S. aureus* (21%) of the total bacteria isolated, This finding was also reported in the studies(Bessa *et al.*, 2015) (Zaman *et al.*, 2017),(Lai *et al.*, 2017)(Pallavali *et al.*, 2017) and disagreement with the study done in korea (Kim *et al.*, 2021) Chronic wounds are typically accompanied by the presence of virulence factors produced by bacteria such as S. aureus and P. aeruginosa. These factors contribute to the continuation of the infection and delay the healing process. Among the virulence factors produced by S. aureus, coagulase, catalase, clumping factor A, and leucocidines are the most clinically relevant (22). Additionally, the production of

elastase by P. aeruginosa has been associated with its pathogenicity in the wound environment (Schmidtchen *et al*, 2003). Therefore, our findings corroborate the presence of the usual microorganisms that are frequently identified in infected wounds.

Besides, it was observed that *Acinetobacter* has the highest resistant microorganism in our study, it was fully resistant to Ceftazidime and Tobramycin (100%), Similarly, a study conducted by Dou in a different area in Libya showed that *Acinetobacter spp.* was the most resistant pathogen (Dau, Tloba and Daw, 2013). The increased rate of resistance of *Acinetobacter* and *P. aeruginosa* to Ceftriaxone in the present study contrasts with previous studies in Ethiopia (Mulu *et al.*, 2012), (Taiwo, Okesina and Onile, 2002). While, increased sensitivity of P. aeruginosa to Colistin in our study, were similar to (Srivastva *et al.*,2014), (De Francesco *et al.*, 2013)(Kirac, Keskin and Yarar, 2018)

Additionally, the majority of Gram-negative bacteria isolated were resistant to septrin, Tobramycin, Gentamycin, Doxycycline, Cefteroxone and Am/sulbactam, Similar results were also shown by other studies nationwide (Srivastva *et al.*, no date) (Kaur Gill and Sharma, 2019), (Duggal *et al.*, 2015) (Trojan, Razdan and Singh, 2016). this high drug resistance may be due to the lack of awareness and absence of effective application of the policy that regulates the use of antibiotics in a country like Libya.

However, moderate susceptibility of Gram-negative bacteria isolated in the present study to Imipenem 171 (68%), and its high activity against Gram-positive bacteria n= 56 (91%). therefore, these antibiotics, are still relevant in the treatment of wound infection in this study area. This finding was compatible in some degree with the study in Ukrainian in the fact that carbapenems still the last choice in the treatment of multi drug resistant bacteria (KON, 2015).MDR strains in our study showed resistance to most current antibiotics, and widely distributed in our hospital. The overall MDR rate in *Pseudomonas aeruginosa* was very high (100%), and it agrees with the study reported by Mulu that demonstrated *Pseudomonas aeruginosa* was (100%) resistant to the majority of antibiotic used (Mulu *et al.*, 2012).

Moreover, the high percentage of *S. aureus* (21%) isolated in the current study is most likely associated with the skin and nasal endogenous flora of the patients as it was explained by (Anguzu and Olila, 2007). According to the results of antimicrobial susceptibility tests carried out on CoNS isolates, 87% of them were found to be resistant to three or more antimicrobial agents, making them multidrug-resistant. This finding is in line with another study conducted by Maleki et al. in which the majority of CoNS isolates were also found to be resistant to multiple antibiotics. (Maleki et al. 2019) (Godebo, Kibru and Tassew, 2013) and (Mulu *et al.*, 2012).

Conclusion:

As antimicrobial resistance is a growing global problem. However, the increased rate of MDR seen in the present study was considered as alarming because only a few treatment options remain for wound infections

Most of bacterial isolates reveal widespread resistance against different antimicrobial classes, treatment of wound infections has to be made based on the culture and susceptibility results. Nevertheless, in emergency cases, ampicillin, penicillin, ampicillin- sulbactam, oxacillin, Azithromycin, doxycycline and Ceftriaxone are not good choices to treat wound infections.

Besides, imipenem still effective treatment for most of bacterial isolated with the exception (Acinetobacter and pseudomonas showed high resistance to it)

Acknowledgments: -
Many thanks to laboratory staff of Microbiology department at AL-Jala hospital for their helpful assistance

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