Molecular Detection of PVL Genes in Methicillin-Resistance Staphylococcus Aureus that Isolated from Ramadi Hospitals Patients

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Abstract

Pantone Valentin Leucocidin (PVL) is a cytotoxin produced by methicillin-resistant Staphylococcus aureus, and it is one of the most dangerous pathogens that threaten health, as it causes infections of the skin and soft tissues. The polymerase chain reaction (PCR) is applied to detect MRS and determine the prevalence of virulence genes, the most important of which are genes luk-pv (lukS-pv,lukF-pv), The species is detected using the 16SrRNA gene. 125 clinical samples were collected from different cases (wounds, burns, urine, suppurations and abscesses of the skin, ear) from the main hospitals in Ramadi (Al Ramadi Teaching, Women and Children Hospital) for the period from month (11-2021 to 1-2022). Twenty-six MRSA isolates were identified by PCR by 16SrRNA gene. The isolates were 96% luk-pv positive. The results showed a diverse profile of resistance to 9 antibiotics, as the total resistance was 100 %against clindamycin, cefoxtin, and erythromycin. It also gave a high resistance to azithromycin by 96.15%. While the resistance was moderate towards lincomycin and tetracycline (42.30%, 46.15%) respectively, whereas the resistance to ceftriaxone, gentamycin and doxycycline was low (11.53%, 11.53%, 3.84%) respectively. While the sensitivity of the isolates was high against gentamycin by 80.76% and moderate against doxycycline by 65.38%. Monitoring and research on MRSA carrying the PVL gene should continue to provide important insight into the spread of these resistant pathogens.

Keywords: Staphylococcus aureus, MRSA, PVL, lukS-pv, lukF-pv

1. Introduction

Staphylococcus aureus is one of the most common pathogens that cause community and hospital diseases and is found in about 30% of the natural environment. (Tang et al., 2013). Its ability to produce many virulence factors helps it to penetrate tissues, generate infections and resist many antibiotics, which makes it a source of threat to patients, especially burns and wounds patients, through its entry into the bloodstream, causing blood poisoning (septicemia) and the situation is worse in patients who have immunodeficiency (Jalil et al.,2017).

Methicillin-resistant Staphylococcus aureus (MRSA) infections are frequent in both community and health care settings (hospital infections) and are associated with morbidity and mortality and require high medical costs (Purrello et al., 2016) Methicillin-resistant Staphylococcus aureus caused a medical danger100 a year ago, as a result of causing lung infections, abscesses, meningitis and septicemia, causing epidemic waves and high deaths (Etinosa et al., 2016).

Toxin Panton-Valentine- Leukacidin(PVL) production in strains of Staphylococcus aureus has been associated with several diseases, from mild skin and soft tissue infections to fatal infections such as necrotizing pneumonia in healthy young adults, who suffer from influenza-like symptoms and have a high mortality rate (Khodamoradi et al.,2019;Tang et al.,2019). PVL It is a poreforming cellular toxin that destroys white blood cells and necroses tissues (Gillety et al., 2002). It is a binary component of cytotoxin that is expressed by some strains of bacteria Staphylococcus aureus and was named in1932 the year of the two scientists (Sirphili Noel Panton and Francis Valentine (Bhatta et al., 2016). It is encoded by two of the co-transcribed genes (lukS-pv and lukF-pv) which are carried by phages (Okolie and James, 2015).

lukS-pv and lukF-pv they are two types of proteins secreted by Staphylococcus aureus bacteria. They first bind lukS-pv a high-affinity receptor on the host cell membrane, then form lukF-pv double bonds that stick to the cell membrane and then release these dimers in an octagonal ring structure and form pores on the host cell, which leads to cell damage (Alonzo and Torres, 2014) as the figure 1.

The aim of our study was to investigate the genes PVL (lukS-pv,lukF-pv) present in methicillin-resistant Staphylococcus aureus bacter.

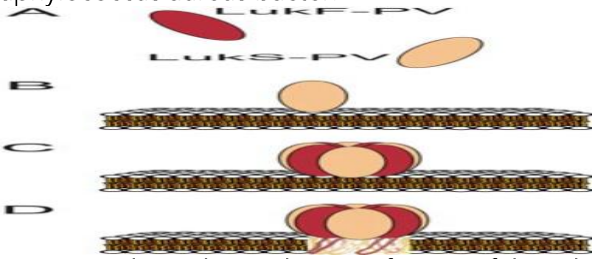

Figure (1): shows the mechanism of action of the pvl toxin genes.

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2. Materials and Method

1-Collection and identification of isolates

125 samples were collected from patients admitted to - Al Ramadi Teaching, Women and Children Hospital in Al-Ramadi for the period from month (11-2021 to 1-2022), where the samples were collected under sterile conditions (wounds, burns, urine, suppurations and abscesses of the skin, ear). Microscopic, biochemical tests and using several media, where the growth was done on mannitol saline medium of Staphylococcus aureus bacteria, as well as it was planted on blood agars to diagnose its culture characteristics, color and shape of the developing colonies, MacConkey agar medium was also used to distinguish positive bacteria from Gram-negative bacteria.

Biochemical tests such as indole, methyl red, foxproskaure, consumption of citrate, oxidase and catalase, blood plasma coagulant production test, DNA degrading enzyme and acetone production test.

2-Genetic diagnosis of methicillin-resistant S. aureus

A-DNA extraction: DNA was extracted and purified using a total DNA extraction kit (Wizard®PCR Preps) According to the instructions manufacturer (Promega). Of the American manufacturer (Promega).

B-Molecular investigation of the 16S rRNA gene and PVL genes using polymerase chain reaction (PCR):

The Universal 16SrRNA gene was used for the final molecular diagnosis of the bacterial isolates under study, by means of a polymerase chain reaction (PCR), and the use of primers for each of the 16SrRNA gene, lukS-pv gene and lukF-pv gene.

Prepare the PCR mixture from 12.5 µl Green Mastermix, 5 µl DNA Template, 1.5 µl F-primer, 1.5 µl R- primer and 4.5 µl Nuclease-Free Water. The final volume is 25 µl, and then the contents of the PCR tubes were mixed well using Vortex and then placed in a PCR device according to the following program, as shown in table (1).

Table (1): Primers sequences used in this study.

Gene	Sequence.	Product	Annealing	Refrance
16srRNA	CGATTCCAGCTTCATGT TGTCGTGAGATGTTGGG	270 bp	47	Stutz et al.,2011
Luks	TTCAGGGTTTTCAACAGTAGCA ACACAATTGCCAGCGGTAAAA	149 bp	52	Deafness
Lukf	GTTTTCGCCAGACCAATAGCC GCCTGTAACTGTGTCTGAAGG	169 bp	53	Deafness

After that, 5 µl of the gene's duplication

Product were transferred for electrophoresis to the prepared agarose gel in concentration 2%, to detect DNA bundles.

3- The Antibiotic Susceptibility

A test of 26 isolates of MRSA was carried out in Mueller-Hinton medium, The diffusion around pits method was used(CLSI, 2020) to measure the areas of inhibition against the slandered concentrations of the following antibiotics:(Ceftriaxone,CEF 30µg),(Erythromicin,E 15 µg),(Azithromicin,AZM 15 µg),(Tetracycline,TETRA 30 µg),(Doxycycline,DOXY30 µg),(Gentamicin,GM10 µg),(Lincomycin,LINC15 µg),(Clindamycin,CD2 µg), (Cefoxitin,CX30 µg).

3. Results and Discussion

1- Collection and identification

The isolates were diagnosed based on the results of bacteriological and biochemical tests and the use of selective culture media, As 105 samples gave positive growth, 80 (76.19) %were positive for gram stained isolates, while the negative isolates for gram stained were 25(23.8) % isolates, The results of the initial diagnosis showed that the positive isolates of gram-positive isolates were 50 of them belonging to Staphylococcus aureus bacteria, where 26 (52) %isolates were resistant to the antibiotic methicillin (MRSA) and 24(48)% isolates are sensitive to it(MSSA) . Colonies of Staphylococcus aureus appeared with a yellow color on the solid saline mannitol medium as in Figure2, indicating the fermentation of manthol salt (Jayasundara, 2014; Bush and Jacoby, 2010). As for the blood agar medium, it was gray in color as in Figure3, A study was conducted in Najaf, Iraq (Al-Mohana et al.,2012) that out of 286 isolates of Staphylococcus aureus, 54(18.8)% isolates were methicillin-resistant (MRSA) and this percentage is agreement to the current study. The researcher also found (Song et al., 2011). In a study conducted in India that the MRSA ratio was 22.6%, and in Korea it was 77.6%, and in Sri Lanka it was 86.5%, and this percentage is agreement to the current study. While the rate of the presence of MRSA in Hong Kung was 56.8% and this percentage is greement to the current study. The researcher also found (Le Van Nam et al., 2019) that the rate of MRSA was 53.5% and this percentage is greement to the current study.

The difference in the rate of the presence of MRSA between different countries is due to the environmental conditions, the place of conducting the study and the time period, as well as the frequency to constantly healthy places and direct contact with people carrying the MRSA bacteria without being careful.

Figure (2): Growth of Staphylococcus aureus on mannitol salt agar

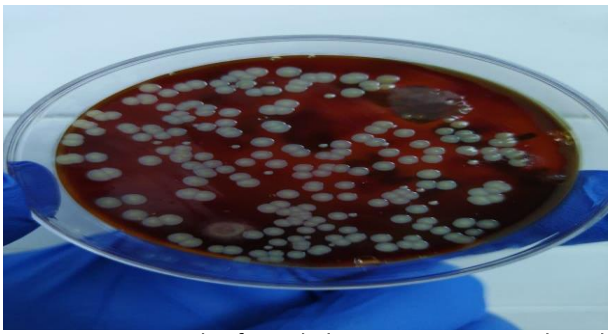

Figure (3): Growth of Staphylococcus aureus on bood agar medium.

And the vital chemo tests shown in the

Following table (2)	
TEST	S.aureus
Coagulase	+
Oxidase	-
Catalase	+
Indol	-
Methyl Red	+
Vogas Proskaur	+
Citrate utilization	+
Acetoin	+
DNase	+
Urease	+
Mannitol	+
Gelatinase	+

2-Genetic diagnosis of methicillin-resistant S. aureus

A-DNA extraction: Total DNA was extracted for S.aureus isolates using genomic DNA extraction Kit (Promega), all the concentration between (11 - 130 ngand µl)

b- Molecular Diagnostics: Staphylococcus aureus isolates were diagnosed using the specific initiator gene by 16S rRNAby PCR using thermal cycles. As the results showed that the isolates belong to Staphylococcus aureus as in Figure 4, 16S rRNA PCRproved to be successful in identifying multiple types of bacteria from multiple types of samples (Matsuda et al., 2017). Also pointed out (Khudhr, 2016; El-Hadedy and El Nour, 2012) the procedure of activating the PCR using the gene 16S rRNA is an identity for Staphylococcus aureus, and in this way the molecular detection of Staphylococcus aureus is done.

In order to detect the PVL (lukS-pv and lukF-pv) genes, the respective primers were used and diagnosed by PCR, where the results of detection for PVL genes were 96% positive.

It was found The researcher (Karim,2016) in Baghdad found that MRSA isolates contain the PVL gene at a ratio of 6.55% and this percentage is agreement to the current studyalso found that the (luk-pv) was 53.48%, and. this percentage is agreement to the current study(Kandala et al.,2017). While the researcher (Al-Hassnawi et al., 2013) found that the percentage of the presence of the (luk-pv) genes is 79%, and this percentage is greement current study. As for the researcher, (Rahama et al., 2017) he found that the (luk-pv) genes are carried by 31.7% percent, and this is agreement current study, While the researcher (Kadhim et al.,2020) found that the (luk-pv) genes have a tolerance of 91.40% and This result is gremeent to the current study.

Been confirmed by analysis of the bands on gel electrophoresis and by comparing their molecular weight with 270 bp DNA Ladder.

This study shows that the (luk-pv) gene is very high, as it is spread by 92% among the MRSA strains in Ramadi patients, PCR technology is highly accurate, and so it is recommended for gene detection to determine MRSA in a routine diagnostic laboratory. Low (luk-pv) genes have been reported in other parts of the world as it was 5% in France, 8.1% in Saudi Arabia, 4 in the United Kingdom and 14.3% in Bangladesh (Afroz et al.,2008) athat there is a great discrepancy in the prevalence of (luk- pv) genes between these regions and other geographical regions, where the researcher Kaure found that India reported 62.85% of the total prevalence of (luk-pv) genes between MRSA and MSSA, If the MRSA ratio is 85.1%, the MSSA ratio is 48.8%

(Jahnsson et al., 2004). The (luk-pv) gene was also found in two isolates, (2.19%) out of 91 patients suffering from skin diseases and in four isolates (7.27%) out of 55 patients with respirat ory infection, and in one isolate (1%) out of 65 patients infected with Staphylococcus aureus.

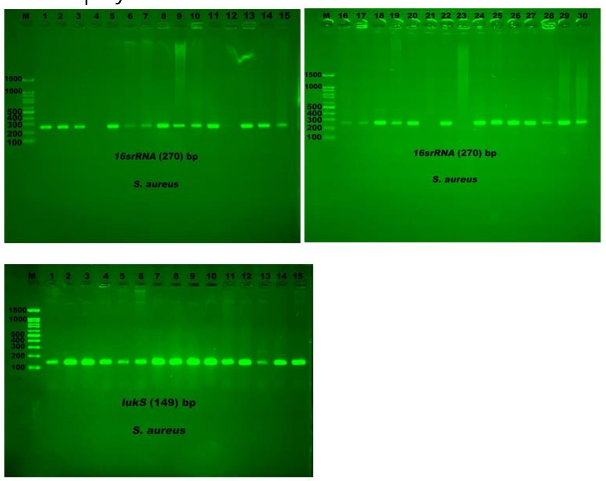

Figure.4: Gel electrophoresis of PCR products for tow targeted genes in Staphylococcus aureus. (2%) agarose gelat 80Vfor 60 mint).

3- The Antibiotic Susceptibility

The susceptibility of isolates of methicillin-resistant

Staphylococcus aureus to (9) antibiotics was tested. As shown in table (3), according to Clinical Laboratory Standards Institute (CLSI) guidelines.

Table (3): Percentages of antimicrobial susceptibility rate of 26 S.aureus isolates against 9 antimicrobial agents.

	S		I		R	
Antibiotics	NO.	%	NO.	%	NO.	%
Ceftriaxone	1	3.84	22	84.61	3	11.53
Erythromicin	0	0	0	0	26	100
Azithromicin	0	0	1	3.84	25	96.15
Tetracycline	0	0	14	53.84	12	46.15
Doxycycline	17	65.38	8	30.76	1	3.84
Gentamicin	21	80.76	2	7.69	3	11.53
Lincomycin	0	0	15	57.69	11	42.30
Clindamycin	0	0	0	0	26	100
Cefoxitin	0	0	0	0	26	100

The reason for MRSA resistance to antibiotic cefoxitin 100% percent is due to its production of broad-spectrum beta-lactamase enzymes, which are either plasma or chromosomal in origin (Shaikh et al., 2015). Also found in a study he conducted in Sokoto Nigeria, The resistance of MRSA to antibiotic was 100% (Adeiza et al., 2019). The antibiotic erythromycin had a resistance rate of 100%, it is due to possessing the resistance genes that are of plasma origin, as it encodes to modify the target site 50S, which is the antigen binding site, and this makes it unable to bind to it, leading to bacterial resistance to it (Reygaert, 2013). Also found the rate of resistance to the antibiotic erythromycin was 100% (Adeiza et al., 2019). While the resistance of the isolates to this antibody was 82.61%, and this resistance is also high (Le Van Nam et al., 2019).

Clindamycin is of the lincosamide family, and this antagonist is of great importance in the treatment of infections caused by methicillin-sensitive Staphylococcus aureus, as well as individuals who are allergic to penicillin (Rayner and Munchof, 20 05) The resistance of MRS to him was by 100%, while found that the resistance of MRS to the anti-clindamycin was by 52.3, and this result does not agree with our study (Giri et al., 2019). The antibiotic azithromycin belongs to the family of macrolides, to which the resistance of the isolates was 96.15, while it was found that the resistance of bacteria to azithromycin was 39.51%, and this result does not agree with our current study.

Tetracycline is a relatively safe and effective antibiotic, and bacterial resistance can return by 46.15% percent due to its frequent use topically for burns and wounds and the treatment of infections, or because of a mutation that has an effect on the exudation of the outer membrane of the antibiotic ((Sevgi et al.,2013).

The results of our study are consistent with the study conducted by the researcher (AL-Taee, 2018) where the resistance ratio was 48.8%. While one of the studies conducted in northern Vietanam indicated that the resistance of bacteria to tetracycline was 73.91, and this study does not agree with our current results (LeVanNam et al., 2019).The resistance to lincomycin and gentamicin was (42.30%, 11.53%) respectively, While a study conducted in Turkey indicated that the resistance of bacteria to lincomycin and gentamycin has reached (42.3%, 90.2%) respectively (Yildiz et al., 2014). Also, the sensitivity ratio for the antigen to gentamycin was 80.76%, while he indicated that the sensitivity of bacteria to this antigen was 8.5% (Yildiz et al., 2014).The sensitivity of bacteria to Doxycycline was 65.38%, and this result is close to the results of the researcher (Giri et al., 2019) the sensitivity of bacteria to this antibiotic was 83.95% (Pickering et al., 2014).

The isolates showed a resistance to the antibiotic ceftriaxone by 11.53%, and their sensitivity to this antibiotic was 3.84%while it was found that the percentage of resistance to this antibody was 60%. While found (AL-Taee, 2018) the resistance of the isolates was 100%. Through our study, it was found that there is a difference in the rates of resistance of isolates to antibiotics between our study and previous studies.

4. Conclusion

This study shows that PVL genes are very high, as they are prevalent in 96% percent among the MRSA strains prevalent in patients in Anbar province.

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