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**Comparison of Matrix-Assisted Laser
Desorption/Ionization-Time of Flight Mass
Spectrometry, VITEK-2 and ID32STAPH for
Species Identification of Clinical Coagulase-
Negative Staphylococci Isolated from Al Shifa
Hospital in Gaza Strip**

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Abstract

Staphylococcal species, notably, coagulase negative staphylococci (CoNS), are considered important causative agents of health-care associated infections (HAI) associated with catheters and implanted medical devices. The increasing incidence of CoNS in HAI confirms the need for an accurate and simple identification method at the species level. Recently, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was introduced as a useful and simple method for the rapid identification of CoNS. In this study we compared MALDI-TOF MS, VITEK-2 and ID32STAPH for phenotypic identification of CoNS at species level. Eighty one clinical isolates of CoNS representing six species that were collected from different clinical samples were analyzed. All CoNS isolates were tested for catalase, Pastorex™, coagulase, *nuc* and *mecA* genes. Our results showed correct species identification by MALDI-TOF MS which was obtained in 98.8% in comparison to 96.3% and 75.3% correct identification for the VITEK-2 and ID32STAPH respectively. Only one strain was identified by MALDI-TOF MS at the genus level. In addition, MALDI-TOF MS identified staphylococci to subspecies level including *Staphylococcus hominis* subsp. *novobiosepticus* and subsp. *hominis*; *S. saprophyticus* subsp. *saprophyticus*. *S. auricularis* was only identified by MALDI-TOF-MS. There were 60 (74.1%) methicillin resistant CoNS (MR-CoNS) that showed *mecA* positive. Using MALDI-TOF MS technique, we were unable to discriminate between MR-CoNS and MS-CoNS, because the topology of dendrogram generated from the spectra of MR-CoNS and MS-CoNS strains were almost the same.

These findings confirm the value of MALDI-TOF MS as simple, accurate and rapid method for phenotypic identification of clinical CoNS isolates.

Keywords:

Coagulase Negative Staphylococci,
Species Identification,
MALDI-TOF MS,
VITEK-2,
ID32STAPH,
Gaza Strip.

1. Introduction:

Hospital-acquired infections associated with catheters and implanted medical devices are most commonly caused by staphylococci, specially coagulase-negative staphylococci (CoNS). They are

responsible for bacteremia, endocarditis, mediastinitis, meningitis and progressive joint destruction.

Current methods for species identification are slow, costly, and sometimes unreliable. To date there are 45 different *Staphylococcus* species with 24 subspecies. The most clinically significant CoNS are *Staphylococcus epidermidis*, *S. lugdunensis*, *S. saprophyticus*, and *S. capitis* (Dupont et al., 2010; Murray, 2010). *S. epidermidis* is probably the major CoNS species causing nosocomial infections, although other CoNS, such as *S. lugdunensis*, *S. schleiferi*, and *S. saprophyticus*, have been reported as even more pathogenic in a range of both nosocomial and community acquired infections (Argemi et al., 2015; Carbonnelle et al., 2007).

Identification of CoNS in the clinical laboratory can be problematic as many of the conventional methods and automated systems currently used do not always distinguish the different CoNS species reliably due to variable expression of biochemical characters. Moreover, molecular methods targeting the *16S rRNA*, *hsp60*, *femA*, *rpoB*, *gap*, *tuf*, and *sodA* are favored for diagnostic purposes but these methods often are expensive and time-consuming techniques (Argemi et al., 2015; Carpaij, Willems, Bonten, & Fluit, 2011; Dubois et al., 2010; Dupont et al., 2010; Carbonnelle et al., 2007). Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can examine the profile of proteins detected directly from the intact bacterial cell surface. This technique, based on relative molecular masses, is a soft-ionization method, allowing desorption of peptides and proteins from cultured bacteria. Ions are separated and detected according to their molecular masses and charges (m/z). This approach yields a reproducible spectrum within minutes, consisting of a series of peaks. Each peak corresponds to a molecular fragment released from the cell surface during laser desorption (Carbonnelle et al., 2007). Recently, MALDI-TOF MS has shown to be a useful and simple method for the rapid identification of CoNS isolated in a routine clinical microbiology laboratory (Argemi et al., 2015; Clark, Kaleta, Arora, & Wolk, 2013; Patel, 2013; Carpaij, Willems, Bonten, & Fluit, 2011; Bizzini, & Greub, 2010; Bizzini et al., 2010; Dubois et al., 2010; Carbonnelle et al., 2007). Starting from whole cells, cell lysates or bacterial extracts, it is possible to identify CoNS to species level within few minutes based on mass analysis of the protein composition of the bacterial cell between 2,000 and 20,000 Dalton. A high quality reference database of spectra generated from well characterized strains and advanced analysis software for spectral pattern matching are essential to obtain accurate species

identification. The output from the MALDI Biotyper, a frequently used MALDI-TOF MS instrument from Bruker®, is a log score in the range of 0 - 3.0, computed by comparison of the peak list for an unknown isolate with the reference main spectra (MSP), containing information on average peak masses, average peak intensities and peak frequencies in the database. Bruker database version 3.1.1.0 includes 120 spectra of 37 different CoNS species (Han, Chang, Hunag, & Chang, 2015; Lee et al., 2015; De Carolis et al., 2014; Dingle, & Butler-Wu, 2013; Martiny et al., 2012).

The aim of this study was to confirm the identification capacities of MALDI-TOF MS to accurately identify clinical CoNS species and to compare its capacity to the phenotypic VITEK-2 and ID32STAPH diagnostic methods. In addition, the study aimed to determine the ability of MALDI-TOF MS to distinguish between methicillin resistant CoNS (MR-CoNS) and methicillin sensitive CoNS (MS-CoNS).

2. Materials and methods:

2.1. Bacterial isolates:

For the present study, 81 unique non-duplicate CoNS isolates representing six species associated with diverse clinical infections, collected from different clinical samples including pus, urine, blood, wound, vaginal and ear swabs from the largest public tertiary referral hospital (Al-Shifa Hospital) in Gaza. These isolates were collected in 2012, out of a total unique 1121 bacterial isolates (March 1–July 31). Isolates were obtained directly from the clinical laboratory of Al-Shifa Hospital, and represent complete capture of all CoNS isolates during the stated collection period. Although a defined sampling strategy was not employed, the collected strains likely reflect the clinical epidemiology of CoNS in Gaza, since Al-Shifa Hospital is the primary referral hospital for patients from all areas of the Gaza Strip. Well typed strains including nineteen reference strains were included (see Table 1). Ethical approval from the Helsinki committee at the ministry of health in Gaza strip (Approval no. PHRC/HC/36/15) was obtained for performing the current study.

Table 1 Included reference strains

Species or subspecies	Strain ID
<i>S. auricularis</i>	ATCC 33753
<i>S. capitis</i> subsp. <i>capitis</i>	ATCC 27840
<i>S. capitis</i> subsp. <i>urealyticus</i>	ATCC 49326
<i>S. cohnii</i> subsp. <i>cohnii</i>	ATCC 29974

<i>S. cohnii</i> subsp. <i>urealyticus</i>	ATCC 49330
<i>S. epidermidis</i>	ATCC 14990
<i>S. epidermidis</i>	ATCC 12228
<i>S. epidermidis</i>	ATCC 14990
<i>S. haemolyticus</i>	ATCC 29970
<i>S. hominis</i> subsp. <i>hominis</i>	ATCC 27844
<i>S. hominis</i> subsp. <i>novobiosepticus</i>	ATCC 700236
<i>S. lugdunensis</i>	ATCC 43809
<i>S. pettenkoferi</i>	CCUG 51270
<i>S. saccharolyticus</i>	ATCC 14953
<i>S. saprophyticus</i> subsp. <i>saprophyticus</i>	ATCC 15305
<i>S. scheiferi</i> subsp. <i>coagulans</i>	ATCC 49545
<i>S. scheiferi</i> subsp. <i>scheiferi</i>	ATCC 43808
<i>S. simulans</i>	ATCC 27848
<i>S. warneri</i>	ATCC 27836

2.2. Identification of CoNS and MR-CoNS:

CoNS isolates were identified phenotypically based on colonial morphology, Gram stain, and by using the following tests: catalase, tube coagulase, Pastorex™ Staph Plus latex agglutination (Bio-Rad, Hercules, California), and the Staph ID 32 API system (bioMérieux, France) according to the manufacturer's instructions.

For VITEK-2 system, bacterial suspensions were prepared by suspending bacterial isolates in 0.45% saline to the equivalent of a 0.5–0.63 McFarland turbidity standard. The ID-GP identification card is a 64-well plastic card that includes 43 tests. Data were analyzed using VITEK-2 database, version 4.03 (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions.

The identification of four CoNS isolates that gave discrepant results at the species level was obtained by sequencing an internal fragment of the *sodA* gene as previously described (Sivadon et al., 2004; Poyart, Quesne, Boumaila, & Trieu-Cuot, 2001). The partial *sodA* gene was amplified and the PCR product sequenced as previously described (Sivadon et al., 2004). The nucleotide sequences were analyzed using the GenBank database of *sodA_{int}* sequences of *Staphylococcus* type strains. A multiplex PCR assay was used for detection of the *nuc* gene to exclude *S. aureus* and *mecA* gene for detection of methicillin-resistance among CoNS isolates (Becker, Roth, & Peters, 1998).

2.3. MALDI-TOF MS:

All isolates were streaked from stocks on Tryptic Soy Agar (Lab M, Lancashire, UK) with 5% horse blood

(E&O, Bonnybridge, Scotland) (TSA+B) and incubated overnight at 37°C. MALDI-TOF MS analysis was performed on a pure subculture on TSA+B incubated overnight at 37°C in a CO₂ incubator.

Only the direct transfer method was used. Briefly, one colony of each bacterial strain was spotted on a ground steel MALDI target plate. Each strain was spotted two times. Spots were allowed to dry at room temperature. Subsequently, 1 µl of matrix (Bruker Daltonik GmbH, Bremen, Germany), a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile (Sigma-Aldrich) and 2.5% trifluoro-acetic acid (Sigma-Aldrich), was added to the spot, which was dried again. Afterwards, the target plate was introduced into the MALDI-TOF MS. Samples were analyzed with a Microflex LT MALDI-TOF MS instrument (Bruker Daltonik GmbH). Parameter settings were ion source (IS) 1 20 kV, IS2 18.5 kV, lens 8.5 kV, pulsed ion extraction 250 ns and no gating. For spectral calibration, the Bruker® bacterial test standard (*E. coli* lysate) was used weekly. The measurements were performed in the automated mode using the software wizard (flexControl version 3.0, MALDI Biotyper version 2.0 and MALDI Biotyper Automation Control version 2.0). Species identification was carried out with library version 3.1.1.0 containing MSP of 3290 reference strains (1805 different species). Ranking tables containing the 10 best matching MSP, sorted according to decreasing log score values, were obtained with the wizard. The acceptance criteria for identification results generally cited in the literature were used: a log score ≥ 1.7 and ≥ 2.0, respectively, are the thresholds for a match at the genus and the species level. Scores of <1.7 were considered an unacceptable identification, according to the manufacturer's recommendations. These thresholds were empirically determined (Argemi et al., 2015).

2.4. Statistics:

Statistics were performed with Analyse-it for Microsoft Excel (version 2.21; Analyse-it Software Ltd, UK). A *P* value of less than 0.05 was considered significant.

3. Results:

Using the three phenotypic methods, all isolates were identified at the species level except for four isolates. Discrepant results (n=4) were reanalysed with MALDI-TOF MS.

For confirmed discrepancies of these four CoNS, the sequencing of the *sodA* gene was performed and

allowed the species identification of these four CoNS clinical isolates.

3.1. Species identification of reference strains:

Reference strains (n = 19) were used to confirm the capacities of the MALD-TOF MS technology for species identification (Table 1). For all tested species at least one reference spectra was present in the Bruker database. MALDI-TOF MS was 100% correct in assigning the species. However, out of the 19 reference strains, six (*S. auricularis*, *S. cohnii* subsp. *cohnii*, *S. pettenkoferi*, *S. saccharolyticus*, *S. schleiferi* subsp. *coagulans*, and *S. schleiferi* subsp. *schleiferi*) were identified only up to genus level and no reliable result was obtained for *S. cohnii* subsp. *urealyticus* (Figure 1).

3.2. Species identification of clinical CoNS strains:

The 81 clinical CoNS isolates were identified using MALDI-TOF-MS, VITEK-2 system and ID32STAPH as described previously. Correct identification (ID) results were obtained for 98.8% (80/81), 96.3% (78/81) and 75.3% (61/81) with MALDI-TOF-MS, VITEK-2 system and ID32STAPH, respectively. The correct ID for both MALDI-TOF-MS and VITEK-2 system was significantly higher than the correct ID of ID32STAPH. Misidentification (Mis-ID) rates were 1.2% (1/81), 2.5% (2/81) and 18.5% (15/81), respectively. The percentage of strains with no-identification (no-ID) was 0.0% (0/81), 1.2% (1/81) and 6.2% (5/81), respectively. A correct ID (100%) of all strains in all

three systems was not achieved. However with MALDI-TOF-MS and VITEK-2 systems, it was obtained for three species, *Staphylococcus hemolyticus*, *Staphylococci epidermidis* and *Staphylococcus warneri* (Table 2).

Only MALDI-TOF MS identified staphylococci to subspecies level including *Staphylococcus hominis* subsp. *novobiosepticus* and subsp. *hominis*; *S. saprophyticus* subsp. *saprophyticus*. Also the single strain of *S. auricularis* was only correctly identified by MALDI-TOF MS and was confirmed by sequencing *sodA* gene PCR product. However, one of the *Staphylococcus hominis* isolates could not be identified using MALDI-TOF MS.

For the CoNS species that most frequently cause diseases in humans or are isolated from human samples, *S. hemolyticus* (n = 33), *S. epidermidis* (n = 26), *S. saprophyticus* (n = 12), and *S. hominis* (n = 07), MALDI-TOF MS gave a correct identification in 98.7% (77/78), VITEK-2 system in 97.4% (76/78) and ID32STAPH in 78.2% (61/78) of isolates (Table 2).

3.3. Differentiation of MR-CoNS from MS-CoNS strains using MALDI-TOF MS

According to the *mecA* PCR results there were 60 (74.1%) MR-CoNS and the rest (21, 25.9%) were MS-CoNS. As shown in figure 2A and 2B, the topology of the dendrogram generated by the MALDI Biotyper 2.0 software from the spectra of MR-CoNS and MS-CoNS strains were almost the same which give low power to discriminate between them.

Table 2 Identification obtained with the matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), VITEK-2 system and ID32STAPH for the 81 clinical coagulase-negative staphylococci (CoNS) isolates

*Species	N.	Identification System								
		MALDI-TOF MS			VITEK-2 system			ID32STAPH		
		ID (%)	Mis-ID (%)	No-ID (%)	ID (%)	Mis-ID (%)	No-ID (%)	ID (%)	Mis-ID (%)	No-ID (%)
<i>S. hemolyticus</i>	33	100	-	-	100	-	-	87.9	9.1	3.0
<i>S. epidermidis</i>	26	100	-	-	100	-	-	88.5	11.5	-
<i>S. saprophyticus</i>	12	100	-	-	91.7	8.3	-	50	50	-
<i>S. hominis</i>	7	85.7	14.3	-	85.7	14.3	-	42.9	42.9	14.3
<i>S. warneri</i>	2	100	-	-	100	-	-	0	-	100
<i>S. auricularis</i>	1	100	-	-	0	-	100	0	-	100
Total strains	81	98.8[§]	1.2	-	96.3[§]	2.5	1.2	75.3	18.5	6.2

N., number of isolates; ID, correct identification; Mis-ID, misidentification; No-ID, no identification.

*Four isolates of CoNS were identified at the species level by sequencing the *sodA* gene (reference standard).

§Statistically significant difference in percentage of correct identification between the identification systems (P-value < 0.05).

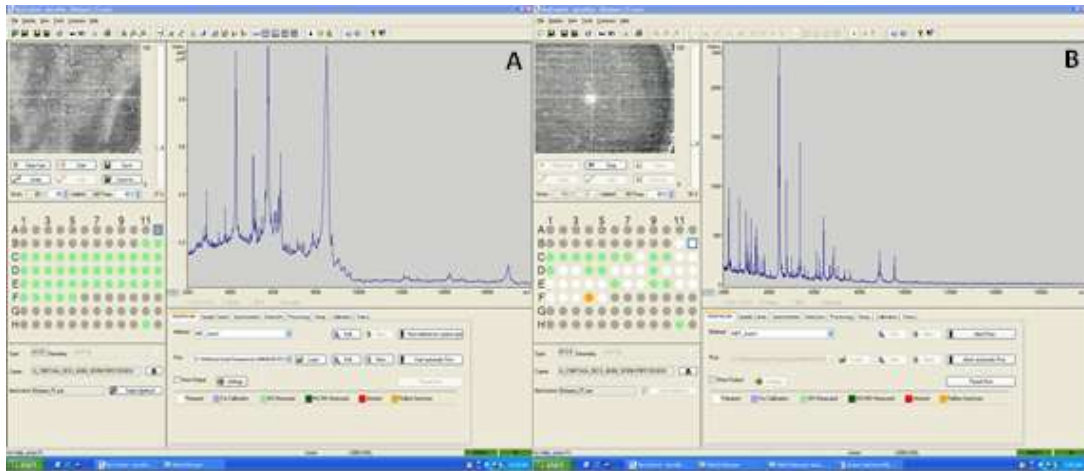


Figure 1 MALDI-TOF MS spectral fingerprints of two different species of CoNS (A, *S. epidermidis* and B, *S. hemolyticus*)

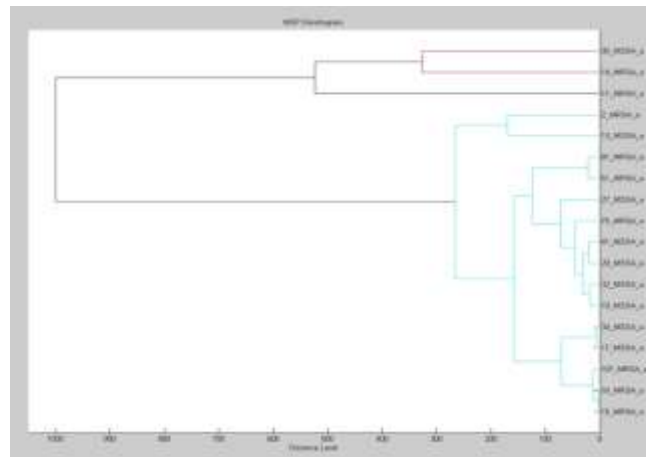


Figure 2A Dendrogram representation of hierarchical cluster analysis generated by the MALDI Biotyper 2.0 software from the spectra of selected MR-CoNS and MS-CoNS strains (without formic acid)

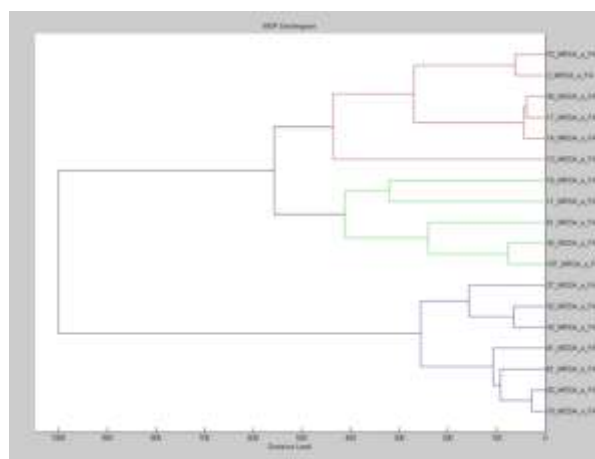


Figure 2B Dendrogram representation of hierarchical cluster analysis generated by the MALDI Biotyper 2.0 software from the spectra of selected MR-CoNS and MS-CoNS strains (with formic acid)

4. Discussion:

The use of MALDI-TOF MS for staphylococcal identification is now considered routine in laboratories compared with the conventional phenotypical methods previously used. This technique allowed the identification of approximately 85% of the CoNS strains, whereas only 14% of the CoNS strains were identified to the species level with phenotypic methods (Argemi et al., 2015). Bacterial identification is routinely achieved using phenotypically based techniques. However, those techniques remain time-consuming and sometimes of limited value (Argemi et al., 2015; Carpaij, Willems, Bonten, & Fluit, 2011; Dubois et al., 2010; Dupont et al., 2010; Carbonnelle et al., 2007), as for example for CoNS, where commercial identification kits identified only 37% of 177 CoNS isolates with the API 20 Staph system (Carbonnelle et al., 2007).

In this study and as first aim, we compared between MALDI-TOF MS, VITEK-2 and ID32STAPH for the identification of CoNS to the species level. Our results showed correct species identification by MALDI-TOF MS which was obtained in 98.8% compared to 96.3% and 75.3% correct identification for the VITEK-2 and ID32STAPH respectively. A relevant research achieved correct identification in 97.4%, 79% and 78.6% using MALDI-TOF-MS, Phoenix and VITEK-2, respectively (Dupont et al., 2010). In our study, Mis-ID rates were 1.2%, 2.5% and 18.5%, respectively. The percentage of strains no-ID was 0.0%, 1.2% and 6.2%, respectively. Comparable findings were achieved in Dupont et al., study where they found that Mis-ID occurred in 1.3%, 21% and 10.3% with MALDI-TOF-MS, Phoenix and VITEK-2, respectively and the rates of no-ID results were low with all systems: 1.3%, 0% and 0.9% with MALDI-TOF-MS, Phoenix and VITEK-2, respectively (Dupont et al., 2010). However, another study obtained a correct identification of only 71.2% for clinical and environmental CoNS with the VITEK-2 ID system and 82.5% when isolates belonging to species that are not included in the automated database were excluded (Delmas et al., 2008). Nevertheless, overall identification accuracy for CoNS with VITEK-2 was in the range of 91–96% (Funke, & Funke-Kissling, 2005; Spanu, 2003; Ligozzi et al., 2011). In another study using MALDI-TOF MS, a total of 151 strains out of 152 (99.3%) CoNS were correctly identified at the species level and only one strain was identified at the genus level (Dubois et al., 2010). A recent study evaluated the

use of MALDI-TOF MS for the identification of 450 blood isolates of the most relevant staphylococcal species, using sequence analysis of the *rpoB* gene as the reference method. A correct species identification by MALDI-TOF MS was obtained in 99.3%, with only three isolates (0.7%) being misidentified (Spanu et al., 2011). These findings are comparable to our results (correct species identification: 98.8%, misidentification: 1.2%). In this study, MALDI-TOF MS correctly identified all six CoNS species to the species level, except for *S. hominis* (85.7%). Many studies showed comparable results, however, one study showed same results except for *S. hemolyticus* (85.8%) (Dupont et al., 2010). Only MALDI-TOF MS identified staphylococcal to subspecies level including *Staphylococcus hominis* subsp. *novobiosepticus* and subsp. *hominis*; *S. saprophyticus* subsp. *saprophyticus*. Same results were obtained by Spanu et al. where MALDI-TOF MS correctly identified all the staphylococcal subspecies studied, including *S. capitis* subsp. *capitis* and subsp. *urealyticus*; *S. cohnii* subsp. *urealyticus*; *S. hominis* subsp. *novobiosepticus* and subsp. *hominis*; *S. saprophyticus* subsp. *saprophyticus*; *S. schleiferi* subsp. *schleiferi* and *S. sciuri* subsp. *sciuri* (Spanu et al., 2011).

The second aim of this report was to determine the ability of MALDI-TOF MS to correctly distinguish between MR-CoNS and MS-CoNS. The topology of dendrogram generated from the spectra of MR-CoNS and MS-CoNS strains were almost the same which give low power to discriminate between them. The molecular available methods used to type strains, including pulsed-field gel electrophoresis and multilocus sequence typing, are laborious and expensive to perform. Several studies have addressed the use of MALDI-TOF MS as a method for typing methicillin-resistant *S. aureus* (Wolters et al., 2011; Jackson, Edwards-Jones, Sutton, & Fox, 2005; Walker, Fox, Edwards-Jones, & Gordon, 2002). But there is no any previous report studying CoNS. While some studies showed the ability of MALDI-TOF MS to distinguish between methicillin-susceptible and resistant *S. aureus* (Majcherczyk, McKenna, Moreillon, & Vaudaux, 2006; Edwards-Jones et al., 2000), contradictory reports have been published on this issue (Wolters et al., 2011; Bernardo et al., 2002). Yet, the MALDI-TOF MS method revealed different clonal lineages of *S. epidermidis* that were of either human or environmental origin, which suggests that the MALDI-TOF MS method could be useful in the profiling of staphylococcal strains (Dubois et al., 2010). On the other hand, a new study showed

that MALDI-TOF MS can distinguish between positive and negative PVL and TSST *S. aureus* isolates by producing specific peaks of 4448 m/z and 5302 m/z (Bittar et al., 2009). But another research indicate that the peaks of 4448 m/z and 5302 m/z are independent of the presence of PVL and TSST as they were found in all 104 MRSA strains with different PFGE in contrast to the aforementioned report (Szabados et al., 2011). Moreover, they did not find evidence for an association of single protein peaks in the m/z range of 3000–10,000 Da with the presence of the toxins sea, seb, sec, sed, see, seg, seh, sei, and sej. So, they suggest that a MALDI-TOF MS-based typing approach in PVL-positive *S. aureus* is clearly inferior and inapplicable compared to well-established molecular typing methods (Szabados et al., 2011).

Finally and because MALDI-TOF MS is easy to perform, fast, and relatively cheap, it is the preferred method for the species identification of clinical CoNS in routine clinical microbiology (Han, Chang, Hunag, & Chang, 2015; Lee et al., 2015; Carpaij, Willems, Bonten, & Fluit, 2011).

5. Conclusion:

Many previously reported studies had demonstrated that MALDI-TOF MS is a powerful tool for the identification of clinically relevant species of CoNS and showed that it is a simple, accurate and rapid method for phenotypic identification of clinical CoNS isolates. Our results obtained in this report demonstrate the higher performance of MALDI-TOF-MS in the identification of CoNS to the species level and even more to the subspecies level compared to VITEK-2 and ID32STAPH. In addition, this study revealed the incapability of MALDI-TOF MS to distinguish between MR-CoNS and MS-CoNS.

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مقارنة بين قدرة تقنيات (MALDI-TOF MS) و (VITEK-2) و (ID32STAPH) في تشخيص أجناس وأنواع المكورات العنقودية السريية سلبية التخثر (CoNS) المعزولة من مستشفى الشفاء في قطاع غزة

كلمات مفتاحية:

المكورات العنقودية السريية سلبية التخثر، التشخيص على مستوى النوع، MALDI-TOF MS, VITEK-2, ID32STAPH, قطاع غزة.

أن المكورات العنقودية، لا سيما، سلبية التخثر منها (CoNS) تعتبر من العوامل المهمة المسبب للعدوى المكتسبة في المستشفيات وخاصة المرتبطة بالقسطرة والأجهزة الطبية المزروعة. أن تزايد حالات الإصابة بهذه البكتيريا يؤكد الحاجة إلى طريقة دقيقة وبسيطة لتشخيصها على مستوى الجنس والنوع. حديثاً، تم استخدام تقنية (MALDI-TOF MS) كوسيلة سهلة وبسيطة لتشخيص المكورات العنقودية سلبية التخثر. لقد تم عزل 81 سلالة بكتيرية من النوع CoNS من عينات سريرية مختلفة من أقسام مستشفى الشفاء وتعريفها باستخدام اختبارات صبغة الجرام وفحص انزيم Catalase و Pastorex وسليبتها للتخثر وامتلاكها للمورثة *mecA*. في هذه الدراسة تمت مقارنة قدرة تقنيات (MALDI-TOF MS) و (VITEK-2) و (ID32STAPH) في تشخيص أجناس وأنواع هذه المكورات العنقودية السريية سلبية التخثر. أظهرت النتائج أن تقنية MALDI-TOF MS أدت إلى تشخيص صحيح في 98.8% من العينات بالمقارنة مع 96.3% و 75.3% لكل من VITEK-2 و ID32STAPH على التوالي. أيضاً كانت تقنية MALDI-TOF MS هي الوحيدة القادرة على تشخيص السلالات إلى تحت النوع (*Staphylococcus hominis subsp. novobiosepticus and subsp. hominis; S. saprophyticus subsp. saprophyticus*). وبالإضافة إلى ذلك، فإن تقنية MALDI-TOF MS هي الوحيدة التي كان لها المقدرة على تشخيص البكتيريا العنقودية الأذنية (*S. auricularis*). كان هناك 60 (74.1%) سلالة مقاومه لعقار الميثيسيلين. حاولنا باستخدام تقنية MALDI-TOF MS التمييز بين السلالات المقاومة والحساسة لعقار الميثيسيلين، ولكن لم ننجح لأن طوبولوجيا الأطياف لكلا النوعين كانت هي نفسها تقريباً. في الختام فإن هذه النتائج تؤكد قيمة تقنية MALDI-TOF MS كطريقة بسيطة ودقيقة وسريعة لتحديد الطرز المظهرية للمكورات العنقودية السريية سلبية التخثر في مختبرات الأحياء الدقيقة الطبية.