CXCR1, TLR-4, AND CXCL8: KEY MEDIATORS OF *PSEUDOMONAS AERUGINOSA* VIRULENCE IN WOUND AND BURN INFECTIONS

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Abstract

Pseudomonas aeruginosa, an opportunistic pathogen of considerable clinical import, presents a formidable challenge to susceptible individuals, particularly within the confines of nosocomial settings. Those with compromised immune systems, indwelling medical devices such as catheters, and extensive thermal injuries are especially vulnerable to its insidious and often devastating effects. This investigation sought to elucidate the roles of Toll-like receptor 4 (TLR-4), Toll-like receptor 5 (TLR-5), the chemokine CXCL8, and its cognate receptor CXCR1 in the context of P. aeruginosa infection. Our findings indicate a significant involvement of TLR-4 and CXCL8 in the host response to this pathogen. Notably, CXCR1 expression was observed to be downregulated both in cellular models and in whole blood samples obtained from patients afflicted with bacterial infections, specifically those caused by P. aeruginosa. Utilizing antibodies targeting these cell surface molecules, we further explored their influence on bacterial adhesion and the modulation of infection. The application of anti-CXCR1 antibodies resulted in a demonstrable increase in bacterial infection in both T24 and A549 cell lines. Conversely, the administration of anti-TLR-4 antibodies exerted an inhibitory effect on P. aeruginosa infection. Anti-CXCL8 antibodies, however, did not elicit a discernible impact on bacterial infection within the initial three hours of exposure. These observations suggest a dichotomous role for these molecules in the host response to P. aeruginosa: CXCR1 appears to function as a negative regulator, while TLR-4 appears to act as a positive regulator in the context of bacterial infection.

Keywords: TLR-4,5 , CXCL8, CXCR1, *P. aeruginosa*, T24 and A549 cells.

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1 Introduction

The development of chronic wounds is frequently linked to the presence of polymicrobial communities. The colonization of the wound site by bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa* [1], and β-hemolytic streptococci, interferes with the physiological healing cascade, ultimately resulting in persistent wounds. These specific pathogens are known to impede wound closure and contribute significantly to the establishment of chronic wound infections[2]. The host immune response to bacterial insult is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs, encompassing microbial constituents such as lipopolysaccharides (LPS), lipoproteins, and nucleic acids, along with endogenous DAMPs like interleukin 1 alpha (IL-1α), high mobility group box 1 (HMGB-1), and interleukin 33 (IL-33), serve as crucial alarm signals, alerting the immune system to both infection and cellular damage[3, 4]. The detection of these molecular patterns is mediated by pattern recognition receptors (PRRs) expressed on or within host cells. Prominent PRRs include toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs). TLRs, exemplified by TLR-1, TLR-2, TLR-4, TLR-5, and TLR-6, are characterized by leucine-rich repeat motifs and constitute integral components of the innate immune system. These TLRs are expressed across a diverse range of cell types, including macrophages[5]. The genus *Pseudomonas* represents a highly diverse and ecologically significant bacterial group, exhibiting a remarkable capacity to thrive in a broad spectrum of environments, including freshwater ecosystems [6]. *Pseudomonas* can exhibit opportunistic pathogenicity, precipitating severe diseases such as haemorrhagic septicaemia and gill necrosis. Studies have demonstrated the induction of the chemokine IL-8 (CXCL8) in response to LPS derived from *P. aeruginosa*[7]. CXCL8 is a cytokine of considerable immunological relevance, characterized by its rapid upregulation in response to PAMPs, pathogen-mediated infections, and cellular stress. This member of the CXC chemokine superfamily plays a crucial role in orchestrating cell recruitment during inflammatory processes[8, 9].

IL-8 receptors (IL-8Rs) are seven-transmembrane domain receptors with an extracellular N-terminus and an intracellular C-terminus. They possess a conserved DRY motif within the second intracellular loop, which is essential for activating G-protein-coupled receptor (GPCR) signaling pathways[10]. Two distinct IL-8Rs, CXCR1 and CXCR2, have been identified, through which IL-8 exerts its signaling effects. These receptors exhibit differential tissue expression in response to PAMPs, pathogens, or cytokines, with CXCR2 demonstrating particularly high expression in muscle tissue compared to traditional immune tissues[11]. However, the protein expression of CXCR2 in salmonids, particularly during early developmental stages, remains to be fully elucidated[12]. Utilizing relevant biomarkers, such as CXCR1, CXCL8, and TLRs, is essential for evaluating the efficacy of immune responses. Therefore, we propose CXCL8 and its cognate receptor as potential immunological markers for early P. aeruginosa infection in developing organisms. This study aims to characterize the modulation (upregulation or downregulation) of various cellular

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molecules in response to a clinical strain of *P. aeruginosa*. Furthermore, we intend to employ specific antibodies to investigate their impact on mitigating bacterial infection.This approach will provide valuable insights into the host-pathogen interactions and contribute to the development of novel therapeutic strategies.

2 **Methodology**

Collection samples:

Specimens were collected from each participant, encompassing serum, whole blood, and a swab sample. Following collection, whole blood samples were immediately cryopreserved, with the addition of triose prior to freezing to ensure RNA integrity. The analysis encompassed 80 blood samples, comprising 55 samples from patients diagnosed with wound and burn infections and 25 samples from healthy control participants. Bacterial colonization of the cotton swab samples was assessed via quantitative culture, employing a previously established protocol. In brief, swab samples were inoculated in triplicate onto Mannitol salt agar, blood agar, and MacConkey agar plates, followed by overnight incubation at 37°C[13]. Subsequent to incubation, colony enumeration was performed, and the concentration of colony-forming units (CFU) per milliliter of effluent was determined. Phenotypic identification of the resultant colonies was conducted utilizing the $VITEK_2$ microbial identification system.

Pseudomonas virulent strain

Clincal samples of *Pseudomonas aeruginosa* was chosen based on the antibiotics resistance and virulence factors. All strains were grown in lysogeny broth medium or agar. All strains were grown an overnight culture and were cultured at 37 °C to reach OD_{600} 0.4. It was equal $1x10^8$ to achieve an appropriate number of bacteria to do biological activity and antibiotics sensitivity.

Antibiotics sensitivity

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer standardized single-disk diffusion method, adhering to established guidelines (Bauer et al., 1966). In brief, Mueller-Hinton agar medium was prepared and sterilized via autoclaving. Bacterial inocula were standardized to a turbidity equivalent to a 0.5 McFarland standard, approximating a cell density of 1.5×10^8 CFU/mL, and subsequently uniformly distributed onto the agar surface using a sterile L-shaped spreader. Commercially prepared antibiotic disks, containing standardized concentrations of antimicrobial agents, were aseptically applied to the inoculated agar plates and gently pressed to ensure uniform contact. Following overnight incubation at 37 °C, the diameters of complete zones of inhibition were measured in millimeters (mm) using a calibrated ruler. The interpretation of susceptibility or resistance to each antimicrobial agent was determined by comparing the observed zone diameters with established interpretive criteria published by the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) (Ferraro, 2000), thereby classifying isolates as susceptible or resistant [14, 15].

Culturing of cell lines

Both A549 human lung epithelial and T24 bladder cell lines were maintained in a humidified incubator at 37°C with a controlled atmosphere of 5-8% CO2.

Subculturing of these cell lines was performed using trypsinization. Briefly, the culture medium was aspirated, and the cell monolayers were washed with 5 mL of Hanks' Balanced Salt Solution (HBSS). Subsequently, 3 mL of 1× Trypsin/EDTA (Lonza) was added, and the cells were incubated for 10-20 minutes to facilitate detachment. Following trypsinization, 7.5 mL of Dulbecco's Modified Eagle's Medium (DMEM), appropriate for the respective cell line's growth requirements, was added to each flask, and the cells were resuspended by repeated pipetting. The resulting cell suspensions were centrifuged at 200 × g for 5 minutes to pellet the cells. The supernatant was then discarded, and the cell pellets were resuspended in 6 mL of complete DMEM. Finally, cell counts were determined using a haemocytometer to ensure accurate seeding densities for subsequent experimental procedures.

Infection assay

A modified kanamycin protection assay was employed to quantify bacterial infection, internalization, and intracellular survival. Bacterial cultures, grown overnight, were harvested by centrifugation, washed, and resuspended in Dulbecco's Modified Eagle Medium (DMEM) to achieve multiplicities of infection (MOIs) of 0.5, 2.5, and 10 for both A549 and T24 cell lines. These cell lines were subsequently infected by incubation with the prepared bacterial suspensions at 37°C in a 5% CO_2 atmosphere for a period of 3 hours. Following the infection period, extracellular, non-adherent bacteria were removed by two washes with Hanks' Balanced Salt Solution (HBSS). To enumerate total cell-associated bacteria (both extracellular and intracellular), a subset of infected wells underwent three washes with HBSS before lysis with a 0.2% (v/v) Triton X-100 solution for 15 minutes. Serial dilutions of the resulting lysates were then plated onto Luria-Bertani (LB) agar and incubated at 37°C for 48 hours. Bacterial colonies were enumerated, and the bacterial load (expressed as colony-forming units per milliliter, CFU/mL) was calculated using the following formula: Total bacterial CFU/mL = (Number of colonies × Dilution factor) / Volume plated (mL)[16].

Total RNA Extraction, cDNA Synthesis, and Quantitative PCR Analysis in *Pseudomonas aeruginosa*-Infected Patients

Whole blood specimens were procured to establish a healthy control group and to define experimental cohorts consisting of patients afflicted with *Pseudomonas aeruginosa* infection. Total RNA was extracted from all samples in accordance with the manufacturer's protocol (Solarbio Life Science). Subsequent to centrifugation at 200 x g for 5 minutes, cellular lysis was performed, and RNA was purified via sequential washes utilizing RPE buffer followed by two washes with WT buffer. Purified RNA was eluted and stored at -20°C pending further processing. Reverse transcription was employed to synthesize complementary DNA (cDNA). Quantitative PCR (qPCR) was performed utilizing Primer Design Precision's 2x qPCR SYBR Green Master Mix. Pre-designed primer/probe sets targeting the gene of interest served as a quality control measure. Amplification was conducted on an Applied Biosystems 7900HT Fast Real-Time PCR System for 40 cycles. The cycle threshold (Ct) values, determined by the fluorescence emission of the amplified

product, were utilized to assess gene expression levels. Ct values obtained pre- and post-infection are presented in the subsequent list, with expression levels exhibiting an inverse correlation with Ct values. These primers were purchased from the South Korean company Macrogen. Relative expression was normalized by comparison to established reference genes. When comparing relative fold expression differences, the Ct technique was utilized [2].

Effect of *Pseudomonas aeruginosa* infection on the levels of surface molecules in A549 and T24 cells

This study investigated the impact of *Pseudomonas aeruginosa* infection on cell surface membrane protein expression in A549 (human lung carcinoma) and T24 (human bladder carcinoma) cell lines using quantitative polymerase chain reaction (qPCR). Cells were seeded in six-well tissue culture plates at a density of $2x\ 10^5$ cells/well and incubated overnight to facilitate adherence. Subsequently, cells were infected with a virulent strain of *P. aeruginosa* at a multiplicity of infection (MOI) of 10 for a duration of three hours. Following the infection period, cells were harvested for RNA extraction and subsequent qPCR analysis. The cell culture medium was aspirated, and cells were washed twice with Hanks' Balanced Salt Solution (HBSS) to remove residual bacteria and media components. Total RNA was extracted, and its concentration was quantified using a NanoDrop Lite spectrophotometer. To eliminate potential genomic DNA contamination, RNA samples were treated with DNase I according to the manufacturer's protocol. Purified RNA samples were then stored at -80°C until further processing. Complementary DNA (cDNA) synthesis was performed using RNA extracted from both infected and uninfected (control) cells. The cDNA was then used as a template for qPCR analysis, adhering to the established laboratory protocol. This enabled the quantification of mRNA transcripts corresponding to cell surface membrane proteins in both infected and control groups, thereby facilitating a comparative analysis of gene expression levels [17].

The Estimation of Human CXCR1, TLR-4, CXCL8

Following a brief centrifugation to clarify serum samples, the quantification of CXCR1, TLR-4, and CXCL8 concentrations was executed via immediate assay. A stock standard solution of 2000 pg/mL was meticulously prepared by reconstituting the lyophilized standard with 1.0 mL of standard sample diluent. A series of twofold serial dilutions, initiated by transferring 500 µL of standard sample diluent into a designated 1000 pg/mL tube and propagated through subsequent tubes, generated the requisite standard curve. The assay procedure commenced with the precise dispensation of 100 µL of either standard or sample into individual wells of a microtiter plate. Following a 90-minute incubation period at 37°C under hermetic conditions, each well underwent three iterative washes with 1x wash buffer, employing cycles of aspiration and replenishment. Subsequently, 100 µL of a working solution comprising biotin-conjugated anti-human CXCR1, TLR-4, and CXCL8 antibodies was introduced into each well, followed by a 30-minute incubation at 37°C. A further three washes with 1x wash buffer were then performed. After resealing the microtiter plate, 100 µL of HRP-avidin working solution was

meticulously dispensed into each well. Following another triplicate wash with 1x wash buffer, the plate was incubated for 30 minutes at 37°C. The chromogenic reaction was initiated by the addition of 100 µL of TMB substrate to each well, followed by gentle agitation and incubation in darkness at 37°C for a duration of 15-20 minutes. The reaction's termination was effected by the addition of 50 µL of stop solution to each well. Optical density measurements were acquired within 30 minutes utilizing a microplate reader at a wavelength of 450 nm. Subsequent to data acquisition and analysis, the concentrations of CXCR1, TLR-4, and CXCL8 within the unknown samples were determined by interpolation against the generated standard curve.

Effect of CXCR1 and TLR-4 antibodies on bacterial infection in A549 and T24 cells

In this investigation, the interaction between select antibodies and the susceptibility of A549 and T24 cell lines to *Pseudomonas aeruginosa* infection was elucidated. Initially, A549 and T24 cells were seeded at a density of 1 x 10^5 cells per well in 96-well tissue culture plates, each well containing 100µl of culture medium. Following an overnight incubation period, allowing for cellular adherence and attainment of approximately 70% confluence, the cells were subjected to a preliminary wash with warmed Hanks' Balanced Salt Solution (HBSS). Subsequently, the cells were exposed to a 1-hour treatment with antibodies, diluted in a buffer consisting of HBSS supplemented with 1% sodium azide and 10% fetal bovine serum (FBS), henceforth referred to as BBN. The antibodies were administered at a concentration of 10µg/ml. Post-antibody treatment, the cells were again washed with HBSS and subsequently challenged with *P. aeruginosa* at a multiplicity of infection (MOI) of 10. The infected cells were incubated for a period of 3 hours, after which they underwent two washes with HBSS to remove non-adherent bacteria. To enumerate the total bacterial burden, encompassing both extracellular and intracellular bacteria, the infected cells were subjected to three washes with HBSS, followed by a 15-minute incubation with 0.1% Triton X-100 to induce cellular lysis. Serial dilutions of the resultant cell lysate were prepared in HBSS and plated onto lysogeny broth (LB) agar. Following a 48-hour incubation period, colony-forming units (CFUs) were enumerated, and bacterial counts were determined using established CFU calculations [18].

Statistical analyses

Statistical analyses are performed using GraphPad Prism.8. IC_{50} was determined using dose response inhibition using non-line fit. Results are presented as mean ± standard error of mean (SEM).

3 **Results**

Study of Biological Activity

Isolation of bacteria

The aim was to examine which species are the most virulent *Pseudomonas aeruginosa* pathogen from burn and wound infection. 55 specimens were collected from patient who suffered burn and wound infection from different hospitals in Al-Najaf and 25 specimens healthy control during (24.07.2024 until 23.09.2024).

Pseudomonas aeruginosa was identified based on phenotyping, biochemical tests, morphology shape and selective media. The results showed that only 3 *Pseudomonas aeruginosa* was detected at burn and wound infection than others bacteria. The prevalence was 09.41% of *Pseudomonas aeruginosa* as demonstrated in (Figure 1).

Pseudomonas aeruginosa **infection in both A549 and T24 cells**

This section was to determine the number of bacteria per number of host cells that can infect and be internalised into two cell lines, A549 human lung epithelial cells and T24 bladder cells. These cell lines were infected with *Pseudomonas aeruginosa* to measure the total bacterial infection after 3hr and 6hrs , and to determine which MOI will be the best in the subsequent investigations. The CFU assay was used to measure the total infection (i.e. adherent) as described in methodology. The MOI used were 0.5, 2, 5 and 10 (bacteria/mammalian cell). The data showed that CFU per host cells was increased when using a higher MOI (Fig 2). For the A549 and T24 cells, MOI 10 was the best ratio of infection compared with other MOI at 3 hrs, because the results were statistically significantly different between MOI 10 compared with other MOI. However, the best MOI for A549 and T24 cells was 5 after 6hr infection, because the data was statistically significantly different between MOI 5 compared to other MOI. The aim of this study was to detect which MOI will be used in the next investigations. Our results showed that the both A549 and T24 number of bacterial infection of MOI 100 was significantly higher than MOI 5 after 3 hr infection. It seems that the appropriate ratio at MOI 5-10 to A549 and T24 cells.

Changes in cell surface molecules gene expression at the mRNA level after infection of A549 and T24 cells

To analyse which gene expression levels are changed during *Pseudomonas aeruginosa* infection, qPCR was used. TLR-4,TLR-5, CXCL8 and CXCR1 gene expression changes were analysed in both cell lines in response to infection by *clincal strian of Pseudomonas aeruginosa*. The fold change was calculated using equation 2^ (-ΔΔCt). All TLR-4,TLR-5, CXCL8 and CXCR1 of A549 and T24 had detectable mRNA levels in uninfected cells, as shown in **Fig 3**. However, only 3 cell surface molecules which are TLR-4, CXCL8 and CXCR1 are involvement in infection, namely, changes in both host cell types and have also changes with patients in response to *Pseudomonas aeruginosa* infection. The results shows there is down-regualation to CXCR1 in response to infection compared to the level of CXCR1 with non infected cells or healthy cases. However, TLR-4 and CXCL8 were up-regulation in response to infection.

The TLR-4, TLR-5, CXCL8 and CXCR1 expression were measured by qPCR in patients and cells have infection with *Pseudomonas aeruginosa*. Expression of these Pro-inflammatory and cell surface molecules in non-infected and infected cells was calculated using the $2^{-\Delta\Delta Ct}$ method following estimation of the house keeping gene **GAPDH**. **TLR-4, TLR-5, CXCL8 and CXCR1** showed changes in A549, T24 and whole blood cells in patients. The significance of differences was tested by

one-way ANOVA, where **** $p<0.0001$ significant; ns=non-significant. The data are the means of 3 separate experiments with duplicate.

Protein expression levels of TLR, CXCL8, and CXCR1

This study investigated the modulation of selected surface molecules at the protein level in response to bacterial infection in patients. Serum levels of TLR-4, CXCL8, and CXCR1 were quantified via ELISA during infection. Notably, infection with a clinical strain of *Pseudomonas aeruginosa* elicited a significant increase in both TLR-4 and CXCL8 levels relative to healthy controls. Conversely, CXCR1 levels exhibited a marked decrease during infection with the same *P. aeruginosa* strain (Fig. 4). These findings suggest a potential role for TLR-4, CXCL8, and CXCR1 not only in bacterial pathogenesis but also in the host response to bacterial infection .

CXCR1 antibody is increase the infection and TLR-4, CXCL8 antibodies are decrease the bacterial infection in A549 and T24 cells

This study investigated the roles of Toll-like receptor 4 (TLR-4), C-X-C chemokine receptor type 1 (CXCR1), and C-X-C chemokine receptor type 8 (CXCR8) in *Pseudomonas aeruginosa* infection through antibody-mediated blockade of these host surface proteins. In brief, cells were pre-treated with the respective antibodies for one hour, followed by two washes with Hanks' Balanced Salt Solution (HBSS) to eliminate non-specific binding. Subsequently, cells were infected with *P. aeruginosa* for three hours, washed twice, and lysed using Triton X-100. Serial dilutions of the lysates were then plated onto lysogeny broth (LB) agar for bacterial enumeration using colony-forming unit (CFU) determination. Data presented in Figure 6 demonstrate that antibodies targeting TLR-4, CXCR1, and CXCR8 modulated bacterial infection and/or internalization compared to cells infected with *P. aeruginosa* alone. This study aimed to elucidate the involvement of TLR-4, CXCR1, and CXCR8 in *P. aeruginosa* infection. **As Fig 5**, The findings reveal a pronounced effect of anti-CXCR1 antibody on bacterial infection in both A549 and T24 cell lines, with a demonstrable increase in bacterial burden in antibody-treated cells. Conversely, anti-TLR-4 antibodies resulted in a reduction in infection compared to untreated controls. However, there is no effect anti-CXCL8 on bacterial infection.

4 **Discussion**

To establish optimal experimental conditions, we first determined the multiplicity of infection (MOI) of *Pseudomonas aeruginosa* required to induce a significant immune response in A549 and T24 cell lines. As depicted in Figure 2, bacterial infection at MOI 100 resulted in significantly higher bacterial loads compared to MOI 5 after 3 hours in both cell lines. These findings suggest an optimal MOI range of 5-10 for both A549 and T24 cells. Our results align with previous studies demonstrating that MOI 2 was effective for infecting RAW 264 macrophages [19] and MOI 1 was suitable for *B. pseudomallei* infection of J774.2 cells [20]. Furthermore, we observed that MOI 10 consistently yielded the highest infection rates in both A549 and T24 cells, corroborating findings by Haraga *et al*. in HeLa cells [21, 22].

Subsequently, we analyzed gene expression profiles in whole blood samples from patients with *Pseudomonas aeruginosa* infection and in infected A549 and T24 cell lines. As shown in Figure 3, CXCL8 and TLR-4 mRNA levels were significantly upregulated in both patient blood and infected cell lines, while CXCR1 expression was downregulated. These findings are consistent with previous reports demonstrating increased CXCL8 expression following *Pseudomonas aeruginosa* infection [23], and elevated TLR4 expression in response to Gram-negative bacterial infections [24].

The pathogenesis of *Pseudomonas aeruginosa* involves a complex interplay of bacterial factors, including flagella, pili, and the type III secretion system, which facilitate direct interactions with host cells [25]. Furthermore, quorum-sensing molecules regulate the release of soluble factors that contribute to the spread of infection [26]. Our findings revealed a significant downregulation of CXCR1 expression in both infected cells and patient blood. Although this downregulation may be more pronounced over longer time periods or in the presence of a larger monocyte population, it suggests a potential regulatory mechanism during infection.

To further investigate the role of these key molecules, we employed neutralizing antibodies against TLR-4, CXCR1, and CXCL8. Notably, anti-CXCR1 antibody treatment significantly increased bacterial burden in both cell lines, while anti-TLR-4 antibodies exhibited a protective effect by reducing infection. In contrast, anti-CXCL8 antibodies had no significant impact on bacterial infection. These findings are supported by previous studies demonstrating that antibodies targeting cell surface molecules can modulate bacterial infection. For example, antibodies against various cell surface molecules have been shown to attenuate Salmonella infection [27], *Neisseria meningitidis* infection [28], and *P. aeruginosa* infection [29]. This study provides evidence supporting the hypothesis that Toll-like receptor 4 (TLR-4), chemokine ligand 8 (CXCL8), and its cognate receptor play pivotal roles in the immune response of infected patients, particularly in the context of *Pseudomonas aeruginosa* infections. This involvement was observed across both the in vitro models utilizing A549 and T24 cell lines. Concordant elevations in both mRNA and protein levels of interleukin 8 (IL-8) and TLR-4 correlate with bacterial infection, suggesting their potential utility as biomarkers for *P. aeruginosa* infection. Conversely, a concomitant decrease in CXCR1 mRNA and protein expression was observed in correlation with bacterial infection, indicating a potential role for CXCR1 modulation in bacterial adhesion at the cell surface. Further investigations are warranted to elucidate the precise molecular mechanisms by which these cell surface molecules contribute to *P. aeruginosa* pathogenesis.

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Declaration

The authors have no discord of interest to declare, and the Funincial support is University of Kufa.

Ethics Clearance

This research does not contain any studies with human subjects or animals done by any of the authors.

ТАБЛИЦЫ

Table 1. Primer disgen in this study was performed from NCBI-Blast.

Primer	Forward	Reverse
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
CXCR1	CTGATCTCTGACTGCAGCTCCT	CAGCAATGGTTTGATCTAACTGAAG
CXCL8	AGTTTTTGAAGAGGGCTGAGA	TGCTTGAAGTTTCACTGGCATC
TLR-4	CCTGCGTGGAGGTGTGAAA	AAAGGCTCCCAGGGCTAAAC
TLR-5	GTCACCAAACCAGGGATGCT	GGGCAAAGTCAATTGCCAGG

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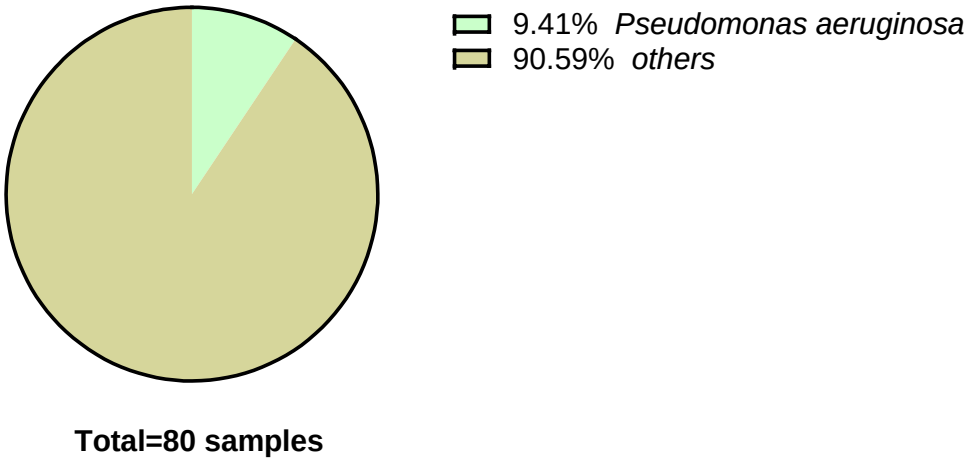
РИСУНКИ

Figure 1. Percentage of different types of bacteria isolated from the burn and wound infection.



A pie chart demonstrating the relative representations of isolated bacterial species from 80 samples from the burn and wound infection from different hospitals in Al-Najaf and Baghdad cities. Pure colonies were isolated and placed in $VITEK_2$ microbial identification. For more accurate validation, manual diagnostic methods have been performed included biochemical and culturing tests.

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Figure 2. *Pseudomonas aeruginosa* infection in T24 and A549 cells at different multiplicities of infection.

Cells were infected at MOI 5, 25, 50 and 100 for T24 cells and A549 human lung cells. The infection (adhered) was measured 3hr and 6hr post-infection using CFU for counting. The significance of the differences between treatments were tested by two-way ANOVA, where * $p<0.05$, *** $p<0.001$ significant; ns=non-significant. The results are the means of 3 separate experiments with 4 replicates

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Figure 3. Upper pannel the level of TLR-4, TLR-5, CXCL8 and CXCR1 at mRNA in patient who diagnosed infected by *Pseudomonas aeruginosa* compared with the level of these cell surface protein in healthy cases.

Lower pannel the level of TLR-4, TLR-5, CXCL8 and CXCR1 at mRNA in both A549 and T24 cells infected by *Pseudomonas aeruginosa* compared with the level of these cell surface protein with non infected cells.

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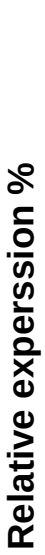
Figure 4. The levels TLR-4, CXCL8 and CXCR1 in patients infected with *Pseudomonas aeruginosa* and healthy cases.



The bars showed changes TLR-4, CXCL8 and CXCR1 in serum. The significance of differences was tested by one-way ANOVA, where **** $p<0.0001$ significant; ns=non-significant. The data are the means of 88 samples of each panels.

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Figure 5. Anti-TLR-4, anti-CXCL8 and anti-CXCR1 antibodies reduce or enhance bacterial infection.

A549 and T24 cells were pre-treated with anti-TLR-4, CXCL8 and CXCR1 antibodies before *Pseudomonas aeruginosa* infection. Bars show the bacterial infection to A549 cells and T24 by clincal strains. The effects of different treatments were tested by one-way ANOVA, where ** significant at $P<0.01$; *** significant at $P<0.001$. The *Pseudomonas aeruginosa* infection are the means of 3 experiments with 4 replicates.

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ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

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Блок 3. Метаданные статьи
CXCR1, TLR-4, AND CXCL8: KEY MEDIATORS OF *PSEUDOMONAS AERUGINOSA* VIRULENCE IN WOUND AND BURN INFECTIONS

Сокращенное название статьи для верхнего колонтитула:
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Keywords: TLR-4,5 , CXCL8, CXCR1, *P. aeruginosa*, T24 and A549 cells**.**

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2	Alrahimi, Jehan	The Role of Tetraspanins in Pseudomonas	The Role of Tetraspanins in Pseudomonas	University of Sheffield	University of Sheffield	https://etheses.whiterose.ac.uk/id/eprint/19680/

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