Detection of macrolide and disinfectant resistance genes in clinical \textit{Staphylococcus aureus} and coagulase-negative staphylococci

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Abstract

\textbf{Background:} \textit{Staphylococcus aureus} and Coagulase-negative staphylococci (CoNS) are a major source of infections associated with indwelling medical devices. Many antiseptic agents are used in hygienic handwash to prevent nosocomial infections by \textit{Staphylococci}. Our aim was to determine the antibiotic susceptibility and resistance to quaternary ammonium compound of 46 \textit{S. aureus} strains and 71 CoNS.

\textbf{Methods:} \textit{S. aureus} (n = 46) isolated from auricular infection and CoNS (n = 71), 22 of the strains isolated from dialysis fluids and 49 of the strains isolated from needles cultures were investigated. Erythromycin resistance genes (\textit{ermA}, \textit{ermB}, \textit{ermC}, \textit{msrA} and \textit{mef}) were analysed by multiplex PCR and disinfectant-resistant genes (\textit{qacA}, \textit{qacB}, and \textit{qacC}) were studied by PCR-RFLP.

\textbf{Results:} The frequency of erythromycin resistance genes in \textit{S. aureus} was: \textit{ermA}+ 7.7\%, \textit{ermB}+ 13.7\%, \textit{ermC}+ 6\% and \textit{msrA}+ 10.2\%. In addition, the number of positive isolates in CoNS was respectively \textit{ermA}+ (9.4\%), \textit{ermB}+ (11.1\%), \textit{ermC}+ (27.4\%), and \textit{msrA}+ (41\%). The MIC analyses revealed that 88 isolates (74\%) were resistant to quaternary ammonium compound-based disinfectant benzalkonium chloride (BC). 56\% of the BC-resistant staphylococcus isolates have at least one of the three resistant disinfectants genes (\textit{qacA}, \textit{qacB} and \textit{qacC}). Nine strains (7.7\%) among the CoNS species and two \textit{S. aureus} strains (2\%) harboured the three-\textit{qac} genes. In addition, the \textit{qacC} were detected in 41 strains.

\textbf{Conclusions:} Multi-resistant strains towards macrolide and disinfectant were recorded. The investigation of antibiotics and antiseptic-resistant CoNS may provide crucial information on the control of nosocomial infections.

\textbf{Keywords:} \textit{Staphylococcus aureus}, Coagulase-negative Staphylococci, Multidrug resistance, \textit{qac}

\textbf{Background}

The increasing number of infections caused by oxacillin-resistant staphylococci makes glycopeptide antibiotics an important choice [1]. The significant prevalence of nosocomial infections caused by multi-resistant \textit{S. aureus} and coagulase-negative staphylococci (CoNS) has been documented [2,3]. These species have the ability to survive in medical devices for months [4]. Resistance to erythromycin in staphylococci is usually associated with resistance to other macrolides. Three genes (\textit{ermA}, \textit{ermB}, and \textit{ermC}) encoding methyltransferases responsible of resistance to macrolides, lincosamides and type B streptogramins (MLS\textsubscript{B} phenotype) by modification of the ribosomal target site have been found in staphylococci [5]. The \textit{msrA} gene displays another mechanism of inducible resistance to erythromycin by encoding an ATP-dependent efflux pump [6]. On the other hand, macrolide efflux is affected by a membrane protein encoded by the \textit{mef} gene [7]. Antiseptic agents include various compounds with different chemical structures [8]. The widespread use of quaternary ammonium compounds (QAC) in hospitals actually contributes to the emergence of disinfectant-resistant bacteria [9,10]. Epidemiological data on antiseptic susceptibility and the distribution of resistance genes are both useful for nosocomial infection control. In several staphylococcal species, \textit{qac}-resistant genes have been identified [11-13].
Found in clinical staphylococci (qacA, qacB, and qacC), these genes are generally carried by plasmids [14-16]. Some of these plasmids (pST6, pSK4, and pSK41) contain antibiotic resistance genes encoding resistance to gentamicin, penicillin, kanamycin, and tobramycin [17,18]. Multidrug resistance pumps have been recognized as mediators of a number of commonly used ammonium antiseptics and detergents [19]. The nearly identical qacA/B gene is normally harboured by large plasmids [20,21]. The relation between qac resistance and penicillin resistance in human clinical staphylococci is quite prevalent [22].

In the present study, we examined the antibiotic susceptibility and resistance to quaternary ammonium compound-based disinfectant benzalkonium chloride (BC) of 46 S. aureus strains isolated from auricular infection and 71 CoNS isolated from dialysis fluids and needles cultures. In order to examine the genetic drug resistance mechanisms, erythromycin resistance genes (ermA, ermB, and ermC) and macrolide efflux genes (msrA and mef) were analysed by PCR multiplex. In addition, quaternary ammonium resistance genes (qacA, qacB, and qacC) were detected by PCR-RFLP.

Methods
Biochemical characterization and antimicrobial susceptibility
A total of 117 clinical staphylococcal strains were isolated from Kairouan, in central of Tunisia including 46 S. aureus strains isolated from auricular infection and 71 CoNS strains isolated from dialysis fluid and needles from a dialysis service. All strains were identified using the Api ID32 Staph system (bioMérieux, Marcy l’Étoile, France) according to the manufacturer’s recommendations and the results were read using an automated microbiological mini-Api (bioMérieux, Marcy l’Étoile, France). Each strain was tested for 18 antibiotics (penicillin, oxacillin, kanamycin, tobramycin, gentamicin, tetracycline, minocyclin, erythromycin, lincomycin, pristinamycin, fosfomycin, nitrofurantoin, pefloxacin, rifampicin, fusidic acid, vancomycin, teicoplanin and cotrimoxazol) using the ATB Staph system (bioMérieux, France) according to the manufacturer’s specifications.

Minimum inhibitory concentration determination
The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of benzalkonium chloride (BC) (Acros organics, USA) (ranging from 0.5 to 256 μg/ml, by serial twofold dilutions) against the tested strains as recommended by the Clinical and Laboratory Standards Institute (CLSI) [23]. An inoculum of $10^4$ - $10^5$ cells ml$^{-1}$ was used. The lowest concentration of antimicrobial agent totally preventing growth after 24 h was taken to be the MIC.

Multiplex PCR for the detection of macrolide-resistance encoding genes
Macrolide resistance genes ermA, ermB, ermC, msrA, and mef were examined in all strains using multiplex PCR, as previously described [24,25]. Multiplex PCR assays were performed in 25 μl PCR mixture 1 and mixture 2. Mixture 1 contained a DNA template (50 ng), 100 μM concentrations (each) of the four dNTPs, 1 U of Go Taq DNA polymerase (Promega, Lyon, France), 5 μl green Go Taq buffer (5X), 25 pM each of forward and reverse primers ermA, ermC, and msrA (Table 1). The PCR mixtures were subjected to thermal cycling (3 min at 96°C, followed by 30 cycles of 30 sec at 95°C for denaturation, 30 sec at 55°C for annealing extension, and extension at 72°C for 2 min). A final elongation at 72°C for 10 min was achieved in a DNA thermal cycler (GenAmp PCR system 9700-Applied Biosystem Int., USA). For mixture 2, the forward and reverse primers (25 pM) of genes ermB and mef were used. PCR products were analysed in agarose gel (1%) electrophoresis in 1X Tris-borate-EDTA buffer (TBE) at pH 8.3. The amplification products were photographed and their size was determined using a 100 bp molecular size marker (Bio-Rad, France).

Detection of qacA, qacB, and qacC genes by PCR-RFLP
Multiplex PCR-RFLP analysis of the qacA/B and qacC genes was achieved as described by Sekiguchi et al. [26]. For the fragment qacA/qacB, the forward primer (corresponding to nucleotides 924-946) and the reverse primer (corresponding to nucleotides 1143-1124) produced 220 bp. For the qacC gene, the forward primer (nucleotides 73-97) and the reverse primer (nucleotides 321-302) produced 249 bp. PCR was performed in a 25 μl reaction volume containing 50 ng of extracted DNA, 5 μl green Go Taq buffer (5X), 200 μM each of deoxynucleoside triphosphates (dNTP), 25 μM each of qacA, qacB, and qacC forward and reverse primers, 1 U of GO Taq DNA polymerase (Promega, USA). Each PCR was performed in duplicate. The reaction mixtures were heated to 94°C for 5 min and were then subjected to 30 denaturation cycles at 94°C for 1.5 min, annealing at 56°C for 0.5 min and extension at 72°C for 1.5 min, ending with a final extension at 72°C for 10 min.

Following PCR, the product was digested with 5 U of Alul at 37°C for 90 min (Promega, France). Ten microlitres of treated product were analysed by gel electrophoresis in 3% agarose gel in 1X Tris-borate-EDTA buffer (TBE, pH 8.3). The amplification products were photographed and their size was determined using a 176 bp fragment and another fragment of 44 bp which is invisible. QacA and qacC genes were not expected to be digested [26].
Table 1 List of primer used for the detection of genes encoding antibiotics and quaternary ammonium compound resistance

| Gene     | Primer 5’-3’                                                                 | Product Size pb | Reference |
|----------|-------------------------------------------------------------------------------|-----------------|-----------|
| ermA     | 5’-TAT CCT ATC GTT GAG AAG GGA TT-3’ 5’-CTA CAC TGG CTC TAG GAT GAA A-3’     | 139             | [22]      |
| ermC     | 5’-GTT TTG TAT CAA ACC CGT ATT C-3’ 5’-ATC TTT TGG TGG TTG ATT G-3’          | 190             | [22]      |
| mstA     | 5’-TCC AAC CAT AGC ACA AAA TC-3’ 5’-AAT TCC CTC TAT TTT GGG TT-3’            | 163             | [22]      |
| mef      | 5’-AGATCATTAATACGACTAGTG-3’ 5’-TCTTCTGATACAAAGG-3’                           | 348             | [23]      |
| qacA, qacB | 5’-TCCTTTAATGCTGCTATACC-3’ 5’-AGCCCTACCTGCTCCACTA-3’                       | 220             | [24]      |
| qacC     | 5’-GGCTTTTCCAATATCCATCTC-3’ 5’-ATGCAGATGTCCGAAATTG-3’                       | 249             | [24]      |

Statistical analysis
Statistical analysis was performed on SPSS v.13.0 statistics software. Pearson’s chi-square test was used to assess inter-group significance. In addition statistical significance was set at P < 0.05.

Results
Biotyping and antimicrobial susceptibility
In this study, 46 S. aureus strains isolated from auricular infection were identified. In addition 71 CoNS were isolated and were subdivided into eight species: S. epidermidis (n = 32) (45%) followed by S. hominis (n = 10) (14.1%), S. haemolyticus (n = 9) (12.7%), S. warneri (n = 5) (7%), S. simulans (n = 6) (8.5%), S. capitis (n = 4) (5.6%), S. chromogenes (n = 3) (4.2%), and S. equorum (n = 2) (2.8%).

The results of the antibiotic susceptibility test confirmed the multi-resistance of 117 staphylococcal strains toward the 18 antibiotics mentioned previously. The majority of these strains were resistant to penicillin (91.1%). The isolated strains were also resistant to kanamycin (45.7%), tetracycline (47.8%), erythromycin (37.7%), lincomycin (21.2%), fosfomycin (20.8%), fusidic acid (17.3%), pefloxacin (18.4%), cotrimoxazol (24.3%), teicoplanin (10.5%), gentamicin (13.4%), rifampicin (12.1%), and tobraycin (16.8%).

Oxacillin-resistant phenotype was found in 9 S. aureus (7.7%) and 24 CoNS (20.5%) isolated strains. All of the strains were susceptible to pristinamycin, nitrofurantoin, and vancomycin (Table 2 and 3).

Resistance to disinfectants agents
The 117 staphylococcus isolates were screened for QAC (BC) resistance. The strains were categorized as BC resistant or sensitive according to the BC MICs. Twenty four (20%) isolates were considered BC highly resistant (BC MICs between 16 and 32 μg/ml), 64 (54%) isolates were resistant to BC (BC MICs between 4 and 8 μg/ml), and 28 (23%) strains were sensitive to BC (BC MICs ≤ 2 μg/ml). 117 staphylococci isolates were analyzed for correlation between BC and antibiotic resistance (Figure 1). This analysis showed that the frequency of erythromycin resistance 71% and oxacillin resistance 84% was higher among the BC-resistant strains.

Multiplex PCR for the detection of genes encoding macrolide resistance
In this study we found that the incidence of the three erythromycin ribosomal methylase genes tested was 9 (7.7%) of S. aureus strains contained ermA, 16 (13.7%) strains harbored ermB gene and 7 (6%) strains were positive for ermC in the total of 117 isolated strains (Figure 2A, B). Furthermore among the CoNS 11 (9.4%) strains contained ermA. In addition, on the total of 117 isolated strains 13 CoNS strains carried the ermB gene, while 32 (27.3%) were positive for ermC. The msrA gene was present in 12 (10.2%) of S. aureus strains and in 48 (41%) CoNS strains (Figure 2A). In contrast, the mef gene was absent in all the staphylococcal strains tested. Sixteen strains of S. epidermidis, eight strains of S. aureus and eighteen strains of CoNS were positive for the mecA gene, yet were susceptible to oxacillin. Furthermore, only nine strains of CoNS (7.7%) and nineteen strains (16.2%) of S. aureus were susceptible to erythromycin, those isolated did not contain any of the erythromycin-resistance genes tested. However, in five S. epidermidis strains (E7, E20, S23, S27, and S40), and in ten S. aureus strains, the ermA and ermC genes were not detected, although it was resistant to erythromycin (Table 2 and 3).

PCR-RFLP detection of qacA, qacB, and qacC genes
Among the tested S. aureus two strains (Sa22 and Sa23) carried the three qac genes (qacA, qacB and qacC)
| Samples | qacA | qacB | qacC | BC<sup>+</sup> | mecA | ermA | ermB | ermC | msrA | Antibiotic          |
|---------|------|------|------|----------------|------|------|------|------|------|---------------------|
| S. epidermidis |      |      |      |                |      |      |      |      |      | S. epidermidis      |
| E10     | +    | -    | +    | 16             | +    | -    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| E11     | -    | -    | +    | 8              | +    | -    | +    | +    | +    | Oxa<sup>R</sup>     |
| E13     | -    | -    | -    | 4              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>     |
| E15     | -    | -    | +    | 8              | +    | -    | +    | +    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| E18     | +    | -    | +    | 16             | +    | -    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| E20     | -    | -    | -    | 2              | +    | -    | -    | -    | +    | Ery<sup>R</sup>     |
| E21     | +    | -    | +    | 8              | +    | -    | -    | +    | +    | Oxa<sup>R</sup>     |
| E24     | -    | -    | -    | 8              | +    | -    | -    | +    | +    | Ery<sup>R</sup>     |
| E4      | -    | -    | -    | 4              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>     |
| E5      | -    | -    | -    | 4              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>     |
| E6      | -    | -    | -    | 2              | +    | -    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| E7      | -    | -    | +    | 8              | -    | -    | -    | -    | -    | Ery<sup>R</sup>     |
| E9      | -    | -    | +    | 16             | +    | -    | -    | +    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S12     | -    | -    | +    | 16             | -    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S15     | -    | -    | +    | 8              | +    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S16     | -    | -    | +    | 8              | +    | -    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S2      | -    | -    | +    | 8              | +    | -    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S21     | +    | +    | +    | 32             | +    | +    | -    | +    | +    | Oxa<sup>R</sup>     |
| S22     | -    | -    | -    | 8              | +    | -    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S23     | -    | -    | -    | 2              | +    | -    | -    | -    | +    | Ery<sup>R</sup>     |
| S25     | -    | -    | +    | 8              | +    | -    | +    | +    | -    | Oxa<sup>R</sup>     |
| S26     | -    | -    | +    | 4              | +    | -    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S27     | -    | -    | -    | 2              | -    | -    | -    | -    | -    | Ery<sup>R</sup>     |
| S33     | -    | -    | -    | 2              | +    | +    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S35     | -    | -    | -    | 2              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S38     | -    | -    | -    | 4              | +    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S40     | -    | -    | +    | 8              | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S43     | -    | -    | -    | 2              | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S48     | -    | -    | -    | 2              | +    | -    | +    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S56     | -    | -    | -    | 2              | +    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S59     | -    | -    | -    | 4              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S9      | +    | +    | +    | 16             | +    | -    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S. hominis |      |      |      |                |      |      |      |      |      | S. hominis          |
| E17     | -    | -    | -    | 16             | +    | -    | -    | +    | +    | Oxa<sup>R</sup>     |
| E2      | -    | -    | -    | 2              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| E27     | -    | -    | -    | 2              | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S18     | +    | +    | +    | 16             | -    | +    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S3      | -    | -    | +    | 8              | +    | -    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S45     | +    | -    | -    | 4              | +    | -    | -    | -    | +    | Ery<sup>R</sup>     |
| S50     | -    | -    | -    | 4              | +    | -    | -    | -    | -    | Ery<sup>R</sup>     |
| S53     | -    | -    | -    | 2              | +    | -    | -    | -    | +    | Ery<sup>R</sup>     |
| S54     | -    | -    | -    | 2              | -    | -    | -    | -    | -    | Ery<sup>R</sup>     |
| S57     | +    | +    | +    | 8              | +    | -    | -    | +    | -    | Oxa<sup>R</sup>     |
| S. equorum |     |      |      |                |      |      |      |      |      | S. equorum          |
| E3      | +    | -    | +    | 8              | +    | -    | -    | +    | +    | Ery<sup>R</sup>     |
| S6      | +    | +    | +    | 16             | +    | -    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
| S. capitis |     |      |      |                |      |      |      |      |      | S. capitis          |
| E25     | +    | +    | +    | 32             | -    | +    | -    | +    | +    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S1      | +    | +    | +    | 32             | -    | +    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S34     | +    | +    | +    | 32             | -    | -    | -    | -    | +    | Ery<sup>R</sup>     |
| S49     | -    | -    | -    | 8              | +    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>S</sup> |
| S. hemolyticus |     |      |      |                |      |      |      |      |      | S. hemolyticus      |
| E16     | -    | -    | +    | 8              | +    | -    | -    | -    | -    | Ery<sup>R</sup>     |
| E19     | +    | +    | +    | 16             | +    | -    | -    | -    | +    | Ery<sup>R</sup>     |
| E22     | -    | -    | +    | 8              | +    | -    | -    | -    | +    | Ery<sup>R</sup>     |

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### Table 2 Distribution of disinfectant and macrolide resistance genes in staphylococci (Continued)

|   | qacA | qacB | qacC | BC<sup>B</sup> | mecA | ermA | ermB | ermC | msrA | Antibiotic |
|---|------|------|------|---------------|------|------|------|------|------|------------|
| S10 | -    | -    | -    | 4             | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
| S13 | +    | +    | -    | 16            | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
| S24 | +    | +    | -    | 16            | +    | -    | -    | -    | +    | Ery<sup>R</sup> |
| S39 | +    | -    | +    | 16            | +    | +    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
| S42 | +    | -    | -    | 16            | +    | -    | +    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
| S58 | -    | -    | -    | 8             | +    | +    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |

+, presence of the gene - absence of the gene; Oxa<sup>R</sup>, oxacillin resistance; Oxa<sup>S</sup>, oxacillin susceptible; BC<sup>B</sup>, MIC of benzalchonium chloride μg/ml.

### Table 3 Distribution of disinfectant and macrolide resistance genes in staphylococci

| Species       | Samples | qacA | qacB | qacC | BC<sup>B</sup> | mecA | ermA | ermB | ermC | msrA | Antibiotic |
|---------------|---------|------|------|------|---------------|------|------|------|------|------|------------|
| S. warnerie   | S11     | -    | -    | +    | 8             | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S14     | -    | -    | -    | 2             | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S29     | -    | +    | -    | 8             | -    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S37     | -    | -    | +    | 16            | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S47     | +    | -    | -    | 4             | +    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
| S. chromogene | S30     | +    | -    | +    | 16            | +    | -    | -    | -    | +    | Ery<sup>R</sup> |
|               | S32     | -    | -    | -    | 2             | -    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S51     | -    | -    | -    | 2             | -    | -    | -    | -    | +    | Ery<sup>R</sup> |
| S. simulans   | E23     | -    | +    | -    | 4             | +    | +    | -    | -    | +    | Ery<sup>R</sup> |
|               | S19     | +    | -    | +    | 16            | +    | -    | -    | -    | +    | Ery<sup>R</sup> |
|               | S20     | -    | -    | -    | 4             | +    | -    | -    | -    | +    | Ery<sup>R</sup> |
|               | S28     | +    | +    | +    | 32            | +    | -    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S36     | +    | -    | -    | 8             | +    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | S5      | +    | +    | +    | 16            | +    | -    | -    | -    | +    | Oxa<sup>R</sup> |
| S. aureus     | Sa1     | -    | -    | +    | 8             | +    | +    | +    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa2     | -    | -    | -    | 4             | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa3     | -    | -    | -    | 8             | -    | -    | +    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa4     | -    | -    | -    | 4             | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa5     | +    | +    | -    | 8             | -    | +    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa6     | -    | -    | -    | 8             | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa7     | -    | -    | -    | 4             | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa8     | -    | -    | -    | 8             | +    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa9     | -    | -    | -    | 2             | -    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa10    | +    | +    | -    | 8             | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa11    | -    | -    | -    | 4             | +    | -    | +    | -    | +    | Ery<sup>R</sup> |
|               | Sa12    | -    | -    | -    | 8             | +    | -    | +    | -    | +    | Ery<sup>R</sup> |
|               | Sa13    | -    | -    | -    | 8             | +    | -    | +    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa14    | -    | -    | -    | 4             | +    | -    | -    | -    | -    | Oxa<sup>R</sup> |
|               | Sa15    | -    | -    | -    | 8             | +    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa16    | -    | -    | -    | 4             | +    | -    | -    | -    | -    | Oxa<sup>R</sup> |
|               | Sa17    | -    | -    | -    | 2             | -    | -    | -    | -    | +    | Ery<sup>R</sup> |
|               | Sa18    | -    | -    | -    | 2             | -    | +    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa19    | +    | -    | +    | 8             | -    | +    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa20    | +    | -    | +    | 4             | -    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa21    | -    | -    | -    | 16            | -    | +    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa22    | +    | +    | +    | 16            | +    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa23    | +    | +    | +    | 16            | +    | +    | +    | +    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa24    | -    | -    | -    | 4             | -    | -    | -    | -    | -    | Ery<sup>R</sup> |
|               | Sa25    | -    | -    | -    | 4             | -    | -    | -    | -    | -    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa26    | -    | -    | -    | 4             | +    | -    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
|               | Sa27    | -    | -    | -    | 2             | +    | +    | -    | -    | +    | Oxa<sup>R</sup>, Ery<sup>R</sup> |
While, nine CoNS strains, contained the three qac genes (qacA, qacB and qacC). In addition, qacC was the most present (35%), followed by qacA (24%), and qacB (15.4%) in the total of 117 isolated strains (Table 2 and 3).

Qac-resistant genes (qacA) were identified in (5.12%) followed by qacC (4.27%) and qacB (3.41%) in the isolated S. aureus strains, while 39 (33%) strains were qac-negative in the total of 117 isolated strains (Figure 4.). On the other hand, among the 32 S. epidermidis strains under study, five were qacA+, two were qacB+, and 16 were qacC+, while 29 strains were qac-negative (Table 2 and 3). The three Qac-resistant genes (qacA, qacB, and qacC) were identified in 9.4% of the total isolated strains. Nine strains (7.7%) of six different CoNS species harboured the three qac-resistant genes.

### Discussion

Recently, the increasing numbers of device-related infections associated with methicillin-resistant staphylococci have raised awareness toward the need for alternative agents to prevent these infections. S. epidermidis species represent the CoNS most recovered from clinical specimens [3]. Among the 71 CoNS isolated in this study, the most prevalent were S. epidermidis (n = 32), S. hominis (n = 10), and S. haemolyticus (n = 9). The antibiotic susceptibility of the 117 staphylococcal (S. aureus and CoNS) isolated in this study confirmed the multi-resistance of these strains toward the 18 antibiotics cited previously. Oxacillin resistance occurred in 7.7% of S. aureus and in 20.5% of CoNS tested by ATB Staph.

Erythromycin resistance in staphylococci is predominantly mediated by erythromycin-resistant methylase encoded by erm genes [27]. The inducible gene ermA is found on the transposon Tn554 and has a single specific site for insertion into the S. aureus chromosome [28]. The ermB gene is found on the transposon Tn551 of a penicillinase plasmid [29]. The ermC gene is responsible for constitutive or inducible resistance to erythromycin and is generally located on small plasmids [5,27,30]. On the other hand, the investigation of the prevalence of ermA, ermB, ermC and msrA genes in Staphylococci showed that only 28 strains of S. aureus (n = 46) and CoNS (n = 71) were found to be susceptible to erythromycin, yet contained erythromycin-resistant gene, and 44 strains (13 S. aureus and 31 CoNS) have at least one of the four genes (ermA, ermB, ermC and msrA) and were susceptible to erythromycin. Similarly, Sekiguchi et al. [26] found discordance among phenotypic susceptibility and the presence of erm genes. They stated that this discordance might be

| Table 3 Distribution of disinfectant and macrolide resistance genes in staphylococci (Continued) |
|--------------------------------------|
| Sa28 | - | - | - | 2 | + | - | - | - | - | Ery² |
| Sa29 | - | - | - | 2 | + | - | - | - | - | Oxa³, Ery⁵ |
| Sa30 | - | - | - | 8 | + | + | + | - | - | Oxa³, Ery⁵ |
| Sa31 | - | - | - | 2 | + | - | + | - | - | Ery⁴ |
| Sa32 | - | - | - | 2 | + | - | + | - | - | Oxa³ |
| Sa33 | - | - | - | 4 | + | - | - | - | - | Ery⁴ |
| Sa34 | - | - | - | 8 | + | - | - | - | - | Oxa³, Ery⁵ |
| Sa35 | - | - | - | 4 | + | - | - | - | - | Oxa³, Ery⁵ |
| Sa36 | - | - | - | 2 | + | - | + | - | - | Oxa³, Ery⁵ |
| Sa37 | - | - | - | 8 | - | - | - | - | - | Oxa³, Ery⁵ |
| Sa38 | - | - | - | 2 | - | - | - | - | - | - |
| Sa42 | - | - | - | 16 | - | - | - | - | - | - |
| Sa41 | - | - | - | 2 | - | - | - | - | - | - |
| Sa40 | - | - | - | 8 | - | - | - | - | - | - |
| Sa46 | - | - | - | 2 | + | - | + | - | - | Oxa⁵, Ery⁵ |

+, presence of the gene - absence of the gene; Oxa⁵, oxacillin resistance; Oxa⁷, oxacillin susceptible; BC⁶, MIC of benzalkonium chloride μg/ml.
due to a mutation in the coding or promoter region of the PCR-detected genes. We noted also that eight strains of S. aureus and CoNS strains were found to be resistant to erythromycin but did not carry any erythromycin resistance gene (Table 1 and 2). This result may be explained by the location of these genes in small plasmids, which were occasionally lost. Fluit et al. [31] demonstrated that the ermC gene responsible for erythromycin resistance is located on a small plasmid. In this study, the incidences of ermA in erythromycin-resistant staphylococci were 7.7% for S. aureus and 3.4% for S. epidermidis. These findings are in disagreement with the study by Eady et al. [5] conducted with coagulase-negative staphylococci (CoNS) in the United Kingdom, in which an incidence of 5.9% for ermA was reported. In a study performed in Denmark, 16% of S. aureus strains were carrying ermA, while only 3% of CoNS strains had this gene [30]. Regarding ermB, we found that this gene was more frequently encountered than ermA in erythromycin-resistant staphylococci with 24.8% (13.7% for S. aureus and 11% for CoNS) of total strains carrying ermB. In the United Kingdom, an incidence of 7.2% for ermB in CoNS has been reported [5]. Staphylococcal strains resistant to macrolides and type-B streptogramins frequently harbour msrA, which encodes an ATP-dependent efflux pump [5]. Erythromycin resistance may be caused by the msrA or ermB gene, as previously reported with staphylococci [5]. Our results are similar to those of a recent study investigating a high level of ermA and ermC genes in CoNS [32].

Staphylococcal multidrug-resistant gene qacA is generally mediated by plasmids mediated resistance to various toxic organic cations and ethidium bromide, as well as...
as a number of commonly used antiseptics and disinfectants, such as benzalkonium chloride and chlorhexidine [19]. The qacA gene also encodes resistance to both monovalent and divalent organic cations. In addition, qacB characteristically differs from qacA by conferring lower or no resistance to divalent organic cations [33]. Some investigations have implied that there is disinfectant cross-resistance with antibiotics [34,35].

Among the tested bacteria, 2 strains of S. aureus and 9 strains of CoNS carried the three qac genes (qacA, qacB, and qacC), as shown in Table 1 and 2. QacC gene was the most present in the 117 isolated strains (35%), followed by qacA (24%), and qacB (15. 4%). Of the 117 isolates investigated in this study, 74% were phenotypically resistant to BC. 56% of the BC-resistant staphylococci isolates have at least one of the three resistant disinfectants genes (qacA/B and qacC). Previous investigators have reported a similar distribution of these three qac resistance genes in clinical S. aureus and CoNS [36,15]. Little is known about the occurrence and possible genetic linkage of qac and antibiotic resistance in staphylococci. Interestingly, we observed that staphylococci resistant to BC were generally more often resistant to antibiotics (Ery and Oxa) than BC-sensitive isolates (Figure 1).

Furthermore, among the nine MRSA isolates, two strains (Sa22 and Sa23) were multi-resistant to antibiotic and harboured the qac genes. MRSA isolates resistant to antiseptics and disinfectants have been reported in Australia and in United Kingdom in the last decade [8]. Sekiguchi et al., [26] have found that among the 65 MRSA isolates 32 (49.23%) were positive for qacA, while one isolate was positive for qacB and seven MRSA 10% were positive for qacC. Three strains of S. hemolyticus harboured qacA and qacB genes. In a recent study, S. hemolyticus isolates were shown to contain both qacA and qacB genes [37]. QacA/B genes are typically located on a transposon of transmissible multidrug-resistant plasmids, such as pSK1 [33]. Staphylococci resistant genes to quaternary ammonium compounds (QACs) have been detected in clinical coagulase-negative staphylococci [36]. QAC resistant Staphylococcus spp. hosting qacA/qacB and smr have been isolated from different environments [38]. Results from a recent study in Norway suggest that qac-resistant genes are common in human clinical staphylococci and that a direct link between resistance to QACs and resistance to penicillin occurs in clinical isolates of human and animal origin as well as in food-related staphylococci [11,22]. Noguchi et al. [16] reported that when the antiseptic susceptibility and the distribution of antiseptic-resistant genes of MRSA isolated in Japan in 1992 were studied, qacA/B were detected in 10.2% (10/98). However, seven years later, qacA/B genes were detected in 47.9% (198/413) in MRSA isolates in Japan.

Conclusion
It appears that the widespread distribution of staphylococci carrying macrolides and qac-resistant genes found in dialysis biomaterial collected in Tunisia may be due to the transfer of resistance plasmids among species and strains, thereby contributing to the dissemination of staphylococcal resistance. Therefore, a closer investigation of antibiotics and antiseptic-resistant CoNS may provide crucial information on the control of nosocomial infections.

Authors’ contributions
TZ was the primary author of the manuscript, assisted in samples collection, antimicrobial susceptibility, detection of resistance genes and assisted in minimum inhibition concentration determination of BC. BK contributed in minimum inhibition concentration determination, assisted in detection of resistance genes and helped in the writing of the manuscript. HM participated in detection of resistance genes, data acquisition and contributed in writing of the manuscript. AB provided funding, supervised the study, and helped to finalize the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Hiramatsu K: Vancomycin resistance in staphylococci. Drug Resist Updat 1998, 1:135-150.
2. Brun-Buisson CH: Les Staphylococcus aureus résistants à la méticilline: Evolution et épidémiologie, impact clinique, prévention. Pathol Biol 1998, 46:227-34.
3. Kloos WE, Bannerman TL: Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev 1994, 7:117-140.
4. Neely AN, Maley MP: Survival of enterococci and staphylococci on hospital fabrics and plastic. J Clin Microbiol 2000, 38:724-726.
5. Eady EA, Ross JI, Tipper JL, Walters CE, Cove JH, Noble WC: Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. J Antimicrob Chemother 1993, 31:211-7.
6. Ross JI, Eady EA, Cove JH, Baumberg S: Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter MsrA may interact. Gene 1995, 153:93-98.
7. Lederer R: Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis 2002, 34:482-492.
8. McDonnell G, Russell AD: Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999, 12:147-179.
9. Revery ME, Bex M, Brun Y, Fleurette J: Evolution of resistance to antibiotics and antiseptics of hospital Staphylococcus aureus strains isolated from 1980 to 1991. Pathol Biol 1993, 41:897-904.
10. Russell AD: Do biocides select for antibiotic resistance? J Pharm Pharmacol 2000, 52:227-233.
11. Anthonisen IL, Sunde M, Steinum TM, Sidhu MS, Sorum H: Organization of the antiseptic resistance gene qacA and Tn552-related ßlactamase genes in multidrug-resistant Staphylococcus haemolyticus strains of animal and human origins. Antimicrob Agents Chemother 2002, 46:3608-3612.
12. Bjorland J, Steinum T, Sunde M, Waage S, Heir E: Novel plasmid-borne gene qacA mediates resistance to quaternary ammonium com pounds in equine Staphylococcus aureus, Staphylococcus simulans, and Staphylococcus intermedius. Antimicrob Agents Chemother 2003, 47:3046-3052.
13. Heir E, Sundheim G, Holck AL: The qacG gene on plasmid pST94 confers resistance to quaternary ammonium compounds in staphylococci isolated from the food industry. J Appl Microbiol 1999, 86:378-388.
14. Leelaporn A, Firth N, Paulsen IT, Hettiaratchi A, Skurray RA: Multidrug resistance plasmid pSK108 from coagulase-negative staphylococci: relationships to Staphylococcus aureus qacA and qacC plasmids. *Plasmid* 1995, 34:62-67.
15. Mayer S, Boos M, Bayer A, Flurt AC, Schmitz FJ: Distribution of the antiseptic resistance genes qacA, qacB and qacC in 497 methicillin-resistant and susceptible European isolates of Staphylococcus aureus. *J Antimicrob Chemother* 2001, 47:896-897.
16. Noguchi N, Hase M, Kitta M, Satassu M, Deguchi K, Kono M: Antisepitic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant Staphylococcus aureus. *FEMS Microbiol Lett* 1999, 172:247-253.
17. Berg T, Firth N, Apisiridej S, Hettiaratchi A, Leelaporn A, Skurray RA: Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multiresistance plasmids. *J Bacteriol* 1998, 180:4350-4359.
18. Sidhu MS, Heir E, Sarum H, Holck A: Genetic linkage between resistance to quaternary ammonium compounds and β-lactam antibiotics in food-related *Staphylococcus aureus* spp. *Microb Drug Resis* 2001, 7:363-371.
19. Littlejohn TG, Paulsen IT, Gillespie MT, Tennent JM, Midgley M, Jones IG, Purewal AS, Skurray RA: Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992, 74:239-245.
20. Littlejohn TG, DiBerardino D, Messerotti LJ, Spiers SJ, Skurray RA: Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *Gene* 1991, 101:59-66.
21. Paulsen IT, Brown MH, Dunstan SJ, Skurray RA: Molecular characterization of the staphylococcal multidrug resistance export protein QacC. *J Bacteriol* 1995, 177:2827-2833.
22. Sidhu MS, Heir E, Leegaard T, Wiger K, Holck A: Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn552 among clinical staphylococci. *Antimicrob Agents Chemother* 2002, 46:2797-2803.
23. CLSI: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard-Seventh Edition CLSI Document M7-A7 2006.
24. Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouellette M, Bergeron MG: Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 2000, 231-238.
25. Lim JA, Kwon AR, Kim SK, Chong Y, Lee K, Choi EC: Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. *J Antimicrob Chemother* 2002, 49:489-95.
26. Sekiguchi J, Hama T, Fujino T, Araake M, Irie A, Saruta K, et al: Detection of the antiseptic- and disinfectant-resistance genes qacA and qacB in methicillin-resistant *Staphylococcus aureus* isolated in a Tokyo hospital. *Jpn J Infect Dis* 2004, 57:288-91.
27. Weisblum B: Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother* 1995, 39:577-585.
28. Murphy E: Nucleotide sequence of ermA, a macrolide-lincosamide streptogramin B determinant in *Staphylococcus aureus*. *J Bacteriol* 1985, 162:636-640.
29. Khan SA, Novick RP: Terminal nucleotide sequences of Tn551, a transposon specifying erythromycin resistance in *Staphylococcus aureus*: homology with Tn2. *Plasmid* 1980, 3:41-48.
30. Westh H, Hougaard DM, Vuatu J, Rosdahl VT: Erm genes in erythromycin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. *APMIS* 1995, 103:225-332.
31. Fluit ADC, Visser MR, Schmitz F: Molecular detection of antimicrobial resistance. *Clin Microbiol Rev* 2001, 14:836-871.
32. Ardic N, Ozyurt M, Sareyyupoglu B, Haznedaroglu T: Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. *Int J Antimicrob Agents* 2005, 26:213-218.
33. Lyon BR, Skurray RA: Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* 1987, 51:88-134.
34. Schweizer HP: Triclosan: a widely used biocide and its link to antibiotics. *FEMS Microbiol Lett* 2001, 202:1-7.
35. Chapman S: Disinfectant resistance mechanisms, cross-resistance and co-resistance. *Int Biodeterior Biodegrad* 2003, 51:271-276.
36. Leelaporn A, Paulsen IT, Tennent JM, Littlejohn TG, Skurray RA: Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. *J Med Microbiol* 1994, 40:214-220.
37. Bjurland J, Stennum T, Kvitlø B, Waage S, Sund B, Heir E: Widespread Distribution of Disinfectant Resistance Genes among *Staphylococcus* of Bovine and Caprine Origin in Norway. *J Clin Microbiol* 2005, 43:63-638.
38. Heir E, Sundheim G, Holck AL: Resistance to quaternary ammonium compounds in *Staphylococcus* spp. Isolated from the food industry and nucleotide sequence of the resistance plasmid pSTB27. *J Appl Bacteriol* 1995, 79:149-156.

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