How to Cite:
Shaker, N. S., & Al-musawi, M. T. (2022). Assessment of the prevalence of Pseudomonas aeruginosa among Iraqi patients with acne and study of their antibiotic susceptibility patterns. *International Journal of Health Sciences*, *6*(S4), 3031–3039. https://doi.org/10.53730/ijhs.v6nS4.9862

Assessment of the prevalence of Pseudomonas aeruginosa among Iraqi patients with acne and study of their antibiotic susceptibility patterns

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***Abstract*---**Background: *Pseudomonas aeruginosa* is found in soil and water all over the world. It prefers moist areas, such as wash basins, toilets, and swimming pools that are insufficiently chlorinated, and expired and ineffective disinfection solutions. Pseudomonas aeruginosa infections range from external infections such as acne. A simple, severe, life-threatening disorder that is highly antibiotic-resistant. Aims: The current study was undertaken to analyze the frequency of *P. aeruginosa* as a microbial skin infection patient with acne and study their antibiotic sensitivity patterns due to a rise in instances of acne among young people in Iraq. Methods: A total of 61 samples were taken from inflamed and pus discharge patients,27 males and 34 females, with acne vulgaris on the face. Samples were cultured on selective, enrichment, and special media then incubated for 18-24 hr. at 37 °C. Bacterial Isolates Identification was done using different culture media, Microscopic Examination with Gram stain, in addition to Biochemical tests. In addition to API 20E identification system and conformation by VITEK 2 compact system. Antibiotic susceptibility test is done using different antibiotics. Results: *Pseudomonas aeruginosa* isolates were found in 23 (37.70%)/ 61 of the samples, with 11 (47.83%) male and 12 (52.17%) female. The highest rate of bacterial infection was within the age group 15- 20 years, followed by the age group less than 15 years and more than 20 years reach to 12 (52.17%), 6 (26.09%), and 5 (21.74%), respectively. All strains have a high resistance to Ceftazidime, Clindamycin, Doxycycline, and Trimethoprim were 23 (100%), resistance to both Penicillin and Erythromycin reached 17 (73.91%) and 16 (69.57%) each respectively, and resistance to

Gentamycin at 15 (65.22%). Conclusion: All isolates of *Pseudomonas aeruginosa* were resistant to antibiotics. This may be due to the indiscriminate use of antibiotics in Iraq.

***Keywords*---**Pseudomonas aeruginosa, acne, antibiotic susceptibility patterns.

Introduction

Acne vulgaris is a very common skin disorder, a pilosebaceous unit inflammation with chronic polymorphic inflammatory lesions (papules, pustules, nodules, and cysts) chiefly on the face, also can accrue on the trunk, upper arms, and back. Acne vulgaris is a self-limiting illness that is most frequent throughout adolescence (Goh *et al.*, 2019). Acne is the eighth most common condition worldwide, with 650 million individuals affected in 2010 (Blaskovich *et al.*, 2019). Although acne affects those aged 12 to 24 years. Severe acne has a significant social and psychological impact, impacting emotions and self-esteem while also raising the risk of depression and suicide (Dekio et al., 2019; Xu and Li 2019). In patients with acne, 85% of all afflicted individuals received extended antimicrobial therapy. Changes in face skin flora and a decrease in Gram-positive (G+ve) bacteria and a proliferation of Gram-negative (G-ve) bacteria cause it. It should be explored in acne patients who have not improved clinically after 3-6 months of antibiotic therapy (Spernovasilis et al., 2021). Colonization and triggering by *Cutibacterium acnes* (*C. acnes*), *C. acnes* is a bacterium that has been linked to the development of acne (Dekio *et al.*, 2019; Xu, and Li, 2019).

Pseudomonas aeruginosa (*P. aeruginosa*) causes a wide variety of skin infections, including acne, *P. aeruginosa* subcutaneous nodules are usually associated with bloodstream infections and occur primarily in immunocompromised hosts (Spernovasilis *et al.*, 2021). Also, *P. aeruginosa* was isolated from patients with acne vulgaris who received prolonged treatment of wide-spectrum antibiotics and from patients who were non-responding to the conventional treatments of acne, the patients had a clinical symptom with Gram- negative folliculitis (GNF) (Eady *et al.*, 1988; Böni and Nehrhoff, 2003; Daniela *et al.*, 2011). Despite regular exposure to antimicrobial chlorine, adolescent swimmers develop acne and are resistant to conventional treatments, and because is found in swimming pools, the researchers postulated that "swimmer acne" is caused by a distinct microbial process, including *P. aeruginosa* (Lynn *et al.*, 2016).

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacteria that may be found in a variety of settings, including water, soil, plants, and animals, and has a proclivity for being found in places where people congregate (Halvorsen *et al.*, 2011). Even though it is a human opportunistic infection, it is associated with severe morbidity and mortality (Sierra-Téllez *et al.*, 2011). In the community and clinical settings, *P. aeruginosa* causes a wide spectrum of infections, with multidrug-resistant and extensively drug-resistant strains causing the latter (Morss- Walton *et al.*, 2022). Due to the increase in cases of

acne among young adults in Iraq, the present study was conducted to assess the prevalence of *P. aeruginosa* as one of the microbial skin infection patients with acne and study their antibiotic susceptibility patterns.

Materials and Methods

Sample collection and culture

A total of 61 samples were taken from inflamed and pus discharge from patients (27 males and 34 females) with acne vulgaris on the face attending the department of dermatology in Baghdad's Al-Shula General Hospital, and private beauty centers in Baghdad province in a period between November 2020 and February 2021. and the patients' ages at 12 to 25 years. A questionnaire sheet was filled out for each individual studied for a history of acne infection, and treatments and excluded patients who took antibiotics seven days prior to sampling. Samples were cultured on selective, enrichment and special media, then incubated for 18-24 hr. at 37℃.

Bacterial Isolates Identification

Macroscopic Examination

Blood agar, MacConkey agar, CHROMagar™ *Pseudomonas*, and Cetrimide agar were used to study the phenotypes of *P. aeruginosa* colonies which include colonial form, shape and color, size, and aroma. (Baron *et al.*, 2007).

Microscopic Examination

Gram stain was applied (Collee *et al.*, 1996).

Biochemical tests

Conventional biochemical tests include: Motility test, Oxidase test, Catalase test, IMViC test (Indole test, Methyl Red-Voges Proskauer, Simmons' citrate agar, Citrate utilization test) (Baron *et al.*, 2007and Collee *et al.*, 1996). In addition to API 20E identification system (BioMerieeux, France) and conformation by VITEK 2 compact system.

Antibiotic susceptibility test (AST)

The AST for *P. aeruginosa* isolates was conducted for 12 antibiotics include (Oxoid Ltd.); (Cefotaxime-CTX 30µg, Penicillin-P10IU, Clindamycin-CM 2µg, Amoxicillin & Clavulanic acid- AMC 20+10µg, Erythromycin- E 15µg, Azithromycin - AZM 10µg, Doxycycline- DO 30µg, Levofloxacin- LVX 5µg, Gentamycin-GM 10µg, Ciprofloxacin-CIP 5µg, Amikacin-AN 30µg, and Trimethoprim- TE 25µg) were investigated using the Kirby-Bauer method and CLSI 2020 guidelines (Collee *et al.*, 1996 and Bauer *et al.*, 1966).

Statistical Analysis

Frequency and percentages were employed to show descriptive statistics for categorical data. To determine the relationship between categorical data, the Chi-square test (x^2) was accomplished. P-values less than 0.05 were statistically significant.

Results and Discussion

Pseudomonas aeruginosa isolates were found in 23 (37.70%)/ 61 of the samples, with 11 (47.83%) male and 12 (52.17%) female Table 1. The highest rate of bacterial infection was within the age group 15- 20 years, followed by the age group less than 15 years and more than 20 years reach to 12 (52.17%), 6 (26.09%), and 5 (21.74%), respectively Table 2.

Table 1: Distribution of *Pseudomonas aeruginosa* according to gender

Male No. (%)	Female No. (%)	Total No. (%)
11 (47.82)	12 (52.17)	23 (100)

Table 2: Distribution of *Pseudomonas aeruginosa* according to age

<15 year No. (%)	15-20 year No. (%)	>20 year No. (%)	Total No. (%)	Chi-Square (x^2)
6 (26.09)	12 (52.17)	5 (21.74)	23 (37.70)	8.63

The diagnosis was based on the form of bacterial isolates grown on the media, where colonies of *P. aeruginosa* isolates appeared on selective media (MacConkey agar) in a pale-yellow color because it is a non-lactose fermenter and this matches the results of previous research (SudhaKar *et al.*, 2015). As for the enriched media (blood agar), the bacteria gave colonies of β-hemolysis type, and this is evidence of the bacteria's ability to produce hemolysin, which breaks down red blood cells on the culture medium.

Regarding the growth of bacteria on Cetrimide agar medium, the bacterial colonies appeared in greenish-yellow color, because most of the colonies produce pyoverdine dye with greenish color, and pyocyanin dye with greenish-blue color, which lights up and fluoresces when exposed to ultraviolet (UV) rays. These dyes are distinguished by their ability to dissolve in water (Del *et al.*, 2020). It should be noted here that Cetrimide agar is a selective and differential medium used to determine the ability of *P. aeruginosa* to grow in the presence of (0.03%) cetrimide, which acts as a detergent and inhibits the growth of most other species of bacteria. From the image result of *P. aeruginosa* colonies on CHROMagar™ *Pseudomonas*, the colonies of *P. aeruginosa* appeared in intense blue-green color. These results are consistent with other studies (Al-Dahmoshi, 2013; Dolan, 2020). Examination using a light microscope showed that *P. aeruginosa* bacteria are G-ve bacilli, positive for oxidase and catalase, negative for MR - VP and urease assays, and these results match with the results of many other researchers (Halvorsen *et al.*, 2011; Tang and Stratton, 2006).

The *P. aeruginosa* can be cultured from skin pustules (Zichichi *et al.*, 2000). With Gram-negative folliculitis (GNF), approximately 80% of patients have superficial pustules with few papules. *P. aeruginosa* is one of the most common bacteria that cause these lesions with *Klebsiella spp.* and *Escherichia coli*, The lesions are deep, nodular, and cyst-like in roughly 20% of GNF patients. Because of the kinds of toxin-producing bacteria and enzymes that break down the epidermis, cysts and abscesses filled with pus can form. Gram-negative bacteria that cause acne lesions are most prevalent around the nose and around the upper lip, spreading to the chin and cheeks. Acne vulgaris is seen in 4% of individuals who use long-term systemic antibiotics and those who swim in hot pools or spas (Diggle and Whiteley, 2020). Additionally, long-term use of tetracycline in the treatment of acne vulgaris can result in folliculitis caused by G-ve bacteria, including *P. aeruginosa* (Boni and Nehrhoff, 2003; Durdu M, Ilkit, 2013). These studies highlight the fact that *P. aeruginosa* folliculitis could arise from sources other than baths and hot.

The present results were recorded highest isolates 37.70% more than the results obtained by Al-Mousawi (Al-Mousawi, 2013) found that a number of secondary school students in Basra Governorate were examined with different ages ranging from 13-17 years, 113 males and 150 females, and the number of *P. aeruginosa* isolates was 2 (0.76%), and the researchers also found that there are other bacterial types, such as *Staphylococcus spp.*, that have caused acne, especially in females, more than it is in males, and the reason may be due to the hormonal changes caused by adolescence and psychological stress in females at that stage have a direct impact on the severity and spread of acne. While the results of the current study were in agreement with the results of the Khaleel, 2022 study in Mosul that 45% of males and 55 % of females, ages 18-23 years, with 1.8 risks of getting acne, and female cases with an irregular menstrual cycle risk of 58 times were among the 150 cases of acne vulgaris studied in Mosul, acne affects the face of 53% of female more than males (46%), and the use of a student's mobile phone has a double risk for developing acne (Khaleel, 2022).

In another study, it was found that 160 acne patients were swabbed, 86 of who were female and 74 of whom were male. Females outnumbered males by 1.16 to 1. The positive cultures were 150 (93.75%) The age group 15-17 years had the largest number of acne sufferers (20%), while the age group 25-34 years had the lowest proportion. Under the age of 12 years was (1.9 %). No *Pseudomonas aeruginosa* bacteria were isolated; however, other types of bacteria and fungi were isolated (Yousif *et al.*, 2016).

In another similar study (Rasool, 2017), the researcher found that 400 patients in Baghdad, whose ages ranged from 12 to 40 years, had their medical condition studied because they suffer from acne on the face. *P. aeruginosa* bacteria recorded the lowest infection rate among young men compared with other types of bacteria. Where was the percentage of its presence in patients, 6 (1.5%); and that the rate of infection with bacteria was in the age group 15-20 years in proportion 122 (64.89 %).

According to the AST results of the current study showed that all *P. aeruginosa* strains were categorized as MDR and Extensive Drug Resistance (XDR) strains (fig.1). Table 3 shows that all strains have a high resistance to Ceftazidime, Clindamycin, Doxycycline, and Trimethoprim were 23 (100%), resistance to both Penicillin and Erythromycin reached 17 (73.91%) and 16 (69.57%) each respectively, and resistance to Gentamycin at 15 (65.22%).

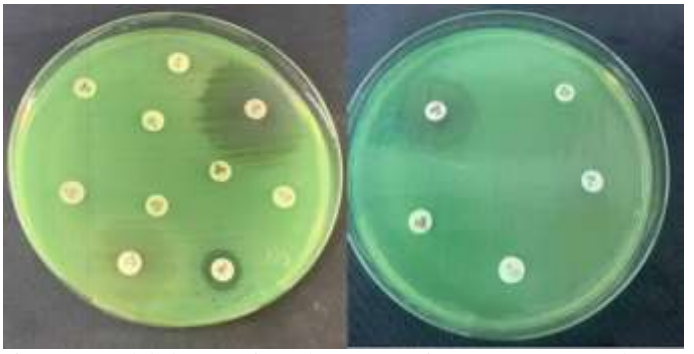

Fig.1: A and B- Sensitivity and resistance of some *P. aeruginosa* isolates to antibiotics

Table 3: Isolates number and percentage of antibiotic resistance of *P. aeruginosa aeruginosa*

Antibiotic	NO. S (%)	NO. I (%)	NO. R (%)	Chi-Square (x^2)
Cefotaxime	0 (0.00%)	0 (0.00%)	23 (100%)	10.25 **
Penicillin	0 (0.00%)	6 (26.09%)	17 (73.91%)	12.49 **
Clindamycin	0 (0.00%)	0 (0.00%)	23 (100%)	10.25 **
Amoxicillin & Clavulanic acid	6 (26.09%)	4 (17.39%)	13 (56.52%)	10.06 **
Erythromycin	2 (8.70%)	5 (21.74%)	16 (69.57%)	12.53 **
Azithromycin	23 (100%)	0 (0.00%)	0 (0.00%)	10.25 **
Doxycycline	0 (0.00%)	0 (0.00%)	23 (100%)	10.25 **
Levofloxacin	2 (8.70%)	13 (56.52%)	8 (34.78%)	11.94 **
Gentamycin	3 (13.04%)	5 (21.74%)	15 (65.22%)	12.58 **
Ciprofloxacin	9 (39.13%)	8 (34.78%)	6 (26.09%)	5.02 *
Amikacin	10 (43.48%)	6 (26.09%)	7 (30.43%)	4.97 *
Trimethoprim	0 (0.00%)	0 (0.00%)	23 (100%)	10.25 **
Chi-Square (x^2)	14.63 **	15.08 **	14.92 **	---

** (P≤0.05), ** (P≤0.01). S= Sensitive, Intermediate =I , Resistant =R*

The MDR strains were defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, While XDR was defined as non-susceptibility to at least one agent in all, but two or fewer antimicrobial categories) (i.e, bacterial isolates remain susceptibility to only one or two categories) (Magiorakos *et al.*, 2012). The results of previous studies showed that *P. aeruginosa* was resistant to many antibiotics, which were originally isolated from

young people suffering from acne. Clindamycin 1%–2%, nadifloxacin 1%, and azithromycin 1% gel and lotion are all available. Other antibiotics, including amoxicillin, erythromycin, and trimethoprim/sulfamethoxazole, are sometimes used, and ciprofloxacin may be administered in *Pseudomonas*-related 'acne' if bacterial overgrowth or infection is masquerading as acne (Sutaria *et al.*, 2022; Kosmadaki and Katsambas, 2017).

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