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Prevalence of Some Causes of Acne Vulgaris and Susceptibility to Antibiotics Against Pathogenic Bacteria in Wasit Province

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ABSTRACT: Acne vulgaris is an achronic inflammatory disease in adolescents. *Staphylococcus epidermidis* (*S. epidermidis*) and *staphylococcus aureus* (*S .aureus*) are the most aerobic and anaerobic bacteria that cause acne *S. epidermidis* is Gram-positive, cocci, facultative aerobic, and coagulase-negative group (CON) of Staphylococci isolated in contrast *S. aureus* which characterized by present of coagulase tow type of pathogens are cultured on blood agar under aerobically. Since the bacteria can form a biofilm to resist antibiotics, the current study aims to investigate the effect of selected antibiotic acne on the pathogens bacteria to avoid resistance by misuse and/or overuse of the drug. Through this accurate study, the skin samples were obtained from acne lesions from a different site (face, shoulder, back, chest) of a total of 105 patients with acne vulgaris attending the Iraqi Dermatology Clinic at AL-Karama Teaching Hospital in Al-Kut province and 41(74.5%) *S. epidermidis* and 3(5,5%) *S. aureus* were the results of aerobic culture, The accurate study was done between July 2023 to February 2024, Samples were cultured on Blood agar, identify the bacteria was performed by staining with Gram stain and trade vitek2 system, antibiotic susceptibility test was done and based on the Clinical and Laboratory Standards Institute (CLSI) 2023.

Additionally, biofilm assay using microtiter plate assay MPA, the results of 41(74.5%) *S. epidermidis* isolates were 24(58.5%) strong,9(22%) moderate,4(9.8%) weak, and only 4(9.8%) non formed. In comparison, all 3(100%) *S. aureus* were moderate. In the actual study, the results of antibiotic susceptibility detected that all 41(100%) isolates of *S. epidermidis* were resistant to ceftazidime, amoxicillin, vancomycin, ampicillin, tetracycline, and penicillin, followed by while all 41(100%) isolates were susceptible to doxycycline followed by 37(90%) sensitive to rifampin, 29 (70.7%) ciprofloxacin, and 8 (68.3%) sensitive to azithromycin. Furthermore, all cases of *S. aureus* (100%) were resistant to ceftazidime, azithromycin, amoxicillin, vancomycin, ampicillin, gentamycin, and penicillin. Conversely, all isolates (100%) were sensitive to doxycycline and clindamycin; however, only 2(66.7%) were sensitive to ciprofloxacin. While (100%) intermediate to rifampin, erythromycin, and tetracycline.

Keywords: acne vulgaris, *Staphylococcus epidermidis*, *Staphylococcus aureus*, biofilm, susceptibile antibiotic

1. INTRODUCTION

Acne vulgaris (AV) is the chronic inflammatory condition known as AV, which affects the sebaceous follicles [1], spreading over the face, shoulder, back, and chest and manifesting clinically as comedones, papules, pustules, nodules, and not often scarring [2].

Although AV does not cause mortality, it can take several years, which leads to psychological morbidity, including anxiety and sadness in those affected, comparable to a chronic illness. Also, permanent scarring could result from moderate to severe acne [3]; its pathogenesis includes follicular hyperkeratinization of abnormal follicular keratinocytes, creating a follicular plug. Sebum secretion increases sebum production triggered by testosterone stimulation follicular colonization by the bacteria acne, such as *Propionibacterium acnes* (*P. acnes*), *S. epidermidis*, and *S. aureus* multiply and induce immune and inflammatory reactions [4]. In addition, AV has pleomorphic lesions, which can be non-inflammatory (open and closed comedones) or inflammatory, such as papules, pustules, and nodules, with varying degrees of severity [5]. According to their severity, acne is categorized into three subcategories these include (a) mild acne, which manifests as comedones and sometimes a few papulopustules; (b) moderate acne, which shows up as more papules and pustules and (c) severe nodulocystic acne, which shows up as nodules larger than 5mm in diameter and is frequently accompanied by noticeable scarring [6]. Moreover, three opportunistic bacteria, such as *S. epidermidis*, *P. acnes*, *and S. aureus*, are considered AV's three most common causes [7].

As well as *S. epidermidis* is an opportunistic, Gram-positive, facultative anaerobe of the cutaneous microbiota [8]. In addition, Nguyen *et al.* [9] clarified that the absence of the blood-clotting enzyme coagulase allows the clinical classification of a diverse staphylococcal species known as coagulase-negative staphylococci (CoNS). Because of the lack of coagulase, they differ from *S. aureus* and a few other coagulase-positive species that are clinically less significant [10]. Moreover, *S. epidermidis* and *S. aureus* are potential bacteria that cause fatality because they can resist antibiotics by forming biofilms. Additionally, it serves as a reservoir for antibiotic-resistant genes that can be horizontally transferred to other organisms [11].

Furthermore, *S. aureus*, the most prevalent member of the skin microbiota, is a pathogen that causes various skin conditions, including folliculitis. Impetigo and additional bacteria have been shown to coexist in acne lesions [12]. The pathogen establishes an extracellular matrix and serum-binding proteins, including fibronectin-binding proteins Alfa and Beta(FnBP-A and FnBP-B) and adhesins [surface protein (*SasG*)], to penetrate the host cell. By attaching themselves to the cellular integrins, these factors facilitate their internalization into the host cells. When the virus penetrates the human skin, it produces many extracellular enzymes, including collagenase, proteases, hyaluronidases, and lipases (*geh1*). These enzymes assist in causing tissue damage, which facilitates the pathogen's spread into deeper tissues [13]. Additionally, during their pathogenic life cycle, they are recognized for producing cytotoxins, including a-, b-, and g-hemolysins, as well as exfoliative toxins such as PantoneValentine leucocidin, leukocidin E-D, *S. aureus* exotoxin, and enterotoxins *AeE* and toxic shock syndrome toxin-1 [13]

A biofilm is a surface-attached microbial population structure, a surface-attached that sticks to biotic or abiotic surfaces and is immersed in an extracellular matrix that the community produces on its own [14]. According to an *in vitro* study, bacteria in the protected microenvironment of biofilms are (50–500) times more resistant to antimicrobial treatments than bacteria floating freely [15]. Biofilms can be examined using electron microscopy and molecular methods (polymerase chain reaction and sonication) for a more precise and sensitive analysis; however, their application is limited by higher operating costs and the need for skilled personnel, necessitating an alternative technique. Therefore, Finding virulence factors might be an essential first step in treating, managing, and preventing infections. These could result in the creation of vaccinations and new or improved antimicrobial medicines, as well as more efficient infection control procedures and a decrease in colonization [12].

The Inflammatory acne Pathophysiology. Composed of four primary factors that influence the emergence of acne:

1. Hyperproliferation of abnormal follicular keratinocytes, resulting in the creation of a follicular plug.
2. Sebaceous follicles' increased production of sebum
3. Microorganisms like P. acnes and S. epidermidis multiply in the sebum.
4. The inflammatory process Many cases have a significant hereditary component. Every type of acne, even comedonal acne, has an inflammatory cause [16]. as shown in Figure 1.1

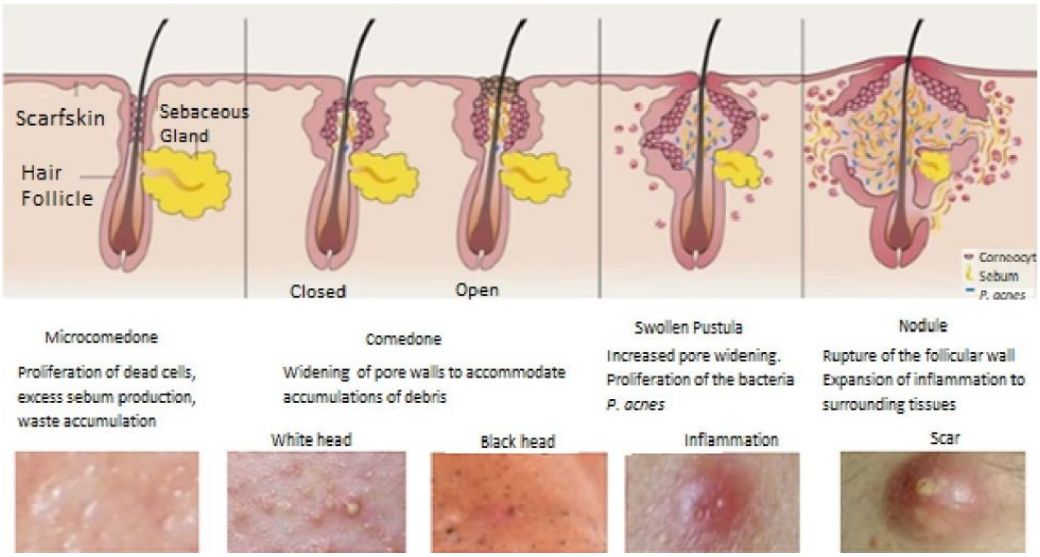

Figure 1. Shows the pathogenesis of acne vulgaris [17]

Moreover, Clinically, acne lesions are categorized with scars, comedones, pustules, nodules, and cysts. Comedones are either closed or open. As open comedones are often called, blackheads get their black look from keratin plug oxidation. The whiteheads are closed comedones [18]. In addition, pustules arise when there is a significant accumulation of neutrophils due to follicular inflammation. Cysts are filled, follicular-lined entities that enlarge. When there is ongoing inflammation, nodules form. These are palpably sore, red lesions in a clinical setting. The follicular structures have burst at this location. After healing, the result may be scarring [3].

Moreover, most acne forms, papule diseases, and nodulocystic diseases can leave scars, so prompt diagnosis and appropriate treatment are essential. Acne scars can also be categorized as mild, moderate, or severe based on various variables[19].

2. MATERIALS AND METHODS

2.1 Specimens collection and culture

In the present cross-sectional study, the skin specimens were obtained from acne lesions on the face, shoulder, back, and chest. 105 specimens were obtained from patients with acne vulgaris who attended the Iraqi Dermatology Clinic at AL-Karama Teaching Hospital in Al-Kut City of Wasit province. The present study was done between July 2023 and February 2024. Specimens were cultured on Blood agar under anaerobic conditions; the bacteria were identified by staining with

Gram stain and trade vitek 2 system. The antibiotic susceptibility test was done using 14 antibiotic and inhibit zone measures based on the Clinical and Laboratory Standards Institute (CLSI) 2023.

2.2 Bacterial identification

Colonies on blood agar were white to yellow smooth, with hemolysis; after Gram's stain under the microscope, they appeared as Gram-positive cocci, arranged either single or clusters, then by trade vitek 2 confirmed the identification.

2.3 Biofilm respect production in Isolates pathogens of acne vulgaris.

A microtiter plate is used in the accurate study to evaluate the biofilm of all isolated samples [20].According to Ruchiatan *et al.*[15], isolates of *staphylococcus* spp. were added to 10 mL Brain-Heart Infusion + 1% glucose (BHIglu) and incubated for a full day at 37°C. After that, TSBglu was added to the incubation product at a ratio of 1:100. The diluted material was split up into 96-well flat-bottom tissue culture plates that had not been treated with 0.2mL of the sample in each well. Each isolate's bacterial suspension in BHIB Fill three wells with 200µL, then incubate the microtitre plate at 37°C for 24 hours for *staphylococcus* spp. The first three wells are the control group, including only BHIB filled, presenting the biofilm development. The quantitative analysis of biofilm was completed as shown in Table 2.1: strongly positive (OD450 > 0.24), mildly positive (0.12 ≤ OD450 < 0.24), or negative (OD450 <0.12)[21].

Table 2.1Analyzing biofilm using the micro-titer plate technique

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Mean OD value	**Biofilm formation**
OD ≤ Odc	None
ODc < OD ≤ 2× ODc	Weak
2× ODc < OD ≤ 4× ODc	Moderate
4× ODc < OD	Strong

***** ODc = optical density cut off ;* OD = optical density

2.4 Antibiotic-resistance

The Kirby-Bauer method, streaking plates, was used to examine the antibiotic susceptibility; the colonies were emulsions from overnight grown culture diluted into the 0.5 McFarland solution standard after adding it to sterile distal water. Thus, an isolate inoculant was created. The suspension of bacteria was streaked on the Muller Hinton agar uniformly using the sterile swab and left to dry. The antibiotic discs have been proven on a plate by using sterile forceps. Kept the cultures for 24 hours at 37°C, then inhibitory zone width measurement was calculated, and the outcomes were interpreted using the CLSI, 2023 standards

however, in present study thirteen of different antibiotics were used,such as, doxycycline (30µg), erythromycin (10µg), azithromycin (15µg), ceftazidime(30µg), ciprofloxacin (10µg), gentamycin (10µg), tetracyclin (10µg), clindamycin(10µg), penicillin (10µg), rifampin (5µg), amoxicillin (2µg), ampicillin (10µg),vancomycin (30µg).

3. RESULTS AND DISCUSSIONS

3.1 Identification of bacteria isolates.

All 55 positive culture specimens were collected from a patient with acne vulgaris in AL-Karamah Teaching Hospital after Gram staining and Vitek 2. The results of the identification of bacteria were shown in Figure 2, in which around three-quarters of patients (74.5%) were found with *S. epidermidis*, followed by only 5(9.1%) for *P. acnes* only 4(7.3%) with Cockrosea spp. and 3(5.5%) with *S.aureus* followed by one (1.8%) of each *Staphylococcus saprophytic* and *Staphylococcus hominins*. In the present study, the most common bacteria that cause acne vulgaris isolated from aerobic cultured on blood agar were *S. epidermidis* 41(74%) and 3(5.5%) *S. aureus*. These results agree with a survey from Iraq Erbil by Muhammed and Dabbagh, who assume the highest percentage of the isolates belonged to *S. epidermidis*, followed by *S. aureus* [22]. Another study is similar to a survey by Elfekki in Egypt, which collected 100 samples from patients with acne and observed that 61% were *S. epidermidis* and 28% *were S. aureus*, considered the most predominant aerobic growth [23]

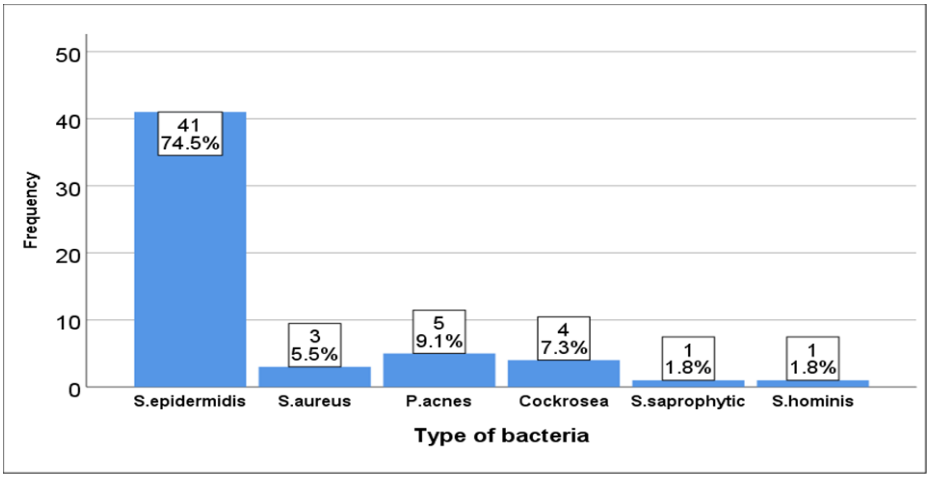

Figure 3.1. Shows the percentage (%) of each organism isolated from a patient with AV.

3.2 Biofilm

The microtiter plate method(MPA) used to assess biofilm formation showed that more than half, 24(58.5%) of *S. epidermidis* had strong biofilm, followed by 9(22%) with moderate biofilm results. Only 4(9.8%) were weak; the same percentage was found for non-biofilm with no statistically significant P value 0.126> 0.001, as shown in Table 1. A study conducted by Saising *et al*. [12], who used two methods, MPA and the Congo red agar method, for both coagulase-positive and CON staphylococci isolated from acne lesions, demonstrated that biofilm forming in CON staphylococci more frequently than coagulase positive a total of 85 isolates *S. epidermidis* 97.7% and 68.8% were biofilm formed of *S. epidermidis* and *S. aureus* respectively. The resistance to antibiotics is not only by forming biofilm-resistant genes or mutation genes.

Table3. 1: Distribution Of *S.epidermidis* Isolates According To Biofilm Production By MPA Method Bacterial.

Bacteria	Biofilm			
	Strong No.(%)	**Moderate No.(%)**	**Weak No.(%)**	**Non No.(%)**
S. epidermidis	24(58.5)	9 (22)	4(9.8)	4(9.8)
S. aureus	0(0)	2(66.7)	0(0)	1(33.3)
P-value = 0.126				

3.3 Antibiotic susceptibility of *S. epidermidis and S. aureus.*

The result of the actual study detected that all 41(100%) isolates of *S. epidermidis* were resistant to ceftazidime, amoxicillin, vancomycin, ampicillin, tetracycline, and penicillin, followed by all 41(100%) isolates were susceptible to doxycycline followed by 37(90%) sensitive to rifampin, 29(70.7%) ciprofloxacin, and 8(68.3%) sensitive to azithromycin. At the same time, 6(14.6%), 12(29.3%), 4(9.8%), 20(48.8%), 22(53.7%), and 28(68.3%) were exhibited intermediate to azithromycin, ciprofloxacin, rifampin, gentamycin, erythromycin, clindamycin respectively with significant statistically *P*-value >0.0001.. As seen in table 3.2

Table 3.2 Antibiotic susceptibility of *S. epidermidis*.

No.	Antibiotic	*S. epidermidis*		
		S No.(%)	I No.(%)	R No(%)
1	Ceftazidime	0(0)	0(0)	41(100)
2	Doxycyclin	41(100)	0(0)	0(0)
3	Azithromycin	28(68.3)	6(14.6)	7(17.1)
4	Amoxicillin	0(0)	0(0)	41(100)
5	Vancomycin	0(0)	0(0)	41(100)
6	Ampicillin	0(0)	0(0)	41(100)
7	Ciprofloxacin	29(70.7)	12(29.3)	0(0)
8	Rifampin	37(90.2)	4(9.8)	0(0)
9	Gentamycim	20(48.8)	20(48.8)	1(2.4)
10	Erythromycin	19(46.3)	22(53.7)	0(0)
11	Tetracyclin	0(0)	0(0)	41(100)
12	Clindamycin	13(31.7)	28(68.3)	0(0)
13	Penicillin	0(0)	0(0)	41(100)
P- value < 0.0001				

However, all cases of *S. aureus* (100%) were resistant to ceftazidime, azithromycin, amoxicillin, vancomycin, ampicillin, gentamycin, and penicillin. Conversely, all isolates (100%) were sensitive to doxycycline and clindamycin; however, only 2(66.7%) were sensitive to ciprofloxacin. While (100%) intermediate to rifampin, erythromycin, and tetracyclines. The *P*- value= 0.006 insignificant statistically

Table 3.3: Culture and sensitivity test for *S. aureus*.

No.	Antibiotic	Sensitivity test		
		S no.(%)	I no.(%)	R no.(%)
1	Ceftazidime	0(0)	0(0)	3(100)
2	Doxycyclin	3(100)	0(0)	0(0)
3	Azithromycin	0(0)	0(0)	3(100)
4	Amoxicillin	0(0)	0(0)	3(100)
5	Vancomycin	0(0)	0(0)	3(100)
6	Ampicillin	0(0)	0(0)	3(100)
7	Ciprofloxacin	2(66.7)	1(33.3)	0(0)
8	Rifampin	0(0)	3(100)	0(0)
9	Gentamycim	0(0)	0(0)	3(100)
10	Erythromycin	0(0)	3(100)	0(0)
11	Tetracyclin	0(0)	3(100)	0(0)
12	Clindamycin	3(100)	0(0)	0(0)
13	Penicillin	0(0)	0(0)	3(100)
P-value =0.006				

Walid *et al*. [19] observed high resistance percentages from 50 staphylococci spp. isolates were detected in 18(36%) of bacterial isolates resistant to erythromycin, followed by 13(26%) of

them were resistant to clindamycin. Furthermore, high susceptibility to doxycycline was detected in 49(98%); these results were less and more similar to the present study, a previous study by Ruchiatan *et al*., who demonstrated all isolates are sensitive to Doxycycline 11(100%) as well as, tetracyclin, followed by clindamycin 6(54.5%); on the other hand, sensitivity to both erythromycin and azithromycin were 4(45%) these results are compatible in some and incompatible the others [15]. The differences may be due to environmental differences and the use of tetracycline as a local antibiotic that increases bacteria resistance.

A study from Egypt investigated the relationship between lipase and antibiotic susceptibility in both *S. epidermidis* and *S. aureus*. It showed inconsistent results out of 9 S. epidermidis isolates antibiotic resistant in clindamycin 7(77.8%) followed by doxycycline and erythromycin as same 6(66.7%) while resistant to penicillin, ciprofloxacin and azithromycin 5(55.6%), 4(44.4%), 3(33.3%) respectively. Additionally, from a total of 19 *S. aureus* isolates highly resistant was to doxycycline 15(83.3%) followed by erythromycin and ciprofloxacin same 13(72.2%), while azithromycin and tetracycline 10(55.6) for both [24]. This dissimilarity may be due to the bacteria strains and the body site of the specimens collected. Furthermore, doxycycline, ciprofloxacin, clindamycin, and azithromycin are lipophilic antibiotics, and when bacteria have active lipase, it leads to resistance.

4. CONCLUSION

Staphylococcus epidermidis and *S. aureus* are the most common bacteria isolated from acne vulgaris by cultured on blood agar under aerobic and anaerobic conditions; however, they are opportunistic bacteria that convert from normal flora into pathogenic bacteria and invasive, and they can resistant antibiotic either biofilm forming or by the presence of lipase target lipophilic antibiotic and lead to resistant to avoid resistant problem antibiotic should be used in mild and severe inflammatory acne and treatment should be no longer than 3-4 month. Furthermore, overusing systemic antibiotics and local antibiotics such as clindamycin and erythromycin may lead to antibiotic resistance.

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