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ORIGINAL ARTICLE

Direct Detection of Carbapenemase and ESBL Producing Organisms in Blood Culture

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Key words:

Carbapenemases, ESBL, Blood cultures, ESBL NDP, Carba NP

ABSTRACT

Background: *Carbapenemases are ß-lactamases that hydrolyze penicillins, cephalosporins, monobactams, and carbapenems. Bacteria producing ß-lactamases may cause serious infections in which the carbapenemase activity renders many ß-lactams ineffective .* ***Objective****: The present study aimed at rapid detection of carbapenemase and Extended-spectrum β-lactamase (ESBL) producing organisms directly from blood culture using ESBL NDP and Carba NP test.* ***Methodology****: 100 positive blood cultures with Gram negative organisms were subjected to routine lab work, enzymatic extraction from bacteria, Carba NP test, ESBL NDP test and finally combination tests were done for all cases.* ***Results:*** *ESBL NDP test detected 33 positive cases 23 were True Positive (TP) and 10 False Positive (FP) and 67 negative cases 65 were True Negative (TN) and 2 False Negative (FN). The test has sensitivity =92%, specificity=86.7% ,PPV=69% , NPV=97% .It diagnosed all cases of Acinetobacter , missed a case of E coli, over diagnosed 6 cases of Klebsiella , and 3 cases of Pseudomonas. Carba NP detected 2 positive cases and 98 negative ones. The test has sensitivity = 40%, specificity =100%, PPV =100%, NPV=96.9%. It missed 2 cases of Acinetobacter, 1 case of Klebsiella, and the two cases correctly diagnosed were Klebsiella.* ***Conclusion****: ESBL NDP test and Carba NP test were both reliable, easy to perform, rapid, inexpensive, cost effective and, easy to interpret.*

INTRODUCTION

Carbapenemases are ß-lactamases with versatile hydrolytic capacities. Carbapenemase-producing Enterobacteriaceae (CPE) isolates have been increasingly reported. The most clinically significant carbapenemases include enzymes belonging to Ambler classes A (KPC, IMI, SME), B (VIM, IMP, NDM), and D (OXA-48). With the exception of OXA-48, they confer high-level resistance to most β-lactams such as penicillins and cephalosporins but variably affect susceptibility to carbapenems[1]. They are either chromosomally encoded (NMC-A, Sme-1 to Sme-3, IMI-1) in *Enterobacter cloacae* and *Serratia marcescens*, or plasmid encoded, (KPC-1) in *Klebsiella pneumoniae (K.Pneumoniae),* and (GES-2) in *Pseudomonas aeruginosa (P. Aeruginosa)*. Class B metalloenzymes are the most clinically significant carbapenemases [2, 3].

Extended-spectrum β-lactamases (ESBLs) are mutant, plasmid-mediated β-lactamases derived from broad-spectrum β-lactamases and confer resistance to penicillins, all extended spectrum cephalosporins and aztreonam, except cephamycins and carbapenems.[4] ESBLs, most commonly encountered in *Klebsiella species and Escherichia coli (E. coli),* have also been detected in *Enterobacter spp., Salmonella spp., Citrobacter spp., Serratia marcescens (S.Marcescens), Proteus spp. and P. Aeruginosa*.[5] ESBLs are susceptible to clavulanic acid.[6]

Class C β-lactamases (AmpC) are cephalosporinases poorly inhibited by clavulanic acid and can be differentiated from ESBLs by their ability to hydrolyse cephamycins[7]. A wide variety of bacterial species, e.g. *E.Coli, K.Pneumoniae , Proteus mirabilis (P.Mirabilis), Enterobacter aerogenes, (E.Aerogenes), Salmonella spp. and Citrobacter freundii (C.Freundii)* have been shown to harbor AmpC β-lactamases.[8] The detection of ESBLs in AmpC-producing Gram Negative Bacteria (GNB) is problematic. Bacterial pathogens producing both ESBLs and AmpC β-lactamases create a requirement for laboratory testing methods that can accurately detect the presence of these enzymes in clinical isolates[9]. The present study aimed at rapid detection of Carbapenemase and ESBL Producing organisms directly from blood culture using ESBL NDP and Carba NP test.

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METHODOLOGY

Specimen collection and procedure:

Blood samples (8-10 ml) from patients were aseptically inoculated into the BACTEC vials at bedside. Inoculated vials were placed in the BACTEC for incubation and monitoring for 21 days. Positive vials were determined and bacterial growth was identified according to conventional standard procedures [10].

Samples:

Positive Blood cultures were subjected to direct film done by gram stain. Gram negative cases were included in the study. Routine lab work was done followed by enzymatic extraction from bacteria then Carba NP test and ESBL NDP test and finally combination test was done for all.

Procedures:

1. Enzymatic extraction from the bacteria:

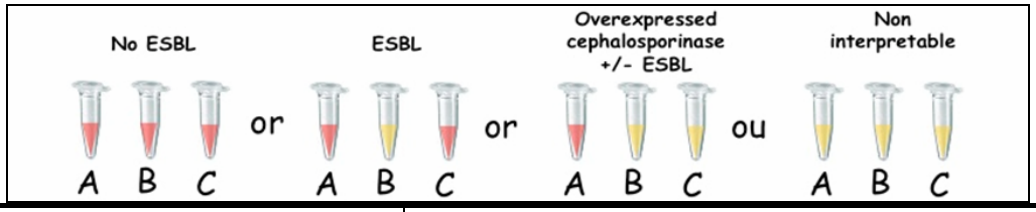
Blood was centrifuged at low speed for 5 minutes to pellet RBCs. The supernatant containing the bacteria was centrifuged at high speed for 15 minutes to pellet the bacteria which was then washed in sterile distilled water and supernatant discarded. The bacterial pellet was resuspended in 100 µL of 20 mmol/L. Tris-HCl lysis buffer and incubated 30 minutes at room temperature. The aliquot was centrifuged at high speed for 5 minutes, then the supernatant was used for testing for Carbapenemase and ESBL enzymes. [11a, 11b]

2. The ESBL NDP (Nordmann-Dortet-Poirel) test:

This is a novel phenotypic detection of ESBL enzymes by colorimetric method. The test identifies the hydrolysis of the lactam ring of cephalosporin (cefotaxime), which generates a carboxyl group, by acidifying the culture media.The change in PH resulting from this hydrolysis is identified by the color change generated using a pH indicator (phenol red). Inhibition of ESBL activity is evidenced by adding tazobactam.[11a]

Solution R (the revelation solution) was prepared by adding 2 ml concentrated red phenol solution 0.5% w/v to 16.6 ml distilled water then pH adjusted at 7.8 by adding drops of NaOH solution. 30 µL of supernatant was added to each of three 1.5 ml eppendorf tubes (A, B and C). 10 µl of tazobactam concentrated solution (4 mg/ml) was added to tube C. 100 µl of Solution R was added to tube A and 100 µl Solution R + cefotaxime 6 mg/ml to tubes B and C. All were incubated at 37°C for 20 mins.

Interpretation:



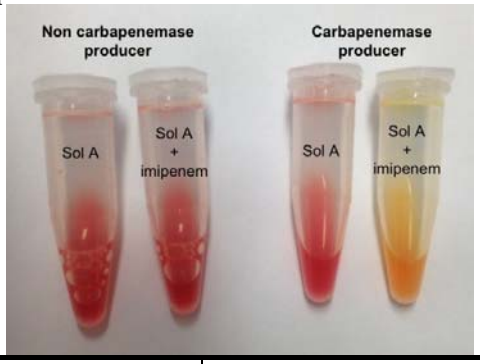
	No antibiotic (tube A)	*Cefotaxime (tube B)*	*Cefotaxime + tazobactam (tube C)*
No ESBL	Red	Red	Red
ESBL	Red	Orange/Yellow	Red
Cephalosporinase or Cephalosporinase + ESBL	Red	Orange/Yellow	Orange/Yellow
Non interpretable	Yellow	Yellow	Yellow

3. The Carba NP (Nordmann-Poirel) test:

The Carba NP test is a novel phenotypic method developed for carbapenemase detection. It is based on in vitro hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red (red to yellow/orange).[11b]

Solution A was prepared by adding 180 µl of ZnSO4 10 mM to solution R to obtain a final concentration of 0.1 mM. 30 µL of supernatant was added to each of two 1.5 ml eppendorf tubes (A and B). 100 µl of Solution A was added to tube A and 100 µl Solution A + imipenem 6 mg/ml to tube B. All were incubated at 37°C for a maximum of 2 hours

Interpretation



	No antibiotic	*Imipenem*
No carbapenemase	Red	Red
Carbapenemase producer	Red	Orange/Yellow
Not interpretable	Yellow	Yellow

4. The routine lab work:

The results were compared with those of the ordinary methods of detection and identification of the organism by the biochemical reactions according to The Manual of Clinical Microbiology 2007[10]. Antimicrobial susceptibility of all isolates was determined by the standard Kirby Bauer disk diffusion method according to CLSI 2014[12]**.** Antibiotic discs included were ampicillin 10µg, cefoxitin 30µg, ceftriaxone 30µg, cefotaxime 30µg, ceftazidime 30µg, cefepime 30µg, amoxicillin-clavulanic 20/10µg, ampicillin-sulbactam 10/10 µg, piperacillin-tazobactam 100/10 µg ,imipenem 10µg, meropenem 10µg, ciprofloxacin 5µg, levofloxacin 5 µg , amikacin 30 µg, gentamicin 10µg

Interpretation:

A. ESBL screening by disc diffusion method was according to CLSI 2011[13]**.** Positive ESBL production was indicated when zone diameter was ≤ 27 mm for CTX and / or ≤ 22 mm for CAZ discs.
B. Screening for the resistance to carbapenems according to CLSI 2012[14] was detected by using imipenem, meropenem disk diffusion method. Results were categorized as sensitive, intermediate and resistant as CLSI 2014 [12] (imipenem 10µg and meropenem: 10µg: S: ≥23, I: 20-22, R: ≤19)

5. Confirmation of β-lactamase production:

A. Combination disc method was according to CLSI, 2011: CTX and CTX/clavulanate 10 µg.discs were used A ≥5 mm increase in zone diameter for either CTX or CAZ discs tested in combination with clavulanic acid, versus its zone diameter when tested alone, confirmed an ESBL-producing organism (Fig 1)[15].
B. Modified double disc synergy test (MDDST)[16]**:** amoxicillin-clavulanic acid (AMC 20/10 µg), piperacillin-tazobactam (TPZ 100/10 µg), CTX 30 µg, CAZ 30 µg, cefepime (FEP 30 µg) discs were used.The organism was considered to be ESBL producer when the zones of inhibition around any of the cephalosporins discs show a clear-cut increase towards the AMC disc (Fig 2).

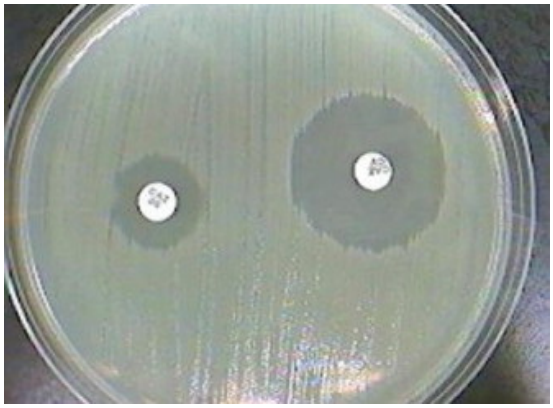


Fig. 1: Combination disc method

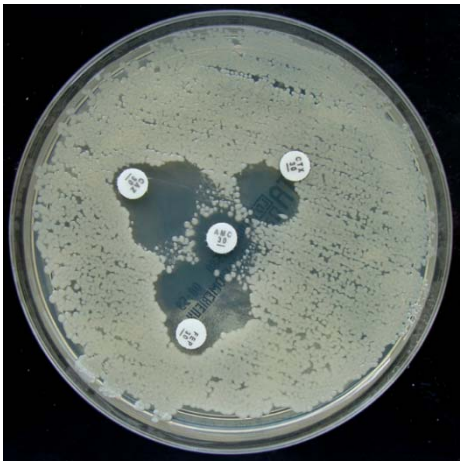


Fig. 2: Double disc synergy

Quality control:

For the routine methods*, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212* were used as control strains for susceptibility testing.Tinam resitant isolate genotipically confirmed as NDM was used as a positive control for the Carba NP test, ESBL isolate genotipically identified as SHV was used as a positive control for the ESBL NDP test,and a known Gram positive strains as a negative control for both.

Our Trials:

1. Tris-HCl lysis buffer for the extraction of the enzyme from the bacteria showed no colorimetric changes in the Carba NP test and ESBL NDP test, so was replaced by [BPERII, Bacterial Protein Extraction Reagent; Thermo Scientific, Pierce Cat : 78260].[11b]
2. Results of extraction by triton method (one ml aliquot of positive blood culture and 10 µl of water.then pellet was directly used) when compared with those of mechanical method were the same in ESBL NDP test but different in CarbaNP test So the mechanical method for bacterial lysis was used.[11b]

Statistical analysis:

Data were statistically described in terms of mean ± standard deviation (± SD), and range, or frequencies (number of cases) and percentages when appropriate. Comparison between the study groups was done using Chi square ($\chi 2$) test was performed. Accuracy was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. P values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 17 for Microsoft Windows.

RESULTS

This study was conducted in the main microbiology Lab of Clinical Pathology Department in Cairo University Hospitals, from September 2014 to February 2015 on 100 positive blood cultures with Gram negative organisms. The study included 58 cases of septicemia, 23 cases of chest infection, 3 cases of lung abscess, 16 cases of disturbed conscious, 1 case of osteomyelitis. All cases were from emergency ICU, internal medicine, neurology ICU, surgery ICU and chest wards. The isolates included 53 *Klebsiella* , 15 *Acinetobacter* ,15 *E coli* , 15 *Pseudomonas*, 2 *Proteus* .

Table 1: ESBL NDP test in relation to Combination disc as a gold standard test

		ESBL NDP		*TOTAL*
Combination Disc		(+)	(-)	
	(+)	TP = 23	FN = 2	(P) = 25
	(-)	FP = 10	TN = 65	(N) = 75
TOTAL		33	67	

ESBL NDP test detected 33 positive cases 23 were TP and 10 FP and 67 negative cases 65 were TN and 2 FN .

Table 2: The Sensitivity and Specificity of ESBL NDP Test

Total cases	*100*
True **P**ositive **R**ate (**Sensitivity)** TPR	92.0%
True **N**egative **R**ate (**Specificity**) SPC	86.7%
Positive Predictive Value (**Precision**) PPV	69.7%
Negative Predictive Value NPV	97.0%
False Positive Rate (**Fall-out**) FPR	13.3%
False Discovery Rate FDR	30.3%
Accuracy ACC	88.0%

ESBL NDP test has sensitivity =92%, Specificity=86.7%, PPV=69%, NPV=97%.

Table 3: ESBL NDP test and Combination disc results for various organisms.

	ESBL NDP Test		***Combination Disk Test***	
	P(+)	N(-)	P(+)	N(-)
Acinetobacter	1	14	1	14
E coli	4	11	5	10
Klebsiella	25	30	19	36
Pseudomonas	4	11	1	14

ESBL NDP test diagnosed all cases of *Acinetobacter,* missed a case of *E coli,* over diagnosed 6 cases of *Klebsiella*, and over diagnosed 3 cases of *Pseudomonas*

Table 4: ESBL NDP test and Combination disc according to cost and time needed for each.

Items	***ESBL NDP test***	***Combination disc***
Time needed	2 hours	48 hours
Labor	moderate	moderate
Cost	450LE/100 specimen 4,5 LE/specimen	2500LE/100LE spécimen 25LE/spécimen

ESBL NDP test was rapid and cost effective than Combination disc.

Quality control results:

In each run we used SHV strain as a positive control and it gave the optimal result (yellowish discoloration in tube B), and we used Gram positive strain as a negative control detecting no colour change.

Table 5: Results of Carba NP test in relation to tienam resistance as a gold standard test.

		Carba NP		*TOTAL*
Tienam resistance	(+)	(+)	(-)	
		TP = 2	FN = 3	(P) = 5
	(-)	FP = 0	TN = 95	(N) = 95
TOTAL		2	98	

Carba NP detected 2 positive cases giving orange discoloration and negative results for 98 cases.

Table 6: The Sensitivity and Specificity of Carba NP test.

Total cases	*100*
True **P**ositive **R**ate (**Sensitivity**) TPR	40.0%
True **N**egative **R**ate (**Specificity**) SPC	100.0%
Positive Predictive Value (**Precision**) PPV	100.0%
Negative Predictive Value NPV	96.9%
False Positive Rate (**Fall-out**) FPR	0.0%
False Discovery Rate FDR	0.0%
Accuracy ACC	97.0%

Carba NP test has Sensitivity = 40%, Specificity =100%, PPV =100%, NPV=96.9%

Table 7: Comparing the results of Carba NP test and tienam resistance in disc diffusion test for various organisms.

	Tienam IMP		***Carba NP Test***	
	S	R	P(+)	N(-)
Acinetobacter	13	2	0	15
E coli	15	0	0	15
Klebsiella	52	3	2	53
Pseudomonas	15	0	0	15

Carba NP test missed 2 cases of *Acinetobacter*, 1 case of *Klebsiella*, and the two cases correctly diagnosed were *Klebsiella*

Table 8: Comparing Carba NP test and disc diffusion test according to the cost and time needed for each.

Items	*Carba Np test*	*Reference method[MHA]*
Time needed	2 hours	48 hours
Labor	moderate	moderate
Cost	400LE/100LE spécimen 4.0LE/spécimen	1500LE/100LE spécimen 25LE 15/spécimen

Carba NP test was rapid and cost effective than disc diffusion method

Quality control results:

In each run we used NDM strain as a positive control and it gave optimal result (yellowish discoloration in tube B), and we used Gram positive strain as a negative control detecting no color change.

DISCUSSION

ESBL-producing Enterobacteriaceae (ESBL-E) are increasing worldwide. Conventional detection of ESBL production remains time-consuming (24 to 48 hours). Rapid detection of those responsible for bacteremia is of utmost importance since successful treatment depends on prompt administration of the appropriate antimicrobial agents. ESBL NDP test has been evaluated in our study for rapid identification of ESBL-E directly from blood culture.

In this study, 100 positive blood cultures showing gram negative bacteria were collected. Isolates were 53 *klebsiella*, 15 *Acinetobacter*, 15 *E coli*, 15 *Pseudomonas*, 2 *Proteus* .Our study revealed that among the 100 blood cultures, the ESBL NDP test was able to detect (33) positive cases (23) of them were TP and (10) were FP and negative results were (67) cases (65) were TN and (2) were FN ,and it diagnosed all cases of *Acinetobacter*, missed a case of *E Coli* , over diagnosed 6 cases of *Klebsiella*, and over diagnosed 3 cases of *Pseudomonas.*

In this study, ESBL NDP test had 92% sensitivity, 86.7% specificity, 69% PPV, 97% NPV, 88, 0% accuracy. Sensitivity of ESBL NDP test was similar to results of *Nordmann P. et al* [11a] applied to 215 ESBL producers and 40 ESBL nonproducers, its sensitivity and specificity were 92.6%. The blood cultures positivity for GNB from 245 patients hospitalized at the Bicêtre hospital, in Paris were detected by using the BacT/Alert system and ESBL NDP test. ESBL NDP test had 100% sensitivity, 100% specificity, 100% PPV and 100% NPV. As a reference standard, they used double-disk synergy test (DDST) and molecular biology techniques to identify the ESBL genes[17]. This may be the cause of disagreement .In our study we used combination disc test according to CLSI 2014[12] but according to EUCAST guidelines[18], ESBL confirmation tests that use cefotaxime as the indicator cephalosporin may be false-positive for *Klebsiella oxytoca* strains with hyperproduction of the chromosomal K1 (OXY-like) β-lactamases[19]. A similar phenotype may also be encountered in *Proteus vulgaris*. Another possible cause of false-positive results is hyperproduction of SHV-1-, TEM-1-or OXA-1-like broad-spectrum β-lactamases combined with altered permeability[20].

The other side of our study discussed the spread of Carbapenemase producing Enterobacteriaceae (CPE) that has been increasingly reported worldwide [21]. Treatment options for infection are extremely limited and effective therapy may be delayed whilst confirmatory results are awaited .Carba NP tests have been recently developed for the early detection of CPE.

Our study revealed that among 100 positive cultures, Carba NP detected only (2) positive cases and it gave negative results for (98) cases. Carba NP test missed 2 cases of *Acinetobacter, 1* case of *Klebsiella* , and the two cases correctly diagnosed were *Klebsiella* .In this study, Carba NP tests had 40% sensitivity, 100% specificity, 100%PPV and 96.9% NPV.The problem of false negative cases was explained by Sahuquillo *J.M. et al.*[3] as their results were less than optimal with some carbapenemase producers, notably harboring OXA-48-like enzymes, GES-5 and NDM. They also found that Carba NP test with *S. marcescens* harboring SME-1 and Enterobacteriaceae harboring GES-5 were often inconsistent, being difficult to interpret with some extracts or giving false-negative results. Thus, the Carba NP test gave suboptimal results compared to those originally described by *Nordmann P et al.*[11]**.** *Nathalie T et al.*[22] confirmed the 100% specificity and PPV of the test, but the sensitivity and NPV were 72.5% and 69.2%. False-negative results were associated with mucoid strains or linked to enzymes with low carbapenemase activity, particularly OXA-48-like enzymes. Carba NP test should be used with caution in areas with high OXA-48 prevalence and should be evaluated in epidemiological settings where carbapenemases with lower hydrolytic activity might be detected, IMP, GES or OXA-198. They also concluded that this test is highly sensitive and specific, easy to perform and interpret, cost-effective, adaptable to any laboratory, and the results obtained within 2 h , but false negative results have been observed using this test, particularly for OXA-48 and GES [3,23].

We compared our results with imipenem Disc as a screening test for CPE according to CLSI 2014[12] that detected (5) resistant cases and (95) sensitive ones. Imipenem should not be used for *Proteus spp., Providencia spp.,* and *Morganella spp*. Meropenem offers the best compromise between sensitivity and specificity in terms of detecting carbapenemase-producers. Imipenem is therefore not recommended for use as a stand-alone screening test compound, due to factors such as low carbapenem MICs and inoculum effects[24]. Many studies used different concentrations of tienam in their work protocol as (3mg) [11]**.** In our study

we used (6mg) tienam in the preparation of Carba NP test [25].

CONCLUSION

ESBL NDP test directly performed on positive blood cultures is a reliable technique to identify ESBL-E within 2 hours. This inexpensive ESBL NDP test may optimize rapid choices of antibiotics for treating bloodstream infections, contribute to avoidance of overuse of carbapenems, enhance identification of ESBLs likely to be the source of nosocomial outbreaks (*K. pneumoniae, Enterobacterspp*.) and facilitate implementation of a rapid strategy for containment.

Identification of carbapenemases by PCR performed on colonies may give results within 4–6 hours with excellent sensibility and specificity. The main disadvantages of molecular-based technologies are their cost, the requirement of trained personnel, and the absence of detection of any novel carbapenemase gene. There is an urgent need for an inexpensive, rapid, sensitive, and specific test for detection of carbapenemase activity. In our study, the Carba NP method was easy to perform, inexpensive, and, easy to interpret, (the color indicator turned orange before 30 min). However, it gave suboptimal results compared to those originally described by *Nordman et al*.[11b]

REFERENCES

1. Dortet L, Bréchard L, Cuzon G, Laurent Poirel L and Nordmann P: Strategy for Rapid Detection of Carbapenemase-Producing Enterobacteriaceae. Antimicrob. Agents Chemother., 2014; 58 (4): 2441-2445
2. Nordmann P and Poirel L: Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect, 2002; 8(6): 321-31.
3. Sahuquillo-Arce JM, Alicia HC, Fernanda YA, Elisa I, Patricia F and Alba R: Carbapenemases: A worldwide threat to antimicrobial therapy. World J Pharmacol 9, 2015; 4(1): 75-95
4. Thomson K.: Controversies about ESBL and AmpC β-lactamases. Emerg. Infect. Dis., 2001; 7: 333-6.
5. Hooper D.: Efflux pumps and nosocomial antibiotic resistance: a primer for hospital epidemiologists. Clin. Infect. Dis., 2005; 40: 1811-7.
6. Chaudhary U. and Aggarwal R.: ESBL, an emerging threat to clinical therapeutics. Indian J. Med. Microbiol., 2004; 22: 75-80
7. Manchanda V. and Singh N.: Occurrence and detection of AmpC β-lactamases among Gram negative clinical isolates using a modified three dimensional test at Guru Tegh Bahadur hospital, Delhi, Indian J. Antimicrob. Chemother., 2003; 51:415-8.
8. Singhal S., Mathur T., Khan S. et al. (2005): Evaluation of methods for AmpC β-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian J. Med. Microbiol. 23:120-4.
9. Pitout D.,Reisbig M.,Venter E. et al.(2003): Modification of the double disc test for detection of Enterobacteriaceae producing ESBL and AmpC β-lactamases. J. Clin. Microbiol.41 (8): 3933-5.
10. Murray PR, Baron EJ, Landry ML, and Michael A.: Editors, Manual of Clinical Microbiology, 9th Edition. 2007 American Society for Microbiology, Washington, DC.
11. a. Nordmann P, Dortet L, and Poirel L: Rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae. J. Clin. Microbiol. 2012a; 50:3016–22.
    b. Nordmann P, Dortet L, and Poirel L: Rapid Detection of Carbapenemase-producing Enterobacteriaceae Emerg Infect. Dis. 2012b; 18(9): 1503–1507.
12. Clinical and Laboratory Standard Institute CLSI: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, M100-S24., Wayne, PA, 2014.
13. Clinical and Laboratory Standards Institute CLSI: Performance standards for antimicrobial susceptibility testing: Twenty-First Informational Supplement., M100-S21. , Wayne, PA, 2011.
14. Clinical and Laboratory Standard Institute CLSI: Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. 2012; M100-S22., Wayne, PA.
15. Ahmed O, El-Hady S, Ahmed T: Detection of blaSHV and bla- CTX-M genes in ESBL producing K. pneumoniae isolated from Egyptian patients with suspected nosocomial infections. Egyptian J. Med. Hum. Gen., 2013; 14(3): 277-83.
16. Khan MK, Thukral SS and Ragni J: Evaluation of a modified double-disc synergy test for detection of extended spectrum β-lactamases in AMPC β-lactamase-producing proteus mirabilis Indian J. of Med. Microbiol., 2008; 26(1):58-61
17. Laurent D, Lorent P and Patrice N: Rapid Detection of ESBL-Producing Enterobacteriaceae in Blood Cultures Emerg Infect Dis. 2015; 21(3): 504–507.
18. EUCAST: The European Committee on Antimicrobial Susceptibility Testing – EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (Version 1.0 December 2013)
19. Stürenburg E, Melanie L, Matthias AH, Rainer L and Dietrich M: Evaluation of the MicroScan ESBL plus confirmation panel for detection of extended-spectrum β-lactamases in clinical isolates of oxyimino-cephalosporin-resistant Gram-negative

bacteria. Journal of Antimicrobial Chemotherapy. 2004; 54(5): 870-875.
20. Spanu T, Sanguinetti M., Tumbarello M. et al.: Evaluation of the New VITEK 2 ESBL test for rapid detection of ESBL production in Enterobacteriaceae isolates. J. Clin. Microbiol., 2006; 44 (9): 3257-62.
21. Queenan AM and Bush K: Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev, 2007; 20(3):440-58.
22. Nathalie T.,David B.,Samir N., Micheal R.,and Roberto G.: Evaluation of the Carba NP Test for Rapid Detection of Carbapenemase-Producing Enterobacteriaceae and Pseudomonas aeruginosa Antimicrob. Agents Chemother, 2013; 57(9): 4578-4580
23. Mitra S, Kazi M, Panchal M, Rodrigues C, Shetty A: Evaluation of Carba NP test for rapid detection of carbapenemase producing Enterobacteriaceae. Indian J of Med. Microbiol., 2015; 33(4) : 603-606.
24. Germán B, Jordi V, Cristina S, and Francisco JC: Detection of carbapenemase-producing Enterobacteriaceae in various scenarios and health settings.Enferm Infec Microbiol Clin. 2014; 32(4):24-32
25. Shawn V, Scott A, Peggy C, Patricia J, Karen L, Mary H and Robin P: Comparison of a Novel, Rapid Chromogenic Biochemical Assay, the Carba NP Test, with the Modified Hodge Test for Detection of Carbapenemase-Producing Gram-Negative Bacilli. J Clin Microbiol. 2013; 51(9): 3097–3101.