Diagnosis Of The Bone And Joint Infections Using Molecular Techniques In Al-Hussein Teaching Hospital At Period From January- August, 2020

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Abstract

A prospective study of 50 samples of patients suffering from different bone and joint infections at Al-Hussien Teaching Hospital at period from January to August 2020. The mean age is 47.98 years, 60% males and 40% females, 24% of the cases were diabetes mellitus, 24% of the cases were immunosuppression, and 16% of the cases were suffering from obesity. These samples examine by conventional culture and by molecular method (PCR and DNA sequences) to evaluate the specificity and sensitivity for each method in the diagnosis of bone and joint infection to know the best and shorter method in the diagnosis. The results revealed the specificity and sensitivity of molecular method are (93.75%) and (88.24%)   respectively, while by cultural method they are (81.25%) and (94.12%) respectively. All the patients were sent for measuring C–Reactive protein, peripheral blood leucocytes and HbA1c to evaluate diabetes mellitus. Also all the patients sent for plain radiography and ultrasound. In this study we evaluate the risk factor for etiology of bone and joint infection such as immunosuppression and obesity. Also evaluate the type of implants, localization of infection, purulence of infection and presence of sinus tract. We proved in this study that the molecular method is more specific but less sensitive than the conventional culture in the diagnosis of bone and joint infections.

Aim of study

This study was conducted to evaluate the specificity and sensitivity of molecular method (PCR and DNA sequences) and compare them with that of Conventional microbiological cultures in diagnosis of bone and joint infections.

Introduction Definition

A musculoskeletal infection is the invasion of an individual's muscles, bones, or joints by disease-causing organisms, their multiplication, and the reaction of the individual's body tissues to these organisms and the toxins they produce. Most musculoskeletal infections are

usually caused by bacteria. Infection in the bones, joints, or muscles may occur at any age and can spread swiftly through an individual's blood, bones, and tissues.

Types of Musculoskeletal Infections
Some of the types of musculoskeletal infections include the following:

Septic arthritis: Septic arthritis, also known as infectious arthritis, is a painful infection of the joint. The infection can occur from bacteria that spread through the bloodstream from another area of the body. Septic arthritis can also occur due to a penetrating injury that delivers germs directly into the joint. Infants and older adults are most likely to develop septic arthritis. Knees are most commonly affected, but septic arthritis also can affect hips, shoulders, and other joints.

Septic bursitis: Septic bursitis is a painful condition that affects the joints. Bursae are fluid-filled sacs that act as a cushion between bones, tendons, joints, and muscles. When these sacs become inflamed due to infection, usually with bacteria, it is called septic bursitis.
Osteomyelitis: Osteomyelitis is an infection of the bone that can occur in any age group. It can be caused by bacteria in the bloodstream from infectious diseases that spread to the bone, an open wound from an injury over a bone, and recent surgery or injection in or around a bone. Smokers and people with diabetes or kidney failure are at increased risk of developing osteomyelitis (figure 1).

Myositis (soft tissue infections): Myositis refers to inflammation of the muscles that can be caused by an infection, injury, or autoimmune disease. There are various types of myositis, the most common being polymyositis and dermatomyositis.

 Polymyositis: causes muscle weakness, mostly in the muscles nearest to the trunk of the body.
Dermatomyositis: causes muscle weakness as well as a skin rash. The main muscles to be affected by myositis are the shoulders, hips, and thighs.

Pyomyositis: Pyomyositis is an acute bacterial infection of the skeletal muscle that results in pain and tenderness of the affected muscle and localized abscess formation. If left untreated, the abscess may extend into the bone and joint or blood poisoning may occur. It most commonly affects the muscles of the limbs and torso. Approximately 90% of cases are caused by the bacterium, Staphylococcus aureus. Treatment generally includes surgical drainage of the abscess and antibiotics.

Infectious tenosynovitis: Tenosynovitis is an infection of a tendon and its protective sheath that results in inflammation of the tendon and synovial sheath. This infection is most common in the finger, hand, or wrist but can occur in any part of the extremities where a tendon glides within a synovial-lined fibro-osseous sheath.

Cellulitis: Cellulitis is a common bacterial infection of the skin and the soft tissues underneath. It occurs when bacteria enter a break in the skin and spreads. It most often affects the skin of the lower legs, although the infection can occur anywhere on your body or face. Without treatment, the infection can spread quickly and may travel to lymph nodes and into the bloodstream leading to a blood infection or permanent damage of lymph vessels.

Abscess: Abscesses are collections of pus in confined tissue spaces, usually caused by a bacterial infection or when germs enter the body through an open wound like a cut. Abscesses can occur in the skin, soft tissue, muscle, or even bones, causing inflammation at the site of infection and a cavity filled with pus.

Risk factors of musculoskeletal infections

1-Glucocorticoid and immunosuppressant treatments. 2-Obesity (body mass index greater than 30 kg/m^2. 3-prolonged surgical time.4- inadequate antibiotic prophylaxis 5-prolonged wound drainage. 6- hematoma. 7-Diabetes mellitus. 8-vascular insufficiency. 9-foreign bodies. 10-cutaneous, urinary, and/or abdominal infections.

Symptoms of Musculoskeletal Infections

Some of the common symptoms associated with musculoskeletal infections include:- 1-Pain and tenderness. 2-Swelling. 3-Fever. 4-Difficulty moving the limbs. 5-Difficulty in weight-bearing. 6-Abscess formation.

Causative Microorganisms of Bone and Joint Infections

Microorganisms involved in BJI according to the expert committee.

The first etiology is staphylococci: in particular Staphylococcus aureus Staphylococci represented the most common cause of Periprosthetic joint infection.

The second etiology is M tuberculosis: with 16 cases of spinal tuberculosis and 4 cases of tuberculous arthritis. neither PCR nor cultures were positive, and the diagnosis of M tuberculosis was based on clinical, magnetic resonance imaging, and histological arguments (figure 3).

The third etiology is Streptococci: with the majority being Streptococcus pneumonia and Enterobacteriaceae infections.

The fourth etiology polymicrobial: due to Staphylococcus epidermidis + Enterococcus faecalis and Escherichia coli + E faecalis. to Mycoplasma spp and Neisseria gonorrhoeae. Musculoskeletal Infections

periprosthetic infection

PJI of the hip joint or arthroplasty-related infection can be defined as the growth of microorganisms in the hip joint in the presence of a hip replacement prosthesis. It represents deep joint infection and does not include isolated superficial wound infection. The affected hip prosthesis may include components of primary or revision THR, hip resurfacing, or hip

hemiarthroplasty. The incidence of PJI is 0.5–2% after primary total hip arthroplasty (THA) and 5–7% after revision THA (figure 2).

The Musculoskeletal Infection Society proposed specific criteria for the definition of PJI during its 21st annual meeting in 2011. These criteria include:-

1-Presence of a sinus tract communicating with the prosthesis; or 2-A pathogen is isolated by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joint; or **Four of the following six criteria exist:**

1- Elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration.
2- Elevated synovial leukocyte count.
3- Elevated synovial neutrophil percentage (PMN %).
4- Presence of purulence in the affected joint.
5- Isolation of a microorganism in one culture of periprosthetic tissue or fluid.
6- Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at 9400 magnification
7-

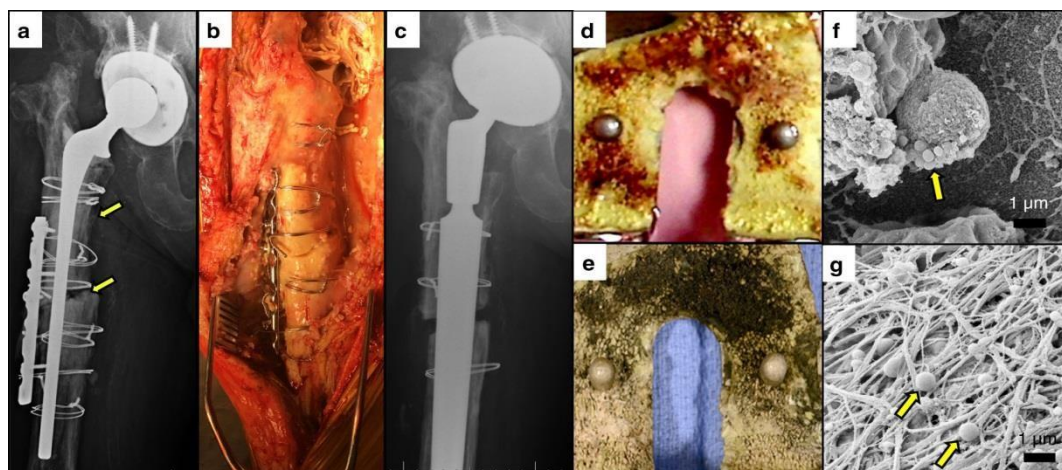Fig. (1) : Biofilm in acute and chronic osteomyelitis.

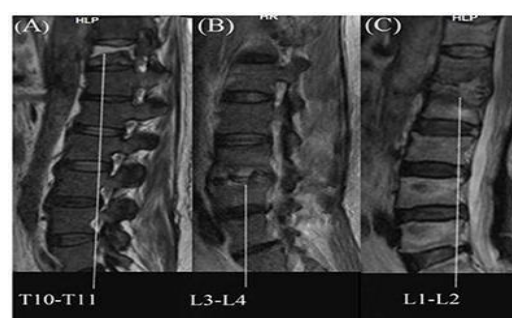Fig. (2) : Periprosthetic infection diagnosis

Fig. (3) : Pyogenic spondylodiscitis

Diagnosis

1-Laboratory tests to check for infection. 2-X-rays to look for changes in the bone. 3-MRI for detailed images of the structures. 4-Ultrasound for detailed images of the soft tissue structures. 5-Bone scan to check the condition of the bones.

Periprosthetic joint infections (PJI) can be broadly classed into two groups: those where there is a strong clinical suspicion of infection and those with clinical uncertainty, including 'aseptic loosening'. Confirmation of infection and identification of the causative organism along with provision of antibiotic susceptibility data are important stages in the management of PJI. Conventional microbiological culture and susceptibility testing is usually sufficient to provide this. However, it may fail due to prior antimicrobial treatment or the presence of unusual and fastidious organisms. Molecular techniques, in particular specific real-time and broad-range PCR, are available for diagnostic use in suspected PJI.

Treatment
Antibiotics are the first-line of treatment for musculoskeletal infections. Bone infections are normally treated with antibiotics for 4 to 6 weeks, whereas joint and muscle infections are usually treated for 3 weeks. Surgery may be required to remove infected material (pus) from the area of infection. This reduces pressure and inflammation and improves blood flow, which makes it easier for the antibiotics to reach the infected area. During surgery to drain the infection, surgeon cleans the inside of the tendon sheath to wash away pus and germs and may remove damaged or dead tissue to allow remaining tissue to heal. In the case of septic arthritis, surgery normally will be required to wash the bacteria out of the joint. In some cases, septic arthritis may be treated by using a needle to draw the infected fluid out of the joint. For severe infections, a person may require surgery more than once to completely remove any infection.

Musculoskeletal Infections Complications
1-Fractures. 2- Arthritis.3- Growth deformity.4- Joint dysfunction.5- Long term morbidity

Patients and Methods
We examine 50 samples of patients suffering from different types of bone and joint infections by conventional culture and molecular method (PCR and DNA sequences) and evaluate and compare the specificity and sensitivity for each of them. All the patients evaluated for age, sex, immunosuppression, obesity and diabetes mellitus. The patients sent for plain radiography and ultrasound, also they sent for C-Reactive protein, peripheral blood leucocytes and HbA1c.

EasyPure® Bacteria Genomic DNA Kit
https://www.transgenbiotech.com/genomic_dna_purification/easypure_bacteria_genomic_dna_kit.html

Gel electrophoresis
PCR products of multi gene were analyzed by using Agarose gel electrophoresis method as following steps:
1- 1% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.
2- Then 2µL of ethidium bromide stain were added into Agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µL of PCR product were added in to each comb well and 5µL of (1500 bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt for 30 min. then 50 volt for 45 min.

5- PCR products were visualized by using ultraviolet trans illuminator.

Component	25µL reaction
10 µM Forward Primer	0.5 µL
10 µM Reverse Primer	0.5 µL
Template DNA	1.5 µL
OneTaq Quick-Load 2X Master Mix with Standard Buffer	12.5 µL
Nuclease-free water	10 µL
Total	25 µL

Table (1): 25 µL reaction

PCR master mix reaction preparation

PCR master mix reaction was prepared by using (OneTaq quick-load)PCR Kit and this master mix done according to company instructions as shown in table (1).

A Protocol for OneTaq® Quick-Load 2X Master Mix with Standard Buffer (M0486)

https://international.neb.com/protocols/2012/09/11/protocol-for-onetaq-quick-load-2xmaster-mix-with-standard-buffer-m0486

After that, these PCR master mix reaction components that mentioned above, placed in standard PCR tubes containing the Multiplex PCR as lyophilized materials containing all other components needed to PCR reaction such as (Taq DNA Polymerase, dNTPs, 6 mM MgCl2, pH 8.7,). Then the tube placed in Exispin vortex centrifuge for 3 minutes. Then transferred in Multigene PCR Thermocycler.

PCR Thermocycling Conditions

PCR Thermocycler conditions for each gene were done by using conventional PCR Thermocycler system as shown in table (2):

steps	Temperatures	time
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C	15-30 seconds
	variable (as mentioned below every photo) 68°C	15-60 seconds
		1 minute per kb
Final Extension	68°C	5 minutes

Hold	4-10°C	

Table (2): Conventional PCR

No.	Gene	Primer Sequences	PCR Product	References
1	**Universal primers 27**	**F 5' AGAGTTTGATCMTGGCTCAG '3 R 5' ACCGCGGCTGCTGGCAC '3**	**(500bp)**	Mohamed Omar a,*, Eduardo M. Suero a, Emmanouil Liodakis a, Moritz Reichling a, Daniel Guenther a, Sebastian Decker a, Meike Stiesch b, Christian Krettek a, Jo¨ rg Eberhard b, et al. Diagnostic performance of swab PCR as an alternative to tissue culture methods for diagnosing infections associated with fracture fixation devices. Injury 2016 ; JINJ-6709.
2	**Primers 16 sRNA1**	**F 5' CAG CAG CCG CGG TAATAC '3 R 5' CAC GAG CTG ACG ACA '3**	**(580bp)**	Rovery C, Greub G, Lepidi H, Casalta JP, Habib G, Collart F, et al. PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. J Clin Microbiol 2005;43:163e7.
3	**Primers 16 sRNA2**	**F 5' GGA GGA AGG TGG GGA TGA CG '3 R 5' ATG GTG TGA CGG GCG GTG TG '3**	**(241bp)**	Saravolatz LD, Manzor O, VanderVelde N, Pawlak J, Belian B. Broad-range bacterial polymerase chain reaction for

				early detection of bacterial meningitis. Clin Infect Dis 2003;36:40e5.

Table (3): Primers of this study
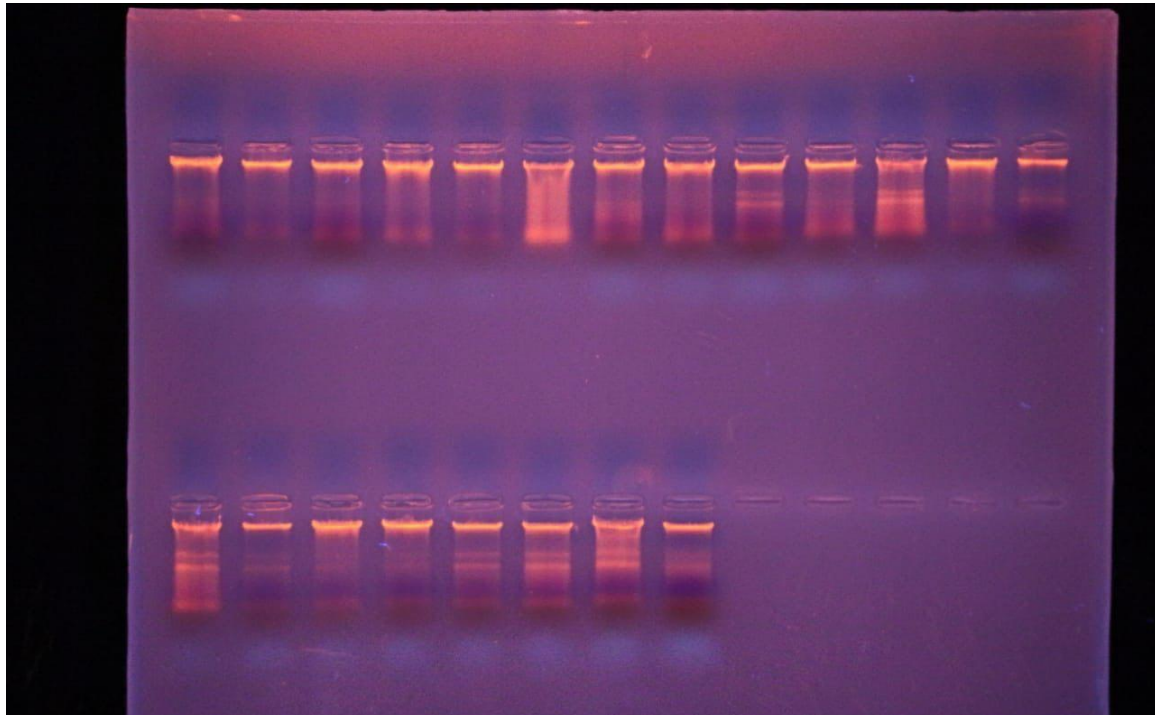
Figure (4): Ethidium Bromide stained agarose gel electrophoresis appearance that displays DNA from bacteria that was extracted.

Figure (5) : Gel electrophoresis for optimization process with different temperatures and different Pimers for PCR product of (Universal 27, 16 sRNA1 and 16 sRNA2) Primers which shows(241bp at 55c, 500bp at 59c and 580bp at 57c) respectively . (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after staining with Ethidium bromide. Lane L : DNA ladder (1500-100)bp

Figure (6) : Gel electrophoresis for PCR product of (Universal primers 27) which show 500bp Primer TM at (59C), (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.) Visualized under U.V light after staining with ethidium bromide. Lane L : DNA ladder (1500-100)bp , Lanes (1-22) represented positive results, Lane (N) represented Negative results .
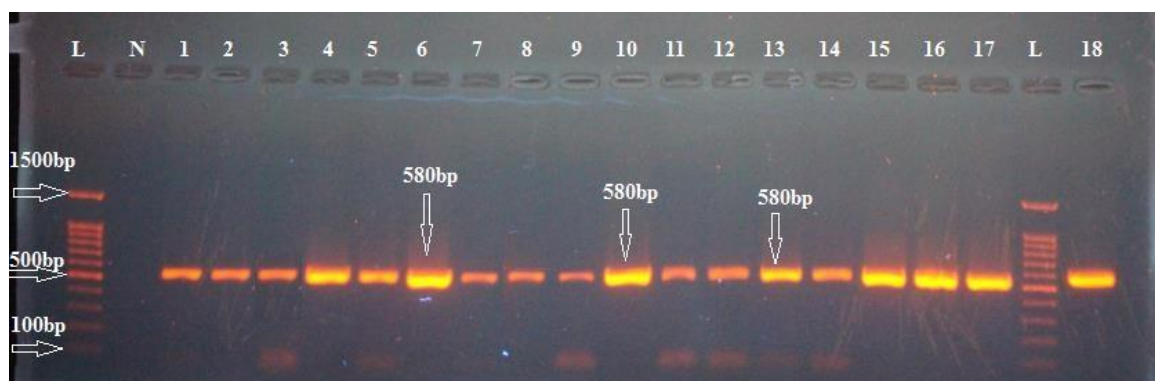
Figure (7) : Gel electrophoresis for PCR product of (16 sRNA1 primer) which show 580bp

Primer TM at (57C), (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after staining with ethidium bromide. Lane L : DNA ladder (1500-100)bp , Lanes (1-18) represented positive results and lane N represent negative control.
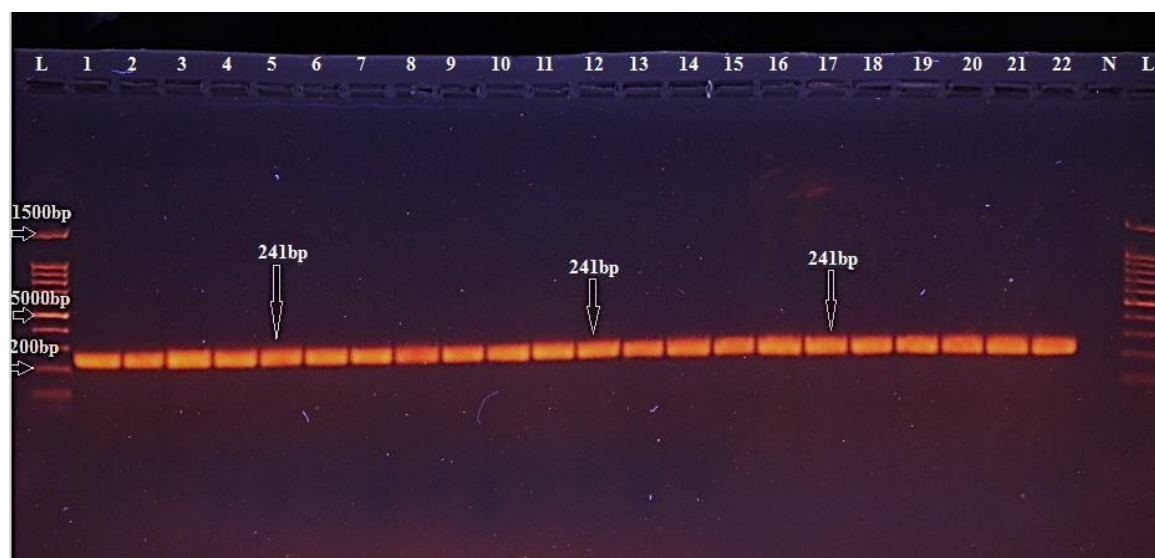
Figure (8) : Gel electrophoresis for PCR product of (16 sRNA2 primer) which show 241bp

Primer TM at (57C), (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after stai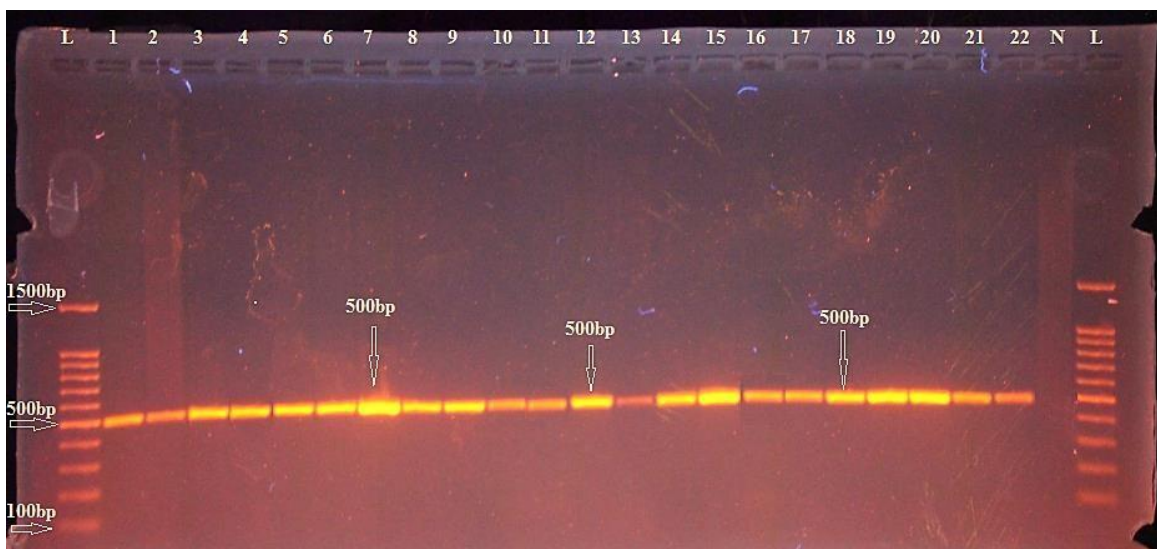ning with ethidium bromide. Lane L : DNA ladder (1500-100)bp , Lanes (1-22) represented positive results and lane N represent negative control.

No.	Primer name	PCR	Temperature	Positive	Negative
1	Universal primers 27	500bp	59	(1-30)	No
2	Primers 16 sRNA1	580bp	57	(1-30)	No
3	Primers 16 sRNA2	241bp	57	(1-30)	No

Table (3): primers with temperature, positive and negative control

Results

In this study we examine 50 samples of patients with the different types of bone and joint infections by conventional culture and molecular methods (PCR and DNA sequences). The results of each of them as in tables (4 and 5).

No.	Sex	Age	Localization	Implant	Purulence	Sinus tract	Conventional Identification Microbial methods	Molecular Identification Swab PCR findings
1	M	30	Humerus	Plate and Screw	Yes	Yes	Escherichia coli Pseudomonas spp Staphylococcus aureus Micrococcus spp	Acinetobacter radioresistens Acinetobacter baumannii Acinetobacter junii Escherichia coli Pseudomonas aeruginos
2	F	35	Supracondylar of humerus	K – Wires	Yes	No	–	Escherichia coli Enterococcus faecium Acinetobacter baumannii Pseudomonas aeruginos
3	M	45	Foot	Screw	No	Yes	Escherichia coli Pseudomonas spp Staphylococcus aureus Enterobacter Morganella morganii	Bacillus spp Escherichia coli Enterococcus faecium Pseudomonas aeruginos Acinetobacter baumannii Enterobacter cloacae
4	F	20	Navicular bone	Screw	No	No	Citrobacter spp Escherichia coli Staphylococcus aureus	Bacillus cereus Enterococcus mundtii Acinetobacter gyllenbergii Acinetobacter nosocomialis Staphylococcus aureus

5	M	25	Lumbar Spine	Plate and Screw	No	No	**Escherichia coli Enterobacter Citrobacter spp.**	–
6	M	30	Tibia	Plate and Screw	Yes	No	**Pseudomonas spp Staphylococcus aureus**	**Pseudomonas aeruginos Staphylococcus aureus Staphylococcus haemolyticus Escherichia coli Salmonella enterica**
7	F	40	Patella	Tension Band Wiring	Yes	Yes	–	**Bacillus anthracis Escherichia coli Staphylococcus aureus Salmonella enterica**
8	M	65	Hip	Total Hip Prosthesis	No	No	**Escherichia coli Pseudomonas spp Staphylococcus aureus Citrobacter spp. Morganella morganii Clostridium spp**	–
9	M	54	Thoracic Spine	Plate and Screw	Yes	Yes	**Staphylococcus aureus Enterobacter sp. Citrobacter spp.**	**Escherichia coli Enterococcus faecium Pseudomonas aeruginos Acinetobacter baumannii**
10	M	45	Knee	Total Knee Prosthesis	Yes	No	**Pseudomonas spp Staphylococcus aureus Klebsiella spp Streptococcus pyogenes**	–
11	M	70	Femur	Plate and Screw	No	No	–	**Bacillus anthracis Bacillus amyloliquefaciens Escherichia coli**

No.	Sex	Age	Localization	Implant	Purulence	Sinus tract	Conventional Identification Microbial methods	Molecular Identification Swab PCR findings
								Enterobacter cloacae
12	F	45	Tibia	Plate and Screw	No	Yes	Escherichia coli Staphylococcus aureus Micrococcus spp Klebsiella spp Citrobacter spp Streptococcus pyogenes	–
13	F	65	Femur	Nail	No	No	Escherichia coli Staphylococcus aureus Klebsiella spp Streptococcus pyogenes	Bacillus anthracis Bacillus amyloliquefaciens Klebsiella pneumoniae
14	M	70	Medial malleolus	Screw	Yes	No	Staphylococcus aureus Klebsiella spp Escherichia coli Pseudomonas spp	–
15	F	42	Tibia	Plate and Screw	No	No	–	Escherichia coli Enterobacter cloacae Staphylococcus aureus Pseudomonas aeruginos
16	M	30	Distal Tibia	Plate and Screw	Yes	Yes	Escherichia coli Pseudomonas spp Staphylococcus aureus Clostridium spp Citrobacter spp Streptococcus pyogenes	Acinetobacter radioresistens Acinetobacter baumannii Staphylococcus haemolyticus Staphylococcus aureus

17	M	45	Hip	Total Hip Prosthesis	No	No	**Micrococcus spp Enterobacter spp Klebsiella spp ilhc aEhcEr hcsE**	–
18	M	38	Foot	Screw	Yes	Yes	–	**Escherichia coli Acinetobacter baumannii Enterobacter cloacae Bacillus cereus**
19	M	50	Supracondylar of humerus	K – Wires	No	No	**Escherichia coli Staphylococcus aureus Citrobacter spp Morganella morganii**	–
20	F	45	Radius and ulna	Plate and Screw	No	No	**Escherichia coli pl scncmrl lPP surPcaschchh l r a l Klebsiella spp**	**Klebsiella pneumoniae Pseudomonas aeruginos Pseudoxanthomonas mexicana**
21	M	42	Femur	Nail	Yes	Yes	–	**Bacillus anthracis Bacillus amyloliquefaciens Bacillus cereus Escherichia coli**
22	M	55	Humerus	Plate and Screw	No	Yes	**ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Enterobacter spp**	–
23	F	35	Femur	Nail	No	No	–	**Escherichia coli Streptococcus pyogenes Enterobacter cloacae**

24	F	30	Thumb	Foreign body	Yes	No	ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Enterobacter spp Klebsiella spp	Klebsiella pneumoniae Pseudomonas aeruginos Pseudoxanthomonas mexicana ilhc aEhcEr hcsE
25	M	65	Hip	Total Hip Prosthesis	No	No	ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Enterobacter spp	–
26	M	30	Tibia	Plate and Screw	Yes	Yes	–	Klebsiella pneumoniae Enterococcus hirae Enterococcus thailandicus Pseudoxanthomonas mexicana
27	M	45	Lumbar Spine	Post Discectomy	No	No	surPcaschchh l r a l Clostridium spp Morganella morganii	–
28	F	65	Knee	Total Knee Prosthesis	Yes	Yes	pl scncmrl lPP surPcaschchh l r a l Micrococcus spp Enterobacter spp Klebsiella spp Citrobacter spp Streptococcus pyogenes	pseudomonas silesiensis Pseudomonas brassicacearum Staphylococcus aureus
29	F	68	Hip	Total Hip Prosthesis	No	Yes	Micrococcus spp Enterobacter spp Bacillus spp ilhc aEhcEr hcsE	–
30	M	40	Lumbar Spine	Post Discectomy	No	No	–	Bacillus amyloliquefaciens Bacillus cereus

									Pseudomonas silesiensis
31	M	50	Tibia	Plate and Screw	No	No		ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Micrococcus	–
32	F	70	Hip	Total Hip Prosthesis	No	No		ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Enterobacter spp Bacillus spp	ilhc aEhcEr hcsE Enterococcus thailandicus Enterococcus termitis Enterococcus plantarum

No.	Sex	Age	Localization	Implant	Purulence	Sinus tract	Conventional Identification Microbial methods	Molecular Identification Swab PCR findings
33	M	36	Lumbar Spine	Plate and Screw	No	No	ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Micrococcus spp Enterobacter spp Klebsiella spp	Acinetobacter radioresistens Acinetobacter baumannii Enterococcus hirae Enterococcus thailandicus Enterococcus termitis Enterococcus plantarum Enterobacter cloacae
34	M	65	Navicular bone	Screw	Yes	No	surPcaschchh l r a l Micrococcus spp Enterobacter spp Klebsiella spp	–
35	F	70	Thoracic Spine	Plate and Screw	No	No	–	Acinetobacter radioresistens Enterococcus thailandicus Enterococcus termitis

									Enterobacter cloacae
36	M	30	Lumbar Spine	Plate and Screw	No	No		**surPcaschchh l r a l Micrococcus spp Enterobacter spp Proteus sppb Kosakonia cowanii**	**shigella sp Escherichia coli Klebsiella pneumoniae Enterobacter cloacae Shigella flexneri**
37	F	55	Knee	Total Knee Prosthesis	No	No		**Micrococcus spp Enterobacter spp Proteus spp**	–
38	M	50	Hip	Total Hip Prosthesis	No	No		–	**Bacillus cereus Enterococcus mundtii Acinetobacter gyllenbergii Acinetobacter nosocomialis Staphylococcus aureus**
39	M	35	Lumbar Spine	Post Discectomy	No	No		**pl scncmrl lPP surPcaschchh l r a l**	–
40	F	55	Knee	Total Knee Prosthesis	No	No		–	**Pseudomonas putida pseudomonas silesiensis Enterococcus raffinosus Enterococcus hirae Bacillus sp Pseudomonas brassicacearum**
41	F	65	Distal Tibia	Plate and Screw	No	No		**ilhc aEhcEr hcsE pl scncmrl lPP Klebsiella spp surPcaschchh l r a l**	–
42	F	60	Patella	Tension Band Wiring	No	No		**pl scncmrl lPP surPcaschchh l r a l Enterobacter spp**	–

43	F	25	Knee	Total Knee Prosthesis	No	No	–	**Acinetobacter radioresistens Escherichia coli Pseudomonas aeruginos**
44	M	45	Thumb	Foreign body	No	No	**ilhc aEhcEr hcsE pl scncmrl lPP Clostridium spp**	**Acinetobacter radioresistens Pseudomonas aeruginos Escherichia coli**
45	M	65	Femur	Nail	Yes	Yes	**ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh l r a l Clostridium spp**	–
46	F	55	Hip	Total Hip Prosthesis	No	No	**Escherichia coli surPcaschchh l r a l Clostridium spp Klebsiella spp**	**ilhc aEhcEr hcsE surPcaschchh l r a l Klebsiella pneumoniae Pseudomonas aeruginos**
47	M	70	Olecranon	Tension Band Wiring	Yes	No	–	**Pseudomonas aeruginos Staphylococcus aureus Staphylococcus haemolyticus Escherichia coli Salmonella enterica**
48	M	28	Lumbar Spine	Plate and Screw	No	No	**r a l surPcaschchh l Clostridium spp Klebsiella spp Streptococcus pyogenes**	**Escherichia coli Salmonella enterica Pseudomonas aeruginos**
49	F	60	Pelvis	Plate and Screw	No	Yes	**ilhc aEhcEr hcsE pl scncmrl lPP surPcaschchh**	–

							l r a l Enterobacter spp Klebsiella spp Streptococcus pyogenes	
							−	Pseudomonas aeruginos Staphylococcus aureus Staphylococcus haemolyticus
50	M	46	Femur	Nail	Yes	Yes		

Table (4): Comparison between Conventional Identification Microbial methods and Molecular Identification Swab PCR findings

Number of Sample	Escherichia coli	Pseudomonas spp	Staphylococcus aureus	Micrococcus spp	Enterobacter spp	Klebsiella spp	Clostridium spp	Citrobacter spp	Proteus spp	Morganella morganii	Streptococcus pyogenes	Bacillus spp
1	+	+	+	+	−	−	−	−	−	−	−	−
2	+	−	−	−	−	−	−	−	−	−	−	−
3	+	+	+	−	−	−	−	−	−	+	−	+
4	+	−	+	−	−	−	−	+	−	−	−	−
5	+	−	+	−	−	−	−	+	−	−	−	−
6	+	+	−	−	−	−	−	−	−	−	−	−
7	+	−	+	−	−	−	−	−	−	−	−	−
8	+	+	+	−	−	−	+	+	−	+	−	−
9	+	−	+	−	−	−	−	+	−	−	−	−
10	−	+	+	−	−	+	−	−	−	−	+	−
11	+	−	−	−	−	−	−	−	−	−	−	−
12	+	−	+	+	−	−	−	+	−	−	+	−
13	+	−	+	−	−	+	−	−	−	−	+	−
14	+	+	+	−	−	+	−	−	−	−	−	−
15	+	−	+	−	−	−	+	−	−	−	−	−
16	+	+	+	−	−	−	−	+	−	−	+	−
17	+	−	−	+	+	+	−	−	−	−	−	−
18	+	−	−	−	−	−	−	−	−	−	−	−
19	+	−	+	−	−	−	−	+	−	+	−	−
20	+	+	+	−	−	+	−	−	−	−	−	−
21	+	−	−	−	−	−	−	−	−	−	−	−

22	+	+	+	−	+	−	−	−	−	−	−	−
23	+	−	−	−	−	−	−	−	−	−	+	−
24	+	+	+	−	+	+	−	−	−	−	−	−
25	+	+	+	−	+	−	−	−	−	−	−	−
26	−	−	−	−	−	−	−	−	−	−	−	−
27	−	−	+	−	−	−	+	−	−	+	−	−
28	−	+	+	+	+	+	−	+	−	−	+	−
29	+	−	−	+	+	−	−	−	−	−	−	+
30	−	−	−	−	−	−	−	−	−	−	−	−
31	+	+	+	−	−	−	−	−	−	−	−	−
32	+	+	+	−	+	−	−	−	−	−	−	+
33	+	+	+	+	+	+	−	−	−	−	−	−
34	−	−	+	+	+	+	−	−	−	−	−	−
35	−	−	−	−	−	−	−	−	−	−	−	−
36	+	−	+	+	+	−	−	−	+	−	−	−
37	−	−	−	+	+	−	−	−	+	−	−	−
38	−	−	+	−	−	−	−	−	−	−	−	−
39	−	+	+	−	−	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	−	−	−	−
41	+	+	+	−	−	+	−	−	−	−	−	−
42	−	+	+	−	+	−	−	−	−	−	−	−
43	+	−	−	−	−	−	−	−	−	−	−	−
44	+	+	−	−	−	−	+	−	−	−	−	−
45	+	+	+	−	−	−	+	−	−	−	−	−
46	+	−	+	−	−	+	+	−	−	−	−	−
47	+	−	+	−	−	−	−	−	−	−	−	−
48	+	−	+	−	−	+	+	−	−	−	+	−
49	+	+	+	−	+	+	−	−	−	−	+	−
50	−	−	+	−	−	−	−	−	−	−	−	−

Table (5): Results of microbiological methods

	Sensitivity %	Specificity %	PPV %*	NPV %**	Accuracy %
PCR	88.24	93.75	96.77	78.95	90
Culture	94.12	81.25	91.43	86.67	90

Table (6): Diagnostic yield of molecular methods and cultures when physician's final decision is considered the gold standard

Where :-

*PPV   : Positive Predictive Value

**NPV : Negative Predictive Value

	Age (years)	Peripheral blood leukocytes (1 μL^{-1})	C – Reactive Protein (mg/L)
Mean	47.98	13.74	37.8
Std. Deviation	± 14.7	± 2.8	± 14

Table (7): The mean and standard deviation of the Age of the patients and their peripheral leukocytes count and C- RP level

Sex	No.	Percentage (%)
Male	30	60 %
Female	20	40 %
Total	50	100 %

Table (8) : Sex distribution of patients in this study

Age group	No.	Percentage (%)
20 – 39 Yrs.	15	30 %
40 – 60 Yrs.	22	44 %
> 60 Yrs.	13	26 %
Total	50	100 %

Table (9) : Age distribution of patients in this study

Etiology	Case	No.	Percentage (%)
Diabetes mellitus (HbA1c)	Yes	12	24 %
	No	38	76 %
Immunosuppression	Yes	12	24 %
	No	38	76 %
Obesity	Yes	8	16 %
	No	42	84 %

Table (10) : Etiology of patients in this study

Discussion

In this prospective study, we evaluated the benefit of molecular methods for the diagnosis of PJI established by adding a specific PCR enhanced the sensitivity and specificity compared with culture.

Molecular methods (PCR and DNA sequences) are more specific than the conventional culture but less sensitive, it means that the molecular method could be diagnosed the negative cases correctly with about 94% of the overall negative cases. While the culture is more sensitive than the molecular method, it means that the culture could be diagnosed

positive cases with about 92% of the overall positive cases, and these results correlate with (Omar and colleagues, 2016) and (Fihman, and his colleagues, 2007) researches.

Benefits of molecular diagnosis

1- The DNA test offers reliable intraoperative detection of all bacterial species within 25 minutes with a sensitivity and specificity comparable with those of conventional microbiological culture of synovial fluid for the detection of bone and joint infection.

2- The PCR followed by sequencing has been successfully developed to identify microorganisms involved in infections when patients have previously received antibiotics or in the presence of slow-growing or intracellular microorganisms. Cultures have limited sensitivity, especially in patients receiving antibiotics.

3- For osteoarticular infections, the studies have shown that the use of this molecular tool increased mainly the identification of Kingella kingae, anaerobic bacteria, and Streptococcus Species.

4- Conventional methods such as microbiological cultures may lack the sensitivity and specificity to establish definitive diagnosis of osteoarticular infections.

5- Herein, we review the general principles and the usefulness of PCR to improve the etiological diagnosis of osteoarticular infection. He reviews the principles and the role of molecular diagnosis in improving the aetiological diagnosis of implant-associated bone and joint infection.

6- Molecular diagnosis has been an important step in the diagnosis of infectious diseases, In implant-associated bone and joint infection, molecular assays have been shown to be useful in complementing culture techniques to identify microorganisms when patients have previously received antibiotics or in the presence of fastidious microorganisms, This molecular tool has allowed not only increasing identification of anaerobic bacteria, such as Finegoldia magna, but also the discovery of the role of Tropheryma whipplei, an aetiological agent of implant-associated bone and joint infection in patients without Whipple's disease.

7- The instrument was simple to use and provided nucleic acids free of PCR inhibitors and free of contamination by foreign bacterial DNA.

8- A prolonged incubation time (10 to 14 days) of periprosthetic tissue samples and sonication fluid is mandatory to optimize the detection of this pathogen by culture.

9- Interestingly, we were able to show that the microbial DNA density (represented as H value) decreases with antimicrobial treatment but remains positive for up to 43 days of treatment. This provides the opportunity to detect the pathogen despite previous antibiotic treatment, a common clinical situation.

10- With additional molecular tests, specific resistance genes, such as the genes conferring resistance to methicillin, quinolones, and rifampin, can be detected in addition, this information is crucial for efficient and targeted antimicrobial therapy in negative cultures.

11- Interestingly, no correlation between the bacterial density in sonication fluid and the DNA quantity was observed. This observation could be the result if some microorganisms were killed by sonication (despite reduced acoustic energy used for this purpose) but the DNA was not affected.

12- We suggest that the specific primer set be modified to include the most common organisms causing periprosthetic joint infection, including low-virulence pathogens, such as P. acnes, Corynebacterium species, Finegoldia magna, and Peptostreptococcus species.

13- The potential of multiplex PCR in the diagnosis of periprosthetic joint infection is especially high in patients who had previously been exposed to antibiotics and have a high probability of false-negative cultures.

14- Patients with orthopedic infections were prospectively included. Phenotypical and genotypical resistance was evaluated in clinical samples (synovial and sonication fluid) where identical pathogens were identified by culture and mPCR.

15- Advantages of this technique include faster availability of results, positive results in the presence of only a few copies of bacterial DNA, and the ability to identify nonviable bacteria, for instance, in those patients already on antibiotic treatment.

Limitations of molecular method

However, it is very important to underline that the interpretation of this molecular tool is critical because of several pitfalls, including:

1- Contamination causing false-positive results, Currently, molecular diagnosis mainly includes conventional PCR .

2- Various bacteria-related factors such as their paucity in joint fluid, highly fastidious growth.

3- The presence of a biofilm.

4- The impact of previous antibiotic therapy have been proposed as reasons for these poor results. Therefore, newer techniques have been sought that improve the yield and accuracy of bacterial identification.

The limitations of these studies are the use of a specific PCR, which is typically able to detect only a single microorganism, or the use of a broad-range (16S ribosomal DNA [rDNA]) PCR, which can detect previously unknown organisms but has lower sensitivity and specificity than specific PCR, requires subsequent sequencing for bacterial identification, and fails to detect mixed infections. These tools are efficient, but several pitfalls exist that necessitate rigor in all steps of the process.

Conclusions

Our prospective study shows that molecular methods(PCR and D.N.A sequences)are clearly beneficial in case of high suspicion of bone and joint infection, they are more specific and less sensitive than the conventional culture in diagnosis of bone and joint infection. culture is more sensitive Compared with PCR methods and has the advantage of detecting polymicrobial or fungal infections. In our opinion PCR could be used in addition to culture method as a screening test to rule out bacterial infection in a much shorter time than culture alone.

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