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Prevalence of multi-drug resistant bacteria in intensive care units at Tripoli University Hospital, Tripoli, Libya

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ABSTRACT

Among hospitalized patients worldwide, infections caused by multidrug-resistant (MDR) bacteria are a major cause of morbidity and mortality. This study aimed to isolate MDR bacteria from five intensive care units (ICUs) at Tripoli University Hospital (TUH). A prospective cross-sectional study was conducted over a seven-month period (September 2022 to March 2023) across five ICUs at TUH. A total of 197 swabs were collected from Patients', healthcare workers' and ICUs equipment. Samples collected from patients were nasal swabs, oral cavity swabs, hand swabs, sputum specimens, skin swabs, umbilical venous catheter swabs, and around cannula. Swabs collected from health care workers were nasal swabs, whereas ICUs equipment's samples were from endotracheal tubes, oxygen masks, and neonatal incubators. Identification and antimicrobial susceptibility test was confirmed by using MicroScan auto SCAN 4 (Beckman Coulter). The most frequent strains were Gram negative bacilli 113 (57.4%) with the predominance of *Acinetobacter baumannii* 50/113 (44%) followed by *Klebsiella pneumoniae* 44/113 (40%) and *Pseudomonas aeruginosa* 6/113 (5.3%). The total Gram positive bacterial strains isolated were 84 (42.6%), coagulase negative *Staphylococci* 55 (66%) with MDRs (89%) were the most common isolates followed by *Staphylococcus aureus* 15 (17.8%). Different antibiotics were used against these isolates; Gram- negative isolates showed high resistance rates to ceftazidime, gentamicin, amikacin and ertapenem. *A. baumannii* were the most frequent MDROs (94%), and the highest resistance rates in Gram-positive strains were observed toward ampicillin, oxacillin, ampicillin/sulbactam and Cefoxitin, representing 90% of total MDR Gram-positive isolates. ESBL and MRS were identified in most of strains. The prevalence of antibiotic resistance was high for both Gram negative and Gram positive isolates. This prevalence requires strict infection prevention and control intervention, continuous monitoring, implementation of effective antibiotic stewardship, immediate, concerted and collaborative action to monitor its prevalence and spread in the hospital.

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Multi-drug resistance; ICU; antimicrobial susceptibility; Tripoli University Hospital; Libya

1. Introduction

Antimicrobial resistance (AMR) is a global health challenge facing health care professionals worldwide, it is responsible for morbidity and mortality and burdens healthcare systems [1], Health care associated Infections are the leading cause of surgical failure, transplant rejection, with antibiotic-resistant organisms responsible for increased morbidity and mortality [1]. Although life-threatening bacterial infections have been treated with antibiotics for decades and greatly improved the well-being and survival of human beings, bacteria have taken a step forward

by resisting antibiotics through various mechanisms [2,3]. Furthermore, report on antimicrobial resistance threats released by center for disease prevention and control (CDC) in 2019 shows that annually; 2.8 million cases of infections caused by antibiotic-resistant bacteria is recorded with 35.000 deaths in the US [4]. The rising phenomenon of antibiotic resistance is a direct result of irrational antibiotic use in humans, animals, and agriculture. This is particularly evident in developing countries, where antibiotics are easily and un-regulatory accessible. The resistance is acquired either naturally or acquired by transfer of resistance genes.

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The continual use of different drugs has exerted selection pressure on bacteria, transforming them into MDR microorganisms [5]. The explosion of MDR bacteria has far-reaching consequences for both clinical outcomes and economic burdens. Nevertheless, the thoughtless use of antibiotics during the COVID-19 pandemics further hastened bacterial resistance, leading to a dramatic increase in morbidity and mortality worldwide [6].

World Health Organization (WHO) published its list of antibiotic-resistant 'priority pathogens' that pose the greatest threat to human health [7]. The list is divided into three categories according to the urgency of needs for new antibiotics: critical, high and medium priorities. The most critical group includes *Acinetobacter*, *Pseudomonas* and *Enterobacterales*, the former include carbapenems resistant and Extended Spectrum Beta-lactamase (ESBL) producing *Enterobacteriaceae*. These microorganisms cause serious and often fatal infections particularly in healthcare settings. The high and medium group contain other Gram positive and negative bacteria such as vancomycin-resistant *Enterococcus faecium*, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA) and *Neisseria gonorrhoeae*, cephalosporins-resistant *Enterobacterales* [7]. MRSA and vancomycin-resistant *Enterococci* (VRE) are the most common MDR Gram-positive bacteria in hospitals. MRSA infections appear to be declining, and therapeutic options are available for both MRSA and VRE infections. On the other hand, MDR Gram-negative bacteria, including *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter*, continue to pose a significant threat [8].

By identifying antibiotic-resistant bacteria colonizing hospitalized patients and healthcare workers, or contaminating equipment, we can implement appropriate contact precautions to prevent the spread of these infections during patient hospitalization. Healthcare facilities are a breeding ground for antibiotic-resistant bacteria, which can easily transfer from the environment or healthcare personnel to patients [9]. Identifying these bacteria and their transmission routes (direct contact with colonized/infected patients or via healthcare worker hands) is crucial. This knowledge allows us to implement appropriate contact precautions and prevent infections during patient hospitalization.

In addition, early identification of antibiotic-resistant pathogens and appropriate targeted antibiotic therapy are the most important factors in reducing mortality from antibiotic resistant pathogens [4]. Growing and widespread emergence of antibiotic resistant bacteria has forced researchers to find alternative treatments for antibiotic resistant bacteria. However, despite global efforts, there are significant gaps in epidemiological data, particularly in low- and middle-income countries (LMICs) including Libya.

There is a paucity data on antibiotic resistant bacteria and antibiotic use in Libya due to the lack of surveillance system for antimicrobial resistance by the Ministry of Health and National Centre for Disease Prevention and Control in Libya along with the low number of studies compared to other countries.

Studies in Libya found carbapenem-resistant bacteria in hospitals. Tripoli metallo- β -lactamase (TMB-1) was detected in *Achromobacter xylosoxidans* isolated from the hospital environment of Tripoli Central Hospital and was found in a class 1 integron of 3kb [10]. This included *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which carried genes like VIM-2, OXA-23, and NDM-1 [11,12]. These bacteria were present in various hospital areas, including intensive care and burn units. Additionally, carbapenem-resistant *Klebsiella pneumoniae* carrying OXA-48 was detected [13]. Worryingly, carbapenem and colistin-resistant bacteria were also isolated from COVID-19 patients in Libya [14]. ESBL and MBL producing Gram-negative bacteria were isolated from operating theaters and ICU of Sabha Medical Center, Sabha city, Libya [15,16].

The current study was conducted prospectively focusing on the prevalence of MDR bacteria among hospitalized patients, healthcare workers and equipment used on patients. The main goal of our study was to determine the most prevalent MDR bacteria and their antibiotic susceptibility pattern trying to fill the gap of knowledge regarding the most frequently encountered MDR microorganisms in health care settings. Our study will highlight the serious concern of antibiotic resistant bacteria and the extent of the problem within the ICUs of the studied health care facility, and will provide useful information on the susceptibility patterns and may guide clinicians in choosing appropriate treatment through constructing antibiogram for resistant bacteria depending on the result of antibiotic susceptibility. The results of the study will be used to inform the Libyan health care system regarding the extent of AMR in a Libyan health care setting.

2. Materials and methods

2.1. Study design and patients

This study was conducted in the microbiology department at the Libyan Biotechnology Research Center in Tripoli, Libya from September 2022 to March 2023. Clinical samples were collected from medical staff, equipment, and patients hospitalized in Tripoli University Hospital (TUH) 's five intensive care units (ICUs). This hospital is the largest hospital in Libya with 1200 beds. The ICUs were; medical ICU (MICU) with 15-bed capacity, neonatal ICU (NICU) with 16-bed capacity, cardiology pediatric care unit (CPCU) with 12-bed capacity, surgical ICU (SICU) with 8-bed

capacity, and pediatric ICU (PICU) with 9-bed capacity. Patient's samples were taken after their informed oral consent.

2.2. Samples collection

This is a cross-sectional study. A total of 197 swabs were collected once from patients, medical staff and ICUs equipment's using moistened (0.9% w/v physiological saline) sterile cotton swabs. Multiple swabs from different sites were sometimes taken from the same patient according to their clinical presentation. Samples collected from patients were; nasal swabs, oral cavity swabs, hand swabs, sputum specimens, swabs from ulcer, surgical wounds, umbilical venous catheter swabs, and around cannula. Swabs collected from health care workers were nasal swabs whereas ICUs disposables and equipment's samples were from; endotracheal tubes, oxygen masks, and neonatal incubators.

2.3. Isolation of bacterial strains

Bacterial swabs were inoculated in Brain Heart Infusion (BHI) and incubated at 37°C for 18–24 h. The samples were then inoculated on MacConkey and MSA agar (Oxoid, UK) and incubated at 37°C for 18–24 h. The initial identification of the bacterial isolates was performed using morphological analysis, Gram staining, and microscopy in accordance with microbiological standards. Each isolate was stored in Trypticase soy broth with 20% glycerol at a temperature of –70°C.

2.4. Identification and antimicrobial susceptibility testing

The identification and antimicrobial susceptibility of the bacterial isolates were determined using a micro scan auto-SCAN 4 panel (Beckman Coulter). The tested antibiotics for Gram-negative bacteria were; amikacin, ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam,

piperacillin/tazobactam, ceftazidime, ceftazidime/clavulanate, cefuroxime, cefepime, aztreonam, imipenem, meropenem, ertapenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, nitrofurantoin, colistin, tigecycline, and trimethoprim/sulphamethoxazole. Extended spectrum beta lactamase (ESBL) production was determined as well as using a Microscan auto-SCAN 4 panel (Beckman Coulter).

The tested antibiotics panel for Gram-positive bacteria were; penicillin, ampicillin, ampicillin/sulbactam, amoxicillin/clavulanate, oxacillin, ceftazidime screen, ceftriaxone, ciprofloxacin, levofloxacin, gentamicin, clindamycin, erythromycin, tetracycline, vancomycin, daptomycin, linezolid, nitrofurantoin, rifampin, quinupristin/dalfopristin teicoplanin, and trimethoprim/sulfamethoxazole.

3. Results

The results showed that the 197 swabs yielded 197 isolates, they were from patients' (131), healthcare workers' (44) and medical equipment's (22), (Table 1, Figure 1). The distribution of bacterial isolates among the five ICUs were as follows; (NICU = 35), (MICU = 66), (PICU = 17), (SICU = 40), and (CPCU = 39) as shown in Table 1.

The prevalence of Gram-negative and Gram-positive isolates among the ICUs is illustrated in Table 2. The results showed that Gram-negative isolates were the most frequent strains (113; 57.4%). The predominant strains were *A. baumannii* 50/113 (44%),

Table 1. Distribution of bacterial isolates among Tripoli University Hospital ICUs.

ICUs	Patients	Healthcare workers	Medical equipment's	Total
NICU	30	3	2	35
MICU	40	17	9	66
PICU	13	2	2	17
SICU	22	14	4	39
CPCU	26	8	5	39
Total	131	44	22	197

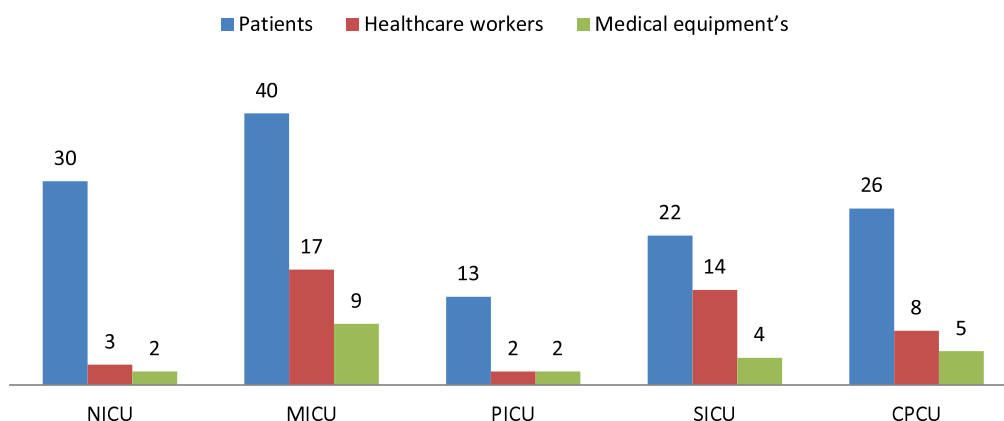


Figure 1. Prevalence of pathogenic bacteria isolated from patients, healthcare workers and ICUs medical equipment's in TUH ICUs. NICU (neonatal ICU), MICU (medical ICU), PICU (pediatric ICU), SICU (surgical ICU) and CPCU (cardiology pediatric care unit).

followed by *K. pneumoniae* 46/113 (40%), *P. aeruginosa* 6/113 (5.3%), *Chryseobacterium indologenes* 3/113 (2.6%), *Serratia fonticola* 3/113 (2.6%), *Myroides* species 2/113 (1.8%), and one strain of *Enterobacter aerogenes*, *Pantoea agglomerans*, and *Pasteurella aerogenes*.

The prevalence of Gram-positive isolates among the ICUs was 84/197 (42.6%), with the commonest strains being *S. epidermidis* 42/84 (50%), followed by *S. aureus* 15/84 (17.8%), *S. haemolyticus* 9/84 (10.7%), *S. sciuri* 4/84 (4.8%), *S. hominis* subsp. *hominis* 2/84 (2.3%) and one strain of *S. xylosus*, *S. auricularis* and *S. simulans*. In general, Coagulase Negative *Staphylococci* (CONS) strains were the most common

isolates, 69/84 (82.2%), followed by Coagulase Positive *Staphylococci* strains 15/84 (17.8%).

Antimicrobial sensitivity was done using a Micro Scan auto SCAN 4 (Beckman Coulter). As shown in Table 3, most Gram negative isolates showed the highest resistance to ceftazidime (96.5%), gentamicin (95%), amikacin (92%), ertapenem (91.2%), ampicillin/sulbactam (90%), imipenem (89.5%) and Piperacillin/Tazobactam (89%). In contrast, the least resistance rate was documented for cefazolin (23%), ceftazidime (26.5%) and moxifloxacin (32%). Extended spectrum β -lactamase (ESBL) was detected by micro scan auto-SCAN 4 panel (Beckman Coulter) using aztreonam, cefotaxime and ceftazidime. However, *A. baumannii* were the most frequent MDROs (94%), followed by

Table 2. Prevalence of Gram-negative and Gram-positive isolates in Tripoli University Hospital ICUs.

Isolate	NICU	MICU	PICU	SICU	CPCU	N0: isolates	%
Gram negative isolates	32	39	15	15	12	113	57.4
<i>K. pneumoniae</i>	13	15	5	8	5	46	40
<i>A.baumannii</i> complex/ <i>haemolyticus</i>	14	13	10	6	7	50	44
<i>P. aeruginosa</i>	0	6	0	0	0	6	5.30
<i>C. indologenes</i>	3	0	0	0	0	3	2.6
<i>S fonticola</i>	1	2	0	0	0	3	2.6
<i>Myroides species</i>	1	0	0	1	0	2	1.8
Others	0	3	0	0	0	3	2.6
Gram positive isolates	3	28	2	24	27	84	42.6
<i>S. aureus</i>	0	2	0	5	8	15	17.8
<i>S. epidermidis</i>	3	17	2	8	12	42	50
<i>S. haemolyticus</i>	0	0	0	4	5	9	10.7
<i>S. sciuri</i>	0	0	0	2	2	4	4.8
<i>S. hominis</i> subsp. <i>hominis</i>	0	1	0	1	0	2	2.3
<i>S. xylosus</i>	0	1	0	0	0	1	1.2
<i>S.auricularis</i>	0	1	0	0	0	1	1.2
<i>S.simulans</i>	0	0	0	1	0	1	1.2
Others	0	6	0	4	0	9	10.7
Total						197	

Table 3. Antibiotic resistance pattern of Gram-negative strains in TUH ICUs.

Antibiotics	NICU n= 32 (%)	MICU n=39(%)	PICU n=15(%)	SICU n=12 (%)	CPICU n=15(%)	Total n=113 (%)
Amikacin	27 (84.4)	35 (89.7)	15 (100)	12 (100)	15 (100)	104 (92.0)
Amoxicillin/clavulanate	14 (43.7)	19 (48.7)	5 (33.3)	5 (41.7)	8 (53.3)	51 (45.1)
Ampicillin/sulbactam	27 (84.4)	33 (84.6)	15 (100)	12 (100)	15 (100)	102(90.3)
Ampicillin	14 (43.75)	20 (51.3)	5 (33.3)	5 (41.7)	8 (53.3)	52 (46.0)
Aztreonam	30 (93.75)	26 (66.7)	15 (100)	12 (100)	15 (100)	98 (86.7)
Cefazolin	19 (59.4)	3 (7.7)	5 (33.3)	0 (0.0)	0 (0.0)	26 (23.0)
Cefepime	29 (90.6)	34 (87.2)	15 (100)	12 (100)	15 (100)	95 (84.1)
Cefotaxime	27 (84.4)	31 (79.5)	5 (33.3)	5 (41.7)	8 (53.3)	76 (67.3)
Cefotaxime/clavulanate	1 (3.12)	5 (12.8)	-	-	-	-
Cefoxitin	3 (9.4)	19 (48.7)	0 (0.0)	0 (0.0)	8 (53.3)	30 (26.5)
Ceftazidime	30 (93.75)	37 (94.9)	15 (100)	12 (100)	15 (100)	109 (96.5)
Ceftazidime/clavulanate	-	3 (7.7)	-	-	-	-
Cefuroxime	13 (40.6)	19 (48.7)	5 (33.3)	5 (41.7)	15 (100)	57 (50.4)
Ciprofloxacin	26 (81.25)	33 (84.6)	10 (66.6)	7 (58.3)	15 (100)	91 (80.5)
Colistin	1 (3.125)	3 (7.7)	-	-	-	-
Ertapenem	30 (93.75)	31 (79.5)	15 (100)	12 (100)	15 (100)	103 (91.2)
Gentamicin	29 (90.6)	37 (94.9)	15 (100)	12 (100)	15 (100)	108 (95.6)
Imipenem	27 (84.4)	33 (84.6)	14 (93.5)	12 (100)	15 (100)	101 (89.4)
Levofloxacin	26 (81.25)	33 (84.6)	10 (66.6)	12 (100)	15 (100)	96 (85.0)
Meropenem	12 (37.50)	22 (56.4)	5 (33.3)	5 (41.7)	8 (53.3)	52 (46.0)
Moxifloxacin	10 (31.25)	18 (46.2)	0 (0.0)	0 (0.0)	8 (53.3)	36 (31.9)
Nitrofurantoin	-	-	-	-	-	-
Norfloxacin	19 (59.4)	23 (59.0)	10 (66.6)	7 (58.3)	7 (46.7)	66 (58.4)
Piperacillin/Tazobactam	31 (96.9)	28 (71.8)	15 (100)	12 (100)	15 (100)	101 (89.4)
Tigecycline	4 (12.5)	0 (0.0)	10 (66.6)	7 (58.3)	7 (46.7)	28 (24.8)
Tobramycin	12 (37.5)	20 (51.3)	5 (33.3)	5 (41.7)	8 (53.3)	50 (44.3)
Trimethoprim/ sulfamethoxazole	13 (40.6)	18 (46.2)	5 (33.3)	5 (41.7)	8 (53.3)	49 (43.4)
ESBL	27 (84.4)	34 (87.2)	15 (100)	12 (100)	14 (93.3)	97 (85.8)

n= number of resistance strains

K. pneumoniae (90%) and *P. aeruginosa* (60%) of the whole MDR Gram-negative isolates in the study. Moreover, ESBL was identified in most of Gram-negative isolates 97/113 (85.8%) (Table 3).

Among Gram-positive strains, the highest resistance rates were toward ampicillin (100%), oxacillin (95%), ampicillin/sulbactam (94%), amoxicillin/clavulanate (93%) and ceftriaxone (93%), while the least resistance rate was toward vancomycin (69%), quinupristin/dalfopristin (69%) and rifampin (75%). Most of Gram-positive strains were MDR (84%) and MRS 74/84 (88%). However, *S. epidermidis* were the most frequent MDROs (96%) followed by *S. aureus* (92%). Additionally, MRSA and MRSE were observed in most strains 18% and 50%, respectively. Notably, resistance to colistin was observed in *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*. In general, Gram-negative and Gram-positive strains that were isolated from PICU, CPICU and SICU were more resistant to majority of antibiotics compared to other ICUs (Tables 3, 4).

4. Discussion

In intensive care units, hospitalized patients are more susceptible to nosocomial pathogens, in particular multidrug-resistant bacteria. These bacteria can spread from patients' health care workers and ICU medical equipment and vice versa, leading to hospital outbreaks by cross-infection and/or cross-contamination [17]. Therefore, the main objective of this study was to determine the MDR profile of pathogenic bacteria isolated from specimens collected and their antibiotic susceptibility patterns in various intensive care units at the TUH.

The incidence of infections caused by multi-drug-resistant pathogens is rising around the world. Several

studies presented different frequencies of MDR among microorganisms in many healthcare settings. In the present study, *A. baumannii* strains were the most frequent MDROs representing (94%) followed by *K. pneumoniae* strains (90%) of the whole MDR gram-negative patient [18] isolates. Our clinical samples results are consistent with several studies. For instance, in Egypt a rate of 57% MDR bacteria were reported in critically ill elderly patients, with Gram-negative bacteria comprised the majority, representing 72% of total MDR isolates [19]. In another study, the dominance of MDR Gram-negative over Gram-positive bacteria has been shown from infected wounds [20]. Furthermore, in a study by Abdelkader MM et. al. on cerebrospinal fluid; 36.6% of the isolates were MDR of which 69.2% were Gram-negatives and 30.8% were Gram-positives [20]. Additionally, in Sudan, 80% of *K. pneumoniae* were reported as MDR [21], and one more study found that there was a high prevalence of MDR *Enterobacteriaceae*; mainly ESBL-positive *E. coli* and *K. pneumoniae* [22], Whereas 18% of *P. aeruginosa* isolates were MDR in a recent work published in 2023 [23], and 89.2% of *S. aureus* were MDR, of them 67.6% were methicillin resistant [24,25].

In another study in Tunisia, a MDR rate of 44% was detected represented mainly by *A. baumannii*, ESBL-producing *Enterobacteriaceae* and *P. aeruginosa* [26]. An Investigation of the carriage of MDR bacteria among hospitalised paediatric patients found that, there was a 7.84% acquisition rate of MDR bacteria on discharge [27]. In Libya, due to lack of national surveillance system and few published studies, little data are available on antimicrobial resistance. Seventy-eight percent (78%) of the GNB exhibit various MDR phenotypes, of these, *K. pneumoniae* showed the highest rates of MDRs (91%) in a study published in 2017

Table 4. Antibiotic resistance pattern of Gram-positive strains in TUH ICUs.

Antibiotics	NICU n=3 (%)	MICU n=27 (%)	PICU n=3 (%)	PCICU n=27 (%)	SICU n=24 (%)	Total n=84 (%)
Amoxicillin/ clavulanate	3 (100)	21 (77.8)	3 (100)	27 (100)	24 (100)	78 (93.0)
Ampicillin/sulbactam	3 (100)	22 (81.5)	3 (100)	27 (100)	24 (100)	79 (94.1)
Ampicillin	3 (100)	27 (100)	3 (100)	27 (100)	24 (100)	84 (100)
Cefoxitin	3 (100)	20 (74.1)	3 (100)	27 (100)	24 (100)	77 (91.7)
Ceftriaxone	3 (100)	21 (77.8)	3 (100)	27 (100)	24 (100)	78 (93.0)
Ciprofloxacin	3 (100)	20 (74.1)	3 (100)	21 (77.8)	24 (100)	71 (84.5)
Clindamycin	3 (100)	22 (81.5)	2 (66.7)	22 (81.5)	24 (100)	73 (86.9)
Daptomycin	-	-	-	-	-	-
Erythromycin	3 (100)	24 (79.5)	3 (100)	23 (82.2)	24 (100)	77 (91.7)
Gentamicin	3 (100)	15 (55.6)	3 (100)	22 (81.5)	24 (100)	67 (79.8)
Levofloxacin	2 (66.7)	18 (66.7)	3 (100)	23 (85.2)	24 (100)	70 (83.3)
Linezolid	3 (100)	16 (59.3)	2 (66.7)	22 (81.5)	24 (100)	67 (79.8)
Nitrofurantoin	-	-	-	-	-	-
Oxacillin	3 (100)	23 (46.2)	3 (100)	27 (100)	24 (100)	80 (95.2)
Penicillin	3 (100)	27 (100)	3 (100)	27 (100)	24 (100)	84 (100)
Rifampin	1 (33.3)	15 (55.6)	2 (66.7)	21 (77.8)	24 (100)	63 (75.0)
Quinupristin/Dalfopristin	3 (100)	15 (55.6)	3 (100)	22 (81.5)	24 (100)	67 (69.8)
Teicoplanin	3 (100)	18 (66.7)	2 (66.7)	22 (81.5)	24 (100)	69 (82.1)
Tetracycline	1 (33.3)	16 (59.3)	3 (100)	22 (81.5)	24 (100)	66 (78.6)
Timethoprim/sulfamethoxazole	2 (66.6)	20 (74.1)	3 (100)	25 (92.6)	24 (100)	74 (88.1)
Vancomycin	3 (100)	17 (63.0)	3 (100)	20 (74.1)	24 (100)	67 (69.8)
MRS	2 (66.7)	21 (77.8)	2 (66.7)	25 (92.6)	24 (100)	74 (88.1)

n= number of resistance strains

[28]. Rate of ESBLs among *E. coli* ranged from 5%-20% and from 11%-18% for *K. pneumoniae* in some studies from Libya [29,30]. These results are nearly in agreement with the our results in which the most common isolates were Gram-negative strains 113 (57.4%) with the predominance of *A. baumannii* 50/113 (44%) followed by *K. pneumoniae* 44/113 (40%), and *P. aeruginosa* 6/113 (5.3%). Gram positive strains were 84 (42.6%) isolates, CoNS strains represented 82.2% as the most commonly isolated strains followed by *S. aureus* (17.8%).

The current findings are also consistent with the recent study that demonstrated the emergence of MDR-gram-negative bacteria in Tripoli and Benghazi hospitals from clinical and non-clinical specimens. *K. pneumoniae* was found to be the most frequently isolated strain being disseminated in hospitals, followed by *E. coli* and *A. baumannii* [31]. Moreover, methicillin resistance (MR) was detected in 35.3% of *S. aureus* and in 75% of coagulase-negative Staphylococci (CNS). However, in our study, coagulase-negative *Staphylococci* isolates (82.2%) were the most common strains, followed by *S. aureus* (17.8%), as shown in Table 2 and Figure 1. In general, *Acinetobacter* strains were the most common pathogens isolates in this investigation, which was consistent with a previous study [32,33]. Conversely, it has been demonstrated in recent study [34,35] that *Klebsiella* spp. are important causative agent of nosocomial infections in intensive care units. This discrepancy could be explained by variations in the patient's immune system, immunological status, nutritional state, and geographical location. In a worldwide research of the incidence of infections in intensive care units, 'Infections in Intensive Care Study,' *Acinetobacter* was similarly found to be the most common pathogen recovered from patients [32,36].

Some Gram-negative species are naturally resistant to colistin. Examples include *Neisseria meningitidis*, Burkholderia species, and *Proteus mirabilis* [37,38]. In this study, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* have acquired resistance to this antibiotic. Colistin resistance is a significant concern because colistin-resistant bacteria emerged due to uncontrolled use of this last line antibiotic for multidrug-resistant Gram-negative bacteria [39,40]. The current findings found that the prevalence of antibiotic resistance in five ICUs at TUH was high for both Gram negative and Gram positive isolates. However, using a program consisting of persuasive stewardship measures, could be useful to control drug-resistant bacteria in a hospital setting [41]. Furthermore, the rises registered recommend that efforts to control the spread of pathogenic and antibiotic-resistant bacteria could require to be strengthened, such as through improved infection control practices and proper use of antibiotics. Moreover, it's essential to continue monitoring these trends to

monitor the success of therapies and spot new trends in resistance [42]. Therefore, these persuasive stewardship measures can be used to reduce MDR and nosocomial infections within our hospital setting.

This study has several limitations. All analyzed strains are not necessarily associated to a clinical relevant infection. However, it may reflect a clinical relevant problem of antimicrobial resistance in the 5 ICUs of Tripoli University Hospital. Also, analyzing bacteria from a single sampling point limits understanding of how these populations change over time. Additionally, not differentiating between staff and patient sources limits identification of transmission routes. Additionally, the study did not analyze particular resistance genes. Despite these limitations, our findings highlight the potential risk posed by the presence of these bacteria and emphasize the importance of ongoing surveillance and infection control measures within the ICUs of Tripoli University Hospital.

5. Conclusions

This study demonstrated the alarming occurrence of multi-drug-resistant bacteria in various intensive care units at TUH, which is considered the largest hospital in Libya with 1200 beds. In this study, Gram-negative isolates were the most frequent strains; *A. baumannii* complex/*haemolyticus* followed by *K. pneumoniae*; and Coagulase Negative *Staphylococci* (CONS) were the major pathogens prevalent strains in the five ICUs. This prevalence and high resistance rate requires immediate, concerted and collaborative action to monitor its prevalence and spread throughout the Libyan hospitals.

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