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Prevalence of multidrug resistant bacteria in Egyptian hospitalized patients

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ABSTRACT

The growth and spread of pathogenic bacteria resistant to commercial antibiotics is one of the biggest issues facing the world today. The aim of this study was to investigate the rates of antibiotic resistance bacteria that cause infections as well as the prevalence and characteristics of antibiotic prescriptions among hospitalized patients. A total of 330 clinical samples including sputum, blood, pus, wound, and urine were collected from hospitalized female and male patients aged 9 to 82 years. Two hundred pathogenic bacteria were isolated and identified from patients and subjected to antibiotic susceptibility testing. Gram-negative bacteria were much more prevalent than Gram-positive bacteria. The most common bacteria were *Klebsiella pneumonia* and *Staphylococcus aureus*. The Gram-negative and Gram-positive isolates were mainly resistant to amikacin, amoxicillin/clavulanic acid, cefazolin, cefaclor, ciprofloxacin, ceftazidime, cefotaxime, cefepime, gentamicin, levofloxacin, ofloxacin, imipenem, meropenem, doxycycline, and tetracycline. Among the 15 antibiotics, most isolated strains were carbapenem-sensitive. All strains were found to be multi-drug resistant (MDR) bacteria. Therefore, these two strains were identified using 16SrRNA sequencing and registered in the GenBank database with accession numbers, CP 072555.1 and MW 453042.1. It could be concluded that MDR bacteria, both Gram-positive and Gram-negative, are common among hospital patients. All of the identified strains were MDR, however they were carbapenem-sensitive. The most common bacteria were *Staphylococcus aureus* and *Klebsiella pneumonia*. These results emphasize the need to search for contemporary antibiotics to combat these antibiotic-resistant microorganisms.

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Introduction

Resistant microbial infections are often associated with hospital environments due to clinical practice; however, they can also arise in the community as a result of selection pressure from both human and animal antibiotic use. Antimicrobial resistance (AMR) is exacerbated by

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the environment's accumulation of antibiotics (Essack 2021, Sridhar et al. 2021). Moreover, multidrug-resistant (MDR) bacteria, sometimes known as "superbugs," are becoming more and more prevalent, posing a hazard to the health of people, animals, and the environment (Aslam et al. 2018). MDR microorganisms generally show resistance to three or more drugs. Eventually, drug-resistant organisms emerged and developed faster than the discovery of antibiotics (Aslam et al. 2018). Antibiotic resistance is recognized as a global concern by various key organizations, including the WHO, and other international agencies (Michael et al. 2014, Rogers Van Katwyk et al. 2020, Spellberg et al. 2016). Tens of millions of lives are currently at risk, and it presents a significant obstacle to global development and the economy (Hashem et al. 2022, Rogers Van Katwyk et al. 2020). Tanwar et al. (2014) highlighted that while MDR is considered natural phenomena, improper use of antibiotics, unsanitary conditions, improper food handling, and ineffective infection prevention and control procedures all play an integral part in the emergence and promotion of MDR. Furthermore, a considerable increase in immune-compromised situations, such as HIV infection, diabetes, organ transplant recipients, and severe burn patients, has rendered the body a prime target for hospital-acquired infectious diseases, which in turn assisted in the spread of multidrug resistance. According to Exner et al. (2017), the emergence of antibiotic resistance, particularly multidrug resistance (MDR), poses a worldwide concern. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of Gram-positive bacterium that often develops resistance to the antibiotic methicillin (Nepal et al. 2017). However, 60% of Gram-negative bacteria develop resistance to all types of antibiotics, including cephalosporin, carbapenems, and fluoroquinolones (Hawkey et al. 2018, Rajivgandhi et al. 2019). The purpose of this study is to explore the rates of antibiotic resistance bacteria that cause bacterial infections as well as the prevalence and quality of antibiotic prescriptions among hospitalized patients.

Material and methods

Collection of clinical samples and isolation of microbes

Approximately 330 clinical specimens including sputum, blood, pus, wound, and urine were collected from individuals diagnosed with infections based on clinical symptoms and admitted to El-Sahel Teaching Hospital and the National Heart Institute, the general organization for teaching hospitals and institutes (GOTHI), Cairo, Egypt. The patients provided informed consent, and the protocol was permitted and accepted by GOTHI (Ethics code: IME00071). Various specimens were obtained from patients diagnosed with infections based on clinical symptoms. Clinical specimens, including sputum, urine, wounds, pus, and blood were collected from patients with complaints of different infections with an average age of 9 - 82 years, 135 females (40.9%) and 195 males (59.1%). The prevalence of MDR bacteria was 200 (60.6%) in 330 clinical samples, which were then included in the overall analysis. The remaining 130 (39.4%) of samples were negative in bacterial growth and were excluded from this study. Recent work in this respect revealed that the highest prevalence of clinical infections was of bacterial origin (Moon et al. 2022).

Laboratory and clinical data for each subject were acquired and recorded. The Clinical and Laboratory Standards Institute's rules and guidelines were adhered to in all laboratory procedures. The collected specimens were inoculated onto appropriate isolation culture media (blood agar, nutrient, MacConky) purchased from Oxoid Ltd. Co. (UK), and incubated at 37 °C for 24 h. Microbial identification was done primarily based on colony characteristics and Gram-stain reactions. Standard microbiological techniques were followed in the biochemical identification of the isolates (Forbes et al. 2016). Further characterization was done by API 20 kits.

Antibiotic susceptibility test for isolated bacteria for determination of MDR isolates

All bacterial isolates were tested for antibiotic susceptibility on the Mueller-Hinton Agar (MHA) medium using the standard Kirby-Bauer disk diffusion technique, as defined by Clinical Laboratory Standard Institute guidelines (2018). The antibiotics for disc diffusion testing for both Gram-positive and Gram-negative isolates were in the following concentrations: Amikacin (30 µg), Amoxicillin/clavulanic acid (30 µg), Cefepime (30 µg), Cefotaxime (10 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Cefazolin (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Levofloxacin (5 µg), Doxycycline (30 µg), Tetracycline (30 µg), Ofloxacin (5 µg), Cefaclor (30 µg), and were obtained from Oxoid Ltd. Co. (UK). Multidrug-resistant (MDR) microbes were defined as resistant to at least one antibiotic in at least three antimicrobial categories (Magiorakos et al. 2012).

Molecular identification by 16S rRNA PCR

Genomic DNA extraction from the selected bacterial cultures

DNA extractions from bacterial colonies were performed using a sodium dodecyl sulfate (SDS)-based method (Natarajan et al. 2016). The bacterial cell suspension was treated with a lysis buffer containing SDS, trisaminomethane hydrochloric acid (Tris HCl), and

ethylene-diamine tetraacetic acid (EDTA). The cell debris and other impurities were removed in several steps sequentially with simultaneous centrifugation. Chilled ethyl alcohol was used to precipitate the genomic DNA.

The precipitated DNA was collected as a pellet by centrifugation. The pellet was dissolved in TE buffer and stored at -40 ^{o}C until the next use. Primers used for *staphylococcus aureus* were:
27F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-TACGGTTACCTTGTTACGACTT-3′.

However, the primers used for *Klebsiella pneumoniae* were:
27F 5`-AGAGTTTGATCCTGGCTCAG-3` and 1492R 5`-TACGGTTACCTTGTTACGACTT-3`

Amplification

Amplification of 16S rRNA gene using PCR for the PCR reaction, the total reaction volume was 50 µl, containing 5 µl of DNA template, 1U Ampli Taq DNA polymerase, and 10 pmol of each primer (forward primer and reverse primer, purchased from Sigma (St. Louis, MO, USA), 200 µmol of each deoxyribonucleoside triphosphate per liter, 1.5 mmol of $MgCl_2$ per liter, 10 mmol of Tris-HCl (pH 8.8) per liter, 50 mmol of KCl per liter, and 0.1% Triton X100. The PCR conditions were initial denaturation for 5 min at 95°C, 35 cycles for 10 s at 94°C, 30 s at 50°C, and 1 min at 72°C; and a final extension for 5 min at 72°C. An aliquot (2 µl) of PCR products was run in an agarose gel to check for amplified fragments.

Sequencing analysis of 16s rRNA gene

The PCR product was purified using a QIAquick gel extraction kit (Sigma). The purified product (15µl) was sequenced using the ABI Prism DNA sequencing kit, Big Dye Terminator Cycle Sequencing (version 3.0), and the ABI Prism 310 genetic analyzer (Applied Biosystems, USA). The sequence was matched to those in a reference database using an identification software that selected the longest recursive matches for optimal sequence alignment. The reference database sequences were obtained and concatenated from the NCBI GenBank database. The final sequence comparisons with the best matches were done manually.

Data management and analysis

The data was collected and entered in an Excel Spreadsheet; data analysis was performed using Statistical Package for the Social Sciences (SPSS) software ^{TM} (version 20.0, IBM). Bacterial isolates, demographic characteristics, and antibiotics susceptibility were summarized as frequencies and percentages. A Chi square test was used for the comparison between groups, and a P-values < 0.05 was regarded as statistically significant.

Results and Discussion

Percentage of microbial isolates in relation to the source of microbial infection

Two hundred clinical pathogens were isolated from different patients from different specimens, Percentage of microbial isolates in relation to the source of microbial infection showed in Fig. 1. The most common source of microbial infection was in wound specimens with a percentage of 40%, followed by 21% urine specimens, 15.5% sputum, 14.5% pus, and 9% blood isolates.

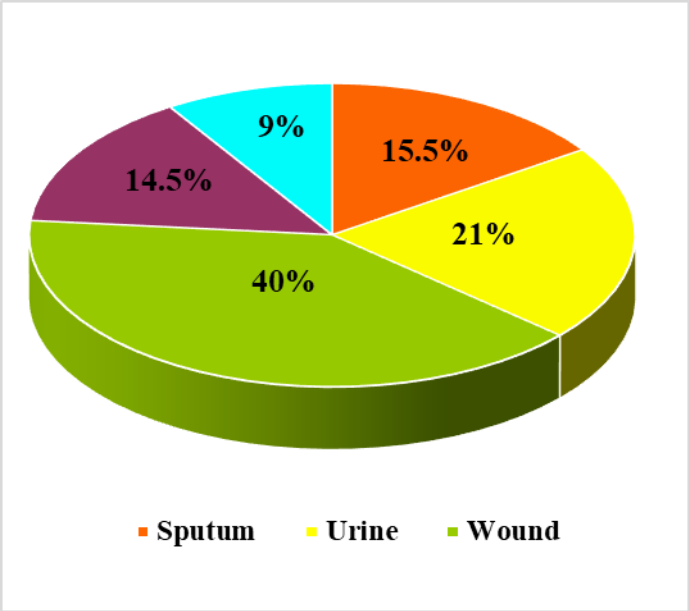

Fig. 1. Percentage of the microbial isolates in relation to the isolation source

Prevalence of microbial Infections in relation to patients' gender

In our study, we examined the prevalence of microbial infections among 200 patients, segregated by gender and type of specimen Table (1) and Fig. (2). The specimens included blood, pus, sputum, urine, and wound samples. For blood samples, out of 18 patients, 10 were male (55.56%) and 8 were female (44.44%). The difference in infection rates between genders was not statistically significant (P=0.739). In the case of pus samples, out of 29 patients, 17 were male (58.62%) and 12 were female (41.38%). Again, the difference in infection rates between genders was not statistically significant (P=0.824). Sputum samples were collected from 31 patients, with a slightly higher prevalence in males (64.52%) compared to females (35.48%). This

Table 1 Prevalence of microbial infections among Patients' Gender[1]

Specimens	Patients	Patients (%)	Male (N)	Female (N)	Male (%)	Female (%)	P-Value
Blood	18	9.00	10	8	55.56	44.44	0.739
Pus	29	14.50	17	12	58.62	41.38	0.294
Sputum	31	15.50	20	11	64.52	35.48	0.042
Urine	42	21.00	25	17	59.52	40.48	0.127
Wound	80	40.00	48	32	60.00	40.00	0.018
Total	200	100.00	120	80	60.00	40.00	

[1] Overall P-Value:0.979

was the only specimen type to show a statistically significant difference between genders (P=0.042). Urine specimens were collected from 42 patients, with males comprising 59.52% and females making up the remaining 40.48%. The difference in infection rates was not statistically significant (P=0.127). Lastly, wound infections were reported in 80 cases, distributed between males and females (60% and 40%, respectively, resulting in no gender disparity in infection rates (P=1). In conclusion, our overall analysis across all specimen types indicated no significant gender-based differences in microbial infection rates among this patient cohort (Overall P-Value=0.979).

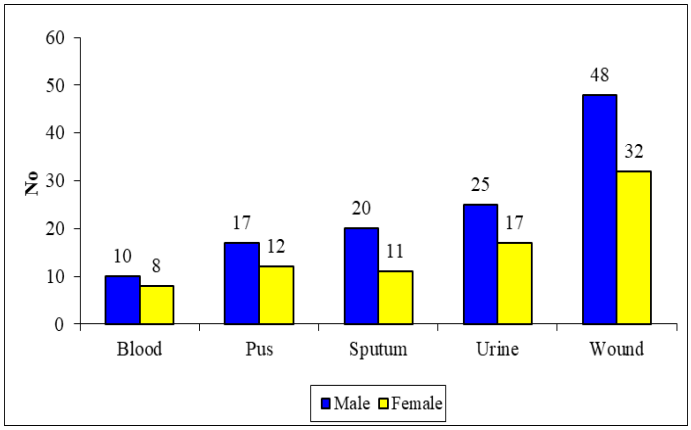

Fig. 2. The prevalence of microbial infections among patients' gender

Distribution of microbial infections in relation to patients' age groups

The age group of 25-40 years, the highest prevalence rate was observed in pus samples at 12 patients with a percentage of 41.00% from all pus samples isolated, followed by blood at 7.00% with 38.8% from blood comparing with the percentage number of patients in other sources of isolates in the same age group. For the age group of 41-55 years, sputum samples showed the highest infection rate at 45.00% of sputum isolate in 6 patients. Interestingly, in the younger age group of 9-24 years, urine samples exhibit a notable prevalence rate at 4.00%. In individuals over 55 years old, pus infections were the most prevalent at 7 samples which represent 24.00%, as shown in Table (2) and Fig. (3).

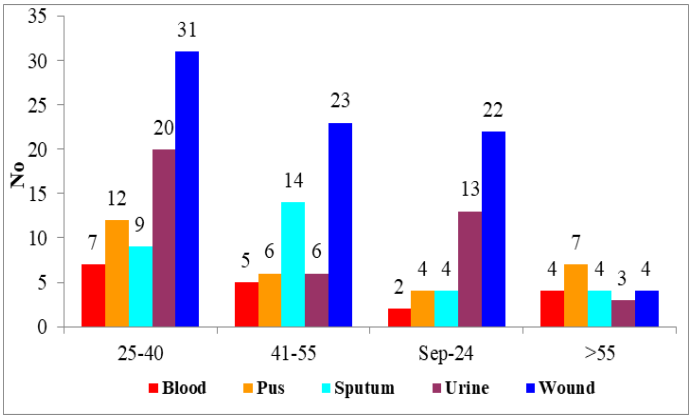

Fig. 3. The prevalence of microbial Infections among patients' age groups

When considering all age groups combined, urine samples demonstrate the highest overall infection rate at 42.00%, suggesting that urinary tract infections may be more common regardless of age compared to other types of infections studied here. The P-values indicate the statistical significance of the differences among different age groups for each specimen type. Only sputum and wound specimens showed statistically significant differences among different age groups (P=0.025 and P=0.021, respectively). This suggests that factors related to these two specimen types may vary significantly with age compared to blood or urine where no significant difference was observed (P=0.434 and P=0.280, respectively). These findings could guide healthcare professionals towards more targeted monitoring and

treatment strategies based on patient's ages for certain types of infections. The overall P-value of 0.021 indicates that there is a statistically significant difference in the prevalence of microbial infections among the different age groups across all specimen types. This further emphasizes the importance of age as a factor in the prevalence of these infections.

Characterization and identification of bacterial isolates

As shown in Table (3), all bacterial isolates were characterized by phenotypic and biological characteristics based on morphological and biochemical characterization (Federal Drug Administration 2001). According to the characterization by biochemical tests and further characteristics obtained by API 20 kits. In view of the presented data from (Fig. 4), the identified bacteria were *Klebsiella pneumonia* represented (31%), followed by *Staphylococcus aureus* (25%), *Pseudomonas aeruginosa* (13.5%), *Escherichia coli* (10.5 %), *Acinetobacter baumannii* (7.5%), *Proteus mirabilis* (5.5 %), *Enterococcus faecalis* (4.5 %), *Proteus vulgaris* (1.5%), and finally *Serratia marcescens* (1 %). All these organisms obtained herein were from clinical specimens as shown in Table (4).

Prevalence the source of bacterial isolates according to bacteria gram staining technique

The Gram-staining technique was performed and reported 29.5% Gram-positive bacterial isolates, 70.5% Gram-negative bacterial isolates as shown in Fig. (5). Table (5) provides a detailed breakdown of the prevalence of various types of microbial isolates across different specimen types. For blood samples, there are 6 (33%) Gram-negative and 12 (67%) Gram-positive isolates. In pus samples, there are 23 (79%) Gram-negative and 6 (21%) Gram-positive isolates. Sputum samples show a distribution of 26 (84%) for Gram-negative and 5 (16%) for Gram-positive bacteria. Urine samples have a distribution of 32 (76%) for Gram-negative and 10 (24%) for Gram-positive bacteria. Wound samples show that there are more Gram-negative than Gram-positive with counts of 54 (68%) for Gram-negative and 26 (33%) for Gram-positive bacteria. The p-value given at the end of the row is noted as '0.002', indicating a statistically significant difference in the distribution of these bacteria across different sample types. This suggests that the type of microbial isolate (Gram-negative or Gram-positive) varies significantly depending on the type of sample (blood, pus, sputum, urine, or wound).

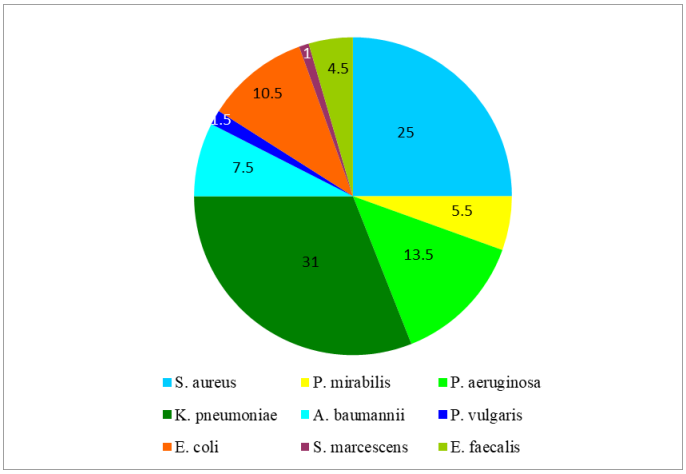

Fig. 4. Percentage of bacterial agents isolated from different clinical samples.

Antibiotic susceptibility pattern of pathogenic bacterial isolates

The increased incidences of infections caused by drug-resistant bacteria become a prime clinical importance (Prestinaci et al. 2015), and the antibiotics that are used for repeated empirical treatment might be the reason for the development of high antibiotic resistance (Ayukekbong et al. 2017). This clearly demonstrated the need to select and identify antibiotic-resistant bacteria to understand their types and their resistance, as antibiotic-resistant bacteria remain a serious health concern worldwide. In the current study, all isolated and identified bacteria were examined against 15 different antibiotics (Table 6). From the antibiotic susceptibility profile of tested isolates, the antibiotic resistance pattern of *S. aureus* showed various resistances to amoxicillin/clavulanic acid, cefazolin, cefotaxime, cefepime, amikacin, ofloxacin, doxycycline, cefaclor, ceftazidime, levofloxacin, ciprofloxacin, gentamicin, and tetracycline. Similar antibiotic resistance profiles of *S. aureus* bacterium were previously published (El-Kattan and Allam 2021, Onyeka et al. 2021, Zheng et al. 2021). *S. aureus* is one of the most frequent multi-drug-resistant bacterial pathogens causing different infections (Mancuso et al. 2021). The mechanism of *S. auraus* resistance was due to genetic factors, mutations, thickening of cell walls, production of β-lactamase, and modification of specific site(s) receptors for antibiotics (Hiramatsu et al. 2014). *E. faecalis* strains showed strong resistance to cefaclor, cefotaxime, ofloxacin, tetracycline, ceftazidime, doxycycline, amikacin, cefazolin, and gentamicin, while 55.6 % of *E. faecalis* were resistant to amoxicillin/clavulanic acid, ciprofloxacin, cefepime, and levofloxacin. Similar results were reported in previous studies (Goda et al. 2023, Abdelkareem et al. 2017). Moreover, Farman et al. (2019) reported that multi-drug

Table 2 Prevalence of microbial Infections among patients' age groups.

Specimens	25-40	41-55	9-24	>55	Total	Patients (%)	P-Value
Blood	7.00	5.00	2.00	4.00	18.00	9.00	0.334
Pus	12.00	6.00	4.00	7.00	29.00	14.50	0.424
Sputum	9.00	14.00	4.00	4.00	31.00	15.50	0.025
Urine	20.00	6.00	13.00	3.00	42.00	21.00	0.280
Wound	31.00	23.00	22.00	4.00	80.00	40.00	<0.001
Total	79.00	54.00	45.00	22.00	200.00	100.00	0.021

Table 3 Biochemical characteristics of bacterial isolates

Group	Isolates Identification	Biochemical characteristics																					
		Catalase	Coagulase	Indole	Methylered	Oxidase	Voguisproskauer	Citrate	H_2S production	Urease	Motility	Lysine	Gelatin	Glucose	Lactose	Maltose	Mannitol	Sucrose	Salicin	L-arbinose	D-Sorbitol	Blood hemolysis	
1	***Staphylococcus aureus***	+	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+	
2	***Enterococcus faecalis***	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	
3	***Escherichia coli***	+	-	+	+	-	-	-	-	-	+	-	-	+	+	+	+	-	-	+	+	-	
4	***Acinetobacter baumannii***	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	
5	***Pseudomonas aeruginosa***	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-	
6	***Klebsiella pneumoniae***	+	-	-	-	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	
7	***Proteus vulgaris***	+	-	+	+	-	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-	
8	***Proteus mirabilis***	+	-	-	+	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	
9	***Serratia marcescens***	+	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+	-	

Table 4 Total count and frequency of bacterial isolates from different clinical specimen

source Isolate	Total Frequency%	Sputum	Urine	Wound	pus	blood
S. aureus	25	5 (2.5)	7 (3.5)	21 (10.5)	5 (2.5)	12 (6)
P. mirabilis	5.5	0	3(1.5)	7 (3.5)	1 (0.5)	0
P. aeruginosa	13.5	0	5 (2.5)	12 (6)	4.5	1 (0.5)
K. pneumoniae	31	21 (10.5)	11 (5.5)	18 (9)	8 (4)	4 (2)
A. baumannii	7.5	0	4 (2)	8 (4)	2 (1)	1 (0.5)
P. vulgaris	1.5	0	0	3 (1.5)	0	0
E. coli	10.5	5 (2.5)	7 (3.5)	6 (3)	3(1.5)	0
S. marcescens	1	0	2 (1)	0	0	0
E. faecalis	4.5	0	3 (1.5)	5 (2.5)	1 (0.5)	0

Table 5. Prevalence of microbial isolates according to bacteria gram staining

Characteristic	Blood N = 18 (%)	Pus N = 29 (%)	Sputum N = 31 (%)	Urine N = 42 (%)	Wound N = 80 (%)	p-value[2]
Isolates						0.002
G-ve	6 (33%)	23 (79%)	26 (84%)	32 (76%)	54 (68%)	
G+ve	12 (67%)	6 (21%)	5 (16%)	10 (24%)	26 (33%)	

[2]Pearson's Chi-squared test

Table 6. Percentage of antibiotic resistance pattern among different bacterial isolates

Antibiotic discs	*S. aureus*	*E. faecalis*	*A. baumannii*	*E. coli*	*K. pneumoniea*	*Pr. mirabilis*	*Pr. vulgaris*	*P. aeruginosa*	*S. marcescens*
Amikacin 30	80	66.7	60	52.4	80.6	36.4	33.3	66.7	0
Amoxicillin/clavulanic acid 30	100	55.6	80	90.5	100	63.6	100	100	100
Cefazolin 30	100	66.7	100	47.6	90.3	54.5	66.7	63	100
Cefaclor 30	90	100	100	42.9	91.9	63.6	100	81.5	50
Ciprofloxacin 5	70	55.6	40	80.9	71	81.8	66.7	88.9	0
Ceftazidime 30	82	88.9	100	100	77.4	63.6	100	66.7	100
Cefotaxime 10	100	100	66.7	85.7	82.3	100	100	100	100
Cefepime 30	100	55.6	60	47.6	79	36.4	33.3	100	0
Gentamicin 10	66	66.7	46.7	95.2	75.8	81.8	100	55.6	100
Levofloxacin 5	76	55.6	53.3	52.4	100	54.5	66.7	92.6	50
Ofloxacin 5	80	100	80	57.1	95.2	100	100	81.5	100
Imipenem 10	28	0	20	38.1	29	9.1	0	33.3	0
Meropenem 10	32	0	26.7	42.9	37.1	18.2	0	11.1	0
Doxycycline 30	80	88.9	100	66.7	66.1	63.6	100	100	50
Tetracycline 30	50	100	93.3	57.1	82.3	90.9	100	100	100

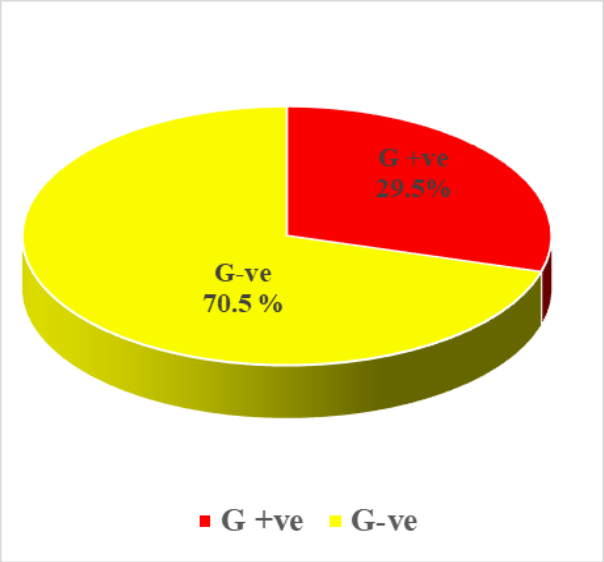

Fig. 5. Prevalence of microbial isolates according to gram staining

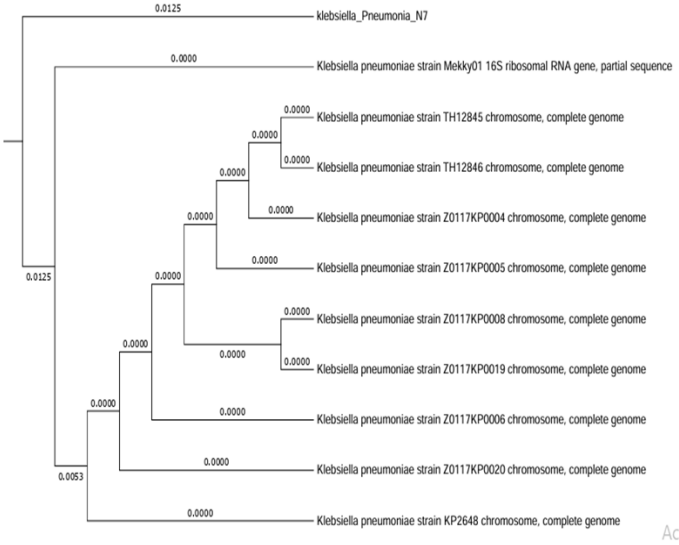

Fig. 6. Phylogenetic tree, demonstrating the species affiliation of the isolated Klebsiella pneumoniae strain based on 16S rRNA gene sequences.

resistance of *E. faecalis* is mostly owing to the strain's ability to acquire and convey the antibiotic resistance genes. On the other hand, *K. pneumoniae* isolates showed the highest resistance to ampicillin, cefaclor, amoxicillin, azithromycin, erythromycin, and cefotaxime. In our study *K. pneumonia* showed high antibiotic resistance to amoxicillin/clavulanic, levofloxacin, ofloxacin, cefaclor, cefazolin, cefotaxime, tetracycline, amikacin, cefepime, ceftazidime, gentamicin, ciprofloxacin and doxycycline, which was consistent with previous findings (Naqid et al.2020; Gomatheswari and Jeyamurugan 2017). According to Bhagirath et al. (2019), *K. pneumoniae* is one of the best-known nosocomial pathogens that has recently been identified as an MDR and pan-drug-resistant problem, indicated a range of findings that were consistent with earlier findings. The antibiotic resistance profile of *P. aeruginosa* isolates exhibited a high antibiotic resistance to amoxicillin/clavulanic acid, cefotaxime, cefepime, doxycycline, tetracycline, levofloxacin, ciprofloxacin, cefaclor, ofloxacin, amikacin, ceftazidime, cefazolin and gentamicin, which was consistent with previous reports (Mohamed et al. 2019, Bhalchandra et al. 2018). *P. aeruginosa* isolates showed high resistance to amoxicillin/clavulanic acid, cefotaxime, cefepime, doxycycline and tetracycline, levofloxacin, ciprofloxacin, cefaclor, ofloxacin, amikacin, ceftazidime and cefazolin which was in agreement with previous findings (El-Kattan et al. 2022).

In this concern, Imanah et al. (2017) found that *P. aeruginosa* strain is resistant to many antibiotics and is capable of easily acquired antibiotic resistance. Moreover, Goda et al. (2023) showed that *P. aeruginosa* has a high potential to evolve multidrug resistance ability. Worldwide, *E. coli* is becoming more resistant to antibiotics. It has shown strong resistance to ceftazidime, gentamicin, amoxicillin/clavulanic acid, cefotaxime, ciprofloxacin, and doxycycline. These results are consistent with those of Saha et al. (2017) and Yakha et al. (2015). The antibiotic profile of *A. baumannii* isolates found high resistance to cefazolin, cefaclor, ceftazidime, doxycycline, tetracycline, amoxicillin/clavulanic acid, ofloxacin, cefotaxime, amikacin, and cefepime, similar to those reported by Bazaid et al. (2022) and Fayyaz et al. (2015). *P. mirabilis* isolates also exhibit varied levels of resistance to the antibacterial drugs, cefotaxime, ofloxacin, tetracycline, ciprofloxacin, gentamicin, amoxicillin/clavulanic acid, cefaclor, ceftazidime, doxycycline, cefazolin, and levofloxacin, similar to the results reported by Mirzaei et al. (2019). *P. mirabilis* may cause several opportunistic and nosocomial infections due to their virulence factors (Hasan et al. 2021). Meanwhile, the antibiotic resistance pattern of *Pr. vulgaris* isolates was noted herein to amoxicillin/clavulanic acid, cefaclor, ceftazidime, cefotaxime, gentamicin, ofloxacin, doxycycline, tetracycline, cefazolin, ciprofloxacin, and levofloxacin. The antibiotic resistance of *Pr. Vulgaris* was reported previously by several authors (Talebi et al. 2023, Zhang et al. 2021). The increasing levels of multi-drug resistance *Proteus* species isolates were reported previously (Ronanki et al. 2022). *Proteus* species are among the most commonly implicated pathogens in hospital as well as community-acquired infections. *S. marcescens* exhibited resistance against amoxicillin/clavulanic acid, cefazolin, ceftazidime, cefotaxime, gentamicin, ofloxacin, and tetracycline; these findings are in agreement with a previous study (Goda et al. 2023). *S. marcescens* was traditionally known as non-pathogenic, however it has emerged as a key opportunistic pathogen identified in nosocomial infection (Ghaith et al. 2018). Moreover, Cristina et al. (2019) reported that *S. marcescens* associated with hospital outbreaks or epidemic events are commonly resistant to several antibiotics. Most isolated strains were sensitive to meropenem and imipenem. Khanam et al. (2018) previously observed that *Carbapenems* are still sensitive to growing resistance in both gram-positive and gram-negative. The current study confirmed that the multidrug resistance ability of the identified bacteria poses a serious concern for public health in the twenty-first century (Jansen and Anderson 2018). ***Molecular characterization by 16S rRNA***

The 16S ribosomal RNA (16S rRNA) sequencing has been reported to be more accurate in the identification of Gram-ve and Gram+ve bacteria (Kumar et al. 2020, Rawy et al. 2020). In the current study, the most frequently bacterial isolates which showed the MDR ability were identified using 16S rRNA gene sequencing methods and the results were compared with the GeneBank database, with a similarity of 97 % of standard *K. pneumoniae* and 99% of standard *S. aureus* (Fig. 6,7). Thereafter, the *K. pneumoniae* and *S. aureus* strains were registered in the GenBank database with accession numbers, CP 072555.1 and MW 453042.1, respectively. This agrees with similar outcomes found in 16S rRNA sequencing used to identify bacterial isolates from different sources (Alsanie et al. 2018; Alsanie 2020, Zakerbostanabad 2019).

Conclusion

The current study identified nine bacterial strains in the various samples. Gram-negative (-) bacteria are far more common than Gram-positive (+) bacteria, and the most common bacteria were *Staphylococcus aureuspneumoniae*. *Klebsiella pneumonia*. With the exception of carbapenem, all of the isolated strains were resistant to all the tested antibiotics. Thus, this study made clear that these bacteria have the potential to cause several infections in Egyptian

patients. Therefore, we must immediately address the multidrug-resistant bacteria, a serious health risk, by employing alternative therapies that would lessen our dependency on antibiotics.

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Ethical approval: All laboratory procedures were performed according to the guidelines and regulations of the Clinical and Laboratory Standards Institute (CLSI). The patients provided informed consent, and the protocol was permitted and approved by the ethics committee of the General Organization for Teaching Hospitals and Institutes, Cairo, Egypt (Ethical approval code: IME00071).

Author's Contributions

Conceptualization: H.M.G., H.I.M., M.A.A.; Investigation: A.M.M., N.E.; Supervision: H.S.G., M.A.A.; Writing-original draft: A.M.M., N.E., H.I.Y., Reviewing, and editing: H.M.G., and M.A.A.

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