Clinical Studies

Is Xpert MRSA/SA SSTI real-time PCR a reliable tool for fast detection of methicillin-resistant coagulase-negative staphylococci in periprosthetic joint infections?

CrossMark

J. Lourtet-Hascoëtt [a,*], A. Bicart-See [a], M.P. Félicé [a], G. Giordano [b], E. Bonnet [c]

[a] Microbiological Laboratory, Hôpital J. Ducuing, 15 rue Varsovie, 31300, Toulouse, France
[b] Traumatology and Orthopaedic Surgery Department, Hôpital J. Ducuing, 15 rue Varsovie, 31300 Toulouse, France
[c] Infectious Diseases Mobile Unit, Hôpital J. Ducuing, 15 rue Varsovie, 31300 Toulouse, France

ARTICLE INFO

ABSTRACT

Periprosthetic joint infections (PJIs) are frequently caused by methicillin-resistant coagulase-negative staphylo-cocci (CoNS). Cultures remain the gold standard but often require a few days. Thus, a rapid test could be interest-ing to guide antibiotic strategy earlier. The purpose of this study was to evaluate the performances of RT-PCR Xpert® MRSA/SA technique for the detection of methicillin-resistant CoNS (MRCoNS) from deep samples in pa-tients with PJIs. RT-PCR was tested on 72 samples. Sensitivity, specificity, positive predictive value, and negative predictive value of RT-PCR method were 0.36, 0.98, 0.90, and 0.74, respectively. Although RT-PCR may allow early microbial diagnosis of PJI due to *Staphylococcus aureus* (MSSA and MRSA), the low sensitivity and the high cost of this method to detect MRCoNS could limit its use in this field.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The incidence rate of prosthetic joint infections (PJIs) for knee or hip prosthesis is 1–3% (Kurtz et al., 2008; Trampuz and Zimmerli, 2005; Zimmerli et al., 2004). Most frequent bacteria found in these infections are staphylococci. In chronic PJIs, the most frequent bacteria isolated are coagulase-negative staphylococci (CoNS) causing 19–40% of infections (Pandey et al., 2000; Segawa et al., 1999; Trampuz and Zimmerli, 2008).

The detection of bacteria in bone and joint infections can be per-formed by different methods.

The culture is the gold standard method; it has been recently im-proved by new methods like sonication or crushing (Achermann et al., 2010; Desplaces, 2002; Trampuz et al., 2007; Vergidis et al., 2011). The main problem is the delay to obtain a result varying from 2 days to 3 weeks (Schafer et al., 2008).

The microbiological diagnosis may be difficult to establish because of many reasons including a long transport, patients under or recently treat-ed with antimicrobial agents, presence of a biofilm, growing of small col-ony variants, and a too small amount of bacteria to get a positive culture (Proctor et al., 1998). Interpretation of culture may also be uneasy due to presence of polymicrobial infections (25%), a mixing of commensal and pathogen bacteria, a monomicrobial infection with various antibiotic susceptibilities, a positive result in only 1 or 2 among 5 or 6 samples, or results positive on subcultures only. Moreover, in monomicrobial

cultures, organisms may express multiple resistance factors, in particular *Staphylococcus epidermidis*, which have a genetic flexibility and continu-ously generate novel variants (Schoenfelder et al., 2010).

Molecular techniques are very sensitive and can detect bacteria in lower quantity than culture. Several studies evaluated the performance of PCR (multiplex PCR, 16S rRNA gene sequencing) in bone and joint in-fections, but the routine use of these methods has been limited previ-ously by technical difficulty, expense, and time required (Achermann et al., 2010; Dempsey et al., 2007; Dora et al., 2008; Fihman et al., 2007; Kobayashi et al., 2009).

A system of PCR, the Xpert® MRSA/SA SSTI RT-PCR (Cepheid, Sunny-vale, CA, USA) has been developed recently for the detection of *Staphylo-coccus aureus* (SA) and *mec*A gene in cutaneous and subcutaneous samples. Performances of the test are excellent with a sensitivity of 94.4% and a specificity of 100% for MRSA (detection of *spa* and *mec*A genes). Moreover, diagnosis is available in less than 1 hour (Wolk et al., 2009).

An empiric treatment by vancomycin or daptomycin is often pre-scribed before obtaining the results of standard microbiological cul-tures. It is interesting to get a fast detection of resistant strains in order to adapt the antibiotic treatment according to antibiotic suscepti-bilities. In case of methicillin-sensitive staphylococci found in culture, treatment should be switched by a beta-lactamin, which has a better ac-tivity on these strains (Dupont et al., 2009; Fernandez Guerrero and de Gorgolas, 2006; Kim et al., 2008).

At this time, the Xpert® MRSA/SA test is neither validated for the de-tection of CoNS nor for the application to bone and joint samples.

The objective of the study was to evaluate this test for the detection of methicillin-resistant CoNS (MRCoNS) harboring only the *mec*A gene in solid and liquid osteoarticular samples.

* Corresponding author. Tel.: +33-648255700.
E-mail addresses: julielourtet@hotmail.com (J. Lourtet-Hascoëtt),
Alain.Bicart-See@wyss.harvard.edu (A. Bicart-See), mpfelice@hjd.asso.fr (M.P. Félicé),
ortho.giordano@gmail.com (G. Giordano), drmaboule@neuf.fr (E. Bonnet).

2. Materials and methods

2.1. Study design

During a 2-year period (from January 2011 to December 2012), we performed a retrospective study in a single orthopedic surgery unit including 32 patients with PJI and 30 negative control patients. The tests were performed on patients diagnosed with staphylococcal PJI with available samples, which were kept frozen. The conservation was performed by a −80 °C freezing of samples directly without a fixative.

2.2. Patients and samples

From 32 patients with a diagnosis of PJI, samples were studied by MRSA/SA PCR. The diagnosis of PJI was based on clinical, radiological, histopathological, and biological data (Gehrke et al., 2013). All most recent PJI cases were included retrospectively: 32 cases were PJI due to CoNS PJI, 10 cases due to SA.

Thirty patients who underwent a same type of surgery but with no clinical symptoms or perioperative negative samples were considered as negative controls.

Culture and Xpert® MRSA/SA were performed on simultaneously for each patient.

2.3. Microbiological culture

After collecting, the intraoperative samples were transferred to microbiology laboratory within 30 minutes. A sample is defined as a specimen collected on 1 site during surgery.

Antibiotics were stopped 15 days prior to surgery.

For each suspect site, solid (bones and joint tissues) and liquid (articular fluids) samples were performed on sterile vials in duplicate to ensure that all doubles could be frozen at −80 °C and kept for 1 year. In our study, the mean duration of frozen conservation was 4.7 months. Articular fluids were all collected from knee joints during surgery. Articular fluids and tissue were both tested in standard culture and RT-PCR on the same patients. As bacteriological cultures were performed differently from solid and liquid samples, bones and tissues and articular fluids were treated separately in the results.

All samples were seeded on rich agar and broths. Liquid samples were inoculated in blood culture bottles (Bactec®; Becton Dickinson,1 Becton Dr, Franklin Lakes New Jersey, USA). All samples were incubated under aerobic with CO_2 and anaerobic atmosphere.

Samples were vortexed in 1-mL saline solution. Gram staining was performed for each sample. Standard cultures were performed on Columbia blood agar, Polyvitex chocolate agar, and BHI solution (bioMérieux®, Marcy l'Etoile, France). Cultures were incubated for 15 days, so that low growing bacteria may be found. Identification was performed by automatized technique on Vitek2 (bioMérieux®) or manual technique on ApiStaph (bioMérieux®).

All patients were included with at least 2 positive perioperative samples with the same CoNS. The CoNS were considered as same bacteria if the same species and antibiotic susceptibilities were found. For all patients with a positive articular fluid, the solid sample was also positive in culture.

Antimicrobial susceptibilities were tested on Vitek2 (bioMérieux®) according to the Committee of Antibiotic Susceptibility from the French Society of Microbiology recommendations. Meticillin resistance was interpreted from oxacillin CMI (Minimum Inhibitory Concentration). Meticillin susceptibility discordant results were confirmed by using a cefoxitin disk. Moxalactam disks were not used because no discordant results between oxacillin and cefoxitin were found.

2.4. Multiplex PCR assay

Samples were first mixed in an elution buffer. The solution was then vortexed during 10 s and transferred in a PCR cartridge according to manufacturer's recommendations.

All samples were analyzed by Xpert® MRSA/SA. This RT-PCR is indicated for the detection of SA and methicillin resistance. The Xpert® system detects 3 different targets: spa gene, mecA gene, and SCCmec gene. The amplification of spa gene indicates the presence of SA. Meticillin resistance is detected by the amplification of mecA gene. SCCmec gene is associated with resistance to methicillin for SA. An internal control (Bacillus globigii) is amplified at each process. The process is complete in 56 minutes.

2.5. Data interpretation

All samples were analyzed by culture during 15 days and then by RT-PCR. A culture result was considered as positive when a Staphylococcus was found on solid agar or broth in less than 10 days. The criteria for a microbiological infection were:

- The same species of CoNS found in culture in at least 2 samples.
- An SA found in culture in at least 1 sample.

A "true-positive" result was defined as a positive mecA gene signal amplified and a positive culture. A "true-negative" result was defined as negative results for mecA gene and culture. A "false-positive" result was defined as a mecA gene signal amplified without a positive culture. A "false-negative" result was defined as a positive culture without a mecA gene signal amplified.

The result was interpreted positive when only mecA gene was amplified. When spa gene and SCCmec were both amplified, SA was suspected.

The diagnosis of bone and joint infections was performed from samples of bones, synovial tissues, and articular fluids. RT-PCR and culture were compared for 72 samples (42 positive and 30 negative samples).

2.6. Data analysis

In our study, sensitivity, specificity, and positive and negative predictive values were calculated. Statistical analysis was performed on Stata, with P values ≤0.05 considered as significant.

3. Results

3.1. Characteristics of the population

Sixty-two patients were included, and 72 samples were performed. Among them, 25% were articular fluids; 57%, knee tissues; and 18%, hip bone and joint tissues. All samples were analyzed both by standard culture and RT-PCR.

3.2. Microbiological results

For all patients included, at least 3 samples per patient were collected (bone, tissue, or articular fluid). Direct examination was positive for 9.7% (95% confidence interval [CI] 4.0–19.0%) of samples.

Among 72 samples, culture was sterile in 30 cases. Thirty-two (44.4%) samples were positive for CoNS (95% CI 32.7–56.6%) and 10

Table 1
Results of culture according to the site of bone and joint infections.

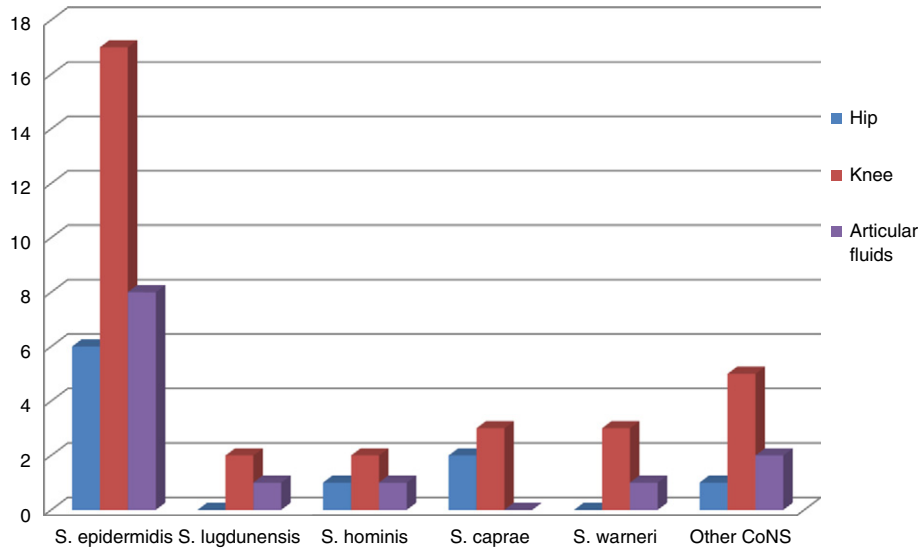| Localization	Negative	MSSA	MSCoNS	MRCoNS
	Culture results			
Hip (bone and joint tissues)	5	1	1	6
Knee (bone and joint tissues)	15	6	5	15
Articular fluid	10	3	1	4
Total	30	10	7	25

Fig. 1. Coagulase-negative staphylococcal species in PJIs.

(13.9%) (95% CI 6.9–24.1%) for SA. Among the 32 CoNS found in culture, 7 (21.8%) (95% CI 9.3–39.9) were sensitive to methicillin (methicillin-susceptible CoNS [MSCoNS]), and 25 (78.1%) (95% CI 60–90.7) were resistant to methicillin (MRCoNS). Details of culture and localizations are presented in Table 1.

Species of CoNS and origin of samples are detailed in Fig. 1.

Among the 32 samples yielding CoNS, we identified 56% *S. epidermidis*, 8% *Staphylococcus caprae*, 7% *Staphylococcus hominis*, 7% *Staphylococcus warneri*, 6% *Staphylococcus lugdunensis*, and 16% other CoNS (*Staphylococcus simulans*, *Staphylococcus capitis*, *Staphylococcus auricularis*, and *Staphylococcus haemolyticus*).

3.3. Comparison between culture and RT-PCR results

Regarding the 30 samples sterile in culture, all results of RT-PCR were negative.

Among 25 samples positive for MRCoNS in culture and RT-PCR, the detection of *mec*A gene was positive in only 9 cases; this is why 16 results are considered as false-negative results. On 1 articular fluid sample, the *mec*A gene signal was amplified but under the validated threshold. The result was interpreted as negative by the GenXpert®, but we can consider the positive signal as a positive result. Regarding articular fluids, the 2 positive RT-PCR results were also positive on knee bone and joint tissues.

All internal controls of RT-PCR were well detected. Among the 7 MSCoNS tested by RT-PCR, 1 false-positive result was found in a knee sample. For this result, culture was positive with SA, but in all other samples, an MRCoNS was found. This false-positive result could thus be considered as a true-positive result due to the presence of an MRCoNS.

Among the 72 samples tested by RT-PCR, 1 test was invalid (no result obtained because of an inhibition of PCR) and considered as negative in our results.

Table 2 shows a comparison of culture and RT-PCR results. Table 3 showed detailed culture and PCR results according to samples localizations.

Sensitivity of RT-PCR method was 0.36 (95% CI 0.18–0.58). Specificity was 0.98 (95% CI 0.89–0.99). Positive predictive value was 0.90 (95% CI 0.56–0.99), and negative predictive value was 0.74 (95% CI 0.62–0.85). The prevalence of MRCoNS on the 72 samples was 0.35. This prevalence was used to calculate the positive and negative predictive values. It is interesting to calculate likelihood ratios in order to evaluate the performances of the RT-PCR. Positive likelihood ratio was 17.1, and negative likelihood ratio, 0.65.

4. Discussion

Adequate antimicrobial treatment for PJI should be initiated as soon as possible after surgery to assume a good recovery. This treatment depends on clinical information, risk factors, and microbiological results.

Staphylococci are the most frequent bacteria isolated from bone and joint samples in patients with PJI. According to the literature, 20% of PJI are caused by SA, and 30%, by CoNS (Pandey et al., 2000; Segawa et al., 1999).

Regarding our experience in PJI, staphylococcal strains are mainly resistant to methicillin (about 65%).

In French certified centers for the management of complex bone and joint infections, the great majority of CoNS are also resistant to methicillin (Sousa et al., 2010).

Therefore, recommendations for empirical treatment of PJI include vancomycin. However, this drug is associated with various side effects including "red man syndrome", thrombosis at the site of infusion, and renal impairment (Sousa et al., 2010).

Table 2
Comparison between culture and PCR results.

Culture results	No. of PCR performed	Positive PCR results
Negative	30	0
MSSA	10	0
MSCoNS	7	1
MRCoNS	25	9

Table 3
PCR and culture results according to localizations.

		Culture			
		Sterile	MSSA	MSCoNS	MRCoNS
Samples	PCR tested	30	10	7	25
Hip periprosthetic tissue	mecA +	0	0	0	2
	mecA −	5	1	1	4
Knee periprosthetic tissue	mecA +	0	0	1	5
	mecA −	15	6	4	10
Articular fluids	mecA +	0	0	0	1 + 1
	mecA −	10	3	1 invalid	2

In SA bacteremia, it has been shown that antistaphylococcal penicillins are more efficient than vancomycin (Kim et al., 2008)

In PJIs due to methicillin-susceptible staphylococci, first-line treatment is based on antistaphylococcal penicillins (Dupont et al., 2009). Microbiological results are crucial to initiate the most adequate antimicrobial treatment. However, the time to obtain positive standard cultures and antimicrobial susceptibility results may vary from 2 to 15 days (Schafer et al., 2008). Regarding CoNS, especially small variant colonies, time before positive culture may be as long as 10 days (Zimmerli et al., 2004). Many PCR tests are time consuming and not appropriate for routine use at this time (Dempsey et al., 2007; Fihman et al., 2007). As shown in previous studies, direct examination of surgical samples lacks sensitivity (Gallo et al., 2008).

In our study, gram stains were only positive in 9% of cases (data not shown). The Xpert® MRSA/SA RT-PCR system has been developed to detect SA in skin and soft tissue samples. It can also determine whether the strain is susceptible to methicillin in less than 1 hour. It has been further used for bone and joint infections. It must be underlined that this technique has been only validated for SA. However, detecting CoNS resistant to methicillin by this technique in samples from PJI in less than 1 hour is an attractive challenge.

The RT-PCR was compared to culture for every sample. PCR results were in accordance with culture for all sterile samples.

Regarding MSCoNS samples, *mec*A gene was not detected for MSSA and MSCoNS.

For MRCoNS, PCR for detection of *mec*A gene was positive for 9 among 25 samples (36%). One PCR result on joint fluid was invalid and considered as negative. Thus, in this study, 16 discordant results were found, showing positive culture and negative PCR. The sensitivity was 0.36; positive likelihood ratio is good compared to negative likelihood ratio. These results seem to indicate that this test is performant for positive results but not for negative results.

Different explanations could be hypothesized. First, more than 50% of the samples were frozen and thaw in order to perform this study; this process could have led to false-negative PCR results. Second, the low bacterial inoculum in the sample and the presence of PCR inhibitors could also have led to false-negative PCR results. (All positive intern controls were positive and well amplifiEd.) Finally, CoNS especially *S. epidermidis* have some genetic flexibility and ability to integrate mobile elements; this could be a reason why *mec*A gene could be not detected.

To avoid contamination, we have usually taken samples for traditional cultures and for PCR. When bacteria are in very low amounts or embedded in a biofilm, they are not evenly distributed and could lead to false-negative results.

Finally, one must keep in mind that Xpert® system has been validated for the diagnosis of skin and soft tissue infections. The thresholds established by the manufacturer did not apply to the detection of staphylococci (SA or MRCoNS) in bone tissue or synovial fluid. Previously, the Xpert® system has been also evaluated on bacteremia or MRSA carriage (Wolk et al., 2009).

However, some authors have applied Xpert® MRSA/SA RT-PCR for the detection of MSSA, MRSA, and MRCoNS in bone and joint tissues (Titecat et al., 2012; Trampuz et al., 2007). In 2012, the prospective study conducted by Titecat et al. (2012) included 30 patients and 104 samples. They tested the Xpert® MRSA/SA SSTI on MSSA, MRSA, and MRCoNS and found a global sensitivity of 84.6% and a negative predictive value of 94.5% (Titecat et al., 2012).

In 2011, Dubouix-Bourandy et al. (2011) evaluated the same test for the detection of MRCoNS in bone and joint tissues. In this study, they analyzed 23 samples for MRCoNS and found a sensitivity of 100% and a specificity of 94.5% (Dubouix-Bourandy et al., 2011).

Compared to these studies, ours showed a lower sensitivity. Despite the advantages of this test in terms of rapidity and automatization, it does not seem to be adapted for the detection of MRCoNS in bone and joint tissues and articular fluid. Other studies are needed to confirm the value of this test in the diagnosis of these specific infections.

In conclusion, standard culture remains the gold standard regarding sensitivity and determination of the susceptibility to antimicrobial agents. Extrapolation of results applied in soft tissues for the detection of MSSA and MRSA to the detection of MRCoNS in bone and joint infected tissues is hazardous.

References

Achermann Y, Vogt M, et al. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. J Clin Microbiol 2010;48(4):1208–14.

Dempsey KE, Riggio MP, et al. Identification of bacteria on the surface of clinically infected and non-infected prosthetic hip joints removed during revision arthroplasties by 16S rRNA gene sequencing and by microbiological culture. Arthritis Res Ther 2007;9(3):R46.

Desplaces C. Diagnostic microbiologique de l'infection sur prothèse articulaire Diagnostic de l'infection sur prothèse articulaire. Tiresias 2002;2:39–45.

Dora C, Altwegg M, et al. Evaluation of conventional microbiological procedures and molecular genetic techniques for diagnosis of infections in patients with implanted orthopedic devices. J Clin Microbiol 2008;46(2):824–5.

Dubouix-Bourandy A, de Ladoucette A, et al. Direct detection of Staphylococcus osteoarticular infections by use of Xpert MRSA/SA SSTI real-time PCR. J Clin Microbiol 2011;49(12):4225–30.

Dupont M, Dutronc H, et al. Recommandations de pratique clinique Infections ostéo-articulaires sur matériel (prothèse, implant, ostéosynthèse); 2009.

Fernandez Guerrero ML, de Gorgolas M. Comparative activity of cloxacillin and vancomycin against methicillin-susceptible Staphylococcus aureus experimental endocarditis. J Antimicrob Chemother 2006;58(5):1066–9.

Fihman V, Hannouche D, et al. Improved diagnosis specificity in bone and joint infections using molecular techniques. J Infect 2007;55(6):510–7.

Gallo J, Kolar M, et al. Culture and PCR analysis of joint fluid in the diagnosis of prosthetic joint infection. New Microbiol 2008;31(1):97–104.

Gehrke T, Parvizi J, et al. Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection. Philadelphia: Musculoskeletal Infection Society; 2013.

Kim SH, Kim KH, et al. Outcome of vancomycin treatment in patients with methicillin-susceptible Staphylococcus aureus bacteremia. Antimicrob Agents Chemother U S 2008;52:192–7.

Kobayashi N, Inaba Y, et al. Rapid and sensitive detection of methicillin-resistant Staphylococcus periprosthetic infections using real-time polymerase chain reaction. Diagn Microbiol Infect Dis 2009;64(2):172–6.

Kurtz SM, Lau E, et al. Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty 2008;23(7):984–91.

Pandey R, Berendt AR, et al. Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. Arch Orthop Trauma Surg 2000;120(10):570–4.

Proctor RA, Kahl B, et al. Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. Clin Infect Dis 1998;27(Suppl. 1):S68–74.

Schafer P, Fink B, et al. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis 2008;47(11):1403–9.

Schoenfelder SM, Lange C, et al. Success through diversity—how Staphylococcus epidermidis establishes as a nosocomial pathogen. Int J Med Microbiol 2010;300(6):380–6.

Segawa H, Tsukayama DT, et al. Infection after total knee arthroplasty. A retrospective study of the treatment of eighty-one infections. J Bone Joint Surg Am 1999;81(10):1434–45.

Sousa R, Pereira A, et al. Empirical antibiotic therapy in prosthetic joint infections. Acta Orthop Belg 2010;76(2):254–9.

Titecat M, Loiez C, et al. Evaluation of rapid mecA gene detection versus standard culture in staphylococcal chronic prosthetic joint infections. Diagn Microbiol Infect Dis 2012; 73(4):318–21.

Trampuz A, Piper KE, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007;357(7):654–63.

Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly 2005;135(17–18):243–51.

Trampuz A, Zimmerli W. Diagnosis and treatment of implant-associated septic arthritis and osteomyelitis. Curr Infect Dis Rep 2008;10(5):394–403.

Vergidis P, Greenwood-Quaintance KE, et al. Implant sonication for the diagnosis of prosthetic elbow infection. J Shoulder Elbow Surg 2011;20(8):1275–81.

Wolk DM, Struelens MJ, et al. Rapid detection of Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. J Clin Microbiol 2009;47(3):823–6.

Zimmerli W, Trampuz A, et al. Prosthetic-joint infections. N Engl J Med 2004;351(16): 1645–54.