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Prevalence of Pathogenic Bacterial Isolates Infecting Wounds and their Antibiotic Sensitivity

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Abstract

Wound infection is one of the health problems that are caused by pathogenic bacteria and antibiotics resistance profiles of Gram negative bacteria increased treatment cost especially in diabetic patients. Thus, the objective of this work was to isolate, purification and identification of pathogenic bacteria from wound infection then determination of susceptibility testing against antibiotics. 41 isolated from wound infections were collected from microbiology laboratory of Zagazig University Hospitals and some private microbiology laboratory Cairo, Egypt. Bacterial isolates were identification by API 20E Enterobacteriaceae. These pathogenic bacterial isolates belong to these genera (*Klebsiella, Enterobacter, Pseudomonas, Providencia, Serratia* and *Citrobacter*). The frequency of the bacterial isolates was 24%, 20%, 14%, 12%, 6% and 6% of the bacterial pathogens isolates in this study respectively. Finally, the antimicrobial susceptibility was performed by disc diffusion method against 12 types of antibiotics covered all mode of action of bacteria.

Keywords: Pathogenic bacterial; Wound infection; Multidrug resistance; Susceptibility patterns; Gram negative bacteria

Introduction

Wounds infection by bacteria and resistance to common antibiotics are the common post-surgical and medical challenges. Wounds bacterial contamination are the common hospital acquired infections causing more than 80% of the mortality [1]. The most common bacterial genera infecting wounds are *Enterococci, Escherichia, Pseudomonas, Klebsiella, Enterobacter, Proteus and Acinetobacter* [2,3]. Wounds infection have been a recognized as the most critical problem especially in the presence of foreign materials that increases the risk of serious infection even with relatively small bacterial infection [4]. Nosocomial infection is usually higher in burn patients that correlate with other factors like nature of burn injury, age of patient, extent of injury and burn depth. Other microbial factors such as type, number of organisms, enzymes, toxins production, colonization of the burn wound site, systemic dissemination of the colonizing organisms, have a strong effect on severity of bacterial wound infection [5,6]. As well as, widespread using of vast groups of antibiotics together with the length of time causes a significant development of antibiotic resistance to wound infecting bacteria [7], that subsequently increase the complications and costs of treatment [8].

Thus, the objective of this work was to isolate and characterize various bacterial isolates infecting wounds, and determination of their susceptibility to various common antibiotics.

Material and Methods

Sample collections

Swabs from abscesses lesions or pus were collected from microbiology lab. It was cultivated immediately without delay. Each sample was inoculated into nutrient agar medium and McConkey agar media. The plate cultures were incubated for 24 h at 30-37°C [5] and the growth was observed thoroughly.

Identification of isolated pathogenic bacteria

Identification of Gram negative pathogenic bacterial isolates were carried out by API 20E Enterobacteriaceae (BioMérieux Co.). API system has been recognized as a rapid test for bacterial identification. The strips were inoculated by single colony in the suspension media and incubated at 35-37 °C for 18-24 h.The results were recorded based on the special chart, three tests were given a code number, the obtained seven digit numbers were expressed to the corresponding organisms regarding to the API index.

Antimicrobial susceptibility of the pathogenic bacterial isolates

The susceptibility of the recovered bacterial isolates to antibiotics was determined using disc diffusion assay, Kirby-Bauer method [9] as described in of guidelines of the National Committee for Clinical Laboratory Standards [10]. Discs of Amikacin 30 µg, Amoxicillin/ Clavulanic 20/10 µg, Ceftazidime 30 µg, Ciprofloxacin 5 µg, Colistinsulphate 10 µg, Cefotaxim 30 µg, Cefepime 30 µg, Nitrofurantoin 300 µg, Levofloxacin 5 µg, Rifampicin 5 µg, Sulphamethoxazole/Trimethoprim 25 µg and Tobramycin 10 µg

(Oxoid, ThermoFisher, Scientific, USA), were used. After incubation, the diameter of inhibition zone around each disk was measured and the organisms were nominated as sensitive (S), resistant (R) and intermediate resistant (MR).

Results and Discussion

Isolation, identification and prevalence of pathogenic bacterial isolates

Bacterial wound contamination is a serious problem in the hospital and the treatment of wound infections remain a significant concern for surgeons. The risk of developing wound infection depends on the number of bacteria colonies on the wound. The problem has been magnified due to the unrestrained and rapidly spreading resistance to the available array of antimicrobial agents. Fifty cases from wound swabs were collected from different types of wounds, cultured on nutrient and MacConkey media. Fifty bacterial isolates were isolated from wounds infection out of the total cases. These bacterial isolates were identified based on the API 20E system. From the results, there was 41 cases (82%) recorded as a positive bacterial growth and only 9 samples were recorded as negative bacterial growth. Gram positive bacteria already isolated in nutrient agar media but the majority for Gram negative bacteria so that used the Gram negative bacteria for application. The identified forty-one pathogenic bacterial isolates were belonging to sex genera (Table 1). These genera were *Klebsiella pneumoniae* (12 isolates; 24%) followed by *Pseudomonas fluorescens* (10 isolates; 20%), *Providencia stuartii* (7 isolates; 14%) and *Enterobacter cloacae* (6 isolates; 12%) and *Serratia rubidaea* and *Citrobacter freundii* (3 isolates; 6%). Similarly, high percentage of microbial growth was reported by Mama et al. 2014 [11], 91.6% of culture was positive microbial growth and 12.7% had no bacterial growth. Coincident results for *K. pneumonia* (24%), *P. fluorescens* (20%), *P. stuartii* (14%) and *E. cloacae* (12%), *S. rubidaea* and *C. freundii* (6%) was reported [12]. Among the recovered isolates, *Pseudomonas* species were the most common isolates (48.9%) followed by *Citrobacter* spp (13.3%), *Enterobacter* spp (11.1%), *Proteus vulgaris* (6.6%), *Klebsiella* spp (2.2%) and *Serratia rubidia* (2.2%) infecting wounds [12].

Bacterial Isolates	Total No.	Percentage %
Klebsiella pneumonia	12	24%
Pseudomonas fluorescence	10	20%
Providencia stuartii	7	14%
Enterobacter cloacae	6	12%
Serratia rubidaea	3	6%
Citrobacter freundii	3	6%

Table 1: The different species of bacteria isolated from wound infection.

Antibiotic susceptibility of the pathogenic bacterial isolates

The antibiotic sensitivity of isolated bacterial strains was carried out by Kirby-Bauer disk diffusion assay against 12 antibiotics (Table 2). Out of the forty-one bacterial isolates, *Pseudomonas fluorescens* was a completely resistance to all the tested antibiotics. Five pathogenic bacterial isolates belong to the genera *Klebsiella, Pseudomonas, Providencia, Enterobacter* and *Serratia* showed a strong resistance to the tested antibiotics by about 91.67%. Seven bacterial isolates belong to *Klebsiella pneumoniae* (No.9), *Pseudomonas fluorescens* (No.16, 19, 21, 22) and Citrobacter freundii (No. 39, 40) showed 83.33% resistance to all tested antibiotics. Whereas four pathogenic bacterial isolates of Gram negative bacilli namely; *K. pneumoniae* (No.3,12), *P. fluorescens* (No. 13) and *E. cloacae* (No. 30) showing 75% resistance to the utilized antibiotic. Four bacterial isolates belonging to *K. pneumoniae* (No. 6), *P. stuartii* (No. 28,29) and *E. cloacae* (No. 34) showed 66.67% resistance to the different tested antibiotics. While three bacterial isolates belong to Gram negative bacilli namely *K. pneumoniae* (No. 7), *P. fluorescens* (No. 20) and *P. stuartii* (No. 24) showed 58.3% resistance to the tested antibiotics. Also, three pathogenic bacterial isolates *K. pneumonia* (No. 10), *E. cloacae* (No. 33) and *C. freundii* (No. 90) showed 50% resistance to the tested antibiotic. Three pathogenic bacterial isolates *K. pneumoniae* (No.4,5) and *P. stuartii* (No. 27) showed 41.67% resistance to the different tested antibiotics and six bacterial isolates namely *K. pneumoniae* (No. 8), *P. fluorescens* (No. 18*), P. stuartii* (No. 25,26) and *S. rubidaea* (No. 38) showed 33.33% resistance. In addition, *K. pneumoniae* (No. 11), *E. cloacae* (No. 35) and *S. rubidaea* (No. 37) showed 25% resistance to the tested antibiotics. While *E. cloacae* (No.32) and *S. rubidaea* (36) showed 16.67% antibiotic resistance.

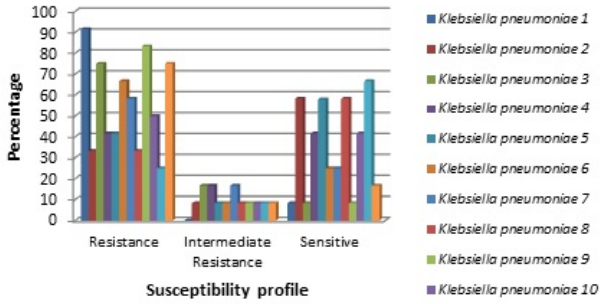

Figure 1: Profile of antibiotics antibiotic susceptibility of *Klebsiella pneumoniae* isolates (Resistance, Intermediate and Sensitive).

The frequency antibiotic resistance of *K. pneumonia* isolates was summarized in Figure 1. *K. pneumoniae* isolate No. 1 was only sensitive to Colistin Sulphate, with moderate resistant to the other experimented antibiotics, while, and isolate No. 2 of *K. pneumoniae* was sensitive to seven antibiotics (Amikacin, Amoxicillin\Clavulanic acid, Ciprofloxacin, Colistin Sulphate, Nitrofurantoin, Levofloxacin and Tobramycin); with moderate resistant to Ceftazidim, Isolate No. 3 of *K. pneumoniae* was sensitive to Colistin Sulphate and moderately resistant to Ciprofloxacin and Levofloxacin. *K. pneumoniae* (No.4) isolate was sensitive to Amikacin, Ciprofloxacin, Colistin Sulphate, Levofloxacin and Tobramycin with moderate resistance to Amoxicillin \Clavulanic acid and Nitrofurantoin. *K. pneumoniae* (No.5) isolate was sensitive to six types of antibiotics (Amikacin, Amoxicillin\Clavulanic acid, Ciprofloxacin, Colistin Sulphate, Levofloxacin and Tobramycin) and moderate resistant to Nitrofurantoin. *K. pneumoniae* (No.6,7) isolates was sensitive (Amoxicillin\Clavulanic acid, Ciprofloxacin and Levofloxacin) while *K. pneumoniae* (No.6) moderate resistant against Nitrofurantoin and K. pneumoniae (No.7) moderately resistant against Cefotaxim and Nitrofurantoin. *K. pneumoniae* (No.8) isolate was

sensitive to seven types of antibiotics (Amikacin, Ceftazidim, Ciprofloxacin, Colistin Sulphate, Nitrofurantoin, Levofloxacin and Tobramycin) and moderately resistant against Amoxicillin\ Clavulanic acid. While *K. pneumoniae* (No.9) isolate was sensitive only to Colistin Sulphate and moderately resistant against Cefepime. Also *K. pneumoniae* (No.10) isolate was sensitive to (Amikacin, Colistin Sulphate, Nitrofurantoin, Levofloxacin and Sulphamethoxazole/ Trimethoprim) and moderately resistant against Ciprofloxacin. *K. pneumoniae* (No.11) isolate was sensitive to eight types of antibiotics (Amikacin, Ceftazidim, Ciprofloxacin, Colistin Sulphate, Cefotaxim, Nitrofurantoin, Cefepime and Levofloxacin) while moderately resistant against Tobramycin. *K. pneumoniae* (No.12) isolate was sensitive to Colistin Sulphate and Nitrofurantoin with moderate resistant to Amikacin.

Isolate No.	Species of bacteria	Mean inhibition zone (mm)\Reaction to antibiotics												species Resistance % (IR+R)
		1	2	3	4	5	6	7	8	9	10	11	12	
		Susceptibility breakpoint												
		AK ≥ 17	AMC ≥ 18	CAZ ≥ 18	CIP ≥ 21	CT ≥ 11	CTX ≥ 23	F ≥ 17	FEP ≥ 18	LEV ≥ 17	RD ≥ 20	SXT ≥ 16	TOB ≥ 15	
1	*Klebsiella pneumoniae*	6\R	7\R	6\R	6\R	13\S	6\R	6\R	6\R	6\R	7\R	6\R	6\R	91.6
2	*Klebsiella pneumoniae*	20\S	19\S	17\IR	31\S	13\S	8\R	21\S	10\R	27\S	12\R	6\R	15\S	33.3
3	*Klebsiella pneumoniae*	6\R	6\R	6\R	19\IR	13\S	6\R	14\R	6\R	16\IR	7\R	6\R	6\R	75
4	*Klebsiella pneumoniae*	21\S	17\IR	10\R	25\S	13\S	6\R	16\IR	10\R	21\S	9\R	6\R	17\S	41.6
5	*Klebsiella pneumoniae*	23\S	18\S	11\R	25\S	12\S	6\R	16\IR	10\R	21\S	9\R	6\R	16\S	41.6
6	*Klebsiella pneumoniae*	6\R	18\S	12\R	22\S	10\R	6\R	16\IR	11\R	20\S	7\R	6\R	6\R	66.6
7	*Klebsiella pneumoniae*	6\R	20\S	14\R	22\S	10\R	16\IR	15\IR	7\R	20\S	7\R	6\R	6\R	58.3
8	*Klebsiella pneumoniae*	19\S	14\IR	20\S	21\S	17\S	6\R	18\S	12\R	20\S	9\R	6\R	20\S	33.3
9	*Klebsiella pneumoniae*	6\R	9\R	6\R	6\R	12\S	13\R	9\R	17\IR	6\R	7\R	6\R	6\R	83.3
10	*Klebsiella pneumoniae*	19\S	8\R	6\R	18\IR	12\S	7\R	17\S	10\R	24\S	9\R	6\S	12\R	50
11	*Klebsiella pneumoniae*	20\S	9\R	20\S	25\S	11\S	25\S	17\S	21\S	25\S	8\R	8\R	14\IR	25
12	*Klebsiella pneumoniae*	15\IR	13\R	14\R	6\R	12\S	6\R	18\S	8\R	7\R	11\R	6\R	6\R	75
13	*Pseudomonas fluorescens*	15\ IR	6\R	11\R	18\ IR	15\S	6\R	6\R	20\S	12\R	18\ IR	6\R	17\S	75
14	*Pseudomonas fluorescens*	6\R	6\R	10\R	15\R	9\R	6\R	6\R	14\R	6\R	6\R	6\R	6\R	100
15	*Pseudomonas fluorescens*	11\R	17\ IR	6\R	6\R	13\S	7\R	8\R	7\R	16\ IR	8\R	6\R	6\R	91.67
16	*Pseudomonas fluorescens*	19\S	6\R	6\R	16\ IR	14\S	6\R	6\R	6\R	8\R	11\R	6\R	10\R	83.33
17	*Pseudomonas fluorescens*	6\R	6\R	6\R	6\R	13\S	6\R	15\ IR	6\R	6\R	7\R	6\R	6\R	91.67
18	*Pseudomonas fluorescens*	18\S	21\S	17\ IR	26\S	6\R	21\ IR	21\S	18\S	18\S	29\S	24\S	12\R	33.33
19	*Pseudomonas fluorescens*	14\R	6\R	9\R	10\R	12\S	6\R	6\R	9\R	7\R	9\R	6\R	15\S	83.33
20	*Pseudomonas fluorescens*	20\S	18\S	21\S	6\R	11\S	6\R	18\S	9\R	8\R	10\R	6\R	11\R	58.33
21	*Pseudomonas fluorescens*	6\R	6\R	6\R	6\R	12\S	7\R	17\S	6\R	6\R	7\R	6\R	6\R	83.33
22	*Pseudomonas fluorescens*	6\R	6\R	12\R	28\S	6\R	6\R	7\R	9\R	25\S	6\R	6\R	6\R	83.33
23	*Providencia stuartii*	15\ IR	16\ IR	6\R	6\R	13\S	6\R	9\R	6\R	15\ IR	11\R	6\R	6\R	91.67
24	*Providencia stuartii*	22\S	6\R	6\R	30\S	8\R	6\R	10\R	6\R	34\S	14\R	22\S	18\S	58.33
25	*Providencia stuartii*	20\S	10\R	18\S	24\S	18\S	23\S	9\R	20\S	21\S	9\R	17\S	13\ IR	33.33
26	*Providencia stuartii*	18\S	12\R	26\S	22\S	6\R	28\S	8\R	25\S	24\S	11\R	21\S	19\S	33.33
27	*Providencia stuartii*	22\S	14\ IR	21\S	30\S	8\R	21\ IR	11\R	25\S	26\S	8\R	20\S	16\S	41.67

28	*Providencia stuartii*	19\S	7\R	6\R	25\S	13\S	7\R	16\ IR	13\R	22\S	6\R	6\R	13\ IR	66.67
29	*Providencia stuartii*	15\ IR	6\R	6\R	27\S	15\S	7\R	19\S	10\R	22\S	6\R	6\R	7\R	66.67
30	*Enterobacter cloacae*	17\S	18\S	6\R	15\R	6\R	14\R	12\R	11\R	15\IR	10\R	6\R	8\R	75
31	*Enterobacter cloacae*	6\R	6\R	6\R	6\R	12\S	6\R	6\R	6\R	6\R	6\R	6\R	6\R	91.6
32	*Enterobacter cloacae*	24\S	16\IR	24\S	17\IR	17\S	26\S	11\R	28\S	21\S	7\R	16\S	17\S	16.6
33	*Enterobacter cloacae*	6\R	17\IR	32\S	18\IR	16\S	6\R	6\R	6\R	22\S	12\R	18\S	8\R	50
34	*Enterobacter cloacae*	13\R	6\R	16\IR	15\R	14\S	6\R	6\R	12\R	12\R	17\IR	6\R	15\S	66.6
35	*Enterobacter cloacae*	18\S	6\R	20\S	16\IR	15\S	16\IR	6\R	22\S	16\IR	17\IR	6\R	18\S	25
36	*Serratia rubidaea*	23\S	18\S	32\S	22\S	14\S	34\S	29\S	30\S	24\S	14\R	14\ IR	15\S	16.67
37	*Serratia rubidaea*	21\S	24\S	25\S	18\ IR	13\S	23\S	17\S	30\S	18\S	8\R	6\R	20\S	25
38	*Serratia rubidaea*	23\S	22\S	29\S	20\ IR	15\S	32\S	16\ IR	30\S	21\S	11\R	6\R	15\S	33.33
39	*Citrobacter freundii*	6\R	6\R	6\R	18\ IR	11\S	7\R	15\ IR	7\R	17\S	8\R	6\R	6\R	83.33
40	*Citrobacter freundii*	17\S	16\ IR	13\R	6\R	11\S	6\R	16\ IR	7\R	6\R	8\R	6\R	11\R	83.33
41	*Citrobacter freundii*	20\S	16\ IR	20\S	6\R	15\S	11\R	21\S	13\R	6\R	7\R	18\S	19\S	50

Table 2: Antimicrobial susceptibility patterns of the pathogenic Gram negative bacilli against different antibiotics by disc diffusion method.

Pseudomonas fluorescens (No.13) was sensitive to (Colistin Sulphate, Cefepime and Tobramycin) and moderately resistant against Amikacin, Ceftazidim, Ciprofloxacin and Rifampicin (Figure 2).

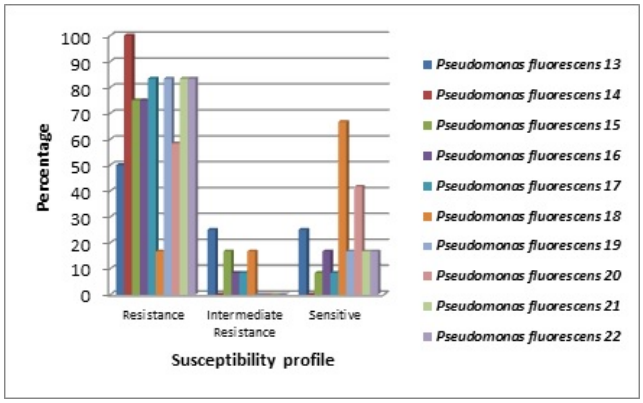

Figure 2: Profile of antibiotic susceptibility of *Pseudomonas fluorescens* (Resistant, Intermediate Resistant and sensitive).

While *P. fluorescens* (No.14) was resistant for all antibiotics. *P. fluorescens* (No.15) was sensitive to Colistin Sulphate only and moderately resistant against two types of antibiotics Amoxicillin/ Clavulanic acid and Levofloxacin. *P. fluorescens* (No.16) was sensitive to Amikacin, Colistin Sulphate and moderately resistant to Ciprofloxacin. Whereas *P. fluorescens* (No.17) was sensitive to Colistin Sulphate and moderately resistant to Nitrofurantoin. *P. fluorescens* No. 18 was sensitive to Amikacin, Amoxicillin/Clavulanic acid, Ciprofloxacin, Nitrofurantoin, Cefepime, Levofloxacin, Rifampicin and Sulphamethoxazole/ Trimethoprim, while it moderately resistant to Ceftazidim and Cefotaxim. Also *P. fluorescens* No.19 was sensitive to Colistin Sulphate and Tobramycin. *P. fluorescens* No.20 was sensitive to Amikacin, Amoxicillin\Clavulanic acid, Ceftazidim, Colistin Sulphate and Nitrofurantoin, while *P. fluorescens* No.21 was sensitive to Colistin Sulphate and Nitrofurantoin and finally *P. fluorescens* No. 22 was sensitive to Ciprofloxacin and Levofloxacin.

Providencia stuartii (No.23) was sensitive to Colistin Sulphate and moderately resistant to Amikacin, Amoxicillin\Clavulanic acid, Levofloxacin and Rifampicin (Figure 3).

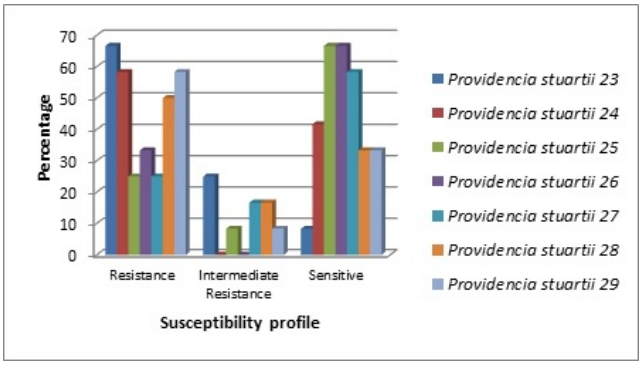

Figure 3: Profile of antibiotic susceptibility of *Providencia stuartii* (Resistant, Intermediate and sensitive).

P. stuartii (No.24) was sensitive to Amikacin, Ciprofloxacin, Levofloxacin, Sulphamethoxazole/ Trimethoprim and Tobramycin without appearance resistant to other antibiotics. While *P. stuartii* (No. 25) was sensitive to eight types of antibiotics (Amikacin, Ceftazidim, Ciprofloxacin, Colistin Sulphate, Cefotaxim, Cefepime, Levofloxacin and Sulphamethoxazole/ Trimethoprim) and moderately resistant to Tobramycin. *P. stuartii* (No.26) was sensitive to Amikacin, Ceftazidim,

Ciprofloxacin, Cefotaxim, Cefepime, Levofloxacin, Sulphamethoxazole/ Trimethoprim and Tobramycin. *P. stuartii* (No.27) was sensitive to Amikacin, Ceftazidim, Ciprofloxacin, Cefepime, Levofloxacin, Sulphamethoxazole/ Trimethoprim and Tobramycin and moderately resistant to Amoxicillin\Clavulanic acid and Cefotaxim. *P. stuartii* (No.28) was sensitive to Amikacin, Ciprofloxacin, Colistin Sulphate and Levofloxacin) and moderately resistant to Nitrofurantoin and Tobramycin. *P. stuartii* (No.29) was sensitive to Ciprofloxacin, Colistin Sulphate, Nitrofurantoin and Levofloxacin and moderately resistant to Amikacin.

Enterobacter cloacae (No.30) was sensitive to (Amikacin and Amoxicillin/Clavulanic acid) and moderately resistant against Levofloxacin (Figure 4).

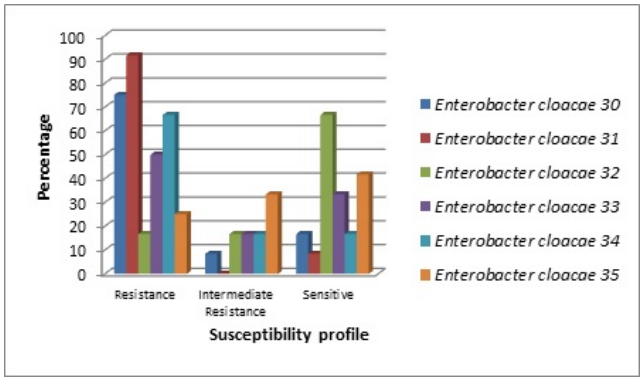

Figure 4: Relative number of susceptibility profile (Resistant, Intermediate Resistant and sensitive) of *Enterobacter cloacae* against antibiotics.

E. cloacae (No.31) was sensitive to only for Colistin Sulphate and without moderately resistant against antibiotics. Also *E. cloacae* (No. 32) were sensitive to (Amikacin, Ceftazidim, Colistin Sulphate, Cefotaxim, Cefepime, Levofloxacin, Sulphamethoxazole/ Trimethoprim and Tobramycin) and moderately resistant against Amoxicillin/Clavulanic acid and Ciprofloxacin. *E. cloacae* (No.33) were sensitive to (Ceftazidim, Colistin Sulphate, Levofloxacin and Sulphamethoxazole/Trimethoprim). While moderately resistant against Amoxicillin/Clavulanic acid and Ciprofloxacin. *E. cloacae* (No. 34) were sensitive to (Colistin Sulphate and Tobramycin) and moderately resistant against Ceftazidim and Rifampicin. Finally, *E. cloacae* (No.35) were sensitive to Amikacin, Ceftazidim, Colistin Sulphate, Cefepime and Tobramycin and moderately resistant to Ciprofloxacin, Cefotaxim, Levofloxacin and Rifampicin.

Serratia rubidaea (No.36) was sensitive to Amikacin, Amoxicillin/ Clavulanic acid, Ceftazidim, Ciprofloxacin, Colistin Sulphate, Cefotaxim, Nitrofurantoin, Cefepime, Levofloxacin and Tobramycin, while moderately resistant to Sulphamethoxazole/ Trimethoprim (Figure 5).

S. rubidaea (No.37) was sensitive to Amikacin, Amoxicillin/ Clavulanic acid, Ceftazidim, Colistin Sulphate, Cefotaxim, Nitrofurantoin, Cefepime, Levofloxacin and Tobramycin and moderately resistant to Ciprofloxacin. *S. rubidaea* (No.38) was sensitive to Amikacin, Amoxicillin/Clavulanic acid, Ceftazidim, Colistin Sulphate, Cefotaxim, Cefepime, Levofloxacin and Tobramycin) and moderately resistant to Ciprofloxacin and Nitrofurantoin.

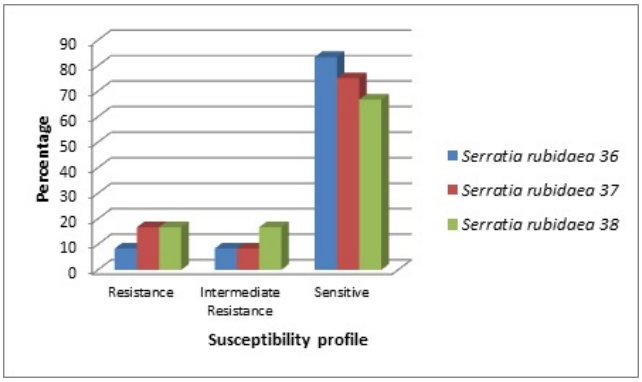

Figure 5: Relative number of susceptibility profile (Resistant, Intermediate Resistant and sensitive) of *Serratia rubidaea* against antibiotics.

Citrobacter freundii (No.39) was sensitive to Colistin Sulphate and Levofloxacin and moderately resistant to Ciprofloxacin and Nitrofurantoin (Figure 6). C. freundii (No.40) was sensitive to Amikacin and Colistin Sulphate while moderately resistant to Amoxicillin\Clavulanic acid and Nitrofurantoin. *C. freundii* (No.41) was sensitive to Amikacin, Ceftazidim, Colistin Sulphate, Nitrofurantoin, Sulphamethoxazole/ Trimethoprim and Tobramycin and moderately resistant to Amoxicillin\ Clavulanic acid.

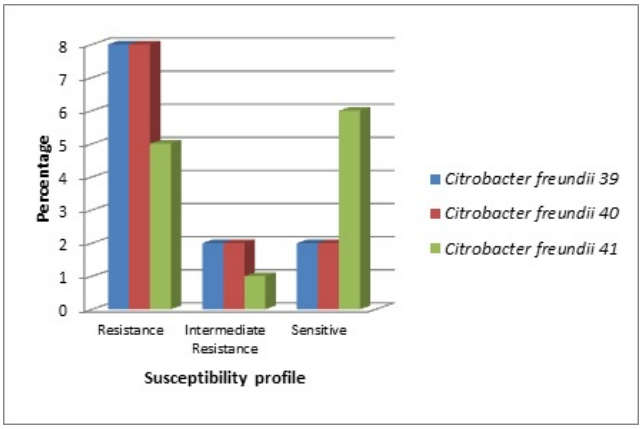

Figure 6: Relative number of susceptibility profile (Resistant, Intermediate Resistant and sensitive) of *Citrobacter freundii* against antibiotics.

Antimicrobial susceptibility pattern of bacterial isolates was tested against selected 12 antibiotics. From Table 2 The results obtained showed that the bacterial isolates varied in their susceptibility to all the antibiotics and showed that maximum sensitivity for Colistin Sulphate (78.1%), both Amikacin and Levofloxacin (53.7%), Ciprofloxacin (46.3%), Tobramycin (39.1%), Ceftazidim (31.7%), Nitrofurantoin (29.3%), both Amoxicillin/ Clavulanic acid and Cefepime (24.4%), Sulphamethoxazole/ Trimethoprim (21.9%), Cefotaxim (17.1%) and Rifampicin (2.4%).

Isolates		Antimicrobial agents (%)											
		AK ≥ 17	AMC ≥ 18	CAZ ≥ 18	CIP ≥ 21	CT ≥ 11	CTX ≥ 23	F ≥ 17	FEP ≥ 18	LEV ≥ 17	RD ≥ 20	SXT ≥ 16	TOB ≥ 15
Klebsiella pneumonia (n=12)	S	6 (50)	4 (33.3)	2 (16.6)	7 (58.3)	10 (83.3)	1 (8.3)	5 (41.7)	1 (8.3)	8 (66.7)	0	1 (8.3)	4 (33.3)
	R	5 (41.7)	6 (50)	9 (75)	3 (25)	2 (16.6)	10 (83.3)	3 (25)	10 (83.3)	3 (25)	12 (100)	11 (91.7)	7 (58.3)
	IR	1 (8.3)	2 (16.6)	1 (8.3)	2 (16.6)	0	1 (8.3)	4 (33.3)	1 (8.3)	1 (8.3)	0	0	1 (8.3)
Pseudomonas fluorescens (n=10)	S	3 (30)	2 (20)	1 (10)	2 (20)	7 (70)	0	3 (30)	2 (20)	2 (20)	1 (10)	1 (10)	2 (20)
	R	6 (60)	7 (70)	8 (80)	6 (60)	3 (30)	9 (90)	6 (60)	8 (80)	7 (70)	8 (80)	9 (90)	8 (80)
	IR	1 (10)	1 (10)	1 (10)	2 (20)	0	1 (10)	1 (10)	0	1 (10)	1 (10)	0	0
Providencia stuartii (n=7)	S	5 (71.4)	0	3 (42.8)	6 (85.7)	4 (57.1)	2 (28.6)	1 (14.3)	3 (42.8)	6 (85.7)	0	4 (57.1)	3 (42.8)
	R	0	5 (71.4)	4 (57.1)	1 (14.3)	3 (42.8)	4 (57.1)	5 (71.4)	4 (57.1)	0	7 (100)	3 (42.8)	2 (28.6)
	IR	2 (28.6)	2 (28.6)	0	0	0	1 (14.3)	1 (14.3)	0	1 (14.3)	0	0	2 (28.6)
Enterobacter cloacae (n=6)	S	3 (50)	1 (16.7)	3 (50)	3 (50)	5 (83.3)	1 (16.7)	0	1 (16.7)	2 (33.3)	0	2 (33.3)	3 (50)
	R	3 (50)	3 (50)	2 (33.3)	3 (50)	1 (16.7)	4 (66.7)	6 (100)	4 (66.7)	2 (33.3)	4 (66.7)	4 (66.7)	3 (50)
	IR	0	2 (33.3)	1 (16.7)	0	0	1 (16.7)	0	1 (16.7)	2 (33.3)	2 (33.3)	0	0
Serratia rubidaea (n=3)	S	3 (100)	3 (100)	3 (100)	1 (33.3)	3 (100)	3 (100)	2 (66.6)	3 (100)	3 (100)	0	0	3 (100)
	R	0	0	0	0	0	0	0	0	0	3 (100)	2 (66.6)	0
	IR	0	0	0	2 (66.6)	0	0	1 (33.3)	0	0	0	1 (33.3)	0
Citrobacter freundii (n=3)	S	2 (66.6)	0	1 (33.3)	0	3 (100)	0	1 (33.3)	0	1 (33.3)	0	1 (33.3)	1 (33.3)
	R	0	1 (33.3)	2 (66.6)	2 (66.6)	0	3 (100)	0	3 (100)	2 (66.6)	3 (100)	2 (66.6)	2 (66.6)
	IR	0	2 (66.6)	0	1 (33.3)	0	0	2 (66.6)	0	0	0	0	0
Total (n=41)	S	22 (53.7)	10 (24.4)	13 (31.7)	19 (46.3)	32 (78.1)	7 (17.1)	12 (29.3)	10 (24.4)	22 (53.7)	1 (2.4)	9 (21.9)	16 (39.1)
	R	14 (34.1)	22 (53.7)	27 (65.9)	15 (36.6)	9 (22)	30 (73.2)	20 (48.8)	29 (70.7)	14 (34.1)	37 (90.2)	31 (75.6)	22 (53.7)
	IR	4 (28.6)	9 (21.9)	3 (7.3)	7 (17.1)	0	4 (28.6)	9 (21.9)	2 (4.9)	5 (12.2)	3 (7.3)	1 (2.4)	3 (7.3)

Table 3: Antibiotic sensitivity/Intermediate resistance pattern (%) of gram negative bacteria isolated from wound infection.

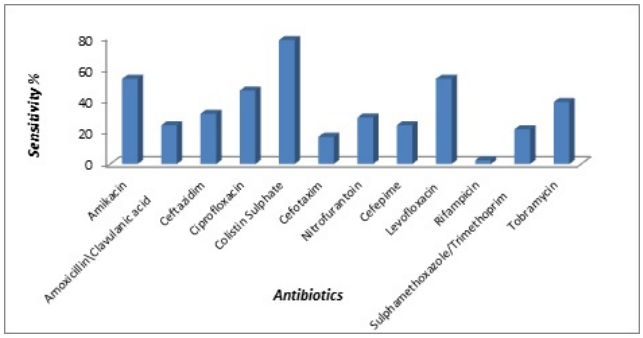

Figure 7: Sensitivity pattern (%) of gram negative bacteria isolated from wound infection.

Whereas bacterial isolates was resistant to Rifampicin (90.2%), Sulphamethoxazole/ Trimethoprim (75.6%), Cefotaxim (73.2%), Cefepime (70.7%), Ceftazidim (65.9%), bacterial isolates was resistant to both (Amoxicillin/Clavulanic acid and Tobramycin) (53.7%), Nitrofurantoin (48.8%), Ciprofloxacin (36.6%), both (Cefotaxim and Sulphamethoxazole/ Trimethoprim) (34.1%) and bacterial isolates was resistant to Colistin Sulphate (22%). The other results of tested Gram negative bacteria are listed in Table 3.

In our study, *Klebsiella pneumoniae* (24%) followed by *Pseudomonas fluorescens* (20%), *Providencia stuartii* (14%) and *Enterobacter cloacae* (12%). Both *Serratia rubidaea* and *Citrobacter freundii* (6%) with agreement [12]. *Pseudomonas* species was found to be the most common isolate (48.9%) followed by *Citrobacter braakii* (13.3%), *Enterobacter* spp. (11.1%), *Proteus vulgaris* (6.66%), *Klebsiella* spp. (2.22%) and *Serratia rubidia* (2.22%). As well as, *Klebsiella pneumoniae* was (83.3%) sensitive to Colistin Sulphate, (66.7%) in Levofloxacin, (58.3%) in Ciprofloxacin, (50%) in Amikacin, (41.7%) in Nitrofurantoin, (33.3%) in both Amoxicillin/Clavulanic acid and Tobramycin. Also (16.6%) in Ceftazidim, finally (8.3%) in both Cefotaxim and Sulphamethoxazole/ Trimethoprim. While the

Klebsiella pneumoniae was (100%) resistance to Rifampicin, (91.7%) in Sulphamethoxazole/ Trimethoprim, (83.3%) in both (Cefotaxim and Cefepime), (75%) in Ceftazidim, (58.3%) in Tobramycin, (50%) in Amoxicillin/ Clavulanic acid (41.7%) in Amikacin, (25%) in both of (Ciprofloxacin, Nitrofurantoin and Levofloxacin) and (16.6%) in Colistin Sulphate. Similar results were reported for sensitivity and resistance of Gram negative bacteria against selected antibiotics (Figure 7).

In conclusion, wound infection by pathogenic bacteria and increasing antibiotics resistance are of the most serious health threats facing the patients, especially diabetic foot patients. Thus, the objective of this work was to isolate and identify pathogenic bacteria infecting wounds. Antimicrobial susceptibility of the isolated pathogenic bacteria to different antibiotics covering all mode of action of antibiotics was conducted.

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