JNI 19es Journées Nationales d'Infectiologie
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Cité des Congrès de Nantes
Nantes
et la région Pays de la Loire

Diagnostic moléculaire des spondylodiscites

Matthieu MILLION

MÉDITERRANÉE INFECTION

Déclaration d'intérêts de 2014 à 2017

- **Intérêts financiers : non applicable**
- **Liens durables ou permanents : non applicable**
- **Interventions ponctuelles : aucunes interventions financées par l'industrie – FMC financé par des organismes ayant l'agrément (TAMARI06) et des associations de médecins généralistes**
- **Intérêts indirects :**
  - **Diagnostic moléculaire KIT OS proposé par l'IHU Méditerranée Infection – développement en cours de techniques moléculaires d'avenir (NGS)**
  - **Responsable des avis diagnostiques et thérapeutiques au CNR Fièvre Q**

Déclaration de liens d'intérêt avec les industries de santé en rapport avec le thème de la présentation (loi du 04/03/2002) :

Intervenant : Million/Matthieu

Titre :Diagnostic moléculaire des spondylodiscites

- L'orateur ne souhaite pas répondre ☑

Consultant ou membre d'un conseil scientifique	☐ OUI	☑ NON
Conférencier ou auteur/rédacteur rémunéré d'articles ou documents	☐ OUI	☑ NON
Prise en charge de frais de voyage, d'hébergement ou d'inscription à des congrès ou autres manifestations	☐ OUI	☑ NON
Investigateur principal d'une recherche ou d'une étude clinique	☐ OUI	☑ NON

Méthodes

Bibliographie

- Pubmed & Google scholar: (spondylodiscitis OR vertebral infection) and (PCR OR Molecular diagnosis)
- Exclusion des études sur les discopathies dégénératives

PCR vs culture

Faut il choisir son camp ?

ARTHRITIS & RHEUMATISM
Vol. 50, No. 9, September 2004, pp 2985–2994
DOI 10.1002/art.20462

The Etiologic Diagnosis of Infectious Discitis Is Improved by Amplification-Based DNA Analysis

Frédéric Lecouvet,[1] Leonid Irenge,[2] Bernard Vandercam,[1] Adrien Nzeusseu,[1] Sandrine Hamels,[3] and Jean-Luc Gala[4]

Lecouvet, Arthritis & Rheumatism, 2004

Table 2. Comparative results of microbiologic and amplification-based DNA analyses*

Patient/ age/sex	History of vertebral surgery	Culture results (methicillin resistance status)	Molecular targets			DNA-based identification (methicillin resistance status)
			16S rDNA, G+/G−	*femA*	*mecA*	
Patients						
1/69/M	Yes	NI	0	1	0	*S simulans* (S)†
2/34/M	Yes	CoNS (S)	Weakly G+	1	0	*S lugdunensis* (S)
3/NA/M	Yes	CoNS (S)	G+	1	0	*S capitis* (S)
4/28/M	Yes	CoNS (S)	G+	1	0	*S epidermidis* (S)
5/46/F	No	*Streptococcus viridans*	G+	0	ND	*Streptococcus viridans (oralis)*
6/74/M	No	NI	G+	0	ND	*Actinomyces israelii*†
7/20/M	No	*S aureus* (S)	G+	1	0	*S aureus* (S)‡
8/74/M	Yes	*Corynebacterium jeikeium* + CoNS (S)	G+	1	0	*S hominis* (S)
9/58/M	Yes	NI	0	1	ND	*S sciuri* (S)†
10/60/M	Yes	CoNS (R)	G+	1	1	*S epidermidis* (R)‡
11/64/F	Yes	CoNS (R)	Weakly G+	1	1	*S capitis* (R)
12/59/M	No	*Ps aeruginosa*	G−	0	ND	*Ps aeruginosa*
13/47/F	Yes	NI	G−	0	ND	*Brucella* species†
14/75/F	No	*Streptococcus mitis*	G+	0	ND	*Streptococcus mitis*
15/46/M	No	*Ps aeruginosa*	G−	0	ND	*Ps aeruginosa*
16/59/F	No	*S aureus* (R)	G+	1	1	*S aureus* (R)‡
17/37/F	Yes	CoNS (S)	G+	1	0	*S epidermidis* (S)
18/72/M	Yes	CoNS (S)	G+	1	0	*S hominis* (S)
19/79/M	No	NI	0	0	0	*M tuberculosis* complex†§
Controls						
1	No	–	0	0	ND	–
2	No	–	0	0	ND	–
3	No	–	0	0	ND	–
4	No	–	0	0	ND	–
5	No	–	0	0	ND	–

19/19 (100%) versus 14/19 (73%) = +27%

Lecouvet, Arthritis & Rheumatism, 2004

Diagn Microbiol Infect Dis. 2014 Jan;78(1):75-8. doi: 10.1016/j.diagmicrobio.2013.10.007. Epub 2013 Oct 14.

Usefulness of a direct 16S rRNA gene PCR assay of percutaneous biopsies or aspirates for etiological diagnosis of vertebral osteomyelitis.

Choi SH[1], Sung H[2], Kim SH[1], Lee SO[1], Lee SH[3], Kim YS[1], Woo JH[1], Kim MN[4].

Author information

Abstract

We performed a prospective study to evaluate the clinical usefulness of a direct 16S rRNA gene (16S rDNA) PCR assay of percutaneous biopsies or aspirates for the etiological diagnosis of vertebral osteomyelitis. During May 2009 to December 2010 and November 2011 to August 2012, consecutive patients with suspected vertebral osteomyelitis who underwent a percutaneous biopsy or aspiration were enrolled. Of 45 patients with vertebral osteomyelitis, 16S rDNA PCR was positive in 24 (53.3%), whereas culture was positive in 13 (28.9%) (P = 0.027). Three of PCR-positive cases (12.5%, 3/24) and 1 of culture-positive case (7.7%, 1/13) were considered to be false-positives. Of 16 patients without prior antimicrobial exposure, 75% of cases (12/16) were positive by either culture (7/16, 43.8%) or PCR (9/16, 56.3%). A 16S rDNA PCR assay with sequencing was more sensitive than routine culture for the etiological diagnosis of vertebral osteomyelitis.

24/45 (53%) versus 13/45 (29%) = + 24% , $p < .05$

Choi, Diagn Microbiol Infect Dis, 2014

Table 2
Results of 16S rRNA gene PCR with sequencing and comparison with the results of conventional culture.

Bacterial DNA by 16S rRNA gene PCR	No. of cases	Culture	
		Positive	Negative
S. aureus	9	4	5
S. epidermidis	3[a]	0	3
E. coli	2	1	1
S. agalactiae	2	1	1
Salmonella enterica ss. *enterica*	1	1	0
M. tuberculosis	1	1	0
Klebsiella pneumoniae	1	0	1
S. dysgalactiae	1	0	1
H. parainfluenzae	1	0	1
Clostridium perfringens	1	0	1
S. capitis	1[b]	1[c]	0
A. xylosoxidans	1[b]	0	1
Negative	21	5[d]	16

[a] Includes 1 PCR false-positive cases.
[b] Finally assessed as false-positives.
[c] Finally assessed as culture false-positive case (*P. acnes*).
[d] Includes 2 *M. tuberculosis*, 1 *S. aureus*, 1 *S. epidermidis*, and 1 *E. faecium*.

Complémentarité (5 culture + PCR -)
5 *S. aureus* négatifs en culture
Même quand résultat identique, délai plus court de la PCR pour certains pathogènes comme *M. tuberculosis* (et *Brucella*)

Choi, Diagn Microbiol Infect Dis, 2014


frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH

Pathogen Identification in Suspected Cases of Pyogenic Spondylodiscitis

Ahmad Farajzadeh Sheikh, Azar D. Khosravi, Hamed Goodarzi, Rouhangiz Nashibi, Alireaza Teimouri, Azim Motamedfar, Reza Ranjbar, Sara Afzalzadeh, Mehrandokht Cyrus and Mohammad Hashemzadeh

57 cas de spondylodiscite à culture négative (abcès épidural, vertèbres, biopsie discale)
21 (37%) positifs par 16S PCR
63% restent inexpliqués

Micro-organisme détecté	N	% des positifs	% des spondylodiscites à culture négative
M. tuberculosis	9	43	16
S. aureus	6	28	11
M. abscessus	5	24	9
M. chelonae	1	5	2

Sheikh, Front Cell Infect Microbiol, 2017


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Sheikh, Front Cell Infect Microbiol, 2017

TABLE 1 | Clinical feature of 21 pyogenic spondylodiscitis patients confirmed by *16S rRNA* PCR.

NO	past medical history	Tuberculosis close contact	Previous Surgery	Direct microbiologic exam of stained smears	Clinical data	Site of Involvement	WBC[a]	ESR[b] Mm/h	CRP[c] Mg/dl	Organism
5	PID[d]	–		Gram positive cocci	Fever and limitation of motion, Sacroiliac abscess	Sacroiliac	14,700	135	10	*S. aureus*
7	Renal failure	+		–	Hemiparesis and weight loss, epidural and paravertebral abscess	T4–T5	4,400	108	44	*M. tuberculosis*
8	Addicts	+		Acid fast bacilli	Low back pain	L3	7,600	102	–	*M. abscessus*
11	Diabetes, Renal failure	–		Acid fast bacilli	Low back pain	L4	12,800	87	11	*M. abscessus*
14	Pulmonary Tuberculosis	–		–	Low back pain	L3–L4	6,700	110	9	*M. tuberculosis*
15	Hypertension	+		–	Local tenderness	T3	8,900	45	–	*M. tuberculosis*
17	Diabetes melitus, metastatic lung cancer	–		–	Low back pain	L3	7,800	70	47	*M. abscessus*
21	–	–		Gram positive cocci	Fever and Low back pain	S1	10,400	78	51	*S. aureus*
23	Hypertension	–		–	Fever and Low back pain, AFB positive, drug hepatitis	L5–T12	11,600	99	39	*M. tuberculosis*
27	–	+		Gram positive cocci	Low back pain, Paravertebral abscess	L3–L4	7,900	98	15	*S. aureus*
28	–	–		Gram positive cocci	Low back pain, weight loss, fever	L3–L4	19,300	37	14	*S. aureus*
31	Extrapulmonary Tuberculosis, Rhinoplasty	–		–	Local tenderness	L3	8,500	38	–	*M. tuberculosis*
35	Diabetes melitus,IHD[e], Pulmonary Tuberculosis	–		–	Low back pain	L3–L4	9,800	58	–	*M. tuberculosis*
36	Pulmonary Tuberculosis	–		Acid fast bacilli	Low back pain	L4–L5	8,500	78	11	*M. tuberculosis*
37	IHD	–		Gram positive cocci	Local tenderness	L2–L3	6,500	68	12	*S. aureus*
41	Diabetes	+		Acid fast bacilli	Low back pain	L4–L5	5,200	79	10	*M. abscessus*
44	–	+		–	Fever and cough and dyspnea, Paravertebral abscess, Lower thoracic abscess	T4	7,900	54	39	*M. tuberculosis*
45	Hypertension, Appendicitis, AKI[f]	–	+	–	Fever and hemiparesis and low back pain	L4	9,300	46	42	*S. aureus*
48		–	–	–	Fever and low back pain, Paraspinal abscess	L3–L4	10,400	77	25	*M. abscessus*
51	–	–	–	–	Fever and low back pain	L1–L2	11,900	98	28	*M.chelonea*
57	–	–	–	–	Low back pain, spasticity and limitation of motion	T10–T11	9,700	54	11	*M. tuberculosis*

Examen direct positif dans seulement 9/21 cas (43%) **Sheikh, Front Cell Infect Microbiol, 2017**

Scand J Infect Dis. 2008;40(10):772-7. doi: 10.1080/00365540802119994.

Broad-range PCR as a supplement to culture for detection of bacterial pathogens in patients with a clinically diagnosed spinal infection.

Fuursted K[1], Arpi M, Lindblad BE, Pedersen LN.

Author information

Abstract

We aimed to evaluate broad-range PCR and subsequent sequencing compared to conventional culture in the diagnosis of spinal infection. The method was a prospective study of all patients admitted to Aarhus University Hospital for surgery during a 12-months period with a clinically diagnosed infection of the spine. Samples from patients undergoing surgery for non-infectious causes (malignancy etc.) were included as control group. Specimens were submitted to conventional culture and molecular investigation with 16S rRNA gene amplification and sequence analysis. 38 patients were included in the study (clinically diagnosed spinal infections=18; non-infectious diseases=20). The specificity was excellent for both culture and PCR (95% and 100%, respectively). A true culture positive result was obtained in 50% of patients (9/18) and 61% was positive (11/18) by broad-range PCR. When combined, culture and PCR allowed for a microbiological diagnosis in 72% of patients (13/18). A positive culture was found only in patients treated < or =7 d compared to < or =16 d for PCR. However, PCR and culture result were equally negatively affected by duration of treatment. The combination of culture and broad-range PCR substantially adds to the number of microbiological diagnoses obtained, and improves the clinician's opportunity to tailor therapy to individual patients.

PCR 16S

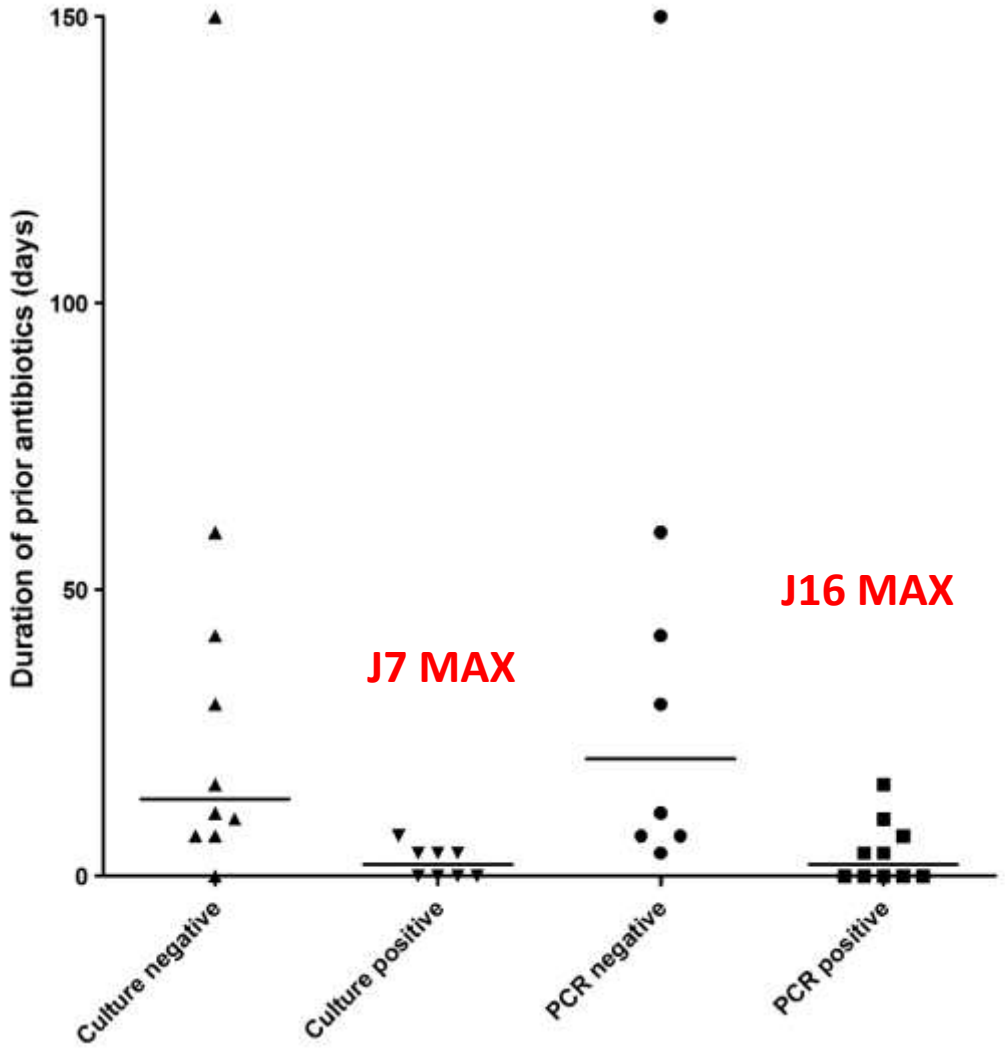
- **Spécificité 100% (cas versus contrôles qui semblent corrects)**
- **11/18 (61%) positif chez les cas contre 9/18 (50%) pour la culture => +11%**

Fuursted, Scand J Infect Dis, 2008

Table I. Clinical and microbiological findings in patients with suspected spinal infections.

Patient No.	Gender/age	Infection diagnosis (level)	Focus of infection	Culture result	PCR result	Blood culture result	Histology (inflammation)
3	M/62	Ostitis (cervical)	Local (External fixation)	S. epidermidis	Mixed infection One was S. epidermidis	Staphylococcus aureus (6 months earlier)	0
5	K/40	Epidural abscess (L4-S1)	Inguinal abscess	Staphylococcus aureus	Negative	Staphylococcus aureus (1.5 y earlier)	Not done
8	M/55	Epidural abscess (T12-L1)	Local (Epidural catheter)	Staphylococcus aureus	Staphylococcus aureus	None	Not done
10	M/67	Spondylodiscitis (T6-7)	Oral (tooth)	Staphylococcus aureus	Streptococcus mutans	Negative	+
11	M/56	Spondylodiscitis (T11-12)	Gastrointestinal	Salmonella. dublin	Salmonella sp.	Salmonella. dublin	+
13	M/53	Spondylodiscitis (C3-4)	Oral (tooth)	Staphylococcus aureus	Staphylococcus aureus	None	+
16	K/53	Discitis (L4-5)	Unknown	Staphylococcus aureus +Pseudomonas aeruginosa	Positive (Mixed infection)	Negative	Not done
20	K/2	Spondylodiscitis (L2-3)	Unknown	Negative	Kingella kingae	Negative	0
23	M/79	Spondylitis (L3-5)	Unknown	Negative	Clostridium histolyticum	None	+
26	K/69	Spondylitis (L1-3)	Endocarditis	Negative	Streptococcus viridans	Streptococcus viridans +Enterococcus. sp	Not done
29	M/64	Spondylitis (L2-4)	Unknown	Negative	Negative	negative	Not done
31	K/72	Epidural abscess (T8)	IV catheter	Negative	Negative	Staphylococcus aureus (2 months earlier)	Not done
32	M/50	Spondylodiscitis (T10-L5)	Local (Epidural catheter)	Staphylococcus aureus	Negative	Staphylococcus aureus	+
35	M/60	Spondylodiscitis (L5-S1)	Unknown	Negative	Negative	Staphylococcus aureus	+
39	M/65	Spondylodiscitis (L4-5)	Unknown	Propionibacterium acnes (Contamination)	Negative	Negative	Not done
40	M/77	Spondylodiscitis (T9-10)	Urinary tract	Negative	Negative	E. coli +Enterococcus faecalis (2 months earlier)	Not done
41	M/72	Spondylodiscitis (L4-5)	Unknown	Staphylococcus aureus	[illegible]	Staphylococcus aureus	Not done
42	M/79	Spondylitis +psoas abscess (T11-L3)	Urinary tract	Negative	Klebsiella pneumoniae	K. pneumoniae (3 months earlier)	Not done

Fuursted, Scand J Infect Dis, 2008



La durée des antibiotiques joue aussi sur la PCR mais moins que sur la culture

Fuursted, Scand J Infect Dis, 2008

Improved diagnosis specificity in bone and joint infections using molecular techniques☆

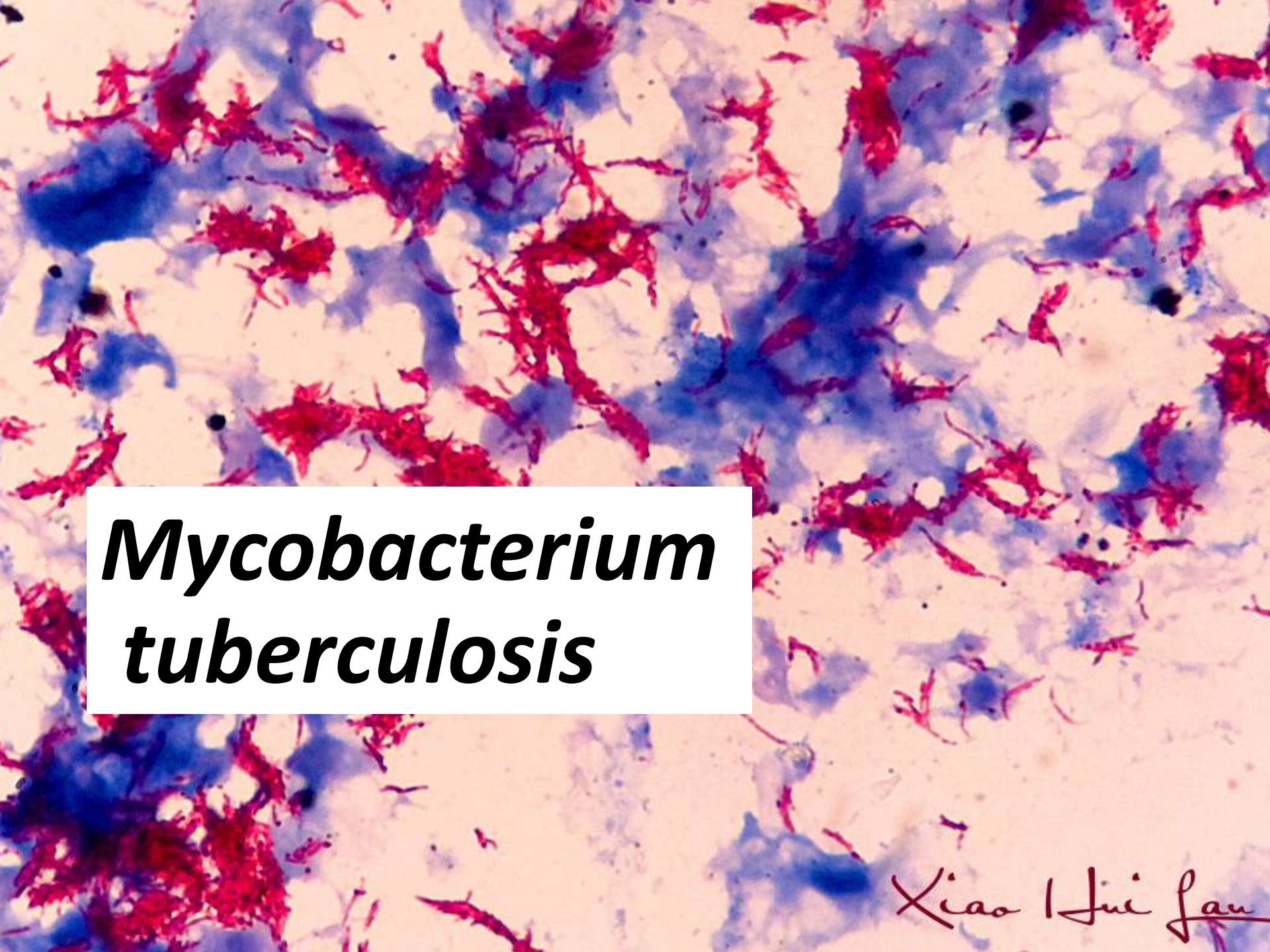
V. Fihman [a,b], D. Hannouche [c], V. Bousson [a,d], T. Bardin [a,e], F. Lioté [a,e], L. Raskine [b], J. Riahi [b], M.J. Sanson-Le Pors [a,b], B. Berçot [a,b,*]

J Infect, 2007

Table 2 Clinical and laboratory results of patients with suspected spondylodiscitis (S), acute septic arthritis (ASA), or control group (C)

Patient	Sex[a]	Age (years)	Condition suspected	No. of samples	Laboratory results[b]					
					Positive cultures		Conventional identification	Positive PCR		Molecular identification
					Solid samples	Synovial fluid		Solid samples	Synovial fluid	
Diagnoses retained by physicians										
21	M	73	S	2	2	–	*S. epidermidis*	0	–	–
22	M	79	S	1	1	–	*E. faecalis*	1	–	*E. faecalis*
23	M	76	S	1	1	–	*S. agalactiae*	1	–	*S. agalactiae*
24	F	46	S	1	1	–	*S. agalactiae*	1	–	*S. agalactiae*
25	M	63	S	1	1	–	*E. coli*	1	–	*E. coli*
26	F	26	S	2	2	–	*S. enterica*	1	–	*S. enterica*
27	M	51	S	2	2	–	*F. nucleatum*	2	–	*F. nucleatum*

Importance de la qualité de la culture sur les prélèvements osseux :
Inoculer au bloc en hémoc aérobie et anaérobie
Ici, PCR inférieure à la culture (6/7)


Mycobacterium
tuberculosis
Xiao Hui Lau

Acta Neurochir (Wien). 2017 Mar;159(3):503-507. doi: 10.1007/s00701-016-3065-0. Epub 2017 Jan 21.

Multiplex PCR as a novel method in the diagnosis of spinal tuberculosis-a pilot study.

Sharma K[1], Meena RK[2], Aggarwal A[3], Chhabra R[3].

Author information

Abstract

BACKGROUND: Establishment of a reliable and rapid diagnosis is of paramount importance in spinal tuberculosis. The available gadgetry of investigations, such as AFB smear, culture of Mycobacterium tuberculosis, and Uniplex PCR, suffers from a lack of adequate sensitivity and/or a lack of rapidity. Therefore, many times a diagnosis is made either very late in the disease process or sometimes empirical therapy has to be started because a definite diagnosis could not be made. All of these are not ideal situations for a clinician. The present study was done with the aim to establish a rapid and reliable diagnosis of M. tuberculosis infection. This was established by identifying M. tuberculosis genes.

METHODS: The study was done on nine consecutive patients who presented with non-traumatic spontaneous vertebral compression collapse. CT-guided aspirate from the involved vertebra was subjected to Multiplex PCR (MPCR) using three primers: IS6110, protein b, and MPB 64. The aspirate was also subjected to smear and culture. The results of MPCR were compared with the final diagnosis.

RESULTS: Seven out of nine patients had a final diagnosis of tuberculosis. MPCR was positive in six of these seven patients, thus showing sensitivity of 85.7% and specificity of 100%. Results of MPCR were obtained within 24 h.

CONCLUSIONS: MPCR using IS6110, protein b, and MPB64 primers has a high sensitivity and specificity in rapid diagnosis of spinal tuberculosis. To the best of our knowledge, this has not been attempted before in spinal tuberculosis. This is particularly useful for paucibacillary infections like spinal tuberculosis. However, further studies using large sample sizes are needed to confirm the practical applicability of this technique.

Sensibilité 86% en 24 heures

Bone Joint J. 2014 Oct;96-B(10):1366-9. doi: 10.1302/0301-620X.96B10.34048.

GeneXpert polymerase chain reaction for spinal tuberculosis: an accurate and rapid diagnostic test.

Held M[1], Laubscher M[1], Zar HJ[2], Dunn RN[1].

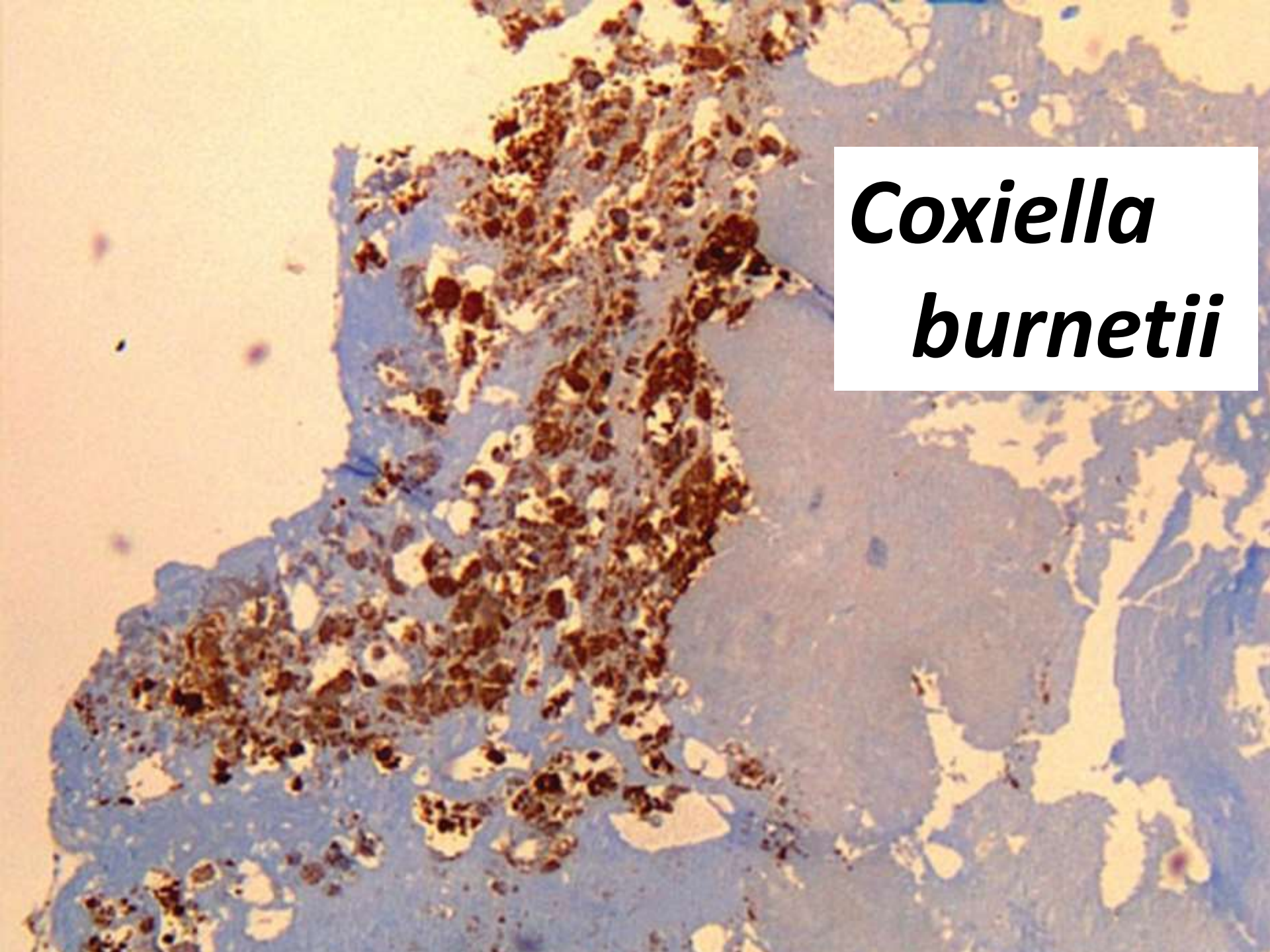
Author information

Abstract

The lack of an accurate, rapid diagnostic test for mycobacterium tuberculosis (TB) is a major handicap in the management of spinal TB. GeneXpert, a new, rapid molecular diagnostic test is recommended as the first line investigation for suspected pulmonary TB in areas with a high prevalence of HIV or drug resistance, yet it has not been validated for the diagnosis of musculoskeletal TB. The aim of this study was to assess the accuracy of GeneXpert in diagnosing spinal TB. A prospective clinical study of 69 consecutive adults with suspected spinal TB was conducted at a tertiary hospital in an area with the highest incidence and prevalence of TB in the world. GeneXpert was used on tissue samples of the enrolled patients and its diagnostic accuracy compared with a reference standard of tissue in liquid culture. A total of 71 spine samples from 69 patients (two re-biopsies) were included in the study. The GeneXpert test showed a sensitivity of 95.6% and specificity of 96.2% for spinal TB. The results of the GeneXpert test were available within 48 hours compared with a median of 35 days (IQR 15 to 43) for cultures. All cases of multi-drug resistant TB (MDR TB) were diagnosed accurately with the GeneXpert test. The MDR TB rate was 5.8%.

GeneXpert

Sensibilité 96% en < 48 heures


Coxiella burnetii

Spondylodiscite à *Coxiella burnetii*

Sur cohorte du CNR n = 2,168 (juin 2016)

40 infections ostéo-articulaires

13 spondylodiscites (32%)

PCR positive dans 100% des cas (12/12) 16S ou IS1111

Associés à une infection vasculaire dans 7/10 cas avec info disponible => 70% des spondylodiscites à *C. burnetii* sont des infections de contiguité

Spondylodiscite à *C. burnetii* sans infection vasculaire cas 1

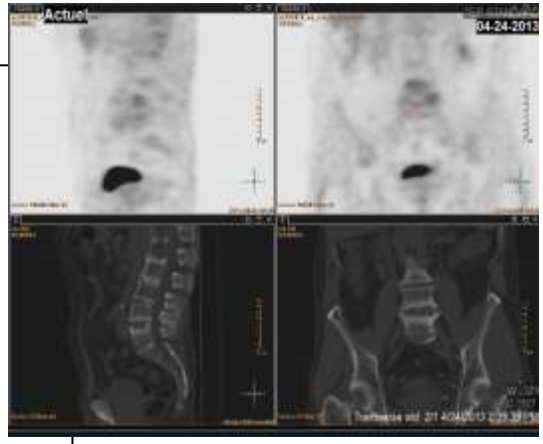
Homme de 80 ans

FIEVRE Q

Diagnostic de fièvre Q déjà connu ❑ non connu ❑
ETT déjà faite lors du premier appel du CNR: oui ❑ non x

X Début de la maladie: octobre 2012
X Fièvre
x Autre: **Spondylarthrite ankylosante** depuis 1986, TVP en 2007

X Forme ganglionnaire ADP cervicales droites et gauches isolées
X TCA allongé 1.15
X IRM du rachis dorso-lombaire: **spondylodiscite L4-L5** d'allure non infectieuse (pas de fuseau para vertébral)
Juillet 2013 PET-TDM: pas d'hyperfixation
Juillet 2013 ETT: pas de valvulopathie



- ❑ Fièvre Q aiguë
- ❑ Endocardite aiguë
- ❑ Suivi de fièvre Q aigue
- ❑ Endocardite possible
- ❑ Endocardite certaine
- ❑ Infection vasculaire possible
- ❑ Infection vasculaire certaine
- ❑ Pseudotumeur pulmonaire
- X Infection ostéo-articulaire Spondylodiscite L4-L5
- ❑ Autres:

TRAITEMENT
Conseil thérapeutique
X oui
Antibiotique utilisé :
D+P
Date début :
Date fin :

Date	Smarlab	IgG	IgM	IgA	IgG	IgM	IgA
14/06/2013	1306846	800	0	0	1600	0	0
27/08/2013	1309395	800	0	0	1600	0	0
26/09/2013	1310569	800	0	0	1600	0	0
	reprise	800	0	0	800	0	0
09/07/2014	4072318075	100	0	0	100	0	0
04/09/2014	4092471470	200	0	0	100	0	0
08/10/2014	4102545341	200	0	0	100	0	0
18/02/2015	5022992826	100	0	0	100	0	0

Biologie moléculaire					
Date	Prelevemen	Smarlab	*IS1111*	*IS30*	*T. extract.*
26/06/2013	serum	1372472	neg		27
18/07/2013	biopsie vert	1374242	30/33	35/neg	27

Courriers	
01/07/2013	biops vert
30/07/2013	D+P

Spondylodiscite à *C. burnetii* sans infection vasculaire cas 2

FIEVRE Q

Femme de 49 ans

Diagnostic de fièvre Q déjà connu ❑ non connu ❑
ETT déjà faite lors du premier appel du CNR: oui x : pas de valvulopathie visible

KIT endocardite réalisé devant une **suspicion de spondylodiscite**

« qui fut ensuite infirmée à l'IRM »

Discopathie dégénérative très inflammatoire

❑ Fièvre Q aiguë ❑ Suivi ❑ Asthénie post-aiguë
❑ Début de la maladie
❑ Fièvre
❑ Fièvre Q aiguë sur terrain à risque ➔ ❑ Valvulopathie
❑ Thrombophlébite ❑ Vasculaire
❑ Péricardite ❑ Myocardite ❑ Immunodépression
x Autre: sous AINS
❑ Forme neurologique ➔ ❑ Méningite
❑ Pneumonie ❑ Méningo-encéphalite
❑ Hépatite aiguë
❑ Forme ganglionnaire
❑ TCA allongé

❑ Fièvre Q aiguë
❑ Endocardite aiguë
❑ Suivi de fièvre Q aigue
❑ Endocardite possible
❑ Endocardite certaine
❑ Infection vasculaire possible
❑ Infection vasculaire certaine
❑ Pseudotumeur pulmonaire
X Infection ostéo-articulaire
❑ Autres:

Sérologie

		Phase I			Phase II		
Date	Smarlab	IgG	IgM	IgA	IgG	IgM	IgA
04/10/2013	1311317	3200	0	0	6400	0	0
24/09/2014	4092535096	6400	0	50	400	0	0
14/10/2015	5100743381	3200	0	0	200	0	0
10/12/2015	5120923100	1600	0	0	1600	0	0
05/01/2016	6010991113	800	0	0	100	0	0
05/02/2016	6021106628	800	0	0	100	0	0
07/03/216	6031201447	800	0	0	100	0	0

CULTURE - PCR
X Culture cellulaire: ec
❑ oui ❑ non
CMI =
❑ Biologie moléculaire
❑oui ❑ non
Second sérum demandé
X IgG anticardioL : 0.16

TRAITEMENT
Conseil thérapeutique
X oui
Antibiotique utilisé :
DP pendant 1an
Date début : 02/10/2014
Date fin :

Biologie moléculaire

Date	Prelevement	Smarlab	*IS1111*	*IS30*	*T. extract.*
04/10/2013	serum	1385859	neg		30
19/11/2015	os	5110838286	35	neg	24
24/11/2015	tissus discal	5110838290	33/35	neg	24
25/11/2015	liq articulaire	5110838299	33	neg	24
07/12/2015	abces TM	5120875415	24/27	33/35	19

Spondylodiscite à *C. burnetii* sans infection vasculaire cas 3

Femme de 48 ans

FIEVRE Q

❑Début de la maladie : février 2012 (abcès rachis lombaire)
❑ Fièvre Q aiguë sur terrain à risque ➔ ❑ Valvulopathie
❑ Thrombophlébite ❑ Vasculaire
❑ Péricardite ❑ Immunodépression : éthylisme chronique
❑ Forme neurologique ➔ ❑ Méningite
X Hépatite
❑ TCA allongé

x Autre tableau clinique : chirurgie rachis en février 2012 pour lombalgies : découverte d'un abcès vertébral : échec de la 1ère mise en culture. Reprise chir en avril 2012 : **PCR positive pour *Coxiella burnetii*** + sérologie positive chez eux.

x : ETT : pas de valvulopathie
X Scanner TAP : pas d'autre localisation (une seule rachis)

❑ Forme inflammatoire
❑ Auto AC
❑ Résistance traitement
❑ Traitement corticoïdes

❑ Suivi
❑ Endocardite possible
❑ Endocardite certaine
❑ Infection vasculaire
❑ Fibrose respiratoire
❑ Pseudotumeur pulmonaire
❑ Cirrhose
x Spondylodiscite
❑ Autres:

CULTURE - PCR
❑ Culture cellulaire
❑ oui ❑ non
CMI =

x Ac anticardioL IgG
01/06/2012 : NEG

TRAITEMENT
Conseil thérapeutique
Antibiotique utilisé :
Doxy + Plaquenil
Date début : avril 2012
Date fin :

Visite fréquente de ferme avec moutons (8 cas suspects autour de cette ferme)

		Phase I			Phase II			PCR	PCR	PCR
Date	Smarlab	IgG	IgM	IgA	IgG	IgM	IgA	serum	ph	selles
22/05/2012	1205149	1600	0	25	3200	0	50	neg		

Spondylodiscite à *C. burnetii* sans infection vasculaire cas 4

Femme de 63 ans

FIEVRE Q

☒ **Fièvre depuis 2mois**

❑ Fièvre Q aiguë sur terrain à risque ➔ **X Vasculaire: angioplastie des artères iliaques**

❑Péricardite ☒ **Immunodépression**

❑ ☒ **PR sous antiTNF+méthotréxate depuis 2000**

☒ **Autre tableau clinique : PR / spondylodiscite à CB en 2005**

☒**ETT : normale**

☒**Reprise anti TNF après 1 an de doxy+plaquenil**

☒**AEG depuis fin 2009+fièvre**

Pas de seuil sérologique parfait !

CULTURE - PCR
❑ Culture cellulaire
❑ oui ❑ non

☒ Biologie moléculaire
☒ oui ❑ non

☒ Pvts à demander sérum

TRAITEMENT
X Conseil thérapeutique

Antibiotique utilisé : **Doxy + plaquenil**
Date début :
Date fin **:FIN 2006**
CMI Doxy =

Date	Smarlab	IgG	IgM	IgA	IgG	IgM	IgA	sang	ph	selles
24/11/1994		neg								
21/10/2005	2501552	400	0	25	800	0	50			
22/09/2005	2510442	400	0	25	800	0	50			
17/01/2006	26000782	400	0	25	800	0	50			
29/06/2010	1006978	400	0	200	800	0	400	neg		
08/07/2010	1007576	200	0	100	400	0	200	neg		

Prélèvement disque intervertébral pos en culture CB le 20/09/2005

Eur J Clin Microbiol Infect Dis (2007) 26:341–347
DOI 10.1007/s10096-007-0285-5

ARTICLE

Q fever osteoarticular infection: four new cases and a review of the literature

C. Landais · F. Fenollar · A. Constantin · C. Cazorla · C. Guilyardi · H. Lepidi · A. Stein · J. M. Rolain · D. Raoult

Table 1 Nineteen cases of osteoarticular infections due to *Coxiella burnetii*

Patient no. [ref.]	Age/sex	Clinical form	Sites involved	Underlying disease	Complications	Diagnosis	Treatment
3	64/M	Spondylodiscitis	Intervertebral disc L2/L3	Rheumatoid arthritis, anti-TNF therapy + methotrexate	Paravertebral abscess & psoas infiltration	Serology, PCR, culture	DOX 200 mg/day & HCQ 600 mg/day, in course
4	47/M	Spondylodiscitis	Intervertebral disc L5/S1	None	Epidural abscess	Serology, PCR	DOX 200 mg/day & HCQ 600 mg/day, in course
5 [17]	7/M	Spondylitis	L3 vertebra	None	CRMO over 8 months: right hip	Serology, culture	DOX 100 mg/day for 2 years, RIF 400 mg/day for 6 months
6 [16]	4/M	Spondylitis	T10 vertebra	None	Extradural extension CRMO over 5 years: both feet, right heel and chest wall	Serology, PCR	DOX 100 mg/day & HCQ 300 mg/day for 3 years
7 [8]	61/F	Spondylitis	Lumbar vertebra	Uterine cancer	NA	Serology	NA
8 [22]	39/M	Spondylitis	L5 vertebra	None	None	Serology	TET for 5 months
9 [22]	76/M	Spondylitis	L1 vertebra	None	Psoas and paravertebral abscess	Serology	Antituberculous therapy for 2 years
10 [15]	67/M	Spondylodiscitis	Intervertebral disc	NA	NA	Serology	DOX

PCR positive à chaque fois qu'elle a été réalisée
Tous les cas avec sérologie positive

Sur 4 cas de spondylodiscites isolées à *Coxiella burnetii* sans infection vasculaire, 2 sont survenus sur une maladie inflammatoire articulaire

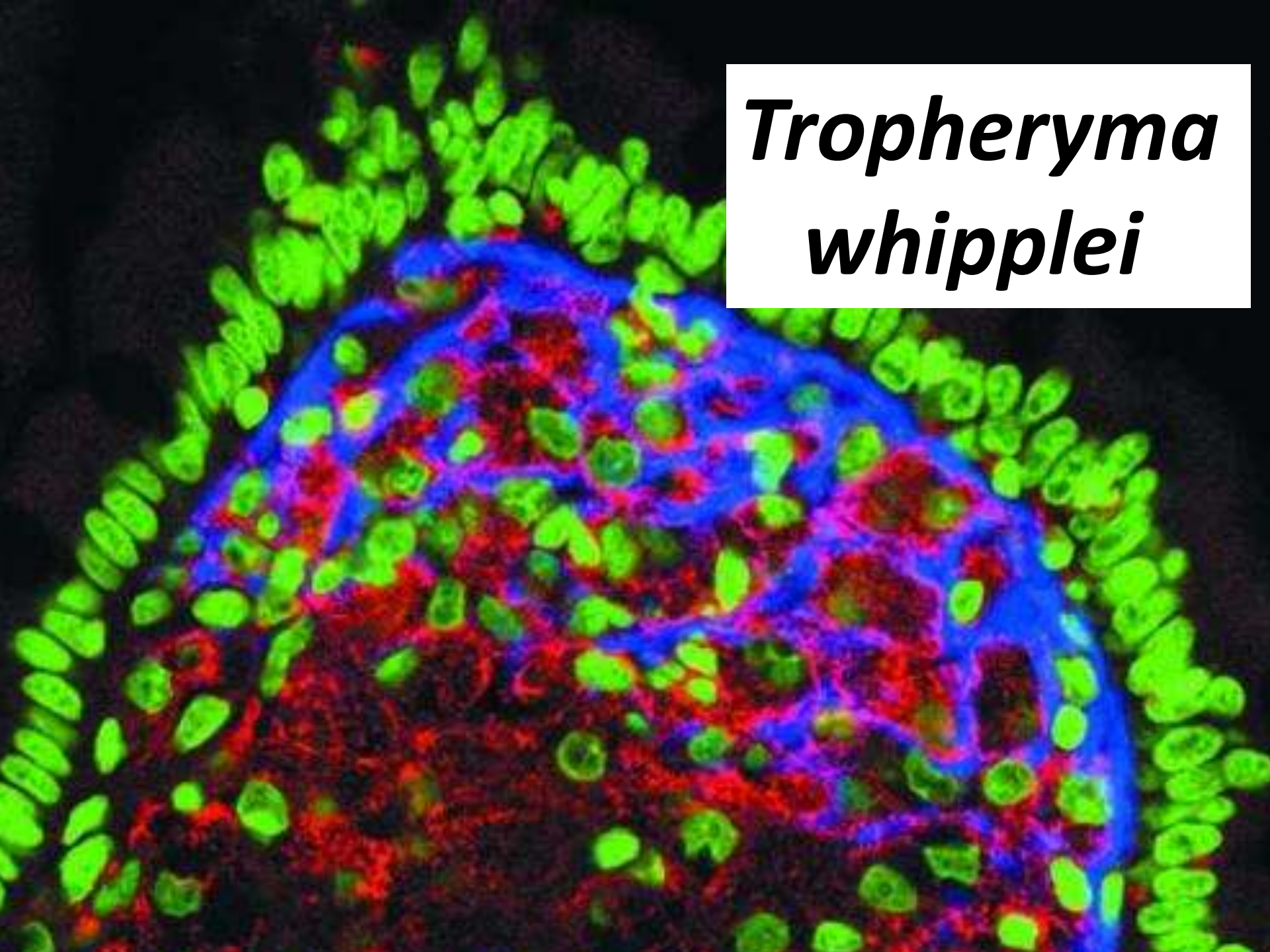
1 polyarthrite rhumatoïde,
1 spondylarthrite ankylosante

Importance d'envoyer au moins 1 couple biopsie serum au CNR pour alimenter la cohorte nationale avec test biologique homogène

Conflit d'intérêt: membre du CNR

Liens d'intérêt & intérêts mutuels : collaboration pour les publications

F. ROBLOT, T. WEITTEN, T. GUIMARD, A. ELSENDORN, E. MERIGLIER, A. SUNDER, B. RAMMAERT, E. CANOUI, A. BOSSERAY, O. EPAULARD, S. BRANGER, B. CHAUDIER, K. BLANC-LASERRE, N. FERREIRA-MALDENT, E. DEMONCHY, J. REYNES, F. DJOSSOU, A. MAHAMAT, A. SOTTO, ……


Tropheryma whipplei

Schweiz Med Wochenschr. 1996 Aug 31;126(35):1495-9.

Spondylodiscitis caused by Tropheryma whippelii.

Altwegg M[1], Fleisch-Marx A, Goldenberger D, Hailemariam S, Schaffner A, Kissling R.

Author information

Abstract

We describe the first case of spondylodiscitis caused by Tropheryma whippelii in which this so far unculturable organism was shown to be present at the site of infection in a patient without significant gastrointestinal symptoms. The methods used included broad-range PCR amplification with universal primers complementary to constant sequences of the gene coding for 16S rRNA, direct sequencing of the amplified fragment, and comparison of the sequence determined with those deposited in sequence databases. In addition to demonstrating the presence of this organism in the affected vertebral body, we found in our patient that the specific PCR is more sensitive than histology for detecting Whipple's bacilli in bowel biopsy specimens. Because histology of small bowel biopsies from the duodenum were-in contrast to PCR from the same site-not diagnostic for Whipple's disease in our patient, we recommend PCR whenever Whipple's disease has to be excluded.

Tout premier cas de spondylodiscite diagnostiqué par PCR dans Pubmed = *T. whipplei* chez un patient sans symptômes gastro-intestinaux

Clin Rheumatol. 2003 Dec;22(6):443-6. Epub 2003 Oct 2.

Spondylodiscitis as the first manifestation of Whipple's disease -a removal worker with chronic low back pain.

Weber U[1], Morf MH, Gubler JG, Altwegg M, Maibach RC.

Author information

Abstract

Whipple's disease is a rare systemic infectious disease caused by the actinobacterium Tropheryma whipplei. Spondylodiscitis is an extremely rare manifestation of the infection and has previously been described in only three case reports. We present a 55-year-old man with persistent lumbago and signs of systemic illness, but without any gastrointestinal symptoms or arthralgia. The signal response in the lumbar spine in magnetic resonance tomography, both native and after intravenous gadolinium administration, was compatible with spondylodiscitis at the L4/L5 level. Culture of a specimen obtained by radiographically guided disc puncture and repeated blood cultures remained sterile. Tropheryma whipplei was detected by PCR amplification in material obtained from the disc specimen, from a biopsy of the terminal ileum and from the stool. The histology of duodenum, terminal ileum, colon and disc material was normal and, in particular, showed no PAS-positive inclusions in macrophages. Long-term antibiotic treatment with sulphamethoxazole and trimethoprim was successful, with marked improvement of the low back pain and normalisation of the systemic inflammatory signs. The possibility of Whipple's disease must be suspected in the case of a 'culture-negative' spondylodiscitis even if there are no gastrointestinal symptoms and no arthralgia present.

IRM: spondylodiscite L4/L5
PCR positive sur le disque, l'iléon terminal et les selles
Histologie normale (duodénum PAS-) !

Kingella kingae

Kingella kingae–Associated Pediatric Osteoarticular Infections: An Overview of 566 Reported Cases

Data on routine laboratory culture techniques with specimens of blood, joint fluids and bone aspirates was available in 411 cases of which only 178 cases (43.3%)were positive.

Detection rate = 81.37% (83 of 102) when specimens were inoculated into blood culture vials (BCVs) regardless the system used.

All the cases (259 out of 259) where PCR technique was used were PCR positive

There were no reported cases of positive cultures for K kingae OAIs with negative PCR (30 spondylodiscitis)

Al Qwbani, Clinical Pediatrics, 2016

Prospective survey of acute osteoarticular infections in a French paediatric orthopedic surgery unit

A. Ferroni[1], H. Al Khoury[2], C. Dana[2], G. Quesne[1], P. Berche[1], C. Glorion[2] and Z. Péjin[2]

1) *Hôpital Necker Enfants-Malades, Laboratoire de Bactériologie and* 2) *Hôpital Necker-Enfants Malades, Unité de Chirurgie Orthopédique Pédiatrique, Paris, France*

6 spondylodiscitis

TABLE 3. Respective contributions of standard cultures in solid media, enrichment in blood culture bottles, PCR and blood culture to the bacteriological diagnosis

	Bacteria recovered by				
Species	Standard culture	Enrichment	Only PCR	Only blood culture	Total
K. kingae	0	4	39	1	44
S. aureus	13	2	3	6	24
S. pyogenes	3	0	1	2	6
S. pneumoniae	2	0	1	0	3
S. agalactiae	0	2	0	0	2
Others	0	2	2	0	4

Case reports

Etude	Examen direct	Culture	PCR	Micro-organisme
Dayan, Spine J, 2007		NA	positive	*Aspergillus*
Hulzebos, Clin Infect Dis, 1999		Négative	positive	*Bartonella henselae*
Garg, BMJ Case Rep, 2018		Négative	positive	*Burkholderia pseudomallei*
Zhang, Mil Med, 2013	Positive	NA	positive	*Brucella abortus*
Navarro-Martinez, J Clin Microbiol, 2008			positive	*Brucella melitensis*
Stokes, Can J Infect Dis Med Microbiol, 2016	Négatif	Négative	positive	*Coxiella burnetii*
Shibata, Intern Med, 2015		Negative	positive	*Escherichia coli*
Sanmilian, Anaerobe, 2013		Positive après	positive	*Fusobacterium necrophorum*
Mediavilla-Santos, Acta Orthop Mex, 2014		Négative	positive	*Fusobacterium nucleatum*
Haddadzadeh, Kardiochir Torakochirurgia Pol, 2014	Négatif	Négative	positive	*Mycobacterium kansasii*
Hasan, SICOT J, 2018	Négatif	Négative	positive	*Mycobacterium tuberculosis*
Jurado, Biomedica, 2015		Négative	positive	*Mycobacterium tuberculosis*
Ribeiro, Braz J Med Biol Res, 2007	Négatif	Négative	positive	*Mycobacterium tuberculosis*
Sobottke, Arch Orthop Trauma Surg, 2008		Negative	positive	*Mycobacterium xenopi*
Fabricius, BMC Infect Dis, 2013		NA	positive	*Treponema pallidum*
Weber, Clin Rheumatol, 2003	Négatif	Négative	positive	*Tropheryma whipplei*
Altwegg, Schweiz Med Wochenschr, 1996	Négatif	Négative	positive	*Tropheryma whipplei*
Spoerl, Orphanet J Rare Dis, 2009	Négatif	Négative	positive	*Tropheryma whipplei*

5 *Mycobacterium*
3 *Tropheryma*
2 *Fusobacterium*
2 *Brucellosis*
Au moins 1 champignon

Am J Med. 2013 Dec;126(12):1143.e25-33. doi: 10.1016/j.amjmed.2013.04.027. Epub 2013 Oct 14.

Systematic PCR detection in culture-negative osteoarticular infections.

Levy PY[1], Fournier PE, Fenollar F, Raoult D.

Author information

Abstract

BACKGROUND: Identification of microorganisms is crucial for the successful treatment of osteoarticular infections. Molecular methods are more sensitive than culture-dependent methods but may suffer from lack of specificity.

METHODS: We studied a large series of 3840 bone and joint culture-negative samples collected from 2308 patients hospitalized in Marseille University Hospitals from November 2007 to October 2009. The samples were systematically cultured for 15 days, and conventional broad-range polymerase chain reaction (PCR) (16S rDNA and 18S rDNA) as well as real-time PCR assays targeting human Bglobin, Staphylococcus aureus, and Kingella kingae were realized on one culture-negative specimen.

RESULTS: Specimens from 741 patients (32.1%) tested positive by culture, including 38 in which bacteria grew only after 6 days of incubation. PCR was positive in 141 (9%) culture-negative specimens. Microorganisms identified by PCR were classified into 2 groups: fastidious bacteria (n = 35), mostly anaerobes in adult patients, and K. kingae in children; and nonfastidious bacteria (n = 106), mostly S. aureus (32.7%). A discrepancy between a positive PCR result for S. aureus and a negative culture were explained by previous antibiotherapy in 31.4% of cases. Our study highlights the usefulness of systematic 16S rDNA gene PCR for the diagnosis of bone and joint infections in culture-negative patients, thus enabling the administration of specific antibiotic treatments.

CONCLUSIONS: We recommend the use of conventional broad-range PCR for culture-negative bone and joint specimens, as well as S. aureus-specific PCR for adults and K. kingae-specific PCR for children. 18S rDNA PCR should be reserved only for specific cases.

70% des discordances entre PCR et culture pour *S. aureus* n'étaient pas expliqués par une antibiothérépie …
***S. aureus* fastidieux / intracellulaire / non cultivable ?**

Limites de la PCR spécifique ….

J Clin Microbiol. 2013 Jan;51(1):366-8. doi: 10.1128/JCM.02524-12. Epub 2012 Nov 7.

Failure of PCR-Based IS6110 analysis to detect vertebral spondylodiscitis caused by Mycobacterium bovis.

Steensels D[1], Fauville-Dufaux M, Boie J, De Beenhouwer H.

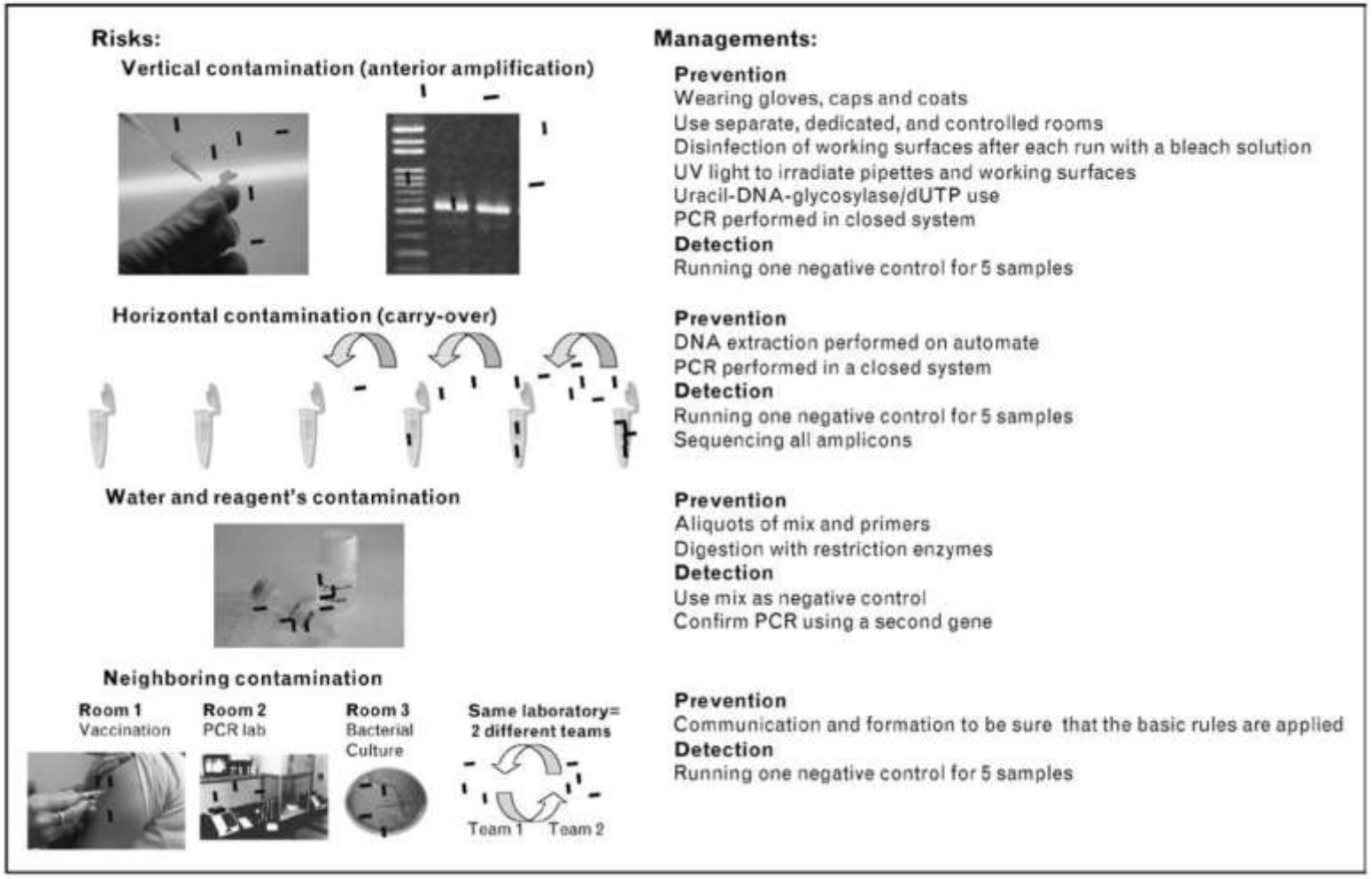
Author information

Abstract

Mycobacterium bovis is responsible for a zoonosis originating in cattle. We report a case of a man with vertebral spondylodiscitis caused by Mycobacterium bovis. Diagnosis was complicated because of the lack of IS6110. These strains are rare, but microbiologists should be aware of their existence.

Primer bias
DNA extraction bias
DNA amplification bias …

Figure 1 Risks and managements of broad-range PCR contamination



Fenollar, Curr Opinion Rheumatol, 2008

KIT infections osseuses

CULTURE

Mutualisés

Liquide de ponction:
1 tube à vis
-culture bactériologie
-culture mycologie
-culture mycobactérie

flacon hémoculture
1 aérobie
1 anaérobie

Biopsies (5 morceaux):
5 tube à vis
-culture bactériologie
-culture mycologie
-culture mycobactérie

BIOLOGIE MOLECULAIRE

si culture négative à 72h
- 16S, CU, *S. aureus*, *C. burnetii*, *T. whipplei*, Mycobactéries, *K. kingae* (pour les enfants de moins de 5 ans)

SEROLOGIE

1 tube jaune gélosé 10 ml

- *Coxiella burnetii, Bartonella henselae, B. quintana,*
- *Brucella melitensis*

Mutualisés

RECHERCHE

Cohorte OS
Liquide de ponction:
1 tube à vis
Biopsie :
1 tube à vis

NON FACTURE

Anatomopathologie Timone 2

1 pot contenant du formol
A l'attention du Pr LEPIDI

RECAPITULATIF PRELEVEMENTS

Liquide de ponction/biopsies
8 tubes à vis
1 flacon hémoculture aérobie
1 flacon hémocultures anaérobie
1 pot formol (biopsie)

Sang
1 tube jaune gélosé 10 ml

Attention séro importante pour *C. burnetii* donc nécessité de prélever un sérum en plus de la biopsie

07 juin 2017

Perspectives…

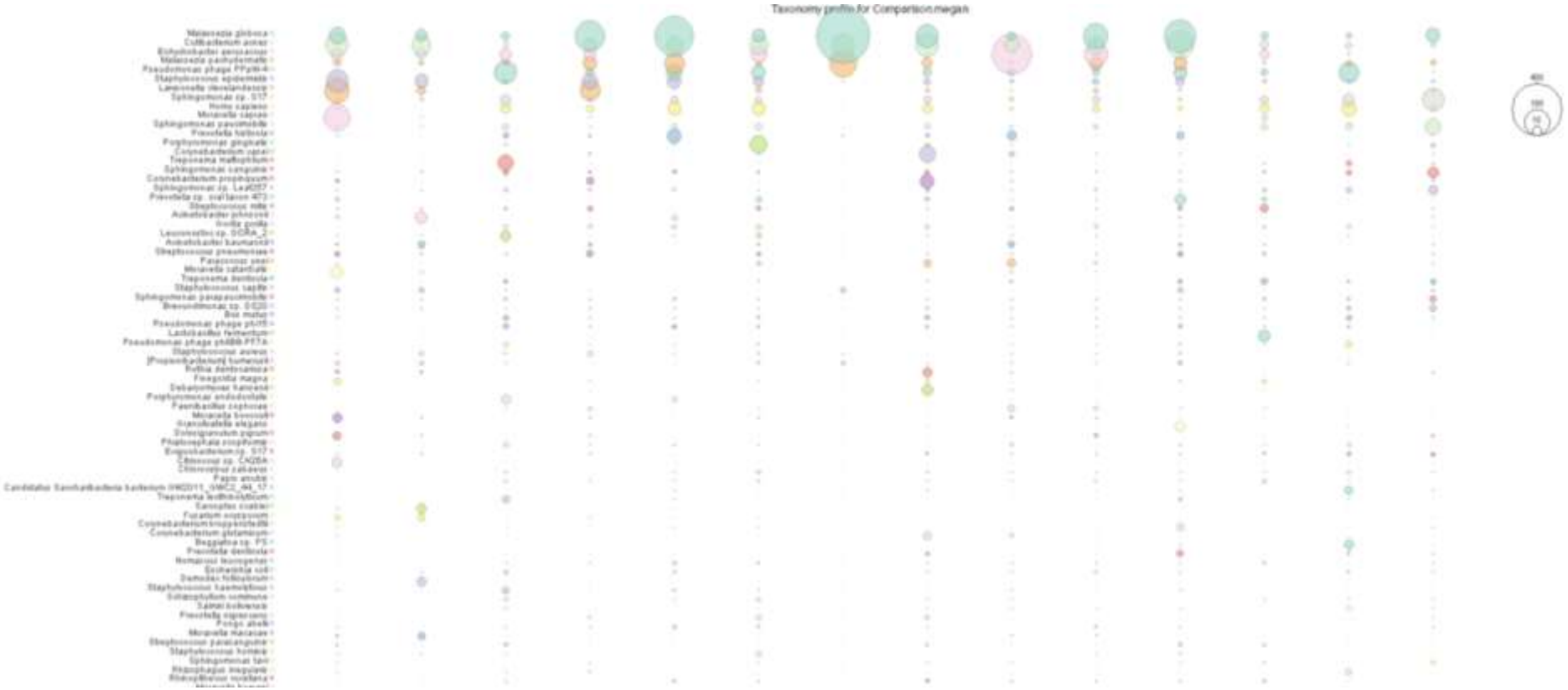
Multiplex

PCR lyophilisée

Next generation sequencing ….

Challenge technique et statistique en raison de la diversité inattendue des séquences procaryotes retrouvées dans n'importe quel prélèvement biologique (y compris dans les mix de séquençage)

Ou comment trouver une aiguille dans une botte de foin…



Exemple de résultats métagénomiques dans le diagnostic sur tissu présumé infecté….
Les contaminants sont majoritaires…
Trouver l'aiguille … ☺

Merci de votre attention