© 2018 IJSRSET | Volume 4 | Issue 4| Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section:Engineering and Technology

Molecular Detection of Carpabenem Resistant Gene (*bla*KPC) among Different Gram Negative Bacteria using Loop Mediated Isothermal Amplification (LAMP) Assay, Khartoum State, Sudan

Mutaz A Elsir[1], Mohammed A. Bakheit[2] , Salma B Satir[3], Amera I Elkhalifa[3], Abdel Rahim M El Hussein[4], Isam M Elkhidir[5], Khalid A Enan[4]*

[1]Department of Microbiology, Faculty of Medical Laboratories, University of Al-Neelain, Khartoum, Sudan
[2]Mast Diagnostica GmbH, Reinfeld, Germany
[3]Department of Microbiology, Faculty of Medical Laboratories, University of Khartoum, Khartoum, Sudan
[4]Central Laboratory, Ministry of High Education and Scientific Research, , Khartoum, Sudan
[5]Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan
*Corresponding Author:Khalid A Enan, Central Laboratory, Ministry of High Education and Scientific Research, P.O.Box:7099, Khartoum, Sudan, Fax:+249-155183855; Email: khalid.enan@gmail.com

ABSTRACT

Background: The rapid emergence and spread of antimicrobial resistance are leading physicians to rely on the carbapenem class of antibiotics to treat the resistant organisms. However, increasing rates of carbapenem resistance have been reported. blaKPC-producing bacteria are extremely resistant to almost all different classes of antibiotics. This study was carried out to detect the presence of carbapenem resistant genes blaKPC among different gram negative bacteria at Khartoum State, Sudan.

Methods: The study was conducted at Khartoum State, during the period from March to July 2017. A total 61 isolates of different gram negative bacteria that were resistant to carbapeneme were screened for the presence of carbapenem resistant gene blaKPC using LAMP assay.

Results: Out of 61 isolates tested, *Acinetobacter bumannii* was the predominant organism. The *Klebsiella pmumonaie* carbapenemase gene blaKPC was detected in 23 (37.7%) of the isolates using LAMP assay.

Conclusion: KPC production is an important mechanism of carbapenem resistance among different gram negative bacteria in Sudan. LAMP assay can provide rapid detection for carbapenem resistant genes.

Keywords: blaKPC ,Multidrug resistance, Carbapenem, LAMP.

I. INTRODUCTION

Multidrug resistance is emerging at an alarming rate among a variety of bacterial species causing both community-acquired and nosocomial infections [(1)].Carbapenem resistance among *Enterobacteriaceae*, in particular among *Klebsiella pneumoniae* and *Escherichia coli*, is an emerging problem worldwide [(2)]. Carbapenemase production, added to other resistance mechanisms already described in enterobacteria such as the extended spectrum beta-lactamases (ESBL) , makes these microorganisms resistant to almost all available antimicrobials[(3)]. One of the most important mechanisms of microbial resistance to β-lactam antibiotics (penicillins, cephalosporins, monobactams,

and carbapenems) is hydrolysis by β-lactamases [4]. Resistance to β-lactams is usually mediated through β-lactamase production or an alteration of porins, although other mechanisms such as target site modifications (e.g., in penicillin binding proteins) and drug efflux pumps have also been reported [9].

Three major classes of carbapenemases are known: 1) metallo-beta-lactamases (imipenemase [IMP], Verona imipenemase [VIM], New Delhi metallo-beta-lactamase [NDM]); 2) oxa-carbapenemases (OXA-48); and 3) *Klebsiella pneumonia* carbapenemase (KPC) [5]. These enzymes are extremely relevant because of their rapid and wide dissemination, once they are encoded by genes located in mobile genetic elements, such as plasmids and transposons [5]. Resistance is acquired by vertical/horizontal transfer of genes such as blaCTX-M, blaKPC and blaNDM, frequently associated with plasmids [6, 7].

Class A carbapenemases,which include blaKPC, NMC, SME-1 to -3, IMI-1, and GES,have been characterized in several genera of the family *Enterobacteriaceae*[8]. blaKPC enzymes, so called because they have been identified mainly in *K. pneumoniae,* have been reported in *Klebsiella oxytoca*, Serratia., Enterobacter spp., Salmonella spp., *Citrobacter freundii*, and *Pseudomonas aeruginosa*[2].

The detection of carbapenemase-producing enterobacteria is extremely important to control infection and prevent dissemination of these resistance. Although phenotypic tests are widely used in many clinical laboratories, it is necessary to confirm the production of enzymes that confer resistance to carbapenems by means of molecular tests, such as the polymerase chain reaction (PCR), which presents high sensitivity and specificity[5].

II. METHODOLOGY

Isolation and Identification of bacteria:

The bacterial isolates used in this study including *Pseudomonas aeruginosa*, *Acinetobacter bumannii*, *Klebsiella pneumoniae*, *Echerichia coli*, *Citrobacter frendii* and *Proteus mirabilis* have been isolated from different hospitals in Khartoum State, Sudan identified in a previous study done by Satir et al[10].(table 2)

Antimicrobial Suscebtibility Testing:

The samples were screened for the following antibiotics; Meropenem (MRP), Imipenem (IMP), Ceftazidime (CAZ), Cefteriaxon (CRO), Cefotaxime (CTX), Cephalexin (CN30), Cefixime (CFM), Penicillin (P), Cefuroxime (CXM) using Kirby-Bauerdisk diffusion method according to CLSI guidelines [10].

Performance Of LAMP Assay:

DNA was extracted form bacterial isolates by the boiling method [10]. Samples were tested for the presence of *Klebsiella pneumoniae* carbapenemase resistant gene blaKPC [10]..

To perform LAMP, primer mix have been prepared as follow; 5µL of each forward outer primer (F3) and backward outer primer (B3), 40µL of each forward inner primer (FIP) and backward inner primer (BIP), 20 µL of both loop forward (LF) and loop backward (LB) primers and 70 µL of distilled water (Table1).

Primer	Sequence
F3	ACT CGA ACA GGA CTT TGG C
B3	CCG TAA CGG ATG GGT GTG
FIP	CCT CAG CGC GGT AAC TTA CAG TGG CTC CAT CGG TGT GTA CG
BIP	TTC CCA CTG TGC TCA TTC ATG CTG CTG GCT GCG AG
LF	GCC TGA GCC GGT ATC CAT
LB	AGG GCT TTC TTG CTG CCG

Table.1: primers sequences for the blaKPC genes.

LAMP master mix for Klebsiella pneumoniae carbapenemase gene blaKPC have been prepared

using Mast tablets. For each tablet 67.2 µL of distilled water, 24 µL of Tris-HCL and 4.8 µL of the primer mix have been added. For each LAMP reaction 8 µL of the prepared master mix were added to 2 µL of the DNA. The LAMP reaction was carried out using Rotor-Gene Q MDx (QIAGEN Hilden, Germany) at constant temperature of 64°C for 60 min in a real-time manner and the results were visualized using FAM channel.

III. RESULTS

Out of 83 isolates, 61 isolates were tested for the presence of the blaKPC gene using LAMP. The majority of the organisms were *Acinetobacter bumannii* with 29 isolates (47.5%), followed by *Klebsiella pneumoniae* 14 (23%), *Pseudomonas aeruginosa 13* (21.3%), *Echerichia coli* 3 (4.9%), *Citrobacter frendii* 1(1.6 %) and *Proteus mirabilis* 1 (1.6 %)[10].

blaKPC was detected in 37.7% (23) isolates, however the gene was mostly detected in *Acinetobacter bumannii* followed by *Klebsiella pneumoniae* (Table 2).

Table 2. The results of the LAMP for the detection of blaKPC in various bacterial isolates

Organisms (n)	blaKPC
Pseudomonas aeruginosa (13)	5
Acintobacter bumannii (29)	10
Klebsiella pneumoniae (14)	7
Echerichia coli (3)	0
C*itrobacter frendii* (1)	1
Proteus mirabilis (1)	0
Total (61)	23 (37.7%)

IV. DISCUSSION

Antimicrobial resistance among gram negative bacteria is a serious public health problem due to the association with different resistance mechanisms. Resistance to a variety of broad-spectrum antimicrobials including β-lactams, is frequently encountered. Carbapenems are the most potent β -lactam antibiotics and are the most commonly used β -lactams for the treatment of serious infections[11]. However, the emergence of Carbapenem resistant bacteria have a great clinical and public health importance[3]. Thus, the recent reports on resistance to carbapenems isolates are of great concern [11].

Rapid identification of these enzymes is thus, essential, and molecular tests able to identify specific carbapenemase-encoding genes must be included in laboratory routines [12]. LAMP was developed for the detection of carbapenem resistant genes with high specificity and sensitivity as reported by Solanki et al [13], Li et al[14], Wang et al[15] and Liu et al[16].Our present study represents the first report on using this technology for detection of blaKPC in Sudan.

In the current study we amplified one of the major carbapenem resistant gene; blaKPC using LAMP assay. blaKPC was detected in 37.7% of isolates (23 isolates).Simillar to the results of satir et al (2016)[10]. It was most commonly found in *Acinetobacter bumannii* followed by *Klebsiella pneumoniae*. The differences in the detection of these genes suggest that they are genetically differrent. Neither *Echerichia coli* nor *Proteus mirabilis* were positive for blaKPC using LAMP. Other carbapenemase resistant genes may be responsible for the resistance of these isolates[10]. Thus it always necessary to look for the other genes and identify the mechanisms of resistance, as the presence of several types of beta-lactamases in enterobacteria makes it difficult to identify individual resistance mechanisms [17].

LAMP assays can generate results more easily and rapidly, with high sensitivity and specificity. LAMP assay is carried out under isothermal conditions and employs four/six specific primers that recognize six/eight distinct regions of the target, thus, increasing sensitivity and specificity. It only requires a simple water bath and hence is cost effective. In addition, the gene amplification can be visualized by the naked eye, either as turbidity in the form of a white precipitate or through a colour change employing a fluorescent intercalating dye such as SYBER®GREEN I[18].

With the application of molecular techniques including LAMP assay, the detection of carbapenemases will become faster and reliable, aiding diagnosis and treatment, as well as providing information that directly influences the control measures for infections caused by these microorganisms.

V. CONCLUSION

KPC production is an important mechanism of carbapenem resistance among different gram negative isolates. Combined use of different diagnostic methods is needed to identify carbapenem resistance in enterobacteria to prevent their spread and to help control infections caused by these organisms. The use of molecular techniques such as LAMP assay can provide a rapid detection for blaKPC genes with high specificity.

VI. REFERENCES

[1]. Spellberg B,Blaser M,GuidosRJ,Boucher HW,Bradley JS,et al.Infectious Diseases Society of America; Combating antimicrobial resistance: policy recommendations to save lives.Clin Infect Dis..2011;52(Suppl 5):S397-428.

[2]. Musa Hindiyeh,Gill Smollen,Zehava Grossman et.al.Rapid Detection of blaKPC Carbapenemase Genes by Real-Time PCR.JOURNAL OF CLINICAL MICROBIOLOGY,Sept.2008,p.2879-2883.

[3]. Li H,Zhang J,Liu Y,et al.Molecular characteristics of carbapenemase producing Enterobacteriaceae in China from 2008 to 2011: predominanceof KPC 2 enzyme.DiagnMicrobiol Infect Dis.2014; 78: 63-5.

[4]. Walsh TR,Bolmstrom A,Qwarnstrom A,Gales A.Evaluation of a new E test for detecting metallo-β-lactamases in routine clinical testing.J Clin Microbiol 2002; 40 : 2755-9.

[5]. Jaciane B.Marques1; Pauline C.Bonez1; Vanessa A.Agertt1 Molecular characterization of Enterobacteriaceae resistant to carbapenem antimicrobials J Bras Patol Med Lab,51,n.3,p.162-165,June 2015

[6]. Stalder T,Barraud O,Casellas M,Dagot C,Ploy MC.Integron involvement in environmental spread of antibiotic resistance.FrontMicrobiol.2012 Apr 9; 3: 119.

[7]. Elgorriaga-Islas E,Guggiana-Nilo P,Dominguez-Yevenes M,et al.Prevalence of plasmid-mediated quinolone resistance determinant aac(6′)-Ib-cr among ESBL producing enterobacteria isolates from Chilean hospitals.EnfermInfeccMicrobiolClin.2012 Oct; 30(8): 466-8.PubMedPMID: 22542083.

[8]. Nordmann,P.,and L.Poirel.2002.Emerging carbapenemases in Gram negative aerobes.Clin.Microbiol.Infect.8:321-331.

[9]. 35.Yigit,H.,G.J.Anderson,J.W.Biddle,C.D.Steward,J.K.Rasheed,L.L.Valera,J.E.McGowan,Jr.,and F.C.Tenover.2002.Carbapenem resistance in a clinical isolate of Enterobacteraerogenes is associated with decreased expression of OmpF and OmpC porin analogs.Antimicrob.Agents Chemother.46:3817-3822.

[10]. Satir SB,Elkhalifa AI,Ali MA,El Hussein ARM,Elkhidir IM,et al.(2016) Detection of Carbepenem resistance genes among selected Gram Negative bacteria isolated from patients in-Khartoum State,Sudan.ClinMicrobiol 5: 266.

[11]. DrorMarchaim,ShiriNavon-Venezia,Mitchell J.Schwaber,and Yehuda Carmeli.Isolation of Imipenem-Resistant Enterobacter Species: Emergence of KPC-2 Carbapenemase,Molecular Characterization,Epidemiology,and Outcomes.Antimicrobial Agents and Chemotheraoy.Apr.2008,p.1413-1418

[12]. Doyle D,Peirano G,Lascols C,et al.Laboratory detection of Enterobacteriaceae that produce carbapenemases.J Clin Microbiol.2012;50(12): 3877-80.

[13]. Rachana Solanki,1LavanyaVanjari,Nagapriyanka Ede,Akhila Gungi,Amarendranath Soory and Lakshmi Vemu.Evaluation of LAMP assay using phenotypic tests and conventional PCR for detection of blaNDM-1 and blaKPC genes among carbapenem-resistant clinical Gram-negative isolates.Journal of Medical Microbiology (2013),62,1540-1544.

[14]. Li P,Niu W,Li H,Lei H,Liu W,et al Zhao X,Guo L,Zou D,Yuan X,Liu H,Yuan J and Bai C (2015) Rapid detection of Acinetobacterbaumannii and molecular epidemiology of carbapenem- resistant A.baumannii in two comprehensive hospitals of Beijing,China.Front.Microbiol.6:997.doi: 10.3389/fmicb.2015.00997

[15]. Huan Wang,Junfu Huang,Han Xia1,Xiaoli Zhang et al. Rapid identification of imipenem-resistant Pseudomonas aeruginosa by loop-mediated isothermal amplification based detection of OprL and OprD2 genes.Int J Clin Exp Med 2016;9(12):23769-23776

[16]. Wei Liu,aDayangZou,a Yan Li,bXuesong Wang ,et al.(2012) Sensitive and Rapid Detection of the New Delhi Metallo-Beta- Lactamase Gene by Loop-Mediated Isothermal Amplification.Journal of Clinical Microbiology p.1580-1585

[17]. Birgy A,Bidet P,Genel N,et al.Phenotypic screening of carbapenemases and associated β-lactamases in carbapenem-resistant Enterobacteriaceae.J Clin Microbiol.2012; 50(4): 1295-1302.

[18]. Notomi,T.,Okayama,H.,Masubuchi,H.,Yonekawa,T.,Watanabe,K.,Amino,N.&Hase,T.(2000).Loop-mediated isothermal amplification of DNA.Nucleic Acids Res 28,E63.