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# Studies on leukocidins toxins and antimicrobial resistance in *Staphylococcus aureus* isolated from various clinical sources

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***Staphylococcus aureus* toxins represent a public health challenge all over the world. This study aims to analyze the prevalence of genes encoding the staphylococcal leukocidins and their correlation with antimicrobial susceptibility and the source of isolation. For this purpose, the susceptibility of 75 *S. aureus* isolates to 12 antimicrobial agents was investigated. The leukocidins genes (*lukD, lukE, lukF* and *lukS*) were detected by polymerase chain reaction (PCR). The ability to express these genes was assessed among 20 isolates by RT-PCR. The most prevalent *luk* genes were *lukF* gene (73.3%), followed by *lukE* (64%), *lukD* (44%) and *lukS* (34.7%). Expression of *lukD, lukE* and *lukS* genes were variable. *lukF* gene was not expressed by any of the tested isolates. A statistically significant association was found between *lukF* occurrence and burn isolates. Besides that, *lukF* gene was more prevalent among amoxicillin-clavulenic acid and amikacin resistant isolates, while *lukE* was predominant with gentamicin resistant isolates. High expression level of *lukD* was found in MRSA and MDR isolates.**

**Key words:** *Staphylococcus aureus*, leukocidins, antimicrobial sensitivity.

## INTRODUCTION

*Staphylococcus aureus* is one of the important human pathogens that causes wide varieties of diseases, ranging from skin infection to bacteraemia and infective endocarditis, beside toxin-mediated diseases (Lowy, 1998). More than 30 extracellular products are produced by *S. aureus* (Rogolsky, 1979). Almost all strains secrete a group of cytotoxins and enzymes such as nucleases, haemolysins, lipases, collagenases, proteases and hyaluronidase. Some strains produce additional exoproteins, which include leukocidins, toxic shock syndrome toxin (TSST-1), the exfoliative toxins and the staphylococcal enterotoxins. Leukocidins and γ-haemolysin are members of a toxin family known as synergohymenotropic toxins, as they act by the synergy of two proteins to form a pore on cell membranes (Dinges et al., 2000).

γ-Hemolysins (*Hlg*) and leukocidins (*Luk*) consist of two classes: F class (molecular weight of about 34 kDa)

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*HlgB*, *LukD*, *LukF*, *LukF-R*, *LukF-PV*, *LukM* and S class (molecular weight of about 32 kDa) *HlgA*, *HlgC*, *LukE*, *LukS*, *LukS-PV* and *LukS-R*. The two classes are important for toxin biological activity (Choorit et al., 1995; Gravet et al., 1998; Qiu et al., 2010).

Most *S. aureus* strains cause necrotizing pneumonia and primary skin infections harbor the Panton-Valentine leukocidin (PVL) determinant (Lina et al., 1999; Gillet et al., 2002). PVL genes were detected of strains associated with finger-pulp infection, cutaneous abscess, cellulitis and furunculosis while they were absent in superficial folliculitis and impetigo associated strains (Lina et al., 1999).

Staphylococcal leukocidins are leukolytic toxins active against human and rabbit macrophages, monocytes and polymorphonuclear cells forming cation-selective transmembrane pores in leukocytes and macrophages. Its mechanism is as follow: three S class molecules of the toxin bind to the specific receptor in the cell membrane leading to conformational changes that allow three F class molecules to bind to the formed S subunit-receptor complex to form a hexameric pore. The formed pore leads to the influx of divalent cations into the cell which stimulates enzymes and inflammatory mediators' secretion then cell lysis (Konig et al., 1994, 1997). The spread of bacterial infection is mediated by the inhibition of phagocyte and macrophage activity due to simultaneous secretion of various leukocidins and hemolysins. Staphylococcal leukocidins has leukotoxic activity (Konig et al., 1997).

In this study, the authors aimed to (i) detect leukocidins genes in *S. aureus* by PCR and evaluate their distribution among isolates from different clinical sources; (ii) determine Leukocidins expression by RT-PCR and (iii) assess the correlation between presence of leukocidins genes and antimicrobial susceptibility. A complete survey on leukocidins and their expression has not been recorded in the Egyptian available literature.

## MATERIALS AND METHODS

### Study population

In this study, 75 strains of S. aureus were collected during a period of 10 months between January 2014 – October 2014 [19 isolates from Mansoura University Children Hospital (MUCH), 11 isolates from Burn and Cosmetic Center (BCC), 31 isolates from Internal Medicine Hospital (IMH) and 14 isolates from Microbiology and Immunity Unit, Faculty of Medicine, Mansoura University]. These isolates were isolated from wounds, burns and sputum.

Following isolation, identification of isolates according to Collee et al. (1996). In this respect, isolates were tested for growth on mannitol salt agar, Gram reaction, catalase production, free and bound coagulase production. The experimental protocol conducted in this study was approved by the Ethics Committee of Faculty of Pharmacy, Mansoura University with code (2015-60). Participants provided their written informed consent to participate in this study. The age of participants for the study ranged between 13-45 years. Written informed consent was obtained from the next of kin, caretakers or guardians on behalf of the minors/children enrolled in our study. Ethics Committee of Faculty of Pharmacy, Mansoura University approved the usage of minors under the age of 18 in the study and approved the consent protocol used for them.

### Antibiotic susceptibility test

*S. aureus* isolates were screened for susceptibility to 12 antimicrobial discs namely; penicillins (oxacillin, 1 µg), cephalosporins (cefoxitin 30 µg, cefotaxime 30 µg, ceftazidime 30 µg, cefepime 30 µg, cephalothin 30 µg), carbapenems (Imipenem 10 µg), β-Lactams combinations (amoxacillin-clavulanic 30 µg, ampicillin-sulbactam 10 µg), aminoglycosides (gentamicin 10 µg, amikacin 30 µg) and quinolones (ciprofloxacin 5 µg) using the standard disc diffusion method and Muller Hinton agar plates (Hoseini Alfatemi et al., 2014). All discs and media were supplied by Oxoid products, UK. Iinterpretation of data was done according to CLSI (2014).
For methicillin resistant *S. aureus* (MRSA) detection, cefoxitin (30 µg) disk was used, where isolates with inhibition zone diameter ≥ 21 were identified as MRSA (CLSI, 2014).

### Molecular techniques

#### *Preparation of the total DNA content of S. aureus isolates*

Isolates were grown overnight at 37°C, then a single colony from each isolate was mixed with 100 µl DNase/RNase-free water to obtain a turbid suspension that was held in a boiling water-bath for 10 min, chilled on ice and centrifuged. For PCR reactions, 5 µl of extracted template DNA were used (Englen and Kelley, 2000).

#### *PCR for leukocidins and mecA genes detection*

The leukocidins toxins genes (*lukF, lukS, lukD* and *lukE*) and methicillin resistance gene (*mecA*) were detected using singleplex PCR reactions (FPROGO2D, Tchne LTD, Oxford Cambridge, U.K.) and specific primers listed in Table 1. The PCR reaction was performed as described previously in Hassan et al. (2012). The program was started with initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing (at the specified temperature for each primer as indicated in Table 1 for 40 s, and extension at 72°C for 1 min followed by final extension at 72°C for 5 min. Negative control was included in each reaction using, $ddH_2O$ instead of DNA extract. The amplified genes were visualized using 2% agarose gel electrophoresis stained with ethidium bromide and compared with a 100 base pair plus (bp) DNA ladder (Thermo scientific). The presence of a band at the expected product size was considered a positive result.

#### *RNA isolation*

RNA was isolated from 20 isolates. Isolates that harbored 2 or more of the tested toxin genes as detected by PCR and isolates sharing the same resistance and toxin gene pattern were selected. RNA was isolated according to Abdel-Rhman (2016) using glass beads and TRI Reagent (Sigma-Aldrich).

The concentration and the purity of RNA for each strain were determined spectrophotometrically by NanoDrop (ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, Delaware, USA) using A260 and A260/280 nm ratio respectively. RNA was considered pure if it has a A260/280 ratio of 1.8:2.1.

#### *Expression of Luk genes*

Isolated RNA was used for preparation of complementary DNA

**Table 1.** Oligonucleotide primers sequences used to amplify the tested genes.

| Gene name | Sequence | | Annealing temperature (°C) | Amplicon size (bp) | References |
|---|---|---|---|---|---|
| *lukE* | Fw | 5'-TGCGTAAATACCAGTTCTAGGG-3' | 60 | 199 | This study |
| | Rv | 5'-TCCAACAGGTTCAGCAAGAG-3' | | | |
| *lukD* | Fw | 5'-ACCAGCATTTGAACTACTTTGT-3' | 60 | 240 | This study |
| | Rv | 5'-TCTAATGGCTTATCAGGTGGAT-3' | | | |
| *lukF* | Fw | 5'-TGTGCTTCTACTTTCCACCAT-3' | 54 | 225 | This study |
| | Rv | 5'-TGTGACTGACTTTGCACCA-3' | | | |
| *lukS* | Fw | 5'-GGTCCATCAACAGGAGGTAAT-3' | 57 | 267 | This study |
| | Rv | 5'-AGGATTGAAACCACTGTGTACT-3' | | | |
| *mecA* | Fw | 5'-TGCTATCCACCCTCAAACAGG-3' | 57 | 286 | (Kondo et al., 2007 |
| | Rv | 5'-AACGTTGTAACCACCCCAAGA-3' | | | |
| *nuc* | Fw | 5'-GCGATTGATGGTGATACGGTI-3', | 55 | 267 | Brakstad et al., 1992 |
| | Rv | 5'-CCAAGCCTTGACGAACTAAAGC-3' | | | |

Fw: Forward primer Rv: reverse primer.

using Quanti-Tect Reverse Transcription kit (QIAGEN, Germany). RT-PCR was performed using Rotor Gene Q thermocycler (QIAGEN, Hilden, Germany) and 5X FIREPol Eva Green, qPCR Mix, ROX Dye (Solis Bio-Dyne, Tartu, Estonia) using the same primers described previously. Program was performed as follows: 95°C for 15 min, then 35 cycles x (denaturation at 95°C for 15 s, annealing as the specified temperature for 30 s and extension at 72°C for 1 min). Target genes expression was normalized to the reference gene *nuc* (encoding nuclease enzyme) expression. The gene expression level in samples was calculated relative to the housekeeping gene using a calibrator sample by the comparative (ΔΔct) method (El-Mowafy et al., 2014). All measurements were performed in triplicate.

#### Statistical analysis

Correlations between data were statistically analyzed using the Graphpad Instat 3. Fisher's exact test was used to evaluate these correlations where a P value ≤0.05 was considered statistically significant.

## RESULTS

### Bacterial isolates

A total of 200 samples were collected from different Mansoura hospitals. Seventy five isolates were identified as *S. aureus*. The clinical origins of these isolates were wound (W, 47 isolates), burn (B, 16 isolates) and sputum (S, 12 isolates).

### Antimicrobial susceptibility test

In the present study, *S. aureus* isolates showed variable resistance towards the 12 antimicrobial agents used as shown in Figure 1. The most effective antibiotic was imipenem as 83% of isolates were sensitive, while ceftazidime was the least effective one as only 8% of isolates were sensitive to it. Methicillin resistance was recorded in 55 isolates (73.3%). Thirty nine isolates were multidrug resistant (MDR) (resistant to 3 or more classes of antimicrobials). The number of antimicrobials resistance per isolate is illustrated in Table 2.

### PCR detection of tested genes

The virulence genes *luk D, luk E, luk S, luk F* and resistance gene *mecA* were amplified from total DNA extracts. Results showed that both *lukF* and *mecA* genes were the predominant genes as they were found in 55 isolates (73.3%), *LukE, lukD* and *lukS* genes were harbored by 64, 44 and 34.6%, respectively.

The toxin gene profile is illustrated in Table 3. The 75 isolates demonstrated 13 different toxin patterns. The most common pattern was T8 which was represented by 16% of isolates followed by patterns T10 and T12 (12 and 10.6% of isolates, respectively).

### RT-PCR analysis of *S. aureus* leukocidins

Relative expression of luk genes were evaluated among tested isolates. The standard curve of the housekeeping gene *nuc* and all expressed genes including *lukD, lukE, lukF* and *lukS* showed $R^2$ values of 0.97-0.99. They showed the same melting profile of pure amplicons which indicated the assay specificity. The relative expression levels of *luk* genes were analyzed using the comparative method ($2^{-\Delta\Delta Ct}$) method.

The relative expression of these genes was investigated in 20 isolates. All isolates showed expression of the housekeeping gene (*nuc*). *lukD* gene

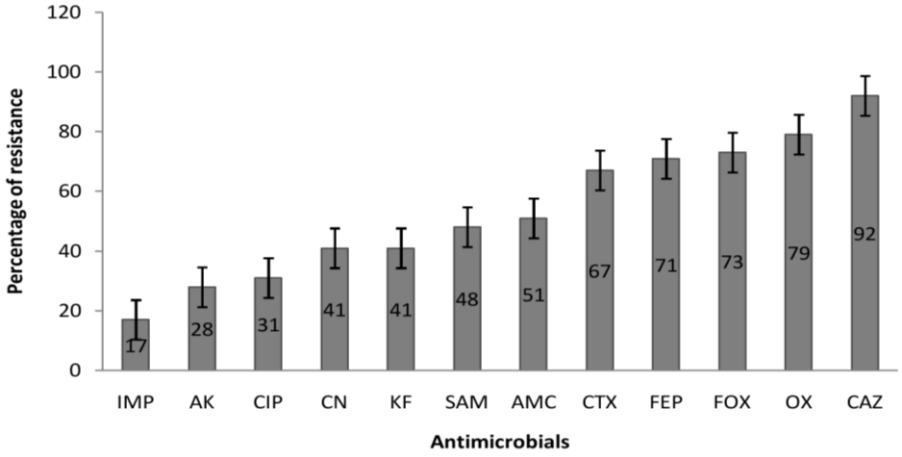


**Figure 1.** The percentage of resistance to different antimicrobials. AK, Amikacin; AMC, Amoxacillin-Clavulanic; CAZ, Ceftazidime; CIP, Ciprofloxacin FEP, Cefepime; CN, Gentamicin; CTX, Cefotaxime; FOX, Cefoxitin; IPM, Imipenem; KF, Cephalothin; OX, Oxacillin; SAM, Ampicillin-Sulbactam.

**Table 2.** Multidrug resistance profile of MSSA and MRSA to different antimicrobials.

| Number of antimicrobials / isolate | No of isolates | | Total | P value |
|---|---|---|---|---|
| | MSSA (n=20) | MRSA (n=55) | | |
| 1 | 0 | 0 | 0 (0%) | |
| 2 | 2 | 0 | 2 (2.6%) | 0.0685 |
| 3 | 6 | 2 | 8 (10.6%) | 0.0037* |
| 4 | 5 | 6 | 11 (14.6%) | 0.1498 |
| 5 | 2 | 12 | 14 (18.6%) | 0.3281 |
| 6 | 4 | 8 | 12 (16%) | 0.7225 |
| 7 | 1 | 11 | 12 (16%) | 0.1636 |
| 8 | 0 | 4 | 4 (5.3%) | 0.5683 |
| 9 | 0 | 1 | 1 (1.3%) | 1.0000 |
| 10 | 0 | 2 | 2 (2.6%) | 1.0000 |
| 11 | 0 | 4 | 4 (5.3%) | 0.5683 |
| 12 | 0 | 5 | 5 (6.6%) | 0.3160 |

was expressed in 14/19 isolates (Figure 2). *lukE* gene was expressed in 13/16 of isolates (Figure 3). *lukS* was expressed in 10/13 of isolates (Figure 4). Although, *lukF* was detected in the 20 isolates tested by PCR, it was not expressed by any of them.

## DISCUSSION

*S. aureus* is an important human pathogen causing nosocomial and community acquired infections (Taiwo et al., 2005). The resistance to antimicrobial agents among *S. aureus* is a growing problem worldwide. Multidrug-resistant staphylococci is a problem for human health. Infections caused by MRSA is a challenge for healthcare institutions (Kurlenda et al., 2009). Besides that, the emergence of virulent MRSA is a serious problem in the treatment and control of staphylococcal infections (Livermore, 2000; Zapun et al., 2008; Duran et al., 2012).

In this study, a total of 75 isolates were isolated from various clinical sources (wound, burn and sputum). These isolates were investigated for their antimicrobial sensitivity to 12 antimicrobials.

For β-lactam class, our results illustrated that imipenem showed the highest activity as only 17% of isolates were resistant in contrast to 64% resistant isolates reported by Kholeif and Mohamed (2009). Cephalosporins showed different activity on *S. auerus* according to its generation. The first generation cephalosporins (cephalothin) showed higher activity toward the isolates (41% of isolates were

**Table 3.** The toxin gene profile among *S. aureus* isolates.

| Toxin pattern | Toxin profile | MDR | NMDR | Total |
|---|---|---|---|---|
| T1 | No toxin | 2 | 4 | 6 |
| T2 | *lukD* | 2 | 1 | 3 |
| T3 | *lukE* | 1 | 3 | 4 |
| T4 | *lukF* | 4 | 3 | 7 |
| T5 | *lukD, lukE* | 2 | 4 | 6 |
| T6 | *lukD, lukF* | 1 | 1 | 2 |
| T7 | *lukE, lukS* | 1 | 1 | 2 |
| T8 | *lukE, lukF* | 9 | 3 | 12 |
| T9 | *lukF, lukS* | 2 | 3 | 5 |
| T10 | *lukD, lukE, lukF* | 7 | 2 | 9 |
| T11 | *lukD, lukF, lukS* | 2 | 2 | 4 |
| T12 | *lukE, lukF, lukS* | 4 | 4 | 8 |
| T13 | *lukD, lukE, lukF, lukS* | 2 | 5 | 7 |

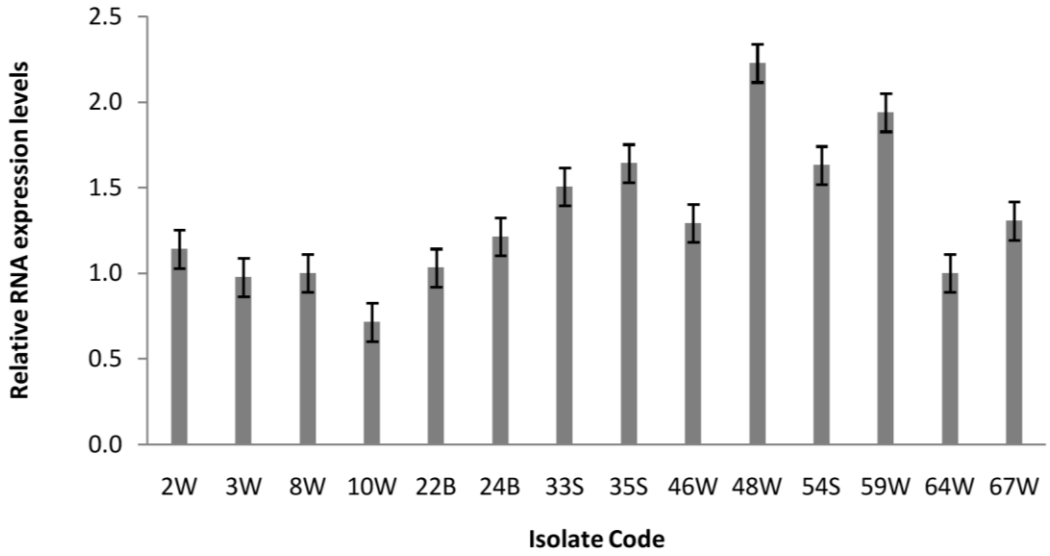


**Figure 2.** Relative *lukD* gene expression levels calculated by the comparative (ΔΔct) method using *nuc* gene as endogenous reference gene.

resistant) and the resistance increases with higher generations of cephalosporin as ceftazidime and cefotaxime (2nd generation) showed high resistance (92 and 67%, respectively). For the 4th generation (cefepime), 71% of isolates were resistant. A previous study was consistent with our results as it showed that 68.4% of isolates were resistant to cefotaxime (Onelum et al., 2015). The study conducted by Kholeif and Mohamed (2009) reported similar results concerning cefepime and cefotaxime while for cephalothin, it showed higher resistance (67%) than ours. On the other hand, another study reported a lower resistance level to cefepime (8%) (Siddiqui et al., 2013).

The resistance of isolates towards β-lactam combinations (ampicillin-sulbactam and amoxicillin – clavulenic acid) was intermediate (48 and 51% respectively). However, Duran et al. (2012) reported low percentage of resistance (23%) to amoxicillin–clavulenic acid. For ampicillin-sulbactam, all tested isolates were sensitive in the study carried out by Ali et al. (2013).

In this study, amikacin showed higher activity toward isolates than gentamicin (28:41%). Thirty one percentage isolates were resistant to ciprofloxacin. This was in accordance with Duran et al. (2012) who reported similar results concerning gentamicin and ciprofloxacin. In contrast, Kitara et al. (2011) showed that all *S. aureus* isolates were sensitive to gentamicin and only 1.6% were resistant to ciprofloxacin.

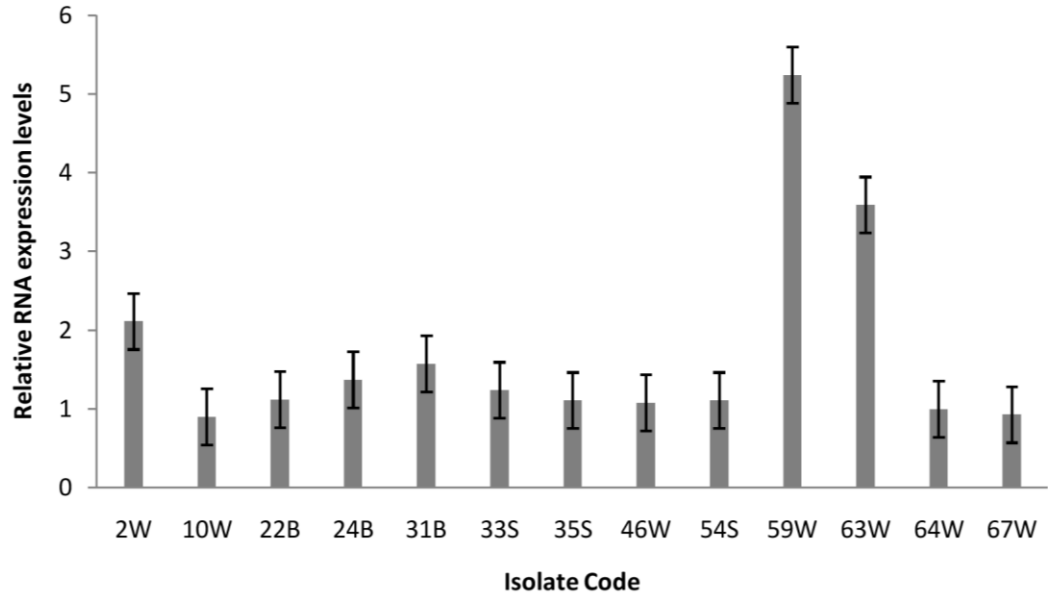


**Figure 3.** Relative *lukE* gene expression levels calculated by the comparative (ΔΔct) method using *nuc* gene as endogenous reference gene.

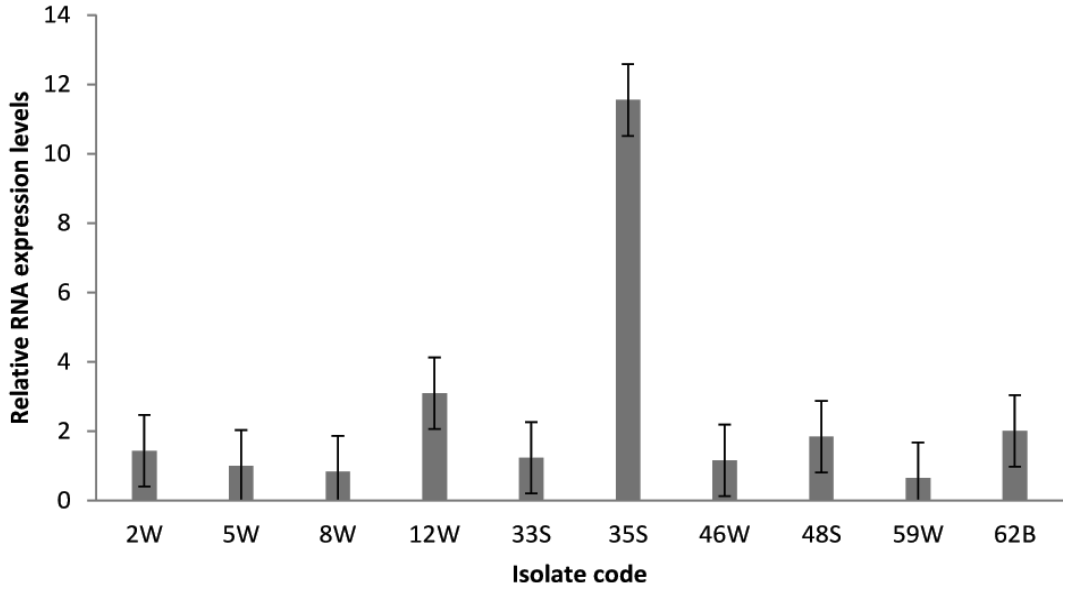


**Figure 4.** Relative *lukS* gene expression levels calculated by the comparative (ΔΔct) method using *nuc* gene as endogenous reference gene.

Concerning cefoxitin disc, 73.3% (55) of the isolates were found to be MRSA. For oxacillin, there were 59 isolates found to be MRSA. These results emphasize that cefoxitin is superior to oxacillin as an indicator of MRSA. Various studies illustrated that the cefoxitin is more sensitive than oxacillin for MRSA detection by disc diffusion method (Skov et al., 2003; Boutiba-Ben Boubaker et al., 2004; Velasco et al., 2005), this may be explained by cefoxitin is an inducer of the *mecA* gene resulting in increased expression of the *mecA*-encoded protein PBP2a (Velasco et al., 2005). The relation between methicillin resistance and clinical sources of *S. aureus* isolates was investigated where only isolates from wounds were significantly associated with MRSA (P value= 0.0136).

Comparing antimicrobial resistance profile of MRSA and MSSA isolates, it was found that resistance to amoxicillin-clavulenic acid, amikacin, imipenem and

cephalothin was significantly correlated to MRSA isolates with P value = 0.001, 0.0018, 0.0154 and 0.0073, respectively. Only isolates resistant to 3 antimicrobials was associated significantly to MSSA (p value= 0.0037).

*S. aureus* possess a remarkable ability to acquire resistance to multiple antibiotics (Jung et al., 2015). Thirty nine *S. aureus* isolates were MDR, 87% of them were MRSA. High level of antibiotic abuse leads to MDR. Many reasons lead to the high level of antibiotic abuse as the self-medication is associated with inappropriate dosage and failure to comply to treatment (Melles et al., 2006). In addition, the use of the over the counter medication with or without prescription increases the antibiotic abuse (Miller et al., 2005; Kholeif and Mohamed, 2009).

In the present study, *S. aureus* isolates were investigated for their leukocidins genes (*lukD, lukE, lukF* and *lukS*) and resistance gene (*mecA*) by PCR. The results show that 55 isolates harbored *mecA* gene. This result was consistent with cefoxitin disc results. The current gold standard for MRSA detection is identification of the *mecA* gene that cannot accurately be measured by disc diffusion or microdilution of oxacillin (Velasco et al., 2005).

In the present work, *lukD* and *luk E,* genes were amplified in 33 (44%) and 48 (64%) of isolates, respectively. The prevelance of *lukD-lukE* was variable in literatures where in a study performed in USA, it was 36.5% (Abdalrahman and Fakhr, 2015). In another study, 82.8%, of isolates were *lukD-lukE* positive (de Almeida et al., 2013).

Concerning *lukF* and *lukS* genes, they were detected in 55 (73.3%) and 26 (34.7%) isolates, respectively. This was consistent with a study performed in northeast Thailand hospitals, as nearly 50% of *S. aureus* isolates were PVL gene positive (Nickerson et al., 2009). In contrast, PVL positive isolates in Malaysia and Bangladesh were 5 and 4.3%, respectively (Neela et al., 2009). A study conducted in Egypt reported that detection of PVL gene was positive in only 23 isolates (39.7%) (Kholeif and Mohamed, 2009).

It was stated that *lukD-lukE* were present at higher percentage than PVL toxins (56:19%) (Baba-Moussa et al., 2010), while their incidence in the present study among isolates was nearly the same (31.4: 34.3%). No correlation was found between the *luk* genes and the isolation source except for *lukF* that was significantly associated with isolates of burn source (P value= 0.046). The *luk* genes were predominant in MRSA with no significant association except for *lukF* that was significantly associated with MRSA (P value= 0.0089). This was consistent with Larsen et al. (2009) who found that 69.4% of CAMRSA were PVL positive.

The correlation between the presence of *luk* genes and the resistance to antimicrobials was studied. *lukF* gene was prevalent among isolates resistant to amoxicillin-calvulenic acid and amikacin (P value= 0.0354 and 0.002, respectively). While for *lukE* gene, it was prevalent among isolates resistant to gentamicin (P value=0.029). In this study, the 75 *S. aureus* isolates demonstrated 13 different toxin patterns. The toxin patten (T1) represents isolates with no leukocidins toxins (6 isolate), while toxin pattern (T13) represent 7 isolates harboring the four toxin genes. T8 was the most common toxin pattern as represent (16%) followed the patterns T10, T12 (12 and 10.6%, respectively). The toxin patterns T4 and T13 have the same distribution between isolates (9.3%). Analysis of toxin pattern distribution among multidrug-resistant and non-multidrug-resistant isolates did not allow the determination of a clear correlation between them.

Detection of toxin genes, does not necessarily mean the ability of toxin expression, this may be attributed to mutation in the gene regulatory region (Indrawattana et al., 2013), so it was important for us to demonstrate the ability to express the studied leukocidins genes among the selected 20 isolates by real-time PCR technique. *lukD* gene was expressed in 73.6% of isolates by variable degrees (Figure 2). In addition, *lukE* gene was expressed in 81.25% isolates that harbored this gene. However, only two of these thirteen isolates showed a relatively very high expression level (Figure 3). For *lukS* gene, it was expressed in 76.9% of the isolates with only one isolate showing very high relative expression level (Figure 4). Regarding *lukF* gene, it was not expressed by any of the tested isolates. This was in accordance with a study by Yu et al. (2013) reporting that the PVL detected by qRT-PCR was expressed in all isolates harboring PVL genes by variable levels suggesting that PVL genes transcription is associated with clinical isolates. Another study showed that PVL genes expression levels are strain dependent, with more than 10-fold variance (Said-Salim et al., 2005). In contrast, a study performed by Kholeif and Mohamed (2009) showed that PVL was positive in 39.7% by real time PCR.

The relation between *luk* genes expression, MRSA and MDR was evaluated. The level of *lukD* expression in MRSA and MDR isolates was higher than the expression levels recorded for MSSA and NMDR isolates. While for *lukE* and *lukS*, thier level of expression were not correlated with MRSA and MDR.

## Conclusion

The present study highlights the prevalence of MRSA among *S. aureus* clinical isolates. High incidence of MRSA isolates were found among isolates of wound infections. A significant association was found between MRSA and MDR isolates. MRSA isolates were significantly resistant to amoxicillin-clavulenic, amikacin, imipenem and cephalothin. These results also demonstrate high prevalence of *luk* genes including PVL genes among *S. aureus* isolates. Leukocidins genes were found to be strain dependent except for *lukF* that was significantly associated with burn isolates. *lukF* was

found to be predominant in MRSA isolates and in isolates resistant to amoxicillin-clavulenic acid and amikacin. *lukE* was prevalent in isolates resistant to gentamicin. High expression levels of *luk* genes were recorded in MRSA and MDR isolates. These results demonstrated the spread of highly resistant *S. aureus* isolates which possess leukocidins toxin in our hospitals and the utmost need for strict guidelines for controlling their spread.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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