Resistance profiles and biofilm formation of coagulase negative staphylococci isolated from clinical specimens in a tertiary care hospital in Palestine

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Abstract

Background: Coagulase-negative staphylococci (CoNS) represent one of the major resistant nosocomial pathogens where its biofilm-related infections often fail to respond to antibiotic chemotherapy. Here, we studied the resistance profiles and biofilm formation in CoNS isolates from clinical specimens at Al Shifa hospital in Gaza, Palestine.

Methods: This study was carried out from March to July 2013 and included 81 clinical isolates. Identification and antibiotic susceptibility testing were performed using VITEK-2 system. The presence of \textit{nuc} and \textit{mecA} genes was performed using multiplex PCR. Qualitative and quantitative biofilm assays were performed using standard methods.

Results: Of the 81 clinical CoNS isolates, \textit{S. haemolyticus} was the most common species (34, 42%), followed by \textit{S. epidermidis} (26, 32.1%) and \textit{S. saprophyticus} (13, 16%). The majority of isolates (83.9%) were from surgery, ICUs, pediatrics and medicine wards and the most common source was pus (28, 34.6%). Antibiotic resistance was highest against aminoglycosides, β-lactams, carbapenems, cephalosporins, fluoroquinolones, fosfomycin and macrolides. Though, no resistance was detected against rifampicin, vancomycin, teicoplanin, nitrofurantoin, linezolid and mupirocin. The antibiotic resistance among MR-CoNS was significantly higher than that among MS-CoNS. Nearly 88.9% of isolates were multidrug resistant with higher percentage among MR-CoNS. Most \textit{S. epidermidis} (76.9%) isolates were biofilm producer, with statistically significant association between methicillin resistance and biofilm production.

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Introduction

Coagulase-negative staphylococci (CoNS) are now representing one of the major nosocomial pathogens and among the most frequently isolated bacteria in the clinical microbiology laboratories [1-5]. They are responsible for bacteremia, endocarditis, mediastinitis, meningitis and progressive joint destruction mainly in patients with neutropenia, indwelling foreign devices, intravascular catheters or other foreign bodies [1, 3, 4]. The clinical most relevant CoNS are *Staphylococcus epidermidis*, *S. lugdunensis*, *S. saprophyticus*, and *S. capitis* [6, 7].

Biofilm production by CoNS, specially *S. epidermidis*, is considered as an important factor in the pathogenesis of implanted medical devices associated infections [1, 2, 8, 9]. Biofilm formation takes place in four successive phases: the attachment of the bacteria to biotic or abiotic surface; the proliferation and accumulation of bacteria in multilayered cell clusters; the growth of biofilm into a thick and structured layer, and finally the detachment and circulation of single cells or cell agglomerates via the bloodstream [1, 2].

Many literature surveys revealed that CoNS showed high resistance against most of the commonly used therapeutic antibacterial agents including methicillin. Irrespective of geographical locations, a worldwide SENTRY study showed that, about 70-75% of CoNS are resistant to methicillin [10]. There is a significant increase in the methicillin-resistant coagulase-negative staphylococci (MR-CoNS) infections and these bacteria have recently started to gain resistance to other widely used antibiotics [11-14].

The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them justify the need to identify various species of CoNS and determine their antibiotic resistance pattern. Epidemiological data about CoNS in Palestine are either scarce or insufficient [15]. To the best of our knowledge, this is the first report describing clinical CoNS infection within the Gaza Strip hospitals. To that end, we conducted this study to determine the frequency of CoNS species isolated from various clinical specimens and to assess their resistance profile to most commonly used antibiotics at Al Shifa hospital in Gaza Strip. Moreover, biofilm forming capacity of *S. epidermidis* isolates was investigated.

Materials and Methods

Study design and setting

This study was conducted for five months between March and July 2013 at the clinical microbiology laboratory of the largest medical complex hospital...
in Gaza Strip, Al-Shifa hospital with 500 acute care beds. The study was approved by the department of human resources and development in the ministry of health (MOH) and by the Helsinki committee at the MOH in Gaza Strip (Approval no. PHRC/HC/36/14).

**Sampling and bacterial isolates**
Eighty one non-duplicate CoNS isolates associated with diverse clinical infections were collected. The isolates represent different sources including pus, urine, blood, sputum, burn and surgical swabs of hospitalized patients in surgery, pediatrics, medicine, burns, gynecology and ICUs departments. These isolates were collected in 2013, out of a total unique 1121 bacterial isolates.

**Isolation and Identification of CoNS and MR-CoNS**

**Conventional techniques**
CoNS isolates were identified phenotypically based on colonial morphology, Gram stain, and by using the following methods: catalase test, tube coagulase test, Pastorex™ Staph Plus latex agglutination (Bio-Rad, Hercules, California), and the Staph ID 32 API system (bioMérieux, France) according to the manufacturer’s instructions. VITEK-2 automated system was used to identify CoNS to the species level using VITEK-2 database, version 4.03 (bioMérieux, Marcy l’Étoile, France). Screening for MR-CoNS was done by testing for oxacillin-resistance among CoNS isolates [17, 18].

**Molecular techniques**

**DNA isolation:** Isolates were overnight subcultured in brain heart infusion broth and pelleted by centrifugation at 5,000 rpm for 15 minutes. After cooling to room temperature, the suspension was centrifuged at 10,000 rpm for 5 minutes, and the supernatant was used directly as template for PCR reactions [16].

**Detection of nuc and mecA genes:** A multiplex PCR assay was used for detection of the nuc gene to exclude any coagulase negative S. aureus isolate and mecA gene for detection of methicillin-resistance among CoNS isolates [17, 18]. Briefly, primers meca-1 (5’-GGGATCATAGCGTCATTATTC-3’) and meca-2 (5’-AACGATTGTGACACGATAGCC-3’) for the gene meca and nuc-1 (5’-TCAGCAAAATGCATCACAACAG-3’) and nuc-2 (5’-CGTAAATGCACTTGGCTTCAGG-3’) for the gene nuc were used in a multiplex PCR reaction on a T100™ Thermal Cycler (BioRad, USA) under the following conditions: an initial 5-min denaturation step at 95°C, followed by 30 cycles of 1 min of denaturation at 95°C, 1 min of annealing at 59°C, and 2 min of extension at 72°C; with a final extension step at 72°C for 10 min. The sizes of the amplicons were 530bp and 280bp for meca and nuc genes, respectively.

**Antimicrobial susceptibility testing**
Antibiotic susceptibility testing for the CoNS isolates was performed with the automated VITEK-2 Compact automated system, using the VITEK-2 Compact Gram Positive Card (bio- Mérieux, Paris, France); Multidrug-resistant (MDR) phenotype was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories used in this study [19].

**Biofilm production**
The biofilm forming capacity of only S. epidermidis strains was investigated qualitatively and quantitatively by methods previously described. For qualitative biofilm production, a simple method using growth on Congo red agar (CRA) for detecting biofilm formation was used [20]. With brain heart infusion agar containing Congo red stain at 0.8 g/liter (BDH Chemicals Ltd., Poole, United Kingdom),
biofilm-positive strains yield black colonies with a dry crystalline surface appearance, while biofilm-negative strains mostly give a red colonies.

For quantification assay of biofilm-forming capacity, tests similar to those described previously were applied [9, 21, 22]. Briefly, *S. epidermidis* strains were cultivated overnight in 10 ml of tryptic soy broth (TSB) supplemented with 0.25% (wt/vol) glucose. Cultures were diluted in TSB supplemented with 0.25% (wt/vol) glucose to a final OD578 of 0.1 and 200 µl of the cell suspension was used to inoculate sterile, 96-well flat-bottom polystyrene microtiter plates (Greiner bio-one; Cellstar). After cultivation for 24 h at 37°C, the wells were emptied and the contents were gently washed three times with 200 µL of PBS (pH 7.2) to remove free-floating planktonic bacteria. The plates were air dried for 10 min., and the remaining surface-adherent cells were stained with 0.1% safranin (Serva) for 30 s. Absorbance at 490 nm (A490) was measured with a Micro-ELISA Autoreader (SpectraMAX GeminiXS; Molecular Devices). Wells containing only sterile TSB served as a background control; their average A490 value was subtracted from all experimental readings. *S. epidermidis* RP62A and *S. carnosus* TM300 were used as positive and negative controls, respectively. Each assay for each strain was performed in quadruplicate.

**Statistical analysis**
The results were tabulated, encoded and statistically analyzed using Statistical Package for Social Sciences (SPSS®) program version 17 (Chicago, IL, USA). Fisher’s exact 2-tailed and Pearson’s χ² tests were used for categorical variables to compare frequencies of CoNS species and MR-CoNS positivity and negativity. Likewise, percentages as of antibiotic resistance profiles of MR-CoNS and MS-CoNS were compared with Pearson’s χ² or Fisher’s exact test as appropriate. *P*-value were calculated and *P* < 0.05 was considered statistically significant.

**Results**

**Identification and distribution of CoNS and MR-CoNS species**
Out of a total 1121 bacterial isolates, 81 strains of CoNS that belong to six species were identified. *S. haemolyticus* was the most common species isolated (34, 42%), followed by *S. epidermidis* (26, 32.1%) and *S. saprophyticus* (13, 16%) (Table 1). CoNS were

| Table 1. Distribution of the 81 CoNS isolates according to gender, ward, type of specimen, species and resistance to methicillin. |
| --- |
| Gender |
| Males | 42 | 51.9 |
| Females | 39 | 48.1 |
| Ward |
| Surgery | 23 | 28.4 |
| ICUs | 17 | 21 |
| Pediatrics | 15 | 18.5 |
| Medicine | 13 | 16 |
| Burns | 10 | 12.3 |
| Gynecology | 3 | 3.7 |
| Specimen |
| Pus | 28 | 34.6 |
| Swab a | 19 | 23.5 |
| Urine | 15 | 18.5 |
| Blood | 12 | 14.8 |
| Sputum | 3 | 3.7 |
| Others b | 4 | 4.9 |

**Specimen**

| Specimen |
| --- |
| *S. haemolyticus* | 34 | 42 |
| *S. epidermidis* | 26 | 32.1 |
| *S. saprophyticus* | 13 | 16 |
| *S. hominis* | 5 | 6.2 |
| *S. warneri* | 2 | 2.5 |
| *S. auricularis* | 1 | 1.2 |

**Specimen**

| Specimen |
| --- |
| Resistant | 60 | 74.1 |
| Susceptible | 21 | 25.9 |
| Total | 81 | 100 |

ICUs: Intensive Care Units. a: Swabs including surgical site infections and burn swabs. b: Others including cerebrospinal, pleural, synovial and ascetic fluids. *: Statistical significant difference.
isolated from 42 (51.9%) males and 39 (48.1%) females. The majority of CoNS isolates (83.9%) were from surgery, ICUs, pediatrics and medicine wards. Of the ten specimen sources, the most common source of CoNS was pus (28, 34.6%), followed by swabs (19, 23.5%), urine (15, 18.5%) and blood (12, 14.8%). Screening for MR-CoNS revealed that 60 (74.1%) were MR-CoNS and 21 (25.9%) were MS-CoNS. There were no statistically significant differences in the distribution of CoNS according to gender, hospital ward and specimen source \((P = 0.47, 0.86, \text{ and } 0.15 \text{ respectively})\). Yet, a statistically significant difference was found between MR-CoNS and MS-CoNS \((P<0.001)\) (Table 1).

As stated in Table 2, the highest numbers of \textit{S. haemolyticus} and \textit{S. epidermidis} strains were recovered from surgery ward (40%, 30.8%, 29.4% respectively), whereas \textit{S. saprophyticus} was mainly recovered from pediatric and surgery wards (23.1% each) (Table 2). As shown in Table 3, most of \textit{S. haemolyticus} and \textit{S. epidermidis} isolates were methicillin resistance (94.1%, 88.5% respectively) with a statistical significant difference in comparison to its counterpart

### Table 2. Distribution of CoNS species according to the source of specimen and hospital ward.

| CoNS spp         | Type of specimen | No. | %     | No. | %     | No. | %     | No. | %     | No. | %     | No. | %     | Total |
|------------------|------------------|-----|--------|-----|--------|-----|--------|-----|--------|-----|--------|-----|--------|--------|
|                  |                  | Pu  | Swab | Uiri | Blood | Spum | Others |     |        | Pu  | Swab | Uiri | Blood | Spum | Others |     |        | Pu  | Swab | Uiri | Blood | Spum | Others |     |        |
| \textit{S. haemolyticus} | 14               | 41.2| 6     | 22   | 17.6  | 5    | 14.7   | 4   | 11.8   | 2   | 5.9   | 3    | 8.8   | 34   | 100   |
| \textit{S. epidermidis} | 9                | 34.6| 7     | 26.9 | 4     | 15.4  | 6    | 23.1  | 0   | 0     | 0    | 0     | 0    | 0     | 26   | 100   |
| \textit{S. saprophyticus} | 1             | 7.7 | 5     | 38.5 | 5     | 38.5  | 2    | 15.4  | 0   | 0     | 0    | 0     | 0    | 0     | 0    | 0     |
| \textit{S. hominis} | 2                | 40  | 0     | 0    | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0    | 0    | 0     |
| \textit{S. auricularis} | 0              | 0   | 0     | 100  | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0    | 0    | 0     |

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\*a: Swabs including surgical site infections and burn swabs. \*b: Others including cerebrospinal, pleural, synovial and ascetic fluids. \*: Statistical significant difference. ICUs: Intensive Care Units. GYN: Gynecology ward.

Table 3. Distribution of CoNS species according to methicillin resistance \((\text{mecA positive, ce}-

\*foxitin & oxacillin resistance.

| CoNS species | MR-CoNS | MS-CoNS | P-value |
|--------------|---------|---------|---------|
| \textit{S. haemolyticus} | 32      | 94.1    | 2       | 5.9    | < 0.0001 |
| \textit{S. epidermidis} | 23      | 88.5    | 3       | 11.5   | < 0.0001 |
| \textit{S. saprophyticus} | 0       | 0       | 0       | 0      | N.A.    |
| \textit{S. hominis} | 4       | 80      | 1       | 20     | 0.07    |
| \textit{S. warneri} | 1       | 50      | 1       | 50     | not applicable |
| \textit{S. auricularis} | 0       | 0       | 1       | 100    | not applicable |
| Total         | 60      | 74.1    | 21      | 25.9   | < 0.001  |
methicillin sensitive ($P< 0.0001$). Surprisingly, all S. saprophyticus isolates were methicillin sensitive.

Antimicrobial resistance profiles

The antimicrobial resistance profile of CoNS isolates to the 28 antibiotics tested was shown in Table 4 and Figure 1. Resistance to multiple classes of antimicrobials was observed among CoNS isolates, including aminoglycosides, β-lactams, carbapenems, cephalosporins, fluoroquinolones, fosfomycin and macrolides. However, no resistance was detected to any of the following antibiotics: rifampicin, vancomycin, teicoplanin, nitrofurantoin, linezolid and mupirocin. Yet, a very low to low resistance rate was observed to tigecycline, moxifloxacin and clindamycin (3.7%, 8.4%, 13.6% respectively). CoNS stra-

### Table 4. Antimicrobial resistance profile of isolated clinical CoNS strains.

| Antimicrobial agents | S. epidermidis | S. saprophyticus | S. hominis | S. warneri | S. auricularis | Total resistance | P-value |
|---------------------|----------------|-----------------|------------|------------|----------------|------------------|---------|
| P                   | 100            | 100             | 100        | 100        | 100            | 98.8             | 1.00    |
| AMP                 | 100            | 100             | 100        | 100        | 100            | 98.8             | 1.00    |
| SAM                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| AMC                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| CXM                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| CEC                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| CTX                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| CRO                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| IPM                 | 84.6           | 23.1            | 80         | 50         | 0              | 77.8             | $<0.001$|
| ERY                 | 30.8           | 69.2            | 100        | 100        | 100            | 71.6             | $<0.001$|
| AZM                 | 30.8           | 69.2            | 100        | 100        | 100            | 71.6             | $<0.001$|
| CLR                 | 30.8           | 69.2            | 100        | 100        | 100            | 71.6             | $<0.001$|
| CLI                 | 19.2           | 0               | 0          | 0          | 0              | 13.6             | 0.51    |
| TET                 | 19.2           | 30.8            | 40         | 0          | 0              | 30.9             | 0.08    |
| TGC                 | 0              | 0               | 0          | 0          | 0              | 3.7              | 0.48    |
| GEN                 | 34.6           | 0               | 0          | 0          | 0              | 45.7             | $<0.001$|
| TOB                 | 42.3           | 0               | 0          | 0          | 0              | 48.1             | $<0.001$|
| LVX                 | 53.8           | 0               | 0          | 0          | 0              | 46.9             | $<0.001$|
| MXF                 | 7.7            | 0               | 0          | 0          | 0              | 8.4              | 0.63    |
| SXT                 | 23.1           | 23.1            | 60         | 0          | 0              | 42               | $<0.001$|
| RIF                 | 0              | 0               | 0          | 0          | 0              | 0                | -       |
| VAN                 | 0              | 0               | 0          | 0          | 0              | 0                | -       |
| TEC                 | 0              | 0               | 0          | 0          | 0              | 0                | -       |
| FOF                 | 7.7            | 100             | 100        | 100        | 100            | 69.1             | $<0.001$|
| FUS                 | 50             | 30.8            | 80         | 0          | 100            | 35.8             | $<0.001$|
| NIT                 | 0              | 0               | 0          | 0          | 0              | 0                | -       |
| LZD                 | 0              | 0               | 0          | 0          | 0              | 0                | -       |
| MUP                 | 0              | 0               | 0          | 0          | 0              | 0                | -       |

*: Values represent percentages P: benzylpenicillin; AMP: ampicillin; SAM: ampicillin/sulbactam; AMC: amoxicillin/clavulanic acid; CXM: cefuroxime sodium; CEC: cefaclor; CTX: cefotaxime; CRO: ceftriaxone; IPM: imipenem; ERY: erythromycin; AZM: azithromycin; CLR: clarithromycin; CLI: clindamycin; TET: tetracycline; GEN: gentamicin; TGC: tigecycline; TOB: tobramycin; LVX: levofloxacin; MXF: moxifloxacin; SXT: trimethoprim-sulfamethoxazole; RIF: rifampin; VAN: vancomycin; TEC: teicoplanin; FOF: fosfomycin; FUS: fusidic acid; NIT: nitrofurantoin; LZD: linezolid; MUP: mupirocin.
ins exhibited nearly complete resistance to penicillin and ampicillin (98.8% each). Rates of resistance to other antibiotics were 77.8% to β-lactam inhibitors, carbapenems and cephalosporins, 71.6% to macrolides, 69.1% to fosfomycin, 48.1% to tobramycin, 46.9% to levofloxacin, 45.7% to gentamicin, 42% to trimethoprim/sulfamethoxazole (SXT), 35.8% to fusidic acid, and 30.9% to tetracycline. *Statistically significant difference in resistance pattern between MR-CoNS and MS-CoNS (P-value < 0.05).
Biofilm production
Out of 26 isolates of *S. epidermidis* examined, a significant number (20, 76.9%) produced biofilms as verified by formation of black colonies with a metallic sheen after 24 h of incubation on CRA plates and by polystyrene microtiter plate assay (P < 0.0001). 82.6% of which were in MR-CoNS, and 33.3% in MS-CoNS. Methicillin resistance was found to be significantly higher in biofilm positive strains (19, 82.6%) than in biofilm negative strains (4, 17.4%) (P < 0.0001) (Table 5). Also, biofilm producer strains were significantly higher among CoNS isolates recovered from pus, swabs and blood specimens (P = 0.0013, P = 0.01, P = 0.028 respectively).

Table 5. Distribution of biofilm-producing *S. epidermidis* according to methicillin resistance and clinical source.

| **S. epidermidis** | Biofilm positive | Biofilm negative | P-value |
|--------------------|-------------------|------------------|---------|
| No. %              | No. %             | No. %            |         |
| MR-CoNS            | 23 88.5           | 19 82.6          | < 0.0001|
| MS-CoNS            | 3 11.5            | 1 33.3           | 0.46    |
| Clinical source    | Pus 9 34.6        | 8 88.9           | 1 11.1  | 0.0013 |
| Swabs              | 7 26.9            | 6 85.7           | 1 14.3  | 0.01   |
| Urine              | 4 15.4            | 1 25             | 3 75    | 0.19   |
| Blood              | 6 23.1            | 5 83.1           | 1 16.7  | 0.028  |
| Total              | 26 100            | 20 76.9          | 6 23.1  | 0.0001 |

Discussion
Data regarding CoNS and MR-CoNS in Palestine are insufficient, with only one report from Nablus, Palestine [15]. Previous reports from Gaza Strip and West Bank of Palestine have mostly focused on epidemiology of *S. aureus* and MRSA both in hospital and community settings [23-27]. Here, we described for the first time the resistance profiles and biofilm formation in CoNS isolated from clinical specimens in Gaza, Palestine. *S. haemolyticus* was the most common species isolated (34, 42%), followed by *S. epidermidis* (26, 32.1%). This finding is comparable to the findings in a study conducted in China where the prevalence of *S. haemolyticus* was the highest (34.1%), followed by *S. epidermidis* (27.4%) [4]. Also in another recent study in Brazil, *S. haemolyticus* was the most prevalent species [9]. However, many studies worldwide showed *S. epidermidis* is the major isolated species followed by *S. haemolyticus* or other CoNS [10, 11, 28-30]. These differences in CoNS species rates from the worldwide literature are depending on the country, hospital specialty, study design and setting. In this study, the most common clinical source of CoNS was pus (34.6%), followed by swabs (23.5%), urine (18.5%) and blood (14.8%). Two Indian studies showed that pus specimens were the most common source of CoNS [31, 32]. Furthermore, these results are nearly similar to the study done in Pakistan in which percentage of CoNS isolates in pus was 36.7%, and in swab was 17.3% but their findings in regard to the blood (45.9%) was higher [12]. Also, other studies revealed higher CoNS isolates from blood cultures as in Pakistan (53.5%) [33], Jordan (30.9%) [11], Tunisia (29%) [34], and Iran (25.4%) [35]. Yet, in a recent study from Jordan and in accordance with our findings, the ICU and surgery wards accounted for the majority of all CoNS isolates obtained (41%) [11]. A plausible explanation for the differences in the isolation rates of CoNS from clinical source and wards between different countries and even some time in the same country may be due in part to differences in sample size or frequency of sampling and using different conventional and/or molecular methods among others for diagnosis. Screening for MR-CoNS revealed that 74.1% were MR-CoNS which is nearly similar to the studies done at Pakistan (70.3%) [12], China (70%) [4], USA (74%) [36], and India (68.4%) [37]. Likewise, prevalence of MR-CoNS in various parts of Europe is in the range of 60-75% [38]. On the other hand, there were significant higher rates of MR-CoNS in studies conducted
in Saudi Arabia and Poland where the prevalence reached 93.6% and 98% respectively [10, 39]. Yet, other studies found less prevalence of MR-CoNS in comparison to our results as from Iran (50%) [30] and India (32.7%) [28].

In this study, the resistance profile of all CoNS isolates showed complete to high resistance to most of the antibiotics tested. This pattern of resistance was shown in previous studies in different countries around the world [4, 8, 10-13, 15]. High resistance to the aforementioned antibiotics could be mainly due to excessive use, misuse, and great prescription of these medication in Gaza for both hospital and community acquired infections and also in agriculture and animal feeding. Moreover, the lack of an antibiotic policy and the availability of antibiotics sold without a medical doctor prescription in Palestine worsen the case [27]. In view of the high resistance rates of CoNS to these aforementioned antibiotics, treatment of infections caused by CoNS, particularly S. epidermidis and S. haemolyticus at Al Shifa hospital with these antibiotics may not be effective. In contrast, no resistance of CoNS isolate was detected to rifampicin, vancomycin, teicoplanin, nitrofurantoin, linezolid and mupirocin, and very low resistance was detected to tigecycline and moxifloxacin (3.7%, 8.4% respectively). Our findings are in agreement with many studies that showed full activity of most of these aforementioned antimicrobials against clinical CoNS isolates [8, 11, 13, 15]. Conversely, many reports from around the world showed different percentage of resistance to vancomycin, teicoplanin and linezolid ranging from 0.8% to 13% [4, 12, 39]. We think that this zero resistance in this study to glycopeptides and linezolid may be due to its limited prescription and use in Gaza Strip hospitals. So, the full sensitivity to vancomycin, teicoplanin and linezolid can work perfectly against clinical CoNS isolated in Gaza hospitals as indicated against S. aureus in recent reports from Gaza Strip [24, 27]. On the other hand, a very low resistance rate was observed to tigecycline and moxifloxacin (3.7% and 8.4% respectively). MR-CoNS showed low to moderate resistance ratios against clindamycin (18.3%), tetracycline (35%), fusidic acid (38.3%) and gentamycin (60%). This picture contradicts a study conducted in Turkey where significantly higher resistance to clindamycin (72%), tetracycline (60%) and gentamycin (90%) was reported. Yet, fusidic acid resistance (25%) was lower than in our findings [40]. The least resistance species found in this study was S. saprophyticus.

With regard to the biofilm production, a significant number of S. epidermidis isolates (20, 76.9%) were producers with significantly higher MR-CoNS (19, 82.6%) than MS-CoNS strains (4, 17.4%) (P < 0.0001) (Table 5). Koksal et al., found 71% of S. epidermidis strains were biofilm positive with statistically significant association (P < 0.001) between MR-CoNS (81%) and MS-CoNS strains (57%) [40].

Our study has some limitations. First, it is worthwhile to mention that a number of our isolates could be contaminants and not real pathogens. Second, we performed this study for short time in one referral hospital in Gaza Strip. So, this may underestimate or overestimate the real prevalence of CoNS species and MR-CoNS. Third, molecular typing was not performed, such that the clonality of the CoNS strains cannot be assessed and also the biofilm associated genes were not investigated.
Conclusion
Several themes of potential concern are raised by our findings. Most noteworthy is the high prevalence of MR-CoNS and its high resistance profile against most agents tested in this study except for new or not commonly used antibiotics in Gaza Strip. Also, most of *S. epidermidis* isolates were biofilm producers. Furthermore, high numbers of CoNS isolates were shown to be MDR. These results highlight the critical need for monitoring and managing the usage of antibiotics in our hospitals and community.

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