Egyptian Journal of Medical Microbiology Volume 26 / No.4 / October 2017 89-96

ORIGINAL ARTICLE

Triton Hodge test for detection of Metallo-β-lactamases Producing *Pseudomonas aeruginosa* Isolates from Egyptian Patients with Infected Diabetic Foot

[1]Shymaa A Mansour*,[1]Marwa A Mansour, [2]Islam M Ibrahim, [3]Jehan Saeed
Departments of [1]Medical Microbiology and Immunology Department, [2] General Surgery, [3]Internal Medicine, Faculty of Medicine, Zagazig University, Egypt.

Key words:
Diabetes mellitus, Foot ulcer, Pseudomonas aeruginosa, Carbapenemases, Metallo-beta-lactamases

Corresponding Author:
Shymaa A Mansour
Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt.
shymaa_abdelazim@yahoo.com
Tel: 01026919619

ABSTRACT

Background: *One of the dreadful complications of diabetes is infected diabetic foot. Accurate detection of carbapenemase-producing Pseudomonas aeruginosa, in the form of metallo-beta-lactamases (MBLs) is necessary to determine the antibiotics for its treatment.* ***Objectives:*** *This study was designed to evaluate the efficacy of Triton Hodge Test (THT) versus MHT for detection of MBLs-producing P. aeruginosa isolates from infected diabetic foot patients.* ***Methodology:*** *Samples were collected from 500 infected diabetic footpatients. They were processed and P. aeruginosa isolates were identified. Then, carbapenem resistant P. aeruginosa (CRPA)isolates weredetected. Modified Hodge test (MHT) and Triton Hodge Test (THT) were used for detectionof MBLs. Furthermore, bla_{IMP}, bla_{VIM} and bla_{NDM} MBLs genes were detected by multiplex and uniplex PCR. Resistance pattern of CRPA isolates was determined.* ***Results:*** *Isolation rate of P. aeruginosa from infected diabetic foot patients was 90/500 (18.00%). CRPA isolates were 16/90 (17.78%). In these isolates, the detection rate of bla_{NDM}, bla_{VIM} and bla_{IMP} genes was (2/16, 12.5%), (10/16,62.5%) and (6/16,37.5%); respectively. The sensitivity, specificity and accuracy of THT in detection of NDM, VIM and IMP carbapenemases are 100 %. On the other hand, the sensitivity of MHT in detection of the studied types are 0.0%, 30.0% and 50.0%; respectively. Its specificity is 100% for all types and its accuracy is 87.5%, 56.3% and 83.1% in detection of these types. No resistance to tigecycline and colistin was detected among CRPA isolates.* ***Conclusion:*** *the THT is a more sensitive and accurate phenotypic test in comparison to MHT for detection of NDM, VIM and IMP carbapenemases in CRPA isolates.*

INTRODUCTION

Diabetes is a group of metabolic disorders characterized by hyperglycemia due to decreased insulin secretion or decreased insulin action or both. Long term hyperglycemia leads to dysfunction and failure of other systems [1].

Diabetes is the eleventh most important cause of premature mortality in Egypt and is responsible for 2.4% of yearly life lost. In addition, it is the sixth most important cause of disability burden in Egypt [2].

Diabetic foot and its related clinical conditions such as foot ulcers and infective complications represent the most common cause of hospitalization in subjects with diabetes [3].

Pseudomonas aeruginosa (P. aeruginosa) is an invasive organism that frequently causes severe tissue damage in infected diabetic foots that may lead to sepsis and amputation [4].

Carbapenems are potent β-lactam antibiotics that are used to treat serious infections. Egypt has been considered among the countries that reported emergence of carbapenem resistance in Gram-negative bacteria[5].

Carbapenem resistance is of critical importance as it threatens the last line of effective antibiotics for carbapenem-resistant strains, namely tigecycline and colistin[6].

In *P. aeruginosa*, carbapenem resistance maybe due to important mutational mechanisms; including derepression of AmpC, upregulation of active efflux, and loss of the carbapenem-specific outer membrane porin OprD. In addition, it can be mediated through carbapenemases[6]. Actually, multiple mechanisms of resistance often exist in combination [7].

Carbapenemases belong to three of the four known classes of ß-lactamases, namely, Ambler Class A (*Klebsiella pneumoniae* carbapenemase (KPC), *Serratia marcescens* enzyme (SME), non metallo-enzyme carbapenemase-A (NMC-A), imipenem hydrolyzing ß-lactamase-1(IMI-1), and some allelic 50 variants of Guiana extended-spectrum ß-lactamase (GES), Class B or metallo-ß-lactamases (MBLs) (Verona integrin encoded MBLs (VIM), Imipenemase (IMP), New Delhi MBLs (NDM), 51 Sao Paolo MBLs (SPM), etc) and

Class D or oxacillinases (OXAs) (OXA-48, OXA-181, etc)[8].

Different tests have been proposed to detect carbapenemases, using either phenotypic or genotypic techniques. One of the phenotypic techniques is Triton Hodge Test(THT) that represents a variant of modified Hodge test (MHT). Pure Triton X-100 reagent is used in THT for solubilization of membrane proteins; NDM carbapenemase that are membrane-bound lipoproteins and so cannot be released into the extracellular medium, like all other known soluble periplasmic carbapenemases[9].

This membrane-bound form is consistent with the presence of a canonical lipidation sequence(LSGC), called lipobox, proximal to the signal peptide of NDM variants [10].

Molecular techniques are considered the gold standard for the optimal identification of different types of carbapenemase-producing Gram-negative bacteria [11].

Currently, there is few data on the carbapenemase producing Gram negative bacterial isolates from diabetic foot infections in Egypt. Hence, our study was designed to evaluate the efficacy of Triton Hodge Test (THT) versus MHT for detection of MBLs-producing *P. aeruginosa* isolates from infected diabetic foot patients.

METHODOLOGY

This study is an observational cross sectional study. It was conducted at Medical Microbiology and Immunology Department and Diabetic Foot Clinic of General Surgery and Internal Medicine Departments, Faculty of Medicine, Zagazig University, Egypt, from May 2015 to March 2017. Approval for performing this study was obtained from Institutional Review Board (IRB) and ethical committee. Also, written informed consent was obtained from each participant.

The present study included total of 500 infected diabetic foot patients suffering from diabetic foot infection; where they categorized according to the Infectious Diseases Society of America (IDSA) classification[12]**.** The patients either had mild; 160/500 (32.0%), moderate; 200/500 (40.0%) or severe; 140/500 (28.0%) infection.

The included cases should have history of diabetes mellitus; fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) or 2-hours post prandial plasma glucose ≥ 200 mg/dL (11.1 mmol/L) (World Health Organization)[13]**.** Patients were previously treated by antidiabetic agents (oral antidiabetic drugs, insulin or both).

We recorded the results of routine laboratory test from patients' reports.

Sample Collection and Processing:

Samples were collected from the deeper portion of the ulcers by using 2 sterile swabs which were dipped in sterile glucose broth. The samples were collected by making a firm, rotatory movement with the swabs. One swab was used for Gram staining and the other was used for cultivation on *Pseudomonas* Cetrimide Agar (Egyptian Diagnostic Media, Egypt) at 37 °C for 24 hours. Patients has been treated with antibiotics were excluded from the study.

Identification of *P. aeruginosa isolates*:

The growing organisms were identified by the standard laboratory techniques including: Gram staining, colony morphology, motility, pigment production, oxidase reaction, growth at 42 °C, gelatin liquefaction test and sugar utilization tests [14]. API 20 NE (bio-Mérieux, France) identification panels for species level identification were used. Only a single isolate per patient was included.

Detection of carbapenem resistant *P. aeruginosa* (CRPA) isolates:

Carbapenem resistance is defined as resistance to at least one of the carbapenem antibiotics (doripenem, imipenem, meropenem, or ertapenem, according to Clinical and Laboratory Standards Institute (CLSI)guidelines[15].The antimicrobial discs which were used were doripenem (10 µg), imipenem (10µg), meropenem (10 µg), or ertapenem (10 µg) (Oxoid, UK). *P. aeruginosa* ATCC 27853 was used as a control strain.

Detection of carbapenemases producing *P. aeruginosa* isolates:

Modified Hodge test (MHT):

This test was performed according to the CLSI guidelines[15]. The presence of an enhanced growth at the intersection of the streak and the zone of inhibition (notable indentation or "cloverleaf" appearance) indicated carbapenemase production [15]**.**

Triton Hodge test (THT):

It was performed on a Mueller-Hinton agar plate (Becton Dickinson, USA) flooded with 50 µL of pure Triton X-100 reagent (Sigma-Aldrich, UK) (0.2% v/v in the MHA plate). Briefly, the detergent was dripped in the center of the plate and quickly distributed by streaking a swab over the entire sterile agar surface 4 to 6 times, until the detergent was completely absorbed. Delays of more than 10 min in streaking the Triton X-100 might alter the agar surface around the Triton X-100 drop. Flooded plates were stored at 4°C until use. Before inoculation with the indicator organism; carbapenem susceptible *E.coli*, excess surface moisture was removed by evaporation at 35°C. *E. coli* positive control strain harbors NDM gene was included in each THT assay to explore the impact of using Triton X-100 in THT versus MHT by releasing the membrane anchoring MBLs [9].

Genotypic detection of MBLs Carbapenemases (Class B):

Polymerase chain reaction (PCR) analysis is considered the gold standards for characterization of the β-lactamases.

DNA extraction:

Total DNAs of the different bacterial isolates were extracted by the DNA extraction kit (Qiagen, Germany) according to manufacturer instructions. The extracted DNA was then stored at -20°C until further processing.

DNA amplification:

For all CRPA isolates, multiplex PCR was used for detection of bla_{IMP} and bla_{VIM}genes[16]while uniplex PCR was used for detection of bla_{NDM}[17]. The primers and PCR programs used in this study were as previously described[16,17]. The three primer pairs (Sigma-Aldrich, Germany) used were listed in (Table 1).

Table 1: Detail of the primers used in the study

Gene	*Sequence*	*bp*	*Ref.*
VIM	**5'-GAT GGT GTT TGG TCG CAT A-3'** **5'-CGA ATG CGC AGC ACC AG-3'**	**390**	**16**
IMP	**5'-GGA ATA GAG TGG CTT AAY TCT C-3'** **5'-CCA AAC YAC TAS GTT ATC T-3'**	**188**	**16**
NDM	**F: CAAATGGAAACTGGCGACCA** **R: GCCTTGCTGTCCTTGATCAG**	**475**	**17**

Antimicrobial susceptibility testing of isolates:

It was done by the Kirby Bauer disc diffusion method, according to CLSI guidelines[15]. The antimicrobial discs which were used were aztreonam (30µg), ciprofloxacin (5µg), amikacin (30µg), piperacillin+tazobactam (100/10 µg), tigecycline (10µg) and colistin (10µg) (Oxoid, UK).

Statistical analysis:

Collected data were computerized and statistically analyzed using the Statistical Package for Social Sciences (SPSS)(version 18). Data were represented as Mean ± SD. Qualitative data were represented as number and percentages. Validity was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Kappa test was used to find agreement between different diagnostic methods. P-value of <0.05 indicates significant results and of <0.01 indicate highly significant result.

RESULTS

A total of 500 infected diabetic footspatients were studied. Table (2) shows that the mean and range of age was 51.1 ± 11.6 (24–72) years. The maximum number of patients was in the age group of 50 to 65 years. Males; 379(75.8%) were more than females 121 (24.2%). Mean duration of diabetes was 13.5±6.16 years.Most of patients was type IIDM; 379 (75.8%), its ulcer site on toes 237(47.4%), had foot skin changes; 201 (83.75%) and neuropathy; 385 (77.0%) of them. Amputation was present in 54 (22.5%) of the patients.Most of patients were treated by insulin alone; 345 (69.0%) and others 155 (31.0%) by oral antidiabetic drugs alone or with insulin.

Isolation rate of *P. aeruginosa*frominfected diabetic foot patients was 90/500 (18.00%).

According to the CLSI criterion for detection of CRPA isolates, they were 16/90 (17.78%) isolates.

In this study, among the investigated CRPA isolates, the detection rate of bla_{NDM}, bla_{VIM},bla_{IMP} genes was (2/16, 12.5%), (10/16,62.5%) and (6/16,37.5%) (Table 3 and Figure 1).

Table (3) shows there is no significant agreement between MHT and the gold standard (PCR) or in between THT and MHT (Figure 2) in detection of NDM and VIM carbapenemases. On the other hand, there is a significant agreement between MHT and PCR and in between THT and MHT in detection of IMP carbapenemases. Moreover, there is a highly significant agreement between THT and PCR in detection of NDM, VIM and IMP carbapenemases.

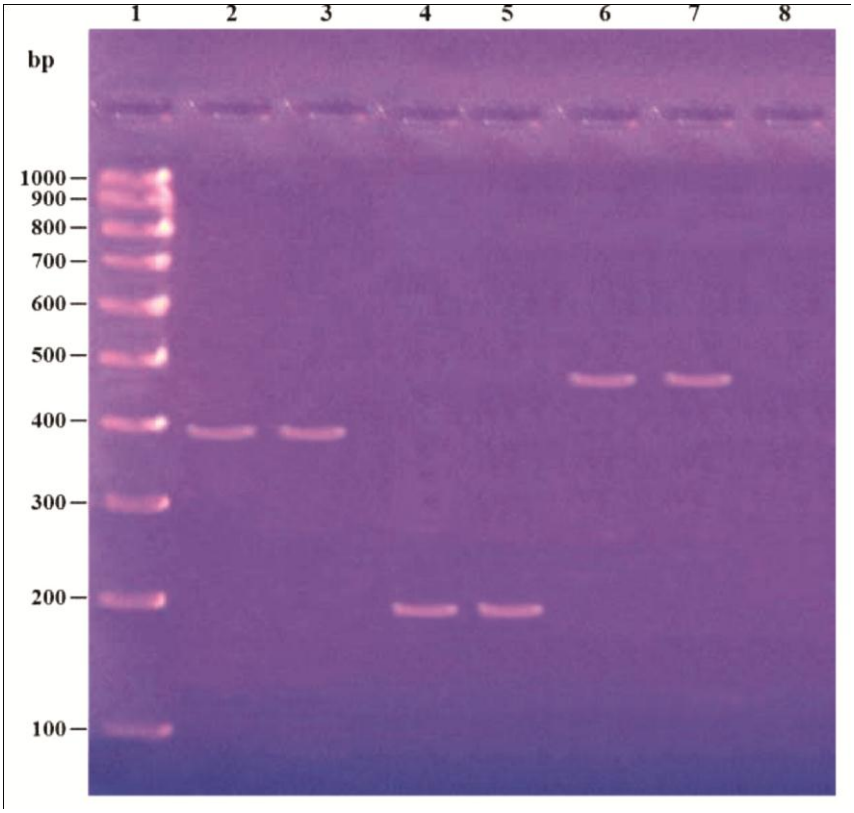

Fig. 1: Gel electrophoresis shows**:** Lane 1: 100-bp DNA ladder marker; lanes 2 & 3: *bla*VIM genes (one band at 390bp); *bla*IMP geneslanes 4 & 5: (at 188 bp); lanes 6 & 7: *bla*NDM genes(one band at 475 bp) and lane 8: negative control (no bands).

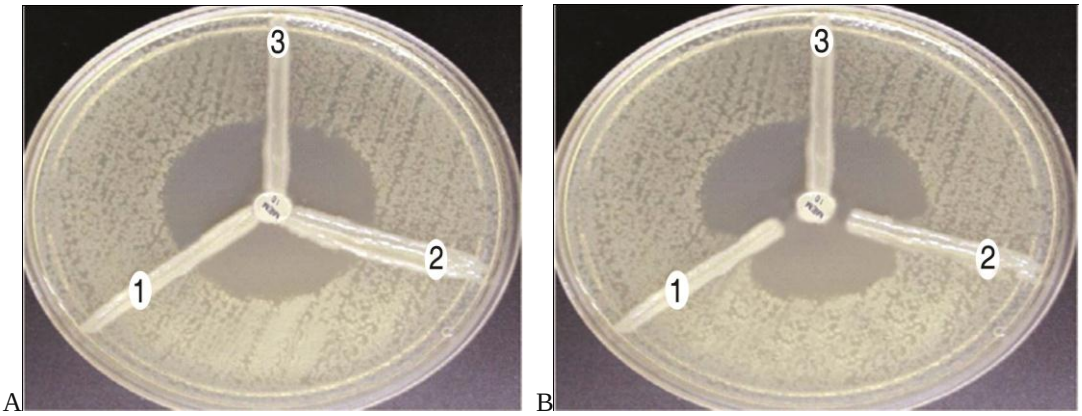

Fig. 2: Comparison between the Modified Hodge test (MHT) versus the Triton Hodge Test (THT) for detection of NDM producing *P.aeruginosa* isolates. Results obtained using a meropenem (MEM) disk (10 µg) as substrate. **(A) MHT** showing **(1)** NDM producing *E.coli* positive control; (The absence of growth of the indicator strain toward the carbapenem disks) **(2)***P.aeruginosa*, positive tested isolate; and **(3)** *P.aeruginosa*, negative tested isolate **(B) THT** showing **(1)** NDM producing *E. coli* positive control; (The growth of the indicator strain is > 3 mm) **(2)** *P.aeruginosa*, positive tested isolate; and **(3)** *P.aeruginosa*, negative tested isolate.

Table (4) shows that the sensitivity, specificity and accuracy of THT in detection of NDM, VIM and IMP carbapenemases are 100%. On the other hand, the sensitivity of MHT in detection of the studied types are 0.0%, 30.0% and 50.0%; respectively, its specificity is 100% for all types and its accuracy is 87.5%, 56.3% and 83.1% in detection of these types

Moreover, Table (5), shows that the resistance pattern of 16CRPA isolates to the following antibiotics;aztreonam,, piperacillin/tazobactam, ciprofloxacin, amikacin, tigecycline and colistin was 75.0%, 68.75%, 50.0%, 25% and 0.00%, and 0.00%; respectively.

Table 2: Patients' clinical & laboratory data.

Variable		*Value*
Age (years)	Mean± SD	51.1 ± 11.6
	(range)	24 –72
Sex	Male N(%)	379 (75.8%)
	FemaleN(%)	121 (24.2%)
Duration of diabetes (years)	Mean± SD	13.5±6.16.
DM type	Type IN(%)	121 (24.2%)
	TypeII N(%)	379 (75.8%)
Ulcer site	ToesN(%)	237 (47.4%)
	Heel &metatarsal headsN(%)	218(43.6%)
	Dorsum of footN(%)	43 (8.6%)
Foot skin changes	(dry skin, corns, callus)N(%)	201 (83.75%)
Amputation	AmputationN(%)	54 (22.5%)
Treatment modality	Insulin alone N(%)	345 (69.0%)
	Oral or mixed N(%)	155 (31.0%)
Co morbidity	Ischemic heart disease N(%)	70 (14.0%)
	Hypertension N(%)	156 (31.2%)
	Diabetic kidney disease N(%)	20 (4.0%)
	Neuropathy N(%)	385 (77.0%)
HBA1c(gm%)	Mean± SD	8.8 ± 1.6
HB(gm%)	Mean± SD	11.3 ±1.7
WBC ($x10^3/mm^3$)	Mean± SD	8.9 ± 2.5
SGPT (U/L)	Mean± SD	25.3±16.9
SGOT (U/L)	Mean± SD	24.3 ±17.9
Creatinine (mg/dL)	Mean± SD	1.3 ±1.7

Table 3: Modified Hodge Tests (MHT) versus Triton Hodge Test (THT) in detection of NDM, VIM and IMP carbapenemase producing *P.aeruginosa*isolates (N=16).

Carbapenemase producers	*N (%) positive by*			*P1*	*P2*	*P3*
	PCR (Gold standard) 16 (100%)	**Modified Hodge Test (MHT)**	**Triton Hodge Test (THT)**			
NDM	2 (12.5%)	0 (0.0%)	2(100%)	0.18 NS	<0.001**	0.18 NS
VIM	10 (62.5%)	3 (30.0%)	10 (100%)	0.14 NS	<0.001**	0.14 NS
IMP	6 (37.5%)	3 (50.0%)	6 (100%)	0.01*	<0.001**	0.01*

Kappa test NS: No significant agreement *: Significant agreement **: Highly significant agreement, P1: Gold standard versus MHT, P2: Gold standard versus THT, P3: MHT versus THT.

Table 4: Validity of the Modified Hodge Tests (MHT) and Triton Hodge Test (THT) in detection of NDM, VIM and IMP carbapenemase producing *P.aeruginosa* isolates.

Carbapenemase producers	*Test*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Accuracy*
NDM	MHT	0	100	0	87.5	87.5
	THT	100	100	100	100	100
VIM	MHT	30	100	100	46.2	56.3
	THT	100	100	100	100	100
IMP	MHT	50	100	100	76.9	83.1
	THT	100	100	100	100	100

Table 5: Resistance pattern of carbapenemase producing *P.aeruginosa* isolates to different antibiotics.

Antibiotic	*Number of Resistant Samples (N=16)*	*Percentage*
Aztreonam	12	75.0%
Ciprofloxacin	8	50.0%
Amikacin	4	25%
Piperacillin+Tazobactam	11	68.75%
Tigecycline	0	0.00%
Colistin	0	0.00%

DISCUSSION

Infected diabetic foots patients have several factors that may be associated with a high risk of carrying antimicrobial resistant microorganisms, such as inappropriate, unnecessary and frequent use of antibiotic treatment, chronic course of the wound and frequent hospital admission[18].

Pseudomonas species are one of the most frequent pathogens contributing to progressive and widespread tissue destruction in case of diabetic foot infections [19].

In this study, the isolation rate of *P.aeruginosa*from infected diabetic footswas90/500(18%) isolates. There are more or less variation in its isolation rate in other studies; Zubair et al.[20]**;** 10.6%, Sivanmaliappan and Sevanan [21]**;**14.3% and Ashish*et al*.[18]**;** 22.22%.

These discrepancies could partly have been due to the differences in the causative organisms, which had occurred over time, geographical variations, or the type and the severity of the infection, as were reported[22].

In this study, we detected 16/90 (17.78%) carbapenemresistant *P.aeruginosa* (CRPA) isolates.

This is in agreement with two studies that have determined the isolation rates of CRPA isolatesfrom university hospitals in Egypt. They were by Hashem et al.[23]**,** 26.5% and El-Domany*et al*. [24],12.2%.

The emergence of MBLs-mediated carbapenem resistance in *P. aeruginosa* is substantially alarming because ofthe ongoing spread and its intrinsic and acquired resistancemechanisms, which limit treatment options of this troublesomepathogen [25].

Effective screening of carbapenemase producers in clinical microbiology laboratories requires the development of sensitive and inexpensive methods [26, 27].

In this work, the higher sensitivity (100 %) of THT than MHT and in detection of NDM (0.0%), VIM (30.0%) and IMP (50.0%) carbapenemases. This may explained by the ability of Triton X-100 to release membrane bound NDM and other soluble periplasmic enzymes (VIM and IMP). Moreover, the long-term stability of Triton-flooded plates (up to 12 weeks) that enables early preparation and fractionation in aliquots as an efficient alternative to daily on-site plate preparation adds to the advantages of THT [28].

In this work, among the investigated CRPA isolates, the detection rate ofbla_{NDM}, bla_{VIM},bla_{IMP} genes was (2/16, 12.5%), (10/16,62.5%) and (6/16,37.5%).

In a study by El Essawy[29]**,** bla_{VIM} and bla_{IMP}genes were (5/22, 22.7%) and (4/22, 18.2%) respectively, while bla_{NDM}gene was not detected in any of the tested CRPA isolates. Another study by Hashem et al.[23] reported that bla_{VIM} and bla_{IMP}genes were (20%) and (4%); respectively. El-Domany *et al*. [24] recorded that bla_{VIM} and bla_{IMP}genes were (8/14, 57%) and (5/14, 35%) respectively.

As regards bla_{VIM} gene, in Iran, there were two studies by Moosavian and Rahimzadeh [30] and Neyestanaki et al.[31] reported prevalence rates of 1.6% and 55%; respectively, compared with 32.89% in a study from Canada, [32]which are less than what we found in the current study.

The 16CRPAisolates showed the following resistance pattern from the highest to no resistance:

aztreonam, piperacillin/tazobactam, ciprofloxacin, amikacin, and colistin: 75.0%, 68.75%, 50.0%, 25% and 0.00%, and 0.00%.

In accordance with our results, El-Domany*et al*[24] found that CRPA clinical isolates exhibited high level of resistance to tested antibiotics. Resistance pattern were 44.7% and 25.5% to ciprofloxacin and amikacin; respectively. By contrast, all clinical isolates were sensitive to colistin so it may be a life saving alternative to carbapenems in the treatment of infections caused by *P. aeruginosa* [33].

In conclusion, this study is the first one in Egypt to evaluate the efficacy of THT for detection of NDM, VIM and IMP carbapenemases in CRPA isolates. The THT is a more sensitive, specific and accurate phenotypic test in comparison to MHT. Hence, these findings will help in detection of carbapenemases which are constraints in antibiotic treatment options and they are also important for application of infection control measures to reduce their spreading.

Recommendations are to perform a national project for surveillance of CRPA isolates especially NDM carbapenemases producing strains that are able to spread in different hospital settings which is of great concern and highlights the need of infection control measures, including antimicrobial management and prompt detection of beta-lactamase-producing isolates.

REFERENCES

1. American Diabetes Association: Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2012;35(1): S64-S71
2. Arafa NA and Amin GE: The epidemiology of diabetes mellitus in Egypt: Results of a National Survey. The Egyptian Journal of Community Medicine 2010;28 (3): 29-34
3. Tuttolomondo A, Maida C, Pinto A: Diabetic foot syndrome: Immune-inflammatory features as possible cardiovascular markers in diabetes. World J Orthop. 2015;6(1):62-76.
4. Mike E and Ali F: The use of antibiotics in diabetics in the diabetic foot. Am J Surg 2004; 187:25-28.
5. Mahmoud A, Zahran W, Hindawi G, Labib A, Galal R.:Prevalence of multidrug-resistant Pseudomonas aeruginosa in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods.J VirolMicrobiol, 2013; 1-13
6. Memish ZA, Assiri A, AlmasriM, RoshdyH, HathoutH, Kaase M, GatermannSören G, and Yezli S: Microbial Drug Resistance 2015; 21(3): 307-314.
7. Juan NC, and Oliver A: Carbapenemases in Pseudomonas spp. Enferm. Infecc. Microbiol. Clin, 2010; 28(1):19–28.
8. Bush K and Jacoby G: Updated functional classification of β-lactamases.Antimicrob. AgentsChemother, 2010; 54:969–976.
9. Pasteran F, Gonzalez LJ, Albornoz E, Bahr G, Vila AJ, Corso A: Triton Hodge test: improved protocol for modified Hodge test for enhanced detection of NDM and other carbapenemase producers. J Clin Microbiol, 2016; 54:640–649
10. King D and Strynadka N: Crystal structure of New Delhi metallo-ß-lactamase reveals molecular basis for antibiotic resistance. Protein Sci., 2011; 20:1484- 1491
11. Nordmann P and Poirel L: Emerging carbapenemases in Gram-negative aerobes. Clin. Microbiol. Infect., 2010; 8:321–331
12. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, LipskyBA:Validation of the Infectious Diseases Society of America's diabetic foot infection classification system.Clin Infect Dis. 2007;44(4):562-565
13. World Health Organization: Definition diagnosis and classification of diabetes mellitus and intermediate hyperglycemia: Report of a WHO Consultation and International Diabetes Federation .Geneva, 2006.
14. Sharma K.: Manual of microbiology: Tools and techniques, 2nd edn. Ann Books Gopaljee Enterprises, Delhi, India., 2008; 163-165; 181-187
15. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fifth informational supplement M100-S25. CLSI, Wayne, PA, USA, 2015.
16. Ellington MJ, KristlerJ, Livermore DM and Woodford N: Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases.J Antimicrob Chemother. 2007; 59(2):321-2
17. MinhasN and Sharma PC: Molecular characterization of Pseudomonas aeruginosa isolates recovered from human patients in Himachal Pradesh (India) for selective genes: extended spectrum β-lactamase (ESBL), ampicillin class c (AMPC) and metallo β-lactamase (MBL) genes. Int J Pharm Sci Res 2016; 7(12): 4905-16.
18. Ashishb J, Sneha H, Geetha B, Bhargavi L., Ramani B and Vishu T: Bacteriological Profile of Diabetic Foot in a Tertiary Care Centre in Trivandrum,India.Int.J.Curr.Microbiol.App.Sci., 2016; 5(6):279-286
19. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC , Chaudhry R: Aclinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care, 2006; 29: 1727-32
20. Zubair M, Malik A, Ahmad J: Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in North India. Biol Med. 2010; 2 (4): 22-34

21. Sivanmaliappan T, Sevanan M: Antimicrobial susceptibility patterns of Pseudomonas aeruginosa from Diabetes Patients with Foot Ulcers. Int J Microbiol.; 2011, Article ID 605195, 4 pages, 2011. doi:10.1155/2011/605195
22. Citron DM, Goldstein EJC, Merriam VC, Lipsky BA: Bacteriology of moderate to severe diabetic foot infections and invitro activity of antimicrobial agents. J Clin Microbiol. 2007; 45 (9):2819–28
23. Hashem H, Hanora A, Abdalla S, Shawky A, Saad A: Carbapenem Susceptibility and Multidrug-Resistance in Pseudomonas aeruginosa Isolates in Egypt. Jundishapur J Microbiol. 2016; 2;9(11):e30257
24. El-Domany, R. A., Emara, M., El-Magd, M. A., Moustafa, W. H., & Abdeltwab, N.M.: Emergence of Imipenem-Resistant Pseudomonas aeruginosa Clinical Isolates from Egypt Coharboring VIM and IMP Carbapenemases. Microbial Drug Resistance. Microb Drug Resist., Jan 2017 13.doi: 10.1089/mdr.2016.0234
25. Hong, D.J., I.K. Bae, I.H. Jang, S.H. Jeong, H.K. Kang, and K. Lee: Epidemiology and characteristics of metallo- beta-lactamase-producing Pseudomonas aeruginosa. Infect. Chemother 2015; 47:81–97
26. Saito R, Koyano S, Dorin M, Higurashi Y, Misawa Y, Nagano N, Kaneko T, Moriya K: Evaluation of a simple phenotypic method for the detection of carbapenemase-producing Enterobacteriaceae. J Microbiol Methods, 2015; 108:45–48
27. Kim HK, Park JS, Sung H, Kim MN: Further modification of the modified Hodge test for detecting metallo-β-lactamase-producing carbapenem-resistant Enterobacteriaceae. Ann Lab Med, 2015; 35:298–305
28. Pasteran F, Veliz O, Rapoport M, Guerriero L, Corso A.: Sensitive and specific modified Hodge test for KPC and metallo-β-lactamase detection in Pseudomonas aeruginosa by use of a novel indicator strain, Klebsiella pneumonia ATCC 700603. J. Clin. Microbiol., 2011; 49:4301-4303
29. El Essawy, AK: Carbapenem-resistant Pseudomonas aeruginosa: Prevalence and impact of carbapenemase-encoding genes in isolates from Kuwait hospitals. Paper presented in 7th World Congress on Microbiology ,Valencia, Spain, 2016.
30. Moosavian, M. and Rahimzadeh M: Molecular detection of metallo-beta-lactamase genes, bla IMP-1, blaVIM-2 and bla SPM-1 in imipenem resistant Pseudomonas aeruginosa isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. Iran J. Microbiol., 2015; 7:2–6
31. Neyestanaki, DK, Mirsalehian A, Rezagholizadeh F, Jabalameli F, Taherikalani M, and Emaneini M: Determination of extended spectrum beta-lactamases, metallo-beta-lactamases and AmpC-beta-lactamases among carbapenem resistant Pseudomonas aeruginosa isolated fromburn patients. Burns., 2014; 40:1556–1561
32. Laupland KB, Parkins MD, Church DL, Gregson DB, Louie TJ, Conly JM, Elsayed S, and Pitout JD: Population-based epidemiological study of infections caused by carbapenem-resistant Pseudomonas aeruginosain the Calgary Health Region: importance of metallo-beta-lactamase (MBL)-producing strains. J. Infect. Dis., 2005;192:1606–1612
33. Mohanty, S., Maurya V., Gaind R., and Deb M: Phenotypic characterization and colistin susceptibilities of carbapenem-resistant of Pseudomonas aeruginosa and Acinetobacterspp. J. Infect. Dev. Ctries., 2013; 7:880–887