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Analysis of GNB Species and Pattern of Resistance Responsible for LRTI in Patients with Cancer

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Abstract: Lower respiratory tract infection (LRTI) is the most lethal infection remains among patients undergoing treatment for cancer. Most of the previous studies with cancer patients have focus on blood stream infections. For that reason the aim of our study was to examine the spectrum and recent trends in antimicrobial resistance of Gram negative bacteria (GNB) recovered from cancer patient having LRTI in Egypt. In addition our objective was to investigate the prevalence and distribution of *Legionella pneumophila* among cancer patients with LRTI. Sputum specimens were collected from 285 cancer patients suspecting of having LRTI. The conventional methods and Microscan Negative Identification panel Type 2 were used for identification of GNB. Susceptibility was assessed for 20 antibiotics in bacterial isolates using agar diffusion method. All the sputum specimens were tested by culture and genus specific PCR for the detection of *Legionella pneumophila*. A total of 130 GNB were isolated. Among these, *Klebsiella pneumoniae* was the most common (35.4 %). We isolated and identified a number of less frequent GNB (17%), whereas no *Legionella pneumophila* was detected. Amikacin was found to be the most effective antimicrobial against GNB. We reported very high percentage of multi-drug resistance GNB (96%). This study reported the development of multidrug resistance Gram negative bacilli in Egypt. Continuous updating of data on antimicrobial susceptibility profiles is required to ensure the efficacy of antimicrobial agents against GNB due to continuous development of antimicrobial resistance patterns among these pathogens.

Keywords: Lower Respiratory Tract Infection, Cancer Patients, Gram-Negative Bacteria, *Legionella Pneumophila*

1. Introduction

Immunocompromised term describes a host who is at increased risk for Life-threatening infection as a consequence of abnormality of the immune system. During the past few decades, the population of immunocompromised patients has developed hugely, blame on the increased use of immunosuppressive drugs [1].

The mortality rates in immunocompromised patients associated with Lower respiratory tract infection (LRTI) are reported between 12% and 50% and the progression from upper respiratory tract infection to LRTI in immunocompromised patients is estimated between 13% and 43% [2]. LRTI covers a wide spectrum of disease including pneumonia, acute bronchitis and aggravation of chronic lung disease [3].

Pneumonia is a recurrent complication in immunocompromised patients, including patients who have hematologic malignancies, have received cytotoxic therapy, or solid organ transplantation [4]. Patients with cancer are at increased risk for Gram negative pneumonia. In hospitalized patients, oropharynx becomes colonized with Gram negative bacilli that are more virulent than normal flora. Gram negative pathogens especially *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae (K. pneumoniae)* are predominant in the first three months, whereas, Gram positive bacteria cause most of the infection seen subsequently [5]. *Legionella* spp. has been reported to be an important cause of nosocomial pneumonia in some centers [6,7]. In addition, outbreaks of nosocomial legionellosis are a frequent problem in hospital environment [8]. *Legionella* species represent normal environmental flora, many cause human disease, most commonly opportunistic pneumonia in

immunocompromised hosts. Approximately 85% of such cases are due to *Legionella pneumophila* [9,10]. Legionella pneumonia can be subclinical or severe and life threatening. The fatality rate can move toward 50% in immunocompromised hosts [11].

In general, infections that are caused by multi-drug resistant Gram negative bacteria (GNB) are associated with up to five times higher mortality rates compared with infections that are caused by susceptible GNB [12, 13]. In addition, infections with multi-drug resistance GNB lead to less desirable outcomes, including longer hospital stays and utmost cost of hospitalization [12].

In the present study, both patients with hematologic malignancies and patients with solid tumors were included. The aim of the study was to search the distribution and antimicrobial resistance of aerobic GNB causing LRTI among cancer patients in Egypt. The study was not limited to the most common GNB, but included less frequent GNB as well. Also the study was done to obtain the insight of the prevalence and distribution of *Legionella pneumophila* among cancer patients in Egypt.

2. Methodology

2.1. Study Specimens Collection

The study was carried out at Microbiology Laboratory of National Cancer Institute, Cairo University, Cairo, Egypt, and Microbiology Laboratory of Faculty of Pharmacy, Al-azhar University, Cairo, Egypt, over a period from December 2012 to July 2014. All hospitalized cancer patients undergoing anti-cancer therapy, with suspected LRTI, were studied. No discrimination was made on the basis of age or gender. A total of 285 sputum specimens were collected, which were submitted to the Microbiology Laboratory of National Cancer Institute (NCI) Cairo, Egypt, for routine culture.

2.2. Isolation and Identification of GNB

Aerobic GNB were isolated and identified using standard methods and biochemical tests [14]. Microscan Negative Identification panel Type 2 (DadeBehring, West Sacramento, USA) was used to confirm the identification of Gram negative isolates. Microscan negative identification panel Type 2, is an in vitro diagnostic method that uses fluorescence technology to detect bacterial growth or metabolic activity and can automatically identify Gram negative bacteria to species level. The system is based upon the reaction obtained with 34 biochemical test dosed and dried into Microscan panel.

2.3. Growth Conditions of Legionella

Reference strains of *Legionella pneumophila* ATCC® 33152 and sputum specimens, washed with a 0.2 M KCl-HCl solution, pH 2.2 [15], were cultured on buffered charcoal yeast extract (BCYE) agar supplemented with α-ketoglutarate. This medium provides iron and L-cysteine, both of which are essential for the growth of legionellae [16].

2.4. Antimicrobial Susceptibility Determination

All aerobic GNB isolates were tested for antibiotic susceptibility by the disk diffusion method described by Kirby Bauer according to the Clinical Laboratory Standard Institute [17]. The antibiotic panel included: Amikacin (30 µg), Ampicillin (10 µg), Amoxicillin /Clavulanic acid (20 µg/ 10 µg), Ampicillin/Sulbactam (20 µg/ 10 µg), Aztreonam (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Meropenem (10 µg), Trimethoprim /Sulfamethoxazole (Co-trimoxazole) (300 µg/ 25 µg), Piperacillin (10 µg), Tobramycin (10 µg), Piperacillin/Tazobactam (100 µg/ 10 µg),Tetracycline (30 µg) and Tigecyclin (15 µg) (Oxoid ltd., Basin Stoke, Hants, England). Resistance to each antibiotic was recorded and the strains resistant to one antimicrobial agent in the three or more antimicrobial categories were defined as multi-drug resistant [18].

2.5. DNA isolation and PCR for Legionella

DNAs from clinical specimens and reference strains of *Legionella pneumophila* were extracted using the QIAamp® DNA Mini Kit (Qiagen, USA) according to the manufacture manuals.

For each DNA sample PCR reaction was performed using forward (5′ - GCT TAA CCT GGG ACG GTC AGA T – 3′) and reverse (5′ -GCG CCA CTA ATT ATT TTC ATA TAA- 3′) oligonucleotides specific primer for *Legionella pneumophila* described previously [19] which amplify 245bp fragments. Primers were prepared by Vivantis, Malaysia. For PCR, Dream Taq Green Master Mix (Thermo scientific, EU) was used. Amplification reactions were performed in a volume of 50 µl with final amounts of 25 µl of Dream Taq Green Master Mix, 0.5 µM of each primer and 500ng of extracted DNA.The thermal cycles were as followed: the reaction mixtures were incubated for 5 min at 94°C for denaturation; 40 cycles of 1min at 94°C; 1min at 53°C and 2 min at 72°C and finally 5 min at 72°C. The PCR products were detected by electrophoresis on 2% agarose gel.

3. Results

In the present study, a total of 130 aerobic Gram negative bacilli were recovered from sputum specimens. All the isolates were identified conventionally and by using semi-automated systems. The main isolated Gram negative bacilli were *K. pneumoniae* (35.4%) followed by *Escherichia coli* (*E. coli*) (20%), *Acinetobacter baumannii* (*A. baumannii*) (17%) then *P. aeruginosa* (10.3%) (Table1).

In the present study, patients were classified into two groups; those with hematological malignancies and those with solid organ malignancies. Out of 130 Gram negative isolates, 77 (59%) isolates were obtained from hematologic malignancies patients, whereas only 53 (41%) isolates were

obtained from solid tumor patients (Table1).

In both hematologic malignancies and solid tumor patients, Gram negative bacteria were mainly *K. pneumoniae* (20% among hematologic malignancies patients and 15.4% among solid-tumor cancer patients) (Table1).

Regarding the detection of *Legionella pneumophila* by culture and PCR, all sputum specimens were negative.

The antimicrobial activity of penicillin derivatives, cephalosporins, monobactams, carbapenems, tetracyclines, quinolones, and aminoglycosides group of antimicrobial agents against aerobic Gram negative bacilli isolated from LRTI of cancer patients is shown (Tables 2 and 3). We reported resistance rates of GNB as 68.5% - 99.2% against penicillin derivatives, 89.2% against monobactam, 46.2% - 51.5% against carbapenems, 79.2% - 95.4% against cephalosporins, 42.3% - 79.2% against aminoglycosides, 65.4% - 68.5% against quinolones and 83% against Co-trimoxazole.

Among all the antimicrobials used, amikacin, imipenem and meropenem showed highest activity against *E.coli*, *K. pneumoniae* and *P.aeruginosa* strains. Among quinolones, levofloxacin has the highest activity. Gentamicin was also found to be effective against *Acinetobacter baumannii* isolates.

Chromobacterium violaceum and *Chryseobacterium meningosepticum* were resistance to all antimicrobial agents used.

In current study, 96% (125/130) of isolated Gram negative bacilli were multi-drug resistant.

4. Discussion

Lower respiratory tract infection complications are a serious cause of morbidity and mortality in cancer patients, especially those with hematological malignancies [20, 21]. By different ways, chemotherapeutic agents predispose bacterial infection [22]. Many of these agents damage the body's immune system [23].

Reemergence of Gram negative infections and increased antimicrobial resistance due to overuse of antibiotics in cancer patients have changed the epidemiology of bacterial infections among these patients [24, 25].

Table 1. *Spectrum of Gram negative bacteria among cancer patients with LRTI.*

Types of Gram negative bacilli	Patients with hematological malignancies	Patients with solid tumor	Total
	N (%)	N (%)	N (%)
Enterobacteriaceae			
Escherichia coli	9 (7)	17 (13)	26 (20)
Escherichia vulneris	1 (0.8)	0(0)	1(0.8)
Klebsiella pneumoniae	26(20)	20(15.4)	46(35.4)
Klebsiella ozaenae	1(0.8)	0(0)	1(0.8)
Serratia marcescens	1(0.8)	2(1.5)	3(2.3)
Enterobacter cloacae	1(0.8)	1(0.8)	2(1.6)
Enterobacter aerogenes	2(1.6)	0(0)	2(1.6)
Citrobacter freundil	2(1.6)	0(0)	2(1.6)
Providencia stuartii	1(0.8)	0(0)	1(0.8)
Non fermentative Gram negative bacilli			
Acinetobacter baumannii	12(9)	10(8)	(17)22
Acinetobacter haemolyticus	1(0.8)	0(0)	1(0.8)
Pseudomonas aeruginosa	11(8)	3(2.3)	14(10.3)
Pseudomonas fluorescens	1(0.8)	0(0)	1(0.8)
Pseudomonas oryzihabitans	1(0.8)	0(0)	1(0.8)
Stenotrophomonas maltophilia	4(3)	0(0)	4(3)
Burkholderia cepacia	1(0.8)	0(0)	1(0.8)
Other Gram negative bacteria			
Chromobacterium violaceum	1(0.8)	0(0)	1(0.8)
Chryseobacterium meningosepticum	1(0.8)	0(0)	1(0.8)
Total	77 (59)	53(41)	130

Table 2. *Antibiotic susceptibility pattern of Enterobacteriaceae.*

AB \ M.O	Escherichia coli		Escherichia vulneris		Klebsiella pneumoniae		Klebsiella ozaenae		Serratia marcescens		Enterobacter cloacae		Enterobacter aerogenes		Citrobacter freundil		Providencia stuartii	
	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)
Ampicillin	0 (0)	26 (100)	0 (0)	1 (100)	0 (0)	46 (100)	1 (100)	0 (0)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Amoxicillin/ Clavulanic acid	2 (8)	24 (92)	1 (100)	0 (0)	8 (17)	38 (83)	1 (100)	0 (0)	1 (33)	2 (67)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Ampicillin/ Sulbactam	0 (0	26 (100)	0 (0)	1 (100)	2 (4)	44 (96)	1 (100)	0 (0)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Piperacillin/ Tazobactam	9 (35)	17 (65)	1 (100)	0 (0)	19 (41)	27 (59)	1 (100)	0 (0)	1 (33)	2 (67)	0 (0)	2 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	1 (100)
Piperacillin	0 (0)	26 (100)	0 (0)	1 (100)	0 (0)	46 (100)	1 (100)	0 (0)	0 (0)	3 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Azteronam	1 (4)	25 (96)	0 (0)	1 (100)	6 (13)	40 (87)	1 (100)	0 (0)	2 (67)	1 (33)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Meropenem	17 (65)	9 (35)	1 (100)	0 (0)	27 (59)	19 (41)	1 (100)	0 (0)	2 (67)	1 (33)	2 (100)	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	1 (100)
Imipenem	20 (77)	6 (23)	1 (100)	0 (0)	28 (61)	18 (39)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	1 (100)
Cefoxitin	10 (38.5)	16 (61.5)	0 (0)	1 (100)	13 (28)	33 (72)	1 (100)	0 (0)	1 (33)	2 (67)	0 (0)	2 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	1 (100)
Ceftazidim	1 (4)	25 (96)	0 (0)	1 (100)	7 (15)	39 (85)	0 (0)	1 (100)	0 (0)	3 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Cefotaxime	2 (8)	24 (92)	0 (0)	1 (100)	9 (20)	37 (80)	0 (0)	1 (100)	2 (67)	1 (33)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Ceftriaxone	1 (4)	25 (96)	0 (0)	1 (100)	3 (6.5)	43 (93.5)	0 (0)	1 (100)	0 (0)	3 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Amikacin	20 (77)	6 (23)	1 (100)	0 (0)	32 (70)	14 (30)	1 (100)	0 (0)	2 (67)	1 (33)	2 (100)	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	1 (100)
Gentamicin	12 (46)	14 (54)	0 (0)	1 (100)	12 (26)	34 (74)	1 (100)	0 (0)	2 (67)	1 (33)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)	1 (100)
Tobramycin	6 (23)	20 (77)	0 (0)	1 (100)	9 (20)	37 (80)	0 (0)	1 (100)	1 (33)	2 (67)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Tetracycline	3 (11.5)	23 (88.5)	0 (0)	1 (100)	4 (9)	42 (91)	1 (100)	0 (0)	0 (0)	3 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Tygacil	11 (42)	15 (58)	1 (100)	0 (0)	24 (52)	22 (48)	1 (100)	0 (0)	1 (33)	2 (67)	1 (50)	1 (50)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)
Ciprofloxacin	6 (23)	20 (77)	0 (0)	1 (100)	17 (37)	29 (63)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	1 (50)	1 (50)	0 (0)	1 (100)
Levofloxacin	8 (31)	18 (69)	0 (0)	1 (100)	17 (37)	29 (63)	1 (100)	0 (0)	3 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	1 (50)	1 (50)	0 (0)	1 (100)
Trimethoprim/ Sulfamethoxazole	3 (11.5)	23 (88.5)	0 (0)	1 (100)	11 (24)	35 (76)	1 (100)	0 (0)	3 (100)	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (100)
Total	26		1		46		1		3		2		2		2		1	

M.O: microorganism; AB: antibiotic disc name; n: number; S: sensitive; R: resistance

Table 3. *Antibiotic susceptibility pattern of Non fermentative Gram negative bacilli.*

AB \ M.O	*Acinetobacter baumannii*		*Acinetobacter hemolyticus*		*Stenotrophoms maltophilia*		*Burkholderia cepacia*		*Pseudomonas aeruginosa*		*Pseudomonas fluorescence*		*Pseudomonas oryzihabitans*	
	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n %)(	S n (%)	R n (%)	S n (%)	R n (%)
Ampicillin	0 (0)	22 (100)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	0 (0)	14 (100)	0 (0)	1 (100)	0 (0)	1 (100)
Amoxicillin/ Clavulanic acid	0 (0)	22 (100)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	0 (0)	14 (100)	0 (0)	1 (100)	1 (100)	0 (0)
Ampicillin/ Sulbactam	1 (4.5)	21 (95.5)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	0 (0)	14 (100)	0 (0)	1 (100)	0 (0)	1 (100)
Piperacillin/ Tazobactam	1 (4.5)	21 (95.5)	0 (0)	1 (100)	1 (25)	3 (75)	0 (0)	1 (100)	6 (43)	8 (57)	0 (0)	1 (100)	1 (100)	0 (0)
Piperacillin	0 (0)	22 (100)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	0 (0)	14 (100)	0 (0)	1 (100)	0 (0)	1 (100)
Azteronam	0	22	0	1	0	4	0	1	2	12	0	1	1	0

AB / M.O	*Acinetobacter baumannii*		*Acinetobacter hemolyticus*		*Stenotrophoms maltophilia*		*Burkholderia cepacia*		*Pseudomonas aeruginosa*		*Pseudomonas fluorescence*		*Pseudomonas oryzihabitans*	
	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n %)(	S n (%)	R n (%)	S n (%)	R n (%)
Imipenem	3 (14)	19 (86)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	7 (50)	7 (50)	1 (100)	0 (0)	1 (100)	0 (0)
Meropenem	3 (14)	19 (86)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	6 (43)	8 (57)	1 (100)	0 (0)	1 (100)	0 (0)
Cefoxitin	0 (0)	22 (100)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	1 (7)	13 (93)	0 (0)	1 (100)	0 (0)	1 (100)
Ceftazidim	1 (4.5)	21 (95.5)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	1 (7)	13 (93)	1 (100)	0 (0)	1 (100)	0 (0)
Cefotaxime	0 (0)	22 (100)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	1 (7)	13 (93)	0 (0)	1 (100)	1 (100)	0 (0)
Ceftriaxone	0 (0)	22 (100)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	0 (0)	14 (100)	1 (100)	0 (0)	0 (0)	1 (100)
Amikacin	3 (14)	19 (86)	0 (0)	1 (100)	1 (25)	3 (75)	0 (0)	1 (100)	8 (57)	6 (43)	1 (100)	0 (0)	1 (100)	0 (0)
Gentamicin	7 (32)	15 (68)	0 (0)	1 (100)	2 (50)	2 (50)	0 (0)	1 (100)	6 (43)	8 (57)	1 (100)	0 (0)	0 (0)	1 (100)
Tobramycin	5 (23)	17 (77)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	3 (21)	11 (79)	0 (0)	1 (100)	1 (100)	0 (0)
Tetracycline	2 (9)	20 (91)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	0 (0)	14 (100)	1 (100)	0 (0)	0 (0)	1 (100)
Tygacil	5 (23)	17 (77)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	4 (29)	10 (71)	0 (0)	1 (100)	0 (0)	1 (100)
Ciprofloxacin	3 (14)	19 (86)	0 (0)	1 (100)	1 (25)	3 (75)	0 (0)	1 (100)	5 (36)	9 (64)	1 (100)	0 (0)	1 (100)	0 (0)
Levofloxacin	3 (14)	19 (86)	0 (0)	1 (100)	2 (50)	2 (50)	0 (0)	1 (100)	6 (43)	8 (57)	1 (100)	0 (0)	1 (100)	0 (0)
Trimethoprim/ Sulfamethoxazole	1 (4.5)	21 (95.5)	0 (0)	1 (100)	0 (0)	4 (100)	0 (0)	1 (100)	2 (14)	12 (86)	0 (0)	1 (100)	0 (0)	1 (100)
Total	22		1		4		1		14		1		1	

M.O: microorganism; AB: antibiotic disc name; n: number; S: sensitive; R: resistance

In the current study, GNB were found associated with LRTI in cancer patients. Among GNB isolated, we observed an increase in Enterobacteriaceae especially *Klebsiella* species. *K. pneumoniae* (35.4%) was the most frequently isolated bacterial strains followed by *E. coli* (20%).

Other studies have also reported that, *Klebsiella* species were among the most frequent Gram negative isolates from respiratory tract infection [26, 27, 28].

P. aeruginosa has also been reported to cause a wide variety of infections in cancer patients as it is a common hospital and opportunistic pathogen [29, 30].

In the present study a number of less-frequent Gram negative bacteria (17%) were isolated and identified (*Acinetobacter haemolyticus*, *Burkholderia cepacia, Chromobacterium violacum, Citrobacter freundil*, *Chryseobacterium meningosepticum*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia vulneris, Klebsiella ozanae, Providencia stuarti*, *Pseudomonas fluorescence*, *Pseudomonas oryzihabitant*, *Serratia marcescens* and *Stenotrophomonas maltophilia*).

Gram negative pathogens of increasing importance in cancer patients include *Stenotrophomonas maltophilia* and emerging pathogen *Burkholderia cepacia* [23]. In this study, 3% of Gram negative bacilli were found to be multidrug resistant *Stenotrophomonas maltophilia*. *Stenotrophomonas maltophilia* is an organism that is frequently isolated from the environment, particularly from water supplies. The incidence of *Stenotrophomonas maltophilia* hospital acquired infections are increasing, particularly in the immunocompromised patient population receiving broad-spectrum antibiotics [23, 31].

The isolation of *Burkholderia cepacia* and other less frequent Gram negative bacilli had been reported as nosocomial infections among immunocompromised patients [32, 33]

The presence or absence of Legionella DNA in specimens might be clinically significant because of that legionella is not part from the human flora [34]. Sputum culture and PCR testing of lower respiratory tract specimens are the most important tools for diagnosis and detection of Legionella infection [35]. In the present study sputum specimens, were cultured on BCYE agar for detection of *Legionella pneumophila* and subjected to DNA extraction with subsequent PCR amplification for detection of specific DNA sequences of *Legionella pneumophila*. Our result revealed that there was no legionella detected by using both methods. We suggest that could be attributed to two reasons first, the studied patients were received antibiotics active against *Legionella* species as empiric therapy without undergoing laboratory testing for *Legionella* species. Second, good

decontaminated procedure of the water system of the hospital (the place which specimens were collected) because other studies suggested that outbreaks or sporadic cases of legionellosis may arise from contaminated water systems of the hospitals [36, 37]

In the present study, amikacin followed by imipenem then meropenem were the most effective drugs against Gram negative bacterial strains. Amikacin appeared to have wider range of activity than tobramycin, gentamicin and other tested antimicrobial agents and this is consistent with other studies [38, 39]. This may be attributed to that amikacin has lower selective pressure due to their restricted use. Other study explained the efficiency of amikacin by the fact that these are very powerful drugs used only in hospital settings and not as first-line therapy (39). With respect to amikacin resistance in our study, it appears that amikacin may be used as the primary antibiotics for the treatment of GNB in Egypt.

Quinolone prophylaxis has been widely used in cancer patients to decrease the risk of Gram negative infections [40]. However, it has been associated with an increased risk of selection of resistant strains [41]. The emergence of lower respiratory tract infections quinolone-resistant Gram negative bacterial was observed in our study. *E. coli* exhibited high resistance to ciprofloxacin (77%) and to levofloxacin (69%). A similar trend was seen with *P. aeruginosa* which exhibited resistance to ciprofloxacin (64%) and to levofloxacin (57%). *K. pneumoniae* exhibited resistance to levofloxacin and ciprofloxacin (63% each). Also, *A. baumannii* exhibited resistance to ciprofloxacin and levofloxacin (86% each).

In general *K. pneumoniae* are resistant to abroad range of antimicrobial agents, and practically always resistance to ampicillin and amoxicillin naturally [42]. In this study, *K. pneumoniae* strains were 100% resistance to ampicillin and piperacillin. The lowest percentage of susceptibility was manifested against ampicillin/sulbactam (4%) and ceftriaxone (6.5%) whereas more susceptibility was observed with amikacin (70%) followed by imipenem (61%) then meropenem (59%). *E. coli* isolates exhibit high resistance pattern. Another study reported that *E. coli* isolates from cancer patients in Egypt exhibited a low susceptibility pattern [43].

Resistance rates of *A. baumannii* were reported as 86% against ciprofloxacin, imipenem, meropenem and amikacin and 95.5% against piperacillin/tazobactam.

We demonstrated that *P. aeruginosa* were resistant to multiple antibiotics, that way rendering current antibiotic therapy ineffective. This could be attributed to the fact that *P. aeruginosa* has intrinsic antibiotics resistance due to low outer membrane permeability, as well as an extensive efflux pump system [44].

In current study, *Acinetobacter* and *Pseudomonas* species exhibited the highest resistance levels to imipenem. *E. coli* and Klebsiella exhibited lower resistance to imipenem. Previous studies in Egypt reported that resistance to imipenem was totally absent or very low [43, 45]. This discrepancy can be attributed to continue development of resistant strains in Egypt and improper use of empirical antibiotics.

The most significant public health threat is the emergence of resistance to multiple antimicrobial agents in pathogenic bacteria [18].

The phenomenon of multi-drug resistant pathogens had emerged in Egypt and worldwide due to excessive antibiotic misuse [43. 46, 47]. Several studies reported an increase in multi-drug resistant GNB in immunocompetent and immunocompromised patients, including patients with malignancies [43, 46, 47].

In our study the most notable finding was the highly increase in multidrug-resistant Gram negative bacilli (96%).

5. Conclusion

Current study demonstrated that *Legionella pneumophila* are not a common cause of lower respiratory tract infection among cancer patients in Egypt. Regarding Gram negative bacilli infection, amikacin would be a discreet choice in high-risk cases. Gram negative bacilli resistant to most classes of antibiotics in this study are due to inappropriate use of these drugs. In order to limit the emergence of multi-drug resistant Gram negative bacteria in Egypt, antimicrobial confined policies should be applied.

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