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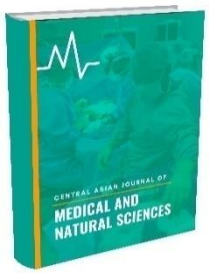

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DETECT SOME GENES (*TETK*, *HLB* AND *NUC*) OF STAPHYLOCOCCUS EPIDERMIDIS ISOLATED FROM ACNE VULGARIS

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Abstract: **Introduction:** Chronic acne vulgaris (AV) is a skin inflammatory condition that affects the pilosebaceous glands. **Aim:** Isolation and identification of Staphylococcus epidermidis from acne vulgaris in Dhi Qar city and study of some genes (tetK, hlb and nuc) of Staphylococcus epidermidis. **Materials & methods:** 210 specimens were collected (113 females and 97 male) from acne patients with age ranged from (14-38) years old. From August to September of 2022, a dermatologist from Thi-Qar province in Iraq used a private clinical lab to make a clinical diagnosis for the patients. Specimen were taken from the forehead, cheek, and chin of the patients. 70% ethanol was used to sanitize the sampling site. Transport media was employed to collect the samples. A lancet was used to make a scrape on the lesion's surface, and the fluid was then removed with light hand pressure in order to remove and extract the closed comedones and the papules. **Results:** It was discovered that 87 (41.4%) of the total samples showed positive results for the growth of S. epidermidis in the best cultured media, including blood agar, mannitol agar, and MacConkey agar. Out of all the samples, 123 (58.6%) showed negative results for S. epidermidis growth. Staphylococcus epidermidis showed total resistance to Penicillin, Oxacillin and Cefoxitin and total sensitive to Nitrofurantoin. The results of PCR assay revealed that 75 (86.2%) of S. epidermidis isolates giving positive results for tetK gene. Despite the fact the hlb gene finds in percentage 21 (24.1%), while 42 (48.3%) of S. epidermidis harbored nuc gene, as represented in Table (4-6). The whole genomic DNA of S. epidermidis isolates.

Key words: S. epidermidis, acne vulgaris, PCR, tetK gene.

Introduction

Acne vulgaris, commonly referred to as acne, is a skin disorder that affects people and is characterized by seborrhea, comedones (black and white spots), papules (pinhead-like growths), nodules (big papules), and scarring (Thappa et al., 2009). Skin with dense sebaceous follicles on the face, chest, and back is affected by acne (Benner and Sammons 2013).

There are two types of acne: inflammatory and non-inflammatory. Androgen stimulation results in lesions because it alters the pilosebaceous units. In the Western world, acne affects 80–90% of teenagers during adolescence; in rural societies, the incidence is reportedly lower (Berlin and Goldberg 2012; Dawson and Dellavalle 2013). The terms papules, scars, comedones, and pustules are all considered forms of acne. Acne vulgaris is the term used to describe the prevalent kind of acne. This kind of acne affects a lot of teens. In acne vulgaris, comedones are visible. Roughly 90% of persons experience it throughout their teens, and occasionally even as adults (Dawson and Dellavalle 2013). Modest to severe cases affect about 20% of persons. According to Spencer et al. (2009), acne is less common in rural locations and may not affect the non-Westernized populations of Paraguay and Papua New Guinea. Compared to men, it affects women 9.8% more frequently than men 9.0%. About 1% of men and 5% of women among persons over 40 experience issues (Dawson and Dellavalle 2013). People of all ethnic groups are affected, and it's unclear whether race has an impact on disease rates (Shah and Alexis 2010; Bhate and Williams 2013). About 3 to 5 million people, or roughly 23% of the population in Australia, and 40 to 50 million people, or roughly 16% of the population in the United States, suffer with acne (White 1998). Many microorganisms have been connected to a variety of skin conditions, such as acne, eczema, burns, and atopic dermatitis (Harder et al., 1997). The coagulase-negative, facultative anaerobic, catalase-positive, nonmotile, Gram-positive Staphylococcus epidermidis (S. epidermidis) bacteria is the source of many illnesses (Oliveira et al., 2017). As the most prevalent bacteria on skin and mucosal surfaces, Staphylococcus epidermidis is one of the emerging pathogens. It can cause nosocomial and inframammary infections in many nations (Hashosh et al., 2022). So that, the current study was aimed to detect some genes (*tetK*, *hlb* and *nuc*) of *Staphylococcus epidermidis* isolated from Acne vulgaris and responsible against bacterial virulence

Materials and methods

Specimens collection

210 specimens from acne patients between the ages of 14 and 38 were gathered, 113 of them were female and 97 were male. From August to September of 2022, a dermatologist from Thi-Qar province in Iraq used a private clinical lab to make a clinical diagnosis for the patients. Samples were taken from the forehead, cheek, and chin of the patients. 70% ethanol was used to sanitize the sampling site. Transport media was employed to collect the samples. A lancet was used to make a scrape on the lesion's surface, and the fluid was then removed with light hand pressure in order to remove and extract the closed comedones and the papules. Every patient's data was entered in accordance with the format specified in the apindex.

Isolation of samples

The collected specimens were inoculated onto Manitol salt agar, which is recognized as a differential and selective medium for the isolation, purification, or identification of Staphylococci. After 24 hours of incubation at 37ºC for each plate, a single pure colony was transferred to BHI agar medium for preservation and to conduct additional biochemical assays that verified the isolates' identities. The isolates were streaked on blood agar and chrom agar and then incubated for 24 hours at 37ºC. The isolates' form, color, and pigments on this medium were evaluated.

Identification of bacterial isolates

Microscopic examination

Gram stain was used to stain the S. epidermidis isolates in order to observe how they responded to the stain and how they were arranged.

Colonial morphology on different media

The colonies' size, shape, color, and blood hemolysis pattern were examined after they were cultivated on blood agar plates. The isolates were cultivated on chrom agar, which was used to identify S. epidermidis by color colonies, while those grown on mannitol salt agar were evaluated for their capacity to ferment mannitol sugar.

Biochemical tests

- ✓ Catalase test
- ✓ Methyl red reagent
- ✓ Oxidase reagent
- ✓ Voges-Proskauer reagent
- ✓ Coagulase test

Extraction of bacterial DNA

Genomic DNA from all *S. epidermidis* isolates were extracted by using Presto™ Mini gDNA Bacteria Kit, according to manufacturer's protocol.

Agarose gel electrophoresis

One and one-half grams of agarose were dissolved in 100 milliliters of 1X TBE buffer to create agarose gel powder (1% or 1.5%). The mixture container was covered with aluminum foil to prevent evaporation and heated on a hot plate with a magnetic stirrer until the agarose was completely dissolved. After the DNA extraction procedure, 1% agarose was utilized for electrophoresis, and 1.5% agarose was used for gene amplification by PCR detection.

PCR detection of target genes

Using primers listed in Table (3–4), the *nuc*, *hlb*, and *tetK* genes were amplified. The reaction tubes' ultimate volume was 20µl, which was made up of 10µl of the Master Mix, 1µl of each gene-specific forward and reverse primer, 2µl of DNA template, and nuclease-free water to finish the volume.

Statistical Analysis

The SPSS software version 20 was utilized to statistically evaluate the study's data. A P value of less than or equal to 0.05 is deemed statistically significant.

Results & Discussion

Samples distribution

For the current study, 210 acne samples were collected (table 1). It was shown that the best cultured media, such as blood agar, mannitol agar, and MacConkey agar, produced positive findings for the growth of S. epidermidis in 87 (41.4%) of the total samples. 123 (58.6%) of the samples had negative S. epidermidis growth results.

Table (1): Distributed of study samples according bacterial growth

Groups	+ve culture	-ve culture	Total No.(%)
Acne swabs	87(41.4%)	123(58.6%)	210(100.0%)

In the province of Thi-Qar, 210 samples from acne patients were gathered. On the mannitol salt agar medium, only 87 isolates (41.4%) were non-mannitol fermenters and showed up as white colonies. On chrom agar, however, the colonies showed up as small, blue colonies that were identified as S. epidermidis, as shown in Fig (1).

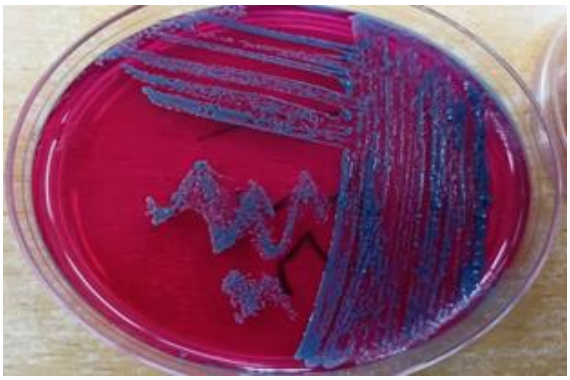

Figure (1): S. epidermidis's morphology on chrom agar

Antibiotic susceptibility test

Staphylococcus epidermidis

Penicillin (P), Oxacillin (OX), and Cefoxitin (CX) all caused complete resistance in *S. epidermidis*. Resistance to gentamicin (CN), nitrofurantoin (F), erythromycin (E), Refampicin (RP), azithromycin (AZM), trimethoprim (TMP), and ciprofloxacin (CIP) is 20%, 0%, 20%, 40%, 10%, 80%, and 20%, respectively as shown in table (2).

Table (2): Testing for antibiotic susceptibility in *Staphylococcus* species.

Antibiotics	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	%
Penicillin (10 U)	R	R	R	R	R	R	R	R	R	R	100
Oxacillin (1mg)	R	R	R	R	R	R	R	R	R	R	100
Gentamicin (10mg)	S	S	S	S	S	S	R	S	R	S	20
nitrofurantoin (300mg)	S	S	S	S	S	S	S	S	S	S	0
Erythromycin (15mg)	S	S	R	S	S	S	R	S	S	S	20
Cefoxitin (30mg)	R	R	R	R	R	R	R	R	R	R	100
azithromycin (15mg)	S	S	S	R	S	R	S	S	R	R	40
Refampicin (5mg)	S	S	S	S	S	S	S	S	R	S	10
trimethoprim (5mg)	R	S	R	R	R	R	R	R	S	R	80
ciprofloxacin (5mg)	S	R	S	S	S	S	S	S	S	R	20

S. epidermidis is a typical component of human skin and mucous membrane flora. However, according to several studies (Sechi et al., 1999; Miller et al., 2005), it is thought to be the most significant opportunistic pathogen in infections linked to the use of medical devices, namely blood stream infections. The S. epidermidis isolates in this investigation exhibited the highest level of resistance to penicillin (100%), out of all the antibiotic kinds tested. This number was less than that of Domínguez et al. (2002), who found that 87% of S. epidermidis that was resistant to antibiotics was isolated from healthy children in Spain. Conversely, in Mymensingh Medical College in Bangladesh, a lower proportion of penicillin-resistant isolates—10% of 30 subjects—was found among healthy individuals (Haque et al. 2009). Not long after penicillin's discovery in 1940, penicillin resistance was first noted (Abraham & Chain 1940). Penicillin abuse and misuse, whether in treatment or in animals raised for food, may be contributing factors to severe resistance cases (Prestinaci et al. 2015). Penicillin, the first antibiotic to be used to treat infections, was widely used worldwide. A strong correlation has been demonstrated between the use of antibiotics and the emergence of bacterial resistance (Olesen et al. 2018). Since S. epidermidis is easily shared between people, frequent exposure to the antibiotic may accelerate the rate at which antibiotic resistant strains spread across the community (Massey et al. 2006). A few European nations, including France, Spain, and Romania, believed that penicillin was no longer needed (Mossialos et al. 2010). In contrast to the earlier work by Eladli et al. (2018), which discovered 40% oxacillin resistance in their healthy patients, the subjects of the current study exhibit a lower level of resistance (20.9%) against S. epidermidis to oxacillin, another type of betalactam antibiotic. Oxacillin works by preventing penicillin-binding proteins (PBPs) from synthesizing bacterial cell walls. The development of resistance to this antibiotic can be attributed to the mecA gene, which codes for modified penicillin-binding protein (abbreviated PBP-2a), rendering β-lactam antibiotics inert against it (Finan et al. 2002). The S. epidermidis isolated from acne vulgaris in this investigation showed a range of antibiotic susceptibility patterns. The isolates have high nitrofurantoin, refampicin, and

erythromycin susceptibility, which is consistent with findings from a number of studies (Aricola et al., 2001; Miller et al., 2005). Therefore, these medications can be used to treat infections brought on by this bacteria and associated with acne vulgaris. However, every isolate was resistant to penicillin.

Molecular diagnosis

The results of PCR assay revealed that 75 (86.2%) of *S. epidermidis* isolates giving positive results for *tetK* gene. Despite the fact the *hlb* gene finds in percentage 21 (24.1%), while 42 (48.3%) of *S. epidermidis* harbored nuc gene, as represented in Table (3). The whole genomic DNA of *S. epidermidis* isolates shown in Fig. (2).

Table (3): Distribution of *tetK, hlb* and *nuc* genes in *S. epidermidis* isolates.

Gene	Positive results No=87
tetK	75 (86.2%)
Hlb	21 (24.1%)
Nuc	42 (48.3%)

According to PCR assay of *tetK* gene, the bands showed in Fig. (3) recognized the size of this gene which was approximately 360bp. Although, Fig. (4) and (5) revealing the size of *hlb* and nuc genes were approximately 833bp, and 423bp, respectively.

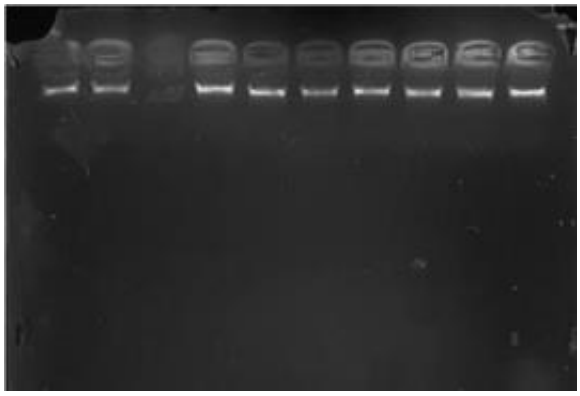

Figure (2): Agarose gel electrophoresis of genomic DNA in *S. epidermidis*.

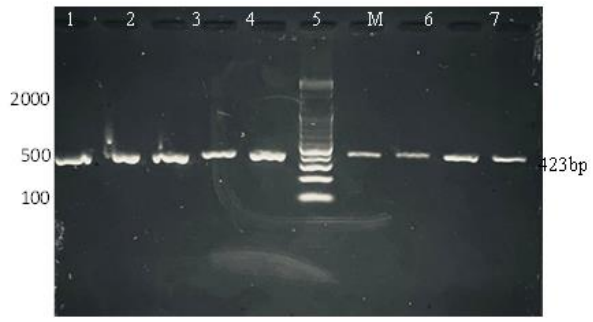

Figure (3): Nucleic gene amplification using agarose gel electrophoresis, where M stands for ladder and 1–9 for positive results.

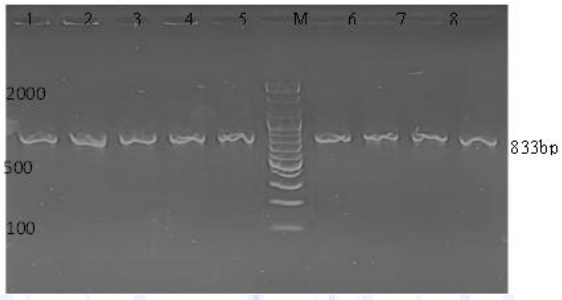

Figure (4): hlb gene amplification using agarose gel electrophoresis, where M stands for ladder and 1–9 indicates favorable results.

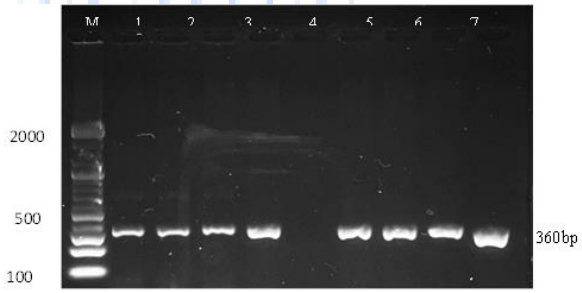

Figure (5): TetK gene amplification using agarose gel electrophoresis, where M stands for ladder, 1-4, 6-9, positive findings, and 5 for negative results.

The amplicons were sized by electrophoresis in 2% agarose gel, and all identified S. epidermidis isolates were screened for tetracycline resistance genes tetK using the primer and PCR conditions as previously published in the current work. Using the PCR approach, it was found that 86.2% of S. epidermidis had the tetK gene, which had a 360 bp product size. These outcomes resembled those of (Duran et al. 2012). Multi-drug resistant bacteria frequently have tetracycline

resistance determinants, which are widespread across bacterial taxa (Jamali et al., 2014). The acquisition of additional genes associated with transposons or conjugative plasmids is often the cause of resistance (Roberts 1996). Because tetracycline resistance genes can be transferred between staphylococcal species, prolonged use may lead to the formation of tetracycline-resistant Staphylococcus species, which poses a major risk to human health (Thorberg et al., 2009). The tetracycline-resistant genes tet(K) linked to an efflux or ribosome protection mechanism were found in the current study (Roberts 1996). The nuc gene, sometimes referred to as the Staphylococcus species specific gene, is amplified in order to identify and measure Staphylococcus epidermidis (Studer et al., 2008). Real-time PCR targeting specific S. epidermidis species is thought to be a helpful tool for expediting S. epidermidis identification, as it can replace the laborious biochemical phenotypic systems now in use. Furthermore, direct PCR identification of Staphylococcus species from dietary and clinical specimens can be carried out under the right conditions (Jos et al., 1996). Lastly, Velasco et al. (2014) suggest that the real-time PCR assay be used as a quick way to identify Staphylococcus species. In this investigation, the PCR reaction was utilized to amplify the gene nuc using its primers for a quick and precise diagnosis and detection of Staphylococcus spp. (Odd et al., 1992). 48.3% of staphylococcal isolates had this gene identified using primers specific for the nuc. Goldfish were identified using conventional diagnostic techniques, whereby 423 bp clear bands of this gene emerged following amplification, electrophoresis, and UV inspection.

Conclusions

Based on current study, *Staph. epidermidis* is characterized by the presence of genes responsible for its virulence, as 75 (86.2%) of S. epidermidis isolates were found giving positive results for tetK gene. Despite the fact the hlb gene was found in percentage 21 (24.1%), while 42 (48.3%) of S. epidermidis harbored nuc gene.

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