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Prevalence, Antibiotic resistance of Staphylococcus aureus, CNS and determination of MRSA, MRCNS strains in clinical samples

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□ ABSTRACT □

A total of 113 strains of *S. aureus* and CNS were isolated from clinical samples from three hospitals in Damascus city between 6/2013 – 8/2014, and were identified by biochemical and molecular tests. The percentage of *S. aureus* was (65.48%), followed *S. epidermidis* (25.66%) then *S. saprophyticus* (4.42%), so the prevalence of *S. aureus* was major in (pus, abscess) which was 28.37%, while the prevalence of *S. epidermidis* was more in CSF which was (31.03%) The molecular methods and antimicrobial resistance were studied in 55 strains of collection of samples (40 *S. aureus* and 15 CNS), where detected *16SrRNA*, *gap*, and *nuc* genes for adjust the identification. Antibiotic resistance tests examined for 8 different antibiotics as follows: AMC (65%), GN (30%), CRO (62.5%), PE (7.5%), OFX (7.5%), AM (70%), VA (2.5%) and AX (55%). The percentage of presence of MRSA strains was 15% and MRCNS was 6.66%.

Key wards: Antibiotic resistance, MRSA, mecA, nuc, Staphylococcus

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انتشار العنقودية الذهبية والعنقوديات سالبة المخثراز ومقاومتها للصادات الحيوية وتحديد سلالات MRCNS, MRSA في العينات المرضية

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(تاريخ الإيداع 30 / 8 / 2015. قبل للنشر في 21 / 1 / 2016)

□ ملخّص □

عزلت 113 سلالة من العنقوديات الذهبية وسالبة المخثراز من العينات المرضية من ثلاث مستشفيات في مدينة دمشق في الفترة ما بين 2013/6 – 2014/8 ، وحددت بالاختبارات الحيوية الكيميائية والجزيئية، وكانت نسبة انتشار العنقودية الذهبية 65,48% تليها العنقودية الجلدية 25,66% ثم العنقودية الرمامة 4,42%، وكان انتشار العنقودية الذهبية كبيراً في القيوح والخراجات حيث بلغ 28,37% بينما انتشار العنقودية الجلدية كان كبيراً في CSF الذي بلغ 31,03%، ودرست الطرائق الجزيئية ومقاومة الصادات الحيوية في 55 سلالة (40 عنقودية ذهبية و 15 عنقوديات سالبة المخثراز، وأجري تحري للمورثات *16SRNA*, *gap*, *nuc*, *mecA* في هذه السلالات، أما نسب مقاومة الصادات الحيوية كانت للأموكسيسيلين كلافولانيك أسيد 65%، والجنتاميسين 30%، والسيفترياكسون 62,5%، والبيفلوكساسين والأوفلوكساسين 7,5%، والأمبيسيلين 70%، والأموكسيسيلين 55%، والفانكوميسين 2,5%، أما نسبة سلالات MRSA كانت 15%، ونسبة سلالات MRCNS كانت 6,66%.

الكلمات المفتاحية: مقاومة الصادات الحيوية، *nuc*، *MRSA*، *mecA*، العنقودية

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Introduction

Staphylococcus aureus is one of the most common causes of bacterial infections, which range in severity from relatively trivial skin infections to lethal invasive disease, and it is responsible for millions of infections in the world each year, most of which are community-acquired infections. (Bauman, 2015; Mahon *et al*., 2015). *S. aureus* also causes hundreds of thousands of health care–associated infections each year, 50 to 60% of it are caused by methicillin-resistant *S. aureus* (MRSA), which has a matter of concern worldwide, in the USA, Europe, and development countries, due to MRSA is associated with a wide range of infections, including bacteremia, surgical wound infections, pneumonia, skin and soft tissue infections like furuncles, carbuncles, impetigo, staphylococcal scalded skin syndrome, and toxic shock syndrome. Forthmore, it is associated with other infections as respiratory tract, UTIs, osteomyelitis, acute endocarditis, acute and chronic cystitis, prostatitis, cervicitis, cerebritis, meningitis, conjunctivitis, otitis media and food poisoning (Wolff and Johnson, 2009; Meikane *et al*., 2009; Burns *et al*., 2010; Goldman and Schafer, 2012).

Coagulase-negative staphylococci (CNS) rarely cause infection unless there is a foreign body in place. They are classic opportunists that only cause infections given a certain host disposition. This is the pathogen most frequently encountered in CNS infections (70–80% of cases) especially *Staphylococcus epidermidis* where cause mainly foreign body infections (McKean *et al.*, 2012; Tortora *et al*., 2013). Examples of the foreign bodies involved are intravasal catheters, continuous ambulant peritoneal dialysis (CAPD) catheters, endoprostheses, metal plates and screws in osteosynthesis, cardiac pacemakers, artificial heart valves (Walker *et al*., 2014). MRSA and CNS are typically resistant to methicillin and multiple other antibiotics, and they are an important reservoir of drug-resistance elements, where they have been known to cause various health care-acquired or nosocomial infections, whereas *Staphylococcus saprophyticus* is associated mainly with urinary tract infections (UTIs), predominately in adolescent girls and young women (Levy and Marshall, 2004; Goldman and Schafer, 2012). Many studies focused on these species, especially MRSA and MRSE (methicillin-resistant Staph. epidermidis). CNS were studied as a cause of infections related to intravascular prosthetic devices, and focused on limitations of present therapy (Schulin and Voss, 2001). In total 177 isolates of CNS other than *S. epidermidis* were tested, and identified by automated ribotyping (Carretto *et al*., 2005). In total 494 isolates of CNS were identified to the species level by biochemical and *sod*A sequencing, also erythromycin resistance phenotypes and specific resistance genes were identified by PCR (Gatermann *et al*., 2007). A rapid detection and sensitivity was showed in MRSA in wound and blood cultures (Wolk *et al*., 2009). A European survey was practiced and the results were reported and showed a broad range of opinion and practice on a variety of issues pertaining to the management of minor and serious MRSA infections (Dryden *et al*., 2010). About 87 strains of CNS were isolated and characterized from food animals by molecular methods and antimicrobial susceptibility were studied (Bhargava and Zhang, 2012). It was investigated of linezolid resistance among methicillin resistant *Staphylococcus aureus* strains isolated from state hospitals in the East and West coast of Malaysia (Pulingam *et al*. 2014). A total sixty *S. aureus* isolates were collected from the Jordan hospital in Amman, and were characterized with Staphylococcus protein A (*spa*) typing, in addition, samples were tested for their susceptibility against seven antimicrobial agents (Bazzoun *et al*., 2014). All isolates of MRSA were collected from soft tissue infection in pediatric hospital in Argentina, and

were showed that all isolates carried Staphylococcal Cassette Chromosome (*SCCmec)* type IV, and 30 of it had *PVL* coding genes (Specht *et al*., 2014).

Aims of the study

This study was performed to determine the prevalence of Staphylococci strains in clinical samples, also the molecular features and antimicrobial resistance in 55 of the strains to determine MRSA, MSSA, and MRCNS. Understanding the epidemiology of MSSA and MRSA infections.

Materials and Methods

Sites and Sampling

The tests were carried out in the laboratories of the plant biology department of Faculty of science in Damascus university. Between 6/2013 – 8/2014 a total of 113 staphylococci strains were isolated from clinical samples (ear, urine, CSF, Wound, Pus, eye, Brain peritoneal shunt, bronchial lavages, pleural effusion, Peritoneal fluid, Abscess, Furuncle, bullous, hand, face) from Damascus city. The samples were collected from three hospitals: Al-Mouwasat, Children hospital and Dermal, Venereal Diseases hospital.

Identification of Staphylococci by Biochemical methods

The isolates were plated without delay on the routine culture media plates, nutrient agar NA(Abtec, England), blood agar BA (Abtec, England), then seeded onto mannitol salt agar MSA (Biolife, Italy) and incubated at 37 °C for 24 h. One hundred and thirteen of Staphylococci were recovered. The strains were identified by colony morphology, Gram stain reactions, catalase test, and positive-negative coagulase using the coagulase slide test (Sigma, Germany) according to the manufacturer's instructions and Novobiocin discs (Bio-analyse, Turkey) resistance. Further biochemical identification of Staphylococci to the species level was performed using API Staph (BioMérieux, France). The strains were stored on NA agar slants at 4 ºC and in glycerol-containing nutrient broth NB (SRL, India) 20% (v/v) at -30 ºC freezer until further analysis and were subcultured on nutrient agar (Isenberg, 2007; De vos *et al*., 2009; Mahon *et al*., 2015).

Molecular Identification of Staphylococci

DNA extraction

Genomic DNA was extracted according to (Japoni *et al*., 2004) with some modifications. Cells were harvested from overnight nutrient broth for 24 h. at 37 ± 1 ºC in shaker incubator (JSR, Korea) at 120 rpm and 10 ml of grown bacteria were centrifuged at 12,000 rpm for 15 min. then, supernatant was discarded. The cells pellet were resuspended in 700 µL of TE buffer for 10 min. Next, 30 µL of 10% SDS added, then mixed thoroughly with proteinase K and 250 µL of 5M NaCl. After the mixture with 600 µL CTAB solution incubated in water bath at 65 ºC for 30 min., then equal volume chloroform/isoamyl alcohol (24:1) were added and mixed by vortex and left 50 min. at room temperature. The supernatant, after centrifugation, were transferred into clean micro-test tube (1.5 mL), then 6 µL RNase added and incubated at 37 ºC. The resulting suspension was precipitated 700 µL cold isopropanol and kept at -20 ºC for twenty minutes. Then, the solution centrifuged for 5 min. at 14,000 rpm, and the supernatant was discarded. The DNA pellet was washed twice with 700 µL 70% ethanol, then dried and resuspended in 50 µL of TE buffer, and stored at -20 ºC.

Detection of 16S rRNA, gap, nuc and mecA genes

The *16S rRNA* was amplified with a pair of specific primers: 16SF1(5` -GGAATTCAAAGGAATTGACGGGGGC-3`) and 16SR2(5` -CGGGATC CCAGGCCCGGGAACGTATTCAC-3`) (Geha *et al*., 1994), and gave (~479 bp). The *gap* gene was amplified with forward primer: GF1(5` -

ATGGTTTTGGTAGAATTGGTCGTTTA-3` ) and reverse primer: GR2(5` - GACATTTCGTTATCATACCAAGCTG-3` ) (Ghebremedhin *et al*., 2008). They gave a PCR product equal to (~933 bp). Finally PCR was performed using *S. aureus* species-specific primers for the *nuc* gene, which codes for thermostable nuclease using the primers, nuc-F (5` GCGATTGATGGTGATACGGTT-3` ) and nuc-R(5` AGCCAAGCCTTGACGAACTAAAGC-3` ) as described by (Thompson *et al*., 2012), generating ~ 270 bp. PCR amplification of *mecA* gene was performed with specific primers -F(5` - AGTTGTAGTTGTCGGGTTT-3` ) -R(5` - AGTGGAACGAAGGTATCATC-3`) (Thompson *et al*., 2012), generating a 533 bp.

The PCR was carried out in 25 µL volumes under the following conditions: 100 ng genomic DNA, 12.5 µL of One PCR^{TM} Master Mix (which contains Taq DNA polymerase, PCR buffer, dNTPs, gel loading dyes and fluorescence dye), 250 nM of each primer, and nuclease free water was added to make up the final volume of 25 µL. Samples were predenaturated at 94 °C for 3 min. and then subjected for 45 consecutive cycles each consisting of 1 min at 94 °C, 1 min primer annealing step at varying temperature of 55, 58 and 60 °C for *nuc* and *16S rRNA*, *mecA* gene and gap respectively, then 2 min at 72 °C. A final elongation at 72 °C for 10 min. was performed. Amplifications were performed in Bioer thermal cycler (China) and the amplified products were separated on 1.25% agarose gels stained with ethidium bromide and visualized under UV light. Molecular sizes of the amplification products were estimated by utilizing a 100 bp DNA ladder, which is ideal for determining the size of double-stranded DNA from 100 -1500 bp.

Antibiotic susceptibility

In vitro antibiotic resistance was determined by disk diffusion with the CLSI (CLSI, 2007) breakpoints. Staphylococci strains were streaked on Mueller-Hinton agar MHA (Merck, Germany) and incubated at 37 ˚C for 24 h. with the following antibiotic: amoxicillin-clavulanic acid (30 µg), gentamicin (10 µg), ceftriaxone (30 µg), pefloxacine (5 µg), ofloxacine (5 µg) and ampicillin (10 µg), vancomycin (30 µg) amoxicillin (25 µg). All discs were obtained from (Bio-analyze, Turkey). Throughout this study, results were interpreted according to the criteria of the Clinical Laboratory Standards Institute National Committee on Clinical Laboratory Standards (NCCLS).

MIC determination

By broth dilution method using CLSI guidelines (CLSI, 2007) the MIC of methicillin and vancomycin (Sigma- Aldrich) were determined. Staphylococci strains were inoculated into nutrient broth NB for 24 h. at 37 ºC. up to (0.5 McFarland), and 1 ml inoculum of overnight growth culture was inoculated into fresh NB containing varying concentrations, serially diluted 256 - 4 µg/mL. All the tubes were incubated for 24 h. at 37 ºC.

Statistical analysis

Data related to the clinical strains and bacterial prevalence were analyzed by chi-square test using SPSS program software version 17 to validate the signification of the results. The results were analyzed in the following data: bacterial prevalence, Prevalence of *S. aureus* and CNS strains between the adults and children, and Prevalence of *S. aureus* and CNS as a source samples. Significance was considered for $P<0.05$.

Results

Isolation and Identification

A total of 113 Staphylococci strains were confirmed both morphological and biochemical tests, representative of 5 species were studied including: (74) *S. aureus*, (29) *S. epidermidis*, (5) *S. saprophyticus*, (2) *S. lentus* and (3) *S. hominis*, and the percentage of appeared of these strains were summarized in (Table 1).

Results of the molecular methods

Of 113 strains and as previously the molecular features and determination of MRSA, MSSA, and MRCNS was carried out in only 55 strains which showed the resistance to antibiotic discs, where (40) *S. aureus*, (6) *S. epidermidis*, (4) *S. saprophyticus*, (2) *S. lentus* and (3) *S. hominis* (Table 2).

The amplicons of the partial *16S rRNA* gene for all of the Staphylococcus species yielded a single band nearly 479 bp (Table 2). While the results of the *gap* gene gave nearly 933 bp and it was not positive in all strains, but was positive in all *S. aureus* strains and negative in CNS strains (Table 2, Figure 1). Whereas the thermostable nuclease (*nuc*) gene were amplified to identify of *S. aureus* strains appeared a single band ~ 270 bp, but it was positive in some strains of CNS like S41, S49, S50, S52, S53, S54, S55 (Table 2, Figure 1).

Amplification of *mecA* gene

The *mecA* gene was detected in all 55 strains by the PCR amplification method. Where for *S. aureus*, PCR amplification of *mecA* gene was positive in 6 of 40 strains (MRSA): *S3, S11, S19, S28, S31, S32*, but the others 34 strains were MSSA Methicillin-sensitive Staph. aureus, where the percentage of presence of *mecA* gene was (15%) (Table 2, Figure 2). While for CNS strains, *mecA* gene was not founded in any strain of *S. epidermidis*. But remain strains of CNS, the *mecA* gene was positive only in one strain of *S. hominis S53* which was isolated from bullous with percentage 6.66%, while the other strains were negative (Table 2, Figure 2).

Table (1): the percentage of prevalence of *S. aureus* and CNS strains as total samples

Staphylococci	Numbers	Percentage %
S. aureus	74	65.48
S epidermidis	29	25.66
S. saprophyticus	5	4.42
S. hominis	3	2.65
S. lentus	2	1.76
Collection	113	100

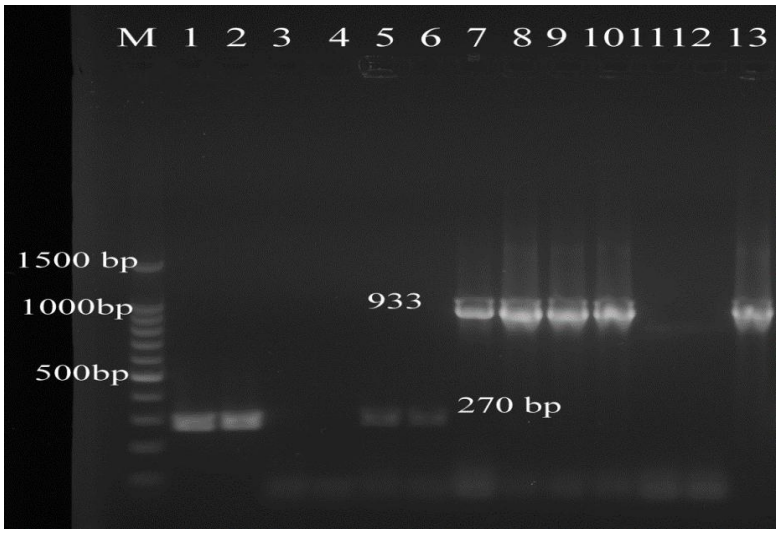

Figure (1): 1.25% Agarose gel electrophoresis for the detection of *nuc* gene (270 bp), *gap* gene (933 bp) in *S. aureus* and CNS by PCR, Lane 1, 2, 3, 4, 5, 6 *nuc* gene , Lane 7, 8, 9, 10, 11, 12, 13 *gap* gene, M: 100 bp DNA Ladder.

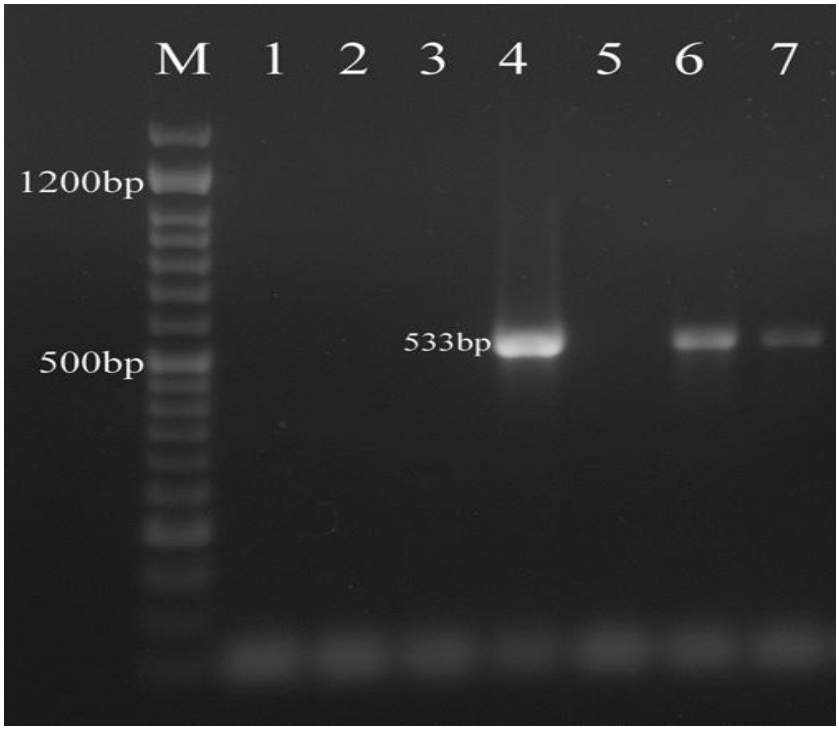

Figure (2): 1.25% Agarose gel electrophoresis for the detection of *mecA* gene (533 bp) in *S. aureus* by PCR, Lane 1, 2, 3, 5 *S. aureus* negative, Lane 4: *S. aureus* 28 from bronchial lavages , Lane 6: *S. aureus* 31 from ear, Lane 7: *S. aureus* S 32 from Conjunctivitis, M: 50 bp DNA Ladder.

Table 2. the results of molecular methods (*nuc* gene, *16S rRNA*, *gap* gene, *mecA* gene) of *S. aureus* and CNS strains

No.	Staphylococci strains	Sources	nuc	16S RNA	gap	mecA
S1	S. aureus	abscess	+	+	+	-
S2	S. aureus	bronchial lavages	+	+	+	-
S3	S. aureus	pus	+	+	+	+
S4	S. aureus	CSF	+	+	+	-
S5	S. aureus	ear	+	+	+	-
S6	S. aureus	pus	+	+	+	-
S7	S. aureus	Pus	+	+	+	-
S8	S. aureus	CSF	+	+	+	-
S9	S. aureus	ear	+	+	+	-
S10	S. aureus	bronchial lavages	+	+	+	-
S11	S. aureus	pus	+	+	+	+
S12	S. aureus	bullous	+	+	+	-
S13	S. aureus	eye	+	+	+	-
S14	S. aureus	urine	+	+	+	-
S15	S. aureus	urine	+	+	+	-
S16	S. aureus	urine	+	+	+	-
S17	S. aureus	abscess	+	+	+	-
S18	S. aureus	eye	+	+	+	-
S19	S. aureus	ear	+	+	+	+

S20	S. aureus	human skin (hand)	+	+	+	-
S21	S. aureus	pus	+	+	+	-
S22	S. aureus	Furuncle	+	+	+	-
S23	S. aureus	Peritoneal fluid	+	+	+	-
S24	S. aureus	Brain Peritoneal shunt	+	+	+	-
S25	S. aureus	urine	+	+	+	-
S26	S. aureus	Pleural effusion	+	+	+	-
S27	S. aureus	wound	+	+	+	-
S28	S. aureus	bronchial lavages	+	+	+	+
S29	S. aureus	CSF	+	+	+	-
S30	S. aureus	CSF	+	+	+	-
S31	S. aureus	ear	+	+	+	+
S32	S. aureus	Conjunctivitis	+	+	+	+
S33	S. aureus	pus	+	+	+	-
S34	S. aureus	human skin (face)	+	+	+	-
S35	S. aureus	abscess	+	+	+	-
S36	S. aureus	pus	+	+	+	-
S37	S. aureus	bullous	+	+	+	-
S38	S. aureus	abscess	+	+	+	-
S39	S. aureus	CSF	+	+	+	-
S40	S. aureus	human skin (face)	+	+	+	-
S41	S. epidermidis	Peritoneal fluid	+	+	-	-
S42	S. epidermidis	CSF	-	+	-	-
S43	S. epidermidis	ear	-	+	-	-
S44	S. epidermidis	CSF	-	+	-	-
S45	S. epidermidis	human skin (face)	-	+	-	-
S46	S. epidermidis	human skin (face)	-	+	-	-
S47	S. saprophyticus	Brain Peritoneal shunt	-	+	-	-
S48	S. saprophyticus	bronchial lavages	-	+	-	-
S49	S. saprophyticus	bronchial lavages	+	+	-	-
S50	S. saprophyticus	pus	+	+	-	-
S51	S. hominis	bullous	-	+	-	-
S52	S. hominis	bullous	+	+	-	-
S53	S. hominis	bullous	+	+	-	+
S54	S.lentus	ear	+	+	-	-
S55	S. lentus	liver lavages	+	+	-	-

Prevalence of *S. aureus* and CNS strains as age group

Table 3 shows that the percentage of distribution of *S. aureus* is more in adults (44.24%) than children (21.23%), followed by *S. epidermidis* which was (14.15%) in adults and was (11.50%) in children, then *S. saprophiticus* (4.42%), *S. lentus* (1.76%), and *S. hominis* (2.65%). There is no significant difference between the percentage of *S . aureus* and CNS in adults and children (*P>0.05*).

Table (3): the percentage of prevalence of *S. aureus* and CNS strains as age group

Staphylococci strains	Adults (numbers, %)	Children (numbers, %)
S. aureus	(50) 44.24	(24) 21.23
S. epidermidis	(16) 14.15	(13) 11.50
S. saprophyticus	(5) 4.42	-
S. lentus	(2) 1.76	-
S. hominis	(3) 2.65	-

Prevalence of S. aureus with percentage as a source samples

Table 4 shows the numbers and percentage of prevalence of *S. aureus* in clinical samples, where was 28.37% in (pus, abscess, wounds), 25.67% in (bullous, ulcers, infection's skin), 16.21% in CSF, 9.45% in ear, 6.75% in urine, and peritoneal shunt 4.05% and the other results were summarized in (Table 4). There is no significant difference between the percentage of *S . aureus* in clinical samples according to source (*P>0.05*).

Prevalence of CNS strain with percentage as a source sample

Table 4 shows the numbers and percentage of prevalence of *S. epidermidis* in clinical samples, where was 31.03% in (CSF), 24.13% in (pus, wounds, abscess), 17.24% in ear, 10.34% in urine, 6.89% in bronchial lavages, 6.89% in peritoneal shunt and 3.44% in newborn, then the remain results were summarized in (Table 4). There is no significant difference between the percentage of CNS in clinical samples according to source (*P>0.05*).

Table (4): the percentage of prevalence of *S. aureus* and CNS strains as source samples (numbers with percentage)

Samples	*S. aureus*%	*S. epidermidis*%	*S. saprophyticus*%	*S. lentus*%	*S. hominis*%
CSF	(12)16.21	(9)31.03	-	(1)50	-
bullous, ulcers, infection's skin	(19)25.67	-	-	-	(3)100
pus, wounds, abscess	(21)28.37	(7)24.13	(1)20	-	-
ear	(7)9.45	(5)17.24	-	(1) 50	-
urine	(5)6.75	(3)10.34	-	-	-
eye	(3)4.05	-	-	-	-
peritoneal shunt	(3)4.05	(2)6.89	(2)40	-	-
bronchial lavages	(3)4.05	(2)6.89	(2)40	-	-
plural effusion	(1)1.35	-	-	-	-
newborn	-	(1)3.44	-	-	-

Antibiotic susceptibility and MICs

Among 55 (40 of *S. aureus* and 15 of CNS), all strains showed different levels of susceptibility against 8 various antibiotics. Inhibition zone diameters were measured depending on NCCLS. The results of antibiotic resistance tests of *S. aureus* was as follows: amoxicillin-clavulanic acid (65%), gentamicin (30%), ceftriaxone (62.5%), pefloxacine (7.5%), ofloxacine (7.5%) ampicillin (70%), vancomycin (2.5%) and amoxicillin (55%) (Table 5). While for CNS strains the results were summarized in (Table 5). The MIC for methicillin of all strains of *S. aureus* was ranging between 16 – 32 μg/mL except the strains which carry *mecA* gene (S3, S11, S19, S28, S31, S32) was >256 μg/mL. Whereas the MIC for methicillin of CNS strains was ranging between 8 – 16 μg/mL except the strain which carry *mecA* gene (S53 *S. hominis*) which isolated from bullous.

Table (5): the percentage of antibiotic resistance and susceptibility for *S. aureus* and CNS strains

Antibiotics	S. aureus (40)	S. epidermidis(6)	S. saprophyticus(4)	S. lentus (2)	S. hominis(3)
	R % S	R % S	R % S	R % S	R % S
Amoxicillin/clavulanic acid AMC 30	65 35	16.66 83.33	100 0	100 0	33.33 66.66
Ampicillin AM 10	70 30	100 -	100 0	100 0	100 0
Amoxicillin AX 25	55 45	33.33 66.66	100 0	100 0	100 0
Vancomycin VA 30	2.5 97.5	- 100	50 50	0 100	0 100
Gentamicin GM 10	30 70	16.66 83.33	50 50	0 100	0 100
Ofloxacine OFX 5	7.5 92.5	- 100	50 50	0 100	0 100
Pefloxacine PE 5	7.5 92.5	- 100	50 50	0 100	0 100
Ceftriaxone CRO 30	62.5 37.5	33.33 66.66	100 0	100 0	33.33 66.66

Discussion

Infections due to Staphylococci strains especially MRSA are becoming a major health issue in the Middle East and the world wide, and yet studies were carried out on the epidemiology and infections which were caused by these bacteria. In addition, that the CNS strains like *S. hominis* and *S. lentus* which isolated from human microflora and widely distributed in environment, mainly in food, farm animals, but recently are frequently associated with human diseases such as infections associated with intravenous catheters, endocarditis, peritonitis, septic shock, infections of the urinary tract, open wounds, renal, and skin infections.

The goal of present research was study the prevalence of *S. aureus* and CNS strains in clinical samples with antibiotic resistance, and investigation of MRSA, and MRCNS in these strains and to our knowledge this research is one of the little studies which carried out in Syria to report on the prevalence of *S. aureus* and CNS strains and detected the MRSA in hospitals

The major prevalence of strains was *S. aureus* (65.48%) followed by *S. epidermidis* (25.66%) then *S. saprophyticus* (4.42%), but the important point that *S. lentus* was isolated from ear and CSF with percentage (2.65%) and *S. hominis* which was isolated from bullous with percentage (1.76%). This observation is in agreement with other studies like (Buzaid *et al*., 2011), and extremely like with study (Keim *et al.*, 2011) where the percentage of *S. epidermidis* was (34%), *S. saprophyticus* was (3%) and *S. hominis* was (2%).

By the prevalence of *S. aureus* and CNS strains between the adults and children, it has observed that the percentage of *S. aureus* was (44.24%) in adults, but in children was (21.23%), while the percentage of CNS strains were close in adults and children (Table 3). Whereas the prevalence of *S. aureus* in clinical samples was major in (pus, wounds, abscess) 28.37%, followed the percentage in (bullous, ulcers, skin's infections) which was 25.67%, then percentage in CSF where was 16.21% (Table 4), hence, the prevalence of *S. epidermidis* in clinical samples was more in CSF (31.03%) than (pus, wounds, abscess) where was 24.13%, followed the percentage in ear which was 17.24, then urine which was

10.34% (Table 4). But the important results that *S. saprophyticus* was isolated from pus, bronchial lavages and peritoneal fluid with ratio 4.42%, also *S. hominis* was isolated from bullous 2.65%, and *S. lentus* was isolated from CSF and ear with percentage 1.76% (Table 1, 4).

These results were close some studies like (Nascimento *et al.*, 2015) which isolated the CNS from health care waste and the percentage of *S. saprophyticus* and *S. lentus* were 1.4%, 1.4% respectively.

Based on the antimicrobial susceptibility patterns observed, for *S. aureus* the major effective antibiotic was vancomycin (97.5%), then pefloxacine and ofloxacine (92.5%) next gentamycin (70%), while AMC, AM, AX were 35%, 30%, 45% respectively, but for CNS strains almost were resistant to AM, AX (Table 5)

For molecular tests and detection MRSA and MRCNS it was chosen four target genes, where the first gene is *gap* which encodes the enzyme (glyceraldehyde-3-phosphate dehydrogenase) and analysis of the enzyme represented a high-throughput reproducible method that allows the identification of distinct Staphylococcus species. The next gene was *16S rRNA* which designing the Staphylococci specific region of the *16S rRNA*. The third gene was *nuc* which codes for thermostable nuclease. Because the *gap*, *16S rRNA* and *nuc* genes are not specific for MRSA, the *mecA* gene was chosen as the fourth target gene that encodes a β-lactam resistant penicillin-binding protein PBP and the gene is inserted in a mobile genetic element, *SCCmec*, which is of fundamental importance in the transmission and epidemiology of bacterial resistance.

Our findings are in line with previous reports from the Mediterranean region and around the world as (Yang *et al*., 2010; Gonzalez *et al.*, 2010; Miko *et al.*, 2013).

By *S. aureus* and based on antibiotic resistance discs there are 28 strains resistant to ampicillin and 22 strains resistant to amoxicillin, but only 6 strains were carried out *mecA* gene, that is to say not all strains of *S. aureus* can be classified MRSA, like S1, S2, S6, S15 which were resistant to (AMC, AM, AX) but it didn't carry *mecA* gene (Table 2, 5). The repression of *mecA* gene and the resulting absence of MR in some of the isolates could be due to several factors. Both genetic and environmental factors play a significant role in the expression of MR. While all strains of *S. epidermidis* were sensitive for all tested antibiotics and this agree with genetic results, where *mecA* gene didn't found in any strains. While for CNS strains most of it were resistant to AM, AX but only *S. hominis* which isolated from bullous was carry *mecA* gene and was resistant to most antibiotic targets, therefore it could be classified MRCNS, and the most of CNS were sensitive to vancomycin, pefloxacine, ofloxacine and gentamycin.

In contrast to previously reported MRSA isolates, Khan *et al.*, (2007) showed that 7 isolates of 17 were carried out by *mecA* gene; Buzaid *et al.*, (2011) reported that 31 % of *S. aureus* were MRSA in tertiary surgical and trauma hospital; Abd-Elhafez *et al.*, (2011) showed that 29 isolates of *S. epidermidis* were positive for the *mecA* gene, and these isolates showed several multidrug-resistance patterns; Thompson *et al.*, (2012) isolated *S. aureus* from untreated hospital wastewaters and showed the presence of *mecA* gene in 99 strains of a total 156 strains; Bhargava and Zhang, (2012) detected the *mecA* gene in 60 of 87 CNS isolates and studied their antimicrobial susceptibility to some antibiotics; Lyer *et al.*, (2014) appeared that about 73 of 100 *S. aureus* were isolated from workers were positive for *mecA* gene; Sergelidis *et al.*, (2014) isolated methicillin-resistant staphylococcus spp. from ready to eat fish products and showed presence the *mecA* gene in two oxacillin-resistant strains and three (23.1%) of *S. epidermidis*, Goering *et al.*, (2008) showed that of 105 strains of *S. aureus* 52 strains were positive for *mecA* gene; Nascimento

et al., (2015) showed that among the oxacillin-resistant strains of CNS the *mecA* gene was detected in two isolates; Moghadam *et al.,* (2014) resulted that among 65 isolated strains of *S. aureus* the *mecA* gene was positive in 40 strains.

Conclusion

As a matter of concern, our results raise issues related to the antibiotic resistance in pathogenic Staphylococci strains carrying important resistance genes. The *16SrRNA*, *gap*, and *nuc* genes are important to adjust the identification of Staphylococci. The *mecA* is the essential gene to determine the resistant genes in *S. aureus* and CNS strains.

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