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Polymerase Chain Reaction Of Oprl And Oprigenes In P. Aeruginosa Isolated From Burn And Wound Infections

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Abstract

Background and objectives: P. aeruginosa owned diverse virulence factors, completely factors contributed to increase its pathogenicity as outer membrane proteins. The goal of the current study was to detect oprI and oprL genes like creditable factors for quick identification of the P.aeruginosa by multiplex PCR technique.

Materials and methods: P.aeruginosa isolates were isolated from 50 burn and wound patientsfrom Teaching Hospital of Mussan province/Iraq during the period from March to May, 2017. The samples were identified by several selective media to detected the morphological characteristic and by biochemical tests.

Result: The using of PCR technique showed all isolates (100%) had oprI gene while 83.33% isolates harbored oprL gene.

Introduction

Burn injury was consideredby way ofone of the noteworthycommunityhealth problems universal, and it was caused nosocomial infections(Hidalgo et al., 2016).The burn patients were rapid acquired different infections in comparing with another patients resulted fromhurt skin barrier and repressedthe immune system, also those patients were stayed in hospital for long time and aggressive therapy and diagnostic methods (Weinstein and Mayhall, 2003).

P.aeruginosawas one of the greatestpublic hospital bacteria, it was classified by means of one of ESKAPE microbes, means group of bacteria had high propensity thatcausedpublic problems, drug resistant, and one of the nosocomial bacteria (Rice, 2008). Also the P.aeruginosawas caused a wide of severe opportunistic infections, principallythe burns patients and immunocompromized patients (Dryden, 2010 and Pachori et al., 2019). The other worldwide problem, the high rate of multidrug-resistance (MDR) isolates hadeffected in the treatedof P.aeruginosa infections (Dogonchi et al., 2018).Also this bacteriawas one of significantand common bacteriawhich infectedburn, patients, since the skin was physical barrier to microbes had been compromised (Lessnau et al., 2012). Moreover, in the severe burns the underlyingtissue of a skin destroyed and also the T cells,didn't reach to infection sites, thus the infection danger increased with the burn size (Akingbade et al., 2012).

The of P. aeruginosa had special outer membrane porins (OprM) was introduced into a laterally replicatedsystem; and it was to intercept the uptake of the antibiotics and the minor resistance mechanisms like energy-dependent multidrug efflux and producing β-lactamase (Schmidt and Kandt, 2012). Also Kutzner et al., (2011) investigatedthe OprM, an OM component of themultidrug efflux pump from P.aeruginosa, as a model ofefflux channels.

Lipoproteins (L and I) were outer membrane proteins of P.aeruginosa, andthere were responsible for the ingrain resistance of the bacteriaagainst antibiotics and antiseptics. The proteins wereexisting only in this bacteria (Douraghi et al., 2014). The present study aimed to detection two virulence genes (opr I, opr L) in P.aeruginosa isolates which recovered from burn and wound infections by PCR technique.

Material and Methods

Bacterial isolates

This study was carried out to collect 50 samples from burn and wound patients during the period from March to May, 2017 from Teaching Hospital of Mussan province/Iraq. The samples were inoculated on selective media like: Blood-agar, Macconky-agar, Muller hinton agar (LAB/ United Kingdom) then incubated on 37◦C for 24h to performed the routine tests of this bacteria such as: pigment production on selective media and oxidase test,indole-test,glucose fermentation, gelatin hydrolysis and the growth at 42◦C (Masuda et al., 1995).**Molecular study**

Extraction of DNA:

Thechromosomal DNA was extracted from fresh cultures of all P.aeruginosaisolates grown aerobically on Brain Heart infusion broth (LAB/ United Kingdom) with using Genomic DNA Extraction kit (Geneaid/Korea).

Primer selection: The primer sequence of genes that used in present study showed in table(1).PCR mixture was carried out in 20µl that consist of 1µl of both forward & reverse primers, 5µl of DNA template, 5µl of master mix (Bioneer/Korea) and the volume was completed with nuclease free water. The thermocycling conditions (multiplex PCR) of both genes as the following: the initial denaturation was 94˚C for 5min followed by 30 cycles of: denaturation at 94˚C for 1min , annealing at 55˚C for 1min and finishing with final extension at 72˚C for 10min. The products of PCR product were visualized by 1.4 % agarose gel electrophoresis,andthe attendance of a 500bp and 250bp band asa positive result for oprL and opI genes,respectively.

Table.1 Oligonucleotide primers sequences for PCR amplified of OprL and OPrl genes.

Gene name	Primer Sequences (5´- 3´)	Length	References
OprL	F: ATGGAAATGCTGAAATTCGGC R: CTTCTTCAGCTCGACGCGACG	500	(De Vos et al., 1997)
OprI	F:ATG AAC AAC GTT CTG AAA TTC TCT GCT R:CTT GCG GCT GGC TTT TTC CAG	250	(De Vos et al., 1997)

Results

The results of existing study showed the proportion of P. aeruginosa isolates was (30/50; 60%) from completely samples which collected from burn and wound infectionpatients. The molecular study showed that 25 isolates (83.33%) of P. aeruginosa harbored oprIgene, while completely isolatesof goal bacteria (100%)had the oprL gene.

The Figs.(1) showed the gel electrophoresis of OprL and OprI genes with the molecular weight of approximately 500 and 250bp, respectively.

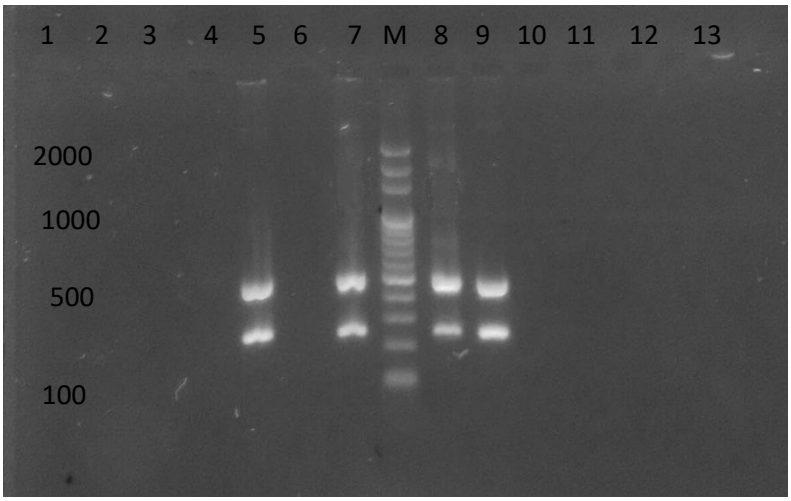

Fig. (1): Agarose gel electrophoresis of oprLand oprIgenes amplification used 1.4% agarose gel, M: DNA ladder, Lanes5,7,8,9: positive results of amplified oprL gene, while 5,7,8,9: positive results of amplified oprI gene; 1,2,3,4,6,10,11,12,13: negative results.

Discussion

The present results showed that 60% of isolates recognized as P.aeruginosaisolated from burn and wound infection samples,which identification according to phenotypic characterization and biochemical tests.Generallygreatest laboratories usedtraditional microbiological methods like culture and biochemical teststo identify of P.aeruginosa in clinical samples. The traditional biologicalprocedureswere timeconsuming and spend numerous days for identifying and confirmatory testing, this consider problem for controlling fatal infections (Kidd et al., 2009;Tahmasebi et al.,2021). The use of PCR technique could enable accurate which consider rapid identification of P.aeruginosa (Anujet al., 2012).The infections by bacteria in burn patients were publicand unmanageable to control. Also P.aeruginosa was one of the most Gram negative bacteria in hospital. The P.aeruginosawasprogressively isolated as a nosocomial bacteria, that causing in height morbidity and mortality rates in burn patients, mechanically ventilated patients.The morphological characteristic and biochemical tests consumes long time to do and need to broad hands-on work by the scientist, both for arrangement and for evaluation (Poh and Yeo, 1993;Shabgah et al.,2021).

The current results showed that theP. aeruginosa occurrenceagreementwith percentage of this bacteria that performed locally, such as(Alkateeb et al., 2016; Alhamdy, 2015)which showed that the highest proportion of bacteria in burn patients wasP.aeruginosa (45%) and (49%). The infection rate

in burn patientswasenormously high of developing countries (Lari et al., 2000). This resultedfrom the occurrence of low level socioeconomic patients whom poor hygienic conditions (Othman et al., 2014).

The molecular detection of oprL gene was 83.33% in P. aeruginosa which detected by PCR technique. While entirely P. aeruginosaisolates hadoprI gene. Numerous methods were developed to quickly and exactly identify ofP.aeruginosa as a medicalsignificant bacteria such as: PCR technique was the potential for identifyingbacterial species quickly by amplification of geneswhich exclusivefor a specific microorganism in clinical samples. Likewise, the molecular detection of P.aeruginosa, were describedthe phenotypic methods for identification of the current bacteria (De Vos et al., 1997;Mohammed and Qasim,2021).(Aljebory, 2018) recorded that 98% ofP. aeruginosa isolates harbored oprL gene; also Lavenir et al., (2007) showed completely of the isolates (100%) were unusually positive for oprI &oprL genes;also the P.aeruginosa produces numerous of virulence factors whose expression was arranged by different systems (Morales-Espinosa et al., 2012); Such as ToxA, exoA , oprL and oprI genes; and those factors may contributed to its pathogenicity (Rhonda et al., 2012). The present results correlated with other local studies such as: Qader et al., (2020) and Khattab et al., (2015) showed 100% of.aeruginosa isolates possessed the opr1 gene.

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