Diffusion method and Vitek machine analysis of ESBLs for *Klebsiella pneumoniae* a comparable study

Hesham M. Mahdy [1], Abdel-Moniem M. Sharaf, Mahmoud M. Al-Aaser [2] and Hamdy M. El-Sayed

Botany Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt
[2.] Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt
[1.] Present address: Biology Department, Faculty of Science and Arts, Al-Baha University, Al-Baha, KSA
mahdyhesham@hotmail.com

Abstract: An investigation concerning comparison methods of agar diffusion and ESBL for *Klebsiella pneumoniae* strains isolated from three biggest hospitals in Egypt. Of 150 different specimens collected along a year; 112 were belonging to *Klebsiella pneumoniae*. *Klebsiella pneumoniae* resistant to ampicillin by 100% followed by cephalothin 94.6% and ceftriaxone 85.7% as recorded by agar diffusion method. A result of Vitek machine was different from agar diffusion in resistant to cephalothin where were 93.75%. Production of ESBLs by agar diffusion (double disc synergy test) was not asserted for H-38, H-40, H-47, D-4, D-5, D-8, K-24, K-26, K-27, K-32, K-38, K-42 and K-47strains while they were producer by Vitek ESBL test.
[Hesham M. Mahdy, Abdel-Moniem M. Sharaf, Mahmoud M. Al-Aaser and Hamdy M. El-Sayed. **Diffusion method and Vitek machine analysis of ESBLs for *Klebsiella pneumoniae* a comparable study.** *Researcher* 2012; 4(12):50-56]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 10

Keywords: ESBL, *Klebsiella pneumonia*, Vitek machine, diffusion method

1. Introduction

Up to the late 1990s pathogenic bacteria was relatively susceptible to first line antibiotics, however several surveillance studies during the 2000s across the world have shown increasing resistance to first line antibiotics including the cephalosporins, fluoroquinolones, and trimethoprim–sulfamethoxazole. The "newer β-lactamases" that consists of plasmid-mediated AmpC β-lactamases (e.g., CMY types), ESBL (e.g., CTX-M types), and carbapenemases (e.g., NDM) are important causes of resistance to β-lactam antibiotics among pathogenic bacteria (Doughari *et al*., 2012).

Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged intensive care unit stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporin use and instrumentation or catheterization (Nathisuwan *et al*., 2001).

Screening of the production of ESBLs is done in most laboratories according to the CLSI guidelines that involve an initial screening with standard cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone discs, followed by a confirmatory test with ceftazidime and cefotaxime disks alone and in combination with clavulanic acid (CLSI, 2008). Augmentation of the zone of inhibition by ≥5 mm is considered a positive test result. The other test for AmpC-typc β-lactamases that involves augmentation of the inhibition zone around ceftazidime and cefotaxime, disks by a boronic acid compound (Yagi *et al*, 2005).

The aim of this manuscript is to demonstrate which reliable method- agar diffusion or Vitek machine- could be used in access the production of ESBL from *Klebsiella pneumoniae*.

2. Materials and Methods

Sample collections: The total of 150 specimens was collected during Nov. 2010-Nov. 2011. Blood, ascitic fluid, sputum, urine, wound swabs, endotracheal tubes, cerebral spinal fluid and other samples were collected from inpatients and outpatients of three hospitals: El-Hussein University, El-Demerdash University, and Kasr El-Ainy University.

Methods of isolation and differentiation of *Klebsiella*: Bacteria were isolated from clinical specimens by agar streak method. An inoculum was spread onto surface plates of readymade media: Endo agar (Difco, England), Blood and MacConkey agar (BBL, USA).*Klebsiella* species identified and differentiated according to their biochemical reactions (Holt *et al*., 1994). The biochemical reactions were glucose fermentation in KIA (Kligler´s iron agar), urease utilization in urea agar, and citrate utilization in citrate agar, lysine decarboxylase production in lysine iron agar and ornithine decarboxylase production in MIO (motility-indole-ornithine) media (BBL). *Klebsiella pneumoniae* identification was confirmed using API strips inoculated and incubated as described by the manufacturer (bio Merieux Vitek System, France). Examination of the strips was conducted after 18-24 h, and the results from the 24 h analysis were used. The results were read and analyzed using analytical profile index, a mini-API instrument (bio Merieux Vitek Systems).

Antimicrobial susceptibility testing: Disc diffusion method was used to investigate the antibiotic resistance of *Klebsiella pneumoniae*. The filter paper discs impregnated with known concentrations of antibiotics are placed on the surface of Müeller Hinton (MH) agar plate, which has been inoculated with a standardized inoculum of the organism to be tested. The antibiotic from the disc diffuses into the agar, resulting in distinct zones of inhibition around these discs. The size of the zone of inhibition (mm) will determine if the bacterium is resistant or susceptible to different antibiotics based on methods recommended by the CLSI (CLSI, 2008). Quality control was carried out according to the recommendations of the CLSI using ATCC strains as controls. Sixteen antibiotics were tested: amikacin (AK), ampicilin (AMP), ampicillin/sulbactam (SAM), aztreonem (ATM), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), cephalothin (CF), chloramphinicol (C), ciprofloxacin (CIP), gentamycin (CN), imipenem (IPM), nalidixic acid (NA), tetracycline (TE), ticracillin/clavulanic acid (TIM) and trimethoprim/ sulphamethoxazole (SXT).

Procedure. With a sterile cotton applicator, 4-5 well isolated colonies were transferred to a saline solution tube following sterile techniques. The inoculums were calibrated with a 0.05 McFarland standard. Using another cotton-tipped-sterile applicator, the Müeller Hinton agar plate was inoculated, streaking the entire surface of the plate, rotating the plate 60° between streaks and ultimately rimming the plate to ensure confluent growth to the edges. After 2-3 minutes, a mechanical dispenser was used to apply the discs. All plates were incubated at 37°C for 18-24 hours before final reading by using a caliber to measure the zone of inhibition. The results were recorded according to CLSI guidelines (Table 1).

Screening of the production of Extended Spectrum β-Lactamases (ESBLs):

A- Double disc synergy test: A standardized suspension of the isolate is inoculated onto a Müeller Hinton agar plate using the antimicrobial discs Cefotaxime (30µg), Cefotaxime/Clavulanic acid (30/10µg), Ceftazidime (30µg), and Ceftazidime/Clavulanic acid (30/10µg). After incubation, the zone of inhibition around each of the discs is measured. A ≥ 5 mm increase zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone, indicates positive for Extended Spectrum Beta Lactamase (ESBL) production (Sanders *et al*., 1996).

B- Vitek ESBL Test: About 3.0 ml of sterile saline (aqueous 0.45% NaCl, pH 7.0) was placed into a clear plastic test tube. Then sufficient numbers of pure bacterial colonies were transferred to the tube containing the saline to make a homogenous suspension with an equivalent density of McFarland ($N^{o.}$ 0.50 to 0.63) using Calibrated VITEK 2 DENSICHEK (biomerieux, France). The tube was then placed in the cassette with the identification card and data entry. In a second tube containing 3.0 ml of saline, 280µl of the suspension prepared for AST-GN (Antibiotic Susceptibility Test-Gram Negative) was then transferred. The tube was then placed in the cassette with the susceptibility card. The identification GN is based on 43 biochemical tests measured carbon source utilization, enzymatic activities and resistance. The identification results were available in approximately eight hours.

Table 1. Interpretive zone diameters of antimicrobial agents for enterobacteriaceae according to CLSI guidelines, 2008.

Antibiotic	Disc cont./µg	Zone diameters (mm)		
		Sensitive	Intermediate	Resistant
AK	30	≥ 17	15 – 16	≤ 14
AMP	10	≥ 17	14 – 16	≤ 13
SAM	10	≥ 15	12 – 14	≤ 11
ATM	30	≥ 22	16 – 21	≤ 15
CTX	30	≥ 23	15 – 22	≤ 14
CAZ	30	≥ 18	15 – 17	≤ 14
CRO	30	≥ 21	14 – 20	≤ 13
CF	30	≥ 17	15 – 16	≤ 14
C	30	≥ 18	13 – 17	≤ 12
CIP	05	≥ 21	16 – 20	≤ 15
CN	10	≥ 15	13 – 14	≤ 12
IPM	10	≥ 16	14 – 15	≤ 13
NA	30	≥ 19	14 – 18	≤ 13
TE	30	≥ 19	15 – 18	≤ 14
TIM	30	≥ 17	15 – 16	≤ 14
SXT	1.25/ 23.75	≥ 16	11 – 15	≤ 10

For the MIC technique, AST card contains 64 Micro wells were used. While control well containing only microbiological culture medium was resident on all cards with the remaining wells containing pre measured amounts of specific antimicrobials combined with culture medium. MIC values were determined for each antimicrobial contained on the card after a defined period of time about 18 hours.

3. Results

3.1. Bacterial isolates: Out of 150 bacterial isolates, which were phenotipically related to *Klebsiella* spp. and reported as *Klebsiella* spp. in a selected hospitals, 112(74.67%) were belonging to *Klebsiella* spp. by differential tubes and only 66(44%) were belonging to *Klebsiella pneumoniae* by API 20E system. All

bacterial isolates were coded according to identification and date of isolation for easily revision.

In total, 66 isolates of *Klebsiella pneumoniae* were collected from different infection sites of patients which include cultures from 32(21.3%) urine, 15(10%) sputum, 15(10%) wound swab, 6(4%) tissue, 19(12.67%) pus, 5(3.3%) stool, 34(22.67%) blood, 8(5.3%) Cerebral Spinal Fluid, 10(6.7%) endotracheal tube, 5(3.3%) skin swab and one culture sample from ascetic fluid (Table 2). The diagnostic clinical symptoms of bacterial infections were examined by physicians in three Egyptian hospitals viz.: El-Hussein University, El-Demerdash University, and Kasr El-Ainy Hospitals as represented for the biggest 3 Universities in Cairo governorate.

Table 2. Bacterial isolates obtained from different infection sources.

Clinical Specimen	Hospital			Total N°. of viable isolates
	El-Hussein	El-Demerdash	Kasr El-Ainy	
Urine	11	12	9	32
Sputum	2	8	5	15
Wound swab	3	5	7	15
Tissue	2	0	4	6
Pus	9	3	7	19
Stool	3	2	0	5
Blood	12	11	11	34
CSF	2	2	4	8
ETT	4	3	3	10
Ascetic fluid	0	1	0	1
Skin	2	3	0	5
Total N°. of viable isolates	50	50	50	150
%	33.33	33.33	33.33	100

* ETT, Endotracheal tube; CSF, Cerebral Spinal Fluid

3.2. Differentiation of bacterial isolates: All members of *Klebsiella pneumoniae* were fermented glucose and other sugars. Generally produced lysine but not ornithine decarboxylase; in addition, they are non-motile; negative for indole production. *Klebsiella* species mostly show positive results for citrate utilization and urease production tests. Table (3) showed total number of *Klebsiella* spp. and other bacterial isolates in each hospital. The net results of biochemical reactions for bacterial isolates were 112(74.67%) isolates belonging to *Klebsiella* spp. and 38(25.33%) belonging to *E. coli* and *Enterobacetr* spp. by differential tubes.

Table 3. Total number of *Klebsiella* spp. and other bacterial isolates in each hospital.

Hospital	*Klebsiella* spp.	Other bacteria
El-Hussein	37	13 isolates
El-Demerdash	38	12 isolates
Kasr El-Ainy	37	13isolates

3.3. Antibiotic susceptibility and extended spectrum β-lactamases for the bacterial species:

A) Antibiotic Susceptibility Test: Antibiotic susceptibility was proceeded manually and automated by Vitek machine. In manual method (agar diffusion method) results indicated that all *Klebsiella pneumoniae* strains (112 isolates) were resistant to ampicillin; sensitive to impenem and showed different susceptibility profiles for other antibiotics. Table (4) shows the results of antimicrobial agents which recorded as sensitive, intermediate sensitive and resistant according to CLSI guidelines (2008).

Notwithstanding, four isolates which isolated from El-Demerdash hospital (D-10, D-14, D-18 and D-22) were resistant to ampicillin only; other isolates such as H-7, H-14, H-27, H-43, D-7, D-30, D-35, D-44, K-3, K-28, and K-44 were resistant to all used antibiotics except impenem.

Table 4. Percentages of antibiotic susceptibility test for *Klebsiella pneumoniae* strains by agar diffusion method

Antibiotic	Susceptibility percentages		
	Sensitive	Intermediate	Resistant
AK	58 (51.8%)	17 (15.2%)	37 (33%)
AMP	0 (0%)	0 (0%)	112(100%)
SAM	11(9.8%)	15(13.4%)	86 (76.8%)
ATM	12 (10.7%)	22 (19.6%)	78 (69.6%)
CTX	11 (9.8.7%)	9 (8%)	92 (82%)
CAZ	32 (28.6%)	13 (11.7%)	67 (59.8%)
CRO	11 (9.8%)	5 (4.5%)	96 (85.7%)
CF	6 (5.4%)	0 (0%)	106 (94.6%)
C	64 (57%)	0 (0%)	48 (42.9%)
CIP	50 (44.6%)	6 (5.4%)	56 (50%)
CN	47 (42%)	2 (1.8%)	63 (56.1%)
IPM	112(100%)	0 (0%)	0 (0%)
NA	43 (38.4%)	7 (6.1%)	62 (55.1%)
TE	19 (17%)	18 (16.1%)	75 (67%)
TIM	17 (15.2%)	13 (11.6%)	82 (73%)
SXT	55 (49%)	4 (3.6%)	53 (47.3%)

Alternatively, susceptibility test result by Vitek machine, table (5), shows some differences from that obtained by manual method. For example 57 isolates were sensitive to amikacin, 10 isolates sensitive to Ampicillin/sulbactam, 91 isolates resistance to cefotaxime, 5 isolates sensitive to cephalothin, 105 isolates resistance to cephalothin, 62

isolates resistance to gentamycin and 16 isolates sensitive to Ticracillin/clavulanic acid. Other antibiotic susceptibility showed no change than agar diffusion method for *Klebsiella pneumoniae* .

Table 5. Percentages of antibiotic susceptibility test for *Klebsiella pneumoniae* strains by Vitek machine.

Antibiotic	Susceptibility percentages		
	Sensitive	Intermediate	Resistant
AK	57 (50.89%)	18 (16.1%)	37 (33%)
AMP	0 (0%)	0 (0%)	112(100%)
SAM	10(8.93 %)	16(14.29%)	86 (76.8%)
ATM	12 (10.7%)	22 (19.6%)	78 (69.6%)
CTX	11 (9.8.7%)	9 (8%)	91(81.25%)
CAZ	32 (28.6%)	13 (11.7%)	67 (59.8%)
CRO	11 (9.8%)	5 (4.5%)	96 (85.7%)
CF	5 (4.46%)	2 (1.8%)	105(93.75%)
C	64 (57%)	0 (0%)	48 (42.9%)
CIP	50 (44.6%)	6 (5.4%)	56 (50%)
CN	47 (42%)	3 (2.68%)	62(55.36%)
IPM	112(100%)	0 (0%)	0 (0%)
NA	43 (38.4%)	7 (6.1%)	62 (55.1%)
TE	19 (17%)	18 (16.1%)	75 (67%)
TIM	16(14.29%)	14 (12.5%)	82 (73%)
SXT	55 (49%)	4 (3.6%)	53 (47.3%)

B) Screening for the production of Extended Spectrum β-Lactamases (ESBLs): A total of 92 (82%) strains were positive for ESBL, of these 78(69.6%) were detected by both double disc synergy test (Figure 1) and Vitek ESBL test. Also, 9(8%) strains were detected only by the antimicrobial discs Ceftazidime, and Ceftazidime/Clavulanic acid of Vitek ESBL test. While remaining 5(4.5%) strains were detected only by the antimicrobial discs Cefotaxime, Cefotaxime/Clavulanic acid of Vitek ESBL test (Table 6).

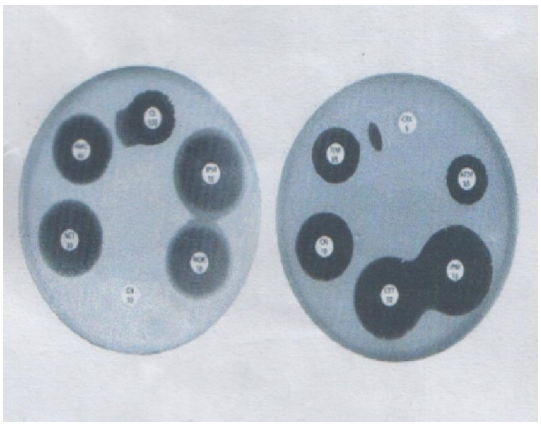

Figure 1. Screening for ESBLs production by double disc synergy test

Table 6. Screening for the production of ESBLs by double disc synergy test and Vitek ESBL test for *Klebsiella pneumoniae* strains.

Code N°.	Double disc synergy test	Vitek ESBL test (mm)				Screening result
		CTX	CTX/CLAV	CAZ	CAZ/CLAV	
H-1	+ve	8	24	15	25	+ve
H-2	+ve	7	21	13	24	+ve
H-4	+ve	7	20	14	25	+ve
H-5	+ve	7	20	11	23	+ve
H-6	+ve	10	22	17	26	+ve
H-7	+ve	8	18	8	21	+ve
H-8	+ve	9	20	16	25	+ve
H-10	+ve	7	17	14	21	+ve
H-11	+ve	6	16	12	24	+ve
H-12	+ve	6	14	9	23	+ve
H-13	+ve	6	17	8	20	+ve
H-14	+ve	8	21	7	20	+ve
H-15	+ve	7	17	14	26	+ve
H-17	+ve	8	18	16	26	+ve
H-19	+ve	7	16	14	23	+ve
H-20	+ve	14	23	10	25	+ve
H-21	-ve	6	6	19	23	-ve
H-22	+ve	7	22	7	21	+ve
H-25	+ve	7	14	12	21	+ve
H-27	+ve	6	21	8	23	+ve
H-28	+ve	6	24	11	23	+ve
H-29	+ve	7	28	13	25	+ve
H-30	+ve	8	24	17	25	+ve
H-31	+ve	9	21	11	24	+ve
H-32	+ve	10	28	12	26	+ve
H-34	+ve	8	22	12	23	+ve
H-35	-ve	14	24	19	22	+ve
H-37	+ve	6	23	6	14	+ve
H-38	-ve	14	23	13	14	+ve
H-40	-ve	13	23	23	25	+ve
H-43	+ve	6	20	6	19	+ve
H-44	+ve	6	21	20	25	+ve
H-45	-ve	16	14	14	14	-ve
H-46	+ve	6	23	15	24	+ve
H-47	-ve	17	31	30	32	+ve
H-48	+ve	9	24	19	27	+ve
H-50	+ve	6	28	20	28	+ve
D-2	+ve	18	31	21	30	+ve
D-3	+ve	6	15	6	19	+ve
D-4	-ve	8	20	22	25	+ve
D-5	-ve	6	11	9	9	+ve

Table 6. Continued.

Code N°.	Double disc synergy test	Vitek ESBL test (mm)				Screening result
		CTX	CTX/CLAV	CAZ	CAZ/CLAV	
D-6	+ve	17	26	11	23	+ve
D-7	+ve	6	22	6	20	+ve
D-8	-ve	6	25	23	25	+ve
D-10	-ve	28	29	28	29	-ve
D-11	-ve	28	29	24	25	-ve
D-12	+ve	8	18	16	25	+ve
D-14	-ve	26	27	24	26	-ve
D-15	-ve	6	6	17	17	-ve
D-16	+ve	6	11	8	26	+ve
H-34	+ve	8	22	12	23	+ve
H-35	-ve	14	24	19	22	+ve
H-37	+ve	6	23	6	14	+ve
H-38	-ve	14	23	13	14	+ve
H-40	-ve	13	23	23	25	+ve
H-43	+ve	6	20	6	19	+ve
H-44	+ve	6	21	20	25	+ve
H-45	-ve	16	14	14	14	-ve
H-46	+ve	6	23	15	24	+ve
H-47	-ve	17	31	30	32	+ve
H-48	+ve	9	24	19	27	+ve
H-50	+ve	6	28	20	28	+ve
D-2	+ve	18	31	21	30	+ve
D-3	+ve	6	15	6	19	+ve
D-4	-ve	8	20	22	25	+ve
D-5	-ve	6	11	9	9	+ve
D-6	+ve	17	26	11	23	+ve
D-7	+ve	6	22	6	20	+ve
D-8	-ve	6	25	23	25	+ve
D-10	-ve	28	29	28	29	-ve
D-11	-ve	28	29	24	25	-ve
D-12	+ve	8	18	16	25	+ve
D-14	-ve	26	27	24	26	-ve
D-15	-ve	6	6	17	17	-ve
D-16	+ve	6	11	8	26	+ve
D-18	-ve	20	22	26	28	-ve
D-19	-ve	29	28	28	29	-ve
D-20	-ve	28	28	27	28	-ve
D-21	+ve	6	13	11	25	+ve
D-22	-ve	28	29	28	28	-ve
D-23	+ve	10	26	20	29	+ve

Table 6. Continued.

Code N°.	Double disc synergy test	Vitek ESBL test (mm)				Screening result
		CTX	CTX/CLAV	CAZ	CAZ/CLAV	
D-24	-ve	29	30	28	29	-ve
D-25	+ve	6	13	11	25	+ve
D-27	+ve	6	11	8	26	+ve
D-28	-ve	16	17	30	32	-ve
D-30	-ve	6	6	6	6	-ve
D-31	-ve	31	30	30	29	-ve
D-33	-ve	24	26	23	23	-ve
D-35	+ve	6	12	8	18	+ve
D-36	-ve	12	11	25	25	-ve
D-37	+ve	18	26	14	24	+ve
D-38	+ve	8	24	15	25	+ve
D-39	+ve	7	21	13	24	+ve
D-41	+ve	7	20	14	25	+ve
D-42	+ve	7	20	11	23	+ve
D-43	+ve	10	22	17	26	+ve
D-44	+ve	8	18	8	21	+ve
D-45	+ve	9	20	16	25	+ve
D-46	+ve	7	17	14	21	+ve
D-47	+ve	6	16	12	24	+ve
K-1	+ve	6	14	9	23	+ve
K-3	+ve	8	21	7	20	+ve
K-5	+ve	7	17	14	26	+ve
K-6	+ve	8	18	16	26	+ve
K-7	+ve	7	16	14	23	+ve
K-8	+ve	14	23	10	25	+ve
K-10	-ve	6	6	19	23	-ve
K-12	+ve	7	22	7	21	+ve
K-13	+ve	7	14	12	21	+ve
K-15	+ve	6	21	8	23	+ve
K-16	+ve	6	24	11	23	+ve
K-18	+ve	7	28	13	25	+ve
K-19	+ve	8	24	17	25	+ve
K-20	+ve	9	21	11	24	+ve
K-21	+ve	10	28	12	26	+ve
K-22	+ve	8	22	12	23	+ve
K-24	-ve	14	24	19	22	+ve
K-25	+ve	6	23	6	14	+ve
K-26	-ve	14	23	13	14	+ve
K-27	-ve	13	23	23	25	+ve
K-29	+ve	6	21	20	25	+ve

Table 6. Continued.

Code N°.	Double disc synergy test	Vitek ESBL test (mm)				Screening result
		CTX	CTX/CLAV	CAZ	CAZ/CLAV	
K-30	-ve	16	14	14	14	-ve
K-31	+ve	6	23	15	24	+ve
K-32	-ve	17	31	30	32	+ve
K-34	+ve	9	24	19	27	+ve
K-35	+ve	6	28	20	28	+ve
K-36	+ve	18	31	21	30	+ve
K-37	+ve	6	15	6	19	+ve
K-38	-ve	8	20	22	25	+ve
K-42	-ve	6	11	9	9	+ve
K-43	+ve	17	26	11	23	+ve
K-44	+ve	6	22	6	20	+ve
K-47	-ve	6	25	23	25	+ve
K-48	-ve	28	29	28	29	-ve
K-50	-ve	28	29	24	25	-ve

4. Discussion: This study shows some virulence factors influence antimicrobial resistant and evaluates two methods used in University hospital laboratories in Cairo for ESBL detection. Those resistant strains isolated from different hospitals in Cairo governorate. We could isolate bacteria from urine, sputum, wound swab, tissue, pus, stool, blood, CSF, ETT, ascetic fluid and skin samples of patients experiencing different complications from three hospitals. The predominant bacterial species isolated was *Klebsiella pneumoniae* due to use selective media in isolation process and also picking up colonies seems like *Klebsiella* morphology.

Foregoing results indicated that *Klebsiella* isolated from 22.67% followed by 21.3% of blood and urine samples, respectively, that could be regard to infections such as pneumonia (an inflammatory illness of the lungs), urinary tract infections (UTI), ankylosing spondylitis (degenerative inflammatory arthritis), septicemia (whole body inflammation) and soft body infections.

Antimicrobial resistance is an increasingly emerging problem worldwide, especially by *Klebsiella* infection. Identifying the resistance pattern of microorganisms in every hospital is the key to success in the appropriate treatment of patients. This study was conducted to evaluate and compare *Klebsiella* infection from environment and patients Admittance Hospital Universities in Capital Cairo-viz.: El-Hussein, El-Demerdash, and Kasr El-Ainy and to determine the susceptibility profile of nosocomial and community acquired *Klebsiella* infection to different antimicrobial agents used.

Beta-lactams are the most widely used antibiotics all over the world, and resistance to this antibiotic has resulted in a major clinical crisis (Mohammadi-mehr and Feizabadi, 2011). With the widespread use of extended-spectrum cephalosporins throughout the world, strains that produce ESBLs have been detected on every inhabited continent. These enzymes are most commonly found in *K. pneumoniae*, but they are increasingly found in Gram-negative bacilli and other Gram-positive. The emergence and spread of ESBL-producing strains have led to questions regarding the optimal therapy for infections caused by ESBL-producing strains. Although many reports have described outbreaks of infections caused by ESBL-producing organisms, until now no randomized prospective study of the treatment of infections caused by ESBL-producing organisms has been conducted **(**De Angelis *et al*., 2012**)**.

Antibiotic susceptibility of our isolates was determined by the disk diffusion method. All the isolates were susceptible to imipenem (and resistant to ampicillin). This antibiotic was, with the chloramphenicol, one of the most active tested antibiotics. This has been observed previously in a study involving 211 clinical strains (Thibault *et al*., 2004) and is of interest because this antibiotic is considered as a good alternative to ceftazidime in the treatment of disseminated disease.

Albeit antimicrobial susceptibility surveillance programs represent one of the main recommendations to control resistant organisms, providing essential information in order to improve the quality of empiric antimicrobial prescribing or guiding development of antimicrobial policies. National and regional distributions of the data are important to enable local prescribing practices (Laure *et al*., 2012). The carbapenem (meropenem, imipenem)? with activity against many bacteria have been the most active broad-spectrum antimicrobial class documented by numerous large surveillance programs (Zhanel *et al*., 2007).

The high levels of antimicrobial resistance in Gram-negative bacteria can be attributed to antibiotic misuse in Egypt (Ashour and El-Sharif, 2009). Policies on the control of antibiotic usage have to be enforced and implemented to avoid the evolution of newer generations of pathogens with higher resistance, not only to the older generation drugs, but also to the relatively new ones. In addition, the entire microbial spectrum in various infection sites, and not just bloodstream pathogens, should be taken into account when initiating empirical antibiotic therapy. More likely, Atif (2006) and Jumaa (2006) reported that *Klebsiella* spp. accounted for up to 10% of all nosocomial bacterial infections placing it among the most important infectious pathogens in hospitals at

over the world including Middle East; in addition to Carrër *et al*. (2010) who isolated multidrug resistant *K. pneumoniae* from Gizah, Egypt (KpE).

In spite the majority of ESBL *K. pneumoniae* (69.6%) was detected by both methods used, it is still 12.5% of strains were detected only by Vitek machine which prove the sensitivity of this method than agar-diffusion method.

Correspondence
Dr. Hesham Mohamed Mahdy
Biology Department,
Faculty of Science and Arts Al-Mikhwah
Al-Baha University
Al-Baha
Kingdom of Saudi Arabia
mahdyhesham@hotmail.com

References

[1] Ashour H A and El-Sharif A. Species distribution and antimicrobial susceptibility of gram-negative aerobic bacteria in hospitalized cancer patients. Journal of Translational Medicine 2009; 7:14.

[2] Atif A. Frequency and antimicrobial susceptibility patterns of bacterial pathogens isolated from septicemic patients in Makkah hospitals, Saudi Medical Journal 2006; 27:361-367.

[3] Carrër A, Poirel L, Yilmaz M, Akan O, Feriha C, Cuzon G, Matar G, Honderlick P and Nordmann P. Spread of OXA-48-Encoding Plasmid in Turkey and Beyond. Antimicrobial Agents and Chemothery 2010; 54: 1369-1373.

[4] CLSI (Clinical laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.

[5] De Angelis G, Restuccia G, Venturiello S, Cauda R, Malhotra-Kumar S, Goossens H, Schrenze J and Tacconelli E. Nosocomial acquisition of methicillin-resistant *Staphyloccocus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) Enterobacteriaceae in hospitalized patients: a prospective multicenter study. BMC Infectious Diseases 2012; 12:74.

[6] Doughari H J, Ndakidemi P, Human I and Benade S. Virulence, resistance genes, and transformation amongst environmental isolates of *Escherichia coli* and *Acinetobacter* spp. Journal of Microbiology and Biotechnology 2012; 22: 25–33.

[7] Holt J, Krieg N, Sneath P, Staley J and Williams S. Bergey's Manual of Determinative Bacteriology, 9th ed., Williams and Wilkins, Baltimore, 1994.

[8] Jumaa P. Frequency of isolation of pathogens from nosocomial bloodstream infection in a tertiary referral hospital in the UAE. International Jorunal of Infectious Diseases 2006; 218.

[9] Laure M, Kempf M, Cavallo J, Chomarat M, Dubreuil L, Maugein J, Muller-Serieys C and Roussel-Delvallez M. Comparative *in vitro* activity of Meropenem, Imipenem and Piperacillin/ tazobactam against 1071 clinical isolates using 2 different methods: a French multicentre study. BMC Infectious Diseases 2012; 10:72.

[10] Mohammadi-mehr M and Feizabadi M M. Antimicrobial resistance pattern of Gram-negative bacilli isolated from patients at ICUs of Army hospitals in Iran. Iranian Journal of Microbiology 2011; 3: 26-30.

[11] Nathisuwan S, Burgess D S and Lewis J S. Epidemiology, Detection and Treatment. Pharmacotherapy 2001; 21: 920-928.

[12] Sanders M, Fille M, Bauernfeind A, Stemplinger I, Amann S, Pfausler B and Fey P. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: Considerations for diagnosis, prevention and drug treatment. Drugs; 1996; 63: 353–365.

[13] Thibault F M, Hernandez E, Vidal D R, Girardet M. and Cavallo J D. Antibiotic susceptibility of 65 isolates of *Burkholderia pseudomallei* and *Burkholderia mallei* to 35 antimicrobial agents. Journal of Antimicrobial and Chemotherapy 2004; 54: 1134–1138.

[14] Yagi T, Wachino J, Knrokawa H, Suznki S, Yamanc K, Doi Y, Shibata N, Kato H, Sliihayama K and Arnkawa Y. Practical methods using boronic acid compounds for identification of class C β-lactamases producing *Klehsiella pneumoniae* and *Escherichia coli.* Journal of Clinical Microbiology 2005; 43: 2551-2558.

[15] Zhanel G G, Dilay W R, Thomson K, Rubinstein E, Hoban J D, Noreddin A M and Karlowsky J A. Comparative review of the carbapenems. Drugs 2007; 67: 1027-1052.

10/15/2012