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ORIGINAL ARTICLE

BACTERIOLOGY OF ACNE

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ABSTRACT: CONTEXT: Acne vulgaris is the most common skin disorder in youth and is of multifactorial etiology. Severe forms of acne are often therapy resistant. **AIMS:** To determine bacteria involved in acne vulgaris and to determine the in vitro antibiotic sensitivity of aerobic isolates. **SETTINGS AND DESIGN:** Cross sectional study. **METHODS AND MATERIALS:** 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. **RESULTS:** High incidence of acne was found in the age group of 13-20 years (76.25%). Males were more affected than females. Pustules (76.25%) were the commonest presentation. Students (71.25%) were more commonly affected. Among aerobic isolates (71.24%), Staphylococcus epidermidis (54.38%) was the most common organism. Among the anaerobes, Propionibacterium acnes (55.17) were the most common. Among the aerobic isolates, most were sensitive to Minocycline followed by Ofloxacin, Azithromycin, Cephalexin, Tetracycline, Cotrimoxazole and Ampicillin. **CONCLUSIONS:** In the present study, acne affected individuals were young males, especially students, with pustules being the commonest presentation. Staphylococcus epidermidis, the dominant organism isolated was susceptible to Ofloxacin, Minocycline and Azithromycin.

KEYWORDS: Acne vulgaris, Pustules, Males, Antibiotic resistance.

INTRODUCTION: An individual is more likely to develop acne than any other disease. [1] Acne is a significant clinical problem with severe social, psychological, and emotional implications. 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] This study was undertaken to determine bacteria involved in acne vulgaris in Visakhapatnam, Andhra Pradesh and to determine the in vitro antibiotic sensitivity of aerobic isolates in acne vulgaris.

MATERIALS AND METHODS: This cross sectional study was carried out on patients referred to the Department of Dermatology, King George Hospital, Visakhapatnam. 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

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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: In the study group, males were 47 (58.75%) (Table 1). High incidence of acne was found in the age group of 13-20 years, in both genders i.e. 61 (76.25%) (Table 2). Males 38/47 (80.85%) were more affected than females 23/33 (69.69%) in the age group of 13-20 years. In both genders, pustular lesions (76.25%) were more common than inflammatory papules (17.5%) and cysts (6.25%) (Table 3).

Students (71.25%) of both sexes were more commonly affected than other occupational groups (Table 3). Pustules were the commonest presentation in all occupational groups followed by inflamed papules and cysts.

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 4). Aerobic isolates (57) were more than the anaerobic (29) isolates. Staphylococcus epidermidis was the most common organism in the aerobic group (Table 5). Among the anaerobes, Propionibacterium acnes was the most common organism. Among all the isolates (aerobic and anaerobic), Staphylococcus epidermidis was most common organism.

Among the aerobic isolates, most were sensitive to Minocycline followed by Ofloxacin, Azithromycin, Cephalexin, Tetracycline, Cotrimoxazole and Ampicillin (Table 6). Staphylococcus epidermidis, the dominant organism was susceptible to Ofloxacin, Minocycline and Azithromycin followed by Cephalexin, Cotrimoxazole, Tetracycline and Ampicillin.

In the present study, there is a high correlation between the direct smear and culture (Table 7). Sixty-three (78.75%) cases were positive by both smear and culture.

DISCUSSION: 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.[1] 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.[1] Males were more affected than females in our study & in the study by Adityan et al.[1] 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]

Acne vulgaris is a polymorphic disease. The primary and the pathognomonic lesion of acne vulgaris is a comedone, which may be open or closed. Closed comedones were the commonest presentation in the study by Adityan et al.[1] In a study by Khunger and Kumar on adult acne revealed that papules and pustules was the usual presentation and mentioned comedonal acne is rare as

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compared to adolescent acne.[6] But the most common type of lesion in our group of acne patients was pustules. It may be due to delay in seeking medical advice only after worsening of acne.

Males tend to show the most severe forms of the disease. In our study, 39 males had pustules and cysts in comparison to 27 females (Table 2). Adityan et al. also observed in their study that male patients had more severe acne vulgaris.[1] In the older age group, women were more affected by acne vulgaris than men.[1,6] In our study, in the age group of 21-30, 10 (30.30%) were females & 9 (19.15%) were males.

Students (71.25%) of both sexes were more commonly affected than other occupational groups. Similarly Adityan et al. also reported that a majority of the patients were college students (33.3%) or school students (33%).[1] The increased incidence of papules and pustules in students in the present study could be related to stress. Patients with acne experienced worsening of disease during examination.[7] 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. Increased acne severity associated with stress may result from factors other than sebum quantity.[8]

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.[4]

In the study group, the percentage of aerobes was 53.75, anaerobe was 26.25, mixed growth was 13.75 and cases that did not yield any isolates were 6.25. Distribution of microbial isolates collates with study by Itzhak Brook et al. where only aerobic or facultative bacteria were recovered in 47% specimens, only anaerobic bacteria in 34% specimens, and mixed aerobic and anaerobic bacteria in 18% specimens.[9]

Staphylococcus epidermidis was the predominant aerobic bacterial isolates & Propionibacterium acnes was the predominant anaerobic bacterial isolates in the present study and also in other studies (Table 8 & 9).[9,10] In the study by Parvin Hassanzadeh et al., the most frequent bacteria isolated from acne patients were Staphylococcus aureus & it was stated that it is possible that acne vulgaris is mainly caused by Staphylococcus aureus rather than Propionibacterium acne. This is in contrast to reports which implicated both Staphylococcus epidermidis and Propionibacterium acnes as bacteria causing acne vulgaris.[3]

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.[2]

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.[11]

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In 80 cases of acne, 11 (13.75%) cases showed mixed bacterial culture. This correlates with the study by Itzhak Brook et al. where mixed growth was seen in 18% of acne lesions.[9]

The occurrence of Proteus sp. in cystic lesions coincides with a study conducted by James J Leyden et al., on Gram negative folliculitis. They examined 50 cases of Gram negative folliculitis and recognized two varieties of Gram negative folliculitis. Type-I, the more common, presented as superficial pustules around the nose. Type-II lesions are characterized by deep nodules and cystic lesions. Proteus sp. appeared to be the causative agent. [12]

Treatment of acne vulgaris often includes combinations of oral and topical agents such as antimicrobials, retinoids, and hormonal agents. Laser and light sources are additional treatment options.[13]

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.[4] 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.[11]

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.[13]

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.[14]

Age group	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 1: Age and sex distribution of study group and control group (n=100)

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Age in years	Papules		Pustules		Cysts		Total
	Males	Females	Males	Females	Males	Females	
13 – 20	6 (%)	4 (%)	30 (%)	19 (%)	2 (%)	-	**61 (76.25%)**
21 – 30	2 (%)	2 (%)	6 (%)	6 (%)	1 (%)	2 (%)	**19 (23.75%)**

Table 2 : Age and sex distribution of acne lesions in study group

Occupation	Papules		Pustules		Cysts		Grand total
	Males	Females	Males	Females	Males	Females	
Students	4	3	30	19	1	-	57
Labourers	2	-	4	-	-	-	6
Housewives	-	1	-	4	-	1	6
Business	1	-	2	-	1	-	4
Call center employees	-	1	-	1	-	1	3
Receptionists	-	1	-	1	-	-	2
Auto Drivers	1	-	-	-	1	-	2
Total	**14**		**61**		**5**		**80**

Table 3: Distribution of acne lesions in various occupational groups

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

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Staphylococcus aureus + Micrococci	3 (27.27%)	-

Table 4 : Distribution of Microbial isolates in study and control groups

Microorganisms	Papules	Pustules	Cysts	Total
Aerobes				**57**
Staphylococcus epidermidis	10	19	2	31
Staphylococcus aureus	3	11	2	16
Micrococci	1	2	-	3
γ haemolytic streptococci	2	-	-	2
Pseudomonas aeruginosa	-	2	-	2
Klebsiella pneumoniae	-	2	-	2
Proteus mirabilis	-	-	1	1
Anaerobes				**29**
Propionibacterium acnes	5	8	3	16
Peptococci	6	3	-	9
Peptostreptococci	1	1	-	2
Fusobacteria	1	1	-	2

Table 5 : Microorganisms isolated from different types of acne lesions

Organism	No. of isolates	Oflo xacin	Mino Cycline	Azithro mycin	Cepha lexin	Tetra cycline	Cotrimo xazole	Ampicillin
Staphylococcus epidermidis	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**

Table 6 : Antibiotic sensitivity pattern of aerobic bacteria in acne

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Category	No. (%)
Smear+ve Culture+ve	63 (78.75%)
Smear-ve Culture+ve	12 (15%)
Smear-ve Culture-ve	5 (6.25%)

Table 7 : Direct smear and culture correlation in study group

Organism	Nishijima S [10]	Brook I [9]	Present study
S.epdermidis	15	12	31
S.aureus	3	7	16
Micrococci	5	--	3
γ haemolytic streptococci	7	5	2
Pseudomonas	4	3	2
Klebsiella	--	--	2
Proteus	--	--	1
Eikenella	--	1	--

Table 8 : Aerobic organisms in acne lesions as reported by different authors

Organism	Nishijima S [10]	Brook I [9]	Present study
Propionibacterium acnes	14	10	16
Peptococci	3	6	9
Pepto streptococci	4	9	2
Fusobacteria	7	3	2
Eubacterium	--	1	-
Bacteroides	2	1	--

Table 9: Anaerobic organisms in acne lesions as reported by different authors

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