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Original article

Antibiotics Resistance and Susceptibility Pattern of *Staphylococcus aureus* and *Staphylococcus epidermidis* associated with Acne

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ABSTRACT

Background and aims. *Pimples (acne) are small skin lesion or inflammations of the skin, mostly caused by the hormonal changes that occur during adolescent and teenage years. Studies have shown that most common bacteria associated with acne are S. epidermidis, S. aureus and Propionibacterium acne. The development of antibiotics resistance by species of bacteria associated with this disease condition has been attributed to the indiscriminate and overuse of certain antibiotics in its treatment. This study was conducted to isolate the incriminating bacteria in acne and to determine their susceptibility to antibiotics.* ***Methods****. A total of 73 samples were collected from male and female patients. The samples obtained were streaked on blood agar and mannitol salt agar plate and then incubated at 37oC for 24 h. Presumptive isolates obtained were subjected to Gram staining and other biochemical tests for identification. The confirmed isolates were further subjected to antibiotics sensitivity tests using disk diffusion method.* ***Results****. The results revealed that S. epidermidis strains were 56(76.7%) and 7(9.5%) were S. aureus strains following morphological and biochemical tests, while 10(13.6%) of the isolates yielded no bacterial growth. Bacterial susceptibility testing was done using disk diffusion method for the 63 isolates (S. aureus and S. epidermidis), and the finding showed that S. epidermidis was susceptible to doxycycline 38(67.86%), and ofloxacin 15(26.79%), while resistance to tetracycline, erythromycin, and clindamycin was observed in 47(82.93%), 40(71.4%), and 22(39.29%) of S. epidermidis isolates respectively. On the other hand, S. aureus was found to be sensitive to clindamycin 7(100%) and doxycycline 7(100%), but it was resistant to ofloxacin 3(42.86%), erythromycin 2(28.57%), and tetracycline 2(28.57%).* ***Conclusion****. The findings of this study revealed presence of antibiotic resistance among Staphylococcus aureus and Staphylococcus epidermidis isolates obtained from patients with Acne vulgaris. This is an indication of the influence the consumption of antibiotics has on the development of antibiotic resistance, which is considered a serious public health problem.*

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INTRODUCTION

Pimples (also called acne) are raised red spots with a white center that develop when blocked hair follicles become inflamed and infected with bacteria. The blockages and inflammation inside the hair follicles produce cyst-like lumps beneath the surface of the skin [1]. It is one of the most common dermatologic diseases, with the onset typically coinciding with puberty, when many physiological changes such as hormone-level changes take place in the body [2]. *Acne vulgarise* is the eight most prevalent disease with studies reporting a global prevalence of 9.4% [3]. The manifestation of acne may range from very mild lesion, called physiologic acne, to very severe form characterized by abscess formation [4]. The development of acne is a multifactorial process involving both endogenous and exogenous factor, including excessive sebum secretion, ductal hypercornification, and changes in the microbial flora especially colonization with *Propionibacterium acne* [5, 6]. Studies have shown that most common bacteria associated with acne are *S. epidermidis, S. aureus and Propionibacterium acne* [7].

Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteraemia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections. Approximately 30% of human population is colonized with *S. aureus*, with mortality rates ranging from 6% to 40 % [8, 9]. *S. aureus* has been implicated as a major organism associated with pus from acne [10]. There is significant *in-vitro* evidence suggesting a possible pathogenic role for *S. aureus* in acne vulgaris [6]. This contrasts with some studies which implicated both *S. epidermidis* and *P. acnes* as bacteria-causing acne vulgaris [6]. *Propionibacterium acnes* and *Staphylococcus epidermidis*, which are normal inhabitants of the skin, have also been isolated from acne vulgaris inflammatory sites of patients [11]. *S. epidermidis* causes an opportunistic infection and has the potential to become a reservoir of antimicrobial resistance genes [12].

Systemic antibacterial drugs have been used in the treatment of acne for many years, and there are several commonly used antibiotics which have established efficacy and safety records. Antibiotics have important roles in the treatment of acne because it can suppress bacterial activities and provide an anti-inflammatory effect [13, 14]. Antibiotic therapy has been integral to the management of acne for many years. However, the widespread use of antibiotics has unfortunately led to the emergence of resistant bacteria [15.

Due to the considerable development of resistance to antibiotics seen among microorganisms causing acne and the differences in species and strains of the microorganisms in different areas, this study was conducted to determine the bacteria incriminated in acne and determine their susceptibility to antibiotics.

METHODS

Sample collection

A total of 73 samples were collected from male and female patient aged between 14 to 28 years old with moderate to severe acne at the Department of Dermatology Hospital, Beer Al-Osta Milad and Tripoli central hospital between May and July 2022. The clinical information includes age, sex, history of acne, history of current or recent antibiotic use, previous or current anti-acne treatments and any past skin infection. The collection was carried out under aseptic condition using sterile swab sticks. The pus of the pimple was pressed out and taken with the sterile swab sticks. Swabs were transported in ice packs to the laboratory within two hours of collection. The swabs containing the inoculum were inoculated onto blood agar plates supplemented with 5% sheep blood and then incubated at 37^{o}C for 24 h.

Identification

In total, the (73) were streaked on mannitol salt agar to observe their characteristics colony morphology. Suspected colonies were identified by gram staining and biochemical tests (catalase, coagulase). In addition, Gram staining test was performed for all isolates strain according to the standard procedure.

Antibiotic sensitivity testing

Antibiotics susceptibilities were tested by disk diffusion method, as recommended by the clinical laboratory standard institute (CLSI). The strain suspension was matched with 0.5 McFarland standard for turbidity. The colonies suspension was streaked on Muller Hinton agar plates. Tetracycline 10 µg, doxycycline 30 µg, erythromycin 15 µg, clindamycin 10 µg and ofloxacin 5 µg were tested by disc diffusion method. antibiotic was placed carefully on Muller Hinton agar plate and incubated for 18 to 24 hours at 37^{o}C. The diameter of zone of inhibition formed around each antibiotic disc was measured. The results were interrupted as resistant, intermediate, or sensitive according to the (CLSI).

Data analysis

The data collected were entered and analyzed using SPSS 17.0 software. The results were expressed based on descriptive analysis into frequencies, and percentages

RESULTS

Patients' demographics

Out of the total 73 patient visiting Hospital Beer Al-Osta Milad and Tripoli central hospital that were sampled, 56 were female and 17 males (Fig 1a). The patients experienced various degrees of severity of acne with moderate acne being the predominant amongst them. Percentage of female patients with severe acne was higher than males. With respect to age, participants withing the age range 22-26 had the highest number of acnes (Fig. 1b).

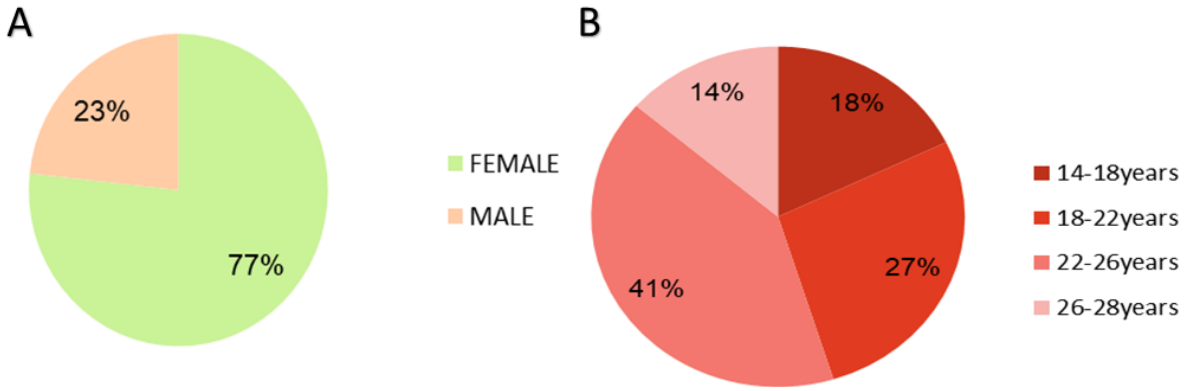

Figure 1. Cases of acne according to gender (A) and age of patients (B).

Identification and cultural characteristics

In this study there was a significant growth of *S. epidermidis* 56 (76.7%) out of the 73 isolates, while *S. aureus* was found in 7 (9.5%) of 73 isolates. An additional 10 (13.6%) of the culture did not yield any bacterial growth and were excluded. Table 1 show the distribution of bacteria isolates according to the sex of patients. The growth morphology of the seven *S. aureus* isolates indicated yellow, convex, smooth, and shiny colonies. The isolates were positive after employing the Gram Staining. Biochemical tests carried out revealed that the isolates were coagulase positive, and catalase positive which are typical of *S. aureus* (Fig. 2 A). The other fifty-six isolates appeared as white, raised, cohesive colonies that were found to be Gram-positive cocci. Similarly, biochemical tests carried out revealed that the isolates were coagulase negative, and catalase positive, which are typical growth features of *S. epidermidis* (Fig. 2 B).

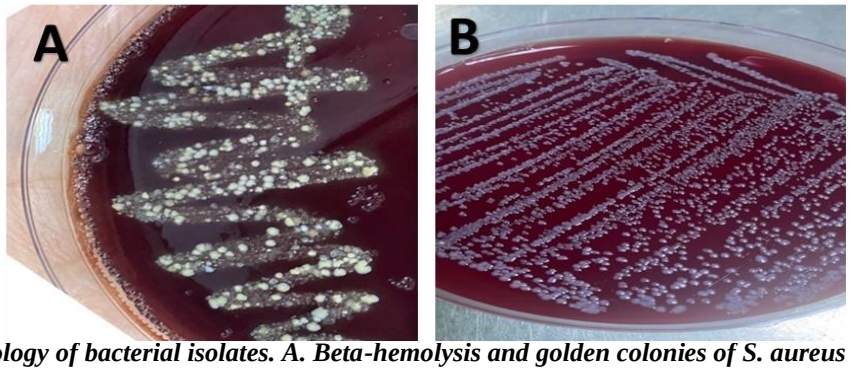

Figure 2. Growth morphology of bacterial isolates. A. Beta-hemolysis and golden colonies of S. aureus seen on blood agar. B. White colonies of S. epidermidis seen on blood agar.

Antibiotics sensitivity tests

Bacterial susceptibility testing was done using disk diffusion method for the 63 isolates of *S. aureus and S. epidermidis*. In this study, 38 (67.86%), 7 (12.5%), 15 (26.79%) and 2 (3.6%) of *S. epidermidis* were found to be susceptible to doxycycline, clindamycin ,ofloxacin and tetracycline respectively. However, the *S. epidermidis* isolates exhibited a high level of resistance to tetracycline with 47 (82.93%) and erythromycin 40 (71.4 %). Similarly, 16 (28.57%), 9 (16.07%) and 22

(39.29%) of the *S. epidermidis* isolates were also resistant to ofloxacin, doxycycline and clindamycin respectively. No *S. epidermidis* was found to be susceptible to erythromycin compared with other antibiotics.

On the other hand, all isolates of *S. aureus* 7 (100%) were susceptible to clindamycin as well as highly susceptible towards doxycycline. A total of 2 (28.57%) *S. aureus* were resistant to tetracycline as well as erythromycin. However, *S. aureus* also showed resistance to ofloxacin 3 (42.86%). Table 2 shows the resistance and susceptibility patterns of the bacteria against the antibiotics tested. While fig 3 shows the antibiotic susceptibility test of *S. epidemidis* using disc diffusion.

Table 1. Showing the resistance and susceptibility patterns of the bacteria against the antibiotics tested.

Microorganisms	Antibiotics	Susceptibility	Intermediate	Resistance
S. aureus	Erythromycin	0 (0%)	5 (71.43%)	2 (28.57%)
	Tetracycline	2 (28.57%)	3 (42.86%)	2 (28.57%)
	Doxycycline	7 (100%)	0 (0%)	0 (0%)
	Ofloxacin	2 (28.57%)	2 (28.57%)	3 (42.86%)
	Clindamycin	7 (100%)	0 (0%)	0 (0%)
S. epidermidis	Erythromycin	0 (0%)	16 (28.6%)	40 (71.4%)
	Tetracycline	2 (3.6%)	7 (12.5%)	47 (82.93%)
	Doxycycline	38 (67.86%)	9 (16.07%)	9 (16.07%)
	Ofloxacin	15 (26.79%)	25 (44.64%)	16 (28.57%)
	Clindamycin	7 (12.5%)	27 (48.21%)	22 (39.29%)

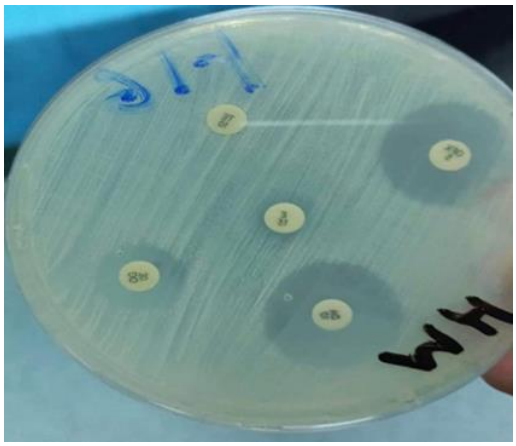

Figure 3. Antibiotic susceptibility test of S. epidemidis using disc diffusion.

DISCUSSION

Acne vulgaris is a non-infectious skin disease condition that occurs more commonly in adolescence (young adults) and less commonly in adults [16]. In this study, it was found that the age group 22-26 years had the highest prevalence of the disease where 41.1% of the participants sampled had the condition, followed by the age group 18–22 years. A similar investigation conducted in Nigerian showed that the age group 21-25 years had the highest prevalence of the disease [17]. Although the prevalence of the disease is gradually increasing among adult population, the condition is largely associated with adolescents, with studies reporting as high as 95% [18]. The reason for this might to be due to an increase in hormonal activity, such as the sex hormones called androgens, the growth hormone (GH) and insulin-like growth factor. Acne is a multifactorial disease of yet incompletely elucidated etiology and pathogenesis [18]. Microbial etiology of acne has been suggested since the beginning of the last century [19]. Elucidating the ambiguous determinants of this condition is of major public health interest. Among the 73 samples studied, *S. epidermidis* was the most isolated bacteria (76.7%) followed by *S. aureus* (9.5%). This contrasts with a study done in India where among 102 isolates, *S. aureus* (65%) was the predominant bacteria isolated [20]. However, in another study in Korea, *S. epidermidis* was the most common bacteria found which is in consonance to the present study [21]. Interestingly, similar finding was recorded in Indonesia where out of 93 swab samples studied, 7.7% were found to be *S. aureus* and 50.5% being *S. epidermidis* [22]. This difference in microbial profile in our study could be explained by variation in geographical location, host factor and antibiotic usage.

Antimicrobial resistance is assuming a center stage as one of the most important public health problems worldwide. The importance of investigating and understanding the antimicrobial resistance profile of bacterial organisms incriminated in acne is because acne vulgaris is the most common skin disease worldwide [3]. In this study, all the *S. aureus* isolates obtained were found to be susceptible to clindamycin and doxycycline 7(100%). In contrast, among 100 isolates investigated in Korea, resistance to doxycycline and clindamycin was found to be 12.5%, and 25% respectively [21]. In another study in India, among 65 *S. aureus* isolates investigated 39.7%, and 59% were found to exhibit resistance to clindamycin and erythromycin respectively [20]. Meanwhile, in this study none of the *S. aureus* were susceptible to erythromycin with 28.57% showing susceptibility to tetracycline and ofloxacin. Moreover, in a related study conducted among 93 isolates in Indonesian 42.9% were susceptible to erythromycin and 71.4% were susceptible to tetracycline [22]. In addition, *S. aureus* was also observed to show resistance to erythromycin in the present study. A similar finding was reported in Indonesian study (28.6%) [23]. However, in the present study, 28,57% were resistance to tetracycline. Similar finding was reported in India where 25% were resistant to tetracycline [24]. Clindamycin and doxycycline were the most effective antibiotics.

On the other hand, in the present study *S. epidermidis* shows high resistance to tetracycline, erythromycin and clindamycin with 82.9%, 71.4% and 39.3% respectively. Similar finding was reported among 93 isolates in Indonesia where resistance to tetracycline (32.6%), erythromycin (65.2%) and clindamycin (52.2%) were reported [22]. Meanwhile, in this study *S. epidermidis* showed low resistance to doxycycline and ofloxacin with 16.07% and 28.57% respectively. Meanwhile, in Korea, 27.3% resistance to doxycycline has been documented [21]. Interestingly, in this study a high susceptibility of *S. epidermidis* against doxycycline was recorded (67.86%). Similar finding was reported in Indonesian study were susceptible to doxycycline (89.1%). It is not surprising that the emergence of antimicrobial resistance among bacterial organisms associated with acne coincided with the introduction of topical antibiotic formulations [25]. This emergence can also be attributed to the extensive use of topical antibiotics including topical clindamycin and topical erythromycin [25]. Unfortunately, one of the persistent driving forces for resistance development in acne is because the condition is routinely treated at the outpatient setting, hence, prescription patterns and regulation of sale of drugs should be considered critical target for antibiotic stewardship efforts.

CONCLUSIONS

The present study revealed that *Staphylococcus epidermidis* and *S. aureus* are among the common pathogenic microorganisms associated with acne in the study area. The study also observed that antibiotics resistance in acne vulgaries persists and the potential for increase is considerable, which will make treatment for patients very difficult. From the antimicrobial susceptibility test, it was also revealed that *S. aureus* showed high sensitivity to clindamycin and doxycycline, Meanwhile *S. epidermidis* showed high sensitivity to doxycycline. Nevertheless, the finding in the present study suggests that the use of tetracycline needs to be limited as most of the *S. epidermidis* isolates exhibited resistance to the drug.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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مقاومة المضادات الحيوية ونمط الحساسية للمكورات العنقودية الذهبية والمكورات العنقودية الجلدية المرتبطة بحب الشباب.

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المستخلص.

الخلفية والاهداف. حب الشباب هو عبارة عن آفة جلدية او التهاب فالجلد تحدث غالبا نتيجة للتغييرات الهرمونية فترة المراهقة والشباب. أظهرت الدراسات ان البكتريا المسببة الأكثر شيوعا هي بكتريا المكورات العنقودية الذهبية والعنقودية البشروية والبكتريا البروبيونية العدية، يتمحور تطور مقاومة البكتيريا الي المضادات الحيوية المرتبطة بهذا المرض الي الاستخدام المفرط والعشوائي للمضادات الحيوية المستخدمة للعلاج. أجريت هذه الدراسة لعزل البكتريا المسببة لحب الشباب وتحديد قابليتها للمضادات الحيوية. **طرق البحث**. ثلاثة وسبعون عينة تم تجميعها من المرضي الذكور والانات. تم زرع العينات في أوساط غدائية وهي اجار الدم واجار ملح المانيتول تم تما وضعهم فالحضانة في درجة حرارة 37° درجة مئوية لمدة 24 ساعة. العزلات المتحصلة عليهم تم اخضاعهم لي اختبار صبغة الجرام واختبارات كيميائية حيوية اخري للتعرف عليها, وأيضا تم اخضاعهم الي اختبار حساسية المضادات الحيوية باستخدام طريقة الانتشار القرصي. **النتائج**. أظهرت أن السلالات العنقودية البشروبة كانت بنسبة 56(76.7%) عزلة وتأتي على التوالي البكتريا المكورات العنقودية الذهبية بنسبة 7(9.5%) بعد الاختبارات المظهرية والكيمائية الحيوية في حين 10(13.6%) عزلات لم تظهر أي نمو بكتيري. تم اخضاع 63 عزلة لاختبار حساسية المضادات الحيوية الانتشار القرصي حيت اظهرت البكتريا البشروية حساسيتها للمضاد الدوكسسيكلين بنسبة 38(67.86%) و اوفوكسسلين بنسبة 15(26.7%). في حين أظهرت البكتريا البشروية مقاومتها لتتراسايكلين والاروسايسين والكليندامايسين بنسبة 47(82.93%) ,40 (71.4%) و22(39.29%) على التوالي. من جهة اخري أظهرت المكورات العنقدية الذهبية حساسيتها للكليندامايسين ودوكسسيكلين بنسبة 7(100%) , في حين كانت مقاومة اوفلوكسسلين 3(42.8%) وللاريترومايسين 2(28.57%) والتتراسايكلين بنسبة 2(28.57%). **الاستنتاج**. كشفت النتائج في هده الدراسة لوجود بكتريا المقاومة للمضادات الحيوية بين المكورات العنقودية الذهبية والعنقودية البشروية التي تم الحصول عليها من المرضي الذي يعانون من حب الشباب وهذا مؤشر الي تأثير استهلاك المضادات الحيوية على تطور مقاومة المضادات الحيوية والتي تعتبر من المشاكل الصحة العامة.

الكلمات الدالة. مقاومة المضادات الحيوية، المكورات العنقودية الذهبية، المكورات العنقودية الجلدية، حب الشباب، الأمراض المعدية.