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ORIGINAL ARTICLE

Prevalence of Bacterial Skin Infections in Baqubah City for the period from December 2021 to April 2022

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ABSTRACT
The current study was conducted on patients with skin infections caused by bacteria in the city of Baqubah for the period from December of the year 2021 to April of the year 2022, where sixty five samples were collected, represented by a smear from boils, impetigo infections, folliculitis, erysipelas, cellulitis and bustular psoriasis, as the clinical diagnosis was made by a specialist dermatologist. The number of swabs for skin infections caused by bacteria was more common among females (38 specimens in females and 27 in males), as folliculitis was the most common, while impetigo was the least prevalent. The bacteria causing these infections were isolated and diagnosed by laboratory methods morphologically, microscopically and biochemically. The results showed 71 bacterial isolates; (27) isolates of Pseudomonas aeruginosa, Staphylococcus aureus (20 isolates), Escherichia coli (14 isolates), Acinetobacter baumannii (6 isolates), Staphylococcus epidermidis (4 isolates).A sensitivity test was conducted to examine the sensitivity of bacteria toward 11 tablets of antibiotics, and the results showed that the highest percentage of resistance was to P. aeruginosa, while Staph. epidermidis has showed less resistance, as Meropenem had the strongest activity against bacterial species, while Gentamicin showed the least activity against bacterial species.
Keywords: Baqubah, Bacterial skin infections, pathogenic bacteria , antibiotics

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INTRODUCTION

The most common factor leading to the development of skin infection involves a break of the skin and multitude of conditions can arise from this process differing mainly by the depthand extent of skin involvement(Levinson, 2016).The classification and management of most common bacterial infections that is commonly caused by *Staphylococcus aureus,Clostridium*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and other Gram negative bacteria are outlined in the following [26]: Impetigo a superficial bacterial infection that can develop either through direct invasion of normal skin (primary) or infection at sites of damaged skin (secondary), Boils and carbuncles are associated with infection of a hair follicle and extend into subcutaneous tissue and antibiotic therapy is only required if there is spreading cellulitis or systemic infection, Folliculitisis usually presents as a crop of pustules affecting areas of moist skin with hair. Cellulitis and erysipelas both of them manifest as spreading areas of skin erythema and warmth. Localized infections are often accompanied by lymphangitis and lymphadenopathy. Some patients can be quite unwell with fevers and features of systemic toxicity. Bacteremia, although uncommon (less than 5%), still occurs.Necrotizing skin infectionsare the best known of which is necrotizing fasciitis, are a medical and surgical emergency that require prompt debridement and appropriate intravenous antibiotics, infection usually involves the necrosis of underlying soft tissues or muscle.

Fujita Melinda *et al.,*[10] showed that bacterial infections can occur in lesions of bustular psoriasis which is a severe rare skin disease characterized by widespread eruption of sterile superficial macro-scopic pustules with or without systemic inflammation. The growing prevalence of human bacterial infections, especially in immunocompromised patients, has resulted in these diseases becoming a worldwide public

health issue and still one of the most infections Baqubah City. The immune status of the host determines the outcome of the disease. Dangerous antibiotic resistant bacteria have been observed with increasing frequency over the past several decades and many factors including economic impact, intrinsic and acquired drug resistance, morbidity and mortality rates, and means of infection were taken into account [9].Therefore, current study aimed to isolation and identification of bacteria from folliculitis, impetigo , cellulitis and erysipelas , boil infection and bustular psoriasis then study the effect of antibiotics on isolated species.

MATERIAL AND METHODS

Sampling: Sixty five specimens of folliculitis swabs, impetigo swabs, cellulitis and erysipelas swabs, boil infection swabs and bustular psoriasis swabs (with more than one sample for some patients) were collected from sixty patients who attended to Baqubah Teaching Hospital and outpatients visitors to the private clinic of professor doctor Khudhair Khalaf Al-Kayali, Diyala Governorate from December 2021 to April 2022. The specimens were collected from different ages ranging from (7 to 60) years old. specimens included both genders (males 27 and38 females).

Bacteriological Investigation

Culturing of the Specimens: Specimens from clinical sources including swabs of folliculitis, impetigo , cellulitis and erysipelas, boil infection and bustular psoriasis were quickly cultured on Blood agar and MacConkey agar. The isolates grown on blood agar were purified on Mannitol salt agar by streaking method. The agar plates were incubated for 24 hrs at 37˚C [25]. Then, biochemical tests and diagnostic tests were performed for the bacteria under study. The laboratory method was performed at Lab. of biotechnology in department of biotechnology /college of sciences/University of Diyalaand the Lab. of bacteriology in educational laboratories of Baqubah Teaching Hospital.

Identification of the Bacteria: Bacterial isolates were diagnosed by studying the morphological properties of the colonies, bacterial cells and microscopic examination which were performed based on Levinson, (2014).

Cultural characterization: Bacterial isolates were diagnosed by studying the phenotypic characteristics of isolates on blood agar, MacConkey agar and Mannitol salt agar depending on the colony's shape and size, colony color, edges, and opacity as well as its ability to hemolysis on blood agar.

Identification of bacterial isolate with the VITEK 2 system: VITEK-2 system was used in this study to diagnose gram positive and negative bacterial isolates, with a high sensitivity (98%) and this device includes 64 biochemical tests that are used to diagnose bacteria and the result of the examination takes about 8 hrs or less (Pincus, 2011).The method steps as the following:

1. Preparation of bacterial suspension: A sterile swab was used to transfer a number of the colonies from a pure culture separately and suspended in 3 ml of sterile saline in clear plastic test tubes. The turbidity was then adjusted up to 0.5 optical densities.

2.Inoculation of identification card: Identification cards were inoculated with the isolates suspension using an integrated vacuum apparatus. A test tube containing the suspension of the isolates were placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests or up to 15 tests. The filled cassette was placed either manually or transported automatically into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the isolates suspension was forced through the transfer tube into micro-channels that fill all the test wells.

3.Card sealing and incubation: Inoculated card was passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types are incubated on-line at 35.5 + 1.0 ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next reading time. Data were collected at 15 minute intervals during the entire incubation period.

Preservation of Identified Bacteria

Short Term Preservation: Bacterial isolates were inoculated after being diagnosed on the nutrient agar in a slant method. The tubes were incubated for 24 hrs at 37˚C and preserved at 4°C for daily use. The isolates were periodically renewed monthly by activating them on the nutrient stock medium and to ensure they remain active they must be replanted on a new slant medium (WHO, 2003).

Long Term Preservation(up to 3 months):the procedure was carried out by culturing the isolated on media containing 20% glycerol. The medium was prepared by adding 2ml of glycerol to 8ml of brain heart infusion broth and thenit is placed in the flask and sterilized by autoclave.Then the tubes cooled, the tubes were inoculated by a pure colony and incubated at 37 ˚C for 24 h. In the end, the tubes were kept in deep freeze (Burnett and Crocker, 2005).

Antibiotics susceptibility test : The sensitivity test procedure was done according to (CLSI, 2019) as the following steps:

Mueller-Hinton agar plates were used for the rapidly growing species in the Kirby- Bauer method. The solvent was sterile in the plates and had a depth of around 4 mm.

Pure culture has been used as inoculum; 2-4 related colonies have been selected and transferred to around 5ml of standard sterile saline. To get an average number equal to 1.5x 10^8 CFU/ml, the turbidity of microbial suspension was compared with the turbidity of the McFarland Standard 0.5.

The sterile cotton swab was immersed into the standard inoculum, streaking was performed 3 times on the entire agar surface of the plate with the swab, rotary the plate between each line at 50 degrees. The inoculum had been allowed to dry with a lid in place for 5-10 minutes and after that, the antibiotics (supplemented from Bioanalyse company ,Turkey)mentioned in the table 1 were distributed on the plates.

The plates were subsequently incubated at 37^{o}C and analyzed 18-24 hrs. Inhibition zones were measured, and the zones' diameters were reported to the nearest millimeter.

Table 1: The Antibiotics Used Throughout The Study

Antibiotics	Symbol	Disk Con. µg/ ml
Clavulonic acid) Augmentin (Amoxicillin	AMC	20/10
Azithromycin	ATH	15
Ceftazidim	CAZ	30
Ceftriaxone	CRO	30
Ciprofloxacin	CIP	5
Doxycycline	DXI	30
Gentamicin	GM	10
Levofloxacin	LEV	5
Ofloxacin	OFX	5
Pipracillin	PRL	100
Meropenem	TN	10

RESULTS AND DISCUSSION

Bacteriological Investigation

Isolation of bacteria : After culturing specimens , the total clinical isolates from 48 specimens were 71 isolates of pathogenic bacteria, table 2 shows the number of specimens according to their source. However, results presented that 16 specimens had no growth, as the smears were taken from the patients; and that may be because of the techniques that used was improper like the smears were not typically sufficient represented to have the contaminants or due to the using of sterilizers and antibiotics (Levinson, 2016).

Table 2: The number of specimens according to their source

Sources	No. of specimens	No. of isolates	No. of no growth specimens	Gender	
				Male	Female
Folliculitis	**26**	**31**	**7**	**11**	15
Bustular psoriasis	**19**	**22**	**5**	**12**	7
Boil infection	**13**	**15**	**0**	**3**	10
Cellulitis and Erysipelas	**4**	**0**	**4**	**0**	4
Impetigo	**3**	**3**	**1**	**1**	2
Total	**65**	**71**	**17**	**27**	38

The results of the current study were not always consistent with previous studies such as with what was stated by Samotij *et al*., (2021) who mentioned in their review that bustular psoriasis is approximately twice as common in women as in men. But, increasing the number of females infected with boils infections was in agreement with Shallcross, (2015).

The percentage of infection difference between the genders may be resulted in the quantity and quality of the normal flora in the bodies of the different sexes, difference in the method of collecting specimens for both sexes, factors that helped reduce the number of males such as war, in addition to the human physiological condition, especially the secretion of female hormones, that differ from males, inaddition to the menstrual cycle, breastfeeding, genetic structure, nutritional quality, psychological state, especially depression, and other stress factors (Joshua *et al.*,2017).

Identification of bacteria: All bacterial isolates were diagnosed firstly by using selective and differential cultures media, as well as microscopic examination, biochemical tests by VITEC2 system was done for confirmation. Results in table 3 shows the number of the isolates for each pathogenic bacteria.All bacterial species were identified morphologically and microscopically according to Levinson, (2016) as follow:

Staphylococcus aureus: *Staph. aureus* isolates were identified by growing on blood agar and Mannitol salt agar under aerobic environments at 37° C for 18-24 hrs. All isolates on blood agar yield clear β-hemolysis around their colonieswhich appear to be medium to large-sized with regular, smooth, convex and shiny rims while in Mannitol salt agar the isolates ferment Mannitol and the color medium wastransformed from pink to yellow (golden)and this distinguishes it from other *Staphylococcus* species that cannot ferment Mannitol, microscopically the isolates were Gram-positive, spherical cells, cluster group grape-like shape through staining with microscopic examination.

***Staphylococcus epidermidis*:** The isolates appeared after culturing on blood agar as a smooth, raised, circular non-hemolytic colony, many isolates yield grey to greyish-white colonies, on Mannitol salt agar, the isolates did not ferment mannitol and appeared as pink colonies without changing in the color of media, microscopic examination showed that the isolates of *Staph. epidermidis* were Gram-positive irregular clusters and non-motile.

***Pseudomonas aeruginosa*:** Morphological identification was done by examining the characteristics of culturing them on MacConkey agar medium, as the colonies appeared a pale color due to their inability fermentation of lactose, while on Blood agar, the colonies showed their ability to bloodβ-hemolysis , A microscopic examination was done, which showed Gram-negative rod cells and motile.

***Escherichia coli*:** *E. coli* isolates were identified after culturing them on MacConkey agar, they appeared smooth, convex with clear rounded ends, pink colonies due to fermenting lactose while on blood agar they appeared hemolysis, the bacteria showed Gram-negative, rod shaped.

Acinetobacter baumannii*:* The most common medium for the growth of *A. baumannii* includes MacConkey agar and Sheep blood agar. The isolates appeared after culturing on blood agar as non-pigmented, white or cream-coloured, smooth to mucoid colonies, the colonies were small translucent and shiny when grew on the MacConkey agar, microscopically appeared Gram-negative coccobacilli and non-motile.

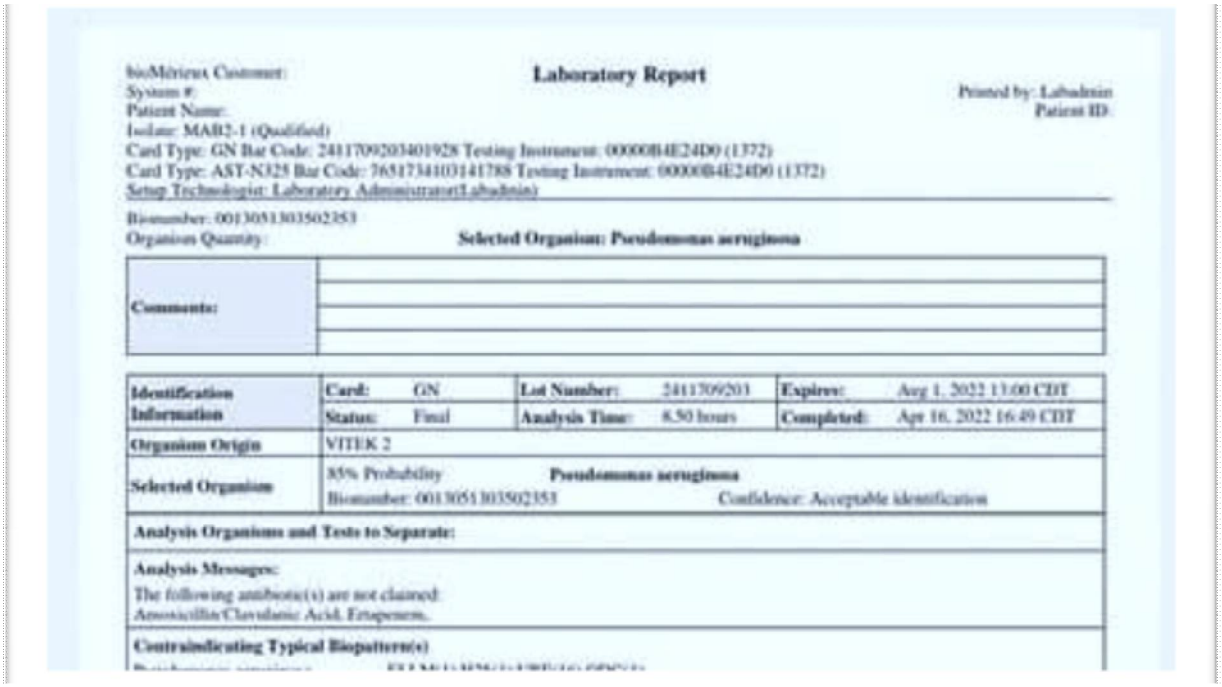
Bacterial isolatesidentification had been confirmed for each S*. aureus, S. epidermidis, P.aeruginosa, E.coli* and *A. baumannii* using Vitek2 system. Results in figure1 shows of a bacterial identification with a high-accuracy diagnosis for *P.aeruginosa*. The results showed that three species belong to the Gram negative bacteria, namely *Pseudomonas aeruginosa, Escherichia coli* and *Acinetobacter baumannii* which were isolated from folliculitis at a ratio of 17 (23.94%), 8 (11.26%) and 2 (2.81%) respectively whereas they were isolated from bustular psoriasis at a ratio of 9 (12.67%), 6 (8.44%) and 3 (4.22%) respectively. While, two species of bacteria belong to the Gram positive, namely; *Staphylococcus aureus* and *Staphylococcus epidermidis* which were at different ratio in infections, *S. aureus* was at ratio 4 (5.63%), 3 (4.22%), 11 (15.49%) and 2 (2.81%) in folliculitis, bustular psoriasis, boil infection and impetigo respectively. While *S. epidermidis* was at ratio 1(1.4%) and 3 (4.22%) in bustular psoriasis and boil infection respectively. cellulitis and erysipelas infection was not caused by any species of bacteria.

Table 3: Number of isolates for each bacteria according to skin disease

Name of skin infection	No. (%) of *Staphylococcus aureus*	No. (%) of *Staphylococcus epidermidis*	No. (%) of *Pseudomonas aeruginosa*	No. (%) of *Escherichia coli*	No. (%) of *Acinetobacter baumannii*
Folliculitis	4(5.63%)	0 (0%)	17(23.94%)	8(11.26%)	2(2.81%)
Bustular psoriasis	3(4.22%)	1(1.4%)	9(12.67%)	6(8.44%)	3(4.22%)

Boil infection	11(15.49%)	3(4.22%)	0 (0%)	0 (0%)	1(1.4%)
Cellulitis and Erysipelas	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Impetigo	2(2.81%)	0 (0%)	1(1.4%)	0 (0%)	0 (0%)
Total	20 (28.16%)	4(5.63%)	27 (38.02%)	14(19.71%)	6 (8.45%)



Laboratory Report

Printed by: Labadmin
Patient ID:

Card Type: GN Bar Code: 2411709203401928 Testing Instrument: 0000084E24D0 (1372)
Card Type: AST-N325 Bar Code: 765173410314178S Testing Instrument: 0000084E24D0 (1372)
Setup Technologist: Laboratory Administrator(Labadmin)

Biomumber: 0013051303502353

Selected Organism: Pseudomonas aeruginosa

Comments:	

Identification Information	**Card:** GN	**Lot Number:** 2411709203	**Expires:** Aug 1, 2022 13:00 CDT		
	Status: Final	**Analysis Time:** 8.50 hours	**Completed:** Apr 16, 2022 16:49 CDT		
Organism Origin	VITEK 2				
Selected Organism	85% Probability	**Pseudomonas aeruginosa**			
	Biomumber: 0013051303502353		Confidence: Acceptable identification		
Analysis Organisms and Tests to Separate:					
Analysis Messages: The following antibiotic(s) are not claimed: Amoxicillin/Clavulanic Acid, Ertapenem.					
Contraindicating Typical Biopattern(s)					

Figure 1: Vitek2 compact system report for *Pseudomonas aeruginosa*

Daniela *et al.* (2011) showed that Gram-negative folliculitis may be the result of prolonged antibacterial treatments in patients with rosacea and acne, it is caused by alteration of facial skin flora and the nasal mucous, a decrease of Gram positive bacteria and a proliferation of Gram-negative bacteria for example *Escherichia coli, Pseudomonas aeruginosa*. The results were in disagreement with Itzhak *et al.,* (2001) who revealed that polymicrobial etiology of secondarily infected bustular psoriasis lesions and the association of bacterial flora such as *E. coli* by two isolates, whereas *S. aureus* by 11 isolates which was isolated from all body sites with the anatomic site of the lesions were demonstrated.

In spite of the systematic review introduced by Gunderson and Martinello, [12] about cases of bacteremia associated with erysipelas and cellulitis which supported the traditional teaching that Streptococcal species and *S. aureus* are the predominant pathogens for both cellulitis and erysipelas, this study were in contrast, there was no bacteremia. Previous studies referred that accounts for most of impetigo cases is caused by *S. aureus* [26-28].

Antibiotics susceptibility test for isolated bacteria: Antibiotics susceptibility test was conducted for five species of pathogenic bacteria as reported by CLSI [7]. Table (4) indicates to the sensitivity of the bacteria to eleven type of antibiotics for each bacteria, to detecting isolates resistance to the antibiotics circulating in health institutions, these antibiotics were chosen for their frequent use in treating bacterial infections.

Antibiotics	Resistant isolates of bacteria (No. / %)									
	Staph. aureus Total No. :20		*Staph. epidermidis* Total No. :4		*P. aeruginosa* Total No. :27		*E. coli* Total No. :14		*A.baumannii* Total No. :6	
	Resist isolates		Resist isolates		Resist isolates		Resist isolates		Resist isolates	
	No.	%	No.	%	No.	%	No.	%	No.	%
Augmentin	16	80	1	25	22	81.48	10	71.42	2	33.33
Azithromycin	16	80	0	0	24	88.88	12	85.71	5	83.33
Ceftazidim	16	80	1	25	9	33.33	5	35.41	2	33.33
Ceftriaxone	13	65	0	0	12	44.44	4	28.57	6	100
Ciprofloxacin	10	50	0	0	14	51.85	3	21.42	5	83.33

Doxycycline	13	65	0	0	21	77.77	7	50	5	83.33
Gentamicin	10	50	0	0	27	100	8	57.14	6	100
Levofloxacin	8	40	1	25	13	48.14	7	50	5	83.33
Ofloxacin	8	40	0	0	25	92.59	9	64.28	3	50
Pipracillin	9	45	0	0	20	74	4	28.57	5	83.33

Meropenem	0	0	0	0	8	29.62	3	21.42	1	16.66

Table 4: The percentages of antibiotic resistance for bacterial isolates

The results of the current study indicated that from the total 20 isolates of *Staph. aureus* 16 isolates showed resistance to Augmentin, Azithromycin and Ceftazidim in 80% ; Ceftriaxone, Ciprofloxacin, Doxycycline and Gentamicin in 65%, 50%, 65% and 50% respectively. Many of previous studies that performed in Iraq revealed indicated to *Staph. aureus* resistance against antibiotics; Yosef, (2019) showed the resistance of *Staph .aureus* isolates to Ciprofloxacin (46.7%) which is approximately agreed with the results of the current study. Also in Iraq, Sahm (2019) reported the resistance of *Staph. aureus* isolates to Ofloxacin, Azithromycin were (44%) and (76%) respectively , these results also agreed with the results of the current study. Resistance to gentamicin (50%) of *S. aureus* detected by most studies such as Al-Geobory, (2011) mentioned that the resistance of *Staph. aureus* isolates to Gentamicin were 49%. In spite of many previous studies that showed the high resistance activity of *Staph. epidermidis* (Abd-Elateef ,2011; Mahmoud,2020), one isolate of this bacteria showed resistance to antibiotics as follow: Augmentin, Ceftazidim, Levofloxacin at 25% for each one, whereas the others were sensitive to all other antibiotics. There are several mechanisms by which *Staphylococcus* can resist antibiotics, including: making the antibiotic ineffective through the ability of bacteria to produce extracellular enzymes such as beta-lactamase enzymes whose task is to convert the antibiotic containing the beta-lactam ring from its active form in effective or alterations in the bacteria's cell wall proteins that keep the antibiotic in the outer environment, or the ability of the microorganism to use secondary metabolic pathways similar to the original pathways that are unaffected by the antibody used by increasing the metabolic activity of the resistance to the antibody. Also, Also, the ability of bacteria to form a thin, stick and mucous layer called the biofilm that prevents the access of the antibiotic to it [16]. Whereas the acquired resistance arises from continuous exposure to high doses of the antibiotic in addition to chromosomal by mutation and extra chromosomal resistance by transfer of type of plasmids that carries genes for resistance to one or more antibiotics among bacteria [15].

All *P. aeruginosa* isolates (27) appeared to most antibiotics in different rates as shown in table 4, all isolates were resistant to Gentamicin (100%), followed by Augmentin and Azithromycin at (81.48 and 88.81%) respectively in addition to other antibiotics. One of the most important causes of these bacteria to resist many of antibiotics is its own R-resistance plasmids which carries different antibiotic resistance genes [12]. Results also showed that most of *E.coli* isolates had a high resistance to Augmentin and Azithromycin at (71.42% and 85.71%) respectively; studies conducted by AL-Nuaeyme, [4] and Mahmoud, [18] referred to ability of this to resist most of antibiotics in high rates. All *A. baumannii* isolates were complete resistant to Ceftriaxone and Gentamicin while high rate of resistance was Azithromycin, Ciprofloxacin, Doxycycline, Levofloxacin and Pipracillin at rates (83.33%) for each one; another previous study mentioned this resistance activity this bacteria [19].

Many previous studies showed the resistance of bacteria that cause skin diseases to a high resistance to antibiotics including gram-negative folliculitis (GNF) for example, that can occur as a complication in patients receiving prolonged treatment with broad spectrum antibiotics for the treatment of acne vulgaris and rosacea [8-12]. Whereas Abrha *et al* [2] mentioned that the clinical efficacy of the topical antibiotics treatments for impetigo is declining at an alarming rate due to the rapid emergence and spread of resistant bacteria. Unfortunately, The spread of multidrug-resistant gram negative bacteria is now seen as a globalized threat and the resistance of this group to several types of antibiotics depends on the administration of the antibiotics extensively in addition to the genetic expression of these bacteria [21].

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