Greener Journal of Medical Sciences

ISSN: 2276-7797 ICV: 5.98

Submitted: 30/09/2016
Accepted: 05/10/2016
Published: 08/11/2016

DOI: http://doi.org/10.15580/GJMS.2016.4.093016149

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ABSTRACT

***Background*: Lower Respiratory Tract Infections (LRTIs) are among the most common infectious diseases affecting humans worldwide and are considered as an important cause of morbidity and mortality for all age groups. Almost three quarters of all antibiotic consumptions are for respiratory tract infections.**

***Methods*: Two hundred and twenty two gram negative bacteria (GNB) were isolated from 763 LRTIs specimens in the period from February 2015 to January 2016 by conventional microbiological methods. Multidrug-resistance (MDR), extensively drug-resistance (XDR) and pan drug-resistance (PDR) for GNB were examined by disc diffusion method. ESβL and MβL GNB suspected strains were studied by screening and confirmatory tests.**

***Results*: The prevalence of culture positive specimens was (65.9%) of the studied specimens, 44.1% of them were aerobic GNB which was distributed as 35.8% of the ward isolates and 60.7% of ICUs isolates. *Klebsiella* spp. (44.6%) was the most common GNB isolated from LRTIs patients followed by *E coli* (20.3%), *Pseudomonas* spp. (18%), *Acinetobacter* spp. (10.8%), *Enterobacter* (4.5%) and *Citrobacter* (1.8%). Total MDR, XDR and PDR GNB were 45.5%, 47.8% and 5.0% respectively. There was statistically significant difference between the studied fermentative GNB and non-fermentative GNB (60.1% Vs. 42%) for ESβL production by Cephalosporin/clavulanate combination disks test (confirmatory test). The highest percentage of MβL production by confirmatory IPM/EDTA was for *Acinetobacter* spp. (62.5%) followed by *Pseudomonas* spp. (60%), *Klebsiella* spp. (52.5%) and *E coli* (40%). The mortality rate was 7.4% and 10.9% in patients who had ESβL or MβL producing isolates respectively.**

***Conclusions*: Multidrug-resistant (MDR) gram-negative bacilli (GNB) are now widespread especially in patients with LRTIs and present a major challenge to modern medical practice. Longer hospital stay, ICU admission, invasive procedures, associated comorbid conditions and empirical antibiotic usage were significantly high risk factors for acquisition of ESβL and MβL.**

Key words: prevalence, GNB, LRTIs, MDR, PDR, ESβL, MβL.

INTRODUCTION

Lower respiratory tract infection (LRTI) is a broad description of a group of disease entities, encompassing acute bronchitis, pneumonia and exacerbations of chronic lung disease *Vishwanath et al (2013).*

LRTIs are among the most common infectious diseases affecting humans worldwide and are important causes of morbidity and mortality for all age groups *Egbe et al (2011).*

Multi drug resistant (MDR) organisms may be associated with either symptomatic respiratory illness or asymptomatic carriage (i.e., colonization). Differentiating colonization from infections can be difficult and requires clinical correlation. Good communication between the treating clinician and the clinical microbiologist will aid in clinical decision making *Vishwanath et al (2013).*

Almost three quarters of all antibiotic consumptions are for respiratory tract infections. The efficacy of Beta-lactam drugs have been increasingly thwarted by dissemination of acquired resistance determinants among pathogenic bacteria as a result of production of extended-spectrum beta-lactamase (ESβL) and metallo-beta-lactamase (MβL) *Shrestha et al (2011).*

Multi-drug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial categories, extensive drug resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan drug resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories *Magiorakos et al (2012).*

The aim of this study was to determine the prevalence of aerobic gram negative bacteria among LRTIs patients and associated risk factors in addition to its effect on patient outcome with declaration of MDR aerobic Gram-negative bacilli (GNB) causing LRTIs, with a special reference to extended-spectrum beta-lactamase (ESβL) and metallo-beta-lactamase (MβL) producing bacterial strains and to study their relation with patient's mortality and morbidity.

MATERIAL AND METHODS

Patients

A cross sectional study was undertaken. This study recruited a total of 763 patients (507 from medical wards and 256 from intensive care units) in Menoufia University Hospitals during the period from February 1st 2015 to January 30th 2016, the study was approved by the Institutional Research Ethics Committee, and informed consent was obtained from all participants. The personal and clinical histories of the patients were taken including age, sex, associated co-morbidities, history of drug administration, duration of hospitalization and exposure to invasive procedure. In the Chest Department, the specimens were taken from patients after complete clinical assessment.

Specimen collection

1. **Sputum** (at least 2 ml) was collected after drinking plenty of fluids, rinsing out the mouth with water and taking three deep breaths before coughing forcefully *Bhattacharya (2006).*

2. Broncho-alveolar lavage fluid (BAL): (With a maximal volume of 200 ml): Sixty ml. four syringes were filled with warmed saline instilled by the bronchoscopy in the right middle lobe bronchus (RML). Gentle suction was performed and repeated for 3 times. BAL fluid was expelled gently into labeled containers already held on melting ice and transported immediately for processing *King (1991).*

3. Transtracheal aspirates (TTA): A needle was inserted through the skin of cricothyroid ligament and catheter was introduced in to the trachea passing to the level of the tracheal bifurcation. The saline was introduced and withdrawn *Kalinske et al (1967)*.

4. Pleural fluid aspiration: The skin, subcutaneous tissue, rib periosteum, intercostal muscles, and parietal pleura should be infiltrated with anesthetic after cleaning with antiseptic solution. The device was inserted over the superior aspect of the rib until pleural fluid was obtained *Broaddus (2010).*

Different specimens were collected according to the patient's condition in the clinician documentation. The specimens were examined and processed in the Microbiology and Immunology Laboratory of Menoufia University hospital.

Isolation and identification of aerobic Gram-negative bacilli (GNB) causing LRTIs:

Each specimen was incubated on nutrient, blood, MacConkey's and chocolate agars and incubated at 37℃ for 18 – 24 hours. GNB were identified by culture characteristics, Gram-staining morphology and positive conventional biochemical reactions (TSI, Simmons' citrate; MIO and oxidase tests) *Cheesbrough (2006).* Duplicate isolates from the same patient were excluded from analysis.

Valid sputum was based on Gram staining (<10 squamous epithelial cells and > 25 pus cells per low power field). *Navaneeth and Belwadi (2002).* BAL fluid was processed by quantitative culture with positive threshold of 10^4 CFU/ml *Chastre et al (2005).*

Clinical isolates were considered pathogenic if there was fever (temperature >38℃), raised leucocytic count (>12.0 × 10^9 cells/L), presence of purulent sputum, positive chest auscultatory findings and radiological findings of chest infection. *Vishwanath et al (2013).*

Antimicrobial susceptibility screening test

Antimicrobial susceptibility screening test were done for GNB isolates by disk diffusion method against different antimicrobial agents (Oxoid); Ampicillin (10 µg), Piperacillin (100 µg), Amoxycillin/clavulinic acid (20/10 µg), Piperacillin-tazobactam (100/10 µg), Amikacin (30 µg), Gentamycin (10 µg), Tobramycin (10 µg), Streptomycin (10 µg), Colistin (10 µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Nalidixic acid (30 µg) , Azteronam (3 0 µ g) ,

Imipenem (10 µg), Meropenem (10 µg), Cefamandole (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Cefepime (30 µg), Cefoxitin (30 µg), Trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg), Chloramphenicol (30 µg), Nitrofurantoin (300 µg) and Tetracycline (30 µg) and interpreted according to the Clinical and Laboratory Standards Institute guidelines *CLSI (2014).*

Screening for ESβLs production

Screening for ESβLs production was performed by disk diffusion test, Suspected ESβL- producing isolates (ceftazidime zone diameter ≤ 22 mm, cefotaxime zone diameter ≤ 27 mm, ceftriaxone zone diameter≤ 25 mm and Aztreonam zone diameter ≤ 27 mm) *CLSI (2014).*

ESβL- producing isolates were confirmed by confirmatory clavulinate combined disks test in which ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10µg) were used. The isolates were considered as ESβL-producer if there was ≥ 5 mm increase in zone diameter of ceftazidime/clavulanic (30/10µg) disk than that of ceftazidime (30µg) disk alone. The same was done with cefotaxime (30µg) and cefotaxime-clavulanate (30/10µg) *CLSI (2014).*

Metalo β lactamases susceptibility testing

This was done by disk diffusion method. GNB isolates were tested against imipenem (IPM 10 µg) and meropenem (MEM10 µg) (Oxoid) by disk diffusion method. The average diameters of zones of inhibition were measured and interpreted according to CLSI guidelines *CLSI (2014).*

Suspected Metalo β lactamases were confirmed by imipenem/EDTA combined disk (IPM/EDTA-CD) test, two (10µg) imipenem disks were placed on the plate at a distance of 15mm apart (center to center) and 5µl of sterile EDTA solution (930µg EDTA) was added to one of the imipenem disk and incubated aerobically at 37°C for 18-24h. The presence of an expanded growth inhibition zone between two discs or increase of zone size more than 7mm in imipenem/EDTA disk than imipenem disk alone was considered as MβL positive *Khosravi et al (2012).*

Statistical analysis

The data were collected, tabulated, and analyzed by SPSS (Statistical Package for Social Science) version 17.0 on IBM compatible computer (SPSS Inc., Chicago, IL, USA). χ^2 (Chi squared test) and Fisher's Exact test were used, according to suitable circumstances, for comparing categorical data.

RESULTS

This study included 763 LRTIs suspected patients (503 males and 260 females) with mean age (41.22±21.32) and ranged from 2 – 73 years, 442 patients from them were men and the other 321 patients were women. From 763 collected specimens, 507 cases were taken from hospital wards and 256 from ICUs patients. Out of them, 503 (65.9%) were culture positive isolates, only 222 (44.1%) of those culture positive isolates were identified as aerobic GNBs. (Table 1).

GNB which were isolated from patients' wards were *Klebsiella* spp. (61.6%), *E coli* (67.5%), *Pseudomonas* spp. (40%), and *Acinetobacter* spp. (29.2%), while from ICUs; *Klebsiella* spp. *E coli*, *Pseudomonas* spp., *Acinetobacter* spp., were 38.4%, 32.5%, 60%, and 70.8% respectively. The predominant isolates of LRTIs infections were *Klebsiella* spp. (44.6%), *Pseudomonas* spp. (20.3%), *E coli* (18%), *Acinetobacter* spp. (10.8%), *Enterobacter* (4.5%) and *Citrobacter (*1.8%). The most common organism isolated from sputum was *Klebsiella* spp. (60.6%), from TTA was *Acinetobacter* spp. and *Citrobacter. (*50%), from BAL was *Citrobacter. (*25%), and from pleural fluid was *Pseudomonas* spp. (12.6%). As regard the types of LRTIs, pneumonia, acute exacerbation of COPD, lung abscess, exacerbation of OSA, interstitial lung disease, and pleural effusion were 40.5%, 20.3%, 15.8%, 10.8%, 7.2%, 5.4% respectively (Table 2).

Table (3) showed that the resistance to azteronam and 3rd generation cephalosporins was (64% - 68%) for *Klebsiella* spp., (60% - 65%) for *E coli.* (60% - 70%) for *Enterobacter*, (25% to 50%) for *Citrobacter*, (31.1% - 68.9%) for *Pseudomonas* spp. and (54.2% - 62.5%) for *Acinetobacter* spp. The most resistance strains to imipenem and meropenem were *Klebsiella* spp. and *Acinetobacter* spp. Nearly 50% of *Klebsiella* spp., *Enterobacter* and *Citrobacter* were resistant to colistin, but lesser resistance was represented on *Pseudomonas* spp. (24.4%) and *Acinetobacter* spp. (37.5%). The resistance of *Pseudomonas* spp. and *Acinetobacter* spp. totigycycline was 77.8% and 66.7% respectively, but the resistance of *Klebsiella* spp., *E coli*. and *Enterobacter* to tigycycline *was* 23.2%, 22.5% and 10% respectively. All *Citrobacter* strains were resistant to ampicillin, piperacillin, gentamycin, tobramycin and streptomycin, but all of them were sensitive to imipenem and meropenem. The resistance of *Acinetobacter* spp. to all agents used ranged from 37.5% to 100%.

The total MDR, XDR and PDR GNB were 45.5%, 47.8% and 5.0% respectively. Also 45.5% of *Klebsiella* spp., 45% of *E coli*, 50% of (*Enterobacter*, *Citrobacter* and *Acinetobacter* spp.) and 42.2% of *Pseudomonas* spp.

were MDR. 49.5% of *Klebsiella* spp., 47.5% of *E coli*, 20% of *Enterobacter*, 53.3% of *Pseudomonas* spp. and 45.8% of *Acinetobacter* spp. were XDR, while 5.1% of *Klebsiella* spp., 7.5% of *E coli*, 4.4% of *Pseudomonas* spp., and 4.2% of *Acinetobacter* spp. were PDR, fig. (1).

There was statistically higher rate of ESβL production in fermentative GNB than non-fermentative GNB using confirmatory cephalosporin/clavulanate combination disks test. Regarding ESβL production, (61.6%) of *Klebsiella* spp., (60%) of *E coli,* (60%) of *Enterobacter*, (25.0%) of *Citrobacter*, (37.8%) of *Pseudomonas* spp. and (50.0%) of *Acinetobacter* spp. were confirmed as ESβL producers, (Table 4 & Fig. 2: a).

Statistically, there was a higher rate of MβL production in fermentative GNB than non-fermentative GNB using confirmatory IPM/EDTA. The highest percentage for MβL production by confirmatory IPM/EDTA was for *Acinetobacter* spp. (62.5%) followed by *Pseudomonas* spp. (60%) and *Klebsiela* spp. (52.5%) as shown in (Table 4 & Fig. 2: b).

There was significantly higher prevalence of ESβL and MβL positive isolates with increasing age, as age group above 50 years represent 45.5% of ESβL positive isolates versus 30.7% of ESβL negative isolates. MβL positive isolates were of significantly higher rate among those with age above 15 years (100%versus 77.7%).

Longer hospital stay, ICU admission, invasive procedures, associated co-morbidities and empirical antibiotic usage posses significantly higher risk for acquisition of ESβL and MβL (P<0.001).

There was a highly significant relation between ESβL or MβL producing isolates and unfavorable patient outcomes.

Table 1: Prevalence of aerobic Gram-negative bacilli among the studied patients with LRTIs

Culture results	The studied patients					
	Ward admission (No = 507)		ICU admission (No = 256)		Total (No = 763)	
	No	%	No	%	No	%
Culture negative	172	33.9	88	34.4	260	34.1
Culture positive	335	66.1	168	65.6	503	65.9
• Aerobic Gm -negative bacilli	120	35.8	102	60.7	222	44.1
• Other growth	215	64.2	66	39.3	281	55.9

Table 2: Distribution of different aerobic Gram-negative bacilli according to site of admission, type of specimens and types of LRTIs

	Isolated aerobic Gram –negative bacilli													
	Klebseilla spp.		*Pseudomonas* spp.		*E coli*		*Acinetobacter* spp.		*Enterobacter*		*Citrobacter*		Total (222)	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Admission Site														
Ward	61	61.6	18	40.0	27	67.5	7	29.2	5	50	2	0.50	120	54.0
ICU	38	38.4	27	60.0	13	32.5	17	70.8	5	50	2	0.50	102	46.0
Type of specimen														
Sputum	60	60.6	18	40.0	20	50.0	9	37.5	4	40	1	25.0	112	50.5
TTA	24	24.2	12	24.4	10	25.0	12	50.0	4	40	2	50.0	64	28.8
BAL	12	12.1	9	20.0	7	17.5	3	12.5	2	20	1	25.0	34	15.3
Pleural fluid	3	3.0	6	15.6	3	7.5	0	0	0	0	0	0.0	12	5.4
Types of LRTIs														
-Pneumonia	30	25.3	19	42.2	19	32.5	16	54.2	4	30	2	50	90	40.5
-Lung abscess	19	19.2	7	15.6	5	12.5	2	8.3	2	20	0	0.0	35	15.8
-Acute exacerbation of COPD	29	29.3	7	15.6	3	7.5	3	12.5	2	20	1	25.0	45	20.3
-Interstitial lung disease	7	7.1	4	8.9	3	7.5	1	4.2	1	10	0	0.0	16	7.2
-Exacerbation of OSA	11	11.1	2	4.4	7	17.5	2	8.3	1	10	1	25.0	24	10.8
-Complicated parapneumonic effusion	3	8.1	6	13.3	3	22.5	0	0	0	0	0	0.0	12	5.4
Total (222)	**99**	**44.6**	**45**	**20.3**	**40**	**18**	**24**	**10.8**	**10**	**4.5**	**4**	**1.8**	**222**	

Table 3: Antibiotic resistance pattern of the isolated aerobic Gram- negative bacilli

Antibiotics	Aerobic Gram- negative bacilli (No=222)											
	Klebsiella spp. (No=99)		*E.coli* (No=40)		*Enterobacter* (No=10)		*Citrobacter* (No=4)		*Pseudomonas* spp. (No=45)		*Acinetobacter* spp. (No=24)	
Resistance pattern	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Ampicillin (10 µg)	95	96	38	95	9	90	4	100	45	100	24	100
Piperacillin (100 µg)	88	88.9	38	95	9	90	4	100	39	86.7	22	91.7
Amoxycillin/clavulinic acid (20/10 µg)	84	84.8	35	87.5	7	70	3	75.0	45	100	24	100
Piperacillin-tazobactam (100/10 µg)	85	85.9	33	82.5	7	70	3	75.0	36	80.0	19	79.2
Amikacin (30 µg)	48	48.5	35	87.5	8	80	3	75.0	29	64.4	17	70.8
Gentamycin (10 µg)	57	57.6	37	92.5	9	90	4	100	32	71.1	19	79.2
Tobramycin (10 µg)	56	56.6	37	92.5	9	90	4	100	31	68.9	19	79.2
Streptomycin (10 µg)	55	55.6	34	85.0	7	70	4	100	30	66.7	16	66.7
Colistin (10 µg)	49	49.5	18	45.0	5	50	2	50.0	11	24.4	9	37.5
Ciprofloxacin (5 µg)	60	60.6	27	67.5	6	60	2	50.0	28	62.0	15	62.5
Norfloxacin (10 µg)	58	58	27	67.5	6	60	2	50.0	24	53.3	15	62.5
Nalidixic acid (30 µg)	62	62	31	77.5	7	70	3	75.0	26	57.8	16	66.7
Azteronam (30 µg)	64	64	24	60.0	7	70	1	25.0	14	31.1	13	54.2
Imipenem (10 µg)	70	70.7	18	45.0	2	20	0	0.0	26	57.8	17	70.8
Meropenem (10 µg)	70	70.7	16	40.0	2	20	0	0.0	22	48.9	16	66.7
Cefamandole (30 µg)	69	69.7	27	67.5	9	90	3	75.0	19	42.2	17	70.8
Ceftriaxone (30 µg)	67	67	25	62.5	7	70	2	50.0	24	53.3	15	62.5
Ceftazidime (30 µg)	68	68	26	65.0	7	70	2	50.0	31	68.9	15	62.5
Cefotaxime (30 µg)	68	68	26	65.0	7	70	2	50.0	22	48.9	15	62.5
Cefepime (30 µg)	64	64	24	60.0	6	60	1	25.0	15	33.3	14	58.3
Cefoxitin (30 µg)	66	66	26	65.0	8	80	2	50.0	16	35.6	12	50.0
Trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg)	82	82	37	92.5	9	90	3	75.0	45	100	22	91.7
Chloramphenicol (30 µg)	80	80.8	36	90.0	8	80	2	50.0	45	100	24	100
Nitrofurantoin (300 µg)	76	76.8	35	87.5	8	80	2	50.0	45	100	24	100
Tetracycline (30 µg)	78	78.8	37	92.5	9	90	3	75.0	45	100	22	91.7
Tigecycline (30 µg)	23	23.2	9	22.5	1	10	0	0.0	35	77.8	16	66.7

Table 4: Multidrug resistance pattern among aerobic gram negative studied bacteria

	Fermentative gram negative bacilli (No= 153)					Non Fermentative gram negative bacilli (No= 69)			Total N = 222	P value#
	Klebsiella No=99	*E.coli No=40*	*Enterobacter No=10*	*Citrobacter No=4*	*TOTAL N=153*	*Pseudomonas spp. No=45*	*Acinetobacter No=24*	TOTAL N=69		
ESβL producers										
Screening disc diffusion	72 (72.7)	29 (72.5)	7 (70)	3 (75.0)	111 (72.5)	27 (60)	19 (79.2)	46 (66.7)	157 (70.7)	0.37
Cephalosporin/clavulanate combination disks test	61 (61.6)	24 (60)	6 (60)	1 (25)	92 (60.1)	17 (37.8)	12 (50.0)	29 (42.0)	121 (54.5)	0.01
P value	0.10	0.24	0.64	0.16	0.02	0.03	0.03	0.004	<0.001	
	Carpabenemase producers									
Screening disc diffusion	70 (70.9)	20 (50)	-	-	90 (58.8)	31 (68.9)	19 (79.2)	50 (72.5)	140 (63.1)	0.05
Class B (metalo β-lactamase) by **IPM/EDTA**	52(52.5)	16 (40)	-	-	68 (44.4)	27 (60)	15 (62.5)	42 (60.9)	110 (49.5)	0.02
P value	0.009	0.37	--	----	0.01	0.38	0.20	0.15	0.004	

IPM/EDTA-CD; Imipenem/ Ethylenediaminetetraacetic acid combined-disk
= Comparison between total fermentative and non fermentative gram negative bacilli

Table 5: risk factors and patients' outcome associated ESBL & MBL positive isolates among aerobic gram negative bacteria LRTIs

	ESβL				P value	MβL				P value
	Positive N = 121		Negative N = 101			Positive N = 110		Negative N = 112		
	No	%	No	%		No	%	No	%	
Age										
< 15 years (25)	2	1.6	23	22.8	<0.001	0	0	25	22.3	<0.001
15– 50 (111)	64	52.9	47	46.5		77	70	34	30.4	
> 50 (86)	55	45.5	31	30.7		33	30	53	47.3	
Sex										
Male (119)	63	52.1	56	55.4	0.26	65	59.1	54	48.2	0.10
Female (103)	58	47.9	45	44.6		45	40.9	58	51.8	
Invasive procedures					0.003					
Yes (152)	93	76.9	59	58.4		97	88.2	55	49.1	<0.001
No (70)	28	23.1	42	41.6		13	11.8	57	50.9	
Comorbidities										
Yes (116)	87	71.9	29	28.7	<0.001	78	70.9	38	33.9	<0.001
No (106)	34	28.1	72	71.3		32	29.1	74	66.1	
Duration of hospital stay										
< 48 hs (78)	19	15.7	59	58.4	<0.001	11	10.0	67	59.8	<0.001
> 48 hs (144)	102	84.3	42	41.6		99	90.0	45	40.2	
ICU Admission										
Yes (102)	89	73.6	13	12.9	<0.001	89	80.9	13	11.6	<0.001
No (120)	32	26.4	88	87.1		21	19.1	99	88.4	
Antibiotic intake										
Yes (187)	111	91.7	76	75.2	<0.001	110	100	77	68.8	<0.001
No (35)	10	8.3	25	24.8		0	0.0	35	31.2	
Outcomes										
1:Improved (79)	41	33.9	38	37.6	<0.001	28	25.5	51	45.5	0.002
2:Discharge against medical advice (127)	71	58.7	56	55.4		70	63.6	57	50.9	
3:Death (16)	9	7.4	7	6.9		12	10.9	4	3.6	

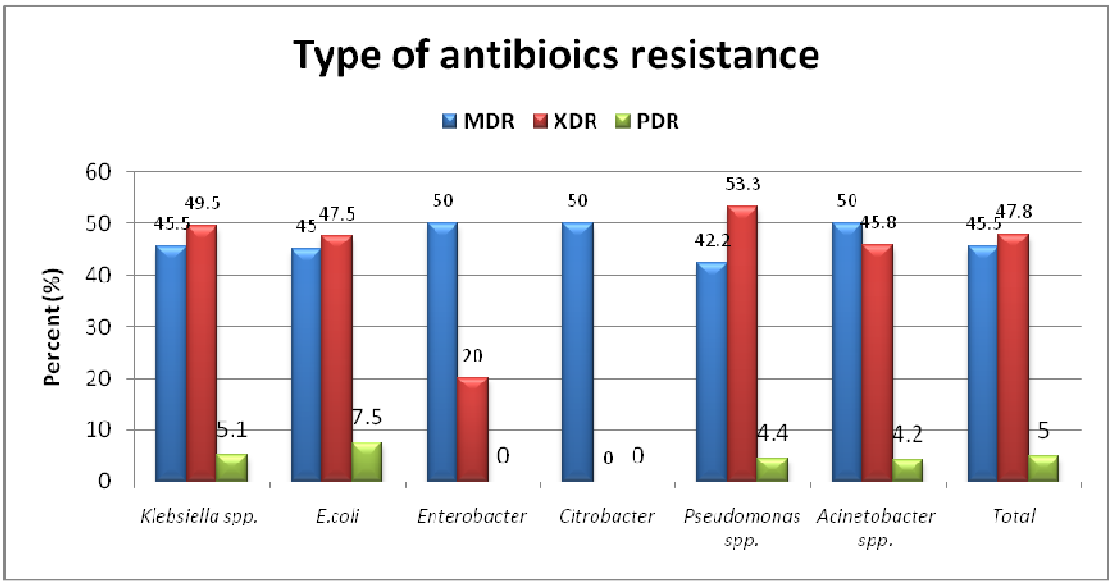

Fig (1): prevalence of MDR, XDR and PDR in the studied aerobic Gram negative bacilli

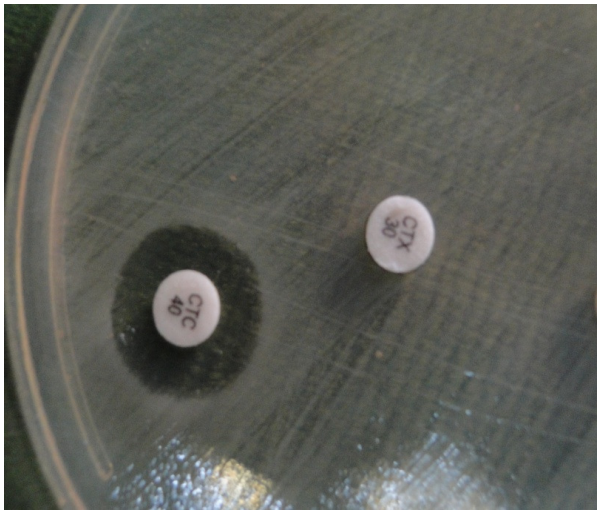

Fig (2:a): Confirmatory ESβL production (ceftazidime-clavulinate combined disks) test

Fig (2:b): Confirmatory MβL production (Imipenem/EDTA combined disks) test

DISCUSSION

Multidrug-resistant (MDR) Gram-negative organisms have become prevalent in hospitals worldwide in recent years and we are now facing infections caused by extensively drug- resistance (XDR) and pan drug-resistance (PDR) that are resistant to most (XDR) or all (PDR) antimicrobial agents available for clinical use *Sader et al (2012).*

The present study focused on determining the prevalence of microbial etiology of LRTIs and their susceptibility profiles. In this study, prevalence of GNB was 44.1% among culture positive LRTIs isolates with prevalence of 35.8% among ward patients, while it was 60.7% among ICU's patients. A nearly similar result was reported by *Patel et al., (2012).*

The higher prevalence was reported by (Navaneeth and Belwadi, 2002; Shrestha et al, 2011) who revealed that 88.4% and 96.5% respectively of the isolates separated from LRTIs were GNB. While Egbe et al (2011) found that only 18.91% of LRTIs isolates were GNB. This can be attributed to the fact that prevalence of the infections varied across geographical locations and regions within the same country, and even over time in the same location and population.

In the present study, GNB were represented as 60.7% from ICUs isolates and this result was supported by Kumari et al, (2007) who demonstrated that GNB were represented as 85.9% in the ICUs isolates where 31.9% of them were NFGNB.

In the current work, the frequency of GNB isolates in ICUs was as follows; *Klebsiella* spp. (38/102), *Pseudomonas* spp. (27/102), *Acinetobacter* spp. (17/102)*, E coli* (13/102) and other organisms were represented in table (2). Nageeb et al, (2014) in the Suez Canal University Hospital in Egypt, reported that the most frequently isolated species from ICUs were *klebsiella* spp*.* (17.4%), *E coli* (10.0%), *proteus* spp. (4.3%) and other Gram negative species (6.0%). Basnet et al (2013) reported that *Pseudomonas* spp., *Acinetobacter* spp. and *Klebsiella* spp. were the pathogens responsible for major problems in ICUs. Also Nageeb et al, (2014) documented that, the most common GNB isolates in the ICU were *Pseudomonas aeruginosa* (21.5%), *Klebsiella* spp. (19%), and *E coli* (12.4%).

The current work showed that the frequencies of isolated GNB from sputum were *Klebsiella* spp. (60%), *E coli (50%)*, *Pseudomonas* spp*.* (40%), *Acinetobacter* spp. (37.5%), *Enterobacter* (40%) then *Citrobacter* (25%) and from TTA was *Klebsiella* spp*.,* (24.2%) *Pseudomonas* spp. (24.4%), *Acinetobacter* spp. (50%), *E coli* (25%), *Enterobacter* (40%) and *Citrobacter* (50%). Anvari et al, (2014) demonstrated another set of frequencies to the organisms that were isolated from sputum; *Acinetobacter* spp. (26.8%), *Pseudomonas aeruginosa* (25%), *E coli* (5.3%), *Klebsiella* spp. (4.1%) and *Citrobacter*(1.5%). Other results were recorded by Kumari et al, (2007) where the sequences of GNB isolated from sputum were *Klebsiella* spp*., Pseudomonas* spp*., E coli*, *Enterobacter*, C*itrobacter* and *Proteus,* while *Pseudomonas* spp*., Klebsiella* spp*., E coli*, *Enterobacter* and *Proteus* were isolated from TTA.

In this study, the frequency of LRTIs types was pneumonia (40.5%), acute exacerbation of COPD (20.3%), lung abscesses (15.8%), exacerbation of OSA (10.8%), ILD (7.2%), and pleural effusion (5.4%). These results were matched with Vishwanath et al, (2013) where pneumonia was represented by 52% followed by COPD disease (31.4%) and Pleural effusion (6.3%).

In the present study, the most predominant organisms isolated from LRTIs were *Klebsiella* spp. (44.6%), *Pseudomonas* spp. (20.3%), *E coli* (18%), *Acinetobacter* spp*.* (10.8%), *Enterobacter* (4.5%) then *Citrobacter* (1.8%). Outcomes that were nearly matched to the results were presented by Vishwanath et al, (2013), where *Klebsiella pneumoniae* (37%) followed by *Pseudomonas aeruginosa* (28.6%)*, and Acinetobacter* spp. (22.7%) were the pathogens which isolated from LRTIs, but an outcome different from the results that were presented by Basnet et al (2013), where the major pathogens causing LRTIs were A*. baumannii* (22.25%), *P. aeruginosa* (20.73%) and then *K. pneumoniae* (20.12%). On the other hand, Patel et al, (2012*)* found that most of the LRTIs were caused by *Pseudomonas aeruginosa* (29.12%). The other organisms were *Klebsiella* spp. (28.08%), *Acinetobacter* spp. (24.72%), *E coli* (19.23%) and others (3.85%).

In the current work, the resistance to 3rd generation cephalosporins ranged from 64% to 68% for *Klebsiella* spp., 62.5% to 65.5% for *E coli.,* 70% for *Enterobacter*, 50% for *Citrobacter*, 48.9% to 68.9% for *Pseudomonas* spp. and 62.5% for *Acinetobacter* spp. The resistance to imipenem and meropenem was (70.7%) for *Klebsiella* spp., (45% and 40%) for *E coli*. (20%) for *Enterobacter*, (57.8% and 48.9%) for *Pseudomonas* spp. and (70.8% and 66.7%) for *Acinetobacter* spp. All *Citrobacter* strains were sensitive to imipenem and meropenem. Another prevalence resistance data was conducted by Anvari et al, (2014) where (22.8-32.7%) of *Klebsiella* spp., (65.7%) of *E coli*, (63.9%) of *Pseudomonas* spp. (91.2%-92.1%) of *Acinetobacter* spp*.* were resistant to 3rd generation cephalosporins. Also, 9.4% and 4.8% of *Pseudomonas* spp., 47.9% and 77.8% of *Acinetobacter* spp. were resistant to imipenem and meropenem respectively. Luckily, none of *Klebsiella* spp., nor *E coli* isolates was resistant to imipenem or meropenem.

Other studies; Gautam et al, (2013) recorded that 65.7% of *E coli* were resistant to ceftriaxone and none of them was resistant to imipenem. Patel et al, (2012) recorded that 13.2% of *Pseudomonas* spp. were imipenem resistant, 2.22% of *Acinetobacter* spp. were imipenem resistant. Shrestha et al, (2013) revealed that 97% of *Acinetobacter* spp. were resistant to cefepime and 83.33% of *Acinetobacter* spp. were resistant to imipenem and meropenem.

(Harris et al, 2015 and Sader et al, 2012) clarified that MDR GNB are now globally widespread and present a major challenge to modern medical practice. The results of this study have proved this clarification as MDR, XDR and PDR were 45.5%, 47.8% and 5.0% respectively. Deotale et al, 2015 reported that 26.9%, 7.4% and 1.9% of resistant GNB were MDR, XDR and PDR respectively. Also, Vishwanath et al, 2013 revealed that 37% among GNB were MDR.

Regarding to each microorganism of this study, 45.5% of *Klebsiella* spp., 45% of *E coli*, 50% of (*Enterobacter*, *Citrobacter* and *Acinetobacter* spp.) and 42.2% of *Pseudomonas* spp. were MDR, while 49.5% of *Klebsiella* spp., 47.5% of *E coli*, 20% of *Enterobacter*, 53.3% of *Pseudomonas* spp. 45.8% of *Acinetobacter* spp. were XDR. 5.1% of *Klebsiella* spp., 7.5% of *E coli*, 4.4% of *Pseudomonas* spp., 4.2% of *Acinetobacter* spp. and none of *Enterobacter* were PDR. *Citrobacter* was not categorized as XDR or PDR. Vishwanath et al, (2013) declared that 48.6% of *Klebsiella pneumoniae* and 33.7% of *Acinetobacter* spp. were the most predominant MDR GNB. High results were derived by Shrestha et al, (2013), where 100%, 97%, 67%, 55% and 50% of MDR GNB were *E coli*, *Acinetobacter*, *K. pneumoniae*, *Pseudomonas* and *Citrobacter* respectively. Lower results of PDR were documented by Kumari et al, (2007) where 1.7% of *Klebsiella* spp., 1.1% of *E coli,* 5% of *Pseudomonas aeruginosa,* 0.8% *of Enterobacter* spp. and 0.2% *of Citrobacter* spp. were PDR.

Extended-spectrum beta-lactamases (ESβLs) have emerged as beta-lactamases is the most common mechanism of bacterial resistance to these antibiotics, Sibhghatulla et al, (2015).

In this study, there was statistically significant difference between the studied fermentative GNB and non-fermentative GNB (72.5% & 60.1% vs. 66.7% & 42.0%) for ESβL production by both screening and confirmatory tests. Nearly similar results were observed by Kumar et al, (2012) where 61% of GNB were confirmed as ESβL producers. Siddiqui et al, (2014) noticed that result of screening ESβL test, (73%) was lesser than ESβL confirmed test (83.42%). The lowest results were demonstrated by Shrestha et al, (2011) where only 12.9% of *Enterobacteriaceae* and 1.3 % of non-fermenting GNB were ESβL producers.

In the present study, 61.6% of *Klebsiella* spp., 60% of both *E coli* and *Enterobacter*, 25% of *Citrobacter* 37.8% of *Pseudomonas* spp. and 50% of *Acinetobacter* spp. were confirmed as ESβL producers. It nearly matched with Siddiqui et al, (2014) results, where 74% of *K. pneumonia*, 62% of *E coli*, 60% of *Enterobacter* and 38% of *Pseudomonas* spp. were confirmed as ESβL producers. Shrestha et al, (2013) found that 67% of *E coli,* 50% of *Citrobacter*, 47% of *K. pneumoniae*, 36% of *Pseudomonas* and 17% of *Acinetobacter* were confirmed as ESβL producers.

Among the β-lactamases, the carbapenemases especially transferrable metallo-β-lactamases (MβLs) are the most feared, because of their ability to hydrolyze virtually all drugs in that class, including the carbapenems Noyal et al, (2009).

This study showed a statistically significant difference between the studied fermentative and non-fermentative GNB for MβL production by screening and confirmatory IPM/EDTA tests. The highest percentage for MβL production by confirmatory IPM/EDTA was for *Acinetobacter* spp. (62.5%) followed by *Pseudomonas* spp. (60%), *Klebsiela* spp. (52.5%) and *E coli* (40%). These results were different from that observed by Shrestha et al, (2013) where 67% of *Acinetobacter*, 3% of K. pneumoniae, 9% of *Pseudomonas* were confirmed as MβL producers. In the study of Shrestha et al, (2011), which declared that 47.2% of *Acinetobacter*, 4.2% of *Klebseilla*, and (2.4%) of Pseudomonas were confirmed as MβL producers.

This study showed that none of *Enterobacter* spp. *or Citrobacter* spp. was a MβL producer. Shrestha et al, (2013) reported that none of *E coli* or Citrobacter isolates was a MβL producer.

In the current work, the highest incidence of ESβL (52.9%) and MβL (70%) positive isolates were represented at age group 15-50 years, followed by the patients over 50 years. These results were supported by Harris et al, (2007) who documented that the Age >60 years targeted to the ESβL producing bacteria and Kumar et al, (2012) who recorded that all MβL positive isolates were from adults.

This study revealed that, longer hospital stay, ICU admission, invasive procedures, associated comorbidities and repeated antibiotics intake were significantly high risk factors for acquisition of ESβL and MβL. This was matched with the Infection Control Fact Sheet of 2007 of a hospital. On the other hand, Kumar et al, (2012) recorded that, there was non-significant difference between MβL positive and MβL negative isolates as regards to duration hospital stay, catheterization, IV lines, previous antibiotic use, mechanical ventilation and endotracheal intubation. Only graft application and surgical intervention were significant risk factors for MβL-positives as compared to MβL negatives. Lim et al, (2014) stated that the presence of a pressure ulcer, non-ambulatory status, medical device in situ, surgical procedure within the previous 12 months, comorbidities are important risk factors for multi-drug resistance.

In the present study, there was a highly significant association between ESβL or MβL producing states and the worst patient outcomes. This was matched with Siddiqui et al, (2014) who found that the infections caused by ESβL-producers were associated with increased morbidity and mortality. The mortality rate in this study was 7.4% and 10.9% in patients that had ESβL or MβL producing isolates respectively, which was lesser than the results recorded by Kumar et al, (2012) where the mortality in patients with MβL positive isolates was 33.33%.

CONCLUSIONS

From the previous results, we can conclude that GNB represent nearly half of LRTIs isolates and the most predominant organisms were *Klebseilla* spp, *Pseudomonas* spp and *Acinetobacter* spp., which are mostly isolated from ICU than medical wards. Tigycyclin and colistin represent higher sensitivity for fermentative and non fermentative GNB respectively. The MDR isolates in respiratory samples were correlated with clinical findings of

patients with referral to their pathogenic role and not merely considered as colonizers. Morbidity increases with LRTIs caused by ESβL or MβL producing isolates.

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Cite this Article: Shymaa A El-A, Amal FM, Gehan AAA, Reda AI, Rabab AE (2016). Prevalence of Aerobic Gram-Negative Bacilli in Lower Respiratory Tract Infections in Menoufia Governorate, Egypt. Greener Journal of Medical Sciences, 6(4): 052-062, http://doi.org/10.15580/GJMS.2016.4.093016149