Nat. Volatiles & Essent. Oils, 2021; 8(4): 15416-15421

NVEO
Natural Volatiles &
Essential Oils

The Molecular Variation Of Wzxand Wzygenes In Multi Drugs Resistant Pseudomonasaeruginosa Isolated From Wounds

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Abstract

Pseudomonas aeruginosa infection is one of the health problems in hospital patients, the multi-drug resistance isolates were enrolled in the present study to detect genetic variation of some B-band LPS synthesis genes, the wzxandwzy genes were detection using monoplex-PCR, the results shows that the wzx was detected in 87.5% of isolates while didn't found in 12.2%, the wzy gene found in the 50% of isolates, the other variations were found both genes (wzx+wzy) in 50%, and 37.5% of isolates have wzx without wzy, and 12.5% have wzy without wzx, The present study concluded that the genetic variants of B-band (wzxandwzy) involvement in the wound infections and multidrug resistance of *P. aeruginosa.*

Key words: MolecularVariation,wzx and wzy Genes, Multi Drugs Resistance, Pseudomonas aeruginosa, Wound

Introduction

The increments of bacterial infections in Iraq in the last years and the high resistance to a wide range of antibiotics, in addition to environment pollutions, and lack of health awareness, all these factors led to increase infection rates of different bacterial isolates (Al-Kadmy et al., 2019).The Pathogenic bacteria characterized by an elaborate assortmentproductions of cell-associated and extracellular bacterial substances forutilizing in the establishment of infection and colonization with a host (Peterson et al., 1996). One of these productions is Lipopolysaccharide (LPS) molecules, its protective factor againstthelysis activity of hosts, the most LPS heterogeneous part is O antigen, moreover, this region is conferred serum resistance to the organism (Farhana and Khan, 2021). The genetic variation of antigen –O based on the genetic polymorphisms of the rfp genes cluster encoded to enzymes utilized in the O-antigen synthesis and construction (Tarr et al.,2000), these genes encoded to enzymes involved in the sugar synthesis, construction sugar and O subunittransferase enzyme, and proteins utilized in the construction O antigen by subunits assembly, these genes arewzx and wzywhichencoded to transporter of O-antigen or flippase and O-antigen polymerase respectively that proved by some studies in different bacterial species (Samuel and Reeves, 2003;Feng et al., 2004;Vinés et al., 2005; Patel et al., 2012).

In somestudies two theories were suggested to explain the lowering C+G percent of heteropolymeric O-antigen encoded gene clusters, The first theory proposed the lateral transferred of genetic materials from lower C+Gpercentorganism (Reeves, 1993, Schnaitman and Klena, 1993). The second theory, dependent on thewzygenesanalysis, utilized the mechanisms of potential translational regulate, regarding to the atypical molar ratio is impacted in the codons utilized atypical for the background, The intriguing schism between the C+Gpercent of interdependent homopolymeric and heteropolymeric O-antigen clusters in the same backgrounds lends credence to the second theory (Rocchetta et al., 1999; Geue et al., 2017).

According to the O antigen role in the Pseudomonasaeruginosa infections and serotyping, the present study suggested to molecular assessment of wzx and wzy in several isolates collected from wound infection in hospitalized patients.

Methodology

Pseudomonas aeruginosaisolates that isolated from wound infection of hospitalized patients were enrolled in the present study, these isolates were diagnosed by macroscopic and microscopic tests, and antibiotics sensitivity was applied, then DNA was isolated from each isolate and electrophoresis were performed for DNA profile of bacteria(Mona et al.,2019). The wzxandwzy detected by monoplex-PCR using the primers wzx F CCG, GGT, TTC, GAT, TTG, TGA, AGG, TTG. R CAC,AAC, AGC, CAC,TAC,TAG, GCA GAA, wzy F GAA, ATT, ATG, CCA, TCT, TGG, CGA, GCG. R CAT, GTG, AAG, CCT, GAA, GGC,AAA, CTC. Using thermo cycler (biometra, German) 94˚C for 5 min, 31 cycles including (94˚C 30 s, 58˚C 30 s, 72˚C 30 s) finally 72˚C for 10 m,The amplification sizes wzx255 bp and wzy450bp which visualized using 1%agaros, 0.5 TBE, 100 V for 30 m(Raad et al., 2021 ;Al-Terehi et al., 2018)..

Results and Discussion

The present output shows that all isolates didn't contain plasmids, the Genetic Variation of wzxandwzyGenesshowsthat the wzx was detected in 87.5% of isolates while didn't found in 12.2%, the wzy gene found in the 50% of isolates, the other variations were found both genes (wzx+wzy) in 50%, and 37.5% of isolates have WZX without wzy, and 12.5% have wzy without wzx (figure 1). The amplification products for both genes are shown in figure (2).

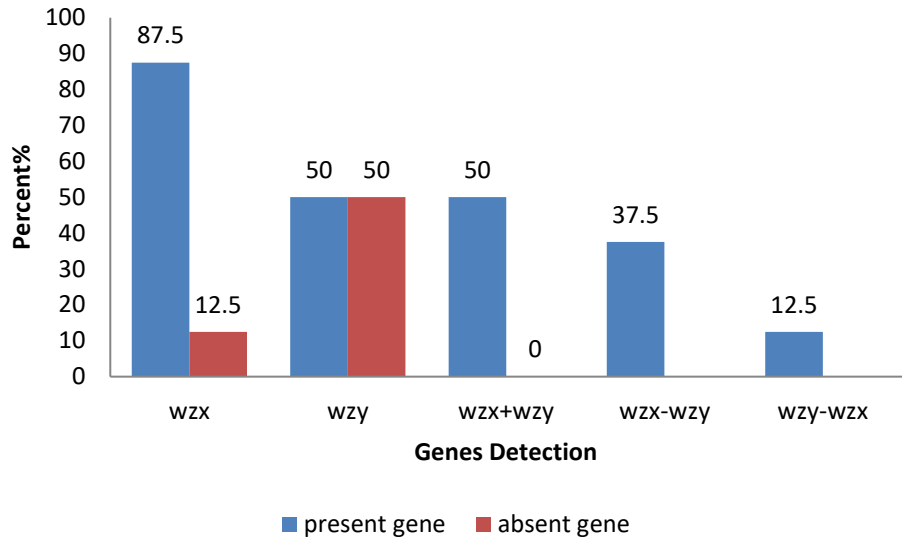

Figure (1) the detection percent's ofwzxandwzyGenes in pseudomonas aeruginosaisolates

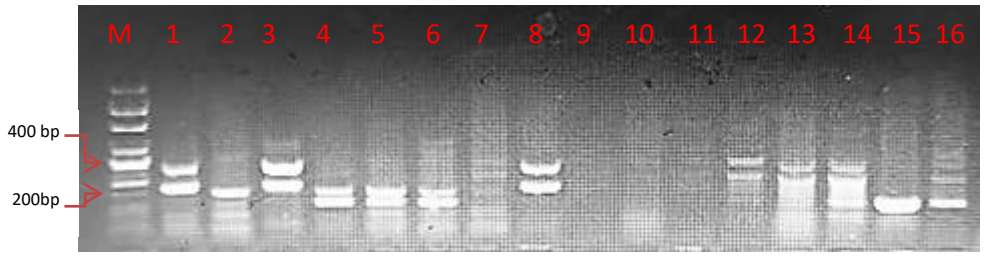

Figure (2) the amplification products of wzxandwzyGenes in pseudomonas aeruginosaisolates, lan m DNA marker (11-1000bp), lanes 1-8 wzx amplification products (255 bp), lanes 9-16 wzyamplification products (450 bp).

The bacterial infection of the wound may lead to severe complications especially with the multidrug resistance isolates ofpseudomonas aeruginosa, thus the detection the virulence factor structure should be studied for drug design and therapy (Al-Terehi et al., 2021).

The O antigen has a major role in the serological distinction, which is one of the most components of LPS that present in different G^{-ve} bacteria, moreover LPS is thermostable and variant among species (Maldonado et al., 2016). The variant of LPS based on the some genetic polymorphisms that encoded to the enzymes and proteins contributed in the LPS synthesis, in the present studywzxandwzyGenesshow different percent of present these genes in pseudomonas aeruginosaisolated from wounds, these differences may be responsible for the virulence of isolates which might be impacted on the LSP structure that have a role in the stimulate the robust pro-inflammatory of the immune system in mammals (Lam et al., 1992)

In pseudomonas aeruginosa the Genes involved in LPS biosynthesis classified into three groups A-band LPS, B-band LPS synthesis, Core oligosaccharide synthesis and housekeeping function impacting in thesynthesisofLPSwzxandwzy under B-band LPS synthesis (Rocchetta et al., 1999), another study found 61.53% ofP. aeruginosa isolates production rough LPS and

serotype O11 LPS when transformed with O-antigen gene (rfb). Therefore the wbp region has acquired mutations, these mutations responsible on the changes in LPS phenotype(Evans et al. 1994).

Other results show two bands of wzxandwzyamplification products, these amplifications may be non-specific amplifications, or two copies of the gene, although of the positive and negative control were applied. Also,it may beresulted from the genetic variations of genes among isolates that produced ranging in amplification size products (Naji Hasan, R. &Abdal Kareem Jasim, S. (2021);Goldberg et al., 1992). On the other hand, the present isolates were multi-resistance drugs, and this resistance belongs tothe genetic variants in the B-band encoded genes, the antibiotic treatmentEffective of P. aeruginosa infections still health problems, this belongs to the high intrinsic resistance to antimicrobial substances which resulted from the outer membrane low permeability (Hancock et al., 1998). The present study concluded that the genetic variants of B-band (wzxandwzy) involvement in the wound infections and multidrug resistance of P. aeruginosa.

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