Molecular study of Methicillin Resistant *Staphylococcus lugdunensis* (MRSL) Isolates in Hilla city, Iraq

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Abstract:

Out of 690 clinical samples collected from different site (wound, burn, blood culture, sub axillary, urine, stool , sputum, throat, ear, skin lesion, high vaginal, and other different swabs), a total of 178 coagulase negative Staphylococci (CoNS) isolates were recovered. Based on phenotypic characteristics, CoNS were identified into 10 different species; 22 isolates were belonged to *Staphylococcus lugdunensis*. Two specific genes for *S. lugdunensis* were used (*tanA* gene and *fbl* gene) to confirm identification. Both of these specific genes were detected in 15 (68.1 %) of 22 isolates that identified phenotypically. The remaining 7 isolates (31.9 %) were re-identified as *S. pseudolugdunensis*. β-lactam resistance screening test showed that 11 (73.3 %) of *S. lugdunensis* isolates were ampicillin resistant. The results of oxacillin screening test and Oxacillin MIC showed that 7 of the 15 (46.6 %) *S. lugdunensis* isolates were oxacillin resistant; all these were resistant to ampicillin. The antibiotic susceptibility test by Disc Diffusion test and MIC to 16 antibiotics showed that resistance rates towards these antibiotics. Eight of fifteen *S. lugdunensis* isolates (53.3 %) were β-lactamase producer. All these isolates were Ampicillin resistant.

Results of *mecA* gene found that *mec A* gene was detected in 6 (40 %) of 15 *S. lugdunensis*. All of these 6 isolates (S1, S2, S3, S4, S5, and S6) were resistant to oxacillin. One isolate (S7) was resistant to Oxacillin but *mecA* was not detected in this isolates. This study is a first record of isolation and characterization of MRSL form clinical samples in Iraq.

الدراسة الجزيئية للميثيسيلين المكورات العنقودية المقاومة للاللقدوننسية(MRSL) يعزل في مدينة الحلة، العراق

مفتاح البحث: الميثيسيلين,المكورات العنقودية المقاومة للاللقدوننسية (MRSL)

الخلاصة:

تم في هذه الدراسة جمع 690 عينة سريرية مختلفة (حروق, جروح, دم, تحت الابط, ادرار, براز, قشع, حنجرة, اذن, افات جلدية, ومسحات مهبلية) كانت من بينها 178 مكورات عنقودية سالبة لانزيم الكوكيوليز . اعتمدت الصفات المظهرية لتشخيص عشرة أنواع مختلفة ، من بينها 22 تعود الى النوع *(Staphylococcus lugdunensis)*. استخدم نوعين من المورثات الخاصة ببكتريا (*S. lugdunensis*) للتاكد من التشخيص المظهري وهما *(fbl وtan A)* على التوالي. تم التحري عن هذه المورثات الخاصة في 15 (68.1 %) من مجموع 22 عزلة التي شخصت على اساس الصفات المظهرية حيث ظهرت عائدية هذه الزلات الى النوع *S. lugdunensis* اما البقية (7عزلات) فقد كانت عائدة للنوع (*S. pseudolugdunensis*) . اظهرت نتائج المسح الاولي للتحري عن العزلات المقاومة للبيتالاكتام مقاومة11 عزلة (73.3 %) للامبيسيلين من بين 15عزلة التي شخصت وراثيا في حين كانت نتائج مقاومة هذه العزلات للاوكساسلين بطريقة المسح الاولي والـ MIC مقاومة (46.6 %) جميعها مقاومة للامبيسلين. أظهرت اختبارات المقاومة لستة عشر نوعا من المضادات الحياتية بطريقة انتشار الاقراصDisc diffusion والتركيز المثبط الأدنى MIC نسب مقاومة لهذه المضادات . اظهرت ثمانية عزلات من بين الخمسة عشر عزلة قدرتها على انتاج انزيم البيتالاكتاميز بطريقة اليود السريعة وكانت جميعها مقاومة للامبيسلين ، .اظهرت نتائج الPCR أن من بين 15 عزلة 6 (40%) كانت حاوية على مورثة *mecA* وجميع هذه العزلات كانت مقاومة للاوكساسلين والامبيسلين في حين وجد ان عزلة واحدة كانت مقاومة للاوكساسلين ولكنها لا تحتوي على مورثة *mecA* . تعد هذه الدراسة هي الاولى في العراق لعزل وتوصيف بكتريا *S. lugdunensis* المقاومة للمثيسللين (MRSL) من عينات سريرية مختلفة.

Introduction

The coagulase-negative staphylococci (CoNS) are a large group of Gram positive bacteria most often found colonizing the skin and mucosal surfaces of humans and other mammals (1). Several species of CoNS are recognized as potential pathogens, mainly causing nosocomial infections, often involved in infections related to implanted medical devices such as intravenous catheters, prosthetic heart valves, and orthopedic implants. *Staphylococcus lugdunensis* is a coagulase-negative staphylococci first described by Freney and his colleagues in 1988 (2). The organism is found as a skin commensal in healthy individuals. *S. lugdunensis* has been implicated in invasive diseases, especially fulminant native and prosthetic-valve endocarditis (3). Other invasive infections include brain abscess and meningitis, skin abscesses and soft tissue infections, spondylodiscitis, foreign body infections, and peritonitis (4). *S. lugdunensis* shares a number of potential virulence factors with *S. aureus.* In particular, *S. lugdunensis* may express a clumping factor and produce a thermostable DNase (5). *S. lugdunensis* produces a tannase (tannin acyl hydrolase) that degrades hydrolysable tannins (6). The phenotypic biological tests, such as the ornithine decarboxylase test and genotypic molecular tests have been developed to identified this bacteria (7). Several nucleic acid targets that permit the differentiation of *S. lugdunensis* from other CoNS using molecular methods. These include the 16S rRNA gene, which was used to confirmative identity of *S. lugdunensis* isolate (8). The *rpoB* gene also is specific to *S. lugdunensis* (4). The *tanA* gene that coded tannase acyle hydrolase were detected in *S. lugdunensis* (6). A fibrinogen-binding protein known as Fbl that encoded by *fbl* gen is specific to *S. lugdunensis* (9). The *mecA* gene has been reported in several data, the first in a neonate with *S. lugdunensis* (MRSL) that produces an alternative penicillin binding protein (PBP2A) (10). Although several researchers have been reported phenotypic and molecular characterization of Methicillin resistant *S. lugdunensis* (MRSL) worldwide, little or no information are available about these resistant bacteria in Iraq.

Therefore the main goals of this study were to isolate and determine the antibiotic resistance patterns of these important bacteria from clinical samples and detecting the presence of *mec*A gene that encodes methicillin resistance to confirm being MRSL.

Materials and methods

Patients and sample collection:

This study included 630 patients (aged 2 days-70 years) suffering from different infections who admitted to four health centers in Al-Hilla city. These patients were admitted to different hospital wards, in addition to swabs taken from private clinics during a period extending from November 2012 to the end of May 2013**.**

Different swabs 690 were generally collected from different site (wound, burn, blood culture, sub axillary, urine, stool , sputum, throat, ear, skin lesion, high vaginal, and other different swabs)**.** Each sample was immediately inoculated on the blood agar plates, and mannitol salt agar. The swab has been inoculated on culture media and incubated aerobically for 24 hours at 37 °C. Information about age, antibiotic usage, residence and hospitalization of patients were taken into consideration.

Bacterial isolates

Staphylococcus lugdunensis isolates were recovered and identified based on their morphology, Gram-staining, catalase test, coagulase test, and ornithine decarboxylation test (11). Identification was confirmed by using two specific genes (*tanA* and *fbl* genes) by PCR assay (12).

Screening of β-Lactam (Ampicillin and Oxacillin) Resistant Isolates:

Fifteen *S. lugdunensis* isolated were subjected to β-lactam resistance screening test as a phenotypic selection test. Preliminary screening of *S. lugdunensis* isolates resistance to β-lactam antibiotics was carried out by using pick and patch method on Muller-Hinton agar plates supplemented with ampicillin. All of 15 *S. lugdunensis* isolates were subjected to oxacillin resistance screening test by using the same method on Muller-Hinton agar plates supplemented with 4% NaCl and oxacillin 6 µg/ml (13).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility patterns of isolates to different antibiotics were determined using Disk Diffusion Test and interpreted according to (13). The following antibiotics were obtained (from Oxoid/U.K, Himedia/India) as standard reference disks as known potency for laboratory use: Ampicillin (10µg), Oxacillin (5µg), Cloxacillin (5µg), Cefoxitin (30µg), Amoxicillin-Clavulanate (20/10 µg), Cefexime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Azithromycin (15 µg) Doxycycline (30 µg). The susceptibility to ampicillin, oxacillin and Vancomycin were also determined using two-fold agar dilution method.

Detection of β-lactamase production:

The present study included 15 isolates were tested to detect their ability to produce β- lactamase. Rapid iodometric method was used for detection of β-lactamse production (14).

Detection of *tanA, fbl* and *mecA* genes

Three genes were detected in present study, first *tanA* gene that coded to tannase acyle hydrolase enzyme that degrades tannin. The 2nd gene was *fbl* gene that coded to fibrinogen binding protein. The third gene that detected in present study was *mecA* that responsible for Oxacillin/Methicillin resistance by coding to Penicillin binding protein (PBP2a) the primer sequence of these genes were (*tanA* F: AGCATGGGCAATAACAGCAGTAA , *tanA* R: GCTGCGCCAATTTGTTCTAAATAT) 239bp, the condition were 95ºC 3min 1x, 94ºC 20sec, 60ºC 20sec 25X, 72ºC ,20 sec., 72ºC 5min 1x (12). (*fbl* F: GTAAATAGCGAGGCACAAGC , *fbl* R: GGTAAATCGTATCTGCCGCT) 425bp , the condition were 94ºC 3min 1x, 94ºC 1min, 60ºC 1min 30x, 72ºC 1min,72ºC 5min 1x (15). (*mecA* F: TCCAGGAATGCAGAAAGACCAAAGC , *mecA* R:GACACGATAGCCATCTTCATGTTGG) 499bp, the condition were 94ºC 3min 1x, 94ºC 1.5min, 55ºC 1min 36x, 72ºC 1min, with final step 72ºC 10min 1x (16).

Results and Discussion

Isolation and Identification of *Staphylococcus lugdunensis* isolates

A total of 690 clinical samples were collected, 602 (87.24%) gave positive growth on blood agar medium, while 88 (12.76%) gave no growth. The reason of negative culture may be attributed to fungal infection, viral infection, or fastidious bacteria that might be lost during transporting or cannot be growing on selective media used in this study.

Out of 393 Gram positive bacteria, 306 (77.8%) were identified as staphylococci based on morphological characteristics and biochemical tests. According to result of coagulase test, the 306 staphylococci isolates were divided into coagulase positive 128 (41.8%) and coagulase negative 178 (58.2%) (Table-1).

Table (1): Numbers, sources and percentage of coagulase negative staphylococci isolates:

Source	No.	(%)	CoNS No.	(%)
wound swabs	105	(17.4)	42	(23.56)
Ear swabs	28	(4.6)	6	(3.37)
Urine swabs	50	(8.3)	11	(6.17)
Skin lesion swabs	25	(4.1)	10	(5.6)
Throat swabs	32	(5.3)	11	(6.17)
Burn swabs	80	(13.2)	15	(8.4)
Blood culture	69	(11.4)	13	(7.3)
Sputum	36	(5.9)	4	(2.24)
Sub axillary swabs*	64	(10.6)	52	(29.2)
HVS**	23	(8.3)	2	(1.12)
Stool swabs	38	(6.3)	2	(1.12)
other	52	(8.6)	10	(5.6)
Total	602	(100)	178	(100)

Result of present study was similar to that of Bouza (17), who found that bacterial isolates from clinical samples included 70.7 % Gram positive , 22.2 % of Gram negative , and 7.2% of yeast, they also found that *S. aureus* and CoNS constituted 40% and 60% respectively. In a local study, Al-Fuadi (18) found that total of 148 bacterial isolates represented by different Gram- positive and Gram-negative bacteria in a percentage of (77%) and (23%) respectively, and they found that a total of 100 *Staphylococcus* isolates, Only 31 (31%) isolates were belonged to *S. aureus*. This difference may be belonged to variation of samples collected in this study. Results also showed that the highest percentages of CoNS in Sub axillary swabs and wound swabs were 29.2% and 23.5% respectively. The high frequency of CoNS in these samples might be due to the fact that *Staphylococcus* species are frequent commensal bacteria on the human skin and mucous surfaces. CoNS were identified depending on phonotypical, biochemical, and physiological tests.

The prevalence of *S. lugdunensis* was 22 (12.3%), which is higher than results of several researchers. This may be due to the fact that depending on phenotypic characteristics alone is insufficient and may result in misidentification of *S. lugdunensis*. So, the present study depended (in addition to phenotypic characteristics) on the genotypic characteristics (PCR) to confirm the result. Depending on PCR results, Out of 22 of CoNS that identified phenotipically as *S. lugdunensis* isolates, 15 (8%) were identified as *S. lugdunensis* while the other seven isolates were belonged to *S. pseudolugdunensis*.

Clinical isolates were as follows: Sub auxiliary swab (4) skin swabs (2), wound (3), burn (1), blood (1), throat (2), ear (1), peritonitis (1), while no *S. lugdunensis* isolates were recovered from urine, sputum swabs, stool swabs, high vaginal swabs. skin swabs represented folliculitis, boils, and abscesses. Researcher (19) found that it is constituted 9 % of CoNS isolates from blood culture, while other researchers found that *S. lugdunensis* constituted only 3.3 % of CoNS collected from different samples (20).

Molecular Characterization of *S. lugdunensis* Isolates:

Definite phenotypic identification of a Gram-positive, catalase-positive coccus as *S. lugdunensis* implies a negative tube coagulase test and positive ornithine decarboxylase activities (21). However, complete hemolytic, yellow pigmentation, and detection of a fibrinogen affinity factor, although not consistently expressed by *S. lugdunensis*, may lead to its misidentification as *S. aureus* (1). *S. lugdunensis* is an unusually virulent coagulase-negative species, associated with severe infection. So, using single-step, speciesspecific PCR protocol for *S. lugdunensis* identification is very important (15).

Detection of *tanA* gene of *Staphylococcus lugdunensis*

The specific *tanA* gene for *S. lugdunensis* was detected in 15 (68.1 %) of 22 isolates that identified phenotypically. These 15 isolates were identified as *S. lugdunensis* (Figure-1). The remaining 7 isolates (31.9 %) were re-identified as *Staphylococcus pseudolugdunensis* (22). Result also found that *S. aureus* and *S. epidermidis* that used as negative control had no *tan*A gene which confirms the result of Nogochi and his co-worker (2010) who found that no gene or protein homologous to *tan*A were found in a similarity search using published databases such as Gen Bank. These results strongly suggest that *tanA* is specific to *S. lugdunensis*.

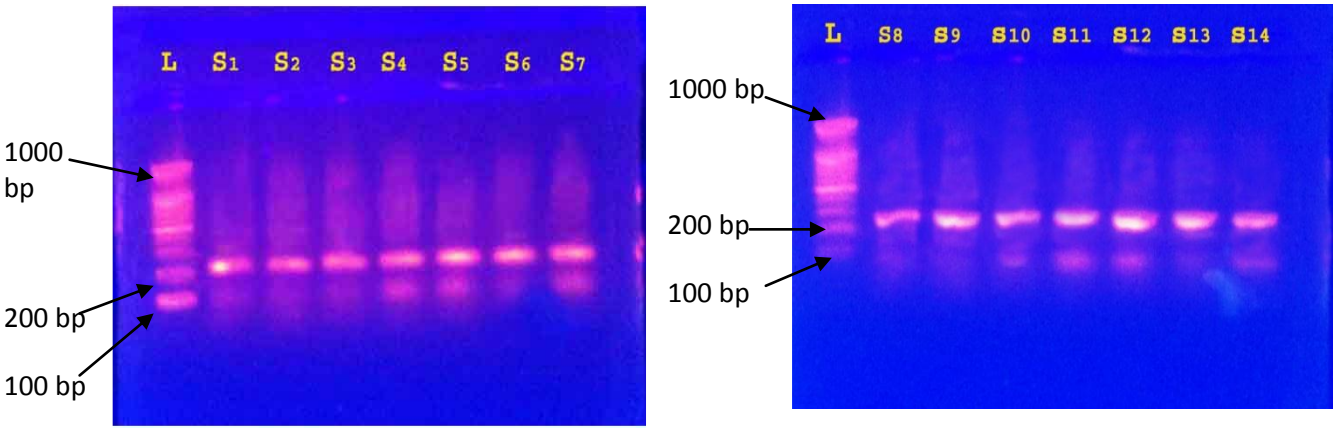

Figure (1): Gel electrophoresis of PCR of *tanA* amplicon (**239bp**) product: Lane L: Ladder (1000-bp ladder), Lanes (S1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14) No. of *S. lugdunensis* isolates from different clinical samples.

Detection of *fbl* gene of *Staphylococcus lugdunensis* isolates:

A suitable nucleic acid target to diagnosed *S. lugdunensis* is *fbl* gene, that encoding a fibrinogen-binding adhesin (15). The gene was detected in all 15 *S. lugdunensis* isolates that was positive to *tanA* in this study (Figure -2), while no amplification product was obtained from *S. aureus* and *S. epidermidis* isolates that used as negative control as in Figure -3.

According to results of PCR, among 22 *S. lugdunensis* that diagnosed phenotypically , 15 isolates were found to be positive to *tanA* and *fbl* genes that were specific to *S. lugdunensis* (4), so other isolates (No.=7) were diagnosed as *S. pseudolugdunensis* (22).

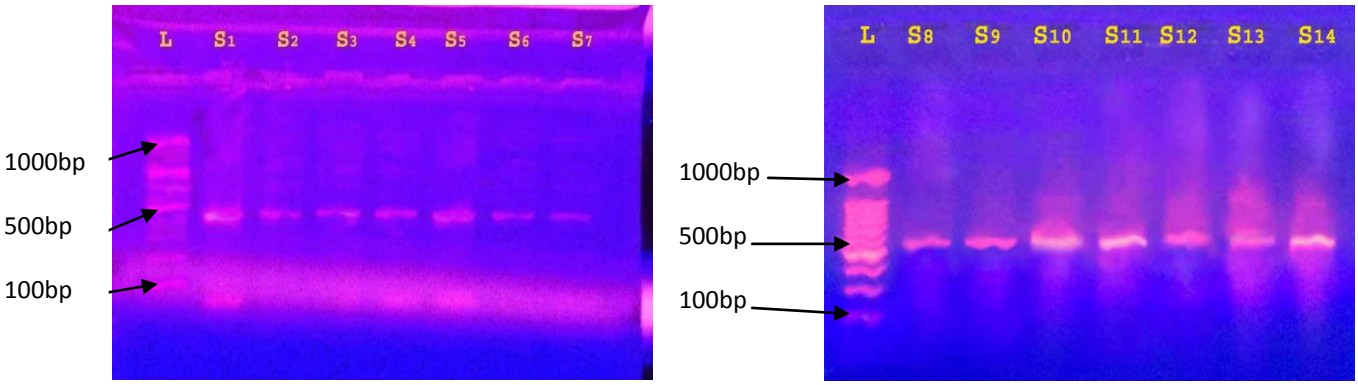

Figure (2): Gel electrophoresis of PCR of *fbl* amplicon (425bp) product: Lane L: Ladder (1000-bp ladder), Lanes (S1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14) No. of *S. lugdunensis* isolates from different clinical samples.

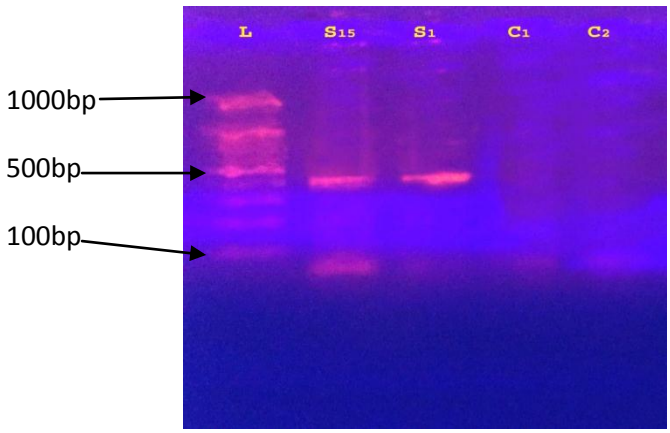

Figure (3): Gel electrophoresis of PCR of *fbl* amplicon (425bp) product: Lane L: Ladder (1000-bp ladder), Lanes (S1, and 15) No. of *S. lugdunensis* isolates. C1*: S. aureus*, C2: *S. epidermidis*.

Primary Screening of β-Lactam (ampicillin and oxacillin) Resistant Isolates:

The results of screening test showed that 11 isolates (73.3 %) of *S. lugdunensis* were resistant to ampicillin, while 4 (26.7 %) were ampicillin sensitive. All these isolates were able to grow normally in the presence of ampicillin, this may be attributed to most of *S. lugdunensis* isolates (about 90% of them) are coming from several infectious sources (nosocomial infections and other anatomical sites) that its resistant to penicillin due to production of β-lactamases that act in the hydrolysis of β-lactam ring of penicillin which is transformed into acid neutralizing its bactericidal effect (23).

The results of oxacillin resistant screening test showed that 7 of the 11 (63.6 %) β-lactam resistant *S. lugdunensis* isolates were oxacillin resistant. Resistance to oxacillin is due to the fact that *S. lugdunensis* isolates have β-lactamase that reduces efficiency of β-lactam antibiotic. This result was in concordance with study of (24) they were referred to identifying methicillin resistance by oxacillin MIC, 76.5% (13 out of 17) and 47.1% (eight out of 17) of strains were considered resistant by the Vitek 2 system and the Wider system.

Susceptibility of *Staphylococcus lugdunensis* to β-Lactam Antibiotics

The results revealed that 11 of 15 *S. lugdunensis* isolates showed high resistance (73.3 %) to ampicillin (Figure-4). Results also showed that the resistance rate to oxacillin, Cloxacillin, were 46.6 %. Methicillin replaces methicillin as oxacillin is stable under storage conditions, and methicillin actually is an excellent inducer of the *mec*A gene. Ezekiel (25) isolate three strains of *S. lugdunensis* of 149 CoNS, all isolates were resistant to oxacillin and other β- lactam antibiotics. Tan (21) in Singapore found that resistance to oxacillin was detected in 5% of isolates.

Results of cefoxitin (2nd generation), ceftriaxone and cefexime (3rd generation), showed that the percentages of *S. lugdunensis* resistant isolates were substantial to these antibiotics: 46.6%, 53.3%, 40%, respectively (Figure-4). These results can be explained by the fact that all staphylococcal strains produce β-lactamase which destroys the β-lactam ring resulting in inactive products (26). Tan (21) found that resistance to cefoxitin was detected in 5% of isolates. The resistance rates to amoxiclav and ceftazidime-clavulanic acid were 60 % and 53.3% respectively. Clavulanic acid can inhibit the action of β-lactamase enzymes that causes decrease in the resistance of bacteria to β-lactam antibiotics (27). Results found that *S.lugdunensis* isolates were susceptible to imipenem (80 %). Imipenem inhibits bacterial cell wall synthesis by binding to and inactivating PBPs (28).

Susceptibility of *Staphylococcus lugdununsis* to non β-Lactam Antibiotics

Result of this study regarding susceptibility to amikacin, found that the isolates showed low level of resistance (46.6 %) to this antibiotic. The resistance rate of azithromycin was 53 %, (Figure-4). This resistance may be attributed to the efflux mechanism in staphylococci which is mediated by *Msr*A; a protein that induced by clarithromycin, azithromycin and telithromycin, and encoded by *msr* A

gene (29). Result of this study regarding susceptibility to clindamycin, found that the isolates showed low level of resistance (46.6 %) to this antibiotic. Researcher (30) found 10%of isolates were resistant to clindamycin. *Staphylococcus lugdunensis* isolates results showed (73%) resistance to doxycycline. Tan (21) in Singapore found that resistance to tetracycline was 12% of isolates. The percentage of resistance for Trimethoprim-sulfamethoxazole was 66%.Sulfonamides inhibit dihydropteroate synthase, which blocks folate biosynthesis. This, in turn, leads to defective thymidine biosynthesis (31). Results of this study revealed that *S. lugdunensis* isolates showed that the level of resistance to rifampicin (66 %). Rifampin acts by interacting specifically with the β subunit of the bacterial RNA polymerase encoded by the rpoB gene. Rifampin resistance in *Escherichia coli* and *S. aureus* is due to alterations in the target leading to a reduced affinity of the enzyme for the antibiotic (32).

Results of antibiotic resistance by MIC

In this study, 11 of 15 (73.3 %) *S. lugdunensis* isolates were resistant to ampicillin (≥ 128µg/ml) while 4 of 15 were having MIC values reached to 2 µg /ml. The MIC values of *S. lugdunensis* isolates against Oxacillin revealed that 5 of 15 isolates reached to32 µg/ml, while MIC value of 2 isolates was ≥ 64µg /ml. Six Oxacillin resistant isolates (S1, S2, S3, S4, S5, and S6) having *mecA* gene, but one (S7) didn't has such gene. Out of 15 *S. lugdunensis* isolates (detected by MIC method), 14 isolates (93.2 %%) were sensitive to Vancomycin, while only one isolate (6.8%) showed reduced susceptibility to vancomycin 8 µg/ml (intermediate resistant). Bourgeois (32) found that 6 of 13 *S. lugdunensis* isolates were tolerant to vancomycin. No isolates showed any degree of resistance to Vancomycin as in many data. The *van*A genes that are responsible for resistance (*van* genes) are inducible and transferable and confer high-level resistance to vancomycin (33).

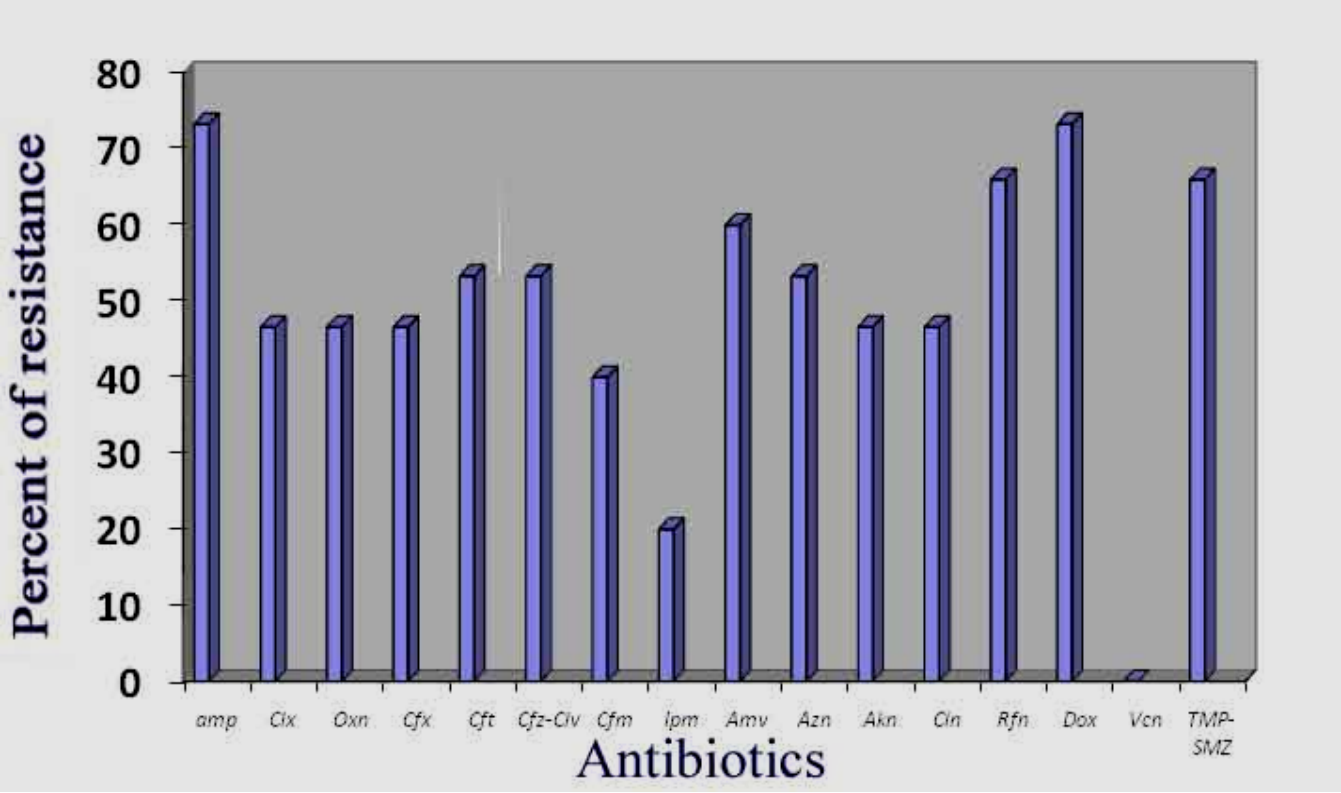

Figure-4: Percentages of antibiotic resistance among *Staphylococcus lugdunensis* isolate

Amp: Ampicillin, Clx: Cloxacillin, Oxn: Oxacillin, Cfx: Cefoxitin, Cft: Ceftriaxone, Cfz-Clv: Ceftazidime-clavulunate, Cfm: Cefixim, Ipm: Imipenem, Amv: Amoxiclav, Azn: Azithromycin, Akn: Amikacin, Cln: Clindamycin, Rfn: Rifampicin, Dox: Doxycycline, Vcn: Vancomycin, TMP-SMZ: Trimethoprime/sulfamethoxazole.

Detection of β-lactamase production:

Eight isolates (53.3 %) were β-lactamase producer. All these isolates were ampicillin resistant, seven of eight β-lactamase producing isolates were oxacillin resistant, while remaining one was oxacillin sensitive. Six of eight were having *mecA* gene (Table -2).

Mateo (24) found that 11.8% of *S. lugdunensis* were β- lactamase producers. Several authors reported that the percentages of β-lactamase-positive *S. lugdunensis* were vary from 24 to 40% in U.S isolates collections (34). Papapetropoulos (35), who isolated 14 *S. lugdunensis* from clinical specimens (abscesses and wound) from (250 beds) in Athens, Greece, 5 (30.2%) of *S. lugdunensis* were β-lactamase positive. The difference between this study and other studies may be due to the fact that the global using of β-lactam antibiotics in Iraq may which results in induction of bacterial resistance to β-lactams via production of β-lactamase (36).

Molecular detection of MRSL isolates

Detection of *mecA* gene

In this study *mec A* gene was detected in 6 (40 %) of 15 *S. lugdunensis* (Figure-5). All of these 6 isolates (S1, S2,S3, S4, S5, and S6) were resistant to oxacilin (Table 2). One isolate (S7) was resistant to oxacillin but *mecA* was not detected in this isolates. This resistant may due to mechanism other than changing PBPs (*mecA)* like hyper production of β-lactamase, efflux mechanism in staphylococci which is mediated by *Msr*A, chemical modification , changing the target of antibiotic ,and/ or changing permeability of membrane (37).

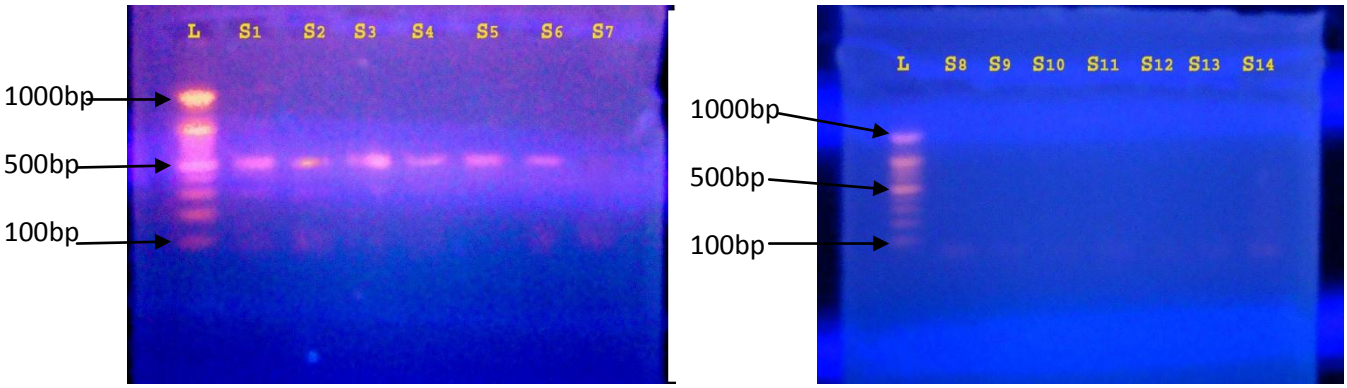

Figure (5): Gel electrophoresis of PCR of *mecA* amplicon (499bp) product: Lane L: Ladder (1000-bp ladder), Lanes (S1,2,3,4,5,6) *mecA* positive, Lanes (S7,8,9,10, 11,12,13, and 14) *mecA* negative samples.

Staphylococcus lugdunensis is generally considered to be susceptible to oxacillin. Several studies reported negative PCR results when screening for *mecA*, but among reports in the English literature *mecA* has been detected in two *S. lugdunensis* isolates (38),(8). (8) reported a case of MRSL causing bloodstream infection in a neonate with an oxacillin MIC>256 mg/L having *mecA* gene. In 2008, Tan (21) found five (4.7%) *S. lugdunensis* strains carrying the *mecA* gene in a collection of 106 clinical isolates.

Table 2: relationship between ampicillin, oxacillin resistant with present of *mecA* gene and β-lactamase production in *Staphylococcus lugdunensis* isolated:

Isolate No.	Ampicillin resistant	Oxacillin resistant	mecA	β-lactamase production
S1	+	+	+	+
S2	+	+	+	+
S3	+	+	+	+
S4	+	+	+	+
S5	+	+	+	+
S6	+	+	+	+
S7	+	+	-	+
S8	+	-	-	-
S9	+	-	-	-
S10	+	-	-	-
S11	+	-	-	+
S12	-	-	-	-
S13	-	-	-	-
S14	-	-	-	-
S15	-	-	-	-

Conclusion

The present study can conclude the following points:

1. The ratio of coagulase negative staphylococci was higher than coagulase positive staphylococci.
2. A highest percentages of *S. lugdunensis* isolate were recovered from sub axillary, wound swabs, and skin swabs, samples, so the results from this study reinforce the propensity of *S. lugdunensis* to be associated with acute cutaneous infections.
3. Although many other reports stated that *mecA* gene presents in low percentage in *S. lugdunensis*, however, in present study, high rate of *mecA* is present in these bacteria.

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