https://doi.org/10.47993/gmb.v46i1.616

Frequency of the mecA gene in methicillin-resistant Staphylococcus aureus in a tertiary hospital in Peru.

Frecuencia del gen mecA en Staphylococcus aureus meticilinorresistente en un hospital de tercer nivel en Perú

Anthony Martín Bustamante-Cabrera[1], Hans Ramón Quiroz-Ruiz[2], Jorge Arturo Vega-Fernandez[3], Marco A. Rivera-Jacinto[4]

Resumen

Objectives: To determine the frequency of the mecA gene in methicillin-resistant Staphylococcus aureus (MRSA) isolates from patients treated at a tertiary level hospital in the Cajamarca region, Peru; additionally, to ascertain which of the two antibiotics used for phenotypic screening is more useful in explaining the presence of said gene. **Methods:** Seventy-one bacterial isolates from samples at the Regional Teaching Hospital of Cajamarca were analyzed, with the identification of S. aureus carried out using the MicroScan system. Phenotypic screening for methicillin resistance was performed using the diffusion technique with cefoxitin and oxacillin disks. DNA extraction was carried out through thermal shock, and the detection of the mecA gene was performed using polymerase chain reaction. Statistical analysis was conducted with SPSS software v.25. **Results:** Of the 71 isolates, 40 (56.3%) were MRSA carriers of the mecA gene, the majority of these isolates corresponded to hospitalized patients 22 (31.0%), being more frequent in bronchial secretion samples 27 (38.0%). Phenotypic screening with the cefoxitin disk better predicted the presence of the mecA gene [P=0.010; Exp(B)= 12.3] compared to the oxacillin disk. **Conclusions:** This study demonstrated a high frequency of mecA positive MRSA in clinical origin samples, mainly from hospitalized patients. It is crucial to establish surveillance measures to identify MRSA in all hospitals in the region.

Palabras claves: Staphylococcus aureus resistente a meticilina, reacción en cadena de la polimerasa, farmacorresistencia microbiana; Perú

Abstract

Objetivos: determinar la frecuencia del gen mecA en Staphylococcus aureus resistente a meticilina (MRSA) aislados de pacientes atendidos en un hospital de tercer nivel en la región Cajamarca, Perú; asimismo, determinar cuál de los dos antibióticos usados como screening fenotípico tiene mayor utilidad para explicar la presencia de dicho gen. **Métodos:** se analizaron 71 aislamientos bacterianos provenientes de muestras del Hospital Regional Docente de Cajamarca, la identificación de S. aureus se llevó a cabo mediante el equipo MicroScan. El screening fenotípico para resistencia a meticilina se realizó mediante la técnica de difusión, con discos de cefoxitina y oxacilina. La extracción de ADN se realizó mediante shock térmico, la detección del gen mecA se realizó mediante reacción en cadena de la polimerasa. El análisis estadístico se realizó con el software SPSS v.25. **Resultados:** de los 71 aislados, 40 (56,3%) fueron MRSA portadores del gen mecA, la mayoría de estos aislamientos correspondieron a pacientes hospitalizados 22 (31,0%), siendo más frecuentes en muestras de secreción bronquial 27 (38,0%). El screening fenotípico con disco de cefoxitina predijo mejor la presencia del gen mecA [P=0,010; Exp(B)= 12,3] en comparación con el disco de oxacilina. **Conclusiones:** este estudio demostró alta frecuencia de MRSA mecA positivo en muestras de origen clínico, principalmente de pacientes hospitalizados. Es importante establecer medidas de vigilancia para identificar MRSA en todos los hospitales de la región.

Keywords: methicillin-resistant *Staphylococcus aureus*, polymerase chain reaction, microbial drug resistance, Peru

Staphylococcus aureus is a gram-positive coccus-shaped bacterium, being the most significant from a clinical perspective. It is present in the usual microbiome of the nasal mucosa in 20-40% of humans[1,2] and is the primary causative agent of pneumonia and other respiratory tract infections, surgical site, artificial joint and cardiovascular infections, as well as a significant number of nosocomial bacteremia cases[3]. Moreover, S. aureus can cause moderately severe skin infections, including boils, abscesses, and wound infections, which generally are not life-threatening but can be accompanied by significant morbidity and pain and due to their frequency represent a considerable burden on public health[4].

Recently, infections caused by S. aureus have become more complex as a consequence of antimicrobial resistance acquired by many strains, among which methicillin-resistant Staphylococcus aureus (MRSA) is the most clinically relevant species[5], with reports increasing both in hospital and community settings[6]. MRSA infections are accompanied by increased morbidity, hospital stay, and mortality, compared to those caused by methicillin-sensitive strains[7].

The molecular mechanism conferring methicillin resistance

[1]Biólogo Biotecnólogo. Laboratorio de Microbiología, Departamento de Ciencias Biológicas, Universidad Nacional de Cajamarca; Laboratorio de Referencia Regional de Salud Pública, Dirección Regional de Salud – Cajamarca, Perú.
https://orcid.org/0000-0003-1545-4753
[2]Maestro en Salud Pública; Biólogo Microbiólogo. Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz Gallo, Lambayeque; Laboratorio de Referencia Regional de Salud Pública, Dirección Regional de Salud – Cajamarca, Perú.
https://orcid.org/0000-0002-8482-8328
[3]Maestro en Microbiología Clínica, Biólogo y Microbiólogo., Huánuco. Facultad de Ciencias Biológicas, Universidad Nacional Pedro Ruiz Gallo, Lambayeque; Laboratorio de Referencia Regional de Salud Pública, Dirección Regional de Salud – Cajamarca, Perú.
https://orcid.org/0000-0003-0073-033X
[4]Doctor en Ciencias Biomédicas; Biólogo Microbiólogo. Laboratorio de Microbiología, Departamento de Ciencias Biológicas, Universidad Nacional de Cajamarca, Cajamarca, Perú. https://orcid.org/0000-0002-9046-5359.
Correspondence to: Anthony Martín Bustamante-Cabrera
Email: anmarbusca12.2000@gmail.com
Received November 29, 2022. Accepted on February 17, 2023.

involves the synthesis of the penicillin-binding protein 2a (PBP2a) encoded by the mecA gene, which has an extremely low affinity for many beta-lactam antibiotics6. By developing this resistance mechanism, S. aureus acquires resistance to antibiotics such as penicillins, cephalosporins up to the fourth generation, and carbapenems[8].

Reports of methicillin resistance in clinical isolates vary by country, reporting rates of over 50% in countries such as the United States and China[9], and 25.7% in Mexico[10]. The first report of MRSA in South America occurred in 2005[11]; since then, various reports have been made across the continent, including hospitals in Peru with rates ranging from 27% to 58%[12,13].

Given the pathogenic role of S. aureus in healthcare-associated infections, an incorrect identification of MRSA can lead to severe consequences in a hospital setting, causing treatment failures and implying high costs; worse still, generating a selective risk of resistance[14]. Given this issue and because reports of this type of resistance in the country are still scarce to date, the aim of the present study was to determine the frequency of the mecA gene in methicillin-resistant Staphylococcus aureus isolated from patients treated at a tertiary level hospital in the Cajamarca region, in northern Peru; also, to evaluate the usefulness of oxacillin (OXA) and cefoxitin (FOX) disks in phenotypic screening to predict the presence of the mecA gene.

Materials and Methods

Bacterial Isolates. A total of 71 isolates from patients treated in various services of the Regional Teaching Hospital of Cajamarca were evaluated, identified as S. aureus by the hospital's Microbiology laboratory using the automated MicroScan autoSCAN4 system. For data collection of each isolate, a form was filled out, recording the origin (hospital and community), type of clinical sample, sex, and age of the patient. The bacterial isolates were transported under cold chain conditions, at a temperature range of 2-8 °C, to the National University of Cajamarca, specifically to the Microbiology Laboratory of the Faculty of Health Sciences to determine their phenotypic resistance pattern and molecular detection of the mecA gene.

Phenotypic Detection of Methicillin Resistance. All isolates were subjected to screening using the diffusion technique with 30 µg FOX and 1 µg OXA discs, on Mueller Hinton agar, with an inoculum equivalent to the 0.5 McFarland nephelometer tube and incubated for 18 hours at 37 °C. S. aureus was considered resistant to FOX when the inhibition halo of the disc had a diameter of less than or equal to 21 mm[15], and resistant to OXA when the diameter of the inhibition halo of the disc was less than or equal to 10 mm[16]. All isolates resistant to either of the two antimicrobials underwent molecular study.

DNA Extraction by Thermal Shock. From the 18-hour bacterial culture, three colonies were suspended in 150 µL of molecular-grade water, vortexed for 5 seconds, and placed in a water bath at 80 °C for 10 minutes, followed by -20 °C for 10 minutes. Subsequently, the sample was thawed and centrifuged for 8 minutes at 10,000 rpm; 60 µL of the supernatant was taken and the purity of the extracted DNA was evaluated using a micro-volume spectrophotometer, NanoDropTM 2000 (Thermo Scientific™).

Detection of the mecA Gene. Polymerase chain reaction (PCR) was performed with a final reaction volume of 50 µL according to the KOD Hot Start DNA Polymerase kit (Novagen®, Toyobo, Merck Millipore). The exact concentrations for PCR were 1 µL of bacterial DNA, 5 µL of dNTP's, 5 µL of 10X buffer, 32 µL of molecular-grade water, 1 µL of KOD polymerase, 1.5 µL of each primer, and 3 µL of 25 mM MgSO4. PCR conditions consisted of an initial polymerase activation at 95 °C for 2 min, followed by 35 cycles with initial denaturation at 95°C for 20 s, primer annealing at 57 °C for 1 min, extension at 72°C for 2 min, and a final extension at 70 °C for 15 s. The primers were mec1 5-'AAAATCGATGGTAAAGGTTGGC'-3 and mec2 5-'AGTTCTGGCACTACCGGATTTGC'-3 (17). Electrophoretic run of the product (533 bp) was carried out on a 1.5% agarose gel and with the DNA Ladder 100bp molecular weight marker (Life Technologies, Invitrogen™). The gel was run at 100 volts for 30 minutes. Bands were evaluated and photographed through the 25 Visi-Blue UV light transilluminator.

Data Analysis and Processing Techniques. Statistical analysis was performed using the SPSS v.25 statistical package. The association between variables (presence of the mecA gene and service, type of sample, sex, age of the patients, resistance to OXA and FOX) was established through the Chi-square test and/or Fisher's exact test. Statistical significance or p-value was set at $p \leq 0.05$, considering a confidence level of 95% and an error of 5%. Additionally, to establish the utility of the discs (FOX or OXA) in predicting phenotypic methicillin resistance with the presence of the mecA gene, binary logistic regression was used.

Results

For the phenotypic screening, 49/71 (69.0%) showed resistance to OXA and 43/71 (60.6%) resistance to FOX; likewise, PCR confirmed the presence of the mecA gene in 40/71 (56.3%) of the isolates. Regarding the type of biological sample, 43/71 (60.6%) corresponded to bronchial secretion, 10/71 (14.1%) to blood cultures, and 4/71 (5.6%) to pharyngeal secretion, and 14/71 (19.7%) to other types of samples (Table 1).

The frequency of mecA positive isolates resistant to OXA and FOX was 39/71 (54.9%) and 37/71 (52.1%), respectively. Of the isolates with the presence of the mecA gene, 22/40 (55%) were isolated from hospitalized patients and 18/40 (45%) from outpatient care. Regarding sex, 25/40 (62.5%) were males and 15/40 (37.5%) females. The Chi-square test showed that there was no association between the type of sample ($p = 0.352$), sex ($p = 0.628$), and the patient's condition (hospitalized or outpatient) ($p = 0.814$) with the presence of the mecA gene. 14.1% of the MRSA isolates detected through screening with OXA did not have the mecA gene, while in 8.5% of isolates

Table 1. Characteristics of S. aureus isolates

Characteristics		Frequencies (%)
Services		
	Hospitalization	58 (58,5)
	Outpatient	46 (46,5)
Sex		
	Male	42 (59,2)
	Female	29 (40,8)
Biological Sample		
	Bronchial secretion	48 (60,8)
	Blood cultures	10 (14,1)
	Pharyngeal secretion	4 (5,6)
	Other	14 (19,7)
Phenotypic screening for methicillin resistance		
	OXA resistance	49 (69,0)
	FOX resistance	43 (60,6)
	No Resistance	22 (31,0)
Molecular detection of methicillin resistance		
	Gen mecA +	40 (56,8)
	Gen mecA -	31 (43,7)

resistant to FOX, the mecA gene was not present (Table 2).

Regarding binary logistic regression, the model correctly classified 87.3% of the cases, being an acceptable model. The positive phenotypic screening for methicillin resistance with FOX disc was more useful for detecting the mecA gene [p = 0.010; Exp(B) = 12.3] compared to the OXA disc (Table 3).

Discussion

The phenotypic method carried out for the detection of MRSA is based on screening with OXA and FOX antibiotics[15].

Table 2. Contrast of mecA gene molecular detection according to type of care, biological sample, sex and phenotypic screening for methicillin resistance.

Indicator		Detection of the resistance gene		
		mecA +	*mecA* –	p value
Type of care				
	Hospitalization	23	16	0,814
	Outpatient	18	15	
Biological Sample				
	Bronchial secretion	27	16	0,352
	Blood cultures	6	4	
	Pharyngeal secretion	2	2	
	Other	5	9	
Sex				
	Male	25	17	0,638
	Female	15	14	
Phenotypic screening with OXA				
	Sensible	1	21	<0,001
	Resistant	39	10	
Phenotypic screening with FOX				
	Sensible	1	25	<0,001
	Resistant	37	6	

Table 3. Binary logistic regression, step 1a: dependency analysis between phenotypic screening with OXA and FOX discs and presence of the mecA gene.

		B	Standard error	Wald	gl	Sig.	Exp(B)	95% CI for EXP(B)	
								Inferior	Superior
Step 1[a]	OXA	2,851	1,841	3,096	1	0,079	17,308	0,758	145,859
	FOX	2,512	0,971	6,688	1	0,010	12,333	1,837	82,789
	Constant	-7,908	2,094	14,265	1	0,000	0,000		

a. Variables specified in step 1: OXA, FOX.

CI: Confidence interval

These antibiotics induce the production of the PBP2a protein, which replaces the native protein in the cell wall[18], generating bacterial resistance to methicillin. Furthermore, the expression of this protein is influenced by the culture conditions, affinity with the antibiotic, and by the expression of the gene encoding this protein. Therefore, confirmatory methodologies such as PCR, a highly sensitive test that specifically detects genes associated with this resistance mechanism, are recommended as the standard detection method for MRSA[19, 20].

This study is the first of its kind conducted in a level III hospital in the Cajamarca Region, in northern Peru. It found the mecA gene present in 40 (56.3%) isolates of S. aureus, a situation comparable to what has been reported in various studies[21,22]; studies conducted in Peru have reported frequencies very similar to those obtained in this study[13].

The mecA gene was found more frequently in isolates from hospitalization, implying a high frequency of MRSA at the hospital level. This is consistent with the literature, as MRSA isolates from hospital settings are characterized by being carriers of the mecA gene[13,20]; however, the results of the present study did not show significant differences between hospitalization isolates and outpatient care, being concerning the high frequency of the mecA gene in patients who were treated on an outpatient basis 18/40 (45%). This demonstrates that hospital-acquired MRSA strains have now spread to other healthcare settings[7], making it imperative to establish measures in hospital environments that interrupt the transmission of MRSA and allow monitoring of resistance levels at the community level[23]. These results do not necessarily indicate that the frequency of MRSA isolates detected at the outpatient level were necessarily acquired in community settings. This high percentage should serve as a basis for studies aimed at detecting the mecA gene in community-acquired MRSA[23].

Regarding the sex of patients with MRSA-positive isolates, we found a higher presence of the mecA gene in male patients. However, similar to other reports[24], sex had no statistically significant effect on the detection of mecA gene-positive strains. Concerning the type of sample obtained, the gene was found more frequently in bronchial samples, followed by blood cultures, results similar to those reported in a study conducted in a hospital in another region of Peru. However, in that study, a large number of mecA-positive isolates came from unspecified secretion samples[13]. The high proportion of the mecA gene from bronchial secretion isolates agrees with other reports showing that patients with MRSA isolated in such secretions have more hospital admissions per year, as well as a longer hospital stay compared to patients with sensitive isolates[7,25].

Regarding the detection of MRSA through phenotypic methods, the results showed that phenotypic screening is significantly associated with the detection of the mecA gene. Due to this, different laboratories conventionally use both discs to detect MRSA[16,26,27]. This study also sought to evaluate which of the two screening methods is more useful for predicting the presence of the mecA gene in MRSA. Binary logistic regression showed that the FOX disc (30 µg) is more useful than the OXA disc (1 µg); these findings agree with studies on the utility of the FOX disc for detecting MRSA, which report up to 93% specificity compared to 74% achieved with the OXA disc[19,28-30].

As shown in Table 2, of the 71 isolates, 10 (14.1%) were resistant to OXA and did not have the mecA gene, a higher value compared to FOX-resistant isolates without the mecA gene. This high proportion of false positives by OXA could be influenced by the hyperproduction of β-lactamases, leading to phenotypic expression of oxacillin resistance but without a genetic resistance mechanism, reducing the specificity of the OXA disc screening method[29]. This has led to the discontinuation of MRSA phenotypic screening with the OXA disc, and the CLSI recommends using the FOX disc for MRSA detection, as it is a better inducer of PBP-2a encoded by the mecA gene[8,15,28], as well as a better inducer of the mecC gene[6]. In addition to the mecA gene, methicillin resistance developed may be mediated by other genes such as mecB and mecC[6,24].

PCR amplification of the mecA gene is recognized as the gold standard test for MRSA detection. However, it is an expensive method not available to most laboratories[8,31], making its application as a routine test in clinical practice impractical. Therefore, laboratories unable to perform molecular detection should use phenotypic detection tests, with the FOX disc being a useful option for detecting mecA gene expression[29]. Based on the results of this study, we advise against using the OXA disc as a screening tool for the detection of mecA positive MRSA.

A limitation of this study was the sample size, as the collection period was short, resulting in a small number of S. aureus isolates. Additionally, this study did not allow for

the analysis of stealth MRSA isolates, that is, those positive for mecA but not showing resistance to cefoxitin and oxacillin.

In conclusion, this study found a high percentage of mecA positive MRSA, mainly in isolates from hospitalized patients. It is important to establish constant surveillance measures for MRSA isolates in all hospitals in the region to reduce the spread among patients and the community, identify carriers and/or patients, and select an appropriate antibiotic treatment. Considering that phenotypic detection is more accessible for laboratories, we have verified that screening with the cefoxitin disc is better for detecting mecA positive MRSA.

Acknowledgments: To the biologist Gladys Esther Huayán Dávila of the Central Laboratory of the Regional Hospital of Cajamarca for facilitating the bacterial isolations.

Conflict of interest: The authors declare that they have no

Bibliographic references

1. Wertheim HFL, Melles DC, Vos MC, Van Leeuwen W, Van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis. 2005;5(12):751-62. Disponible en: https://doi.org/10.1016/S1473-3099(05)70295-4

2. Becker K, Schaumburg F, Fegeler C, Friedrich AW, Köck R, Prevalence of Multiresistant Microorganisms PMM Study. Staphylococcus aureus from the German general population is highly diverse. Int J Med Microbiol. 2017;307(1):21-7. Disponible en: https://doi.org/10.1016/j.ijmm.2016.11.007

3. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603-61. Disponible en: https://doi.org/10.1128/CMR.00134-14

4. McCaig LF, McDonald LC, Mandal S, Jernigan DB. Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis. 2006;12(11):1715-23. Disponible en: https://pubmed.ncbi.nlm.nih.gov/17283622/

5. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, Holland TL, Fowler VG Jr. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nat Rev Microbiol. 2019;17(4):203-18. Disponible en: https://doi.org/10.1038/s41579-018-0147-4

6. Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al. Methicillin-resistant Staphylococcus aureus. Nat Rev Dis Primers. 2018;4:18033. Disponible en: https://doi.org/10.1038/nrdp.2018.33

7. Ippolito G, Leone S, Lauria FN, Nicastri E, Wenzel RP. Methicillin-resistant Staphylococcus aureus: the superbug. Int J Infect Dis. 2010;14 Suppl 4:S7-11. Disponible en: https://doi.org/10.1016/j.ijid.2010.05.003

8. Madhavan A, Sachu A, Balakrishnan A, Vasudevan A, Balakrishnan S, Vasudevapanicker J. Comparison of PCR and phenotypic methods for the detection of methicillin resistant Staphylococcus aureus. Iran J Microbiol. 2021;13(1):31-6. Disponible en: https://doi.org/10.18502/ijm.v13i1.5489

9. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents. 2012;39(4):273-82. Disponible en: https://doi.org/10.1016/j.ijantimicag.2011.09.030

10. Martínez-Medina RM, Montalvo-Sandoval FD, Magaña-Aquino M, Terán-Figueroa Y, Pérez-Urizar JT. Prevalencia y caracterización genotípica de cepas de Staphylococcus aureus resistente a meticilina aisladas en un hospital regional mexicano. Rev Chilena Infectol. 2020;37(1):37-44. Disponible en: http://dx.doi.org/10.4067/S0716-10182020000100037

11. Ribeiro A, Dias C, Silva-Carvalho MC, Berquó L, Ferreira FA, Soares Santos RN, et al. First report of infection with community-acquired methicillin-resistant Staphylococcus aureus in South America. J Clin Microbiol. 2005;43(4):1985-8. Disponible en: https://doi.org/10.1128/JCM.43.4.1985-1988.2005

12. Tamariz J, Horna G, Tapia E, Vicente W, Silva M, Zerpa R, et al. Staphylococcus aureus resistente a meticilina adquirido en la comunidad aislados en tres hospitales de Lima-Perú. Rev Med Hered. 2010;21(1). Disponible en: https://doi.org/10.20453/rmh.v21i1.1139

13. Cabrejos-Hirashima L, Vives-Kufoy C, Inga-Salazar J, Astocondor L, Hinostroza N, García C. Frecuencia de Staphylococcus aureus meticilinorresistente adquirido en la comunidad en un hospital de tercer nivel en Perú. Rev perú med exp salud pública. 2021;38(2):313-7. Disponible en: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1726-46342021000200313&lng=es&nrm=iso&tlng=es

14. Salazar de Vegas E, Tiappa A, Marval J, Gámez L, Trujillo D, Guzmán M, et al. Detección del gen mecA en cepas de Staphylococcus aureus aisladas de pacientes atendidos en el Servicio Autónomo Hospital Universitario "Antonio Patricio de Alcalá" Cumaná, estado Sucre. Rev Soc Ven Microbiol. 2017;37(2):44-6. Disponible en: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S1315-25562017000200003

15. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.

16. Morosini MI, Cercenado E, Ardanuy C, Torres C. Detección fenotípica de mecanismos de resistencia en microorganismos grampositivos. Enferm Infecc Microbiol Clin. 2012;30(6):325-32. https://doi.org/10.1016/j.eimc.2011.09.009

17. Cotaquispe R, Sarmiento R, Lovón S, Rodriguez J. Caracterización fenotípica y genotípica de Staphylococcus spp con resistencia a meticilina en pollos comerciales. Rev investig vet Perú. 2021;32(3):e20395. Disponible en: https://doi.org/10.15381/rivep.v32i3.20395

18. Yin S, Daum RS, Boyle-Vavra S. VraSR two-component regulatory system and its role in induction of pbp2 and vraSR expression by cell wall antimicrobials in Staphylococcus aureus. Antimicrob Agents Chemother. 2006;50(1):336-43. Disponible en: https://doi.org/10.1128/aac.50.1.336-343.2006

19. Torres C, Cercenado E. Lectura interpretada del antibiograma de cocos gram positivos. Enferm Infecc Microbiol. Clín. 2010;28(8):541-53. Disponible en: https://doi.org/10.1016/j.eimc.2010.02.003

20. Cikman A, Aydin M, Gulhan B, Karakecili F, Kurtoglu MG, Yuksekkaya S, et al. Absence of the mecC gene in methicillin-resistant Staphylococcus aureus isolated from various clinical samples: The first multi-centered study in Turkey. J Infect Public Health. 2019;12(4):528-33. Disponible en: https://doi.org/10.1016/j.jiph.2019.01.063

21. Abente S, Carpinelli L, Guillén R, Rodríguez F, Fariña N, Laspina F, et al. Frecuencia de Staphylococcus aureus meticilino resistente y del factor de virulencia PVL en pacientes ambulatorios con infección de piel y partes blandas de Asunción, Paraguay. Mem Inst Investig Cienc Salud. 2016;14(2):8-16. Disponible en: https://doi.org/10.18004/Mem.iics/1812-9528/2016.014(02)08-016

22. Hussein N, Salih RS, Rasheed NA. Prevalence of Methicillin-Resistant Staphylococcus aureus in Hospitals and Community in Duhok, Kurdistan Region of Iraq. Int J Infect. 2019;6(2):e89636. Disponible en: https://dx.doi.org/10.5812/iji.89636

23. Raupach-Rosin H, Rübsamen N, Szkopek S, Schmalz O, Karch A, Mikolajczyk R, et al. Care for MRSA carriers in the outpatient sector: a survey among MRSA carriers and physicians in two regions in Germany. BMC Infect Dis. 2016;16:184. Disponible en: https://doi.org/10.1186/s12879-016-1503-5

24. Abimana JB, Kato CD, Bazira J. Methicillin-Resistant Staphylococcus aureus Nasal Colonization among Healthcare Workers at Kampala International University Teaching Hospital, Southwestern Uganda. Can J Infect Dis

Med Microbiol. 2019;2019:4157869. Disponible en: https://doi.org/10.1155/2019/4157869

25. Mohamed SOO, Ali AHM, Ibrahim AAH, Elnil M, Elhassan ABE, Salman MST, et al. Prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) infection in patients with Cystic Fibrosis: a meta-analysis. 2019. Disponible en: https://doi.org/10.21203/rs.2.11668/v1

26. Pillai MM, Latha R, Sarkar G. Detection of methicillin resistance in Staphylococcus aureus by polymerase chain reaction and conventional methods: a comparative study. J Lab Physicians. 2012;4(2):83-8. Disponible en: https://doi.org/10.4103/0974-2727.105587

27. Panda RK, Mahapatra A, Mallick B, Chayani N. Evaluation of Genotypic and Phenotypic Methods for Detection of Methicillin Resistant Staphylococcus aureus in a Tertiary Care Hospital of Eastern Odisha. J Clin Diagn Res. 2016;10(2):DC19-21. Disponible en. https://doi.org/10.7860/jcdr/2016/17476.7278

28. Vyas A, Sharma M, Kumar S, Kumar M, Mehra SK. A comparative study of Oxacillin screen agar, Oxacillin disc diffusion and Cefoxitin disc diffusion, Oxacillin E-test method for routine screening of methicillin-resistant Staphylococcus aureus. Intl J Cur Res Rev. 2015;7(10):55-60. Disponible en: https://ijcrr.com/article_html.php?did=547

29. Ramandinianto SC, Khairullah AR, Effendi MH. MecA gene and methicillin-resistant Staphylococcus aureus (MRSA) isolated from dairy farms in East Java, Indonesia. Biodiversitas. 2020;21(8):3562-8. Disponible en: https://doi.org/10.13057/biodiv/d210819

30. Rajeswarie S, Pradha V, D'Souza AO, Vinod R. Comparison of Phenotypic and Genotypic Characterization Methods for the Detection of Methicillin-Resistant Staphylococcus aureus. Cureus. 2022;14(3):e23396. Disponible en: https://doi.org/10.7759/cureus.23396

31. Pramodhini S, Thenmozhivalli PR, Selvi R, Dillirani V, Vasumathi A, Agatha D. Comparison of Various Phenotypic Methods and mecA Based PCR for the Detection of MRSA. J Clin Diag Res. 2011;5:1359–62. Disponible en: https://www.jcdr.net/articles/pdf/1659/3269_f.pdf