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Detection Of Panton-Valentine Leukocidin Toxin Produced By Methicillin-Resistant Staphylococcus Aureus In Tonsillitis Patients At Basra Province/Iraq

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Abstract

Panton-Valentine leucocidin (PVL) is a toxin composed of two components, Luks-PV and LukF-PV. the two components are formed just before the assembling into a pore-forming heptamer on the immune cell neutrophil membranes, causing the neutrophil to be lysis.In the present study, the aim is s to molecularlyidentify MRSA and for producing PVL.A total of (4) MRSA isolate were obtained from tonsillitis patient from Assist.prof.SaadShakir Mahdi at University of Basra college of science, The percentage of PVL – positive was in only two isolates.

Keywords: MRSA,PVL,tonsillitis,Staphylococcus aureus

Introduction

Staphylococcus aureus is a pathogenthat is carried on the human body normally. one of the most important speciesis Methicillin-resistant S. aureus (MRSA) which has gen that made the isolatecapable of resistance to methicillin and other beta-lactam antibiotics[1]. These bacteria consider a major health care problem,Staphylococcus aureus is one of the most frequent pathogens in the etiology of tonsillitis and its relevance is due to its antimicrobial resistance and persistence in the internal tissues of the tonsils[2]S. aureus have been detected in both external and internal tissues of the tonsils [3]MRSA was primarily recorded as nosocomial pathogen in human hospitals,[4]MRSA have important virulence factorsthat arepanton-valentine leukocidin (PVL)which PVL was described by panton and valentine in 1932 [5].PVL is composied of two major subunits Luk F – PV and luk S –PV that can form pores at the polymorphonuclear neutrophils (PMN) membrane[6] The diagnosis protocol for MRSAPVL-positive is using molecular detection by using polymerase chain reaction (PCR) and nucleic acid hybridization kit to detectPVLgene[7]

Materials and Methods

Bacterial isolates

A total of 4S.aureus isolates were obtainedfrom tonsillitis patients by Assist.prof.SaadShakir Mahdi at the University of Basra college of science the isolates werecharacterized morphologically to insure the presence of S.aureus by inoculated on a CHROM agar plate,the result was read after 24-48 hrs of incubation at 37c the growth of colonies showing mauve color (pink to blue) coloration was considered to be positive staphylococcus aureus [8]

Genetic profiling

DNA extraction:

The DNA was extracted by using the DNA Bacteria kit (Geneaid,Korea) according to the manufactures specifications.

16S rRNA gene PCR

The extracted DNA was amplified with specific primers with product size 756 bp according to Makgotlho et al.,2009[9]to confirm the presence of MRSA. The GoTaqPromega master mix (Promega, USA) was used according to the manufactures specifications.(Staph 756F` 5 -AACTCTGTTATTAGGGAAGAACA-3` /Staph 750R 5 -CCACCTTCCTCCGGTTTGTCACC-3`) and PCR program wasinitial denaturation at 94c for 10 min and 35 cycles of denaturation at 94 c for 45sec., annealing at 55c for 45 sec. and extension at 72 c for75 seconds and final extension was performed at 72c for10 min. Amplified products electrophoresis in 1.5% agarose gel and visualized by using UV gel documentation system.

PCR assay for detection of PVL

To confirm thepresence of PVL in the isolates we used a specific primer according to (Makgotlho et al.,2009)

(pvl-Forward` 5-ATCATTAGGTAAAATGTCTGCACATGATCCA -3` and pvl-Reverse` 5-

GCATCAASTGTATTGGATAGCCAAAAGC -3`). and PCR program was:: initial denaturation at 95c for 5 min and 35 cycles of denaturation at 94 c for 1min., annealing at 51c for I min. and extension at 72 c for1 min and final extension was performed at 72c for10 min. Amplified products electrophoresis in 1% agarose gel and visualized by using UV gel documentation system.

Result

Gel migration results of the total DNA extract of Staphylococcus aureusMigration results of bacterial DNA shown in Figure (1) showed the appearance of the DNA strand bands extracted from the samples under study when migrated with agarose gel of 0.8% concentration using the ultraviolet imaging adevice.

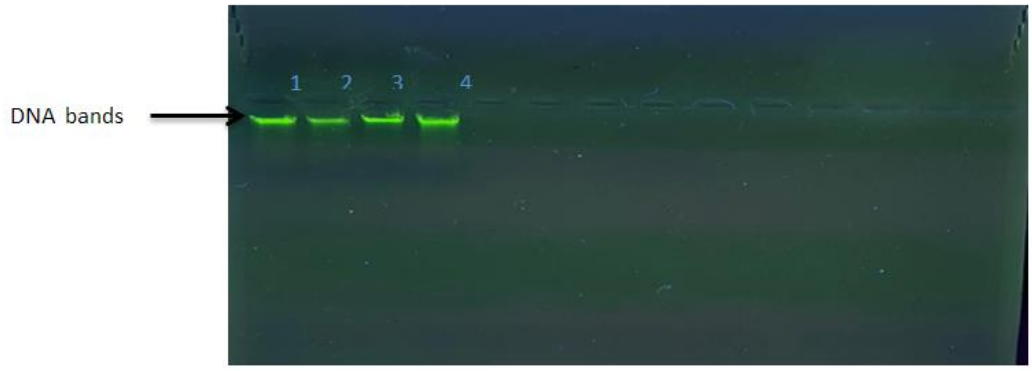

Figure 1: S.aureus DNA bands on agarose gel

Results of amplification of bacterial DNA extracted by polymerase chain reaction technique for the 16sRNA gene of Staphylococcus aureus.The results of amplification of the Universal 16s RNA gene for Staphylococcus aureus showed the appearance of bands at a molecular weight of 756 kDa when migrated with 1% agarose gel using a UV imager as shown in figure (2)

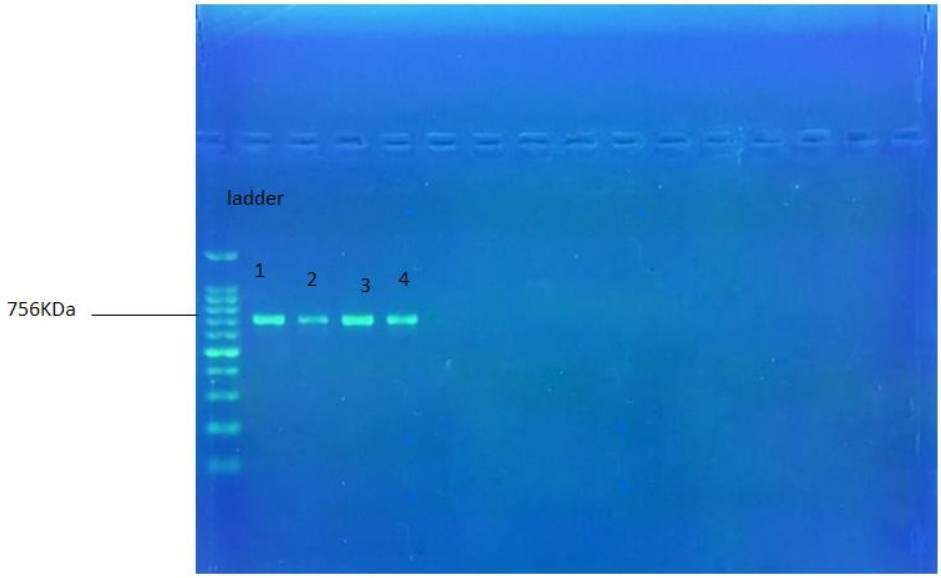

figure (2) Universal 16s RNA gene packages in 1% agarose gel using 1k ladder

All isolates of MRSA(4 isolates) were tested for the presence of pvl genes using Polymerase Chain Reaction assay. The results showed that only two of them are PVL-positive for tonsillitis patients as shown in figure(3)

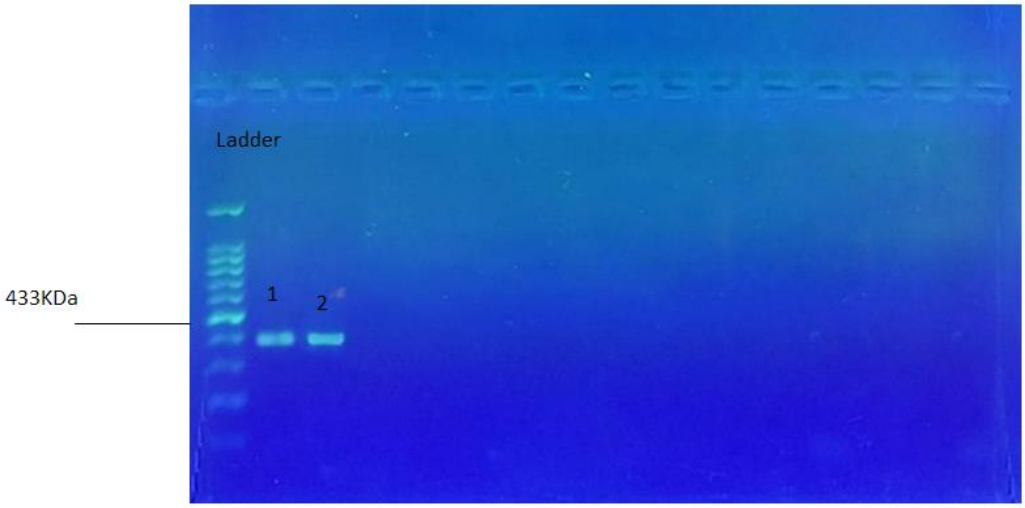

figure 3: The PVL gene was packaged at 433 kDa with 1% agarose gel using a 1k Lader

DISCUSSION

All MRSA staphylococcus aureus isolates found in tonsillitis were tested for PVL production and together the result was 100% potent for two isolates only.It was found that the bacterial toxin has multiple pathological effects in the events of various diseases, as it was found that 4.6% of the samples taken from the skin and soft tissues contained PVL-resistant Staphylococcus aureus bacteria[10] It also has a role in the events of necrotizing hemorrhagic pneumonia[11] and when it spreads in the lung tissue, it works to destroy it, and 75% of deaths were recorded due to PVL bacterial toxin [12]. PVL causes necrotizing pneumonia and sepsis when it spreads in the respiratory system [13], and the regions of western and central Africa are witnessing a sharp increase in the

spread of isolates of PVL-producing methicillin-resistant Staphylococcus aureus [5]In a study, PVL-producing staphylococcus aureus was found in samples taken from tonsils with medium resistance to antibiotics, and two isolates of methicillin-resistant Staphylococcus aureus (3.3%) were found in the same samples. Methicillin-resistant staphylococcus (1.3%) out of 76 samples in patients with recurrent [2] Strains containing pvl genes increase disease severity, and potential adaptation in the hospital environment would lead to outbreaks of serious nosocomial infections. This perspective is of great importance in a country already suffering from high rates of infection due to multidrug-resistant organisms[14]The presence of PVL-producing staphylococcus aureus varies according to geographic regions and population distribution, as its distribution was found to vary by regions of the world 5% in France and in the United Kingdom 4.9% and 8.1% in Saudi Arabia and Bangladesh it is 14.3%[15][16].Lina et al. found that 50%-93% of S. aureus responsible for cutaneous abscess, cellulitis, or furunculosis and 85% of those responsible for community-acquired pneumonia harbored the genes encoding for PVL compared with none of those causing diseases such as nosocomial pneumonia, infective endocarditis, urinary tract infection, enterocolitis, or toxic-shock syndrome [15]. Gillet et al. found that 16 patients with community-acquired pneumonia attributable to PVL-positive S. aureus were younger (median age 14.8 years), had fewer underlying disorders, and had more often had influenza-like syndromes or furuncles than 36 patients with community-acquired pneumonia due to S. aureus without PVL genes. The patients in the first group also had a more severe disease course and 75% of them died; in the other group, 47% died [12]. Dufour et al. reported on 14 cases of community-acquired infections due to PVL-positive MRSA, suggesting the community emergence of a new

superadapted S. aureus strain [1].[17] in Iraq,reported frequency of18 (100 %) were pvlpositive,alsostudy of [18]was shownpresent ofpvl positive in 14(21.21%) isolatesStaphylococcus aureusout of 150 clinical swabs sample.

Conclusion

The presence of PVL-producing staphylococcus aureus in tonsils maybe play a very important marker for chronic tonsillitis and lose the ability to treat the inflammation only by tonsillectomy due to the antibiotic resistance

References

1. Dufour P, Gillet Y, Bes M, et al (2002) . Community-acquired methicillin-resistant Staphylococcus aureus infections in France: the emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis.;35:819– 824.
2. Cavalcanti, V. P., Camargo, L. A. de, Moura, F. S., Fernandes, E. J. de M., Lamaro-Cardoso, J., Braga, C. A. da S. B., & André, M. C. P. (2019). Staphylococcus aureus in tonsils of patients with recurrent tonsillitis: prevalence, susceptibility profile, and genotypic characterization. The Brazilian Journal of Infectious Diseases
3. Zautner A.E., M. Krause, G. Stropahl, et al(2010).Intracellular persisting Staphylococcus aureus is the major pathogen in recurrent tonsillitisPLoS ONE, 5 , p. e9452.
4. Boyle-Vavra, S. and Daum, RS. (2007). Community-acquired methicillin-resistant Staphylococcus aureus: the role of Panton-Valentine leukocidin. Lab Invest, 87 (1): 3–9.
5. Kaneko J. and Kamio Y. (2004). Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes". BiosciBiotechnolBiochem 68 (5): 981–1003. Narita , S.,Kaneko , J , Chiba , J ; Piemont , Y and Kamio ,Y . (2001). Phage conversion of panton-valentine leukocidin in S,aureus . Molecular analysis. Gene, 268: 195 – 206.

6. Tacconelli, E.; De Angelis, G.; Cataldo, MA.; Pozzi, E. and Cauda, R. (2008). Does antibiotic exposure increase the risk of methicillin-resistant Staphylococcus aureus (MRSA) isolation? A systematic review and meta-analysis. J. Antimicrob.Chemother., 61 (1): 26–38.
7. Tang,Y.W.; Kilic,A. ;Yang,Q. ; Haijing , Li ; Miller,R.S. ; et al . (2007) .Staphplex system for rapid and simultaneous identification of antibiotic resistance determinants and panton – valentine leukocidin detection. J .Clin. Microbiol, 45 (6): 18667 – 1873
8. Diederen, B. ; van Duijn, A. ;van Belkum, P.;Willemse, P. ;van Keulen,T. and Kluytmans,J. (2005). Performance of CHROMagar MRSA medium for detection of methicillin-resistant Staphylococcus aureus. J. Clin. Microbiol. 43:2581-2583.
9. Makgotlho, P. E. (2009). Molecular Characterization of Methicillin-Resistant Staphylococcusaureus strains, MSC. University of Pretoria, South Africa. Marais, E.;Aithma, N.;Perovic, O.;Oosthuysen, WF.;Musenge, E.;Dusé, AG. (2009). Antimicrobial susceptibility of Methicillin-Resistant Staphylococcusaureus isolates from South Africa. J S Afr Med, 99(3):170-3.
10. Holmes, A.; Ganner, M.; M.c and Guane, S. (2005).Staphylococcus aureus isolates carrying Panton-Valentine leukocidin genes in England and Wales: frequency,characterization, and association with clinical disease. J. Clin. Microbiol 43 (5): 2384-90.
11. McGrath B, Rutledge,F. and E. Broadfield, (2008) “Necrotising pneumonia, Staphylococcus aureus, and Panton-Valentine leukocidin,” The Journal of the Intensive Care Society, vol. 9, pp. 170– 172.
12. Gillet Y, Issartel B, Vanhems P, et al. (2002)Association between Staphylococcus aureus strains carrying the gene for Panton-Valentine leukocidin and highly lethal necrotizing pneumonia in young immunocompetent patients. Lancet;59:753–9.
13. Maltezou HC, Giamarellou H. (2006)Community-acquired methicillin-resistant Staphylococcus aureus infections. Int J Antimicrob Agents.;27:87–96.
14. Aires**,** D. M.; Bartzavali, C.; Spiliopoulou, I.; Sanches ,I.S.; Crisostomo, M.I.and de Lencastre, H.(2003). Two international methicillin-resistant Staphylococcus aureus clones endemic in a university hospital in Patras, Greece. J.Clin.Microbiol.;41(5):2027-32.
15. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. (1999)Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis.;29:1128–32.
16. Afroz S, Kobayashi N, Nagashima S, Alam MM, Hossain ABMB, Rahman MA, et al. (2008)Genetic characterization of Staphylococcus aureus isolates carrying Panton-Valentine Leukocidin genes in Bangladesh. Jpn J Infect Dis.;61:393–6.
17. Tahrir H. Gadban, Saad S. Al-Amara,Hanadi A. Jasim(2020)Screening the frequency of panton-valentine leukocidin(pvl) gene between methicillin resistant Staphylococcus aureus isolated from diabetic foot patients in Al-Basrahgovernorate, south of Iraq . Systematic Reviews in Pharmacy Vol 11, Issue 11.
18. Saad S. Al-Amara(2021). Comparison between phenotype and molecular resistance characteristic in Staphylococcus epidermidis isolates from wound infections in Al-Basrah province, Iraq-.Periodicals of Engineering and Natural Sciences Vol. 9, No. 2,pp.897-903897