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The Genetic Tree of Seven *Cutibacterium acnes* Strains and their Susceptibility to Common Antibiotics

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Abstract

This study was conducted to investigate one of the most prominent bacteria that causes acne (*Cutibacterium acnes*), studying the spread of the phenomenon of resistance to the various antibiotics used to treat acne and studying the evolutionary relationship between isolates of *Cutibacterium acnes*. 125 samples were collected from patients with acne vulgaris cases in Mosul city, from dermatology consultations at Al-Salam Teaching Hospital and Mosul General Hospital, the diagnosis was made for samples through routine examinations (macroscopic and microscopic) and through molecular diagnosis based on universal *16s rRNA gene*, the results of the isolation and diagnosis were obtaining seven isolates of *Cutibacterium acnes* and one isolate of *Cutibacterium avidum* with (5.6%) and (0.8%) respectively from all samples.

Eight antibiotics were chosen to perform the susceptibility tests by disc diffusion assay, the study showed that seven of C. acnes isolates were resistant to fusidic acid and azithromycin with 85.7%, tetracycline with 71.4%, clindamycin, and gentamycin with 57.1% respectively and vancomycin 42.8% the isolates were sensitive 100% to imipenem and levofloxacin. In this study, an evolutionary tree was created depending on the degree of neighbor- joining among these molecularly diagnosed isolates based on the MEGA11 program strains 103, 107, and 111 each showed a genetic similarity with 93%, and their cluster formed a 91% similarity with strain 112, which in turn clustered with 89% with strain 118 and their similarity with strain 117 decreased to 74%. In turn, it clustered with strain 110 at a rate of 99%. *C. avidum* strain was distant from *C. acnes* strain. Naturally, the *Staphylococcus aureus* strain was at the end of the tree, which was used to create a root for the genetic tree of the strains .

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1. Introduction:

Cutibacterium acnes (C. acnes) one of the most prevalent skin microorganisms identified in the sebaceous gland has been shown to contribute to the development of acne vulgaris [1]. *C. acnes* is a gram-positive, diphtheroid or coryneform bacterium, rod-shaped and slightly curved, measuring 0.4 to 0.7 µm in width and 3 to 5 µm in length [2]. Anaerobic bacteria cannot thrive on solid medium in the presence of air oxygen However, *C. acnes* is classified as an aerotolerant anaerobe because it has enzymatic systems that can detoxify oxygen, enabling it to remain on the skin's surface [3]. Expression of the cytochrome d oxidase gene promotes tolerance exposure to oxygen for several hours and enables development even under limited oxygen circumstances [4]. Acne vulgaris is a chronic inflammation affecting the pilosebaceous unit, usually after a prolonged course. It often starts around adolescence by *C. acnes* a bacterial species [5]. When this condition appears, it usually shows up as pustules, papules, nodules on the face), although it can influence the trunk upper arms, and back. the pathogenesis of acne vulgaris includes

the interaction of several factors that eventually lead to the creation of its primary lesion, known as comedo. Although acne vulgaris is widespread among adolescents, it may affect individuals varied in age so it is not restricted to age group, this condition can vary in severity, varying in severity from less severe forms with few comedones to more severe forms with deformity-causing inflammatory manifestations which can lead to scarring, hyperpigmentation and adverse psychological effects [6] [7] [8].

Antimicrobial resistance has become known as a serious danger to public health worldwide. Resistance to antimicrobial drugs may be accurately and rapidly detected and subsequent appropriate antimicrobial therapy. Along with antimicrobial management, are necessary for reducing the establishment and spread of antibiotic resistance [9]. Antibiotics are used to control the development of bacteria. The usual course of treatment for moderate acne, particularly for situations where topical combinations [10]. *C. acnes* resistance to commonly used antibiotics, such as erythromycin and clindamycin, [11]. bacterial phylogenetic analyses are widely done to study evolutionary relationships among distinct bacterial species and genera using their *16S rRNA gene* sequences [12].162 genome sequences representing 10 of the 16 species of *Propionibacterium* are hosted by NCBI under the taxonomic ID corresponding to the *propionibacteria* as of February 2016. *Propionibacteria's* taxonomy can now be reevaluated, and an initial look at how they have evolved to adapt to various habitats. the *16S rRNA* gene sequences, genome sizes, and DNA GC content of these traditional groups were found to differ significantly from one another. thus showing gene adaption in the cutaneous *propionibacteria* to the human host; as a result, these cutaneous species were claimed to be members of the unique genus *Cutibacterium*, leading to the reclassification of *P. acnes* as *C. acnes* [13]. Acne vulgaris is a chronic inflammation affecting the pilosebaceous unit, usually after a prolonged course. It often starts around adolescence with *C. acnes* a bacterial species whereas dehydroepiandrosterone levels are normally circulating [14].

In order to shed light on the bacteria *C. acnes*, the research aimed to isolate and identify *C. acnes*, investigate its response to common treatments, and determine its genetic relationship to other strains based on 16S rRNA gene sequences

2. Materials and methods

2.1 Samples collection:

swabs were taken from 125 patients who had acne, their ages ranged from 15 to 20 years from different schools and hospitals (Al-Salam Teaching Hospital and Mosul General Hospital) in Mosul city (from 1/October/2023 to 2/January/2024. Based on the approval of the scientific and ethical research committee at its session numbered (247) held on 13/September/2023 on the research project numbered (2023155). After sterilizing the affected area with 70% ethanol, samples were taken using a sterile swab from pustules and whiteheads then transferred the samples into sterile Thioglycollate broth and then placed in anaerobic incubation using a jar containing a gas pack (Himedia, India), the incubation period was 5-7 days in 37º C. after that cultured were transferred to brucella agar supplemented with vitamin k and sheep blood [15].

2.2 Identification of bacteria:

2.2.1 Routine Identification:

The phenotypic features of the colonies on Brucella agar were observed; Colony characteristics included shape, dimension, texture, and color.

the Gram stain reaction was accomplished and examined under oil immersion with a 1000X objective lens to observe cell forms and groupings and their color.

Some biochemical tests were carried out in order to approach their diagnosis and exclude other bacteria from among these tests: catalase test, detection of urease enzyme, and indole test.

2.2.2 Molecular diagnosis:

DNA was extracted using a geneaid DNA extraction kit (Geneaid, Taiwan) following the steps recommended by the manufacturing company. the concentration and purity of DNA were measured using a Nano drop device
(biodrop, England). Universal 16s rRNA gene primers were used to diagnose the bacterial isolates accurately at molecular levels obtained from (Macrogene, Korea). the sequence of primer as demonstrated in table (1) according to [16]. Each primer was prepared by adding 250µl of TAE buffer to reverse and forward tube primes to obtain the stock solution(100µM), and prepare working solution was by adding 10µl of stock solution to 90µl of TAE buffer to obtain 10µm then stored at -20 until used. PCR reaction was carried out in a 25µl reaction containing 12.5µl of Green Master Mix (1X) (Promega, USA), 1µl of each primer (Macrogene, Korea), 5.5µl nuclease free water and 5µl of DNA sample extract [17] as demonstrated in table (2), then thermos cycling conditions were as demonstrated in table (3) according to [18].

Table1. Universal 16S rRNA primer sequence.

Gene	Primer sequence (5'-3')	Base pair	reference
Forward	AGAGTTTGATCMTGGCTCAG	1495 bp	[16]
Reverse	AAGGAGGTGATCCARCCGCA		

Table 2. Reaction mixture of PCR with final concentration.

Components	Volume in microliters	Final concentration
Master mix	12.5	1X
Reverse primer	1	0.25 µm
Forward primer	1	0.25 µm
DNA sample	5	<250 µg
nuclease free water	5.5	
Total volume	25	

Table 3. PCR program for 16s rRNA

Reaction stages	Number of cycles	Temperature (C)	Time (minute)
Initial denaturation	1	94.0	5
Denaturation 2		94.0	1
Annealing	30	55.0	1
Extension 1		72.0	1
Final Extension	1	72.0	7

Analysis of PCR products by Electrophoresis using red safe day and ladder (Transgene biotech, china) at 50 volts for 45 minutes was done.

●Nucleic acid sequencing

PCR products for *16s rRNA* gene were sent sequencing at Psomagene sequencing company (USA) the results were then received in the form of data for the sequences of the nitrogenous bases for each sample, and the data was entered through the National Center for Biotechnology Information (NCBI) and Local Alignment Search Tool (BLAST) was used to compare our results with DNA sequence of another global registered strain to identify the bacterial species.

2.3 The susceptibility test of antibiotics:

The Kirby-Bauer disk diffusion technique was used to determine the susceptibility of seven isolates of *C. acnes* to eight antibiotics (Clindamycin 10µg, Vancomycin 30µg, Tetracycline 10µg, Imepinem 10µg, Gentamycin10µg, Levofloxacin 5µg, Fusidic acid 30µg and Azithromycin 15µg), bacterial suspension prepared to produce a bacterial suspension compared with 0.5 of stander McFarland this approximately equals to $1.5x10^8$ CFU/ml [19]. 100µl of bacterial suspension was spread on Mueller-Hinton agar medium supplemented with vitamin K and Sheep blood, the antibiotic discs were placed on the agar with sterile forceps. later the plates were inverted and incubated anaerobically at 37˚C for 3 days [20]. Inhibition zones that developed around the discs were measured by millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute (CLSI, 2023) [21] and (Eucast2024) [22].

2.4 Phylogenetic tree:

All seven strains of *C. acnes* as well as *C. avidum* were tested the detect the similarity between each. Staphylococcus aureus (which was isolated from acne vulgaris from this study and diagnosed based on the 16s rRNA gene) was used to make the tree. The analysis by DNA alignment was accomplished for matching the strain above using the Mega 11 software program to detect the similarity and draw the phylogenetic tree according to neighbor joining.

3. Results and discussion:

3.1 Isolation and Identification of Bacteria

Seven strains of *C. acnes* and one isolate of *C. avidum* were obtained with (5.6%) and (0.8%) respectively from all samples.

3.1.1 Routine Identification

After cultivation and purification of all the samples obtained from acne vulgaris, the colonies on brucella agar medium supplemented with (vitamin K, sheep blood) where appeared, white to gray in color, convex, semi-opaque, and glistening as shown in Figure (1), as described by (Yuan) [23].

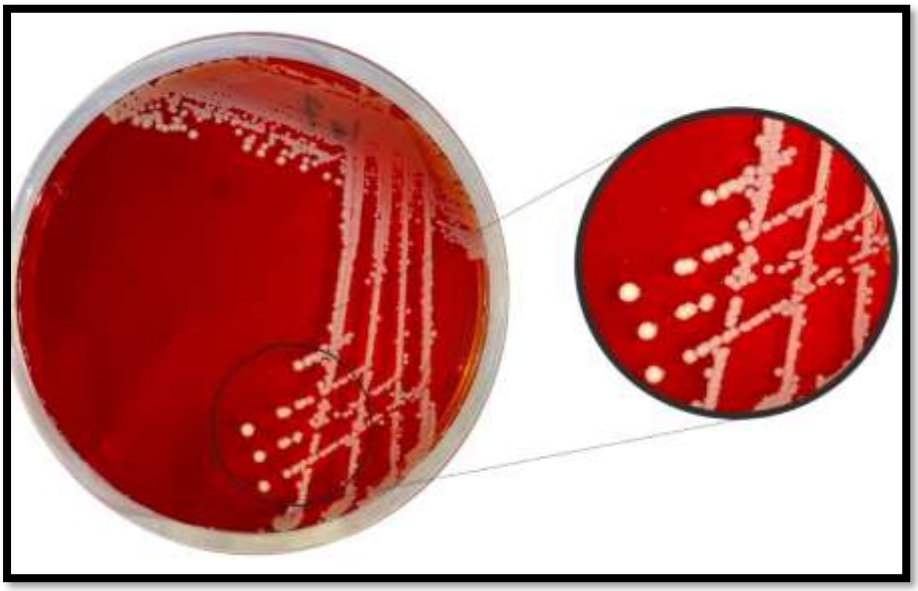


Figure (1). Colonies of *C.acne* in brucella agar with blood.

The features of *C. acnes* colonies (brain heart infusion with blood agar) are described by (Yuan) as follows: after 2-3 days after inoculation, pad-shaped, white or gray in color, glossy, translucent or opaque, and pinprick-sized colonies to colonies 0.5mm in diameter on the surface of rabbit or horse blood agar [23]. The nutritional requirements of *C. acnes* is basal salts medium supplemented with glucose, biotin, and amino acids Other nutrients that were not absolute requirements, but which significantly improved the growth of this species, included the purines, Tween 80, which served as a source of oleic acid, sodium L lactate, a-ketoglutarate, and pyruvate. heme and vitamin K are added if there is no growth [24].

C. acnes appeared as Gram-positive bacilli, as shown in the figure (2). As mentioned by Sriharat [2], *C. acnes* appeared gram positive, non-spore former, extremely pleomorphic, irregular rods with either curved, clubbed, or pointed ends and have diphtheroid or coryneform shaped these characteristics.

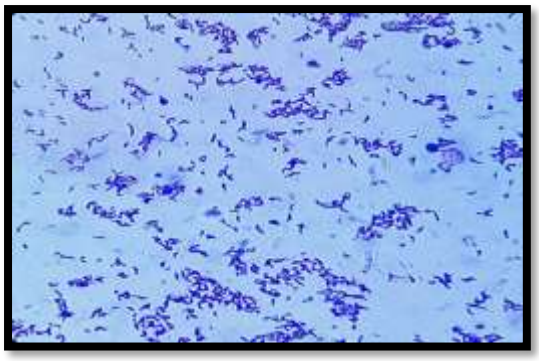
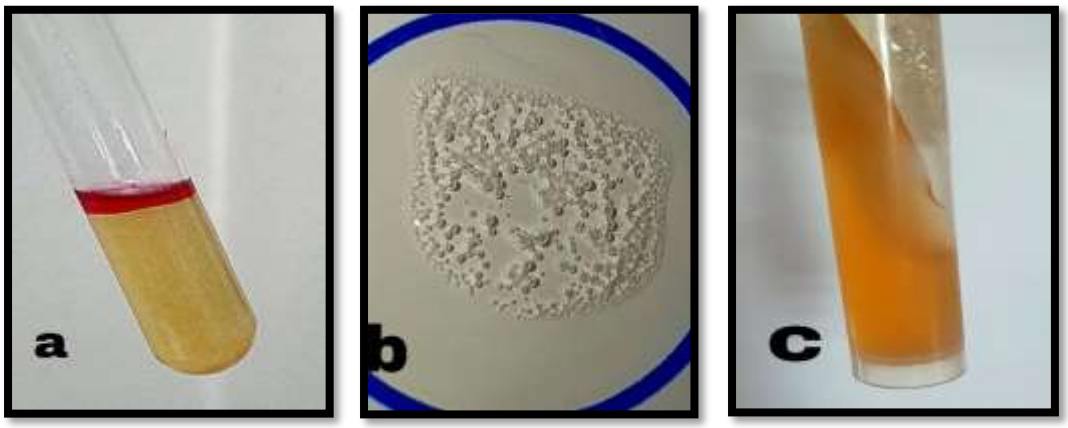


Figure (2). Cell morphology of *C. acnes* at (1000X).

● Biochemical tests:

Indole test appeared in a positive result after adding three drops of Kovac's reagent to bacterial culture on tryptone broth medium, a red ring appeared as shown in figure (3 a) [25]. For the Catalase test, the result was positive appeared clearly by the development of air bubbles shown in Figure (3 b). The urease test was negative because there was no change in the medium's color as shown in Figure (3 c). the urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease. ammonia is formed with resulting alkalinity in the presence of the enzyme, and the increased pH is detected by a pH indicator (phenol red) [26].



Figure(3). the biochemical tests: a-indole test (+ve) b-catalase test (+ve) c-urease test(-ve)

3.1.2 Molecular Identification:

After DNA extraction, the concentration and purity were measured by a Nanodrop device. the concentrations of DNA isolates ranged between (100-200) ng/µl and the purity was between (1.8 _ 2). After using the PCR program for the *16s rRNA* gene, the product was placed in an electrophoresis device and the result showed the presence of bands with an estimated molecular size depending on the radiations of the ladder that approximately 1500 base pairs as shown in figure (4).

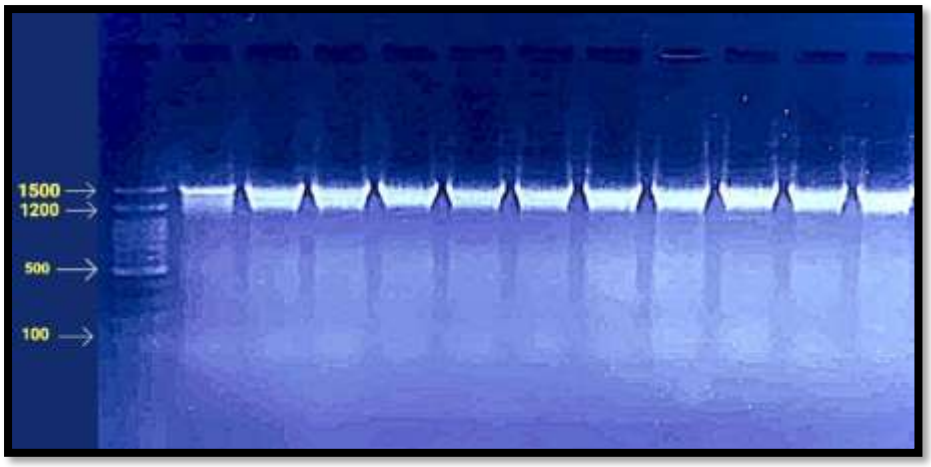


Figure (4). Agarose gel electrophoresis of PCR products for universal *16 s rRNA* gene (1495bp) on the 2% of agarose at 50 v in 45 minutes.

The gene *16 rRNA* is one of the most conserved among bacterial rRNA genes, shared by all bacterial species. It is around 1500 base pairs long and is made up of conserved nucleotide sequences interspersed with nine variable sections that are distinct to each genus or species. Microbes' phylogenetic categorization is based on the genetic sequences of their variable regions. By selecting conserved sections for PCR primers and flanking variable regions, it is feasible to create broad-range PCRs capable of detecting DNA from any bacterial species. The identification of bacteria at the species or genus level is completed by sequencing the amplified PCR product, including variable sections, and comparing it with known bacterial sequences in microbial databases., The features of the 16S rRNA gene and the enormous quantity of database information available make it a viable target for broad-range molecular investigation [27].

●Nucleic acid sequencing:

The results of sequence helped in the diagnosis of our local isolates based on NCBI as shown in figures (5) and (6) for two species *C. acnes* and *C. avidum* respectively. The percentage identity of our local strain with the registered global bacterial strains appeared to be between (87.5-100) % as demonstrated in Table (4).

We obtained seven isolates of *C.acnes* and one belongs to *C.avidum* depended on the NCBI website as shown in figure (5)and(6)

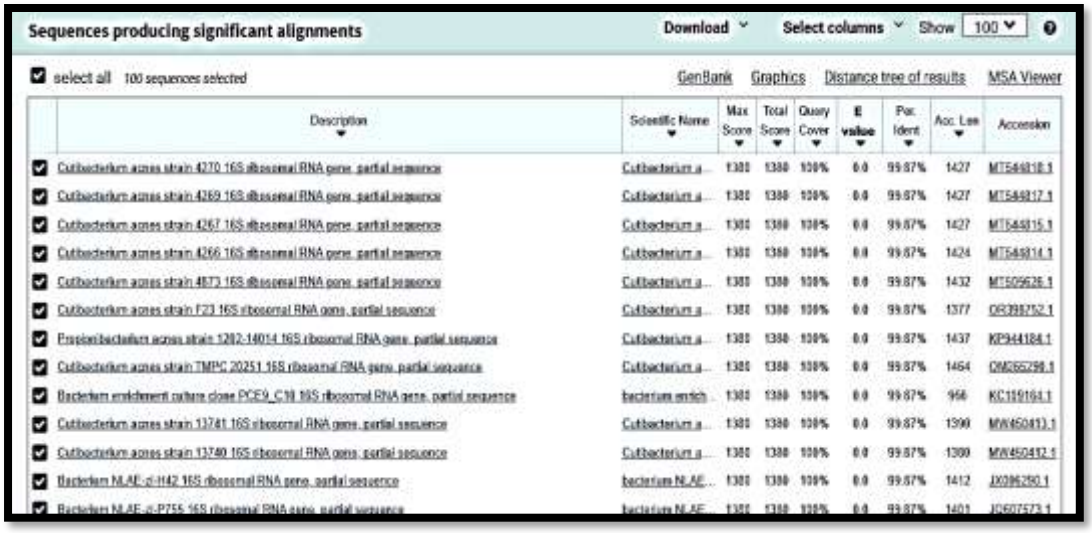


Figure (5). Identity of *C. acnes* understudy with global strains in NCBI

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Cutibacterium avidum strain 39 16S ribosomal RNA gene, partial sequence	Cutibacterium avidum	1432	1432	100%	0.0	100.00%	1414	MK455360.1
Cutibacterium avidum strain PCH-192 16S ribosomal RNA gene, partial sequence	Cutibacterium avidum	1432	1432	100%	0.0	100.00%	830	MF099824.1
Uncultured bacterium clone nbu591d12c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF070748.1
Uncultured bacterium clone nbu590b11c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1343	KF070731.1
Uncultured bacterium clone nbu590a04c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF070636.1
Uncultured bacterium clone nbu591f12c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF070667.1
Uncultured bacterium clone nbu591d08c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF070499.1
Uncultured bacterium clone nbu551e10c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1343	KF069742.1
Uncultured bacterium clone nbu551c12c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1343	KF069740.1
Uncultured bacterium clone nbu551h04c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF069574.1
Uncultured bacterium clone nbu554g10c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1343	KF069155.1
Uncultured bacterium clone nbu451f06c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF063841.1
Uncultured bacterium clone nbu452d11c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF063788.1
Uncultured bacterium clone nbu451f05c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1338	KF063741.1
Uncultured bacterium clone nbu447g08c1 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1432	1432	100%	0.0	100.00%	1344	KF063712.1

Figure (6). Identity of *C. avidum* understudy with global strains in NCBI

Table (4): the percentage identity for seven strains of *C. acnes* and one strain of *C. avidum*.

NO	Local strain name	% identity
1	*C. acnes* 103	99.87
2	*C. acnes* 107	87.54
3	*C. acnes* 110	99.16
4	*C. acnes* 111	99
5	*C. acnes* 112	99.3
6	*C. acnes* 117	99.7
7	*C. acnes* 118	98.4
8	*C. avidum* 113	100

One of the *C. acnes* isolates was identical to the isolate *C. acnes* strain 4269 16S rRNA gene with 99.3% isolated in China and *C. acnes* strain 4270 with 99.74 isolated in China, others were identical to the *C. acnes* strain KCOM 1861 with 87.5% isolated in Korea and *C. avidum* were identical to the *C. avidum* strain 39 with 100% isolated in France, depended on global isolates recorded in NCBI website.

The percentages of bacterial isolates in the NCBI database may differ from those recorded globally in DNA sequences for several reasons:

1. Sample and Source: The samples collected and recorded in the NCBI database may differ from those collected in DNA sequencing studies. This can affect the genetic diversity present in their corded samples.
2. Testing and Recording Methodologies: Different laboratory studies may use varied methodologies for isolating and recording bacteria, potentially resulting in species identification through different or varying degrees of accuracy.
3. Biological Diversity: The actual biological diversity of the studied environment, influenced by environmental, genetic, and evolutionary factors, can lead to variations in percentage compositions across different locations.
4. Analytical and Statistical Analysis: The methods used for data analysis and statistical procedures can impact the recorded result leading to differences in recorded percentages.

3.2Antibiotic Sensitivity of C. acnes:

Eight antibiotics were chosen to perform the susceptibility tests by disc diffusion assay. According to the standard values (CLSI,2023) [21] and (Eucast2024) [22] the findings indicated differences in the antibiotic resistance patterns. the study showed that seven of *C. acnes* isolates were resistant to fusidic acid and azithromycin at 85.7%, tetracycline at 71.4%, clindamycin and gentamycin at 57.1% respectively, and vancomycin at 42.8% the isolates were sensitive 100% to imipenem and levofloxacin, as shown at Table (5).

Table (5). The antibiotic sensitivity of *C. acnes*

Type of isolation	**Type of antibiotics**							
	DA	**VA**	**TE**	**IPM**	**CN**	**LEV**	**FA**	**AZM**
C. acnes 103	S	R	R	S	R	S	R	R
C. acnes 107	R	S	R	S	S	S	R	R
C. acnes 110	R	S	I	S	S	S	R	R
C. acnes 111	S	R	R	S	R	S	R	R
C. acnes 112	S	I	R	S	R	S	R	R
C. acnes 117	R	R	S	S	R	S	R	S
C. acnes 118	R	S	R	S	S	S	S	R
% of the resistant strain	57.1%	42.8%	71.7%	0%	57.1%	0%	85.7%	85.7%

R: resistance I: intermediate S: susceptibility

DA: Clindamycin _ VA: Vancomycin _ TE: Tetracycline _ IPM: Imepinem _ CN: Gentamycin _ LEV: Levofloxacin FA: Fusidic acid _ AZM: Azithromycin

Research has shown that antibiotics work effectively to treat inflammatory acne, But the excessive use of antibiotics has led to an increase in bacterial resistance to antibiotic drugs in up to 40% of *C. acnes* strains (resistance to clindamycin, tetracycline, and erythromycin), increasing the possibility of treatment failure [28]. Interestingly, the anaerobic species may have inherent resistance due to a lack of molecular processes involved in the absorption of the antibiotic[29] *C. acnes* resistance to commonly used antibiotics, such as erythromycin and clindamycin, has been found in high prevalence in Mediterranean nations, primarily owing to antibiotic overuse. For example, researchers in Spain showed 91 and 92.4% resistance to both medicines. In Greece, resistance to both antibiotics was 75.3%, whereas in Italy, it was 59.5. The growing number of *C. acnes* drug-resistant strains has raised global worries about the diminishing number of antibiotics available to treat this widespread illness [11]. *Citrbacter spp* has shown resistance to tetracycline and sensitivity to levofloxacin and variability against gentamycin [30].

3.3 Phylogenetic tree:

After relying on the MEGA 11 program to determine the extent of the relatedness among the strains under study, the sequences of the nitrogenous bases were added to all the isolates, and a phylogenetic tree was created depending on the neighbor joining as shown in the following figure (7).

A phylogenetic tree following analysis of 9 nucleotide sequences of 1495 bp of the gene coding for 16S rRNA gene in different strains of *C. acnes, C. avidum,* and staph aureus were drawn according to the neighbor-joining tree method using the MEGA11 software package. Strains 103 and 107 each showed a genetic affinity of 93%, and their clusters formed a 92% affinity with strain 111, their clusters formed an 89% affinity with strain 112 which in turn clustered 86% with strain 118. The similarity of the latter to strain 117 decreased to 74%. In turn, it clustered with strain 110 at a rate of 99%. The *C. avidum* strain was distant from the *C. acnes* strain. Naturally, the *Staphylococcus aureus* strain was at the end of the tree, which was used to create a root for the genetic tree of the strains. These differences in the percentage of affinity between strains of the same species are perhaps due to the different factors affecting the formation of acne, as well as a result of the occurrence of mutations that cause genetic variation.

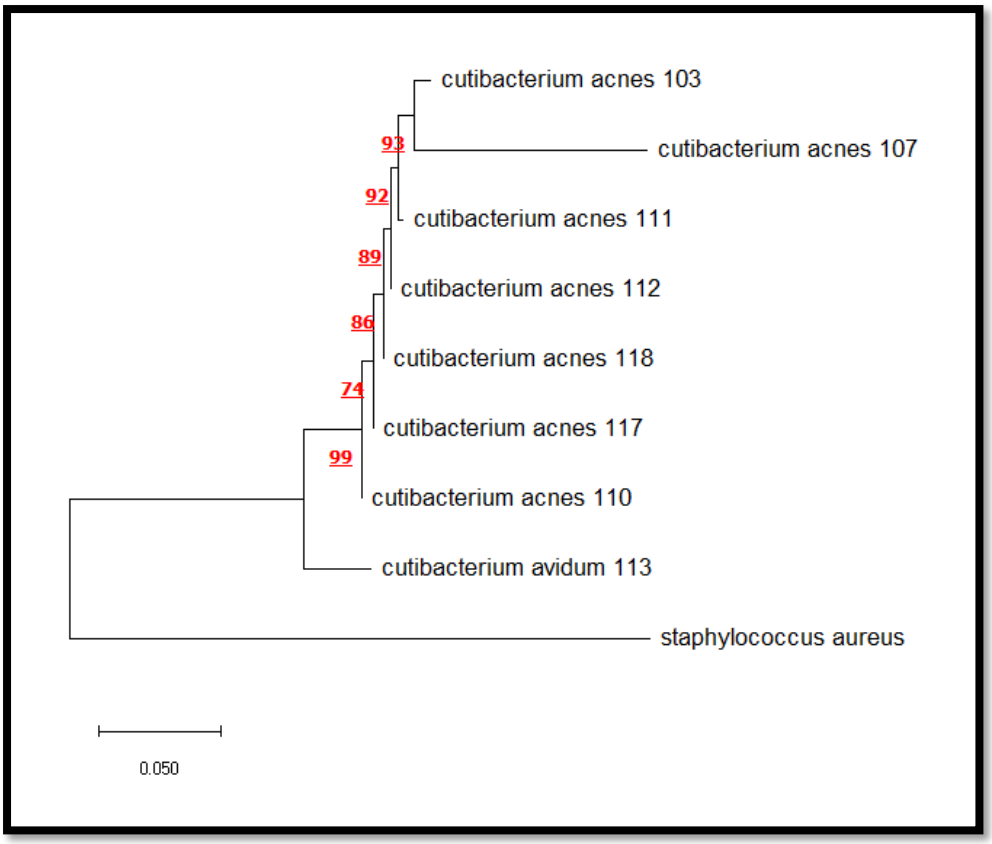


Figure (7). Phylogenetic tree of *C. acnes*

4. Conclusions:

In our study, we used universal 16s rRNA for two purposes. Firstly, we used it to diagnose bacteria, and secondly, we used it to determine the extent of genetic relatedness between bacterial isolates. From our study, we concluded that comparing nucleotide sequences between our local isolates of the bacterium C. acnes and global isolates is an efficient method for diagnosing these bacteria. It is not necessary to rely on a specific gene for diagnosis. Additionally, the study revealed genetic differences ranging from 74% to 99% between our local isolates of C. acnes bacteria, as determined from the genetic tree resulting from general gene sequencing comparisons. Finally, all of our local isolates showed absolute sensitivity to the two antibiotics (Imepinem and Levofloxacin), which gives a clear conclusion about the efficiency of these two antibiotics in treating *C. acnes.*

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الشجرة الوراثية لسبع سلالات من *Cutibacterium acnes* وحساسيتها للمضادات الحيوية الشائعة

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المستخلص:

أجريت هذه الدراسة بهدف التعرف على إحدى أبرز البكتيريا المسببة لحب الشباب *Cutibacterium acnes* ودراسة انتشار ظاهرة المقاومة للمضادات الحيوية المختلفة المستخدمة لعلاج حب الشباب ودراسة العلاقة التطورية بين عزلات *Cutibacterium acnes*, تم جمع 125 عينة من المرضى الذين يعانون حالات حب الشباب في مدينة الموصل من استشاريات الأمراض الجلدية في مستشفى السلام التعليمي ومستشفى الموصل العام، وتم تشخيص العينات من خلال الفحوصات الروتينية ومن خلال التشخيص الجزيئي على أساس universal 16s rRNA، وكانت نتائج العزل والتشخيص الحصول على سبع عزلات من بكتريا *Cutibacterium acnes* وعزلة واحدة من بكتريا *Cutibacterium avidum* بنسبة (5.6%) و (0.8%) على التوالي من جميع العينات. تم اختيار ثمانية مضادات حيوية لإجراء اختبارات الحساسية بالاعتماد على طريقة disc diffusion assay، وأظهرت الدراسة أن سبعة من عزلات C. acnes كانت مقاومة للمضادين fusidic acid و azithromycinبنسبة 85.7%، tetracyclineبنسبة 71.4%، clindamycin و gentamycinبنسبة 57.1% على التوالي والفانكومايسين بنسبة 42.8%. كانت العزلات حساسة بنسبة 100% imipenemو levofloxacin. في هذه الدراسة تم إنشاء شجرة تطورية تعتمد على درجة التقارب بين هذه العزلات المشخصة جزيئيا اعتمادا على برنامج MEGA11 أظهرت كل من السلالات 103 و107 و111 تقاربا وراثيا بنسبة 93%، وشكل عنقودها تقاربا مع السلالة 112 بنسبة91% وهو بدوره تعنقد بنسبة 89% مع السلالة 118 وهذا الأخير قلت نسبة تقاربه مع السلالة 117 الى 74% بدوره تم التعنقد مع السلالة 110 بنسبة 99% جاءت سلالة *C. avidum* بعيدة عن سلالات C. acnes ومن الطبيعي ان كانت سلالة *Staphylococcus aureus* في نهاية الشجرة التي استخدمت لعمل جذر لشجرة الوراثية لسلالات المحلية.