Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2013; 1(6):724-727 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2013.v01i06.0017

Research Article

Study of Microbiological Spectrum in Acne Vulgaris: An *In Vitro* Study

KS Dhillon[1], Krati R Varshney[2*]
[1]Professor, Department of Dermatology, Era's Lucknow Medical College & Hospital, Lucknow , Uttar Pradesh, India .
[2]Resident, Department of Microbiology, Era's Lucknow Medical College & Hospital, Lucknow, UP, India.

***Corresponding author**
Dr Krati R Varshney
Email: krativarshney2001@yahoo.co.in, krati_vrshny19@hotmail.com

Abstract: Acne vulgaris is one of the most common skin disorders in young adults especially during Puberty. The widespread and long-term use of antibiotics in the treatment of acne has resulted in the spread of resistant bacterial strains and treatment failure. We examined the bacteriology of acne vulgaris and evaluated its susceptibility to the antibiotics widely used for acne. Samples were collected from nodulocystic and pustular skin lesions of fifty young adults amongst college students in the age range of 18-27 years old from Dermatology OPD. The specimes were cultured individually on blood agar and Muller-Hinton media. The cultures were then incubated under both aerobic and anaerobic conditions for 2 to 7 days. Bacteria were identified and their resistance to common antibiotics was evaluated according to the standard procedures. In aerobic culture of pustular and nodulocystic skin lesions, *Staphylococcus aureus* was present in 45% of subjects, *Staphylococcus epidermidis* in 49% and *Micrococcus spp* in 45% of subjects. In anaerobic bacterial culture of pustular and nodulocystic skin lesions, *Staphylococcus aureus* was present in 41%, *Propionibacterium acne in* 32% and *Staphylococcus epidermidis* in 20% of subjects. This study revealed that clindamycin and erythromycin were the least effective antibiotics for *Propionibacterium acne* while tetracycline was the least effective for *Staphylococcus aureus.* A synergic effect of benzoyl peroxide, erythromycin or clindamycin was noticed. Rifampin was the most effective antibiotic *in vitro*.
Keywords: Acne vulgaris, antibiotic resistance, rifampin

INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosabaceous follicles that affects more than 85% of adolescents and young adults [1]. Acne vulgaris is a multifactorial, pleomorphic skin disease of the pilosebaceous follicles (PSFs) characterized by a variety of non-inflamed (open and closed comedones) and inflamed (macules, papules, pustules and nodules) lesions [2]. 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 [3]. Microcomedones (earliest subclinical lesions) are thought to be the precursor lesions that can then develop into non-inflamed and/or inflamed lesions. Although a common disease, the aetiology of acne is not yet fully elucidated. It carries an enormous financial and psychosocial impact [2]. Despite extensive research on acne pathogenesis, the exact sequence of events and their possible mechanisms leading to the development of a microcomedone and its transformation into an inflamed lesion has remained unclear [2]. It seems that several factors influence acne including diet, menstruation, sweating, stress, ultra violet radiation and occupation [4]. It was also reported that a low-glycemic-load diet improves symptoms in acne vulgaris patients [5]. 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*, *Staphylococcus epidermidis* and *Malassezia furfur* [3]. Depending on the severity of the disease, the acne patients receive topical or systemic therapy, or a combination [6, 7]. Pathogenesis of microorganism originates from production of proinflammatory mediators (e.g. IL-1, TNFα) as well as many lipases. Increased number of *Propionibacterium acne* was reported in acne patients, but their number was not correlated with the clinical severity [8]. This study was undertaken to determine bacteria involved in acne vulgaris in Lucknow, Uttar Pradesh and to highlight the *in vitro* antibiotic sensitivity in acne vulgaris.

MATERIALS AND METHODS

50 (35males,15females) patients of acne vulgaris with pustular and nodulocystic skin lesions were selected amongst college students, in the age group of 18-27 years from October 2012 to March 2013, were included in the study. Patients with pregnancy or endocrinal problems like hirsutism, menstrual dysfunction or adrenal dysfunction and those taking drugs or contraceptives were not included .The subjects were carefully examined in Department of Dermatology. The samples were immediately sent to Microbiology Department where they were cultured individually on blood agar and Muller-Hinton media. The cultures were then incubated at 37°C under both aerobic and anaerobic conditions for 2 to 7 days. The

colonies species were determined morphologically by specific culture media such as mannitol, indole and sorbitol media and specific standard microbial tests such as oxidase, catalase, and coagulase tests [10].

Identification of *P. acnes*: All bacteria anaerobically isolated were subjected to further identification. Antimicrobial susceptibility testing was performed per colony morphology type isolated. Gram's stain, catalase, and indole test spot results were used as reference parameter to identify P. acnes among morphologically identical colonies isolated from additional media or from additional specimens from the same patient [9]. The sensitivity of bacteria to antibiotics was determined according to the method of Kirbauy [10].

RESULTS

The micro-organisms in pustular and nodulocystic skin lesions were grown both aerobically and anaerobically as presented in Table 1. The different bacteria in pustular and nodulocystic skin lesions in both genders were grown aerobically and anaerobically. Aerobically, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acne* and *Micrococcus spp* were detected in 45%, 49%, 0%, 45% of samples respectively while these figures anaerobically were 41%, 20%, 32% and 0% respectively. When the effects of different antibiotics on *Propionibacterium acne, Micrococcus spp, Staphylococcus epidermidis, and Staphylococcus aureus* were tested (Table 2), *Propionibacterium acne, Staphylococcus epidermidis* and *Staphylococcus aureus* were more sensitive to rifampin compared to other drugs. As shown in Table 2, the combined inhibitory effect of clindamycin or erythromycin with benzoyl peroxide was less than rifampin alone. Table 2 shows the effects of different antibiotics on isolated bacteria from pustular and nodulocystic skin lesions. The diameter of inhibition zone (mm) by each antibiotic was studied. According to the manual instruction of Padtan Tab Co., inhibition zone less than 17 mm® was considered as resistance to antibiotic.

Table 1: Analysis of bacteria in samples obtained from youngsters

Cultures	Samples	*S. aureus*	*S. epidermidis*	*P. acne*	*Micrococcus spp*
Aerobic	Pustular and nodulocystic skin lesions	45	49	-	45
Anaerobic	Pustular and nodulocystic skin lesions	41	20	32	-

Table 2 : The effects of different antibiotics on isolated bacteria of acne vulgaris

Name of antibiotic	Sensitivity %	Resistance %
Clindamycin	50	50
Doxycycline	72	28
Amoxycillin	60	40
Tetracycline	63	37
Erythromycin	48	52
Cephalothin	50	50
Gentamicin	50	50
Kanamycin	39	61
Rifampin	87	13
Neomycin	17	83
Benzoyl-Peroxide	75	25
Clindamycin + Benzoyl-Peroxide	66	34
Erythromycin + Benzoyl-Peroxide	69	31

DISCUSSION

In this study, more *Staphylococcus aureus* and *Micrococcus spp* were found in aerobic cultures while more *Staphylococcus aureus* and *Propionibacterium acne* responsible for acne, were found in anaerobic cultures. Since the most frequent bacteria isolated from acne patients were *Staphylococcus aureus*, it is possible that acne vulgaris is mainly caused by *Staphylococcus*

aureus rather than *Propionibacterium acne* [12]. This is in contrast to some reports which implicated both *Staphylococcus epidermidis* and *Propionibacterium acnes* as bacteria causing acne vulgaris [13].We have attempted to take a balanced view of most of the available evidence, including the microbiological data, and shown that the central role of *P. acnes* in the initiation of acne lesions is not yet irrefutable [2]. It may be concluded that geographical regions affect the bacteria involved in acne vulgaris. Since bacterial resistance to conventional antibiotics such as erythromycin and tetracycline was reported to have an increasingly trend [11], research on finding the effective antibiotics seems indispensable. In this geographical area with *Staphylococcus aureus* as primary casual agent in acne development, *Staphylococcus aureus* was resistant to tetracycline, erythromycin and clindamycin which is consistent with reports by some other investigators, [14-16] but was highly sensitive to Rifampin. Since these antibiotic agents were previously used to treat acne, the results indicate that the widespread use of antibiotics can lead us to antimicrobial resistance with serious problems not limited to *P. acnes*, but also to other bacterial species [17]. The choice of antibacterial agents should take into account the severity of acne, cost-effectiveness, benefit-risk ratios, and the potential for the development of resistance [18]. The treatment options in acne are far from ideal, [19] therefore the improved understanding of acne pathogenesis should lead to a logical therapy to successfully treat this skin disease.

CONCLUSION

We believe that because of changing drug-sensitivity of bacterial strains, it seems important to perform assessment of bacterial flora and antibiotic susceptibility of isolates in acne cases, especially in clinically severe and resistant to treat. Beside the presence of resistant strains of *S. aureus*, *S.epidermidis*, *P.acne* to various antibiotics as in this study, it emphasizes the need to discourage antibiotics' abuse and the implementation strategies for elimination of carriage of *S. aureus*. We suggest that rifampin is a suitable antibiotic for acne patients, but to achieve a better result, combination of rifampin with other antibiotics seems necessary. Also we suggest an *in vivo* study to be performed for better evaluation acne vulgaris treated by rifampin.

ACKNOWLEDGEMENT

We would like to thank all the volunteers for their co-operation during data collection.

REFERENCES

1. Hanna S, Sharma J, Klotz J; Acne vulgaris: More than skin deep. Dermatol Online J., 2003; 9(3): 8.
2. Shaheen B, Gonzalez M; Acne sans *P. acnes.* J Eur Acad Dermatol Venereol., 2013; 27(1): 1–10.
3. Simpson NB; Disorders of the sebaceous glands. In Burn T, Breathnach S, Cox N, Grifiths C editors; Rook's Textbook of Dermatology. 7th edition, Oxford: Blackwell Science, 2004: 43.15.
4. Firooz A, Sarhangnejad R, Davoudi SM, Nassiri-Kashani M; Acne and smoking: Is there a relationship? BMC Dermatol., 2005; 5: 2.
5. Smith RN, Mann NJ, Braue A, Mäkeläinen H, Varigos GA; A low-glycemic-load diet improves symptoms in acne vulgaris patients: A randomized controlled trial. Am J Clin Nutr., 2007; 86(1): 107–115
6. Gollnick HP, Krautheim A; Topical treatment in acne: current status and future aspects. Dermatology, 2003; 206(1): 29–36.
7. Stein RH, Lebwohl M; Acne therapy: Clinical pearls. Semin Cutan Med Surg., 2001; 20(3): 184–189.
8. Zaenglein AL, Thiboutot DM; Acne vulgaris. In Bolognia JL, Jorizzo JL, Rapini RP editors; Dermatology. 2nd edition, Toronto: Mozby; 2003: 532.
9. Zamiri I; Corynebacterium. In Collee Gerald J, Marimon Barrie P, Fraser Andrew G, Anthony S editors; Mackie & McCartney, Practical Medical Microbiology, 14th edition, Elsevier, 2007: 305.
10. Baron EJ, Finegold SM; Diagnosis microbiology. 8th edition, The CV Mosby Company, Methods for testing antimicrobial effectiveness; 1990: 171–194.
11. Ashkenazi H, Malik Z, Harth Y, Nitzan Y; Eradication of Propionibacterium acnes by its endogenic porphyrins after illumination with high intensity blue light. FEMS Immunol Med Microbiol., 2003; 35(1): 17–24.
12. Toyoda M, Morohashi M; An overview of topical antibiotic for acne treatment. Dermatology, 1998; 196(1): 130–134.
13. Thiboutot D; New treatments and therapeutic strategies for acne. Arch Fam Med., 2000; 9(2): 179–187.
14. Cunliffe WJ, Baron SE, Coulson IH; A clinical and therapeutic study of 29 patients with infantile acne. Br J Dermatol. 2001; 145(3): 463–466.
15. Noyon V, Legallou F, Richet H, Dreno B; The resistance of Propionibacterium acnes and Staphylococcus epidermidis to cyclones. Ann Dermatol Venereol., 1998; 125(12): 885–887.
16. Tan HH, Goh CL, Yeo MG, Tan ML; Antibiotic sensitivity of Propionibacterium acnes isolates from patients with acne vulgaris in a tertiary dermatological referral centre in Singapore. Ann Acad Med Singapore, 2001; 30(1): 22–25.
17. Patel M, Bowe WP, Heughebaert C, Shalita AR; The development of antimicrobial

resistance due to the antibiotic treatment of acne vulgaris: A review. J Drugs Dermatol., 2010; 9(6): 655-664.

18. Tan HH; Antibacterial therapy for acne: A guide to selection and use of systemic agents. Am J Clin Dermatol., 2003; 4(5): 307-314
19. Lomholt HB, Kilian M; Is acne caused by colonization with the "wrong" strain of Propionibacterium acnes? A review of the role of Propionibacterium acnes in acne. Ugeskr Laeger., 2008; 170(15): 1234-1237.