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DETECTION OF KPC GENE IN ANTIBIOTIC RESISTANT *KLEBSIELLAPNEUMONIA* CAUSING URINARY TRACT INFECTION

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ABSTRACT

Klebsiella pneumonia carbapenemases (KPCs) are class A variant of β –lactamase enzymes capable of hydrolyzing all known β –lactam antibiotics. Worldwide spread of KPC producing strain makes them a potential threat for current antibiotic based therapy. In this study, we isolated the *K. pneumoniae* from the urine samples and screened for antibiotic resistance. Then molecular amplification techniques PCR was used for identification of KPC gene in these isolated samples. The PCR results coordinate with the antibiotic susceptibility testing and KPC positive isolates showed a multidrug resistant.Considering the potential for rapid horizontal and vertical transmission of KPC genes, prompt recognition is critical. This study validated a rapid, sensitive, and specific PCR assay for the detection of KPC gene.

KEYWORDS: ***K. pneumonia,*****Carbapenemases,KPC, PCR**

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INTRODUCTION

Klebsiellapneumoniae is a non-motile enteric gram-negative bacterium that can be found in the natural environment and often found as a commensal resident of the human gastrointestinal tract[1]. *K. pneumoniae* prominentlycolonize in healthy human's gastrointestinal tract, eyes, respiratory tract, and genitourinary tract. *K. pneumoniae* strains considered as opportunistic rather than true pathogens because they mostly affect debilitated patients[2]. β-Lactamsare mainly used for treatment for serious infections, and the most active of these are the carbapenems. These are used for the treatment of infections caused by extended-spectrum beta -lactamase (ESBL)-producing *Enterobacteriaceae*like*K. pneumoniae*[3]. Resistance tocarbapenems is mainly associated with acquired carbapenem-hydrolyzing β-lactamases such as *K. pneumonia* carbapenemase (KPC) enzymes that were first found in *K.pneumonia*[4, 5]. KPC-producing organisms show resistance to a broad range of antimicrobial agents, including all available β-lactams, fluoroquinolones, and aminoglycosides which challenge clinicians for successful treatment[6-9]. The treatment against KPCs often limited to tigecycline and colistin, but some isolated have been observed recently, showing resistance to these drugs. These colistin and tigecyclineresistance strains are defined as extremely drug-resistant (XDR) strains because all available standard antibiotics are ineffective against them[10].KPC-producing*Enterobacteriaceae* infection causes critical illness, immunosuppression, and multiple comorbid conditions which is associated with high mortality rates[11]. In one study, infection of KPC-positive strains showed 47% mortality rate[12]. KPCs have now spread all over the globe and reached endemic proportions in countries other than USA[13]. In this study *K.pneumonia* was isolated from patients detected with urinary tract infection, and developed highly sensitive PCR technique for detection of KPC gene.

MATERIALS AND METHODS

Isolation of K.pneumonia

Total seven urine samples from patients with urinary tract infection were collected from the various diagnostic Centers in Bangalore. The samples were serially diluted and spread on nutrient agar plates. The single isolated colonies were selected and identified on the basis of morphology (shape, size, structure, texture, appearance, elevation and color). Further identification was done on the basis of gram staining and biochemical characterization.

Antibiotic sensitivity test

Antibiotic sensitivity of isolated samples was done using disc (Himedia, India). The bacterial isolate was spread on the solid MH media and the antibiotic disc was placed carefully on the plate and incubated at37^{o}C for 24 hrs. The clearance zone was noted after incubation for determination of antibiotic resistance.

Isolation of DNA

DNA was isolated from the samples as described previously[14] and checked on 0.8% agarose gel.

The quantity of the isolated DNA was checked in UV-VIS spectrophotomer (Vivaspec Biophotometer, Germany).

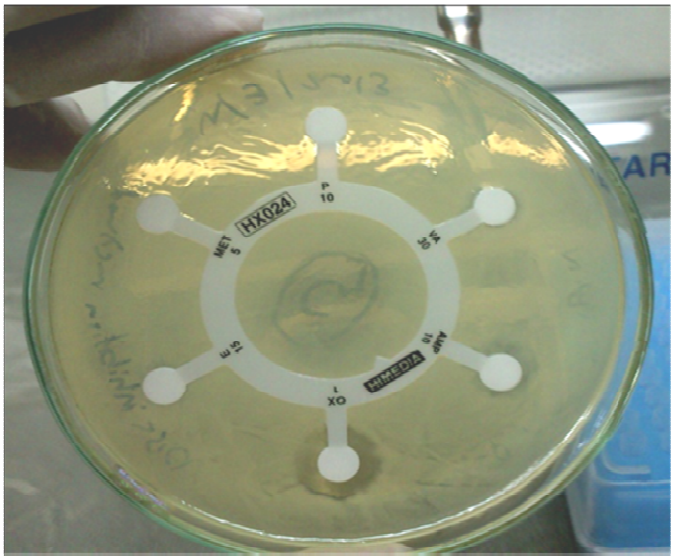
PCR

The specific primers were designed for KPC gene using Primer3 Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). The designed oligonucleotides were synthesized in Sigma Corporation USA. PCR amplification was then performed with 20 µl of reaction. The PCR conditions used were 30 cycles of amplification at a denaturation temperature of 94°C for 60 s, an annealing temperature of 56°C for 60 sec and an extension temperature of 72°C for 60 sec. This step was followed by a final extension at 72°C for 6 min. PCR products were resolved on 1.5% agarose gels, stained with ethidium bromide, and photographed with UV illumination

RESULTS AND DISCUSSION

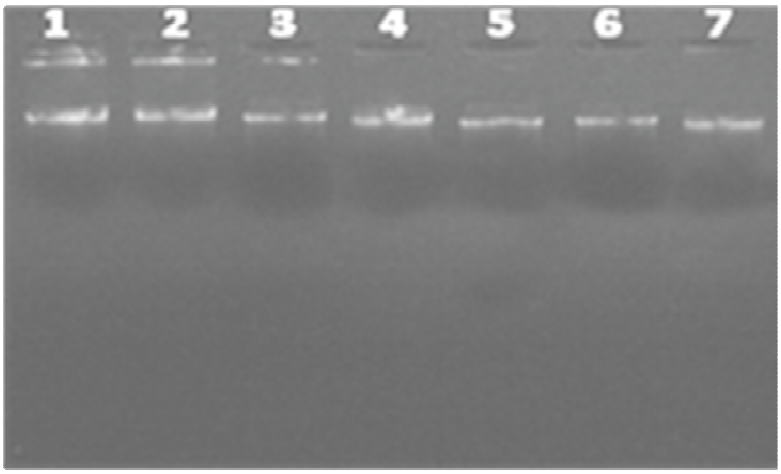
From the collected urine samples *K.pneumoniae*were isolated and screened for antibiotic resistance using the disc.The antibiotic resistance pattern was showed in figure 1 for one sample. Among the seven samples three samples showed antibiotic resistance against β- lactum antibiotics and others were sensitive.

Figure 1
Antibiotic resistance pattern of K. pneumonia determined by disc.



The *K. pneumonia* samples were cultured in the LB broth media and DNA was isolated. The isolated DNA was electrophorized in 0.8 % Agarosegel as showed in figure 2.

Figure 2
Isolated DNA on 0.8 % agarose gel



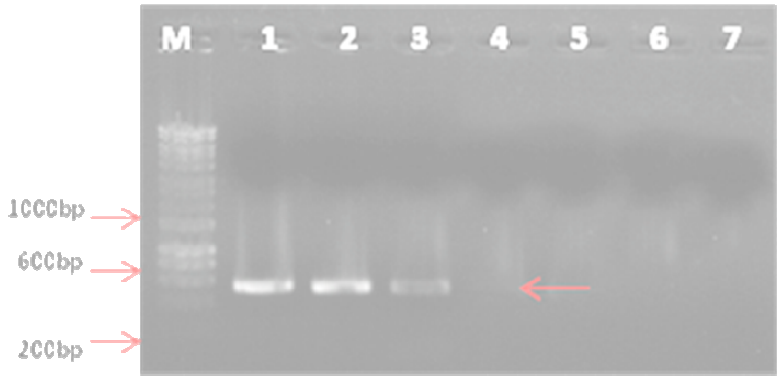
The quantity and quality of DNA was analyzed by UV visible spectrophotometer and the data was showed in the table 1.

Table 1
DNA concentration and purity of isolated DNA

DNA Sample	Concentration (ng/ µl)	A260/280
1	201	1.72
2	222	1.87
3	120	1.84
4	143	1.80
5	300	1.92
6	225	1.83
7	286	1.91

The designed primers were validated initially *in silico* and subsequently in wet lab. The primers yielded an expected amplicon of the ~600 bp size as showed in figure 3.

Figure 3
Amplification of KPC gene by PCR



Only three samples produced the expected product and other four samples didn't show any amplification. The PCR results coordinate with the susceptibility testing and KPC positive isolates showed a multidrug resistant. Earlier studies have reported that carbapenemresistant may be incorrectly identified which results in inappropriate therapy[15]. DNA sequencing is the most definitive method but impractical for large number of samples, hence, molecular detection of KPC genes by PCR is quick and reliable method. The reported assay is rapid and simple to perform, with a high degree of sensitivity and specificity.

CONCLUSION

Molecular detection of KPC gene by PCR provided a quick method to identify the presence of this important resistance determinant.

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