ORIGINAL ARTICLE

Phenotypic and Genetic Detection of Healthcare Associated Multi-drug Resistant *Staphylococcus aureus* Infection: A Guideline Step of Infection Control Strategy in an Outbreak Prevention

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ABSTRACT

Background: *Methicillin-resistant Staphylococcus aureus (MRSA) represents a serious causal agent in nosocomial infections that are becoming increasingly difficult to cure due to their emerging drug resistance. Usage of vancomycin for treatment of serious infections caused by MRSA strains become vancomycin-resistant Staphylococcus aureus (VRSA) in clinical settings. Therefore, it becomes essential to understand the epidemiology of MRSA and VRSA.* ***Objectives:*** *This study aims to early detection of MRSA and VRSA infections either phenotypic or genotypic consider a guideline step of infection control strategy in outbreak prevention, Screen the magnitude of multidrug resistant S. aureus infections in Aswan university hospitals and to determine possibilities for future antibiotic treatment.* ***Methodology****: 182 different samples were collected from patients and 74 from environmental sources of infection. MRSA isolates were screened by ORSAB media, Oxacillin and Cefoxitin disk diffusion method then confirmed by detection of MecA gene by PCR, VRSA isolates were screened by Vancomycin disk diffusion method then confirmed by detection of VanA and VanB genes by PCR.* ***Results:*** *MRSA isolates were identified in 22 patients and 4 environmental sources of infection. None of them were VRSA .Each ward revealed infection with multiple strains indicating multiple sources of infection while certain strains were found multiple patients and multiple wards.* ***Conclusions:*** *Studying the epidemiology of MRSA and VRSA is very important. Suspected sources of infection including HCWs should be seriously considered, not only as a source of infection, but also as a major cause for transmitting infection between patients in different wards.*

INTRODUCTION

Antimicrobial resistance (AMR) is an imperative open wellbeing concern shared by created and creating nations. The burden of irresistible illnesses is heavier and compounding in creating nations compared to created nations due to constrained get to, inaccessibility and unaffordability of antimicrobials required for treating bacterial diseases caused by AMR living beings[1].

The development of AMR was regularly considered as an issue predominantly concerning hospitals and care facilities, however in the recent years resistant bacteria have been seen to spread in the wider community as well, leading to an increase in both the population at risk and the number of resistant infections[2]. AMR could be a worldwide instead of a nearby issue, as AMR can spread between nations or landmasses[3].

A WHO first worldwide report on anti-microbial resistance distributed in 2014 famous that, within the African locale, there exists a major crevice in observing and following anti-microbial resistance, with information assembled in as it were constrained number of nations over the landmass[4].

In expansion, there's agreement that the development of AMR too undermines life sparing restorative innovations such as organ transplants where results are subordinate on avoidance of surgical location contaminations[5].

High antimicrobial use in suspected COVID-19 cases, we have yet to discover the impact of COVID-19 on global AMR rates[6].

Staphylococcus aureus (*S. aureus*) diseases are around the world in dissemination and their rate is tall particularly in low-resource nations where wellbeing cleanliness is regularly compromised[7].

S. aureus is one of the critical causes of bacterial diseases in clinics and community settings. It can cause a wide run of contaminations shifting from gentle contaminations such as furuncles to life threatening contaminations such as pneumonia and serious sepsis[8].

MRSA is presently mindful for diseases with significant horribleness and mortality and has created resistance to numerous antimicrobial operators complicating the clinical treatment of diseases. Fast and exact discovery of methicillin safe strains in *staphylococci* is exceptionally basic in arrange to select suitable treatment, to avoid pointless utilize of glycopeptides antimicrobials and to require essential measures for contamination control[9]

The frequency of healthcare related contaminations caused by MRSA proceeds to extend around the world. Contaminations caused by MRSA strains are related with longer healing center remain, delayed anti-microbial organization, and higher costs than diseases caused by methicillin sensitive *S. aureus* (MSSA) strains[10].

Since the rise of vancomycin resistance in enterococci in 1988 and in vitro shows that its resistance qualities (Van A and Van B) are transmissible to other bacterial species counting *S.aureus*. The danger of vancomycin resistance in *S. aureus* has been of extraordinary concern and a risk to public health [11].

The foremost proficient way to avoid the spread of MRSA is questionable. Contact segregation of MRSA patients, hand cleanliness expanded surface cleansing and topical anti-microbial treatments are common measures to diminish transmission[12]. In spite of the fact that there's a considerable advance within the understanding of vancomycin resistance in *staphylococci*, more inquire about ought to be done to discover the perfect treatment and control of multi medicate safe VRSA contaminations. [13].

Screening for MRSA has the particular reason of identifying colonization in patients and in flare-up circumstances, in therapeutic faculty who have coordinate contact with colonized or tainted patients[14]. The reconnaissance of MRSA (both from contaminations and colonization) is pivotal for the usage of viable treatment conventions and contamination control measures[15].

The aim of the present work is to detect MRSA and VRSA infections either phenotypic or genotypic as a guideline step of infection control strategy in outbreak prevention and screen the magnitude of multidrug resistant *S. aureus* infections.

METHODOLOGY

Subjects:

This study was carried out on 256 samples obtained either from patients admitted to Aswan University Hospitals or from suspected sources of infection. There was 22 MRSA isolates recovered from (182) different samples collected from inpatients with nosocomial infections in different departments and units, the majority of samples were from General Surgery Department of Aswan University Hospitals. Also, 4 MRSA isolates were recovered from 74 environmental sources of infection; these were 18 Health care workers (HCWs) nasal swabs, 14 Hands and nails swabs, 10 HCWs Mobile phones swabs, 10 swabs from Mobile phones of patients and 22 swabs from bed side tables, dressing tables, linens, surgical dressing, surgical instruments and equipments.

Isolation and identification of *Staphylococcus aureus*:

Based on primary isolation of different specimens on mannitol salt, blood and nutrient agar, isolates were identified by standard methods. Identification of *S. aureus* done by morphological character and biochemical reactions including Gram stain , catalase test and both slide and tube coagulase tests, DNase test[16].

Antimicrobial susceptibility testing:

The antimicrobial susceptibilities of *S.aureus* isolates were determined using the disk diffusion method (modified Kirby-Bauer) on Muller-Hinton agar, as recommended by The European Committee on Antimicrobial Susceptibility Testing[17].

Identification of Methicillin Resistant *Staph aureus* (MRSA):

Methicillin Resistant *Staph aureus* (MRSA) isolates were screened and confirmed by: ORSAB, Oxacillin and Cefoxitin disks diffusion susceptibility method according to the Clinical and Laboratory Standards Institute standard guidelines[17].

Detection of susceptibility of the *Staph aureus* isolates to vancomycin:

Vancomycin-resistant *Staph aureus* (VRSA) isolates were screened and confirmed by using the Vancomycin disc diffusion method in accordance with clinical and laboratory standard institute guidelines[17]. The antibiotic disk used was vancomycin (30µg) from Sigma, USA.

Molecular study for detection of vancomycin resistance genes (VanA and VanB) using PCR:

DNA extraction:

- DNA extraction kit (Jena Bioscience; Germany).
- PCR Master Mix 2x (Thermo Scientific):

PCR Master Mix is a concentrated 2X solution containing Taq DNase, dNTPs (deoxynucleotide triphosphates), reaction buffer, MgCl2, and all other PCR components except the DNA template and primers. Primer solution (Thermo Scientific): The sequences of the primers used were in Table 1.

Table 1: The primers used for detection of MRSA and VRSA

Target	Sequence (5′ –3′)	Amplicon size (bps)	References
mecA (F) mecA (R)	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	533	[9].
vanA (F) vanA (R)	CATGAATAGAATAAAAGTTGCAATA CCCCTTTAACGCTAATACGACGATCAA	1032	[18].
vanB (F) vanB (R)	ACGGAATGGGAAGCCGA TGCACCCGATTTCGTTC	647	[19].

PCR conditions for mecA, vanA and vanB genes: Suppl. table 1.

Preparation of PCR mix: Taq green PCR Master Mix (2x) was gently and briefly vortexed after thawing. Thin walled PCR tube was placed on ice and the following components were added for each 50 µl reaction. Amplification reaction: Suppl. table 2

Equipment:

Thermal cycler (Biometra, Germany)

Gel electrophoresis Reagents:

- Agarose powder (Fermentas, Germany).
- Ethidium bromide dye (Sigma, USA): 10 mg/ml water stock solution maintained at room temperature in aluminum foil-wrapped vials.
- The electrophoresis buffer is Tris Borate EDTA (TBE).
- Fermentas (Germany) DNA molecular weight (MW) marker (100-1300 bp).
- PCR products were electrophoresed on a 1.5% Agarose gel and visualized using ethidium bromide under ultraviolet light.
- The electrophoresis chamber's agarose gel was coated with 1x Tris borate buffer.
- The first well of the gel was loaded with a molecular weight marker (a 100 base pair DNA ladder).
- PCR product of each sample, positive and negative controls were mixed with loading buffer and loaded to wells of the gel separately.
- The gel runs at 100 volts for 10min then 80 volts for 25 min.
- The position of the PCR bands was compared with marker: mecA gene band at (533 bp), vanA gene band at (1032 bp) and vanB gene band at (647 bp).
- Thermal cycler (Applied Biosystem; Model: vertiti) program was adjusted.
- Agarose gel electrophoresis of the amplified DNA was done and ethidium bromide stained bands in gel were visualized on long wave length ultraviolet transilluminator, and photographed.

Statistical Analysis:

The acquired data were tabulated and analyzed by computer with the "Statistical Package for the Social Sciences" (SPSS) version 16. Data analysis was carried out using descriptive statistics such as frequency, mean, and standard deviation. To determine the relationships between the research variables, appropriate statistical tests of significance were utilized. Chi square and correlations were used to compare differences in frequency distributions across groups. It was considered significant when the p-value was less than 0.05, and it was used to determine the importance of relationships, associations, and interactions between variables.

RESULTS

The distribution of *Staphylococcus aureus* as shown in Table (2) was 88 isolates (34.3%) from 182 total isolates: 74 (40.6%) out of 182 patient's samples and 14 (18.9%) out of 74 suspected sources of infection.

Table 2: Distribution of *S. aureus* found in different specimens

Bacterial growth		From patient samples		From environmental sources		Total	
		No	%	No	%	No	%
Staphylococcus aureus		74	40.6	14	18.9	88	34.3
Coagulase negative staphylococci		39	21.4	4	5.4	43	16.8
Non staphylococci		69	37.9	32	43.2	101	39.4
No bacterial growth		0	0	24	32.4	24	9.3
Total		182	100	74	100	256	100
Chi-square	**X2**	88.643		22.365			
	p- value	P1: 0.001*		P1: 0.001*			

P-value: P1: 0.001* means there is statistically significant difference between types of bacterial growth isolated from patients samples with significant increase in *S.aureus*.

P2: 0.001* means there is statistically significant difference between types of bacterial growth isolated from environmental sources of infection.

Table 3 and Figure 1 show the distribution of MRSA isolates among whole *S.aureus* isolated from patients' samples and environmental sources of infection.

Table 3: Distribution of MRSA isolates among whole *S.aureus* isolated

Specimen		**MRSA**	**MSSA**	**Total *S.aureus***	**P**	
From environmental sources of infection	**No.**	4	10	14	P1	0.312
	%	28.6	71.4	15.9		
From patients	**No.**	22	52	74	P2	0.001*
	%	29.7	70.3	84.1		
Chi-square	**X2**	7.071				
	P-value	0.006*				

P-value: P1: 0.312 means there is statistically insignificant difference between MRSA and MSSA in environmental sources.
P2: 0.001* means there is statistically significant increase in MRSA than MSSA in patients samples.

Antibiogram typing of all MRSA isolates showed the 10 different resistance patterns identified in the MRSA isolates according to antibiotics susceptibility testing results. The most common were the first 3 types.

Table 4: Antibiogram typing of all MRSA isolates:

Antibiogram types		**Resistance pattern**	**No. of MRSA isolates**	**%**
1		SXT+TE+CIP	7	26.92
2		SXT+E+DA+AK+CN+TE+CIP+R	5	19.32
3		CN+TE	4	15.38
4		AK+CN	2	7.69
5		AK+CN+TE	2	7.69
6		E+AK+CN	2	7.69
7		E	1	3.84
8		E+ AK+CN+TE	1	3.84
9		E+DA+TE	1	3.84
10		C+E+DA+TE+CIP	1	3.84
		Total	26	100.00
Chi-square	X^2	21.167		
	P value	0.001*		

Septrin (SXT) 25mcg/disc, Ciprofloxacin (CIP) 5 mcg/disc, Tetracycline (TE) 10 mcg/disc, Erythromycin (E) 15 mcg/disc, Amikacin (AK) 30 mcg/disc, Chloramphenicol (C) 30 mcg/disc, Gentamicin (CN) 10 mcg/disc, Rifampicin (R) 5 mcg/disc, Clindamycin (DA) 2 mcg/disc.
P value: 0.001* means that there is there is statistically significant difference between different antibiogram types with significant increase in type1&2.

Genotypic detection of MRSA and VRSA by PCR: All *S. aureus* isolates (88) were analyzed by PCR to detect mecA, vanA and vanB genes. Twenty six *S. aureus* isolates had mecA gene and none of them had van A and van B as shown in table 5, Figure 2 and Suppl. table 3, Suppl. Figure 1, 2**.**

Table 5: Detection of all MRSA isolates phenotypically and compared genotypically by detection of MecA gene by PCR:

Oxacillin disc diffusion method		**Cefoxitin disc diffusion method**		**ORSAB**		**Confirmed by MecA gene**	
R N (%)	S **N(%)**	R **N (%)**	S **N(%)**	+ve **N (%)**	-ve **N(%)**	+ve **N (%)**	-ve **N(%)**
25(96.1%)	63(3.9%)	26(100%)	62(0%)	26(100%)	62(100%)	26(100%)	62(100%)

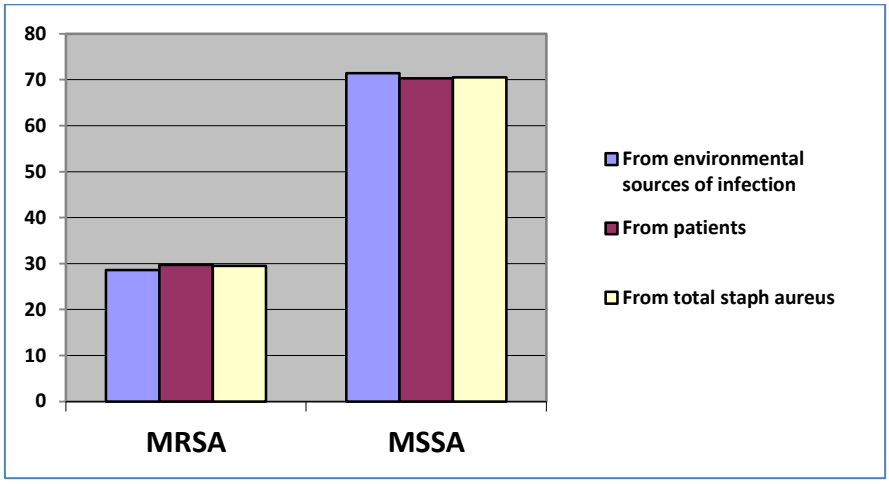

Fig. 1: Prevalence of MRSA in different specimens from patients and environmental sources of infections

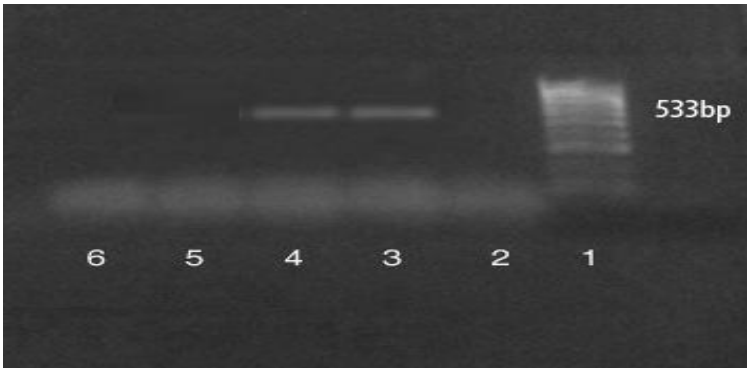

Fig. 2: Gel electrophoresis of PCR amplification for mecA gene in S. aureus isolates (533 bp): Lane 1:marker (100bp) - Lane 3 and 4: positive cases - Lane 5 and 6: negative case- Lane 2: negative control.

DISCUSSION

Antimicrobial resistance develops as a result of spontaneous genetic alterations[20]. However, abuse of antibiotics in medicine, agriculture, and the food sector (meat production) hastens these genetic modifications[21]. It has the potential to spread among humans as well as from animals. Antimicrobial resistance presently accounts for approximately 7 million fatalities per year, and it is estimated to reach around 10 million deaths by 2050, with an associated cost of about 100 trillion USD globally[20].

In this study 88 (34.3%) isolates proved to be *S.aureus* of all 256 samples which were 74 (40.6%) out of 182 patients samples and 14 (18.9%) out of 74 suspected sources of infection.

In the present study, the rate of *S. aureus* isolation from different specimens from patients , the highest rate was from Postoperative wound Swab (37.8%), followed by endotracheal (17.6%) and Bronco alveolar lavage (BAL) (14.9 %) while the lowest rate of isolation was from sputum (6.8%) blood (9.5%) and urine (13.5%) with statistically significant difference.

Comparable results were reported by Reza Sharif et al[23] who found that 75.9% of *S. aureus* were from blood, 18.9% were from urine and 5.2% were from respiratory tract samples. Regarding MRSA, they found that 75.9% of the isolates were from blood, 18.9% were from urine, 51.7% were from respiratory tract. While Elrefahi et al[22] found that the highest rate was from drains (33.1%) followed by wound (22.76%), urine (13.7%), sputum (13.7%), blood (6.8%), and ascetic fluid (6.8%) and endotracheal tubes (6.8%), Kosowska-Shick et al[24] found that S. aureus was frequently isolated from wound (60%), sputum (20.7%), blood (9.7%) and ascetic fluid (2.4%).

Concerning hospital acquired MRSA percentage in our study 26 (29.7 %) isolates were MRSA, This was similar to the result of Falagas et al[25] (24%), and were close to Hurley et al[26] found that (65.7%) of hospital patients had MSSA bacteremia and (34.3%) had MRSA bacteremia. These results agree with the present results. In reverse the study of Borg et al[27] which found that more than (50%) of the *S. aureus* blood cultures isolates were MRSA. Also Thati et al[28] found that (79.61%) of *S. aureus*, were identified as MRSA, Dubey et at[29] reported (81.73%) of *S. aureus* isolates were MRSA and

also lower than that obtained by and Mendes et al[30] who demonstrated that (73%) of the clinical *S. aureus* isolates from two hospitals in Korea were MRSA and., but higher than Havaei et al[31] (16%) and Patil SS et al[32] (18%). Silveira et al[33] Reported that several European countries have national surveillance data with very low rates of MRSA. These low prevalence rates in their studies may be probably due to proper hand hygiene, antibiotic stewardship and surveillance programs.

The gold standard for MRSA confirmation is the detection of the mecA gene using PCR. The molecular detection methods are more desirable, advantageous, and accurate than the phenotypic method[15].

The Cefoxitin disc diffusion test for MRSA detection was assessed in the current investigation. All 26 MRSA isolates were Cefoxitin resistant, with 100% sensitivity and specificity. Similar outcomes with 100% sensitivity and specificity for the Cefoxitin disc diffusion test were reported by Velasco et al[35] and Anand et al[36]. The findings, however, only partially concur with the research of Broekema[37] who reported 97.3% sensitivity, and Taha[38] who reported 94.4% sensitivity.

This study revealed that MRSA was (96.2%) by Oxacillin disc diffusion (25 of 26). Lower results were mentioned by Pillai et al[34] who reported MRSA in (45.45%) by Oxacillin screen agar and (37.3%) by Oxacillin disc diffusion. They also added that the Oxacillin disc diffusion test, which was used routinely earlier, is showing low specificity (56%).Also, Jain et al[39] discovered that 75.26% of *S.aureus* isolates were MRSA. The sensitivity and specificity of the Oxacillin disc diffusion technique were 100% and 58.33 percent, respectively. While, Becker et al[40] used ORSAB to detect MRSA, 102 out of 104 MRSA-positive clinical specimens (98%) were correctly identified and this matched with the current work.

CONCLUSION

HCWs are considered the most important source of infection through the nasal carriage of MRSA. Detection of MRSA among patients in different wards within the same unit indicates the intradepartmental spread of these strains between different wards. The implementation of rapid diagnostic techniques in clinical microbiology laboratories to aid the choice of drug therapy is another emerging facet of antimicrobial stewardship. Moreover, AMR was associated in different studies with an increased risk of longer hospitalization and mortality. The clinical and economic burdens of AMR are huge and call for antibiotic stewardship programs to be taken and for increasing global surveillance networks. Early detect of MRSA and VRSA infections as a Guideline Step of Infection Control Strategy in Outbreak prevention. Investigation of the susceptibility patterns and the spread of antibiotic resistance among *S. aureus* hospital associated isolates are very important to determine possibilities for future antibiotic treatment.

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Authors contribution: Conceptualization, A. Sadek and A.S Yassin.; methodology, M.A. Ibrahim; software, M.M.; validation, E.M. Fahmy, A.A. Ezzat and M.R. Saad ; formal analysis, A.S Yassin.; investigation, N.M. Kamel; resources, A. Sadek.; data curation, M.M. Amin; writing original draft preparation, A.S. Yassin; writing review and editing, M.M. Amin; visualization, E.M. Fahmy; supervision, A. Sadek.; project administration, A. Sadek. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Zulkeflle, Siti Norayuni Mohd, Yus Amira Yusaimi, Norio Sugiura, Koji Iwamoto, Masafumi Goto, Motoo Utsumi, Nor'Azizi Bin Othman, Zuriati Zakaria, and Hirofumi Hara: "Phenotypic and Genetic Characterization of Multidrug-Resistant *Staphylococcus aureus* in the Tropics of Southeast Asia." *Microbiology (United Kingdom)* 2016;162 (12): 2064-2074.
2. Giacomini, Elisa, Valentina Perrone, Davide Alessandrini, Daniela Paoli, Carmela Nappi, and Luca Degli Esposti.: "Evidence of Antibiotic Resistance from Population-Based Studies: A Narrative Review." *Infection and Drug Resistance* 2021;14: 849-858.
3. Song, Jae Uk, Hye Kyeong Park, Hyung Koo Kang, and Jonghoo Lee. (2017): "Proposed Risk Factors for Infection with Multidrug-Resistant Pathogens in Hemodialysis Patients Hospitalized with Pneumonia." *BMC Infectious Diseases* 2017; 17 (1): 1–9

4. Shekarabi, Marjan, Bahareh Hajikhani, Alireza Salimi Chirani, Maryam Fazeli, and Mehdi Goudarzi. (2017): "Molecular Characterization of Vancomycin-Resistant *Staphylococcus aureus* Strains Isolated from Clinical Samples: A Three Year Study in Tehran, Iran."2017 Aug 30;12(8):e0183607
5. Garoy, Eyob Yohaness, Yacob Berhane Gebreab, Oliver Okoth Achila, Daniel Goitom Tekeste, Robel Kesete, Robel Ghirmay, Ruta Kiflay, and Thomas Tesfu.: "Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara , Eritrea can J Infec Dis Med Microbio. 2019 Feb;6: 8321834.
6. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 28; 395:1054-1062.
7. Sani. Iliya, Jonathan Mwangi, Ronald. Maathai, Mary. Muriuki, and Christopher Wainaina. Molecular Detection of Panton Valentine Leukocidin Toxin in Clinical Isolates of *Staphylococcus aureus* from Kiambu County, Kenya." *International Journal of Microbiology* 2020 Aug 27: 3106747.
8. Shristi Raut, Kishor Bajracharya, Janak Adhikari, Sushama Suresh Pant, and Bipin Adhikari. Prevalence of Methicillin Resistant *Staphylococcus aureus* in Lumbini Medical College and Teaching Hospital , Palpa , Western." *BMC Research Notes* 2017; 10: 187
9. Amany Gamal Thabit, Ahmed Sadek Ahmed, and E. H. Ahmed. Molecular Detection of Panton - Valentine Leukocidin (PVL) and Methicillin Resistance in Staphylococcus Aureus Pathogen," Egypt. J. Med. Microbiol. 2017; 26 (3): 19-24.
10. Hashim, Alaa Eldeen m., Fathia m. et al., Nasal colonization of methicillin-resistant Staphylococcus aureus among medical residents in Al-Azhar University Hospital, Damietta branch, Al-Azhar Assiut Med J 2018;16 (1): 87-95.
11. Bamigboye, Bosede Titilope, Olugbenga Adekunle Olowe, and Samuel Sunday Taiwo.Phenotypic and Molecular Identification of Vancomycin Resistance in Clinical *Staphylococcus aureus* Isolates in Osogbo , Nigeria Euro J of Microbiol and Imm 2018; 8 (March): 25–30.
12. Moosavian, Mojtaba, Paria Baratian Dehkordi, and Mohammad Hashemzadeh. Characterization of Sccmec, Spa Types and Multidrug Resistant of Methicillin-Resistant *Staphylococcus aureus* Isolates in Ahvaz, Iran. Infection and Drug Resistance 2020 April 9; 13: 1033-144.
13. Tarek El Saeed El Banna, Fatma Ibrahim Sonbol, A. A. Abd El Aziz, and Engy. El Ekhnawy. Prevalence and Antimicrobial Susceptibility of Vancomycin Resistant Staphylococci in an Egyptian University Hospital," J. Med. Microbiol. Diagnosis 2018; 07(01):1–8.
14. F. Cyr Doscoph Afle, A. J. Agbankpe, R. C. Johnson, O. Houngbégnon, S. C. Houssou, and H. S. Bankole. Healthcare-associated infections: bacteriological characterization of the hospital surfaces in the University Hospital of Abomey-Calavi/so-ava in South Benin (West Africa). BMC Infect. Dis. 2019; 19(1): 28.
15. SM Samy, FA Tkeli, EA Eisa, A. M. Amin, HH Dawood, and M Z Hussein. Genotyping of Nosocomial Methicillin Resistant Staphylococcus aureus with tracing the Source of Infection: A Guideline Step in Infection Control Strategy at General Surgery Department of Tanta University Hospital. Egyptian Journal of Medical Microbiology2018; 27 (4): 27-35.
16. Ashraf A. Abdel-Tawab, S. F. Darwish, F. I. El – Hofy, and E. M. Shoieb, "Phenotypic and Genotypic Characterization of Coagulase Negative S. Aureus Isolated from Different Sources. Benha Vet. Med. J. 2018; 34(3): 129-149.
17. Clinical and Laboratory Standards Institute (CLSI): CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition. 2021; 40.
18. Rehab El-Baky. Prevalence and Conjugal Transfer of Vancomycin Resistance among Clinical Isolates of Staphylococcus aureus, Adv. Res. 2014; 2(1):12-23.
19. El-banna, Tarek El-saeid, Fatma Ibrahim Sonbol, and Ahmed Ahmed Abd El-aziz. Original Research Article Characterization of Vancomycin Resistant *Staphylococcus aureus* in Tanta University Hospital. International Journal of Current Microbiology and Applied Sciences 2015; 4 (10): 1-11.
20. Yadav, Shailendra, and Atya Kapley. Antibiotic Resistance: Global Health Crisis and Metagenomics. Biotechnology Reports 2021;29: e00604.
21. Koen, B, S Ben, and V Julie. Antibiotic Resistance, Stewardship, and Consumption.The Lancet Planetary Health 2019; 3(2); e66
22. Elrefahi H.B., Ghoneim E.M., Elhendawy G. R., Abd elmotelab T.M. and Hassan H.A. Characterization of vancomycin resistant *S.aureus* (VRSA) in National Liver Institute. Menoufia Med. J. 2014; 27(4):825.

23. Reza Sharif, Mohammad, Javad Alizargar, and Alireza Sharif. Prevalence and Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Isolates at Shahidbeheshti Hospital. World Journal of Medical Sciences 2013;9(2): 84-87.
24. Kosowska-Shick, Klaudia, Lois M. Ednie, Pamela McGhee, Kathy Smith, Cynthia D. Todd, Amanda Wehler, and Peter C. Appelbaum. Incidence and Characteristics of Vancomycin Nonsusceptible Strains of Methicillin-Resistant *Staphylococcus aureus* at Hershey Medical Center. Antimicrobial Agents and Chemotherapy 2008;52(12): 4510-4513.
25. Falagas, Matthew E., Drosos E. Karageorgopoulos, John Leptidis, and Ioanna P. Korbila. MRSA in Africa: Filling the Global Map of Antimicrobial Resistance. PLoS ONE 2013;l 8(7)e68024.
26. Hurley, James C., Sara E. Cosgrove, and Y. Carmeli. Comparison of Mortality Associated with Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* Bacteremia: An Ecological Analysis [with Reply] (Multiple Letters). Clinical Infectious Diseases 2003;37(6): 866-869.
27. Borg, Michael A., Marlieke De Kraker, Elizabeth Scicluna, Nienke van de Sande-Bruinsma, Edine Tiemersma, Jos Monen, Hajo Grundmann. Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Invasive Isolates from Southern and Eastern Mediterranean Countries. Journal of Antimicrobial Chemotherapy 2007; 60(6):1310–1315.
28. Thati, Venubabu, Channappa T. Shivannavar, and Subhaschandra M. Gaddad. Vancomycin Resistance among Methicillin Resistant *Staphylococcus aureus* Isolates from Intensive Care Units of Tertiary Care Hospitals in Hyderabad. Indian Journal of Medical Research 2011;134(11):704-708.
29. Dubey, Debasmita, Shakti Rath, Mahesh C. Sahu, Lolly Pattnaik, Nagen K. Debata, and Rabindra N. Padhy. Surveillance of Infection Status of Drug Resistant *Staphylococcus aureus* in an Indian Teaching Hospital. Asian Pacific Journal of Tropical Disease 2013; 3 (2): 133-42.
30. Mendes, Rodrigo E., Myrna Mendoza, Kirnpal K. Banga Singh, Mariana Castanheira, Jan M. Bell, John D. Turnidge, Stephen S.F. Lin, and Ronald N. Jones. Regional Resistance Surveillance Program Results for 12 Asia-Pacific Nations (2011). Antimicrobial Agents and Chemotherapy 2013; 57(11): 5721-5726.
31. Havaei, Seyed Asghar, Fahimeh Ghanbari, Ali Asghar Rastegari, Amir Azimian, Farzad Khademi, Nafiseh Hosseini, Amirmorteza Ebrahimzadeh Namvar, Hamid Vaez, Seyed Mehdi Havaei, and Mojtaba Shahin. (2014): " Molecular Typing of Hospital-Acquired *Staphylococcus aureus* Isolated from Isfahan, Iran. International Scholarly Research Notices 2014: 2014:1-6.
32. Patil SS, Suresh KP, Shinduja R, Amachawadi RG, Chandrashekar S, Pradeep S, Kollur SP, Syed A, Sood R, Roy P, Shivamallu C. Prevalence of Methicillin-resistant *Staphylococcus aureus* in India: A Systematic Review and Meta-analysis. Oman Med J. 2022 Jul 31; 37(4): e440.
33. Silveira, Alessandro C.O., Gabriela R. Cunha, Juliana Caierão, Caio M. de Cordova, and Pedro A. d'Azevedo. (2015): "MRSA from Santa Catarina State, Southern Brazil: Intriguing Epidemiological Differences Compared to Other Brazilian Regions. Brazilian Journal of Infectious Diseases 2015;19 (4): 384-389.
34. Pillai, Manju M, Ragunathan Latha, and Gautam Sarkar. Detection of Methicillin Resistance in *Staphylococcus aureus* by Polymerase Chain Reaction and Conventional Methods: A Comparative Study. Journal of Laboratory Physicians 2012; 4(02): 083-088.
35. Velasco, David, Maria del Mar Tomas, Monica Cartelle, Alejandro Beceiro, Astrid Perez, Francisca Molina, Rita Moure, Rosa Villanueva, and German Bou. Evaluation of Different Methods for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* Aureus. Journal of Antimicrobial Chemotherapy 2005; 55(3):379-382.
36. Anand, K., 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 2009; 27(1):27-29.
37. Broekema M.N., Van T.T., Monson T.A. Marshall SA, Warshauer DM. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in *Staphylococcus aureus* in a large-scale study. J. Clin. Micrbiol. 2009; 47(1):217–219.
38. Taha, Samaa, Noha Kamel, and Lobna Metwally. Mupirocin Resistance among Methicillin Resistant *Staphylococcus aureus* Isolates in an Egyptian Hospital July 2022; 3(3): 51–55.
39. Jain, Amita, Astha Agarwal, and Raj Kumar Verma. Cefoxitin Disc Diffusion Test for Detection of Meticillin-Resistant Staphylococci. Journal of Medical Microbiology 2008;57(8): 957-961.
40. Becker, Andrea, Dietmar H. Forster, and Eberhard Kniehl. Oxacillin Resistance Screening Agar Base for Detection of Methicillin-Resistant *Staphylococcus aureus*. Journal of Clinical Microbiology 2002;40(11): 4400-4401.