The factors essential of MRSA (Fem gen) and (Mac gen) in Methilicine resistance Staphylococcus aureus

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Abstract
Background: Skin infections account for a significant portion of dermatologic diseases a disruption in the skins. The dermatophytes or ringworm infections are confined to stratum corneum or keratinise structures derived from epidermises, such as nails or hair, and these superficial infections are considered in this chapter. The natural course of infection is variable, and, in many instances, spontaneous remission occurs. Infections with Trichophyton rubrum, the commonest cause of dermatophytosis. Common skin infections include cellulitis, erysipelas, impetigo, folliculitis, and furuncles and carbuncles. Cellulitis is an infection of the dermis and subcutaneous tissue that has poorly demarcated borders and is usually caused by Streptococcus or Staphylococcus species. Erysipelas is a superficial form of cellulitis with sharply demarcated borders and is caused almost exclusively by Streptococcus. Impetigo is also caused by Streptococcus or staphylococcus and can lead to the lifting of the stratum corneum resulting in the commonly seen bullous effect. Folliculitis is an inflammation of the hair follicles.Aim of the study: using primers for amplification of to detect mecA gen **and Fem**A isolates of MRSA from skin infection and found the significant correlation between them or not Patients and methods: Skin infections with bacterial and fungal at isolation hospitals in Iraq (Al-Anbar provinces) were included in this study.skin swab and skin scrapping specimens were Committee/the University of Anbar.Results: A total of Seventy (70) patients both male and female were showing symptoms of The number of methillicine resisten staphylococcus aureus(MRSA) was(54),while methillicine sensitive staphylococcus aureys(MSSA) was(8),while staphylococcus epidermis was(8). While Tinea corporis was (70).
keywards: Methillicine Resistance Staphylococcus aureas (MRSA) , skin infection , dermatophytes ,MecA gene , Fem gen.

1. Introduction

Methicillin Resistant Staphylococcus aureus (MRSA), a significant pathogen associated infection acquired in the hospital (nosocomial infections), has become one of the worldwide health problems(1). A strain of S. aureus known as MRSA is resistant to the beta-lactam antibiotic class, which includes penicillin, cephalosporin, monobactam, and carbapenem. (2). Due to inappropriate antibiotic medication exposure, genetic alterations have resulted in this resistance. MRSA strains were first discovered in the UK in 1961. (UK)(3). To identify antibiotic resistance genes like mecA, genotypes for MRSA have been studied. (4). The gold standard for determining MRSA genotypes is to find conserved genes (fixed/preserved) consistently identified in the mecA gene, which is within the range of a certain chromosome in Staphylococcal Cassette Chromosome (SCCmec)(5). The mutant penicillin-binding protein 2a (PBP2a or PBP2') protein encoded by the mecA gene is what causes MRSA resistance. PBP is a collection of enzymes found in the cell membrane of S. aureus that catalyzes the trans-peptidation required to create peptidoglycan chains (crosslinkages). MRSA survives in heavy antibiotic exposure because PBP2a's affinity is so low. (6). The gold standard for the detection of mecA is polymerase chain reaction (PCR), which can be used to amplify mecA. (7). There is no information available on mecA on MRSA distribution in North Sumatra. In S. aureus, there is a specific gene called femA that encodes a 48 kDa protein involved in the formation of cell wall Methicillin-resistant Staphylococcus aureus (MRSA) a structural gene on the chromosome, the femA and femB(fem) genes encode proteins that affect the degree of methicillin resistance in S. aureus. (8).

2. Methods

Patient study and sample collection:

A total of 70 specimens obtained from patients admitted to dermatology Private clinics were studied during the period from December 2021 to April 2022.The patients were of different sex, out of 70 specimens, 55.5% specimens were female while the male gender were 45.5% specimens.
Isolation of MRSA/Sample Collection Total 70 samples will be collected from Ramadi Teaching

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Hospital in Ramadi the isolation of Methicillin-resistant Staphylococcus aureus (MRSA). A proper specimen collection is essential for accurate diagnosis and initiation of appropriate therapy. The sample for the study will pus swabs, wound swabs for the isolation of MRSA. The first step of the sample collection was through cleaning of infected site with normal saline. Sterile dry cotton swabs were used for the sample collection. for the collection of pus swab and wound swab, the swab was rolled gently and firmly on the base of the wound for the collection of pus swab from active wound. One sterile cotton swab for culture. Collected samples will be transported immediately to the laboratory and processed. the colonies of Gram-positive bacteria in cluster will be further confirmed by. The blood Agar media will be prepared by adding 10% blood in blood Agar base media. The swabs were inoculated on blood Agar and incubated at 37 C for 24 hrs. Staphylococcus aureus will be identified and differentiate from related organisms on the basis of colony morphology, on blood agar plates, colonies of Staphylococcus aureus are frequently surrounded by zones of clear beta-hemolysis. The golden appearance of colonies of some strains is the etymological root the bacteria's name; aureus meaning "golden" colonybacteria are grown on Mannitol Salt Agar of colony morphology, Staphylococcus aureus produces yellow colonies with yellow zones, whereas other coagulase-negative staphylococci produce small pink or red colonies with no color change to the medium. If an organism can ferment mannitol, an acidic by product is formed that causes the phenol red in the agar to turn yellow and then diagnosis byVitek compact 2

PCR detection of MecA and Fem A gene

The primers were designed based on the National Center for Biotechnology Information NCBI and provided by the Company as a lyophilized product of various concentrations of picomol. Solution Final concentration of 10 pmol/µl was prepared separately by dissolving 10µl of stock solution for each primer and added to 90µl free nuclease distilled water un-ionic(ddH2O), mixed well and kept in (-20oC). They were mixed by vortex to homogenize before use. The sequences used in the study for (Mec gene and Fem gene) listed in Table (1– 1).

Table (1 –1) Sequence of PCR primer and molecular size of PCR products.

Gene	Sequence of forward and reverse (primer 3/-5/)	Product (bp)	Ref
Mec	F/AAAATCGATGGAAAGGTTGGC	533	(9)
	R/AGTTCTGCAGTACCGGATTTGC		
Fem	F/AGACAAATAGGAGTAATGAT	509	(9)
	R/AAATCTAACACTGAGTGATA		

3. Result and discussion

MecA gene of methicillin resistance *Staphylococcus aureus*

Amplification of mecA gene target by polymerase chain reaction and electrophoresis by agarose gel electrophoresis, showed that, out of 54 specimens of Staph. aureus 41(76%) were positive for MecA gene while 13(24%) were negative. figure (1 – 1).

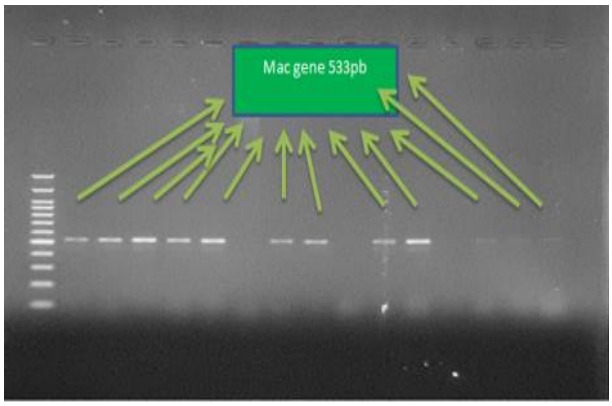

Figure (1 - 1): Gel electrophoresis Mac gen in staphylococcus aureus.gene size 533pb.

FemA gene of methicillin resistance *Staphylococcus aureus*

After amplification of femA gene target by polymerase chain reaction, this study showed that, out of 54 specimens of *Staphylococcus aureus,* 20 (37%) were positive for femA gene, while 34 (63%) were negative. figure (1 – 2).

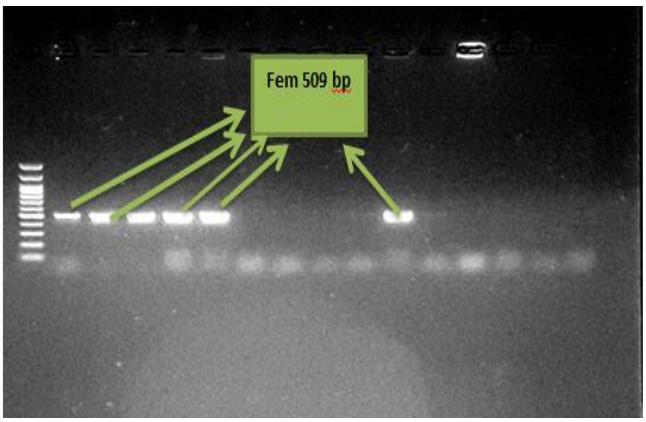

Figure (1 - 2): Gel electrophoresis Fem gene in staphylococcus aureus.gene size 509pb.

Methicillin-resistant Staphylococcus aureus (MRSA) is characterized by the structural gene mecA, and the genes femA and femB(fem) encode proteins that affect the degree of methicillin resistance in S. aureus. Therefore, the detection of femA and femB coupled with mecA by PCR was thought to be a more reliable indicator to identify MRSA by separating it from mecA-positive CNS than single detection of mecA in order to test efficacy of detecting mecA and fem genes in identification of MRSA.

1,069 S. aureus isolates, including 576 mecA-positive (mecA+) isolates, which are MRSA by definition, and 493 mecA-negative (mecA) isolates, which are MSSA, were examined for clonal connections and antibiotic susceptibilities. To examine isolates, these isolates were chosen from various sources. (10).

MRSA and MSSA with low levels of resistance, high levels of MRSA expressed femA at higher levels. MRSA likely contains a femA regulatory gene. The

expression of high levels of methicillin resistance appears to need femA (11).

The polymerase chain reaction amplification of the mecA gene target revealed that 50 (81.97%) were positive for the mecA gene, whereas 11 (18.33%) were negative for the mecA gene, bringing this study close to our result. (12). 114 (26.54%) of the 429 Staphylococcus aureus isolates were MRSA strains. For the PCR experiment, MSRA strains were chosen. 81 MRSA isolates (71.05%) were mec 33 (28.95%) MRSA strains were mec positive and a gene positive. On 1% agarose gel electrophoresis, a negative was seen. The PCR assay for the detection of mec was a quick and precise method. Using a gene of MRSA strains, as opposed to traditional techniques like cutler, biochemical, and microscopy, takes less time and is more effective for managing infections. (13).Results obtained by conventional methods .Methicillin-susceptible S. aureus(n =11) have (Fem gene 100%) and no Mec gene while Methicillin-resistant S. aureus (n=4) have both Fem and mec gene (14).Although mecA is the prerequisite of methicillin resistance and femA shows more correlation than other fem genes with methicillin resistance (15). other factors also influence the expression of methicillin resistance (16). However, methicillin resistance of such strains may be induced by b-lactam antibiotics (17).

According to the results there is a relationship between the presence of femA gene and resistance to methicillin in Staph aureus. Results is compatible with those observed by (9)who documented that the PCR product of femA gene was obtained from almost all the Staph. aureus 200 specimens' strains except for five oxacillin-resistant strains (2.5%), neither of these genes were detected. The result is incompatible with those observed who considered that the femA gene is essential for methicillin resistance(18). also concluded that out of 45 specimens, 34 strains contained the femA gene as detected. Phenotyping analysis showed that these 34 strains (femA-positive) were Staph. aureus (19).. Our result was incompatible with reported that the production of the femA fragment occurred in all Staph. aureus strains (20).

They include 70 specimens taken from skin infections. From this specimen 54(77%) were diagnosed as by laboratory steps MRSA Mec gene and Fem gene frequency showed that Mec gene &Fem gene negative 13(24%), Mec&Fem gene positive 20(37%) and Fem gene negative 34(63%), Fem gen positive 20(37%), Mec gene negative 13(24%), Mec gene positive 41(76%). table(1-2).

Table(1 - 2) MacA gene and FemA gene extraction by pcr frequency.

MRSA	MecA gene (+) ve No. (%)	MecA gene (-) ve No. (%)	FemA gene (+) ve No. (%)	FemA gene (-) ve No. (%)	MecA & FemA genes positive No. (%)	MecA & FemA genes (-) ve No.(%)
NO: 54	41	13	20	34	20	13
Percentage	76%	24%	37%	63%	37%	24%

There were two main reasons that the latter antimicrobial agent is effective on MRSA, the first one is that mecA gene which encodes the low-affinity penicillin-binding protein 2a, methicillin resistance in Staphylococci is due to the acquisition of the mecA gene, presence of the mecA gene defines the Staphylococcus as methicillin resistance (21). The other main reason is that femA gene is involved with biosynthesis of cell wall of bacterial cells, the femA gene is essential for the expression of methicillin resistance in Staph. aureus and is universally present only in Staph. aureus isolates, this gene has been implicated in cell wall metabolism and is present in large amounts in actively growing cultures (22). Transposons are genetic elements that contain several kbp of DNA, including the information necessary for their migration from one genetic locus to another. In doing so, they create insertion mutations (23). Transposon-mediated inactivation of methicillin resistance can be exploited as a tool to identify factors involved in cell wall metabolism (24).Transposon may play a role to interpret the results, Tn551 inactivation has identified several determinants femA gene that, in addition to the mecA gene, are also critical for the expression of high-level and homogeneous resistance to methicillin(25).

Results showed that there was no mecA positive in MSSA. This result was in agreement with those observed by (Arbefeville, and associates, (2011) who concluded that MSSA has no mecA gene and consider novel methods for the detection and differentiation of MSSA(26). On the other hand, results disagree with those observed by Rallapalli, and co-workers, (2008) who concluded that out of 55 Staph. aureus strains, three strains demonstrated mecA gene, which appeared to be oxacillin sensitive by disc diffusion method(27) . This may be due to agar disk diffusion which is the most used antibiotic susceptibility assay in clinical microbiology laboratories, but it is not particularly accurate because it may demonstrate a lack of reproducibility and sensitivity (28). The results disagree with those observed by Wielders et al., (2002) who concluded that several MSSA, have mecA gene (29). The presence of mecA gene is generally to indicate the potential resistance to beta-lactam group and used as a marker to identify MRSA. In this study, PCR product was shown as 533 bp in all resistant isolates using a primer designed by Pournajat et al. (2015) (30) . The similar result showed by Sudigdoadi (2010) in which all 45 isolates of MRSA were investigated, certainly has a mecA gene which is found in a 20-100 kb called staphylococcal cassette chromosome (SCCmec) (31, 32). MRSA resistance to methicillin and all beta-lactam group antimicrobial is due to

changes in normal penicillin binding protein PBP 2 to PBP 2a. Mutation to PBP 2a showed that the change in the binding site resulted in lower affinity to beta-lactam group (33)therefore if the bacteria are cultured in medium containing a high concentration of beta-lactams, they still survive and grow.

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