"CLINICO-BACTERIOLOGICAL STUDY OF ACNE VULGARIS PATIENTS ATTENDING DERMATOLOGY OUT PATIENT DEPARTMENT, WITH SPECIAL REFERENCE TO ANAEROBES- A ONE YEAR CROSS SECTIONAL STUDY."

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This is to certify that the dissertation entitled **"CLINICO-BACTERIOLOGICAL STUDY OF ACNE VULGARIS PATIENTS ATTENDING DERMATOLOGY OUT PATIENT DEPARTMENT, WITH SPECIAL REFERENCE TO ANAEROBES- A ONE YEAR CROSS SECTIONAL STUDY"** is a bonafide research work done by ***REG NO: BI0116001.***

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LIST OF ABBREVIATIONS

AV	-	Acne Vulgaris
BPO	-	Benzoyl peroxide
Cd	-	Clindamycin
COX	-	Cyclooxygenase
DAMP	-	Damage associated molecular patterns
FDA	-	Food and Drug Administration
GPB	-	Gram Positive Bacilli
IL	-	Interleukin
LOX	-	Lipoxygenase
MIC	-	Minimum Inhibitory Concentration
MUFA	-	Monounsaturated fatty acids
NF	-	Nuclear factor
PAMPs	-	Pathogen-associated molecular patterns
PAPA	-	Pyogenic arthritis, pyoderma gangrenosum, and acne conglobata
PASH	-	Pyoderma gangrenosum, acne and suppurative hiradenitis
PCR	-	Polymerase Chain Reaction
PIA	-	Polysaccharide intercellular adhesion
PPAR	-	Peroxisome proliferator-activated receptors
PRR	-	Pattern recognition receptors
Spp.	-	Species
TLR	-	Toll like receptors
TNF	-	Tumor necrosis factor
VBNC	-	Viable but non culturable

ABSTRACT

INTRODUCTION:

Acne vulgaris is a chronic inflammatory disorder affecting the skin of 70-90% young adults especially during puberty. Although *Propionibacterium acnes (P.acnes)* is known to predominate over other bacteria, the spectrum of others has been infrequently studied.

OBJECTIVES:

1. To isolate and identify the aerobic and anaerobic bacteria associated with acne vulgaris patients and correlate them with clinical findings.
2. To carry out the antibiotic sensitivity of the aerobic and anaerobic bacteria involved in acne vulgaris patients.

MATERIALS AND METHODS:

After Ethical Committee approval, the study was conducted from January 2017 to December 2017 at Department of Microbiology, Jawaharlal Nehru Medical College (in collaboration with Department of Dermatology), KLE Society's Dr.Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka. Eighty-five samples, from patients of all age groups suffering from acne vulgaris, were obtained after informed consent under strict asepsis using a comedone-extractor. The sample was transferred to thioglycollate medium and transported to the Microbiology Laboratory. It was subjected to Gram's stain and was put up on blood agar, propionibacterium agar and chocolate agar for aerobic and anaerobic cultures.

RESULTS:

Overall, the majority of patients belonged to grade one acne vulgaris and the highest prevalence was seen between 15-25 years of age (71.8%). Women were marginally more affected than men (51.8%). Gram's stain predominantly showed

gram positive cocci. Sixty seven of eighty five samples yielded growth. Out of 67 culture positives, 32 (47.8%) grew a single type of bacteria and 35(52.2%) showed mixed growth. Among 32 pure isolates, 21 (65.6%) cultured positive for *P.acnes* while 10 (31.3%) were *S.epidermidis* and only 1 (3.1%) showed positive for *S.aureus*. Out of 35 mixed isolates, there were 24 (68.6%) samples with concurrent growth of *P.acnes* and *S. epidermidis* while 11 (31.4%) were *P.acnes* and *S.aureus*. Overall, 56 *P.acnes*(83.6%), 34*S.epidermidis*(50.7%) and 12 *S.*aureus (17.9%) were isolated.

Among all *P. acnes* growths, over 66% had mild lesions (grade 1). Likewise, most of the concomitant growths (*P. acnes* with *S. epidermidis* and *S. aureus*) were also grade1 lesions.

Aerobic bacterial isolates were found to be mainly resistant to Clindamycin and Erythromycin whereas Gentamicin and Doxycycline were found to act well against them. Among the anaerobes, 50% of the isolates were resistant to Clindamycin.

CONCLUSION:

Although *P.acnes* was the dominant organism isolated from acne lesions, however, co-existence with *Staphylococcal species* also appears prevalent. The emergence of a resistance pattern amongst anaerobes, as reported by many studies, is also reflected in this study. Knowing the susceptibility pattern of these pathogenic bacteria will help in effective treatment thereby, preventing complications.

Key words: Acne vulgaris, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, Antibiotic sensitivity test

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INTRODUCTION

Acne vulgaris (AV), a chronic inflammatory disorder of the pilosebaceous follicles, affects more than 85% of adolescents and young adults.[1]Although the disease is not life threatening, it affects the quality of life by creating a psychological burden due to the manifestation of multifarious lesions on the face, chest, shoulders and back.[2]Spontaneous regression is common, but in few cases acne persists beyond the age of 25 and extends into the fourth and fifth decades of life.[3]It is characterized by a variety of non-inflamed (open and closed comedones) and inflamed (macules, papules, pustules and nodules) lesions. 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 (P.acnes)*, and (iv) inflammation.[4] Microcomedones (earliest subclinical lesions) are thought to be the precursor lesions that can then develop into non-inflammatory and/or inflammatory lesions. Although a common disease, the aetiology of acne is not yet fully understood.[5]

Among the bacteria species that colonize normal skin as resident flora, only those able to colonize the follicular duct and multiply there can be pathogenic for acne.[6] Various studies done previously reports that three major organisms could be associated with the development of acne lesions: *Propionibacterium acnes*, *Staphylococcus epidermidis (S.epidermidis)* and *Malassezia furfur*.[4] However, acne have not shown any improvement after antifungal treatment, so yeasts could not be associated with the pathogenesis of acne. *Staphylococci* have also developed antibiotic resistance in most patients, scientific interest has therefore been focused on *Propionibacteria.*[6]

In the last years, several studies have documented that both aerobes and anaerobes are found in Acne vulgaris. Aerobically, *Staphylococcus* species are the most common isolates and *P.acnes* are the predominant anaerobic bacterial isolates. Other anaerobes found are *Peptococci, Peptostreptococci* and *Fusobacterium* species.[7]

Propionibacterium acnes is the microorganism reported to cause acne, but their presence with other microbes in acne lesions seems to be a puzzle, as they have their own role in causing other diseases[8].*P. acnes* is an anaerobic, non-motile, non-sporulating, pleomorphic Gram-positive bacillus (GPB), predominantly inhabits the sebaceous region.[9]*P.acnes* play a central role in acne pathogenesis. This bacterium 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 responses.[10]

Amongst the aerobic bacteria, *Staphylococcus species* are commonly encountered. Lipases, fatty acid modifying enzyme, polysaccharide intercellular adhesion (PIA), and polyglutamicacid are the virulence factors in *Staphylococcus epidermidis*.[11,12]Adhesins, fibronectin binding protein (FnBp)-A, FnBPB, proteases, lipases, and hyaluronidases are the virulence factors in *Staphylococcus aureus*.[13,14] Thus, each pathogen follows its own pathogenic strategy, with a diverse and unique set of genes/factors operating in a concerted manner to cause disease in the host.[8]

Although acne is not a life-threatening disease, it has significant physical and psychological consequences such as permanent scarring, poor self-image, social desolation, depression and anxiety.[15] Therefore, the primary goals of acne treatment

are prevention of scarring and alleviation of clinical symptoms. Depending on the severity of disease, acne patients receive topical or systemic therapy or a combination of both. The treatment of acne involves the empirical use of antimicrobials.[16]

In view of the most common skin disorder of the adolescents and young adults, the fact that it is caused by both aerobic and anaerobic bacterial pathogens and also due to the emergence of antibiotic resistant bacteria, this study was carried out to isolate and identify the causative bacteria involved in acne vulgaris and their antibiogram was determined. The knowledge of pathogenic bacteria involved in causing acne lesions and carrying out their antibiogram will help in developing a treatment strategy, prevention and also making a rational choice of both empirical and definitive antibiotic therapy for acne vulgaris.

OBJECTIVES OF THE STUDY

1. To isolate and identify the aerobic and anaerobic bacteria associated with acne vulgaris patients and correlate them with clinical findings.
2. To carry out the antibiotic sensitivity of the aerobic and anaerobic bacteria involved in acne vulgaris patients.

REVIEW OF LITERATURE

In addition to serving as a defensive barrier, the human skin also fosters several symbiotic bacteria, fungi and parasites.[17]The defensive properties of the skin are augmented in the presence of locally manufactured enzymes (proteases, lysozymes) and antimicrobial peptides.[8]

The epidermal layer of the skin blocks microbial penetration and the dermis possesses several appendages including hair follicles, sebaceous, apocrine, and sweat glands which are responsible for the irregular appearance of the skin.[18, 19]Hair follicles are flooded by secretions (sebum) produced by the sebaceous glands. The products of sweat glands include fatty (apocrine) and salty fluids (eccrine). The latter is involved in regulating body temperature.[20]In addition to the above, the pH, temperature and moisture contribute in providing viable conditions for the growth of a wide spectrum of microorgansisms – the microbiota of the human skin.[8]

The bacterial members of this diverse network predominantly include Staphylococcus, Corynebacterium, and Propionibacterium among others which, belong to Actinobacteria, Proteobacteria, Bacteroidetes, and Firmicutes.[20,22] The normally cordial ties between humans and the amiable bacteria can be overturned in certain situations. This can and has been demonstrated to result in illness and infirmities of the skin and tissues elsewhere.[21,23]

- **<u>Acne vulgaris and its clinical features</u>**

AV is a chronic inflammatory disease of the pilosebaceous unit (sebaceous glands and hair follicles) usually occurring in a younger population. It is a disorder of

adolescence, but it persists until the middle age in a minority of individuals. Acne vulgaris occurs in several clinical forms and the peculiar clinical presentations include seborrhoea, comedones, erythematous papules and pustules, less frequently nodules, deep pustules or pseudocysts, and ultimate scarring in few of them.[24]Acne has four main pathogenetic mechanisms *viz.* increased sebum productions, follicular hyperkeratinization, *Propionibacterium acne* colonization, and the products of inflammation. It is commonly seen on the face and upper trunk, sites with well-developed sebaceous glands.[25]

- **Non-inflammatory acne.**

Non-inflammatory acne is characterized by clogged pores and follicles that may or may not have been infected by the *P. acnes* strain.

- Closed comedones (whiteheads) are small (~1 mm), skin-colored papules without an obvious follicular opening.

- Open comedones (blackheads) have a dilated follicular opening filled with a keratin plug. The black color of the open comedones is due to oxidized lipids and melanin. However, non-inflammatory acne can become infected, especially if the whiteheads and blackheads are squeezed with dirty tools or fingers.[25]

- **Inflammatory acne.**

The characteristic features of inflammatory acne include erythematous papules and pustules due to colonization of the closed comedones by *P. acnes*. Furthermore nodules and cysts filled with pus or serosanguinous fluid coalesce to form sinus tracts

thus breaking down sebum into free fatty acids. It irritates the follicular epithelium eliciting an inflammatory response by neutrophils and thereafter lymphocytes, which further disrupts the epithelium. The inflamed follicle ruptures into the dermis (sometimes precipitated by physical manipulation or harsh scrubbing), where the comedone contents elicit a further local inflammatory reaction, producing papules. If the inflammation is intense, grossly purulent pustules occur. A severe form of acne inflammation is acne conglobata (severe nodulocystic acne) which is classified in the follicular occlusion tetrad along with dissecting cellulitis of the scalp, hidradenitis suppurativa, and pilonidal cysts. It is also a part of pyogenic arthritis, pyodermagangrenosum, and acne conglobata (PAPA) and pyodermagangrenosum, acne, and suppurative hidradenitis (PASH) syndromes. The consequence of inflammatory acne is post-inflammatory hyper pigmentation, which is especially seen in patients with darker skin. However, it fades slowly over time. Nodulocystic acne often leads to pitted or hypertrophic scars.[25]

Post-Adolescent Acne

Although acne is principally a disorder of adolescence, current research indicates that the prevalence of adult patients with acne is increasing. According to the time of onset, two subtypes of adult acne are recognized: persistent and late-onset. Persistent acne is a continuation or relapse of the disease from adolescence into adulthood and middle age, while the late onset type involves patients aged twenty-five years and older who have not previously been affected by acne vulgaris. Both subtypes more frequently affect women and are often associated with inflammation which tends to flare in the week prior to menstruation, changes in pigmentation, and scarring. Late-onset acne is thought to be less common than persistent acne.

Papulonodules on the lower face, jawline, and neck characterizes this particular phenomenon. Women with hyperandrogenism are more prone to post-adolescent acne vulgaris.[25]

Clinical grading of acne vulgaris

To assess the clinical severity of acne, a grading method is commonly used. According to James and Tisserand, the severity of acne can be graded as:

- Grade 1 - Simple non-inflammatory acne - comedones and few papules.
- Grade 2 - Comedones, papules being the predominant and few pustules.
- Grade 3 - Larger inflammatory papules, pustules and few cysts. Pustules are the predominant lesions. It is a more severe form involving the face, neck and upper portions of the trunk.
- Grade 4 - More severe, where cyst becomes confluent, nodulo-cystic.[26]

Pathogenesis of acne

The pathogenesis of acne, the most common skin disease, is multifactorial. Together with other genetic and environmental factors, cytokines play an important role in the pathogenesis of AV. Another predisposing factor for acne susceptibility with no apparent relation to its severity is the tumor necrosis factor-α-308 gene polymorphism.[27]Pathogenesis manifests in the pilosebaceous follicle, currently attributed to increased sebum production, alteration of the quality of sebum lipids, regulation of cutaneous steroidogenesis, androgen activity, interaction with neuropeptides, an exhibition of pro- and anti-inflammatory properties, follicular hyperkeratinization and the proliferation of *P. acnes* within the follicle. Therefore, AV develops as a result of an interplay between the following four factors.[28, 29]

- Release of inflammatory mediators
- Follicular hyperkeratinization with follicular plugging
- *Propionibacteriun acnes* colonization of the follicle
- Excess sebum production

In addition, research in the areas of diet and nutrition, genetics and oxidative stress have also yielded some interesting insights into the development of acne.[30]

Release of inflammatory mediators

Inflammation is considered to play a crucial role in the pathogenesis of acne.[31] Increase in the activity of the pro-inflammatory cytokine, interleukin (IL)-1, is observed before the beginning of hyperproliferation around the uninvolved follicles and is thought to trigger the activation of keratinocyte proliferation.[32]Nuclear factor kappa beta (NF- κβ) regulated mRNA gene levels of the cytokines- tumour necrosis factor (TNF)-α, IL-1 β, IL-8 and IL-10 are significantly upregulated in acne-involved skin, compared to the uninvolved normal adjacent skin. Many pro-inflammatory cytokine genes including those of matrix metalloproteinases, β-defensin 4, IL-8 and granulysin are also involved in inflammatory acne lesions.[33] It has been shown that elevated expression of the chemokine IL-8 and the activated protein, activator protein (AP)-1, attracts circulating inflammatory cells into the tissue. Inflammation is further characterized by the action of active lipid mediators such as leukotrienes, prostaglandins and 15-hydroxyeicosatetraenoic acids (15-HETE). These molecules are synthesized from arachidonic acid or linolenic acid by the enzyme lipoxygenase (LOX) and cyclooxygenase (COX), respectively. Expression of both COX isozymes, COX-1 and COX-2, along with 5-LOX are in human sebocytes have been observed in vitro. In particular, COX-2 expression is selectively upregulated in acne-involved

sebaceous glands *in vivo*. Phosphodiesterases lower the intracytoplasmic levels of cAMP, leading to the preferential expression of pro-inflammatory cytokines such as TNF-α, IL-1, IL-8, IL-12 and IL-23.[34, 35] Interleukin-1 triggers remodeling of the pilosebaceous unit and promotion of comedogenesis. Neutrophils are attracted by interleukin-8 to the site of inflammation in the pilosebaceous unit. Interleukin-12 is the major pro-inflammatory cytokine produced by monocytes in response to invading gram-positive micro-organisms. It induces expression of antimicrobial peptides such as defensins, which have been implicated in the evolution of the acne lesion.[36] Psoriasin, a member of the S100 gene family, was shown to be highly expressed in the epidermis and the ductus seboglandularis of acne-involved skin.[37]

Toll-like receptors

Toll-like receptors (TLRs) are transmembrane proteins that are crucial players in the innate immune response to microbial and other invaders. Ten TLRs have recently been described in humans that are mainly expressed on immune cells, such as monocytes, macrophages, dendritic cells and granulocytes. Toll-like receptors are a subtype of pattern recognition receptors (PRRs) that can activate innate immune responses through keratinocytes, neutrophils, monocytes/macrophages, natural killer cells and dendritic cells (including Langerhans cells). Toll-like receptors (TLR) TLR-2 and TLR-4 appear to be specific for acne pathogenesis. Microbial ligands (such as *Propionibacterium acnes*) can activate several pathways that ultimately set off nuclear factor (NF)-κβ transcription factor which causes the release of inflammatory cytokines (IL-1, IL-6, IL-8, IL-10, IL-12 and TNF-α). Toll-like receptor activation also leads to the release of antimicrobial peptides (human β defensin 1 and human β defensin 2) that play an important role in innate immune responses.[38]Toll-like

receptor-mediated cytokines additionally induce matrix metallo-proteinases that contribute to acne inflammation, dermal matrix destruction and scar formation.[39]TLR stimulation mimics the action of IL-1a and promotes the production of proinflammatory cytokines, prostaglandins, leukotrienes.

Follicular hyperkeratinization with follicular plugging

The initiating steps in comedogenesis critical to the pathophysiology of acne are the activation of Toll-like receptor and secretion of IL-1α from keratinocytes which may be the initiating steps in comedogenesis.Interleukin-1α is released from the infundibular keratinocyte in response to *P. acnes*- mediated TLR activation and is an important step in the complex natural evolution of the acne lesion.[40] Moreover, IL-1α may contribute to both the creation of a comedogenic cytokine milieu, as well as eventual sebocytehypercornification, characteristic of acne lesions. Microcomedone, the precursor lesion in acne, results from both follicular keratinization and reduced desquamation of keratinocytes in the infundibulum, thereby forming a keratin plug at the follicular infundibulum.[41] Epithelial hyperproliferation (comedo formation) is driven by increased levels or sensitivity to androgens, changes in sebum lipid composition, *P. acnes* over growth and local cytokine milieu. Biofilm, a complex aggregation of microorganisms encased within an extracellular polysaccharide lining secreted by bacteria, has a role in the formation of a microcomedo by acting as a biological glue; *de novo* formation of inflammatory lesions has also been proven.[42]

***Propionibacteriun acnes* colonization of the follicle**

P. acnes, a Gram-positive anaerobic bacteria normally found in the sebaceous follicle, play an important role, both directly and indirectly, in the development of

inflammatory acne. Other propionibacteria that may have a role include *Propionibacterium granulosum* and *Propionibacterium avidum*. *P. acnes* release many enzymes such as proteinases, lipases, hyaluronidases and chemotactic factors that are integral in the inflammatory cascade.[43] It directs immune reactions by modulation of the T helper 1/T helper 2 response and induction of monocyte-derived dendritic cell maturation.[44]*P. acnes* stimulate the host innate immune response by activating Toll-like receptors and recognizing pathogen-associated molecular patterns (PAMPs).[45, 46]*P. acnes* also stimulate inflammasome formation, which are large complexes formed when PAMPs are sensed by DAMP (damage- associated molecular patterns) from the host leading to the activation of caspase-1, IL-1β and IL-18 which produce the inflammatory papules of acne.[47, 48]

Role of sebum in acne

Sebaceous glands produce and secrete sebum, a group of complex oils including triglycerides and fatty acid breakdown products, cholesterol esters, wax esters, squalene, and cholesterol.[49] The main events in acne pathogenesis are increased sebum excretion, alteration of lipid composition and changes in the oxidant/antioxidant ratio characteristic of the skin surface lipids.[50] Decreased levels of linoleic acid have been found in skin lipids of acne patients.[51] The presence of lipoperoxides is an important hallmark of sebum in acne patients, mainly due to the peroxidation of squalene and a decrease in the level of vitamin E, the major sebum antioxidant. Lipoperoxides and monounsaturated fatty acids (MUFA) are capable of inducing the alteration in keratinocyte proliferation and differentiation, whereas peroxides are capable of inducing production of pro-inflammatory cytokines and activation of peroxisome proliferator-activated receptors (PPAR).The biological

function of sebocytes is further regulated by several factors including the ligands of receptors expressed in sebocytes; such as androgens and estrogens, PPAR ligands and neuropeptides (NP), liver-X receptor ligands, histamines, retinoids and vitamin D. Sebaceous function can also be modified by histamine and conversely, antihistamines, since histamine receptors have been identified in human sebaceous gland cells.[53] Retinoids also affect the biological function of sebocytes. Retinoic acid receptors (isotypes α and γ) and retinoid X receptors (isotypes α, β, γ) are expressed in human sebocytes. All isoforms of all-trans-retinoic acid exhibit anti-proliferative effects and inhibit sebocyte differentiation and lipid synthesis.[54, 55] Neuropeptides (with hormonal and non-hormonal actions) can also control the development of clinical inflammation in acne (neurogenic co-control). Substance P can be identified in numerous immune-reactive nerve fibers of acne skin and sebaceous glands respond to it with the synthesis of the neutral endopeptidase.[56]

Vitamin D receptor, vitamin D-25-hydroxylase, 25-hydroxyvitamin D-1alpha-hydroxylase and 1, 25-dihydroxyvitamin D-24-hydroxylase are expressed in SZ95 sebocytes *in vitro*. Furthermore, incubation of SZ95 sebocytes with 1, 25 $(OH)_2$ D3 leads to a dose-dependent modulation of cell proliferation, cell cycle regulation, lipid content and IL-6/IL-8 secretion *in vitro*.[57]

Bacteriology of acne vulgaris

It is difficult to establish the importance of microorganisms in the pathogenesis of acne because all of the organisms implicated are also common commensals on healthy skin. A number of potential microbial pathogens, singly and in combinations, have been implicated to cause acne vulgaris. The list of possible agents continues to expand and includes members of a number of genera, including

P.acnes, S.epidermidis, S.aureus, Klebsiella pneumonia, Steptopcoccus, Enterobacter etc.[2, 58]

<u>Propionibacterium acnes</u>

P. acnes, is an opportunistic pathogen that plays an important role in the progression of inflammatory acne vulgaris. Bacterial colonization of the pilosebaceous unit by *P. acnes*, a common anaerobic GPB is commensal on normal skin. Increased sebum production and follicular hyperkeratosis are thought to be initial events leading to a change of the pilosebaceous milieu that favors the proliferation of *P. acnes*. It is ubiquitously present within the sebaceous follicles of the human skin. These acne-causing bacteria are usually gram positive, non-motile, fat splitting microorganisms, having the ability to grow under different oxygen tensions. They exclusively occupy the follicular canal and clog the hair follicle. The bacterium aids in the rupturing of the follicular walls, using their secretory enzymes with degradative properties.[59-61]These bacteria also target other skin cells, namely, keratinocytes and phagocytic cells like macrophages, stimulating the cells to produce proinflammatory cytokines, including interleukin (IL)-1b, IL-8, IL-12, and tumor necrosis factor-a, leading in the inflammatory acne disease.[61-64] The genomic information clearly highlights that the products of the *P. acnes* have a major impact on the acne process, but not the invasiveness of the organism. The notable virulence genes involved in the pathogenesis of acne are *camp5, gehA, tly, sialidases, neuraminidases, endoglycoceramidases, lipases,* and *hemolysins*.[60, 65] The lipoglycan-based cell envelope and their extracellular secreted lipase, particularly triacylglycerol lipase, encoded by the *gehA* gene assists in the adherence and the colonization of the bacterium to the sebaceous follicle.

The other product which aids in the acne process by destroying the host tissue includes porphyrins, hyaluronatelyase, endoglycoceramidase, sialidases/ neuramidase, cardiolipinsynthetase, and calicineurin like phosphoesterase.[65, 66]The organism further possesses several proteins associated with cell invasion, which are secreted by genes, namely *PAmce, PAp60,* and cell surface antigen, which are produced by htaA and hsp20.[67] This help the pathogen to invade the host cell further and makes it highly immune-reactive, thereby establishing high virulence. [8]

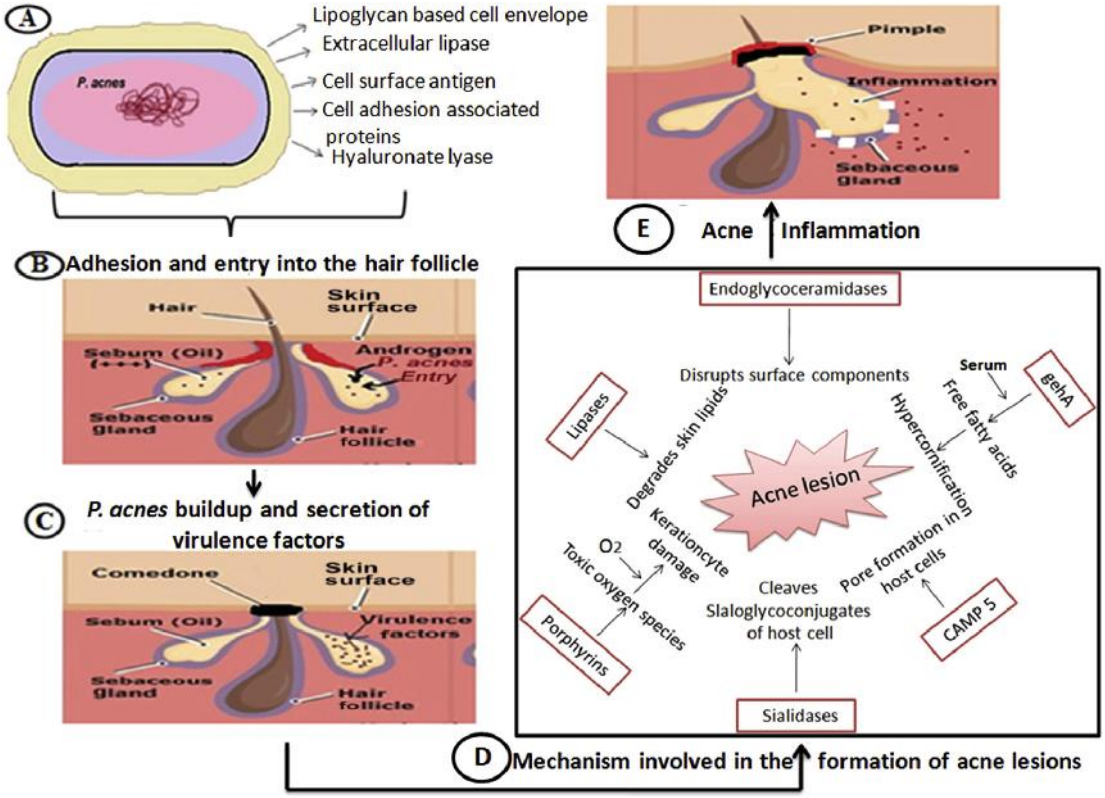


Figure 1 Schematic representation showing the steps involved in the pathogenesis of Propionibacterium acnes in acne disease progression: (A) important factors within P. acnes contributing acne pathogenesis; (B) adhesion and entry into the hair follicle; (C) P. acnes build up and secretion of virulence factors; (D) mechanism involved in the formation of acne lesions.[68]

Staphylococcus epidermidis

S. epidermidis are non-pathogenic resident micro-organism of the human skin microbiome. However, it turns into an infectious agent due to extrinsic factors when the immune system is compromised.[69] The first and foremost virulence factor produced by this organism is a fatty acid modifying enzyme which esterifies the fatty acids in the skin to cholesterol, as fatty acids are bactericidal for the organism to survive.[70]The bacterium possesses several adhesion factors for its attachment to the skin surface, like surface anchored proteins, fibrinogen binding protein, autolysin protein, PIA, and poly-N-succinyl-glucosamine, helping as a probable attachment factor.[12,71,72]The potentially virulent *S.epidermidis* also has the ability for biofilm formation and is a reservoir of antibiotic resistance genes, which get horizontally transferred to other organisms.[71, 73]In the process of acne development, the lipases (*geh1*gene) and the delta-haemolysin (*hld* gene) are two virulence factors that have an impact in acne inflammation.[12]Although they have such virulence characteristics, they have been found to rarely damage the keratinocytes in the skin. This has shown that *S.epidermidis* secreted the exopolysaccharide intercellular adhesin (PIA), which is responsible for biofilm formation and protects them against major components of human innate host defence.[71-73]This biofilm provides the favourable anaerobic conditions to grow *P. acne* in an easy manner.

According to Pathak et al.,[17]the population of *S. epidermidis* and *P. acnes* were found to be increased by ~70% and ~82%, respectively, in acne patients compared with controls. The microbial load of these microbes was found to be increased simultaneously in the case of acne, which indicates some important role of these two bacteria in the development and regulation of acne disease. On the basis of

the above evidence, we can say that *S. epidermidis* plays an important role in acne pathogenesis not in a direct manner but in an indirect manner.[17, 23]A potential research including RNA sequencing and quantitative whole-cell proteome analysis of *S. epidermidis* as well as affected tissue at different stages of disease development might help to better understand the role of this bacterium in acne pathogenesis.[8]

Staphylococcus aureus

S. aureus, the most prominent member of the skin microbiota, plays a role as a pathogen in many skin infections such as folliculitis and impetigo. Furthermore, its co-existence with other microbes in acne lesions has also been reported.[74, 75]It produces extracellular matrix and serum binding proteins such as adhesins [surface protein (SasG)] and the fibronectin binding proteins FnBP-A and FnBP-B help in the invasion process. These factors help in their internalization into the host cells by connecting them to the cellular integrins. Once it invades the human skin as a pathogen, it starts producing several extracellular enzymes such as proteases, lipases (*geh1*), hyaluronidases, and collagenase, that aid in tissue damage and thus helps the pathogen to spread into the deeper tissues.[75, 76]They are known for their production of exfoliative toxins, such as enterotoxins A-E, toxic shock syndrome toxin-1, Pantone Valentine leucocidin, leukocidin E-D, *S. aureus* exotoxin, and cytotoxins such as α-, β-, γ- hemolysins which are known to be pathogenic determinants.[77,78]It also produces enzymes, namely staphylokinase (sak) and aureolysin: the first enzyme binds to defensins preventing them to act against the pathogen, while the latter one binds and cleaves human cathelicidin LL-37, offering further protection for the pathogen to establish their pathogenicity in the human system.[77, 79]

Laboratory Diagnosis of acne

Diagnostics of acne is based on the clinical picture and laboratory evidence of *Propionibacterium* and *Staphylococcus* bacteria. The technique of sampling is very important[24]-According to Zandi et al, the skin is first wiped with 70% ethanol and the material is squeezed using a comedone extractor from skin lesions and collected by sterile swab sticks. These swabs are then transferred into the transport anaerobic Thioglycollate medium. A direct smear is also made using these swabs.

Samples are transported to the microbiology laboratory immediately. Each specimen from the transport media is inoculated into two plates. Blood agar (Conda, Spania), one of which is incubated in aerobic condition at 37°C for 24h and the other one in anaerobic conditions for one week. After one week if *P. acnes* culture is negative, a subculture is grown, material from Thioglycollate medium is prepared again. *P. acnes* colonies are determined by Gram stain and specific tests such as catalase, indole, gelatine and esculine are carried out.[80]

According to another author, the specimens are inoculated onto 5% sheep's blood agar, MacConkey agar, and brain-heart infusion agar (HiMedia, Mumbai, India) supplemented with 5 g/L glucose and 2 mg/L furazolidone. Plates are incubated at 37°C under both aerobic and anaerobic conditions for 2–7 days and examined for growth. Anaerobic culture is performed using the Gaspak system (HiMedia Labs., Mumbai, India).[81]

Aerobic and anaerobic bacteria are identified by Gram stain, colony morphology, and standard biochemical tests.[82] *P. acnes* strains were presumptively identified as GPB grown anaerobically with positive indole, catalase, and nitrate

reduction tests. Final identification is confirmed by the automated VITEK2 Compact (Biomerieux, Marcy l'Etoile, France) system.[81]

Molecular methods

In recent years, our knowledge regarding microorganisms has been depended on the culture technique, while culture method is able to identify little minority (less than 1%) of the bacteria (Grice et al., 2008).[83] For the purpose of identification of the viable but non culturable (VBNC), microorganisms which are not capable of growing in the usual culture media (Kong and Segre, 2012; Rhoads et al., 2012),[84, 85]microorganisms growing slowly (Millar et al., 2007)[86] and, also, obligate anaerobes micro-organisms (Rhoads et al., 2012)[85] the molecular techniques have a lot of applications.

Usage of the techniques relied on nucleic acid has obviated the limitations available on the way of identification of microorganisms through culture technique (Taravati et al., 2013).[87] In the molecular methods, little quantity of the microorganism's DNA is identifiable and living of the studied microorganism is not to be required (Kong and Segre, 2012;Rhoads et al., 2012;).[84, 85]Also, in order to carry out PCR, samples such as blood and liquids of the body can be used, and preparation of the fresh sample is not required. Before sampling, antibiotic consumption doesn't influence the result achieved from PCR, while it leads to the establishment of the false negative response in the results obtained from culture.[88]

P. acnes is a fastidious bacterium which requires enrich medium, special nutrient factors and anaerobic conditions in order to grow. Due to its slow growth, identification of the *P. acnes* through culture is not so much possible.[88] In 2003, a

research was conducted by Le Page et al aiming at diagnosis of the vascular prosthesis infection through method amplification and sequencing of *16S rDNA*.[89] In this study, of 20 studied samples, 5 positive samples (25%) of the *P. acnes* were identified with the assistance of the PCR method (Le Page et al., 2003). In 2006, a research was conducted on the individuals with endophthalmitis by Bagyalakshmi et al.[90] Thirty samples were collected from the studied patients and using the mPCR method, 4 positive samples (13.3%) of the *P. acnes* were diagnosed (Bagyalakshmi et al., 2006).

In 2013, a study was carried out by Rollason et al,[91] on the patients with herniated waist disk surgery. Of total 64 studied samples, the presence of the *P. acnes* in 24 samples (38%) was confirmed with aid of analysis of *recA* gene sequence (Rollason, et al., 2013). In 2014, a study was carried out by Bunker et al on the individuals with shoulder infection following the orthopaedic surgery. Of 10 studied patients, 6 positive samples (60%) of *P. acnes* were diagnosed by use of the PCR method (Bunker et al., 2014).[92]

Some discrepancies are observed among the results achieved from the researches, this discrepancy may be related to sampling place, type of the studied sample (acne or other infections resulted from *P. acnes*) and conditions of PCR performance. Usage of the molecular methods for the purpose of identification of the biologic factors compared to the traditional methods of diagnosis leads to time and cost economizing.[88] Despite the day increasing improvements of molecular techniques for the purpose of diagnosis of a variety of infections, culture method as a standard method remains to be a special value and position (Kong and Segre, 2012; Taravati et al., 2013).[84, 87]

Treatment

Strategies for successful acne management

For an effective and efficient management of acne, a careful patient evaluation followed by consideration of several "patient factors" and "medication factors" is required in choosing a particular therapeutic regimen.[16]

Patient Factors

Majority of the patients present with non-inflammatory and inflammatory lesions. The predominance of one type, along with the number of lesions, plays a role in determining the severity of acne. Other factors to be considered include age, skin type (dry, oily, or combination), coexisting conditions, patient motivation, lifestyle, menstrual regularity and premenstrual flare-ups, evidence of hirsutism, the effect of acne, and potential therapies on the patient's quality of life. If the patient is taking birth control pills, it is important to determine the brand, as certain formulations contain agents (eg, androgenic progestins) that may provoke acne. Exposure to comedogenic substances such as tars, polyvinyl chloride, or other substances used for hair care should also be determined. Other medications that may cause acne include corticosteroids, androgens, iodides, bromides, lithium, trimethadione, halothane, vitamin B12, and hyperalimentation therapy. In addition, mechanical trauma can aggravate a patient's acne. Incorporation of these factors into the choice of a specific therapeutic regimen can enhance patient compliance, which is essential for the success of acne treatment.[93]

While acne is generally considered an affliction of adolescence, a steadily increasing number of older patients (particularly women) have been seeking treatment

for acne.[94] This is a more demanding, articulate group of patients with high expectations for improvement. Furthermore, they may have a low tolerance for adverse effects of therapy, such as erythema or scaling, and greater concern about scarring and post-inflammatory hyperpigmentation.[95]

Medication Factors

The patient's skin type and preferences should be considered in the choice of vehicle for topical agents. Those vehicles with a higher proportion of alcohol (eg, solutions and some gels) are often preferred by patients with oily skin; patients with dry skin may prefer a vehicle that offers greater moisturization, such as a cream, lotion, or ointment.[95]There are also questions regarding the compatibility of the various vehicles and agents with cosmetics, which may be important to some patients.[93]In addition, solutions and washes can be more easily applied to large areas of the skin such as the back, even though they are drying. Suggesting how the recommended therapy can be incorporated into the patient's skin care regimen is important because patients often have questions regarding the application of various medications. Tailoring treatment recommendations to fit within the patient's lifestyle will increase the likelihood of compliance. Patient education regarding the use of the drug, the rationale for the specific therapy chosen, and realistic expectations for improvement are also key to treatment success.

Overview of existing therapies

AV often represents a therapeutic problem. Historically, the existing storehouse of anti-acne agents has included topically applied or systemically administered antimicrobials or retinoids. The ideal agent would target each of the

pathogenic factors without producing adverse effects; however, the anti-acne agents currently available target only 1 or 2 of the pathogenic factors. Apart from isotretinoin, there is no agent with broad-spectrum action in acne. There is a clear-cut need for new, safe, and effective agents in the treatment of acne.[95]

Comedolytic Agents

In mild forms of the disease, the local therapy is advantageous, mostly a very good therapeutic effect. Topical tretinoin (all-trans-retinoic acid) is a highly effective comedolytic agent. It normalizes follicular keratinization, promotes drainage of pre-existing comedones, and inhibits the formation of new ones.[96, 97]There also may be a decrease in inflammatory lesions due to inhibition of microcomedone formation. Maximal clinical improvement may not be apparent until after 3 or 4 months of use. Tretinoin is effective as monotherapy for non-inflammatory acne or mild to moderate inflammatory acne. It has also been used effectively in combination with either topical antibiotics, benzoyl peroxide (BPO), or systemic antibiotics, presumably because of its ability to increase the penetration and enhance the efficacy of other agents.[97-99]Another benefit of such combinations is the apparent decrease in the irritation from tretinoin by the addition of a topical antimicrobial agent.[98]

The most common adverse effect associated with existing topical tretinoin preparations is the local irritation. Patients may also experience erythema, dryness, and peeling. These effects often resolve after approximately 3 weeks.[95]Tretinoin should only be used in combination with BPO when applied 1 to 2 hours before or after application of BPO to avoid irritation and to increase efficacy. Tretinoin also induces a mild thinning of the stratum corneum that may increase sensitivity to sunlight, necessitating proper sunscreen use.[100] Finally, an exacerbation of

inflammatory lesions (pustular flare) within 2 to 4 weeks of initiation of therapy may also occur.[98]To minimize local irritation, therapy should start with a mild formulation and the concentration should be gradually increased. In summary, topical tretinoin is a mainstay in the treatment of acne, but its adverse effect profile warrants careful patient management to optimize efficacy and tolerability.[16]

Antimicrobial Agents

Benzoyl peroxide is a potent topical bactericidal agent that reduces the population of *P. acnes* by generating reactive oxygen species in the sebaceous follicle.[98] It rapidly improves both inflammatory and non-inflammatory lesions and has therefore been a first-line choice in the therapy of mild acne and a mainstay in acne therapy in general. It is available in a wide variety of concentrations and preparations. Benzoyl peroxide is very effective in combination with either topical antibiotics or tretinoin.[99]When combined with tretinoin or 3% erythromycin, BPO can have a synergistic effect on inflammatory acne.[98]The major adverse effect of BPO is local irritation, which is often seen during therapy initiation. Erythema and dryness may also occur. Allergic contact dermatitis, necessitating therapy discontinuation, has been reported in approximately 1% to 3% of patients.[100]This drug may cause bleaching of clothing and bed linens, therefore, patients should be informed regarding the same. This may present a problem, particularly when it is applied to the chest or back.[16]

Antibiotics

Topical Antibiotics

Topical antibiotics are useful in the treatment of mild to moderate inflammatory acne. They reduce the population of *P. acnes* in sebaceous follicles and also demonstrate anti-inflammatory properties by suppressing chemotaxis and decreasing the percentage of pro-inflammatory free fatty acids in surface lipids.[98]Topical antibiotics available for use include erythromycin, clindamycin, sodium sulfacetamide, and salicylic acid. Erythromycin and clindamycin have been shown to be of equivalent efficacy for treating moderate acne.[98, 100]They are often used effectively in combination with BPO or tretinoin. Sodium sulfacetamide is an antibacterial agent that has been used in anti-acne preparations for many years. Until recently, it has only been available in combination with sulphur 5% as a keratolytic agent. Salicylic acid is effective against comedones and inflammatory lesions but may be less effective in patients who cannot tolerate topical tretinoin. All topicals should be applied to the entire face rather than to individual lesions.[16]

The development of resistance of *P. acnes* to topical antibiotics has become more prevalent and may result in loss of efficacy.[100-102]Studies have demonstrated that resistance to erythromycin may be reduced by using the drug in combination with BPO.[98, 100]

Systemic Antibiotics

Systemic antibiotics such as erythromycin and tetracycline, or the derivatives doxycycline and minocycline, are most often used for moderate to severe inflammatory acne not responding to topical combinations, acne involvement of areas

where topical agents cannot be easily applied (back), or for acne with high scarring potential. The primary mechanism of action of these agents in acne treatment is the suppression of *P. acnes* growth, which reduces the production of inflammatory factors.[100]Many of these agents, such as tetracycline and erythromycin, also possess intrinsic anti-inflammatory activity.[95, 100]

Erythromycin

Both oral erythromycin and tetracycline are comparable in its therapeutic effect on acne,[95, 100]although the resistance of *P. acnes* to erythromycin seems to be more common than that produced by tetracyclines.[101, 102]The most common adverse effect associated with erythromycin is gastrointestinal (GI) tract irritation, which may be alleviated to some degree by taking the drug with food or milk.

Tetracycline, Doxycycline and Minocycline

Tetracycline and its derivatives are the most commonly used oral medications for acne vulgaris. Tetracycline hydrochloride is known to penetrate sebocytes and keratinocytes to reach the follicular canal.[95]With the topical antibiotics, an inherent issue is the evolution of resistance of *P. acnes* to tetracycline,[102, 103]and this should be suspected if a patient's acne worsens after several months of treatment. Doxycycline is a lipophilic tetracycline derivative with demonstrated efficacy in the treatment of inflammatory acne. Like tetracycline, the resistance of *P. acnes* to doxycycline has been reported.[102] Minocycline, also a lipophilic derivative of tetracycline, achieves excellent penetration into the follicular canal and is often effective in cases of acne that have not responded to treatment with other oral antibiotics.[95]There are fewer reports of resistance of *P. acnes* to minocycline than with tetracycline and

doxycycline. While tetracycline should be taken on an empty stomach, doxycycline and minocycline can be taken with food, which should decrease the GI tract upset.

In a nut shell, orally administered erythromycin or tetracycline are effective in the management of moderate to severe acne. However, as systemic agents, they are associated with more significant and diverse adverse effect profiles than many topical agents. These agents are increasingly associated with the development of resistance to *P. acnes*.[95]

Clindamycin /BPO Topical Gel

This new anti-acne treatment is a combination product of 1% clindamycin and 5% BPO. The advantage of combination gel is that it does not require refrigeration like other antibiotic/BPO combination products. It is currently awaiting approval from the FDA.[16]

Recently, the combination of clindamycin and BPO has been reported to be efficacious in controlling acne and superior to either individual agent used alone.[104] In these double-blind, randomized, parallel, vehicle-controlled trials, patients were treated for 11 weeks with a nightly application of 1% clindamycin gel, 5% BPO gel, or the combination gel. Efficacy was determined by lesion counts and assessment of global response. With the combination product, the mean reduction of inflammatory lesions was significantly greater than vehicle at weeks 2, 5, 8, and 11 and significantly greater than each of the individual ingredients at weeks 8 and 11. In this study, safety was evaluated by reporting adverse effects and scoring of irritancy parameters. All of the study preparations were well tolerated and received excellent overall tolerance

ratings from 95% of the patients. The only adverse event reported in the clindoxyl group was a transient, slight stinging on initial application.[16]

New Anti-acne Agents

In recent years, there are several new agents that are available for the treatment of patients with acne vulgaris. These include new retinoids, new tretinoin formulations, azelaic acid, a new formulation of sodium sulfacetamide, and an oral contraceptive containing a second-generation progestin.[95]

Hormonal treatments

Hormonal treatments of acne consist of the ovarian suppression of androgen production by oral contraceptives, androgen receptor blockers: cyproterone acetate and spironolactone, adrenal suppression of androgen production by corticosteroids and inhibitors of 5-α-reductase. Hormones are not the first choice of treatment, only for women with mild acne or signs of hyperandrogenism who ask for contraception. Pills with an extremely low androgenic progestin concentration, or– even more efficient – with a low dose of 2 mg of cyproterone acetate should be used.[105]

Vaccination

Another approach to the therapy of acne is the application of auto- or stockvaccines containing inactivated strains of *Propionibacteria* and/or *Staphylococci*.[24]

Their effect is based on the non-specific modulation ofthe immune system of patients.[24, 106]The vaccinotherapy is usually applied together with external treatment and follows the systemic antibiotic therapy when the pronounced inflammatory

manifestations are eliminated. Contrarily to wide-specter antibiotics that can cause the recession of the disease without any effect on possible recurrent attacks after the end of their application, the use of oral vaccines reveals a long-term favourable effect.[24]

The Teaching Hospital in Olomouc has a long history of vaccinotherapy use. Whole cell stockvaccines of the standard composition are preferred (*Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* in the ratio 1:1:1). They are prepared using the cellophane technique with following inactivation of obtained germs and their lyophilization. They can be applied as subcutaneous injection or in oral forms of drops, capsules or tablets. Based on the experience, it can be stated that the vaccinotherapy shows a very good effect without unwanted side effects in most patients. The therapy failed at less than 2 % of patients, no worsening of the state was registered. The differences in the effectiveness of individual drug forms (injections, tablets, capsules and drops) were not described.[107]It was confirmed that the proper onset of vaccination in mild forms of acne prevents the development of severe cases with permanent consequences. Vaccines can be applied in all cases of acne papulopustulosa, conglobata, induration or abscess forms. Priors to the vaccine therapy, biochemical, hematological and immunological examinations are to be performed. When a clinical improvement is reached as the result of antibiotic therapy, it is recommendable to continue with vaccinotherapy, eventually with another immunotherapy, according to the results of immunological tests.[24, 108]

METHODOLOGY

Study Centre: The present study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi.

Source of Data: Patients of Acne vulgaris (all age groups) attending the Out Patient Department of Dermatology at KLE Society's Dr. PrabhakarKore Hospital and Medical Research Centre, Belagavi over a period of one year from January 2017 to December 2017 were included in this study.

Method of collection of data

- **Study design:** Cross-sectional study.
- **Study period**: Period of one year from January 2017 to December 2017
- **Sample size calculation:**

  $n= 4pq/ d^2$

  n= sample size

  p= 55 (isolation rate)

  q= 45 (100-p)

  d= absolute error = 20% of p = 11

  $n=4 x55 x 45/ (11)^2$

  =81.8 ~ 85

- **Sample size:**85 (85 samples were collected in the study)
- **Sampling Procedure:** Universal sampling method

- **Inclusion Criteria**:

  1) All the patients with inflamed (macules, papules, pustules and nodules) and non-inflamed (open and closed comedones) acne vulgaris of all age groups.

  2) Patients who were not on systemic and topical antibiotics for 6 weeks prior to study.

- **Exclusion Criteria:** Patients with Drug induced acne were excluded from the study.

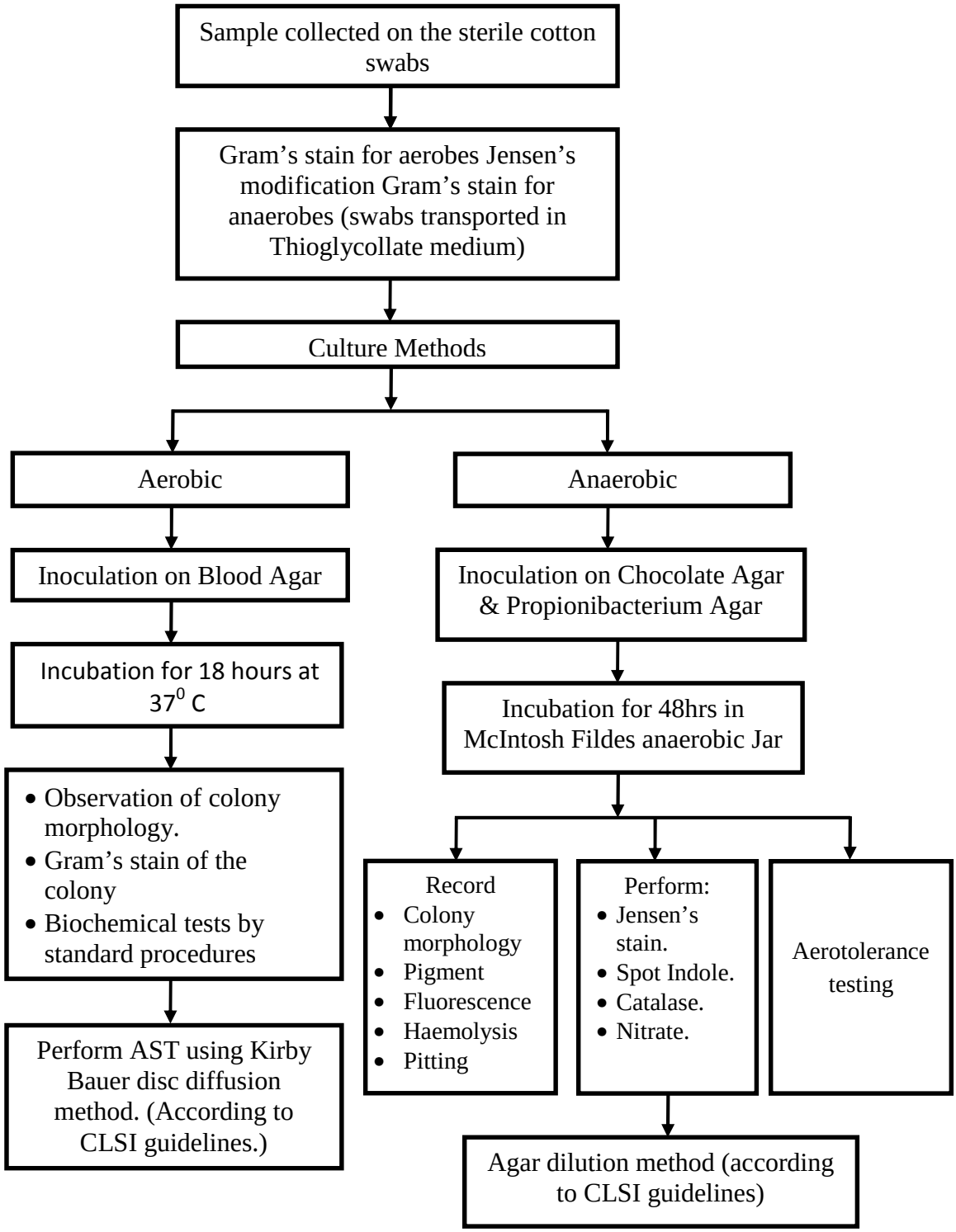
- **Collection of sample**

  ✓ After obtaining informed written consent from the patients selected for the study, a short history on familiar occurrences, cosmetics used, menstrual cycles irregularities (for women) indicating hyperandrogenism, drugs like vitamin B complex, Lithium, Phenytoin, steroids, anti-tubercular, endocrinological and metabolic diseases were taken.

  ✓ Area was cleansed using 70% ethanol, samples from skin lesions (using a sterile extractor) were collected on the sterile cotton swabs. The samples were then transferred to thioglycollate medium and transported to the Microbiology laboratory at Jawaharlal Nehru Medical College.

  ✓ **Sample processing** – Direct smears were made by using the swab. The smear was stained by Gram's stain (Hucker's modification[109] for aerobes and Jensen's modification Gram's stain for anaerobes) for presumptive identification of the number and types of microorganisms present in the sample. Morphology of the organism and other observations in the gram stained smear were recorded.

Figure 2: Flow chart showing the methodology of sample collection and processing



1. **Culture:**

Culture was done for both aerobic and anaerobic bacteria.

a) Aerobic culture:

The swab was inoculated onto-

1) 5% sheep blood agar

- The inoculated culture plates were incubated at 37^{o}C for 24 hours aerobically.
- The isolates were identified and characterized biochemically by standard operative procedures.[110]

b) Antibiogram of aerobic bacteria:

The antibiotic susceptibility testing was done for aerobic bacterial isolates by disk diffusion method as described by Kirby-Bauer, on Mueller Hinton agar (MHA) plates. The following are the antibiotics which were tested:

Antibiotics	**Concentration per disc (µg)**
1) Ampicillin (Amp)	10
2) Ciprofloxacin (Cip)	5
3) Clindamycin (Cd)	2
4) Cotrimoxazole (Cot)	25
5) Erythromycin (E)	15
6) Gentamicin (Gen)	10
7) Doxycycline (Do)	30
8) Penicillin (P)	10

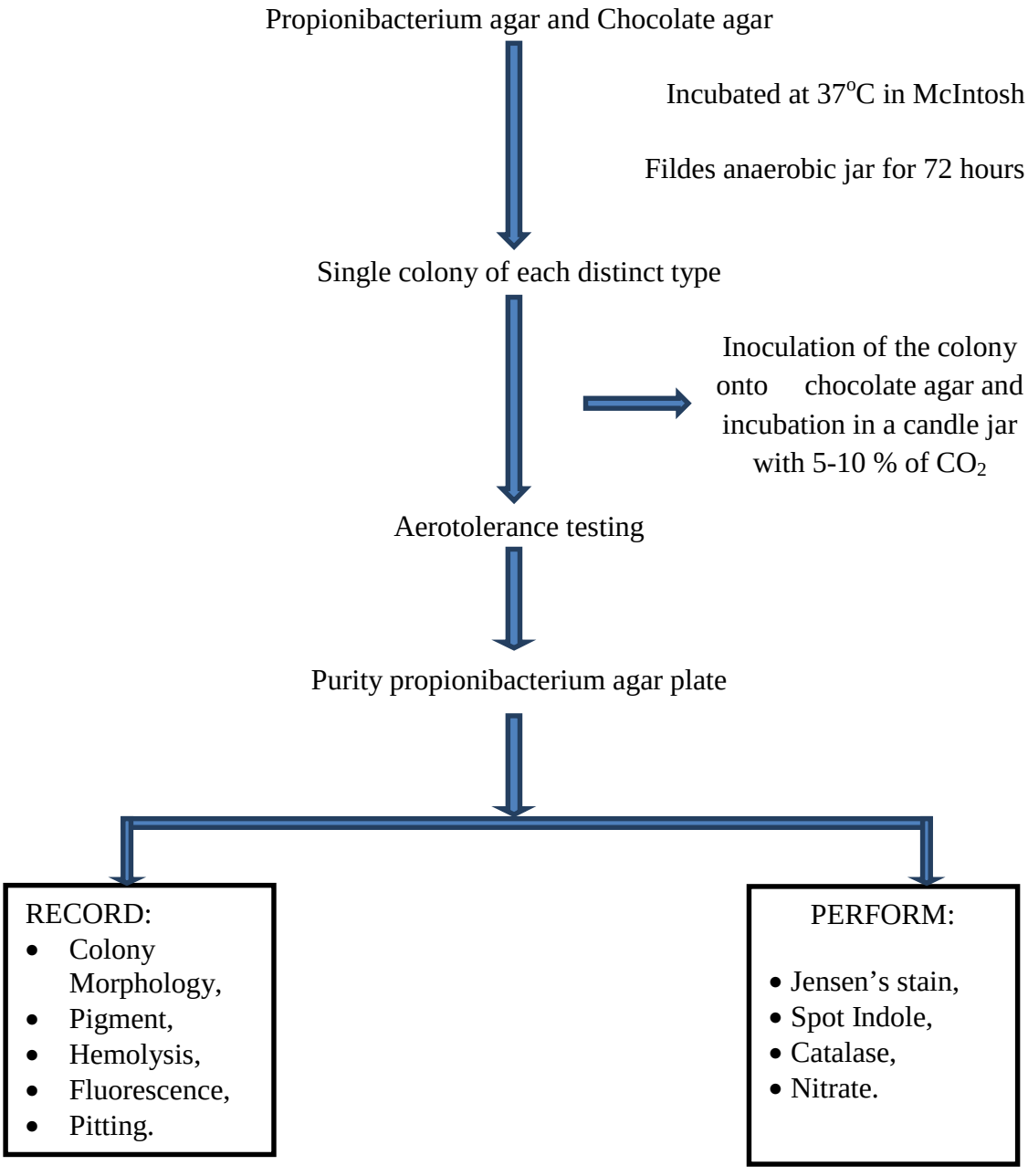
Method: For antibiotic susceptibility testing, a single colony was inoculated in peptone water and incubated at 37^{o}C for 4-6 hours. Then its turbidity was adjusted to McFarland's 0.5 standard and using a sterile cotton swab, lawn culture of the inoculum was done on to MHA plate and antibiotic disks were placed. This plate was

incubated over night at 37°C. Zone of inhibition was measured on the next day. Interpretation was recorded according to the Kirby-Bauer chart.[111]

Control strain used was *Staphylococcus aureus* ATCC 25923.

c) Culture of anaerobic bacteria: [112]

Each swab which was transported in Thioglycollate medium was inoculated onto:



Method used to obtain anaerobiosis: in the jar was "Internal gas generating system" described by Laxminarayana and Vaidhyalingam.[113]

After 72 hours of incubation at 37°C the anaerobic jar was opened. The plates were examined for the presence of bacterial colonies. Each predominant distinct colony was sub cultured onto purity propionibacterium agar plate (PAP). From a pure culture on a PAP, following were recorded.

- Colony morphology, including size, shape, color, internal appearance (such as speckling) and general appearance (ex: mucoid, transparent, opaque)
- Pigment
- Hemolysis
- Pitting of agar
- Fluorescence

- **Aerotolerance test-**
  - Chocolate agar plate was inoculated for incubation in candle jar at 37°C to test for aerotolerance.
  - If there was no growth on plates after 72 hours of anaerobic incubation, the plates were reincubated for an additional period of 48 hours and for a maximum period of 1 week.

The following tests were done from the purity plate.

- **Catalase test:** Growth was removed from propionibacterium agar plate to a drop of 15% hydrogen peroxide on a glass slide and observed for evolution of bubbles.

- **Spot Indole test:** A loopful of growth from pure culture on a propionibacterium agar plate was removed and this growth was smeared on a filter paper that had been saturated with 1% paradimethylaminocinnamaldehyde in 10% concentrated hydrochloric acid.

- A positive reaction was indicated by the rapid development of blue colour around the growth. Negative reaction gave no colour change or a pinkish colour.

- **Nitrate test:** This test was done by using nitrate discs. The disc was removed from surface of plate and placed in a clean petridish. One drop each of reagents A and B were added. Development of pink to red colour indicated that nitrate had been reduced to nitrite. If no colour developed in few minutes, a small amount of zinc dust was added and waited for 5 minutes.

- Development of red colour indicated that nitrate was not reduced. If no colour developed, it was taken as positive test.

Preparation of Nitrate reagents

Solution A-

Sulfanilic acid- 0.5 g

Glacial acetic acid- 30.0 ml

Distilled water- 120.0 ml

Solution B-

1, 6-Cleve's acid 0.2 g (5-amino-2-naphthalenesulfonic acid)

Glacial acetic acid- 30.0 ml

Distilled water- 120.0 ml

Anaerobic isolates were stored in Robertson cooked meat medium.

d) Antibiotic susceptibility testing for anaerobic bacteria:

Antibiotic susceptibility testing for anaerobic bacterial isolates was performed using agar dilution method for Clindamycin according to CLSI guidelines M11-A6.[114]

Background information:

- Antibiotic tested: Clindamycin[115]
- Potency: 91µg/mg
- Solvent: distilled water
- Diluent: distilled water
- Number of petri plates required per dilution is: 1
- Volume of culture medium per plate: 20 ml
- (40 ml medium was prepared for each dilution)
- MIC range tested: 0.125 to 32 µg/ ml

- **Quantity of Antimicrobial powder required-**

$$\text{Weight (mg)} = \frac{\text{volume (ml) x concentration (µg/ ml)}}{\text{Assay potency (µg/ mg)}}$$

Example: to prepare 10 ml of a stock solution of clindamycin containing 1,000 µg/ ml with potency 91µg/ mg,

$$\text{Quantity of the drug required is} = \frac{\text{10 ml x 1000 µg/ ml}}{\text{91µg/ mg}} = 109.89 \text{ mg}$$

ii) Preparation of stock solution[116]-

Preparation of 1,000 µg/ ml stock solution:

Required volume is 0.6 ml but a stock solution of 1,000 µg/ ml can be prepared in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	10.99
2 ml	21.98
3 ml	32.97
4 ml	43.96
5 ml	54.95
10 ml	109.89

Preparation of 100 µg/ ml stock solution:

Required volume is 0.35 ml but a stock solution of 100 µg/ ml can be prepared in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	1.1
2 ml	2.2
3 ml	3.3
4 ml	4.4
5 ml	5.49
10 ml	10.99

The required amount of antibiotic was weighed and dissolved in distilled water. Final volume was made by adding the diluent (Distilled water).

iii) **Preparation of antibiotic dilution plates-**

40 ml of Brucella blood agar with vitamin K and hemin was prepared and allowed to cool in a water bath between 45^{o}C and 50^{o}C. Antibiotic from the stock solution was added using micropipette with sterile tips.

Dilution µg /ml	Volume taken (ml)	Stock solution
32	1.28	1,000 µg /ml
16	0.64	1,000 µg /ml
8	0.32	1,000 µg /ml
4	0.16	1,000 µg /ml
2	0.08	1,000 µg /ml
1	0.04	1,000 µg /ml
0.5	0.2	100 µg /ml
0.25	0.1	100 µg /ml
0.125	0.05	100 µg /ml

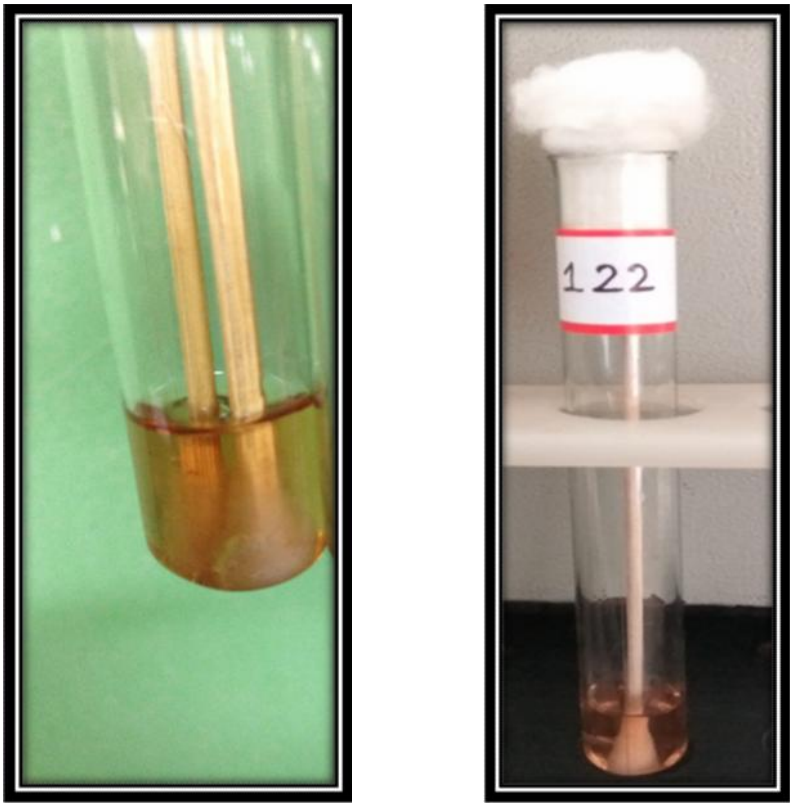
- ✓ The flask was swirled to thoroughly mix the preparation which was subsequently poured into the petri plates on a level surface to a depth of 3-4 mm. The plates were allowed to solidify at room temperature and their antibiotic concentration was labelled on them.

iv) **Procedure for MIC determination by agar dilution[114]-**

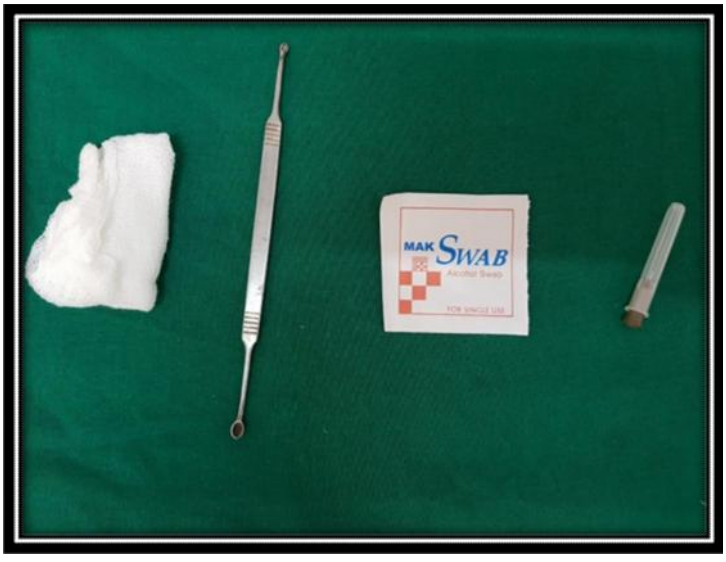
All the plates were brought to room temperature and allowed to dry. A plain medium without antibiotic was used as control.

- A suspension of test organism was prepared in Thioglycollate broth with vitamin K and hemin without indicator and its turbidity adjusted was to 0.5 McFarland standards.
- This suspension was diluted 10 times (1 in 10 dilution) using sterile saline. This suspension was tested within 15 minutes.
- The plates were arranged in increasing concentrations and the test tubes were kept as per the grid markings.
- 1-2 µl of this inoculum was transferred onto the agar plate in such a way that it formed a spot of 5-8 mm. The final inoculum on plate was 10^4cfu/ml.
- The spots on the plate were allowed to dry (10 minutes). They were then inverted and incubated in McIntosh Fildes jar for 48 – 72 hrs.
- Following incubation, the aerobic control plate was checked for the absence of growth on all the spots. The control organism growth was recorded in each plate.
- The end point, the first negative, was read at the point where a marked change in growth appeared as compared with growth on control plate.
- The concentration of antibiotic that had completely inhibited bacterial growth was taken as MIC.
- *Clostridium difficile* ATCC 700057 strain was used as control strain.
- Susceptibility MIC range of this ATCC strain for clindamycin was 2-8 µg/ml.

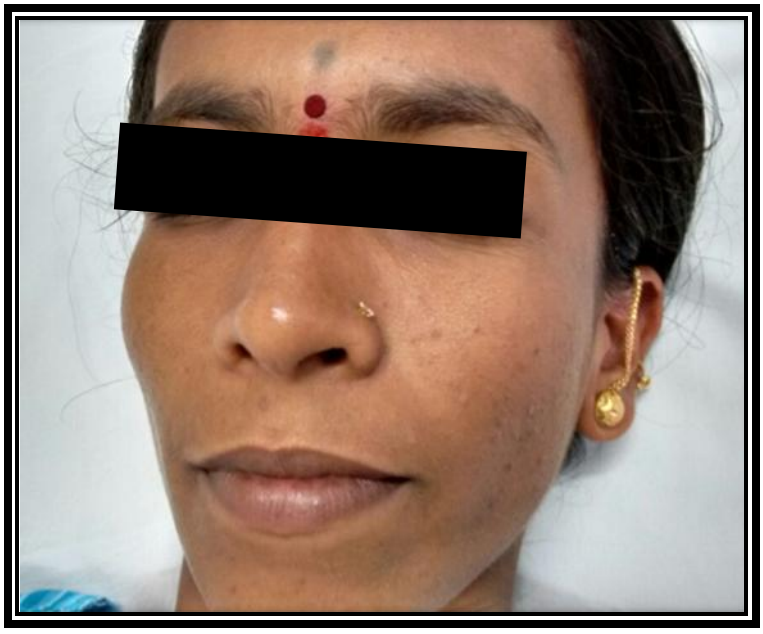
PHOTOGRAPHS



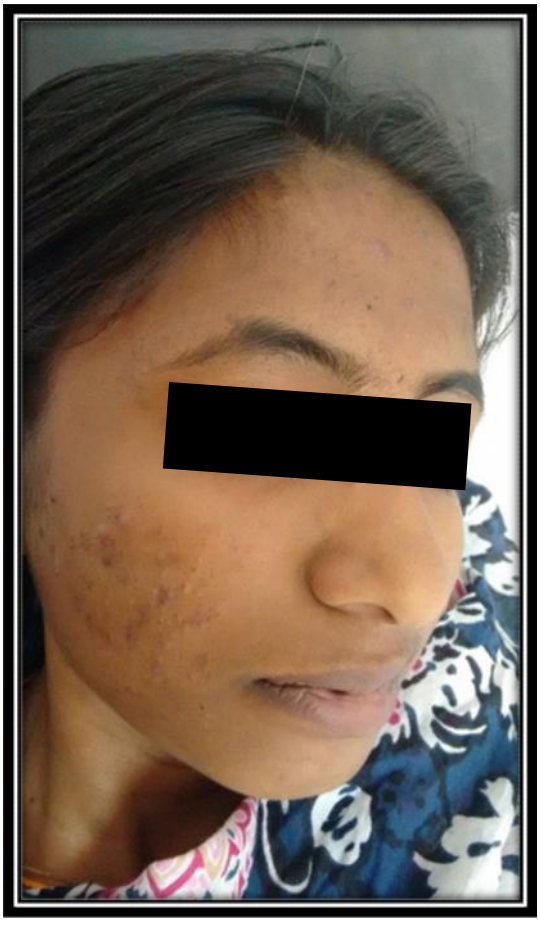
Photograph 1: Swabs for aerobic and anaerobic cultures in Thioglycollate medium



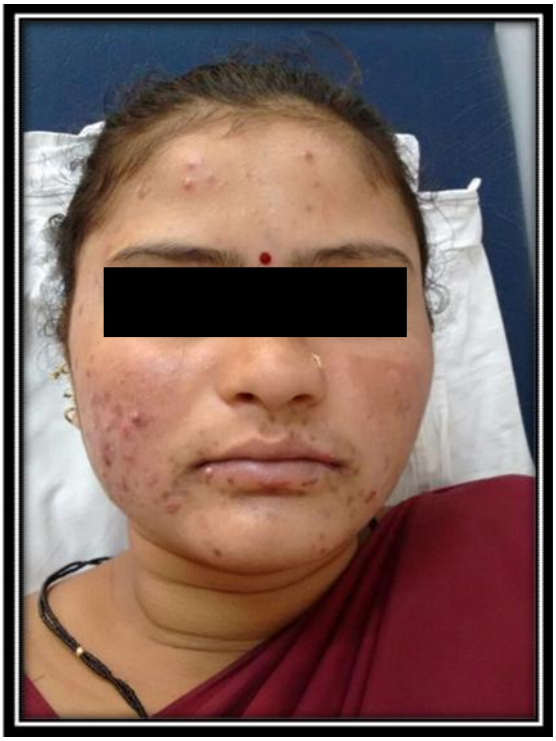
Photograph 2: Armamentarium for sample collection



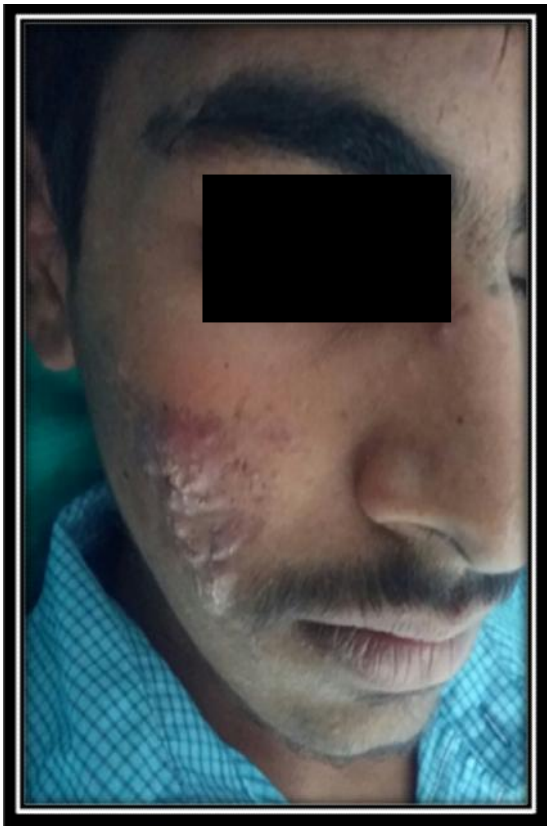
Photograph 3: Grade 1 Acne vulgaris



Photograph 4: Grade 2 Acne vulgaris



Photograph 5: Grade 3 Acne vulgaris



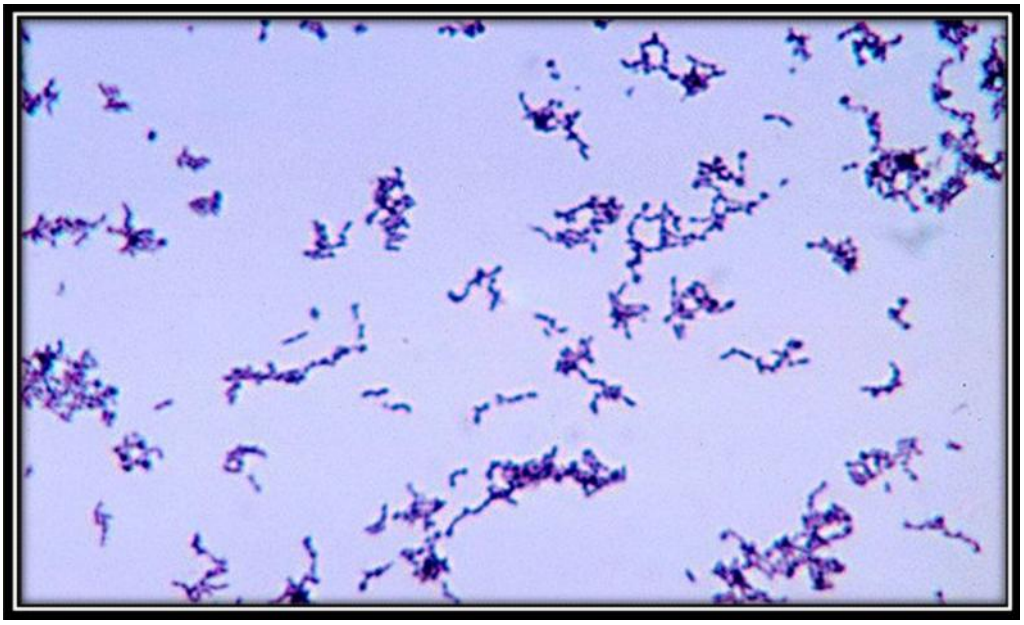
Photograph 6: Grade 4 Acne vulgaris

IDENTIFICATION OF ANAEROBIC BACTERIA

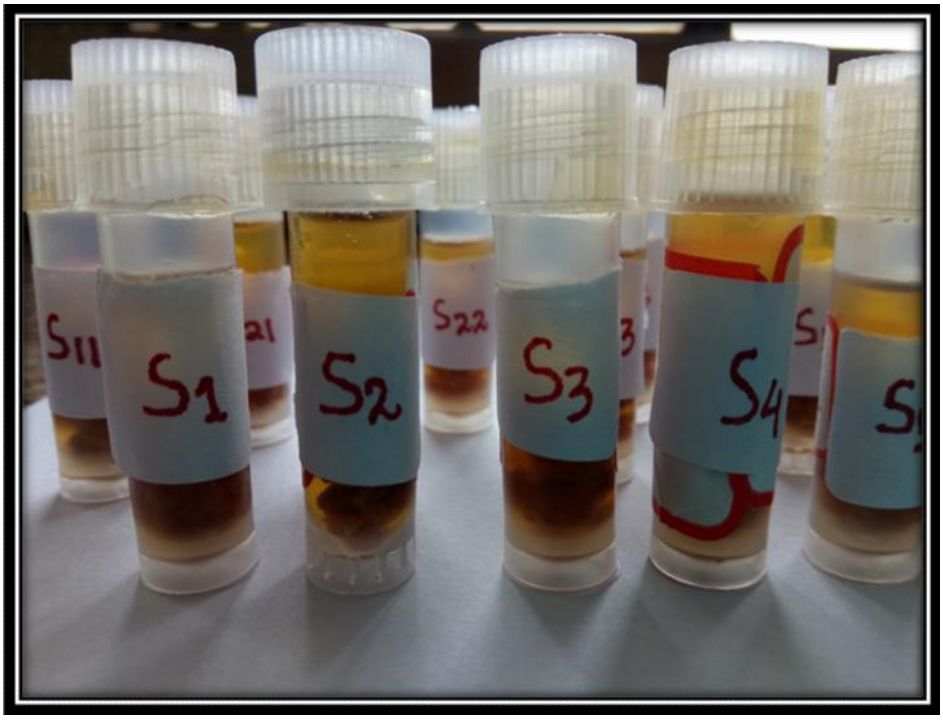
Photograph 7: McIntosh Filde's anaerobic jar

Photograph 8: Small white opaque colonies of *P.acnes* on Propionibacterium agar

Photograph 9: Small pigmented opaque colonies of *P.acnes* on Chocolate agar



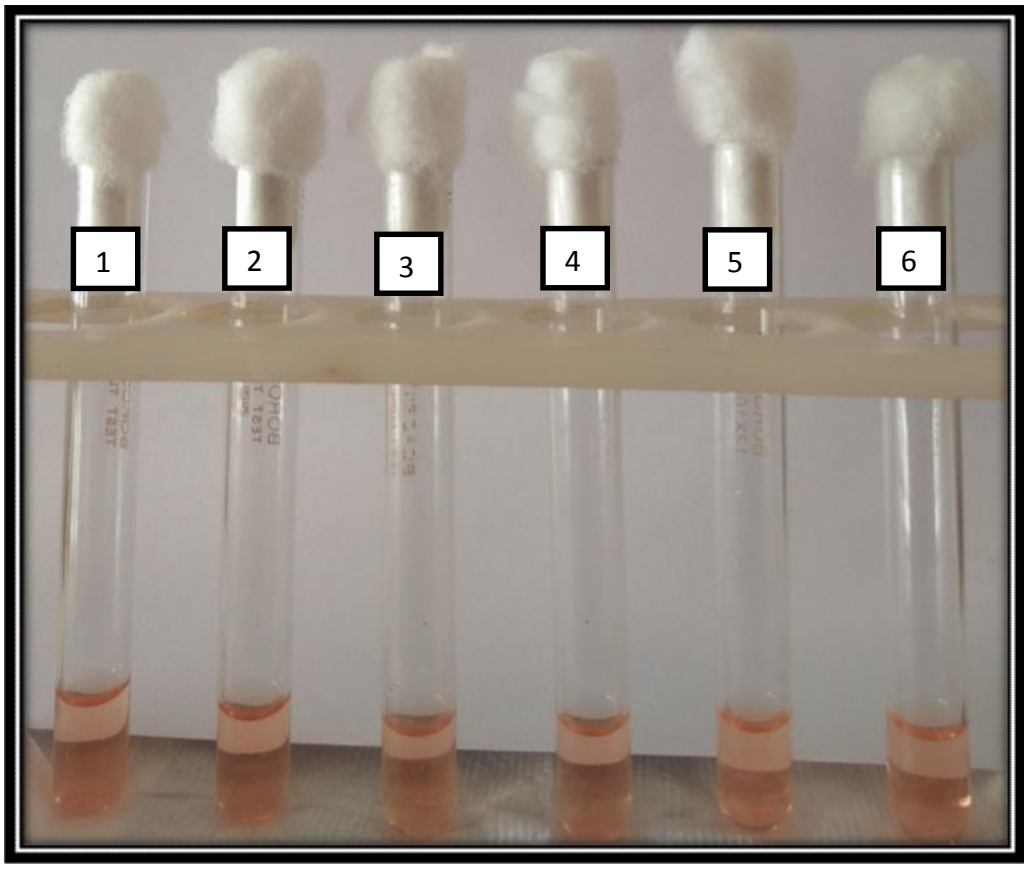
Photograph 10: Gram stained smear of *P. acnes* showing pleomorphic appearance of GPB



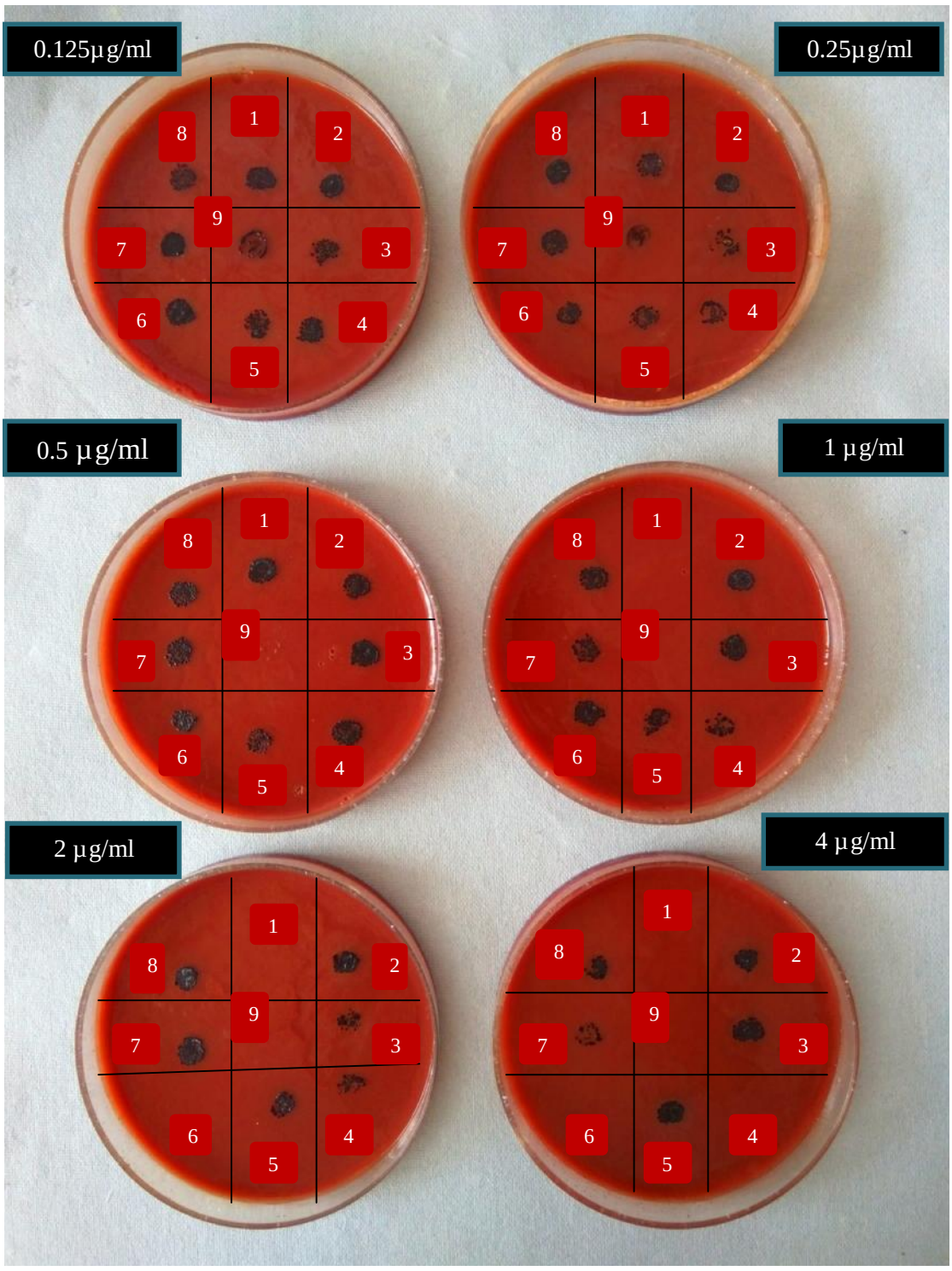
Photograph 11: Stock of *P.acnes* in Robertson cooked meat medium

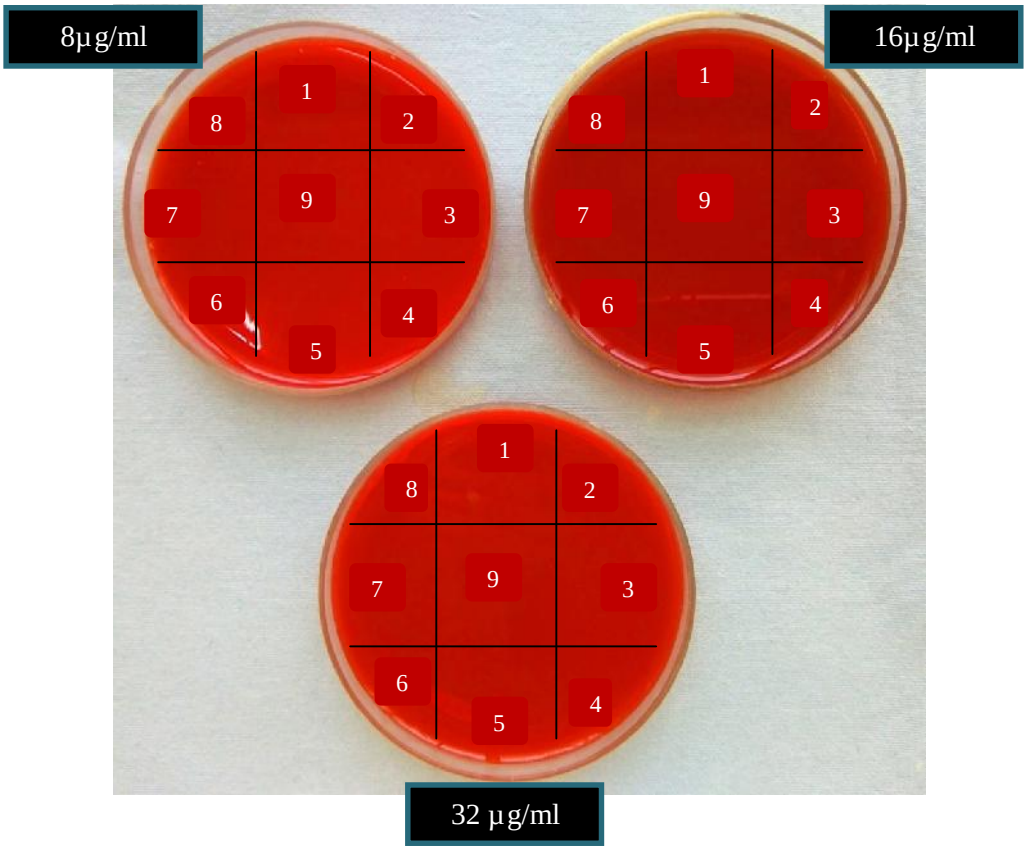
ANTIBIOTIC SUSCEPTIBILITY TESTING OF THE ANAEROBIC BACTERIAL ISOLATES

Photograph 12: Stock solutions of Clindamycin



Photograph 13: Suspension of anaerobic bacterial isolates- *P.acnes* (*1 to 6*) in Thioglycollate broth


0.125µg/ml
0.25µg/ml
0.5 µg/ml
1 µg/ml
2 µg/ml
4 µg/ml



MIC of isolate 9= 0.5µg/ml, MIC of isolate 1= 1 µg/ml

MIC of isolate 6 = 2 µg/ml, MIC of isolate 4=4 µg/ml

MIC of isolates 2,3,5,7 and 8= 8 µg/ml

Antibiogram of Clindamycin

Isolates 1,6 and are sensitive

Isolate 4 is an intermediate

Isolates2,3,5,7 and 8 are resistant

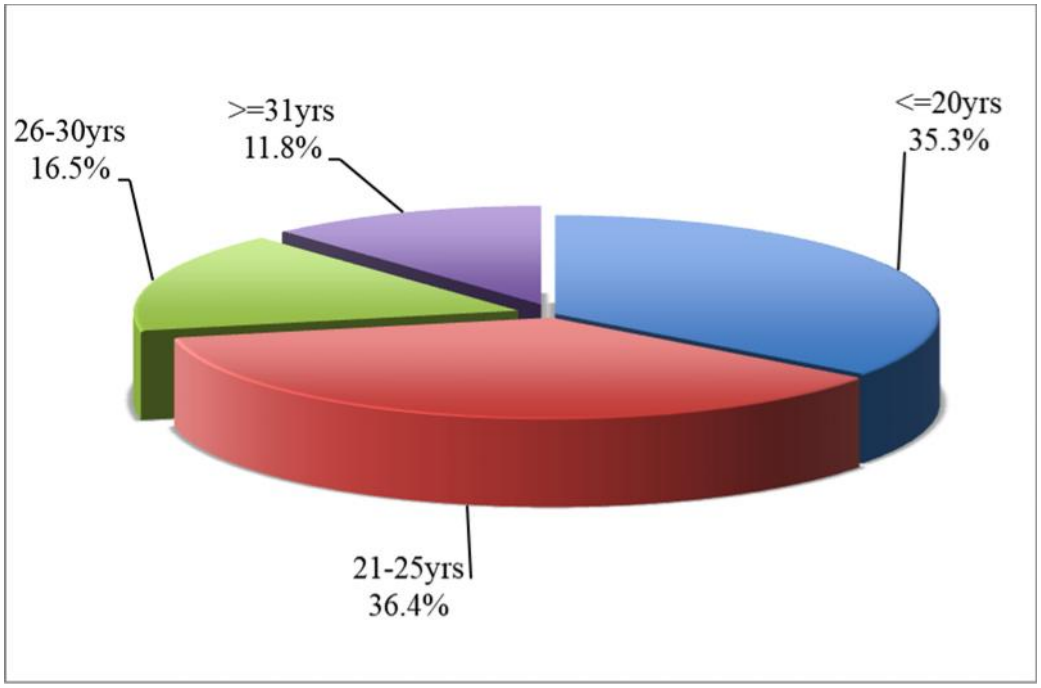
Photograph 13: Agar dilution method used to test susceptibility of *P. acnes to Clindamycin*

RESULTS

A total of 85 samples from skin lesions of patients with Acne vulgaris presenting to the Dermatology outpatient department at Dr. Prabhakar Kore Charitable Hospital and Research Centre were collected. Samples were obtained between January 2017 and December 2017.

The samples were processed for isolation, identification and antibiotic sensitivity of both aerobic and anaerobic bacteria.

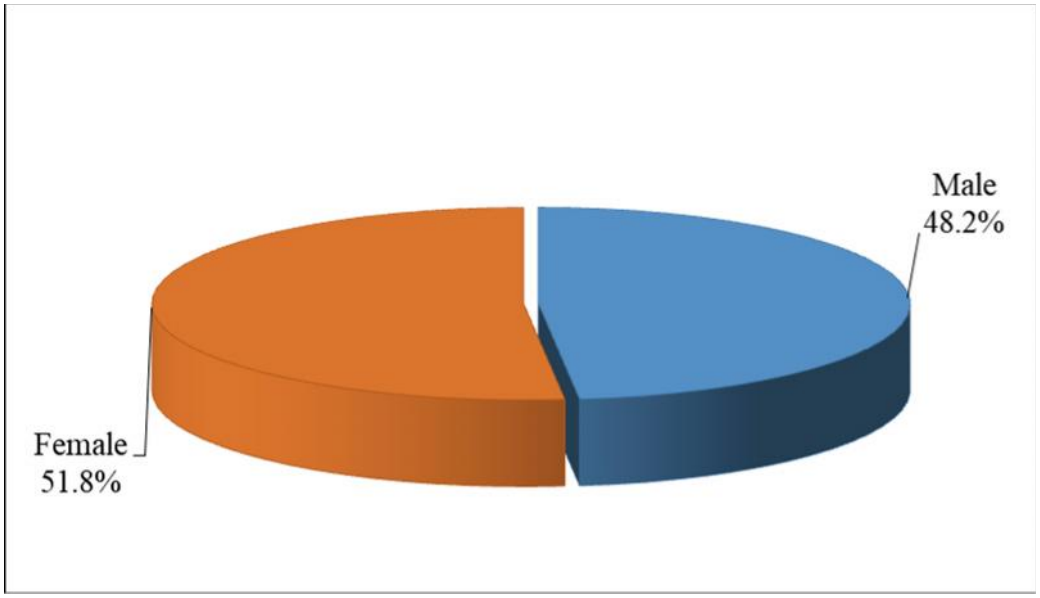
GRAPH 1: AGE DISTRIBUTION



In the present study,

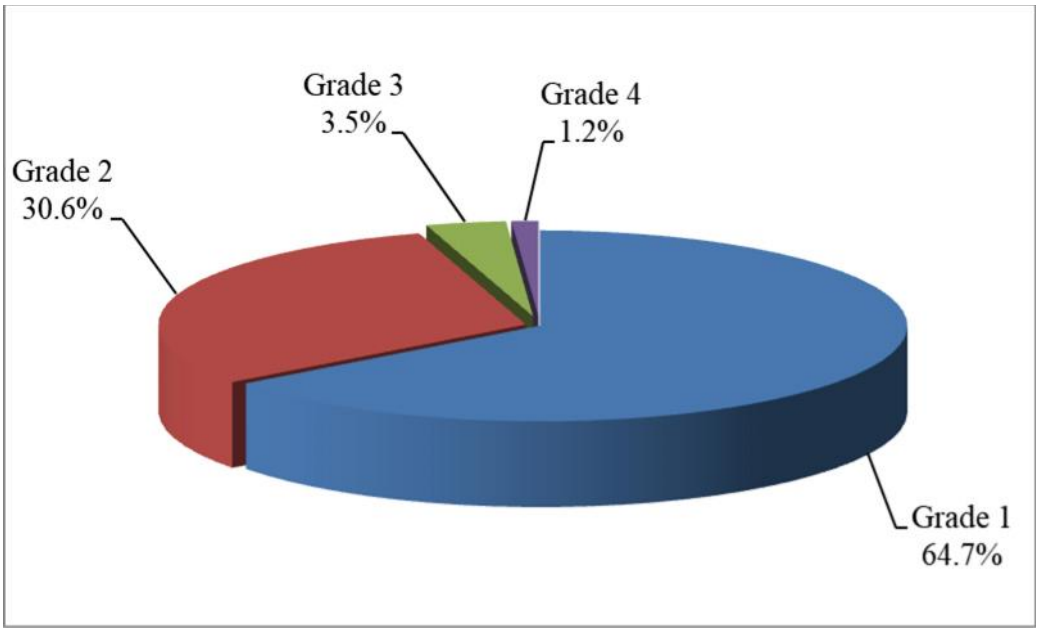
- The mean age of participants (ranging from 16 to 38 years) was 23.3 years.
- The majority of our patients (71.8%) were between 15 and 25 years old.
- There was only 1 patient above the age of 35 (38 years).

GRAPH 2: GENDER DISTRIBUTION



- The study population of 85 patients comprised of almost equal number of men (41) and women (44).

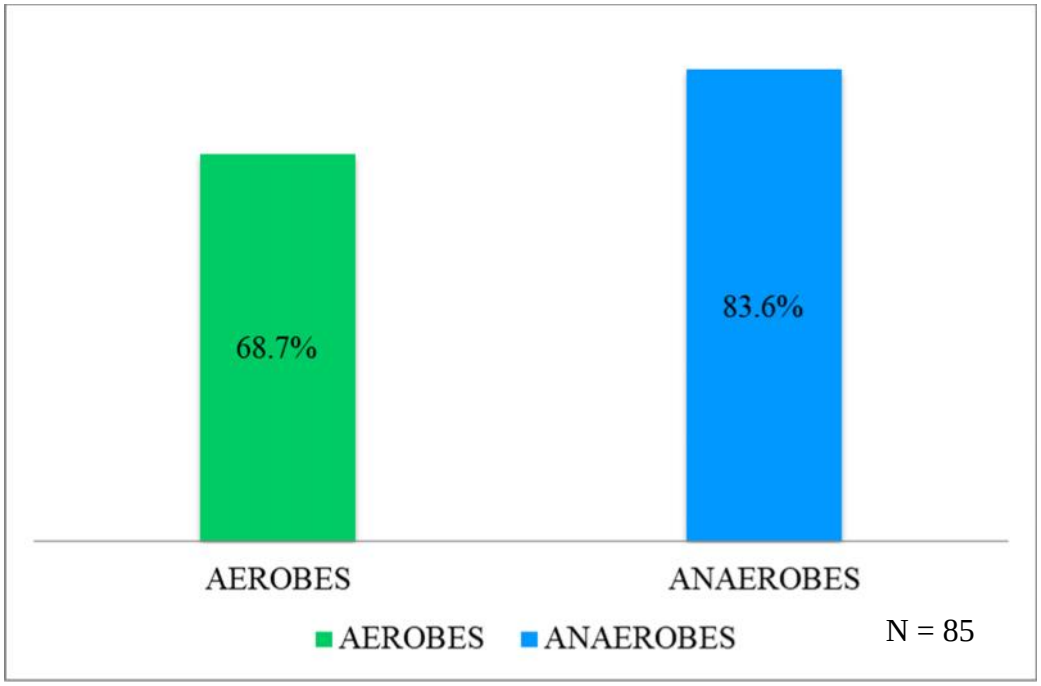
GRAPH 3: GRADES OF ACNE VULGARIS



In the present study,

- Over 64% patients were diagnosed as having grade 1 acne vulgaris.
- There were only 4 (4.7%) patients with grade 3 and 4 acne lesions.

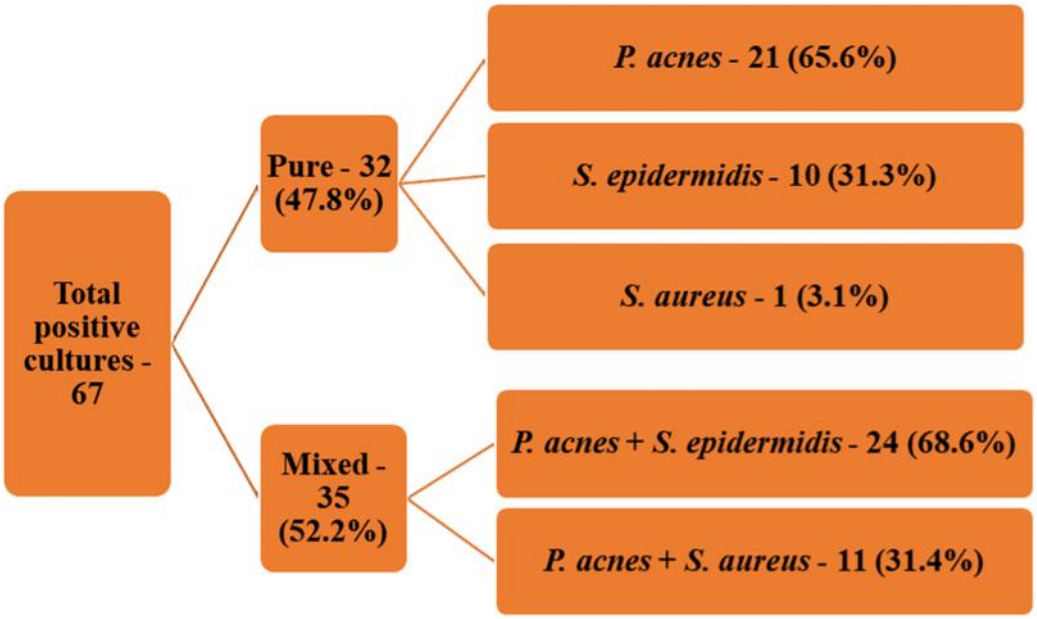
GRAPH4: TYPES OF BACTERIAL ISOLATES



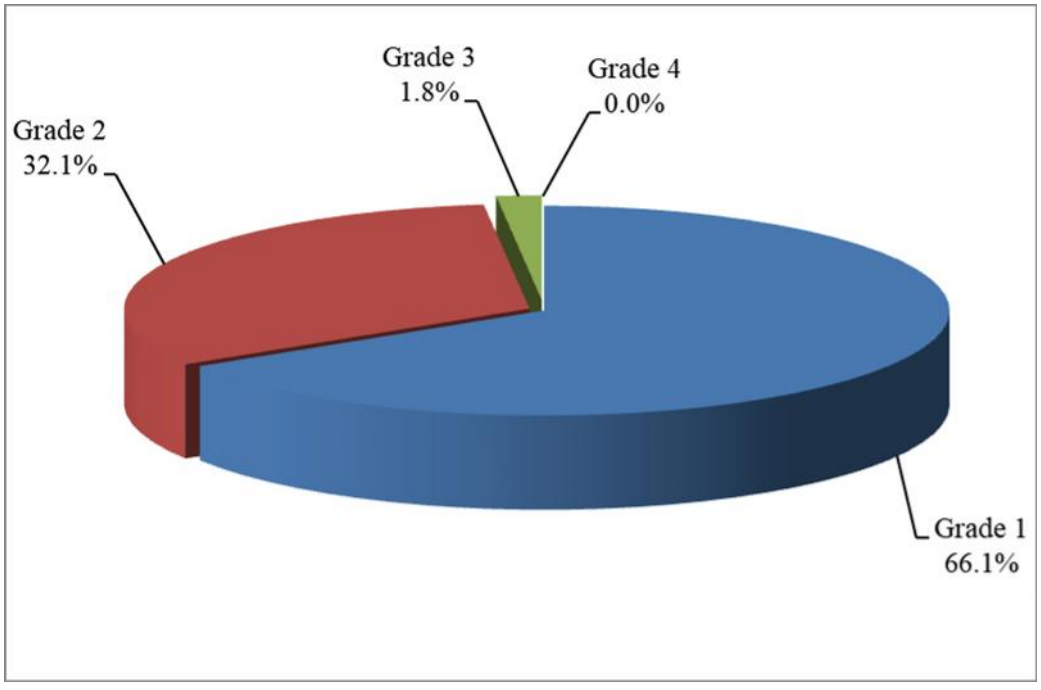
In the present study,

- Among 85 tested samples, 67 showed positive cultures (78.8%).
- Out of the 67 positive cultures, aerobic and anaerobic bacteria were observed in 46 and 56 cases respectively.
- Among 32 pure isolates, 21 cultured positive for *P. acnes* while *S. epidermidis* and *S.aureus* were seen in 10 and 1 samples respectively.
- There were 24 samples with concurrent growth of *P. acnes* and *S. epidermidis* while *P. acnes* and *S. aureus* were simultaneously isolated in 11 cases.

GRAPH 5: PURE AND MIXED ISOLATES



GRAPH 6: GRADES OF ACNE VULGARIS GROWING P. ACNES



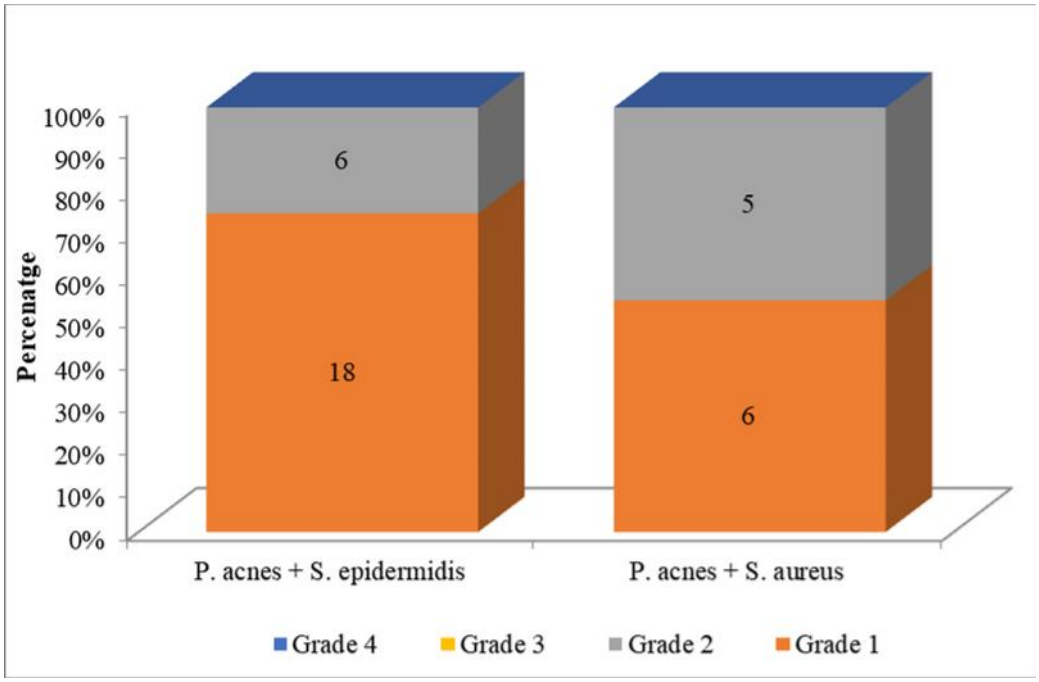
Most patients (66.1%) exclusively culturing positive for *P. acnes* belonged to grade 1 while only 1 patient had grade 3 lesions.

TABLE 1: COMPARISON OF MIXED GROWTH WITH CLINICAL GRADES OF ACNE VULGARIS

Grade	*P. acnes+S.epidermidis*	Percentage	*P. acnes* + *S.aureus*	Percentage
Grade 1	18	75%	6	54.5%
Grade 2	6	25%	5	45.4%
Grade 3	0	0%	0	0%
Grade 4	0	0%	0	0%

(*Chi-square (corrected)= 0.1500, P = 0.6990*)

GRAPH7: COMPARISON OF MIXED GROWTH WITH CLINICAL GRADESOF ACNE VULGARIS

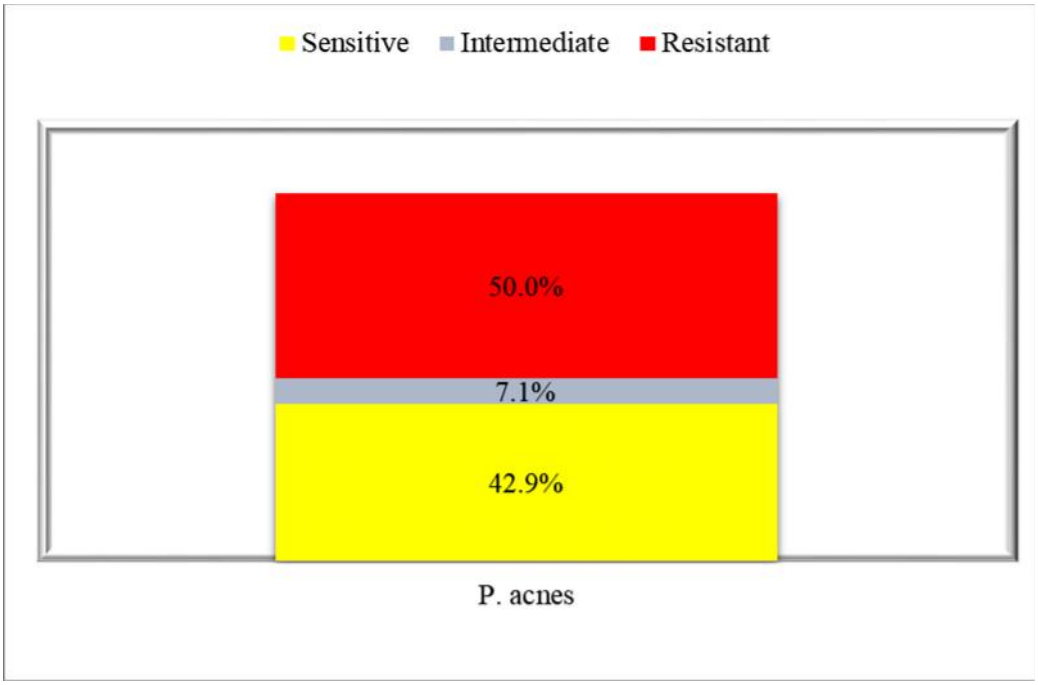


Similar to the above trends, concomitant growth of *P. acnes* with *S. epidermidis* and *S. aureus* was mostly seen in grade 1 lesions. All the remaining lesions were grade 2 in severity.

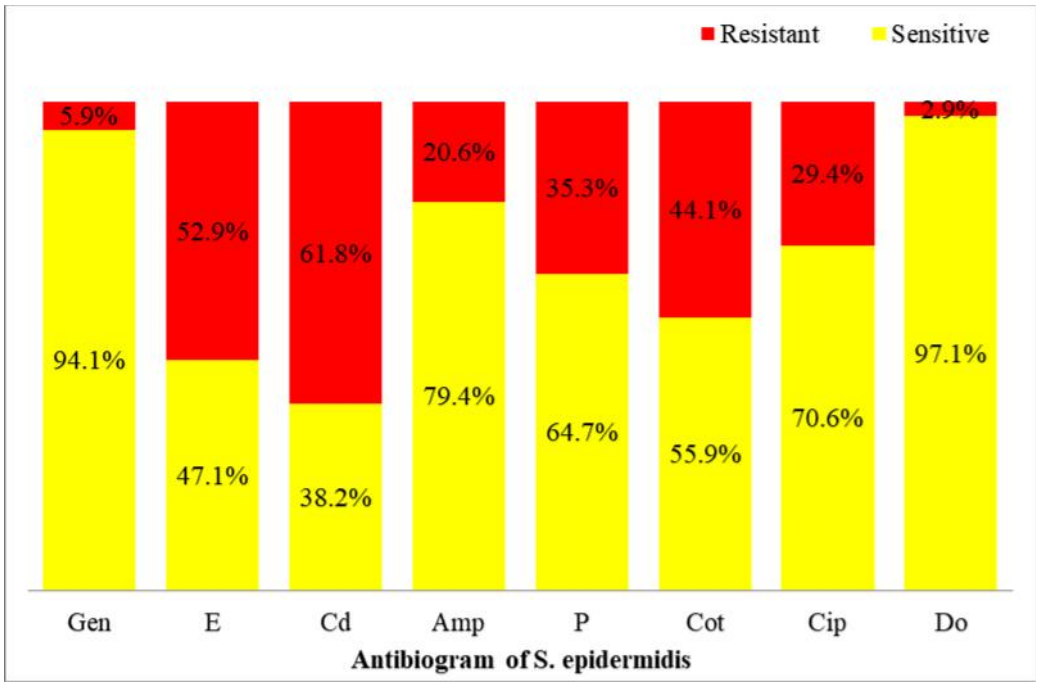
In the present study,

- Overall, 50% of the 56 samples with *P. acnes* positive growths were resistant to Clindamycin. While 24 samples were sensitive, 4 were intermediately sensitive

GRAPH 8: P. ACNES SENSITIVITY AND RESITANCE WITH CLINDAMYCIN



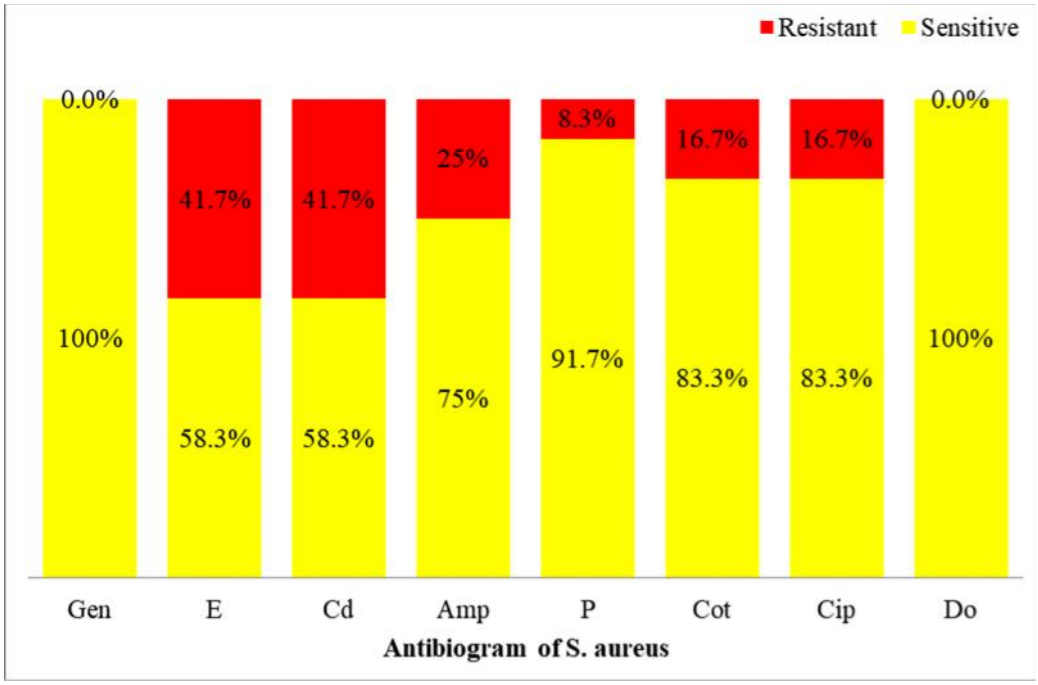
GRAPH 9: ANTIBIOGRAM OF *S. EPIDERMIDIS*



Results from AST analysis of *S. epidermidis* revealed;

- Over 97% sensitivity of bacteria towards Doxycycline and 94.10% towards Gentamicin.
- Only 38.20% of the *S. epidermidis* cultured were sensitive to Clindamycin which was followed by Erythromycin (in terms of greatest resistance).

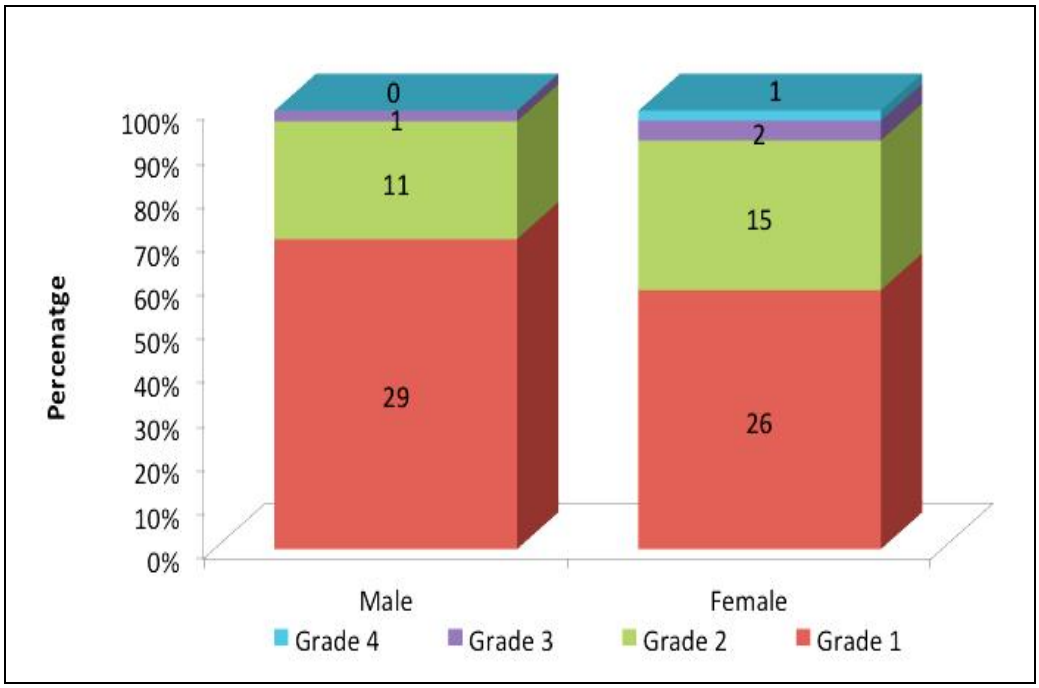
GRAPH 10: ANTIBIOGRAM OF S. AUREUS



The antibiogram of *S. aureus* revealed;

- A 100% sensitivity of bacteria towards Doxycycline and Gentamicin while lowest sensitivities were, again, observed with Erythromycin and Clindamycin (58.3%).

GRAPH 11: ASSOCIATION BETWEEN GENDER AND CLINICAL GRADES OF ACNE VULGARIS



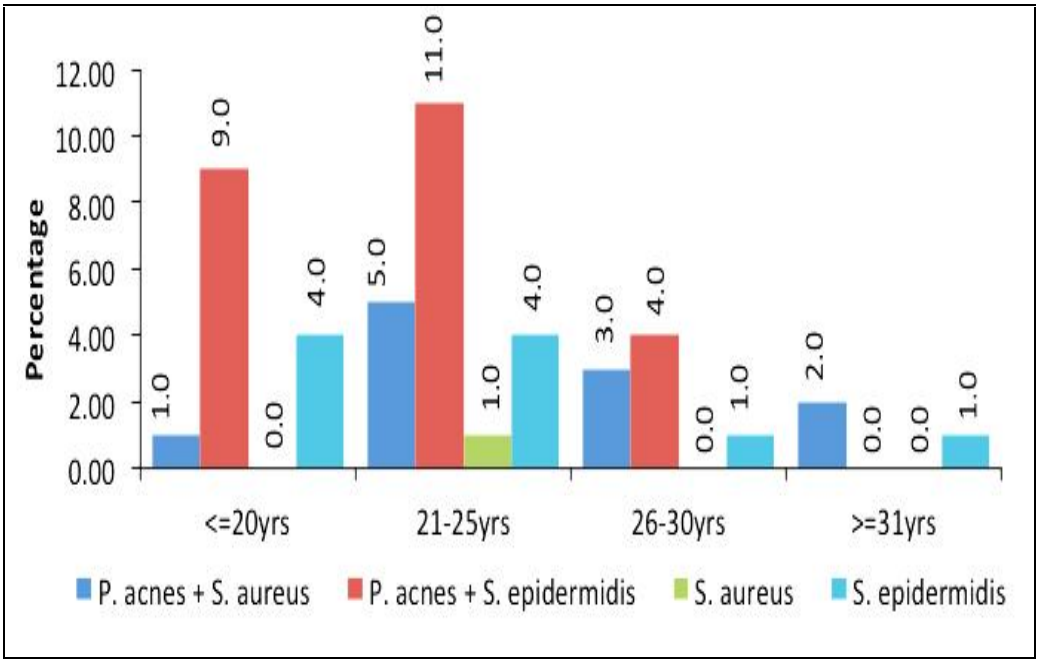
Further analysis revealed no statistical correlation (*Chi-square= 1.6753 P = 0.4332)*between gender and clinical grades of Acne Vulgaris.

TABLE 2: ASSOCIATION BETWEEN AGE GROUPS AND CLINICAL GRADES OF ACNE VULGARIS

Age groups	Grade 1	%	Grade 2	%	Grade 3	%	Grade 4	%	Total
<=20yrs	21	70.0	8	26.7	1	3.3	0	0.0	30
21-25yrs	20	64.5	8	25.8	2	6.5	1	3.2	31
26-30yrs	7	50.0	7	50.0	0	0.0	0	0.0	14
>=31yrs	7	70.0	3	30.0	0	0.0	0	0.0	10
Total	55	64.7	26	30.6	3	3.5	1	1.2	85
Chi-square= 1.8173 P = 0.6112									

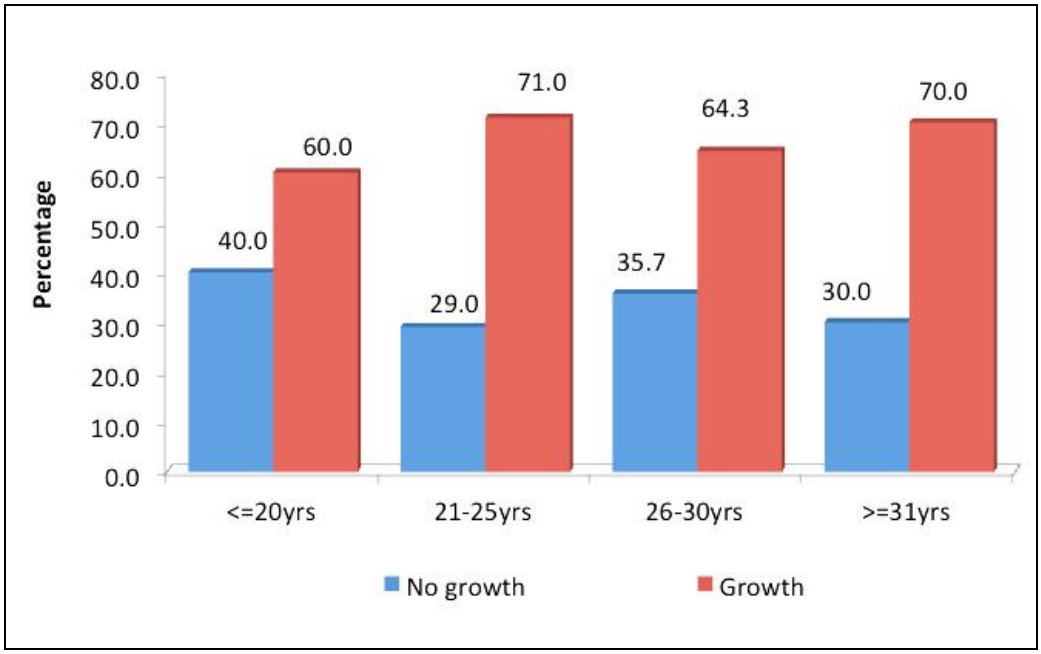
Although grade 1 lesions were the predominant type in all age groups,this did not statistically correlate (*Chi-square= 1.8173 P = 0.6112).*

GRAPH 12: ASSOCIATION BETWEEN AGE GROUPS AND BACTERIAL ISOLATES



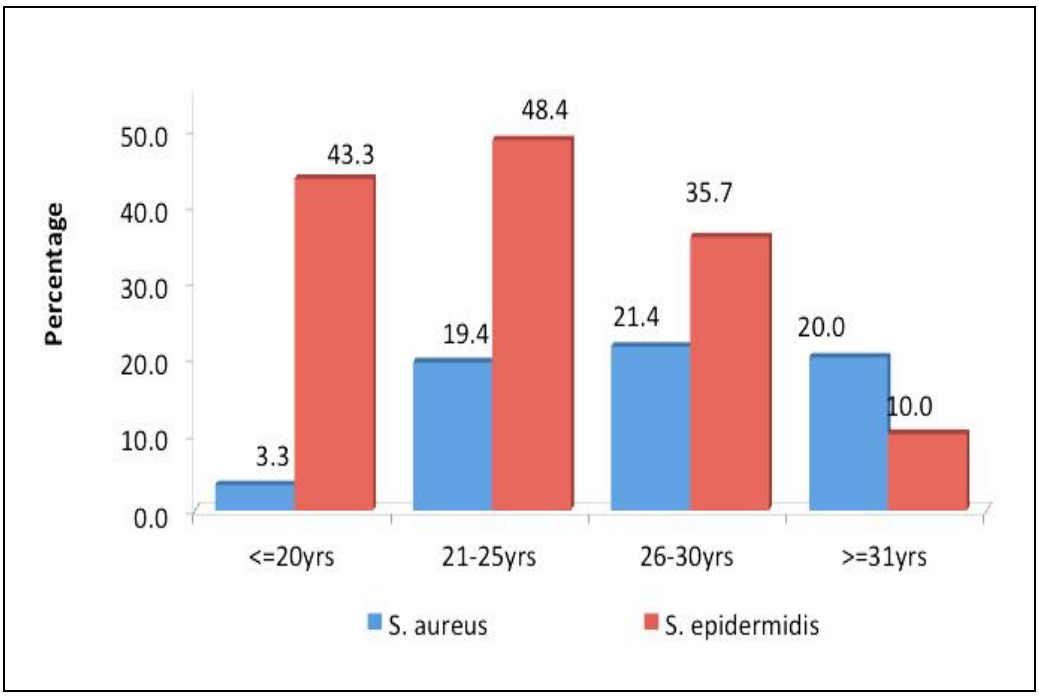
Interestingly, *S. epidermidis* and *S. aureus* were significantly associated with the co-existence of *P. acnes* in people younger than 26 years when compared with older patients (*Chi-square= 8.8861 P = 0.0310**).

GRAPH 13: ASSOCIATION BETWEEN AGE GROUPS AND GROWTH OF ANAEROBIC BACTERIA –P. ACNES



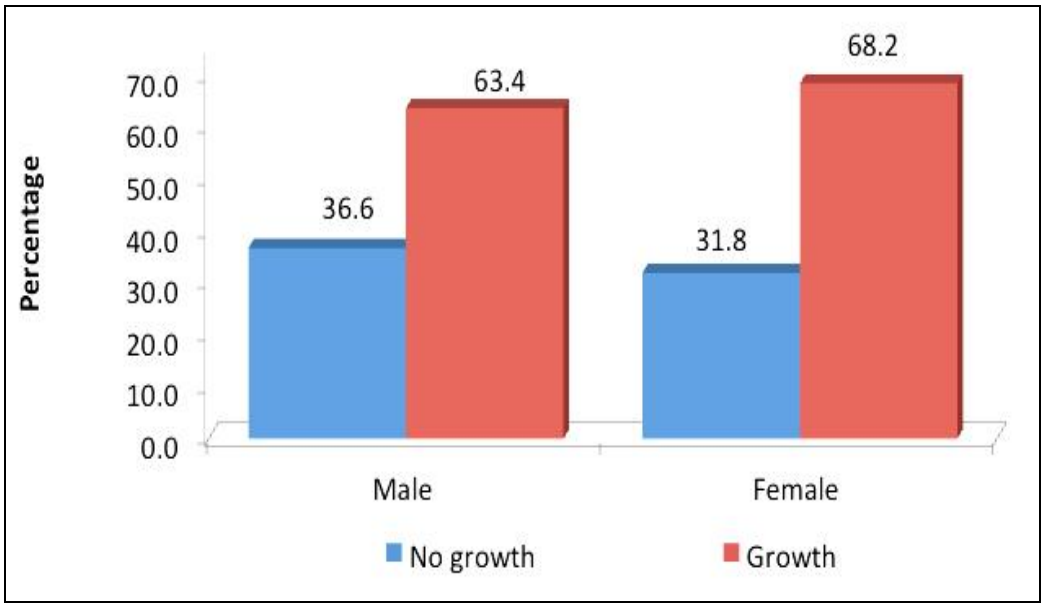
Various age groups were not statistically predictive for the growth of anaerobic bacteria (*P.acnes*) (*Chi-square= 0.9103 P = 0.8232).* Growth rates observed in 21-25 years and 30 years and above were the highest (71% and 70% respectively).

GRAPH 14: ASSOCIATION BETWEEN AGE GROUPS AND GROWTH OF AEROBIC BACTERIA

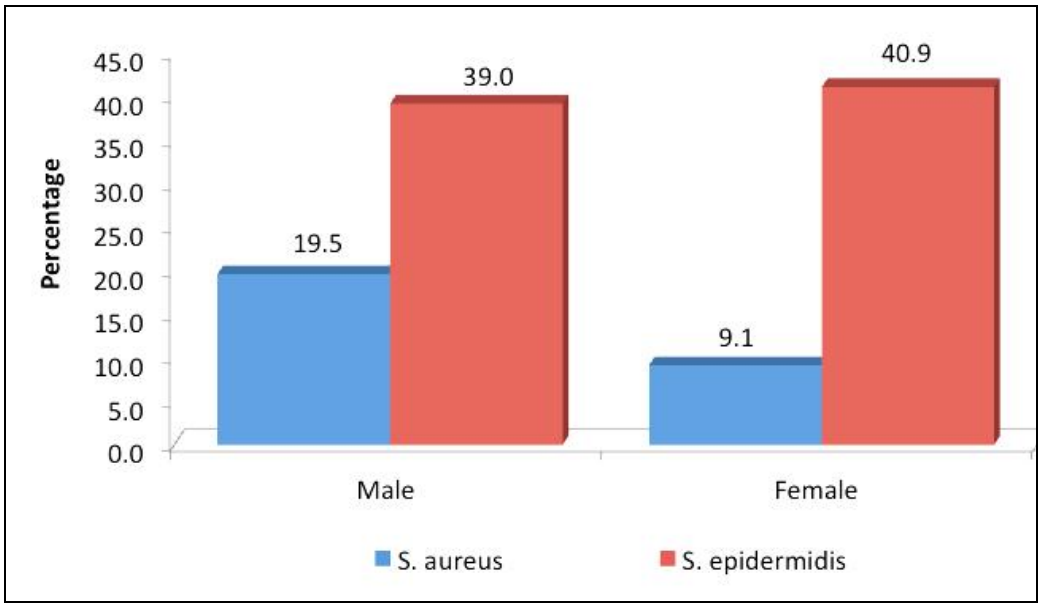


Similarly, the growth of aerobic bacteria did not correlate statistically with different age groups (*Chi-square= 5.7763 P = 0.1232).*

GRAPH 15: ASSOCIATION BETWEEN GENDER AND GROWTH OF ANAEROBIC BACTERIA



GRAPH 16: ASSOCIATION BETWEEN GENDER AND GROWTH OF AEROBIC BACTERIA



Both genders were equally likely of growing anaerobic and aerobic bacteria (*Chi-square=0.2150 P = 0.6431, Chi-square= 1.9893 P = 0.3702)*

DISCUSSION

Acne vulgaris is a chronic condition that is virtually universal in adolescence.[4]

In our study, samples from skin lesions of 85 patients with Acne vulgaris presenting to the Dermatology OPD at Prabhakar Kore Hospital, Belagavi, Karnataka were collected for analysis. The mean age at presentation was 23.3 years and over 70% of the patients were aged 25 or less. On reviewing published literature, mean ages of patients with acne ranged from 15.9 to 25.6 years, which was in accordance with our findings.[81, 117-119]

TABLE 3: Age distribution in various studies

Authors	Sample size	Year of study	Age range (years)	Mean age
Kane et al[117]	93	2002-03	14-46	25.58
Adityan et al[118]	309	2006-08	10-33	15.9
Nakase et al[119]	91	2009-10	-	25.5
Biswal et al[81]	102	2010-12	11-29	18.7
Present study	**85**	**2017**	**16-38**	**23.3**

Among the participants in our study, we did not observe any gender predilection as both men (48%) and women (52%) seemed to be equally affected. Similar observations have been made by Jahns et al[120]in Lithuanian and Swedish patients (2012) and Adityan et al[118] from Pondicherry (2006-08).While others have

observed a strong association of acne in women in over 80% patients (Kane et al and Nakase et al),[117, 119]a few have reported men to be the more frequent sufferers (Biswal et al).[81]

TABLE 4 : Gender distribution in various studies

Authors	Sample size	Year of study	Men	Women
Kane et al[117]	93	2002-03	24.7%	75.3%
Adityan et al[118]	309	2006-08	55.7%	44.3%
Nakase et al[119]	91	2009-10	19.8%	80.2%
Biswal et al [81]	102	2010-12	63.7%	36.3%
Jahns et al[120]	38	2012	52.6%	47.4%
Present study	**85**	**2017**	**48.2%**	**51.8%**

According to the widely followed classification of Acne by James and Tisserland, a spectrum of lesions has been described from grade 1 (comedones and few papules) to grade 4 (severe nodulo-cystic forms).[26]

In the study by Adityan and Thappa, grade 1 was the predominant type occurring in over 60% cases. The least common variety observed by the authors was grade 3seen in only 2.6% patients.[118]Evaluating the clinical profile of Acne vulgaris in a semi-urban population, Saxena et al reported predominance of grade 2 lesions followed by grade 1, while grades 3 and 4 were seen in 5.1% and 3.7% respectively.[121]

Using a similar grading system (by Pillsbury),[122]Biswal et al[81]reported a more equitable distribution of patients between grades 1, 2 and 3 (32%, 26%, and 30% respectively) while 12% patients had grade 4 lesions.

TABLE 5 : Grades of Acne vulgaris

Authors	Sample size	Year of study	Grade (%)			
			1	**2**	**3**	4
Adityan et al[118]	309	2006-08	60.2%	27.5%	2.6%	9.7%
Biswal et al[81]	102	2010-12	32%	26%	30%	12%
SaxenaK et al[121]	429	2011	25.2%	66%	5.1%	3.7%
Present study	**85**	**2017**	**64.7%**	**30.6%**	**3.5%**	**1.2%**

Among 85 samples collected, 67 (78.8%) showed positive cultures. Of these, 46 (68.7%) yielded aerobic bacteria while 56 (83.6%) showed anaerobic bacteria. Aerobically, *S.epidermidis* and *S.aureus* were detected while *P.acnes* was the only anaerobe that could be cultured. Among 67 culture positives, 32 (47.8%) were single type of bacteria and 35 (52.2%) showed mixed growth.

Out of 32 pure isolates, 21 cultures positive for *P.acnes* while *S.epidermidis* and *S.aureus* were seen in 10 and 1 samples respectively. There were 24 samples with concurrent growth of *P.acnes* and *S.epidermidis* while *P.acnes* and *S.aureus* were simultaneously isolated in 11 cases. Hence, the total bacterial isolates were as follows: 56 (83.6%) *P.acnes*, 34 (50.7%) *S.epidermidis*, 12 (17.9%) *S.aureus*. In a microbiological analysis of acne lesions among college students by Dhillon et al,[5]

49% cultures grew *S. epidermidis* while *S.aureus* and *P.acnes* were seen in 45% and 32% patients respectively. In another study by Biswal et al[81] on 102 acne patients, the microbial growth consisted of 65% *S.aureus*, 5% *S.epidermidis* among aerobes and 66% of *P.acnes* in anaerobic cultures. There were 39% samples that showed mixed growth of *P.acnes* and *S. aureus* and only 3% had *P. acne* and *S. epidermidis*. Another study conducted by Hassanzadeh et al[123] showed 51% *S.aureus*, 53% *S.epidermidis* aerobically and 33% *P.acnes* were grown anaerobically.

According to Nishijima et al,[124] 21 strains of *P.acnes* were isolated from 21 acne lesions, whereas only 2 strains of *P.acnes* were isolated alone. 26 strains of *S.epidermidis* were isolated from 26 acne lesions, but 1 strain was isolated alone. There were 17 lesions out of 30 cases that showed *P.acnes* and *S.epidermidis*.

TABLE 6 : Bacteriological profile of acne patients in published literature

Author	Sample size	Year of study	Aerobic bacteria		Anaerobic bacteria (*P. acnes*)	Mixed growth	
			S.epidermidis	*S.aureus*		*P.acnes*+ *S.epidermidis*	*P.acnes* +*S. aureus*
Nishijima et al [124]	30	1996-97	26	-	21	17	-
Hassanzadeh et al[123]	100	2004-05	53%	51%	33%	-	-
Dhillon KS et al[5]	50	2012-13	49%	45%	32%	-	-
Biswal et al[81]	102	2010-12	5%	65%	66%	3%	39%
Present study	**85**	**2017**	**50.7%**	**17.9%**	**83.6%**	**68.6%**	**31.4%**

Out of 85 samples, 56 *P.acnes* were isolated. Among them, 37 isolates were from Grade 1, 18 were from grade 2, 1 isolate was from grade 3 whereas no *P.acnes* was isolated from grade 4 lesions. Concomitant growth of *P. acnes* and *S. epidermidis* was observed in 75% grade 1 lesions, whereas the remaining 25% were seen in grade 2 lesions. The respective figures for *P. acnes* and *S. aureus* in grades 1 and 2 were 54.5% and 45.5%. Although statistical analysis didn't reveal significance, a majority of combined aerobic and anaerobic cultures were seen in grade 1 lesions. In a paper by Dhillon et al,[5] severe forms of acne (pustular and nodulocystic lesions) cultured positive for *S. aureus* (45%), *S. epidermidis* (49%) and micrococcus (45%) in aerobic cultures. Anaerobic lesions, on the other hand, grew *S. aureus*, *P. acne*, *S. epidermidis* in 41%, 32% and 20% cultures respectively. A grade-wise sub-characterization was not available in the paper and therefore, didn't allow further comparison. Results from other similar analyses are mentioned below in the table.

TABLE 7 : Bacteriological profile of acne patients according to different grades in published literature - anaerobes

Study	Grade	*P. acne* growth Percentage (%)
Nishijima S et al[124]	3	6.7%
Hassanzadeh P et al[123]	3-4	33%
Dhillon KS[5]	3-4	32%
Present study	Grade 1	66.1%
	Grade 2	32.1%
	Grade 3	1.8%
	Grade 4	0%

TABLE 8 : Bacteriological profile of acne patients according to different grades in published literature – aerobes and anaerobes

Authors	Grade	*P. acnes* + *S. epidermidis*	Percentage	*P. acnes* + *S. aureus*	Percentage
Nishijima S et al[124]	1	1	3.3%	0	0
	2	2	6.6%	0	0
	3	12	40%	1	3.3%
	4	0	0	0	0
Present study	Grade 1	18	75%	6	54.5%
	Grade 2	6	25%	5	45.4%
	Grade 3	0	0%	0	0%
	Grade 4	0	0%	0	0%

We observed an overall 42.9% sensitivity of *P. acnes* towards Clindamycin in the anaerobic growths of our samples. While 50% of the 56 strains were resistant to the same, an intermediate sensitivity was observed in 7.1% patients. On comparing this with published literature, a wide range of resistance towards clindamycin has been observed in the past. Schafer et al[125] in their study evaluating antibiotic resistance patterns towards various drugs (including clindamycin, doxycycline, co-trimoxazole, erythromycin and tetracycline) in skin samples from 83 Chilean acne patients reported an overall resistance of 33.7%. Of this, 7.5% was seen towards clindamycin. A similar analysis from Hong Kong by Luk et al[126] reported 53.5% resistance towards clindamycin. Details from other studies have revealed varying rates of resistance and can be studied from the table below.

TABLE 9 : Susceptibility pattern of *P.acnes* to Clindamycin in published literature

Authors	Year	*P. acnes* (number of strains)	Clindamycin		
			Sensitive	Intermediate	Resistant
Nakase K et al[119]	2009-10	69 (out of 91)	-		**18.8%**
Luk NM et al[126]	2013	86 (out of 111)	-		**46 (53.5%)**
Mendoza N et al[127]	2013	100	-		**15 (15%)**
Schafer F et al[125]	2013	80 (out of 83)	-		**7.5%**
Present study		**56 (out of 85)**	**24 (42.9%)**	**4 (7.1%)**	**28 50%)**

Analysis of antibiotic susceptibility of *S. epidermidis* strains from our samples revealed 97% sensitivity towards doxycycline and 94.10% towards Gentamycin and the bacteria were least sensitive (38.20%) to clindamycin. Srikanth and colleagues in a recent paper from Vishakapatnam observed in acne patients that maximum (100%) sensitivity was seen for *S.epidermidis* with ofloxacin while co-trimoxazole and ampicillin demonstrated least sensitivities of 43.4% and 19.4% respectively.[7]

The antibiogram of *S. aureus* strains from our study showed better overall sensitivity of bacteria towards Doxycycline and Gentamycin (100%) while lowest sensitivities were, again, observed with Erythromycin and Clindamycin (58.30%). On comparing these results with those of Srikanth et al,[7] best sensitivity was seen for minocycline (56.3%) while none of the 16 strains of *S. aureus* were sensitive to co-trimoxazole and ampicillin.

TABLE 10 : Antibiotic susceptibility pattern of aerobic isolates in published literature

Authors	Drug / Bacteria	Most sensitive	Least sensitive
Srikanth et al[7]	*S. epidermidis* – 31	Ofloxacin (100%)	Co-trimoxazole (43.4%), ampicillin (19.4%)
	S. aureus – 16	Minocycline (56.3%)	Co-trimoxazole (0%), ampicillin (0%)
Present study	*S. epidermidis* – 34	Doxycycline (97%) Gentamycin (94.10%)	Clindamycin (38.20%)
	S. aureus – 12	Doxycycline and Gentamycin (100%)	Erythromycin and Clindamycin (58.30%)

This study brings out the clinical profile of acne vulgaris in a tertiary care hospital in Belgaum. For a successful management of acne, a meticulous pairing of individual patients with the appropriate antiacne agents is required along with educating the patients simultaneously. The patient should be thoroughly evaluated on the grounds of acne severity, predominant lesion type, age and type of the skin. Antibiotic resistance is a significant problem. Long term use of antibiotics has led to the emergence of resistant bacterial strains and treatment failure. To track the patterns of *P. acnes* resistance to antimicrobials, an epidemiological study must be carried out. This will be helpful in guiding the researchers to design therapeutic regimens that can minimize the development of resistance.[16]

CONCLUSION

Our study showed that Acne vulgaris, an inflammatory disorder of sebaceous follicles commonly affecting the youth, can also persist in adults. It is a multifactorial disease in which both aerobic and anaerobic bacteria play important roles. In this study, *Staphylococcus epidermidis* was more commonly isolated in aerobic cultures while *Propionibacterium acnes* was observed in all anaerobic cultures. Overall, among the 67 positively grown cultures, anaerobic bacteria appeared to outnumber the aerobes by 14.9%.

Women and men were almost equally affected (1.07:1) and the majority of patients belonged to the age group of 15-25 years most commonly suffering from grade 1 lesions. Among all *P. acnes* growths, over 66% had mild lesions (grade 1). Likewise, most of the concomitant growths (*P. acnes* with *S. epidermidis* and *S. aureus*) were also grade 1 lesions.

Aerobic bacterial isolates were found to be mainly resistant to Clindamycin and Erythromycin whereas they had a higher sensitivity to Gentamicin and Doxycycline. Clindamycin resistant anaerobic bacteria were found to be prevalent.

Antibiotic resistance in acne vulgaris is a growing concern worldwide, making it difficult to treat these patients. There are several recommendations to limit the emergence and outbreak of the antibacterial resistance of *P.acnes*. It is advisable to discourage antibiotics' abuse and implement the usage of non-antibiotic agents such as benzoyl peroxide or retinoids wherever possible. Periodic antibiogram of the bacterial strains should be carried out to be aware of the changes in their resistance

pattern. This will help us in formulating a better empirical or definitive therapy for treating patients of acne vulgaris with various grades.

Lastly, the clinicians should educate and sufficiently inform the patients about the dose, duration, the form of administration of the treatment and also the importance of good adherence to acne therapy.

SUMMARY

- The present study was conducted on 85 patients with acne vulgaris of all age groups, attending the Out Patient Department of Dermatology at KLE'S Dr. Prabhakar Kore Charitable Hospital and Reference Centre, Belagavi between the period of January 2017 to December 2017.
- Samples from skin lesions (using a sterile extractor) were collected on the sterile cotton swabs from these patients and transferred to thioglycollate medium. These were then processed in the Department of Microbiology, JNMC, Belagavi.
- Most of the patients (71.8%) were between 15 and 25 years old and the group comprised of almost equal number of men (41) and women (44).
- Out of 67 culture positive, 68.7% were aerobic isolates and 83.6% were anaerobic bacteria. All the anaerobic isolates were *P. acnes*.
- Among 32 pure isolates, 65.6% cultured positive for *P. acnes* while *S. epidermidis* and S. *aureus* were seen in 31.3% and 3.1% samples respectively.
- There were 24 (68.6%) samples with concurrent growth *of P. acnes* and *S. epidermidis* while P. *acnes* and *S. aureus* were simultaneously isolated in 11 (31.4%) cases.
- Out of 85 patients, over 64% patients were diagnosed as having grade 1 acne vulgaris.
- Among all *P. acnes* growths, over 66% had mild lesions (grade 1). Likewise, most of the concomitant growths (*P. acnes* with *S. epidermidis* and *S. aureus*) were also grade 1 lesions.

- Most of the aerobic isolates showed resistance to Clindamycin and Erythromycin but they showed better sensitivity to Gentamicin and Doxycycline
- Amongst the 56 anaerobic bacterial isolates, 28 (50%) of them were resistant to Clindamycin while24 (42.9%) were sensitive and only 4 (7.1%) had intermediate MIC.

APPENDIX

1. Gram stain Procedure: Hucker's modification: [109]

Principle: After treatment with decolorizing agents, gram positive bacteria retain para- rosaniline dyes and appear violet color while gram negative bacteria lose the dye and take up counter stain and appear pink in color.

Procedure:

a) A clean grease free glass slide was labelled and a thin smear was made on it using the first high vaginal swab and allowed to air dry.

b) The smear was fixed by passing the slide three to four times through the flame of a Bunsen burner.

c) Slide was then placed on the slide rack and the smear overlaid with crystal violet solution.

d) After 20 seconds, the slide was washed thoroughly with tap water.

e) Subsequently, the smear was overlaid with Gram iodine solution for 20 seconds and washed again with water.

f) The smear was held between the thumb and fore finger and the surface flooded with a few drops of acetone-alcohol decolorizer, until no color washed off.

g) The smear was washed with running water and placed back on the staining rack. Surface of the smear was overlaid with safranin (counter stain) for 10 seconds and washed with running water.

h) The slide was placed in an upright position in a rack, allowing excess water to drain off.

i) The stained smear, after being dried was examined under 100 X (oil) immersion objective lens.

Quality control: Gram positive: *Staphylococcus aureus* ATCC 25923

Gram negative: *Escherichia coli* ATCC 25922

2. Jensen's Gram stain Procedure:[110]

This method uses alcohol as decolorizer and weak neutral red as counterstain. It is recommended for examination of smears for gonococci.

a) A thin smear was made from the first swab, dried, fixed and then placed on the staining rack.
b) The smear was covered with 0.5% methyl violet for 30 seconds.
c) The slide was tilted and sufficient Lugol's (1%) iodine was used to wash away the stain. The smear was covered with fresh iodine for 30 seconds.
d) The slide was tilted and iodine was washed off with ethanol till color ceased to come out of the smear.
e) The slide was then washed with running water.
f) Neutral red (0.1%) was the counterstain used. The smear was covered with it for 2 min.
g) The slide was washed with running water and blotted to dry.
h) It was then examined under oil immersion objective.

3. Method used for obtaining anaerobiosis : in the jar was by " internal gas generating system" described by Lakshminarayana and Vaidhyalingam.[113]

Principle of internal gas generating system:

In this system, hydrogen and carbon dioxide gas mixture required for creating anaerobiosis is obtained from the following reactions:

a) Citric acid + sodium bicarbonate ⟶ Sodium citrate + carbon dioxide

$C_6H_8O_7 + 3NaHCO_3 \longrightarrow Na_3C_6H_5O_7 + 3H_2O + 3CO_2$

b) Sodium borohydride + water ⟶ Sodium metaborate + Hydrogen

$NaBH_4 + 2\ H_2O \longrightarrow NaBO_2 + 4H_2$

Operation of the internal gas generator system:

a) 1 g of Sodium borohydride was taken in a 30 ml test tube.

b) 1 g of Sodium bicarbonate and 1 g of citric acid were taken in a 5 ml test tube, which was placed inside the 30 ml test tube.

c) The stem of a 20 ml funnel was plugged lightly with cotton to control the flow of water. The funnel was placed in 30 ml test tube in such a way that the stem of funnel dips into 5 ml test tube. Entire unit was kept inside the anaerobic jar with the indicator. 20 ml of distilled water was poured in the funnel just before closing the lid of the jar.

The water poured into the funnel drips into the 5 ml test tube liberating CO_2. Carbon di oxide being heavier stays within displacing the air. Once the 5 ml test tube is filled with water, it overflows into the 30 ml test tube liberating hydrogen, which being the lighter gas, rushes out with CO_2.

d) The palladium catalyst reduces the oxygen present within the jar to form water. Catalyst is exothermic, so warming of the lid of the jar can be felt.

Catalyst:

Cold catalyst which contained pellets of alumina coated with finely divided palladium (Baker platinum Ltd., London) was used. It was reactivated every time before use by drying at 150^oC- 160^oC for 1-2 hours.

Indicator for anaerobiosis:

- **Fildes and McIntosh indicator**- 3 Stock solutions were prepared:

a) A solution of 6% glucose in distilled water

b) 6 ml of 0.1 N NaOH diluted to 100 ml with distilled water.

c) 3 ml of 0.5% w/v solution of methylene blue diluted to 100 ml of distilled water.

- Each time the indicator solution was required equal parts of the 3 stock solutions were mixed together in a test tube and the mixture was boiled until methylene blue was reduced.
- The tube of colorless methylene blue was immediately placed in anaerobic jar
- If anaerobic conditions were secured and maintained the indicator solution remained colorless.

- **Bacteriological indicator**- *Pseudomonas aeruginosa* ATCC 25922 was inoculated on to a nutrient agar plate and kept inside the jar along with the other plates. This bacterium is aerobic. A growth free culture plate indicates a successful anaerobiosis.

4. **Catalase test [112]:** Growth from the purity blood agar was removed onto a glass slide. A drop of 15% hydrogen peroxide was added and observed for evolution of bubbles.
5. **Spot indole test [112]:** A loopful of growth from a pure culture on the blood agar plate was removed and smeared on filter paper that was saturated with 1% paradimethylaminocinnamaldehyde in 10% (V/V) concentrated HCl.

A positive reaction was indicated by the rapid development of blue color around the growth. Negative reaction gave no color change or a pinkish color.

6. **Nitrate test [112]:** Test was done using nitrate disk. The disk was removed from the surface of the blood agar plate and placed in a clean petridish. One drop each of Nitrate reagents A and B were added. Development of pink to red color indicated that nitrate has been reduced to nitrite.

If no color developed in few minutes, a small amount of zinc dust was added and waited for 5 minutes. Development of red color indicated that nitrate was not reduced. If no color developed, nitrate was reduced beyond nitrite (positive test).

Preparation of Nitrate reagents:

Solution A-

Sulfanilic acid- 0.5 g

Glacial acetic acid- 30 ml

Distilled water- 120 ml

Solution B-

1, 6- Cleve's acid- 0.2 g

(5-amino-2-naphthalenesulfonic acid)

Glacial acetic acid-30 ml

Distilled water- 120 ml

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ANNEXURES

ANNEXURE I - ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
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Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 88

Date: 17/10/2016

To,

[redacted]

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"CLINICO-BACTERIOLOGICAL STUDY OF ACNE VULGARIS PATIENTS ATTENDING DERMATOLOGY OUT PATIENT DEPARTMENT WITH SPECIAL REFERENCE TO ANAEROBES – A CROSS SECTIONAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Ganga Pilli)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE II – CONSENT FORM

CONSENT FOR THE PARTICIPATION IN RESEARCH

TITLE: Clinico-Bacteriological Study of Acne Vulgaris patients attending Dermatology Out Patient Department, with special reference to Anaerobes - A cross sectional study

Study Investigator

Guide

Co-guide

INTRODUCTION:

Acne vulgaris is an extremely common, chronic inflammatory disorder of the skin affecting almost all adolescents. It is a multifactorial skin disease characterized by a variety of non-inflamed and inflamed lesions. The severity of acne vulgaris is done on the basis of numerous grading methods.

In the pathogenesis of acne, various bacteria, both Aerobic (*staphylococcus* species) and Anaerobic (*Propionibacterium acnes*, *Peptococci*, *Peptostreptococci*, and *Fusobacterium*species) are involved where *Propionibacterium acnes* play an important role. Long term and rampant use of antimicrobials lead to the treatment failure and cause both social and psychological impact.

OBJECTIVES OF THE STUDY:

The purpose of research is to isolate and identify the bacteria (aerobes and anaerobes) causing acne vulgaris and to correlate it clinically and also to determine the antibiotic susceptibility of the isolated bacteria, both aerobes and anaerobes.

PROCEDURE INVOLVED:

You are requested to participate in this study which will help you in providing an appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

If you agree to be a part of this study, you will be subjected to sample collection with the help of a sterile extractor. The sample will be collected on the sterile cotton swabs and will be transported to the Microbiology laboratory for further processing.

RISKS AND BENEFITS:

There are no risks involved and benefit is to know the causative bacteria and which antibiotics it is susceptible to, so that appropriate treatment can be given.

ALTERNATIVES:

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with the treating physician and the treatment shall remain unaffected. If you decide to participate, you are free to withdraw at any time.

PRIVACY AND CONFIDENTIALITY:

The only people to know that you are a research subject are the members of the research team. No information about you or provided by you during the research will be disclosed to others without your written permission, except in emergency to protect your rights and welfare.

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed in a conference, no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not be paid /offered any gifts/incentives for participating in the study. You will not be reimbursed for expenses.

In case you have any questions about your rights as a participant, you can contact Dr. Ganga S Pilli, Professor of Pathology and Chairman of Institutional Ethics Committee, JNMC (Phone No: 9480275601 Ext: 4052)

CONSENT STATEMENT

I, the undersigned _____have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Name

Witness Name signature

Experimenter's Name signature

Date:

Place:

ANNEXURE-III

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

Name: Age/Sex:

OPD No: Occupation:

Education: Address:

Chief Complaints:

Onset:

Duration:

Past History:

Treatment taken, if any:

Family History:

Personal History:

Diet:

Appetite: Sleep:

Bowel and bladder habits:

Menstrual History:

General Physical Examination:

Pulse rate: Blood Pressure:

Temperature: Weight:

Oedema, Pallor, Icterus, Clubbing:

Lymphadenopathy:

Oral mucosa:

Cutaneous Examination:

No. of lesions:

Site of lesions:

Type of lesions:

Comedones	Nodules
Papules	Cysts
Pustules	Scars

Hair:

Nails:

Associated dermatological lesions:

Systemic Examination:

C.V.S –

R.S –

G.I.T –

C.N.S –

Grading of acne vulgaris (According to James and Tisserand's grading for the severity of acne vulgaris grading)

Clinical Diagnosis:

Laboratory investigation:

- Hucker's modification of Gram's stain
- Culture for aerobes:

  Blood agar:
- Biochemical reactions:
- Catalase, Coagulase, Oxidase, Indole, Urease, Citrate utilization, Mannitol fermentation, TSI
- Antibiogram using disc diffusion method (According to CLSI guidelines)
- Culture of Anaerobes
- Growth on Chocolate Agar and Propionibacterium Agar
- Colony morphology
- Pigment
- Haemolysis
- Fluorescence
- Pitting
- Jensen's modification Gram's stain of individual colony
- Catalase test
- Spot Indole test
- Nitrate test
- Antibiogram using agar dilution method (According to CLSI guidelines)

ANNEXURE – IV

MASTER CHART

Sl no.	Ip no.	Lab no.	AGE	SEX	CLINICAL DIAGNOSIS	ANAEROBES	AST - Cd	AEROBES	Gen	E	Cd	Amp	P	Cot	Cip	Do	NOGC
1	3565142	B/5397/17	22	F	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	S	R	S	S	S	-
2	4231866	B/5399/17	22	F	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
3	4232510	B/5401/17	20	F	Acne Vulgaris-Grade 1	P. acnes	R	-	-	-	-	-	-	-	-	-	-
4	4215240	B/5403/17	20	M	Acne Vulgaris-Grade 1	-	-	S. epidermidis	S	S	R	S	S	S	S	S	-
5	4215256	B/5405/17	19	M	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	S	R	S	S	S	-
6	4215853	B/5407/17	20	M	Acne Vulgaris-Grade 1	P. acnes	R	-	-	-	-	-	-	-	-	-	-
7	4215290	B/5409/17	19	F	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	R	S	S	S	-
8	4273732	B/10111/17	21	F	Acne Vulgaris-Grade 1	-	-	S. aureus	S	R	R	R	S	S	S	S	-
9	3647029	B10113/17	21	F	Acne Vulgaris-Grade 1	-	-	S. epidermidis	S	S	S	S	R	S	S	S	-
10	4216280	B/10115/17	19	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
11	4289697	B/13814/17	21	M	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	S	R	R	S	S	-
12	4269954	B/13816/17	20	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
13	4215417	B/13818/17	19	M	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	R	R	S	R	S	-
14	4296049	B/13820/17	19	F	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	S	S	S	S	-
15	3463806	B/16202/17	19	F	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
16	4035989	B/16200/17	26	F	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	R	S	S	S	S	-
17	4337043	B/22092/17	19	F	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
18	4339498	B/22094/17	21	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
19	3427584	B/22096/17	20	F	Acne Vulgaris-Grade 1	P. acnes	R	-	-	-	-	-	-	-	-	-	-
20	4349382	B/22098/17	17	F	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
21	4419631	B/32823/17	23	F	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	S	R	R	S	-
22	4421629	B/32825/17	23	F	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	S	S	S	S	-
23	4421621	B/32827/17	26	M	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	S	R	S	S	R	R	S	-
24	4403651	B/32829/17	24	F	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	S	R	S	S	-
25	2618268	B/44492/17	17	M	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	S	R	S	S	-
26	40010029	B/44494/17	38	M	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
27	4101119	B/44496/17	31	F	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
28	400940	B/44498/17	33	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
29	4415194	B/44500/17	16	F	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	S	R	S	S	S	-
30	4075986	B/44502/17	19	M	Acne Vulgaris-Grade 1	-	-	S. epidermidis	S	R	R	S	S	S	R	S	-
31	4436868	B/43991/17	20	F	Acne Vulgaris-Grade 2	P. acnes	R	S. aureus	S	R	R	R	S	S	R	S	-
32	4436865	B/43993/17	20	F	Acne Vulgaris-Grade 2	P. acnes	R	S. epidermidis	S	R	R	R	S	R	R	S	-

33	3435022	B/43995/17	18	M	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	R	S	S	R	R	R	R	S	-
34	3435021	B/43997/17	18	M	Acne Vulgaris-Grade 2	-	-	S. epidermidis	S	S	S	R	S	S	S	S	-
35	821599	B/43999/17	21	M	Acne Vulgaris-Grade 1	P. acnes	R	-	-	-	-	-	-	-	-	-	-
36	1907883	B/44001/17	22	M	Acne Vulgaris-Grade 1	P. acnes	R	S. aureus	S	R	R	S	S	R	S	S	-
37	2322274	B/44003/17	22	F	Acne Vulgaris-Grade 2	P. acnes	S	-	-	-	-	-	-	-	-	-	-
38	4374802	B/44005/17	20	M	Acne Vulgaris-Grade 3	-	-	-	-	-	-	-	-	-	-	-	NOGC
39	4429623	B/44007/17	20	F	Acne Vulgaris-Grade 2	P. acnes	R	-	-	-	-	-	-	-	-	-	-
40	4210986	B/44009/17	21	F	Acne Vulgaris-Grade 3	-	-	-	-	-	-	-	-	-	-	-	NOGC
41	4162739	B/48895/17	23	F	Acne Vulgaris-Grade 2	P. acnes	R	S. epidermidis	S	S	R	S	R	S	S	S	-
42	3187311	B/48897/17	22	F	Acne Vulgaris-Grade 1	P. acnes	R	-	-	-	-	-	-	-	-	-	-
43	3882078	B/48899/17	27	M	Acne Vulgaris-Grade 2	P. acnes	I	-	-	-	-	-	-	-	-	-	-
44	4516401	B/48901/17	24	M	Acne Vulgaris-Grade 2	P. acnes	S	S. epidermidis	R	S	S	R	R	S	S	S	-
45	4505743	B/48903/17	25	F	Acne Vulgaris-Grade 2	-	-	S. epidermidis	S	S	S	S	R	R	S	S	-
46	4532634	B/48907/17	27	F	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
47	4505757	B/48909/17	27	F	Acne Vulgaris-Grade 2	P. acnes	I	S. epidermidis	S	R	R	S	S	R	R	S	-
48	4516088	B/48911/17	32	M	Acne Vulgaris-Grade 1	P. acnes	I	S. aureus	S	R	R	R	S	S	S	S	-
49	4017975	B/48913/17	28	M	Acne Vulgaris-Grade 2	-	-	-	-	-	-	-	-	-	-	-	NOGC
50	4515989	B/48915/17	18	F	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
51	4594439	B/60845/17	32	M	Acne Vulgaris-Grade 1	P. acnes	R	-	-	-	-	-	-	-	-	-	-
52	4432862	B/60847/17	21	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
53	4417207	B/60849/17	31	F	Acne Vulgaris-Grade 2	P. acnes	R	-	-	-	-	-	-	-	-	-	-
54	2740599	B/60851/17	21	F	Acne Vulgaris-Grade 2	P. acnes	R	S. epidermidis	S	S	S	S	S	R	R	S	-
55	4435937	B/60853/17	31	F	Acne Vulgaris-Grade 1	-	-	S. epidermidis	S	R	R	R	S	R	S	S	-
56	4618149	B/62054/17	25	M	Acne Vulgaris-Grade 1	P. acnes	S	S. aureus	S	S	S	S	S	S	S	S	-
57	4204885	B/62056/17	18	M	Acne Vulgaris-Grade 2	P. acnes	R	S. epidermidis	S	R	R	S	S	R	S	S	-
58	3434991	B/62058/17	27	F	Acne Vulgaris-Grade 2	-	-	-	-	-	-	-	-	-	-	-	NOGC
59	4609982	B/62060/17	17	M	Acne Vulgaris-Grade 2	-	-	-	-	-	-	-	-	-	-	-	NOGC
60	4606526	B/62062/17	20	F	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
61	4608576	B/62064/17	30	M	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	R	R	S	S	-
62	4154876	B/62066/17	19	F	Acne Vulgaris-Grade 2	-	-	-	-	-	-	-	-	-	-	-	NOGC
63	4583810	B/62068/17	32	F	Acne Vulgaris-Grade 2	P. acnes	S	S. aureus	S	S	S	S	S	S	R	S	-
64	4162739	B/62071/17	23	F	Acne Vulgaris-Grade 2	-	-	S. epidermidis	S	R	R	S	S	S	R	R	-
65	3754786	B/62073/17	33	F	Acne Vulgaris-Grade 2	P. acnes	R	-	-	-	-	-	-	-	-	-	-
66	4463108	B/63611/17	21	F	Acne Vulgaris-Grade 3	P. acnes	R	-	-	-	-	-	-	-	-	-	-
67	4421629	B/63613/17	23	F	Acne Vulgaris-Grade 4	-	-	S. epidermidis	S	R	R	S	S	R	S	S	-
68	4509711	B/63615/17	20	F	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
69	4015542	B/63617/17	18	M	Acne Vulgaris-Grade 1	-	-	S. epidermidis	S	R	R	S	S	S	R	S	-

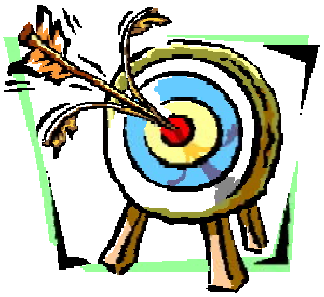
70	4417166	B/63619/17	26	M	Acne Vulgaris-Grade 2	P. acnes	S	-	-	-	-	-	-	-	-	-	-
71	4509648	B/63621/17	22	F	Acne Vulgaris-Grade 1	P. acnes	S	-	-	-	-	-	-	-	-	-	-
72	3757152	B/63623/17	30	M	Acne Vulgaris-Grade 2	P. acnes	S	S. aureus	S	S	S	S	R	R	S	S	-
73	4509133	B/63625/17	30	F	Acne Vulgaris-Grade 1	-	-	S. epidermidis	S	R	R	S	S	R	S	S	-
74	4509110	B/63627/17	31	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
75	4432690	B/63629/17	18	F	Acne Vulgaris-Grade 2	P. acnes	R	-	-	-	-	-	-	-	-	-	-
76	4608427	B/39299/18	25	M	Acne Vulgaris-Grade 1	-	-	-	-	-	-	-	-	-	-	-	NOGC
77	4608678	B/39301/18	26	M	Acne Vulgaris-Grade 2	-	-	-	-	-	-	-	-	-	-	-	NOGC
78	4512740	B/39303/18	24	F	Acne Vulgaris-Grade 1	P. acnes	S	S. aureus	S	S	S	S	S	S	S	S	-
79	4612231	B/39305/18	24	M	Acne Vulgaris-Grade 2	P. acnes	S	S. aureus	S	S	S	S	S	S	S	S	-
80	4516144	B/39307/18	25	M	Acne Vulgaris-Grade 2	P. acnes	I	S. aureus	S	S	S	S	S	S	S	S	-
81	3030836	B/39309/18	25	M	Acne Vulgaris-Grade 1	P. acnes	R	S. epidermidis	S	R	R	S	S	S	S	S	-
82	4597784	B/39311/18	23	M	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	S	S	S	S	S	-
83	3795545	B/39313/18	27	M	Acne Vulgaris-Grade 1	P. acnes	R	S. aureus	S	R	R	S	S	S	S	S	-
84	4600212	B/39315/18	25	M	Acne Vulgaris-Grade 1	P. acnes	S	S. epidermidis	S	S	S	S	S	S	S	S	-
85	4603992	B/39317/18	30	M	Acne Vulgaris-Grade 1	P. acnes	S	S. aureus	S	S	S	S	S	S	S	S	-

ANNEXURE – V

KEY TO MASTER CHART

Amp	-	Ampicillin
Cd	-	Clindamycin
Cip	-	Ciprofloxacin
Cot	-	Cotrimoxazole
Do	-	Doxycycline
E	-	Erythromycin
Gen	-	Gentamycin
P	-	Penicillin
M	-	Male
F	-	Female
NOGC	-	No organisms grown on culture
S	-	Sensitive
I	-	Intermediate
R	-	Resistant

Introduction



Objectives

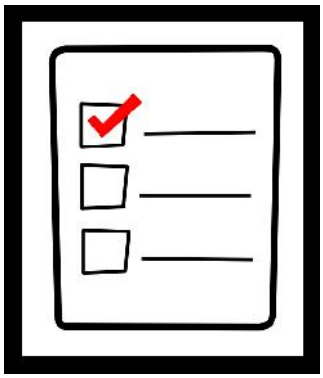
Review of Literature

Methodology

Results

Discussion

Conclusion

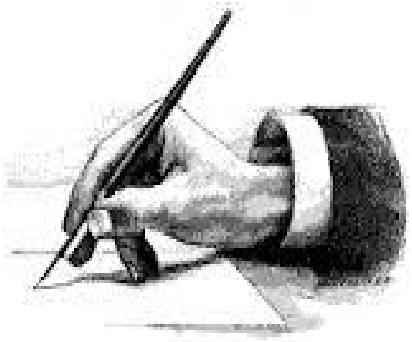


Limitations

Recommendations

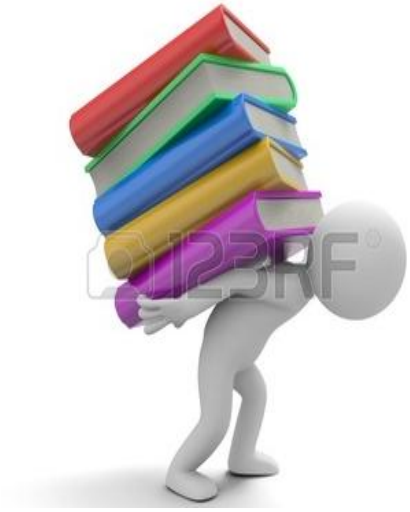
Summary

Bibliography



Annexure-I

Annexure-II



Annexure-III

Annexure-IV

Annexure-V