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Frequency and Antimicrobial Resistance Pattern among Bacterial Clinical Isolates Recovered from Different Specimens in Egypt

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Abstract: Antimicrobial resistance (AMR) is a global public health threat resulting in high mortality rates. Current study aimed to identify the most prevalent pathogens among variable infection sites and their AMR pattern. Data concerning cultures and antibiotic susceptibilities were retrieved from Microbiology Department's records and statistically analyzed. Out of 554 bacterial isolates, Gram negative isolates (68.4%) were predominant. Urine specimens showed the highest incidence of recovery of total isolates (41.5%, n=230) followed by blood (23.1%, n=128), while sputum specimens exhibited the least frequency (17%, n=94). *E. coli* (30.7%, n=170), *S. aureus* (21.1%, n=117) and Klebsiella spp (20.9%, n=116) were the most frequently isolated pathogens. Recovery of isolates was significantly more frequent among males (P<0.05) except in case of urine specimens. Highest incidence of resistance in both Gram positive and Gram negative isolates was recorded in case of cephalosporins and penicillin/β-lactamase. Gram positive isolates exhibited the least resistance to linezolid (10.8%) and vancomycin (9.5%) whereas colistin was the most effective against Gram negative isolates as it recorded 16.4% resistance. Higher frequency of multiple drug resistance (MDR) was also observed in Gram negative isolates compared to Gram positive ones. Resistance to uropathogens and MDR were significantly more frequent in males. Although *E. coli* was the most prevalent uropathogen but it showed the least incidence of MDR however Pseudomonas spp exhibited the highest MDR rate. The high incidence of resistance in the current study is alarming and highlights the necessity of routinely monitoring the local prevalence of resistance for selecting the best antimicrobial treatment and as a guide for empirical therapy.

Keywords: Prevalence, Multiple Drug Resistance, Pattern, Egypt

1. Introduction

Emergence of antimicrobial resistance to different antibiotics is a critical problem that leads to a real danger of post antibiotic era [1]. During the last decade, many reports have documented the doubling or even tripling in the resistance rates of nearly all groups of serious pathogens [2] in addition to the progressive emergence of MDR isolates [3]. The lack of proper and early identification of the causative pathogens especially in patients with serious infection led to the administration of broad spectrum antibiotics. Such issue resulted in dramatic emergence of resistant strains that the magnified the problem of resistance [1]. The Center for Disease Control and Prevention (CDC) reported that high rates of infection with resistant pathogens all over the world resulted in passive influence on the global economy, loss in productivity and elevated death rates [4]. Data concerning the endemic antimicrobial resistance are usually unavailable especially in the areas of the world where antibiotics are available over the counter [5]. Despite that many reports demonstrated the incidence and the resistance patterns of many pathogens, few studies are available to

estimate the endemic antimicrobial resistance profile in low and middle income countries [6]. Thus an evidence based knowledge regarding the local antimicrobial resistance pattern is considered an essential guide for treatment of specific pathogens as well as for empirical antimicrobial therapy [5]. This guide is also of significant importance in the implementation of the effective antimicrobial stewardship [1] as well as in the design of national and international research programs [5]. Therefore, the present study aimed to identify the microbial spectrum and the antimicrobial resistance pattern of the most prevalent pathogens recovered from variable infection sites in addition to determination of the prevalence of multiple drug resistance.

2. Materials and Methods

2.1. Study Design

Retrospective study was conducted utilizing the microbiology laboratory records of in-patients in an Egyptian hospital in Cairo. Microbiology records were reviewed and records showing mixed cultures or unidentified microorganisms as well as duplicate records for the same patients were excluded [7]. Records for specimens other than blood, sputum, urine and wound specimens were also excluded. Information regarding the identified bacterial isolate, specimen type, patient's gender and antimicrobial susceptibility was collected and recorded.

2.2. Identification of the Isolated Organism

Sample processing, identification of the microorganism to the genus and/or species level was performed according to the standard operating procedures of the hospital in place. Briefly, bacterial isolates were identified based on morphological characters, Gram stain and confirmatory biochemical test. Gram positive bacteria were identified using catalase reaction, coagulase test as well as via testing the hemolytic activity on blood agar. Identification of Gram-negative bacteria was carried out through inoculation on MacConkey agar plates, followed by biochemical tests such as oxidase and urease tests.

2.3. Antimicrobial Susceptibility

Antimicrobial susceptibilities of the bacterial isolates were determined using Kirby-Bauer disk diffusion method using Mueller Hinton agar plates according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The tested antimicrobial discs were routinely supplied from Oxoid and Bioanalyse.

2.4. Multiple Drug Resistance (MDR)

MDR isolates were identified according to the guidelines recommended by European Centre for Disease Prevention and Control (ECDC) and the CDC. MDR isolates were identified as isolates showing resistance to at least one antimicrobial agent in three or more antimicrobial classes [8].

2.5. Statistical Analysis

Data were presented as counts and percentage. Statistical analysis was performed using statistical package for social sciences (SPSS) computer software (version 25), IBM software, USA. Pearson Chi-square test was performed to identify the significant effect of each antibiotic on different isolates as well as the relation between gender and prevalence of different isolates. Chi-square and Fisher exact tests were used to test the association between gender and resistance to different antibiotics. Statistically significant difference was considered at p value ≤ 0.05.

3. Results

3.1. Antibiotic Resistance Pattern

Among total isolates (n=554), Gram negative isolates (68.4%, n=379) were more prevalent compared to Gram positive ones (31.6%, n=175). Urine specimens showed the highest frequency of recovery of total isolates (41.5%, n=230) followed by blood (23.1%, n=128), while sputum specimens exhibited the least percentage (17%, n=94) of recovery. *E. coli* (30.7%, n=170), *S. aureus* (21.1%, n=117) and Klebsiella spp (20.9%, n=116) were the most frequently isolated pathogens.

Table 1. *Frequency of the predominant Gram positive pathogens among different specimens.*

Isolates	Blood			Sputum			Urine			Wound			Total		
	% (n)	% M	% F	% (n)	% M	% F	% (n)	% M	% F	% (n)	% M	% F	%[a]	% M	% F
S. aureus (n=117)	57.3 (67)	71.6	28.4	11.1 (13)	76.9	23.1	13.7 (16)	43.8	56.2	17.9 (21)	66.7	33.3	66.9	67.5	32.5
Streptococcus spp (n=36)	30.6 (11)	54.5	45.5	19.4 (7)	85.7	14.3	41.7 (15)	46.7	53.3	8.3 (3)	66.7	33.3	20.6	58.3	41.7
CoNS (n=22)	40.9 (9)	44.4	55.6	22.7 (5)	60	40	18.2 (4)	50	50	18.2 (4)	75	25	12.6	54.5	45.5
Total	49.7 (87)	66.7*	33.3*	14.3 (25)	76*	24*	20 (35)	45.7	54.3	16 (28)	67.9*	32.1*	N=175	64*	36*

CoNS; coagulase-negative Staphylococcus, N; Total number of Gram positive isolates, % (n); percentage of each isolate relative to the total number of isolates, % M; Percentage of isolates recovered from males, % F: Percentage of isolates recovered from females, %[a]: percentage of each isolate relative to N, *; Statistically significant difference between the incidence of recovery of isolates from males and females.

Regarding total Gram positive isolates, blood specimens is the major source for recovery of Gram positive isolates as it presented 49.7%. *S. aureus* was the most common pathogen (66.9%), followed by Streptococcus spp (20.6%), while coagulase-negative Staphylococcus (CoNS) recorded a frequency of 12.6%. Among different specimens, *S. aureus* (57.3%) and CoNS (40.9%) were most frequently recovered from blood. Meanwhile, Streptococcus spp exhibited the

highest incidence of recovery from urine specimens (41.7%). Gram positive isolates were more common in males (64%) compared to females (36%). Assessment of the recovery of these pathogens among blood, sputum and wound specimens followed the same pattern except that there was no statistically significant difference (P>0.05) between the frequency of recovery of uropathogens from females (54.3%) compared to males (45.7%) (Table 1).

Gram negative isolates showed predominance of *E. coli* (44.9%, n=170) followed by Klebsiella spp (30.6%, n=116) and Pseudomonas spp (10.6%, n=40). On the other side, Proteus spp (6.3%, n=24), non-lactose fermenters (NLF) (6.1%, n=23) and Acinetobacter spp (1.6%, n=6) were less frequent. The majority of Gram negative isolates were recovered from urine (51.5%, n=195) with the predominance of *E. coli* (58.2%, n=99) and Klebsiella spp (55.2%, n=64). Wound and sputum specimens were also found as another source for recovery of *E.coli*, where they showed frequency of recovery in the order of 18.2% and 14.7%, respectively. Pseudomonas spp also showed high incidence of recovery from urine (40%, n=16) followed by wound (27.5%, n=11). Recorded data revealed higher incidence of total Gram negative isolates (60.7%) in male patients. Gram negative isolates in male patients was significantly (P<0.05) more common than females among different specimens except in case of uropathogens where the difference between the incidence of recovery of these isolates was statistically non-significant among male and female patients (Table 2).

Table 2. *The spectrum of Gram negative isolates in different specimens.*

Isolates	Blood			Sputum			Urine			Wound			Total		
	% (n)	% M	% F	% (n)	% M	% F	% (n)	% M	% F	% (n)	% M	% F	% [a]	% M	% F
E. coli (n=170)	8.8 (15)	73.3	26.7	14.7 (25)	88	12	58.2 (99)	39.4	60.6	18.2 (31)	90.3	9.7	44.9	58.8	41.2
Pseudomonas spp (n=40)	15 (6)	66.7	33.3	17.5 (7)	71.4	28.6	40 (16)	50	50	27.5 (11)	72.7	27.3	10.6	62.5	37.5
Klebsiella spp (n=116)	13.8 (16)	50	50	20.7 (24)	70.8	29.2	55.2 (64)	46.9	53.1	10.3 (12)	91.7	8.3	30.6	56.9	43.1
Proteus spp (n=24)	4.2 (1)	100	0	12.5 (3)	100	0	16.7 (4)	75	25	66.7 (16)	75	25	6.3	79.2	20.8
NLF (n=23)	4.3 (1)	100	0	34.8 (8)	50	50	47.8 (11)	72.7	27.3	13.0 (3)	100	0	6.1	69.6	30.4
Acinetobacter spp (n=6)	33.3 (2)	100	0	33.3 (2)	50	50	16.7 (1)	0	100	16.7 (1)	100	0	1.6	66.7	33.3
Total	10.8 (41)	65.9*	34.1*	18.2 (69)	75.4*	24.6*	51.5 (195)	45.1	54.9	19.5 (74)	85.1*	14.9*	N=379	60.7*	39.3*

NLF; Non-lactose fermenters, N; Total number of Gram negative isolates, % (n); percentage of each isolate relative to the total number of isolates, % M; Percentage of isolates recovered from males, % F: Percentage of isolates recovered from females, %[a]: percentage of each isolate relative to N, *; Statistically significant difference between the incidence of recovery of isolates from males and females.

3.2. Antibiotic Resistance Pattern of Isolates Recovered from Various Infection Sites

3.2.1. Gram Positive Isolates

Highest incidence of resistance was recorded to third generation cephalosporins especially in case of ceftazidime (100%, n=22) and cefixime (97.9%, n=48) with lower resistance to ceftriaxone (56.3%, n=135) and cefotaxime (44.4%, n=27). Whereas, cefoperazone showed the least resistance either alone (27.8%, n=18) or in combination with sulbactam (25%, n=72). High resistance rate was also recorded in case of first, second and fourth generation cephalosporins. The combination between penicillin and β-lactamase inhibitors exhibited high incidence of resistance especially in case of amoxicillin/clavulanic acid (70.2%, n=121) followed by ampicillin/sulbactam (55.8%, n=104). While piperacillin/tazobactam retained most of its antimicrobial activity where it showed only 25% resistance (n=16). Regarding macrolides, azithromycin exhibited a resistance rate in the order of 57.1% (n=35). Clindamycin, fusidic acid and fluoroquinolones showed moderate resistance, but lower resistance was recorded in case of carbapenems and teicoplanin. On the other side, the least resistance was observed towards nitrofurantoin (11.8%, n=34), linezolid (10.8%, n=65) and vancomycin (9.5%, n=84) (Table 3).

Regarding different isolates, *S. aureus* exhibited high resistance rates to most antibiotics, whereas it showed lower resistance to vancomycin (10.6%, n=66), linezolid (14%, n=43), teicoplanin (22.4%, n=58) and cefoperazone/sulbactam (20.8%, n=48) in addition to 100% susceptibility to nitrofurantoin. Streptococcus spp and CoNS followed similar pattern but Streptococcus spp was 100% susceptible to both vancomycin (n=10) and piperacillin/tazobactam (n=2). Moreover, all CoNS isolates were susceptible to linezolid (n=10), imipenem (n=8) and piperacillin/tazobactam (n=3). Data also revealed a statistically significant difference in the antimicrobial potentials to different isolates in case of amoxicillin/clavulanic acid, ceftriaxone, cefixime, cefoperazone/sulbactam and glycopeptide antibiotics (Table 3).

Table 3. *Resistance profile among Gram positive isolates recovered from different specimens.*

Antimicrobial classes	Antimicrobial agent	% Resistance (n)				P value
		S. aureus	Streptococci	CoNS	Total isolates	
1-Penicillin/β-lactamase inhibitors	Amox/clavu	76.8 (82)	69.2 (26)	30.8 (13)	70.2* (121)	0.03
	Amp/sulb	53.7 (67)	54.5 (22)	66.7 (15)	55.8 (104)	0.21

Antimicrobial classes	Antimicrobial agent	% Resistance (n)				P value
		S. aureus	Streptococci	CoNS	Total isolates	
	Piper/tazob	36.4 (11)	0 (2)	0 (3)	25 (16)	0.25
2-Cephalosporin 1st G	Cefazolin	63.6 (11)	0 (1)	66.7 (3)	60 (15)	0.40
	Cephradine	78.6 (14)	80 (5)	50 (2)	76.2 (21)	0.7
	Cephalexin	83.3 (12)	50 (4)	80 (5)	76.2 (21)	0.08
Cephalosporin 2nd G	Cefuroxime	42.2 (45)	46.2 (13)	25 (8)	40.9 (66)	0.22
	Cefaclor	69.2 (13)	62.5 (8)	50 (2)	65.2 (23)	0.77
Cephalosporin 3rd G	Ceftriaxone	53.3 (92)	75 (28)	25 (16)	56.3* (135)	0.04
	Ceftazidime	100 (12)	100 (7)	100 (2)	100 (22)	-
	Cefotaxime	38.9 (18)	40 (5)	75 (4)	44.4 (27)	0.26
	Cefixime	97.5 (40)	100 (5)	100 (3)	97.9* (48)	0.04
	Cefoperazone	25 (12)	25 (4)	50 (2)	27.8 (18)	0.53
Cephalosporin 4th G	Cefepime	83.3 (12)	55.6 (9)	60 (5)	69.2 (26)	0.18
3. Cephalosporin/β-lactamase inhibitors	Cefoperazone/Sulb	20.8 (48)	31.3 (16)	37.5 (8)	25* (72)	0.03
4-Carbapenems	Meropenem	27.4 (84)	47.6 (21)	18.2 (11)	30.2 (116)	0.17
	Imipenem	33.3 (18)	55.6 (9)	0 (8)	31.4 (35)	0.08
5-Glycopeptide antibiotics	Teicoplanin	22.4 (58)	66.7 (12)	16.7 (12)	28.0* (82)	0.02
	Vancomycin	10.6 (66)	0 (10)	12.5 (8)	9.5* (84)	0.04
6- Macrolide	Azithromycin	63.6 (22)	44.4 (9)	50 (4)	57.1 (35)	0.07
7- Oxazolidinones	Linezolid	14.0 (43)	8.3 (12)	0 (10)	10.8 (65)	0.13
8- Lincosamides	Clindamycin	53.2 (47)	70 (10)	33.3 (6)	54.0 (63)	0.5
9-Tetracycline	Doxycycline	27.8 (72)	45.8 (24)	50 (10)	33.9 (106)	0.07
10-Fusidane	Fusidic acid	64.7 (17)	50 (2)	50 (6)	40 (25)	0.49
11- Fluoroquinolone	Norfloxacin	42.9 (14)	75 (8)	NT	54.2 (22)	0.17
	Ofloxacin	33.3 (15)	50 (10)	75 (4)	44.8 (29)	0.14
	Ciprofloxacin	50 (14)	37.5 (8)	66.7 (6)	50 (28)	0.76
	Levofloxacin	37.8 (37)	25 (8)	66.7 (9)	40.7 (54)	0.06
12- Nitrofurans	Nitrofurantoin	0 (14)	20 (15)	20 (5)	11.8 (34)	0.17

Amox/clavu; amoxicillin/clavulanic acid, Amp/sulb; ampicillin/sulbactam, Piper/tazob; piperacillin/tazobactam, G; generation, NT; not tested, n; number of bacterial isolates tested against each antimicrobial agent, *; statistically significant difference between the effect of each antibiotic on different isolates.

3.2.2. Gram Negative Isolates

Gram negative isolates exhibited high resistance rates to most antibiotic classes such as cephalosporins. However, the combination between cefoperazone and sulbactam reduced the resistance to cefoperazone from 77.2% to 42.4%. Penicillin/β-lactamase inhibitors also showed high degree of resistance especially in case of amoxicillin/clavulanic acid (92.3%, n=78) followed by ampicillin/sulbactam (71.7%, n=329), while the combination between piperacillin and tazobactam showed lower resistance (57.2%, n=173). Sulfamethoxazole either alone or in combination with trimethoprim resulted also in high resistance rates in the order of 85.7% and 88.8%, respectively. High incidence of resistance was also recorded in case of azteronam (81.1%, n=90), azithromycin (73.2%, n=71) as well as towards fluoroquinolones. Similar pattern was also observed in case of gentamicin (66.4%, n=122) and doxycycline (62.8%, n=121), imipenem (44.9%, n=78) and meropenem (38%, n=284) (Table 4).

Nitrofurantoin showed potential antimicrobial activity against *E. coli*, where the percentage of resistance against it was 17.2%. Meanwhile, Klebsiella spp, NLF, Pseudomonas spp and Proteus spp recorded high resistance rates to nitrofurantoin in the order of 60.7%, 77.8%, 92.9% and 100%, respectively. Moreover, *E. coli* showed high resistance rates to most antimicrobial classes with lower resistance to piperacillin/tazobactam (43.3%, n=67) and amikacin (34.6%, n= 104), followed by cefoperazone/sulbactam (27.8%, n=90) and meropenem (23.3%, n=120). On the other side, the least resistance was observed in case of colistin (6.7%, n=15). Higher degree of resistance was recorded in case of Klebsiella spp compared to *E. coli.* Pseudomonas spp also followed similar resistance profile with the least resistance to both imipenem and colistin (12.5%, n=8). In addition, an elevated resistance in case of other bacterial isolates such as Proteus spp, Acinetobacter spp and NLF was also recorded. It was also obvious that, colistin was the most promising antimicrobial agent either against each Gram negative isolate or in case of total isolates, where it exhibited a resistance rate in the order of 16.4%. Data also revealed that some antimicrobials showed a statistically significant difference in their antimicrobial activities to different bacterial isolates as presented in Table 4.

Table 4. *Resistance pattern in Gram negative isolates recovered from variable specimens.*

Antimicrobial classes	Antimicrobial agent	% Resistance (n)						Total isolates	P value
		E. coli	Klebsiella	Pseudomonas	Proteus	Acinetobacter	NLF		
1-Penicillin/β-lactamase inhibitors	Amox/clavu	81.8 (33)	100 (22)	100 (11)	100 (3)	100 (2)	100 (7)	92.3 (78)	0.14
	Amp/sulb	62.5 (144)	82.5 (103)	81.8 (33)	80 (25)	50 (6)	61.1 (18)	71.7* (329)	0.006

Antimicrobial classes	Antimicrobial agent	% Resistance (n)						Total isolates	P value
		E. coli	Klebsiella	Pseudomonas	Proteus	Acinetobacter	NLF		
	Piper/tazob	43.3 (67)	76 (50)	43.5 (23)	66.7 (15)	83.3 (6)	58.3 (12)	57.2* (173)	0.004
2-Cephalosporin 1st G	Cefazolin	100 (14)	100 (11)	100 (2)	100 (2)	NT	100 (1)	100 (30)	-
	Cephradine	81.5 (27)	81.3 (16)	100 (4)	100 (3)	NT	100 (3)	84.9 (53)	0.6
	Cephalexin	92.9 (14)	100 (22)	83.3 (6)	100 (6)	100 (1)	100 (2)	96.1 (51)	0.84
Cephalosporin 2nd G	Cefuroxime	64.7 (17)	100 (13)	83.3 (6)	100 (1)	100 (1)	100 (2)	82.5 (40)	0.15
	Cefaclor	84.1 (44)	93.5 (31)	100 (7)	100 (6)	100 (1)	100 (6)	90.5 (95)	0.45
Cephalosporin 3rd G	Ceftriaxone	72.6 (117)	83.7 (86)	86.2 (29)	100 (17)	80 (5)	82.4 (17)	80.1* (271)	0.008
	Ceftazidime	84.8 (33)	89.7 (29)	66.7 (12)	100 (5)	100 (3)	71.4 (7)	84.3 (89)	0.27
	Cefotaxime	63.5 (52)	87.1 (31)	61.5 (13)	60 (5)	100 (2)	100 (5)	72.2* (108)	0.02
	Cefixime	78.3 (23)	100 (17)	100 (11)	83.3 (6)	100 (2)	100 (6)	90.8 (65)	0.15
	Cefoperazone	78 (50)	84.8 (33)	47.1 (17)	85.7 (7)	100 (3)	100 (4)	77.2* (114)	0.04
Cephalosporin 4th G	Cefipeme	80 (20)	93.8 (16)	75 (8)	85.7 (7)	NT	50 (2)	83 (53)	0.43
3. Cephalosporin/β-lactamase inhibitors	Cefoperazone/ Sulb	27.8 (90)	62.9 (62)	41.2 (17)	46.7 (15)	100 (1)	38.5 (13)	42.4* (198)	0.001
4- Monobactam	Aztreonam	65.4 (26)	96.7 (30)	61.1 (18)	100 (8)	100 (4)	100 (4)	81.1* (90)	0.004
5-Carbapenems	Meropenem	23.3 (120)	40.4 (89)	51.7 (29)	51.7 (21)	83.3 (6)	63.2 (19)	38* (284)	< 0.001
	Imipenem	39.3 (28)	48.4 (31)	12.5 (8)	83.3 (6)	50 (2)	66.7 (3)	44.9* (78)	0.048
6-Aminoglycosides	Gentamicin	48.8 (43)	65.9 (41)	87.5 (16)	81.8 (11)	100 (3)	87.5 (8)	66.4* (122)	0.04
	Amikacin	34.6 (104)	57.3 (82)	37.1 (35)	60.9 (23)	57.1 (7)	52.9 (17)	45.9 (268)	0.12
7- Macrolide	Azithromycin	60.7 (28)	83.3 (24)	75 (8)	80 (5)	100 (2)	75 (4)	73.2* (71)	0.485
8-Tetracycline	Doxycycline	56.7 (60)	61.8 (34)	82.4 (17)	80 (5)	50 (2)	66.7 (3)	62.8 (121)	0.27
9- Fluoroquinolones	Norfloxacin	64.3 (70)	78.3 (46)	64.3 (14)	40 (5)	NT	91.7 (12)	70.1 (147)	0.11
	Ofloxacin	63.6 (55)	72.1 (43)	76.5 (17)	66.7 (9)	50 (2)	100 (9)	70.4 (135)	0.33
	Ciprofloxacin	63.6 (77)	70.7 (58)	50 (16)	64.3 (14)	100 (3)	70.6 (17)	65.9* (185)	0.004
	Levofloxacin	66.7 (78)	82 (50)	54.5 (22)	80 (15)	100 (3)	77.8 (9)	71.8 (177)	0.31
10- Synthetic quinolone	Nalidixic acid	75 (12)	85.7 (7)	100 (3)	NT	0 (1)	100 (1)	79.2 (24)	0.32
11- Polymyxins	Colistin	6.7 (15)	10.5 (19)	12.5 (8)	NT	0 (2)	25 (4)	16.4* (55)	0.03
12- Sulfonamide	Sulfamethoxazole	85 (20)	92.3 (13)	NT	100 (1)	NT	0 (1)	85.7 (35)	0.17
	Trimeth/Sulfa	90.2 (41)	82.3 (23)	83.3 (6)	100 (4)	100 (1)	100 (5)	88.8 (80)	0.66
13- Nitrofurans	Nitrofurantoin	17.2 (87)	60.7 (61)	92.9 (14)	100 (6)	0 (1)	77.8 (9)	43.8* (178)	< 0.001

Amox/clavu; amoxicillin/clavulanic acid, Amp/sulb; ampicillin/sulbactam, Piper/tazob; piperacillin/tazobactam, Trimeth/Sulfa; trimethoprim/sulfamethoxazole, G; generation, NT; not tested, n; number of bacterial isolates tested against each antimicrobial agent, *; Statistically significant difference between the effect of each antibiotic on different isolates.

3.3. Multiple Drug Resistance

Recorded data revealed that MDR occurs in 58.9% of total Gram positive isolates with a significant prevalence of MDR in males (66%). *S. aureus* exhibited the highest incidence of MDR (59.8 %), followed by Streptococcus spp (58.3%) and CoNS (54.5%). MDR was more common in blood (60.9%, n=87) and urine (62.9%, n=35) isolates, with lower frequency in wound (50%, n=28) and sputum (56%, n=25). Isolates recovered from blood, sputum and wound showed also a significant higher frequency of MDR among male patients, except in case of urine isolates where there was no significant difference between the prevalence of MDR among male and female patients (Table 5).

Table 5. *Multiple drug resistance pattern in Gram positive isolates.*

Bacterial isolates	Blood			Sputum			Urine			Wound			Total		
	% MDR	% M	% F	% MDR	% M	% F	% MDR	% M	% F	% MDR	% M	% F	% MDR	% M	% F
S. aureus	59.7	72.5	27.5	76.9	80	20	62.5	50	50	47.6	63.6	36.4	59.8	68.6	31.4
Streptococcus spp	72.7	62.5	37.5	28.6	100	0	60	55.6	44.4	66.7	50	50	58.3	61.9	38.1
CoNS	55.6	40.0	60.0	40	100	0	75	33.3	66.7	50	100	0	54.5	58.3	41.7
Total	60.9	67.9*	32.1*	56	85.7*	14.3*	62.9	50	50	50	68.8*	31.3*	58.9	66.0*	34.0*

CoNS; coagulase-negative Staphylococcus, *; Statistically significant difference between the incidence of MDR among male and female patients.

Higher incidence of MDR (77%) was recorded in Gram negative isolates compared to Gram positive ones. *E. coli* showed the least percentage of MDR (67.6%), while Pseudomonas spp exhibited the highest incidence of MDR (95%). A significant higher frequency of MDR was observed in males (63%) compared to females (37%). Blood, sputum and wound isolates exhibited similar profile but the difference between the incidence of MDR in male and female uropathogens was non-significant (p>0.05) (Table 6).

Table 6. *Multiple drug resistance profile among Gram negative isolates.*

Bacterial isolates	Blood			Sputum			Urine			Wound			Total		
	% MDR	% M	% F	% MDR	% M	% F	% MDR	% M	% F	% MDR	% M	% F	% MDR	% M	% F
E. coli	80	75	25	68	94.1	5.9	67.7	44.8	55.2	61.3	89.5	10.5	67.6	62.6	37.4
Pseudomonas spp	66.7	75	25	100	71.4	28.6	100	50.0	50	100	72.7	27.3	95	63.2	36.8
Klebsiella spp	75	41.7	58.3	91.7	72.7	27.3	79.7	51.0	49	75	88.9	11.1	81	58.5	41.5
Proteus spp	100	100	0	100	100	0	100	75.0	25	81.3	69.2	30.8	87.5	76.2	23.8
NLF	100	100	0	100	50	50	81.8	77.8	22.2	33.3	100	0.0	82.6	68.4	31.6
Acinetobacter spp	100	100	0	100	50	50	0	0	0	100	100	0.0	83.3	80	20
Total	78	65.6*	34.4*	85.5	76.3*	23.7*	75.4	50.3	49.7	73	81.5*	18.5*	77	63*	37*

NLF; Non-lactose fermenters, *; Statistically significant difference between the frequency of MDR among male and female patients

3.4. Prevalence of Resistance Among Uropathogens

Higher incidence of resistance to most antibiotics was significantly observed in isolates that were recovered from males compared to females (Table 7).

Table 7. *Antibiotic resistance pattern in Gram positive and Gram negative uropathogens relative to gender.*

	% Resistance							
	Gram positive				Gram negative			
Antimicrobial agent	Male	Female	Total no.	P value	Male	Female	Total no.	P value
Amox/clavu	75	36.4	23	0.098	88.9	85.7	46	1
Amp/sulb	71.4	36.4	18	0.367	75.7*	61*	170	0.018
Piper/tazob	0	0	2	-	53.8*	25*	50	0.019
Cefazolin	100	-	1	1	100	100	13	-
Cephradine	66.7	100	8	0.375	100*	64.7*	34	0.02
Cephalexin	60	66.7	8	1	100	93.8	29	1
Cefuroxime	75*	0*	10	0.033	80	75	22	1
Cefaclor	75	50	8	1	90.5	84.8	54	0.691
Ceftriaxone	90*	50*	22	0.019	83.1*	61.6*	132	0.002
Ceftazidime	100	100	8	-	66.7	77.8	27	0.609
Cefotaxime	50	50	4	1	85.2*	40.9*	49	< 0.001
Cefixime	100	-	1	1	100	100	9	-
Cefoperazone	100	-	1	1	89.7	75	57	0.089
Cefepime	100	100	2	-	71.4	85.7	14	1
Cefoperazone/sulb	42.9*	9.1*	18	0.046	45.2*	19.6*	98	0.003
Meropenem	60*	25*	22	0.045	30.1*	10*	143	< 0.001
Imipenem	33.3	0	4	1	33.3	22.2	33	0.491
Aztreonam	-	-	-	-	84.6	75	21	1
Vancomycin	0	-	1	-	-	-	-	-
Azithromycin	-	-	-	-	100	28.6	8	0.167
Gentamicin	-	-	-	-	60	50	25	0.569
Linezolid	0%	-	2	-	-	-	-	-
Clindamycin	100	100	2	-	-	-	-	-
Doxycycline	33.3	55.6	21	0.161	64.9	56.3	69	0.41
Amikacin	-	-	-	-	41.5*	20*	103	0.012
Norfloxacin	62.5	45.5	19	0.387	79.7*	56.1*	125	0.004
Ofloxacin	40	14.3	12	0.559	72.2	60.5	74	0.109
Ciprofloxacin	50	42.9	11	1	68.1*	54.9*	95	0.025
Levofloxacin	0	33.3	11	0.491	77.1*	57.5*	75	0.050
Nitrofurantoin	7.7	10	33	1	54.7*	34*	175	0.002
Nalidixic acid	-	-	-	-	100	75	21	0.113
Colistin	-	-	-	-	0	20	8	0.385
Sulphamethoxazole	-	-	-	-	91.7	84.2	31	1
Trimeth/Sulfa	-	-	-	-	87.1	88.1	73	0.98

Amox/clavu; amoxicillin/clavulanic acid, Amp/sulb; ampicillin/sulbactam, Piper/tazob; piperacillin/tazobactam, Trimeth/Sulfa; trimethoprim/sulfamethoxazole, n; number of Gram positive bacterial isolates, *; statistically significant difference between male and female isolates.

4. Discussion

Evaluating the altitudes of the problem of AMR is a challenge as the levels of antimicrobial resistance vary among healthcare settings and geographical regions. Infections with MDR pathogens result in postponed therapy which causes negative impact on the patient's health especially in case of immunocompromised individuals [9]. Moreover, adequate recognition of the proper use of antibiotics in each

community is a key factor in the progress of resistance [10]. Current study aimed to determine the most predominant pathogens in our community and their antimicrobial resistance pattern.

In the present study, urinary tract infection was the most prevalent followed by blood stream infection with least frequency in case of respiratory tract infection. Gram negative isolates were mostly involved in urinary tract infections while Gram positive isolates were responsible for blood stream infection (BSI). Resembling our findings, a study reported that all the recovered uropathogens were Gram negative whereas 60% of the isolates causing BSI were Gram positive with highest incidence of *S. aureus* [2]. In the mean context, it was reported that urine specimens contributes in the recovery of 55.2% of bacterial isolates whereas blood, wound and sputum cultures were responsible for 25.3%, 16.2%, and 3.3% of isolates, respectively [11]. Moreover, a study demonstrated that Gram negative isolates were more common (61.3%, n=57) with the predominance of *E. coli* (n=36) [2]. *S. aureus* (22.8%, n=100), *Klebsiella pneumoniae* (14.8%, n=65) and *E. coli* (9.3%, n=41) were also reported as the most common pathogens among variable specimens in another study [12]. In agreement with the current study *E. coli* and *Klebsiella pneumoniae* weren't only the most frequently isolated pathogens among Gram negative isolates [13] but they also represented the most predominant pathogens relative to other uropathogens [14-16].

Although many studies reported that *E. coli* was as the most predominant isolate recovered from urine specimens but on the contrary to our results Klebsiella spp was categorized as the sixth most common uropathogen in one study [17] and *S. aureus* was the second pathogen involved in urinary tract infection (UTI) in another study [18]. The similarities as well as the variation in the type and frequency of these pathogens among different studies could be related to many factors such as environmental conditions, health practices, patient conditions, personal hygiene, number of patients involved in each study and laboratory procedures [19].

E.coli is not only one of the major pathogens responsible for UTI but it also plays a key role in wound and respiratory tract infection. Similar to current findings, *E. coli* was the most frequently isolated from urine specimens (85.9%) followed by wound (8.4%) and sputum specimens (5.6%) [20].

In the same context to the current results, Pseudomonas spp was one of the most prevalent Gram negative pathogens associated with urinary tract infections as well as in surgical sites [21]. In addition to other studies which reported that pus is the major source from which pseudomonas could be recovered [10, 22].

Regarding BSI, the current data highlights the participation of Gram positive pathogens in this type of infection with higher rate of recovery of Gram positive isolates by about 2 folds compared to Gram negative isolates. Whereas the incidence of MDR among BSI was higher in Gram negative isolates compared to Gram positive ones. That was also supported by a study which demonstrated that among BSI, 59% of bacterial isolates were Gram positive however the frequency of MDR in Gram positive isolates was low (19.4%) compared to that in case of Gram negative isolates (34.2%) [23].

It is also important to point out the involvement of *S. aureus* and CoNS in BSI where both pathogens were reported as the most frequently isolated from blood specimens [17, 12], respectively. Despite that our study revealed the superior contribution of *S. aureus* in the occurrence of BSI compared to CoNS. Another study demonstrated that among Gram positive isolates participating in BSI, CoNS (38.8%, n=72) was the most common pathogen followed by *S. aureus* (20.8%) [23].

Concerning the antimicrobial resistance pattern, the effectiveness of vancomycin against *S. aureus* was obvious in the current study in addition to other studies [11]. For example, it was reported that all *S. aureus* isolates were 100% susceptible to vancomycin [24].

In coincidence with the present study, higher incidence of resistance was recorded in Gram negative isolates compared to Gram positive ones [10]. In addition, *E. coli* demonstrated elevated resistance rates to ciprofloxacin and third generation cephalosporins compared to lower resistance towards nitrofurantoin [12]. In another study, *E. coli* exhibited elevated resistance to nalidixic acid and ceftriaxone [20]. Also in a study carried out in Mansoura University Hospitals (Cairo), it was observed that *E. coli* was highly resistant to cefuroxime (96%), ceftriaxone (92%), cefaclor (90%) and ciprofloxacin (76%) whereas lower resistance was recorded against meropenem (40%), imipenem (30%) and amikacin (16%) [25]. Also in agreement with our results, it was reported that *E. coli* exhibited the lowest percentage of MDR despite that it was the predominant uropathogen [18].

Resembling our findings, resistance to β-lactam antibiotics was reported as a major problem in a study carried out by Ibrahim and Hameed [13]. But on the contrary to the present study, they demonstrated lower resistance levels of Gram negative bacteria to amikacin, gentamicin and doxycycline in addition to high sensitivity of Gram positive isolates to macrolides and clindamycin [10]. The variation in the resistance pattern between the current study and other studies indicates this profile is influenced by variable determinants such as the diversity among different geographical regions [23], time during which each study was carried out as well as the study population [11].

Regarding resistance profile of Pseudomonas species and in agreement with the present study an elevated resistance rate was recorded against piperacillin/tazobactam and cefipime whereas higher sensitivity was observed to amikacin in addition to maximum sensitivity to imipenem [26]. Despite that another study reported that *Pseudomonas aeruginosa* was most commonly isolated from male patients, but it showed no resistance either to imipenem or colistin. The same study pointed out low antimicrobial resistance towards ceftazidime, piperacillin/tazobactam and cefipime [22], but these records weren't consistent with the current

findings. This may be attributed to the variation between the detected pathogens in both studies and may indicate emergence of resistance in our community.

The recorded high incidence of MDR among Pseudomonas spp may be justified by the reported selective pressure due to mutations in chromosomal genes that led to production of extended spectrum β-lactamases (ESBL) as well as hyper expression of AmpC gene and the role of the efflux pumps. In addition to another resistance mechanism which is mediated through horizontal transfer of transposable elements that are coding for metallo-β-lactamases. Pseudomonas spp may also gain resistance to antibiotics as a consequence of interference with antibiotic permeability to the cell surface due to biofilm formation [21].

Elevated incidence of resistance to third generation cephalosporins and aztreonam as well as lower resistance rates to carbapenems in the present study might indicate the emergence of ESBL producing organisms in our community due to antibiotic abuse [10]. This is dependent on the fact that ESBLs are defined as Gram-negative bacteria that produce β-lactamases resulting in resistance to first, second and third generation cephalosporins as well as aztreonam whereas they aren't able to confer resistance to carbapenems. ESBLs are also antagonized by inhibitors of β-lactamase such as clavulanic acid [27]. This could justify the obvious decrease in resistance which was recorded in the current study when cefoperazone (third generation cephalosporin) was combined with sulbactam (β-lactamase inhibitor) compared to the recorded elevated resistance against cefoperazone alone.

On the other side, the resistance to carbapenems may be related to efflux pumps and mutations in penicillin binding proteins. These mechanisms might enhance the resistance in case of *Klebsiella pneumoniae*, *P. aeruginosa* and *Acinetobacter baumannii* [28]. Thus the recorded higher resistance in case of Klebsiella spp compared to *E. coli* in the present study may be related to infections with Klebsiella-producing carbapenemase-2 (KPC-2) or Metalloproteinase-1 producing *K. pneumoniae* [11].

Current study also recorded the emergence of resistance against colistin although it is considered the last line of defense against carbapenemase-producing Enterobacteriaceae. That might be attributed to the expression of plasmid-mediated colistin-resistant genes [29]. Also in consistence with our study, the bacterial uropathogens that were recovered from males showed higher incidence of resistance compared to females [18].

The rapid emergence of resistance is a global disaster that coincides with the regression in the discovery of new antibiotics [30]. It is worth to highlight that unreasonable consumption of antibiotics as well as transmission of resistant isolates among patients accounted for the progress in AMR rates [20]. Thus effective infection control measures [31], identification of the resistance mechanisms and the rational use of antibiotics through implementing effective antimicrobial stewardship are essential concerns. This stewardship should depend on assessment of the local prevalence of pathogens and their resistance profile so it could potentially manage the danger of AMR through reducing the selective pressure exerted on sensitive strains [32].

5. Conclusions

Gram negative isolates were more prevalent compared to Gram positive ones. Urinary tract infection was the most common followed by blood stream infection with highest incidence of *E. coli*, *S. aureus* and Klebsiella spp among total isolates. *E. coli* was the most common isolate accounting for urinary tract and wound infection whereas *S. aureus* was most frequently associated with blood stream infection. Males were more frequently subjected to different types of infections compared to females.

Highest incidence of resistance was associated with cephalosporins, followed by penicillin/β-lactamase inhibitors. However Gram positive isolates exhibited the lowest resistance to linezolid and vancomycin whereas colistin was the most effective antimicrobial agent against Gram negative isolates. Despite that the discovery of nitrofurantoin isn't recent but it retained most of its potentials especially against *E. coli* as well as Gram positive isolates.

Elevated frequency of MDR was obvious among Gram negative isolates. Although *E. coli* was the most prevalent pathogen but it showed the least incidence of MDR. Contrarily, Pseudomonas spp exhibited the highest MDR rate. Prevalence of MDR was higher in males except in case of uropathogens. The elevated resistance rates in case of pathogens that were recovered from males reflect the necessity of considering the patient's gender in case of empirical prescription of antimicrobials. Also, the emerging resistance to carbapenems and colistin should also be taken into account and spot light on the importance of effective control measures.

It is necessary to note that antimicrobial therapy should take into account the data regarding the local prevalence of causative pathogens and their antimicrobial resistance profile rather than the universal guidelines. The present study presents a whole vision regarding the antimicrobial resistance pattern for the most frequent bacterial isolates among different specimens as well as essential considerations during empirical antimicrobial therapy. This local prevalence will also aid in establishing an effective antimicrobial stewardship to preserve the potentials of the current antimicrobial agents.

Conflict of Interest

The authors declare that they have no competing interests.

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