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Validation of a novel molecular diagnostic panel for pediatric musculoskeletal infections: Integration of the Cepheid Xpert MRSA/SA SSTI and laboratory-developed real-time PCR assays for clindamycin resistance genes and *Kingella kingae* detection

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ARTICLE INFO

Summary of Main Point: A novel molecular diagnostic panel combining the commercially available Xpert MRSA/SA SSTI (Cepheid) and laboratory-developed real-time polymerase chain reactions (PCR) for the clindamycin resistance-inducing *erm* genes and *K. kingae* detection is a sensitive and specific diagnostic tool with potential to improve pathogen identification in pediatric musculoskeletal infections.

Keywords:
Osteomyelitis
Septic arthritis
Pediatric
Infection
Diagnostics

ABSTRACT

Background: Pathogen detection in pediatric patients with musculoskeletal infections relies on conventional bacterial culture, which is slow and can delay antimicrobial optimization. The ability to rapidly identify causative agents and antimicrobial resistance genes in these infections may improve clinical care.
Methods: Convenience specimens from bone and joint samples submitted for culture to Children's Hospital Colorado (CHCO) from June 2012 to October 2016 were evaluated using a "Musculoskeletal Diagnostic Panel" (MDP) consisting of the Xpert MRSA/SA SSTI real-time PCR (qPCR, Cepheid) and laboratory-developed qPCRs for *Kingella kingae* detection and *erm* genes A, B, and C which confer clindamycin resistance. Results from the MDP were compared to culture and antimicrobial susceptibility testing (AST) results.
Results: A total of 184 source specimens from 125 patients were tested. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert MRSA/SA SSTI compared to culture and AST results were 85%, 98%, 93%, and 95% respectively for MSSA and 82%, 100%, 100%, and 99% for MRSA. Compared to phenotypic clindamycin resistance in *S. aureus* isolates, the *erm* A, B, and C gene PCRs collectively demonstrated a sensitivity, specificity, PPV, and NPV of 80%, 96%, 67%, and 98%. In comparison to clinical truth, *Kingella* PCR had a sensitivity, specificity, PPV, and NPV of 100%, 99.5%, 100%, and 100%.
Conclusions: This novel MDP offers a rapid, sensitive, and specific option for pathogen detection in pediatric patients with musculoskeletal infections.

1. Introduction

Acute and chronic musculoskeletal (MSK) infections in children are a common cause of hospitalization, with an incidence of approximately 6 cases per 1000 pediatric hospitalizations (Dahl et al., 1998; Arnold et al., 2006). *Staphylococcus aureus* is the most commonly isolated pathogen in acute pediatric MSK infections (Arnold et al., 2006; Copley, 2009; Paakkonen et al., 2013; Peltola and Paakkonen, 2014; Arnold and Bradley, 2015; Spruiell et al., 2017). Among patients less than 4 years of age, *Kingella kingae* has been described as a common pathogen as well, though it is difficult to recover by culture (Yagupsky et al., 1993; Yagupsky and Dagan, 1997; Verdier et al., 2005; Yagupsky et al., 2011; Yagupsky, 2015). Pathogen identification in MSK infections allows for targeted antimicrobial therapy (Gafur et al., 2008; Funk and Copley, 2017). Current pathogen identification techniques rely on the culture of blood and source specimens; however culture fails to identify a pathogen in 21–68% of pediatric MSK infections (Arnold et al., 2006; Paakkonen et al., 2013; Arnold and Bradley, 2015; Spruiell et al.,

Abbreviations: CHCO, Children's Hospital Colorado; MDP, Musculoskeletal Diagnostic Panel; qPCR, real-time polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value

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2017). Additionally, culture takes 1–5 days to recover pathogens and often 24–72 h longer for antimicrobial susceptibility test (AST) results. Real-time polymerase chain reaction (qPCR) has increased the yield and speed of pathogen identification in other infectious diseases such as bacteremia, meningitis, and gastroenteritis (Altun et al., 2013, Binnicker, 2015, Leber et al., 2016, Messacar et al., 2016, Messacar et al., 2017, Murphy et al., 2017, Timbrook et al., 2017) and has been investigated for adult patients with MSK infections (Dubouix-Bourandy et al., 2011; Titecat et al., 2012; Valour et al., 2014; Lourtet-Hascoett et al., 2015; Sambri et al., 2017; Titecat et al., 2017). Given the characteristic epidemiology and bacterial resistance patterns for acute pediatric MSK infections, an ideal rapid diagnostic tool would target *S. aureus*, including methicillin and clindamycin resistance, as well as *K. kingae* (Arnold et al., 2006; Copley, 2009; Arnold and Bradley, 2015).

The Xpert MRSA/SA SSTI qPCR (Cepheid) is cleared by the U.S. Food and Drug Administration for rapid identification of *S. aureus* and genotypic detection of methicillin resistance from cutaneous and subcutaneous swabs, and has been evaluated in Europe for use with bone and joint specimens from adults with MSK infections (Wolk et al., 2009; Dubouix-Bourandy et al., 2011; Titecat et al., 2012; Valour et al., 2014; Sambri et al., 2017; Titecat et al., 2017). We designed and evaluated a novel musculoskeletal diagnostic panel (MDP) that incorporates three components: the Xpert MRSA/SA SSTI assay, a qPCR for the *erm* A, B, and C genes that confer clindamycin resistance among several bacterial species most notably *Staphylococcus aureus* (Weisblum, 1995; Leclercq, 2002; Woods, 2009), and a qPCR for *K. kingae*. This novel panel was specifically designed to provide rapid results for the common pathogens in children with MSK infections. The purpose of this study was to validate the clinical utility of this novel MDP.

2. Materials and methods

2.1. Patients and specimens

The study was conducted at the Children's Hospital Colorado's (CHCO) microbiology laboratory (Aurora, CO). CHCO is a 444-bed academic, quaternary care, free-standing children's hospital. Eligible specimens were from patients who had been seen at CHCO from June 2012 to December 2016 and had bacterial cultures of source specimens obtained from deep MSK sources (e.g. bone, joint, muscle, deep abscess). Some banked specimens were collected from patients who had bacterial cultures ordered but had low clinical suspicion for an underlying infectious process. After bacterial cultures and AST were performed for patient care, any remaining specimen from bone or joint source samples (convenience specimens) were banked and stored at −80 °C prior to validation testing. The electronic medical records for patients with banked specimens were retrospectively reviewed for demographic, clinical, and microbiologic data.

2.2. Conventional microbiological methods

Specimens were cultured aerobically on blood (TSA with sheep blood), MacConkey, and Chocolate agar as well as anaerobically (Brucella Anaerobic Agar and BBE/LKV Bi-plate). All specimens (tissues and aspirates) were also placed into a blood culture bottle (PedsPlus/F), termed “broth-enriched” culture, which was incubated for 5 days on a Bactec 9120/9240 prior to 2014 or Bactec FX from 2014 onward (Becton Dickinson, Sparks, MD). To be inoculated into blood culture bottles, firm tissue specimens (bone, synovial tissue, etc) were first ground in sterile saline using a closed tissue grinder system (Cardinal Health, Waukegan, IL, USA); an aspirate of the homogenized fluid was then used for inoculation. Bacteria isolated from aerobic, anaerobic, and “broth-enriched” culture techniques were identified using standard microbiology methods. We identified *Staphylococcus* species, enteric gram-negative bacteria, *Pseudomonas aeruginosa*, and *Enterococcus* spp. isolates by a Microscan panel prior to 8/2014 (Siemens Healthcare Diagnostics, Inc., West Sacramento, CA) and MALDI-TOF after 8/2014 (Bruker Corp., Billerica, MA). Nonfermenters, *Haemophilus* spp. and *Neisseria* spp. were identified using RapID NF and RapID NH tests (Remel Inc., Lenexa, KS). Methicillin resistance was determined by penicillin binding protein 2′ latex agglutination test (Oxoid, Basingstoke, UK) or MicroScan panel (Siemens Healthcare Diagnostics, Inc., West Sacramento, CA). Clindamycin susceptibilities were conducted using D-test by either disk diffusion method or MicroScan and were reported as directed by the Clinical and Laboratory Standards Institute (CLSI). A D-test was performed routinely on all *Staphylococcus aureus* isolates and a positive D-test was reported as “clindamycin resistant” and considered clindamycin resistant for the *erm* gene analysis.

2.3. Specimen processing for the MDP

Banked specimens were thawed at room temperature. Bone or synovial tissues were brought to 1 mL with sterile saline. Firm tissues (23/184, 13%) were ground in a closed tissue grinder system (Cardinal Health, Waukegan, IL, USA), vortexed for 60 s with glass beads (Baxter, McGaw Park, IL, USA), and large fragments were allowed to settle. Aspirates or synovial fluid were diluted with sterile saline to 500 µL, vortexed for 60 s, and centrifuged for 5 s at 2000 × *g*. All Xpert MRSA/SA SSTI testing was performed shortly after the above specimen processing. An aliquot of each supernatant was also removed and underwent DNA extraction for the *erm* A/B/C and *K. kingae* qPCRs. Extractions were performed on a Qiagen© EZ1 instrument, using the tissue kit with 200 µL input and 100 µL elution volumes. The nucleic acids were frozen at −80 °C for later testing using *erm* A/B/C and *K. kingae* qPCRs.

2.4. MDP testing and interpretation

The Xpert MRSA/SA SSTI component of the MDP is a self-contained, fully automated system for DNA extraction and qPCR that can also be performed on-demand. It detects genes for the staphylococcal protein A (*spa*), methicillin-resistance (*mecA*), the staphylococcal chromosomal cassette (SCC), and the internal sample processing control (SPC). Detection of spa, *mecA*, and the *SCC* indicates the presence of methicillin-resistant *S. aureus* (MRSA). Detection of spa without mecA, with or without SCC, indicates *S. aureus*. Negative SPC results reflect the presence of a qPCR inhibitor, so all results in such a case are considered invalid.

For the Xpert assay, the swab from the specimen collection kit (Copan, Murietta CA) was placed directly into each processed specimen fluid for 60 s, transferred to the kit elution buffer, then capped and vortexed for 10 s. The resulting solution was pipetted into the Xpert MRSA/SA SSTI test cartridge and tested on a GeneXpert Infinity instrument, which provides results in approximately 60 min. Per manufacturer instructions, specimens with invalid results were aspirated out of the test cartridge, transferred into a fresh cartridge, and re-run. If the second run was also invalid, no MRSA/SA results were included for that specimen.

The *erm* gene component of the MDP consisted of individual qPCR reactions for the *erm* A, *erm* B, and *erm* C genes. Primers and probes (Supplementary Table S1) were designed using the Primer3 program and assessed by NCBI BLASTN 2.7 analysis (He, 2016, O'Brien et al., 2016, Consortium, 2017, Sato et al., 2017). Each 25 µL reaction contained 5 µL of nucleic acid extract, 15.25 µL of master mix (Qiagen) containing 0.25 µL of an internal processing control (Applied Biosystems), 1 uL of each primer (all primer solutions at 10 µM), 1 µL of probe (all probe solutions at 5 µM), and 1.75 µL water. Thermocycling consisted of 50 °C for 2 min and 95 °C for 10 min, followed by 95 °C for 15 s and 60 °C for 60 s for a total of 45 cycles on an ABI 7500 thermocycler (Thermofisher). All specimens were tested in duplicate and considered positive if crossing points were 40 cycles or lower. Bacterial DNA containing or lacking *erm* A and *erm* B or the *erm* C plasmid served as

controls for each run.

The laboratory-developed *K. kingae* qPCR component of the MDP was designed to detect the *rtx*-A gene and initially assessed as described above. Reactions were similarly prepared and amplified, except *K. kingae*-specific primers and probes were used (Supplementary Table S1). All specimens were tested in duplicate and considered positive if crossing points were 38 cycles or lower. Reactions containing or lacking *K. kingae* 23,330 DNA were used as positive or negative controls, respectively. Personnel performing the *erm* and *K. kingae* PCRs were blinded to the Xpert, culture, and AST results.

2.5. MDP limit of detection

Analytical sensitivity (limit of detection, LOD) for the Xpert MRSA/SA SSTI assay was assessed by spiking serially-diluted spectroscopy-quantified MRSA strain 4330 and *S. aureus* 29,213 (American Type Culture Collection) into culture-negative joint fluid and homogenized bone tissue, adsorbing the material onto swabs and testing as described above. The LOD was defined as lowest number of bacteria per test detected by all 3 replicates of a dilution on multiple runs. Specificity was assessed by testing *K. kingae* 23,330 and 23,332, *Propionibacterium acnes* 11,827, *Staphylococcus epidermidis* 12,228; *Streptococcus pneumoniae* 49,615 and 799,677, and *Streptococcus pyogenes* 19,615.

Analytical sensitivities for the *erm* PCRs were established by testing culture-negative joint fluid and tissue containing serial dilutions of *S. aureus* BA977 which contains the *erm* A gene, *S. pneumoniae* 799,677 which contain *erm* B gene, or an *erm* C plasmid. Each LOD was defined as the fewest organisms per reaction detected by all 20 replicates of a dilution on multiple runs. Specificity was further established by testing *S. aureus* 29,213 which lacks the *erm*-A gene, *S. pneumoniae* 49,615 which lacks the *erm*-B gene, and the MRSA, *K. kingae*, *P. acnes*, *S. epidermidis*, and *S. pyogenes* strains listed above. Accuracy was further assessed by testing 20 banked clinical isolates of MRSA and *S. aureus* known by the D-test to express or lack inducible clindamycin resistance.

Analytical sensitivity for the *K. kingae* PCR was determined by testing culture-negative joint fluid and tissue containing serially-diluted *K. kingae* 23,330 on multiple run and was defined as the lowest number of bacteria per reaction detected in all 20 replicates of a dilution on multiple runs. *K. kingae* strain 23,332 was also tested. Specificity was established by testing MRSA, and the strains of *S. aureus*, *P. acnes*, *S. epidermidis*, *S. pneumoniae*, and *S. pyogenes* listed above.

2.6. Data analysis

Results from all assays and data extracted from a retrospective review of patient charts were collected and managed using REDCap electronic data capture tools hosted at Children's Hospital Colorado (Harris et al., 2009). Sensitivity, specificity, PPV and NPV for each component of the MDP were calculated based on each sample's original culture growth and antimicrobial susceptibility testing using standard statistical methods. Use of clinical specimens and data were approved by the Colorado Multiple Institutional Review Board.

3. Results

3.1. Patient demographics and culture results

During the evaluation period, there were a total of 488 musculoskeletal specimens from 340 patients submitted to the CHCO laboratory for culture. A total of 184 (38%) specimens on 125 (37%) unique patients had remaining convenience specimen banked and were included in the study (Fig. 1). The characteristics of patients included in the study are summarized in Table 1. Most (54%) were previously healthy, though 46% had conditions that predispose to infection, including implanted hardware, severe neurologic impairment, or immunosuppression; some patients had multiple comorbidities. Sixty-four percent had a diagnosis of osteomyelitis and/or septic arthritis consistent with deep musculoskeletal infection, 4% of patients were diagnosed with superficial skin infection or superficial abscess only. Twenty five percent were given a non-infectious diagnosis, mostly of rheumatologic disease.

3.2. Analytical sensitivity (LOD) results

The analytical sensitivity (LOD) of the Xpert MRSA/SA SSTI component was estimated at 10^3 *S. aureus* per test in joint fluid and bone tissue. The LOD of MRSA in joint fluid was approximately 10^4 bacteria per test but was only 10^6 per test in tissue. When lower amounts of MRSA were present in tissue specimens, Xpert testing detected *S. aureus* but failed to detect genotypic methicillin resistance. There was no detection of high concentrations (10^5 organisms per test) of *P. acnes*, *S. epidermidis*, *K. kingae*, *S. pneumoniae*, or *S. pyogenes* with the Xpert MRSA/SA SSTI analytical sensitivity testing.

Analytical sensitivities for the *erm* components of the panel were approximately 50 bacteria per reaction for the *erm* A PCR, 5 bacteria per reaction for the *erm* B PCR, and 1 copy per reaction for the *erm* C PCR in joint fluid and bone. High concentrations of *P. acnes*, *S. epidermidis*, *K. kingae*, *S. pyogenes* or clindamycin-susceptible *S. aureus* and *S. pneumoniae* were not detected during the analytical sensitivity testing of the *erm* PCR. In addition, all *erm* PCR results and D-test results (which indirectly indicate the presence of an *erm* gene) correlated perfectly for 10 clindamycin susceptible and 10 clindamycin resistant MRSA clinical isolates.

Analytical sensitivity of the *Kingella* PCR component of the panel was approximately 50 bacteria per reaction in joint fluid and 500 bacteria per reaction in bone. Reference *K. kingae* strain 2332 was also detected. There was no detection of high concentrations of MRSA, *S aureus*, *S. epidermidis*, *P. acnes*, *S. pyogenes*, or *S. pneumoniae* during the analytical sensitivity testing of the *K. kingae* PCR.

3.3. Comparison of culture to Xpert MRSA/SA SSTI

Xpert MRSA/SA SSTI qPCR results were valid on 182 (99%) of the 184 specimens evaluated. Two of the 184 samples were invalid on Xpert MRSA/SA SSTI assay after repeated attempts and were excluded from the Xpert MRSA/SA portion of the MDP analysis. In total, combined culture identified methicillin-sensitive *S. aureus* (MSSA) alone in 45 of the 182 (25%) source specimens and methicillin-resistant *S. aureus* (MRSA) alone in 10/182 (5%) of source specimens (Fig. 1, Tables 2 and 3a). Three specimens with MSSA and 1 specimen with MRSA grew multiple bacterial pathogens; these 4 polymicrobial specimens were considered positive for MSSA or MRSA for comparison with the Xpert test.

Of the 48 total specimens with MSSA identified by culture, 41 (85%) had *S. aureus* detected by Xpert MRSA/SA SSTI PCR assay. Of the 7 specimens with MSSA identified on culture that did not have *S. aureus* detected by PCR, 4/7 specimens (57%) had MSSA isolated on broth-enriched culture alone (Fig. 1, Table 3a). Three of these 4 had been pretreated with antimicrobials within 72 h of source culture collection. Two specimens that were negative for *S. aureus* on combined culture were positive for *S. aureus* by Xpert MRSA/SA SSTI assay. Both specimens came from the same patient with culture-confirmed MSSA musculoskeletal infection 1-month prior at the same anatomic site. The Xpert assay detected 9 (82%) of the 11 specimens that grew MRSA, including one which was polymicrobial (Fig. 1, Table 3a). One (9%) of the 11 MRSA culture-positive specimens was positive for the *spa* target but neither mecA or SCC, indicating the presence of *S. aureus* but not MRSA. One (9%) of the 11 specimens positive for MRSA only in broth-enriched culture was negative by PCR for all targets.

Overall, comparison of the Xpert MRSA/SA SSTI assay to combined culture results and AST resulted in a sensitivity and specificity of 85.4/98% for MSSA and 81.8/100% for MRSA. Sensitivity increased to 93%

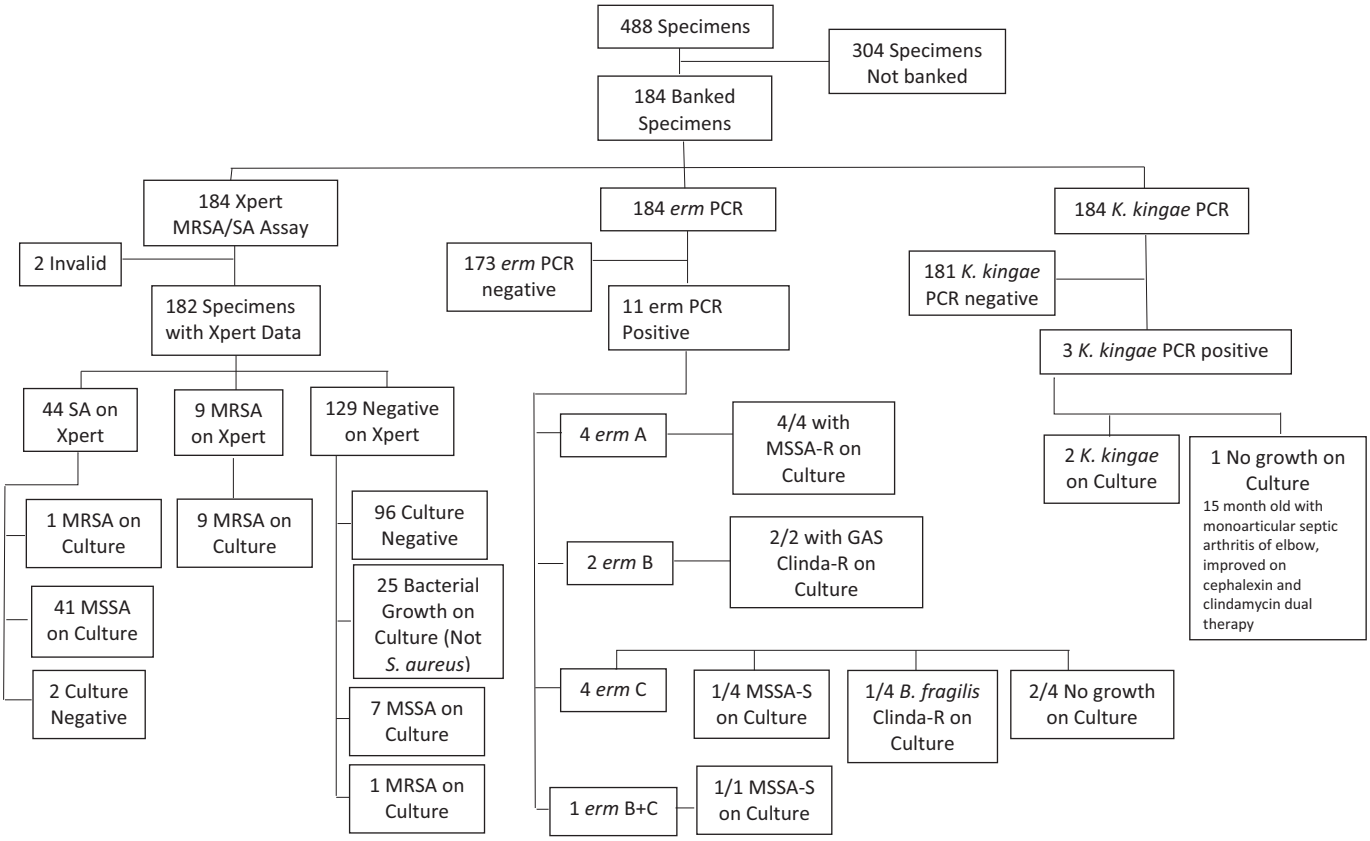

Fig. 1. Musculoskeletal diagnostic panel testing on included specimens.

Table 1
Patient and Specimen Characteristics.

Demographics	*N* = 125 Patients	%
Male	79	63%
Age (Median)	10.6 years	
Comorbidities	N = 125 Patients	
None	67	54%
Rheumatologic Diagnosis	22	18%
Underlying Bone Disease	16	13%
Preceding Trauma	13	10%
MSK Hardware in Place	13	10%
Severe Neurologic Impairment	9	7%
Immunosuppressed	7	6%
Specimen Type	*N* = 184 Specimens	%
Bone Tissue/Bone Biopsy Tract Aspirate	73	40%
Synovial Fluid	70	38%
Soft Tissue Abscess	15	8%
Synovial Tissue	8	4%
Subperiosteal Abscess	8	4%
Soft Tissue Mass/Cyst	7	4%
Muscle Aspirate	3	2%
Antimicrobial Pretreatment	N = 184 Specimens	
Antibiotic Exposure within 72 h of Sample Collection	60	33%
Discharge Diagnoses	*N* = 128 Admissions	
Osteomyelitis	42	33%
Septic Arthritis	22	17%
OM + SA	18	14%
Chronic Recurrent Multifocal Osteomyelitis	16	13%
Non-Infectious	24	19%
Superficial Skin Infection	3	2%
Superficial Abscess Only	3	2%

Table 2
Culture results.

Pathogen	N = 184	%
Staphylococcus aureus	56	30%
MSSA-Clindamycin Sensitive	42	23%
MRSA-Clindamycin Sensitive	9	5%
MSSA-Clindamycin Resistant	4	2%
MRSA-Clindamycin Resistant	1	0.5%
Other Bacteria	13*	7%
Polymicrobial	9**	5%
Group A *Streptococcus*	7	4%
Kingella kingae	2	1%
Culture Negative	97	53%

* Other bacteria detected only by culture were Coagulase-negative *Staphylococcus* (3), *Abiotrophia* spp. (1), *B. fragilis* (1), *Bifidobacterium* spp. (1), *Coccidioides immitis* (1), *E. coli* (1), *Enterobacter cloacae* (1), *Fusobacterium* spp. (1), *Moraxella* spp. (1), *P. acnes* (1), *S. anginosus* (1).

** 4/9 polymicrobial cultures included *S. aureus* (3 MSSA and 1 MRSA).

for MSSA and 90% for MRSA when specimens positive only in broth-enriched cultures were considered culture negative (Table 3a).

3.4. *erm* A/B/C gene PCR

Results for *erm* A/B/C PCR were valid on all 184 specimens. Five of the 184 (3%) samples had a clindamycin-resistant *S. aureus* identified by AST (Table 2). Eleven of the 184 (6%) samples were positive for an *erm* gene by PCR, 4/11 (36%) of which had *erm*A detected (Table 3b, Fig. 1). All *erm*A positive samples were from patients who had clindamycin resistant MSSA by AST. Two of the 11 *erm* PCR-positive specimens had *erm*B identified, both of which had clindamycin-resistant *Streptococcus pyogenes* identified on culture suggesting appropriate identification of *erm*B. Four of the 11 (36%) *erm* PCR-positive samples

Table 3a
Comparison of Xpert MRSA/SA SSTI Assay to Culture Results.

	Combined Culture Results					Xpert MRSA/SA SSTI Assay Performance			
		MSSA	MRSA	Non-SA Bacterial Growth	No Growth	Total	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Xpert MRSA/SA SSTI qPCR Results	SA	41	1	2	0	44	85.4% (72–94%)	98% (94–100%)	93% (81–98%)	95% (90–97%)
	MRSA	0	9	0	0	9	81.8% (48–98%)	100% (98–100%)	100% (100%)	98.9% (96–100%)
	Negative	7	1	25	96	129				
	Total	48	11	27	96	182				

had *erm*C detected, none of which grew clindamycin-resistant *S. aureus*. One of these 4 (25%) *erm*C positive samples grew clindamycin-resistant *Bacteroides fragilis* and 2/4 (50%) were culture negative. The fourth *erm*C positive specimen had clindamycin-susceptible MSSA isolated. Finally, one of the 11 (9%) *erm* PCR-positive samples had both *erm*B and *erm*C detected, although the culture yielded clindamycin-susceptible MSSA. One of the 5 (20%) specimens with clindamycin-resistant *S. aureus* did not have any of the three *erm* genes identified. Only the broth culture of this specimen grew, and Gram-positive cocci in groups (GPCs) were identified; this organism was presumed to be clindamycin-resistant MSSA which was not further identified and did not undergo separate AST as the patient had previously grown this organism from two other anatomic sites. The other two clindamycin-resistant MSSA specimens from this same patient had *ermA* detected by PCR. For our MDP, the *erm* A/B/C qPCR component was envisioned as being most useful in the common clinical scenario of isolating *S. aureus* (both MSSA and MRSA) from culture of an MSK source specimen. The performance of the *erm*A/B/C PCR on *S. aureus* positive samples alone collectively demonstrated a sensitivity, specificity, positive and negative predictive value of 80%, 96%, 67%, and 98% respectively (Table 3b).

3.5. *Kingella kingae* PCR

K. kingae PCR results were valid on all 184 samples tested. Of these, only 2/184 (1%) samples had *K. kingae* identified by culture, both of which had *K. kingae* appropriately identified by PCR testing. A third specimen had a positive *K. kingae* PCR but was culture negative (Fig. 1, Table 3c). Compared to culture, the *K. kingae* PCR had a sensitivity, specificity, positive and negative predictive value of 100%, 99.5%, 66%, and 100% respectively (Table 3c).

4. Discussion

Decreasing the time to pathogen identification and susceptibility for pediatric musculoskeletal infections can lead to faster appropriate antimicrobial therapy and improved clinical outcomes (Copley, 2009; Paakkonen et al., 2013; Peltola and Paakkonen, 2014; Spruiell et al., 2017). PCR-based diagnostic tools that target common bacteria have shown promise in adult osteoarticular infections (Dubouix-Bourandy et al., 2011; Titecat et al., 2012; Valour et al., 2014; Lourtet-Hascoett et al., 2015; Sambri et al., 2017; Titecat et al., 2017). In particular, the Xpert MRSA/SA SSTI assay has been evaluated on perioperative bone and joint specimens in adult patients with excellent sensitivity and specificity for MSSA, MRSA, and methicillin-resistant coagulase-negative *Staphylococcus* (100% and 98.3%, 100% and 100%, and 100% and 95.3% respectively) (Dubouix-Bourandy et al., 2011). This study demonstrates encouraging performance of our Musculoskeletal Diagnostic Panel (MDP) to rapidly detect MSSA, MRSA, and *K. kingae*, with considerable clinical utility among pediatric patients.

The Xpert MRSA/SA SSTI component had an 85% and 82% sensitivity for *S. aureus* and MRSA respectively, compared to combined culture. These values are slightly lower than the reported sensitivity (94.4–100%) of the assay compared to culture on solid media in adult osteoarticular infections or the 96–100% positive agreement when testing swabs from skin and soft tissue infections (the FDA-cleared use of the assay) (Wolk et al., 2009; Dubouix-Bourandy et al., 2011; Valour et al., 2014). This somewhat lower sensitivity in our study is most likely due to including broth as a component of our combined culture technique. Indeed, broth culture has been shown to increase sensitivity for pathogen detection compared to culture on solid media alone in adult osteoarticular infections (Blackmur et al., 2014). When broth culture growth alone is considered culture negative, the sensitivity of the Xpert MRSA/SA SSTI for *S. aureus* and MRSA increases (93% and 90% respectively), and approaches results reported in adult patients. The high sensitivity of broth-enriched culture described in this study suggests that inoculation of bone and joint specimens into blood culture bottles offers the most sensitive, although not rapid, means to pathogen identification and should be continued as part of the diagnostic workup for these valuable specimens. In addition, the analytical sensitivity of the Xpert MRSA/SA assay for detection of MRSA in bone tissue was lower than for joint fluid and for *S. aureus* in bone or joint samples. This issue likely contributed to the lower sensitivity we observed for MRSA with the MDP.

A further advantage of the Xpert MRSA/SA assay is its ability to rapidly infer the presence of MSSA, MRSA, and methicillin-resistant coagulase negative staphylococci based on the amplification profiles of the 3 gene targets (*spa*, *mecA*, and SCC). Although the test is designed and cleared to report only *S. aureus* and MRSA, genotypic presence of *S. aureus* in the absence of genotypic methicillin resistance suggests presence of MSSA. Among our isolates, 41/42 samples that had *spa* alone detected with no genotypic methicillin resistance on Xpert MRSA/SA assay were phenotypically identified as MSSA on standard culture techniques. Only 2/182 (1%) of specimens were identified on Xpert MRSA/SA assay as *S. aureus* that did not have MSSA/MRSA on original culture results. However, both specimens were from the same patient who had been admitted 1-month prior with a MSSA musculoskeletal infection, suggesting that this “false positive” result from the Cepheid testing was likely a “true positive.” Although the Xpert MRSA/SA SSTI test was not more sensitive than standard microbiologic culture, it clearly provides reliable rapid pathogen identification within several hours of sample collection and allows for detection of *S. aureus* in patients who have been pretreated with antibiotics prior to obtaining cultures (33% of specimens in this cohort). Overall, our retrospective results suggest that the Xpert MRSA/SA SSTI assay has considerable utility for rapid pathogen identification in pediatric MSK infections, although further studies are needed to determine its potential clinical impact in the routine care of such patients.

There is increasing interest in using molecular diagnostic techniques to provide antimicrobial susceptibility results. Detection of clindamycin resistance among acute MSK pathogens is clinically important as clindamycin is a frequently used oral antimicrobial option to treat acute MSK infections. Therefore, genotypic evaluation of the *erm* gene, which infers the MLS-B phenotype, was included in the MDP (Weisblum, 1995; Leclercq, 2002; Woods, 2009). The *erm*A and *erm*B PCRs both had

Table 3b
Comparison of *erm*A/B/C PCR to Antimicrobial Susceptibility Testing.

		Clindamycin Resistance Results			*erm* A/B/C PCR Performance			
		Clindamycin Resistant	No Clindamycin Resistance	Total	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
erm A/B/C qPCR Results	*erm* Gene Detected	7	4	11	88% (47–99%)	98% (94–100%)	64% (39–83%)	99% (96–100%)
	erm Gene Not Detected	1	172	173				
	Total	8	176	184				

promising results in our study. Of the 11 *erm*-positive specimens, three specimens had pathogens other than *S. aureus* identified on combined culture. Two *erm*B positive samples were from cultures that had clindamycin-resistant *Streptococcus pyogenes* identified. *S. pyogenes* is well-described as having the ability to obtain the *erm*B gene to express MLS-B phenotypic resistance (Plainvert et al., 2015; Michos et al., 2016). One *erm*C specimen was found on combined culture to have clindamycin-resistant *Bacteroides fragilis*. There are descriptions of *B. fragilis* obtaining an *erm*F gene; suggesting that the positive *erm*C PCR in this sample was cross-reacting with an *ermF* gene in this clindamycin-resistant *B. fragilis* sample (Quesada-Gomez, 2011; Plainvert et al., 2015; Michos et al., 2016; Soki et al., 2016). Since all three non-*S. aureus* isolates were phenotypically clindamycin-resistant pathogens that have had documented ability for *erm* gene acquisition, these 3/11 isolates were considered “true positives” in our statistical analysis.

The *erm*C gene PCR did not perform well; none of the 5 samples with *erm*C detected had MSSA-R/MRSA-R on combined culture. There are several possible explanations for the *erm*C gene assay's low PPV. It is possible that genotypic presence of an *erm* gene does not always confer phenotypic clindamycin-resistance (Arthur et al., 1987; Weisblum, 1995; Maravic, 2004; Woods, 2009; Pereira et al., 2016). While *erm* genes are known to be expressed in either a constitutive or inducible manner, standard microbiologic testing using a D-test to assess inducible resistance (CLSI gold standard testing) was negative on the MSSA-S/MRSA-S samples in which *erm*C was detected. To our knowledge, the description of an *erm* gene repressor mechanism has not been previously described (Woods, 2009). Although designed to minimize this possibility, the *erm*C primers might have cross-reacted with a sequence other than the *erm*C target in these specimens.

The final component of our MDP, the *K. kingae* PCR, had excellent sensitivity, specificity, positive and negative predictive values when compared to clinical truth, although only three *K. kingae* PCR-positive specimens were identified. One of these specimens was culture-negative but obtained from a 15-month old patient with septic arthritis of the elbow and adjacent osteomyelitis of the radius who improved with dual therapy of cephalexin and clindamycin. This clinical scenario is consistent with a *K. kingae* MSK infection. Using clinical truth as supportive evidence, our study adds further support to previous reports that PCR is a more sensitive assay for diagnosing *K. kingae* infections (Verdier et al., 2005; Chometon et al., 2007; Yagupsky, 2015). The overall *K. kingae* prevalence in our population, however, was surprisingly low given epidemiologic descriptions of *K. kingae* from 7 to 65% among patients less than 4 years of age with acute MSK infections (Yagupsky et al., 1993; Yagupsky and Dagan, 1997; Yagupsky et al., 2011; Yagupsky, 2015). Our low prevalence of *K. kingae* can partially be explained by including specimens from patients diagnosed with non-infectious processes. In addition, as samples were from a subset of all MSK patients admitted to CHCO during this time, disproportionate banking of samples from older children may have falsely decreased the *K. kingae* prevalence. Twenty nine of the 125 patients were less than or equal to 4 years of age, of which 20 had an acute MSK infection. Limiting our analysis to this subset of patients, 3/20 (15%) had *K. kingae* identified by culture and/or MDP PCR, which is more consistent with published *K. kingae* reviews in this age group. Alternatively, significant regional variations in *K. kingae* epidemiology might exist and our results may reflect a lower prevalence of *K. kingae* musculoskeletal infections than has been described elsewhere.

When considering the utility of the MDP as a collective diagnostic tool, our findings suggest that incorporation of the Xpert MRSA/SA SSTI assay with the *K. kingae* PCR could offer a rapid and reliable method for microbiological testing in pediatric patients with MSK infections. Based on the low PPV for the *erm*C component in particular, the *erm* A/B/C PCR testing will require further evaluation before inclusion in the MDP diagnostic tool.

This study had several limitations. The MDP was performed on banked, frozen specimens which may have resulted in decreased assay

Table 3c
Comparison of *Kingella kingae* PCR and Culture Results.

	K. kingae Combined Culture Results				*K. kingae* PCR Performance			
		K. kingae Isolated	No *K. kingae* Isolated	Total	Sensitivity	Specificity	PPV	NPV
K. kingae qPCR Results	*K. kingae* PCR Positive	2	1	3	100%	99.5%	66%	100%
	K. kingae PCR Negative	0	181	181				
	Total	2	182	184				

performance compared to using fresh specimens. Similarly, specimens with low volumes were diluted to obtain appropriate sample volumes required for our assays which may have decreased sensitivity. Similarly, because the study was limited to specimens that had remnants remaining, our results may not reflect the performance of MDP in the entire pediatric population with MSK infections. As we relied on availability of convenience specimens, there is the potential for selection bias towards larger specimens from likely older patients which may not be truly representative of acute pediatric musculoskeletal infections.

5. Conclusion

Overall our MDP, particularly the Xpert MRSA/SA SSTI and *Kingella*-specific PCR components, demonstrated excellent sensitivity and specificity. Importantly, these assays can provide pathogen detection up to 3 days sooner than culture and may have considerable value as adjunctive tools to detect the most common pathogens in children with MSK infections (Dubouix-Bourandy et al., 2011). Prospective studies are needed to assess the usefulness of these assays to diagnose MSK infections in children and their impact on clinical care.

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References

Altun, O., Almuhayawi, M., Ullberg, M., Ozenci, V., 2013. Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J. Clin. Microbiol. 51 (12), 4130–4136.
Arnold, J.C., Bradley, J.S., 2015. Osteoarticular infections in children. Infect. Dis. Clin. N. Am. 29 (3), 557–574.
Arnold, S.R., Elias, D., Buckingham, S.C., Thomas, E.D., Novais, E., Arkader, A., Howard, C., 2006. Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant Staphylococcus aureus. J. Pediatr. Orthop. 26 (6), 703–708.
Arthur, M., Brisson-Noel, A., Courvalin, P., 1987. Origin and evolution of genes specifying resistance to macrolide, lincosamide and streptogramin antibiotics: data and hypotheses. J. Antimicrob. Chemother. 20 (6), 783–802.
Binnicker, M.J., 2015. Multiplex molecular panels for diagnosis of gastrointestinal infection: performance, result interpretation, and cost-effectiveness. J. Clin. Microbiol. 53 (12), 3723–3728.
Blackmur, J.P., Tang, E.Y., Dave, J., Simpson, A.H., 2014. Use of broth cultures perioperatively to optimise the microbiological diagnosis of musculoskeletal implant infections. Bone Joint J. 96-B (11), 1566–1570.
Chometon, S., Benito, Y., Chaker, M., Boisset, S., Ploton, C., Berard, J., Vandenesch, F., Freydiere, A.M., 2007. Specific real-time polymerase chain reaction places Kingella kingae as the most common cause of osteoarticular infections in young children. Pediatr. Infect. Dis. J. 26 (5), 377–381.
Consortium, U.G., 2017. Urmite.
Copley, L.A., 2009. Pediatric musculoskeletal infection: trends and antibiotic recommendations. J. Am. Acad. Orthop. Surg. 17 (10), 618–626.
Dahl, L.B., Hoyland, A.L., Dramsdahl, H., Kaaresen, P.I., 1998. Acute osteomyelitis in children: a population-based retrospective study 1965 to 1994. Scand. J. Infect. Dis. 30 (6), 573–577.
Dubouix-Bourandy, A., de Ladoucette, A., Pietri, V., Mehdi, N., Benzaquen, D., Guinand, R., Gandois, J.M., 2011. Direct detection of Staphylococcus osteoarticular infections by use of Xpert MRSA/SA SSTI real-time PCR. J. Clin. Microbiol. 49 (12), 4225–4230.
Funk, S.S., Copley, L.A., 2017. Acute hematogenous osteomyelitis in children: pathogenesis, diagnosis, and treatment. Orthop. Clin. North Am. 48 (2), 199–208.
Gafur, O.A., Copley, L.A., Hollmig, S.T., Browne, R.H., Thornton, L.A., Crawford, S.E., 2008. The impact of the current epidemiology of pediatric musculoskeletal infection on evaluation and treatment guidelines. J. Pediatr. Orthop. 28 (7), 777–785.
Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., Conde, J.G., 2009. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J. Biomed. Inform. 42 (2), 377–381.
He, S.A.Z.F., 2016. Distributional Pattern and Adaptive Evolution of Antibiotic-Resistent *Staphylococcus aureus* From Different Cities in China.
Leber, A.L., Everhart, K., Balada-Llasat, J.M., Cullison, J., Daly, J., Holt, S., Lephart, P., Salimnia, H., Schreckenberger, P.C., Desjarlais, S., Reed, S.L., Chapin, K.C., Leblanc, L., Johnson, J.K., Soliven, N.L., Carroll, K.C., Miller, J.A., Dien Bard, J., Mestas, J., Bankowski, M., Enomoto, T., Hemmert, A.C., Bourzac, K.M., 2016. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. J. Clin. Microbiol. 54 (9), 2251–2261.
Leclercq, R., 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. 34 (4), 482–492.
Lourtet-Hascoett, J., Bicart-See, A., Felice, M.P., Giordano, G., Bonnet, E., 2015. Is Xpert MRSA/SA SSTI real-time PCR a reliable tool for fast detection of methicillin-resistant coagulase-negative staphylococci in periprosthetic joint infections? Diagn. Microbiol. Infect. Dis. 83 (1), 59–62.
Maravic, G., 2004. Macrolide resistance based on the Erm-mediated rRNA methylation. Curr. Drug Targets Infect Disord. 4 (3), 193–202.
Messacar, K., Breazeale, G., Robinson, C.C., Dominguez, S.R., 2016. Potential clinical impact of the film array meningitis encephalitis panel in children with suspected central nervous system infections. Diagn. Microbiol. Infect. Dis. 86 (1), 118–120.
Messacar, K., Hurst, A.L., Child, J., Campbell, K., Palmer, C., Hamilton, S., Dowell, E., Robinson, C.C., Parker, S.K., Dominguez, S.R., 2017 Sep. Clinical impact and provider acceptability of real-time antimicrobial stewardship decision support for rapid diagnostics in children with positive blood culture results. J. Pediatric Infect. Dis. Soc. 6 (3), 267–274.
Michos, A., Koutouzi, F.I., Tsakris, A., Chatzichristou, P., Koutouzis, E.I., Daikos, G.L., Stathi, A., Syriopoulou, V.P., 2016. Molecular analysis of Streptococcus pyogenes macrolide resistance of paediatric isolates during a 7 year period (2007–13). J. Antimicrob. Chemother. 71 (8), 2113–2117.
Murphy, C.N., Fowler, R.C., Iwen, P.C., Fey, P.D., 2017. Evaluation of the BioFire FilmArray(R) Gastrointestinalpanel in a midwestern academic hospital. Eur. J. Clin. Microbiol. Infect. Dis. 36 (4), 747–754.
O'Brien, F.G., Zaini, Z., Grubb, W.B., 2016. The Complete Sequence of Plasmid pWBG738 from the ST1-MRSA-IV Lineage of Community Methicillin-Resistant *Staphylococcus aureus*.
Paakkonen, M., Kallio, M.J., Kallio, P.E., Peltola, H., 2013. Significance of negative Cultures in the Treatment of Acute Hematogenous Bone and Joint Infections in Children. J. Pediatric Infect Dis. Soc. 2 (2), 119–125.
Peltola, H., Paakkonen, M., 2014. Acute osteomyelitis in children. N. Engl. J. Med. 370 (4), 352–360.
Pereira, J.N., Rabelo, M.A., Lima, J.L., Neto, A.M., Lopes, A.C., Maciel, M.A., 2016. Phenotypic and molecular characterization of resistance to macrolides, lincosamides and type B streptogramin of clinical isolates of Staphylococcus spp. of a university hospital in Recife, Pernambuco, Brazil. Braz. J. Infect. Dis. 20 (3), 276–281.
Plainvert, C., Martin, C., Loubinoux, J., Touak, G., Dmytruk, N., Collobert, G., Fouet, A., Ploy, M.C., Poyart, C., 2015. Highly virulent M1 Streptococcus pyogenes isolates

resistant to clindamycin. Med. Mal. Infect. 45 (11 − 12), 470–474.
Quesada-Gomez, C., 2011. Bacteroides mobilizable and conjugative genetic elements: antibiotic resistance among clinical isolates. Rev. Esp. Quimioter 24 (4), 184–190.
Sambri, A., Pignatti, G., Romagnoli, M., Donati, D., Marcacci, M., Cadossi, M., 2017. Intraoperative diagnosis of Staphylococcus aureus and coagulase-negative Staphylococcus using Xpert MRSA/SA SSTI assay in prosthetic joint infection. New Microbiol. 40 (2).
Sato, T., Wada, T., Ohkoshi, Y., Murabayashi, H., Takakuwa, Y., Nishiyama, Y., Nakajima, C., Suzuki, Y., Yokota, S., 2017. Complete Genome Sequence of Invasive Multidrug-Resistant Streptococcus Pneumoniae Serotype19F in Sapporo, Japan.
Soki, J., Hedberg, M., Patrick, S., Balint, B., Herczeg, R., Nagy, I., Hecht, D.W., Nagy, E., Urban, E., 2016. Emergence and evolution of an international cluster of MDR Bacteroides fragilis isolates. J. Antimicrob. Chemother. 71 (9), 2441–2448.
Spruiell, M.D., Searns, J.B., Heare, T.C., Roberts, J.L., Wylie, E., Pyle, L., Donaldson, N., Stewart, J.R., Heizer, H., Reese, J., Scott, H.F., Pearce, K., Anderson, C.J., Erickson, M., Parker, S.K., 2017 Sep. Clinical care guideline for improving pediatric acute musculoskeletal infection outcomes. J. Pediatric Infect. Dis. Soc. 6 (3), e86–e93.
Timbrook, T.T., Morton, J.B., McConeghy, K.W., Caffrey, A.R., Mylonakis, E., Laplante, K.L., 2017. The effect of molecular rapid diagnostic testing on clinical outcomes in bloodstream infections: a systematic review and meta-analysis. Clin. Infect. Dis. 64 (1), 15–23.
Titecat, M., Loiez, C., Senneville, E., Wallet, F., Dezeque, H., Legout, L., Migaud, H., Courcol, R.J., 2012. Evaluation of rapid mecA gene detection versus standard culture in staphylococcal chronic prosthetic joint infections. Diagn. Microbiol. Infect. Dis. 73 (4), 318–321.
Titecat, M., Wallet, F., Robineau, O., Valette, M., Migaud, H., Senneville, E., Loiez, C., 2017. Focus on MRSA/SA SSTI assay failure in prosthetic joint infections: 213 consecutive patients later. J. Clin. Microbiol. 55 (2), 635–637.
Valour, F., Blanc-Pattin, V., Freydiere, A.M., Bouaziz, A., Chanard, E., Lustig, S., Ferry, T., Laurent, F., Lyon Bone Joint Infection Study, G., 2014. Rapid detection of *Staphylococcus aureus* and methicillin resistance in bone and joint infection samples: evaluation of the GeneXpert MRSA/SA SSTI assay. Diagn. Microbiol. Infect. Dis. 78 (3), 313–315.
Verdier, I., Gayet-Ageron, A., Ploton, C., Taylor, P., Benito, Y., Freydiere, A.M., Chotel, F., Berard, J., Vanhems, P., Vandenesch, F., 2005. Contribution of a broad range polymerase chain reaction to the diagnosis of osteoarticular infections caused by Kingella kingae: description of twenty-four recent pediatric diagnoses. Pediatr. Infect. Dis. J. 24 (8), 692–696.
Weisblum, B., 1995. Erythromycin resistance by ribosome modification. Antimicrob. Agents Chemother. 39 (3), 577–585.
Wolk, D.M., Struelens, M.J., Pancholi, P., Davis, T., Della-Latta, P., Fuller, D., Picton, E., Dickenson, R., Denis, O., Johnson, D., Chapin, K., 2009. 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. 47 (3), 823–826.
Woods, C.R., 2009. Macrolide-inducible resistance to clindamycin and the D-test. Pediatr. Infect. Dis. J. 28 (12), 1115–1118.
Yagupsky, P., 2015. Kingella kingae: carriage, transmission, and disease. Clin. Microbiol. Rev. 28 (1), 54–79.
Yagupsky, P., Dagan, R., 1997. Kingella kingae: an emerging cause of invasive infections in young children. Clin. Infect. Dis. 24 (5), 860–866.
Yagupsky, P., Dagan, R., Howard, C.B., Einhorn, M., Kassis, I., Simu, A., 1993. Clinical features and epidemiology of invasive Kingella kingae infections in southern Israel. Pediatrics 92 (6), 800–804.
Yagupsky, P., Porsch, E., St Geme 3rd, J.W., 2011. Kingella kingae: an emerging pathogen in young children. Pediatrics 127 (3), 557–565.