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Original Article

Biotechnology Section

PCR Based Molecular Detection of the Gyr-B-2 Gene from the Klebsiella Sp. Isolates from Patients who were Suffering with Pneumonia and Urinary Tract Infections (UTIs)

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ABSTRACT

Purpose: Detection of the virulence gene is a key component in determining the pathogenicity of any isolates, because these genes act multi-functionally and multi-factorially. A gyrase specific gene primer, in combination with the PCR technology, allows the precise detection of the DNA *gyrase* subunit B2 gene (*gyr*-B-2) from different virulent microorganisms. In the present study, forward and reverse primers with lengths of 20bp and 21bp were used for the detection of the *gyr*-B-2 genes in the clinical isolates of the *Klebsiella* sp. which were collected from patients who were suffering from pneumonia and urinary tract infections (UTIs).

Materials and Methods: A total of 14 isolates *viz.*, K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11, K12, K13 and K14 were isolated from 3 different private medical colleges of Sylhet city.

Results: The *gyr*-B-2 gene which was amplified in 12 isolates *viz.*, K1, K2, K3, K4, K5, K6, K7, K8, K10, K11, K12 and K14 gave the expected 411bp PCR product after its visualization under a gel documentation system in a 1.2% agarose gel.

Conclusions: The present study was undertaken to detect the gyrB2 gene from Klebsiella sp, which will be helpful for further scientific studies. This PCR was outstanding in the detection of the *gyb*-B-2 gene in pneumonia and urinary tract infections in patients, which were caused by the *Klebsiella* species.

Key Words: Virulence gene, gyr-B-2, PCR amplification, Visualization

INTRODUCTION

The molecular techniques which have been used in recent years for the detection of pathogens and their virulent genes, have proven to be promising in the disease diagnosis and the prophylaxis. Among them, the Polymerase Chain Reaction (PCR) has been found to be the most significant one in the last couple of years [1]. The *Klebsiella* species are gram negative, rod shaped, non-motile bacteria that are found in the environment and also in the human intestinal tract [2,3]. Pneumonia is the commonest infection among the patients in the intensive care facilities across the world. It ranks worst among the patient morbidity and mortality cases in hospital acquired infections [4]. *K. pneumoniae* is the most common species which is isolated from hospital patients. In most of the patients, a variety of sites are colonized, mostly the urinary tract, with or without a serious infection [5]. Rarely can *K. pneumoniae* cause severe pneumonia in susceptible individuals. The clinical features of a bacteraemic infection with *K. pneumoniae* are due to the virulence factors which are expressed by the organism [3]. Bajaj *et* al. (1999) reported that the *Klebsiella* species caused urinary tract infections in a maximum number of cases (124, 37.35%), followed by *Escherichia coli* (114, 34.4%), *Pseudomonas aeruginosa* 32 (9.64%) and *Staphylococcus aureus* 23 (6.93%) [6]. Several genes are responsible for the virulence properties of the *Klebsiella* species, but the DNA *gyrase* subunit B2 gene (gyr-B-2) is the principle one that has been reported by many researchers [7]. DNA gyrase is a prokaryotic type II topoisomerase which cuts both strands of the DNA helix simultaneously in order to manage the DNA tangles and the supercoils and it is a major target of the quinolone antibacterials [7, 8]. A majority of the mutations which confer resistance to the quinolones arise within the quinolone resistance-determining region of gyrA, close to the active site (Tyr^{122}), where DNA is bound and cleaved [7]. However, some quinolone resistance mutations are known to exist in gyrB. Liu *et* al. (2008) detected *Klebsiella pneumoniae* by PCR in infants by using the16S-23S internal transcribed spacer [1]. Catherine Dauga (2002) amplified the gyrB gene and performed a molecular phylogenetic analysis in the Enterobacteriaceae, which included *Klebsiella pneumonia* and *Klebsiella terrigena* [6].

MATERIALS AND METHODS

Collection of the Bacterial Isolates

The *Klebsiella* sp. bacterial isolates were collected from three different medical colleges and hospitals of the Sylhet district, Bangladesh viz. the M. A. G. Osmani Medical College Hospital, the Ibn Sina Hospital and the Ragib-Rabeya Medical College and Hospital, Bangladesh. The isolates were collected from patients who were suffering from pneumonia and urinary tract infections.

The Culture Conditions

The bacterial isolates were streaked on nutrient agar plates from a previous plate and they were incubated at 37ºC overnight for the appropriate colony formation. After the incubation, a single colony

of each plate was selected for re-isolation to get a pure culture in a new nutrient agar plate.

Isolates	Collected from	Type of patient
K1	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K2	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	Pneumonia
K3	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K4	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K5	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	Pneumonia
K6	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K7	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K8	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K9	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K10	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	UTI
K11	Ibn Sina Hospital, Sylhet, Bangladesh	UTI
K12	Ragib-Rabeya Medical College and Hospital, Sylhet, Bangladesh	Pneumonia
K13	Ibn Sina Hospital, Sylhet, Bangladesh	UTI
K14	M. A. G. Osmani Medical College and Hospital, Sylhet, Bangladesh	Pneumonia

[Table/Fig-1]: *Klebsiella* sp. isolates with their isolation history

DNA Extraction

A total of 13 bacterial isolates were inoculated into nutrient broth and they were incubated overnight at 37ºC and at 120rpm in a shaker incubator. The bacterial genomic DNA was extracted by using a commercial ATP Genomic DNA Extraction Kit and the extracted DNA was preserved at -20ºC in an ultra freezer [Table/Fig-1].

The PCR Reaction Mixture Set Up

The PCR was performed in 25µl reaction mixtures which contained 1.2µl of the DNA template (genomic DNA of the bacteria) , 1µl of 25 mM $MgCl_2$, 5µl of the 5x colourless reaction buffer, 0.5 µl concentration of each deoxynucleotide Triphosphate (dNTP), 1.2µl of each forward primer and reverse primer and 0.15µl of DNA polymerase along with its amplification buffer. The amplifications were carried out in a MultiGene gradient thermal cycler (Labnet International Inc. USA) [Table/Fig-2].

Primer Sequence (5' to 3')	PCR product length	Pathogen
Gyr-B-2F TCCGGCGGTCTGCACGGCGT Gyr-B-2R TTGTCCGGGTTGTACTCGTC	411bp	*Klebsiella* sp.

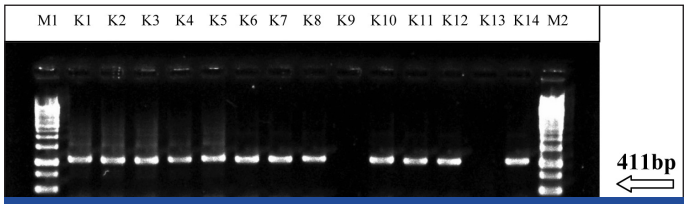
[Table/Fig-2]: Primer used for present study

The Amplification Conditions

The PCR reaction was optimized with the following parameters: An initial denaturation step at 94°C for 4 min; a denaturation step at 94°C for 1 minute, annealing at 62°C for 1 minute, extension at 72°C for 90 s; and a final extension step at 72°C for 10 minutes. 35 serial cycles of reactions were performed.

RESULTS

The amplified PCR products were detected by the agarose gel electrophoresis of each amplification mixture in 1.2% agarose gels in 1% Tris-acetate-EDTA buffer. The gels were stained with an ethidium bromide solution (10mg/ml) for 20 minutes. The position of each band on the gels were then visualized and they were documented in a gel documentation system. Among the 13 isolates of *Klebsiella* sp., the *gyr*-B-2 gene was amplified in 12 isolates *viz.*, K1, K2, K3, K4, K5, K6, K7, K8, K10, K11, K12 and K14. The sizes of the amplification products which were obtained by PCR were identical to those which were predicted from the target *gyr*-B-2 primers [Table/Fig-3].



[Table/Fig-3]: PCR amplification of gyr-B-2 gene in *Klebsiella* sp. with expected product length of 411bp in 1.2% agarose gel.

DISCUSSION

The *Klebsiella* sp. has been reported as a common pathogen for human associated pneumonia and other diseases. Earlier studies had reported that the gyrase subunit B2 gene contributed the major virulence properties of many bacterial species and it had been used as a molecular tool for the identification of the bacterial species [8] and for the phylogenetic analysis [9-11]. It was reported that the pathogenesis of the *Klebsiella* sp. was multifactorial, that the mechanisms were not clearly understood and that the site of attachment and the penetration were not known [2]. In this study, we examined whether the gyr-B-2 gene was amplified in the *Klebsiella* sp. and whether it had a direct role in the *Klebsiella* sp. associated diseases. Podschun and Ullmann reported the contribution of the *gyr*-B-2 gene in the progression of the *Klebsiella* spp. and other Enterobactericeae associated diseases [2]. The *gyrB* gene is a single-copy gene which is present in all the bacteria which encode the ATPase domain of DNA gyrase, which is an enzyme which is essential for DNA replication [12]. In the present study, 12 isolates were found to be positive for the *gyr*-B-2 gene, thus revealing the distribution and the virulence properties of this gene in the *Klebsiella* species. This study confirmed the detection of the gyr-B-2 gene product of 411bp by using PCR as a taxonomic marker.

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