Molecular and Phenotypic Detection of Some of the Coded Genes for Virulence Factors in Mrsa and Mssa Isolated From Different Clinical Cases

Noora Hamid Ibrahim[1], Ahmed Mohammed Turki[2] , Jamal Abd Al-Rahman[3]

[1] Post graduate / Department of Biology, College of Education for pure Sciences, University of Anbar, Iraq, [2]Assist prof. Dr. Department of Biology, College of Science, University of Anbar, Iraq, [3]Assist prof. Dr. Department of Biology, College of Education for pure Sciences, University of Anbar, Iraq

Abstract

The current study included the collection of 100 clinical samples obtained of patients reviewed in the laboratories of Ramadi hospital for women and children and Hit General Hospital and some civil laboratories within the city of Ramadi, including Bacterimea samples, Urine, abscesses, heart inflammation, wounds, rhinitis, osteoporosis, otitis ,Laryngitis, sputum (35, 20, 5, 6, 7, 5, 8, 7, 4, 3) samples respectively to investigate the presence of *staphylococcus aureas* . The results of diagnosis of bacterial isolates showed that there were 55 bacterial isolates belonging to the *S.aureus* bacteria after they were grown on the culture media and examined microscopic as well as the use of some of the tests on the chemical, and the diagnosis was confirmed using the Vitek2-compact system .The diagnostic results were confirmed by the use of genetic diagnosis. The results of the phenotypic investigation were identical to the results of the genetic investigation using the polymerase chain reaction technique in terms of possessing isolates producing both hemolycin (α, β), on the *HlA* encoding genes for the production of type $\alpha\alpha$ and hlB encoded for type β production, For plasma blood on the *co-a* gene, and the resistance isolates of methylation on the *mecA* gene.

Keywords: *virulence factors; phenotypic detection; MRSA; MSSA;*

Introduction

S.aureus is a public pathological cause that causes serious infections and is often life-threatening in the community and hospital.Its antibiotics have become more difficult to eliminate because of their high resistance to these antibiotics [(1)]. These bacteria cause infections ranging from simple pimples, impetigo, boils and abscess, to acute infections such as bacteremia and Toxic shock syndrome [(2)]. *S.aureus* is due to its ability to multiply rapidly and possess many virulence factors, such as the production of toxins and enzymes that have an important role in host tissue invasion and bacterial spread, among which the most important toxin is hemolysine, which is one of the most virulent elements, has been able to cause holes in the cell walls of the cells[(3)], The lactamase enzymes used by *S.aureus* contribute to the resistance of many antibiotics [(4)] It is characterized by the formation of biofilms and responsible for chronic injuries to resist the process of phagocytosis[(5)]. Methicilin Resistant *S.aureus* (MRSA) has shown high resistance to this antioxidant and after using it in treating the injuries in this bacteriaits and yet[(6)]. the *mecA* gene is responsible for the resistance of staphylococci to the penicillin group[(7)].

Materials and working methods: Samples Collection: 100 samples were collected from patients in Al Ramadi Teaching Hospital, Women and Children Hospital, Hit General Hospital, Health Centers and some private labs in Ramadi for the period from 15-7-2018 to10-12-2018.The samples included cases of endocarditis, bone marrow, ear, nose and throat.

Corresponding author:
Noora Hamid Ibrahim[1]
noonooha93@gmail.com

Suppurations and boils of the skin. Sputum bacteremia was collected under sterile conditions and of both sexes at different ages They were taken to the laboratory for culture.

Identification

Bacterial isolates were diagnosed using the methods used by [8], And by using culture, microscopic and biochemical phenotypes as well as Vitek 2Compact System.

Minimum inhibitory concentration (MIC) followed the preparation [9] of the double dilution method to determine the concentrations of the antigens under study for calculating the MIC of antagonists (Cefitraxone, Erythromycin, Amikacin, Vancomycin, Tetracycline, Gentamycin, Ciprofloxacin, Oxacillin,Trimethprim,Rifampcin)by using Microtitration plates, concentrations starting at (1/2) and ending at (256) mg / ml were obtained for all antidotes under study except Methicillin and Cefoxitin used for diagnosis.

Phenotypic investigation of some bacterial virulence factors The productivity of some virulence factors, including the production of hemolysin, the capsule, the biofilm, the coagulase and the DNA enzyme, was investigated using various laboratory culture media.

Molecular investigation of some agents of virulence in bacterial isolates polymerase chain reaction (PCR) A-

The single polymerase chain reaction (PCR) was investigated for some of the genes responsible for virulence factors in Methicillin resistant *Staphylococcus aureus* (MRSA) bacteria, using DNA fragments with a limited number of Oligonucleotide nucleotide, which acts as primers for virulence genes in the MRSA strain, which included the genes of *coa, mecA, hla.hlb*.

Bacterial Genomic DNA Extraction B- The extraction was carried out according to the Bacterial Genomic DNA extraction kit and its instructions from Geneaid. **C- Preparation of PCR Master Mix** Using the PCR PreMix AccuPower kit and its instructions from Bioneer (South Korea), he prepared the polymerase chain reaction mixture. **Preparation of Agarose Gel D-** Prepared by[10].

Electrophoresis Agarose Gel E- The electrophoresis of the prepared acarose gel was carried out by 1.5% under a difference of voltage (100) volts and current (80) milliamps at a time of 60 minutes for the purpose of detecting the DNA Bands bundles extracted representing PCR products (PCR products) according to the method [10].

F- Primers Especially Special prefixes for diagnostic, virulence and antibiotic resistance genes were used by the researcher using NCBI GeneBank and Primer 3 plus prefixes, as well as processing of these prefixes by Bioneer / South Korea, as in a table(1).

Table (1) used prefixes under study, sequence of nitrogenous bases and volumes of amplification products

Amplification output volume (bp)	Sequence of nitrogenous bases3'-5'		Name of initiator	Initiator type
456	F	TAAAGAAAATGGCATGCACAAAA	hlA gene	Agents of Virulence factors
	R	GGTCCCCAATTTTGATTCACC		
428	F	ACGATGGAACATTGGATATGA	Co A	
	R	CCCATATGTCGCAGTACCATC		
527	F	TTTGCTTCTATTTTGTTGTAAGCTAT	hlB gene	
	R	GCTATCATTATCGAATCCACAAC		
466	F	ATCAAAATTGGGTACAAGATGATACCT	mecA gene	
	R	AATTCACCTGTTTGAGGGTGGAT		
R: Reverse primer F: Forward primer				

Result and discussion Isolation and diagnosis of *Staphylococcus aureuas* a-

The results of bacterial transplantation showed 70 isolates out of 100 samples where growth occurred, while 30 samples did not show growth until after 48 hours incubation period. The samples were distributed (35, 20, 5, 6, 7, 5, 8, 7, 4, 3), The cases, (bacteremia, Urine, abscesses, heart inflammation, wounds, rhinitis, osteoporosis, otitis,Laryngitis,sputum) respectively. The lack of growth may be due to the small number of bacterial cells or the absence in the sample taken by cotton swabs and encountered occasional power outages or the fact that the bacteria need anaerobic an aerobic lap conditions where these negative cases were concentrated in the samples of cases of bacteremia, pyoderma, boils and endocarditis Heart, wounds, osteomyelitis and otolaryngitis The results of isolation and diagnosis showed the prevalence of positive bacteria of Melon Cram on negative bacteria, where the number of positive isolates reached 61 isolates representing 87% of the total isolates that gave positive growth, while the number of negative isolates of Melon Cram The isolation and diagnosis results indicate that the staphylococcus aureus is prevalent on the rest of the staphylococcus species as it reached 98.2% of the total isolated staphylococcus species [(11)] *s. aureus* isolated by 56.8%.

The results of the sensitivity test for S. aureus isolates under study against (10) antibiotics showed that the isolates of these bacteria varied in their resistance to all antibiotics under test as all isolates were 100% resistant to the antibiotic Methicilin, while the resistance was towards (Ciprofloxacin. , Vancomycin, Tetracyclin, Erythromycin, Trimethoprim / sulfamethoxazole, Ciftraxone, Gentamycin),in the rate of (91%, 51%% 76, 80%, 27%, 36%, 58%) respectively, while Rifampcin and Amikacin were 100% sensitive

Phenotypic detection of some virulence factors b-Production of Haemolysin Formation

The results of the present study showed that 50 isolates out of 55 isolates (92.7 %) produced this enzyme, The results of the present study are consistent with the findings of [(12)]where all *S. aureus* isolates of hemolycin were produced by 100%.

Capsaule Formation

The susceptibility of *S.aureus* staphylococcus aureus to the composition of the capsule under study reached 27.2% of the total of 55 isolates.the results were in agreement with the result[(13)] which recorded 42% of S.aureus.

Biofilm formation by the isolates of S aureus bacteria under study

The results showed that all the bacterial isolates under study (55) isolates arecapable of producing the biofilm using Microtitration plates method (MTP).where the results of our current study agreed with the results of researchers[(14)].

Table2: Susceptibility of S. aureus isolates to MTP formation

Isolates source	Isolates number	Absorption values for the number of isolates										Mean
Blood	26	.244	124.	160.	147.	394.	418.	520.	412.	581.	486.	263.
		.416	.130	150.	300.	340.	132.	135.	166.	170.	200.	
		.140	125.	220.	341.	201.	221.					
Wound swab	4	160.	073.	105.	106.							364.
Urine	11	147.	120.	121.	407.	170.	123.	130.	320.	330.	405.	220.
		193.										
Bone marrow	2	120.	107.									167.
Endocarditis	4	105.	100.	107.	103.							336.
Throat swab	2	164.										156.
Sputum	1	213.										213.
Ear swab	2	102.	087.									095.
Pus abscess	3	081.	102.	085.								587.
Nasal swab	1	090.										090.

Plasma coagulation enzyme Formation Coagulase

All the isolates of S. aureus bacteria under study showed that they have the ability to produce plasma coagulant enzyme (90.9%). Our results are in line with the results of[15] which produced 100% isolates for plasma coagulant enzyme.

DNase Formation

The results of this enzyme revealed that 43 of the 55 isolates were isolated by 78%.our results were agreement with the results[16].

Molecular detection of virulence genes c-

The results of the detection of the gene responsible for the production of coenzyme Coagulase (*Co a*) ، that the proportion of the isolation of MRSA isolates of the *Coa* gene is (95%),that came with results[17] and the proportion of the presence of the gene encoded *coa* gene for the production of cookies in the current study is expected as researchers[18] Production of all the strains of *S. aureus* bacteria, including MRSA. The *mecA* antibiotic resistance gene (*mecA*) showed that all 40 MRSA isolates (100%) possessed the *mecA*-resistant antibiotic gene *mecA* (Figure 1). 74.1% of *mecA*-containing isolates were consistent with the results [19], which obtained 100% of the presence of *mecA*

gene in its isolates, while the detection of the gene responsible for total erythrocyte degradation α) *hla* gene) The percentage of *hla* gene in the current study is (97.5%) where these results are consistent with [20], which possessed isolation belonging to your Tria S. aureus on the *hla* gene 100% As for the gene responsible for partial erythrocyte degradation (l) gene (*hlb*), the results showed that the proportion of its presence in isolates under study is (75%) and this was contrary to what reached [21] that got a 40% presence of the *hlb* gene in *S. aureus*.

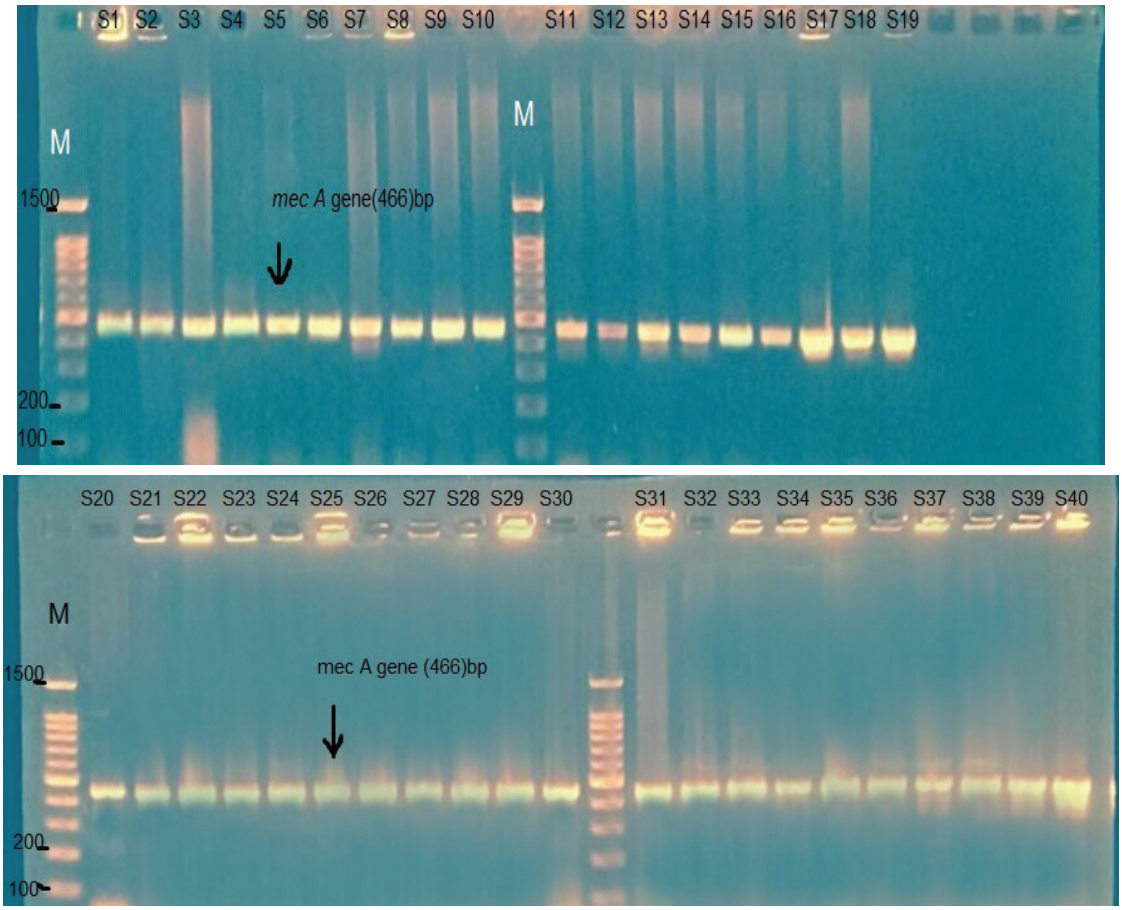

Figure (1) *mecA* gene amplification products for Staphylococcus aureus bacteria and electrically carried on the acarose gel (1.5%) and voltages (100) for an hour using PCR technique. Since M = DNA Ladder marker (100-1500bp), S = Staphylococcus aureus, all isolates showed a positive result of the mecA gene responsible for methicillin resistance in MRSA

Genetic detection of genotypes of some virulence factors for each type of staphylococcus under study, including the genotype responsible for the production of both types of hemolysin, the methicillin-resistant gene, and the blood plasma coagulation gene, was confirmed by the results above which confirmed the phenotypic detection results. Determinants (virulence factors) In addition, the above explanations regarding the results of phenotypic detection are fully consistent with the results of genetic detection

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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