Isolation and Diagnosis of Staphylococcus Aureus and Candida Albicans from Impetigo, Ecthyma, Bullous Impetigo Patients

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Abstract
One of the skin infections that affect the skin is impetigo, Ecthyma, Bullous impetigo . Impetigo is a highly contagious superficial skin infection. It is a common skin infection in children and is most often caused by Staphylococcus aureus. Impetigo and ecthyma are common bacterial infections of the skin commonly caused by S. aureus and / or Group A streptoccus . Staphylococcus aureus has become the most common causative microbial pathogen of this prevalent childhood disease Impetigo can be bullous or non-bullous. A total of 120 specimens from clinically proven cases of bacterial skin infections, Impetigo (74) (61.7%) , Ecthyma (40) (33.3%), Bullous (6) (5.0%). in the Dermatological Consultation Unit in Marjan Hospital / Babylon Governorate. For the period from September 2022 to December 2022. Pathogens were diagnosed using direct examination, culture on differential media, and biochemical tests. Staphylococcus aureus (18%), Candida albican(18%),Staphylococcus epidermidis (9%) , Staphylococcus saprophyticus(10 %) , Psedomonas. aeruginosa (7%). The aim of this study is Isolation and diagnosis of Staphylococcus aureus and candida albicans from impetigo , Ecthyma , Bullous impetigo patients .
Keywords: Staphylococcus aureus , Candida .albicans , skin infections , impetigo , Ecthyma , Bullous impetigo .

1. Introduction

Impetigo is a highly contagious bacterial infection of the surface layer of the skin. Impetigo usually begins as erythematous macules on the skin, which rapidly develop into a thin, fluid-filled vesicle that becomes purulent, ruptures, and forms a thin, honey-colored crust(Bangert et al ., 2012 ; Miller et al ., 2022). It is most prevalent in children aged 2-5 years old but can occur at any age. The peak incidence is during summer and fall. Bullous impetigo is more common in infants.
Impetigo clinically presents as a bullous or non bullous type. Non bullous impetigo is the most common form of impetigo and is caused by S. aureus in 80% of cases and group A β-hemolytic Streptococcus alone or in combination with S aureus in the remainder (Cole and Gazewood, 2007 ; Nardi and Schaefer , 2017).
Bullous impetigo is a common skin infection of early childhood, resulting from by S. aureus exfoliative toxins. Due to compromised barrier function and immune dysregulation children (Mannschreck et al ., 2020). Bullous or blistering diseases are caused by a variety of traumatic, infectious, inflammatory, and autoimmune conditions (Horlings et al .2020). Bullous impetigo is almost exclusively caused by S aureus (Cole and Gazewood, 2007 ; Hartman , 2014).
Ecthyma is a form of ulcerative impetigo which erodes through epidermidis and dermis. Mostly, the crusts are found on the lesions, and when the crust is removed, there is a purulent ulcer at the bottom(Karaaslan et al., 2022). Ecthyma is often referred to as deep impetigo because it extends into the dermis. It begins with a small, pus-flled blister and red border , which eventually leaves a crusty ulcer underneath. Ecthyma is characterized by purulent, shallow ulcers with a punched-out appearancee. Overlying the ulcer is a thick, brown-black crust and surrounding erythema (Potter et al, ,2016).
Bacterial skin infections represent one of the major healthcare issues affecting people worldwide (Tognetti et al 2012). The skin microbiome is an ecosystem comprised of a multitude of microbial species interacting with their surroundings, including other microbes and host epithelial and immune cells(Laurice and Grice2020). S. aureus and S. epidermidis may inhabit the human skin and other microbes(Skowron et al 2021). 2021).
Common infections of the skin such as impetigo and scabies represent a large burden of disease globally, being particularly prevalent in tropical and resource-limited settings(Taiaroa et al.,2021).
Microbial diversity is reduced in AD and inversely correlates with diseas e severity. Skin commensal microbes, including coagulase-negative staphylococci (CoNS), may aid skin homeostasis and provide protection against S. aureus. Thus, the diminution of commensal skin microbiota with flares may promote S. aureus colonization and infection in AD. During flares of paediatric AD, both Staphylococcus epidermidis and S. aureus are increased, suggesting a compensatory role for S. epidermidis (Alexander et al., 2020).

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impetigo is a common skin infection in children and is frequently caused by Staphylococcus aureus. Each year in the United States, about 400,000 patients in hospitals become infected with S. aureus, The S. aureus is a gram-positive organism with aerobic to facultative anaerobic lifestyle and colonizes skin, nares, and axillae of humans. S. aureus is a catalase-positive organism with most strains secreting coagulase and it ferments mannitol sugar to lactic acid. Testing for catalase is an important criterion to distinguish Staphylococci from Streptococci and coagulase test for distinguishing S aureus from S epidermidis. Staphylococcus aureus is a non-spore forming coccus. The pathogenesis of S .aureus is caused by many virulence factors and their mechanisms into invasion and inflammation, which include colonization, synthesis of extracellular molecules, which promote adherence, and the ability to avoid host defenses; secreted virulence factors such as toxins (Al-Mebairik et al., 2016). S. aureus causes several forms of human infections and syndromes, especially infections of the skin and soft tissue (Kobayashi et al.2015).

2. Material and method

Bacteriological analyses and strains

The S. aureus was identified in all samples by conventional methods (21). A group of 120 strains were selected, previously collected at Marjan Medical Hospital in Babel Governorate/Iraq. All originated from patients suffering from skin infections or bullous impetigo.

All specimens and samples were transferred in sterile transport swabs and inoculated on culture of selective medium, namely Blood and Mannitol salt agar , Sabouraud Dextrose Agar , using the direct method of inoculation. They were then inoculated at 37°C for 18-24 hours .

3. Results and Discussions

3.1 Demography of the study populations

A total 120 clinical specimens were collected in the current study from patients attending the medical consultation department, dermatology unit at (Marjan Medical City) in Babylon Province.

Table (1) show that less than two third (65%) of types of disease were impetigo as represented in figure (1).

Table 1: Frequency and percentage of types of disease

		Frequency	Percent
Types of disease	Impetigo	78	65.0
	Ecthyma	36	30.0
	Bullous	6	5.0
	Total	120	100.0

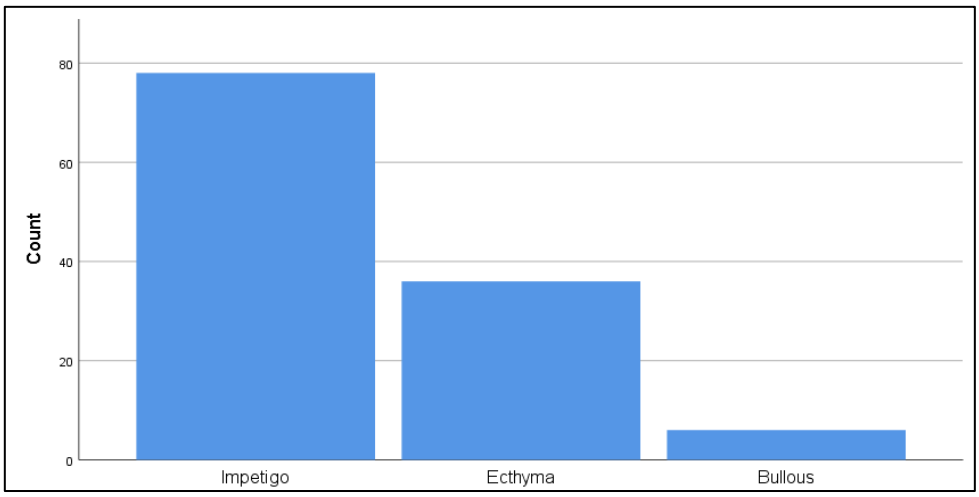

Figure 1: Distribution of types of impetigo in studied population

Bullous impetigo is a bacterial skin infection caused by Staphylococcus aureus that results in the formation of large blisters called bullae, usually in areas with skin folds like the armpit, groin, between the fingers or toes, beneath the breast, and between the buttocks. It accounts for 30% of cases of impetigo, the other 70% being non-bullous impetigo (Hartman-Adams et al .,2014). Bullous impetigo is only caused by S. aureus and accounts for approximately 10% of cases, most often seen in infants (Russell et al.,2019)

Impetigo is a highly contagious, superficial skin infection that most commonly affects children two to five years of age. The two types of impetigo are nonbullous impetigo (i.e., impetigo contagiosa) and bullous impetigo (Brown et al., 2003). Ecthyma is a deep tissue form of impetigo. It is characterized by crusted sores beneath which ulcers form with a "punched out" appearance and it is more common in children, older people and immunocompromised people or in conditions of poor hygiene and hot humid weather. Streptococcus pyogenes and Staphylococcus aureus are the bacteria responsible for ecthyma (Demircioglu and Oren, 2008).

The results in Table (3) and Figure (3) provides an overview of the distribution of different types of infections among different age groups, showing the distribution of different types of infection (Impetigo, Ecthyma, Bullous impetigo) among various age groups. The table also includes the number and percentage of cases for each type of infection within each age group. Age Group 1-10 Impetigo: 42 cases

(35%), Ecthyma: 12 cases (10%) and Bullous impetigo: 4 cases (3.3%) in case of Age Group 11-20: Impetigo: 8 cases (6.7%) , Ecthyma: 8 cases (6.7%), Bullous impetigo: 0 cases (0%) while in Age Group 21-30: Impetigo: 6 cases (5%), Ecthyma: 6 cases (5%) and Bullous impetigo: 2 cases (1.7%). There are three types of impetigo: non-bullous, bullous, and ecthyma. The most common form is non-bullous impetigo (NBI), also called impetigo contagiosa, accounting for almost 70% of cases (Cole and Gazewood, 2007).

Table 2: distribution of infection type according to Age group

Age Group	Type of infection						Total
	Impetigo		Ecthyma		Bullous impetigo		
	No.	%	No.	%	No.	%	
1-10	42	35	12	10	4	3.3	58
11-20	8	6.7	8	6.7	0	0	16
21-30	6	5	6	5	2	1.7	14
31-40	12	10	4	3.3	0	0	16
41-50	4	3.3	8	6.7	0	0	12
51-60	2	1.7	2	1.7	0	0	4
Total	74	61.7	40	33.3	6	5	120

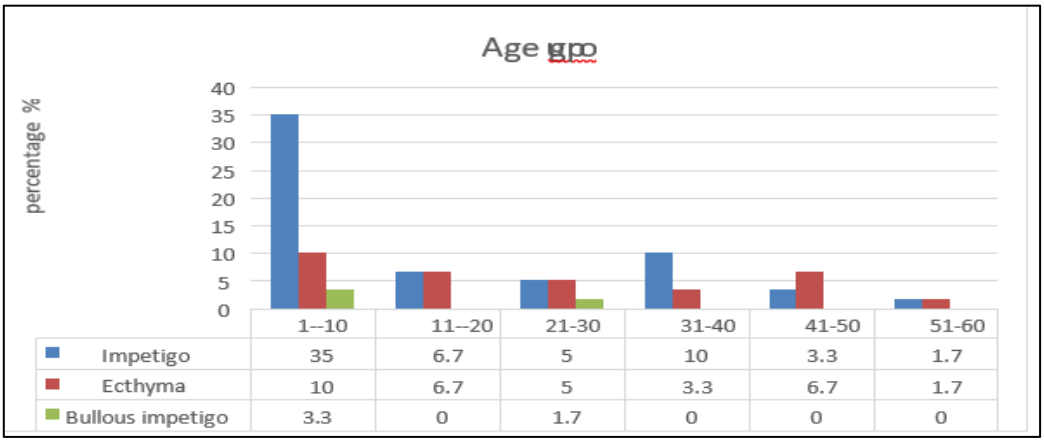

Figure 2: distribution of infection according to age group

Impetigo is the most common infection among all age groups, accounting for 61.7% of the total cases (74 out of 120). It is predominant in all age categories, with the highest number of cases in the (1-10) age group (42 cases). This finding suggests that Impetigo is a common skin infection that affects individuals across different age ranges, but it is more prevalent among children. The global prevalence of impetigo is estimated to be 11.2%, being 2.5-fold higher in children (12.3%) than adults (4.9%) (Hay et al.2014).

In study done by Elisa et al (2022) found that only 6% of children in Italy have at least an episode of NBI, a lower prevalence than that reported in the international literature. Furthermore, our results show a significantly decreasing IR trend over time with the highest value in children 1–4 years old. Different from ours, all the above-mentioned studies considered a broad definition of impetigo, including the bullous form, which seems to be more prevalent in children aged 2–16 years and could reflect a higher IR (Shallcross et al,2013).

Ecthyma is the second most common type of infection, constituting 33.3% of the total cases (40 out of 120). It is distributed relatively evenly across the age groups, with a higher concentration in the 1-10 and 11-20 age groups (both with 8 cases each). This data suggests that while Ecthyma is not as prevalent as Impetigo, it affects individuals of various age groups. Bullous impetigo has the lowest number of cases, making up only 5% of the total (6 out of 120). It is primarily found in the 1-10 age group, with 4 cases reported. This indicates that Bullous impetigo is less common compared to the other two types of infection, and it is most commonly seen in children.

2. Biochemical test and virulence factors for microorganisms

Biochemical tests are essential in microbiology to identify and differentiate various microorganisms based on their metabolic activities. Virulence factors are specific characteristics or molecules possessed by certain microorganisms that enable them to cause disease or infection. The result in table (4) shows the biochemical test use for identification of Candida albicans and Staph aureus. The most important virulence is the biofilm formation all Staph aureus isolates were positive while 22 isolates of Candida albicans were positive.

Table 3: Biochemical test for *Candida albicans* and *Staph aureus*

Biochemical test	*Candida albicans*		Total
	Positive	Negative	
Hemolysis	25	0	25
Biofilm formation	22	3	25
	Staph aureus		
Hemolysis	25	0	25
Congo agar (Biofilm)	25	0	25
Coagulase test	25	0	25
Catalase test	25	0	25
Oxidase test	0	25	25
Urease	25	0	25

Identification of all Clinical Samples causative microorganisms was performed by classical microbiological methods for 120 specimens. There were 70% of specimens were positive (growth) and 30% were negative(non-growth) (fig 4).

Staph. aureus was 18%, Streptococcus 14%, P .aeruginosa 7%,S. saprophyticus 10%, S. epidermis

9% and Candida albicans 18% as show in fig (5).

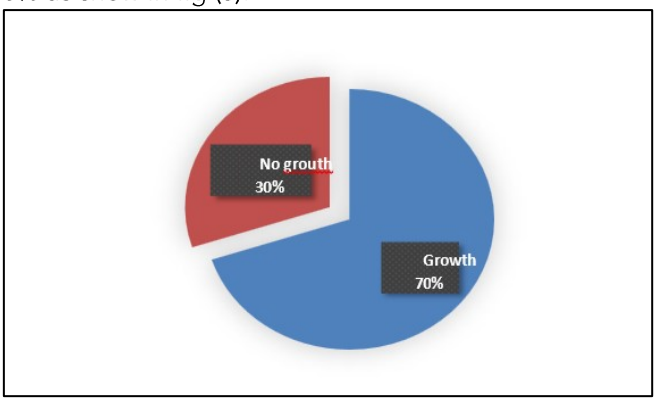

Figure 3: Percentage of bacterial growth in patients

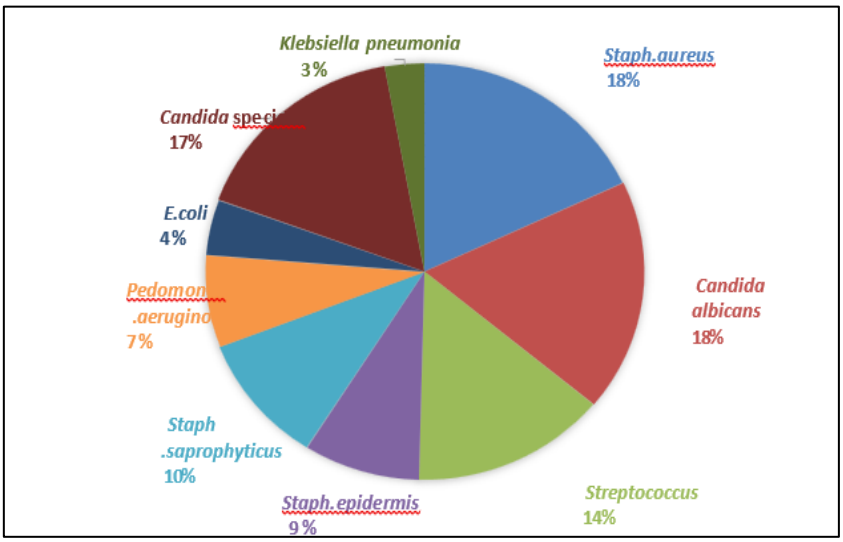

Figure 4: percentage of bacterial isolate (n=9 from 70 %)

The results in fig (6) show that 40% of Staph aureus isolates were strong biofilm 24% were intermediate while 36% were week formation of biofilm. In fig (7) the result shows the percentage intensity of Biofilm formation for Candida albicans were 52%, 20% intermediate, 16% week and 12% negative biofilm formation.

Staphylococcus aureus can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression throughout (Nathan et al.2011). The S. aureus responsible for biofilm-associated infections can have different genetic backgrounds and, therefore, express a different spectrum of virulence factors during infection (Jarraud et al. 2002).

Candida albicans were shown to affect biofilm formation in vitro (Shin et al., 2002; Douglas, 2003; Hajjeh et al., 2004).

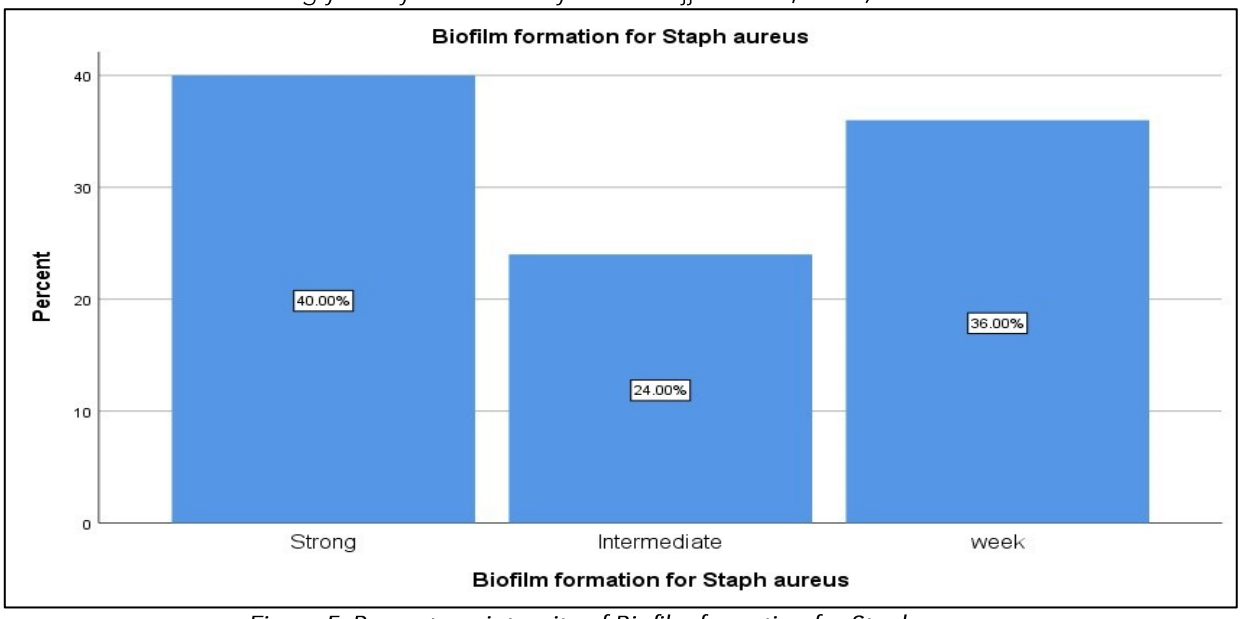

Figure 5: Percentage intensity of Biofilm formation for Staph aureus

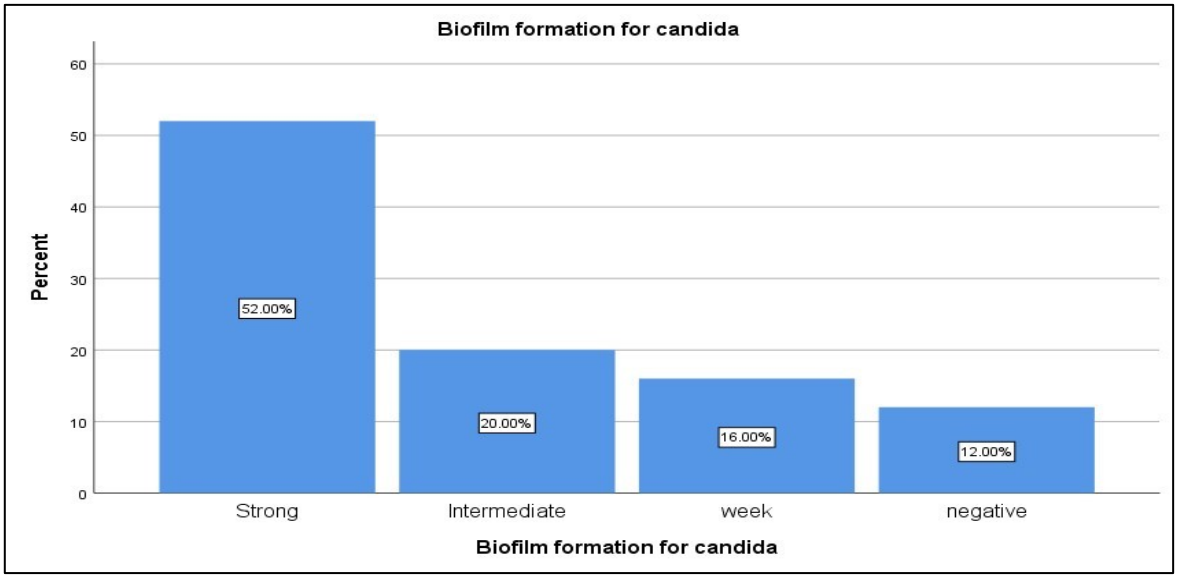

Figure 6: Percentage intensity of Biofilm formation for Candida albicans

References

Alexander, H., Paller, A. S., Traidl-Hoffmann, C., Beck, L. A., De Benedetto, A., Dhar, S., ... & Flohr, C. (2020). The role of bacterial skin infections in atopic dermatitis: expert statement and review from the International Eczema Council Skin Infection Group. *British Journal of Dermatology*, *182*(6), 1331-1342.

Al-Mebairik, N. F., El-Kersh, T. A., *ALS*heikh, Y. A., & Marie, M. A. M. (2016). A review of virulence factors, pathogenesis, and antibiotic resistance in *Staphylococcus aureus*. *Reviews in Medical Microbiology*, *27*(2), 50-56.

Bangert, S., Levy, M., & Hebert, A. A. (2012). Bacterial resistance and impetigo treatment trends: a review. *Pediatric dermatology*, *29*(3), 243-248.

Brown J, Shriner DL, Schwartz RA, Janniger CK. Impetigo: an update. *Int J Dermatol*. 2003; 42:251-5.

Cole, C., & Gazewood, J. D. (2007). Diagnosis and treatment of impetigo. *American family physician*, *75*(6), 859-864.

Demircioğlu, F., & Ören, H. (2008). Ecthyma gangrenosum: sign of Pseudomonas aeruginosa bacteremia. *Pediatric Hematology and Oncology*, *25*(4), 369-370.

Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends in microbiology*, *11*(1), 30-36.

Hajjeh, R. A., Sofair, A. N., Harrison, L. H., Lyon, G. M., Arthington-Skaggs, B. A., Mirza, S. A., ... & Warnock, D. W. (2004). Incidence of bloodstream infections due to Candida species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. *Journal of clinical microbiology*, *42*(4), 1519-1527.

Hartman-Adams, H., Banvard, C., & Juckett, G. (2014). Impetigo: diagnosis and treatment. *American family physician*, *90*(4), 229-235

Hay, R. J., Johns, N. E., Williams, H. C., Bolliger, I. W., Dellavalle, R. P., Margolis, D. J., ... & Naghavi, M. (2014). The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *Journal of Investigative Dermatology*, *134*(6), 1527-1534.

Horlings, R. K., Mangold, A. R., & Jonkman, M. F. (2020). Bullous Diseases. In *Skin Disorders in Migrants* (pp. 75-85). Springer, Cham.

Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., ... & Vandenesch, F. (2002). Relationships between Staphylococcus aureus genetic background, virulence factors, agr groups (alleles), and human disease. *Infection and immunity*, *70*(2), 631-641.

Karaaslan, A., Cretin, C., Akin, Y., Adak, A., Gulhan, T., & Karatas, O. (2020). Ecthyma due to Streptococcus Pyogenes Mimicking Cutaneous Anthrax/Deri Sarbonu Suphesi Uyandiran Streptococcus Pyogenes'e Bagli Gelisen Ektima. *Southern Clinics of Istanbul Eurasia (SCIE)*, *31*(1), 84-87.

Kobayashi S D, Malachowa N, De00Leo F R.,2015, Pathogenesis of *Staphylococcus aureus* abscesses. The American journal of pathology. 185(6): 1518-1527.0

Laurice, & Grice, E. A. (2020). The skin microbiota: balancing risk and reward. Cell host & microbe, 28(2), 190-200.

Mannschreck, D., Feig, J., Selph, J., & Cohen, B. (2020). Disseminated bullous impetigo and atopic dermatitis: Case series and literature review. *Pediatric Dermatology*, *37*(1), 103-108.

Miller, K. M., Carapetis, J. R., Cherian, T., Hay, R., Marks, M., Pickering, J., ... & Bowen, A. C. (2022, September). Standardization of Epidemiological Surveillance of Group a Streptococcal Impetigo. In *Open Forum Infectious Diseases* (Vol. 9, No. Supplement_1, pp. S15-S24). US: Oxford University Press.

Nardi, N. M., & Schaefer, T. J. (2017). Impetigo.

Nathan K Archer,[1,2] Mark J Mazaitis,[3] J William Costerton,[4] Jeff G Leid,[5] Mary Elizabeth Powers,[6] and Mark E Shirtliff Staphylococcus aureus biofilms Virulence. 2011 Sep-Oct; 2(5): 445–459.

Potter, T., Danoff, R., & Tjiattas-Saleski, L. (2016). Ecthyma. *Osteopathic Family Physician*, *8*(6).

Russell, J (Ed), Ryan, R (Ed). Common dermatologic conditions in primary care. Springer Nature Switzerland: Humana Press Inc 2019. Available from: https://www.springer.com/gp/book/9783030180645

Shallcross, L. J., Petersen, I., Rosenthal, J., Johnson,

A. M., Freemantle, N., & Hayward, A. C. (2013). Use of primary care data for detecting impetigo trends, United Kingdom, 1995–2010. *Emerging Infectious Diseases*, *19*(10), 1646.

Shin, J. H., Kee, S. J., Shin, M. G., Kim, S. H., Shin, D. H., Lee, S. K., ... & Ryang, D. W. (2002). Biofilm production by isolates of Candida species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. *Journal of clinical microbiology*, *40*(4), 1244-1248.

Skowron, K., Bauza-Kaszewska, J., Kraszewska, Z., Wiktorczyk-Kapischke, N., Grudlewska-Buda, K., Kwiecińska-Piróg, J., ... & Gospodarek-Komkowska, E. (2021). Human skin microbiome: impact of intrinsic and extrinsic factors on skin microbiota. *Microorganisms*, *9*(3), 543.

Taiaroa, G., Matalavea, B., Tafuna'i, M., Lacey, J. A., Price, D. J., Isaia, L., ... & Jack, S. (2021). Scabies and impetigo in Samoa: a school-based clinical and molecular epidemiological study. *The Lancet Regional Health-Western Pacific*, *6*, 100081.

Tognetti, L., Martinelli, C., Berti, S., Hercogova, J., Lotti, T., Leoncini, F., & Moretti, S. (2012). Bacterial skin and soft tissue infections: review of the epidemiology, microbiology, aetiopathogenesis and treatment: a collaboration between dermatologists and infectivologists. *Journal of the European Academy of Dermatology and Venereology*, *26*(8), 931-941.