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Research Article,

Detection and Frequency of Hld Gene among Methicillin Resistant Staphylococcus Aureus (Mrsa)

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Abstract:

Summary

This study was conducted to determine source of Methicillin Resistant *Staphylococcus aureus* (MRSA) in hospital environment and molecular detection of *Hld* gene in MRSA isolates and to investigate relationship of MRSA carriage with environmental, household, medical, and occupational exposures among patients with wound infection admitted to Al-Basrah and Al-Sadder Teaching Hospitals and determine *Hld* gene sequencing of MRSA. The results revealed that there were 46 *Staphylococcus aureus* ,only 39 isolates (84.8%) showed resistant to the Methicillin while 7 isolates (15.2%) were sensitive to the Methicillin.The *Hld* gene was found in 24/39(61.5%) of MRSA.

Key words: Methicillin Resistant Staphylococcus aureus (MRSA), Hld gene

Introduction:

Methicillin resistant Staphylococcus aureus (MRSA), one of the most common pathogens associated with an increase of antimicrobial-resistance. (Suhaili et al. 2018), Now a day's number of cases of MRSA infections worldwide were increased, about 90% of human Staph.aureus (Hussain, Naqvi, and Sharaz 2019) and (Garazi et al. 2009) MRSA is usually resistant to wide variety antibiotic including's ; macrolides, aminoglycoside, lincosamide & all β-lactams, the occurrence of multidrug resistance (MDR) among MRSA, make it difficult to treat (Mahmoudi et al. 2019) and Gupta, et.al., 2019), MRSA colonization is usually asymptomatic in healthy peoples (Yang et al. 2019) The pattern and prevalence of MRSA varies greatly in hospitals between different countries or within the same country. (Hussain et al. 2019) MRSA was received a great interest in medical research and practice due to the increasing challenges in controlling and treating it. (Alkharsah et al. 2018) .In 2001 first complete genomes of Staph aureus were sequenced. (Yan et al. 2016) The Staph aureus genome can be divided into three segments: the first one is core genome, the second one is core variable genes, and the third one is mobile genetic element (MGE), the core genome is highly conserved among isolates; however, a slight difference occurs as single nucleotide polymorphisms (SNPs) that arise from mutations, Core variable genes with known functions include surface proteins and their associated regulatory genes, (MGEs) are the most altered part of the Staph aureus genome and comprise about 25% of all genetic material in the isolate of Staph aureus (Hau 2017) Core genome is largely preserved, and the similarity of genes between isolates is about 98–100% (Kırmusaoğlu 2017) Staph aureus was developing rapidly by horizontal gene transfer (Dai et al. 2019) . The most known PI of Staph aureus is (SaPI1) ,Staph aureus not only carried (SaPIs) but also variant surface antigens that encoded by the (vSa-gene family) that encoded approximately 50% of virulence factors and toxin, vSa1 contains enterotoxin coding genes such as Tst and Seb, whereas vSa2 contains genes that encoded enterotoxin gene such as Sec, some genes such as antibiotic resistance genes prophages play an active role in pathogenesis of Staph aureus due to causing horizontal gene transfe (HGT) (King 2020);(Pain 2020);(Kırmusaoğlu 2017);(Úbeda et

al. 2003) and (Lindsay et al. 1998) There was three type of Staph aureus plasmids based on their ability to conjugate and size; class I-III, Class I plasmids are the smallest in size (less than 5kb) and have the highest copy number (Shien 2014) Expression of the virulence factors controlled by the density of bacterial cells and multiple environmental factors such as pH, oxygen, and carbon dioxide levels, these factors operate through different regulatory systems (Zhu 2010) In general, different combinations of the virulence genes may determine different outcomes of infection. (Luo et al. 2018).Hemolysins rupture red blood cells within the host, and there are several types of hemolysin that differ in terms of the type of hemolysis that occurs and how it occurs, which are Alpha, Beta, Gamma, and Delta. (Kessel 2017) The Alpha (α), Beta (β), Delta (δ) and Gamma (γ) hemolysin are coded by hla, hlb, hld and hlg genes respectively, these toxins are very important in causing a Staph aureus infections (Hoseini Alfatemi et al. 2014) The gene examined at in this study was hld gene. Delta hemolycin was identified over 60 years ago and they make hemolysis by several mechanism:

• Connect the cell surface and aggregate at the surface to form pores across the membrane.
• Curvature of the membrane by binding to the cell surface, thus destabilizing the plasma membrane
• Act as a membrane cleaner at high concentration. (Vandenesch, Lina, and Henry 2012)

Materials and methods:

Samplling

A total of 484 samples collected from patients (Skin swab, Nasal swab and Wound swab), Health Care Workers (Skin swab and Nasal swab), hospital words (Orthopedic and Surgical words) and Operation Theater (various places of Operation Theater before and after sterilization). Samples were collected in the period Between November 2018 and August 2019, from two locations, Al-Basrah Teaching Hospital and Al-Saddr Teaching Hospital. Each swab was transferred in to enrichment medium (brain heart infusion broth (BHIB)) for 2-4 hour at 37^{o}C. (Nicholas P. Vitko and Anthony R. Richardson 2014)

Culturing and identification:

Staphylococcus grows easily on most routine media at aerobic or micro-aerophilic conditions. It was quickly grows at (37°C), and the ideal temperature in which the pigment is formed is 20-25°C Staph aureus usually forms grey to golden yellow colonies due to carotenoids, Produces β-haemolysis on horse, sheep or human blood agar plates (Suzuki et al. 2012) and (Gillet et al. 2002) The bacterial morphology was observed microscopically as Gram-positive cocci arranged in grape-like irregular clusters (Gillet et al. 2002) All Staph aureus strains produce coagulase enzyme. Staph aureus are catalase positive and oxidase negative (Suzuki et al. 2012) Staph aureus express a clumping factor (fibrinogen affinity factor) (Reddy, Srirama, and Dirisala 2017) Staphylococcus can grow in a medium with a high salt concentration, so they can grow easily in MSA. The acidity of the medium changes as the bacteria ferments mannitol and turn phenol red pH-indicator; Staph aureus changes color of MSA from the alkaline (red)to the acidic(yellow), while the rest of the Staphylococcus will grow without changing the color of the medium. (Gillet et al. 2002)

Detection of MRSA:

Cefoxitin disc diffusion, Significant method to detect MRSA, by testing MRSA resistance to the cefoxitin disc, culture was done on MHA plate , incubation temperatures at 35-36 ^{0}C and times of 18-24 hour strains of Staph aureus having zone of inhibition less than 19mm defined as MRSA. (Brown et al. 2005)

PCR Methods, Strains of Staph aureus harboring MecA gene defined as MRSA (Brown et al. 2005)

Genomic DNA extraction:

As the instruction of manufacturer (promega company) Polymerase Chain Reaction (PCR) according to the manufacturer's instructions, the DNA were detected by gel electrophoresis, the samples were loaded in 0.8% agarose gel 1×TBE (54 g Tris-base, 0.5M EDTA, 1-l distilled water, pH=8 and diluted with 400 ml of distilled water) and electrophoresed at 60 V for 30 min.

Polymerase chain reaction technique:

PCR is a very effective method to amplify a particular DNA as many copies of a specific DNA (Bartlett 2003), all MRSA isolates were assayed for the presence of the *Tst* gene by PCR using previously described primers, for PCR used diluted forward and reverse primers to reach (100 pmol/µl) concentration as stock solution, distilled water was used as the negative control.

Table-1 PCR mastermix Volum:

PCR mix		Volum
Promega Green Master Mix		12.5µL
DNA template		2.5µL
Primer	Forward Primer	1.5 µL
	Reverse Primer	1.5 µL
nuclease free water		7 µL
Total		25 µL

Table-2 Oligonucleotide Sequences and Amplicon Size of Each Gene Used in This Study

Gene	Oligonucleotide Sequence (5'-3')	Amplicon Size, bp	Reference
MecA		147	Funaki, *et al.*,2019
MECA-1	GTGAAGATA TACCAAGTGATT		
MECA-2	ATGCGCTA TAGATTGAAAGGAT		
Hld		111	Rossato, *et al.*, 2018
HLD-1	AAGAATTTTTATC TTAATTAAGGAAGGAGTC		
HLD-2	TTAGTGAATTT GTTCACTGTGTCGA		

Table-3 Thermal Cycler Programs Used in This Study Statistical analysis

Gene	Temperature (°C)/Time					Cycle No.
	Initial denaturation	Cycling condition			Final extension	
		denaturation	annealing	extension		
MecA	94/5 min	95/30 sec	50/45 sec	72/1 min	72/7min	30
Hld	95/2 min	95/15sec	56/30 sec	72/20sec	72/5 min	30

Statistical analysis:

Statistical analysis was done using SPSS V. 20, Experimental data were presented in terms of observed numbers and percentage frequencies, and then analyzed by using Chi-square ($\chi 2$) test to determine the relationship between the variables, P value $\leq$ 0.05 was considered statistically significant.

Results:

Identification of bacterial isolates:

Samples were collected in the period between November 2018 and August 2019 from two locations, Al-Basrah Teaching Hospital and Al-Sadder Teaching Hospital. Out of 485 samples only 46 (9.48 %) were identified as coagulase positive staphylococci, as shown in table (3-1) PCR product was electrophoresed in 1.5 % agarose gel, Stained with ethidium bromide, , 7 µL of PCR products and promega DNA ladder (50-1000bp) carefully loaded in the wells and electric current was matched (65 volt for 45 h). The gel was then observed under a UV light and compare with ladder (50-1000bp).

Detection of MRSA isolate:

Cefoxitin resistance staph aureus isolate harboring mecA gene (MRSA) was detected in 39from 46(73.4%) S. aureus isolates. 10(25.6%) isolates of MRSA were from wound samples, 6 (15.4%) from patient skin swab and 5 (12.8%) from patient noses, 4(10.3%) from hospital words, 4(10.3%) from health care workers hands, 7(17.9%) from health care workers noses and 3 (7.7%) from hospitals theaters samples.

Table-4 Number of Staph aureus isolates MecA gene detactions:

Type of specimens	*Staph aureus*				Total *Staph aureus* isolate	
	MRSA No.	%	MSSA No.	%	No.	%
Hospital wards	4	10.3	1	14.3	5	10.9
Hospital theatres before sterilization	3	7.7	1	14.3	4	8.7
H.C.Ws hands	4	10.3	1	14.3	5	10.9
H.C.Ws nasal swab	7	17.9	0	0	7	15.2
Wound swab	10	25.6	1	9.1	11	23.9
Patient skin swab	6	15.4	2	28.6	8	17.4
Patient nasal swab	5	12.8	1	14.3	6	13.0
Total	39	100	7	100	46	100

df=6, P Value =0.851, χ^2 =2.654

Staphylococcus MecA gene presence in all MRSA isolates, to detect Staph aureus isolates with MecA gene, it was subjected to PCR technique, MecA gene band detected at 147bp region.

Hld gene detections:

Most of MRSA isolates harboring *Hld* gene (61.5%), *Hld* gene band detected at 111bp as showed in

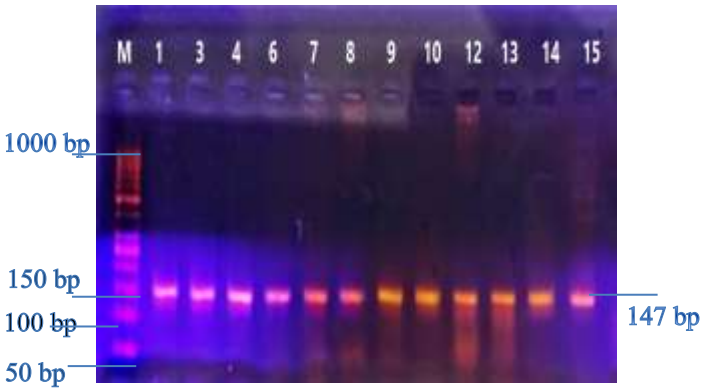

Figure 1: Amplified *MecA* gene (147bp) of *Staph aureus* isolates in PCR technique Agarose gel (1.5%), (95voltage for 45minutes) **M: DNA Ladder (50-1000bp). All MRSA was positive for *MecA* gene**

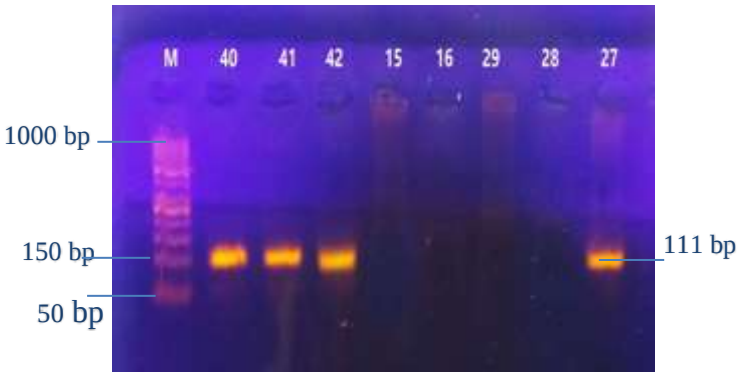

Figure-2: Amplified PCR products of *Hld* gene (111bp) of *Staph aureus* isolates in PCR technique, Agarose gel (1.5%), (95voltage for 45minutes), M: DNA Ladder (50-1000bp), 40,41,42,27 samples harboring *Hld* gene, 15,16,19,25,28,29,30 are negative samples.

Table-5 Prevalence of various genes of MRSA isolated from different specimens.

Source of isolates	MRSA genes	
	MecA	***Hid***
Hospital wards	4 (100%)	4(100%)
Operating Theaters	3 (100%)	3(100%)
H.C.Ws hand	4 (100%)	0
H.C.Ws nasal swab	7 (100%)	5(71.4%)
Wound swab	10 (100%)	6(60%)
Patients skin swab	6 (100%)	2(33.3%)
Patients nasal swab	5 (100%)	4(80%)
Total of positive result	39 (100%)	24(61.5%)
Negative result	0	15(38.5%)
df	-	**6**
P value	-	**0.032**
χ^2	-	**5.913**

Sequencing for Hld gene:

Figure3 showed DNA sequence of Hld gene, Figure 4 showed the alignment

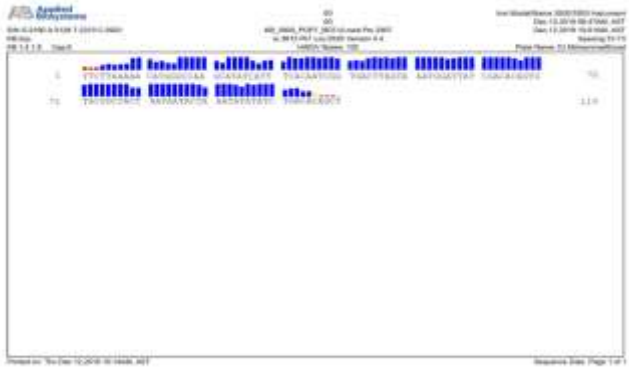

Figure 3; DNA Sequence for *Hld* gene

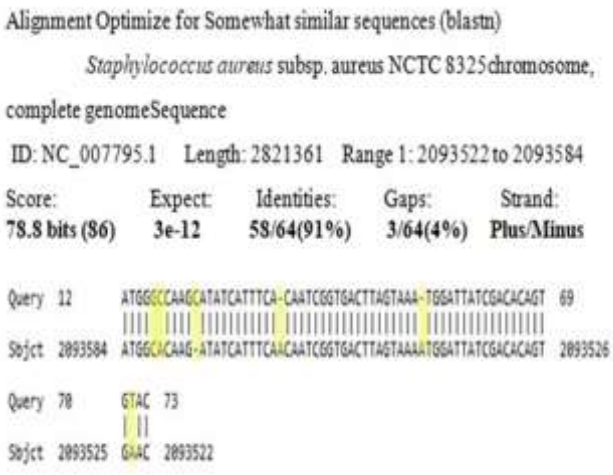

Figure 4: Alignment of *Hld* gene

Query isolate (our isolate) begin from (12-73) bp when compared with subject isolate (Stander isolate) begin from (2093584-2093522) bp, where the compatibility occur between the two isolates for identification Query isolate, identities was 91%; There was a mismatch in six places; three substitution in nitrogen base in three locations; replacement of Guanine (G) in Query isolate in instead of Cytosine (C), Cytosine (C) in Query isolate in instead of Adenine(A) and Thymine in Query isolate in instead of Adenine (A), Deletion in Adenine (A) in two locations and insertion in Cytosine (C), this difference may be due to point mutation.

Discussion:

isolation showed high prevalence range of MRSA strains 85% (39/46) of the total *staph aureus* isolated from various samples, higher rate of MRSA isolations from H.C.W nasal swabs 100% (7/7), followed by wound swabs 90.9% (10/11), Nasal swab from patient 83% (5/6), H.C.Ws hand swabs and hospital words 80% (4/5) for each, lowest rate

were recorded for the patient Skin swab (6/8) and operative rooms (3/4) 75% each. The finding about high prevelance of MRSA is not surprising and is also in line with several studies carried out in Iraq. (Al-azawi et al. 2016) ; (Al-dahbi and Al-mathkhury 2013) and (Al-Maliki 2009)The ratio of MRSA was relatively low in a study conducted in Kurdistan region of Iraq, in 2015 where the MRSA prevalence was 53% (Hussein et al. 2015) In another study in Iran was 69% (Jahanshahi, Zeighami, and Haghi 2018) while in a study conducted in India, the percentage was much lower 16.6% (Goud et al. 2011) MRSA prevalence 51.4% at the Korean hospital from the *Staph aureus* collected from blood and nasal colonizers (Peck et al. 2009) In general MRSA was highly prevalent in Asian countries (Hussain et al. 2019) In the German study there was a decrease in MRSA rate (Schubert, Kämpf, Wahl, et al., 2019b) In Turkey 2017, high rates of *staph aureus* high resiste to penicillin and ampicillin (Yılmaz and Aslantaş 2017) A study in Isfahan, Iran, in 2018 showed a nasal carriage of MRSA 51.9% among patient and 16% among health workers (Moshtagheian et al. 2018) MRSA prevalence in wounds was high, and this is consistent with previous studies, 76.9% (Khanal and Jha 2010); 44% (Tyagi, Kapil, and Singh 2008); 60.1% (Orrett and Land 2006); 34.8% (Hafeez, Chughtai, and Aslam 2004) The presence of MRSA in wounds delays healing (Solomkin 2001). In other study the average of MRSA rate for Wound Infections After Cardiothoracic Surgery was 54% in a three-year period from 2007 to 2010 (Walsh, Greene, and Kirshner 2011) During the present study, MRSA isolate from HCWs noses was (19.4%) Which corresponds to previous studies (Caceres 2011); (Shittu et al. 2011) and (Munoz et al. 2008); 12.7% (Shibabaw, Abebe, and Mihret 2013) 12% in (Ibarra et al. 2008); 14.3% (Radhakrishna et al. 2013), MRSA rate was low in other studies; only 5.3% in Iran study (Askarian et al. 2009); and it was 0% in Kenya (Omuse, Kariuki, and Revathi 2012) With increasing of MRSA colonization rate, there is greater risk in developing drug-resistant wound infections. Therefore, it is necessary to avoid infection as much as possible. Previous reserchs have shown a large infections rate due to cross-contamination by hands of health staff (Rotter and Koller 1991) One of the most efficient ways to detect MRSA isolates is to investigate the *MecA* gene. (Dive et al. 2013); (Sobhy et al. 2012); (Anand et al. 2009); (Shittu, Lin, and Morrison 2007) and (Van Leeuwen et al. 1999) The *MecA* gene located in the *SCC*mec resistance island, encodes for alter protein (PBP2a), which has the lowest β-lactam potential (Hiramatsu et al. 2014) All MRSA isolates were tested for presence of *Hld* gene. All isolates and 100% contained *MecA* gene, Followed by *Hld* (61%) , All MRSA isolate in our study harboring *MecA* gene. This result agreed with many other studies that showed all MRSA isolate harboring *MecA* gene (Yang et al. 2020); (Mussa and Al-mathkhury 2018); (Karmakar, Dua, and Ghosh 2016)(Al-Charrakh, Al-Hassnawi, and Al-Khafaji 2015); (Dağı et al. 2015) Other studies considered that Methicillin resistance can be happen in *MecA* absence, MRSA could have other mechanism(s) for resistance; e.g., altered target site or may be reduced drug accumulation. *MecA* gene absence may also due to a technical error upon detection. (Mahdi et al. 2016); (Carpaij et al. 2011) and (Wielders et al. 2002) Concerning the *Hld* gene ,Delta hemolysins is 26 peptide amino acids encoded by the hld gene, the mechanism of secreting delta toxin was not yet been understood (Shumba, Mairpady Shambat, and Siemens 2019) high prevalent of Hld gene reported in our study (61.5%), The result was similar to (Motallebi et al. 2019) and (84.2%) (Hoseini Alfatemi et al. 2014) The highest rate of *Hld* prevalence in our study was in the hospital environment (100%) in (Wards and Operating Theaters). The percentage 100% was recorded also in many studies (Shukla et al. 2010) and (Kateete et al. 2011)

References:

[1.] Al-azawi, Noor, K. Mohammed Ali, Alaa Alwan, S. B. I. Al-rifai, and A. Al-bayati. 2016. "Antibiotic Resistance Pattern of HA-MRSA Strains Isolated from Leukemia Patients in Baghdad, Iraq." *International Journal of Infectious Diseases* 45:81–82.

[2.] Al-Charrakh, Alaa H., Huda H. Al-Hassnawi, and Jawad K. Al-Khafaji. 2015. "Molecular Characteristics of Community-Associated Methicillin-Resistant Staphylococcus Aureus (CA-MRSA) Isolates from Clinical Specimens in Iraq." *British Microbiology Research Journal* 5(3):227.

[3.] Al-dahbi, Ali M. and Harith J. Al-mathkhury. 2013. "Distribution of Methicillin Resistant Staphylococcus Aureus in Iraqi Patients and Healthcare Workers." *Iraqui Journal of Science* 54(2):293–300.

[4.] Al-Maliki, A. A. A. 2009. "A Study of Some Methicillin-Resistant Staphylococci (MRSA) and (MRSE) Isolated from Baghdad Hospital Patients."

[5.] Alkharsah, Khaled R., Suriya Rehman, Fatimah Alkhamis, Amani Alnimr, Asim Diab, and Amein K. Al-Ali. 2018. "Comparative and Molecular Analysis of MRSA Isolates from Infection Sites and Carrier Colonization Sites." *Annals of Clinical Microbiology and Antimicrobials* 17(1):1–11.

[6.] AL-SAIMARY IHSAN E. ANTIBIOGRAM AND MULTIDRUG RESISTANCE PATTERNS OF STAPHYLOCOCCUS AUREUS (MDRSA) ASSOCIATED WITH POST OPERATIVE WOUND INFECTIONS IN BASRAH – IRAQ. JOURNAL OF ISLAMIC WORLD ACADEMY OF SCIENCES 20:2, 57-66, 2012

[7.] AL-SAIMARY IHSAN E . PREVALENCE OF B-LACTAMASE PRODUCING AND NON-PRODUCING STAPHYLOCOCCUS AUREUS ASSOCIATED WITH PATIENTS IN INTENSIVE CARE UNITE. MEDICAL JOURNAL OF ISLAMIC WORLD ACADEMY OF SCIENCES 20:1, 17-28, 2012.

[8.] Anand, K. B., P. Agrawal, S. Kumar, and K. Kapila. 2009. "Comparison of Cefoxitin Disc Diffusion Test, Oxacillin Screen Agar, and PCR for MecA Gene for Detection of MRSA." *Indian Journal of Medical Microbiology* 27(1):27.

[9.] Askarian, Mehrdad, Alihosein Zeinalzadeh, Aziz Japoni, Abdolvahab Alborzi, and Ziad A. Memish. 2009. "Prevalence of Nasal Carriage of Methicillin-Resistant Staphylococcus Aureus and Its Antibiotic Susceptibility Pattern in Healthcare Workers at Namazi Hospital, Shiraz, Iran." *International Journal of Infectious Diseases* 13(5):e241–47.

[10.] Bartlett, john M. S. and David Stirling. 2003. *PCR Protocols*. Vol. 226.

[11.] Brown, Derek F. J., David I. Edwards, Peter M. Hawkey, Donald Morrison, Geoffrey L. Ridgway, Kevin J. Towner, and Michael W. D. Wren. 2005. "Guidelines for the Laboratory Diagnosis and Susceptibility Testing of Methicillin-Resistant Staphylococcus Aureus (MRSA)." *Journal of Antimicrobial Chemotherapy* 56(6):1000–1018.

[12.] Caceres, Mercedes. 2011. "Frequency of Nasal Carriers of Methicillin-Resistant Staphylococcus Aureus among Health Workers in Nicaraguan Hospitals." *Revista Panamericana de Salud Publica= Pan American Journal of Public Health* 30(6):610–14.

[13.] Carpaij, Neeltje, Rob J. L. Willems, Thomas W. Rice, Robert A. Weinstein, Jason Hinds, Adam A. Witney, Jodi A. Lindsay, Marc J. M. Bonten, and Ad C. Fluit. 2011. "Genetic Variation in Spatio-Temporal Confined USA300 Community-Associated MRSA Isolates: A Shift from Clonal Dispersion to Genetic Evolution?" *PLoS One* 6(2).

[14.] Dağı, Hatice Türk, Duygu Fındık, Gamze Demirel, and Uğur Arslan. 2015. "Detection of Methicillin Resistance and Various Virulence Factors in Staphylococcus Aureus Strains Isolated from Nasal Carriers." *Balkan Medical Journal* 32(2):171–75.

[15.] Dai, Yingxin, Junlan Liu, Wei Guo, Hongwei Meng, Qian Huang, Lei He, Qianqian Gao, Huiying Lv, Yao Liu, and Yanan Wang. 2019. "Decreasing Methicillin-Resistant Staphylococcus Aureus (MRSA) Infections Is Attributable to the Disappearance of Predominant MRSA ST239 Clones, Shanghai, 2008–2017." *Emerging Microbes & Infections* 8(1):471–78.

[16.] Dive, Georges, Camille Bouillon, Aline Sliwa, Bénédicte Valet, Olivier Verlaine, Eric Sauvage, and Jacqueline Marchand-Brynaert. 2013. "Macrocycle-Embedded β-Lactams as Novel Inhibitors of the Penicillin Binding Protein PBP2a from MRSA." *European Journal of Medicinal Chemistry* 64:365–76.

[17.] Garazi, Michele, Barbara Edwards, Donna Caccavale, Charles Auerbach, and Gisele Wolf-Klein. 2009. "Nursing Homes as Reservoirs of MRSA: Myth or Reality?" *Journal of the American Medical Directors Association* 10(6):414–18.

[18.] Gillet, Yves, Bertrand Issartel, Philippe Vanhems, Jean Christophe Fournet, Gerard Lina, Michèle Bes, François Vandenesch, Yves Piémont, Nicole Brousse, Daniel Floret, and Jerome Etienne. 2002. "Association between Staphylococcus Aureus Strains Carrying Gene for Panton-Valentine Leukocidin and Highly Lethal Necrotising Pneumonia in Young Immunocompetent Patients." *Lancet* 359(9308):753–59.

[19.] Goud, Rajendra, Soham Gupta, Ujjwal Neogi, Deepali Agarwal, Kesava Naidu, Raju Chalannavar, and Gaddad Subhaschandra. 2011. "Community Prevalence of Methicillin and Vancomycin Resistant Staphylococcus Aureus in and around Bangalore, Southern India." *Revista Da Sociedade Brasileira de Medicina Tropical* 44(3):309–12.

[20.] Hafeez, Rubeena, A. S. Chughtai, and M. Aslam. 2004. "Prevalence and Antimicrobial Susceptibility of Methicillin Resistant Staphylococcus Aureus (MRSA)." *Int J Pathol* 2(1):10–15.

[21.] Hau, Samantha Jean. 2017. "Genetic and Phenotypic Characterization of Livestock Associated Methicillin Resistant *Staphylococcus Aureus* Sequence Type (ST) 5 in Comparison with Clinical ST5 Isolates from Humans." *ProQuest Dissertations and Theses* 238.

[22.] Hiramatsu, K., Yuki Katayama, M. Matsuo, T. Sasaki, Y. Morimoto, A. Sekiguchi, and T. Baba. 2014. "Multi-Drug-Resistant Staphylococcus Aureus and Future Chemotherapy." *Journal of Infection and Chemotherapy* 20(10):593–601.

[23.] Hoseini Alfatemi, Seyedeh Mahsan, Mohammad Motamedifar, Nahal Hadi, and Hadi Sedigh Ebrahim Saraie. 2014. "Analysis of Virulence Genes among Methicillin Resistant Staphylococcus Aureus (MRSA) Strains." *Jundishapur Journal of Microbiology* 7(6):1–10.

[24.] Hussain, Muhammad Shahbaz, Abbas Naqvi, and Muhammad Sharaz. 2019. "METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)." *The Professional Medical Journal* 26(01):122–27.

[25.] Hussein, Nawfal R., Ahmed Alyas, Marwan Majeed, and Mahde S. Assafi. 2015. "Prevalence Rate and Prevalent Genotypes of Ca-Mrsa in Kurdistan Region: First Report from Iraq." *International Journal of Pure and Applied Sciences and Technology* 27(1):44.

[26.] Ibarra, Maria, Tristan Flatt, Diane Van Maele, Aisha Ahmed, Jaime Fergie, and Kevin Purcell. 2008. "Prevalence of Methicillin-Resistant Staphylococcus Aureus Nasal Carriage in Healthcare Workers." *The Pediatric Infectious Disease Journal* 27(12):1109–11.

[27.] Jahanshahi, Aidin, Habib Zeighami, and Fakhri Haghi. 2018. "Molecular Characterization of Methicillin and Vancomycin Resistant Staphylococcus Aureus Strains Isolated from Hospitalized Patients." *Microbial Drug Resistance* 24(10):1529–36.

[28.] Karmakar, Amit, Parimal Dua, and Chandradipa Ghosh. 2016. "Biochemical and Molecular Analysis of Karmakar, A., Dua, P., & Ghosh, C. (2016). Biochemical and Molecular Analysis of Staphylococcus Aureus Clinical Isolates from Hospitalized Patients. Canadian Journal of Infectious Diseases and Medical Microbiology, 20." *Canadian Journal of Infectious Diseases and Medical Microbiology* 2016.

[29.] Kateete, David P., Sylvia Namazzi, Moses Okee, Alfred Okeng, Hannington Baluku, Nathan L. Musisi, Fred A. Katabazi, Moses L. Joloba, Robert Ssentongo, and Florence C. Najjuka. 2011. "High Prevalence of Methicillin Resistant Staphylococcus Aureus in the Surgical Units of Mulago Hospital in Kampala, Uganda." *BMC Research Notes* 4(1):326.

[30.] Kessel, Carol I. 2017. "MOLECULAR SUBTYPING OF STAPHYLOCOCCUS AUREUS ISOLATES FROM THE UP COMMUNITY FOR THE PRESENCE OF TOXIN-ENCODING GENES."

[31.] Khanal, L. K. and B. K. Jha. 2010. "Prevalence of Methicillin Resistant Staphylococcus Aureus (MRSA) among Skin Infection Cases at a Hospital in Chitwan, Nepal." *Nepal Medical College Journal : NMCJ* 12(4):224–28.

[32.] King, Alyssa N. 2020. "Transcription Factors, Sigma Factors, and Regulatory RNAs Coordinate and Integrate the Staphylococcus Aureus Stress Response."

[33.] Kırmusaoğlu, Sahra. 2017. "MRSA and MSSA: The Mechanism of Methicillin Resistance and the Influence of Methicillin Resistance on Biofilm Phenotype of Staphylococcus Aureus." *The Rise of Virulence and Antibiotic Resistance in Staphylococcus Aureus; Enany, S., Ed* 25–41.

[34.] Van Leeuwen, Willem B., Cindy Van Pelt, A. D. Luijendijk, Henri A. Verbrugh, and Wil H. F. Goessens. 1999. "Rapid Detection of Methicillin Resistance in Staphylococcus Aureus Isolates by the MRSA-Screen Latex Agglutination Test." *Journal of Clinical Microbiology* 37(9):3029–30.

[35.] Lindsay, Jodi A., Alexey Ruzin, Hope F. Ross, Natasha Kurepina, and Richard P. Novick. 1998. "The Gene for Toxic Shock Toxin Is Carried by a Family of Mobile Pathogenicity Islands in Staphylococcus Aureus." *Molecular Microbiology* 29(2):527–43.

[36.] Luo, Kui, Fuye Shao, Kadijatu N. Kamara, Shuaiyin Chen, Rongguang Zhang, Guangcai Duan, and Haiyan Yang. 2018. "Molecular Characteristics of Antimicrobial Resistance and Virulence Determinants of Staphylococcus Aureus Isolates Derived from Clinical Infection and Food." *Journal of Clinical Laboratory Analysis* 32(7):e22456.

[37.] Mahdi, W. K., Noha A. Hassuna, Mona A. Esmail, Safaa S. Hammad, and Sayed F. Abdelwahab. 2016. "Molecular Typing of Methicillin Resistant Staphylococcus Aureus Colonizing Egyptian Healthcare Workers and Patients." *Int. J. Curr. Microbiol. App. Sci* 5(6):687–98.

[38.] Mahmoudi, Shima, Setareh Mamishi, Mohsen Mohammadi, Maryam Banar, Mohammad Taghi Haghi Ashtiani, Masoumeh Mahzari, Abbas Bahador, and Babak Pourakbari. 2019. "Phenotypic and Genotypic Determinants of Mupirocin Resistance among Staphylococcus Aureus Isolates Recovered from Clinical Samples of Children: An Iranian Hospital-Based Study." *Infection and Drug Resistance* 12:137.

[39.] Moshtagheian, S., M. Halaji, H. Sedaghat, M. Shahin, B. Nasr Esfahani, S. R. Havaei, and S. A. Havaei. 2018. "Molecular Characteristics of Methicillin-Resistant Staphylococcus Aureus Nasal Carriage from Hospitalized Patients and Medical Staff in Isfahan, Iran." *Ann. Ig* 30(3):237–44.

[40.] Motallebi, Mitra, Fereshteh Jabalameli, Reza Beigverdi, and Mohammad Emaneini. 2019. "High Prevalence of Direct Repeat Unit Types of 10di, 8 h and 8i among Methicillin Resistant Staphylococcus Aureus Strains with Staphylococcal Cassette Chromosome Mec Type IIIA Isolated in Tehran, Iran." *Antimicrobial Resistance & Infection Control* 8(1):50.

[41.] Munoz, P., J. Hortal, M. Giannella, J. M. Barrio, M. Rodríguez-Créixems, M. J. Pérez, C. Rincón, and E. Bouza. 2008. "Nasal Carriage of S. Aureus Increases the Risk of Surgical Site Infection after Major Heart Surgery." *Journal of Hospital Infection* 68(1):25–31.

[42.] Mussa, Ali A. and Harith Jabbar Fahad Al-mathkhury. 2018. "Incidence of Ciprofloxacin-Resistant of Methicillin Resistant Staphylococcus Aureus Isolated from Iraqi Patients." *Iraqi Journal of Science* 59(3A):1225–30.

[43.] Nicholas P. Vitko and Anthony R. Richardson. 2014. "The Levels of Use of Opioids, Amphetamines and Cocaine and Associated Levels of Harm: Summary of Scientific Evidence." (March 2014):1–21.

[44.] Omuse, Geoffrey, S. Kariuki, and Gunturu Revathi. 2012. "Unexpected Absence of Meticillin-Resistant Staphylococcus Aureus Nasal Carriage by Healthcare Workers in a Tertiary Hospital in Kenya." *Journal of Hospital Infection* 80(1):71–73.

[45.] Orrett, Fitzroy A. and Michael Land. 2006. "Methicillin-Resistant Staphylococcus Aureus Prevalence: Current Susceptibility Patterns in Trinidad." *BMC Infectious Diseases* 6:1–6.

[46.] Pain, Maria Charlene Ronessen. 2020. "Exploring the Pangenome of Staphylococcus Haemolyticus.

Colonisation, Hospital Adaption, Pathogenicity and Novel Species Identification."

[47.] Peck, Kyong Ran, Jin Yang Baek, Jae-Hoon Song, and Kwan Soo Ko. 2009. "Comparison of Genotypes and Enterotoxin Genes between Staphylococcus Aureus Isolates from Blood and Nasal Colonizers in a Korean Hospital." *Journal of Korean Medical Science* 24(4):585–91.

[48.] Radhakrishna, M., Monalisa D'Souza, Subbannayya Kotigadde, Vishwas Saralaya, and Shashidar Kotian. 2013. "Prevalence of Methicillin Resistant Staphylococcus Aureus Carriage amongst Health Care Workers of Critical Care Units in Kasturba Medical College Hospital, Mangalore, India." *Journal of Clinical and Diagnostic Research: JCDR* 7(12):2697.

[49.] Reddy, Prakash Narayana, Krupanidhi Srirama, and Vijaya R. Dirisala. 2017. " An Update on Clinical Burden, Diagnostic Tools, and Therapeutic Options of Staphylococcus Aureus ." *Infectious Diseases: Research and Treatment* 10:117991611770399.

[50.] Rotter, M. L. and W. Koller. 1991. "European Test for the Evaluation of the Efficacy of Procedures for the Antiseptic Handwash." *Hyg. Med* 16:4–12.

[51.] Schubert, Melanie, Daniel Kämpf, Marlena Wahl, Samuel Hofmann, Maria Girbig, Lutz Jatzwauk, Claudia Peters, Albert Nienhaus, and Andreas Seidler. 2019. "MRSA Point Prevalence among Health Care Workers in German Rehabilitation Centers: A Multi-Center, Cross-Sectional Study in a Non-Outbreak Setting." *International Journal of Environmental Research and Public Health* 16(9):1660.

[52.] Shibabaw, Agumas, Tamrat Abebe, and Adane Mihret. 2013. "Nasal Carriage Rate of Methicillin Resistant Staphylococcus Aureus among Dessie Referral Hospital Health Care Workers; Dessie, Northeast Ethiopia." *Antimicrobial Resistance and Infection Control* 2(1):25.

[53.] Shien, Lee Lian. 2014. "Comparative Genomic of Methicillin Resistant Staphylococcus Aureus Pr01 (MRSA PR01) and Methicillin Sensitive Staphylococcus Aureus SA D22901 (MSSA SAD22901) and Methicillin Resistant Derivatives of the Latter."

[54.] Shittu, Adebayo, Johnson Lin, and Donald Morrison. 2007. "Molecular Identification and Characterization of Mannitol-Negative Methicillin-Resistant Staphylococcus Aureus." *Diagnostic Microbiology and Infectious Disease* 57(1):93–95.

[55.] Shittu, Adebayo O., Kenneth Okon, Solayide Adesida, Omotayo Oyedara, Wolfgang Witte, Birgit Strommenger, Franziska Layer, and Ulrich Nübel. 2011. "Antibiotic Resistance and Molecular Epidemiology of Staphylococcus Aureusin Nigeria." *BMC Microbiology* 11(1):92.

[56.] Shukla, Sanjay K., Mary E. Karow, Jennifer M. Brady, Mary E. Stemper, Jennifer Kislow, Natalie Moore, Katherine Wroblewski, Po-Huang Chyou, David M. Warshauer, and Kurt D. Reed. 2010. "Virulence Genes and Genotypic Associations in Nasal Carriage, Community-Associated Methicillin-Susceptible and Methicillin-Resistant USA400 Staphylococcus Aureus Isolates." *Journal of Clinical Microbiology* 48(10):3582–92.

[57.] Shumba, Patience, Srikanth Mairpady Shambat, and Nikolai Siemens. 2019. "The Role of Streptococcal and Staphylococcal Exotoxins and Proteases in Human Necrotizing Soft Tissue Infections." *Toxins* 11(6):332.

[58.] Sobhy, Nagat, Fatma Aly, Ola Abd El Kader, Abeer Ghazal, and Amira Elbaradei. 2012. "Community-Acquired Methicillin-Resistant Staphylococcus Aureus from Skin and Soft Tissue Infections (in a Sample of Egyptian Population): Analysis of Mec Gene and Staphylococcal Cassette Chromosome." *Brazilian Journal of Infectious Diseases* 16(5):426–31.

[59.] Solomkin, Joseph S. 2001. "Antibiotic Resistance in Postoperative Infections." *Critical Care Medicine* 29(4):N97–99.

[60.] Suhaili, Zarizal, Putri' Amira Rafee, Norhidayah Mat Azis, Chew Chieng Yeo, Syafinaz Amin Nordin, Abdul Rachman Abdul Rahim, Mazen M. Jami. Al-Obaidi, and Mohd Nasir Mohd Desa. 2018.

"Characterization of Resistance to Selected Antibiotics and Panton-Valentine Leukocidin-Positive Staphylococcus Aureus in a Healthy Student Population at a Malaysian University." *Germs* 8(1):21–30.

[61.] Suzuki, Haruo, Tristan Lefébure, Paulina P. Bitar, and Michael J. Stanhope. 2012. "Comparative Genomic Analysis of the Genus Staphylococcus Including Staphylococcus Aureus and Its Newly Described Sister Species Staphylococcus Simiae." *BMC Genomics* 13(1).

[62.] Tyagi, Arti, Arti Kapil, and Padma Singh. 2008. "Incidence of Methicillin Resistant Stahylococcus Aureus (MRSA) in Pus Samples at a Tertiary Care Hospital, AIIMS, New Delhi." *Journal, Indian Academy of Clinical Medicine* 9(1):33–35.

[63.] Úbeda, Carles, Ma Ángeles Tormo, Carme Cucarella, Pilar Trotonda, Timothy J. Foster, Íñigo Lasa, and José R. Penadés. 2003. "Sip, an Integrase Protein with Excision, Circularization and Integration Activities, Defines a New Family of Mobile Staphylococcus Aureus Pathogenicity Islands." *Molecular Microbiology* 49(1):193–210.

[64.] Vandenesch, François, G. Lina, and Thomas Henry. 2012. "Staphylococcus Aureus Hemolysins, Bi-Component Leukocidins, and Cytolytic Peptides: A Redundant Arsenal of Membrane-Damaging Virulence Factors?" *Frontiers in Cellular and Infection Microbiology* 2(February):12.

[65.] Walsh, Edward E., Linda Greene, and Ronald Kirshner. 2011. "Sustained Reduction in Methicillin-Resistant Staphylococcus Aureus Wound Infections after Cardiothoracic Surgery." *Archives of Internal Medicine* 171(1):68–73.

[66.] Wielders, C. L. C., A. C. Fluit, S. Brisse, J. Verhoef, and F. J. Schmitz. 2002. "MecA Gene Is Widely Disseminated in Staphylococcus Aureus Population." *Journal of Clinical Microbiology* 40(11):3970–75.

[67.] Yan, X., L. M. Schouls, G. N. Pluister, X. Tao, X. Yu, J. Yin, Y. Song, S. Hu, F. Luo, W. Hu, L. He, F. Meng, T. Donker, E. Tsompanidou, J. M. van Dijl, J. Zhang, and H. Grundmann. 2016. "The Population Structure of Staphylococcus Aureus in China and Europe Assessed by Multiple-Locus Variable Number Tandem Repeat Analysis; Clues to Geographical Origins of Emergence and Dissemination." *Clinical Microbiology and Infection* 22(1):60.e1-60.e8.

[68.] Yang, Dingyi, Yin Xin Ho, Laura M. Cowell, Iqra Jilani, Simon J. Foster, and Lynne R. Prince. 2019. "A Genome-Wide Screen Identifies Factors Involved in S. Aureus-Induced Human Neutrophil Cell Death and Pathogenesis." *Frontiers in Immunology* 10:45.

[69.] Yang, Feng, Shidong Zhang, Xiaofei Shang, Hongsheng Li, Hang Zhang, Dongan Cui, Xurong Wang, Ling Wang, Zuoting Yan, and Yan Sun. 2020. "Short Communication: Detection and Molecular Characterization of Methicillin-Resistant Staphylococcus Aureus Isolated from Subclinical Bovine Mastitis Cases in China." *Journal of Dairy Science* 103(1):840–45.

[70.] Yılmaz, Ebru Şebnem and Özkan Aslantaş. 2017. "Antimicrobial Resistance and Underlying Mechanisms in Staphylococcus Aureus Isolates." *Asian Pacific Journal of Tropical Medicine* 10(11):1059–64.

[71.] Zhu, Yefei. 2010. "Staphylococcus Aureus Virulence Factors Synthesis Is Controlled by Central Metabolism."