The Role of Antibiotic-Killed *Staphylococcus aureus* and Its DNA to Cause Arthritis in Rats

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Abstract

The purpose of this study is to investigate the capability of bacterial DNA compared to bacterial lysate in stimulating arthritis using rat model. One hundred mid-stream urine specimens were collected during November 2012 to January 2013, from patients suffering from urinary tract infections attending hospitals in Baghdad, Iraq. Susceptibility of *Staphylococcus aureus* isolates to antibiotics was examined. Twenty five isolates were identified as *S. aureus* and they developed multi drug resistance. *S. aureus* S_1 lyaste and its DNA were intra-articulary injected in rats. The levels of IL-6, anti-ds DNA Ab and leukocytes count were measured. In general, IL-6, anti-ds DNA Ab and leukocytes count were significantly higher in sera of rats injected with cell lysate. Moreover, time period significantly ($P< 0.05$) affected the immunological aspects and joints damage. In a conclusion, bacterial lysate caused more damage to rat joints than DNA. Furthermore, bacterial lysate evoked immunological parameters more than DNA did.

Keywords: Arthritis, *Staphylococcus aureus*, Bacterial lysate, DNA, IL-6 and Anti-ds DNA Ab.

دور خلايا *Staphylococcus aureus* المقتولة بالمضادات الحياتية والدنا المستخلص منها في احداث التهاب المفاصل في الجرذان

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الخلاصة

الغرض من هذه الدراسة هو التحري عن قدرة الحمض النووي البكتيري مقارنة بالبكتريا المتحللة في تحفيز التهاب المفاصل باستعمال نموذج الفئران. تم جمع مائة عينة إدرار وسط المجرى للفترة من تشرين الثاني 2012 لغاية كانون الثاني2013 من مرضى يعانون من التهاب المجاري البولية من مستشفى اليرموك التعليمي والمختبرات التعليمية لمدينة الطب. تم فحص حساسية عزلات العنقوديات الذهبيه لمجموعة من مضادات الحياتية. وقد تم تحديد خمسة وعشرين عزلة تابعة للعنقوديات الذهبية واعطت مقاومة متعددة للمضادات الحياتية. تم حقن الخلايا المتحللة ودنا بكتريا S_1 *S. aureus* في مفصل الجرذان وقد تم قياس مستويات IL −6 ، و Anti−ds DNA Ab و العدد الكلي لكريات الدم البيض. . بشكل عام ، كان تركيز IL −6 ، و Anti−ds DNA Ab والعدد الكلي لكريات الدم البيض أعلى بكثير في مصول الجرذان المحقونة بالخلايا المتحللة. علاوة على ذلك، المدة الزمنية كان لها تأثيرمعنوي ($P< 0.05$) في الجوانب المناعية و

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الضرر في المفاصل. نستنتج من هذه الدراسة ان البكتريا المتحللة سببت المزيد من الضرر لمفاصل الجرذان اكثر من الحمض النووي. علاوة على ذلك، أثارت البكتريا المتحللة المعلمات المناعية أكثر مما فعل الحمض النووي.

Introduction

Septic arthritis is a disease where an infectious agent, most commonly a bacterium, causes an inflammatory response in the joint [1]. The main route by which the pathogen reaches the joint is through the blood [2] and therefore the arthritis is often accompanied by septic symptoms. Other routes for pathogens reach the joints are direct inoculation due to trauma or an iatrogenic event, infected contiguous foci or a neighboring soft-tissue sepsis [3] and urinary tract infections [4,5].*Staphylococcus aureus* is, by far, the organism most frequently responsible for cases of Septic arthritis of the facet joints (SAFJ), and the most common form of dissemination is hematogenous. Potential sources of propagation are the skin, intravenous catheters, and more rarely, respiratory and urinary tract infections [5].

Staphylococcus aureus is a commensal occur on the human body as a part of the normal flora It is an opportunistic pathogen capable of causing a broad range of human diseases and regarded as a leading cause of community and hospital infections [6]. Methicillin-resistant *Staphylococcus* aureus (MRSA) is a growing worldwide problem accounting for at least 25-50% of infectious *S .aureus* isolates [7]. Bacterial lysate was shown to induce a non-specific response (i.e. intensification of phagocytosis) but also to orchestrate both cellular (B, T cell stimulation) and humoral responses (antibodies and proinflammatory cytokines production) [8]. Staphylococcal components have been shown to be inflammatory, and may contribute to sepsis [9]. Bacterial DNA is cellular component with high stimulatory potential for the innate immune system [10]. Krieg suggested that DNA of bacteria multiplying in a joint as a possible cause of joint destruction in septic arthritis [11]. The requirements for immune stimulation by bacterial DNA were defined to be dependent upon short sequences of CpG dinucleotides [10, 12]. Bacterial DNA fragments have the ability to bind to Toll-like receptors and stimulate immune cells. They induce natural killer cell activity and proinflammatory cytokines release from mononuclear cells. DNA is readily taken up by leukocytes, it is logical to hypothesize that bacterial DNA, and specifically unmethylated CpG oligonucleotides, are capable of causing inflammation in the joints and may contribute to disease progression and morbidity [13]. Recently, bacterial DNA was detected in the synovial fluid sample [14].

On this basis, the present work was undertaken to identify the role of bacterial components, especially bacterial DNA compared with bacterial lysate in the excitability of the immune system.

Materials and Methods

Specimen collections

Mid-stream urine specimens were collected from 100 patients (age: 20 - 70 years) presented with urinary tract infections during the period from November 2012 to January 2013. All specimens were collected from patient of AL-Yarmouk Teaching Hospital and the Educational Laboratories of Baghdad Medical City. Patients were carefully educated to collect a proper specimen by themselves; sterile dry wide necked leak proof containers were used for urine collection, collected mid-stream urine and directly were transferred to the laboratory.

Specimens processing

The collected specimens were streaked directly on mannitol salt agar (Hi-media) and incubated at 37ºC for 24 hrs then a single pure isolated colony was transferred to nutrient agar medium for the preservation and to carry out other biochemical tests that confirmed the identification of isolates. Staphylococci were identified depending on the morphological features on culture media and biochemical tests [6,15].

Antibiotic susceptibility test

This test was done to obtain the most sensitive isolate. All *S. aureus* isolates were tested for detecting susceptibility of the isolates to the commonly used antibiotics; Ampicillin (10 µg/ml), Amoxicillin (25 µg/ml), Cefotaxime (30 µg/ml), Cefoxitin (30 µg/ml), Ceftriaxone (30 µg/ml), Cephalothin (25 µg/ml) by Kirby- Bauer method on Muller-Hinton agar (MHA) (Hi-media, India) [16]. Isolates were assigned as resistant, intermediate or susceptible in accordance to the Clinical and Laboratory Standards Institute (2013). Cefotaxime susceptibility performed in broth dilution test, the

results were compared with standard break points values; sensitive (≤ 8 µg/ml), intermediate (16-32µg/ml) and resistant (≥ 64µg/ml) according to (CLSI) recommendations [17].

DNA Extraction from *Staphylococcus aureus*

Staphylococcus aureus S1 DNA were extracted using a Wizard genomic DNA purification kit (Promega, USA).

Preparation of *Staphylococcus aureus* lysate

After culturing the bacteria in brain-heart infusion broth (Himedia, India) for 24 hrs at 37°C, the bacterial cells were harvested by centrifugation for 15 mins at 3000 rpm, washed twice with sterile saline, resuspended in normal saline and then adjusted the bacterial suspension turbidity to a concentration of 1.5×10^8 cells/ml by comparing with 0.5 McFarland. In order to obtain lysate cell, a Mueller Hinton broth containing 160 µg/ml cefotaxime (20X MIC) was inoculated with *S. aureus* cells to obtain density comparable to 0.5 McFarland standard and incubated at 37°C for 24 hrs. In order to check the viability and efficacy of cefotaxime, the broth culture was streaked with the sterile swab on the surface of nutrient agar plates and incubated at 37°C for 24 hrs [18].

The injection protocol

Swiss white male rats were used (age 6-8 weeks) obtained from High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Baghdad. They were housed in standard plastic cages, kept in a well-ventilated room, temperature of 24-28°C. The Rats had free access to tap water and dry pellets (*ad libitum*) obtained from local market. All animals were grouped randomly into five groups and each group includes three animals table-1.

Table 1-Animals groups used in this study

Group	Description
Control -1	Injected with 100 µl of TE buffer and blood was collected after 1 day.
Control -14	Injected with 100 µl of TE buffer and blood was collected after 14 days.
Group-1	Injected with 100 µl of *S. aureus* lysate cell and blood was collected after 1 day.
Group-2	Injected with 100 µl of *S.aureus* lysate cell and blood was collected after 14 days.
Group-3	Injected with 100 µl of *S. aureus* DNA at 30 µg / 100 µl and blood was collected after 1 day.
Group-4	Injected with 100 µl of *S. aureus* DNA at 30 µg / 100µl and blood was collected after 14 days.

All rats were anesthetized with chloroform and the right knees were sterilized carefully with alcohol. Afterward, they were injected intra-articularly either with 100 µl TE buffer, 100 µl of cell lysate, or 100 µl of bacterial DNA (30 µg/100 µl) dissolved in TE buffer [13]. Blood was collected by heart puncture after 1 and 14 days of injection for the estimation of IL-6, anti-ds DNA antibodies and Leucocyte total count (by hemocytometer).

Estimation of IL-6

Interleukin-6 levels were measured using Quantikine®ELISA, USA kit according to the protocol stated by the kit manufacturer.

Estimation of anti-ds DNA antibodies

Anti-dsDNA antibodies were measured using Rat anti-double stranded DNA, dsDNA ELISA kit, China kit according to the protocol stated by the kit manufacturer.

Results and discussion

Antibiotic Susceptibility of *Staphylococcus aureus* isolates

Susceptibility tests were determined for *S. aureus* isolates to 6 different antibiotics by disc diffusion method recommended by CLSI guidelines [17]. These antibiotics included β-lactam antibiotics (Penicillins and Cephalosporins) β-lactam antibiotics were used in this study due to their mode of action inhibiting cell wall synthesis which cause the release of the bacterial cell DNA into the surroundings [19]. From obtained results various levels of susceptibilities to different antibiotics among isolates was observed. All *S. aureus* isolates were resistance to Ampicillin (100 %), Amoxicillin (68%) this results different from results obtained in other local study done by Zeidan [20], who showed that isolates with high level of resistant to this antibiotic which was more than (90%), these differences in results may due to the source of isolates. Furthermore, resistance to Cephalosporins and Ceftriaxone reached 26.66%, while resistance to Cephalothin (25%), Cefoxitin

and Cefotaxime (20%). were also noticed.Such resistance perhaps was due to the production of β-lactamase enzyme that destroyed the β-lactam ring and inactivated the Penicillin antibiotic and this enzyme was encoded by plasmid that easy to transfer among strains [21]. Furthermore, it may be attributed to the prevalence of exogenous gene called *mecA* carried by a mobile genetic element, *SCCmec*, which *S. aureus* has acquired from an as yet unknown bacterial species by lateral gene transfer [22]. This gene is coding for Penicillin- binding proteins (PBP2a) with very low affinity to β-lactam antibiotics including Methicillin [23]. The isolate S1 was susceptible to the highest number of antibiotics table-2; hence, it was selected for *in vivo* study.

Table 2- The susceptibility of *S. aureus* isolates to β-lactam antibiotics.

***S. aureus* Code**	**Cefoxitine**	**Ceftriaxone**	**Cefotaxime**	**Ampicillin**	**Amoxicillin**	**Cephalothin**
S_1	S	S	S	R	S	S
S_2	S	I	S	R	I	S
S_3	S	S	S	R	I	S
S_4	S	I	S	R	S	S
S_5	R	R	R	R	R	R
S_6	S	I	I	R	I	S
S_7	S	I	S	R	I	S
S_8	S	I	S	R	R	S
S_9	S	I	S	R	I	S
S_{10}	S	S	S	R	I	S
S_{11}	S	S	S	R	R	S
S_{12}	R	R	R	R	R	R
S_{13}	R	R	R	R	R	R
S_{14}	S	S	S	R	R	S
S_{15}	S	R	S	R	I	S

R= Resistant, I= Intermediate susceptible, S= Sensitive

Results of Preparation *Staphylococcus aureus* lysate

No growth was found in nutrient agar plate after incubation at 37°C for 24 hrs. Which mean the Cefotaxime was killed *S. aureus* bacteria and we have cell lysate.

Concentration and Purity of DNA

The DNA was extracted from *S. aureus* S_1 isolate which develop high sensitivity to a number of β-lactam antibiotics, using Wizard genomic DNA purification kit. The spectrophotometer apparatus was used to determine the absorbance of DNA solutions with the use of D.W. as a blank. The absorbance of DNA at 260nm and 280nm were 0.0124, 0.0068 respectively. Consequently, the concentration of *S. aureus* S_1 DNA was 62 µg/ ml and the purity value of the extracted DNA (1.82) was demonstrated to be within the accepted range of 1.7-2.0 [24]. Several samples were mixed together in order to obtain the required concentration (30µg/100µl) for the subsequent steps.

DNA Electrophoresis

Agarose gel electrophoresis was used to verify DNA extraction and confirm the purity of the DNA. The results of electrophoresis certified the presence of one band of DNA as visualized under UV light.

Concentration of Interleukin-6

As it is shown in figure-1, both bacterial DNA and cell lysate significantly (P=0.000438) elevated the level of IL-6 in injected animals. However, *S. aureus* cell lysate induced more IL-6 concentration than DNA effect. On the other hand, the concentration of IL-6 in rat injected with cell lysate after 14day is much more than in rat injected with *S. aureus* lysate cell after 1day. Interestingly, there were insignificant differences ($P > 0.05$) between groups treated with DNA after 1 day and 14 days of injection and their spouses of cell lysate.

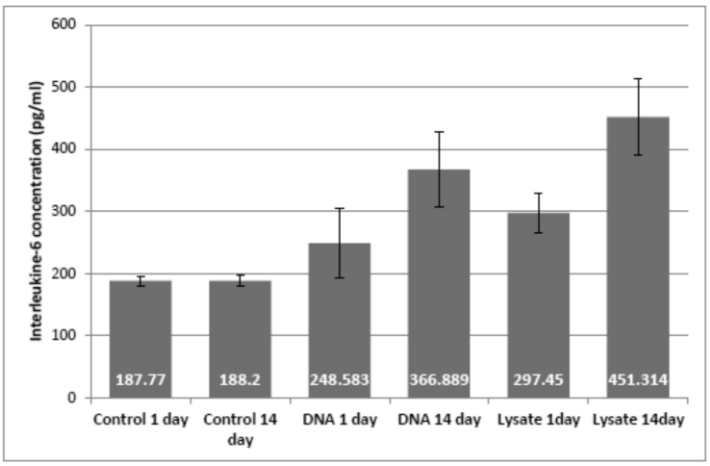

Figure 1- Interlukein-6 level in rat groups' serum. Time period indicates the period after injection to specimen collection. P=0.000438. LSD0.05 = 78.77. Error bars represent standard deviation.

Our result agreed with Tissi *et al.* [25] findings in the mouse model of *S. aureus* arthritis, high IL 6 production systemically was observed throughout the course of arthritis. This is, perhaps, due to that the cell lysate contained stimulatory cell wall fragments such as lipoteichoic acid, lipoproteins and peptidoglycan [26] which led to the activation of the immune system in rat through the interaction of the bacterial constituents with receptors on the surface of mononuclear cells such as TLR2 which led for induction of proinflammatory cytokine, IL-1, IL-6, and TNF-α by monocytes and macrophages [27]. Mattsson *et al*. [28] found that peptidoglycan molecules are able to stimulate the production of proinflammatory cytokines from leukocytes. This increase in the concentration of IL-6 is due to the important role of bacterial components in stimulating the immune system to release cytokines, and thus the rat's immune system became more active after 14 days of injections rather than 1day, which leads to an increase in the concentration of IL-6. The IL-6concentration difference in rat groups which injected with *S. aureus* cell lysate after 1 and 14 day may be due to stimulation activity of cell lysate increased with the time. As a result, the cytokine must be regulated to control both the magnitude and duration of response. Furthermore, differences observed between cell lysate and bacterial DNA could attributed to that bacterial lysate have many stimulatory components more than *S. aureus* DNA such as cell wall-associated and secreted proteins (e.g., protein A, hemolysins, and phenol-soluble modulin) and cell wall components which stimulate the immune system to produce IL-6. IL-6 is essential for regulation of the immune process; however, overproduction of the cytokine leads to inflammation and disease [29]. Tissi *et al.* [25] found that intravenous inoculation of CD1 mice with 10^7 CFU of type IV group B *Streptococcus* (GBS IV) results in a high incidence of diffuse septic arthritis. High levels of IL-1β and IL-6, but not TNF-α, were detected in the joints of mice injected with GBS IV from 5 to 15 days after infection, when articular lesions were most frequent and severe. IL-1β and IL-6 concentrations in the joints significantly ($P< 0.001$) exceeded those detected in the serum, confirming a strong local production. It is well known that bacterial DNA includes a repeated series of unmethylated CpG motifs that bind to the toll-like receptor 9, thus becoming a potent activator of cells of the innate immune system, namely macrophages, dendritic cells, and natural killer cells [30]. Previous studies have shown that bacterial DNA directly activates macrophages [31].The first step of activation comprises the uptake of bacterial DNA or synthetic oligonucleotides by macrophages in a saturable, sequence-independent, temperature- and energy-dependent manner into an acidified intracellular compartment, where DNA degraded to oligodeoxynucleotides [32]. Once there, unmethylated CpG dinucleotides activate the stress–kinase/Jun pathway within minutes, yielding transcriptionally active activating protein 1 and nuclear factor kB. These transcription factors control the mRNA expression of a variety of cytokines and the secretion of proinflammatory cytokines, such as TNFα, IL-1β, IL-6, and IL-12. These cytokines are considered to exert proinflammatory activities in septic and aseptic arthritis, being able to mediate cartilage and bone destruction [33].

Furthermore, CpG DNA triggers B cells to proliferate and secrete immunoglobulins and cytokines, both of which contribute to stronger humoral responses [12]. CpG DNA also directly activates monocytes, macrophages, and dendritic cells to secrete various Th1 cytokines [30], which in turn activate T and NK cells to secrete a broad range of cytokines [34]. The recognition of CpG motifs by

the innate immune system requires engagement of Toll-like receptor 9, which induces cell signaling and subsequently triggers an immune response [35]. IL-6 is produced by various cells, including Th2 cells, B cells, monocyte/ macrophages, fibroblasts, and endothelial. It can be induced by TNFαand IL-1, and it can induce acute-phase responses cells [36]. High levels of TNFα, interleukin-1 ,and IL-6 are present in the joints in rheumatoid arthritis and experimental arthritis, such as collagen-induced arthritis [37].When bacterial DNA or synthetic DNA, unmethylated oligonucleotides containing CpG motifs were injected into the knee joint of mice, arthritis developed quickly, while methylated DNA had no significant effect. Also, the affected tissue was characterized by monocyte and macrophage influx with the release of their associated cytokines and chemokines. Host inflammatory cytokines, including (IL-1) and IL-6, are released into the joint fluid by synovial cells [38]. Furthermore, Al-Mathkhury *et al*. [39] estimated IL-6 in rats' sera after injection the animal model with high GC content DNA and Low GC content DNA, DNA activates the immune system causing the production of proinflammatory cytokines. Bacterial DNA succeeded in stimulating the immune system of rats to produce IL-6. Actually, substantial evidence exists that bacterial CpG motifs induce the immune response against challenge with a wide variety of pathogens and have the rapeutic activity in murine models and in human clinical trials [40].

Assessment the concentration of Anti-Double strand Antibody

The concentration of Anti-double strand antibody for all samples is illustrated in figure-2. It was found that there were highly significant differences (P>0.05) between control groups and other groups. Cell lysate succeeded in inducing anti-dsDNA Ab in rat sera after 14 days of injection more than other groups. Remarkably, the present study indicated insignificant differences (P>0.05) between DNA 1 day, DNA 14 days, and cell lysate 1 day, 14 days. The concentrations of anti-ds DNA Ab in rat groups injected with *S. aureus* cell lyaste and DNA after 14 days are slightly more than in rat groups injected with cell lyaste and DNA after 1 day. Such finding could be assigned to that the rats immune system was stimulated to produce anti-DNA Ab directly after injected knee joints with bacterial lyaste and DNA. This is my be due to over production of IL-6 after 1 day injected rat group led to B cell activation to produce anti-ds DNA ab. Since IL-6 plays an important role in the differentiation of antigen plus CD40 co-activated B cells into antibody- secreting cells, the lack of IgG anti-DNA/chromatin auto-antibodies in IL-6-deficient mice may reflect an absolute requirement for IL-6 in the differentiation of germinal center B cells into anti-DNA/chromatin secreting plasma cells. This interpretation is consistent with previous observations that IL-6 is crucial for the development of anti-DNA/chromatin antibodies in spontaneous murine lupus models [41].

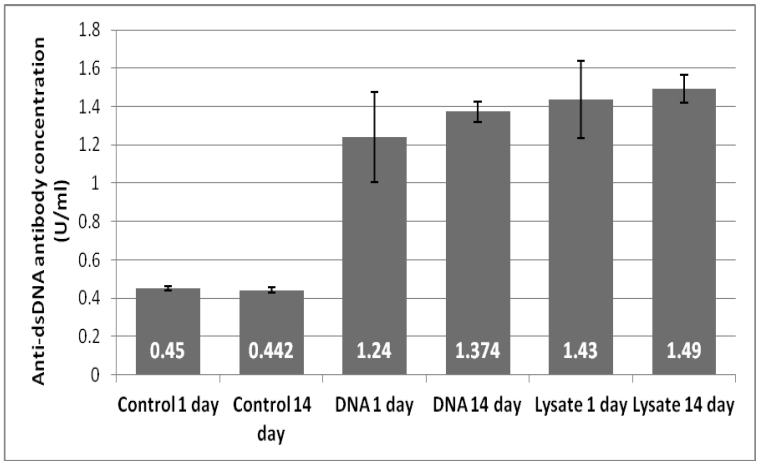

Figure 2- Anti-dsDNA Ab level in rat group's serum. Time period indicates the period after injection to specimen collection. P= 2.5 X 10-5. LSD= 0.239. Error bars represent standard deviation.

This result was not agreed with the results obtained by Deng and Tarkowski [13], as they stated that serum levels of IgG and IgM antibodies specific for dsDNA and ssDNA were low in bacterial DNA injected mice, and in comparison with the auto-antibody levels of control mice, showed no difference. One of the most striking features of their *S. aureus* arthritis model is the occur rence of polyclonal B-cell activation. Immunoglobulin production, especially of the IgG class, as well as auto-antibody production, including that of IgG RF and IgG anti-ssDNA antibodies, was increased up to20-fold.

Such findings could be attributed to that bacterial DNA, by virtue of characteristic sequence motifs, can activate the immune system and drive the production of antibodies to sequential as opposed to backbone DNA determinants. In its antigenic properties, foreign DNA resembles foreign proteins in that it has an epitope structure based on non-conserved sequences that are absent from the host and that are therefore not subject to tolerance [42]. A study done by Pisetsky [43] mentioned that bacterial DNA induces abundant antibody production. By using bacterial dsDNA as the immunogen, the induced antibodies bind only to bacterial dsDNA without cross-reactivity to mammalian dsDNA. Gilkeson *et al.* [44] in their study results suggested that anti-DNA antibodies induced by bacterial DNA bind to DNA structures dependent on both the base and the sugar phosphate moieties of the nucleic acid antigen and may resemble some anti-DNA antibodies expressed in spontaneous autoimmune disease in these binding properties. Furthermore, Al- Mathkhury *et al.* [38] demonstrated that the rats' immune system was stimulated to produce anti DNA antibodies after intraperitoneally injection with bacterial DNA. The concentration of anti-ds Ab in rat injected with *S. aureus* cell lysate after 14 days is slightly more than in rat injected with *S. aureus* lysate cell after 1 day. Such finding could be assigned to that the rats' immune system was stimulated to produce anti DNA antibodies after injection in knee joints with bacterial lysate.

References

1. Tarkowski A. **2006**. Infection and musculoskeletal conditions: Infectious arthritis. *Best. Pract. Res. Clin. Rheumatol*. 20(6), pp:1029-1044.
2. Morgan D.S. Fisher, D. Marianos, A. and Currie, B.J. **1996**. An 18 year clinical review of septic arthritis from tropical Australia. *Epidemiol. Infect*. 117(3), pp:423-428.
3. Garcia-Arias, M. Balsa, A. and Mola, E.M. **2011**. Septic arthritis. *Best. Pract. Res. Clin. Rheumatol*. 25(3), pp:407-421.
4. Baysoy G. Gürel, S. Çakıcı, H. and Uyan, A. **2006**. Concurrent septic arthritis and urinary tract infection in a patient with nephrocalcinosis and vesicoureteral reflux. *Turk. J. Pediatr*. 48, pp:275-278.
5. Herrero J. and Aparicio, J. **2011**. *Escherichia coli* septic arthritis of a lumbar facet joint following urinary tract infection. *Int. J. Inf. Dis*. 15, pp:63–65.
6. Forbes B.E.; Sahm, D.F. and Weissfeld, A.S. **2007**. *Bailey & Scott's Diagnostic Microbiology*. pp. 627.12th ed. Mosby Elsiver.
7. Otto, M. **2012**. MRSA virulence and spread. *Cell. Microbiol*. 14(10), pp:1513-1521.
8. Chorostowska-Wynimko, J. and Roży, A. **2008**. Bacterial immunostimulants mechanism of action and clinical application in respiratory diseases. *Pneumonol. Alergol. Pol*. 76(5), pp:353–359.
9. Fournier, B.and Philpott, D.J. **2005**. Recognition of Staphylococcus aureus by the Innate Immune System. *Clin. Microbiol. Rev*. 18(3), pp:521-540.
10. Kim, D. Jinwon, J. Younghee, L. and Hyung-Joo, K. **2011**. Novel Immunostimulatory Phosphodiester Oligodeoxynucleotides with CpT Sequences Instead of CpG motifs. *Mol. Immunol*. 48(12-13), pp:1494-504.
11. Krieg, A.M. **1999**. Mechanisms and applications of immune stimulatory CpG oligodeoxynucleotides. *Biochim. Biophys. Acta*. 1489(1), pp:107-116.
12. Krieg, A.M. Yi, A.K. Matson, S. Waldschmidt, T.J. Bishop, G.A., Teasdale, R. Koretzky, G.A. and Klinman, D.M. **1995**. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature*. 374(6522), pp:546-549.
13. Deng, G.M. and Tarkowski, A. **2000**. The features of arthritis induced by CpG motifes in bacterial DNA. *Arthritis Rheum*. 43(2), pp:356–364.
14. Temoin, S. Chakaki, A. Askari, A. El-Halaby, A.; Fitzgerald, S. Marcus, R.E. Han, Y.W. and Bissada, N.F. **2012**. Identification of oral bacterial DNA in synovial fluid of arthritis patients with native and failed prosthetic joints. *J. Clin. Rheumatol*. 18(3), pp:117–121.
15. Holt, J.G. Krieg, N.R. Sneath, P.H.A. Staley, J.J. and Williams, S.T. **1994**. *Bergey's manual of determinative bacteriology*. pp. 542. 9th ed. Williams & Wilkins. Baltimore, Maryland, USA.
16. Morello, J.A. Mizer, H.E. and Granato, P.A. **2006**. *Laboratory manual and workbook in microbiology applications to patient care*. p. 95-99.18th ed. The McGraw-Hill Companies, Inc., New York.

17. Clinical and Laboratory Standards Institute (CLSI). **2013**. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-second Informational Supplement. pp. 32. CLSI document M100-S22.

18. Van langevelde, P. Ravensbergen, E. Grashoff, P. Beekhuizen, H. Groeneveld, P. H. P. And Van dissel, J. T. **1999.** Antibiotic- induced cell wall fragment of *Staphylococcus aureus* increase endothelial chemokine secretion and adhesiveness for granulocytes. *Antimicrob. Agents. Chemother*. 34(12), pp:2984-2989.

19. Beers, M.H. **2003**.*The merck manual of medical information* (2nd ed). Merck & Co. Inc. Whitehouse Station. Pp:1561-1568.

20. Zeidan, I.A. **2005**. Genetic and bacteriologic study to *Staphylococcus aureus* isolated from clinical specimens and resistant to vancomycin antibiotic. MSc. Thesis. University of Baghdad, Iraq.

21. Brooks, G.F. Carroll, K.C. and Morse, S.A. **2007**. *Jawetz Melnick and Adelberg`s Medical microbiology*. pp. 818. 24th ed. The McGraw-Hill. New York.

22. Zhaxybayeva, O. and Doolittle, W.F. **2011**. Lateral gene transfer. *Curr. Biol*. 21(7), pp:242-246.

23. Zetola, N. Francis, J.S.; Nuermberger, E.L. and Bishai, W.R. **2005**. Community-acquired methicillin-resistant *Staphylococcus aureus*, an emerging threat. *Lancet Infect Dis*. 5(5), pp:275-286.

24. Chaudhuri, S.R. Pattanayak, A.K. and Thakur, A.R. **2006**. Microbial DNA extraction from samples of varied origin. *Cur. Sci*. 91(12), pp:1697-1700.

25. Tissi, L. Manuela, P. Roberta, B. Graziella, O. Christina Von, H. and Francesco, B. **1999.** Role of tumor necrosis factor alpha, interleukin-1β, and interleukin-6 in a mouse model of group B Streptococcal Arthritis. *Infect. Immun*. 67(9), pp:4545-4550.

26. Van Langevelde, P. Ravensbergen, E. Grashoff, P. and Van Dissel, J.T. **1999**. Antibiotic-Induced cell wall fragments of *Staphylococcus aureus* increase endothelial chemokine secretion and adhesiveness for granulocytes. *Antimicrob. Agents. Chemother*. 43(12), pp:2984-2989.

27. Wang, J.E. Jorgensen, P.F. Almlof, M. Thiemermann, C. Foster, S.J. Aasen, A.O. and Solberg, R. **2000**. Peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 production in both T cells and monocytes in a human whole blood model. *Infect*. *Immunol*. 68(7),pp:3965-3970.

28. Mattsson, E. Harwald, H. Bjorck, L. and Egesten, A. **2002**. Peptidoglycan from *Staphylococcus aureus* induces tissue factor expression and procoagulant activity in human monocytes. *Infect. Immun*. 70(6), pp:3030-3039.

29. Kishimoto, T. **2006**. Interleukin-6: discovery of a pleiotropic cytokine. *Arthritis. Res. Ther*. 8, Suppl 2:S2.

30. Wagner, H. **2002**. Interactions between bacterial CpG-DNA and TLR9 bridge innate and adaptive immunity. *Curr. Opin. Microbiol*. 5(1), pp:62–69.

31. Stacey, K.J. Sweet, M.J. and Hume, D.A. **1996.** Macrophages ingest and are activated by bacterial DNA. *J. Immunol*. 157(5), pp:2116–2122.

32. Zhao, Q. Waldschmidt, T. Fisher, E. Herrera, C.J. and Krieg, A.M. **1994.** Stage specific oligonucleotide uptake in murine bone marrow B-cell precursors. *Blood*. 84(11),pp: 3660–3666.

33. Joosten, L.A. Lubberts, E. Helsen, M.M. and Van den Berg, W.B. **1997**. Dual role of IL-12 in early and late stages of murine collagen type II arthritis. *J. Immunol*. 159(8),pp:4094–4102.

34. Chace, J.H.; Hooker, N.A. Mildenstein, K.L.; Krieg, A.M. and Cowdery, J.S. **1997**. Bacterial DNA-induced NK cell IFN-gamma production is dependent on macrophage secretion of IL-12. *Clin. Immunol. Immunopathol*. 84(2), pp:185-193.

35. Hemmi, H. Takeuchi, O. Kawai, T. Kaisho, T. Sato, S. Sanjo, H. Matsumoto, M. Hoshino, K. Wagner, H. Takeda, K. and Akira, S. **2000**. A Toll-like receptor recognizes bacterial DNA. *Nature*. 408(6813), pp:740-745.

36. Van Sink, J. **1990**. Interlukine-6: An overview. *Annu. Rev. Immunol*. 8, pp:233-278.

37. Feldman, M. Brennan, F. and Maini, R. **1996**. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol*. 14, pp:397-440.

38. Shirtliff, M.E. and Mader, J.T. **2002**. Acute septic arthritis. *Clin. Microbiol. Rev*. 15(4), pp:527-44.

39. Al-Mathkhury, H.J.F. Utba, N.M. and Al-Alusi, N.S. **2012**. Low GC and high GC bacterial DNA impact on anti DNA antibodies, IL-6 and IL-12 level in rats. *Int. J. Sci. Technol.* 7(2), pp:90-94.
40. Rachmilewitz, D. Karmeli, F. Takabayashi, K. Hayashi, T. Leider-Trejo, L. Lee, J. Leoni, L.M. and Raz, E. **2002**. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. *Gastroenterology*. 122(5), pp:1428-1441.
41. Alarcon Riquelme, M.E. Moller, G. and Fernandez, C. **1993**. Macrophage depletion decreases IgG anti-DNA in cultures from (NZB × NZW) F1 spleen cells by eliminating the main source of IL-6. *Clin. Exp. Immunol.* 91(2), pp:220–225.
42. Pisetsky, D.S. **1996.** Immune activation by bacterial DNA: a new genetic code. *Immunity*.5(4), pp:303–310.
43. Pisetsky, D.S. **1998.** Antibody responses to DNA in normal immunity and aberrant immunity. *Clin. Diagn. Lab. Immunol*. 5(1), pp:1-6.
44. Gilkeson, G.S.; Pritchard, A.J. and Pisetsky, D.S. **1991**. Specificity of anti-DNA antibodies induced in normal mice by immunization with bacterial DNA. *Clin. Immunol. Immunopathol*. 59(2), pp:288-300.