Irish Journal of Medical Science (1971 -) (2019) 188:1289–1295
https://doi.org/10.1007/s11845-019-01973-1

ORIGINAL ARTICLE

The role of real-time PCR testing in the investigation of paediatric patients with community-onset osteomyelitis and septic arthritis

Sadhbh O'Rourke[1] · Mary Meehan[2] · Désirée Bennett[2] · Nicola O'Sullivan[2] · Robert Cunney[2,3] · Patrick Gavin[4] · Roisin McNamara[5] · Noelle Cassidy[6] · Stephanie Ryan[7] · Kathryn Harris[8] · Richard Drew[2,9,10]

Received: 9 December 2018 / Accepted: 19 January 2019 / Published online: 31 January 2019

Abstract

Background Culture yield in osteomyelitis and septic arthritis is low, emphasising the role for molecular techniques.
Aims The purpose of this study was to review the laboratory investigation of childhood osteomyelitis and septic arthritis.
Methods A retrospective review was undertaken in an acute tertiary referral paediatric hospital from January 2010 to December 2016. Cases were only included if they had a positive culture or bacterial PCR result from a bone/joint specimen or blood culture, or had radiographic evidence of osteomyelitis.
Results Seventy-eight patients met the case definition; 52 (66%) were male. The median age was 4.8 years. Blood cultures were positive in 16 of 56 cases (29%), with 11 deemed clinically significant (*Staphylococcus aureus* = 8, group A *Streptococcus* = 3). Thirty-seven of 78 (47%) bone/joint samples were positive by culture with *S. aureus* ($n = 16$), coagulase-negative *Staphylococcus* ($n = 9$) and group A *Streptococcus* ($n = 4$), being the most common organisms. Sixteen culture-negative samples were sent for bacterial PCR, and four were positive (*Kingella kingae* = 2, *Streptococcus pneumoniae* = 1, group A *Streptococcus* = 1).
Conclusions Sequential culture and PCR testing can improve the detection rate of causative organisms in paediatric bone and joint infections, particularly for fastidious microorganisms such as *K. kingae*. PCR testing can be reserved for cases where culture is negative after 48 h. These results have been used to develop a standardised diagnostic test panel for bone and joint infections at our institution.

Keywords Osteomyelitis · Paediatric · Polymerase chain reaction · Septic arthritis

Introduction

Osteomyelitis and septic arthritis in children can still lead to significant morbidity and lifelong disability, so early diagnosis and appropriate management are important in order to prevent and reduce complications [1, 2]. Obtaining a microbiological diagnosis is important to guide treatment. Bone and joint infections in children are primarily haematogenous in origin. However, the diagnostic yield with traditional microscopy and culture of blood or operative specimens is low, emphasising the role for new molecular techniques to improve identification of the responsible pathogens. While *Staphylococcus aureus* is

✉ Sadhbh O'Rourke
orourkemicro@gmail.com

[1] Department of Clinical Microbiology, Temple Street Children's University Hospital, Dublin 1, Ireland

[2] Irish Meningitis and Sepsis Reference Laboratory, Temple Street Children's University Hospital, Dublin 1, Ireland

[3] Health Protection Surveillance Centre, Dublin 1, Ireland

[4] Department of Infectious Diseases, Temple Street Children's University Hospital, Dublin 1, Ireland

[5] Emergency Department, Temple Street Children's University Hospital, Dublin 1, Ireland

[6] Department of Orthopaedics, Temple Street Children's University Hospital, Dublin 1, Ireland

[7] Department of Radiology, Temple Street Children's University Hospital, Dublin 1, Ireland

[8] Department of Microbiology, Virology and Infection Prevention and Control, Great Ormond Street NHS Foundation Trust, London, UK

[9] Department of Clinical Microbiology, Royal College of Surgeons, Dublin 2, Ireland

[10] Clinical Innovation Unit, Rotunda Hospital, Dublin 1, Ireland

generally considered to be the most common causative organism implicated, improvements in traditional culture techniques and the availability of molecular testing methods are changing our understanding of the aetiology of these infections [3–6]. The frequency of identification of oropharyngeal flora such as *Kingella kingae* as a causative pathogen particularly in infants and young children has increased [7, 8]. There is little data regarding causative organisms of paediatric bone and joint infections in the UK and Ireland and, as a consequence, no definitive national guidelines; however, recent European guidelines have been produced [9]. The primary aim of the study was to evaluate the current practice of laboratory investigation of children with suspected osteomyelitis or septic arthritis in a tertiary paediatric hospital and to use the data obtained to inform a national microbiology diagnostic algorithm-incorporating PCR requesting guidelines for the Irish Meningitis and Sepsis Reference Laboratory (IMSRL).

Methods

Study design

A retrospective review was performed in order to evaluate the assessment and diagnosis of paediatric osteomyelitis or septic arthritis cases admitted to Temple Street Children's University Hospital, Dublin (TSCUH) over a 7-year period, from January 2010 to December 2016. TSCUH is an acute tertiary referral paediatric hospital which cares for approximately 145,000 children per year with approximately 55,000 Emergency Department attendances. The hospital has an on-site 24-h-a-day microbiology laboratory. The IMSRL is also located on-site and provides a national non-culture diagnostic service using real-time PCR-based testing for a range of invasive disease-associated bacterial pathogens.

Selection of cases

Cases reviewed in this study were identified via the Emergency Department Electronic Patient Record System (Symphony®, EMIS Health, Leeds, UK). A retrospective search was performed for cases presenting to the Emergency Department at TSCUH using the following diagnostic codes: arthritis, discitis, non-specific back pain, limp, osteomyelitis, septic arthritis and "other orthopaedic problems". Further additional cases were identified from the IMSRL in-house assay database and from the TSCUH Infectious Diseases and Clinical Microbiology consults database.

Cases were excluded from analysis if the patient was not admitted, no bone or joint specimen was processed by the laboratory, the clinical presentation was not in keeping with osteomyelitis or septic arthritis, an alternative diagnosis was made, or the patient was transferred to another hospital directly from the Emergency Department.

Data collection and analysis

Baseline characteristics collected included patient demographic data (age, gender), clinical features (temperature, presenting symptoms and signs, bone or joint affected), laboratory indices (white cell count (WCC), neutrophils, platelets, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)), microbiology results (blood cultures, bone and/or joint specimen cultures, throat swab, methicillin-resistant *Staphylococcus aureus* (MRSA) screen and PCR results) and imaging performed (plain radiograph, ultrasound scan, isotope bone scan, CT and/or magnetic resonance imaging (MRI)). Data from eligible study participants was collated and managed using Excel® (Microsoft, Redmonton WA, USA). Non-parametric data were analysed using the Mann-Whitney test (GraphPad Prism®, version 6).

Case definition

Cases were included for analysis where the patient was admitted between 1 January 2010 and 31 December 2016, and was less than 16 years of age at the time of presentation. Osteomyelitis or septic arthritis was defined as a positive culture or PCR result from blood, joint fluid, bone aspirate or biopsy, or imaging findings consistent with osteomyelitis or septic arthritis, in the presence of compatible clinical findings.

Laboratory methods

Blood cultures were performed by inoculating whole blood into paediatric blood culture bottles with subsequent incubation in a BacT/ALERT® 3D Blood Culture System (bioMérieux, Marcy l'Étoile, France) in the TSCUH clinical microbiology laboratory. For any cultures signalling positive, an aliquot was removed and inoculated onto Columbia blood agar and chocolate agar media and incubated for 48 h at 35–37 °C in 5–10% CO_2 and in anaerobic conditions or in 5–10% CO_2, respectively. Joint fluid, bone aspirate and bone biopsy specimens were cultured using a similar process, with inoculation into blood culture bottles where possible, and/or inoculation directly onto blood, chocolate and MacConkey agars and incubated as described.

Targeted real-time PCR assays for *S. aureus*, group A *Streptococcus*, *S. pneumoniae* and *K. kingae*, and a broad-range bacterial 16S rRNA gene PCR assay on joint fluid, bone aspirate and biopsy specimens were performed by the IMSRL and the Microbiology Department at Great Ormond Street Hospital for Children (GOSH), NHS Foundation Trust, UK, and the original methods are included in the references [10–14]. These assays were in-house validated assays and

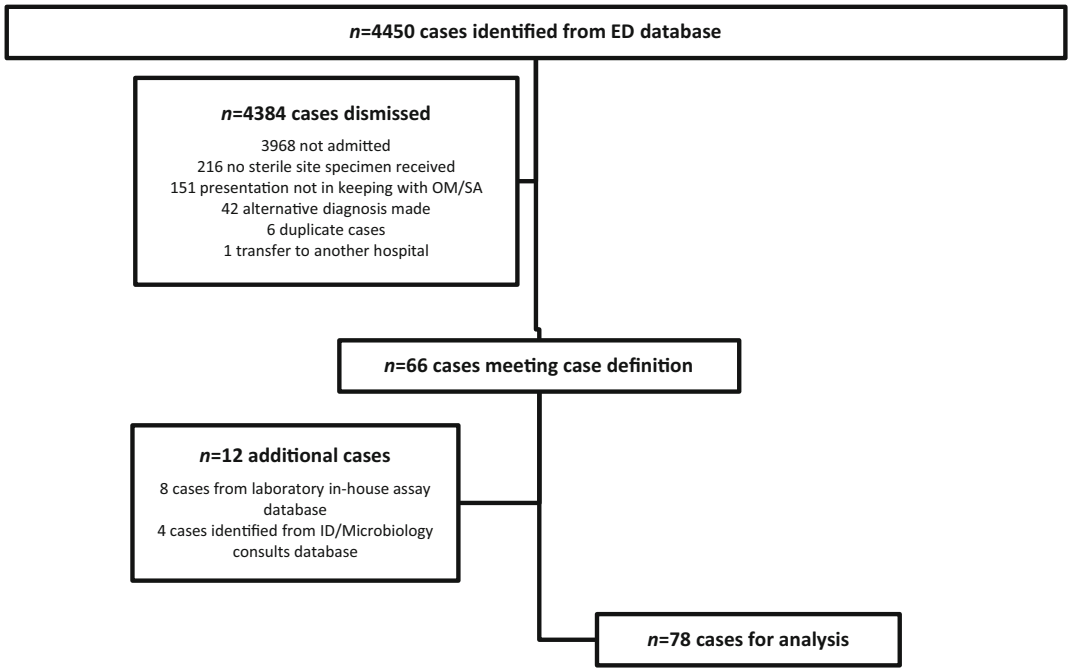

Fig. 1 Flow diagram of osteomyelitis and septic arthritis cases identified for analysis. ED, Emergency Department; OM, osteomyelitis; SA, septic arthritis; ID, infectious diseases

did not use commercial kits. The organisms were chosen to be included in the panel, as they represented the most common causes of community-acquired osteomyelitis and septic arthritis in both the adult and paediatric population. The assays were performed where possible in the IMSRL and sent to GOSH if required. Of note, the 16S rRNA service was only performed in GOSH. Tissue samples were first liquefied by Proteinase K digestion and, along with fluid samples, subjected to bead

Table 1 Baseline laboratory investigations

Test	No.	Median	Interquartile range	Range
C-reactive protein (mg/L)	78	39.5	30–103	1–366
Reference range 0–10 mg/L				
Erythrocyte sedimentation rate (mm/h)	73	43	19–55.8	4–132
Reference range 3–13 mm/h				
White cell count ($\times 10^9$/L)	78	11.8	8.7–14.7	3.5–25.6
Reference ranges				
≤ 7 days, 10–26 $\times 10^9$/L				
≤ 1 year, 6–18 $\times 10^9$/L				
≤ 8 years, 5–15 $\times 10^9$/L				
≤ 13 years, 4.5–13.5 $\times 10^9$/L				
> 13 years, 4–11 $\times 10^9$/L				
Neutrophils ($\times 10^9$/L)	78	6.3	4.3–9	1.2–20.8
Reference ranges				
≤ 1 day, 5–13 $\times 10^9$/L				
≤ 3 days, 1.5–7 $\times 10^9$/L				
≤ 2 years, 1–8.5 $\times 10^9$/L				
≤ 6 years, 1.5–8.5 $\times 10^9$/L				
≤ 12 years, 1.5–8 $\times 10^9$/L				
≤ 16 years, 1.8–8 $\times 10^9$/L				
> 16 years, 2–7 $\times 10^9$/L				
Platelets ($\times 10^9$/L)	78	321	269–401	64–659
Reference range 150–450 $\times 10^9$/L				

beating with Lysing matrix B (MP Bio, Cambridge, UK) to ensure complete lysis of bacterial cells. Total nucleic acid was extracted and PCR set-up performed using the QIAsymphony SP/AS instrument (Qiagen, Manchester, UK).

Four real-time PCR targets plus an internal positive control were amplified in two separate multiplexed reactions on a 7500 Fast Real-Time PCR system (Life Technologies, Paisley, UK) using previously published primer and probe sequences, and all other assay conditions were as previously described [11, 15]. Broad-range 16S rRNA gene PCR was performed as previously described [12], with the addition of a second primer pair that target a different region of the 16S rRNA gene [12, 16].

Results

A total of 4450 Emergency Department presentations were initially identified, and 66 (1.5%) of these fulfilled the case definition and were included in the analysis. A further 12 cases were identified from the IMSRL and the TSCUH Infectious Diseases and Clinical Microbiology consults databases. Overall, 78 cases were included (Fig. 1) in the data analysis. The median age was 4.8 years (range 0.1–14.9 years) and 52 (66%) patients were male. Eighteen of 73 (24.6%) of patients had a pyrexia of ≥ 38.0 °C at the point of triage (not recorded = 5). Septic arthritis was present in 35 (45%) cases with the affected joint being the hip in 19 cases, knee in 14 cases, and a single case in an ankle and one case in a toe. Osteomyelitis was present in 27 (35%) cases with the bones being affected including femur ($n = 9$), calcaneus ($n = 4$), tibia ($n = 4$), digit ($n = 3$), pelvis ($n = 2$), ulna ($n = 2$), cuboid ($n = 1$), radius ($n = 1$) and a single case of multifocal osteomyelitis. Sixteen (21%) cases were considered to have both osteomyelitis and septic arthritis present, and the joints affected were hip ($n = 5$), knee ($n = 5$), ankle ($n = 3$), elbow ($n = 1$), shoulder ($n = 1$) and multiple joints ($n = 1$).

Results of laboratory investigations are shown in Table 1 and Fig. 2. Four of the sixteen culture-negative samples detected a pathogen (*K. kingae* = 2, *S. pneumoniae* = 1, group A *Streptococcus* = 1) when tested by targeted real-time PCR. The broad-range 16S rRNA gene PCR did not detect any additional pathogens in the culture-negative specimens. A subgroup analysis of CRP level by organism showed that

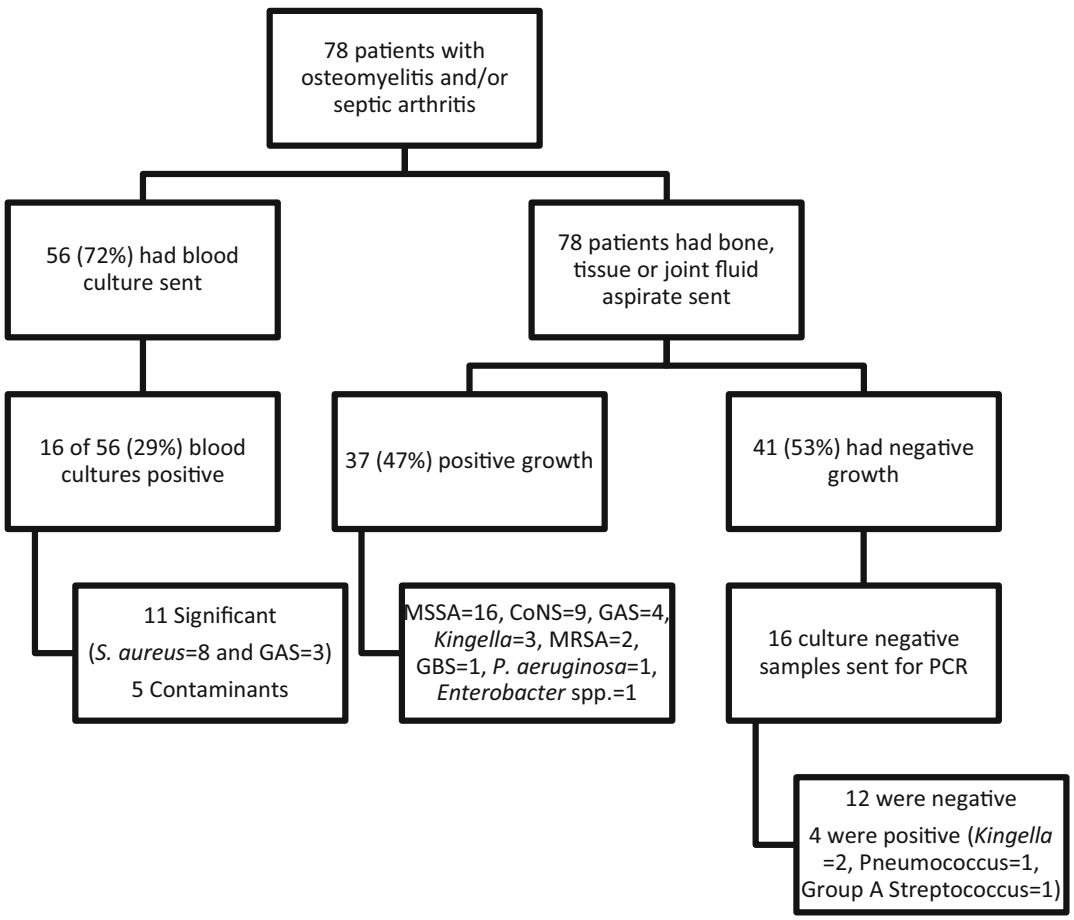

Fig. 2 Summary of microbiology results. GAS, group A *Streptococcus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; CoNS, coagulase-negative *Staphylococci*; MRSA, methicillin-resistant *Staphylococcus aureus*; GBS, group B *Streptococcus*; PCR, polymerase chain reaction

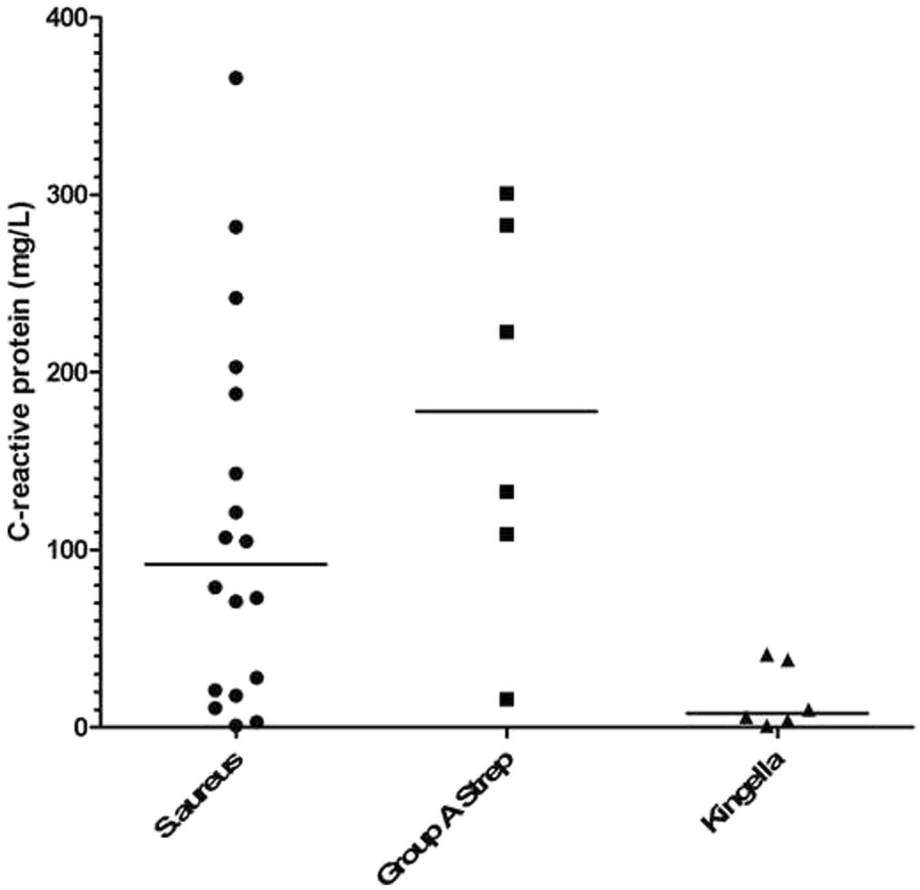

Fig. 3 C-reactive protein depending on the causative organism

the CRP for patients with infections due to *K. kingae* was significantly lower than for patients with infections due to *S. aureus* (Mann-Whitney $U = 18.5$, $p < 0.05$) or group A *Streptococcus* ($U = 2.0$, $p < 0.01$) (Fig. 3). The imaging modalities that were performed varied considerably between patients (Table 2); however, all patients had plain film radiographs performed. Twenty-nine (37%) of the 78 patients had plain film radiographs which were reported as normal, which may reflect the acuity of the clinical presentation.

Discussion

This retrospective review confirms that in cases of paediatric osteomyelitis and septic arthritis, even where appropriate specimens are obtained for traditional microbiological culture, a causative organism is frequently not identified. Blood cultures were useful as 11 of 56 patients (19.6%) had a clinically significant blood culture result. Bone/joint aspirates or biopsies were positive in 47% of cases, and this emphasises the need for early sampling.

Targeted real-time PCR assays for *S. aureus*, group A *Streptococcus*, *S. pneumoniae* and *K. kingae*, and a broad-range bacterial 16S rRNA gene PCR assay were utilised. The organisms chosen for targeted PCR represent the most common pathogens causing childhood bone and joint infections. *K. kingae* is known to be an emerging pathogen, particularly in children under the age of 5 years. Its detection has become more prevalent since the advent of PCR, as it is a fastidious organism and is often not detected using culture alone [9, 15]. Recent European guidelines advise that

Table 2 Radiological investigations

Imaging in addition to plain film X-rays		
Modality	No.	%
MRI only	19	24%
No further imaging	16	21%
USS and MRI	12	15%
USS only	10	13%
Bone scan only	7	9%
USS and bone scan	7	9%
Bone scan and MRI	2	3%
USS and MRI	2	3%
USS, bone scan and MRI	2	3%
CT and MRI	1	1%

MRI magnetic resonance imaging, *USS* ultrasound scan

"antimicrobials with activity against *Kingella* should be considered in children <5 years of age, especially in areas with high rates" [9]. For culture-negative cases in this study, appropriate PCR testing improved the detection rate of causative organisms in four of sixteen cases, suggesting PCR may have a role as a second line investigation, especially when considering the infections due to *K. kingae*. In this study, there was no additional yield from the utilisation of 16S rRNA PCR in the determination of a causative organism, compared to the use of targeted real-time PCR for the most common pathogens. This suggests that targeted PCR is sufficient in most cases and that 16S rRNA PCR could be reserved for use in cases where there is no pathogen identified following culture and targeted PCR, and for the identification of rare and unusual pathogens.

Other laboratory parameters were also useful; the median white blood cell count was 11.8×10^9/L, and ESR and CRP were elevated on average with a median of 43 mm/h and 39 mg/L, respectively, suggesting that ESR and CRP may be more discriminatory diagnostic markers at presentation than WCC. This has been demonstrated in other studies, particularly where *K. kingae* was found to be the causative organism [4, 11, 15]. Our study has also shown that the CRP in patients with *K. kingae* infections is significantly lower than for infections due to group A *Streptococcus* and *S. aureus*. The approach to radiological investigations beyond plain film radiographs varied between patients, and in light of recent European Society for Paediatric Infectious Diseases (ESPID) guidelines, there is a need to reassess the benefits of each modality [9].

PCR relies upon the detection of bacterial DNA, and so may be helpful where cultures are negative and where patients have received antibiotics prior to specimen collection. Unfortunately, due to the retrospective nature of this study, it was not possible to determine whether antibiotics had been prescribed prior to specimen collection, and this is a limitation of the study. This would be an important aspect to explore in similar future studies, as receipt of antibiotics prior to specimen collection has the potential to impact on positive culture yield and increase reliance on the utility of PCR.

Conclusions

In conclusion, this retrospective review suggests that appropriate PCR testing should be performed in culture-negative cases of suspected paediatric bone or joint infection, as it can improve the rate of identification of a causative organism. PCR testing could be delayed, pending 48 h culture results, thus reserving PCR testing for culture-negative cases. By limiting the use of PCR for these cases, it is possible to rationalise testing to those who are likely to benefit the most. All patients with suspected osteomyelitis or septic arthritis should have blood cultures performed at presentation given that almost 20% of patients had a clinically significant positive blood culture on admission. The results of this study underline the benefit of PCR testing in suspected paediatric bone and joint infection, and will help inform the development of a syndromic diagnostic test panel for bone and joint infections at the IMSRL.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the TSCUH Research and Ethics Committee as a retrospective review (Reference 16.052). For this type of study, formal consent is not required. This article does not contain any studies with animals performed by any of the authors.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Cohen E, Lifshitz K, Fruchtman Y, Eidelman M, Leibovitz E (2016) Current data on acute haematogenous osteomyelitis in children in southern Israel: epidemiology, microbiology, clinics and therapeutic consequences. Int Orthop 40(9):1987–1994. https://doi.org/10.1007/s00264-016-3211-6
2. Martin AC, Anderson D, Lucey J, Guttinger R, Jacoby PA, Mok TJ, Whitmore TJ, Whitewood CN, Burgner DP, Blyth CC (2016) Predictors of outcome in pediatric osteomyelitis: five years experience in a single tertiary center. Pediatr Infect Dis J 35(4):387–391. https://doi.org/10.1097/INF.0000000000001031
3. Chiappini E, Camposampiero C, Lazzeri S, et al. (2017) Epidemiology and management of acute haematogenous osteomyelitis in a tertiary paediatric center. Int J Environ Res Public Health 14(5). https://doi.org/10.3390/ijerph14050477
4. Grote V, Silier CC, Voit AM et al (2017) Bacterial osteomyelitis or nonbacterial osteitis in children: a study involving the German surveillance unit for rare diseases in childhood. Pediatr Infect Dis J 36(5):451–456. https://doi.org/10.1097/INF.0000000000001469
5. Kerr DL, Loraas EK, Links AC et al (2017) Toxic shock in children with bone and joint infections: a review of seven years of patients admitted to one intensive care unit. J Child Orthop 11(5):387–392. https://doi.org/10.1302/1863-2548.11.170058
6. Osei L, El Houmami N, Minodier P et al (2017) Paediatric bone and joint infections in French Guiana: a 6 year retrospective review. J Trop Pediatr 63(5):380–388. https://doi.org/10.1093/tropej/fmw102
7. Al-Qwbani M, Jiang N, Yu B (2016) Kingella kingae-associated pediatric osteoarticular infections: an overview of 566 reported cases. Clin Pediatr (Phila) 55(14):1328–1337. https://doi.org/10.1177/0009922816629620
8. Gravel J, Ceroni D, Lacroix L, Renaud C, Grimard G, Samara E, Cherkaoui A, Renzi G, Schrenzel J, Manzano S (2017) Association between oropharyngeal carriage of Kingella kingae and osteoarticular infection in young children: a case-control study. CMAJ 189(35):E1107–E1e11. https://doi.org/10.1503/cmaj.170127
9. Saavedra-Lozano J, Falup-Pecurariu O, Faust SN, Girschick H, Hartwig N, Kaplan S, Lorrot M, Mantadakis E, Peltola H, Rojo P,

Zaoutis T, LeMair A (2017) Bone and joint infections. Pediatr Infect Dis J 36(8):788–799. https://doi.org/10.1097/INF.0000000000001635

10. Harris KA, Hartley JC (2003) Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. J Med Microbiol 52(Pt 8):685–691
11. Tann CJ, Nkurunziza P, Nakakeeto M, Oweka J, Kurinczuk JJ, Were J, Nyombi N, Hughes P, Willey BA, Elliott AM, Robertson NJ, Klein N, Harris KA (2014) Prevalence of bloodstream pathogens is higher in neonatal encephalopathy cases vs. controls using a novel panel of real-time PCR assays. PLoS One 9(5):e97259. https://doi.org/10.1371/journal.pone.0097259
12. Harris KA, Yam T, Jalili S, Williams OM, Alshafi K, Gouliouris T, Munthali P, NiRiain U, Hartley JC (2014) Service evaluation to establish the sensitivity, specificity and additional value of broad-range 16S rDNA PCR for the diagnosis of infective endocarditis from resected endocardial material in patients from eight UK and Ireland hospitals. Eur J Clin Microbiol Infect Dis 33(11):2061–2066. https://doi.org/10.1007/s10096-014-2145-4
13. Lehours P, Freydiere AM, Richer O, Burucoa C, Boisset S, Lanotte P, Prere MF, Ferroni A, Lafuente C, Vandenesch F, Megraud F, Menard A (2011) The rtxA toxin gene of Kingella kingae: a pertinent target for molecular diagnosis of osteoarticular infections. J Clin Microbiol 49(4):1245–1250. https://doi.org/10.1128/JCM.01657-10
14. Harris KA, Turner P, Green EA, Hartley JC (2008) Duplex real-time PCR assay for detection of Streptococcus pneumoniae in clinical samples and determination of penicillin susceptibility. J Clin Microbiol 46(8):2751–2758. https://doi.org/10.1128/JCM.02462-07
15. Chometon S, Benito Y, Chaker M, Boisset S, Ploton C, Bérard J, Vandenesch F, Freydiere AM (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
16. Doyle R, Gondwe A, Fan YM, Maleta K, Ashorn P, Klein N, Harris K (2018) Lactobacillus-deficient vaginal microbiota dominate postpartum women in rural Malawi. Appl Environ Microbiol 84: e02150–e02117. https://doi.org/10.1128/AEM.02150-17