Isolation and identification of Klebsiella pneumoniae bacteria and study of virulence gene expression using RT-PCR and its effect on some immunological variables

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Abstract

Eighty samples were collected from inpatients and healthy individuals in health facilities in Salah al-Din Governorate. The sample consisted of 48 patients (55%) and 32 healthy individuals (45%). Forty-four (44) urine samples and thirty-six (36) samples from burns and wounds were collected between March 2025 and July 2025. The patients' ages ranged from 5 to 65 years. All samples were subjected to bacterial culture, and the results showed that 65 (55%) of the samples were positive for bacterial culture, while 32 (45%) of the samples were negative for bacterial culture. Subsequently, blood samples were collected from the group of patients and healthy individuals and separated by centrifugation. Then, the biochemical variables, which included (IL-6, TNF-α), were measured. The results showed a significant increase in the group of patients infected with Klebsiella pneumoniae compared to the control group (healthy individuals) at a probability level of $P \leq 0.05$. Also, virulence factors were investigated phenotypically, and the results of genetic detection using polymerase chain reaction (PCR) showed the presence of the biofilm of Klebsiella pneumoniae.

Keywords / Klebsiella pneumoniae, RT-PCR, IL-6, TNF-α.

Introduction

Klebsiella pneumoniae is an opportunistic pathogen. It is a Gram-negative bacterium belonging to the Enterobacteriaceae family. It has a capsule and is non-motile (1). Klebsiella pneumoniae is one of the most important types of Klebsiella bacteria, which are naturally found in the human digestive system and can cause many opportunistic infections. It is directly associated with patients with urinary tract infections, as well as patients with wounds, burns, and pneumonia (2). The age groups most susceptible to infection with this type of bacteria are newborns and the elderly, because this bacteria is highly contagious and is primarily responsible for an increasing number of community-acquired infections (3). These bacteria infect the mucous surfaces of the digestive system and pharynx in humans and can exhibit high degrees of virulence and antibiotic resistance, as they can resist a range of antibiotics and are called multidrug resistant (4,5).

K. pneumoniae is an opportunistic pathogen, as it is the main culprit in catheter-associated urinary tract infections of patients in hospitals and health institutions, because infection with it

occurs in hospitals, as it is transmitted and infected from person to person through contact or direct contact with patients involved, as well as through wounds and burns contaminated with K. pneumoniae bacteria (6,7).

K. pneumoniae possesses virulence factors that help it resist antibiotics. The co-existence and expression of virulence factors and the genetic drives for antibiotic resistance all complicate treatment outcomes. Therefore, the development of multidrug-resistant K. pneumoniae (MDR) poses a significant risk to the healthcare system in hospitals and health institutions (8).K. pneumoniae causes severe pathological changes in various host organs, especially in lung tissue. There is a vast difference between pathogenicity and virulence, as pathogenicity is the ability of bacteria to cause disease.As for virulence, it means the virulence of the bacterial species, i.e., the extent of its ability to cause disease. The virulence factors vary according to the location of the infection in the human body. The virulence factors of the strains that cause acquired urinary tract infections differ from the virulence factors possessed by the strains isolated from hospitalized pneumonia patients and health institutions (9,10).

The main cause of urinary tract infections is K. pneumoniae bacteria, which accounted for more than 90% of cases, whether they were Gram-negative or Gram-positive bacteria, in addition to some types of viruses and parasites that can cause direct infection, which often occurs through contact via the urinary tract, such as denovirus (11). The causes of urinary tract infections include common pathogenic bacteria found in intensive care units in hospitals and health institutions, whether these causative bacteria are Gram-negative or Gram-positive (12).

The term UTIs refers to many urinary tract diseases, such as kidney and ureteritis, as well as urethritis, and is detected by the presence of bacteria in the urine that lead to infections, estimated at more than 510 cells/ml after culturing a urine sample (13). Urinary tract infections (UTIs) occur at a higher rate in females than in males. The reason for this difference and infection is due to hormonal and anatomical differences, as the length of the urethra in males, as well as the secretions of the prostate, all play a role in inhibiting the growth of pathogenic bacteria, thus leading to a reduction in the infection rate in males. Circumcision plays a fundamental and important role in preventing infections, as uncircumcised children have a higher infection rate than circumcised children (14,15).In addition to the existence of several factors that contribute to the infection, including age, gender, the condition of the organs that collect and store urine, social status, and the level of education of the parents, the urinary system represents and excretes it to the outside and includes the kidney, ureter, bladder, and urethra (16). Interleukins play an important and effective role in stimulating proteins in the body to perform their function in resisting infections, through white blood cells that can destroy harmful bacteria and other harmful bodies that enter the body. It can trigger a series of reactions that arm the body's leukocytes against burns and wounds, and K. pneumoniae can release leukocytes and detect the presence of bacteria at the site of injury. Then these cells release interleukin-6, which

in turn gives a signal to white blood cells to stimulate T-cells, which in turn release interleukin-6, which stimulates various immune system cells to defend the body (17, 18).

TNF-α is an important cytokine that is secreted during inflammation caused by burns and wounds caused by K. pneumoniae bacteria.It is an angiogenic factor found mainly in the lining of connective tissues and in T cells, which stimulates endothelial cells and increases their ability to divide and affects pro-angiogenic elements and has the ability to treat advanced inflammations caused by urinary tract infections (19, 20).Based on the results of this study, the current research aims to isolate and identify Klebsiella pneumoniae bacteria and to study the gene expression of virulence genes using RT-PCR technology and its effect on some immune variables.

Material and Methods

Collection of specimens

Eighty samples were collected from inpatients and healthy individuals in health facilities in Salah al-Din Governorate. The sample consisted of 48 patients (55%) and 32 healthy individuals (45%). The samples included urine (44), wound/burn swabs (36), and blood samples taken from patients and healthy individuals between March 2025 and July 2025. Patient ages ranged from 5 to 65 years. All samples were subjected to bacterial culture, and the results showed that 65 (55%) samples were positive for bacterial culture, while 32 (45%) samples were negative for bacterial culture. Then, blood was collected from the group of patients and healthy individuals and separated by centrifugation. Then, the biochemical variables, which included (IL-6, TNF-α), were measured.

Diagnosis of bacterial isolates

K. pneumoniae bacteria were identified by colonies growing on MacConkey agar medium and by observing the shape of the colonies through color, borders and mucous texture (21). Then the colonies were taken and placed on a glass slide and mixed with normal saline solution, and left to dry. After that, they were quickly passed over the flame two to three times to fix the colonies, then stained with Gram stain and examined under the microscope to observe their shape, color, and location of aggregation (22).

Morphological detection of the biofilm of K. pneumoniae bacteria

The micro-calibration plate method was used to detect biofilm production according to the method of (Lamichhane et al.) (23).

Molecular study of K. pneumoniae bacteria

DNA was extracted from bacterial cells appearing on a medium of brain and heart fluid (cerebrospinal fluid). These cells were grown on this medium for 24 hours at a temperature of 37°C. The extraction process was carried out according to the ABIOpure Extraction protocol and its specific steps.

Estimation of (IL-6, TNF-α) levels in the study groups

The level of (IL-6, TNF-α) was estimated using ELISA technology and a ready-made kit supplied by the Chinese company (Melsin Medical)).

Statistical Analysis

The SPSS statistical program was used to find the mean and standard deviation value SD±. The means for the patient group were also determined compared to the control group (healthy individuals) using the t-test at a probability level of ($P \geq 0.05$).

Result and Desiccation

Isolation and diagnosis of Klebsiella pneumoniae bacteria

Eighty samples were collected from inpatients and healthy individuals in health facilities in Salah al-Din Governorate. The sample consisted of 48 patients (55%) and 32 healthy individuals (45%). The samples included urine (44) and wound/burn swabs (36). Blood samples were also taken from patients and healthy individuals for the period from March 2025 to July 2025, and the ages of the patients ranged between (5-65) years. These samples were cultured on MacConkey Agar and Blood Agar. The culture results showed that (48) samples, representing (55%), showed clear bacterial growth on the culture medium, and (32) samples, representing (45%), did not give any bacterial growth, as shown in Figure (1).

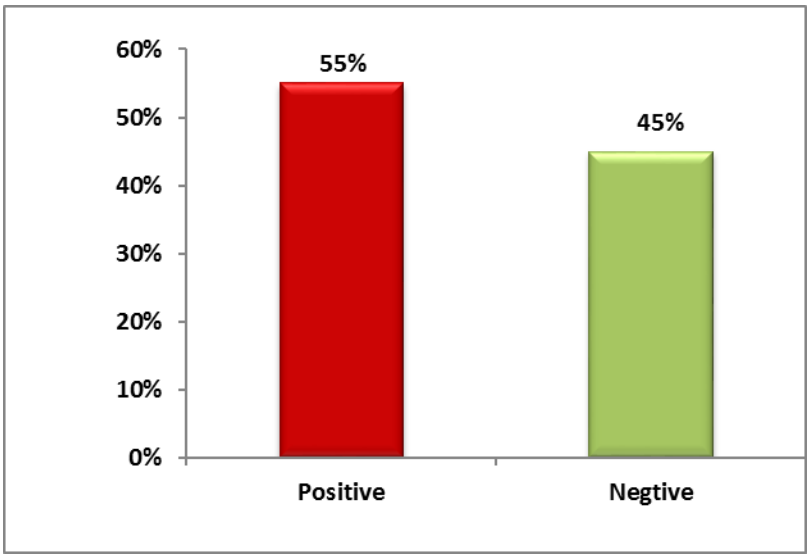

Figure (1) shows the percentage of bacterial growth on the culture medium

The results of bacterial culture on MacConkey medium were as follows, according to the source of the sample: urine from a total of (80) samples (44) and a sample of wound and burn swabs (36). Blood samples were also taken from patients and healthy individuals. The present study shows that the percentage of infections that appeared as bacterial infections was higher in urine samples than in all samples collected from different clinical sources. This is consistent with what Al-Azzi (2019) (24) and Al-Saadi (25) concluded, and they showed in the results of their study that the percentage of urine samples was positive at higher percentage than all the other samples studied.

Phenotypic detection of Biofilm

Biofilm production of the 15 multidrug-resistant K. pneumoniae isolates under study was detected using the Microtiter Plate Method. The present study showed that all isolates were biofilm-producing at a rate of 100%, and the absorbance values of the control pits were (0.3). The biofilm-producing isolates were distributed into moderate producers, as there were 12 isolates out of 17 isolates, at a rate of 71.48%, and 5 strong biofilm-producing isolates, at a rate of 28.52%. The classification of biofilms was based on the absorbance values of the pits in which the isolates were distributed, after the average of the three couplings for each isolate was extracted and compared with the absorbance values of the control pits, as shown in Figure (2).

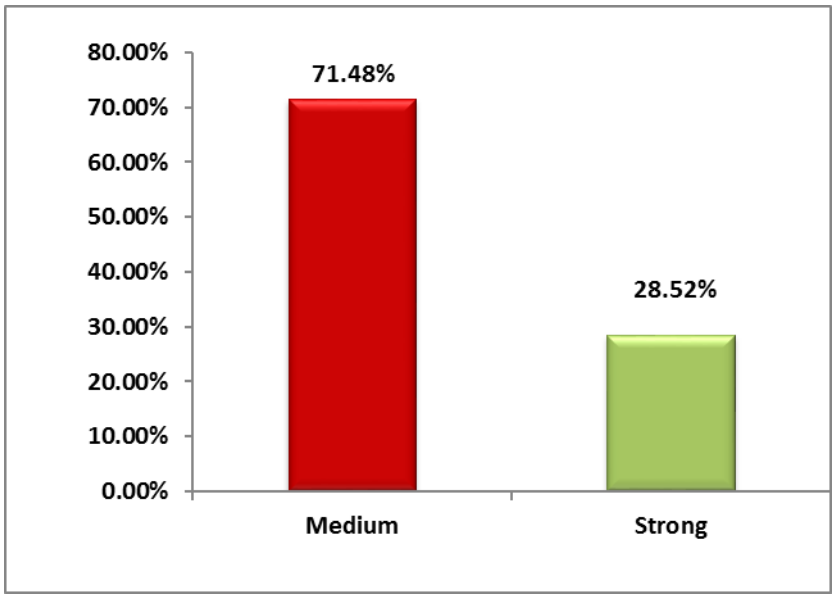

Figure (2) The intensity of biofilm synthesis in K. pneumoniae bacteria.

The results are agree with the result of Al-Rubyaie (26), who showed in his study results that all K. pneumoniae bacterial isolates are biofilm-producing at a rate of 100%, because the biofilm has an important and significant role in the virulence of K. pneumoniae bacteria, as it is responsible for continuous infection due to its resistance to phagocytosis and killing due to humoral and cellular immunity and resistance to antibiotics (27). Furthermore, the ability of bacteria to produce biofilms varies from one isolate to another due to the physical and chemical properties of K. pneumoniae bacteria, the physical interaction between the components, the type of surface to which the biofilms adhere, as well as temperature and pH (28).

Molecular detection of biofilm genes in K. pneumoniae bacteria

The 17 isolates of multidrug-resistant pneumoniae. K were subjected to molecular detection of some biofilm genes using primers specific to these genes and polymerase chain reaction technology. The genes that were detected are (lexStrat.mrkD). The results showed that all isolates were carriers of the luxS gene, which is a type II quorum-sensing gene, as shown in Figure (3).

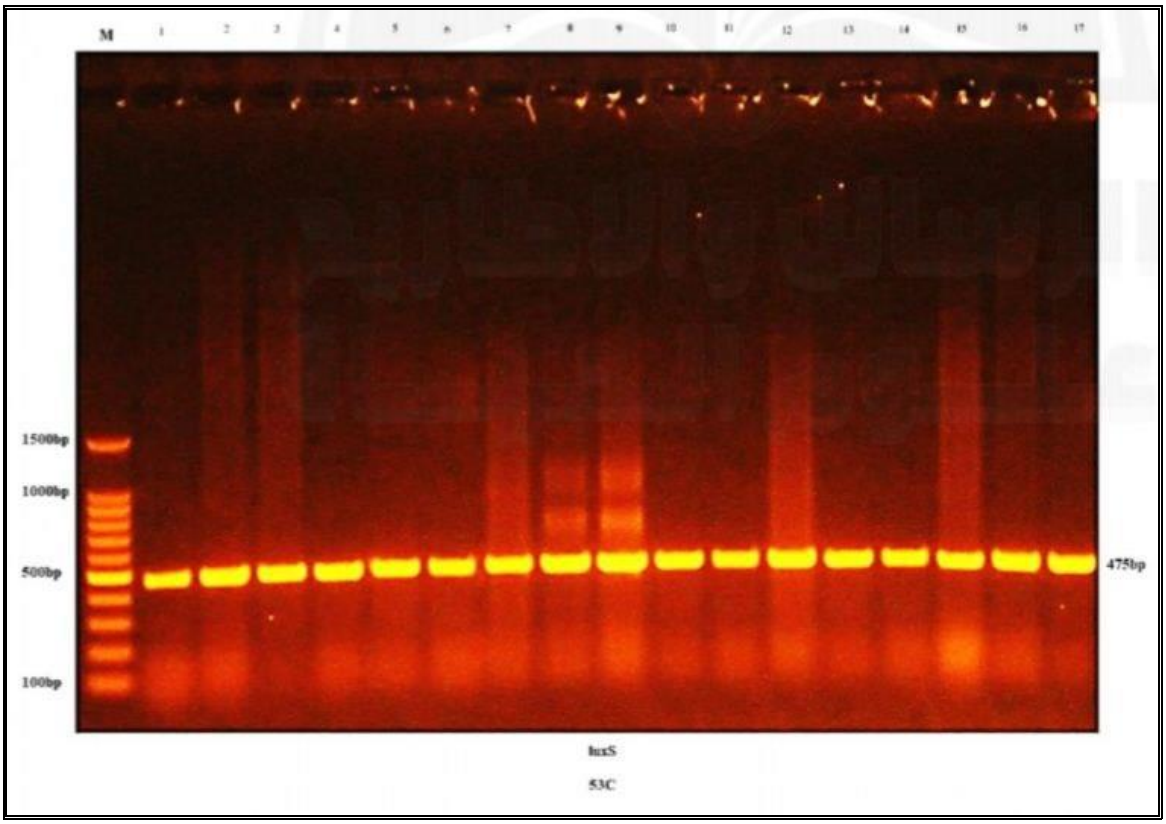

Figure (3) shows the electrophoresis of the PCR reaction product for the luxs gene using 1.5% acarose gel with Ethidium Bromide stain (90 minutes, 100 V/cm2). The M path is a volumetric indicator measuring 100 base pairs (100 bp).

The results of the current study are consistent with those of Mohamed (29) and Shadkam et al. (30), who demonstrated that all isolates under study carried this gene at a rate of 100%. This is because K. pneumoniae bacteria form biofilms on various surfaces, and cell adhesion depends on the production of exopolysaccharides and type III adhesive proteins (31). Furthermore, a type II functional quorum-sensing system (QS) was clearly identified in a clinically isolated, multidrug-resistant strain of K. pneumoniae. It was found that there is a relationship between the hexs gene, which is one of the important genes for the synthesis of type II autotrophs 2-AL, and mutations in quorum sensing, and that genes associated with quorum sensing cause changes in biofilm formation and in the expression of lipopolysaccharides (32). The second biofilm gene, mrkD, was detected, and the primer specific to it was used. The results appear the presence of the gene in the K. pneumoniae bacterial isolates under study at a rate of (97%), as shown in Figure (4).

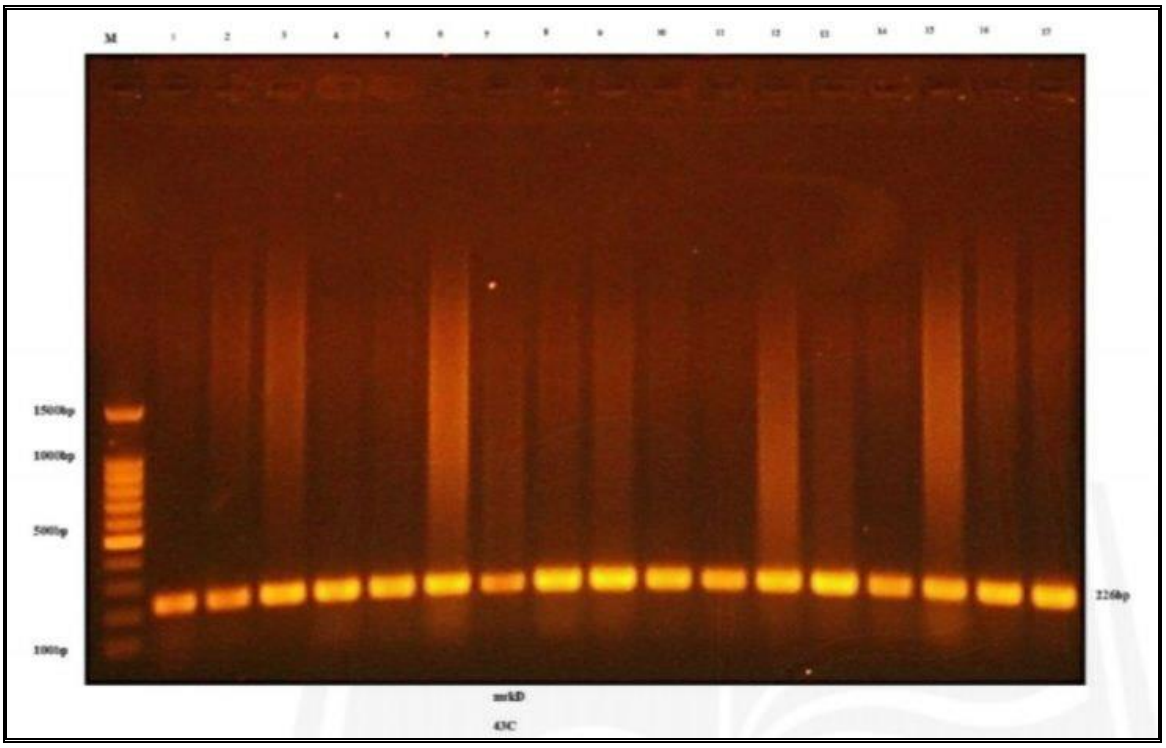

Figure (4) shows the electrophoresis of the PCR reaction product for the mrKD gene using 1.5% acarose gel with Ethidium Bromide stain (90 minutes, 100 V/cm2). The M path is a volumetric indicator measuring 100 base pairs (100 bp).

The results of the present study are agree with the results of the study by both (Saady) (33) and the results of the study by (Rastegae et al.) (34), who indicated in their study results the presence of the mrkD gene in all multidrug-resistant K. pneumoniae isolates at a rate of 100% and (94.5%) respectively.

Immunological variables of blood samples in both groups

Table (1) shows the mean ± S.D of the immunological parameter for the studied samples in both groups.

Groups	Mean ± SD		
	Control	Patients	P-Value
Parameter	n=24	n=24	
IL-6 (Pg/ml)	27.15± 19.62	33.03± 15.97	P ≤ 0.05
TNF-α (pg/ml)	51.71± 41.746	69.03± 51.04	P ≤ 0.05

The results of the current study showed a significant elevated in the levels of both IL-6 and TNF-α, measured in picograms/ml, in the patient group compared to the control group, at ($P \leq 0.05$), as shown in the following figures.

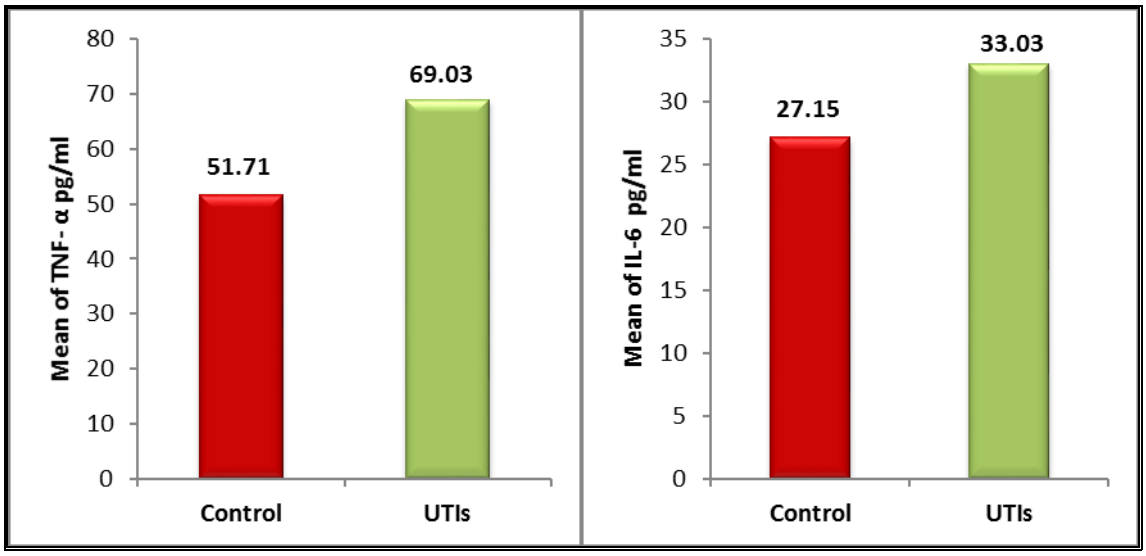

Figure (5) The concentration of immunological variants in urinary tract patients caused by K. pneumoniae bacteria.

Urinary tract infections (UTIs) are among the most common and impactful infections in the body and are directly linked to healthcare facilities and hospitals worldwide (18). Approximately 80% of UTIs are caused by inactivated urinary catheters, specifically those containing the bacterium K. pneumoniae (35).

Interleukin-6 is considered one of the important cytokines for the immune response of people with urinary tract infections (UTIs) caused by K. pneumoniae bacteria. This cytokine is important in the early control of infected individuals and in maintaining bladder control in the future. Identifying its role in the immune system through bacteria that enable a protective response to IL-6 is crucial for exploring how we can exploit this mechanism for new infection control strategies (36). The finding of elevated interleukin-6 levels in patients with urinary tract infections was consistent with Al-Tamimi and his group (37).

As for TNF-α, its results showed a significant rise in the serum of patients with urinary tract infections compared to the control group. This result was agree with (Lu Yu) and his group (38). The reason for the increase is due to the increase in proteins that are responsible for increasing the immune activity of cells several times above normal rates (39). Here, the immune system may be unable to control and stop them, and in that case, these proteins spread rapidly to different parts of the body, not just to the areas affected by the urinary tract infection. It begins to attack healthy cells, devour red and white blood cells, and destroy the liver and other body

organs one by one. At that time, the walls of the blood vessels can allow immune cells to enter the surrounding tissues in the body (40).

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