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Original Article

Molecular detection of *K. pneumoniae* by using *16srRNA* gene isolated from Tigris River

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ABSTRACT

The present work was aimed to detect the prevalence of *klebsiella pneumonia* in surface water which is a part of feacal coliform bacteria that consider one of microbial pollution indicators in water, and the detection carried through different tests but the most effective, reliable and easy application was PCR technique by using specific primers for the detection of *16srRNA* gene. The results showed all of 40 isolates of *klebsiella pneumonia* were tested for the presence of the *16srRNA* gene by PCR was carrying this gene.

Keywords: *16srRNA, k. pneumonia, Microbial*

INTRODUCTION

K. pneumoniae is a rod-shaped, non-motile, gram negative bacterium mostly consider as a community-acquired and a hospital-acquired pathogen that encountered by physicians worldwide and recently environmental acquired pathogen [7, 8]. Klebsiella spp. are commonly found in both clinical and nonclinical settings and account for up to 8% of all nosocomial infections in the Western world, placing them among the eight most infectious agents in hospitals. Their nonclinical habitats encompass the gastrointestinal tracts of mammals, as well as environmental sources, such as soil, surface waters, and plants [12]. The classification of Klebsiella species was based on their pathogenic features or origin. Later the classification of these bacteria depend on medical importance of the genus and its being subdivided into three species corresponding to the diseases they caused: *K. pneumoniae*, *K. ozaenae*, and *K. rhinoscleromatis* [11]. Furthermore, taxonomic keys were proposed that included characteristics such as substrate utilization and enzymatic activities. The genus Klebsiella consists of a number of species, including *K. pneumoniae*, *K. oxytoca*, *K. planticola* and *K. terrigena* [9]. 16S ribosomal RNA gene have considered an extremely useful tool in defining bacterial relationships (including those of Klebsiella) and especially in identifying environmental isolates[10]. Water is considered as vehicle for these bacteria which harbored antibiotic resistant gene, also sewage are the ones, which have been identified as reservoirs of enteric bacteria for spread of resistance factors [13]. Natural reservoirs of resistance genes may also provide a source of transferable traits for emerging pathogens [4]. The aim of this study was to detection *Klebsiella pneumonia* using *16srRNA* gene isolated from natural surface waters of Tigris River in Baghdad City by using Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

Tigris River play vital role in using their water for domestic purpose, most of the urban centers in Iraq are located along and near the Tigris River. Three locations were selected on Tigris River are: northern area of Ghera'att City (S1) (upstream), the area of Baghdad medical city (S2) (middle), the area near Al-Jadiriah Bridge (S3)(downstream) (fig2).

Sample collection: Water sample were collected monthly from November 2013 to October 2014.at three different sites. Samples were collected by using sterilized glass bottles, the collected samples were transport to the laboratory by ice box for analysis.

Isolation and Identification of klebsiella pneumonia: *Klebsiella* isolated by using membrane filtration procedure according to [5] and

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used HiCrome *Klebsiella* Selective Agar Base (HiMedia), then *Klebsiella* isolates were submitted to commercial multi test system of gram negative bacteria and API 20E system (BioMrrieux, France), also the VITEK 2 system (Biomerieux,France) was used in this study to confirm the identification of *Klebsiella* spp.

Extraction of Genomic DNA: Extraction Genomic DNA was done using a commercial Wizard® Genomic DNA Purification Kit (Promega, U.S.A) according to the Manufacturer Company instructions.

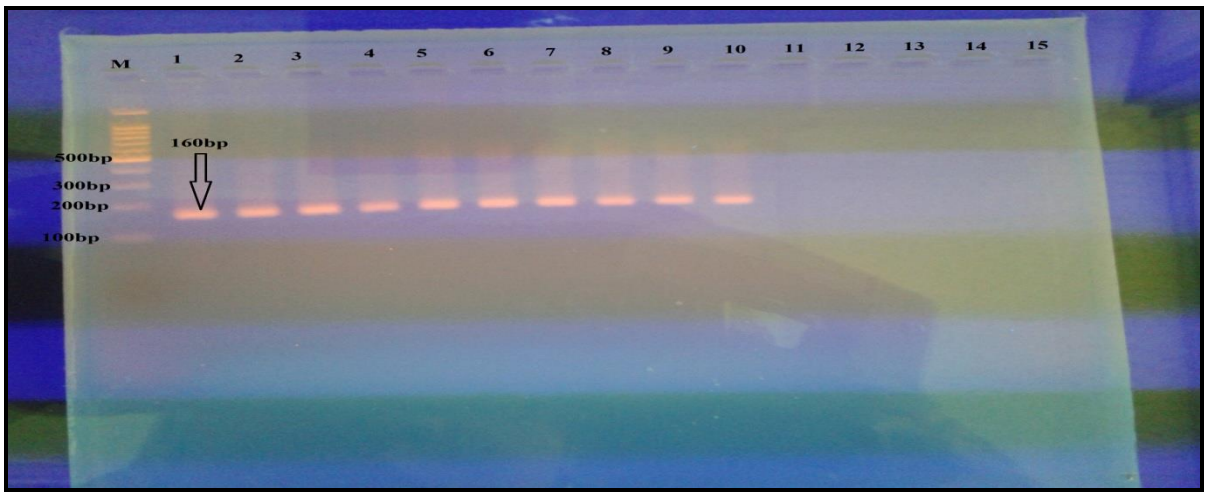
Detection 16s rRNA gene by PCR: A PCR reactions with specific primer were performed to identify *16s rRNA* are F(TGGAGCATGTGGTTTAATTCGA) R(TGCGGGACTTAACCCAACA) [14]. PCR was carried in 25µl of PCR reaction volumes contained 1.25(10pmol/µl) for each F and R primer(Alfa DNA/Montreal), 5 µl of DNA template, nuclease free water 5 µl, Green Go *Taq* Master Mix 12.5µl pH (8) (Promega, USA) . Amplification of DNA was performed using Thermal Cycler (Thermoelectron industries, France). The PCR was carried out under the following conditions: initial denaturation at 94 C◦/5 min, followed by 30 cycles of denaturation at 94 C◦ for 30s, primer annealing at 55C◦/1 min, and primer elongation at 72 C◦ for 1 min and final extension at 72 C◦/5 min.

The PCR products were mixed with 10 µl of loading dye and analyzed by electrophoresis in 1% agarose gels (for 1 hours using 1X TBE running buffer. 1000 bp DNA ladder was included in each run, and DNA bands were viewed under UV transilliuminator and then photographed.

RESULTS AND DISCUSSION

All 40 isolates of *K. pneumoniae* submitted to molecular identification through PCR amplification of *16srRNA* forward and reverse, the amplified DNA with the *16srRNA* primers resulting in PCR product with band of molecular size about 160 bp as showed in figure (1) and all isolates carried this gene and used for rapid diagnosis for this bacteria. Water from Tigris River had been used as raw water for potable water supply and industrial and domestic purposes. The river water is also used for irrigation, fishing, tourism and other beneficial uses [1]. Microbial pathogens are considered pollutants and according to the ward health organization, the mortality of water associated diseases exceeds 5 million people per year and more than 50% of these people suffered from microbial intestinal infections [5, 15].

So in this work had been used Polymerase Chain Reaction (PCR) as useful tool for detection environmental isolates. On the other hand, Klebsiella can be classificated depending on *16S rRNA* genes [3]. So the rRNA sequences play a central role in the study of microbial evolution, particularly, the *16S rRNA* genes have become the standard for the determination of the diversity in the specific populations [6]. The *16S rRNA* is universally distributed, allowing the comparison of phylogenetic relationships among all extant organisms. In conclusion, the prevalence of Klebsiella spp. especially *K. pneumonia* in surface water which a part of feacal coliform bacteria and we can consider it one of microbial pollution indicators in water as *E.coli*. So PCR considered a powerful and potential tool for the routine clinical identification of *Klebsiella* species.



Figure(1):Agarose gel electrophoresis (1% agarose, 7 V/cm in 90min) for *16S rRNA* gene in *K. pneumoniae* (Clinical (C) and Environmental (E)) isolates, line M : 100bp DNA ladder, lines (1E,2E,3E,4E,5E,6C,7C,8C,9C,10C) PCR products for *16S rRNA* gene (160bp), line (11) the negative controls.

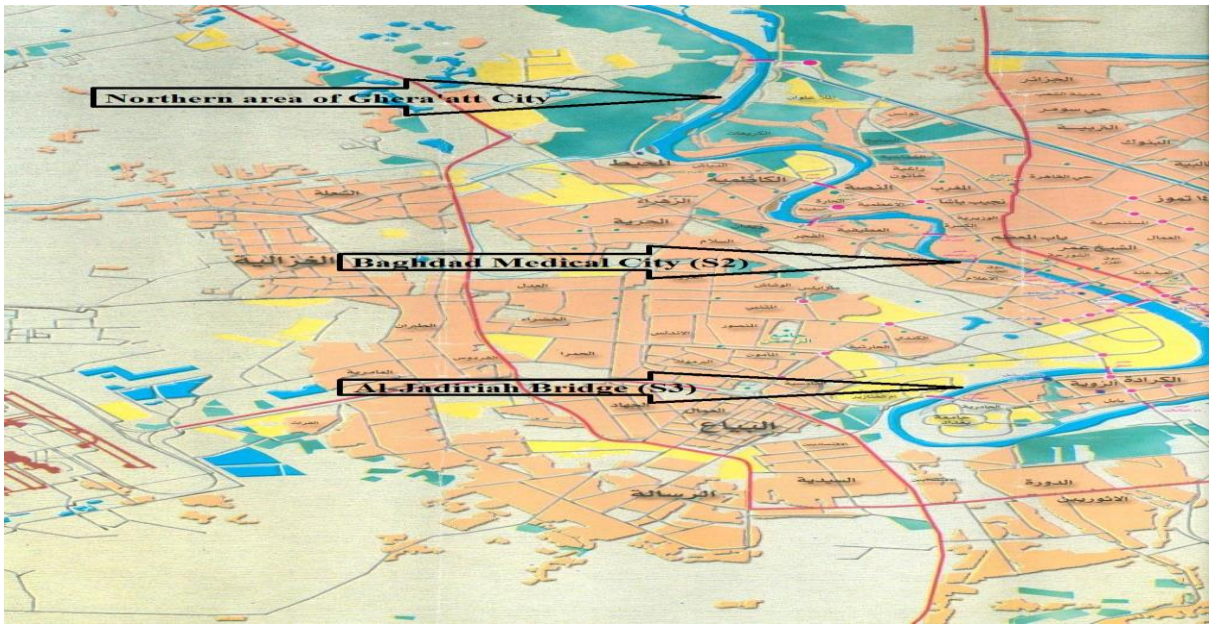


Figure2. Tigris River map and the location of sample station

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