Evaluation of Modified Oxidase, Cover-Glass Test, Antibiotic Sensitivity for Differentiation of *Staphylococcus* spp. from Other Related Genera

لبية الاختبارات الاوكسيديز المحور النمو تحت غطاء الشريحة والحساسية تجاه المضادات الحيوية في تفريق أفراد جنس *Staphylococcus* spp. عن بقية الاجناس المتقارية الأخرى

**Aws Ibrahim Sulaiman**

**Basima Ahmed Abdullah**

كلية العلوم/ جامعة الموصل

**Abstract**

To comparison between modified oxidase, cover-glass, antibiotic sensitivity (Nitrofurantoin, Furazolidone) to evaluate which of them are most useful for differentiation *Staphylococcus* spp. from other related genera. A total of 115 clinical isolates out of them 38 isolates belongs to *Staphylococcus* spp. were collected from pus, urine and blood cultures of patients for both gender from period March to July 2012 at Al-Salam General Teaching Hospital in Mosul city. All isolates were diagnosed and identified classically and using API test. The result indicated that Imipenem is highly effective against *Staphylococcus* spp. and all isolates were negative to modified oxidase test except one isolate. All other isolates were given good growth under cover-glass test except one. 67.5% of isolates were resistant to Nitrofurantoin and 32.5% were sensitive but 21.6% of isolates were resistant to Furazolidone and 78.4% were sensitive. The above tests are the most tests in differentiation between *Staphylococcus* spp. and other related Genera.

**Key wards:** Modified Oxidase, Cover-Glass, Nitrofurantoin, Furazolidone

**Introduction**

*Staphylococci* produce round, smooth, white, creamy colonies on BA after 18 to 24 h of incubation at 35° to 37°C. *Staph aureus* may produce haemolytic zones around the colonies and may exhibit pigment production (yellow) with extended incubation. *Staph epidermidis* colonies are usually small to medium sized, non-haemolytic, white to gray colonies, some may be weakly haemolytic. *Staph. saprophyticus* forms slightly larger colonies, with about 50% of the strains producing a yellow pigment. *Staph haemolyticus* produces medium sized colonies, with moderate or weak haemolysis and variable pigment production. Colonies of *Staph lugdunensis* are often haemolytic and medium sized, although small colony variants can occur. Staphylococci ferment glucose, growth in NaCl agar 6.5,10 and 15 %, catalase positive resistant to bacitracin, urease differential, novobiocin differential, oxidase negative and differential to VP test, the species are established as opportunistic pathogens are *Staph. capitis* and *Staph.hominis* these organisms have been associated to endocarditis, septicemia and wound infections [1].

The Oxidase reaction test is the most useful for characterizing of gram negative bacteria and some gram positive bacteria especially in differentiation Staphylococci from Micrococcii [2, 3, 4].
Since the reaction is affected by the pH of growth medium, the age of the culture, the reagent used and the timing of the reaction and standardization of the test procedure [4,5]. The cover-glass test is the new test for differentiation Staphylococci and other related genera like Micrococcii, this test is rapid, highly specific, and easy to perform and interpret, it should be used in combination with Furazolidone and Bacitracin discs in both primary and secondary culture as useful tool for differentiating Staph. aureus and other Staphylococcus spp. of human origin from Micrococcus spp. or related genera [6].

The member of the genus Staphylococcus differ from those of Micrococcus by being facultative anaerobic with a G+C content of 20 to 39%, containing cytochrome a and b, peptidoglycan and teichoic acids in their cell walls with oligoglycine peptides in the inter-peptide bridge of their cell walls and being susceptible to Furazolidone and resistant to Bacitracin [7, 8].

Staphylococci are generally susceptible to lysostaphin, Nitrofurantoin, Furazolidone and resistant to erythromycin, bacitracin a low levels. In the laboratory routine rapid distinction of Staphylococci from Micrococcii can be made demonstrating susceptibility of staphylococci to 100 μg Furazolidone, 100 μg Nitrofurantoin and resistant to 0.04 μg Bacitracin, further more Staphylococci exhibit a negative reaction with the rapid modified Oxidase test, whereas other related genera especially Micrococcii are positive for this test [9,10].

The aim of this study was to differentiate Staphylococcus spp. from other related Genera.

Materials and Method

Samples and isolation of Bacteria

A total of 115 isolates from clinical specimen, thirty eight out of them belongs to Staphylococcus spp. were collected from March to July 2012 in Al-Salam General Teaching Hospital in Mosul city, the specimens included pus, urine and blood culture for both gender. Screening test for genus Staphylococcus spp. depending on gram stain, coagulase test, cell shape, nitrate reduction, novobiocin sensitivity test urease test, growth in (6.5,10 and 15)% NaCl, haemolysis on blood agar, catalase, oxidase, growth on CLED medium, Voges-Proskauer test (VP), growth on Vogel-Johanson agar (VJ) Micro and Molecular Biology, Mannitol Salt Fermentation (MSF) and Crystal Violet agar (CVA) [11]. Identification was confirmed at the species level by API ID 32 STAPH system (Bio Merieux, Marcy l’Etoile, FRANCE) according to manufacturer's instructions.

Modified Oxidase Test

The test was performed with simple modified test, in briefly a 6% solution of Tetra-Methyl-Phenelyne-Diamine (TMPD) in Di-Methyl-Sulphoxide (DMSO), filtered by Whatman No.1 paper test and placed in petri dish and wetted with 0.5ml of TMPD-DMSO, one heavy isolated colony was picked up with wooden applicator stick and streaking on the wet filter paper, the color in the positive reaction to blue-purple was record after 10,15 and 30s [2,4,10].

Cover-Glass Test

A sterile cover-glass was placed with a pair of sterile tweezers onto heavily inoculated areas of the blood agar or chocolate agar, with a clinical specimen or bacterial culture and incubation at 37°C for 24h, the plate was checked for pigmented colonies or bacterial biomass underneath the cover-glass [6].

Antibiotic Sensitivity Test (AST)

All isolates of Staphylococcus spp. Tested against 23 antibiotics discs according to Bioanalyzer Company, TURKEY. Table (1) below express these antibiotics.

Table (1): The Antibiotics Discs

<table>
<thead>
<tr>
<th>Antibiotics and abbreviation</th>
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<tr>
<td>Penicillin (P 10)</td>
<td>Trimethoprim (TMP 10)</td>
<td>Rifampicin (RA 5)</td>
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<tr>
<td>Tobramycin (TOB 10)</td>
<td>Methicillin (ME 10)</td>
<td>Clindamyacin (DA 10)</td>
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<tr>
<td>Oxacillin (OX 10)</td>
<td>Tetracycline (TE 10)</td>
<td>Imipenem (IPM 10)</td>
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<td>Erythromycin (E 15)</td>
<td>Ciprofloxacin (CIP 10)</td>
<td>Nitrofurantoin (F 100)</td>
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<tr>
<td>Amoxicillin-Clavulanic (AMC 30)</td>
<td>Norfloxacin (NOR 10)</td>
<td>Ceftriaxone (CRO 10)</td>
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<td>Cefotaxime (CTX 10)</td>
<td>Amikacin (AK 10)</td>
<td>Doxyxycline (DO 10)</td>
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<td>Vancomycin (VA 10)</td>
<td>Chloramphenicol (C 10)</td>
<td>Azithromycin (AZM 15)</td>
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<td>Gentamicin (CN 30)</td>
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Furazolidone prepare locally from Furadon medicine oral suspension (Diamond Pharma, SYRIA). Taking with micropipette 0.1mg (100 µg) and immerged in disc of filter paper size (6mm) in diameter under sterile condition, the antimicrobial resistance patterns were determined by the Kirby-Bauer disk diffusion test performed according to recommendations and after incubation at 37°C for 18h. The inhibition zone around the disc was measured about Nitrofurantoin if less than 14mm/ disc its resistant, between 15-16mm/disc is intermediate, more than 17mm/disc is sensitive. Furazolidone if less than 9mm/disc is refer to resistant but more than 12mm/disc this means sensitive [12].

**Result and Discussion**

Identification of (38) clinical isolates of *Staphylococcus spp.* was carried out depending on biochemical reactions and 16 out of them identified by API ID 32 STAPH system. *Staph. aureus* (10), *Staph. epidermidis* (5), *Staph. lugdunensis* (5), *Staph. haemolyticus* (3), *Staph. hominis* (3), *Staph. saprophyticus* (3), *Staph. xylosus* (3), *Staph. cohnii* (2), *Staph. sciuri* (2), *Staph. capitis* (1) and *Staph. intermedius* (1). Table (2) shows the results of biochemical tests and Figure (1,2) Also.

These results agree with (Wesley and others) when they studies on identification of *Staphylococcus spp.* observed that all biochemical tests are useful to conventional methods for differentiate the *Staphylococcus spp.* from each other and from other Genera [13].

Table No. (2): Identification Scheme for Clinical Isolates of *Staphylococcus spp.*

<table>
<thead>
<tr>
<th>Nitrate Reduction</th>
<th>Urease Test</th>
<th>Growth in NaCl %</th>
<th>Hemolysis type on Blood agar and colonies color</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Vogel-Johnson agar (VJ)</th>
<th>Voges-Proskauer agar (VP)</th>
<th>Crystal Violet agar (CVA)</th>
<th>Mannitol Salt Fermentation</th>
<th>Growth on CLED Medium</th>
<th>Novobiocin Disc</th>
<th>Staphylococcus spp.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+</td>
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<td>-</td>
<td>Y</td>
<td>+</td>
<td>+</td>
<td>Staph. aureus</td>
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<td>d</td>
<td>G</td>
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<td>Staph. hominis</td>
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<td>Staph. intermedius</td>
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<td>d</td>
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<td>Staph. capitis</td>
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<td>Staph. sciuri</td>
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<td>Staph. xylosus</td>
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<td>+</td>
<td>+</td>
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<td>G</td>
<td>Staph. cohnii</td>
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<td>+</td>
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<td>Staph. sciuri</td>
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<td>+</td>
<td>G</td>
<td>S</td>
<td>Staph. intermedius</td>
<td></td>
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</tbody>
</table>

*Haemolysis type on Blood agar and colonies color: w=white, y=yellow.*

**Vogel-Johnson (VJ):** + = coagulase positive (Black colonies, Yellow halos around Black colonies, - = Negative to coagulase (Black colonies without Yellow halos)

***Crystal Violet agar (CVA):*** haemolytic and coagulating strains appear purple= p or yellow = y and non-coagulating strains appear white = w

****Growth on CLED Medium:*** green = G and yellow colonies= Y

*****Novobiocin disc:*** less than or equal 15mm/disc resistant, more than 15 mm/disc sensitive.
Antibiotic Profiles of *Staphylococcus* spp.

The antimicrobial resistance patterns of *Staphylococcus* spp. Clinical isolates were highly resistant to Penicillin (92.2) % and low resistant to Imipenem(13.1)%. Shape (1) shows the antibiotic profiles *Staphylococcus spp.*

Our results regarding antibiotics profiles test found that the most species are resistant to penicillin among other antibiotics. These results are similar to (Franca and his colleagues) upon studies of *Staphylococcus* spp. resistant to antibiotics, they found that all species have high resistant to amoxicillin (50%) followed by streptomycin (43%), tetracycline (40.5%), lincomycin (39%), erythromycin (33.8%), rifampicin (16.7%), oxacillin (16.7%), norfloxacin (13.8%), doxycycline (12.0%), ciprofloxacin (8.5%), enrofloxacin (8.1%) and gentamicin (7.6%), these results similar to our results in some antibiotics and differs from others, this may be depending on sites of the researchers were working in Brazil therefore the strains are different phenotypically and genotypically from our strains.[14]

Routine testing of Staphylococcal isolates can be easily performed in the laboratory using standard guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) formally known as National Committee for Clinical Laboratory Standards (NCCLS). When using commercial tests laboratories need to adhere to the manufactures recommended procedure. [1]
**Modified Oxidase Test**

The all isolates were negative to modified oxidase test except *Staph. haemolyticus* isolate was positive to the test.

Modified oxidase results appear that all species except one are negative to this test, these results are very similar to the results of [3, 4] when were studies about rapid test for differentiation Staphylococci from Micrococcoci they found that all strains of Micrococcoci were positive and Staphylococci were negative, but this test have disadvantage because the TMPD derivatives solubilized in DMSO were stable at room temperature for several weeks, when the solution was protected against light, different media also affected the oxidase reaction better growth could be observed on agars containing glucose them on glucose-deficient media and the oxidase reaction was more pronounced.

The modified oxidase test is recommend from UK standards for Microbiology Investigations, since negative to this test mean that the organism closely to *Staphylococcus spp.* more than *Micrococcus spp.* [10].

**Cover-Glass Test**

Depending on the results most of clinical isolates were able to grow under cover-glass test accept *Staph. intermedius* failed to grow after 24h of incubation Figure (3).

Cover-Glass test in our study appeared that all isolates were growing well except *Staph. intermedius* failed to grow and this result is very similar to LabMedica when researchers were studies about cover-glass test for differentiation between Staphylococci and Micrococcoci [6].

![Figure No. (3): Cover-Glass Test, Left (Positive) Staph. lugdunensis and Right (Negative) Staph. intermedius](image)

**References**


