Use of PCR to Detection *Pseudomonas aeruginosa* from Clinical Samples in Hilla Teaching Hospital

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Abstract

Wounds, burns, urinary tract infection (UTI) and ear infections are often difficult to treat due to various bacterial pathogens. *Pseudomonas aeruginosa* is one of the common invaders of this infection . Precise diagnosis of this etiological agent in this infection is of critical importance particularly in treatment of problematic cases. The existing diagnostic methods have certain limitations particularly related to specificity. This study was aimed to reliable diagnosis of *P. aeruginosa* from samples enrichment in nutrient broth PCR was used to detect *P. aeruginosa* with two primers targeted one gene saved in housekeeping gene (*gyr b*). The first primer (gyr b190) amplified 190-bp fragment , whereas other bacteria did not yield any 190-bp fragment. The specificity of the assay was 100% . PCR method depending on primer gyr b190 is rapid and more accurate than other diagnostic methods for the identification of *P. aeruginosa* strains, and it can be used to detect *P. aeruginosa* from clinical samples without using a selective medium or additional biochemical tests. While second primer gyr b222 amplified *gyr b* with fragment 222bp only when using a selective medium, but in nutrient broth get false negative.

Keywords: ***gyrB;*** *Pseudomonas aeruginosa*; PCR; clinical

الخلاصة

الجروح، والحروق، والتهاب المسالك البولية (UTI)، والتهاب الأذن غالبا ما يصعب علاجها ويرجع ذلك الى مختلف مسببات الأمراض البكتيرية. الزائفة الزنجارية ***Pseudomonas aeruginosa*** هي واحدة من الغزاة المشتركة لهذه العدوى. التشخيص الدقيق لهذا العامل المسبب للمرض في هذه الالتهابات ذو أهمية حاسمة خاصة في علاج الحالات المستعصية . الأساليب التشخيصية الموجودة لديها بعض القيود المتعلقة بشكل خاص للخصوصية. وتهدف هذه الدراسة إلى تشخيص موثوق للزائفة الزنجارية من عينات نميت في وسط معزز للنمو(Nutrient broth). تم استخدام تفاعل البلمرة المتسلسل PCR للكشف الزائفة الزنجارية مع اثنين من البوادء استهدفت أحد الجينات المحفوظة (Housekeeping genes) وهو (*gyr b* gene). البادء الأول (gyr b190) ضخم قطعه قدرها 190 bp ، في حين أن البكتيريا الأخرى لم تسفر عن أي قطعة عند 190 bp. كانت خصوصية الفحص 100٪. طريقة PCR التي اعتمدت على البادء gyr b190 سريعة وأكثر دقة من الطرق التشخيصية الأخرى لتحديد سلالات الزائفة الزنجارية ، ويمكن استخدامه للكشف عن الزائفة الزنجارية من العينات السريرية دون استخدام وسيلة انتقائية أو الاختبارات البيوكيميائية إلاضافية. في حين البادء الثاني gyr b222 ضخم قطعه قدرها 222bp فقط عند استخدام اوساط زرعية انتقائية ، ولكن في الاوساط المعززة للنمو اعطى نتيجة سلبية كاذبة.

الكلمات المفتاحية: الجينات المحفوظة ***gyrB***، الزائفة الزنجارية، تفاعل البلمرة المتسلسل ، سريري.

Introduction

Pseudomonas aeruginosa is a Gram-negative rod bacterium, which has a remarkable ability to adapt and thrive in a variety of environments: water (Karadzic *et al*.,2006) clinical settings and hospital (Schwartz *et al*.,2006). *P. aeruginosa*, a common cause of nosocomial urinary tract infections (UTI's) (Ferroni *et al*.,1998), burn , Wounds, endocarditis and otitis externa (Alammary.,2013). *P. aeruginosa* infection is the primary and most important cause of morbidity and mortality in people with the genetic disease cystic fibrosis (CF) (West *et al*.,2002). Wound infections are complications caused by bacteria which result in belated healing and can sometimes be even life-threatening(Bryan *et al*.,1983). These infections also

considerably contribute to increased health care costs. *P. aeruginosa* has been recognized as a frequent inhabitant of chronic non-healing wounds(James *et al*.,2008) and is one of the foremost opportunistic bacteria isolated from wounds which cause high morbidity and mortality despite antimicrobial therapy (Lau *et al*.,2005). *P. aeruginosa* infections are generally detected by standard microbiological chain reaction (PCR) are rapid and reliable for the identification of microbial pathogens, many PCR based diagnostic methods have been developed for *P. aeruginosa* (de Vos *et al*.,1997: Motoshima *et al*.,2007).

Materials and Methods

The present work includes investigation of 86 clinical samples were collected by sterile swab from surface of burns , flooring of burns unit and Tools of burns unit in hospital , wound ,ear and urine samples. Then, swab injected in 15 ml nutrient broth and incubated for overnight at 37^{o}C. Isolated colonies were identified by using the oxidase test, and using Api – 20 system (Forbes *et al*.,2007) . Types of clinical samples and numbers as shown in table number (1).

A total of eighty-six samples from Hilla Teaching Hospital and Merjan Teaching Hospital, starting with the beginning of November 2013 and ending with the end of April 2014.

Swabs were cultured on nutrient broth and incubate overnight then DNA extracted (in order to get all types of DNA for all bacteria grown in nutrient broth from swab) . On the other hand, swab cultured on cetrimide agar overnight and identified *Pseudomonas aeruginosa* by using standard biochemical tests and then DNA was extracted from pure culture of *Pseudomonas aeruginosa* . The way of working as shown in diagram below.

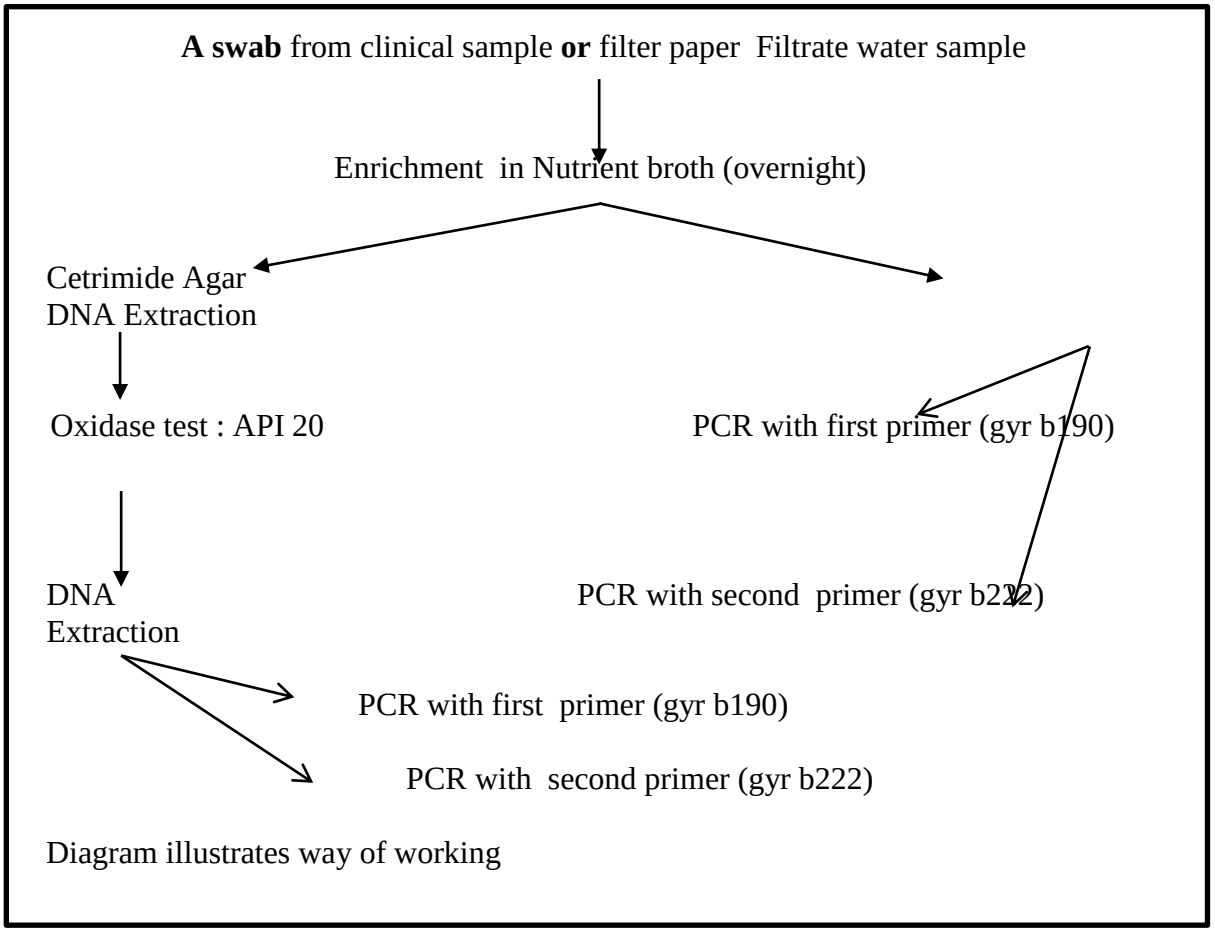


Diagram illustrates way of working

Clinical specimens included from burns 30 , flooring and tools of burns unit 14, handles laundries in water cycle 11, urine samples from people with urinary tract infection 11, wounds, ear infection 12.

Bacterial DNA preparation

Cells were harvested from 1 ml bacterial cultures, from Nutrient broth washed once with TE (10 mM Tris-HCl, 1 mM EDTA) buffer, pH 8.0, and resuspended in 10 ml of sterile distilled water.

DNA extraction

Samples were inoculated in nutrient broth culture and incubated overnight at 37°C . The bacterial genomic DNA was extracted by using commercial DNA extraction kit (Presto™ Mini gDNA Bacteria Kit. Korea) and extracted DNA was preserved at -20°C in an ultra freezer.

Primer selection

The primers used in this study are given in Table (2). The primers (gyr b190), (gyr b222) specific to *P. aeruginosa* were chosen from the published sequence (Lee *et al*.,2011: Qin *et al*.,2003).

Table 2. Primer used for detacting *P. aeruginosa*

Primer	Sequence 5" to 3"	PCR product length bp
gyr b190	F GGCGTGGGTGTGGAAGTC R TGGTGGCGATCTTGAACTTCTT	190
gyr b222	F CCT GAC CAT CCG TCG CCA CAA C R CGC AGC AGG ATG CCG ACG CC	222

Amplification condition

PCR reaction was optimized with the following parameters: an initial denaturation step of 94°C for 5 min; a denaturation step of 94°C for 1 min, annealing at (gyr b190= 60°C for 1 min)(gyr b222=68 for 1 min), and extension at 72°C for 1 min; and a final extension step of 72°C for 10 min. 35 serial cycles of reaction was performed.Used Master mix PCR (Bioneer : Korea). Five microliters of the reaction mixture was analyzed by standard gel electrophoresis (1.5 % µg/ml agarose) and the reaction products were visualized by staining with ethidium bromide (0.5 µg/ml in the running buffer). As shown in figure(1).

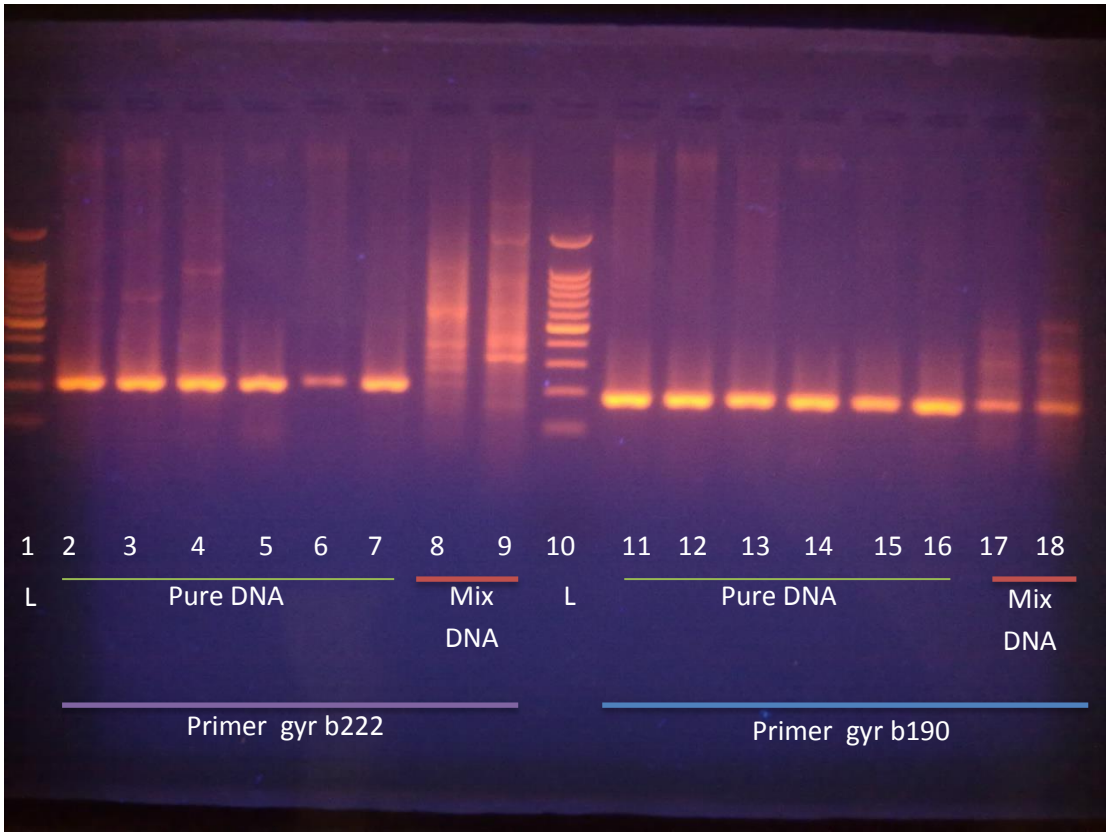
Results and discussion

Clinical samples which gave positive results of *Pseudomonas aeruginosa* in routine tests are identical with the result of PCR with primer gyr b190, and *Pseudomonas aeruginosa* gave positive results from burns (No.18= 60%) , flooring and Tools of burns unit (No. 4=28.5%), handles laundries in in water cycle (No. 10 =90%), urine samples from people with urinary tract infection (No.2 =18.18 %), wounds(No. 2 = 20%), ear infection (No. 3 = 25%). As shown in table (1).

Table (1): Clinical samples number, positive isolate for *Pseudomonas aeruginosa* and percentage of positive isolates.

Types of clinical samples	No.	Positive	Percentage
Burns	30	18	60 %
Flooring and Tools of burns unit	14	04	28.5 %
Handles laundries in in water cycle	11	10	90.0 %
Urine samples from people with urinary tract infection	11	02	18.18 %
Wounds	10	02	20.0 %
Ear infection	12	03	25 %
Total	86	39	45.3 %

Advances in molecular techniques have a significant impact on the study of microbiology. As a result of advanced technologies, further discoveries in research and demands have increased; the time and cost to perform these molecular methods are slowly decreasing. However, first of all, the identity of the organism must be confirmed to decrease costs of reagents and time involved in wrongly characterizing non *P. aeruginosa*
isolates (Coering *et al*.,2008). Through mentioned above, in this study targeted *gyr b* gene based on two primers used to reach rapid and accurate diagnosis of this bacteria and the results were excellent within first primers gyrb 190 and It can be used to detect *P. aeruginosa* from clinical samples without using a selective medium or additional biochemical tests. While second primer gyr b222 amplified *gyr b* gene with fragment 222bp only when using a selective medium, but in nutrient broth get false negative. As shown in figure(1).



Figure(1) : PCR amplification of primer gyrB190 and gyr b222 in 1.5% agarose gel. L= 100 bp DNA ladder. From 2 to 7 and from 11 to 16 pure DNA of *Pseudomonas aeruginosa* . 6,7, 17 and18 mix DNA of sample. Samples from 2 to 9 repeated them selfe from 11 to 18.

16S rRNA gene is the center of the bacterial classification. However, it is known that the *16S rRNA* gene is highly conserved which poses the question if this gene is suitable to differentiate very closely related species (Valverde *et al*.,2006: Dutta and Gachhui.,2007: Rivas *et al*.,2007). Therefore, other genes have been used to aim in a more detailed phylogeny such as *recA, atpD, carA, gyrB, rpoB and rpoD*, whose usefulness for species differentiation has been verified in the genus Pseudomonas. For instance, the genetic distances calculated from the concatenation of *gyrB* and *rpoD* could be less erroneous than the whole 16S rRNA sequences by presenting higher number of base substitutions outside the variable regions (Yamamoto et al.,2000).

In addition, the *gyr b* gene is rarely transmitted horizontally, its molecular evolution rate is higher than that of 16S rRNA, and the gene is distributed ubiquitously molecular evolution rate is higher than that of 16S rRNA (Kasai *et al*.,1998).

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