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Original article

Screening and activity evaluation of some antibiotics against bacteria that were isolated from pustules in patients suffering from treatment-resistant acne vulgaris subsequent to COVID-19, Basrah/Iraq

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ABSTRACT

Background: Acne is among the most common skin diseases. Bacteria contribute to inflammatory lesions in acne. By using antibiotics in the management, a problem is increasing resistance to these antibiotics.**Aims**: To evaluate the activity of some antibiotics against bacteria involved in acne vulgaris that were isolated from patients suffering from treatment-resistant acne vulgaris after COVID-19 **Methods:** The study included sixty-two patients with treatment-resistant pustules. Samples were collected from the patients using sterile swabs to culture them on culture media under aerobic and anaerobic conditions. All isolates were subjected to routine tests to identify bacterial species, and Antibacterial Sensitivity Tests were done to test twenty-seven antibacterial agents. **Results**: The main causative microbial agent was *Staphylococcus epidermidis* (90%), *Staphylococcus aureus* (8.4%), and (1.6%)for both *Escherichia coli* and *Proteus*. All isolates of *S.epidermidis* were completely resistant to some Macrolides, such as Azithromycin and Erythromycin and other beta-lactam antibiotics, such as Cefixime, Penicillins, and Amoxicillin. In contrast, most isolates resisted Clarithromycin, Cephoxitin, Doxycycline, Tetracycline and Gentamicin. Other isolates show sensitivity towards some antibiotics, including Cephotaxin, Amikacin, and Levofloxacin.Most isolatesof *S. aureus* were resistant to Azthromycin, Clindamycin , Erythromycin but susceptible to Cephalothin and Cephoxitin.**Conclusions:** Bacteria causing acne vulgaris in this study were resistant to several antibiotics used in the treatment regimen but sensitive to other antibiotics not used for treatment, according to in vitro drug sensitivity testing. The antibiotics used for treatment should be reevaluated.

Introduction

Acne Vulgaris is a chronic inflammatory condition of the pilosebaceous follicles. It isn't fatal, but it can leave the sufferer with scars, irritation, and significant psychological effects [1]. Acne is a common skin disease affecting adolescents treated by dermatologists. It also can be seen in adults [2]. The causes of acne have been widely investigated; genetic background, gender, smoking, and certain diets are predisposing factors for the disease flare-up [3]. Although acne is not an infectious disease, bacteria contribute significantly to inflammatory, anaerobic bacterium such as *Propionibacterium acnes* and aerobic bacterium [4] such as

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Staphylococcus aureus [5] and *Staphylococcus epidermidis* [6]. So, several studies have indicated the use of topical and or systemic antibiotics in managing mild, moderate, and severe inflammation [7]. Therefore, many of these antibiotics are used for acne treatment, such as macrolides, Tetracyclines, Clindamycin, Trimethoprim/sulfamethoxazole8Sulfonamides and Dapsone [7,9].

A considerable amount of literature has been published on the abuse or incorrect use of local and systemic antibiotics worldwide; this is associated with a lack of ethical consideration in prescribing and dispensing antibiotics. Furthermore, the low-cost availability of different dosage forms facilitates the reaching of antibiotics to consumers in Iraq. It has raised a major health problem in recent years of antibiotic resistance [10]. However, this problem has globally increased over time, mostly explained by the misuse of antibiotics in treating a wide range of infectious diseases without any restriction or time limit duration that makes such antibiotics ineffective. Therefore, a growing body of reports from different countries around the world talks about antibiotic-resistant strains of bacteria associated with acne, as *Propionibacterium acnesStaphylococcus spp* [11].

Today, especially after overutilization and improper use of existing antibiotics during the COVID-19 pandemic, different antibiotics for acne treatment, such as Erythromycin, Clindamycin, and Tetracyclines, are ineffective and develop emergency strains of bacteria that are resistant to antibiotics [12].

So far, such resistance is poorly understood, and the evidence for the relationship is unexplained. So, this study aimed to investigate and clarify antibiotics resistance ratespost-COVID-19 that cause management failure of patients with acne vulgarisinBasrah City, Iraq.

Materials and methods

Patients

This study was conducted at the Abu Al-Khasib General Hospital in Basrah Governorate. Samples were collected from patients of a dermatology consultant under the supervision of a dermatologist from December 2021 to July 2022. The study included seven male and fifty-five female patients with treatment-resistant pustules. Their ages ranged from 15 to 65 years old, and 82% of them were under thirty years old.

Sample collection

Sterile swabs were used to collect samples from patients. After sterilizing the pustules using 70% ethyl alcohol and squeezing the contents of the pustules, two samples were collected using two sterile swabs.The swabs were inoculated onto various culture media including blood agar, MacConkey's agar, and chocolate agar (HiMedia Laboratories **Private Limited**,India). They were incubated directly (for each sample, one swab was kept under aerobic conditions and the other under anaerobic conditions) at a temperature of 37Cº. After the culture results appeared, all isolates were subjected to a set of routine tests to identify bacterial species.

Antibacterial Sensitivity Tests

The Kirby-Bauer disc diffusion method was used to test the antimicrobial sensitivity of all isolates on Mueller-Hinton agar(**HiMedia Laboratories Private Limited,India)** and commercial antibiotic discs for twenty-seven antibiotics(**HiMedia Laboratories Private Limited,India**and Liofilchem,Italy).

Suspension of the tested bacteria was matched with 0.5 McFarland standards (1.5 × 108 CFU/mL) that were spread onto culture medium, and the antibiotic discs were placed onto the agar surface of the plate for incubation at 37C∘. Subsequently, the diameters of the resulting zones of inhibition were measured from the diameter of the zone of inhibition (in millimeters) and interpreted using the CLSI zone diameter interpretative standards (CLSI, 2020) in addition to using Vitek.

Statistical analysis

Spss (v.26) was used to analyze the data .the ANOVA test was used to determine the significant differences.$P<0.05$.

Results

Microbial analysis of samples taken from acne pustules indicates that all samples showed bacterial growth (100%). The main causative agent was *S. epidermidis* with fifty-five samples (90%) belonging to this bacterium. Additionally, five samples (8.4%) were found to contain *S. aureus* while one sample (1.6%) exhibited the presence of both *Escherichia coli* and *Proteus*.

The results of Antibacterial Sensitivity Tests against *S. epidermidis* indicated that all tested isolates were completely resistant to some macrolides as Azithromycin (AZM) and

Erythromycin (E) and other beta-lactam antibiotics as Cefixime (CFM), Penicillins(P) and Amoxillin (Ax) while most isolates showed resistance to Clarithromycin (CLR) Cephoxitin (FOX), Doxycycline (DO) Tetracycline (TE) and Gentamicin (GEN). Other isolates show sensitivity towards some antibiotics, including (FOX), Amikacin (AK), and Levofloxacin (LEV). Additionally, other antibiotics had a milder effect on these bacteria, such as Neomycin (N) and Vancomycin (VA) as evidenced in **Table 1**.

By analyzing data to determine significant differences between groups of antibiotics consisting of more than one antibiotic that tested against*S.epidermidis*, it was found that there were notable disparities in the resistance to antibiotics among different types, the macrolide antibiotics group (p =0.003) **Table 2** the Beta-lactam antibiotic 1st, 2nd, and 3rd generation of Cephalosporins group (p =0.002) **Table3**, the Aminoglycoside group (p=0.019) **Table 4** and the Beta-lactam antibiotics group (p =0.050) **Table 5**, all exhibited significant differences in this regard. Notably, there were also sizeable differences between the isolates sensitive to antibiotics within the Beta-lactam antibiotics 1st, 2nd, and 3rd generation of Cephalosporins (p=0.006) **Table 2**.

The Antibacterial Sensitivity Tests conducted on isolates of *S.aureus*revealed that all isolates were resistant to (AZM), Clindamycin (DA), (CFM), (E), Tobramycin (TOB), (TE), (GEN), and (Ax) except for one isolate which showed sensitivity to (Azm) and (DA). Furthermore, all isolates were susceptible to Cephalothin (Kf) and (FOX) except for one isolate. Concerning the sample shared between *E. coli* and *Proteus*, the results showed that *E. coli* was resistant to (AZM), (CFM), (CTR), (DO), (TCC), Trimethoprim (TMP), and Trimethoprim Sulphethozole (SXT) but sensitive to (LEV). On the other hand, *Proteus* exhibited resistance to (Azm), (KF), Ticarcillin (TCC), (TMP), and Cephepime (CFP), but was sensitive to (TOB).

Table1. The Antimicrobial Sensitivitytest of *Staphylococcus epidermidis, in brackets* abbreviation of the disk content antibiotic and concentration (µg).

Macrolide antibiotics			
Antibiotic (abbreviation)	Resistant %	Intermediate %	Sensitive %
Azthromycin (AZM 15)	100	0	0
Erythromycin (E 10)	100	0	0
Erythromycin (E15)	100	0	0
Clarithromycin (CLR 15)	85.6	37.9	3.4
Beta-lactam antibiotic 1^{st},2^{nd}&3^{rd} –generation of Cephalosporins			
Cephalexin (CI 30)	46.2	34.6	19.2
Cefixime (CFM 5)	100	0	0
Cephepime (CFP 30)	17.4	26.1	56.6
Cephalothin (KF30)	36.8	7.9	55.3
Cephoxitin (FOX 30)	87.5	0	12.5
Cefotaxime (CTX)	16.7	22.2	61.1
Cefpodoxime (CDP 10)	57.1	28.6	14.3
Ceftriaxone, (CTR)	44.4	0	55.6
beta-lactam antibiotics			
Ticarcillin (TCC 15)	14.8	33.3	51.9
Penicillins (P 10)	100	0	0
Amoxillin (AX)	100	0	0
Piperacillin (PRL 100)	50	0	50
Sulfonamides antibiotics			
TrimethoprimSulphethozole (SXT 25)	45.8	6.25	47.9
lincomycin antibiotics.			
Clindamycin (D10)	73.9	13	13
Diaminopyrimidines antibiotics			
Trimethoprim (TMP 5)	42	6	52
Tetracycline class antibiotics			
Doxycycline (DO 30)	62.5	9.4	28.1
Tetracycline (TE 30)	88.2	0	11.7

Aminoglycoside antibiotics			
T*obramycin* (TOB 30)	47.7	20.5	31.8
Neomycin (N10)	26.7	73.3	0
Amikacin (AK)	28.6	7.1	64.3
Gentamicin (GEN 10)	65.4	3.8	30.8
Fluoroquinolone antibiotics			
Levofloxacin (LEV)	33.3	0	66.7
Glycopeptide antibiotics			
Vancomycin (VA 30)	0	47.4	52.6

Table 2. Macrolide antibiotics sensitivity test, in brackets abbreviation of the disk content antibiotic and concentration (µg).

Macrolide antibiotics			
Antibiotic	Resistant %	Intermediate %	Sensitive %
Clarithromycin(CLR15)	58.6	37.9	3.4
Azthromycin (AZM 15)	100	0	0
Erythromycin (E 10)	100	0	0
Erythromycin (E 15)	100	0	0
p-value	0.003*	0.391	0.391

* represent significantly difference among antibiotics P <0.05 level.

Table 3. Beta –lactam antibiotic 1st,2nd and 3rd –generation of cephalosoprins Sensitivity Test, in brackets abbreviation of the disk content antibiotic and concentration (µg).

Beta lactam antibiotics1st,2nd&3rd –generation of Cephalosporins			
Antibiotic	Resistant %	Intermediate %	Sensitive %
Cephalexin (CI30)	46.2	34.6	19.2
Cefixime (CFM5)	100	0	0
Cephepime (CFP 30)	17.4	26.1	56.6
Cephalothin (KF 30)	36.8	7.9	55.3
Cephoxitin (FOX 30)	87.5	0	12.5
Cefotaxime (CTX)	16.7	22.2	61.1
Cefpodoxime (CDP 10)	57.1	28.6	14.3
Ceftriaxone (CTR)	44.4	0	55.6
p-value	0.002*	0.023*	0.006*

* represent significantly difference among antibiotics P <0.05 level.

Table 4. Aminoglycoside antibiotic sensitivity test, in brackets abbreviation of the disk content antibiotic and concentration (µg).

Aminoglycoside antibiotic			
Antibiotic	Resistant %	Intermediate %	Sensitive %
T*obramycin* (TOB 30)	47.7	20.5	31.8
Neomycin (N10)	26.7	73.3	0
Amikacin (AK)	28.6	7.1	64.3
Gentamicin (GEN 10)	65.4	3.8	30.8
p-value	0.019*	0.203	0.094

* represent significantly difference among antibiotics P <0.05 level

Table 5. Beta-lactam antibioticsensitivity test, in bracketsabbreviation of the disk content antibiotic and concentration (µg).

Beta-lactam antibiotics			
Antibiotic	Resistant %	Intermediate %	Sensitive %
Ticarcillin (TCC 15)	14.8	33.3	51.9
Penicillins (P 10)	100	0	0
Amoxillin (AX)	100	0	0
Piperacillin (PRL 100)	50	0	50
p-value	0.050*	0.391	0.182

* represent significantly difference among antibiotics P <0.05 level

Discussion

S.epidermidis, is one ofthe skin commensals that plays a role in the physiopathology of skin diseases like acne [13]. The results of this study show that *S.epdermidis* was present in 90% of specimens taken from pustules whereas *S. aureus* was isolated in only 8% of specimens accordance with findings of Moon *et al* [6],Soady and Karomi in Kirkuk[14] ,Rasool in Baghdad [15] and Innam and Yousif in Erbil[16]which contradicts Al-Musawi's results, who only isolated *S. aureus* of acne in adolescents in the Basrah governorate [17] while the percentage of *E. coli* isolates was very identical to what was Rasool recorded [15].

Macrolid includes antibiotics that have been used as a treatment for acne [18].The results of this study show that all the *S. epidermidis* isolates were resistant to (AZM). This finding contradicts previous studies that have shown the effectiveness of (AZM) in treating acne, as demonstrated in previous studies in Basrah [19,20]On the other hand, some studies have suggested that (AZM) is a well-tolerated option for acne vulgaris treatment [21] The emergence of resistance to (AZM) may be attributed to its widespread use in treating COVID-19 infections worldwide[22] including in Iraq, as mentioned in the study by Abbas *et al*., who referred to the role of Hydroxychloroquine and (AZM) in promoting the recovery of COVID-19 patients [23].Another study by Ali and Othman found that the use of plasma and (AZM) was the best therapy for the COVID-19 pandemic[24].These findings are consistent with the study by Abdelmalek and Mousa in Jordan, where they found a threefold increase in (AZM) use during the COVID-19 pandemic, coupled with misuse, leading to resistance [25].

In the present study, all isolates of *S. epidermidis* were resistant to (E) which is higher than was recorded by Bernadette *et al*. (65.2%) [26] and Moon *et al.* (58.3%) [6] ,this resistance of *S.epidermidis* to (E) may be due to the prevalence of the resistance genes ermA and ermC of *S.epidermidis* in Basrah province [27] The use of (CLR) may impact the microbiota and lead to the development of long-lasting resistant *S.epidermidis* isolates[28], while the resistance to (P) has been attributed to the presence of antibiotic resistance genes[29]. Additionally, all *S.epidermidis* isolates, in the present study, were found to be resistant to (AX) and (CFM) consistent with earlier research [30,31],where as 87.5% of isolates were resistant to (FOX) compared with 58% recorded by Hellmark *et al*.[32]

On the other hand, (DO) and (TE) were administered as a first-line for acne treatment[33] but emergence resistance toward them may attributed to the presence of resistance genes of some *S.epidermidis* strains [29].

Aminoglycosides are broad-spectrum antibiotics commonly utilized to treat Staphylococci infections, particularly those caused by *S. aureus* and *S. epidermidis, but* production of aminoglycoside modifying enzymes by staphylococci strains is considered the main mechanism for emergence resistance[34] as observed in the present study 65.4% of *S. epidermidis* isolates wereresistant to (GEN).

Most *S. aureus* isolates, in the present study, were resistant to (DA) and (E), consistent with the study of Moon *et al*[6]. On the other hand, contrary to the results of Adejuwon *et al*.[34], the majority of isolates showed resistance to (TE), (Gen), and (Ax). Although The Tetracycline class antibiotics should generally be the first-line therapy for moderate to severe acne [8]the resistance to (TE) may be due to the concentration of (TE) in inflamed lesions, which might act by inhibiting neutrophils chemotaxis rather than its usual antibacterial actions [35]. Consistent with the results reported by Arshad *et al*. [36] both *S.aureus* and *Escherichia coli* were resistant to (CFM).

It appears that the acne-causing bacteria's resistance to antibiotics, especially those used in

regime therapy, may be increasing as a result of heightened exposure to disinfectants and non-pharmaceutical agents during the COVID-19 pandemic. This exposure may trigger the development of mechanisms that induce mutations, leading to a rise in antibacterial resistance. Additionally, the spread of COVID-19 has impeded the diagnosis and treatment of patients with infectious diseases that necessitate antibiotic usage[12].

In current study, (AK), (VA) and (LEV) were exhibited high activity against *S. epidermidis* isolates that which aligns with the findings of Calderón-Jaimes *et al.*[37], Hellmark *et al.* [32]and Sari *et al.* [38] respectively. It is interesting to note, that although (SXT) is efficacious in treating acne, it was frequently used as a third-line treatment option with some patients[33].(KF) exhibits strong action against *S. aureus*[39] as well as (FOX) has activity against *S.aureus* that is isolated from clinical specimens [40] .Cefotaxime (CTX) seems to be effective in treating infections caused by staphylococci[41] including skin infections caused by *S.epidermidis and S.aureus* [42]by current study.

One of the study's limitations is that it only used one Basrah medical facility to gather samples from patients with acne that was resistant to therapy. As a result, we recommend that the research's scope be extended in the future to encompass additional Basra medical facilities.

Conclusions

Numerous antibiotics were evaluated in this investigation. A number of them, including (AZM),(TE),(DA),(E),(Do), and (TMP), were registered as antibiotics for the treatment of acne vulgaris, but others were not. The bacteria causing acne vulgaris in this study was found to be resistant to several antibiotics used in the treatment regimen, but sensitive to other antibiotics that are not used for treatment, according to in vitro drug sensitivity testing. The antibiotics used for treatment should be reevaluated.

Funding

None declared.

Conflict of Interest

There are no significant conflicts of interest among the authors relevant to this research subject.

Data availability

Data is available on request from the corresponding author.

Author Contributions

The corresponding author , Ban Anas Sabbar contributed to funding acquisition, Investigation, methodology , resources, validation, visualization and writing – original draft. The second author , Abdulla Ayob Yaqoub contributed contributed to conceptualization, funding acquisition,investigation, investigation, resources, visualization and writing – review & editing. The third author, Ausama Ayob Jaccob contributed to conceptualization, funding acquisition,investigation, methodology , Project administration investigation, resources, software, supervision visualization and writing – review & editing

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