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Determination of the Varied Resistance Abilitiesthat are Possessed by *staphylococcus Aureus*

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Abstract

Background: *Staphylococcus aureus* is constantly evolving and possesses new mechanisms of resistance to antibiotics. Antibiotics that unable to eliminate bacteria may cause them to become more virulent.Effort should be continuous to find new antibiotics, and accurate diagnostic techniques and tests that are absolutely relied upon in knowing the characteristics of the diagnosed bacteria to find the appropriate treatment.

Methods:*Staphylococcus aureus* isolates were diagnosed by morphological, biochemical, and molecular methods. *S. aureus* sensitivity to antibiotics was tested by Disk Diffusion Method and Vitek®2 system, . Also, D-test was performed for both food and clinical samples.

Result: The differences were found between the two tests (disk diffusion method and Vitek®2 system) which were used to sensitivity test. Food isolateshad an inducible MLSB phenotype with 80% and clinical isolates had it with 33.3%.All *S. aureus* isolates had*blaZ* gene. The largest percentage of isolates were having the *mecA* gene.

Conclusion: There is no test can be considered absolutely accurate. Food sample isolates are the most tolerant to the altered conditions . The resistance to each one of antibiotics is controlled by many and different genes anddifferent materials then, if one of these genes became inactive, the other genes or materials can do its action.

Keywords:*Staphylococcus aureus,* Erythromycin, Clindamycin, Methicillin, Vitek®2 system, and D- test.

Introduction

It is normal that frequent use of methicillin antibiotic to treat *S. aureus* , has big role to methicillin resistant *S. aureus* (MRSA) emergence(1). Especially, if it is known theabundant use of antibiotic can be considered as an incentive for bacteria to develop themselves(2).It is also common for MRSA to appear in hospital(HA-MRSA) as a result of exposure to health risk factors. But it is interesting that MRSA appears in healthy people (CA-MRSA) without exposure to surgery, catheters, dialysis, or others tools used in the hospitals(3).

Presence of CA-MRSA will become a familiar as HA-MRSA, if the capacity of *S. aureus* to obtain or develop several mechanisms is demonstrated(4). Particularly, *S. aureus* is found in different ambiences and adapts to varying conditions(5).Effect of antibiotics on

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Staphylococcus differs according to useantibiotics, source or environment in which *Staphylococcus* is found such as foods and their types, hospital and workers who work in it,in addition to animals and those dealing with them. Where antibiotics have various mechanisms to influence bacteria, on the other hand bacteria develop their own mechanisms or acquire a new mechanism through mobile genetic elements like plasmids and transposons as a means to protect these bacteria from the antibiotics[6].

Where *S. aureus* withstands penicillin by producing penicillinase, which has ability to cleave β- Lactam ring of penicillin and make it inefficient. And this bacterium resists methicillin (which didn't effect by penicillinase) by earning *mec* gene[7]. As this gene is responsible for preventing methicillin from interacting with the target of producing PBP2a [8]. Also *S. aureus* invalidate the effect of aminoglycoside antibiotics such as gentamycin and kanamycin by modifying enzymes[9] .The resistance is transmitted not only from bacterial species to other species within the same genus, but also among bacterial genesis, and Vancomycin resistance transmission from *Enterococcus* to *Staphylococcus* through Tn1546 is the best example[10 and 11]. Hence, there is an urgent need to develop new types of antibiotics to eliminate resistant bacteria[6].

Material and methods

Samples were included in this study are food and clinical specimens.Themedium was chosen to isolate *S. aureus*, was either Paired Parker agar or mannitol salt agar according to source of *S. aureus*. Identification of *S. aureus* with biochemical and molecular methods result in *S.aureus* was founded in 37 food and clinical samples.*S. aureus* sensitivity to antibiotics was tested by Disk Diffusion Method[12] and Vitek®2 system, following the company instructions was supplied with discs that specific Vitek®2 test . Also,the D- test was performed for both food and clinical samplesaccording to[13].

β-lactamase was investigated by PCR assay using primers which are F: AAG AGA TTT GCC TAT GCT TC and R:GCT TGA CCA CTT TTA TCA GC[14]to amplify the*blaZ* gene with size 517bp(*blaZ*gene is responsible for β- Lactamase enzyme) . The amplification was performed under the conditions: Temperature 94 ºC as initial denaturation for 4 minutes ,35 cycles in order to denaturation with 94ºC , annealing (55ºC) , extension(72 ºC) and each one of these stages is accompanied with one minute , final extension (72 ºC) for 7 minutes.

The ability to methicillin resistantwas examined by Polymerase Chain Reaction using primers, F:AAAATCGATGGTAAAGGTTGGC and R:AGTTCTGGAGTACCGGATTTGC[15]to amplify *mecA* gene where the product must be with size 533bp with the program consists of initial denaturation with 95ºC for 3minutes and 33 cycles for denaturation with 94 ºC for 1minutes, annealing with 53 ºC for 30 seconds, and extension with 72 ºC for 1 minutes also one cycle for final extension with 72 ºC for 6 minutes.

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Results and discussion

Isolated *S. aureus* from food and clinical samples appeared as jet black colonies surrounded by a transparent area on the Paired Parker agar figure (1), and as golden-yellow colonies on mannitol salt agar figure (2).

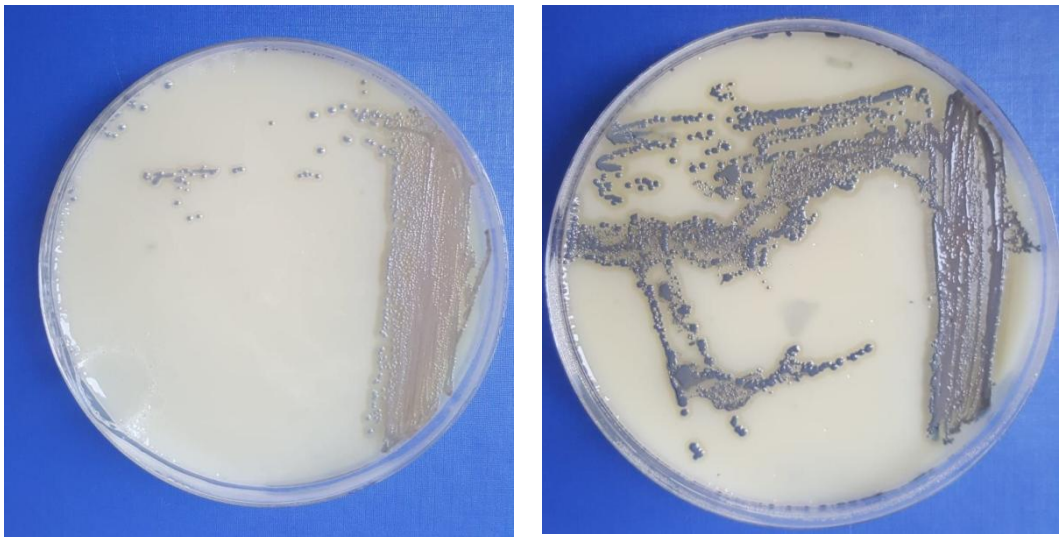

Figure (1): *S. aureus* on Paired Parker agar

Figure (2): *S. aureus* on mannitol salt agar

Sensitivity of *S. aureus* isolates were examined by Disk diffusion method and Vitek®2 system , the results were slightly different between two tests, especiallyto these related with clinical sample table (1).This result indicates to the fact, thesetwo tests are relative .

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Table (1): *Staphylococcus aureus* sensitivity test of Disk method and Vitek®2 system

Food isolates (19 isolates of *S. aureus*)						
Antibiotics	Disk Diffusion method			Vitek®2 system		
	Resistant%	Intermediate %	Sensitive %	Resistant%	Intermediate%	Sensitive %
Cefoxitin	19 (100%)	0 (0.0%)	0 (0.0%)	17(89.5%)	0 (0.0%)	2(10.5%)
Oxacillin	19 (100%)	0 (0.0%)	0 (0.0%)	17(89.5%)	0 (0.0%)	2(10.5%)
Erythromycin	5 (26.3%)	4 (21.1%)	10 (52.6%)	5 (26.3%)	2(10.5%)	12(63.2%)
Clindamycin	0 (0.0%)	0 (0.0%)	19 (100%)	4 (21.1%)	0 (0.0%)	15(78.9%)
Clinical isolates (18 isolates of *S. aureus*)						
Antibiotics	Disk Diffusion method			Vitek®2 system		
	Resistant%	Intermediate%	Sensitive %	Resistant%	Intermediate%	Sensitive %
Cefoxitin	18 (100%)	0 (0.0%)	0 (0.0%)	12(66.7%)	0 (0.0%)	6 (33.3%)
Oxacillin	17 (94.4%)	0 (0.0%)	1(5.6%)	13(72.2%)	0 (0.0%)	5 (27.8%)
Erythromycin	9 (50%)	7(38.9%)	2(11.1%)	13(72.2%)	2(11.1%)	3(16.7%)
Clindamycin	0 (0.0%)	4(22.2%)	14(77.8%)	7 (38.9%)	1(5.6%)	10 (55.6%)

Inhibition zone around clindamycin disk took D- shape when the isolate was positive to this test , figure (3) . The results showed that inducible MLSB percent in food and clinical samples were 80% and 33.3% respectively table(2).Thus, food isolates is more virulentthanclinical isolates, this is supported by[16]. Regarding to*S. aureus* with phenotype Macrolides- Lincosamides- Streptogramins B (MLSB), Macrolides, Lincosamides, and Streptogramins B became useless todiscourage it for presence genes responsible for proteins that work to resist these antibiotic effects[17].

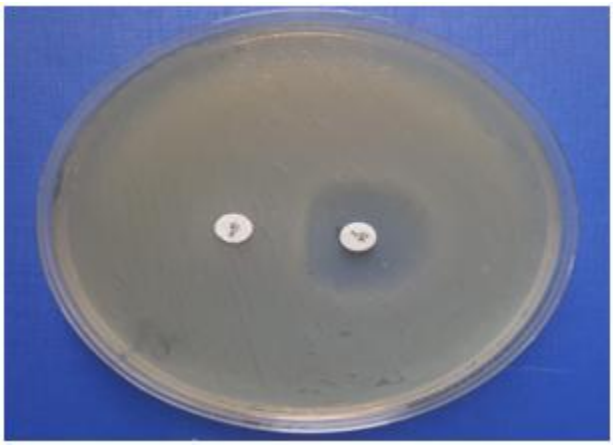

Figure (3): D- test

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Table(2):Properties of *S. aureus* isolates resistant to erythromycin in terms of sensitivity to clindamycin

Food isolates			Clinical isolates		
Susceptibility pattern	Number of isolates	%	Susceptibility pattern	Number of isolates	%
MS	1	20	MS	4	66.7
Inducible MLSB	4	80	Inducible MLSB	2	33.3
Constitutive MLSB	0	0.0	Constitutive MLSB	0	0.0
Total	5		Total	6	

MS: Erythromycin (Resistant), Clindamycin(Sensitive), and D-test (-)
Inducible MLSB: Erythromycin (Resistant), Clindamycin(Sensitive), and D-test (+)
Constitutive MLSB :Erythromycin (Resistant), Clindamycin(Resistant)

Polymerase Chain Reaction technique appeared that all *S. aureus* werecontaining a *blaZ*gene figure (4). Also, most of these isolates wereacquiring mecA gene figure (5).

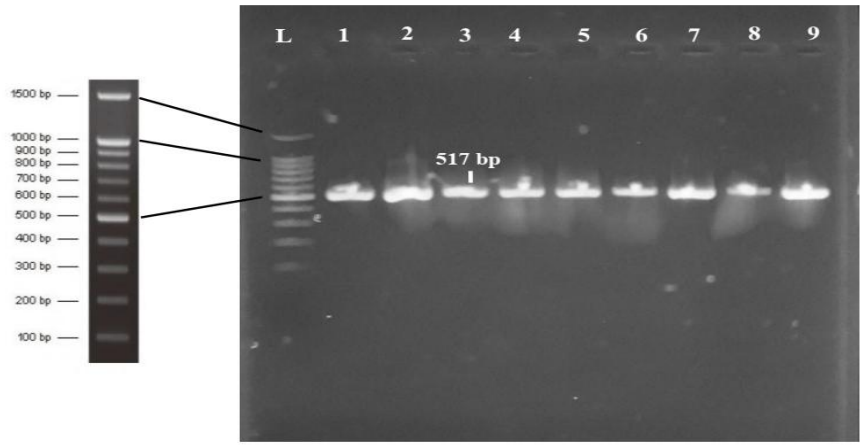

Figure (4):*blaZ* gene product with (517bp) size on gel electrophoresis

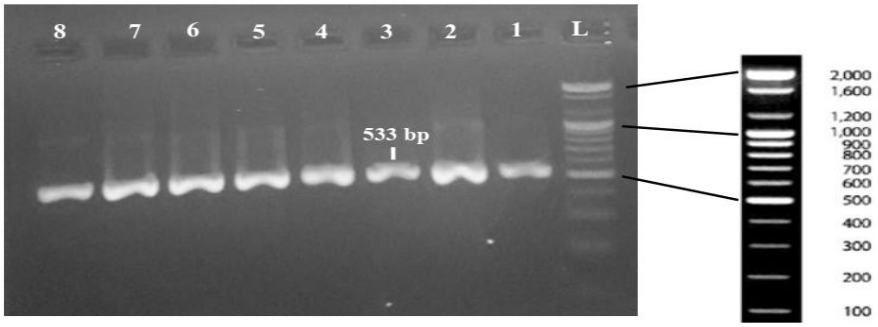

Figure (5): Product of *mecA* gene with (533bp) size on gel electrophoresis

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Acquisition of *mecA* gene by*S. aureus* will be reduced or finish efficiency ofβ-lactam antibiotics that use as a treatment to eliminate this bacteria[18]. And β-lactamase enzyme alone (that is encoded by *blaZ* gene) maight be able to neutralize β-Lactam antibiotic effect.

There are different tests and techniques to describe *S. aureus* characteristics in terms of virulence factors , toxicity , and resistance against drugs …etc. But each one of thesetests and techniques requires several conditions, and the environmental conditions of country in which these methods were invented can be one of the requirements. Also the technical person plays a major role in the accuracy of the test results , therefore, all the matters should be considered to get accurate results. Even with the accuracy results , there are specific techniques are neededespecially for the matters that are related with the several activities such as RT-PCR technique .

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