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EXPLORING STREPTOCOCCUS SPECIES IN UNMARRIED FEMALE ACNE PATIENTS: ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION IN QUETTA CITY

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Abstract

The skin, body's largest organ, which contains millions of microorganisms, is susceptible to various conditions, including acne vulgaris. While most skin microorganisms are beneficial, certain strains may contribute to dermatological issues. Acne, particularly prevalent among teenagers, involves inflammatory responses in the sebaceous glands. This study focused on unmarried female acne patients in Quetta City, aiming to identify bacterial pathogens associated with acne lesions. Pus samples from 100 patients were collected at BMC Quetta's dermatology OPDs. Both PCR and conventional methods were employed for pathogen identification. Results indicated P. acne isolation in 56% of patients through conventional methods and in 65% of patients through PCR methods. S. epidermidis was identified in 71% and 79% of the samples using conventional and PCR methods, while S. aureus detection rates were 76% (conventional method) and 83% (PCR). Furthermore, S. pyogenes was detected in 47% of patients through PCR and 32% of patients using conventional methods. These findings underscore the significance of specific streptococcus species in acne pathogenesis. Such insights contribute to enhance dermatological understanding and targeted treatment approaches.

Keywords: *Acne vulgaris, Dermatology, Female acne, Molecular detection, Pathogen identification, Patients, Quetta city, Skin infections, Streptococcus species*

INTRODUCTION

Acne vulgaris, the most prevalent dermatological condition encountered by dermatologists (1), manifests as a spectrum of skin lesions encompassing comedones, papules, pustules, and scarring. Although commonly associated with adolescence, research reveals its persistence into adulthood (2). Notably, women tend to experience this condition more than men. In women, acne presents as a severe inflammatory skin disorder, primarily affecting areas rich in sebaceous glands, such as the face, upper chest, neck, shoulders, and back (3, 4). The onset of acne is often linked to increased androgen levels during puberty, contributing to its exacerbation. Various forms of acne exist, ranging from scaly, red skin (seborrhea) to the presence of whiteheads, blackheads (comedones), papules, nodules, pimples, and scarring (4). Acne may manifest as either inflammatory or non-inflammatory, with nodulocystic acne exemplifying the former, characterized by the formation of cysts within hair follicles and typically affecting regions such as the groin, buttocks, armpits, and sweat ducts (3). Non-inflammatory acne presents as either closed or open comedones, with the latter known as blackheads, displaying dark hyperkeratotic plugs due to melanin oxidation within follicular openings, and the former, whiteheads, appearing as white to flesh-toned lesions with no open pores (5). The pathogenesis of acne involves several bacterial species, with *Propionibacterium acnes* primarily associated with inflammation, contributing to microcomedo or comedone formation as well as inflammatory lesions like pustules, nodules, and papules (2).

The aim of this study is to employ conventional and molecular techniques to identify and characterize bacterial pathogens isolated from unmarried female acne patients. Acne research builds on existing knowledge

to refine treatment approaches and help address different aspects of this common skin disease. The knowledge accumulated from research contributes to a more comprehensive and nuanced understanding of acne and its treatment. The significance of identifying bacterial pathogens in acne lesions is crucial for guiding treatment decisions, addressing antibiotic resistance concerns, developing personalized therapeutic approaches, and advancing our understanding of the complex factors contributing to acne. This information has practical implications for both clinical practice and ongoing research in dermatology. *P. acne*, a gram-positive, anaerobic, slow-growing rod, plays a pivotal role in the development of inflammatory acne by inhabiting pilosebaceous follicles. Additionally, it may exert a physiological role in inhibiting pathogenic bacteria such as *S. aureus* and group A *S. pyogenes*, allowing other commensal strains like *S. epidermidis* to thrive (6). *S. epidermidis*, co-agulase negative gram-positive cocci, is a common inhabitant of human skin and mucosal surfaces. While typically nonpathogenic, it may cause infections, particularly in individuals with compromised immune systems (7). The diagnosis of acne is primarily clinical, and treatments vary from topical agents such as clindamycin and erythromycin, oral retinoids for severe cases, and oral antibiotics like tetracyclines and erythromycin, targeting *P. acnes*. Acne scarring is amenable to various treatments, including collagen infusions, autologous fat exchange, laser resurfacing, and surgical interventions (8).

In Pakistan, similar studies were conducted in Quetta and Islamabad focusing on identifying different risk factors linked to an infection of acne vulgaris (9), as well as a correct comprehension of the pathogenicity of acne and how it may be effectively managed (10). A cross-sectional survey was carried out in 2010 among medical students to find out what the undergraduates in Karachi thought about acne (11). Another study on the frequency of various acne-causing bacteria was carried out in Haripur (12).

MATERIALS AND METHODS

The study was conducted in Quetta, the capital of Balochistan, with research sites including Bolan Medical Hospital and the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB) at the University of Balochistan. Pus samples originating from acne-afflicted individuals seeking care at the Dermatology Department outpatient clinics of Bolan Medical Hospital were the focal point of data collection. For the study, a cohort of 100 unmarried female patients, ranging in age from 14 to 30, was carefully chosen. Acne is most common during adolescence and the teenage years. It usually begins during puberty, when hormonal changes stimulate the sebaceous (oil) glands in the skin. These glands become more active, leading to an increased production of sebum, which can clog pores and contribute to the development of acne. Some individuals may continue to experience acne into their 20s, 30s, and even beyond. Adult-onset acne is not uncommon, and factors such as hormonal fluctuations, genetics, and certain lifestyle factors can contribute to its development in adults. Due to some ethical considerations only females patients were selected and as hormonal fluctuations differ between males and females thus only one gender was selected. Acne is often influenced by hormonal changes, and unmarried females may have more stable hormonal profiles compared to those who are pregnant or using hormonal contraceptive. Patients were provided detailed information about the research, including its objectives, procedures, potential risks, benefits, and the use of their samples. They were given the opportunity to ask questions and make an informed decision about whether to participate. Pus samples were taken from patients on sterile swabs. These samples were then brought to the University of Balochistan's CASVAB's Bacteriological Laboratory for examination. The acne samples were put into 5 ml Falcon tubes containing nutrient broth in the lab. The samples were thoroughly vortexed for proper mixing and then divided equally. Half of the sample volume (2.5 ml) was transferred to another Falcon tube and placed within an anaerobic environment, achieved using an anaerobic jar. The remaining 2.5 ml was maintained in an aerobic environment. The samples from both sets were subsequently incubated for 24 hours at 37 °C.

BIOCHEMICAL IDENTIFICATION

Bacterial pathogens were then identified through a combination of staining techniques and biochemical assays. Following the 24-hour incubation period, bacterial cultures were aseptically retrieved from the nutrient broth using sterilized loops. Subsequently, these cultures were carefully inoculated onto specialized agar plates, including Reinforced Clostridium Medium (RCM), Mannitol Salt Agar (MSA), and blood agar plates. Incubation

ensued for an additional 24 hours, meticulously maintained at a temperature of 37ºC. Diverging environmental conditions were provided for the aerobic and anaerobic samples, with the former placed within an exclusively aerobic environment and the latter secured within an anaerobic jar. Following this incubation period, growth patterns were meticulously observed on the agar plates. The comprehensive panel of conventional biochemical tests encompassed Catalase, Oxidase, Indole, Methyl Red, Voges-Proskauer, and Nitrate Reduction assays, alongside assessments of sugar fermentation profiles (13). These tests facilitated the precise identification of the bacterial isolates under investigation.

MOLECULAR IDENTIFICATION

In the molecular identification process, DNA extraction was performed using the boiling method. Colonies from agar plates were mixed with 300 µl of 1X TE buffer in Eppendorf tubes, followed by boiling for 10 minutes at 95°C. After centrifugation, 60 µl of supernatants were preserved at -20°C until PCR analysis. PCR was carried out using specific primers targeting organisms of interest: *S. epidermidis*, *P. acnes*, *S. aureus*, and *S. pyogenes*. The PCR reagent concentrations included 15 µl of PCR master mix, 2 µl of DNA, 1 µl of each primer (forward and reverse), and 6 µl of molecular grade water, resulting in a total volume of 25 µl. PCR cycling conditions varied for each organism, with a touchdown PCR method employed. Following PCR, gene amplification was confirmed through 1% agarose gel electrophoresis using a 50 bp DNA ladder (14) Amplified products were stored at -20°C, and positive controls were sourced from CASVAB Quetta.

RESULTS AND DISCUSSION

In this study, a total of 100 acne patients were observed who visited the dermatology department of BMCH Quetta. Samples were collected and analyzed through both conventional and molecular methods to detect pathogens that were involved in acne pathogenicity.

Table I. The detection rate of acne pathogens in isolated samples

Total (n=100)	*P. acne*	*S. epidermidis*	*S. aureus*	*S. pyogenes*
Biochemical Identification	P= 56	P= 71	P= 76	P= 32
	N= 44	N= 29	N= 24	N= 68
	56%	71%	76%	32%
Molecular Identification	P= 65	P= 79	P= 83	P= 47
	N= 35	N= 21	N= 17	N= 53
	*9	*8	*7	*15
	65%	79%	83%	47%

* Number of samples that did not show growth on culture method; P = positive, N = negative

Molecular methods are more sensitive and reliable methods for identifying bacterial pathogens, focusing on the analysis of genetic material at the molecular level and offering high sensitivity and specificity. On the other hand, conventional methods are lengthy, time-consuming, involve a wide range of techniques, may not include molecular components, and may have low specificity and sensitivity. The results of this study are consistent with those of a study conducted in Baghdad, where PCR showed positive growth rates of 50% for *P. acnes*, 29% for *S.epidermidis*, and 24% for *S.aureus* (15). According to the findings, colonies of *P. acne* were round, opaque, white and yellow in size, 1–2 mm, and exhibited Gram-positive bacilli under the microscope. The *S. epidermidis* colonies appeared as white, opaque, smooth Gram-positive cocci, and the *S. aureus* colonies appeared as smooth golden-yellow Gram-positive cocci with grape-like clumps and were mannitol-fermenting bacteria. A study conducted in Iraq found that colonies of *S. pyogenes* on blood and chocolate agar exhibited beta-hemolysis, were negative for catalase, and showed Gram-positive cocci under the microscope (16). In Syria, *S. aureus* colonies isolated from acne samples were yellow in color and exhibited Gram-positive cocci and fermented maltose under the microscope (17).

As most females are affected by acne, the current study was simply exposed to females. In Pakistan, a high prevalence of acne was found in females. A study from Quetta and Karachi showed 64% and 78.4% of acne patients were females (9, 11). These findings showed that females were more likely to be affected by acne than males; it may be because of hormonal changes in females that are associated with acne. An imbalanced level of

hormones disturbs the sebum production of the body, and variations in estrogen and androgen levels can also cause acne. Women experience more hormonal acne than men, mostly in puberty or a week before their periods. Other factors that are involved in the pathogenesis of acne are environmental factors: cosmetics, hygiene, diet and certain hair or skin products can clog pores and cause comedonal acne (18).

Table II. Sociodemographic details of patients involved in this study

Features	Type	Percentage (%)
Types of acne	Mild	31.0
	Moderate	52.0
	Severe	17.0
Type of skin	Oily	78.0
	Dry	22.0
Family history of acne	Yes	27.0
	No	73.0
Types of treatments applied	Topical and systemic	17.0
	Topical	24.0
	Non	59.0

Human skin is of different types relying upon different internal (genetic) or external (environmental) factors. On the basis of these factors human skin is classified into; normal, oily and dry skin. It has been found that acne is mostly found on oily skin because these types of skin have high sebaceous glands. Many people have oily skin and under each pore, there are sebaceous glands that produce sebum. More production of sebum creates oily skin. Sebum mixes with the dead cells of the skin and blocks the pores, which causes acne pathogens to grow and as a result, it may cause acne (9). Table II shows that 78% of acne patients had oily genetic or environmental factors. It may be transferred from parents to their children. Table II shows skin and 22% had dry skin. Contrary to our findings, a review conducted in the same city showed that 70% of patients had oily skin, 15% had dry skin, and 15% had normal skin type (9). 62% of patients had oily skin and 38% had dry skin (12). Acne might be caused by that 27% of patients had a family history with respect to acne, and 73% had no family history. The results of our study are strongly similar to those of previously published studies (19, 20).

The majority of patients with acne were using topical antibiotics, such as erythromycin and clindamycin, which come in a variety of forms; including gels, lotions, solutions, saturated pads, and combinations with benzoyl peroxide. These antibiotics reduce the amount of bacteria that cause acne which are found in pilosebaceous follicles. Additionally, patients utilized minocycline, doxycycline, erythromycin, clindamycin, and cotrimoxazole as systematic antibiotics, all of which target the pathogens that cause acne (21). Retinoids and isotritinion were also being used by patients as systematic treatments. In 1970s, when all previous therapies for nodulocystic acne failed, patients responded to systemic isotretinoin therapy; therefore, this would be the ideal systematic treatment for severe acne (22). Biochemical tests like Catalase, Oxidase, Indole, Methyl Red, Voges Proskauer's, Nitrate Reduction and Sugar fermentation tests were applied for identification of these pathogens.

Table III. Biochemical tests for the identification of *P. acne*

S. no	Biochemical tests	*P. acne*
1	Catalase test	+
2	Indole test	+

BIOCHEMICAL IDENTIFICATION

The *P. acne* isolates were catalase-positive and Indole-positive (Fig. 1), similar to previous studies conducted in Japan and Iraq (23, 24).

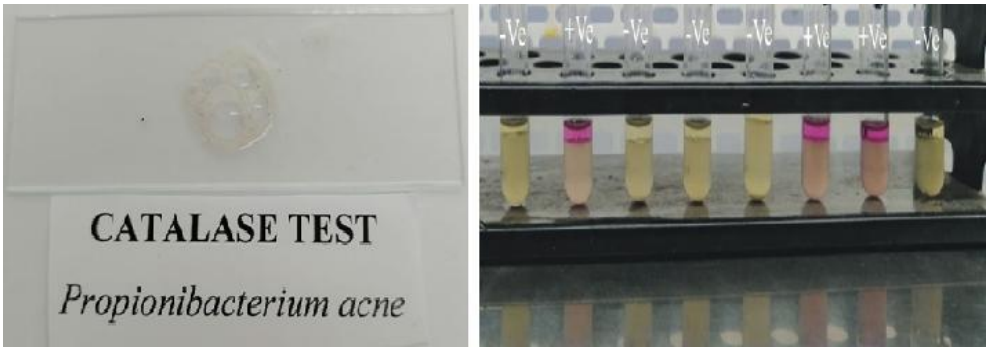

Fig. 1. Catalase test and Indole test for identification of *P. acne*

The results of the biochemical tests shown in Table IV for *S. epidermidis, S. aureus and S. pyogenes* were similar to different studies conducted in Australia (25), Bangladesh (26), and Egypt (27).

Table IV. Biochemical tests for the identification of *S. epidermidis, S. aureus and S. pyogenes*

S.no	Biochemical test	*S. epidermidis*	*S. aureus*	*S. pyogenes*
1	Catalase test	+	+	-
2	Oxidase test	-	-	-
3	Co-agulase test	-	-	NA
4	Nitrate Reductase test	+	+	NA
5	Methly-Red test	-	+	+
6	Vegos-Proskauer test	+	+	-
7	Indole Test	NA	-	-
8	Galactose	+	NA	+
9	Maltose	+	+	+
10	Fructose	+	+	+
11	Arabinose	-	-	-
12	Lactose	+	+	+
13	Mannose	+	+	+
14	Sucrose	+	+	+
15	Xylose	-	-	-
16	Glucose	+	+	+
17	Hemolysis	NA	NA	β hemolysis

The findings of this study revealed 56% positive results for *P. acne* isolates through conventional methods; similar studies in Egypt and Jordan revealed 65% and 87% positive results for *P. acne* (19, 28). According to current findings, the detection rate of the *S. aureus* pathogen was 56%; similarly, studies in India showed 65.65% and 78.75% positive results for *S. aureus* through the conventional method (29, 30). And the detection rate of *S. pyogenes* in acne patients was 32% through the conventional method, which closely resembles a previous study conducted in Egypt (27).

MOLECULAR IDENTIFICATION

Molecular method is an appropriate and a reliable method for identification of several pathogens isolated from acne samples. In this study, PCR method was used for identification of *P. acne, S. aureus, S. epidermidis and S.pyogenes* (31). The current study shows a total 65% identification rate for *P. acne,* which is similar to the results obtained from China (26), Iraq (32) using the same primers (PRA) with the same amplification size of 1202 bp, and Israel (33). In this study, a total of 79% of samples showed the presence of *S. epidermidis* through PCR. Similar results of 81.5% and 68.75% were found in two different studies conducted in Iraq (23, 31). **Fig 2 (a).** Gel electrophoresis (2% agarose) showed an amplified product of *P. acne* (1202 bp), lanes 1 to 19 positive for *P. acne*. **Fig 2 (b).** Gel electrophoresis (2% agarose) showed the amplified products of *S. epidermidis* (194 bp), Lanes 1 to 13 positive for *S. epidermidis*. **Fig 2 (c).** Agarose gel (2%) electrophoresis shows the amplified product of *S. aureus* (270 bp) by PCR. Lanes 1, 2, 4, and 6 were positive for *S. aureus*, PC: Positive control, NC: Negative control. **Fig 2 (d).** Gel electrophoresis (2% agarose) showed amplified products of *S. pyogenes* (407bp) by PCR. Lanes 1 to 11 positive for *S. pyogenes*, PC: Positive control, NC: Negative control.

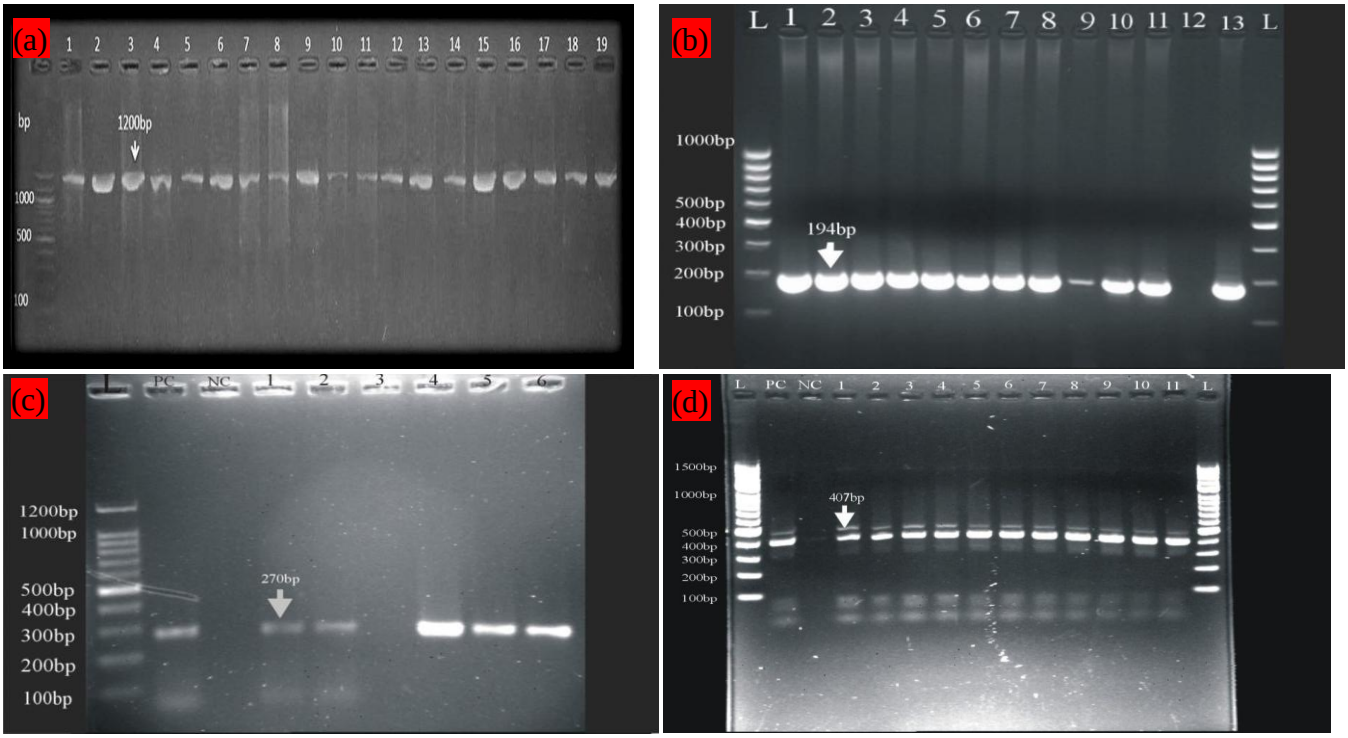

Fig. 2 (a). PCR based detection of *P. acne* in acne samples. The 1202bp amplification of targeted gene of *P. acne* is shown in sample number 1-19. DNA ladder is denoted by L. **(b).** PCR based detection of *S. epidermidis* in acne samples. The 194bp amplification of targeted gene of *S. epidermidis* is shown in sample number 1-11 and13. DNA ladder is denoted by L. **(c).** PCR based detection of *S. aureus* in acne samples. The 270bp amplification of targeted gene of *S. aureus* is shown in sample number 1,2,4,5,6. DNA ladder is denoted by L while PC and NC represents positive and negative control respectively. **(d).** PCR based detection of *S. pyogenes* in acne samples. The 407bp amplification of targeted gene of *S. pyogenes* is shown in sample number 1-11. DNA ladder is denoted by L while PC and NC represent positive and negative control respectively.

CONCLUSION

In conclusion, our research sheds light on the pervasive impact of acne lesions within the Quetta population, transcending urban and rural boundaries. Beyond the spectrum of benign non-inflammatory manifestations, such as whiteheads and blackheads, our study underscores the escalation of this condition to more severe and distressing inflammatory forms, including papules, pustules, and nodules. Importantly, this research goes beyond the technicalities, emphasizing the profound influence of these microorganisms on individuals' lives. The prevalence of these problematic microorganisms in our samples signifies their relevance as a pertinent concern for the community.

It is paramount to recognize that untreated acne can pose substantial health risks. Our findings necessitate an urgent understanding of the potential complications and the importance of proactive management. This study serves as a crucial reminder, emphasizing the need for preventative measures to mitigate the impact of these troublesome organisms. Specifically, for women, our research holds profound implications, urging vigilance and providing insights into mitigating the presence and impact of these bacteria. The reality, illuminated by our results, is that these microorganisms are not confined to clinical settings but pervade various aspects of daily life, potentially leading to complications if left unattended.

In essence, our research aspires to provide more than scientific insights; it aims to empower individuals, especially women, with the knowledge to safeguard their well-being and quality of life. By framing the study in this context, we intend to make our findings not only informative but also engaging and relevant to a broad readership. Our goal is to communicate the vital importance of proactive measures and responsible healthcare practices in daily life, ultimately contributing to the enhancement of dermatological health and overall well-being.

Based on our research, it is evident that acne is more prevalent among teenagers, emphasizing the urgency of early treatment to control these pathogens and prevent significant damage to the skin. We recommend the use of authentic facewash or cleansing agents to prevent sebum production and acne formation. Oral and topical antibiotics should only be employed under the recommendation of dermatologists.

Moving forward, future research should explore the long-term impact of different dermatological interventions and their effectiveness in diverse demographic groups. Additionally, investigating the socio-economic factors influencing access to dermatological care and preventative measures could provide valuable insights. By addressing these limitations and unanswered questions, future research can contribute to the development of targeted and accessible dermatological health practices, fostering improved public health outcomes.

Conflict of Interest:

All authors declare no conflict of interest

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