GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Diagnosis of bacteria isolated from patients with cutaneous eczema by PCR technique at Azadi Hospital, Kirkuk, Iraq

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GSC Biological and Pharmaceutical Sciences, 2024, 28(03), 240–247

Publication history: Received on 13 August 2024; revised on 20 September 2024; accepted on 23 September 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.3.0338

## Abstract

Eczema is a common skin condition that can be worsened or caused by a bacterial infection. This study aimed to investigate the bacterial pathogens associated with skin eczema in patients admitted to Azadi Hospital in Kirkuk using polymerase chain reaction (PCR) technology. The goal was to enhance the accuracy and efficiency of bacteria identification compared to traditional methods. A total of [300] patients suffering from symptoms of skin eczema between the ages of 1-40 years, females and males, were included. Bacterial isolates were obtained from skin samples, and conventional microbiological techniques were initially used for identification. Subsequently, molecular analysis using PCR was performed to confirm and characterize the bacterial species. The study included 165 male patients represented (55%), 135 female (45%), of participants who had symptoms of skin eczema. The PCR test targeted specific genes associated with common bacteria implicated in skin infections, such as *Staphylococcus. aureus* (33 %), *Staphylococcus epidermidis* (28%), *Klebsiella pneumoniae* (20 %) , *pseudomonas aeruginosa* (13 %), respectively, and the remaining( 6 %). of the samples were natural flora. The genetic fingerprints of these bacterial species were identified, an image of them was taken on an agarose gel electrophoreses , and the results were compared with those obtained through traditional methods to evaluate the sensitivity and specificity of PCR in diagnosing bacterial infections in cases of skin eczema. Preliminary results indicated that PCR significantly improved the accuracy and speed of bacterial identification compared to traditional methods. It has allowed the detection of specific bacterial species that might be missed using conventional methods. The prevalence and diversity of bacterial species causing skin eczema in Azadi Hospital in Kirkuk were also explored. This study contributes valuable insights into the microbial etiology of cutaneous eczema, emphasizing the importance of molecular techniques in accurate and rapid diagnosis. The findings may guide more targeted and effective treatment strategies for patients with bacteria-associated skin eczema, ultimately improving patient outcomes and reducing the risk of complications.

**Keywords:** (PCR) for skin eczema; Multiplex DNA fingerprinting; Gene primers

## 1. Introduction

Many different types of microorganisms, the majority of which are gram-positive, reside in the skin. . Most of the these bacteria are found on the outer surface layers of the skin as natural micro flora [1]. These microbes on the skin live as a diverse group in environments that vary in temperature, acidity, humidity, and oil content. The skin, as an ecosystem, includes living biological and physical components that occupy diverse habitats for microorganisms, and can help us understand the delicate balance that exists between microorganisms and the host. [2]. Eczema can begin in childhood, adolescence, or adulthood and can be mild, moderate, or severe. Newborn babies may develop dermatitis in the first few weeks and months after birth. [3]. Extremely dry and irritated skin patches that can lead to blisters or infections appear on the skin from excessive scratching in young children. [4]. However, adults may also be affected and it usually appears at different ages in people over 40 years of age or in their 30s [5]. The term "eczema" refers to a group of inflammatory skin disorders that can lead to the appearance of blisters, scaly areas, rashes, dry skin, itching, and various

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infections. There are seven types of dermatitis, which are atopic dermatitis, contact dermatitis, dyshidrotic eczema, scaly eczema, and dermatitis. Oily skin, stasis dermatitis. [6]. Eczema can appear as red, irritated skin in people with light skin and appear grey, brown, or purple in people with dark skin. [7]. Atopic dermatitis may lead to cracked skin and the appearance of ulcers if it is not diagnosed correctly and early so as not to affect the health and psychological state of the patient [8]. Eczema can have multifactorial origins. Bacterial infections are recognized as potential aggravating factors or major causes of this dermatitis, which is a long-term disease but is not contagious. [9]. The skin is considered the skin's first line of defense against the environment and acts as a barrier to protect it. Most people with eczema have inherited problems with the epidermis, or outer layer of skin. [10] However, healthy skin significantly prevents water absorption by the outer layer of the skin and the entry of microorganisms and environmental pollutants into the human body. [11] In cases of eczema, this barrier is less effective and more permeable [12]. Thus, compromised skin barriers, microbial or allergic colonies on the skin, and decreased immune responses are major causes of skin eczema. [13]. In the context of cutaneous eczema, bacterial colonization can complicate the clinical presentation and contribute to the persistence of symptoms. Patients of different ages visit Azadi Hospital in Kirkuk with skin eczema who seek medical care, necessitating a comprehensive understanding of the bacterial pathogens involved to identify bacterial strains associated with eczema and to help develop diagnostic methods or medications to protect the patient from infection [14]. Bacterial colonization of eczematous lesions not only complicates the clinical course but also presents challenges in terms of accurate and timely diagnosis. Traditional microbiological methods involve culturing bacterial isolates from clinical specimens, a process that requires extended incubation periods and may ignore highly susceptible or slow-growing bacterial species. The advent of molecular diagnostic techniques, especially polymerase chain reaction (PCR), has revolutionized the field of microbiology by allowing rapid, targeted amplification of specific DNA sequences. The application of PCR in dermatology has shown promising results in improving the diagnostic landscape, providing a molecular-level understanding of the microbial factors involved in skin eczema. Eczema screening methods include screening for candidate genes, genome sequencing, and genome-wide analysis. and phenotypes compatible with other complex characteristics. Monogenic genetic mutations associated with eczema-like manifestations [15]. This study sought to investigate the bacterial profile associated with skin eczema in patients at Azadi Hospital in Kirkuk, using PCR as a diagnostic tool. By targeting specific genes associated with common bacterial pathogens, PCR enables a more targeted and efficient identification process, potentially revealing microbial agents that may be overlooked using traditional microbiological methods. [16]. As a result, many skin microorganisms can be identified and classified using 16S metagenomic sequencing in a culture-independent manner. [17]. However, the 16S ribosomal subunit possesses multiple hypervariable regions that allow taxonomic classification of bacteria, although they are conserved across the same lineages and species and differ from prokaryotes in other ways. [18]. DNA extraction and analysis is crucial to performing DNA analysis and is essential to the analysis of this procedure. [19]. PCR methods, especially Multiplex PCR and RAPD-PCR, offer the possibility of accelerating the identification of many bacteria. [20]. The present study used a multiplex PCR method for molecular identification and confirmation of frequently occurring pathogens in bacteremia in cutaneous eczema. To prevent nonspecific primers, a unique sequence was chosen for each bacterium. [21]. The purpose of this study was to use multiplex PCR to identify common bacteria causing skin eczema Understanding the microbial landscape of skin eczema at Azadi Hospital is critical to inform personalized treatment strategies, improve patient care, and reduce the risk of complications associated with bacterial infections. The incorporation of PCR technology into routine diagnostic protocols holds the potential to revolutionize the accuracy and speed of bacterial identification in cases of skin eczema, ultimately improving patient outcomes and contributing to the comprehensive management of this skin condition.

## 2. Methodology

### 2.1. Study design and participate

In this study, the researcher collected study samples from patients with septic eczema coming to Azadi Hospital, both males and females. The number of research samples was 300 samples, as the ages of the patients ranged from 1-40 years, and the period that the researcher worked for was from January to May ( 2023) . Swabs were taken from the eczema-affected parts of the face, arm, children’s eczema, and fingers, as shown in Figure 1)

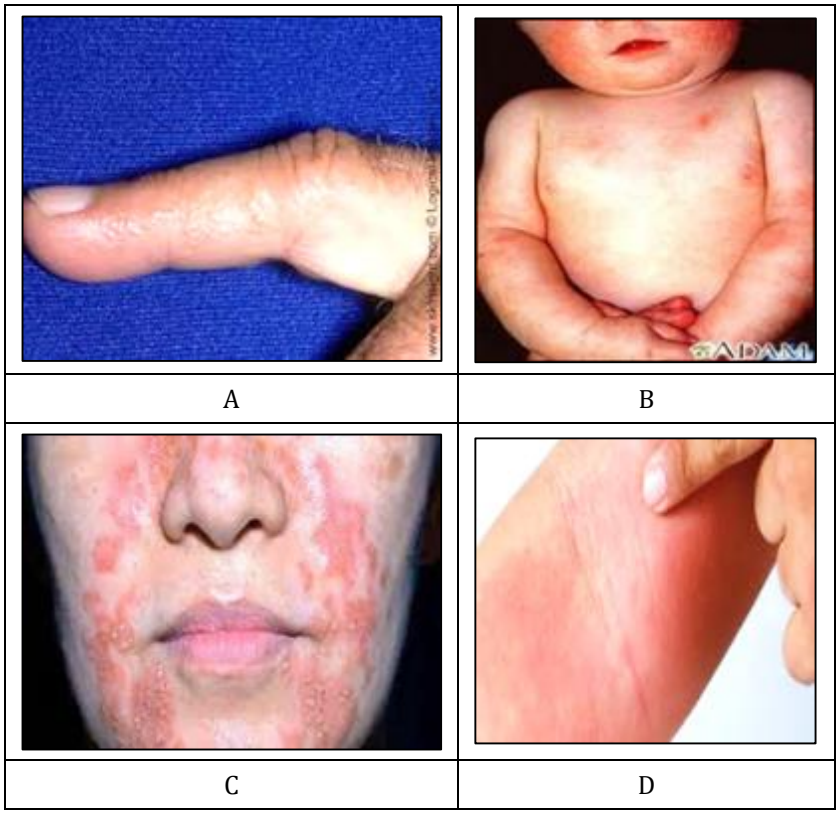


**Figure 1** A- finger Eczema, B- baby Eczema, C-faces Eczema, D-Flexural Eczema

Patients with cutaneous eczema aged 1–40 years, male and female, had varying rates of infection; In children, the incidence was higher than in adults, but in adults [23]. These samples did not include patients who used antibiotics, calcineurin inhibitors, corticosteroids, or immunosuppressive medications in the two weeks before the screening procedures. In addition, breastfeeding and pregnant women were excluded. Before taking samples, patients were asked not to shower for twelve hours and to stop using any personal antimicrobial products for forty-eight hours in order to obtain healthy results in the process of isolating the microbes that cause the eczema condition.

### 2.2. Methodology of taking sample

Microbial skin samples were obtained from a skin lesion, preferably in the vicinity of the antecubital fold. In the absence of antecubital lesions, the most prevalent skin lesion was chosen. Scrub and swab samples were the two types of samples that were gathered. These two samples were concurrently obtained from non-overlapping sections of the same skin lesion. After being rubbed along the antecubital crease for 30 seconds, a sterile, dry, flock-flocked nylon swab (Copan480CE) was first inserted back into the tube containing the liquid Amies Medium[23]. The liquid medium was also extracted using an eppendorf tube. Second, a modified scrub technique was used, which was based on the methodology of Williamson and Kligman.12,21 A sterile PVC sampling ring with an internal diameter of 4 cm was placed just below the antecubital crease. To it, 0.85% NaCl, 0.1% bacteriological peptone, and 0.1% Tween 80 sterile wash fluid (0,75 mL) were added. [25] After using a sterile swab to scrub the skin inside the ring for a minute, the fluid was collected in a 2 ml eppendorf tube. The samples were processed further for DNA analysis and then stored at -80 °C. Regular procedures were used to prepare the bacterial cultures. Isolate cultures were then grown on Nutrient Agar and MacConkey Agar, and they were kept isolated and brooding for 24 to 48 hours at 37 °C. Identification of the bacterial isolates was done by morphological identification, biochemical testing, and first identification of the bacterial species using specific phenotypic traits such as Gram stain and culture characteristics along with antibiotics. Bacterial sensitivity analysis was conducted using the conventional Kirby-Bauer diffusion method[26].

### 2.3. DNA isolation and PCR technique

The bacterial sample was drawn for PCR by scratching cells off a nutrient agar plate using a sterile needle . After that, 1 ml of 1X Tris-EDTA buffer was used to wash the cells twice. The pellet was suspended again in 0.5 ml of distilled antiseptic water and boiled for 10-15 minutes. After boiling, the cells were incubated for ten minutes on ice. Subsequently, centrifugation at 11,500 x g for 5 minutes was done. DNA density was estimated using a spectrophotometer, and the samples were stored at -20ºC until they were used for DNA analysis. After elution, each

sample was processed by multiplex PCR using forward and backward primers. While the reference strains of bacterial gene primers were determined, which present in the following (table 1):

**Table 1** Test for amplification through the used primers

| **Primers name** | **Nucleotide Sequences5\ , 3** | **Targeted species of bacteria** | **Amplicon Size bp** | **References** |
|---|---|---|---|---|
| Fem F<br>Fem R | (5′AGACAAATAGGAGTAATGAT3′)<br>(5′ TCTGTTTATCCTCATTTACTA 3′) | *Staphylococcus aureus* | 723 | Mehrotra *et al*. 2000) |
| Sep F<br>SepR | (5′ CTCAAGTTGGCGAACCTCAG 3′)<br>(5′ GAGTTCAACCGCATTGGAGTC3′) | *Staphylococcus epidermidis* | 651 | J. Soares *el al* 2013 |
| Gyr-B-2F<br>Gyr-B-2R | (5′TCCGGCGGTCTGCACGGCGT 3′)<br>(5′AGGCCGCCAGACGTGCCGCA3′) | *Klebsiella pneumonia* | 411 | Foysal MJ, Rahman MM, Prodhan MS (2013) |
| IntI F<br>IntI R | (5′ GCCTTGCTGTTCTTCTACGG 3′)<br>(5′CGGAACGACAAGAAGATGCC3′) | | 135 | Firoozeh F, el al 2001. |

Here, sequences of DNA oligonucleotide were considered as primers, DNA size prediction for PCR products was done using multiplex PCR for bacteria diagnosis amplification as well as Multiplex PCR was conducted as follows: the multiplex PCR test conditions were standardized by adapting the concentration of each bacterial strain four primer pairs, the optimal temperature strength (55C), Each PCR reaction mixture was prepared in a volume of 25 ml, consisting of 12.5 ml of PCR premix with magnesium chloride, taking an amount of each primer that equal 0.8 mM, 150-200 mg of DNA pattern. The master mix was prepared by adjusting the dNTPs concentration to 0.4 mM, magnesium chloride to 3 mM, and 1.45 U of DNA polymerase was added ., after that amplifying condition of Standardized DNA was obtained from isolated samples. The following criteria have been used to optimize the PCR reaction: Following a denaturation stage that lasted two minutes at 93°C, there was an annealing step that lasted two minutes at 60°C, an extension phase that lasted eighty seconds at 70°C, and an eight-minute supplement step that concluded at 73°C. 35 cycles of successive reactions were carried out. After that, PCR yield analysis was carried out using gel electrophoresis on 1.5% agarose gel that had been treated with Ethidium bromide. Following that, a gel image was selected. Using a fingerprint of DNA as a basis for diagnosing pathogen bacterial strains according to distinct band numbers [27].

## 3. Results

The 300 cases in this study were between the ages of 1 and 40 years, with 165 male patients (representing 55% of the total) and 135 female patients (45%). While a small percentage of the isolates had negative results, the majority showed positive results for cultures. were recognized by means of the conventional system for identifying microbial cultures and isolations (chemical and biological tests). Of the total isolates that could be identified, 99 (33%) *Staphylococci aureus* bacteria, 84 (28%) of the samples contained *Staphylococcus epidermidis*, 60 (20%) contained *Klebsiella pneumoniae* ,39 (13%) contained *Pseudomonas aeruginosa* and 18 (6%) of the samples that were left contained non-pathogenic microorganisms, (normal flora) ( Figure 2) shows Staphylococci aureus bacteria growing on blood agar and (Table 2) shows The number & percentages of bacteria found on eczema patients skin . The isolates obtained from the two sample collection techniques scanning and scraping were identical, indicating that the diagnosis is the same.

**Figure 2** *Staphylococci* cultures on blood agar

**Table 2** The number & percentages of bacteria found on eczema patients skin

| **Bacterial strains** | **Number of patients** | **Percentage** |
|---|---|---|
| *Staphylococcus aureus* | 99 | 33 % |
| *Staphylococcus epidermidis* | 84 | 28 % |
| *Klebsiella pneumonia* | 60 | 20 % |
| *pseudomonas aeruginosa* | 39 | 13 % |
| *natural flora* | 18 | 6 % |
| Total | 300 | 100% |

The process of diagnosing the genes causing the disease by bacteria isolated from those with septic eczema, both males and females, and at specific ages, comes after the isolation and diagnosis by conventional methods. This procedure makes use of electrophoresis, where the molecular weight density of the gene determines how quickly the DNA bands migrate. It's employed in Agarose gel treated with ethidium bromide (ETBR) dye is used in this procedure. For the DNA bands to show up during the migration process, the dye has a radioactive ingredient in it. The study's PCR test is appropriate for amplifying the genes causing the disease factor.( Fem F and Fem R) (Gyr-B-2 F, Gyr-B-2 R), (Sep F, Sep R), (IntI F, IntI R) primers were employed in this procedure. regarding every DNA molecule extracted using the Multiplex PCR technique. The following were the outcomes: Every instance resulted in the Lan 1 molecule being amplified all primers were applied to all isolates, and the final products were (723 bp for *Staphylococcus aureus* ,( 651 bp for *Staphylococcus epidermis* ,( 411 bp for *Klebsellia pneumonia* ) and (135 bp for *Pseudomonas aeruginosa*), as shown in ( figure 2) for one of the following reasons: Lan 2: four primers were used for one isolate, yielding the result (723 bp *Staphylococcus auraus*), Lane 3: four primers were also used the result (*Staphylococcus epidermis* 651 bp), Lan 4: four primers were used the result (411 bp *Klebsellia pneumonia* ) and Lan five: in the same manner,( 135 bp *Pseudomonas aeruginos*a) But since Lane N was a negative result, there was no band as shown in ( figure 2) . The difference between the two methods is that the first method produces faster results than the second method, which is using the four primers and isolates together, and it gave the same results.

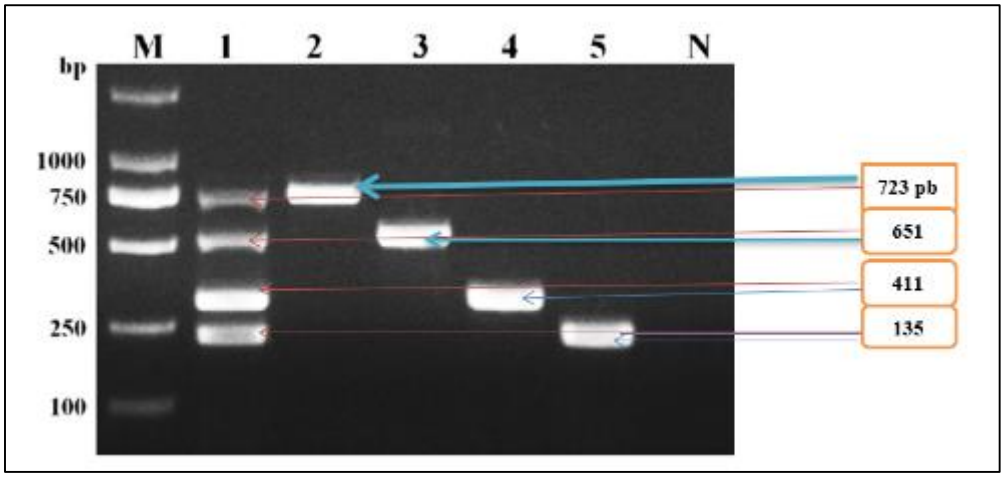


**Figure 3** The following is DNA gel electrophoresis picture of the Bactria strains which created by Multiplex-PCR

Figure (3) Finger printing of the four primer pairs used in multiplex PCR tests for *Staphylococcus aureus, Staphylococcus epidermidis. Klebsellia pneumonia and Pseudomonas aeruginosa,* Multiplex-PCR of all four strains is shown in Lane 1; of *Staphylococcus aureus* (723 bp), *Staphylococcus epidermis* (651 bp), *Klebsellia pneumonia* (411 bp), *Pseudomonas aeruginosa* (135 bp), and Lane N (negative control) .

## 4. Discussion

This work offers a thorough examination of bacterial isolates taken from septic eczema patients, emphasizing the identification of particular bacterial strains and the use of molecular methods for gene diagnosis. Distribution of Bacterial Strains *Staphylococcus aureus* is the most common strain, followed by *Staphylococcus epidermidis, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and non-pathogenic bacteria (flora) . The study also identifies these bacterial strains that are frequently found in patients with septic eczema and this distribution provides insight into the microbiological composition associated with this skin disease. The author used conventional identification techniques (chemical and biological tests), among other conventional techniques, to identify microbial cultures and isolates. Although these techniques are crucial for preliminary identification, they might not have the same level of specificity and precision as molecular methods . Molecular diagnosis was the study incorporates molecular techniques, particularly polymerase chain reaction (PCR), to diagnose the genes responsible for the disease-causing bacteria. By amplifying specific gene regions using primers designed for each bacterial strain, the researcher can achieve targeted and accurate diagnosis. The use of multiplex PCR allows for simultaneous amplification of multiple target genes, enhancing efficiency. Using gel electrophoresis and gel imaging to see the amplified DNA fragments, particular bacterial strains can be identified according to the size of the DNA bands. Each bacterial strain is represented by distinct bands visible in the gel imaging, allowing for a quick and accurate diagnosis. Methods comparison of molecular techniques are compared with traditional culture-based methods for accuracy and efficiency. Although the results from both approaches are reliable, molecular diagnosis has advantages in terms of speed and specificity. Specifically, the multiplex PCR approach simplifies the diagnostic process by enabling the simultaneous detection of multiple bacterial strains in a single assay. Implications for clinical practice management strategies, such as antibiotic selection and infection control measures, can be informed by knowledge of the microbial profile and genetic properties of bacteria linked to septic eczema. Molecular techniques provide a fast and precise diagnosis that allows for the appropriate treatment to be started right away, potentially improving patient outcomes. All things considered, this work emphasizes how crucial it is to combine traditional microbiological techniques with molecular diagnostics for the detection and characterization of bacterial infections, especially in dermatological disorders such as septic eczema. The findings open the door for more focused therapeutic interventions in clinical practice and advance our knowledge of disease pathogenesis

## 5. Conclusion

The microbial makeup and genetic traits of the bacterial strains linked to septic eczema are better understood thanks to this study. The most commonly isolated strain of bacteria found in septic eczema patients was *Staphylococcus aureus*, which the researchers identified using a combination of traditional microbiological techniques and molecular diagnostics. When it comes to identifying the genes responsible for a disease, molecular techniques specifically, multiplex PCR have shown to be effective and faster than traditional methods. The complementary nature of molecular

diagnostics and traditional culture-based techniques is demonstrated by a comparison; molecular methods provide fast and precise diagnosis, which is crucial for prompt clinical management. The results highlight the significance of incorporating molecular techniques into standard diagnostic procedures, improving our comprehension of disease pathogenesis and directing patients' customized treatment plans for septic eczema. Subsequent investigation into the molecular mechanisms of bacterial infections in dermatological conditions such as septic eczema is necessary. Furthermore, by refining diagnosis algorithms and treatment regimens, attempts to integrate these discoveries into clinical practice can enhance patient outcomes and advance the field of infectious disease management.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest is to be disclosed.

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