



Role of DNA Isolated from Gut Microbiota *Escherichia coli* in Mice Joints Inflammation

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Abstract: The gastrointestinal microbiome is the largest and most diverse reservoir of all the human body niches. The aim of the study relationship between gut microbiota *Escherichia coli* in mice joints inflammation. In this study, 100 samples of stool were gathered from healthy individual and 20 urine samples from people with recurrent Urinary Tract Infection (UTI) and both types of samples were proceeded accordingly to isolate *E. coli* strains where 92 strains were isolated from stool samples and 10 from urine samples. The isolated strains were subjected to antibiotic sensitivity test and the results indicated that 39.2% out of the total number of the isolates were multidrug resistance while all the pathogenic strains were multidrug resistant. According to the sensitivity results three isolates were chosen to DNA isolation, two of them isolated from stool samples (Sensitive and Resistant) and one from urine samples. The extracted DNA was divided to two parts one of them was subjected to cleavage by EcoRI restriction enzyme and the other remains without treatment as a whole DNA, both were injected directly to mice knee joints to study the histopathological effects of bacterial cell free DNA on knee joints. I was conclude that indicated no effect where all the tested tissues were similar to those of group control.

Keywords: *E. coli*, gut microbiota, sensitivity test, DNA isolation, mice knee.

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Introduction

The innate immune system is programmed to attack foreign objects including microorganisms which are not recognized as self-components of the host. However, most of the bacterial populations inside human body are non-pathogenic where they presented itself as an essential component of human health and development and exert many vital functions in human hosts when it comes to the metabolism of nutrients and drugs, maintenance of integrity of the gastro-intestinal mucosal barrier, immune-modulatory roles and even protection against exogenous pathogens(1).

The gastrointestinal microbiome is the largest and most diverse reservoir of all the human body niches. From the

mouth to the anal cavity, each digestive organ section provides a specific environment that allows the growth and colonization of organisms (2). Using prebiotic dietary microbial supplements to modify gut populations, cause-and-effect relationships between the gut microbiome and systemic inflammation, metabolic dysregulation, and host illness have been discovered (3). Several studies found that cell free DNA (CFDNA) levels rise in body fluids during different pathological conditions, including trauma, inflammatory disorders and cancers. In addition, researches that study autoimmune diseases, especially in rheumatoid arthritis (RA): have shown that increased level of CFDNA in plasma correlates with disease

progression, therefore, such condition considered as a marker to the response of some autoimmune diseases therapeutic processes (4).

Between bacterial DNA and vertebrate DNA, there are a number of significant distinctions. One of these is that bacterial DNA contains far more non-methylated CpG oligonucleotides than human DNA, especially in base contexts known as "CpG motifs" (5). It is logical to hypothesize that bacterial DNA, and specifically non-methylated CpG oligonucleotides, are capable of producing inflammation in the joints and may contribute to the disease progression since DNA is readily taken up by leukocytes (6).

Septic arthritis can result from localized bacterial infections in the joints. Other destructive joint diseases, including autoimmune diseases like rheumatoid arthritis, are linked to an increased prevalence of bacterial arthritis. In humans, bacterial arthritis is a rapidly progressing and very destructive joint disease (7).

Materials and methods

One hundred stool samples were gathered from healthy individual to isolate *E. coli* from the gut microbiota, the age range was between 2 to 40 years old. Freshly collected samples were brought to the lab in a cooled environment and streaked on a Macconkey agar plate before being incubated at 37°C for 24 h. (8)

Twenty urine samples from a patient with recurrent urinary tract infections were collected in a sterile 50 ml plastic cups, transported to the lab, spread on Macconkey agar, and incubated for 24 h. at 37°C (9).

Biochemical testing was used for further identification and conformation.

Antibiotic susceptibility test

The susceptibility test was conducted according to Kirby-Bauer disc diffusion method, where the inoculum was prepared by moving three to five colonies from the primary culture plate to a sterile saline tube and compared to the 0.5 McFarland turbidity standard, the turbidity was adjusted when needed.

Using a sterilized swab, the inoculum was streaked onto a Mueller Hinton agar plate and allowed to dry at room temperature, then, using a pair of sterile forceps, the suitable antimicrobial discs from 10 different antibiotics (Table 1) were placed on the inoculation plates and incubated for 18 h. at 35 °C, the resulted inhibition zones was recorded(10).

Guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2020) were used to interpret the results(11).

Isolating of genomic DNA

Wizard genomic purification kit (Promega, USA) was used to extract the genomic DNA from *E. coli* isolates.

Measurement and purity of DNA

The absorbance using a UV spectrophotometer at 260 and 280 nm wavelengths were used to determine the purity and concentration of the DNA samples, where the reading of OD (260) was used to calculate the DNA concentration, while the ratio of OD₂₆₀/280 reading was used to measure the purity of DNA (12).

Cleavage and purification of DNA

The cleavage of DNA was performed using EcoR1 restriction enzyme produced by Promega corporation and according to the procedure mentioned in the leaflet provided by the company while the purification of cleaved DNA was performed using wizard SV Gel and

PCR clean-up system kit produced by Promega corporation and according to the protocol provided in the kit.

The injection protocol

The knees of the mice were sterilized with alcohol and the right knee was intraarticularly injected with 100 μ l of DNA dissolved in Tris-EDTA buffer (TE) while the control mice were only injected with 100 μ l of only TE. The animals were divided into groups according to the type of treatment, the first group received injections of whole DNA, the second one received injections of cleaved DNA, and the

control group were treated as mentioned above. Three replicates were used for each treatment, all the injections were carried out on sedated mice and animals were kept in their cages for 24 h. before sacrificed.

Histopathological examination

Following customary fixation, decalcification, and paraffin embedding, Hematoxylin and eosin was used to stain sections. All of the slides were coded and evaluated and histopathologic alterations in joints were examined (13).

Table (1): The types of Antibiotics used in this study.

No	Antibiotic Kind	Sensitivity determinants			Mechanism of action
		R	I	S	
1	Azithromycin 15 (AZM)	≤ 12	-----	≥ 13	Protein synthesis
2	Amikacin 30 (AK)	≤ 14	15-16	≥ 17	Cell wall
3	Augmentin 20/10 (AMC)	≤ 13	14-17	≥ 18	Cell wall
4	Ceftriaxone 30 (CRO)	≤ 19	20-22	≥ 23	Cell wall
5	Ceftazidime 30 (CAZ)	≤ 17	18-20	≥ 21	Cell wall
6	Ciprofloxacin 5 (CIP)	≤ 21	22-25	≥ 26	DNA synthesis
7	Cefotaxime 30 (CTX)	≤ 22	23-25	≥ 26	Cell wall
8	Imipenem 10 (IMP)	≤ 19	20-22	≥ 23	Cell wall
9	Gentamicin 10 (CN)	≤ 12	13-14	≥ 15	Cell wall
10	Tetracycline 30 (TE)	≤ 11	12-14	≥ 15	Protein synthesis

Results and discussion

Isolation of microbiota *E. coli* from stool samples

According to the results of chromogenic agar, microscopic

examination and the biochemical tests (Table 2) ninety-two *E. coli* isolates were gathered from stool samples and ten from urine samples.

Table (2): The results of biochemical tests.

Test	Result
Growing on MacConkey agar	Pink color solid colony
Growing on EMB	Green metallic sheen
Lactose fermentation	+
Gram stain reaction	+
Indole	+
Methyl red	+
Voges-Proskauer	-
Citrate	-
TSI	Acid/Acid, with gas, No H ₂ S
Urease	-
Catalase	+
Oxidase	-
Motility	+

Antibiotic susceptibility test Susceptibility of gut microbiota *E. coli*

The result showed that 39.2% out of the total number of the isolates were multidrug resistance where they resist at least three antibiotics from three different groups, 8.7% resist three antibiotics from two different groups, 21.7% resist two antibiotics and 30.47% resist one antibiotic and none of them were susceptible to all of the used antibiotics. In addition, the results indicated that all of the isolates resist (AMC) while all of them showed a 100% susceptibility to (AK, IPM). The percentage of response to the other antibiotics was varying, whereas the greatest susceptibility rate was to (CN) with a 97.8%, while the greatest resistant rate was to (AZM) with a 43.5% (Figure 1).

Susceptibility test for pathogenic *E. coli*

All the pathogenic isolates tested in this study were multidrug resistant where the result indicated that 10% of them resist eight antibiotics, 60% resist seven antibiotics, 20% resist six antibiotics and 10% resist five antibiotics. All the tested isolates were

resistant to the (AMC, CTX, CAZ and CRO) antibiotics, while all of them were susceptible to (AK and IPM). The greatest susceptibility for the rest of antibiotics was for (AZM) with 70%, while the greatest resistance was to (CIP) with a percentage of 90% (Figure 2).

Susceptibility of gut microbiota isolates compared to pathogenic ones

Both types of *E. coli* isolates were sensitive to (AK, IPM) and resist (AMC). While 90% of PA were resistant to (CIP): the majority of gut microbiota were susceptible to the same antibiotic with a percentage of 60%. Another difference appeared between the two types in the response towards cephalosporins (CTX, CAZ and CRO) where the PA were totally resistant to this group while the results of gut microbiota was uneven, with highest susceptibility to (CAZ) followed by (CRO) then (CTX). Furthermore, the difference in results between pathogenic and gut microbiota was more obvious in response to (CN) where 70% of pathogenic isolates were resistant, while 97.8% of the gut microbiota were susceptible (Figure 3).

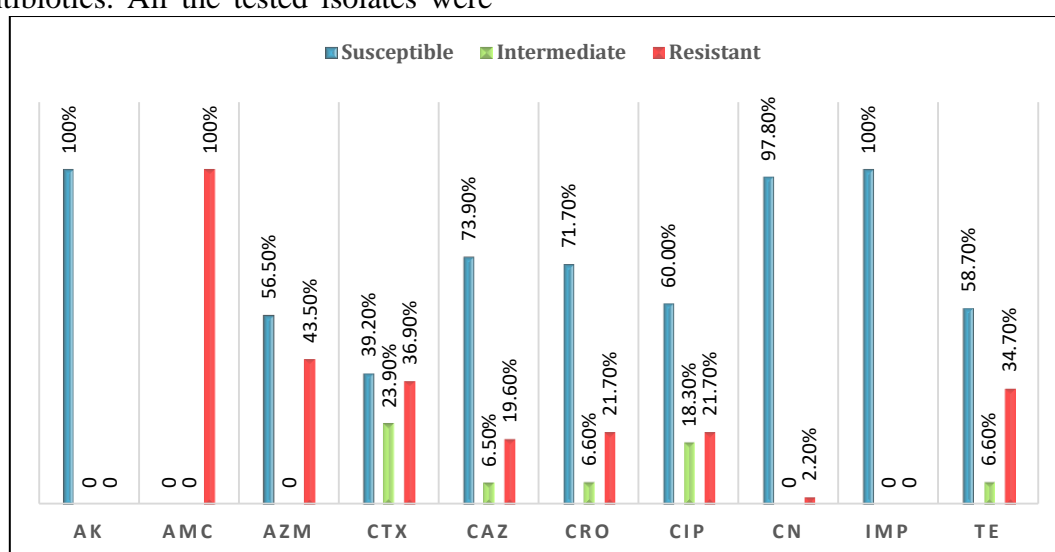


Figure (1): Susceptibility test for gut microbiota *E. coli*

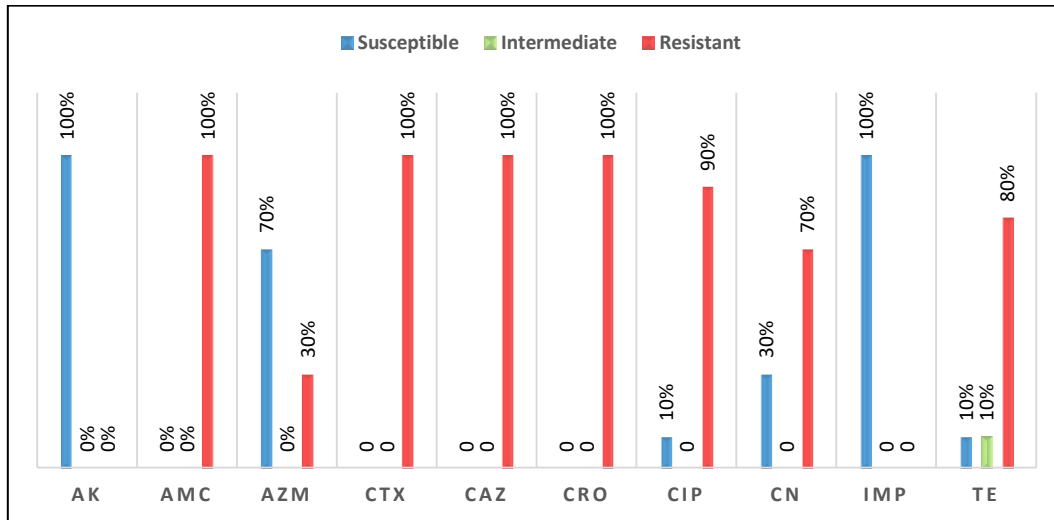


Figure (2): Susceptibility test for pathogenic *E. coli*

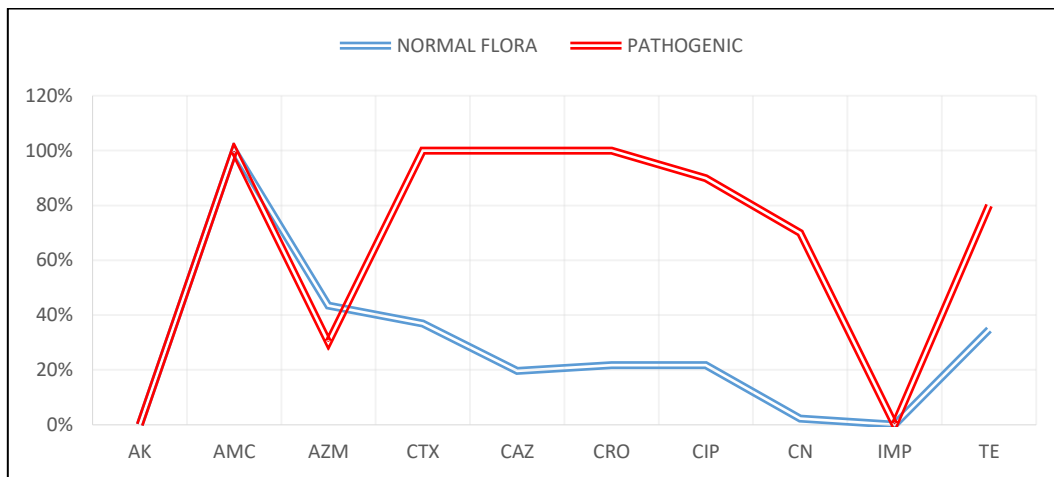


Figure (3): Comparison between the susceptibility results of gut microbiota and pathogenic isolates

DNA extraction

Deoxyribonucleic acid was extracted from an overnight culture of *E. coli* from three isolates one of them was isolated from urine sample and showed resistance to antibiotics (U/R) the other two were isolated from stool samples, one of them was resistant to antibiotics (Sm/R) and the other was sensitive (Sm/S) using Wizard genomic DNA purification kit Promega which proofed its high accuracy in extraction of DNA from Gram-negative bacteria with high purity. Genomic DNA was further analyzed quantitatively and qualitatively by gel electrophoresis

(Figure 4) which showed one band of DNA for each type. This indicates the methods used to extract and purify the DNA were effective.

Estimation of the purity and concentration of DNA

The purity value of the extracted DNA from the three isolate were (U/R- 1.8): (Sm/S- 1.9): (Sm/R- 1.8). the result demonstrated to be within the accepted range of 1.7-2.0 (14). The bacterial DNAs were then adjusted to the required concentrations as a final concentration used in the subsequent steps.

Histopathological

The histopathological results of knee joint indicated that the isolated DNA in both status (break and non-

break) tested showed no effect on the knee joints of the mouse were all the tested tissues appeared similar to those of the control group (Figures 4,5).

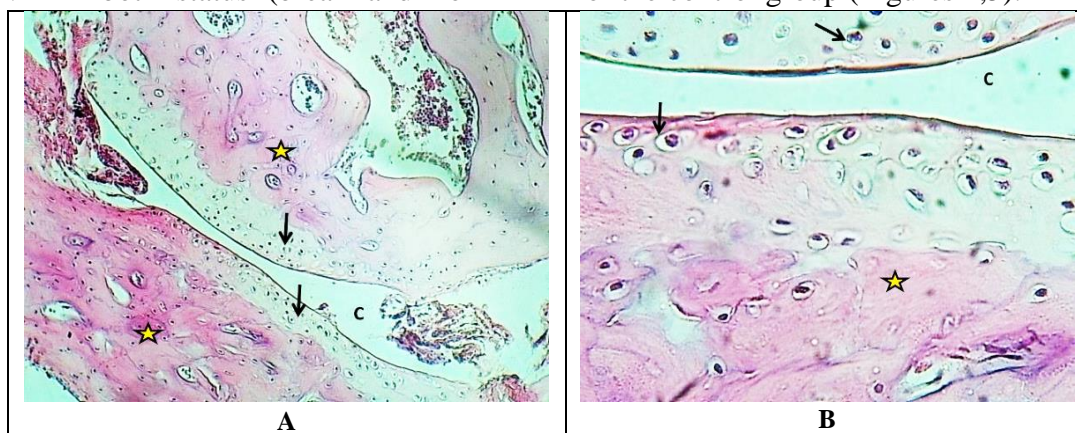


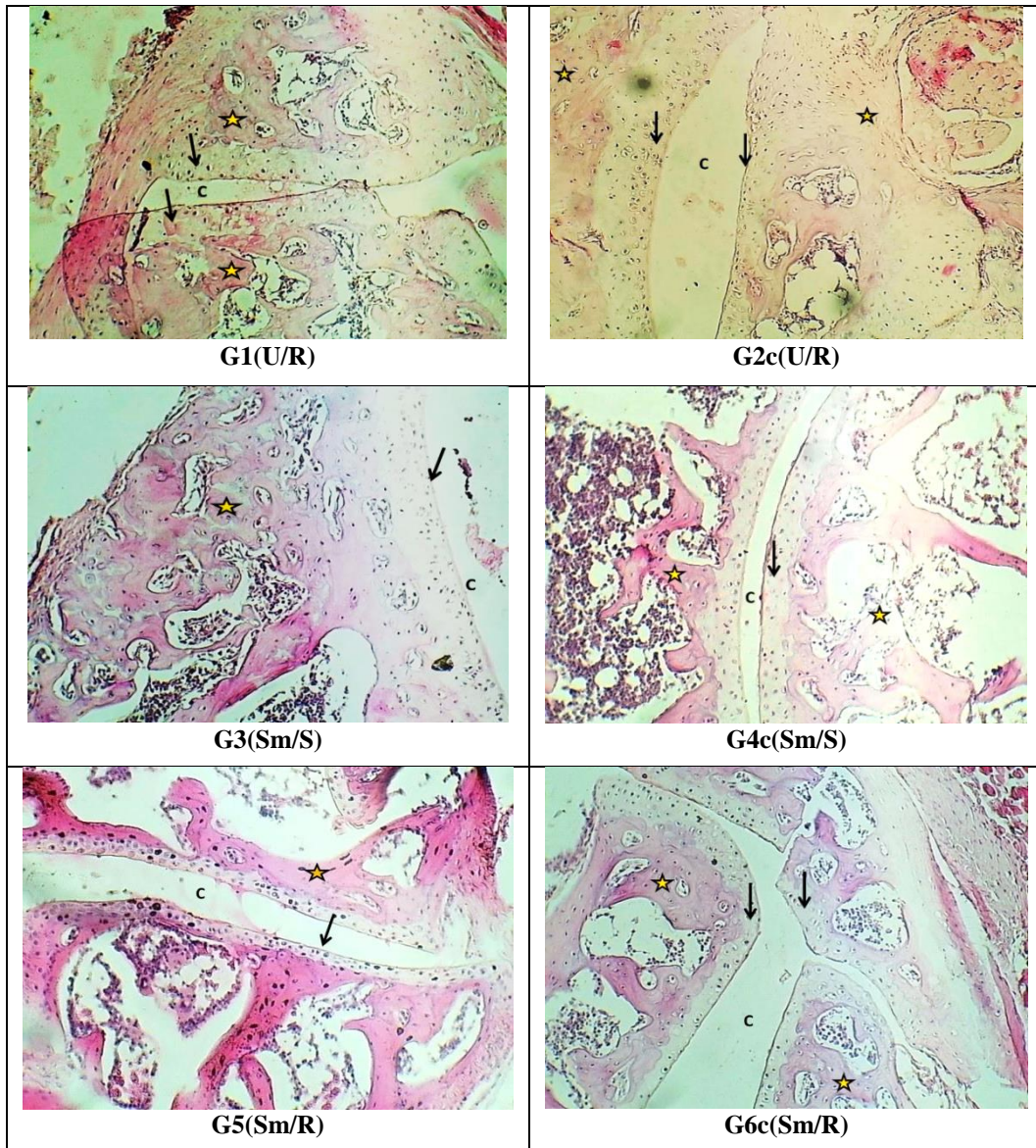
Figure (4):

- (A): Section of joint (control group) shows: normal of synovial cavity (C,); bone epiphysis (Asterisk): articular hyaline cartilage (arrows.) HandE stain.100x.
 (B): Section of joint (control group) shows: normal of synovial cavity (C,); bone epiphysis (Asterisk) and articular hyaline cartilage with normal chondrocytes (arrows).HandE stain.400x.

From the results mentioned above, it has been proved that the resistance ability is common among pathogenic and gut microbiota *E. coli* isolates with several isolates resist multiple drugs from different groups. similar results found in many studies (15,16,17,18,19 and 20).

According to Vardanyan and Hruby (21): Imipenem has an Alpha-hydroxyethyl side chain, which significantly increases its resistance to hydrolysis by beta-lactamases in contrast to penicillins and cephalosporins, which have a side aminoacyl group side chain. This may explain the results gathered in this study where the (IMP) was very effective against pathogenic and gut microbiota isolates. Jacoby (22) mentioned that chromosomes of many Enterobacteriaceae contain genes coded

for AmpC beta-lactamases enzymes, these genes located on transmissible plasmids therefore they can appear in bacteria lacking these kinds of genes like *E. coli*, such enzymes confer resistance to beta-lactamase inhibitor beta-lactam combination and over expression of these enzymes mediate resistance to cephalosporins including ceftriaxone, cefotaxime and ceftazidime. On the other hand, Carbapenems usually used to treat infections caused by AmpC beta-lactamases enzymes producing bacteria. Furthermore, Shamki *et al.*; (23) conducted a study on 325 fecal specimens collected from children with diarrhea, they found that 28 isolate were *E. coli*, all of them were multi-drug resistant. However, none of them were found resistant to imipenem.

**Figure (5):**

- G1(U/R):** Group 1 injected with whole DNA isolated from urine sample/multidrug resistant shows: normal of synovial cavity (C,): bone epiphysis (Asterisk): articular hyaline cartilage (arrows). HandE stain.100x.
- G2c(U/R):** Group 2 injected with cleaved DNA isolated from urine sample/multidrug resistant shows: normal of synovial cavity (C,): bone epiphysis (Asterisk): articular hyaline cartilage (arrows). HandE stain.100x.
- G3(Sm/S):** Group 3 injected with whole DNA isolated from microbiota sample/sensitive shows: normal of synovial cavity (C,): bone epiphysis (Asterisk): articular hyaline cartilage (arrows). HandE stain.100x.
- G4c(Sm/S):** Group 4 injected with cleaved DNA isolated from microbiota sample/sensitive shows: normal of synovial cavity (C,): bone epiphysis (Asterisk): articular hyaline cartilage (arrows). HandE stain.100x.
- G5(Sm/R):** Group 5 injected with whole DNA isolated from microbiota sample/multidrug resistant shows: normal of synovial cavity (C,): bone epiphysis (Asterisk): articular hyaline cartilage (arrows). HandE stain.100x.
- G6c(Sm/R):** Group 6 injected with cleaved DNA isolated from microbiota sample/multidrug resistant shows: normal of synovial cavity (C,): bone epiphysis (Asterisk): articular hyaline cartilage (arrows). HandE stain.100x.

Similar results were noticed in this study where most of the isolates were resistance to (AMC) and cephalosporins (CTX, CAZ, CRO) while they were susceptible to (IMP) which may indicate that some of the tested isolates harbor the bla(AmpC) gene.

Many studies revealed that bacterial DNA has a direct link with arthritis, Malalah *et al.*, (24) tested the effect of purified DNA extracted from *Staphylococcus aureus* on knee joints of Rats and the histopathological results showed degeneration of joint space, aggregation of lymphocyte and necrosis. Also, Kadhim *et al.*, (25) clarified that purified DNA isolated from *Proteus mirabilis* caused congestion, inflammatory cells infiltration, edema and necrotic cells when injected in knee joints of a rat model. Deng and Tarkowski (26) stated that bacterial cell free DNA might induce arthritis. They injected the knee joints with bacterial DNA and the results indicated that arthritis was induced by bacterial DNA. Zeuner *et al.*, (27) clarified that the CpG oligonucleotides induced arthritis where their histological results indicated that several changes including perivascular infiltration by mononuclear cells and hyperplasia of the synovial lining after injection of oligonucleotides. Deng *et al.*, (28) 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.*, (29) also agreed with the previous studies. The results indicated from this study contradict the previous result where after 24 hours' injection of bacterial DNA in knee joints of mice the histological tests indicated no effect

where the tissues were similar to those of control group.

Cukrowska *et al.* (30) stated that intestinal microbiota doesn't induce immune responses that may lead to arthritis. However, as a result of dysregulation of intestinal microbiota, normal microbiota can act as an external antigen to stimulate lymphocyte proliferation and differentiation. Activated lymphocytes can then release various cytokines, such as IL-1, IL-6, IL-17, and TNF- α . IL-1 and TNF- α prompt white blood cells to accumulate in the articular cavity and stimulate production of small molecule inflammatory mediators, thereby leading to cartilage damage and changes in bone. But they mentioned that whether the bacterial DNA is involved in Osteoarthritis pathogenesis remains controversial. Which might explain the results obtained from this study where the injection time (24 hours) was not enough to generate pathological effects.

Conclusion

The results of this study has shown that the multidrug resistance ability are spread among gut microbiota *E. coli*. Furthermore, the ability of free bacterial DNA in arthritis development and progression remains controversial and need further studies to elucidate the complete picture.

Recommendations

More research is needed in these fields to face the challenge raise by the disruptions of antibiotic resistant ability among gut microbiota and the role of bacterial free DNA in pathogenicity and diseases progression

References

1. Ramires, L. C.; Santos, G. S.; Ramires, R. P.; Furtado, L.; Jeyaraman, M.; Muthu, S., et al. (2022). The Association between Gut Microbiota and Osteoarthritis: Does the Disease Begin in the Gut. International Journal of Molecular Sciences, 23(3): 1494.

2. Barko, P. C.; McMichael, M. A.; Swanson, K. S. and Williams, D. A. (2018). The Gastrointestinal Microbiome: A Review. *Journal of Veterinary Internal Medicine*, 32(1): 9-25.
3. Schott, E. M.; Farnsworth, C. W.; Grier, A.; Lillis, J. A.; Soniwala, S.; Dadourian, G. H., et al. (2018). Targeting the gut microbiome to treat the osteoarthritis of obesity. *JCI Insight*, 3(8): e95997.
4. Pietrzak, B.; Kawacka, I.; Olejnik-Schmidt, A. and Schmidt, M. (2023). Circulating Microbial Cell-Free DNA in Health and Disease. *International Journal of Molecular Sciences*, 24(3): 3051.
5. Duvvuri, B.; and Lood, C. (2019). Cell-Free DNA as a Biomarker in Autoimmune Rheumatic Diseases. *Frontiers in Immunology*, 10, 502.
6. Dong, C.; Liu, Y.; Sun, C.; Liang, H.; Dai, L.; Shen, J., et al. (2020). Identification of Specific Joint-Inflammatogenic Cell-Free DNA Molecules from Synovial Fluids of Patients with Rheumatoid Arthritis. *Frontiers in Immunology*, 11, 662.
7. Goldenberg, D. L. (1998). Septic arthritis. *The Lancet*, 351(9097): 197–202.
8. Ogunrinola, G.; Oyewale, J.; Oshamika, O. and Olasehinde, G. I. (2020). The Human Microbiome and Its Impacts on Health. *International Journal of Microbiology*, 12, 8045646.
9. Singh, A.; Das, S.; Singh, S.; Gajamer, V.; Pradhan, N.; Lepcha, Y., et al. (2018). Prevalence of Antibiotic Resistance in Commensal *Escherichia Coli* among the Children in Rural Hill Communities of North East India. *PLoS One*, 13(6): e0199179.
10. Hudzicki, J. (2016). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *ASM*, 8, 2009, 1–23.
11. CLSI. (2020). Performance standards for antimicrobial susceptibility testing 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.
12. El-Ashram, S.; Al Nasr, I. and Suo, X. (2016). Nucleic acid protocols: Extraction and optimization. *Biotechnology Reports (Amsterdam)*: 5: 12:33-39.
13. Wei, P.; and Bao, R. (2023). Intra-Articular Mesenchymal Stem Cell Injection for Knee Osteoarthritis: Mechanisms and Clinical Evidence. *International Journal of Molecular Sciences*, 24(1): 59.
14. Chaudhuri, S. R.; Pattanayak, A. K. and Thakur, A. R. (2006). Microbial DNA Extraction from Samples of Varied Origin. *Current Science*, 91: 1697-1700.
15. Akter, S.; Chowdhury, A. and Mina, S. A. (2021). Antibiotic Resistance and Plasmid Profiling of *Escherichia coli* Isolated from Human Sewage Samples. *Microbiology Insights*, 14: 1–6.
16. Issa, A.; Almayah, A. and Ibrahim, H. (2020). New virulence factor of normal Flora *E. Coli*. *Systematic Reviews in Pharmacy*, 11(2): 71–76.
17. Jafri, S.; Qasim, M.; Masoud, M.; Rahman, M.; Izhar, M. and Kazmi, S. (2014). Antibiotic resistance of *E. coli* isolates from urine samples of Urinary Tract Infection (UTI) patients in Pakistan. *Bioinformatics*, 10(7): 419–422.
18. AL-Jubouri, S. S., & Shami, A. M. (2022). Molecular Detection of Cephalosporin Resistance Genes in *Escherichia coli* Isolated from Urinary Tract Infections in Baghdad Hospitals. *Iraqi Journal of Biotechnology*, 21(2), 145-152.
19. Roberts, M. (2004). Tetracycline Resistance. *Encyclopedia of Medical Genomics and Proteomics*, 50(1): 1262–1265.
20. Al-Taai, H. R. (2018). Antibiotic resistance patterns and adhesion ability of uropathogenic *Escherichia coli* in children. *Iraqi Journal of Biotechnology*, 17(1).
21. Vardanyan, R. and Hruby, V. (2006). *Synthesis of Essential Drugs (1st Edition)*. Elsevier, Amsterdam.
22. Ali, F. A. (2018). Distribution of CTX-M gene among *Escherichia coli* strains isolated from different clinical samples in Erbil City. *Iraqi Journal of Biotechnology*, 17(1).
23. Shamki, J. A.; Al-Charrakh, A. H. and Al-Khafaji, J. K. (2012). Detection of ESBLs in Enteropathogenic *E. coli* (EPEC) isolates associated with infantile diarrhea in Kut City. *Medical Journal of Babylon*, 9(2): 403-412.
24. Malalah S; Utba, N. and AL-Mathkury, H. (2014). The Role of Antibiotic-Killed *Staphylococcus aureus* and Its DNA to Cause Arthritis in Rats. *Iraqi Journal of Science*, 55, 4B:1802-1810.
25. Kadhim, A; AL-Mathkury, H. and Obaid, H. (2014). Role of *Proteus mirabilis* DNA in Comparison to *Candida albicans* DNA in Rats' Joints Infection. *Iraqi J. Sci.*, Vol 55, No.3B, pp:1170-1182.

26. Deng, G. M. and Tarkowski, A. (2000). The Features of Arthritis Induced by CpG Motifs in Bacterial DNA. *Arthritis and Rheumatism*, 43(2): 356–364.
27. Zeuner, R. A.; Ishii, K. J.; Lizak, M. J.; Gursel, I.; Yamada, H.; Klinman, D. M., *et al.* (2002). Reduction of CpG-Induced Arthritis by Suppressive Oligodeoxynucleotides. *Arthritis and Rheumatism*, 46(8): 2219–2224.
28. Deng, G. M.; Nilsson, M.; Verdrengh, M.; Collins, V.; and Tarkowski, A. (2001). Intra-articularly localized bacterial DNA containing CpG motifs induces arthritis. *Nature Medicine*, 5(6): 702 – 705.
29. Ohshima, S.; Saeki, Y.; Mima, T.; Sasai, M.; Nishioka, K.; Nomura, S., *et al.* (1998). Interleukin 6 plays a key role in the development of antigen-induced arthritis. *Proceedings of the National Academy of Sciences*, 95(14): 8222–8226.
30. Cukrowska, B.; Kozakova, H.; Rehakova, Z.; Sinkora, J. and Tlaskalova-Hogenova, H. (2001). Specific antibody and immunoglobulin responses after intestinal colonization of germ-free piglets with non-pathogenic *Escherichia coli* O86. *Immunobiology*, 204(4): 425-433.