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Original Article

Microbiology Section

Bacterial Aetiology and Susceptibility of Pathogens Associated with Acne Vulgaris

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ABSTRACT

Introduction: Acne vulgaris is a pilosebaceous disorder among youth during puberty and also in young adults. Hormonal imbalance, bacterial activity and inflammation can cause acne formation. Due to some anti-inflammatory effects and temporary self treatment of acne using some antibiotics, there is overuse/improper use of antimicrobial agents which leads to resistance in bacterial strains.

Aim: To provide an overview of bacteria that may cause acne and determine their susceptibility to antibiotics and reduce the overuse of antibiotics which leads to antibiotic resistance.

Materials and Methods: This was a cross-sectional study done on samples from from 100 patients with chief complaints of acne, attending Dermatology Department at Konaseema Institute of Medical Sciences and Research Foundation (KIMS RF). This study was done between November 2019 to February 2021. Acne pustules were cultured on all the basic media like blood agar and Mac Conkey agar, under aerobic and anaerobic conditions. Bacterial identification was performed by gram staining and relevant biochemical tests. Antibiotic susceptibility was done based on the Clinical and Laboratory Standards Institute (CLSI) 2019. Data was calculated and analysed statistically by Statistical Package for Social Science (SPSS) version 21.0.

Results: Total 100 participants were taken for the study. Females (63%) were more commonly affected as compared to males (37%) and the most commonly affected age group was teenage i.e., 10 to 20 years in both male and females. Among 100 subjects, 77 cases showed bacterial growth by aerobic and anaerobic culture and 23 cases showed negative for bacterial culture. All isolates were susceptible to rifampin (100%) ($p<0.001$). *Propionibacterium acnes (P. acnes)* was susceptible to doxycycline (88.8%) and minocycline (88.8%), while 22.3% was resistant to erythromycin, clindamycin, and tetracycline while 33.3% showed resistance to ofloxacin. Gram positive cocci like *Staphylococcus epidermidis (S.epidermidis)* was susceptible to the antibiotic minocycline and rifampin (100%); but resistant to quinolines like ofloxacin (52.5%) macrolides like erythromycin (62.5%), clindamycin (47.5%) and tetracycline (17.5%). The susceptibility of gram positive cocci like *S. epidermidis* to doxycycline was (87.5%), which was lower than that of *P. acnes* (88.8%). *Staphylococcus aureus (S. aureus)* was found to be sensitive to minocycline (90.9%), doxycycline (72.7%), clindamycin (63.6%), and tetracycline (63.6%); but it was resistant to macrolide like erythromycin (45.5%) and quinolines like ofloxacin (36.5%).

Conclusion: This study suggested that bacterial culture and antibiotic sensitivity can be better suggested if the culture and sensitivity is available so that, sensitivity and resistant pattern is known. The use of clindamycin and macrolide (erythromycin) needs to be reduced as most gram positive cocci like *S. epidermidis* were resistant to both.

Keywords: Antibiotics, *Clostridium* species, *Propionibacterium acnes*, *Staphylococcus aureus, Staphylococcus epidermidis*

INTRODUCTION

Around 85% of adolescents and young adults affected by chronic inflammatory disorder of pilosebaceous follicles over face which is called as Acne vulgaris [1,2]. Pathogenesis mainly includes increased oily secretions like sebum, hypercornification of the pilosebaceous duct, colonisation of the duct with normal microbial flora which includes *P. acnes* and the production of infection and inflammation [3]. Other factors which influence the acne include fatty diet intake, excessive sweating, hormonal changes during menstruation, stress, occupation, exposure to ultraviolet radiation [4]. Most of the times acne is genetically determined and host response pattern, bacterial colonisation and inflammatory conditions are also being triggered [5].

Acne does not spread from one person to another. It is mainly due to blocking of pilosebaceous duct by microflora like *P. acnes* and *S. epidermidis* [3]. Depending on the severity and pattern of the disease, the acne patients are given topical treatment or topical and systemic combination of antibiotics. Due to development of a resistance to antibiotics and production of new strains in microorganisms, it is better to do culture and sensitivity and give the appropriate drug to the patient [6,7]. This study was undertaken to determine bacteria responsible for acne vulgaris and also to find out in-vitro antibiotic sensitivity to give proper care to the patient.

MATERIALS AND METHODS

This was a cross-sectional study done by taking samples from 100 patients with chief complaints of acne attending Dermatology Department at Konaseema Institute of Medical Sciences and Research Foundation (KIMS and RF). The study was conducted from November 2019 to February 2021. Before initiation of study, Institutional Ethics Committee (IEC) approval in KIMS RF (IEC/PR/2018-002/08.06.2021) was taken and also written consent from patients was also obtained.

Inclusion criteria: The different types of acne lesions are papules, pustules, painful nodules and nodulocystic lesions were included.

Exclusion criteria: The single acne lesions which are produced during menstruation were excluded.

Samples from the pustular and nodulocystic skin lesions were collected from 100 patients. The samples were immediately cultured on all the basic media like blood agar and Mac Conkey agar, under aerobic at 37°C for about 24 hours to 48 hours and for anaerobic

conditions also 37°C for 2 to 7 days. The colonies species were determined by gram staining and cultured under specific culture media and the appropriate biochemical tests such as mannitol, indole and Methyl Red (MR) and Voges-Proskauer (VP) and specific standard microbial tests such as oxidase, catalase and coagulase tests. [8] The sensitivity patterns of bacterial strains to antimicrobials was determined according to the method of disc diffusion method by Kirby bauer test [8]. The susceptibility of the microorganisms was interpreted according to the standard breakpoints determined by the CLSI guidelines 2019, [9] and according to the method used in an earlier studies done by Nakase K et al., for other bacterial isolates [10].

STATISTICAL ANALYSIS

The data was calculated and arranged into different tables and cross tables using SSPS version 21.0 and Chi-square test was used for significance.

RESULTS

Analysis of bacteria in both aerobic and anaerobic cultures: Most common type of acne lesions observed in this study was acne as painful nodules in 42 cases. Other types of lesions were papules, pustules and nodulocystic lesions [Table/Fig-1]. The most common site of acne lesions in the study were besides nose and on cheeks. The most commonly affected were females (63%) compared to male (37%) and the most commonly affected age group was teenage i.e., 10 to 20 years in 29 cases of both males and females as shown in [Table/Fig-2]. Out of 100 patients, 77 cases showed bacterial growth by aerobic and anaerobic culture and 23 cases showed sterile or negative bacterial culture [Table/Fig-3]. Aerobic and anaerobic growth of organisms is shown in [Table/Fig-4].

Type of acne lesions	Number of cases
Painful nodules	42
Papules	6
Pustules	24
Nodulocystic lesions	28

[Table/Fig-1]: Types of acne lesions.

Age group (years)	No. of males	No. of females
10-20	18	29
21-30	11	19
31-40	6	11
41-50	2	4
TOTAL	37	63

[Table/Fig-2]: Age and gender distribution.

Culture results	Study group (n=Number of cases)	p-value
Positive for bacterial culture	77	<0.0001
Negative for bacterial culture	23	<0.0001
Aerobes	56	-
Anaerobes	21	-

[Table/Fig-3]: Aerobic and anaerobic culture details. p-value is calculated using Chi-square test

Antibiotics resistance and susceptibility of *P. acnes, S. aureus,* and *S. epidermidis* and *Klebsiella spps* to antibiotics: Bacterial susceptibility testing was done using Kirby-bauer test under both aerobic and anaerobic conditions. [Table/Fig-5] shows the resistance patterns of the bacteria against several antibiotics. All isolates were susceptible to rifampin (100%). *Propionibacterium acnes* isolates were susceptible to antibiotics doxycycline (88.8%) and minocycline (88.8%), while 13% showed resistant to erythromycin, clindamycin and tetracycline. 33.3% showed resistance to ofloxacin.

Type of isolate	n=Number of cases
Aerobic (n=56)	
S.epidermidis	40
S.aureus	11
Klebsiella spp	5
Anaerobic (n=21)	
Propionibacterium acnes	9
Bacteroides	12
Sterile	23

[Table/Fig-4]: Aerobic and Anaerobic isolates in study group.

Antibiotic	*S.epidermidis* (40)	*S.aureus* (11)	*Klebsiella* spp (5)	*P.acnes* (9)
Rifampin	40 (100%)	11 (100%)	5 (100%)	9 (100%)
Minocycline	40 (100%)	10 (90.9%)	4 (80.%)	8 (88.8%)
Doxycycline	35 (87.5%)	8 (72.7%)	4 (80.%)	8 (88.8%)
Clindamycin	21 (52.5%)	7 (63.6%)	3 (60%)	7 (77.7%)
Tetracycline	33 (82.5%)	7 (63.6%)	3 (60%)	7 (77.7%)
Erythromycin	15 (37.5%)	6 (54.5%)	4 (80%)	7 (77.7%)
Ofloxacin	19 (47.5%)	7 (63.5%)	3 (60%)	6 (66.6%)

[Table/Fig-5]: Antibiotic sensitivity pattern.

S. epidermidis was susceptible to minocycline and rifampin (100%); but resistant to ofloxacin (52.5%), erythromycin (62.5%) and clindamycin (47.5%). The susceptibility of *S. epidermidis* to doxycycline was (87.5%), which was lower than that of *P. acnes* (88.8%). *S. aureus* was found to be sensitive to minocycline (90.9%), doxycycline (72.7%) and clindamycin (63.6%), but it was resistant to erythromycin (45.5%) and ofloxacin (36.4%). The usage of rifampin showed statistically significance difference p<0.001 (Chi-square test).

DISCUSSION

Acne vulgaris is one of the chronic skin disease that not only occurs in teenage group but also in young adults [11]. The pathogenesis of acne is mainly by disturbance of hormonal imbalance which occurs in teenage. Clinical features of acne may include redness, itching, pain, comedones, papules, pustules, and nodules. Even though acne can be healed by itself, it can cause some dismorphy of skin like atrophic and hypertrophic scarring that may decrease patient's cosmetic sense [11,12]. It is important for all the Dermatologists to go through the issue carefully and treat acne properly because it can come out into acute outbreaks or in a slow onset, prolonged-relapse. For treatment of acne, there is increasing evidence that treatment of acne with the combination of both topical applications and oral antimicrobial agents is effective as a first-line therapy for acne that is inflammation and non inflammation related [11-14]. The excessive use of oral antimicrobial agents will lead to the emergence of antibiotic resistance in many bacterial isolates and also in the commensal flora of the body. Thus it can only be recommended in moderate to severe acne to prevent antibiotic resistance strains [11,15].

Acne vulgaris is a chronic skin disease with many symptoms. The primary and the pathognomonic lesion of acne vulgaris is a comedone, which may be open or closed. Closed comedones were the most common presentation in the study by Adityan B and Thappa DM [16]. In a study by Khunger N and Kumar C on adult acne revealed that papules and pustules was the usual presentation in their study and mentioned comedonal acne is rare as compared to adolescent acne [17]. In this study, also most common type of lesions was painful nodules which correlates with the study of Adityan B and Thappa DM [16].

Acne is not an infectious disease; however, the blocked pilosebaceous ducts get infected with secondary bacteria and cause inflammation besides the nose and on the cheeks with *Propionibacterium* which are trapped by hardened sebaceous plugs within the ducts. The three major

organisms isolated from the surface of the skin and the pilosebaceous ducts of patients with acne are *Propionibacterium acnes, S. epidermidis* and *Malassezia furfur* in some studies [18]. The most common bacterial isolates obtained from the acne lesions are *S. epidermidis, Clostridium* spp, *S. aureus, P. acnes* and *Klebsiella* spp. Resistance genes in *S. epidermidis* are seen and these genes can also be transmitted highly and are also capable to remain dormant in skin and cause re infections [15,19]. The other bacteria, *S. aureus*, is usually a commensal bacterium of human skin and is capable of causing acne [20].

In this study, *Staphylococcus epidermidis* was the most commonly isolated bacteria species (40%) which correlates with studies of Sitohang IBS et al., (50.5%) and Srikanth M et al., and Sylvia L (63.6%) [21-23]. Barira S [20] found 30% and Moon SH et al., found 28% of *Staphylococcus epidermidis* isolates [20,24]. Differences in methods of specimen collection might be reason for differences in *S.epidermidis* proportions and other reason could be due to the fact that it is a commensal bacterium in acne [19]. The most common bacteria found in Korean acne patients in 2012 was found to be *S.epidermidis*. Though *Staphylococcus epidermidis* can increase inflammation of the skin with unknown causes [24]. In this study, *S. epidermidis* was most susceptible to antibiotics like minocycline and rifampin and was resistant to macrolide like erythromycin. It is believed that *S. epidermidis* has capability of producing many resistance strains [13]. This was again confirmed by present study that showed resistance to erythromycin (62.5%), clindamycin (57.5%), and tetracycline (27.5%) are found in higher proportions in *S. epidermidis* which is also correlating with study of Sitohang IBS et al., i.e resistance to macrolide like erythromycin (65.2%), clindamycin (52.2%), and tetracycline (32.6%) [21].

S. aureus was isolated in 11% of subjects. This result coincided to that of Sylvia L [23], (i.e., 9.1%) with a almost same percentage and Sitohang IBS et al., also got near by percentage result i.e 7.7% and Srikanth M et al., 13% [21-23]. A recent study showed that *S. aureus* is involved in acne pathogenesis [15]. *S. epidermidis* is capable of transferring its resistance genes to *S. aureus* via plasmids [15]. However, in this study *S. aureus* did not seem to be a potential acne promoter. It was found to be susceptible bacteria to minocycline but *S. aureus* showed resistance to macrolide like erythromycin. *S. aureus* showed 100% susceptibility to rifampin which is almost matching to Hassanzadeh P et al., [25] and the other drug to which it showed susceptibility is minocycline (90.9%) but for tetracycline (63.6%) it is showing less susceptibility and the susceptibility to tetracycline was matching to the results from Khorvash F et al., [26] which showed that the susceptibility of *S. aureus* to tetracycline was 69.4%. Sitohang IBS et al., [21] also showed similar susceptibility i.e higher susceptibility to minocycline (100%) than tetracycline (71.4%).

It is still unclear whether the prior antibiotic treatment history can bring any significance in the resistance patterns. *P. acnes* has capability of causing inflammation associated with acne [13]. It was predicted to be the most prevalent species to cause acne inflammation. However, it was present in only 9% of isolates which showed almost same results as Sitohang IBS et al., study i.e 11%. Barira S et al., [20] found that *P. acnes* was present in 38% of samples, while Sylvia L [23] found that 78.8% of samples showed positive for *P. acnes*. The low prevalence of *P. acnes* in this study was likely related to a high proportion of other bacteria like Clostridium and *Staphylococcus* spp. This microbe has the ability to adapt to in most of the environmental conditions easily and inhibit other bacteria [15,19]. However, González R et al., [27] isolated *P. acnes* from all specimens. In their study, the specimens were taken from both inflammatory lesions (papules and pustules) and non inflammatory lesions (open and closed comedone). These were then directly inoculated into the suitable medium. This method increases the isolate rate of *P. acnes* [11]. The number of samples for aerobic culture are more as compared to anaerobic cultures due to difficulties in growing anaerobic bacteria, and even sample collection is also very difficult because it should be collected without exposure to air.

Propionibacterium acnes growth and multiplication are most affected by acne inflammation. From other studies, it is known that *P. acnes* is highly resistant to macrolide like erythromycin; and most of the strains are cross-resistant to clindamycin [15]. The pattern of resistance of *P. acnes* is also based on the patients genes and the severity of acne infection [14]. This study showed that *Propionibacterium acnes* was sensitive to doxycycline and minocycline. This result was almost same as the results of Sitohang IBS et al., [21] Barira S [20] which showed 94.7% susceptibility to minocycline and it can be concluded that the proportion of these two antibiotics doxycycline and minocycline were almost same, thus the susceptibility of *P. acnes* to doxycycline and minocycline very high. *Propionibacterium acnes* is generally susceptible to tetracycline but its becoming resistance being most used antibiotic systemically.

P. acnes were resistant to erythromycin, clindamycin, and tetracycline where to Sitohang IBS et al., [21] and Barira S [20] who found almost same results that *P. acnes* was most resistant to erythromycin (63.2%), followed by clindamycin (57.9%), and tetracycline (47.4%). ER Eady et al., [28] found 73 resistant isolates of *Propionibacterium acnes* however, due to the development of resistance strains, studies on antimicrobial susceptibility should be done regularly updated and repeated every 5 to 10 years over all regions and antibiotic resistance should be checked in clinics where ever facilities are available [26].

In this study, Bacteroides were 12% which is showing distinct pathogen to other studies but 5% isolates of Bacteroides were also identified in Dreno B et al., study but due to its growth difficulty antibiotic susceptibility testing was not done [15]. This study also showed 5% *Klebsiella* isolates which showed similar results with Srikanth M et al., study and the percentage of *Klebsiella* isolates was 2% [22]. Antibiotic sensitivity showed 100% sensitivity to rifampin and 60% of sensitivity to tetracycline but for Srikanth M et al., study tetracycline showed total resistance [22]. The most preferable oral antibiotics for acne vulgaris which were included in this study, which are rifampin, minocycline and doxycycline. Rifampin showed 100% susceptibility to all types of organism. This study correlated with Hassanzadeh P et al., which states *Staphylococcus aureus* and *epidermidis* were resistant to tetracycline, erythromycin and clindamycin but were highly sensitive to rifampin [25].

It was found that patients with acne treated with antibiotics had 2.15 times higher risk of developing upper respiratory infection [11]. The use of antibiotics should be limited because it may cause collateral damage. The effectiveness of treatment should target multiple pathogenic factors instead of primarily focused on treating the bacteria [24].

Limitation(s)

A limitation of this study was the relatively small number of samples for the period of study. A bigger sample number will lead to more isolates so that these results are more valid.

CONCLUSION(S)

This study suggests that bacterial culture and antibiotic sensitivity can be better suggested if the culture and sensitivity is available so that we can know the sensitivity patterns and also the resistant strains. On the basis of the results, it is suggested that rifampin will be a better antibiotic for acne patients, but to get much more better results, combination of rifampin with other antibiotics works good. All isolated bacteria were more sensitive to doxycycline compared to tetracycline. The use of tetracycline, clindamycin and erythromycin

needs to be limited since most of the isolates were resistant to these agents. Therefore, periodical resistance monitoring over time is suggested in the future to prescribe the antibiotics which are not resistant.

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