



ORIGINAL ARTICLE

Macrolide-lincosamide-streptogramin B resistance phenotypes and their associated genotypes in *Staphylococcus aureus* isolates from a tertiary level public hospital of Uruguay

Lorena Pardo^a, Virginia Machado^a, Dianna Cuello^a, Paula Aguerrebere^a, Verónica Seija^{b,c}, Valeria Braga^a, Gustavo Varela^{a,*}

^a Bacteriology and Virology Department, Medicine School, Universidad de la República, Montevideo Alfredo Navarro 3051 (south entrance), 11800, Uruguay

^b Bacteriology Laboratory, "Hospital Pasteur", Montevideo, Larravide 2458, 11800, Uruguay

^c Laboratory of Public Health, Alfredo Navarro 3051 (north-entrance), Montevideo, Uruguay

Received 16 July 2019; accepted 21 October 2019

Available online 9 January 2020

KEYWORDS

Staphylococcus aureus;
MLS_B resistance;
D-test;
ermA;
ermC;
msrA/B;
SmaI-PFGE

Abstract This study was undertaken to investigate the resistance phenotypes to macrolide-lincosamide-streptogramin B (MLS_B) antibiotics and their associated genotypes in isolates of *Staphylococcus aureus*. We analyzed one hundred, consecutive, non-duplicate isolates (methicillin-susceptible MSSA, n = 53 and methicillin-resistant MRSA, n = 47) obtained from various clinical samples between July 2012 to December 2013. The resistance profile to MLS_B antibiotics was determined by phenotypic methods and the resistance genes were detected by PCR assays. All of the isolates were subjected to pulsed-field gel electrophoresis (*SmaI*-PFGE). The overall prevalence of resistance to MLS_B antibiotics was 38% and the resistance phenotype distribution was as follows: cMLS_B, 22%; iMLS_B, 10%; MS_B, 5% and L, 1%. We detected *ermA*, *ermC*, *ermB* and *msrA/B* genes in these resistant isolates. The single *ermA* gene was commonly observed mainly in those with a cMLS_B R phenotype, whereas the combination *ermA* and *ermC* was more commonly observed in isolates with inducible expression. The patterns of *SmaI*-PFGE suggest a great genetic diversity in both MRSA and MSSA resistant to MLS_B antibiotics. The results demonstrate the local presence of *S. aureus* resistant to MLS_B antibiotics and its most frequently described responsible genes. Some of these isolates, especially those with the iMLS_B phenotype, may be associated with therapeutic failure. Therefore, efforts should be directed to the correct detection of all MLS_B resistant isolates using appropriate laboratory tests. PFGE results reveal a wide spread of resistance genes rather than the circulation of *S. aureus* clones resistant to MLS_B antibiotics.

© 2019 Asociación Argentina de Microbiología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail addresses: gvarela@higiene.edu.uy, gvarela1961@gmail.com (G. Varela).

PALABRAS CLAVE

Staphylococcus aureus;
Resistencia a MLS_B;
D-test;
ermA;
ermC;
msrA/B;
Smal-PFGE

Fenotipos de resistencia a macrólidos lincosamidas-estreptograminas B y sus genotipos asociados en *Staphylococcus aureus* aislados en un hospital público de nivel terciario en Uruguay

Resumen Los objetivos de este estudio fueron investigar en *Staphylococcus aureus* la presencia de fenotipos resistentes a los antibióticos macrólidos, lincosamidas y estreptograminas tipo B (MLS_B) y conocer sus genotipos responsables. Analizamos 100 aislamientos consecutivos, no duplicados (53 sensibles a meticilina [MSSA] y 47 resistentes a meticilina [MRSA]), obtenidos entre 2012 y 2013 a partir de diferentes muestras clínicas. El perfil de resistencia a los antibióticos MLS_B fue determinado por métodos fenotípicos y los genes de resistencia se detectaron por PCR. Todos los aislamientos fueron comparados por Smal-PFGE. La prevalencia global de resistencia a los antibióticos MLS_B fue del 38% y la distribución de los fenotipos de resistencia fue la siguiente: cMLS_B, 22%; iMLS_B, 10%; MS_B, 5%; L, 1%. Se detectaron los genes *ermA*, *ermC* y *msrA/B* en los aislamientos resistentes. El gen *ermA* se observó, sobre todo, en aislamientos con fenotipo resistente constitutivo R (cMLS_B), mientras que la combinación *ermA* y *ermC* se detectó principalmente en aislamientos con resistencia inducible (iMLS_B). Los patrones de Smal-PFGE sugieren una gran diversidad genética en los aislamientos resistentes a los antibióticos MLS_B, tanto MRSA como MSSA. Los resultados demuestran la presencia local de *S. aureus* resistentes a los antibióticos MLS_B y de sus genes responsables más frecuentemente descritos. Estos cultivos, especialmente aquellos con fenotipo resistente iMLS_B, pueden asociarse con fallos terapéuticos. Por lo tanto, los esfuerzos deben dirigirse a la correcta detección de todos los cultivos resistentes a MLS_B utilizando pruebas de laboratorio adecuadas. Los resultados de Smal-PFGE sugieren una amplia diseminación de genes de resistencia, más que la circulación de clones resistentes a los antibióticos MLS_B.

© 2019 Asociación Argentina de Microbiología. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Staphylococcus aureus is a versatile microorganism that is found in the human microbiota and is also responsible for both hospital- or community-acquired illnesses. The diseases that it causes range from mild lesions that compromise skin and soft tissues to more severe conditions such as pneumonia, septic arthritis, endocarditis, osteomyelitis, and sepsis^{12,31,33}. A biological characteristic of *S. aureus* is its ability to acquire exogenous genetic material that encodes several resistance mechanisms for different antibiotics. The mobile staphylococcal chromosomal cassette *mec* (SCC*mec*) that carries the *mecA* gene which encodes PBP2a, which is responsible for methicillin-resistance (MRSA), was likely acquired by horizontal transfer from a related species⁸. Three categories of MRSA are currently recognized, healthcare-associated (HA-MRSA), community-associated (CA-MRSA) and livestock-associated MRSA (LA-MRSA) according to epidemiological criteria, site of infection acquisition (community, hospital or animal contact), associated antibiotic susceptibility profile and genotype, including SCC element type^{11,34,35}. *S. aureus* belonging to the three aforementioned categories have been implicated around the world in both sporadic cases and outbreaks of human staphylococcal diseases^{4,16,37}. Macrolides, lincosamides, and type B streptogramin (MLS_B) antibiotics can be alternatives for treatment of MRSA infections³⁶. However, the increase in macrolide, lincosamide and type B streptogramin resistant isolates complicates the empirical antimicrobial

treatment selection in *S. aureus* infections¹⁴. A fairly common resistance mechanism observed in *S. aureus* is due to the action of efflux pumps, encoded by the *msrA* and *msrB* genes responsible for pumping macrolide and streptogramin B antibiotics out of the bacteria (MS_B resistance phenotype). On the other hand, the exclusive resistance to lincosamides (L resistance phenotype) mediated by lincomycin nucleotidyl-transferase enzymes and codified by genes *lnuA-F*, is occasionally observed. Isolates that carry *lnu* genes resist high levels of lincomycin but can be susceptible to clindamycin in the disk induction test^{18,23}. In *S. aureus* and other gram-positive bacteria, resistance to MLS_B antibiotics is mainly due to the 23S rRNA modification by adenine-N methyltransferase enzymes. This modification compromises macrolide, lincosamide and type B streptogramin activity. Genes encoding these methylases have been designated as *erm* genes and their expression can be constitutive (cMLS_B resistance phenotype) or inducible (iMLS_B resistance phenotype)¹³. Isolates with the iMLS_B phenotype show in vitro resistance to erythromycin, but can falsely seem to be susceptible to clindamycin. These isolates have a high rate of spontaneous mutation to constitutive resistance phenotypes that can be selected by the usage of clindamycin, resulting in therapeutic failure. This fact highlights the importance of performing a correct laboratory identification using any of the recommended tests^{3,6}. In our region, data about the prevalence of resistance to MLS_B antibiotics in isolates of *S. aureus* recovered from adult individuals are scarce. This study aimed to investigate the presence

of the different erythromycin and clindamycin resistance phenotypic profiles and also to determine the responsible genotypes in a set of *S. aureus* isolates recovered in a public, tertiary level hospital of Montevideo city, Uruguay.

Materials and methods

We study 100 consecutive, non-duplicate *S. aureus* isolates obtained from patients admitted at the "Hospital Pasteur" (HP) from July 2012 to December 2013. *Setting* The HP is a 250-bed tertiary level public and teaching hospital for all medical and surgical specialties. Patients receiving medical care at the HP are adults (>16 years of age) and often come from low-income households.

Bacterial isolates, source, identification and antimicrobial susceptibility testing.

S. aureus isolates were identified using the automated system VITEK 2 version 7.01 and classified as methicillin-resistant (MRSA) or methicillin-susceptible (MSSA) according to the obtained MIC value to oxacillin and the result of the cefoxitin test. The site of infection was taken from the laboratory records. Erythromycin (ERY) and clindamycin (CLI) phenotypes were assessed and interpreted using the D-zone test published by Steward et al.³² (D phenotype: D-shaped clear zone around the CLI disk near the ERY disk; D+ phenotype: like the previous one but with small colonies growing close to the CLI disk in an otherwise clear zone; HD phenotype: two zones of growth around the CLI disk: one zone is a light growth extending from the CLI disk to the second zone, where the growth is much thicker; R phenotype: growth to the edge of both CLI and ERY disks; MS_B phenotype: a clear zone around the CLI disk; L phenotype: growth only up to the CLI disk; S phenotype: susceptible to both the CLI and ERY disks)^{3,2}.

The IHSA 23 strain of *S. aureus* was used as a positive control for the D-test. Minimum inhibitory concentration (MIC) to erythromycin, clindamycin, vancomycin, teicoplanin and oxacillin was determined by the agar dilution method according to the Clinical and Laboratory Standards Institute recommendations⁶. The susceptibility patterns to ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, tetracycline and rifampicin were taken from the VITEK report. Broth macrodilution assays (BMA) that included combinations of CLI plus ERY were conducted with minor modifications in accordance with the general procedure previously described by Steward et al.³². Briefly, the combinations were tested using CLI at final concentrations ranging from 0.5 to 8 µg/ml plus ERY at final concentrations ranging from 0.03 to 0.12 µg/ml. Reading was done after 18–24 h of incubation at 35–37 °C. This procedure was applied to those isolates that showed the iMLS_B, cMLS_B, MS_B and S phenotypes by disk diffusion tests. *S. aureus* ATCC 29213 (CLI and ERY-susceptible) was included as control. The isolates were stored at –20 °C in skimmed milk for further studies.

Molecular typing and antibiotic resistance gene detection. Bacterial DNA was obtained from isolated colonies using the Wizard genomic DNA preparation kit (Promega, Madison, Wis, USA) adding 20 mg/ml lysostaphin (Sigma Chemical) in the cell lysis step. Detection of the *mecA* gene and SCCmec typing was performed in all MRSA isolates by a multiplex PCR assay previously described by Oliveira DC and

H. de Lencastre²⁰. MRSA isolates that carried the SCCmec type IV were arbitrarily taken as community-associated methicillin-resistant *S. aureus* (CA-MRSA) while those that carried the SCCmec type I, II, or III were grouped as health-care associated methicillin-resistant *S. aureus* (HA-MRSA). PCR detection of the *ermA*, *ermB*, *ermC*, *msrA* and *msrB* genes was performed on all isolates that showed the following resistance phenotypes: iMLS_B (D and D+); cMLS_B (HD and R); and MS_B using standard protocols and previously described primers¹⁵. *S. aureus* MM122-35 was used as positive control for the *ermA* gene, *S. aureus* MM2 was used as a positive control for the *ermB* gene, and *S. aureus* MM523 for the *ermC* gene. *S. aureus* MM3627 was included as positive control for both *msrA* and *B* genes. All the strains were kindly provided by Genoveva Pensado (Bacteriology and Virology department). Ultrapure RNase/DNase free water was used as negative control in all PCR reactions.

Pulsed field gel electrophoresis (PFGE). DNA macrorestriction with the *SmaI* enzyme and further separation of fragments by pulsed field gel electrophoresis (PFGE) were carried out using a previously described protocol²⁴. The obtained profiles were analyzed by the unweighted pair group method with arithmetic average (UPGMA) using *BioNumerics* v.7.1. The isolates that showed ≥80% similarity were included into the same pulsogroup (PG), while those that showed ≥95% similarity were considered to be of identical pulsotype (PT)⁹. *S. aureus* subsp. *aureus* (strain NCTC 8325) was included as reference size marker.

Results

Source of analyzed *S. aureus* isolates and resistance phenotypes

We studied 100 isolates of *S. aureus*. Twenty-three of them were recovered from invasive diseases including bacteremia (n=10; 43.4%), ventilator-associated pneumonia (n=8; 35%), deep abscesses (n=4; 17.3%) and acute meningococcalitis (n=1; 4.3%). On the other hand, 77 isolates were recovered from skin and soft tissue infections (SSTIs) including superficial abscesses (n=39; 50%), wounds (n=22; 28.5%), cellulitis (n=11; 14.2%) and whitlows (n=5; 6.4%).

Fifty-three isolates were classified as MSSA and the remaining 47 as MRSA (all of them carried the *mecA* gene); 25 corresponded to CA-MRSA and 22 to HA-MRSA, according to the above described abbreviated criterion. The distribution of the SCCmec types in HA-MRSA was as follows: SCCmec type I, 5 isolates; SCCmec type II, 15 isolates and SCCmec type III, 2 isolates. Fifteen percent of the MSSA isolates were resistant to MLS_B antibiotics, whereas this figure increased to 63.8% in the MRSA (CA-MRSA and HA-MRSA) isolates. All *S. aureus* isolates included in this study were susceptible to vancomycin and teicoplanin. Four isolates (4%) were resistant to rifampicin, all of them carrying the SCCmec type II. Twenty two out of 47 (46.8%) MRSA showed resistance to ciprofloxacin (HA-MRSA, n=21; CA-MRSA, n=1). However, only 4 out of 53 (7.5%) MSSA were resistant to this antibiotic. Eight *S. aureus* isolates (8%) showed resistance to gentamicin, 4 (7.5%) corresponded to MSSA isolates and 4 (8.5%) to MRSA (HA-MRSA, n=3; CA-MRSA, n=1). One methicillin-susceptible isolate was resistant

Table 1 Distribution of phenotypes to MLS_B antibiotics in *S. aureus* isolates using the D-zone test.

	MRSA with SCCmec type				Subtotal	MSSA n (%)	Total
	I n (%)	II n (%)	III n (%)	IV n (%)			
iMLS _B ^a	0 (0%)	0 (0%)	0 (0%)	5 (5%)	5 (5%)	5 (5%)	10 (10%)
cMLS _B ^b	4 (4%)	14 (14%)	2 (2%)	1 (1%)	21 (21%)	1 (1%)	22 (22%)
MS _B ^c	1 (1%)	1 (1%)	0 (0%)	1 (1%)	3 (3%)	2 (2%)	5 (5%)
L ^d	0 (0%)	0 (0%)	0 (0%)	1 (1%)	1 (1%)	0 (0%)	1 (1%)
S ^e	0 (0%)	0 (0%)	0 (0%)	17 (17%)	17 (17%)	45 (45%)	62 (62%)
Total	5 (5%)	15 (15%)	2 (2%)	25 (25%)	47 (47%)	53 (53%)	100 (100%)

^a iMLS_B: inducible resistance to MLS_B antibiotics.^b cMLS_B: constitutive resistance to MLS_B antibiotics.^c MS_B: resistance to erythromycin.^d L: resistance only to clindamycin.^e S: erythromycin and clindamycin susceptible.**Table 2** Resistance to MLS_B antibiotics in *S. aureus* isolates.

% of resistance to	MRSA with SCCmec IV (n = 25)	MRSA with SCCmec I, II, III (n = 22)	MSSA (n = 53)
Macrolides(iMLS _B , cMLS _B and MS _B resistance phenotypes)	28%	100%	15.1%
Lincosamides(iMLS _B , cMLS _B and L resistance phenotypes)	28%	90.9%	11.3%

to tetracycline. The only *S. aureus* isolate that showed resistance to trimethoprim-sulfamethoxazole belonged to the HA-MRSA group. **Table 1** shows the resistant phenotypes to MLS_B antibiotics found in MRSA and MSSA isolates. **Table 2** shows the percentage of resistance to macrolides and lincosamides found in MRSA (HA-MRSA, CA-MRSA) and MSSA isolates. Three out of the 10 isolates with iMLS_B resistance showed the D+ phenotype in the disk-diffusion test. The isolates with the iMLS_B resistance phenotype (n = 10; 5 CA-MRSA and 5 MSSA) showed MICs for CLI ≤ 0.125 $\mu\text{g/ml}$ and those with phenotype S (n = 62; MSSA 45 and CA-MRSA 17) had MICs for CLI ≤ 0.25 $\mu\text{g/ml}$. In those isolates that exhibited the cMLS_B resistance phenotype (n = 22; HA-MRSA, 21 and MSSA, 1 isolate) MICs for CLI were ≥ 256 $\mu\text{g/ml}$. As shown in **Table 3**, the combination of 2 $\mu\text{g/ml}$ of CLI plus 0.12 $\mu\text{g/ml}$ of ERY in the BM assay allowed us to differentiate between isolates displaying iMLS_B or cMLS_B resistance phenotypes and those with the MS_B or S phenotype. Seven out of 10 isolates that showed the iMLS_B resistant phenotype (D, 4 isolates and D+, 3 isolates) grew in the presence of CLI at 8 $\mu\text{g/ml}$ plus ERY 0.12 $\mu\text{g/ml}$ and all the isolates (n = 22) that showed the cMLS_B resistance phenotype (HD and R) grew in the presence of the combination 8 $\mu\text{g/ml}$ of CLI plus ERY 0.12 $\mu\text{g/ml}$.

Distribution of the resistance genes to MLS_B antibiotics among *S. aureus* isolates

As shown in **Table 4**, all the isolates that showed the iMLS_B resistance phenotype in the D-test carried the combination of the *ermA* and *ermC* genes. Instead, in those isolates that displayed the cMLS_B resistance phenotype (HD or R) the predominant genotype was *ermA* (alone or in combination with other resistance genes). In those isolates that showed

the cMLS_B (HD) phenotype, the combination of the *ermA* and *mrs* genes was seen more frequently (6 out of 8) than in those that displayed the cMLS_B (R) phenotype (2 in 14) (**Table 4**). The *ermB* gene was found in just one isolate with the cMLS_B resistance phenotype. The responsible genotype was not studied in the CA-MRSA isolate that showed the L resistance phenotype.

Smal-PFGE profiles in MRSA and MSSA isolates. As shown in **Figure 1**, the Smal-PFGE profile analysis of MRSA isolates revealed a wide variety of PTs (n = 41) and 3 clusters (pulsogroups, PG: A, B and C, with $\geq 80\%$ similarity and more than 2 isolates in each). Cluster A included 6 isolates, one with SCCmec IV, one with SCCmec III, one with SCCmec I and finally 3 isolates that carried the SCCmec II element with the cMLS_B resistance phenotype that showed 100% identity and were recovered from 3 inpatients located in the same surgery ward. The only isolate with the L resistance phenotype recovered in this study was located in this PG. Cluster B (9 isolates) included 8 CA-MRSA isolates. Three of them (including an isolate carrying the SCCmec type II) showed 100% similarity and were visually identical to the main pulsotype found in local CA-MRSA isolates recovered in Uruguay between 2001 and 2002 (PFGE profile A1, type USA1100). Other 3 isolates in this PG showed 100% similarity among them (including the single CA-MRSA isolate with the cMLS_B resistance phenotype recovered in this study) and were also visually similar to the CA-MRSA PFGE profile A1. Cluster C also included CA-MRSA isolates. The 5 CA-MRSA isolates that displayed the iMLS_B resistance phenotype belonged to 5 different PTs (see **Fig. 1**). In the MSSA isolates, 52 PTs and 3 PGs (A, B and C, $\geq 80\%$ similarity, more than 2 isolates in each) (see **Fig. 2**) were recognized. Only 2 isolates showed 95% similarity and were located in the PG C. This PG included 7 isolates, 2 of them with the iMLS_B phenotype (see **Fig. 2**).

Table 3 Results of Broth Macrodilution Assays (BMA).

	Disk diffusion phenotype results				
	iMLS _B ^a		cMLS _B ^b (HD/R)	MS _B ^c	S ^d
	D	D+			
No. of isolates	7	3	22 (8/14)	5	62
No. of isolates that grew with					
<i>CLI 8 µg/ml plus</i>					
ERY 0.12 µg/ml	4	3	22	0	0
ERY 0.06 µg/ml	4	3	22	0	0
ERY 0.03 µg/ml	4	3	22	0	0
<i>CLI 4 µg/ml plus</i>					
ERY 0.12 µg/ml	4	3	22	0	0
ERY 0.06 µg/ml	4	3	22	0	0
ERY 0.03 µg/ml	4	3	22	0	0
<i>CLI 2 µg/ml plus</i>					
ERY 0.12 µg/ml	7	3	22	0	0
ERY 0.06 µg/ml	7	3	22	0	0
ERY 0.03 µg/ml	7	3	22	0	0
<i>CLI 1 µg/ml plus</i>					
ERY 4 µg/ml	7	3	22	0	0
ERY 1 µg/ml	7	3	22	0	0
<i>CLI 0.5 µg/ml plus</i>					
ERY 1 µg/ml	7	3	22	0	0
<i>MIC100 to CLI without ERY</i>	≤0.125 µg/ml		≥250 µg/ml	≤0.125 µg/ml	≤0.250 µg/ml

^a Inducible resistance to MLS_B antibiotics, D: clear zone around the CLI disk near to the ERY disk; D+: like the previous one but with small colonies growing up to the CLI disk in an otherwise clear zone.

^b cMLS_B (HD/R) Constitutive resistance to MLS_B antibiotics, HD: two zones of growth appear around the CLI disk, one zone is a light growth extending from the CLI disk to the second zone where the growth is much thicker; R: growth to the edge of both the CLI and ERY disks.

^c MS_B clear zone around the CLI disk.

^d S susceptible to both the CLI and ERY disks.

Table 4 Genotypes found in isolates of *S. aureus* resistant to MLS_B antibiotics.

Genotype	Resistance phenotype in the D-test assay				
	iMLS _B ^a n = 10	cMLS _B (HD) ^b n = 8	cMLS _B (R) ^c n = 14	MS _B ^d n = 5	L ^e n = 1
	<i>ermA</i> /10 <i>ermC</i> /10 <i>msrB</i> /3	<i>ermA</i> /1 <i>ermB</i> /1 <i>ermA</i> + <i>ermC</i> + <i>msrA</i> + <i>msrB</i> /4 <i>ermA</i> + <i>msrA</i> /2	<i>ermA</i> /11 <i>ermC</i> /1 <i>ermA</i> + <i>msrA</i> /1 <i>ermA</i> + <i>msrB</i> /1	<i>msrA</i> /5 <i>msrB</i> /5	ND ^f

^a iMLS_B: inducible resistance to MLS_B antibiotics.

^b cMLS_B (HD): constitutive resistance to MLS_B antibiotics (two zones of growth appear around the CLI disk, one zone is a light growth extending from the CLI disk to the second zone where the growth is much thicker).

^c cMLS_B (R): constitutive resistance to MLS_B antibiotics (growth to the edge of both the CLI and ERY disks).

^d MS_B: resistance only to erythromycin.

^e L: growth only up to the CLI disk.

^f ND, not done, unknown.

Discussion

Clindamycin has been used for the treatment of severe staphylococcal diseases, taking into account some of its pharmacological properties. However, resistance can

develop in isolates with the iMLS_B phenotype, and spontaneous constitutively resistant mutants have arisen from such isolates during clindamycin therapy, determining therapeutic failures in some patients¹⁰. This is the first local report about the distribution of resistance phenotypes to

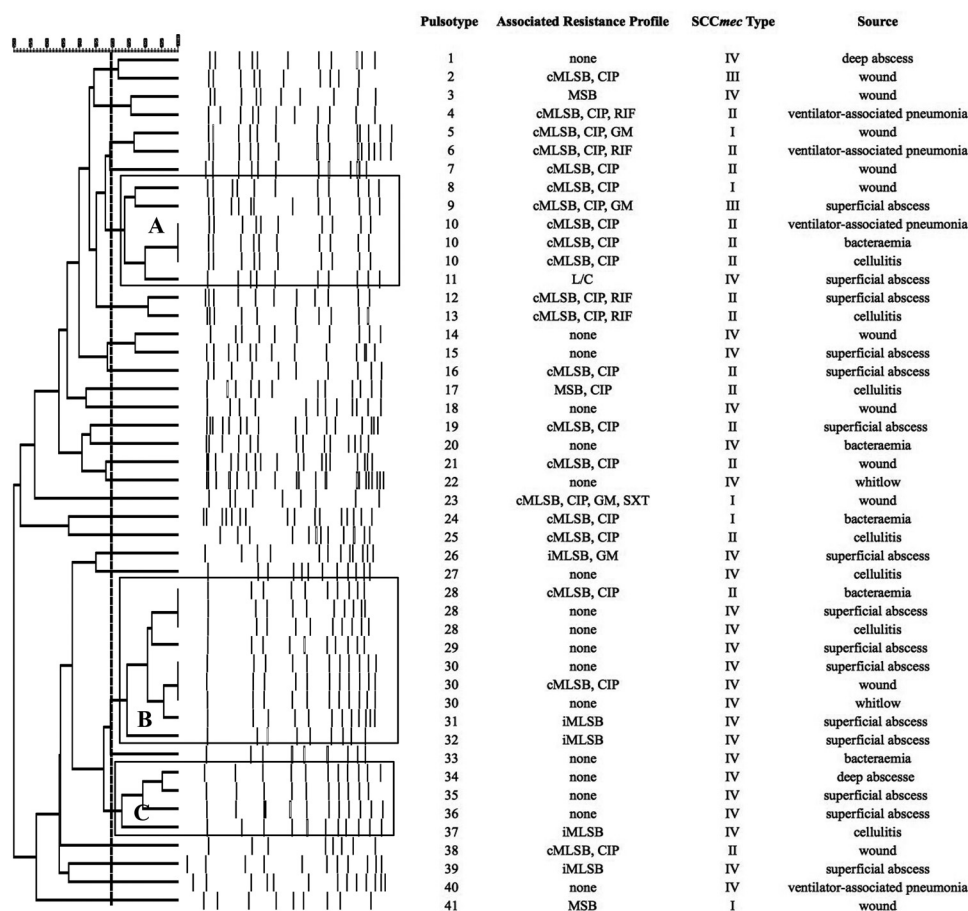


Figure 1 Pulsed-field gel electrophoresis (PFGE) dendrogram of methicillin-resistant *S. aureus* isolates showing PTs, PGs, associated profile of resistance, SCCmec type and sample source. Dashed line indicates PGs (named as A, B and C, with $\geq 80\%$ similarity and more than 2 isolates in each). cMLS_B, constitutive resistance to macrolide, lincosamide and type B streptogramin; iMLS_B, inducible resistance to macrolide, lincosamide and type B streptogramin; MS_B, resistance to macrolide and streptogramin B; L, resistance to lincosamides; CIP, resistance to ciprofloxacin; GM, resistance to gentamicin; RIF, resistance to rifampicin; SXT, resistance to trimethoprim-sulfamethoxazole; None, susceptible to all tested antibiotics.

MLS_B antibiotics and their responsible genes in a set of *S. aureus* isolates recovered from adult inpatients in a public hospital. The overall prevalence of resistance to MLS_B antibiotics was 38%. This figure is similar to that reported by Abbas et al.¹ (40%) in a study conducted in India that included 500 isolates of *S. aureus* recovered in a tertiary hospital and otherwise higher than that also found in India by Prabhu et al. (28.42%)²⁵. Similarly to what happened in Serbia and India, in the current study most *S. aureus* isolates (62%) showed susceptibility to both macrolide and lincosamide antibiotics. However, the distribution of the resistance phenotypes was different from that reported by those authors. In Serbia, iMLS_B was the most frequent resistance phenotype detected (31.5–33.7%); instead in India it was MS_B (17%) followed by both cMLS_B and iMLS_B resistance phenotypes (11.6% and 10.8%, respectively)^{21,25}. In this study the overall data show that the predominant resistance phenotype was cMLS_B (22%) followed by iMLS_B (10%), MS_B (5%) and L (1%). Similar figures were reported by Sedaghat in the Iranian teaching hospital and also by Paz Pereira et al. in neighboring Brazil^{3,28}. The cMLS_B resistance phenotype prevailed among HA-MRSA isolates (SCCmec I, II and III) (20/22;

91%), which is in concordance with similar studies carried out in other countries^{2,5,29,39}. In this work the prevalence of the iMLS_B resistance phenotype was similar among MRSA (CA-MRSA and HA-MRSA) (5/47; 10.6%) and MSSA (5/53; 9%) isolates. This result is not in concordance with the findings reported by Abbas et al.¹ or Rahbar et al.²⁶, which showed a higher prevalence of the iMLS_B resistance phenotype in MSSA isolates. Furthermore, in contrast to the findings of Mišić et al.²¹ in Serbia, none of the HA-MRSA (SCCmec I, II and III) isolates studied here showed the iMLS_B resistance phenotype (Table 1). The prevalence of MSSA isolates with the iMLS_B resistance phenotype found in our study (9%) was lower than that reported by Shoji et al. in Japan (25.4%)³⁰. As we will comment below, the results obtained by Smal-PFGE showed great genetic diversity in both MSSA and MRSA isolates with iMLS_B and cMLS_B resistance phenotypes (Figs. 1 and 2). This suggests a broad diffusion of the responsible resistance genes rather than the circulation of a predominant PT. The overall prevalence of the MS_B resistance phenotype was 5%; this figure being lower than that reported by Mišić et al.²¹ in Serbia (17.6%) but similar to the results (6%) obtained by Sedaghat et al.²⁸ in the

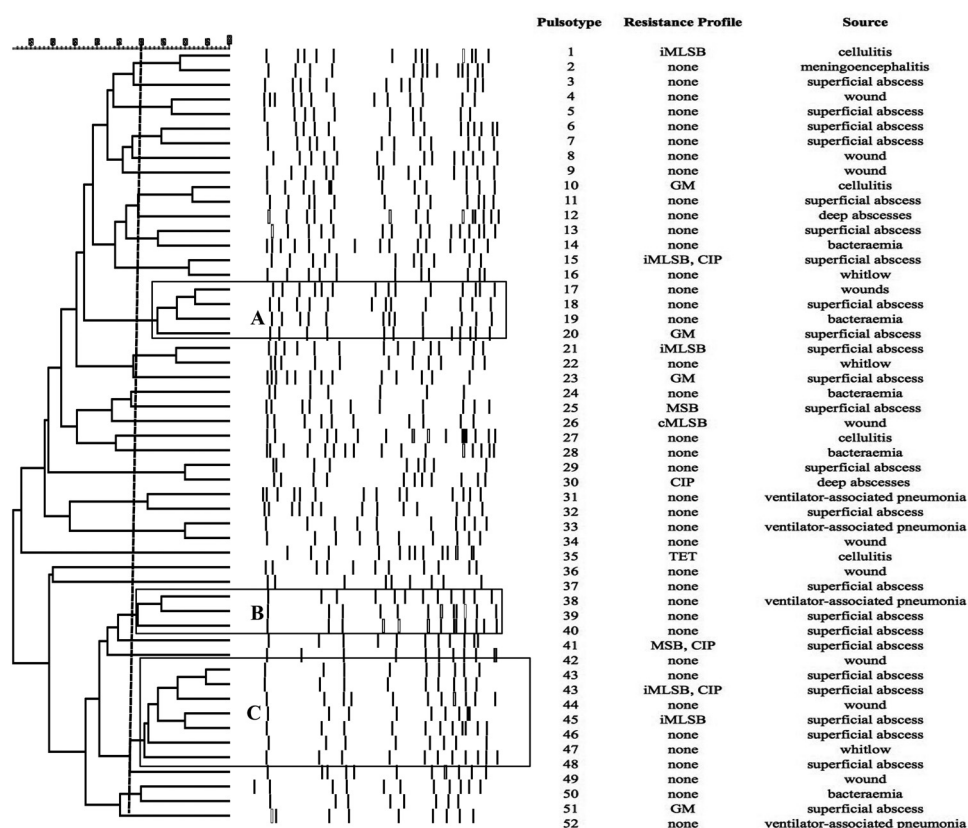


Figure 2 Pulsed-field gel electrophoresis (PFGE) dendrogram of methicillin-susceptible *S. aureus* isolates showing PTs, PGs, associated profile of resistance and sample source. Dashed line indicates PGs (named as A, B and C, with $\geq 80\%$ similarity and more than 2 isolates in each). cMLSB, constitutive resistance to macrolide, lincosamide and type B streptogramin; iMLSB, inducible resistance to macrolide, lincosamide and type B streptogramin; MSB, resistance to macrolide and streptogramin B; L, resistance to lincosamides; CIP, resistance to ciprofloxacin; GM, resistance to gentamicin; RIF, resistance to rifampicin; TET, resistance to tetracycline; None, susceptible to all tested antibiotics.

Iranian teaching hospitals^{21,28}. The combination of *msrA* and *msrB* genes was detected in all (5) isolates that showed this resistance phenotype. Only one isolate showed the L resistance phenotype; as formerly seen in Argentina, which corresponded to a CA-MRSA isolate carrying SCCmec type IV¹⁸. The occurrence of this resistance type remains low at the HP hospital (1%) as was described previously^{17,21,23}. The prevalence of resistance phenotypes to MLS_B antibiotics vary depending on the analyzed country, geographic area and even the type of patient (inpatients vs. outpatients; hospital vs. community origin; children vs. adults; public vs. private institutions; patients or healthcare workers included, among others). This global trend highlights the need for each country, region or hospital to use its own bacterial resistance surveillance program^{2,5,26,29}. The obtained results in the BM assay combining 2 µg/ml of CLI plus 0.12 µg/ml of ERY allowed us to differentiate between isolates displaying iMLSB or cMLSB phenotypes and those with the MSB or the S phenotype. However, in contrast with Steward et al.'s findings³², in this study some *S. aureus* isolates with an iMLSB resistance phenotype and positive D test were able to grow at higher concentrations of clindamycin. This could be partly explained by the fact that these isolates with the D or D+ phenotype carry the combination of *erm* and *msr* genes, as we discussed further³². All analyzed *S. aureus* isolates

(n = 100) were susceptible to vancomycin and teicoplanin. However, it is necessary to conduct an active surveillance to early detect the presence of glycopeptide-resistant strains. As expected, most of the *S. aureus* isolates grouped in this study as HA-MRSA (21 of 22) showed ciprofloxacin resistance. Only 4 of 100 isolates were resistant to rifampicin and all of them corresponded to HA-MRSA.

Resistance genes to MLS_B antibiotics and genetic comparison by SmaI-PFGE

The PCR analysis identified the *ermA* gene as the most frequent MLS_B resistance gene followed by *ermC*. The *ermA* gene was detected individually or in combination with other genes such as *ermC*, *msrA* and *msrB* in 30 (79%) out of 38 *S. aureus* resistant to MLS_B antibiotics. In 12 of them, especially in HA-MRSA (11/14), it was individually detected and all these isolates showed a cMLSB resistance phenotype (R) (see Table 4). This result is in concordance with previous findings obtained in European hospitals²⁷. In general, the *ermA* genes are mainly detected in MRSA isolates and are located in transposons related to Tn554, while the *ermC* genes are frequently responsible for resistance in MSSA and are located in plasmids. Based on the SmaI PFGE results, we

can rule out the hypothesis of a broad hospital spread of a single pulsotype with a cMLS_B resistance phenotype carrying the *ermA* gene. Only three isolates belonging to cluster A (Fig. 1) showed 100% similarity; they carried the SCCmec type II and were recovered from three patients of the same ward. This probably corresponded to a case of limited intra-hospital cross contamination. In the two above cited studies, there are no data about the genetic comparison of the included strains; therefore, it cannot be ruled out that they pertained to prevalent clones. In similar studies conducted in Brazil and European countries the *ermC* gene predominated over the *ermA* gene^{19,22,37}. As it occurred in Brazil, we merely detected one resistant isolate that carried the *ermB* gene and showed the cMLS_B resistance phenotype⁷. This low prevalence (2.6%) was expected, taking into account that we only included isolates collected from adult patients. This gene is frequently found in *S. aureus* isolates from animal origin and also in pediatric CA-MRSA isolates^{7,38}. The PCR analysis identified the *msrA* and *msrB* genes, alone or combined, in 16 isolates belonging to the MSSA, CA-MRSA or HA-MRSA categories with iMLS_B, cMLS_B and MS_B resistance phenotypes. An interesting result of this study was the high rate of combination of MLS_B resistance genes. The most commonly detected profile was *ermA* plus *ermC* (n = 14), followed by the combination of *ermA* plus *msrA* (n = 7), many of which also bore the *msrB* gene. All isolates with the iMLS_B resistance phenotype (n = 10) carried both *ermA* and *ermC* genes, and three of them also harbored the *msrB* gene (Table 4). The *Sma*I-PFGE results showed great genetic diversity in both MSSA and MRSA isolates with iMLS_B and cMLS_B resistance phenotypes. They support the wide spread of the resistance genes to MLS_B antibiotics in these studied clinical *S. aureus* isolates rather than the circulation of a predominant PT. This study has two limitations: MRSA isolates were considered to be CA-MRSA or HA-MRSA only according to the SCCmec type that they carried and the use of PCR for *erm* genes does not allow to differentiate between isolates with iMLS_B or cMLS_B resistance phenotypes.

Conclusions

The overall prevalence of resistance to MLS_B antibiotics in this group of clinical *S. aureus* isolates was 38% and the resistance phenotype distribution was as follows: cMLS_B, 22%; iMLS_B, 10%; MS_B, 5% and L, 1%. In these resistant isolates we detected, *ermA*, *ermC*, *ermB* and *msrA/B* genes. The single *ermA* gene was commonly found in HA-MRSA (SCCmec I, II and III) isolates, mainly in those with a cMLS_B R resistance phenotype; whereas the combination *ermA* and *ermC* was more commonly observed in MSSA or CA-MRSA (SCCmec IV) isolates with inducible resistance expression (iMLS_B phenotype). There was a high rate of combination of MLS_B resistance genes and the obtained patterns by *Sma*I-PFGE suggest a great genetic diversity in this group of MRSA and MSSA MLS_B resistant isolates. The obtained results demonstrate the local presence of *S. aureus* resistant to MLS_B antibiotics and its most frequently described responsible genes. These isolates (especially those with the inducible resistance phenotype, iMLS_B) may be associated with therapeutic failures; therefore, efforts should be directed toward their correct detection using

appropriate laboratory tests, particularly among those recovered from hospitalized individuals suffering from comorbidities or receiving immunosuppressive treatment.

Funding

This work was supported by a grant of the Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República [program I + D group 290].

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We thank Dr. Felipe Schelotto for the critical review of the manuscript.

References

1. Abbas A, Srivastava P, Prem Singh N. Prevalence of MLS_B resistance and observation of *ermA* and *ermC* genes at a tertiary care hospital. *J Clin Diagn Res*. 2015;9:DC08–10.
2. Adhikari RP, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant *Staphylococcus aureus* in a tertiary care hospital, Kathmandu, Nepal. *BMC Infect Dis*. 2017;17:483.
3. Ajantha G, Kulkarni R, Shetty J, Shubhada C, Jain P. Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates by using the lower limit of recommended inter-disk distance. *Indian J Pathol Microbiol*. 2008;5:376–8.
4. Bosch T, Verkade E, van Luit M, Landman F, Kluytmans J, Schouls LM. Transmission and persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* among veterinarians and their household members. *Appl Environ Microbiol*. 2015;81:124–9.
5. Bottega A, Rodrigues MA, Carvalho F, Wagner TF, Leal IA, Santos SO, Rampelotto RF, Hörner R. Evaluation of constitutive and inducible resistance to clindamycin in clinical samples of *Staphylococcus aureus* from a tertiary hospital. *Rev Soc Bras Med Trop*. 2014;47:589–92.
6. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2018.
7. Coutinho V de LS, Paiva RM, Reiter KC, de-Paris F, Barth AL, Machado ABMP. Distribution of *erm* genes and low prevalence of inducible resistance to clindamycin among *staphylococci* isolates. *Braz J Infect Dis*. 2010;14:564–8.
8. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*. 2010;23:616–87.
9. Goolam Mahomed T, Kock M, Masekela R, Hoosien E, Ehlers M. Genetic relatedness of *Staphylococcus aureus* isolates obtained from cystic fibrosis patients at a tertiary academic hospital in Pretoria, South Africa. *Sci Rep*. 2018;8:12222.
10. Gudiol C, Cuervo G, Shaw E, Pujol M, Carratalà J. Pharmacotherapeutic options for treating *Staphylococcus aureus* bacteremia. *Expert Opin Pharmacother*. 2017;18:1947–63.
11. Jevons MP, Coe AW, Parker MT. Methicillin resistance in *Staphylococci*. *Lancet*. 1963;281:904–7.
12. Kaspar U, Kriegeskorte A, Schubert T, Peters G, Rudack C, Pieper DH, Wos-Oxley M, Becker K. The culturome of the human nose

- habitats reveals individual bacterial fingerprint patterns. *Environ Microbiol.* 2016;18:2130–42.
13. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis.* 2002;34:482–92.
14. Lewis Ii JS, Jorgensen JH. Inducible clindamycin resistance in *staphylococci*: should clinicians and microbiologists be concerned? *Clin Infect Dis.* 2005;40:280–5.
15. Lina G, Quaglia A, Reverdy M, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among *Staphylococci*. *Antimicrob Agents Chemother.* 1999;43:1062–6.
16. Loewen K, Schreiber Y, Kirlow M, Bocking N, Kelly L. Community-associated methicillin-resistant *Staphylococcus aureus* infection: Literature review and clinical update. *Can Fam Physician.* 2017;63:512–20.
17. Lozano C, Aspiroz C, Sáenz Y, Ruiz-García M, Royo-García G, Gómez-Sanz E, et al. Genetic environment and location of the *lnu(A)* and *lnu(B)* genes in methicillin-resistant *Staphylococcus aureus* and other staphylococci of animal and human origin. *J Antimicrob Chemother.* 2012;67:2804–8.
18. Mendez Ede L, Roldan ML, Baroni MR, Mendosa MA, Cristobal SA, Virgolini SM, Faccone D. A case of familial transmission of community-acquired methicillin-resistant *Staphylococcus aureus* carrying the *lnu(A)* gene in Santa Fe city, Argentina. *Rev Argent Microbiol.* 2012;44:303–5.
19. Merino-Díaz L, Cantos de la Casa A, Torres-Sánchez MJ, Aznar-Martín J. Detection of inducible resistance to clindamycin in cutaneous isolates of *Staphylococcus* spp. by phenotypic and genotypic methods. *Enferm Infecc Microbiol Clin.* 2007;25:77–81.
20. Milheirico C, Oliveira D, De Lencastre H. Multiplex P.C.R strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: “SCCmec IV multiplex”. *J Antimicrob Chemother.* 2007;60:42–8.
21. Mišić M, Čukić J, Vidanović D, Šekler M, Matić S, Vukašinović M, Baskić D. Prevalence of genotypes that determine resistance of *staphylococci* to macrolides and lincosamides in Serbia. *Front Public Health.* 2017;5:200.
22. Pereira JNP, Rabelo MA, Lima JLC, Neto AMB, Lopes ACS, Maciel MAV. Phenotypic and molecular characterization of resistance to macrolides, lincosamides and type B streptogramin of clinical isolates of *Staphylococcus* spp. of a university hospital in Recife, Pernambuco, Brazil. *Braz J Infect Dis.* 2016;20:276–81.
23. Novotná G, Adamková V, Janata J, Melter O, Spizek J. Prevalence of resistance mechanisms against macrolides and lincosamides in methicillin-resistant coagulase-negative staphylococci in the Czech Republic and occurrence of an undefined mechanism of resistance to lincosamides. *Antimicrob Agents Chemother.* 2005;49:3586358–9.
24. Pardo L, Vola M, Macedo-Viñas M, Machado V, Cuello D, Mollerach M, Castro M, Pérez C, Varela G, Algorta G. Community-associated methicillin-resistant *Staphylococcus aureus* in children treated in Uruguay. *J Infect Dev Ctries.* 2013;7:10–6.
25. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. *J Lab Physicians.* 2011;3:25–7.
26. Rahbar M, Hajia M. Inducible clindamycin resistance in *Staphylococcus aureus*: a cross-sectional report. *Pak J Biol Sci.* 2007;10:189–92.
27. Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Köhrer K, Verhoef J, Fluit AC. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. *J Antimicrob Chemother.* 2000;45:891–4.
28. Sedaghat H, Esfahani BN, Mobasherizadeh S, Jazi AS, Halaji M, Sadeghi P, et al. Phenotypic and genotypic characterization of macrolide resistance among *Staphylococcus aureus* isolates in Isfahan, Iran. *Iran J Microbiol.* 2017;9:264–70.
29. Seifi N, Kahani N, Askari E, Mahdipour S, Naderi Nasab M. Inducible clindamycin resistance in *Staphylococcus aureus* isolates recovered from Mashhad, Iran. *Iran J Microbiol.* 2012;4:82–6.
30. Shoji K, Shinjoh M, Horikoshi Y, Tang J, Watanabe Y, Sugita K, Tame T, Iwata S, Miyairi I, Saitoh A. High rate of inducible clindamycin resistance in *Staphylococcus aureus* isolates e A multicenter study in Tokyo, Japan. *J Infect Chemother.* 2015;21:81–3.
31. Sibbald MJJB, Ziebandt AK, Engelmann S, Hecker M, de Jong A, Harmsen HJM, Raangs GC, Stokroos I, Arends JP, Dubois JYF, van Dijk JM. Mapping the pathways to staphylococcal pathogenesis by comparative secretomics. *Microbiol Mol Biol Rev.* 2006;70:755–88.
32. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, McGowan JE Jr, Tenover FC. Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. *J Clin Microbiol.* 2005;43:1716–21.
33. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev.* 2015;28:603–61.
34. Uhlemann A-C, Otto M, Lowy FD, DeLeo FR. Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. *Infect Genet Evol.* 2014;21:563–74.
35. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis.* 2005;11:1965–6.
36. Wang W, Chen W, Liu Y, Siemieniuk RAC, Li L, Martínez JPD, Guyatt GH, Sun X. Antibiotics for uncomplicated skin abscesses: systematic review and network meta-analysis. *BMJ Open.* 2018;8:e020991.
37. Xie X, Bao Y, Ouyang N, Dai X, Pan K, Chen B, Deng Y, Wu X, Xu F, Li H, Huang S. Molecular epidemiology and characteristic of virulence gene of community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus* isolates in sun Yat-sen memorial hospital, Guangzhou, southern China. *BMC Infect Dis.* 2016;16:339.
38. Zehra A, Singh R, Kaur S, Gill JPS. Molecular characterization of antibiotic-resistant *Staphylococcus aureus* from livestock (bovine and swine). *Vet World.* 2017;10:598–604.
39. Zorgani A, Shaverf O, Tawil K, El-Turki E, Ghenghesh K. Inducible clindamycin resistance among *staphylococci* isolated from burn patients. *Libyan J Med.* 2009;1:104–6.