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Detection the Susceptibility Diversity of Commonly Used Antibiotics Against *Staphylococcus* Species Isolated from Iraqi Acne Patients

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Abstract :

Context: Acne vulgaris (AV) is a chronic inflammatory disorder that primarily affects adolescents and young adults, one of the causing agent of acne is *Staphylococcus aureus.*

Aims: Isolate and diagnose bacteria from acne lesions on the face, as well as conduct sensitivity tests using commonly prescribed antibiotics for acne treatment. The study also seeks to analyze the distribution of acne in both male and females.

Materials and methods: the bacteria Subjects in the case group were 360 acne vulgaris patients A total of 280 were identify as of *Staphylococcus aureus*. the rest samples 80 were identify as *Staphylococcus epidermis*, diagnosed using colonies appearance on media and the biochemical test, antibiotics sensitivity test was conducted on all of *S.aureus* isolates to determine the best antibiotics that works to eliminate acne vulgaris and evaluating the distribution of acne in male and females .

Statistical analysis: Chi-square and percentage calculations were used in order to determine the significance level, or P-value, between the various study parameters. To determine the difference in drug resistance levels, the Fisher tests (95% confidence interval) were utilized. Statistically non-significant values of p>0.05 were regarded as such, whereas statistically significant values of p0.05 were. SPSS (v 20) was used to conduct the statistical analysis.

Results: Results showed in the male group of 140 isolate, blackhead acne type is the highest percentage 69.8% than females 47.7% . Tested the efficacy of several commonly used antibiotics against staph for the treatment of acne, which are Ampicillin, Tetracycline, Erythromycin, trimethoprim-sulfamethoxazole, Clindamycin, Oxacillin, Rifampicin and Vancomycin. The highest rate of resistance to ampicillin was recorded (54.64%), while the vancomycin antibiotic recorded the lowest rate of resistance (5.36%).

Conclusions: the outcomes were encouraging. Our findings, when compared to those of national and worldwide investigations, revealed that the percentage of aberrations in antibiotic resistance is quite low. As a result, ongoing antibiotic renewal may be necessary to combat these resistant strains.

Key-words: Acne, Antibiotic resistance, *Staphylococcus aureus. Staphylococcus epidermidis.*

الكشف عن تنوع حساسية المضادات الحيوية الشائعة الاستخدام ضد أنواع المكورات العنقودية المعزولة من مرضى حب الشباب العراقيين

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مستخلص

السياق: حب الشباب هو اضطراب التهابي مزمن يصيب في المقام الأول المراهقين والشباب، وأحد العوامل المسببة لـحب الشباب هو المكورات العنقودية الذهبية.

الأهداف: عزل وتشخيص البكتيريا من آفات حب الشباب في الوجه، وكذلك إجراء اختبارات الحساسية باستخدام المضادات الحيوية الموصوفة عادة لعلاج حب الشباب. كما تسعى الدراسة إلى تحليل توزيع حب الشباب عند الذكور والإناث على حد سواء.

الطرق والمواد: كانت البكتيريا في مجموعة الحالات 360 مريضاً مصاباً بحب الشباب، وتم تحديد 280 منهم على أنهم من المكورات العنقودية. المذهبة. وتم تحديد العينات المتبقية وعددها 80 على أنها المكورات العنقودية. البشرة، تم تشخيصها باستخدام مظهر المستعمرات على الوسط والاختبار البيوكيميائي، وتم إجراء اختبار الحساسية للمضادات الحيوية على جميع عزلات بكتريا المكورة العنقودية البرتقالية لتحديد أفضل المضادات الحيوية التي تعمل على القضاء على حب الشباب وتقييم توزيع حب الشباب عند الذكور والإناث.

التحليل الإحصائي: تم استخدام حسابات مربع كاي والنسبة المئوية لتحديد مستوى الأهمية، أو القيمة P، بين معلمات الدراسة المختلفة. لتحديد الفرق في مستويات مقاومة الأدوية، تم استخدام اختبارات فيشر (فاصل الثقة 95٪). تم اعتبار القيم غير المهمة إحصائيًا البالغة p>0.05 على هذا النحو، في حين تم اعتبار القيم ذات دلالة إحصائية البالغة p0.05. تم استخدام برنامج SPSS (الإصدار 20) لإجراء التحليل الإحصائي.

النتائج: أظهرت النتائج في مجموعة الذكور المكونة من 140 عزلة أن نوع حب الشباب ذو الرؤوس السوداء هو الأعلى بنسبة 69.8٪ مقارنة بالإناث 47.7٪. تم اختبار فعالية العديد من المضادات الحيوية شائعة الاستخدام ضد المكورات العنقودية لعلاج حب الشباب، وهي الأمبيسلين، التتراسيكلين، الاريثروميسين، تريميثوبريم-سلفاميثوكسازول، كليندامايسين، أوكساسيلين، ريفامبيسين وفانكومايسين. سجلت أعلى نسبة مقاومة للأمبيسلين (54.64٪)، بينما سجل المضاد الحيوي الفانكومايسين أقل نسبة مقاومة (5.36٪).

الاستنتاجات: كانت النتائج مشجعة. كشفت النتائج التي توصلنا إليها، عند مقارنتها بتلك التي توصلت إليها التحقيقات الوطنية والعالمية، أن نسبة الانحرافات في مقاومة المضادات الحيوية منخفضة للغاية. ونتيجة لذلك، قد يكون التجديد المستمر للمضادات الحيوية ضروريًا لمكافحة هذه السلالات المقاومة..

1. Introduction

Teenagers and young adults are most commonly affected with acne vulgaris (AV), a chronic inflammatory illness that can manifest as comedones, papules, pustules, nodules, and erythema. It is a complex illness brought on by the interaction of environmental and hereditary variables(Hazarika, 2021) . There are two categories for factors, The major four causes of AV are increased sebum secretion, pilosebaceous unit growth, inflammation, and *Cutibacterium acnes (Propionibacterium acnes)* bacterial growth(Karadag *et al*., 2021). The pathogenesis of AV is also influenced by other bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*(Claudel *et al*., 2019). genetic predisposition (family history of severe acne), obesity, oily/seborrheic skin, higher skin surface pH, emotional stress, repetitive mechanical trauma, exposure to excessive sunlight, pre-menstruation, topical application of greasy products or occlusive preparations, and medications are some of the minor predisposing factors of AV (Yang *et al*., 2020) . *Staphylococcus aureus* is one of the AV's major causative agents. The microorganisms responsible for causing a variety of human illnesses, such as AV, skin and soft tissue infections, impetigo, foliculitis, carbuncles, mastitis, and others(Taylor & Unakal, 2017) . Because long-term antibiotic medication, oral (e.g., tetracycline, erythromycin, trimethoprim sulfamethoxazole) is a standard of care in the treatment of AV, patients with AV are frequently exposed to antibiotics for prolonged periods of time (Abdu-Allah *et al*., 2020) . Because they can reduce bacterial activity and have an anti-inflammatory impact, antibiotics are crucial in the treatment of acne. Both topical and systemic formulations of antibiotics are available. Only moderate-severe acne is treated with oral antibiotics, which are given for a maximum of 12-18 weeks. On mild-to-moderately severe acne, oral antibiotics are coupled with topical antibiotics (Sitohang *et al*., 2019).

Due to the rising incidence of bacteria with antibiotic resistance, using antibiotics to treat acne has recently run into difficulties. The spread of antibiotic resistance in *P. acnes* has an effect on the entire world. *Propionibacterium sp*. was shown to be resistant to erythromycin (47.94%) and tetracycline (20.55%) in 73 instances in France,

Germany, Japan, Australia, the United States of America, and England, according to (Eady *et al*., 2003) .

The aim of this research is to isolate and diagnose bacteria from acne lesions on the face, as well as conduct sensitivity tests using commonly prescribed antibiotics for acne treatment. The study also seeks to analyze the distribution of acne in both male and females.

2. Related Works

2.1. Ethical approval

All participants agreed to provide the investigator with the specimens. The ethics committee of College of Science, Mustansiriyah University approved this work, Informed consent according to the Declaration of Helsinki was obtained from all participants (Ref.:BCSMU/1221/00011M)

2.2. Samples Collection

360 acne swab samples with ages ranging from 14 to 30 years old were gathered from the Allergy and Asthma Center in Baghdad between the months of August 2022 and April 2023. A dermatologist examined patients to determine whether they have acne using the Global Acne Grading System (GAGS) (Bez *et al*., 2011). Within two hours after collection, all swabs were sent to the laboratory using the normal procedure.

2.3. Samples Identification

Each swab sample was directly inoculated onto a sterilized blood agar plate, MacConkey agar, and Mannitol salt agar and Nutrient agar then incubated at 37°C for 24 hours to make a diagnosis based on cultural characteristics, microscopic features, and biochemical tests such as gram stain, catalase test, coagulase test and oxidase test.

2.4. Antibiotic susceptibility test

According to the Clinical Laboratory Standards Institute (CLSI), all of the *S.aureus* isolates (140 from male patients and 140 from female patients) were tested for antibiotic susceptibility using the disc diffusion method (Janarthanan *et al*., 2023) . In the sensitivity testing, the following antibiotics were used: Rifampicin (RA) 5 µg, Erythromycin (E) 10 µg, Clindamycin (DA) 10 µg Tetracycline (TE) 10 µg, Vancomycin (VA) 30 µg, trimethoprim-sulfamethoxazole (SXT) 25 µg, ampicillin (AM) 25 µg and oxacillin (OX) 5 µg. The strain's suspension was matched with 0.5 McFarland standards to produce a concentration of 1.5 10^8 CFU/

mL for the susceptibility test on Mueller-Hinton agar (MHA).

2.5. Statistical analysis

Chi-square and percentage calculations were used in order to determine the significance level, or P-value, between the various study parameters. To determine the difference in drug resistance levels, the Fisher tests (95% confidence interval) were utilized. Statistically non-significant values of $p > 0.05$ were regarded as such, whereas statistically significant values of p0.05 were. SPSS (v 20) was used to conduct the statistical analysis.

3. Results and discussion

3.1. Identification of the isolates

In this study, *Staphylococcus aureus* was the most often isolated bacterium from acne patients. Out of a total of 360 isolates, 280 were identified as *Staphylococcus aureus*, while the remaining 80 were identified as *Staphylococcus. epidermis* determined by:

3.1.1. Microscopic Features

Both *Staph. aureus* and *Staph. epidermidis* are Gram-positive bacteria with positive catalase test results. *S.aureus* is coagulase-positive, unlike *S.epidermidis*, and both are oxidase-negative. On agar plates, neither grew on MacConkey agar, but both displayed growth on blood agar. *S. aureus* showed β-hemolysis, while *S. epidermidis* did not exhibit hemolysis. In Mannitol Salt Agar, both grew, with *S. aureus* producing a golden color and *S.epidermidis* exhibiting a pink color as showed in table 1.

Table 1: Types and Characteristic features of the bacteria that isolated from all types of AV

Characteristic features	*Staphylococcus. aureus*	*Staphylococcus. epidermis*
blood agar	light to golden yellow pigment	white pigment
MacConkey agar	does not grow	does not grow
Nutrient agar	large yellow or white colonies	cream-colored to white colonies
Gram stain	gram-positive	gram-positive
catalase test	Positive (+ve)	Positive (+ve)
coagulase test	Positive (+ve)	Negative (-ve)
Oxidase	Negative (-ve)	Negative (-ve)
Mannitol salt agar fermentation	Positive (+ve)	Negative (-ve)
Hemolysis	β-hemolytic	Nonhemolytic

3.2. Antibiotic susceptibility testing

The current The recent investigation relied on the count of antibiotics to which the bacteria exhibited resistance as an indication of antimicrobial resistance. The pace of discovering new antimicrobial medications for treating these ailments has decelerated, contributing to an upswing in resistant pathogens, thereby fueling the escalating resistance trajectory. While each of these occurrences is disconcerting in its own right, their concurrent manifestation poses a dual threat, potentially impacting both public health and the economy.

In Table 2 of the antibiogram results, all tested S. aureus isolates demonstrated affirmative resistance to multiple antibiotics employed in this research.

Table 2: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolates from male and female group against a selection of eight antibiotics

Male/140	DA_10	VA_30	TE_10	E_10	AM_25	SXT_25	OX_5	RA_5	p value
S No.	120	92	54	57	56	100	98	108	0.01
S %	42.86	32.86	19.29	20.36	20.00	35.71	35.00	38.57	
R No.	20	11	62	49	79	40	26	18	0.01
R %	7.14	3.93	22.14	17.50	28.21	14.29	9.29	6.43	
I No.	0	37	28	34	5	0	16	14	0.01
I %	0.00	13.21	10.00	12.14	1.79	0.00	5.71	5.00	
Female/140	DA_10	VA_30	TE_10	E_10	AM_25	SXT_25	OX_5	RA_5	p value
S No.	110	101	40	63	52	90	106	106	0.01
S %	39.29	36.07	14.29	22.50	18.57	32.14	37.86	37.86	
R No.	28	4	70	57	74	40	20	24	0.01
R %	10.00	1.43	25.00	20.36	26.43	14.29	7.14	8.57	
I No.	2	35	30	20	14	10	14	10	0.01
I %	0.71	12.50	10.71	7.14	5.00	3.57	5.00	3.57	

Ampicillin AM ,Tetracycline TE, Erythromycin E ,trimethoprim-sulfamethoxazole SXT ,Clindamycin DA ,oxacillin OX ,Rifampicin RA ,Vancomycin VA .

***S.Sensitive,I.Intermedate, R.Resistante**

Based on these findings, we believe that the global spread of antibiotic-resistant bacteria has reached a concern-

ing level, particularly in developing nations (Amann *et al*., 2019).

The following were the results for the resistance value from the total of (280), regardless of the group being male or female and regardless of the type of AV:

Specifically, clindamycin DA (17.14%), oxacillin OX (16.43%), rifampicin RA (15.00%), vancomycin VA (5.36%), tetracycline TE (47.14%), erythromycin E (37.86%), trimethoprim-sulfamethoxazole SXT (28.57%), and ampicillin AM (54.64%), respectively.

The results of table 3

Table 3: Sensitivity profile regardless the group male or female and regardless type of AV

ALL PATIENTS 280	DA_10	VA_30	TE_10	E_10	AM_25	SXT_25	OX_5	RA_5	p value
S No.	230	193	94	120	108	190	204	214	0.001
S %	82.14	68.93	33.57	42.86	38.57	67.86	72.86	76.43	
R No.	48	15	132	106	153	80	46	42	0.001
R %	17.14	5.36	47.14	37.86	54.64	28.57	16.43	15.00	
I No.	2	72	58	54	19	10	30	24	0.001
I %	0.71	25.71	20.71	19.29	6.79	3.57	10.71	8.57	

clearly show that staphylococcal isolates from various forms of acne and from various age groups have high resistance to numerous antibiotics routinely used to treat acne, and this resistance varies in percentages. For instance, a global investigation that was based on 243 acne patients and confirmed the usefulness of ampicillin in treating acne and lowering its damage observed ampicillin resistance (54.64%) (Barber *et al*., 2019) . In addition to the fact that a local investigation (Fareed *et al*., 2023) confirmed that Staphlococcus aureus had a 40% resistance rate to ampicillin and that there were 150 germs present, BlaZ, the gene that produces -lactamase, mediates the staphylococcal mechanisms resistance to ampecillin. When staphylococci are exposed to -lactam antibiotics, an enzyme that is primarily extracellular is produced. This enzyme hydrolyzes the -lactam ring, rendering

the -lactam inactive. Two nearby regulatory genes, the repressor blaI and the antirepressor blaR1, regulate blaZ (Kernodle, 2000) The majority of S. aureus isolates were found to be resistant to tetracycline, erythromycin, and trimethoprim-sulfamethoxazole in another section of the present investigation, and these findings are consistent with those of (Delost *et al*., 2016) and (Biswas *et al*., 2015) .

A global investigation on the level of concerns about tetracycline resistance on Cutibacterium revealed that tetracycline used to treat acne is less effective (Swallow *et al*., 2022) where the proportion was (up to 37.0%) for acne isolates (Boyanova, 2023) .

Furthermore, this investigation revealed the lowest levels of resistance to clindamycin (17.14%), oxacillin (16.43%), rifampicin (15.00%), and vancomycin (5.36%), contradicting (Filimon *et al*., 2009) claim that vancomycin-resistant bacteria exist. A strain of S. aureus has evolved that is resistant to the glycopeptides. Japan reported the first vancomycin-resistant S. aureus case in 1996, but it wasn't until 2002 when glycopeptide antibiotic-resistant S. aureus was first identified. Infection with VRSA has been documented in three cases in the US.F. (Menichetti, 2005) .

To develop resistance to antimicrobial agents, bacteria primarily use one or more of four main pathways. The antibiotics themselves are rendered inactive through enzymatic degradation, as is the case with b-lactamase-producing bacteria, or the target molecules are structurally altered to prevent antibiotic binding to the target, as is the case with unmodified tetracyclines, changes in cell wall permeability that prevent antibiotic entry into the cell interior, as is the case with fluoroquinolones and b-lactams, or antibiotics are expelled from the cell by efflux pumps, These alterations in bacteria that confer resistance can result from genetic processes that alter the bacterial genome, like point mutations and genetic mutations, or they can happen as a result of horizontal gene transfer between bacteria, both within and between species (though this is a rare occurrence between species) via transformation, bacteriophage-mediated transduction, or plasmid-mediated conjugation (Sun *et al*., 2013) .

All of the aforementioned antibiotics are used for the following reasons: This shows that bacteria are becoming

more resistant to antibiotics while also showing that some medications work very well at impacting the bacteria

3.3. Acne vulgaris isolates Distribution among male and female

the results show that there was no significant difference in value between male and female isolates that were normally distributed and well-selected. However, the value did show a significant difference in the case of cyst and mild in the age mean as showed in table 4.

Table 4: Distribution of the different types of AV isolated from 140 male and 140 female

Tested groups	*Age Statistic*	*Acne type (No)*							*P value*
		Blackheads	*Cysts*	*Mild*	*Nodules*	*Papules*	*Pustules*	*Whiteheads*	
Male 140	*Mean*	20.88	20	20	19.06	23	19.14	19.48	*NSIG*
	SD	4.26	4.04	4.28	4.71	2.3	4.03	4.16	
	Median	22	20	18	17		17.5	18	
Female 140	*Mean*	21.63	23	25.37	19	23.88	22	20.03	*NSIG*
	SD	4.36	4.63	3.81	2.75	4.67	3.53	4.2	
	Median	22	25	27	19	25	22	22	
P value		NSIG	0.05	0.05	NSIG	NSIG	NSIG	NSIG	

SD = standard deviation, NS = No significant

The results also showed that in the male group of 140 isolate the mean value where at the highest number in the blackhead acne type due to the Hormonal activity in puberty is one of the causes for acne. During puberty, the increase in male sex hormones called androgens causes the sebaceous gland, which is located around a hair follicle, to grow larger and produce more sebum. Sebaceous glands are affected when their activity become hyperactive compared with normal activity in which P. acnes, will attack the sebum that is trapped under the skin surface when the hair follicles become blocked. (Ramli *et al*., 2012) .also Male produce more of the hormone testosterone than women, which results in thick and oily skin. The production of sebum, or oil, is also higher in males because their sebaceous glands are more active (from the testosterone), which can lead to clogged pores, causing breakouts of

blackheads another reason is the facial hair can trap oil and act as a breeding ground for the bacteria that exacerbates pimples and acne (Akdoğan *et al*., 2018).Sweating may increase the severity, especially during the warmer months and after exercising, especially from wearing nylon fabrics and the addition of supplements to enhance muscle growth may also promote and enhance underlying acne(Aguirre, 2015). The highest age mean value from 140 isolates in the female group was associated with mild acne, and the similar pattern was seen in (Karadag *et al*., 2021). Mild acne occurred more frequently in females while severe acne occurred more frequently in males with a p value of (0.001) due to the activity of cyclical fluctuations in oestrogen and/or progesterone in which the effects of oestrogen on the skin has provided evidence to suggest that oestrogen is associated with increases in skin thickness and dermal water content, improved barrier function, and enhanced wound healing. According to studies on the effects of progesterone, the prevalence of various dermatoses coincides with peak levels of progesterone. Acne and irritating dermatitis are two dermatoses that are aggravated during menstruation. Exacerbations occur during the menstrual cycle when progesterone levels are at their highest. Reduced immunological and barrier functioning as a result of cyclical changes in oestrogen and/or progesterone are among the underlying processes. The most well-known perimenstrual cutaneous reactions to hormones released throughout the menstrual cycle are autoimmune progesterone and oestrogen dermatitis (Raghunath *et al*., 2015) .

Clinical and biochemical hyperandrogenism was found in a considerable proportion of acne-prone women, with post-adolescent acne having a higher frequency than adolescent acne (Dhaher *et al*., 2022) demonstrated the outcomes.

Table 5: Distribution of Acne Vulgaris between male and female included in the study

Tested groups	Acne types distribution	Black-heads	Cysts	Mild	Nod-ules	Pap-ules	Pus-tules	White-heads	P value
Male 140	Number	9	20	7	15	2	14	73	0.001
	% Within the group	6.4	14.3	5	10.8	1.4	10	52.1	
	% be-tween the group	3.2	7.2	2.5	5.4	0.7	5	26.1	
Female 140	Number	11	19	8	6	10	17	69	0.001
	% within the group	7.8	13.6	5.7	4.3	7.2	12.1	49.3	
	% be-tween the group	3.9	6.8	2.9	2.1	3.6	6.1	24.5	
P value		NSIG	NSIG	NSIG	0.01	0.01	NSIG	NSIG	

Table 5 shows the results of our investigation, which reveal a high significant difference in values due to the various percent and type of acne. In descending order, the male group had whiteheads, cysts, nodules, pustules, blackheads, mild, and papules, with the whitehead value being the highest 73 (52.1%) in male group and (26.1%) between the group, and the pustule value being the lowest 2 (1.4%) in male group and (0.7%) between the group. This can be explained by the link between male hormones and the development of acne, such as testosterone, which plays an important role in(Makrantonaki *et al*., 2011) . When these hormone levels rise, it can cause an overproduction of oils and sebum, which can clog the sebaceous glands and contribute to the formation of acne. The same findings were found in an international study that provided evidence and information by analyzing the relationship between male hormones and acne formation (Zhang *et al*., 2022) . While the female group included whiteheads, cysts, pasules, blackheads, papules, mild, and nodules in declining order. The maximum value for whiteheads was 69 (49.3%) in the female group and (24.5%) between the groups, while the lowest value for nodules was 6 (4.3%) in the female group

and (2.1%) between the groups. The study reveals that elevated levels of luteinizing hormone (LH) and testosterone (TEST) hormones may be linked to the development of acne, similar to the findings of a local study (Yunos *et al*., 2023) .

4. Conclusion

This study was motivated by the urgent need to identify the most efficient antibiotics for the treatment of acne because each antibiotic has a unique mechanism of action and aids in the elimination of the bacteria that cause acne. Over time, microbes change and adapt to their surroundings, and Because some acne-causing bacteria strains have evolved to be resistant to previous antibiotics, the efficacy of treatments has decreased , A uncivilized phenomenon-the routine use of medications by patients even without obtaining a prescription from the specialist doctor-began in Iraq as a result of the general state of the people's economy and the harm done to the standard of living due to the wars and the destruction that befell it. As a result, the outcomes of this must be reviewed. Find out the amount of the harm caused by an increase in the proportion of bacteria that are resistant to antibiotics by studying with worldwide studies. Because of the rise in awareness, the cessation of antibiotic overuse, and the relative improvement in the economy, the outcomes were encouraging. Our findings, when compared to those of national and worldwide investigations, revealed that the percentage of aberrations in antibiotic resistance is quite low. As a result, ongoing antibiotic renewal may be necessary to combat these resistant strains.

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