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

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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 are being reported. blaKPC-producing bacteria are extremely resistant to almost different classes of antibiotics. This study was carried out to detect the presence of carbapenem resistant genes blaKPC among different gram negative isolates 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 genes blaKPC using LAMP assay.
Results: Out of 61 isolates tested, Acintobacter bumannii was the predominant organism. The Klebsiella pmumonaie carbapenemase gene blaKPC was detected in 37.7% (23) 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, Carbapenemases, 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 pneumonia 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 betalactamases (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, Acintobacter bumannii, Klebsiella pneumonia, Echerichia coli, Citrobacter frendii and Proteus mirabilis have been isolated from different hospitals in Khartoum state, Sudan and identified in a previous study done by Satir et al[10].(table 1)

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 pneumonia carbapenemase resistant gene blaKPC.

To perform LAMP, primer mix have been performed 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 (LF) primers and 70 µL of distilled water. LAMP master mix for Klebsiella pneumonia carbapenemas gene blaKPC have been prepared using Mast taps. For each tap 67.2 µL of distilled water, 24µL 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 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. The majority of the organisms were Acintobacter bumannii 29 isolates (47.5%), followed by Klebsiella pneumonia 14 (23%), Pseudomonas aeruginosa 13 (21.3%), Echerichia coli 3 (4.9%), Citrobacter frendii 1(1.6 %) and Proteus mirabilis 1 (1.6 %).

blaKPC was detected in 37.7% (23) isolates, however the gene was mostly detected in Acintobacter bumannii followed by Klebsiella pneumonia (Table1).

Table 1. 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 pneumonia (14)	7
Echerichia coli (3)	0
Citrobacter frendii (1)	1
Proteus mirabilis (1)	0
Total (61)	23 (37.7%)

IV. DISCUSSION

Antimicrobial resistance among gram negative bacteriais 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 RachanaSolanki [13], PuyuanLi[14], Huan Wang[15] and Wei Liu[16].Our present study represents the first report on using this technology for detection of blaKPC in Sudan.

In the current study we amplified two of the major carbapenemase resistant gene; blaKPC using LAMP assay.blaKPC was detected in 37.7% of isolates (23 isolates). It was most commonly found in Acintobacter bumannii followed by Klebsiella pneumonia. The differences in the detection of theses genes suggest that they are genetically differ. 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. It is 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 employ four/six specific primers that's 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 the methods is needed to identify carbapenem resistance in enterobacteria to prevent their spread and control the 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.

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