Journal of Basrah Researches ((Sciences)) Vol. (47). No. 1 (2021)

Available online at: https://www.iasj.net/iasj/journal/260/issues

ISSN –1817 –2695

Received date: 9-11-2021 Accepted date: 14-12-2021 Available online date: 31-12-2021

Panton-valentine leukocidin (pvl) gene prevalence among methicillin-resistant *Staphylococcus aureus* isolated from oral infection in Basrah Governoratel-Basrah, Iraq

Sadeq O. Hattab [1], Saad S. Al-Amara[2]

1. Senior bacteriology in pathological Analysis, Department of public health laboratory, Basrah, Iraq.

2. Department of Biology, College of Science, University of Basrah, Iraq

Abstract:

Panton and Valentine classified PVL as a synergy-menotropic poison virulence factor in 1932. The pvl is a synergy-homeotropic toxin that is made up of two distinct components that work together on the cellular membrane. The lukS-PV and lukF-PV mediated genes, which have been utilized in epidemiological research to diagnose and track the prevalence of Panton-Valentine, generate the toxin. Leukocytes are the main cellular targets of PVL. The *PVL* protein interacts with complement receptors on the membranous surfaces of these cells, causing the cells to deteriorate. The toxin stimulates neutrophils to produce proinflammatory cytokines and is a major pathogen of infections.

Background:

Staphylococcus aureus isolates that are positive for Valentine leucocidin (*pvl*) are becoming more linked to many types of infections.

Methods:

One hundred and fifty swab samples were collected from patients with dental infections who attended a health center in Al-Basrah, Iraq. The blood agar and mannitol salt agar, as well as coagulation testing, were used to identify *S. aureus* isolates. Vitek®2 technique to confirm the identification of isolates. Oxacillin and cefoxitin discs were followed for identification of the methicillin-resistant isolates. The polymerase chain reaction technique was used to detect *PVL* and *mec A* genes.

Results:

Out of one hundred and fifty swab samples the 15 isolates (15.8%) were identified as *S. aureus*. All (n=15) *S. aureus* isolates were categorized as MRSA depending on the criteria of inhibitory resistance against oxacillin and cefoxitin disc. Furthermore, all isolates were shown a positive result for the *pvl* and *mecA* gene.

Conclusion: The PCR investigation was successful and the best methods can be used in identifying the *pvl* and *mecA* genes. The study present showed ower rate of *S. aureus* infections of the oral cavity.

Introduction:

There are many types of oral infections that can occur in a patient's mouth. Because of the moisture, warmth, and nutritional content (fats, carbohydrates, and protein), the mouth cavity serves as a growing substrate for pathogens. The *Staphylococcus aureus* is an opportunist and can be considered pathogen a commensal bacteria in the oral cavity that causes systemic infections and oral infections in the human being as an body (Akwa *et al*., 2020). Many oral illnesses are caused by *S. aureus.* Treatment of *Staphylococcus aureus* infection of complicated due to the increase of rate of resistance to many categuries of antibiotic including methicillin antibiotics (ADIL GHALIB FADIL, 2016; Tong *et al*., 2015). The spread of methicillin-resistant *Staphylococcus aureus* (MRSA), is a known health concern and financial burden worldwide (Al Bshabshe *et al*., 2020).

According to genetic data, methicillin resistance is attributed to *mecA.* The *mecA* genes encode the penicillin-binding protein (PBP2a) resulting in resistance to entire class of antibiotics. (Carretto *et al*., 2018; Turner *et al*., 2019; Ismail *et al*., 2021). The global spread of methicillin-resistant *Staphylococcus aureus* (MRSA) means that it is now a pathogen of public health concern worldwide (Chmagh & Abd Al-Abbas, 2019).In addition, an increasing number of studies from different countries show that pvl dominance among MRSA isolates is on the rise (Bhatta *et al*., 2016; Bakthavatchalam *et al*., 2017). The current study aimed to identify the panton-valentine leukocidin (pvl) gene prevalence among MRSA isolated from dental infected patients in Al-Basrah, Iraq (Gadban *et al*., 2020).

Material and Methods

Sample Collection:

During the period from December 2020 to January 2021, one hundred and fifty swabs were collected from dental infections patients who were admitted to Al-Matiha Health Center and Al-Qaleej Al-Arabi Health Center, and (75) samples were collected for each center. The ages ranged from 10 to 75 years, and the average age was 47 years, as well as included both sexes.

All patients were selected depending on their clinical examination by specialist doctors. Samples were collected from a decayed tooth with a cotton-sterile swab. All samples were transported to a microbiological laboratory at the University of Basrah - a college of Science /department of biology for isolation, identification of bacteria, and for the further investigations test as showing table (1).

Table (1) Percentage and Number of Patients According to Demographic of sex and age groups.

Variables	*Class*	*Groups*	
		No.	%
Sex	male	77	51
	female	73	49
	total	**150**	**100**
Age	10 - 32 y.	45	30
	33 - 54 y.	65	43
	55 - 75 y.	40	27
	Total	**150**	**100**

.Isolation and Identification:

The swab sample was first cultured in brain heart infusion broth for 24 hrs. at 37°C. The broth that gave positive bacterial growth was transferred to mannitol salt agar and blood agar plates and kept at 37°C for 24 hrs. The properties of the isolates were then used to detify it. The strains with *Staphylococcus aureus* characteristics were chosen, and the coagulase test was performed (Harley and Prescott, 2002). Through using Vitek® 2 system version 07.01 device, the isolation identification was confirmed.

Screening of *Staphylococcus aureus* Methicillin-Resistant (MRSA)

MRSA was identified using the oxacillin and cefoxitin diffusion disc method according to (Datta *et al*., 2011; Sharma *et al*., 2017). The inhibition zone diameter was measured and interpreted according to (CLSI, 2018).

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 rDNA gene PCR:

The isolated DNA was amplified using specified primers with a product size of 756 bp.

Molecular detection of *mecA* and *pvl* genes: According to (Lina *et al*., 1999), universal primers were employed for amplification of the *mecA* and *PVL* genes, respectively such as a table (2).

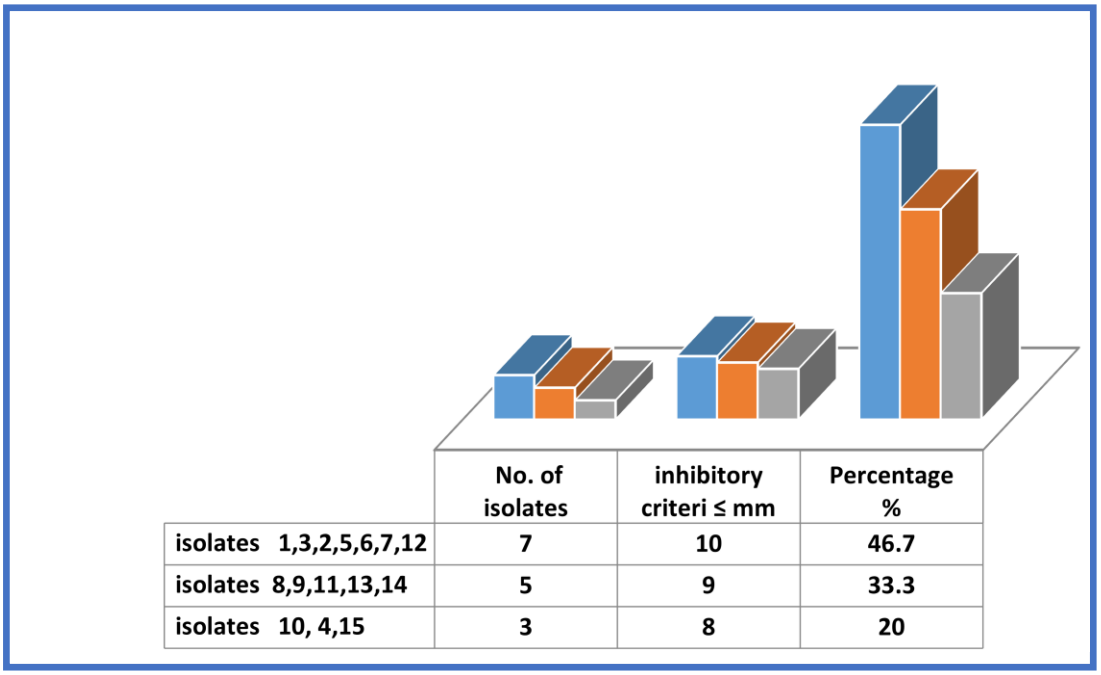
Table (2) The *16SrDNA*,*mecA* and *PVL* Specific primers used in PCR

primers	Sequence	length	Size bp	Optimizing TA*
Staph.*16SrDNA*	F:5'-AACTCTGTTATTAGGGAAGAACA-3'	23	756	55°C
	R:5'-CCACCTTCCTCCGGTTTGTCACC-3'	23		55°C
mecA	5'-AAAATCGATGGTAAAGGTTGGC-3'	22	533	53 °C
	5'-AGTTCTGGAGTACCGGATTTGC-3'	22		53 °C
PVL	5'ATCATTAGGTAAAATGTCTGGACATGATCCA-3'	31	433	55 °C
	5'-GCATCAACTGTATTGGATAGCAAAAGC-3'	27		55 °C

RESULT

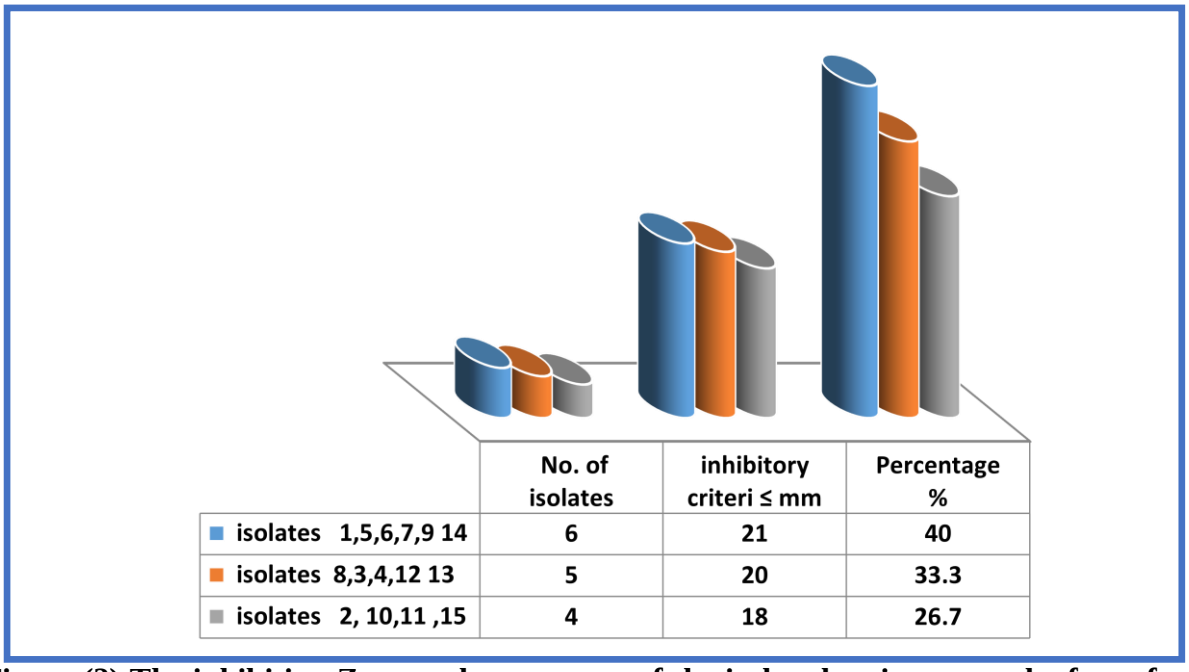
Only 15(10%) *Staphylococcus aureus* isolates were identified out of 150 swab samples collected December 2020 to January 2021. Mannitol fermentation, type of on hemolysis blood *agar*, and the coagulation production test results are used to identify *S.aureus* isolates from mouth infections. The Vitek®2 system was used for confirm the identification. The results showed that all (n=15).

S. aureus isolates classified as MRSA isolates. through given the inhibitory resistant criteria for oxacillin disc 1µg. The results showed that identified with the numbers (1,3,2,5,6,7and12), (8,9,11,13and14) and (10, 4and15) gave the inhibition zone less than (10m, 9mm and 8mm) in percentage 46.7% ,33.3% and 20% respectively (Fig. 1). Also, inhibitory resistant criteria detect based on the interpretation of CLIS, all *S.aureus* isolates (n=15) were tested against cefoxitin (30µg) antibiotic disc. The isolates with the numbers (1,5,6,7,9 and14),(8,3,4,12 and13) and (2, 10,11 and15) gave the inhibition zone less than (21mm, 20mm and 18mm) with percentages 40%,33.3% and 26.7% respectively, as shown in (Fig. 2).



	No. of isolates	inhibitory criteri ≤ mm	Percentage %
isolates 1,3,2,5,6,7,12	7	10	46.7
isolates 8,9,11,13,14	5	9	33.3
isolates 10, 4,15	3	8	20

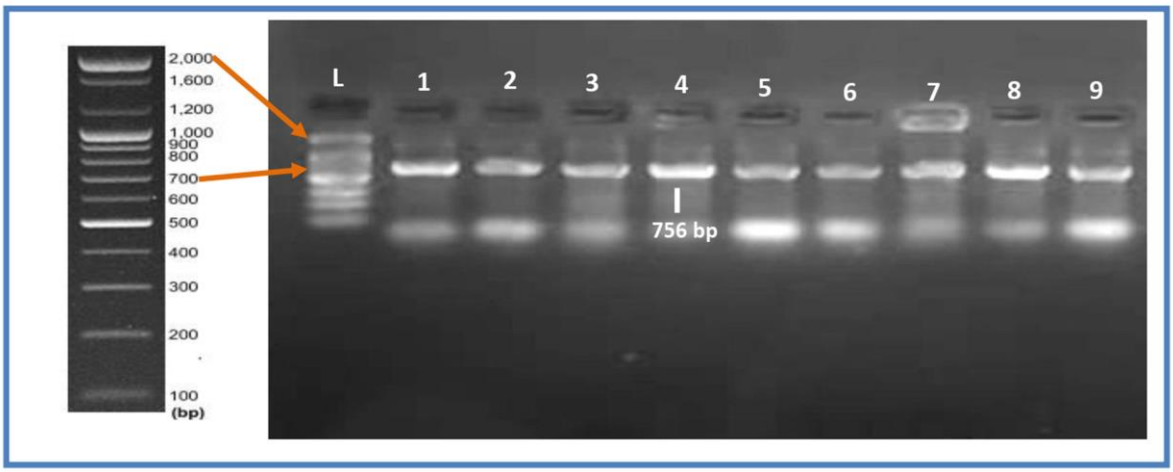
Figure (1) The inhibition Zone and percentage of the isolated resistant results for oxacillin 1µg antibiotic disc.



	No. of isolates	inhibitory criteri ≤ mm	Percentage %
isolates 1,5,6,7,9 14	6	21	40
isolates 8,3,4,12 13	5	20	33.3
isolates 2, 10,11 ,15	4	18	26.7

Figure (2) The inhibition Zone and percentage of the isolated resistant results for cefoxitin 30µg antibiotic disc.

Furthermore, genetic profiling results of amplifying *16S rDNA* were shown a positive result for all (n=15) isolates exhibited a positive result with the single *16S rDNA* band viewed in (756 bp) (Fig. 3). Also, the amplification results of the *mecA* gene were shown a positive result for all (n=15) isolates through given a single band at 533 bp (Fig. 4). While *PVL*gene amplification was shown a positive result for all (n=15) isolates through given a single ring 433 bp (Fig. 5). The standard DNA molecular ladder was used to compare all amplified genes (2000 bp).



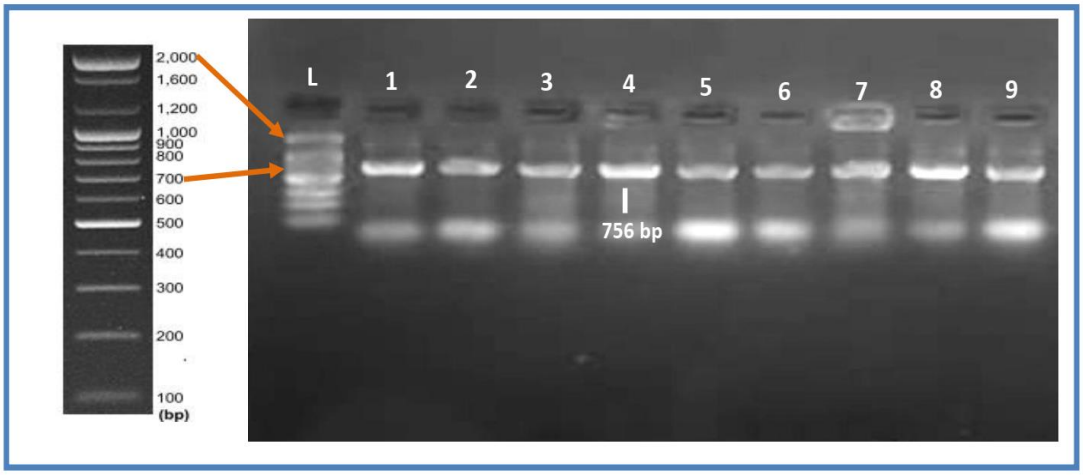


Figure (3) Gel electrophoresis patterns show PCR amplified products of 16S rDNA gene. (2000 bp DNA ladder), (756 bp 16S rDNA gene) band of bacterial isolates.

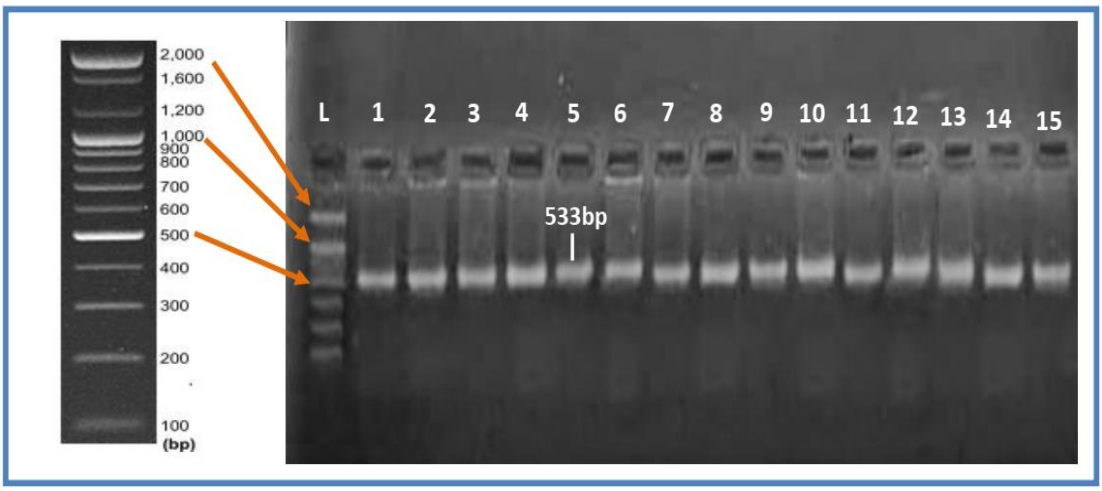


Figure (4) Gel electrophoresis patterns show PCR amplified products of the *mec A* gene. (2000 bp DNA ladder), (533 bp *mec A* gene) band of bacterial isolates.

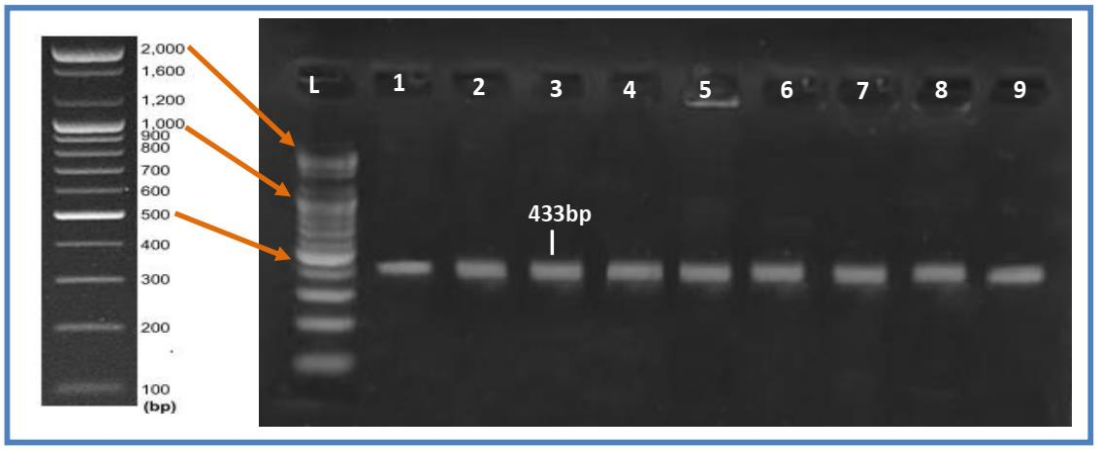


Figure (5) Gel electrophoresis patterns show PCR amplified products of pvl gene. (2000 bp DNA ladder), (433 bp *pvl* gene) band of bacterial isolates.

DISCUSSION

16S rDNA nucleotide sequence data for each isolate, fifteen alignments of (15) bacterial isolates were identified from (15) isolates from dental injury patients. It is a double-stranded chromosomal DNA containing fixed non-coding sites and non-protein producing and everything that does not code for protein becomes distinctive in giving the identification identity of the bacteria, and this gene is used to detect the genus of the bacteria not its species.

The present study shows lower rate of infection caused by S. aureus (10%) comparsed *Salmonella typhoid* of Manisha *et al*,(2019) reported from (64) samples were collected from patients with dental infection the present 40(62.5%) *S.aureus* isolates were found, also on other studies of (Ohara-Nemoto *et al*.,2008; Mc Cormack *et al*.,2015; Kim and Lee 2015) were reported the *S. aureus* found in 46.4%, 18%, 36.6% respectively. Ansari *et al*, (2011) study was reported from (250) samples were collected from patients with dental infection the results given *S.aureus* (56%), *E.coli* (25%) *Pseudomonas spp*. (13%), *S.typhoid* (4%) and *Shigella* (2%) . and also the results have shown the *S.aureus* isolates were resistant to various antibiotics. A study by Kzar *et al*, (2020) reported from (30) pus samples were collected from patients with dental infection in the presence of *S. aureus* (26.2%). Other studies by Donkor & Kotey, (2020) showed the appearance of MRSA in the mouth can be enhanced by the widespread by using of antibiotic prophylaxis among high-risk dental procedure candidates. These changes in the epidemiology of MRSA it has important implications for MRSA preventive strategies.

Also, the results showed a very high susceptibility of isolates to amoxicillin (97%) followed by cefotaxime and imipenem (94%). Whereas the lowest sensitivity was detected to clindamycin (18%). The study of Hanumanthappa *et al*, (2018) reported that in vitro culture and antibiotic sensitivity test (AST) utilizing methicillin, oxacillin, and Cefoxitin screen disk diffusion (DD) are the most widely used procedures in laboratories for quickly identifying *Staphylococci* resistant to methicillin. In this study of Access,(2017) the ability to determine methicillin resistance is crucial in determining the prognosis of *S. aureus* infection. Phenotypic detection methods are less expensive than genotyping procedures, however, they are dependent on environmental circumstances. Polymerase Chain Reaction (PCR) detection of the *mecA* gene was the gold standard for identifying MRSA.

The results revealed that all S.aureus isolates in this investigation gave a positive result for mecA gene identification using PCR techniques

According to Aboud and Khudaier, (2019) pointed that the resistance is caused by a mutant penicillin-binding protein, PBP2a, which is produced by *mecA* gene alleles. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains has acquired the gene that makes it resistant to almost all beta-lactam antibiotics. Resistance to other antibiotics is also common MRSA (Aboud & Khudaier, 2019). These organisms are considered dangerous pathogens in hospitals, and they are effective treatment can be difficult (Lina *et al.*, 1999). MRSA infections have increased at an alarming rate in recent years. MRSA infection has become a major health concern worldwide. No longer restricted to patients in medical settings, MRSA infections are expected to rise (Singh *et al*., 2019). First molecular surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) at Canselor Tuanku Muhriz Hospital (HCTM), a Malaysian teaching hospital, was performed in 2009. The dominant version is identified as MRSA (Abbas *et al.*, 2017; Ismail *et al*., 2021).

.

The virulence of MRSA is due to the toxin ponton valentine (*PVL*).To MRSA strains are in increasing threating the health (Zhang *et al*, 2018). Leukocytes are the main cellular targets of *PVL*. The *PVL* protein interacts to complement receptors on the membranous surfaces of these cells, causing the cells to deteriorate. The toxin stimulates neutrophils to produce pro-inflammatory cytokines and is a major pathogen of infection. (Shields *et al*., 2016; Saeed *et al*., 2018). Furthermore, *PVL* predominance among MRSA isolates is on the rise, according to a growing number of investigatio *PVL*-positive in recent years, *S. aureus* has gotten a lot of

attention. The *PVL* gene was found in 100 percent of *S. aureus* strains in this stream, and the majority of these were linked to dental infections. However, positive *PVL* and mecA findings for *Staph aureus* identefecation result in enhanced virulence, making these isolates a genuine threat to persons with oral injuries. ns from various nations. (Bhatta *et al*., 2016 ; Bakthavatchalam *et al*., 2017).

PVL is a pore-forming toxin produced by strains of *S. aureus*, which have been predominantly associated with skin and soft tissue infections but can also cause lethal necrotizing pneumonia (Saeed *et al*., 2018; Divyakolu *et al*., 2019). Chaya *et al*, (2018), mentioned that skin infection with *pvl*-positive MRSA is rapidly increasing, and can recur with multiple lesions, necessitating appropriate antibiotic treatment and surgical drainage. In another study, for Schaumburg *et al*, (2014) MRSA indicated the transmission of *S.aureus* high prevalence USA 300 related isolates between women and their newborn neonates. *S. aureus* insulates 56.7 % of the *S. aureus* har bour genes that code *pvl* gene.

CONCLUSION

The PCR investigation was successful and the best method can be used in identifying the *PVL* and *mecA* genes.

REFERENCES

(CLSI), C. and L. S. I. (2018). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. Quality, 3(4), 1–14.

Abbas, B. A., Khudaier, B. Y., & Khudaier, A. M. (2017). Studies on mecA gene in methicillin resistant Staphylococcus aureus isolates. Jokull, 67(6).

Aboud, W. A. A., & Khudaier, B. Y. (2019). Molecular Detection of Vancomycin and Methicillin-Resistant Genes in Staphylococcus aureus in Basrah, Southern Iraq. Basrah Journal of Science, 1(5), 1–3.

Access, O. (2017). detection methods : analytical comparative study. 8688, 1–7.

ADIL GHALIB FADIL. (2016). Knowledge and Practice Regarding Infection Control Among Dental Students At Basra University College of Dentistry, Iraq. International Journal of

General Medicine and Pharmacy (IJGMP) , 5(6), 35–46. http://www.iaset.us/view_archives.php?year=2016&id=51&jtype=2&page=3

Akwa, A. A. Y., Zabara, A. Q. M. Q., Al-Shamahy, H. A., Al-labani, M. A., Al-Ghaffari, K. M., Al-Mortada, A. M., Al-Haddad, A. M., & Al-Sharani, A. A. (2020). Prevalence of Staphylococcus Aureus in Dental Infections and the Occurrence of Mrsa in Isolates. Universal Journal of Pharmaceutical Research, May. https://doi.org/10.22270/ujpr.v5i2.384

Al Bshabshe, A., Joseph, M. R. P., Awad El-Gied, A. A., Fadul, A. N., Chandramoorthy, H. C., & Hamid, M. E. (2020). Clinical Relevance and Antimicrobial Profiling of Methicillin-Resistant Staphylococcus aureus (MRSA) on Routine Antibiotics and Ethanol Extract of Mango Kernel (Mangifera indica L.). BioMed Research International, 2020. https://doi.org/10.1155/2020/4150678

Ansari, S. A., Baqai, R., Memon, M. R., Aziz, M., & Khan, M. K. (2011). Biofilm Formation and Isolation of Staphylococcus Aureus From Blood Cultures of Dental Patients Undergoing Oral Surgical Procedures. Journal of the Pakistan Dental Association, 20(03), 154–158.

Bakthavatchalam, Y. D. et al. (2017) ' Diagnosis and management of Panton-Valentine leukocidin toxin associated Staphylococcus aureus infection: an update ', Virulence. Taylor & Francis, 0(0), pp. 00–00. doi: 10.1080/21505594.2017.1362532.

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.

Carretto, E., Visiello, R., & Nardini, P. (2018). Methicillin Resistance in Staphylococcus aureus. Pet-to-Man Travelling Staphylococci: A World in Progress, 85(Pt 1), 225–235. https://doi.org/10.1016/B978-0-12-813547-1.00017-0

Chaya, A., Sato, M., Namkoong, H., & Wada, N. (2018). Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus infection of the lip. International Journal of Infectious Diseases, 75, 80–81. https://doi.org/10.1016/j.ijid.2018.07.026

Chmagh, A. A., & Abd Al-Abbas, M. J. (2019). PCR-RFLP by AluI for coa gene of methicillin-resistant Staphylococcus aureus (MRSA) isolated from burn wounds, pneumonia and otitis media. Gene Reports, 15(February). https://doi.org/10.1016/j.genrep.2019.100390

Clinical and Laboratory Standards Institute (CLSI), (2007) Performance Standards for

Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. Seventeen, Clinical and Laboratory Standards Institute. Seventeen. Clinical and Laboratory Standards Institute consensus process. doi: 1-56238-525-5.

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.

DeLeo, F. R. et al. (2010) 'Community-associated meticillin-resistant Staphylococcus aureus', The Lancet. Elsevier Ltd, 375(9725), pp. 1557–1568. doi: 10.1016/S0140-6736(09)61999-1.

Divyakolu, S., Chikkala, R., Ratnakar, K. S., & Sritharan, V. (2019). Hemolysins of <i>Staphylococcus aureus</i>—An Update on Their Biology, Role in Pathogenesis and as Targets for Anti-Virulence Therapy. Advances in Infectious Diseases, 09(02), 80–104. https://doi.org/10.4236/aid.2019.92007

Donkor, E. S., & Kotey, F. C. (2020). Methicillin-Resistant Staphylococcus aureus in the Oral Cavity: Implications for Antibiotic Prophylaxis and Surveillance . Infectious Diseases: Research and Treatment, 13, 117863372097658. https://doi.org/10.1177/1178633720976581

Gadban, T. H., Al-Amara, S. S., & Jasim, H. A. (2020). 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. Systematic Reviews in Pharmacy, 11(11), 285–290. https://doi.org/10.31838/srp.2020.11.42

Hanumanthappa, A. R., Chandrappa, N. R., & Rajasekharappa, M. G. (2018). Prevalence of methicillin-resistant Staphylococcus aureus in Karnataka. Indian Journal of Pathology and Microbiology, 8(3), 31–38.

Ismail, M. A. H., Kamarudin, N., Samat, M. N. A., Rahman, R. M. F. R. A., Saimun, S., Tan, T. L., & Neoh, H. M. (2021). Methicillin-resistant staphylococcus aureus (Mrsa) clonal replacement in a malaysian teaching hospital: Findings from an eight-year interval molecular surveillance. Antibiotics, 10(3). https://doi.org/10.3390/antibiotics10030320

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.

Lina, G., Quaglia, A., Reverdy, M. E., Leclercq, R., Vandenesch, F., & Etienne, J. (1999). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins

among staphylococci. Antimicrobial Agents and Chemotherapy, 43(5), 1062–1066. https://doi.org/10.1128/aac.43.5.1062

Luna, C. M., Rodríguez-Noriega, E., Bavestrello, L., & Gotuzzo, E. (2010). Treatment of methicillin-resistant Staphylococcus aureus in Latin America. Brazilian Journal of Infectious Diseases, 14(SUPPL. 2). https://doi.org/10.1590/s1413-86702010000800007

Manisha, D., Abdullah, A. M. S., Zobayda, F. H., Nanda, B., Amrita, P., Md., M. M., Md., F. R. K., & Sukumar, S. (2019). man characterization of Staphylococcus aureus isolated from huental infection. African Journal of Microbiology Research, 13(14), 273–278. https://doi.org/10.5897/ajmr2019.9076

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.

Monecke, S. et al. (2012) 'Rapid microarray-based identification of different mecA alleles in staphylococci', Antimicrobial Agents and Chemotherapy, 56(11), pp. 5547–5554. doi: 10.1128/AAC.00574-12.

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.

Prescott, J. P. H. and L. M. (2002) Harley- Prescott: Laboratory 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.

Saeed, K. et al. (2018) 'Panton-Valentine leukocidin-positive Staphylococcus aureus: a position statement from the International Society of Chemotherapy', International Journal of Antimicrobial Agents. Elsevier B.V., 51(1), pp. 16– 25. doi: 10.1016/j.ijantimicag.2017.11.002.

Salgueiro, V., Manageiro, V., Bandarra, N. M., Ferreira, E., Clemente, L., & Caniça, M. (2020). Genetic relatedness and diversity of Staphylococcus aureus from different reservoirs: Humans and animals of livestock, poultry, zoo, and aquaculture. Microorganisms, 8(9), 1–15. https://doi.org/10.3390/microorganisms8091345

Schaumburg, F. et al. (2014) 'Transmission of Staphylococcus aureus between mothers and infants

in an African setting', Clinical Microbiology and Infection, 20(6). doi: 10.1111/1469-0691.12417.

Shallcross, L. J. et al. (2013) 'The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: A systematic review and meta-analysis, The Lancet Infectious Diseases. Elsevier Ltd, 13(1), pp. 43–54. doi: 10.1016/S1473-3099(12)70238-4.

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.

Shay, K. (2002). Infectious complications of dental and periodontal diseases in the elderly population. Clinical Infectious Diseases, 34(9), 1215–1223. https://doi.org/10.1086/339865

Shields, B. E., Tschetter, A. J. and Wanat, K. A. (2016) 'Staphylococcus simulans An emerging cutaneous pathogen', JAAD Case Reports. Elsevier Inc, 2(6), pp. 428–429. doi: 10.1016/j.jdcr.2016.08.015.

Singh, K. et al. (2019) 'Methicillin-Resistant Staphylococcus aureus Infections in Patients With Renal Disorders: A Review ', World Journal of Nephrology and Urology, 8(1), pp. 8–13. doi: 10.14740/wjnu384.

Tong, S. Y. C., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. (2015). Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603–661. https://doi.org/10.1128/CMR.00134-14

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., Holland, T. L., & Fowler, V. G. (2019). Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nature Reviews Microbiology, 17(4), 203–218. https://doi.org/10.1038/s41579-018-0147-4