Role of Proteus mirabilis DNA in Comparison to Candida albicans DNA in Rats' Joints Infection

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Abstract
In This study a comparison between *Proteus mirabilis* DNA and *Candida albicans* DNA in Arthritis. Fourteen *Proteus* isolates (11.6%) were collected from 120 specimens collected from midstream urine of patients of both sex and different ages attending different hospitals in Baghdad. Antibiotic sensitivity assay showed that All *Proteus mirabilis* recovered from UTI developed multidrug resistance and variable degree of resistance. Histopathological changes in model treated with *Proteus mirabilis DNA* revealed congestion, inflammatory cells infiltration, oedema, hemorrhagic exudates as well as necrotic cells. Furthermore, articular joints damage has been noticed with articular tissue in ligament and lining epithelium. In addition, an aggregation of lymphocyte was observed as well. While rats treated with *Candida albicans* DNA depict a congestion and a mild infiltration of inflammatory cells with oedema and augmented infiltration reaction inside the joint space. Serum Interleukine-6 (IL-6) levels peaked within 24 hours after injection of *P. mirabilis* DNA intraarticularly, while serum IL-6 levels did not increase in rats injected with *C. albicans* DNA and TE. The serum level of Anti-double strand antibody IgM (Anti-ds Ab) was assessed after 14 days and results revealed that Anti-ds Ab concentration was significantly higher ($P< 0.01$) in the animal model treated with *Proteus mirabilis* DNA than those treated with *Candida albicans* DNA. Total white blood cell count has been assessed, Animal model treated with 30µg/100 µl *P. mirabilis* DNA showed a significant ($P< 0.05$) increasing in total count of WBC, while there were insignificant differences between animals treated with 30µg/100 µl *C. albicans* and control animal.

Keywords: Septic arthritis, Unmethylated DNA.

دور دنا بكتريا *Proteus mirabilis* مقارنه بدنا الخميره *Candida albicans* في اصابه مفاصل الجرذان

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الخلاصه:
في هذه الدراسه اجريت مقارنه بين دنا بكتريا *Proteus mirabilis* ودنا الخميره *Candida albicans* المسببه لالتهاب المفاصل اربعة عشر عزله (11,6 %) تم الحصول عليها من (120) عينه جمعت من مرضى مصابين بالتهاب المجاري البوليه لكلا الجنسين ومن مختلف الاعمار من مستشفيات مختلفه في بغداد. اختبار الحساسيه اظهر مقاومه متعدده للمضادات الحيويه لكل عزلات *Proteus mirabilis*. التغييرات

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النسيجيه في النموذج المعامل مع دنا البكتريا اظهرت ترشيح خلايا التهابيه ، وذمه، خراجات دمويه وكذلك خلايا ميته. فضلا عن ذلك، لوحظ ضرر في المفصل، النسيج، الاربطه، والخلايا الطلائيه المبطنه للمفصل. بالاضافه الى ذلك لوحظ تجمع للخلايا اللمفاويه . بينما اظهرت الجرذان المعامله بدنا الخميره ترشيح بسيط للخلايا الالتهابيه مع وذمه .ارتفع مستوى Interleukin−6 في مصل الجرذان خلال 24 ساعه بعد حقن دنا البكتريا في داخل المفصل بينما لم يزداد تركيزه في الجرذان المعامله بدنا الخميره والدارئTE Buffer. مستوى Anti−ds DNA antibody تم تقديره بعد 14 يوم واظهرت النتيجه زياده معنويه ($P<0.01$) في النوذج الحيواني المعامل مع دنا البكتريا مقارنه بتلك التي تم معاملتها بدنا الخميره . مجموع كريات الدم البيضاء تم تقييمه في النوذج الحيواني المعامل بدنا البكتريا والتي اظهرت زياده معنويه ($P<0.05$) في مجموع كريات الدم البيضاء، بينما التغيير غير معنوي بين الحيوانات المعامله بدنا الخميره وحيوانات السيطره(Control).

Introduction

Septic arthritis is a key consideration in adults presenting with acute monoarticular arthritis. Failure to initiate appropriate antibiotic therapy within the first 24 to 48 hours of onset can cause subchondral bone loss and permanent joint dysfunction [1].

Bacterial DNA differs from vertebrate DNA in having a much higher content of unmethylated CpG dinucleotides, in particular, base contexts that are termed 'CpG motifs'. The vertebrate immune system appears to have evolved pattern recognition molecules that recognize 'CpG motifs' as foreign, and trigger protective immune responses which are strongly Th1-biased [2]. It has been initially expected that there would be a cell surface receptor for CpG DNA; it appears instead that the recognition of CpG motifs is accomplished through one or more intracellular CpG binding proteins [3]. Although many cell types are able to bind DNA on their surface, this binding appears to be non-sequence specific [4]. DNA is then taken up by cells, also in a non-sequence specific fashion, into an endosomal compartment where the DNA is acidified and digested by nucleases [5, 6, 7]. It appears that this endosomal acidification of CpG DNA may be required for its immune stimulatory activities since inhibition of endosomal maturation with specific inhibitors such as chloroquine completely blocks the downstream signaling pathways induced by CpG [8,9].

Bacterial DNA and synthetic oligonucleotides that express these CpG motifs rapidly stimulate B cells, T cells, and macrophages to proliferate, secrete Abs, and/or produce a variety of Th1-associated immunomodulatory cytokines, including IFN-g, IL-12, IL-6, IL-18, and TNF-a [10]. Further, CpG motifs may facilitate the development of Ag-specific immunity by initiating an innate, Ag-nonspecific inflammatory response at the site of vaccination [11]. Since DNA is readily taken up by leukocytes, it is logical to hypothesize that bacterial DNA, and specifically nonmethylated CpG oligonucleotides, are capable of causing inflammation in the joints and may contribute to disease progression and morbidity [12]. Bacterial infections can be localized to the joints, causing septic arthritis. Bacterial arthritis is a rapidly progressive and highly destructive joint disease in humans, other destructive joint diseases, including autoimmune disorders such as rheumatoid arthritis, are connected to an increased incidence of bacterial arthritis [12,13].

Patients with arthritis can develop anti-ds DNA antibodies; however they are usually treatment related. Anti-TNFα biological therapies, such as adalimumab, infliximab and etanercept, can often induce the production of anti-dsDNA antibodies. They are usually low avidity and are only detectable transiently after treatment [14,15].

Material and Methods

From September 2012 to February 2013, one hundred and twenty specimens were collected from patients, suffering from urinary tract infections, attending Al-Kindy teaching hospital, Al-Yarmouk hospital, the central health laboratories and educational laboratories/medical city in Baghdad.

The collected specimens were streaked directly on MacConkey agar, blood agar and sabouraud agar; thereafter, incubated at 37°C for 24 hr. The pale non lactose fermenters colonies were selected then a single colony was streaked on blood agar for the activation, detection of bacterial ability to lyse red blood cells and observation swarming phenomena. All specimens were identified according to morphology, biochemical tests and confirmed by Vitek 2 system.

Candida albicans was collected for mid stream urine from patients suffering UTI, these isolates were identified depending on the morphological features on culture medium and germ tube formation with the use of API-Candida system

Antibiotic susceptibility of *Proteus mirabilis*

This test was done by disc diffusion method according to Kirby-Bauer test [16]. After incubation, the diameter of each inhibition zone was measured in millimeter (mm) using a ruler. And the isolate was interpreted as either susceptible, intermediate, or resistant to a particular drug by comparison with standards inhibition zone as in table 1.

Table 1- Inhibition zone diameter standards (CLSI, 2012)

Antibiotics	Concentration (µg/disc)	Diameter of inhibition zone (mm)		
		R	I	S
Amikacin	30	≤ 14	15–16	≥ 17
Tobromycin	10	≤ 12	13–14	≥ 15
Imipenem	10	≤ 19	20–22	≥ 23
Ciprofloxacin	5	≤ 15	16–20	≥ 21
Ceftazidime	30	≤ 17	18–20	≥ 21
Gentamicin	10	≤ 12	13–14	≥ 15
Trimethoprim-Sulfamethoxazole	1.25/23.75	≤ 10	11–15	≥ 16

R= resistant, I= Intermediate, S= Sensitive

Isolating of Genomic DNA from *Proteus mirabilis* and *Candida albicans*

Proteus mirabilis DNA was extracted and purified using Wizard genomic DNA purification kit (Promega, USA) according to the protocol stated by the kit manufacturer. While *Candida albicans* DNA was extracted and purified using EZ-10 Spin column fungal genomic DNA Mini-Preps Kit (BIO BASIC INC.) according to the protocol stated by the kit manufacturer.

Measurement and purity of DNA

The concentration and the purity of the DNA samples were determined by measuring the absorbance using a UV spectrophotometer at 260 nm and 280 nm wavelengths; the D.W. was used as blank. The OD_{260} reading was considered as the concentration of DNA while the $OD_{260/280}$ ratio reading was considered as the purity of DNA [12].

The injection protocol

DNA dissolved in Tris-EDTA buffer (TE) in a volume of 100µl were all injected intraarticularly in the knee joints, these knees were first sterilized with alcohol.

The right knee received an intraarticular injection of DNA (30 µg/100 µl), while the control was injected with 100 µl TE buffer only. All the intraarticular injections were performed on anesthetized Rats [12].

Blood sample collection

EDTA anticoagulated blood has been used for counting white cells; the count was performed within 6 hrs. And non- anticoagulated blood collected in a plain tube to obtain serum.

Histopathologcal examination

Histopathologic changes in joints was performed after routine fixation, decalcification, and paraffin embedding. Sections were cut and stained with hematoxylin and eosin. All the slides were coded and evaluated blindly[12].

Interleukin 6 level

Interlekin-6 levels were measured in rats' sera using Quantikine®ELISA kit according to the protocol stated by the kit manufacturer.

Anti DNA antibodies

Rat Anti-double strand DNA antibody were measured in rats' sera according to AESKULISA dsDNA-M kit using protocol stated by the kit manufacturer

Total white blood cells count

Whole blood was diluted 1 in 20 in a WBC diluting fluid which haemolyzes the red cells, the white cells left to be counted.

White cells were counted microscopically using a counting chamber (haemocytometer) and the number of WBCs of blood calculated in four corners of the chamber.

Results and discussion

Isolation and Identification of *Proteus*

Fourteen *Proteus* isolates (11.6%) were obtained from 120 specimens collected from midstream urine of patients of both sexes and different ages attending deferent hospitals in Baghdad. Identification was achieved depending on cultural characteristics, microscopic examination, biochemical tests and confirmed by vitek 2 compact system.

Fourteen isolates were suspected to belong to the genus *Proteus* appeared as small pale colonies, little convex and circular with smooth edges with a distinguishing fishy odour on MacConkey agar plates. MacConkey agar was used for growing *Proteus* isolates because it differentiates them from other gram negative species and it contains all required nutrients for *Proteus* growth.

Proteus isolates represented differences in some biochemical characteristics as shown in table 2. All *Proteus* isolates were oxidase negative, catalase positive, and urease positive. But variable results were noticed in indole test, and citrate utilization test. Biochemical tests were confirmed by VITEK® 2 SYSTEM which gave similar results.

Table 2:- Results of Biochemical tests of *Proteus* species

Id	Biochemical test	*P. mirabilis*	*P. vulgaris*
1	Oxidase	-	-
2	Catalase	+	+
3	Indole	-	+
4	Citrate Utilization	v	v
5	Triple-Sugar Iron Agar	K/A ++	K/A ++
6	Urease	+	+
7	Methyl Red	+	+
8	Vogas-Proskauer	-	-

(+) positive result, (-) negative result, (v) variable result, K= Alkaline, A= Acidic

Among *Proteus* isolates, *P. mirabilis* performed 85.7% (12 isolates) of total *Proteus* isolates, while *P. vulgaris* appeared only in 14.3% (2 isolates) of total *Proteus* isolates. The present study results are in agreement with a study done in Baghdad by Al-Rahho [17]; in which she mentioned that *P. mirabilis* performed 86.6% and *P. vulgaris* covered 13.3% of the total *Proteus* isolates in urine with patient suffering from UTI. Also in a study accomplished in Nigeria by Mordi and Momoh [18], *P. mirabilis* was the commonest followed by *P. vulgaris.*

Identification of *Candida albicans*

Four Candidal isolates were identified depending on the morphological features on culture medium and germ tube formation with the use of API-Candida system. The morphology of *C. albicans* colonies on sabouraud dextrose agar were white to creamy, round, soft, and smooth to wrinkled, with a characteristic yeast odor. *C. albicans* isolates showed a positive result since the formation of germ tubes was seen as long tube-like projections extending from the yeast cells. There was no constriction at the point of attachment to the yeast cells. These tubular extensions represent an early stage in the formation of true hyphae.

Antibiotic Susceptibility of *Proteus mirabilis*

Susceptibility test was done for all *P. mirabilis* isolates against seven antibiotics widely used for the treatment of *Proteus* infections; included Amikacin, Tobromycin, Imipenem, Ciprofloxacin, Ceftazidime, Gentamicin and Trimethoprim-Sulfamethoxazole. All *Proteus mirabilis* recovered from UTI developed multidrug resistance and variable degree of resistance toward Trimethoprim-

Sulfamethoxazole (100%), Ciprofloxacin (66.6%), Ceftazidime (58%), Gentamicin (58%),Imipenem (25%), Tobromycin (16.6%) and Amikacin (16.6%) figure-1.
A review of the antimicrobial susceptibility profile of *P. mirabilis* from the clinical urine specimens showed that Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Ceftazidime and Gentamicin were the less effective antibiotics (efficacy 0% - 42%), while the highest efficacy (75% – 83.4 %) appeared in Imipenem,Tobromycin and Amikacin.

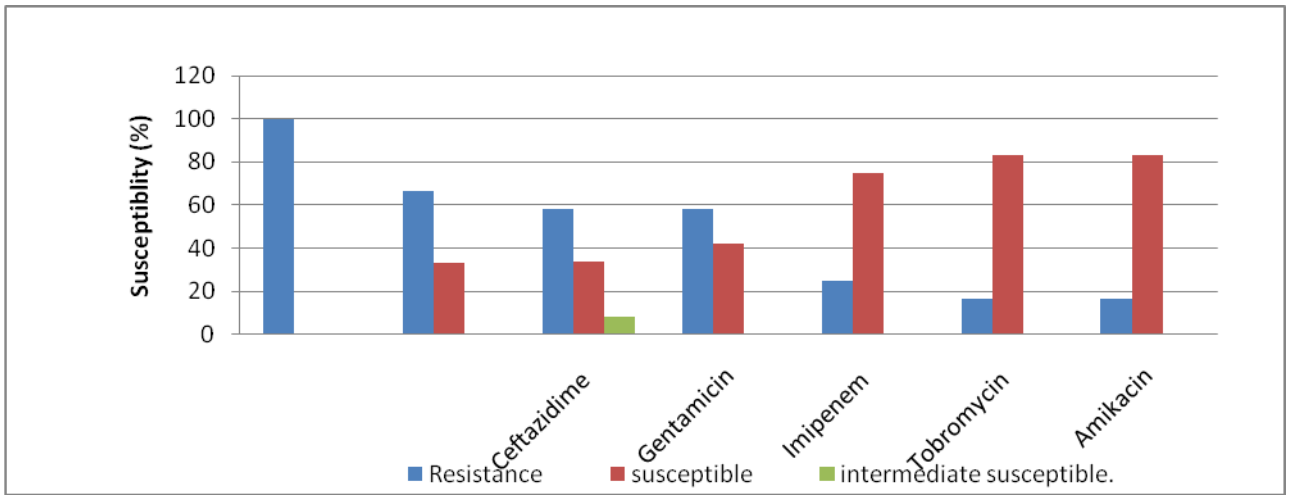


Figure 1- Multidrug resistance of *P. mirabilis* isolates

A recent study achieved in Tikrit-Iraq presented by Al-Jebouri and Mdish [19] showed a similarity to Amikacin resistance, while no agreement appeared against Gentamicin and Ciprofloxacin. Nevertheless, Al-Jawadi [20] in Mosul-Iraq, agreed with our study in respect to Gentamicin and Amikacin susceptibility. In other study completed by Alsaimary *et al.* [21], no agreement appeared to Trimethoprim-Sulfamethoxazole.
Resistance to Ciprofloxacin, Ceftazidime and Gentamicin developed by this study isolates were similar to studies done by Jombo *et al*. [22] in Nigeria, Manik *et al.*[23] in India, respectively. However, susceptibility of *P. mirabilis* to Tobromycin, Amikacin and Trimethoprim-Sulfamethoxazole were in disagreement with findings of Amin *et al*. [24] in Iran.

DNA Extraction

The DNA was isolated from *P. mirabilis* isolate which developed the highest sensitivity towards the tested antibiotics. *P. mirabilis* DNA was isolated using a Wizard genomic DNA purification kit method. Whereas, *C. albicans* DNA was isolated using EZ-10 Spin column fungal genomic DNA Mini-Preps Kit, Genomic DNA was further analyzed quantitatively and qualitatively by gel electrophoresis, which showed one band of DNA. This indicates the methods utilities used in extraction and the purification of DNA figure-2.

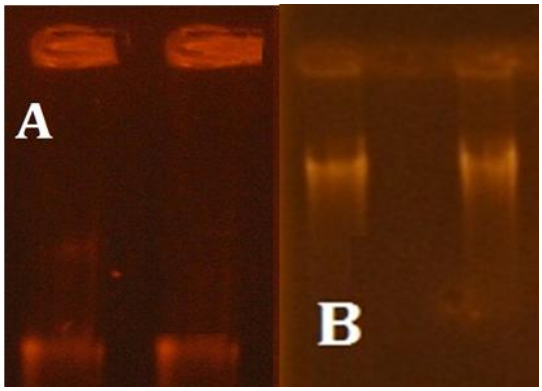


Figure 2- Electrophoresis of two repeats of *P. mirabilis* isolate DNA (A) and two repeats of *C. albicans* isolate DNA (B). Running conditions: 0.8% Agarose gel, 5 volt/cm for 2 hrs, stained with ethidium bromide dye and visualised by UV light.

Concentration and Purity of DNA

The spectrophotometer apparatus was used to determine the absorbance of DNA solutions with the use of distilled water as blank.

The absorbance of *P. mirabilis* DNA at 260nm and 280nm were 0.323 and 0.182, respectively. While the absorbance of *C. albicans* DNA at 260 nm and 280 nm were 0.361 and 0.211, respectively. Accordingly, the concentration of *P. mirabilis* DNA was 161.5µg/µl and the purity ($OD_{260/280}$ ratio) was 1.77. While the concentration of *C. albicans* DNA was 180.5µg/µl and the purity was 1.71. As a consequence, the extracted DNA was considered pure given that it was within the expected range of 1.7 to 2 [25].

Histopathological study

The histological changes caused by purified DNA from *Proteus mirabilis* and *Candida albicans* were investigated using rat model. Normal appearance was observed in the knee joints of control rat as it is shown in figure 3.

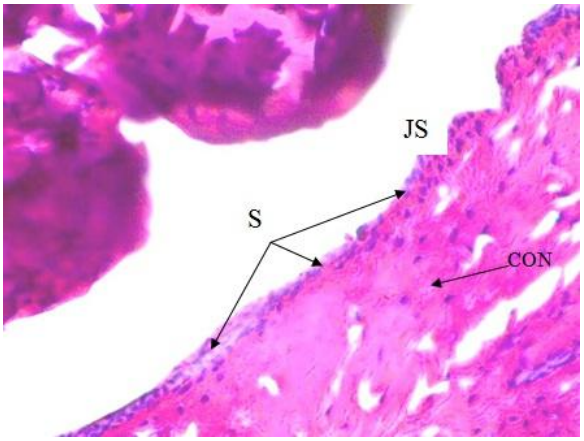
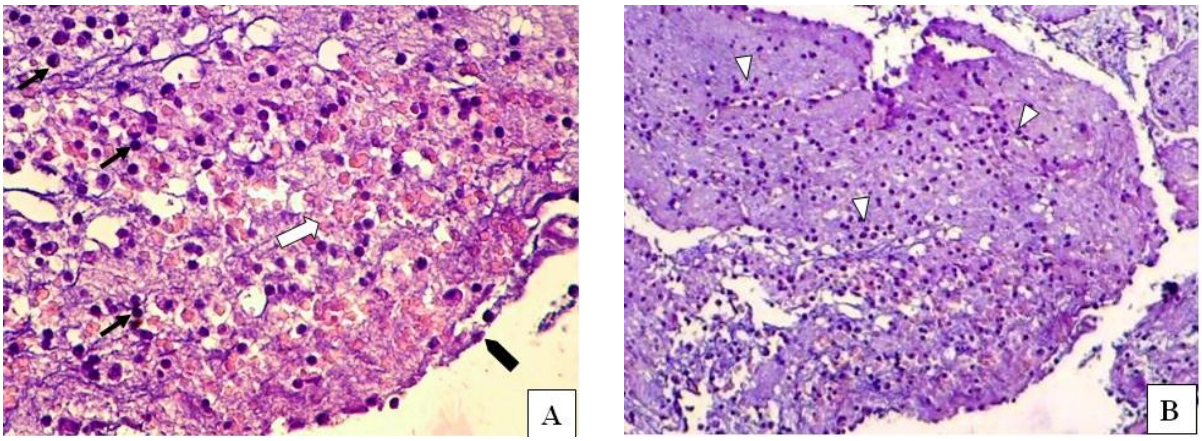


Figure 3- Histological section of control rat joint 3 days after injected with 0.1ml of TE buffer shows a normal structure appearance of synovial lining tissue(S), joint space (JS) and connective tissue (Con). H & E. X200

Histopathological examination of knee joints from rat intraarticulary treated with 30 µg/100 µl of *P. mirabilis* DNA revealed inflammatory cells infiltration, oedema, hemorrhagic exudates due to the lysis of blood vessels, necrotic cells, articular joints damage has been noticed with articular tissue in ligament and lining epithelium cells, and an aggregation of lymphocyte was observed as well figure-4.



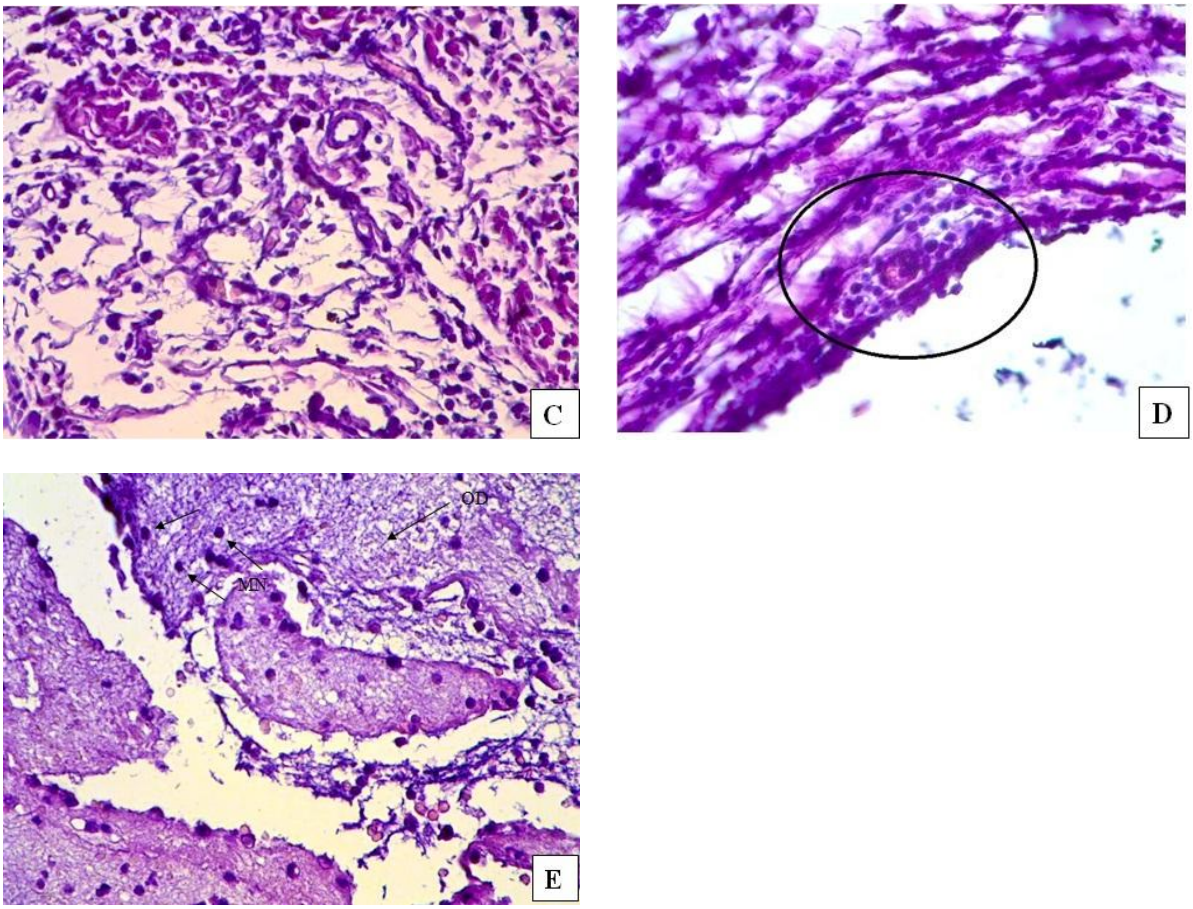


Figure 4- Histological section of rat joint 3 days after injected with 0.1ml of 30 µg/100 µl of *P. mirabilis* DNA showing: A) synovial membrane damage (pentagon), inflammatory cells infiltration (black arrows), hemorrhagic exudates (white arrow) (X200). B) Necrotic cells (white triangles) (X100). C) Damage in the ligament and lining epithelium cells (X200). D) Aggregation of lymphocyte (X200). E) Odema (OD) and mononuclear cells (MN) H & E (X200).

The histological sections of rat joint that have been treated with 30 µg/100 µl DNA of *C. albicans* depict a congestion and a mild infiltration of inflammatory cells with oedema and augmented infiltration reaction inside the joint space as it is shown in figure 5.

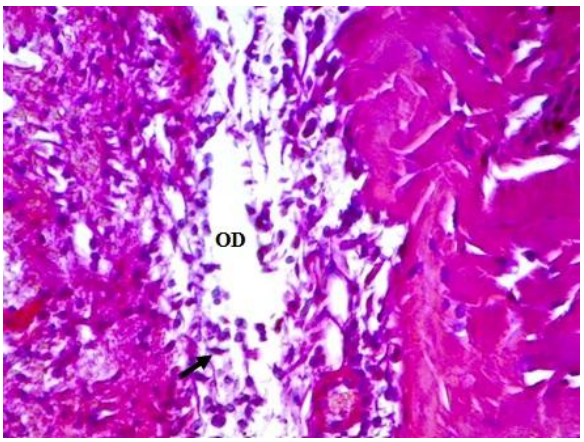


Figure 5- Histological section of rat joint 3 days after injected with 0.1ml of 30 µg/100 µl of *C. albicans* DNA showing augmented infiltration reaction which consist of infiltered cells (black arrow) and oedema (OD) inside the space of joint tissue ligament. H & E. X200.

Taken together, the groups of rat treated with *P. mirabilis* and *C. albicans* DNA developed different histopathological changes with features relatively less intense in *C. albicans* than *P. mirabilis.*

The bacterial DNA differs from eukaryotic DNA in having a much higher content of unmethylated CpG dinucleotides. The vertebrate immune system appears to have evolved pattern recognition molecules that recognize unmethylated 'CpG motifs' as foreign, and trigger protective immune responses [2]. Moreover, many studies have also mention that Unmethylated CpG motifs were responsible for this induction of arthritis, as oligonucleotides containing these motifs produced the arthritis [25].

Deng and Tarkowski [12] in their study hypothesized that bacterial DNA might induce arthritis. They injected bacterial DNA and CpG Oligodinucleotide directly into knee joints and they found that arthritis was induced by bacterial DNA or CpG ODN. This joint inflammation was caused by ssDNA as well as by dsDNA, in contrast, oligonucleotides lacking CpG or being methylated did not give rise to arthritis.

The histopathological changes in joints treated with *P. mirabilis* DNA in our study, showed a strong agreement with by Deng and Tarkowski [12], there was synovial hypertrophy and infiltrating cells in the synovial lining cell layer, deep in the sublining space, as well as in surrounding synovial vessels in arthritic joint sections. In contrast, cartilage and bone destruction were not observed throughout the entire observation period. Zeuner *et al.* [26], in his study mentioned that induction of arthritis by CpG ODN, CpG-induced arthritis was characterized histological changes that included perivascular infiltration by mononuclear cells and hyperplasia of the synovial lining. Deng *et al*. [25]; showed that unmethylated CpG motifs were responsible for the induction of arthritis, as oligonucleotides containing these motifs produced the arthritis and indicate an important pathogenic role for bacterial DNA in septic arthritis. Ohshima *et al.*, [27] in their study of arthritis, a severe arthritis induced which characterized by marked lining layer damage and dense mononuclear cell infiltration as well.

In another local study carried out by Al-Mathkhury *et al.*, [28], a marked inflammatory reactions appeared with nonmethylated DNA of *Pseudomonas aeruginosa* to rat lungs represented by infiltration of various inflammatory cells; macrophages, neutrophils and lymphocytes.

Also Al-Mathkhury and Abdul-Ghaffar [29] intraurethreally injected a similar concentration of both *S. aureus* and *C. albicans* DNA in mice. Histological sections of the kidney treated with *S. aureus* revealed congestion and mild degenerative changes of renal tubules with mild inflammatory cells infiltration, the bladder showed edema with infiltration of inflammatory cells, while sections of kidney challenged with *C. albicans* DNA showed normal kidney and bladder structure. However, Al-Mathkhury and Al-Shaybany reported that the high GC ratio *P. acnes* DNA developed higher inflammatory effects in skin of mice than the low GC ratio *S. aureus* DNA [30].

Assessment the Concentration of Interleukin-6

The serum level of Interleukin-6 (IL-6) was assessed in three groups of rats, first group was treated with 30µg/100 µl of *P. mirabilis* DNA, the second group was treated with 30µg/100 µl of *C. albicans* DNA and the control group was treated with 100µl of TE buffer. Serum IL-6 levels peaked within 24 hours after injection of *P. mirabilis* DNA intraarticularly, while serum IL-6 levels did not increase in rats injected with *C. albicans* DNA and TE Buffer, as appeared in figure 6.

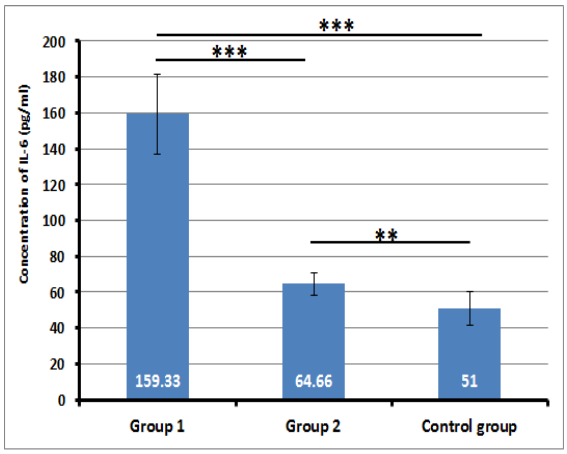


Figure 6- Interlukein-6 level in rat serum. Group 1, Group 2, and Control group represent rat groups intraarticularly injected with 100 µl of 30µg/100 µl of *P. mirabilis* DNA, 100 µl of 30µg/100 µl of *C. albicans* DNA and 100µl of TE buffer, respectively.

In this regard, previous studie has shown that bacterial DNA directly activates macrophages [31]. The first step of activation encompasses 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]. 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 [33]. These cytokines are considered to exert proinflammatory activities in septic and aseptic arthritis, being able to mediate cartilage and bone destruction [34].

Deng and Tarkowski, [12] showed that bacterial DNA can induce macrophages and lymphocytes to release IL-6 in serum of animal injected intraarticulary with CpG motifs in bacterial DNA, which serum IL-6 levels within 24 hours after injection has been increased while Serum IL-6 levels were not increased in model injected with calf thymus DNA (eukaryotic DNA) and control model as well.

Ohshima *et al.* [27] in their study concluded that IL-6 plays a key role in the development of arthritis at both the induction phase and the effector phase, and the blockade of IL-6 is possibly beneficial in the treatment of arthritis.

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. Hostinflammatory cytokines, including interleukin-1 (IL-1) and IL-6, are released into the joint fluid by synovial cells [35].

Furthermore, Al- Mathkhury *et al.* [36] 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.

Assessment of Anti-Double strand Antibody

The serum level of Anti-double strand antibody (Anti-ds DNA Ab) was assessed in three groups of rats referred to as 1, 2, and 3 after 14 days of intraarticularly injection with 30µg/100 µl of *P. mirabilis* DNA, 30µg/100 µl of *C. albicans* DNA and 100µl of TE buffer, the latter considered as a control group. Results revealed that Anti-ds Ab concentration was significantly higher ($P< 0.01$) in the group 1 than other two groups, as it is presented in figure 7.

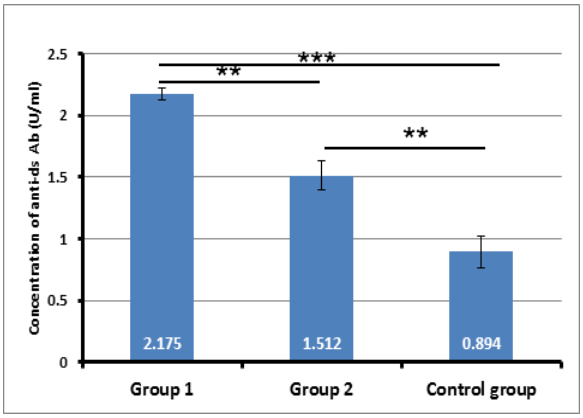


Figure 7- Anti-ds Ab level in rat serum. Group 1, Group 2, and Control group represent rat groups intraarticularly injected with 100 µl of 30µg/100 µl of *P. mirabilis* DNA, 100 µl of 30µg/100 µl of *C. albicans* DNA and 100µl of TE buffer, respectively

Because of the role of anti-DNA antibody in the pathogenesis of disease, we studied and measured anti-ds Ab in the sera of rats injected intraarticularly with pure eukaryotic and prokaryotic DNA. The strong association of anti-DNA with autoimmunity has been confirmed in experiments replicating lupus by immunizing normal mice with DNA. However, even when coupled to a protein carrier and presented in adjuvant, mammalian DNA fails to elicit significant antibody production [37]. On the other hand, 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 [38].

Subsequent studies on the specificities of these responses have demonstrated that Normal human serum can bind to DNA from many bacterial species, although, interestingly, they do not bind to DNA from *Escherichia coli*. This binding is very species specific. Thus, antibodies that bind to the DNA of one bacterial species do not bind cross-reactively to the DNA of another bacterial species [39]. Moreover, Pisetsky [37] 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.* [40] 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.

Also, Pyun *et al*. [41] demonstrated that anti-DNA antibodies from normal mice, although induced by bacterial DNA, may display a broad range of antigen recognition and thus resemble lupus anti-DNA antibodies, many of which are polyspecific, in their pattern of cross-reactivity.

Gilkeson *et al.* [42] reported that immunization of normal mice with bacterial DNA induces a significant anti-DNA response that includes antibodies resembling some lupus anti-DNA in their binding properties, although lacking specificity for mammalian dsDNA.

Furthermore, Al- Mathkhury *et al.* [28] demonstrated that the rats' immune system was stimulated to produce anti DNA antibodies after intraperitoneally injection with bacterial DNA.

Total White Blood Cells count

Rat blood was collected after 24 hrs. From the three rats' groups and the total count of WBC is depicted in figure 8:

Animal model treated with 30µg/100 µl *P. mirabilis* DNA showed a significant ($P< 0.05$) increase in total count of WBC, while there were insignificant differences between animals treated with 30µg/100 µl *C. albicans* and control animal.

Bacterial DNA was reported to have an immunostimulatory effect on leukocytes [43], since these molecules mediate leukocyte rolling, and thus control early steps of extra vasation of leukocytes during inflammation [44]. Elkins *et al.*, (1999) demonstrated that DNA contains unmethylated CpG dinucleotides in particular base contexts (CpG motifs) are rapidly activate leukocytes and this activation are triggered by CpG motifs.

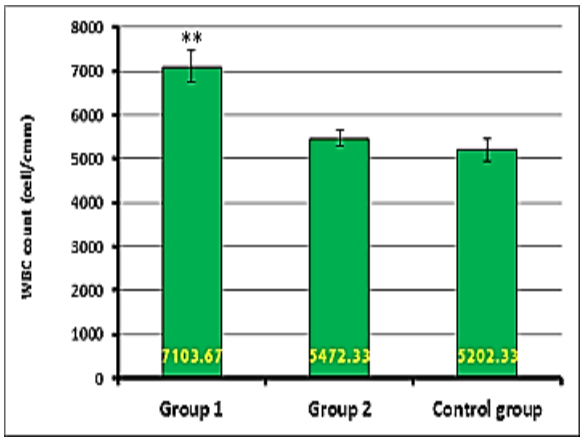


Figure 8- White Blood cell count in rat groups. Group 1, Group 2, and Control group represent rat groups after 24 hrs. intraarticularly injected with 100 µl of 30µg/100 µl of *P. mirabilis* DNA, 100 µl of 30µg/100 µl of *C. albicans* DNA and 100µl of TE buffer, respectively

Comparison between *P. mirabilis* DNA and *C. albicans* DNA

The results of the present study indicate that histological changes caused by *C. albicans* DNA were generally with a lower potencythan that of *P. mirabilis* DNA. Nevertheless, they were of approximately similar GC content DNA organism the approximate similarity in GC% between *P. mirabilis* DNA (38.9%GC) and *C. albicans* (35.1%GC) (Pearson *et al.*, 2008). Hence, the differences

observed in histological changes, IL-6, and Anti-ds Ab were not due to the difference in frequency of CG (fCG). As that the dinucleotide [CG] showed a frequency as expected from the individual G+C content and that an increase in CG went along with increased immunestimulation [46] [47] [48].
Candida albican DNA has mitogenic properties for immune cells that are similar to those of bacterial DNA, which correlated with the presence of nonmethylated CG dinucleotides and suggest that the CpG motif-containing DNA may contribute to the development of inflammatory responses after infection with *C. albicans*. In addition *C. albicans* DNA may contain as yet unidentified immunostimulatory structure other than CpG motifs that may be present at a higher frequency in this organism [49][50].

Several explanations may clarify why *C. albicans* DNA may not be a strong inflammatory agent include: the degree of methylation affects the immunostimulatory nature of DNA. *C. albicans* DNA contains about 0.1% 5-methylcytosine during growth in the yeast phase and significantly less during mycelial growth, a methylation known to diminish the activity of CpG DNA via interaction with TLR9 [51-53]. Ramirez-Ortiz *et al.* [54] reported that the fungal pathogen *Aspergillus fumigates* DNA contains unmethylated CpG sequences. It stimulated the production of proinflammatory cytokines; however it was less than bacterial DNA based on the methylation content since CpG motifs in *A. fumigates* DNA had a low level of methylation. This result could reflect the differences in CG content in *C. albicans* and *P. mirabilis* DNA since the amounts of stimulatory induced by DNA were strongly correlated with the frequency of CG dinucleotides in the genome. TLR-9 recognizes unmethylated CpG dinucleotides which abundant in prokaryotic DNA and rare in eukaryotic DNA [55].

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