

Occurrence of *Staphylococcus aureus* among Inpatients of a Tertiary Hospital in Jalingo, Taraba State, Nigeria

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ABSTRACT

Patients stay longer on admission as a result of being infected by certain pathogens like *S.aureus*. One hundred isolates were obtained from clinical specimens of patients who had stayed for not less than 72 hours in male and female wards of Specialist hospital, Jalingo. The specimens used were sputum, nasalswab, throat swab and wound swab. Standard procedures were used in the isolation and identification of the bacteria as well as in determining the antimicrobial resistance profile. However, resistance to cefoxitin was used to identify the MRSA. Nevertheless, 21% of the isolates were *S. aureus* while 61.9% of the *S.aureus* were MRSA..Though, the prevalence of *S.aureus* was highest (23.1%) in the nasal swab of inpatients aged 46-55($p=0.054$),wound swab(17.4%) of inpatients aged 26-35($p=0.254$),throat swab(6.5%) of inpatients aged 36-45 years ($p=0.935$) and sputum(3.0%) of inpatients aged 36-45 and 76-85($p=0.009$), all the *S.aureus* isolated from the nares were MRSA. All the *S.aureus* were multi drug resistant-they were respectively 76.2%,90.5%,81.0%,85.7%,47.6% and 52.3% resistant to gentamycin (10 μ g), ciprofloxacin (20 μ g), streptomycin (10 μ g), erythromycin(10 μ g), cefuroxime (30 μ g) and levofloxacin (20 μ g).However,100% resistance was observed in Ampiclox(30 μ g), Amoxicillin(30 μ g). Zinnocef (20 μ g), Ceftriaxone (20 μ g) and cefoxime (10 μ g).It is therefore recommended that further study be carried out to find out whether there is a relationship between the prevalence of MRSA and the site of isolation(specimen). Also, there is need to find out if there are conditions in the nares that promote the acquisition of mec A gene by *S.aureus*.

Key Words: *Staphylococcus aureus*, Inpatients, Specimens, Age, Antibiotic Resistance, sputum, wounds, nares. throat swab.

INTRODUCTION

Staphylococcus aureus is one of the main causes of life threatening blood stream infections such as sepsis and endocarditis (Kwiencki and Horswill,2020).MRSA , a superbug, causes antibiotic resistance in both hospital and community acquired infections.*Staphylococcus aureus*, an opportunistic bacterium, becomes methicillin resistant when the gene responsible for methicillin resistance, *mec A*, which is carried by a DNA fragment known as staphylococcal cassette chromosome *mec* (SCC*mec*) is acquired. This gene encodes a protein called penicillin-binding protein (PBP-2a). The PBP-2a binds β -lactams with lower avidity, which results in resistance to this class of antibiotic agents (Bhatta *et al.*,2016; Hussein ,2016)

Staphylococcus aureus is of public health significance due to the combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Qiu *et al.*, 2010). Although it may be part of the normal human microbiota, it can cause wide range of diseases from skin and soft-tissue infections (STIs) to severe invasive disease such as infective endocarditis, osteomyelitis, and toxic shock syndrome (Corrado *et al.*,2016).*Staphylococcus aureus* is also a major cause of food-borne illness worldwide (Hennekinne *et al.*,2012).

Staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element, carries the *mec A* gene which is responsible for methicillin resistance. Due to the structural organization and genetic content of SCC*mec*, they are classified into 11 different types (SCC*mec*I-XI) (Furuno *et al.*, 2005; Ito *et al.*, 2004). *Staphylococcus* is one of the most common causes of nosocomial and community-acquired infections (Asadollahi *et al.*, 2018), while MRSA is one of the most important nosocomial pathogens that can cause healthcare-associated infections (Chen *et al.*, 2014; Monecke *et al.*, 2014).

Hospital-acquired infections (healthcare-associated infections) are infections that are not present or incubating at the time of admission to a hospital (Monegro *et al.*, 2023). Patients are said to have been infected with nosocomial bacteria such as MRSA when they have stayed in the hospital for at least three days and a disease which was neither there or incubating at the time of admission surfaced. Bacteria usually implicated in hospital acquired infections include MRSA, extended spectrum beta lactamase producing *Escherichia coli*. Patients that are vulnerable to hospital acquired infections such as MRSA are those that have stayed for a long time in the hospital, the aged, immunocompromised etc (Sydnor and Perl, 2011).

Community associated MRSA (CA-MRSA) infections can occur in healthy individuals (Herold *et al.*, 1998), suggesting that these strains have greater virulence. Skin and soft-tissue infections represent about 90% of cases of CA-MRSA infection, mostly characterized by abscesses or cellulitis with purulent drainage (DeLeo *et al.*, 2010).

Virulence in *Staphylococcus* is linked with the ability to secrete the exotoxin Pantone-Valentine leukocidin (PVL) which induces lysis of monocytes and neutrophil granulocytes (Loffler *et al.*, 2010). PVL is one of the most important virulence factors of *S. aureus*. This beta pore forming cytotoxin is associated with tissue necrosis and also causes disruption of leukocyte membranes (Shrestha *et al.*, 2014). PVL carrying *S. aureus* is responsible for different life-threatening invasive diseases, and also skin and soft tissue infections. PVL-SA infected skin is red and inflamed with pus. It can have different other appearances like cellulitis, abscesses, boils, folliculitis, etc. At first, PVL carrying *S. aureus* infects skin and soft tissues but the infection gradually spreads to the lung and disrupts the lung tissues, causing hemorrhagic necrotizing pneumonia, one of the most lethal diseases caused by *S. aureus* (Mc Grath *et al.*, 2008). Additionally, there is evidence that PVL-positive methicillin susceptible *Staphylococcus aureus* (MSSA) may be reservoirs for the development of PVL-positive MRSA via the integration of the staphylococcal cassette chromosome *mec* (SCC*mec*) elements including the *mecA* gene conferring methicillin resistance [19] (Rasigade *et al.*, 2010).

The occurrence of *Staphylococcus aureus* in hospital is not a case that should be overlooked because of its ability to cause morbidity and mortality among patients. Hence the aim of this study, to assess the occurrence of *S. aureus* among inpatients in specialist hospital, Jalingo, Taraba State.

Study area

Specialist hospital, Jalingo, located in the outskirts of Jalingo Metropolis was the study area. This hospital is a state government owned tertiary hospital well patronized by residents of Jalingo and its environs. Referrals are also made to the Specialist hospital.

Study Design

The study was an experimental design. The subjects who were all inpatients who had been hospitalized for at least three days were randomly selected after receiving their informed consent.

Study Population

All the inpatients in male and female wards who had been hospitalized for a minimum of three days within

the study period constituted the population of study.

Specimen Collection

Specimens were collected from the anterior nares and throats of the patients using sterile polyester swabs with a standard rotating technique. The swabs were placed in 0.5 ml of sterile water and 0.1 ml of the resulting suspension was streaked onto blood agar. Also, specimens were collected from sputum and wound of patients.

Identification of the Isolates

All the specimen were separately cultured on blood agar (one plate for each specimen) and incubated at 37°C for 18-24 hours. The morphology of the colonies were noted and biochemical tests were carried on the pure cultures. Then, the bacterial organisms were gram stained and viewed under the microscope.

Isolation and Identification of *S. aureus*

The young cultures of the specimens were inoculated onto mannitol salt agar and incubated for 18-24 hours at 37°C [20]. All yellow pigmented colonies were inoculated on nutrient agar. The resulting growth from respective plates of media were again examined for colony characteristic and morphology. Each bacterium was Gram stained and tested for production of catalase, free coagulase, yellow pigment, and thermo nuclease (TNase) according to method described by Lancette and Tatini (1992).

Antimicrobial Sensitivity of Isolates

Three colonies of each 24 hours pure culture on Nutrient agar was emulsified and adjusted to 0.5 McFarland standard. Each standard inoculum suspension was used to flood the surface of Mueller Hinton agar plate and allowed to dry for 4 minutes. A sterile standard antibiotic discs of peflacine (10µg), gentamicin (10µg), ampiclox (30µg), Zinnocef (20µg), amoxicillin (30µg), Rocephin (25mg), ciprofloxacin (20µg), streptomycin (10µg), septrim, erythromycin (10µg), ceftriaxone (20µg), Imipenem (10µg), cefuroxime (30µg), cefoxime (10µg) and, Levofloxacin (20µg), was placed on the inoculated plates. A maximum of six discs were placed on each plate. The zone of inhibition were measured using a transparent meter rule. The sensitivity of each bacterium to each antibiotic was interpreted as sensitive and resistance.

Detection of MRSA by cefoxitin sensitivity assays

Each colony of the *S. aureus* on nutrient agar was suspended in 4ml sterile normal saline and the inoculums density adjusted to 0.5 McFarland turbidity standards. The surface of Mueller Hinton agar was flooded with the standardized bacterial suspension and allowed to dry for 4 minutes. A sterile standard discs of cefoxitin (30µg) was placed on the inoculated plates. The plates were incubated at 37°C for 24 hours. MRSA was identified on the basis of measurement of zone of inhibition produced by cefoxitin (<21 mm). ATCC culture of *S. aureus* (43300) was used as a positive control (Clinical Laboratory Standards Institute, 2007). The MRSA obtained from the hospitalized patients were regarded as health care acquired HA-MRSA

Statistical analysis

The data obtained was presented in frequency and percentage and the inferential statistics determined by Chi-square test using the statistical package for social sciences (SPSS v.22).

Ethical Clearance

Ethical approval was obtained from the Taraba State ministry of Health as well as the the management of

Taraba State Specialist hospital.

Informed Consent

The informed consent of the study participants were obtained.

RESULTS

The prevalence of *S.aureus* was 21%. However, 8 (61.5%) of the isolates from the nares were *S.aureus*; the highest being 15.4% from adults aged 26-35 and 18-25 ($p=0.054$) while 2 (6.1%) of the isolates from the sputum were *S.aureus*. Nevertheless, 5 (16.1%) of the isolates obtained from throat swabs were *S.aureus* while the highest number of the isolates (2) were separately obtained from the age groups 18-25 and 36-45 (0.935). Though the prevalence of the *S.aureus* obtained from the wounds were 26.1%, the highest percentage (17.4%) was obtained from the age group 26-35 as presented in table 1

AGES	NASAL SWAB		SPUTUM		THROAT		WOUND	
	NO	YES	NO	YES	NO	YES	NO	YES
18-25	0	2(15.4%)	8(24.2%)	0	11(35.5%)	2(6.5%)	6(26.1%)	1(4.3%)
26-35	0	2(15.4%)	6(18.2%)	0	3(9.7%)	1(3.2%)	3(13.0%)	4(17.4%)
36-45	1(3.0%)	1(7.7%)	9(27.3%)	1(3.0%)	5(16.1%)	2(6.5%)	5(38.5%)	1(4.3%)
46-55	0	3(23.1%)	1(30.3%)	0	1(3.2%)	0	2(8.7%)	0
56-65	2(6.1%)	0	3(9.1%)	0	2(6.5%)	0	1(4.3%)	0
66-75	2(6.1%)	0	4(12.12%)	0	1(3.2%)	0	0	0
76-85	0	0	0	1(3.0%)	1(3.2%)	0	0	0
Above 85	0	0	0	0	2(6.5%)	0	0	0
TOTAL	5(38.5%)	8(61.5%)	31(93.9%)	2(6.1%)	26(83.9%)	5(16.1%)	17(73.9%)	6(26.1%)
PVALUE	0.054		0.009		0.935		0.254	

The prevalence of MRSA was 52.4%, However, all (100%) of the *S.aureus* isolated from the nares were MRSA while no MRSA was obtained from the sputum. Meanwhile, 2 (20%) and 3 (50%) MRSA were isolated from the throats and wounds respectively. This is presented in Table 2.

Table 2: The Prevalence of MRSA isolated from male and female inpatients in Specialist Hospital Jalingo

Specimen	Gender	Total Number of isolates	<i>Staphylococcus aureus</i>	MRSA
Nasal	Male	8(61.5%)	4(50%)	4(100%)
Swab	Female	5(38.5%)	4(80%)	4(100%)
Sputum	Male	13(39.4%)	1(7.7%)	0
	Female	20(60.6%)	1(5%)	0
Throat	Male	17(54.8%)	2(11.8%)	2(100%)

	Female	14(45.2%)	3(21.4%)	0
wound	Male	12(52.2%)	2(16.7%)	2(100%)
	Female	11(47.8%)	4(36.4%)	1{25%}
Total		100 (100%)	21(21%)	11(52.4%)

The *S.aureus* were respectively 76.2%,90.5%,81.0%,85.7%,47.6% and 52.3% resistant to gentamycin (10µg), ciprofloxacin (20µg) ,streptomycin (10µg), erythromycin (10µg), cefuroxime and levofloxacin. However, 100% resistance was observed in Ampiclox (30µg), Amoxicillin (30µg). Zinnocef, Seprtim, Cefriaxone (20µg) and cefoxime as presented in Table 3 below.

Table 3: The antibiotic profile of the *Staphylococcus aureus* isolated from inpatients in Specialist Hospital Jalingo

ANTIBIOTICS(µg}	SENSITIVE	RESISTANCE
PEFLACINE(10µg)	0%	21(100%)
GENTAMYCIN(10 µg)	5(23.8%)	16(76.2%)
AMPICLOX(30µg)	0%	21(100%)
ZINNOCEF(20µg)	0%	21(100%)
AMOXACILLIN(30µg)	0%	21(100%)
ROCEPHIN (25mg)	2(9.5%)	19(90.5%)
CIPROFLOXACIN(20µg)	2(9.5%)	19(90.5%)
STREPTOMYCIN (10µg)	4(19.0%)	17(81.0%)
ERYTHROMYCIN(10µg)	3(14.3%)	18(85.7%)
CEFRIAXONE(20µg)	0%	21(100%)
IMIPENEM(10µg)	3(14,3%)	18(85.7%)
CEFUROXIME(30µg)	11(52.3%)	10(47.6%)
CEFOXIME(10µg)	0%	21(100%)
LEVOFLOXACIN(20µg)	10(47.6%)	13(61.9%)
AUGMENTIN (20/10µg)	6(28.6%)	15(71.4%)
PENICILLIN(30µg)	0%	21(100%)

DISCUSSIONS

The prevalence of *S.aureus* among inpatients in male and female wards of the specialist hospital, Jalingo was 21%.The prevalence in the nares , sputums ,throats and wounds were respectively 61.5% ,6.1% ,16.1% and 26.1%.The prevalence was highest in the nares because the nose is one of the natural habitats of *S.aureus*. Moreso, *S.aureus* was more prevalent among younger adults(18-45years) compared to the older adults(46 and above)(Table 1).This could be because younger adults are more active and are more likely to expose themselves to conditions or environments that can get them contaminated. Even among the younger adults ,the prevalence was higher among among the specimen collected from the nares and the wounds. *S.aureus*

was hardly isolated from the sputum even among the younger adults. The researchers therefore are questioning whether there is/are chemical/s or conditions in the sputum that hinder *S.aureus* the growth or survival of *S.aureus*. Udobiet *al.*,(2013) , reported that 44(23.8 %) of the *S.aureus* isolated from Ahmadu bello university Zaria were obtained from wounds. However, in this study, it was observed that 6(28.6%) of the *S.aureus* were obtained from wounds. The differences in the values obtained in the two studies can be attributed to the differences in the sample sizes. Moreover, in this study, samples were collected only from inpatients who had been hospitalized for more than 72 hours. On the other hand, Adeiza *et al.*,(2020) reported the prevalence of *S.aureus* to be 61.8% which is much higher than the prevalence obtained in this study. The reason for this difference could be because only nasal swabs were used in the previous study. On the other hand ,considering only the isolates from the nasal swab, the prevalence of *S.aureus* was 61.5% which is in agreement to a previous report (61.8%) by Adeiza *et al.*,(2020) .In a another similar study, Okedo *et al.*,(2020) reported having isolated 17(33.3%) *Staphylococcus aureus* which is higher than the value(21%) obtained in this current study .One can therefore say that the prevalence of *S.aureus* varies from hospital to hospital and across specimens and individuals.

The prevalence of MRSA in specialist hospital ,Jalingo was 61.9%.The Highest prevalence,100% was obtained from the nasal swabs(Table 2).This could be because the nose is a natural habitat of *S.aureus*..This makes the researchers to raise the eyebrows: Is there any factor/condition in the nares that encourages the acquisition of mec A gene. This notwithstanding, the only two *S.aureus* obtained from the sputum were MSSA. However, Okon *et al.*,(2013) also reported the isolation of 1(1%) of *S.aureus* from sputum. The researchers therefore recommends that much more sputum be sampled for the presence *S.aureus* as well as MRSA. For this will help in drawing conclusion on the prevalence of *S.aureus* and MRSA in sputum. Nevertheless, 2(100%) of the *S.aureus* isolates from the wound swab collected from the male inpatients were MRSA. Though, out of the 4 (36.4%) *S.aureus* isolated from the female inpatients only one(25%) was MRSA.

Udobi, Obajuluwa and Onaokpo,(2013),reported that of the 44(23.8%) *S.aureus* obtained from wounds,33(75%) were MRSA. This differs from the 3(50%) MRSA obtained from the *S.aureus* isolated from wounds. This difference could aside being attributed to the sample sizes, the gap in the time of the studies could have played a major role too. Since recently(2023) more people; patients, patients' relatives and health workers are more knowledgeable on infection prevention and control compared to then(2013). Also, Abdullahi and Iregbu(2018)reported having obtained 97(26.9%) MRSA from 360 *S.aureus* isolates obtained from patients in National hospitals Abuja while in this study,13(61.9%) was obtained from 21(21%) *S.aureus* isolated from male and female inpatients. The differences in the “figures” can be attributed to the differences in the sample size as well as in the facilities in the hospitals. They (Abudullahi and Iregbu,2018) also reported that 25(25.8%) of the isolated MRSA were from wounds while in this study 3(14.3%) of the MRSA isolates were obtained from the wounds and this could have been because the nasal swabs were among the specimens with most of the MRSA isolates being obtained from there. Adeiza, Onalapo and Olayinka,(2020) reported the prevalence MRSA as 46.9% which is lower than 51.9% obtained in this study. The difference in the data could be because only inpatients who had been hospitalized for not less than 72 hours were used in this study. In another similar study carried out in a hospital by Okedo-Alex *et al.*,(2020),the

MRSA obtained among the *S.aureus* was 52.9%(9/17) which is lower than 61.9% obtained in this study. the difference could be because in this stud, inpatients were used.

The *S.aureus* were respectively 76.2%,90.5%,81.0%,85.7%,47.6% and 52.3% resistant to gentamycin (10µg),ciprofloxacin(20µg), streptomycin (10µg), erythromycin(10µg), cefuroxime and levofloxacin. However, 100% resistance was observed in Ampiclox(30µg), Amoxacillin(30µg). Zinnocef, Septrim, Cefriaxone(20µg) and cefoxime. The multidrug resistance observed in the *S.aureus* isolated from the

inpatients was most likely because the bacteria must have acquired antimicrobial resistant plasmids. It could also be because of exposure to various antibiotics used in hospitals. Some of them also possessed intrinsic resistance mechanism. This study agrees with the report of Onwubiko and Sadiq(2024) that *S.aureus*, are multi drug resistance,

The *S.aureus* isolates were 100% resistant to penicillin while Okon *et al.*, [26] reported 92.1% and Abdullahi and Iregbu(2018) reported 100% resistance too. The resistance to gentamicin was 76.2%. However, Okon *et al.*, (2013) reported 14.6% susceptibility while Abdullahi and Iregbu(2018) reported 53.6% resistance. The high resistance observed in gentamicin could be because gentamicin is readily available and very cheap, therefore, it's abuse is very high. Also, a high resistance(90.5%) was observed in ciprofloxacin. This buttresses the previous report by Okon *et al.*, (2013) and Abdullahi and Iregbu(2018). In the same vein, 100% resistance was observed in ampiclox, zinnocof, amoxicillin and cefoxime. In summary, all the *S.aureus* isolates obtained in this study were multidrug resistant. This could be because of the over extensive use of the antibiotics. Moreover, the antibiotics are readily available over the counter.

Yes, the prevalence of *Staphylococcus aureus* and MRSA in the various sites of the body of inpatients was studied. However, the study failed to compare this prevalence to that of outpatients or individuals from the general public. This could have helped to determine whether the prevalence was influenced by the long stay in the health facility. Moreover, no comparison was carried out to compare the influence of the length of stay in hospital to the infection/colonization by *Staphylococcus aureus* /MRSA among the inpatients. Aside this, the researchers did not take note of the condition of the patients (i.e whether they were colonized/infected with *S.aureus*/MRSA) before admission into the hospital.

CONCLUSION

S.aureus colonizes inpatients, some of the *S.aureus* were MRSA. However, all the *S. aureus* isolates were multidrug resistant. Though the results of these findings may or may not be generalized across hospitals depending on the policies already on ground in the health facility as well as the category/class of the health facility. There is still need for infection prevention and control to be intensified in hospitals to curb the spread of these bacteria.

SUGGESTION S FOR FUTURE RESEARCH

It is recommended that the risk factors for *S.aureus* and MRSA colonization of the nares be extensively studied. Moreover, there is need to compare the prevalence of *S.aureus* and MRSA among inpatients to that of the general public/assumed healthy individuals and outpatients.

Conflict Of Interest: The authors declare no conflict of interest.

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REFERENCES

1. Abdullahi N and., Iregbu K. C.(2018) Methicillin resistant *Staphylococcus aureus* in a central Nigeria Tertiary Hospital. *Ann Trop. Pathol.* 9: 6-10. Available from <https://www.atpjournals.org/text.asp?2018/9/1/6/234154>. Doi :10.4103/atp.atp-37-17

2. Adeiza, S. S., Onalapo, J. A. and Olayinka, B. O.(2020). Prevalence, risk factors and antimicrobial susceptibility profile of methicillin resistant *Staphylococcus aureus* (MRSA) obtained from nares of patients and staff of state owned hospitals in Nigeria. *GMSHyg Infect Control*.15;2020 Doi:10.3205/dg000360
3. Asadollahi, P., Farahani, N.N., Mirzaii M, Khoramrooz, S.S., van Belkum, A., Asadollahi, K., Belkum, A. V., Asadollahi, K., Dadashi, M. and Sarokhalif, D. D. (2018) Distribution of the most prevalent spa types among clinical isolates of methicillin-resistant and -susceptible *Staphylococcus aureus* around the world: a review. *Frontiers in Microbiology*, 9 (163): 1–16. doi: 10.3389/fmicb.2018.00163. *International Journal of Infections*, 3 (2) , Article e35375
4. Bhatta, D. R., Cavaco, L. M. and Bhatta, D. R.. (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*, 16: 199
5. Chen, Y., Liu, Z., Duo, L., Xiong, J., Gong, Y., Yang, J. and Wang, Z.(2014). Characterization of *Staphylococcus aureus* from distinct geographic locations in China: an increasing prevalence of spa-t030 and SCCmec type III. *PLoS ONE*. 9(4):e96255. doi: 10.1371/journal.pone.0096255.
6. Clinical Laboratory Standards Institute, Performance Standard for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement M100-S17, Clinical Laboratory Standards Institute, Wayne, Pa, USA, 2007.
7. Corrado, A., Gregorio, E., Bertholeti, S., Seubert. A., Bagnoli, F., Bensi, G. and Chiarot, E.(2016) “*Staphylococcus aureus*-dependent septic arthritis in murine knee joints: Local immune response and beneficial effects of vaccination,” *Scientific Reports*, 6, 38043;doi:10.1038/srep38043
8. DeLeo, F.R., Otto, M., Kreiswirth, B. N. and Chambers, H. F. (2010) Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet*.;375:1557–1568.
9. Furuno, J. P., Perencevich, E. N., Johnson, J. A., Wright, M. O., Mc Gregor, J.C., Morris, J.G., Jr, Strauss, S. M., Roughman, M., Nemoy, L. L., Standiford, H. C., Hebden, J. N. and Harris, A. D.(2005) Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci co-colonization. *Emerging Infectious Diseases*,11(10):1539–1544. doi: 10.3201/eid1110.050508.
10. Hennekinne, J. A., De Buyser M. L, and Dragacci, S.(2012) “*Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation,” *FEMS Microbiology Reviews*, (36)(4): 815–836.
11. Herold, B. C., Immergluck, L. C., Maranan, M. C., Lauderdale, D. S., Gaskin, R. E., Boyle-Vavra, S., Leitch C. D., Daum, R. S (1998) Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA*.;279:593–598.doi:10.1001/jama.279.8.593
12. Hussein, N.R. (2016)Prevalent Genotypes of *Staphylococcus aureus* Strains Isolated From Healthcare Workers in Duhok City, Kurdistan Region, Iraq
13. Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H. and Hiramatsu, K.(2004) Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. *Antimicrobial Agents and Chemotherapy*, 48(7):2637–2651. doi: 10.1128/AAC.48.7.2637-2651.2004.
14. Karmakar A, Jana D, Dutta K, Dua P, Ghosh C.(2018) Prevalence of Panton-Valentine Leukocidin Gene among Community Acquired *Staphylococcus aureus*: A Real-Time PCR Study. *J Pathog*. 2;2018:4518541. doi: 10.1155/2018/4518541. PMID: 30245888; PMCID: PMC6139182.
15. Kwiecinski, J.M. and Horswill, A.R.(2020). *Staphylococcus aureus* bloodstream infections: pathogenesis and regulatory mechanisms. *Curr Opin Microbiol*. 53:51-60. doi: 10.1016/j.mib.2020.02.005. Epub 2020 Mar 12. PMID: 32172183; PMCID: PMC7244392.
16. Lancette ,G. A. and Tatini, S. R. (1992).*Staphylococcus aureus*. In Vanderzant C. Splittstoesser D.F. editors, *Compendium of Methods for the Microbiological Examination of foods*.3rd Washington DC, USA. American Public Health Association.pp533 -550.
17. Loffler, B., Hussain, M., Grundmeier, M., Bruck, M., Holzinger, D., Varga, G., Roth, J., Kahl, B. C.,

- Proctor, R. A.. (2010) Staphylococcus aureus panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathogenesis* 2010;6:e1000715.
18. McGrath, B., Rutledge, F. and Broadfield, E.(2008) “Necrotising Pneumonia,,” *Journal of the Intensive Care Society*, 9, (2):170–172.
19. Monecke, S., Muller, E., Dorneanu, O. S., Vremera, T. and Ehricht, R.(2014) Molecular typing of MRSA and of clinical Staphylococcus aureus isolates from Iasi, Romania. *PLoS ONE*, 9(5):e97833. doi: 10.1371/journal.pone.0097833. 7.
20. Monegro, A.F., Muppidi. V. and Regunath, H.(2023) Hospital-Acquired Infections. In: *Stat Pearls* [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2024 Jan–. PMID: 28722887.
21. Okedo-Alex, I., Ezeanosike, O., Ojide, K., Akamike, I., Ogah, E and Chinwe, O.(2020)Prevalence of Methicillin Resistant Staphylococcus aureus among health care workers in the neonatal unit of a tertiary healthcare setting in Nigeria. *International Journal of Infectious Diseases* .101(1):76
22. Okon, K. O. Shittu, A. O., Usman, H., Adamu, N., Balogun, S. T. and Adesina, O. O. (2013). Epidemiology and Antibiotic susceptibility Pattern of Methicillin -Resistant Staphylococcus aureus recovered from tertiary Hospitals in North Eastern Nigeria. *Journal of Medicine and Medical Sciences*.4(5) : 214-220. Available online: <http://www.interestjournals.org/JMMS>
23. Onwubiko. E. N (and Sadiq. M. N. (2024). Antibiotic Sensitivity Pattern of Staphylococcus aureus from clinical Isolates in a tertiary health Institution in Kano Northwestern Nigeria. *PAMJ* 47/
24. Qiu, J., Feng, H., Lu, J., Xiang, H., Wang, J., WangX., Liu, J., Deng, X.(2010), “Eugenol reduces the expression of virulence-related exoproteins in Staphylococcus aureus,” *Applied and Environmental Microbiology*, 76(17): 5846–5851.
25. Rasigade, J.P., Laurent, F., Lina, G., Meugnier, H., Bes, M., Vandenesch, F., Etienne, J. and Tristan, A. (2010) Global distribution and evolution of Panton-Valentine leukocidin-0positive methicillin-susceptible Staphylococcus aureus, 1981-2007. *Journal of Infectious Diseases*,201:1589–97.
26. Shrestha, B.,W. Singh, V. S. Raj, B. M. Pokhrel, and T. M. Mohapatra, (2014) “High Prevalence of Panton-Valentine leukocidin (PVL) genes in nosocomial-acquired staphylococcus aureus isolated from tertiary care hospitals in Nepal,” *BioMed Research International*, vol. 2014, Article ID 790350, 7 pages.
27. Sydnor, E. R. and Perl. T.M.(2011). Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev.* 24(1):141-73. doi: 10.1128/CMR.00027-10. PMID: 21233510; PMCID: PMC3021207.
28. Udobi, C. E., Obajuluwa, A. F. and Onalapo, J. A. (2013). Prevalence and antibiotic resistant pattern of methicillin resistant Staphylococcus aureus from an Orthopaedic hospital in Nigeria. *Biomed Research International*.vol. 2013. doi.org /10.1155/2013/860467.