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

Tahrir H. Gadban[1], Saad S. Al-Amara[2], Hanadi A. Jasim[3]

1. Senior technician in pathological analysis, almawany general hospital, Al-Basrah, Iraq.
2. Department of biology, college of science, Al-Basrah university, Iraq.
3. Department of microbiology, college of Medicine, Al-Basrah university, Iraq.

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 37°C 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 37°C. 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

inhibition zone diameter was measured and interpreted according to criteria of CLSI, (2007).

Genetic profiling
DNA extraction:
The DNA Presto Mini gDNA Bacteria kit (Geneaid,USA) was used to extract genomic DNA according to the manufactures specifications.

16S rRNA gene PCR
The extracted DNA was amplified with specific primers with product size 756 bp according to (McClure *et al.*, 2006). The GoTaq promega master mix (promega, USA) was used according to the manufactures specifications. It was performed with: a 1 cycle denaturation step (5 min. at 95°C) and ended with final extension step (5 min. at 72°C), 35(30s at 95°C; 30s at 55°C;1 min. at 72°C). Amplified products electrophoresis in 1.5% agarose gel and visualized with ethidium bromide by using UV gel documentation system.

Molecular detection of *mecA* and *pvl* genes
Specific primers used for amplification of *mecA* and *pvl* gene according to (Pournajaf *et al.*, 2014) , and (Lina *et al.*, 1999) respectively .

The reaction of PCR for *S. aureus* (*mecA* and *pvl*)
The GoTaq Promega master mix (Promega, USA) was used in the PCR reaction. It was performed for *mecA* genes: a 1 cycle denaturation step (3 min at 95°C) and ended with a final extension step (6 min.. at 72°C), 30(1min. at 94°C; 30s at 53°C;1 min. at 72°C), while *pvl* gene : a 1 cycle denaturation step (1 min at 95°C) and ended with a final extension step (1 min. at 72°C), 35(30s at 94°C; 30s at 55°C;1 min. at 72°C) with product size 533 bp and 433 repectivly. Amplified products electrophoresis in 1.5% agarose gel and visualized with ethidium bromide by using UV gel documentation system.

RESULT
Out of 150 swab samples from March-2018 to February-2019, only 21 isolates which include 18(85.7%) isolates was identified as *S. aureus* , while the other 3(14.3%) isolate was identified as *Staphylococcus spp.* . The criteria that depended to identify *S. aureus* included ferment mannitol with changing the colour of the indicator from red to yellow by producing acid when grown on mannitol agar. Also, colonies surrounded by areas of clear β-hemolysis when grown on blood agar and given positive results in a coagulase production test. The vitek ®2 was used to support and confirm the results of the identification tests that used for identification of *S. aureus* isolates from diabetic foot patients. The result of vitek ® 2 showed that all (18) isolates were identified as *S. aureus* .

Staphylococcus aureus methicillin resistant (MRSA)
The results in the (Figure 1) showed that all (n=18) *S. aureus* isolates given the inhibitory resistant criteria for oxacillin disc (1μg) and all these isolates classified as MRSA . The results show that isolates with the numbers (3,10,16,18), (2,6,7,11), (4,9,14), (12,15), (5, 13), (1, 8) and (17) gave the inhibition zone less than (9mm, 8mm, 10mm, 7mm, 6mm, 5mm and 4mm) in percentage 22.2%, 22.2%, 16.7%, 11.1%, 11.1%, 11.1% and 5.6% respectively.*.*
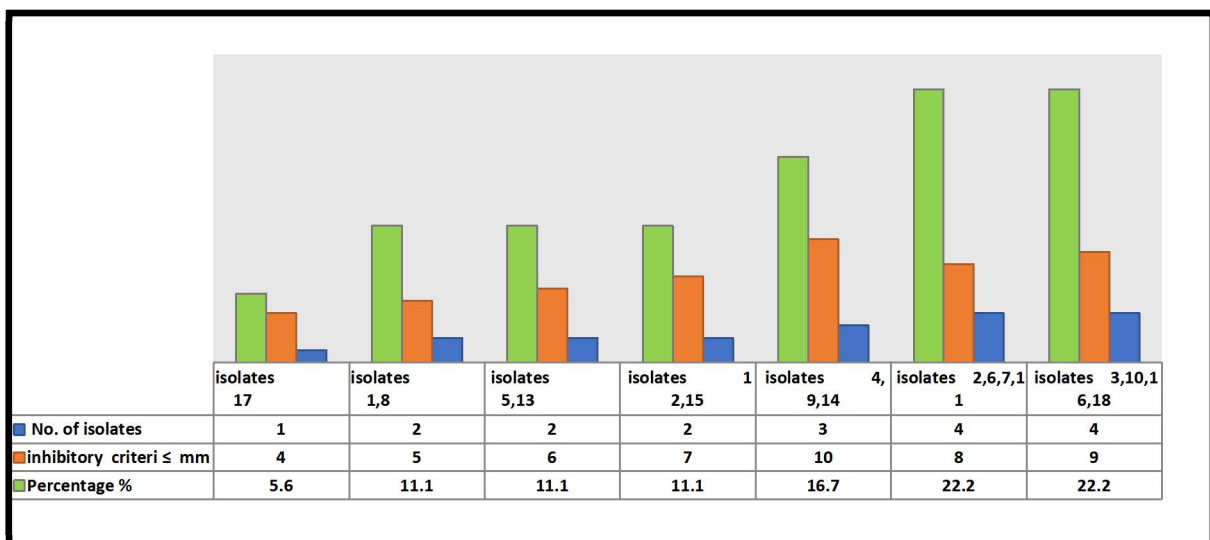

	isolates 17	isolates 1,8	isolates 5,13	isolates 2,15	isolates 4, 9,14	isolates 2,6,7,11	isolates 3,10,16,18
No. of isolates	1	2	2	2	3	4	4
inhibitory criteri ≤ mm	4	5	6	7	10	8	9
Percentage %	5.6	11.1	11.1	11.1	16.7	22.2	22.2

Figure (1) The percentages of *S. aureus* isolates that resist for Oxacillin (1 μg) antibiotic disc

Also, all of *S. aureus* isolates (n=18) were tested against the cefoxitin (30 μg) antibiotic disc to detect the inhibitory resistant criteria and indicated resistance to cefoxitin and the isolates classified as a MRSA . In figure-2 the results showed the isolates with the numbers (7,9,12,13,14,17), (1,4,6,10,7,16), (8), (3,5,11) and (2,15) gave the inhibition zone less than (21m, 20mm, 18mm, 16mm, and 14mm) with percentage 33.3%, 33.3%, 5.6%, 16.7, and 11.1% respectively.

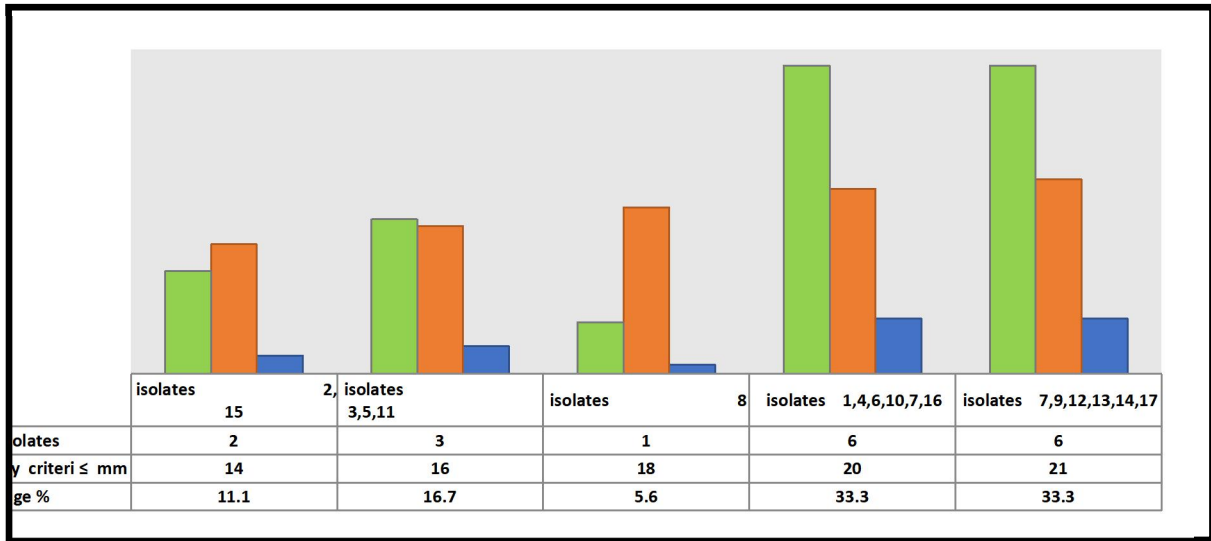|	isolates 2, 15	isolates 3,5,11	isolates 8	isolates 1,4,6,10,7,16	isolates 7,9,12,13,14,17
olates	2	3	1	6	6
y criteri ≤ mm	14	16	18	20	21
ge %	11.1	16.7	5.6	33.3	33.3

Figure (2) The percentage of the *Staph aureus* isolates that resist for Cefoxitin (30 µg) antibiotic disc

Genetic profiling
16S ribosomal RNA.
The result of amplifying 16S rRNA was showed the all 18 isolates gave the positive result and single band viewed in (750bp) when compared to a typical molecular DNA ladder (2000 bp) , (Figures 3).

Molecular detection of *mecA* and *pvl* genes
Amplifying *mecA* gene was showed that all 18 isolates gave the positive result as single band in 533 bp (Figure 4).While the amplifying *pvl* gene also showed with all 18 isolates and gave the positive result as a single band in 433 bp (Figure 5). All amplified genes were compared with standard molecular DNA ladder (2000 bp).
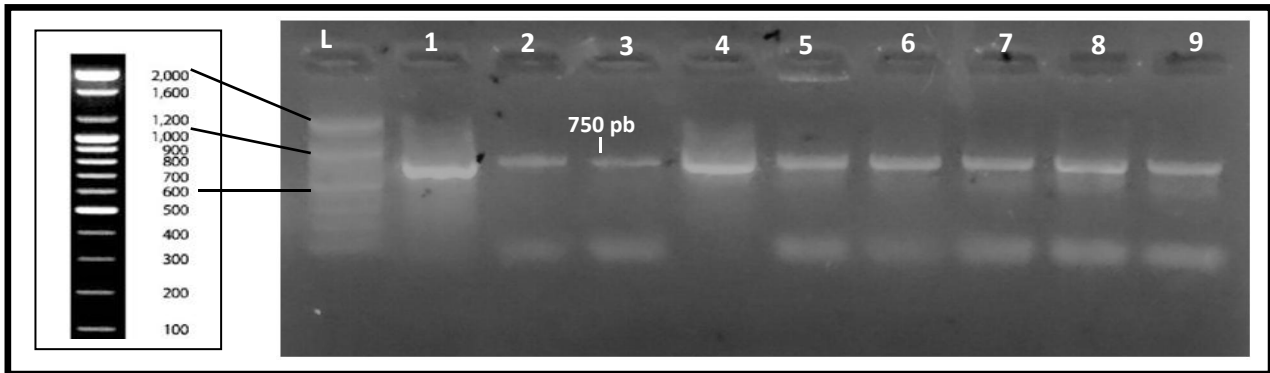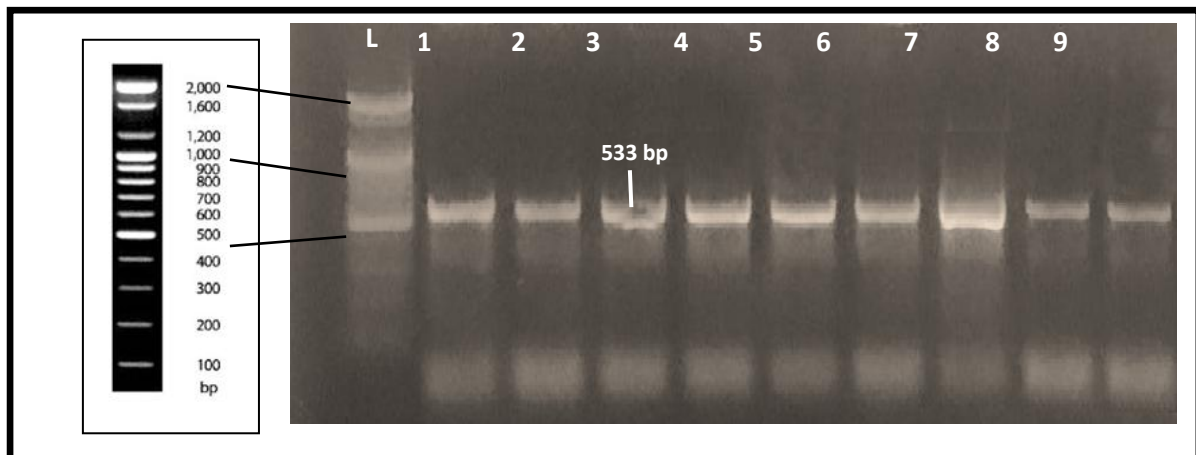

Figure (3) Gel electrophoresis patterns show PCR amplified products of 16S rRNA. Lane L:(2000 bp DNA ladder) , Lane:(No. 1-9) 16S rRNA band of bacterial isolates.

Figure (4) Gel electrophoresis patterns indicate products amplified with PCR of *mecA* gene . Lane L: (2000 bp DNA ladder) , Lane: (No. 1-9) *mecA* gene bands of *S.aureus* isolates .
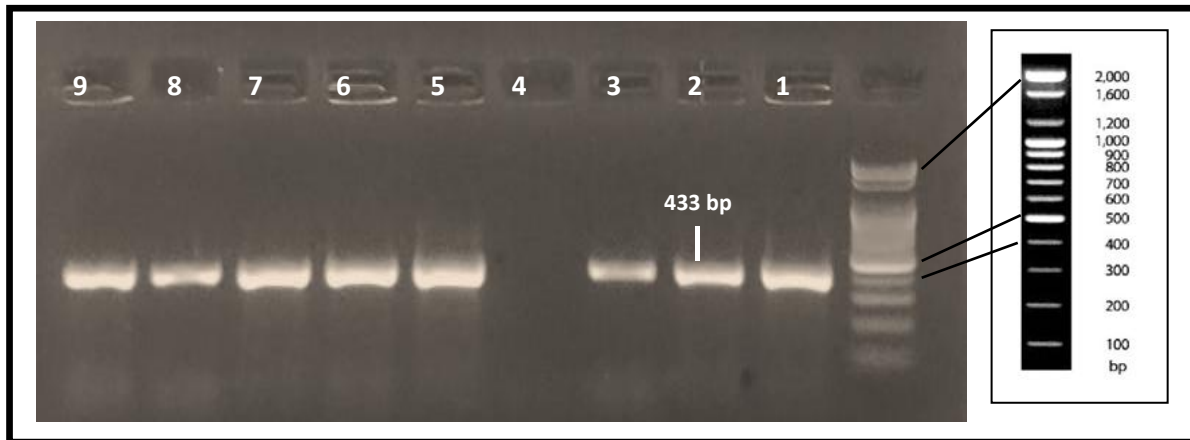

Figure (5) Gel electrophoresis patterns indicate products amplified with PCR of *pvl* gene . Lane L: (2000 bp DNA ladder), Lane: (No. 1-9) *pvl* gene band of *S.aureus* isolates.

DISCUSSION

MRSA has emerged as a critical and widespread challenge for diabetic foot ulcer patients (Tentolouris *et al.*, 1999;Game and Jeffcoate, 2004)**.** In both neighborhood and hospital procedures, *S.aureus* often was the isolated pathogen. *S. aureus* change antimicrobial pattern sensitivity, particularly in developing countries,it increasingly making antimicrobial agents fewer effective(Bukhari *et al.*, 2004). This study, found the prevalence of *S. aureus* in diabetic foot ulcers was 18(12%) isolated from 150 diabetic foot ulcers swab samples . This finding is in line with the study conducted in (8.4%) in Bandar Abbas District, Southern Iran (Ahmadishooli *et al.*, 2020) and (11%) in Malaysia (Goh *et al.*, 2020). However, the prevalence reported in the current study is lower than a study reported (20%) in Brasil (Pontes *et al.*, 2020), (28.8%) in Manchester, UK (Tentolouris *et al.*, 1999),(34%) in Babyloncity, Iraq(Al-Allak *et al.*, 2019), (43.8%) in England (Nelson *et al.*, 2018), (51%) in Portugal (Mendes *et al.*, 2011) and (54.7%) in Sulaimani city, Iraq(Qadir *et al.*, 2020). The difference in prevalence may be due to difference in the experimental data obtained, a time taken to research and the procedure used to detect *S. aureus* .

Also in this study, the prevalence of MRSA was 100% in both antibiotic and molecular detection methods that use to detect MRSA. the prevalence reported in the current study is higher than a study reported in (63.4%) in china (Wang *et al.*, 2010), (29.8%) in USA and(24.5%) in Portugal (Mendes *et al.*, 2011) (Lavery *et al.*, 2014). Excessive and uncontrolled used of antibiotics in Iraq is one of the biggest challeng that lead to the emergence of significant levels of multi-drug resistance. In addition to that, many patients resort to taking many antibiotics without medical advice, especially broad-spectrum antibiotics. Therefore, we suppose that the high percentage of MRSA between isolated of *S.aureus* is a natural result of the heavy use of broad-spectrum antibiotics. Treating MRSA for any diabetic foot infection is likely to lead to an increase in resistance as well as an increase in the cost burden for health care. Infections with MRSA involving other sites, prior hospitalizations, nursing homes, immunocompromised patients, prior

antibiotic treatment (Demling and Waterhouse, 2007;Ding *et al.*, 2012;Lavery *et al.*, 2014).

Panton-Valentine leukocidin is consist of two-component and coded by two cotrans-cribed genes, *lukS-PV* and *lukF-PV*, which exist on a prophage and induce tissue necrosis and leukocyte destruction (Finck-Barbançon *et al.*, 1993;Prevost *et al.*, 1995). The *S.aureus* have a variety virulence factors which promote soft tissue and bone infections,however *pvl* is considerd as a one of those virulence characteristics, and it tends to be associated with extreme DFUs (Ambrosch *et al.*, 2011). In our study, the *pvl* gene present among all *S.aureus* that isolates from the DFU patients. This finding is in line with the study reported (100%) in France (Naas *et al.*, 2005). The frequency of *pvl* in *S.aureus* over the last years given the widly distribution of *pvl*-producing MRSA strains(Esmaielzadeh *et al.*, 2018) .

However, a few studies not shown any relationship between *pvl* gene and virulence (Campbell *et al.*, 2008;Bae *et al.*, 2009). In these studies, explanation this finding in clinical results may be affected by the efficacy of the administered antibiotic treatment. The *pvl* gene is use widely as a marker for MRSA acquired in the population, responsible for deep dermal infections and soft tissue (Rieg *et al.*, 2005;Havaei *et al.*, 2010). On the other hand the pvl situation among different isolates from MRSA varies. Statistics from different areas show the growing number of pvl predominance between MRSA isolates. Bhatta *et al.*, (2016) in western Nepal, reported (63.3 %) of *pvl* prevalence among MRSA ,also study by D'Souza *et al.*, (2010) in India, reported frequency of 64 % positive *pvl* isolates among MRSA while ,Rossney *et al.*, (2007) from Ireland show the 76% of pvl-positive for MRSA. In oure study show the 100% of MRSA isolates have *pvl* gene.Therefor, PCR analysis effective of detecting *pvl* and *mecA* genes and also being 100% accurate and effective. Besides that, in any microbiology laboratory with PCR capability, this assay is easily suitable for practical clinical use(McClure *et al.*, 2006).

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

REFERENCES

1. Clinical and Laboratory Standars Institute (CLSI), (2007) Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. Seventeent, Clinical and Laboratory Standars Institute. Seventeent. Clinical and Laboratory Standards Institute consensus process. doi: 1-56238-525-5.

2. Abbot, C.A, Garrow A, Carrington A, Morris J, Van Ross E, B. A. (2005) 'Foot ulcer risk is lower in South-Asian and African-Carribean compared with European diabetic patients in the U.K', *Diabetes Care*, 8(8), pp. 1869–1875. doi: 28/8/1869.

3. Ahmadishooli, A. *et al.* (2020) 'Frequency and Antimicrobial Susceptibility Patterns of Diabetic Foot Infection of Patients from Bandar Abbas District, Southern Iran', *Journal of Pathogens*, 2020, pp. 1–10. doi: 10.1155/2020/1057167.

4. Al-Allak, M. H., Al-Khafaji, N. S. K. and Al-Dahmoshi, H. O. M. (2019) 'Microbial and resistance profile among diabetic foot-infections', *EurAsian Journal of BioSciences*, 13(2), pp. 1953–1959.

5. Ambrosch, A. *et al.* (2011) 'Diabetic foot infections: Microbiological aspects, current and future antibiotic therapy focusing on methicillin-resistant Staphylococcus aureus', *International Wound Journal*, 8(6), pp. 567–577. doi: 10.1111/j.1742-481X.2011.00849.x.

6. Bae, I. G. *et al.* (2009) 'Presence of genes encoding the Panton-Valentine leukocidin exotoxin is not the primary determinant of outcome in patients with complicated skin and skin structure infections due to methicillin-resistant Staphylococcus aureus: Results of a multinational trial', *Journal of Clinical Microbiology*, 47(12), pp. 3952–3957. doi: 10.1128/JCM.01643-09.

7. Bhatta, Dharm R. *et al.* (2016) 'Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant Staphylococcus aureus (MRSA) in Western Nepal: A matter of concern for community infections (a hospital based prospective study)', *BMC Infectious Diseases*. BMC Infectious Diseases, 16(1), pp. 1–6. doi: 10.1186/S12879-016-1531-1.

8. Bukhari, M. H. *et al.* (2004) 'A laboratory study of susceptibility of methicillin resistant Staphylococcus aureus (MRSA)', *Pakistan Journal of Medical Sciences*, 20(3), pp. 229–233.

9. Campbell, S. J. *et al.* (2008) 'Genotypic characteristics of Staphylococcus aureus isolates from a multinational trial of complicated skin and skin structure infections', *Journal of Clinical Microbiology*, 46(2), pp. 678–684. doi: 10.1128/JCM.01822-07.

10. D'Souza, N., Rodrigues, C. and Mehta, A. (2010) 'Molecular characterization of methicillin-resistant Staphylococcus aureus with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India', *Journal of Clinical Microbiology*, 48(5), pp. 1806–1811. doi: 10.1128/JCM.01867-09.

11. Datta, P. *et al.* (2011) 'Evaluation of various methods for the detection of meticillin-resistant Staphylococcus aureus strains and susceptibility patterns', *Journal of Medical Microbiology*, 60(11), pp. 1613–1616. doi: 10.1099/jmm.0.032219-0.

12. Demling, R. H. and Waterhouse, B. (2007) 'The increasing problem of wound bacterial burden and infection in acute and chronic soft-tissue wounds caused by methicillin-resistant Staphylococcus aureus.', *Journal of burns and wounds*, 7, pp. 86–98.

13. Ding, Q. *et al.* (2012) 'Risk factors for infections of methicillin-resistant Staphylococci in diabetic foot patients', *Zhonghua yi xue za zhi*, 92(4), pp. 228–231.

14. Esmaielzadeh, N. *et al.* (2018) 'isolated from Burn Wounds in Tabriz , Iran', (4), pp. 54–59.

15. Finck-Barbançon, V. *et al.* (1993) 'Pore formation by a two-component leukocidin from Staphyloccocus aureus within the membrane of human polymorphonuclear leukocytes', *BBA - Molecular Basis of Disease*, 1182(3), pp. 275–282. doi: 10.1016/0925-4439(93)90069-D.

16. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, C. R. A. (2006) 'A Clinico-microbiological Study of Diabetic Foot Ulcers in an Indian Tertiary Care', *DIABETES CARE*, 29(8), pp. 1727–1732. doi: 10.2337/dc06-0116.

17. Game, F. and Jeffcoate, W. (2004) 'MRSA and osteomyelitis of the foot in diabetes', *Diabetic Medicine*. Wiley Online Library, 21, pp. 16–19.

18. Goh, T. C. *et al.* (2020) 'Clinical and bacteriological profile of diabetic foot infections in a tertiary care', *Journal of Foot and Ankle Research*. Journal of Foot and Ankle Research, 13(1), pp. 1–8. doi: 10.1186/s13047-020-00406-y.

19. Hartemann-Heurtier, A. *et al.* (2004) 'Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact', *Diabetic Medicine*. Wiley Online Library, 21(7), pp. 710–715.

20. Havaei, S. A. *et al.* (2010) 'Prevalence of genes encoding bi-component leukocidins among clinical isolates of methicillin-resistant Staphylococcus aureus', *Iranian Journal of Public Health*, 39(1), pp. 8–14.

21. Lavery, L. A. *et al.* (2014) 'Risk factors for methicillin-resistant Staphylococcus aureus in diabetic foot infections', *Diabetic Foot and Ankle*, 5. doi: 10.3402/dfa.v5.23575.

22. Leung, P. C. (2007) 'Diabetic foot ulcers - a comprehensive review', *journal of the Royal Colleges of Surgeons of Edinburgh and Ireland*, 5(4), pp. 219–231.

23. Lina, G. *et al.* (1999) 'Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci', *Antimicrobial Agents and Chemotherapy*, 43(5), pp. 1062–1066. doi: 10.1128/aac.43.5.1062.

24. McClure, J. A. *et al.* (2006) 'Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci', *Journal of Clinical Microbiology*, 44(3), pp. 1141–1144. doi: 10.1128/JCM.44.3.1141-1144.2006.

25. Mendes, J. J. *et al.* (2011) 'Clinical and bacteriological survey of diabetic foot infections in Lisbon', *Diabetes Research and Clinical Practice*. Elsevier Ireland Ltd, 95(1), pp. 153–161. doi: 10.1016/j.diabres.2011.10.001.

26. Naas, T. *et al.* (2005) 'Three-year survey of community-acquired methicillin-resistant

Staphylococcus aureus producing Panton-Valentine leukocidin in a French university hospital', *Journal of Hospital Infection*, 61(4), pp. 321–329. doi: 10.1016/j.jhin.2005.01.027.

27. Nelson, A. *et al.* (2018) 'CODIFI (Concordance in Diabetic Foot Ulcer Infection): A cross-sectional study of wound swab versus tissue sampling in infected diabetic foot ulcers in England', *BMJ Open*, 8(1), pp. 1–11. doi: 10.1136/bmjopen-2017-019437.

28. Pontes, Dê. G. *et al.* (2020) 'Microbiologic characteristics and antibiotic resistance rates of diabetic foot infections', *Revista do Colegio Brasileiro de Cirurgioes*, 47, pp. 2–10. doi: 10.1590/0100-6991e-20202471.

29. Pournajaf, A. *et al.* (2014) 'PCR-based identification of methicillin-resistant Staphylococcus aureus strains and their antibiotic resistance profiles', *Asian Pacific Journal of Tropical Biomedicine*, 4(1067), pp. S293–S297. doi: 10.12980/APJTB.4.2014C423.

30. Prescott, J. P. H. and L. M. (2002) *Harley-Prescott :Laboraory Exercises in Microbiology*. fifth, *Journal of Chemical Information and Modeling*. fifth. Edited by L. M. P. John P. Harley. The McGraw–Hill Companies. doi: 10.1017/CBO9781107415324.004.

31. Prevost, G. *et al.* (1995) 'Panton-valentine leucocidin and gamma-hemolysin from Staphylococcus aureus ATCC 49775 are encoded by distinct genetic loci and have different biological activities', *Infection and Immunity*, 63(10), pp. 4121–4129. doi: 10.1128/iai.63.10.4121-4129.1995.

32. Qadir, A. N. *et al.* (2020) 'Prevalence of Microorganisms and Antibiotic Sensitivity Among Patients with Diabetic Foot Ulcer in Sulaimani City, Iraq', *Hospital Practices and Research*, 5(2), pp. 56–63. doi: 10.34172/hpr.2020.11.

33. Rieg, G. *et al.* (2005) 'Necrotizing Fasciitis Caused by Community-Associated Methicillin-Resistant', 352, pp. 1445–1453.

34. Rossney, A. S. *et al.* (2007) 'The emergence and importation of diverse genotypes of methicillin-resistant Staphylococcus aureus (MRSA) harboring the panton-valentine leukocidin gene (pvl) reveal that pvl is a poor marker for community-acquired MRSA strains in Ireland', *Journal of Clinical Microbiology*, 45(8), pp. 2554–2563. doi: 10.1128/JCM.00245-07.

35. Sandhu, S., Rathnayake, I. and Huygens, F. (2014) 'Prevalence of methicillin resistance and virulence determinants of <i>Staphylococcus aureus</i> in diabetic foot ulcers', *International Journal of Basic & Clinical Pharmacology*, 3(6), p. 978. doi: 10.5455/2319-2003.ijbcp20141201.

36. Sharma, S. *et al.* (2017) 'Evaluation of different phenotypic methods for the detection of methicillin resistant Staphylococcus aureus and antimicrobial susceptibility pattern of MRSA', *International Journal Of Community Medicine And Public Health*, 4(9), p. 3297. doi: 10.18203/2394-6040.ijcmph20173832.

37. Tentolouris, N. *et al.* (1999) 'Methicillin-resistant Staphylococcus aureus : an increasing problem in a diabetic foot clinic', 16, pp. 767–771.

38. Wang, S. H. *et al.* (2010) 'Meticillin-resistant Staphylococcus aureus isolated from foot ulcers in diabetic patients in a Chinese care hospital: Risk factors for infection and prevalence', *Journal of Medical Microbiology*, 59(10), pp. 1219–1224. doi: 10.1099/jmm.0.020537-0.