Bacterial Isolates and their Antibiotic Susceptibility Patterns among Patients Admitted to Zliten Teaching General Hospital Libya

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العزلات البكتيرية وأنماط حساسيتها للمضادات الحيوية بين المرضى النزلاء بمستشفى زليتن العام التعليمي. زليتن، ليبيا

الملخص:

الغرض من الدراسة: لاتزال مشكلة العدوى بالبكتيريا المقاومة للعديد من المضادات الحيوية مشكلة حقيقية في العديد من الدول النامية والمرتبطة بالاستخدام السيئ للمضادات الحيوية في العلاج. تهدف هذه الدراسة إلى مراجعة ونشر نتائج دراسة سابقة أجريت لعزل وتعريف البكتيريا المسببة للعدوى واختبار مدى مقاومتها للمضادات الحيوية للمرضى بمستشفى زليتن التعليمي.

الطريقة: أجريت هذه الدراسة على المرضى المتواجدين بمستشفى زليتن التعليمي، تم أخذ عينات من المرضى بالمستشفى وتم عزل وتعريف البكتريا واختبار مدى حساسية العزلات البكتيرية لمجموعة من المضادات الحيوية باستخدام جهاز فينوكس (The BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks, MD, USA). تم جمع البيانات وتحليلها باستخدام برنامج SPSS version 20 software . وتحديد ما إذا كانت هذه النتائج معنوية عند مستوى الثقة 5% (P- value ≤ 0.05).

النتائج: شملت الدراسة 443 عينة سريرية، 205 (46.3%) كانت موجبة (أظهرت نمواً بكتيرياً). كانت نسبة البكتيريا السالبة لصبغة جرام (74.6%) أعلى من نسبة البكتيريا الموجبة لصبغة جرام (25.4%). من بين الأنواع السالبة لصبغة جرام المعزولة كانت بكتيريا *إشريشياكولاي* (*Escherichia coli*) وأنواع *كليبسيلا* (*Klebsiella*spp.) هي الأكثر عزلاً، حيث عزلت بنسبة (47.4) و(7.4%) على التوالي. في حين أن بكتيريا *ستافيلوكوسأوريوس* (*Staphylococcus aureus*) (12.6%) هي الأكثر شيوعا من البكتيريا الموجبة لصبغة جرام. بشكل عام (67.6 %) من البكتيريا المعزولة كانت مقاومة للعديد من المضادات الحيوية. بكتيريا *سيدوموناس أيروجينوزا* (*Pseudomonas aeruginosa*) وبكتيريا *ستافيلوكوكوس هيمولتكوس* (*Staphylococcus haemolyticus*) كانت نسبة المقاومة لأكثر من مضادين حيويين MDR فيها 100%.

الكلمات المفتاحية: *مقاومة المضادات الحيوية، مستشفى زليتن التعليمي، عينات سريرية، عزلات بكتيرية*

Abstract

Purpose: Infections with multi-drug resistant (MDR) bacteria are serious problem to many devolving countries associated with the widespread use of antibiotics. This study was aimed to review of results of previous study that carried out to determine the bacterial isolates and their antibiotic profiles from patients admitted to Zliten Teaching General Hospital, Libya.

Methods: This study was conducted at Zliten teaching hospital. Various clinical specimens were sampled from patients, and using standard microbiological techniques we examined different specimens collected from 443 patients during October 2012 to December 2012. The BD Phoenix Automated Microbiology System were used to identify and determine the susceptibility of bacterial isolates against antimicrobial agents. Socio-demographic and laboratory results were collected and analyzed using SPSS version 20 software. P- value ≤ 0.05 was considered statically significant.

Results: A total of 443 study subjects were included in the study, 205 (46.3%) were culture-positive for bacterial pathogens. One hundred fifty three (74.6%) were gram negative bacteria and 52 (25.4%) were gram positive bacteria. *Escherichia coli* 50 (47.4%) and of *Klebsiella* spp. 37 (7.4%) were the predominant Gram- negative isolates, while *Staphylococcus aureus* 22 (12.6%) and *S. haemolyticus* 13 (10.4%) were from Gram-positive bacteria. Overall, (67.6%) of the isolates were found to be MDR. The rate of MDR was 100% for *Pseudomonas aeruginosa*and *Staphylococcus haemolyticus.*

Keywords: *bacterial isolates, antibiogram, clinical specimen, Zliten, Libya*

Introduction:

Nosocomial infections represent a major health problem all over the world and associated with high morbidity and mortality worldwide. Various reviews have estimated that nosocomial infections account for approximately 10 to 15% and more than 40% of hospitalized patients in developed and developing countries, respectively (Ali *et al* 2014).

It was estimated that a wide spectrum of organisms has been directly associated with nosocomial infections. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Salmonella* spp.are the commonly isolated organisms from infections in the clinical and community settings and the most common pathogens responsible for nosocomial infections. They are also the current most serious antibiotic-resistant bacterial pathogens (Mancuso *et al* 2021).

Resistance to antimicrobial agents is a major health issue that affects the whole world, in practical low come countries. Infections with multi-drug resistant (MDR) bacteria associated with overuse and misuse of antibiotics have been reported from several developing countries .In Libya, misuse of antimicrobial agents by the public is widespread, and antimicrobials can be purchased from pharmacies without prescription (Ghenghesh *et al.,* 2013). However, the most common MDR nosocomial pathogens have been reported were methicillin-resistant *Staphylococcus aureus* (MRSA) and drug-resistant gram negative bacteria. So, the Hospitals provide a suitable environment to MDR bacteria, making options of the treatment very expensive and limited (Yang, *et al* 2021). Furthermore, A few documented data on the bacterial isolates and antibiotic resistance profiles in Zliten Teaching General Hospital were published. Therefore, the present study aimed to review of results of previous study that was conducted to isolate the pathogenic bacteria from patients admitted to Zliten Teaching General Hospital, and evaluate the important causative agents of bacterial infections and their antibiotic susceptibility profiles which may be imperative as it guides to a successful selection of an effective treatment plan.

Methods:

Collection of samples: This study was done at Zliten teaching hospital. Samples were collected from patients hospitalized in different hospital wards. samples was collected from the most likely site of infection: blood, urine, cerebrospinal fluid (CSF), respiratory tract secretions, wound

secretions,or other clinically relevant sites.To determine the most important contributing factors to antibiotic resistance, demographic data was recorded for patients with isolates found to be resistant.

Laboratory methods: Using standard microbiological techniques we examined different specimens collected from 443 patients during October 2012 to December 2012 (Miller, 1985, Jorgensen, 2009). The BD Phoenix Automated Microbiology System (PAMS), MSBD Biosciences, Sparks Md, USA) was used to identify of the isolated bacteria and determine their susceptibility to a variety of antimicrobial agents according to the manufacturer's instructions. combination panels for identification (ID) and antimicrobial susceptibility testing (AST) of bacteria were used (Hussain, 2001).

Analysis: The Socio-demographic and laboratory results were collected and analyzed using SPSS version 20 software. P -values ≤ 0.05 were considered as statisticallysignificant.

Results

Socio-Demographic Characteristics

A total of 443 patient specimens were included and processed. Among the total samples, 263 (59.4%) were females and 180 (40.6%)were males with the age range of several days to 79 years. Most of the patients (37.6%) were in the age group of 15–29 years (Table 1).

Table1. Demographic Characteristic and Culture-Positive Status of Patients at Zliten Teaching General Hospital

Variables	Category	Total *N*(%)	Culture Result		Isolates Gram-Reaction	
			Negative *N*(%)	Positive *N*(%)	Gram-Negative *N*(%)	Gram-Positive *N*(%)
Sex	Female[1]	263 (59.4)	144 (54.7)	119 (58)	91 (76.5)	28 (23.5)
	Male	180 (40.6)	94 (89.7)	86 (42)	62 (72)	24 (28)
Age (years)	0–14	82 (18.6)	43 (52.4)	39(47.6)	33 (84.7)	6 (15.3)
	15–29	167 (37.6)	87 (52)	80(48)	59 (73.7)	21 (26.3)
	30–44	106 (24)	59 (55.7)	47(44.3)	32 (68)	15 (32)
	45–59	55 (12.4)	30 (54.5)	25(45.5)	18 (72)	7 (28)
	≥60	33 (7.4)	19 (57.6)	14(42.4)	11 (78.6)	3 (21.4)
Total		443 (100)	238 (53.7)	205 (46.3)	153 (74.6)	52 (25.4)

[1] The proportion of bacterial isolates was significantly higher in females **(*P*< 0. 03)**

Among 443 of the specimens collected, bacterial pathogens were isolatedfrom 205 patients with the isolation rate of 46.3%.From the total 205 positive patients, the proportion of bacterial isolates was significantly higher in females, 119 (58%), than males, 86 (42%) (*P*=0.03), one hundred fifty three (74.6%) of the isolates were gram negative and 52 (25.4%) were gram positive (Table 1.). About 145/ 205 (70.8%) of the total isolates were *Escherichia coli*, *Klebsiella* spp, *Proteus* spp, *Staphylococcus aureus*, and *Staphylococcus haemolyticus*. Among these, *E. coli* accounts (29 %) followed by *Klebsiella* spp. (22%),*Proteus* spp, (13%), *S. aureus* (13%), and *S. haemolyticus* (8%) (Figure 1).

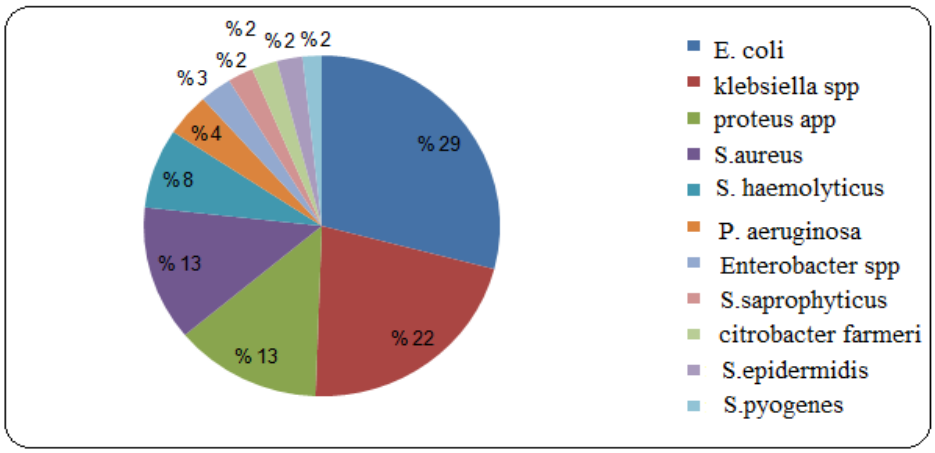

Figure.1: Bacteria isolated from patients at Zliten Teaching General Hospital.

Antimicrobial Resistance Profiles of Bacterial Isolates

Gram-Negative Bacteria

Most of antibiotics sensitivity testes showed that the bacterial isolates were resistant to many of the antibiotics tested. where that Gram-negative bacteria in 319 (34.5%) of antibiotics tests were resistant to those antibiotics. The k2resistance results were showed in 84% ampicillin, followed 39.1% for clindamycin, 35.5% for amoxicillin+clavulanic acid, 33.3% for gentamycin; least resistant being 2.5% for amikacin. Among *Pseudomonas aeruginosa* isolates, antibiotic resistance was observed in 62.5% (35/56 test). These isolates exhibited 100% resistance to ampicillin, ciprofloxacin, and nitrofurantoin 85.7% to amoxicillin+clavulanic acid. *Citrobacter fermeri* showed an overall resistance of 50%. *Klebsiella* spp, Proteus spp. And *E. coli* isolates demonstrated an overall resistance of 38%, 30%, and 29% of tests respectively. Free low-resistance rates were observed to nitrofurantoin (2.7%), (28.6%) among *Klebsiella* spp and . *Pseudomonas aeruginosa* respectively (Table 2).

Table 2: Antimicrobial Resistance Profiles of Gram-Negative Bacterium Species at Zletin Teaching General Hospital

	E.coli **(n= 50)**		***Klebsiella*** **spp. (n= 37)**		***Proteus*** **spp (n=23)**		***Pseudomonas aeruginosa*****(n= 7)**		***Citrobacter Fermeri*****(n=4)**		***Total***	
	#T	**R**	**#T**	**R**	**#T**	**R**	**#T**	**R**	**#T**	**R**	**#T**	**R**
Ampecillin	**49**	**39**	**33**	**33**	**13**	**8**	**7**	**7**	**4**	**2**	**106**	**89**
Amikacin	**49**	**0**	**37**	**1**	**23**	**0**	**7**	**2**	**0**	**0**	**116**	**3**
Amoxiclav	**50**	**21**	**37**	**13**	**23**	**1**	**7**	**6**	**4**	**2**	**121**	**43**
Ceftriaxone	**50**	**4**	**36**	**8**	**23**	**5**	**7**	**7**	**4**	**2**	**120**	**26**
Cipofluxacin	**48**	**9**	**36**	**17**	**23**	**7**	**7**	**3**	**4**	**2**	**118**	**38**
Clindamycin	**50**	**20**	**36**	**14**	**23**	**10**	**7**	**1**	**4**	**2**	**120**	**47**
Gentamycin	**50**	**15**	**36**	**14**	**23**	**7**	**7**	**2**	**4**	**2**	**120**	**40**
Nitrofurantoin	**48**	**6**	**36**	**9**	**12**	**11**	**7**	**7**	**0**	**0**	**103**	**33**
Total	**394**	**114**	**287**	**109**	**163**	**49**	**56**	**35**	**24**	**12**	**924**	**319**

Abbreviations: **# T**, number of isolates tested against each antimicrobial agent; **R**, isolates resistance to antimicrobial agents

Gram-Positive Bacteria

The most frequent Four Gram-positive bacteria isolated from clinical specimens in this study were tested for antibiotic suitability test. Overall, 52 (25.4%) Gram-positive bacteria were resistant to the tested antibiotics. Resistance rates of 75.6%, 78.4%, 75% and 62.5% were recorded for cefoxitin, tetracycline, erythromycin and gentamicin, respectively. The high resistance rates among total *S. aureus* isolates were recorded, where that resistance rates of 85.7% for tetracycline, 80% for each of erythromycin and ciprofloxacin and an overall rate of 67% **(**Table 3).

Table 3: Antimicrobial Resistance Profiles of Gram-Positive Bacterium Species Among Patients at Zletin Teaching General Hospital

Antimicrobials	*S.aureus* (n=22)		*S.haemolytics* (n= 13)		*S.saprophyticus* (n=4)		*S. epidermidis* (n=4)		Total	
	#T	R	#T	R	#T	R	#T	R	#T	R
Cefoxitin	13	9	12	11	4	3	4	3	33	26
Clindamycin	21	4	13	4	4	1	4	0	42	9
Ciprofloxacin	21	1	13	5	4	2	4	0	42	8
Erythromycin	21	2	13	12	4	3	4	0	42	17
Gentamycin	20	4	13	5	4	0	4	2	41	11
Nitrofurantoin	22	0	13	0	4	0	4	0	43	0
Penicillin G	16	8	10	2	3	1	2	1	31	12
Tetracycline	19	5	13	4	3	0	4	2	39	11
Oxacillin	20	11	13	10	4	1	4	1	41	23
Total	173	44	113	53	34	11	34	9	354	117

Abbreviations: **#T**, number of isolates tested against each antimicrobial agent; **R**, isolates resistance to antimicrobial

Multi-Drug Resistance Profiles of the Isolates

From the total isolates, 19 (11.3) were susceptible to all the tested antibiotics. The majority of the tested isolates were resistant to two or more antimicrobials tested. In general, 115(67.6%) of bacterial species were MDR. Among resistant isolates to more than one antibiotic, the most common pathogen was *Pseudomonas aeruginosa*, and *Staphylococcus haemolyticus* (100%); followed by *Proteus* spp (82.6%) (Table 4).

Table4: Multi-Drug Resistance Profiles Among Bacterial Isolates at Zliten Teaching General Hospital

BacterialIsolates	R0	R1	R2	R3	R4	≥ R5	Overall MDR
E.coli(*n*=50)	11	9	6	11	2	11	30
*Klebsiella*spp.(*n*=37)	3	12	1	3	11	7	22
*Proteus*spp.(*n*=23)	0	4	6	3	6	4	19
S.aureus (*n*=22)	4	7	2	5	1	3	14
S.haemolyticus (n= 13)	0	0	2	2	2	7	13
P. aeruginosa (n=7)	0	0	0	0	2	5	7
S.saprophyticus(*n*=4)	0	1	0	1	0	2	3
C.fermeri(*n*=4)	0	1	1	0	0	2	3
S.epidermidis(*n*=4)	1	1	0	0	1	1	2
S.pyogenes(*n*=3)	0	1	0	0	1	1	2
Others (38)	-	-	-	-	-	-	-
Total(*n*=205)	19	36	18	25	26	43	115

Abbreviations: R0, susceptible to all antimicrobials tested; R1, R2, R3, R4, resistance to one, two, three, four, and ≥ R5, resistance to five, and more than five antibiotics taken from different classes, respectively. MDR, Resistance to two or more antibiotics.

Discussion

Antibiotic resistance is one of the most serious global threats to the treatment of infectious diseases. Resistance to multiple antibiotics has developed for many common gram positive and gram negative bacterial pathogens, such as staphylococci, pneumococci and *pseudomonas* spp. Consequently, the therapeutic options for treatment of some infections are limited, especially in the developing countries where second- and third-line antibiotics are unavailable (Sharma, 2004).

In the present study, among the 443 study subjects, bacterial pathogens were isolated from 205-patients with the isolation rate of 46.3%, and this was lower than the results reported by **(**Muluye *et al*., 2014) where they found the bacterial isolation rate was 70.2%.However, countries and hospitals with the fewest controls on antibiotic prescribing before collection of samples have lowest isolation rate from cultures.

The prevalence of the culture-confirmed bacterial infection was significantly higher in females (58%) than males (42%).The results was in agreement with a report by (Yitayeh *et al.*,2021) which may be correlated to more urine specimens were taken from females with urinary tract infection symptoms.The majority of the patients who were positive for bacterial culture were in the age range of 15 to 29 years, which was 80 (39%), followed by in the age range of 30- 44 years, which was 47 (23%); age range of 0-14 years which was 39 (19%). However, no significant differences were found in the isolation rates of pathogens in relation to the age of patients.

Among the total isolates, 153 (74.6%) were gram negative bacteria and 52 (25.4%) were gram positive, this was consistent with (Magiorakos, *et al*., 2012) where Gram- negatives bacteria were more frequently found compared to Gram- positive isolates. The predominant isolates in this study was found to be *E. coli*, which was 29%, this was similar to studies by **(**Krishna *et al*.,2018**).**This could be due to the abundance of *E. coli* in urinary tract infection. *Klebsiella spp* was the second most isolated pathogen, which was analogous to similar studies by(Iliyasu *et al*.,2018).

Furthermore, *Proteus* spp, and S. *aureus* were the other frequently isolated organisms in the present study, which is similar to the studies by **(**TenHove *et al*,. 2017), and (Krishna *et al*., 2018), which could be associated with their frequent existence in the hospital wards.

In this study, overall high levels of resistance were demonstrated against ampicillin, cifoxitin, amoxicillin+clavulanic, erythromycin and gentamicin. These were consistent with resistance rates obtained from studies in Ethiopia and India. However, majority of bacteria isolates revealed lower levels of resistance against ciprofloxacin and amikacin. Moreover, Gram positive bacteria showed high levels of resistance (84.3, 56%, 40.4%) to cefoxitin, oxacillin and erythromycin respectively. This finding is similar with studies carried out by (Prakash *et al* 2013, Godebo, *et al* 2013).

In the present study .the resistant to different antibiotic was observed. Resistance to ampicillin was observed in (84%) isolates, to clindamycin in 39.1%, to amoxicillin+clavulanic acid in 35.5%, to gentamycin in 33.3% among gram-negative isolated bacteria. Other investigators reported relatively similar findings in previous studies (Ali *et al* 2014 and Yitayeh *et al*,. 2021) Bacteria resistant to antimicrobial agents have been frequently isolated, and it is steadily increasing around the world, possibly as a result of their indiscriminate use, where widespread use of antibiotics provides the selective pressure favoring propagation of the resistant organisms (Conly, 2002)

In the present study it was found that amikacin was the most effective antibiotics against gram-negative, whereas, Nitrofurantoin (100%), Ciprofloxacin (81%) and Clindamycin (78.5%) demonstrated high activity against gram-positive bacteria. The low resistance rates detected for these antibiotics may be attributed to their uncommon use in the treatment, and the use of these antibiotics is only in hospitalized patients according to culture results. A striking finding was the high MDR rates among some bacterial isolates. Among resistant isolates to more than two antibiotic (MDR), the most common pathogens were *Pseudomonas spp*. And *S. haemolyticus*, 100%; followed by Proteus spp. 82.6%; *S. saprophyticus* and *C. fermeri* 75%. Nineteen (11.3%) of the isolates were sensitive to all drugs tested and 36 (21.5%) were resistance to only one drug. However, among the total isolates, 115 (68.8%) were resistant for two or more drugs tested and 43(25.7%) were resistant to more than five antibiotics used in the study. our finding was supported by other studies done by (Abera *et al*, 2014) where 63.3–85% MDR rate was reported.

Conclusion

Infections with bacterial isolates resistant to a majority of antibiotics are still a global issue specially in developing countries. We can control on bacterial resistance by practicing better infection control procedures, keep check routinely on antibiotic use and its resistance, adopting antibiotic control policy. Efficient surveillance system can play its critical part at national and international level. Efforts are required by all stakeholders to control and prevent MDR bacterial infections.

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