Sys Rev Pharm 2020;11(11):285-290
A multifaceted review journal in the field of pharmacy

Screening the frequency of panton-valentine leukocidin (pvl) gene between methicillin resistant Staphylococcus aureus isolated from diabetic foot patients in Al-Basrah governorate, south of Iraq

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ABSTRACT

Background:
Staphylococcus aureus with methicillin resistant is an important pathogen associated with diabetic foot ulcers (DFUs).The (pvl) gene is considered a marker for MRSA that used widely as acquired in the population, it's responsible for deep dermal infections and soft tissue. The current research aimed to establish the panton-valentine leukocidin (pvl) frequency of gene between MRSA isolated from infected DFUs individuals in Al-Basrah governorate, south of Iraq.
Methods:
One hundred fifty swab sample from diabetic foot ulcers (DFUs) patients who admitted to two local hospitals in Basrah governorate.The S. aureus isolates were identified by criteria of growth on both blood agar and mannitol salt agar, and then it tested for coagulase test. The isolates confirmed as S.aureus by using the vitek®2 system for identifying the Staphylococcus aureus. MRSA had been confirmed by testing the isolates against oxacillin and cefoxitin disc.The 16S rRNA was used to confirm the identification of S.aureus isolates.the PCR was used to detect the production of pvl genes.
Results:
Out of 150 swab samples only 21 isolates which include 18(85.7%) isolates was identified as S. aureus, the other 3(14.3%) isolate was identified as Staphylococcus spp. The result of vitek ® 2 showed all 18(100%)isolates were identified as S. aureus. On the other hand all (18) S. aureus isolates given the inhibitory resistant criteria for oxacillin disc 1µg and cefoxitin disc 30 µg, these isolates classified as MRSA. Furthermore, the result of amplifying 16S rRNA was given the positive result to identify S.aureus . MRSA was found in all isolates, the 18 (100 %) were pvl positive.
Conclusion:
The frequency of pvl gene found in all MRSA isolates in this study. It can be used as community acquired-MRSA marker. The PCR analysis effective in detecting pvl & mecA genes

Keywords: MRSA, diabetic foot ulcers (DFUs), pvl, Staphylococcus aureus.

INTRODUCTION

Diabetes mellitus is a common, chronic disease. Often it causes wounds to recover later, such as diabetic foot ulcers (DFUs) . It increasing globally incidence mainly in the elderly (Leung, 2007) .The diabetes wound in 25% of cases develop to ulcers infection (Abbot *et al.*, 2005;Lavery *et al.*, 2014). Furthermore the DFUs infections significantly medical , economic issue and social in diabetes patients. If infectious agents are not treated properly, these are related to the amputation of the infected foot (Hartemann-Heurtier *et al.*, 2004).On otherhande the DFUs causes by diverse mixed from Gram- negative and positive bacterial , but most frequently species is *S. aureus* (Wang *et al.*, 2010; Mendes *et al.*, 2011). Almost the 50% from *S. aureus* are Meticillin-resistant. Several studies found its emergence 15–30% in diabetic wounds (Gadepalli *et al.*, 2006;Wang *et al.*, 2010;Sandhu *et al.*, 2014). Already virulence *S.aureus* present in DFUs is a major factor that contributes to the based on the severity of a wound, because these factors allow the pathogen to enter and kill the host tissue(Sandhu *et al.*, 2014). The current research aimed to establish the panton-valentine leukocidin (*pvl*) frequency of gene between MRSA isolated from infected DFUs individuals in Al-Basrah governorate, south of Iraq.

MATERIAL AND METHODS

Sample collection :
From March-2018 to February-2019, 150 swab samples were collected from diabetic foot ulcers (DFUs) patients who admitted to two local hospitals in Al-Basrah governorate, south of Iraq.

Isolation and Identification of bacteria:
Swab sample was cultivated firstly for 24h. at 37ºC in brain heart infusion broth . The broth media that given the positive growth were streaked on both mannitol salt agar and blood agar plates and incubated for 24h. at 37ºC. The isolates that given the characteristic of *Staphylococcus aureus* was selected and checked according to (Harley and Prescott, 2002) for coagulase test.The vitek® 2 system version- 07.01 device was used The test card of Gram positive bacteria (VITEK® 2 GP ID-P Reference number 21342 , bioMérieux , USA) for identification the *S. aureus* isolates.

Screening of *Staphylococcus aureus* methicillin resistant (MRSA)
The oxacillin and cefoxitin diffusion disc methods was used to detected the MRSA. Both tests were prefromed according to (Datta *et al*., 2011;Sharma *et al*., 2017).The