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RESEARCH ARTICLE

Molecular Detection of *Pseudomonas aeruginosa* Isolated from Intrauterine Devices.

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Abstract

Pseudomonas aeruginosa is a common bacterium in the environment. In humans, it is an opportunistic pathogen that is a prevalent cause of hospital-acquired infections. Currently, routine bacteriological culture on selective/non-selective culture media is the cornerstone of microbiological detection. The aim of this study was to compare isolation rates of *P. aeruginosa* by conventional culture and molecular (PCR) detection of *P. aeruginosa* isolated from intrauterine devices. Out of the 98 intrauterine devices collected from women in three hospitals in governorate Al-Najaf, Iraq, only 20 (30.5%) isolates belonged to *P. aeruginosa*, identified by diagnostic tests including cultural characters, biochemical tests and confirmed by PCR technique. The PCR method of gene *algD GDP mannose* was the best methods for diagnosis, which has led to isolate and diagnosis 20 isolates of *P. aeruginosa* from intrauterine device.

Introduction:-

Pseudomonas aeruginosa is a common bacterium that can cause disease in animals and humans. It is found in soil, water, skin flora and most man-made environments throughout the world. It is an opportunistic pathogen for both humans and plants (Fang *et al*., 2012). All members of the *Pseudomonas* genus are Gram-negative rods that are characterized by a positive oxidase reaction with some members capable of producing pigments (Meyer, 2000). Pseudomonads are metabolically and physiologically versatile allowing survival in a diverse range of biotic and abiotic habitats and are especially important organisms in human disease and as biotechnological agents (Silby *et al*., 2011).

Some strains of *P. aeruginosa* overproduce the extracellular polysaccharide alginate, which gives rise to a mucoid phenotype, the production of alginate supports biofilm formation (Driscoll *et al*., 2007). Although classed as an obligate aerobe, in the absence of oxygen *P. aeruginosa* can use nitrate, nitrite or nitrous oxide as final electron acceptors (Davies *et al*., 1989). When nitrate or nitrites are not available, arginine can be used for anaerobic growth via fermentation (Vander *et al.*, 1984). This ability allows *P. aeruginosa* to adapt to the oxygen-restricted anaerobic environment found in biofilm layers (Yoon *et al*., 2002). Physiological characteristics of *P. aeruginosa* include swimming, twitching and swarming motility by means of a single polar flagella (Leone *et al*., 2008)

The genome of *P. aeruginosa* is relatively large compared to other bacteria that have been sequenced. Its size varies considerably between strains, ranging from 5.5 to 7 Mbp (Lee *et al*., 2006). The core genome is composed of highly conserved regions including housekeeping genes that have a low nucleotide divergence of 0.5 - 0.7 % (Klockgether *et al*., 2011). The accessory genome, representing more than 20 % of the total genome size, is responsible for the variance in genome size and diversity between strains (Klockgether *et al*., 2006). The *P. aeruginosa* accessory genome consists of extrachromosomal components such as plasmids, islands and blocks of DNA that are integrated into the chromosome at various sites (Klockgether *et al*., 2011). These components may be acquired from other species of bacteria through mechanisms such as horizontal gene transfer. The ‘mosaic’ and plastic genome structure of *P. aeruginosa* provides this bacterium with the ability to modify, cause infection and adapt to a wide range of

habitats (Norgaard-Gron, 2010). Intrauterine device (IUD) is one of the most convenient contraceptive procedures used by women of Asian and African countries. Previous surveys have revealed that 75% of the IUDs recovered from patients suffering from reproductive tract infections (RTIs) were covered with a consortium of microbes (Pruthi *et al*., 2003).
The aim of present study was to molecular confirmation of *P. aeruginosa* isolates.

Materials and Methods:-

Bacterial Isolation:-

Samples were transported quickly to bacteriological laboratory, each sample was placed in sterilized container containing brain heart infusion broth. After that, the container was incubated at 37°C for 24 hours (Ryder, 2005). And each specimen was inoculated using direct method of inoculation on a selective media namely MacConkey agar and Pseudomonas Isolation Agar then inoculated at 37°C for 24 hours. The next day the single colony test for biochemical test and Gram stain procedure according to (Macfaddin, 2000).

Extraction of Bacterial DNA:-

The template DNA prepared from 1.5 ml of fresh cultures of bacterial isolates grown at 37 C° in Luria Bertani broth (Green and Sambrook, 2012). DNA was extracted using genomic DNA extraction kit / Genaid according to the manufacture protocol. The extracted DNA solution was stored at 20- C°.

DNA Concentration and Purity Measurement:-

The concentration of DNA was measured by Nanodrop spectrophotometer according to the Nanodrop Optizen /Korea manual, DNA purity was measured depending on the ratio of sample absorbance at wave lengths 260 and 280 nm. A ratio of ~1.8 is considered as pure DNA (Green and Sambrook, 2012).

PCR Amplification:-

Oligonucleotide primers amplified region of a 915 bp segment of *algD GDP mannose* of *Pseudomonas aeruginosa* and not producing fragment from other related bacteria, the primer sequences for the upper and lower oligonucleotides were as follows: forward primer is GGGGGATCTTCGGACCTCA and the revers primer is TCCTTAGAGTGCCCACCC (Spilker *et al*., 2004).

The reaction was run for initial denaturation at 94 C° for 5 min followed by 30 cycles of 1 min at 94 C° (denaturation), 1 min at 50 C° (annealing), 1 min at 72 C° (extension) and 5min at 72 C° (final extension) in a thermocycler. Products of the PCR were electrophoresed on a 1% agarose gel containing ethidium bromide and photographed. Positive results were indicated by the presence of a 915 bp band seen on gel with an ultraviolet transilluminator.

Results and Discussion:-

Isolation and Identification:-

A total of 98 intrauterine devices were collected from women in three hospitals, Najaf, Iraq. The results revealed that 20 clinical samples were positive for *P. aeruginosa* isolates. The thread attached to the tail of the IUDs is perhaps one of the routes of microbial migration from vagina to the uterus, they proposed that the tail act as a wick to allow bacteria travel by capillary action, in addition to the presence of the IUD gives a solid surface for attachment (Pruthi *et al*., 2003).

Molecular Identification of *Pseudomonas aeruginosa* Isolates:-

A total of 20 of *P. aeruginosa* isolates were recovered from IUDs samples. All isolates were subjected to molecular identification through PCR amplification of *P. aeruginosa algD GDP mannose*. All isolates gives positive results (915 bp) bands, and identified as *P. aeruginosa*. The results of the specific PCR reactions are shown in figure (1). Molecular tests based on polymerase chain reactions are considered faster, high sensitivity and more accurate procedures than culture techniques for microbial identification and can be used for diagnostic and epidemiological purposes (Ferreira *et al*., 2011), while molecular genetic techniques need careful treatment of samples to provide good quality DNA for analysis, sheared or degraded DNA can be used for the PCR (Maddox and Fales, 1991). The main advantage of PCR technique has over conventional methods is that it can provide results in 24 hours whereas routine culture followed by biochemical tests need 36-48 hours (Jamil *et al*., 2007).

A large, well designed study in Indonesia by Hatta *et al.,* (2007) reported a sensitivity of 94.5% by PCR from clinical and environmental samples. It is, therefore, concluded that the PCR method was much superior to others tests yielding very high sensitivity and specificity. Although the PCR method requires extensive infrastructure and specialized skilled personnel, and cannot be made available everywhere, especially in developing countries, it can be made available to the reference centers for utilizations by other healthcare facilities following referral system. In fact, due to the rapid and definitive diagnosis, hospital admission of the patient can be avoided, reducing patients suffering, save working days and unnecessary expenditure on unrelated and misdirected treatment which may be many times more than the cost of PCR (Hatta *et al*., 2007).

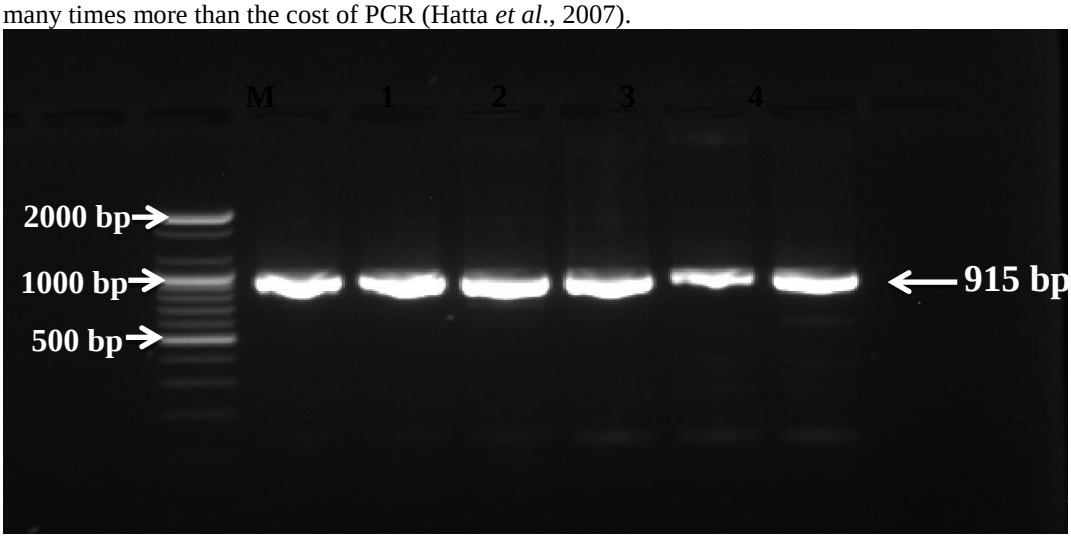

Figure (1): Gel Electrophoresis of Amplified the *algD GDP mannose* Gene Using *P. aeruginosa* Primers.

Electrophoresis was performed on 1.5 % agarose gel and run with a 70 volt current for 2 hrs. Lane M= Molecular standard (100-2000 bp ladder). Lane 1, 2, 3 , 4 , 5 , 6 = *P. aeruginosa* isolates.

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