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MOLECULAR AND BACTERIOLOGICAL STUDY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DIFFERENT SOURCES

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Abstract

The study included collection of 186 different samples in period between October to December 2018 from different hospitals (Alyarmok medical city and Tikrit teaching hospital). In order to isolate different types of Staphylococcus which are isolated from different sources like wound infection, burn infection, urinary tract infection, otitis media infection and contamination of operations halls). In order to know its ability to methicillin resistant and partial investigation of two specialist genes which are MacA, coa. The study shows about 126 samples which gives a positive result in 67.78% from it, about 26.34% from wound infection, 21.5% from otitis media infection 10.8% from urinary tract infection, 2.15% from burn infection 6.99% from operations halls contamination. The virulence factors that the results have shown the ability of *S. aureus* to product hemolycin in 86.76%, DNase in 89.7%, Urease in 98%, Lipase in 52.94%, Lecithanase in 47.0%, protase in 58.82%, B-Lactamease in 47.6%, coagulase enzyme in 83.82%. 11 types of antibiotic have been chosen S. aureus more resistant to Methicillin and cefachlor antibiotic and less resistant to levofloxacin antibiotic. 17 isolations were selected, depending on their resistant to antibiotic and their production of most of virulence factors then to evaluate each gene, MecA, Coa by specialized prefixes in polymer chain reaction (PCR) technology where they appeared, the PCR results showed that the presence of gene MecA in 9 isolations of *S. aureus* in 34.62%, while present the gene Coa in 73.1% in 19 isolations from *S. aureus*. They found that there is no relationship between the gene and injury sites.

Key words : *Staphylococcus aureus,* methicillin resistant, PCR, MecA, Coa gene.

Introduction

Staphylococcus aureus is considered to be one of the most important species in the medial family and is characterized by its wide range Spread in nature, as it is present in the air, soil, mucous membranes, skin, upper respiratory tract and human digestive tract (Herbert *et al*., 2001).

This is a bacterial type Characterized by the emergence of strains resistant to antibiotics, so it became a major cause of Nosocomial infections and high rates worldwide (Kozio³-Montewka *et al.,* 2006).

Methicillin Resistant *S. aureus* (MRSA) is one of the most serious skin and soft tissue pathogens and is often resistant to most antibiotics. Some healthy individuals may be carriers of this bacterial pathogen (Ibler & Kromann, 2014).

The cause of resistance to *Staphylococcus aureus*, especially to the antibodies of the beta lactam group comes from their ability to produce beta-lactase enzyme in addition to its ability to generate internal resistance by owning a MecA gene, which reduces the amount of Penicillin binding protein and this protein is not familiar with the association with anti-beta lactam (Chan *et al.*, 2016)

PCR technique has been used to detect some of the virulence and antibiotic resistance as well as genetic elements related to pathogens without resorting to testing of antibiotic sensitivity, isolation and diagnosis (Nassif and Sansonetti, 1989).

The present study aims to isolate and identify of

Staphylococcus aureus (*S. aureus*) from different sources like wound infection, burn infection, urinary tract infection, otitis media infection and contamination of operations halls). Polymerase chain reaction PCR was tested and used to determine some of the virulence factors by Specific primers.

Materials and Methods

Collection of *S. aureus* isolates

A total of 186 patients, aged 1 to 82 years, were taken from Al-Yarmouk Hospital and the City of Medicine in Baghdad city, Tikrit Educational Hospital in Salah al-Din, and between October -December 2018, of which 70 were wound, 52 were flexible Otitis media, 43 samples of urinary tract infection, 8 samples of burns, and 13 samples of contamination of operating theaters. The samples were distributed to the Mannitol salt Agar and incubated at 37 ° C for 24 hours (Brown *et al.,* 2005).

Identification of *S.aureus*

The selection of staphylococci was on the basis of colony morphology, Gram staining, biochemical tests which are Mannitol test, catalase test, Oxidase test, Coagulase Test (Harley& dan Prescott,2002) Confirm diagnostics using a device Vetik 2 compact system.

Antimicrobial susceptibility testing

The sensitivity of eleven types of antibiotics was tested, depending on the disc diffusion method as recommended by (NCCLS,2004)The antibiotics used in the current study are (Vancomycin(VA/5), Ampicillin sulbactam(AM/20), Cefachlor(CFC/30), Ciprofloxacin(CIP/5), Amikacin(AK/10), Gentamicin(CN/30), Nitrofurantoin(F,100), Oxacillin(OX/5), Levofloxacin(LEV/5), Trimethoprim(TMP/5), Methicillin(MEC/10).

Determination of Virulence Factors

Some virulence factors (Blood Hemolysin, DNase, Protease, Urease, Lecithinase and Lipase and Betalactamase test) were investigated and the tests were applied on ten isolates that identified as Methicillin resistant(Morello *et al.*,2006).

Genomic DNA Extraction

26 isolates of bacteria were selected based on their resistance to antibiotics and their production for most factors The severity was selected by 10 isolates from wounds, 7 from middle ear infection, and 5 from infection Lounges, 2 burns and 2 UTIs for the purpose of DNA extraction(Onasanya *et al.*,2003)**.**

Determination of concentration of bacterial DNA

Using the Nano drop device, where 1 microliter was taken from the sample and placed in the place assigned to the device. After the operation of the device, the concentration and purity were recorded. The diluted DNA absorption was measured at 260 and 280 nm (Al- Noaami, 2014)

Polymerase chain reaction

Used primers that target the quality of common sequences of genes (Mec A, Coa)

Amplification program for, Mec A gene the reactions mixtures Depending on the (Ameen, 2014) included an initial denaturation at 95°C for 5 min consisted of 30 cycles of 95 ºC for 1 min, specific annealing temperature 58æ% C for 1 min, 72 ºC for 1 min and a final cycle of primer extension at 72°C for 5 min in a Thermal Cycler. Coa gene the reactions mixtures Depending on the(Tiwari *et al.*,2008) included an initial denaturation at 94°C for 4 min. specific annealing temperature 54 ºC for 1 min. and 72 ºC for 2 min, and a final cycle of primer extension at 72°C for 5 min.

Results and Discussion

The results have appeared in table 2 showed that (126) samples and 67.78% Showed bacterial growth while 60 samples and 32.22% did not produce bacterial growth, and no bacterial growth which may be due to the use of antibiotics, or the pathogen may not be germs and studied, or the pathogen may be difficult to detect in normal ways (Eykan, 2001)

The wounds infections were highest isolation rate for *Staphylococcus aureus* as in the Fig. (1) and at 38%, and agreed with what he found (Kluymans *et al.,* 1997). Followed by middle ear infection (34%) due to the high resistance to *S. aureus* bacteria to several types of antibiotics or may be present natural opportunistic infections and become pathogenic in case of low body resistance (Zhu *et al.*,2008). the spread of infection in hospitals due to close contact and the point of hospital staff and patients carrying *S. aureus*, especially resistance to methylene in their noses or on their skin (Afrough *et al.*, 2013).

Table 3 shows the most important biochemical tests of *Staphylococcus aureus* and the diagnostic step using the Vetik 2 is a complementary and confirmed diagnostic.

All these results of the detection of some virulence factors produced by Staphylococcal isolates are illustrated in Table 4. Shows the production of Hemolysin, DNase, Urease, Lecithinase, lipase, protease, B-Lactamase and coagulase production were detected in this study.

Table 5 showed an extremely high rate (80%) of resistance to Cefachlor, Methicillin, Ampicillin sulbactam

Table 1: Specific primers used.

Gene	Primers	Primers sequence(5-3)	Refrains
mec A	Forward	AAAATCGATGGTAAAGGTTGGC	[12]
	reverse	AGTTCTGCAGTACCGGATTTGC	
Coa	Forward	GATTTTGGATGAAGCGGATT	[13]
	reverse	ATACTCAACCGACGACACCG	

Table 2: Numbers and percentages of isolated samples.

Type of samples	Total samples		Positive samples		Negative samples	
	No.	%	No.	%	No.	%
wound infection	70	37.60	49	26.34	21	11.30
burn infection	8	4.30	4	2.15	4	2.11
urinary tract infection	43	23.10	20	10.8	23	12.36
otitis media infection	52	28.0	40	21.5	12	6.45
contamination of operations halls	13	7.0	13	6.99	0	0

Table 3: The biochemical tests of *Staphylococcus aureus.*

Test	Result
Oxidase	-
Catalase	+
Coagulase	+
Mannitol fermentation	+
Lipase	+
Proteinase	+
Lecithinase	+
DNase	+
Urease	+
Hemolysin	+β and α

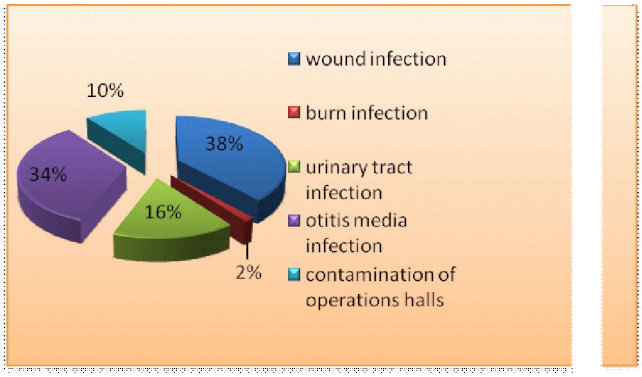

Fig. 1: Isolation percentage of *S. aureus.*

and Oxacillin These levels of resistance are comparable to levels obtained in a previous study was observed Most studies suggest that bacteria are resistant to most antibiotics especially beta-lactam (Belongia *et al.*, 2005, Hajo Grundmann *et al.*, 2010).

Staphylococcus aureus resists to Methicillin through the production of penicillin-binding protein (PBP) protein, which lowers the direction of other beta-lactam (Lowy, 2003).

While isolates are sensitive to Trimethoprim, Ciprofloxacin, Levofloxacin, Amikacin and Vancomycin was observed rates reach to 51.92%, 50%, 44.23%, 40.38% and 38.46% respectively. These results agreed with many other studies in the world similar findings were reported in (Nimmo *et al.*, 2000, Hsu Li-Yang *et al.*, 2005).

The Ciprofloxacin is a broad spectrum which inhibits the efficacy of DNA gyres in bacteria, can increase with the enzyme complex and decompose two DNA sequences.

The results showed relationship between (MRSA &MSSA) that 87.36% of the

Table 4: Relationship between virulence factors and site of injury.

Antibiotics	No.	Resistant (%)	Susceptible (%)	Intermediate (%)
CFC	52	48(92.31)	4(7.69)	0
MEC	52	45(86.54)	5(9.62)	2(3.85)
AM	52	44(84.62)	1(1.92)	7(13.46)
F	52	40(76.93)	6(11.54)	6(11.54)
OX	52	43(82.69)	9(17.31)	0
VA	52	30(57.69)	20(38.46)	2(3.85)
TMP	52	25(48.1)	27(51.92)	0
CN	52	28(53.84)	19(36.53)	5(9.62)
AK	52	29(55.77)	21(40.38)	2(3.85)
CIP	52	22(42.22)	26(50)	4(7.69)
Lev	52	26(50)	23(44.23)	3(5.78)

isolates were resistant to Methicillin. *Staphylococcus aureus* was resistant to this antibiotic through the production of penicillin-binding protein (PBP), which decreased the direction of the other beta-lactam antagonists, symbolized by pBp2a table 5 showed (MRSA) high resistance to most of the antibiotic under study compared with (MSSA), which showed low resistance. The resistance is due to cross Resistance because pBp2a is Little familiarity for association with beta-lactam and cephalosporins on the cell wall MRSA (Lowy, 2003).

Detection of mecA gene or its protein-binding protein (PBP2a) by PCR is the gold standard for MRSA detection. In this study, the table 6 show 9 positive isolates of total 26 isolates recorded in the current study, and can explain the lack of expression of the gene in return for the creation of disease through several mechanisms. These mechanisms are: 1- Excessive production of penicillinase and 2 -the presence of modulation in pbp2

Table 5: Antibiotic Susceptibility of *S. aureus.*

Virulence factors	wound infection		burn infection		urinary tract infection		otitis media infection		contamination of operations halls		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hemolysin	25	36.8	1	1.5	9	13.2	19	27.9	5	7.4	59	86.8
DNA	24	35.3	1	1.5	9	13.2	20	29.4	7	10.3	61	89.7
Urease	26	38.2	1	1.5	11	16.2	22	32.4	7	10.3	67	98.5
Lecithinase	15	22.0	1	1.5	5	7.4	8	11.7	3	4.4	32	47.0
Lipase	16	23.5	1	1.5	7	10.3	10	14.7	5	7.4	36	52.9
Protease	17	25.0	1	1.5	7	10.3	10	14.7	5	7.4	40	58.8
B-Lactamase	10	14.7	0	0	6	8.8	11	16.2	3	4.4	30	47.6
Coagulase	21	30.9	1	1.5	7	10.3	20	29.4	5	7.8	57	83.8

Table 6: Percentage of MecA gene spread in *Staphylococcus aureus* by location of infection.

Source of isolation	No.	%
wound infection	5	19.2
otitis media infection	1	3.90
contamination of operations halls	3	11.52
Total	9	34.62

Table 7: Percentage of Coa gene spread in *Staphylococcus aureus* by location of infection.

Source of isolation	No.	%
wound infection	7	43.75
otitis media infection	4	25
contamination of operations halls	4	25
urinary tract infection	1	6.25
Total	16	100

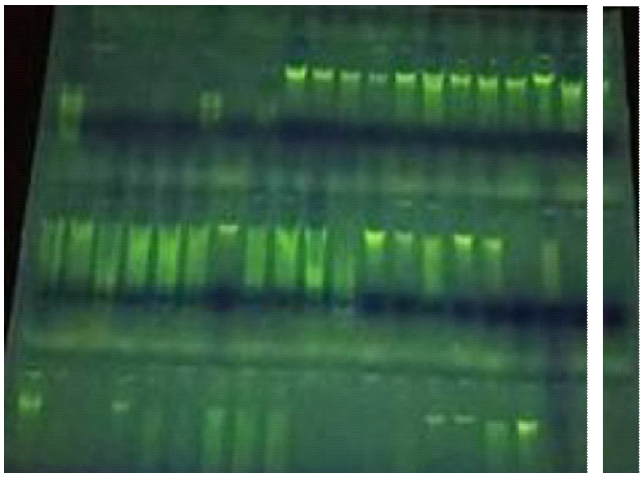

Fig. 2: Packaged DNA for Staphylococcus isolates after being carried on a 0.8% gel.

or it may be due to inability to express of mecA gene (Santos *et al.*, 1999).

Path 1 100pb DNA leader.

Paths 2, 3, 6, 7, and 8 are the result of MecA gene from the DNA of wound isolates.

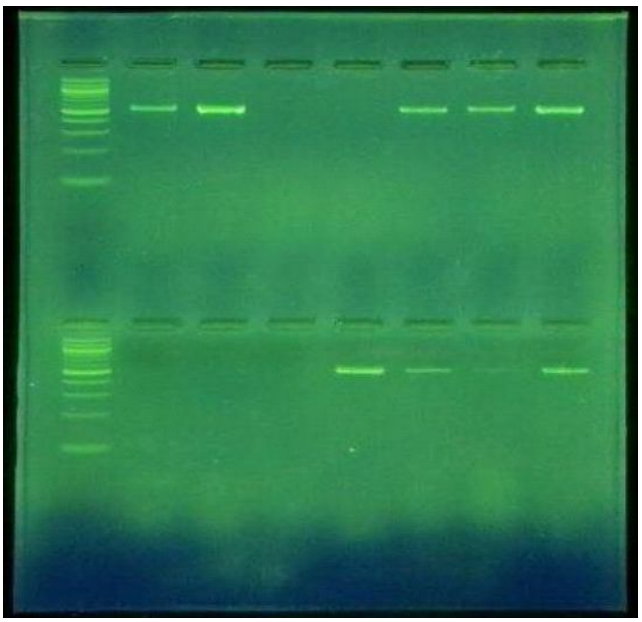

Fig. 3: Results of DNA polymerase chain reaction for *S. aureus* isolated from wound and otitis media infection and contamination of operating theater for MecA gene.

Path 13, 14 and 15 MecA gene from DNA isolates contamination of operations halls.

Path 16 is the result of MecA gene of DNA isolated from the otitis media infection

The table 7 shows 16 positive isolates recorded in the current study, the Coa is virulence factor of staphylococcus aureus (Motamedi *et al.,* 2015). The end of 3 of the Coa gene contains a sequence of tandem duplicates 81- bp, which varies between the staphylococcus strains (Aslantas *et al.,* 2006), the coa classification which was considered a simple and accurate method for the diagnosis of *S. aureus* isolated from a different source (Bukowski *et al.,* 2015).

Path 1 100pb DNA leader.

Path 2and 3 the process of amplifying the Coa gene from the DNA of wound isolates

Path 4 The process of amplifying Jin Coa of DNA isolates burns

Track 5 process amplifies the Coa gene from the DNA isolates of the urinary tract

Track 6 process amplifies the Coa gene from the DNA of the middle ear isolates

The path 7 and 8 process amplify the Coa gene from the DNA isolates of the operating theaters

Conclusion

Staphylococcus aureus are more common in different sources, where the wounds ranked first in terms of isolation and then the otitis media infection, urinary tract infection, contamination of operations halls and burn infection, and showed high resistance to Cefachlor, Methicillin and less resistant to Levofloxacin.

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