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Research Article

TO STUDY BACTERIAL CULTURE AND SENSITIVITY PROFILE IN ACNE INFECTION PATIENTS IN SOUTHINDIA

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ABSTRACT

Acne vulgaris is a chronic inflammatory disorder of Pilosebaceous follicle. Acne is one of the most common skin disorders throughout the world, affecting 67%–95% of adolescents. Actually, acne is clearly a chronic inflammatory skin disease and not primarily an infectious disease. It is characterised by pleomorphic lesions, including comedones, pustules, papules, nodules and cysts. To determine antimicrobial susceptibility patterns of different organisms to frequently used drugs. Samples from skin lesions of study group (80 patients) and normal skin of control group (20 patients) were collected by sterile swabs. The specimens were used for aerobic and anaerobic culture at 37°C. The isolates were subjected to biochemical tests for identification. All the aerobic isolates were subjected to antimicrobial sensitivity testing by disc diffusion method.

Keywords: Acne vulgaris, bacterial culture, Pustules, Males, Antibiotic resistance.

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INTRODUCTION

Acne vulgaris is a common skin disorder which is of multifactorial etiology. It is a chronic disorder of pilosebaceous follicle and affects youth especially during puberty and adolescent age group. [1, 2] The clinical lesions are non inflammatory open and closed comedones of varying degree and/or papules, pustules and nodules of varying degree of inflammation and depth. A mainly genetically determined host response pattern combined with bacterial “triggering” is generally accepted as being important for the apparently unbalanced inflammatory

activity. [2] Due to development of resistance in microorganisms causing acne to common antibiotics and the differences in species and strains of the microorganisms in different regions, a research in the method of therapy seems indispensable [3].

Its pathogenesis is multifactorial and includes abnormal sebum secretion, follicular hyperkeratinisation, inflammation and immunity *P. acnes* plays a vital role in the pathogenesis of acne by activating the innate and adaptive immunity. Chemotactic factors and proinflammatory cytokines are produced by immune reactions, resulting in local inflammation and potential scarring. Anti-inflammatory and antimicrobial medications are the basis of acne therapy. Therefore, antibiotics are widely used in patients with acne, inhibiting or eradicating the *P. acnes* colonisation, and reducing the production of proinflammatory mediators. Topical and systemic antibiotics are frequently used in the treatment of acne. For the past 30 years, a decrease in the percentage of susceptibility of *P. acnes* strains to these antibiotics has been reported in many countries, indicating that antibiotic-resistant *P. acnes* among patients with acne is a global problem. With routine and

long-term use of antibiotics, the resistance profile of *P. acnes* has been gradually altered, and varies greatly from one region to another. Our study aim is to identify the bacterial etiology of acne To determine the antibiotic susceptibility profile of pathogenic isolates.

MATERIAL AND METHODS

This study was carried out on patients referred to the Department of Dermatology, Sri Lakshmi Naryana Institute of Medical Sciences, Pondicherry. Samples from normal skin (From the forehead of control group of 20 patients) and skin lesions (Of study group of 80 patients) were collected by two sterile swabs moistened with nutrient broth after wiping first with 70% ethanol. Impression smears were taken on a clean slide for Grams staining. The samples were immediately inoculated individually on blood agar, Mac Conkey's agar and were incubated both aerobically and anaerobically at 37°C. The samples were also inoculated into Robertson's cooked meat broth and sealed with molten paraffin. Only culture positive, Robertson's cooked meat broth tubes were sub cultured and incubated under anaerobic

conditions. The isolates were subjected to a battery of relevant tests for identification. All the aerobic isolates were tested for sensitivity to antimicrobial agents by Kirby-Bauer disc diffusion method.

RESULTS AND DISCUSSION:

Table 1: Shown in the study group, males were 47 (58.75%) .

Table 2: Shown Among the isolates from the study group, aerobes were 43(53.75%), anaerobes were 21 (26.25%) and mixed growth was 11(13.75%) and cases that did not yield any isolates were 5 (6.25%).

Table 3: Among the aerobic isolates, most were sensitive to Minocycline followed by Ofloxacin, Azithromycin, Cephalexin, Tetracycline, Cotrimoxazole and Ampicillin. *Staphylococcus epidermidis*, the dominant organism was susceptible to Ofloxacin, Minocycline and Azithromycin followed by Cephalexin, Cotrimoxazole, Tetracycline and Ampicillin.

The pathogenesis of acne appears to be multifactorial, although it is yet only partly understood.

Table 1: Age and sex distribution

Age	Study group (n=80)		Control group (n=20)		Total
	Males	Females	Males	Females	
13 – 20	38	23	11	4	76
21 – 30	9	10	2	3	24
Total	47	33	13	7	100

Table 2: Distribution of Microbial isolates in study and control groups

Types of isolates	Study Group (n=80)	Control Group (n=20)
Aerobes	43 (53.75%)	8 (40%)
<i>Staphylococcus epidermidis</i>	23 (53.48%)	8 (40%)
<i>Staphylococcus aureus</i>	13 (30.23%)	-
γ haemolytic streptococci	2 (4.65%)	-
<i>Pseudomonas aeruginosa</i>	2 (4.65%)	-
<i>Klebsiella pneumoniae</i>	2 (4.65%)	-
<i>Proteus mirabilis</i>	1 (2.32%)	-
Anaerobes	21 (26.25%)	4 (20%)
<i>Propionibacterium acnes</i>	12 (57.14%)	4 (20%)
Peptococci	5 (23.81%)	-
Peptostreptococci	2 (9.52%)	-
<i>Fusobacterium</i> sp.	2 (9.52%)	-
Mixed growth	11 (13.75%)	-
<i>Staphylococcus epidermidis</i> + <i>Propionibacterium acnes</i>	4 (36.36%)	-
<i>Staphylococcus epidermidis</i> + Peptococci	4 (36.36%)	-
<i>Staphylococcus aureus</i> + Micrococci	3 (27.27%)	-

Table 3 : Antibiotic sensitivity pattern of aerobic bacteria in acne

No. of isolates	Ofloxacin	Minocycline	Azithromycin	Cephalexin	Tetracycline	Cotrimoxazole	Ampicillin	
<i>Staphylococcus epidermidis</i>	31	31(100%)	29 (93.54%)	26 (83.87%)	23 (74.19%)	16 (51.61%)	15 (43.38%)	6 (19.35%)

Staphylococcus aureus	16	6(37.5%)	9(56.25%)	7(43.75%)	3(18.75%)	2(12.5%)	R	R
Micrococci	3	3(100%)	3(100%)	3(100%)	2(66.66%)	1 (33.33%)	2(66.66%)	1(33.33%)
γ haemolytic streptococci	2	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)	1(50%)	1(50%)
Pseudomonas	2	2 (100%)	2 (100%)	2 (100%)	R	R	R	1 (50%)
Klebsiella	2	2(100%)	2(100%)	2(100%)	1(50%)	R	1(50%)	R
Proteus	1	1(100%)	1(100%)	1(100%)	1(100%)	R	1(100%)	R
Total sensitive isolates	47	48	43	32	21	20	9	

A mainly genetically determined host response pattern combined with bacterial “triggering” is generally accepted as being important for the apparently unbalanced inflammatory activity. Acne is a chronic inflammatory disease of the pilosebaceous units. It is characterized by seborrhoea, the formation of open and closed comedones, erythematous papules and pustules and in more severe cases nodules, deep pustules and pseudocysts. In many cases a degree of scarring will ensue. Four major factors are involved in the pathogenesis: (i) increased sebum production, (ii) hypercornification of the pilosebaceous duct, (iii) abnormality of the microbial flora especially colonization of the duct with *Propionibacterium acnes*, and (iv) inflammation [4]

Acne usually starts in adolescence, peaks at the ages of 14 to 19 years and frequently resolves by mid-twenties [3]. High incidence of acne was found in the age group of 13-20 years, in both genders i.e. 61 (76.25%) in present study. The most common age groups to be involved in acne vulgaris were 16-20 years (59.8%) in a hospital-based study from South India [3]. Males were more affected than females in our study & in the study by Adityan et al [3]. In general, androgens stimulate the formation of sebum, while estrogens reveal a suppressive effect on it. The activity of sebaceous glands is thus dependent on the ratio of estrogens and androgens. The increased level of androgens in adolescence is known to be a starting point for the development of juvenile acne [5].

In any case, the current treatment options are far from ideal. The use of broad-spectrum antibiotics has led to widespread resistance, and the alternative treatment, isotretinoin, is highly teratogenic and induces pronounced subjective side effects when it is used at therapeutic doses. A better understanding of the etiology of acne is essential to develop more efficient treatment options with fewer side effects.

The increased incidence of papules and pustules in the present study could be related to stress. Patients with acne experienced worsening of disease during examination [6]. In adolescents, psychological stress did not appear to affect the quantity of sebum production, but

significantly affected the severity of acne papulopustulosa, especially in males. Therefore, the possibility exists that previously uncultured bacteria resident in the sebaceous follicles play an etiologic role in the development of acne. This hypothesis was evaluated in the present study by mapping the microbial diversity of sebaceous follicles of acne-affected and healthy individuals.

Increased acne severity associated with stress may result from factors other than sebum quantity [7]. Its pathogenesis is multifactorial and includes abnormal sebum secretion, follicular hyperkeratinisation, *Propionibacterium acnes* (*P. acnes*) hypercolonisation, inflammation and immunity. Four major factors are involved in the pathogenesis including increased sebum production, hypercornification of the pilosebaceous duct, an abnormality of the microbial flora especially colonization of the duct with *Propionibacterium acnes*, and the production of inflammation. It seems that several factors influence acne including diet, menstruation, sweating, stress, ultra violet radiation and occupation. Positive association between intake of milk and acne was reported and this findings support earlier studies and suggests that the metabolic effects of milk are sufficient to elicit biological responses in consumers. It was also reported that a low-glycemic-load diet improves symptoms in acne vulgaris patients. Acne is not an infectious disease, but three major organisms were isolated from the surface of the skin and the pilosebaceous duct of patients with acne including *Propionibacterium*. Acne is not an infectious disease in the classical sense; however, inflammatory acne can be viewed as an infection of the blocked pilosebaceous ducts with *Propionibacteria* which are trapped by cornified plugs within the follicular ducts. The three major organisms isolated from the surface of the skin and the pilosebaceous ducts of patients with acne are *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Malassezia furfur*.

By using a genetically based strategy (Analysis of 16S rRNA genes) with sensitivity and discriminatory power surpassing those of culture-based methods, Bek-Thomsen et al. demonstrated that the bacterial microbiota

of follicles from acne-affected subjects showed more, although still very limited, diversity. The microbiota was dominated by *Propionibacterium acnes* and *Staphylococcus epidermidis*, which were the only species consistently found. These results clearly exclude the possibility that yet-uncultured bacteria are associated with acne-affected skin follicles [8]. As a validation of the method, the overall composition of the microbiota of superficial acne-affected skin that was demonstrated was very similar to that previously shown both for healthy human skin by a standard culture technique and for skin from the forearm and the forehead by the sequence-based technique used generally.

A possible alternative or additional explanation may be that the inflammatory reaction in the skin follicles of acne patients interferes with their apparently uniquely potent antimicrobial defense mechanisms. The presence of *S. epidermidis* exclusively in acne-affected follicles raises the question of the potential role of this species. Previous studies excluded staphylococci as agents that play a role in the pathogenesis of acne on the basis of their rapid development of resistance to therapeutic antibiotics. *Propionibacterium acnes* play a central role in acne pathogenesis. Not only does this anaerobic bacterium produce lipases, proteases, and other extracellular enzymes, it also secretes chemotactic factors attracting polymorphonuclear leukocytes, lymphocytes, and macrophages. The inflammatory response initiated by these extracellular products stimulates the classical and alternative complement pathways and other immune response [9].

Oral antibiotics are the most widely prescribed agents in acne and are indicated for severe acne, moderate facial acne not responding to topical therapies and/or extensive truncal acne. Cyclines (Tetracycline, oxytetracycline, doxycycline, lymecycline, minocycline) have excellent efficacy and are the antibiotics of choice [10]. In the present study, most of the aerobic isolates were sensitive to Minocycline (84.21%). Only 36.84% of the isolates were sensitive to tetracycline. Resistance to tetracycline in *Staphylococcus aureus* has been reported in the present study and in the study by Hassanzadeh et al [3]. It may be due to extensive tetracycline usage in the past. It has been suggested that tetracycline, which becomes concentrated in inflamed lesions and has been the mainstay of acne treatment for two decades, could act by inhibiting neutrophil chemotaxis rather than its antibacterial actions [9].

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Antibiotic resistance is a growing concern worldwide and should be suspected in patients unresponsive to appropriate antibiotic therapy after 6 weeks of treatment. To prevent resistance, prescribers should avoid antibiotic monotherapy, limit long-term use of antibiotics and combine usage with benzoyl peroxide whenever possible [11]. In this geographical area, *Staphylococcus aureus* was highly sensitive to Mupirocin and Fusidic acid among topical and to Doxycycline and Ciprofloxacin among systemic agents. On the basis of these results, we suggest that Mupirocin and Fusidic acid among topical and Doxycycline and Ciprofloxacin among systemic agents are suitable antibiotics for acne patients.

Many antibiotics from different classes have been successfully used in acne treatment, including tetracyclines, clindamycin, macrolides, and trimethoprim-sulfamethoxazole. Several mechanisms have been proposed to explain the salutary effects of these antibiotics, including antianabolic effects on sebaceous glands, antilipolytic effects on bacterial lipase, anti-inflammatory effects on host cells, and lastly antibiotic effects on bacteria. The reason that antibiotics are effective in these skin diseases is not clear. Without convincing evidence of a specific microbe to blame, emphasis has been placed on host cell effects; however, the fact that many different classes of antibiotics are active in these diseases argues for an antibacterial mechanism [12]. We suggest that indiscriminate use of antibiotics, which predisposes individual to development of antibiotic resistant pathogenic strains, should be avoided. Acne therapy varies according to severity of disease, topical medications are generally enough in clearing comedonal acne, while inflammatory acne usually requires systemic antibiotics.

CONCLUSION

Staphylococcus epidermidis, the dominant organism isolated was susceptible to Ofloxacin, Minocycline and Azithromycin. Clindamycin and Azithromycin are most commonly prescribed drugs for acne treatment but in this study we have found many cases showing resistance to above two drugs. Knowledge of causative organism and antibiotic susceptibility pattern is essential to give proper antibiotic therapy and to avoid resistance to ineffective drugs. These results suggest that dermatologists should be more prudent in prescribing antibiotics for acne.

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