Infection (2019) 47:195–200
https://doi.org/10.1007/s15010-018-1192-7

ORIGINAL PAPER

Evaluation of the multiplex PCR based assay Unyvero implant and tissue infection application for pathogen and antibiotic resistance gene detection in children and neonates

Cihan Papan[1,2] · Melanie Meyer-Buehn[1] · Gudrun Laniado[1] · Johannes Huebner[1]

Received: 26 June 2018 / Accepted: 11 August 2018 / Published online: 21 August 2018

Abstract

Background Skin and soft tissue infections have a high disease burden in children. The emergence of multidrug-resistant bacteria over the last decades has heavily influenced hospitalization rates, morbidity and mortality. In addition, with increased survival rates in neonatology and oncology, health-care associated infections are more frequently encountered. There is a growing need for fast and feasible diagnostic tools for the recognition of microorganisms and drug resistances.

Methods In this prospective study, we compared results of routine culture with the multiplex PCR based Unyvero Implant and Tissue Infection (ITI) application. Specimens were obtained from different sources from neonates and children.

Results We analyzed specimens from 29 patients (72.4% male) with a median age of 8.1 years (range 0.03–15.2). Concordance between Unyvero ITI and culture was reached in 16 of 29 samples (55.2%). Unyvero ITI yielded an overall sensitivity and specificity of 76.3% and 96.5%, respectively. Accuracies were best for non-fermenting bacteria, for which sensitivity was 100% and specificity 98.2%. Detection rates were lower for Gram-positive bacteria (68.8 and 95.2%, respectively). Unyvero correctly detected one $bla_{OXA-24/40}$ producing *Acinetobacter baumannii*, while none of the six *gyrA87* had a correlate in antimicrobial susceptibility testing.

Conclusions Unyvero ITI quickly provides additional information relevant for clinical decision-makers. Sensitivity of the PCR must be improved especially for Gram-positive bacteria, and further studies are needed to assess the impact on clinical decision-making and outcome.

Keywords Children · Neonates · Skin and soft tissue infections · Bite and burn wounds · Health-care associated infections · Multiplex PCR.

Abbreviations

AST	Antimicrobial susceptibility testing
CI	Confidence interval
CN	Concordantly negative
CNS	Coagulase negative *Staphylococci*
CP	Concordantly positive
ITI	Implant and tissue infection
MRSA	Methicillin-resistant *Staphylococcus* aureus
NPV	Negative predictive value
PCR	Polymerase chain reaction
PJI	Prosthetic joint infection
PPV	Positive predictive value
SSTI	Skin and soft tissue infection
UN	Unyvero negative
UP	Unyvero positive

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s15010-018-1192-7) contains supplementary material, which is available to authorized users.

✉ Cihan Papan
cihan.papan@medma.uni-heidelberg.de

[1] University Children's Hospital at Dr. von Haunersches Kinderspital, Ludwig Maximilians University, Munich, Germany

[2] Pediatric Infectious Diseases, University Children's Hospital Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany

Background

Skin and soft tissue infections (SSTI) are frequently encountered in both adults and children [1, 2]. Beside a substantial morbidity and mortality, SSTI also have a major socioeconomic impact [3]. The emergence of multidrug-resistant

bacteria, mainly methicillin-resistant *Staphylococcus aureus* (MRSA), has led to increased hospitalization rates [4]. One major obstacle in the management of SSTI is the lack of a rapid and reliable diagnostic tool that enables clinicians to initiate an appropriate therapy, especially in the face of multidrug-resistant organisms. Due to continuous medical progress in neonatology or oncology, immunocompromised children are increasingly prone to health-care associated infections of intravascular catheters, ventilation therapy, and surgical procedures [5, 6]. The broad use of prophylactic antimicrobials not only facilitates growth of drug-resistant organisms, but also further complicates diagnosis of possible infections by inhibiting bacterial growth in routine culture. Altogether, SSTI and health-care associated infections necessitate a quick and reliable diagnostic tool which not only detects the responsible microorganisms, but also yields information on antimicrobial resistance determinants. The Unyvero ITI application, based on multiplex PCR, has been developed by Curetis AG (Holzgerlingen, Germany) to diagnose implant, catheter-related, osteoarticular, skin, and soft tissue infections with a short turnaround time of approximately 5 h. Previous studies in adults with prosthetic joint infections showed high specificity [7–9]. However, there are no data for children. We sought to evaluate Unyvero ITI in children and neonates with SSTI or health-care associated infections and to compare the findings with routine culture methods.

Methods

We prospectively compared findings of routine microbiology with findings of the Unyvero ITI in hospitalized children and neonates in whom diagnostics was performed due to suspected infection. Materials were obtained from children from the following sites: intraoperative swabs from abdominal surgical sites; wound swabs; swabs from surgically opened skin abscesses, burn or bite wounds or synovial fluids. All specimens underwent routine diagnostic analysis according to standard microbiology laboratory procedures [10] and as described before [11]. The sample remnants were irreversibly anonymized and analyzed by Unyvero ITI application according to manufacturer's instructions as explained elsewhere [12, 13]. This application is intended to detect gene products of 23 pathogens: *Staphylococcus aureus*, Coagulase-negative Staphylococci, *Streptococcus agalactiae, Streptococcus pyogenes, Granulicatella adiacens, Abiotrophia defectiva, Enterococcus* spp., *Enterococcus faecalis, Escherichia coli, Enterobacter cloacae* complex, *Enterobacter aerogenes, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus* spp., *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa, Propionibacterium acnes, Propionibacterium avidum/granulosum, Finegoldia magna, Bacteroides fragilis group, Corynebacterium* spp., *Candida albicans, and Candida parapsilosis*. In addition, the following 20 resistance genes are tested: *ermA, ermC, mecA, mecC, vanA, vanB, rpoB, aacA4, aac(6´)aph(2´´), bla*$_{\text{CTX-M}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24/40}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{VIM}}$, *gyrA83* and *gyrA87*. Data were collected and analyzed in Microsoft Excel (Microsoft Corporation, Richmond, USA) and GraphPad Prism (GraphPad Software, Inc.). Apart from age, sex and the designation of the material and its source, no further clinical data were collected. Results from conventional microbiology testing and Unyvero ITI were compared in terms of detected microorganisms; detected antibiotic resistance genes were correlated with findings from the antimicrobial susceptibility testing (AST). Concordance rates for each pathogen defined by identical results between the results from both techniques were calculated. Results were regarded as concordantly positive (CP) or concordantly negative (CN) when both, Unyvero and culture were positive or negative; Unyvero positive (UP) when Unyvero was positive and culture was negative; and Unyvero negative (UN) when Unyvero was negative and culture was positive. The performance of Unyvero ITI per pathogen was evaluated by calculating prevalence, sensitivity, specificity, positive predictive value, and negative predictive value. 95% confidence intervals were determined in compliance with the efficient score method [14, 15]. Furthermore, we assessed the correlation between bacterial quantification, designated as "many, moderate, or little", and the Unyvero signal strength for each pathogen. Data were excluded when all PCR chambers were invalid or if the failure concerned chambers containing organisms that yielded positive results in the corresponding culture. All specimens were obtained between February 2015 and December 2015. This prospective and observational study was reviewed, approved, and declared unobjectionable by the ethic committee of the Ludwig-Maximilians-Universität München (UE Nr. 012–14). The need for informed consent was waived since no additional patient samples were obtained.

Results

Samples of a total of 29 patients were analyzed, of which the majority of ten specimens were intraabdominal swabs from surgical sites (34.5%), and eight specimens were from skin wounds (27.6%). Five samples were from abscesses (17.2%), three from burn wounds (10.3%), and one each from a bite wound, synovial fluid, and a thoracic drainage catheter (3.4% each). No analysis had to be excluded due to completely invalid runs (Table 1). Partially invalid runs occurred in three cases, but the respective failure was not related to the pathogen detected in culture.

Table 1 Descriptive data

Descriptive data	Number (percentage)
Cases included	29
Cases excluded due to complete invalidity	0
Partially invalid findings	3 (10.3%)
Neonates	3 (10.3%)
Male	21 (72.4%)
Age, median in years (range)	8.1 (0.03–15.2)
Age, mean in years	6.9

Concordance rates

Positive results in both the routine method and Unyvero were found in 21/29 (72.4%) specimens, while only ten of those were concordantly positive. In two cases, the Unyvero failed to detect the culturally proven pathogen. In 6/29 (20.7%) specimens, both methods were concordantly negative. Total concordance was thus reached in 16/29 (55.2%). Unyvero yielded no pathogens in eight specimens, one pathogen in nine specimens, two pathogens in seven specimens, and three or more pathogens in five specimens (Supplementary Table).

Pathogen identification

Cumulatively, Unyvero ITI showed a sensitivity of 76.3% (95% CI 59.4–88.0%) and specificity of 96.5% (95% CI 94.2–97.9%), while positive predictive and negative predictive values were 64.4% (95% CI 48.7–77.7%) and 98.0% (95% CI 96.0–99.0%), respectively (Table 2). Considering concordantly positive findings, the most frequently detected pathogens were *Escherichia coli* in 8/29 cases (27.6%), *Staphylococcus aureus* in 6/29 cases (20.7%), and *Bacteroides fragilis* in 4/29 cases (13.8%). When both, concordantly positive and Unyvero positive findings were taken into account, the most prevalent pathogens were still *Escherichia coli* with 9/45 (20%), *Staphylococcus aureus* and *Bacteroides fragilis* each with 6/45 (13.3%), and *Enterococcus* spp. with 5/45 (11.1%).

Test performances for Gram-negative bacteria were best, especially for the non-fermenting bacteria *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The sensitivity for this group, i.e. the rate of culture-positive specimens that were detected by Unyvero, was 100% (3/3; 95% CI 31–100%). The specificity, i.e. the rate of negative results in Unyvero conforming with the culture, was 98.2% (54/55; 95% CI 89-99.9%). For Enterobacterales, sensitivity was

Table 2 Pathogen and group specific results

Pathogen	CP	UN	UP	CN	Prev	Sensitivity	Specificity	PPV	NPV
S. aureus	6	0	0	23	20.7	100.0 (51.7–100.0)	100.0 (82.2–100.0)	100.0 (51.7–100.0)	100.0 (82.2–100.0)
CNS	2	2	1	24	6.9	50.0 (9.2–90.8)	96.0 (82.0–96.0)	66.7 (12.5–98.2)	92.3 (73.4–98.7)
S. pyogenes	2	1	0	26	6.9	66.7 (12.5–98.2)	100.0 (83.9–100.0)	100.0 (19.8–100.0)	96.3 (79.1–99.8)
Granulicatella adiacens	0	0	1	28	0	–	96.6 (80.3–99.8)	0.0 (0.0–94.5)	100.0 (85.0–100.0)
Abiotrophia defectiva	0	0	1	28	0	–	96.6 (80.3–99.8)	0.0 (0.0–94.5)	100.0 (85.0–100.0)
Enterococcus spp	1	1	4	23	3.4	50.0 (2.7–97.3)	85.2 (65.3–95.1)	20.0 (1.1–70.1)	95.8 (76.9–99.8)
Enterococcus faecalis	0	1	2	26	0	0.0 (0.0–94.5)	92.9 (75.0–98.8)	0.0 (0.0-80.2)	96.2 (79.1–99.8)
E. coli	8	1	1	19	27.6	88.9 (50.7–99.4)	95.0 (73.1–99.7)	88.9 (50.7–99.4)	95.0 (73.1–99.7)
E. cloacae complex	1	0	0	28	3.4	100.0 (5.5–100.0)	100.0 (85.0–100.0)	100.0 (5.5–100.0)	100.0 (85.0–100.0)
Enterobacter spp	0	0	0	29	0	–	100.0 (85.4–100.0)	–	100.0 (85.4–100.0)
K. pneumoniae	0	0	1	28	0	–	96.6 (80.3–99.8)	0.0 (0.0–94.5)	100.0 (85.0–100.0)
K. oxytoca	2	0	0	27	6.9	100.0 (19.9–100.0)	100.0 (84.5–100.0)	100.0 (19.8–100.0)	100.0 (84.5–100.0)
A. baumannii	1	0	1	27	3.4	100.0 (5.5–100.0)	96.4 (79.8–99.8)	50.0 (2.7–97.3)	100.0 (84.5–100.0)
P. aeruginosa	2	0	0	27	6.9	100.0 (19.8–100.0)	100.0 (84.5–100.0)	100.0 (19.8–100.0)	100.0 (84.5–100.0)
Propionibacterium acnes	0	0	2	27	0	–	93.1 (75.8–98.8)	0.0 (0.0–80.2)	100.0 (84.5–100.0)
Bacteroides fragilis	4	1	2	22	13.8	80.0 (29.9–98.9)	91.7 (71.5–98.5)	66.7 (24.1–94.0)	95.7 (76.0–99.8)
Candida albicans	0	2	0	27	0	0.0 (0.0–80.2)	100.0 (84.5–100.0)	–	95.3 (75.8–98.8)
Total	29	9	16	439	100	76.3 (59.4–88.0)	96.5 (94.2–97.9)	64.4 (48.7–77.7)	98.0 (96.0–99.0)
Grampositive	11	5	9	178	37.9	68.8 (41.5–87.9)	95.2 (90.8–97.6)	55.0 (32.0–76.2)	97.3 (93.4–99.0)
Non-Fermenter	3	0	1	54	10.3	100.0 (31.0–100.0)	98.2 (89.0–99.9)	75.0 (21.9–98.7)	100.0 (91.7–100.0)
Enterobacterales	11	1	2	131	37.9	91.2 (59.8–99.6)	98.5 (94.1–99.7)	84.6 (53.7–97.3)	99.2 (95.2–99.9)
Anaerobic	4	1	4	49	13.8	80.0 (29.9–98.9)	92.5 (80.9–97.6)	50.0 (17.4–82.6)	0.98 (88.0-99.9)

CP, UN, UP and CN as defined in the materials and methods section, given as absolutes; Prevalence (Prev), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) given as accuracy measures, in percent; 95% confidence intervals in brackets

91.7% (11/12; 95% CI 59.8–99.6%), and specificity was 98.5% (131/133; 95% CI 94.1–99.7%). Furthermore, Unyvero detected anaerobic bacteria with a sensitivity of 80% (4/5; 95% CI 29.9–98.9%) and a lower specificity of 92.5% (49/53; 95% CI 80.9–97.6%), influenced by a high rate of presumably false-positive findings. The performance for Gram-positive bacteria was even poorer, yielding a sensitivity of only 68.8% (11/16; 95% CI 41.5–87.9%), while specificity was 95.2% (178/187; 95% CI 90.8–97.6%). Interestingly, the detection of *Staphylococcus aureus* was perfect (sensitivity and specificity of 100%), while the failure of Unyvero to detect CNS and *Streptococcus pyogenes* accounted for the poor overall performance for Gram-positive bacteria. Overall, *Candida albicans* was the only pathogen that Unyvero failed to detect completely (2 specimens). On the other hand, Unyvero detected bacteria in five culture-negative specimens, namely *Propionibacterium acnes, Granulicatella adiacens, Abiotrophia defectiva*, and *Klebsiella pneumoniae*.

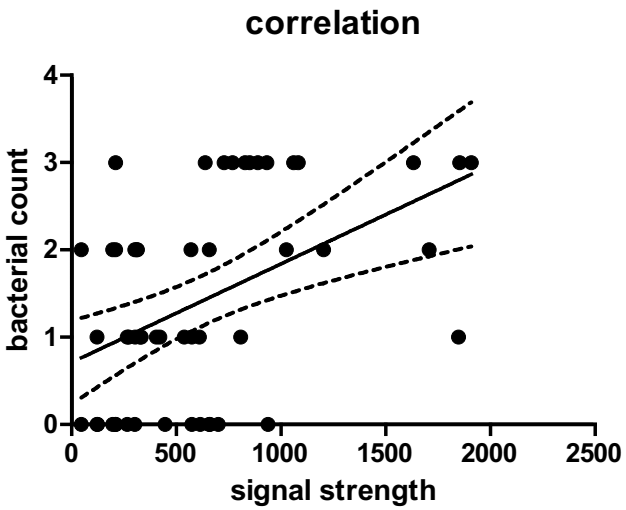

Fig. 1 Correlation between signal strength measured by Unyvero and bacterial count assessed by the routine method; bacterial count 1: little; 2: moderate; 3: many; signal strength: no specified unit. Dotted lines: 95% confidence interval

Resistance markers

In total, 12 resistance genes were detected by Unyvero in 11 specimens. The most frequently encountered resistance gene was *gyrA87* in six cases; followed by *ermC* in three specimens, *aac(6´)aph(2``)* in two specimens, and $bla_{\text{OXA-24/40}}$ in one specimen. None of the six *gyrA87* was associated with chinolone resistance in AST; only one of the *ermC* findings correlated with macrolide and clindamycin resistance in the according AST. None of the *aac(6´)aph(2``)* findings were associated with aminoglycoside resistance in AST. The only detected carbapenemase $bla_{\text{OXA-24/40}}$ was found in a carbapenem-resistant *Acinetobacter baumannii*.

Correlation between signal strength and microbial count

We tested for congruency between the Unyvero and the routine method by comparing signal strength and microbial counts. Overall, correlation between the signal strength of Unyvero and the microbial count was weak (Spearman $r = 0.48$), while for higher signal strengths, the correlation was slightly stronger (Fig. 1). In addition, we observed lower means (404.5 vs. 723.2) of signal counts in specimens in which culture had yielded no pathogen.

Discussion

In this study, we prospectively evaluated the point-of-care multiplex PCR Unyvero ITI in children and neonates, and compared it to routine microbiological culture methods. Unyvero successfully detected all culture-positive cases of *Staphylococcus aureus*, a major cause of not only skin and soft tissue infections, but also osteoarticular, and foreign body infections [16]; non-fermenting bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, an important pathogen of hospital-acquired infections [6]; and some Enterobacterales such as *Klebsiella oxytoca* and *Enterobacter cloacae* complex. Detection rate for all Enterobacterales, including *Escherichia coli*, was not as good as for non-fermenting bacteria, but still above 90%. In 16 findings (55.2%), Unyvero detected a pathogen while the corresponding culture remained negative for the respective pathogen. This might indicate a higher precision of PCR, which can detect traces of genetic material also from bacterial debris, hence leading to specificity lower than 100%. The clinical relevance of these findings remains unclear, yet for some organisms that are difficult to grow in culture this may offer an advantage. Specificity as it is calculated in our analysis is influenced by negative findings, and could be increased, by adding more especially rare and irrelevant pathogens into the assay. Yet, sensitivity remains the clinically more important variable in our analysis, since it shows as in how far pathogens detected by culture are also reliably detected by Unyvero. Overall, Unyvero delivered a sensitivity of 76.3%, missing pathogens grown in culture in nine cases, among those both cases of *Candida albicans*. Furthermore, sensitivity was low for Gram-positive bacteria such as Coagulase negative Staphylococci (important cause of catheter-related infections [17]), and *S. pyogenes*, a major pathogen associated with SSTI [18]. A biological explanation for this finding may be that Gram-positive bacteria possess a thicker cell wall, which might affect the lysis during PCR, a phenomenon that has recently been observed for the Unyvero

pneumonia assay [11]. Our findings confirm previous studies, all of which were carried out in adults with prosthetic joint infections (PJI). In a pilot study by Borde et al., a concordance rate of 82% between the Unyvero and the routine method [7] was found. In two other prospective studies, sensitivities ranged from 60.5 to 66.7%, and specificities from 98.0 to 100%, the latter being mainly due to concordantly negative findings [8, 9]. In a more recent study, a Swiss group tested the Unyvero ITI assay on culture negative specimens from bone cement spacers, and found no advantages of the assay [19]. Villa et al. evaluated the system for early and late PJI, resulting in a sensitivity of 34.2% before and 81.6% after broth enrichment, in comparison with routine culture [20]. Finally, in a large French trial published in 2018, the Unyvero ITI assay was compared to routine and to 16S RNA PCR in patients with PJI, yielding concordance rates of 58.1% and 70.1%, respectively [21].

Besides the clinically important detection of a carbapenemase with a matching finding in AST in our study, Unyvero ITI detected multiple resistance genes, e.g. *gyrA87*, without correlate in the routine sensitivity testing. This finding is not entirely surprising since the mere presence of genes does not necessarily implicate expression of the enzyme and subsequent resistance. Yet, the clinical relevance of these findings remains unclear and no additional data on therapy and outcome were available for these patients.

One limitation of this study was the low number of specimens. In addition, no clinical data were available for correlating findings with e.g. antimicrobial therapy or disease severity, both of which may influence detection of pathogens with routine methods. Every multiplex PCR assay covers a specific selection of possible pathogens. For pediatric patients, it would be advisable to include especially organisms that are difficult to culture, e.g. *Kingella kingae*, a pathogen encountered especially in children with osteoarticular infections [22], or *Mycobacterium tuberculosis*, which can cause skeletal tuberculosis [23].

No specimen had to be excluded from our analysis due to complete failure of all PCR chambers, while partially invalid probes were found in three cases. Of the former studies of the Unyvero ITI application in adults, Hischebeth [8] reported on one failed chamber out of 62 specimens. In comparison with earlier studies with the Unyvero pneumonia application with invalidity rates ranging from 6.1 to 34.3% [11–13, 24–26], we consider this rate as an improvement of the system.

Conclusions

In summary, our data indicate that Unyvero might facilitate fast diagnosis in cases of SSTI, osteoarticular or health-care associated infections. However, it needs to be reassessed in patients undergoing antimicrobial therapy prior to diagnosis. Sensitivity for some clinically important Gram-positive bacteria, but also fungal pathogens needs to be improved. Our results need confirmation in further studies with special emphasis on correlation with clinical data, incorporation in clinical decision algorithms, and cost efficiency analysis.

Acknowledgements We would like to thank Hannelore Zeilhofer, Petra Aydin, Petra Kappel, and Birgit Hanings for excellent technical assistance. Curetis GmbH (Holzgerlingen, Germany) provided the Unyvero system, cartridges, and technical support, but had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Funding This work received no specific grant from any funding agency.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interests.

Ethical statement This prospective and observational study was reviewed, approved, and declared unobjectionable by the ethic committee of the Ludwig-Maximilians-Universität München (UE Nr. 012–14). The need for informed consent was waived since no additional patient samples were obtained.

References

1. Esposito S, Noviello S, Leone S. Epidemiology and microbiology of skin and soft tissue infections. Curr Opin Infect Dis. 2016;29:109–15.
2. Sanders JE, Garcia SE. Evidence-based management of skin and soft-tissue infections in pediatric patients in the emergency department. Pediatr Emerg Med Pract. 2015;12:1–23. (quiz 23–24)
3. Pallin DJ, Egan DJ, Pelletier AJ, Espinola JA, Hooper DC, Camargo CA. Increased US emergency department visits for skin and soft tissue infections, and changes in antibiotic choices, during the emergence of community-associated methicillin-resistant Staphylococcus aureus. Anna Emerg Med. 2008;51:291–8.
4. Edelsberg J, Taneja C, Zervos M, Haque N, Moore C, Reyes K, Spalding J, Jiang J, Oster G. Trends in US hospital admissions for skin and soft tissue infections. Emerg Infect Dis. 2009;15:1516–8.
5. Zingg W, Hopkins S, Gayet-Ageron A, Holmes A, Sharland M, Suetens C. Health-care-associated infections in neonates, children, and adolescents: an analysis of paediatric data from the European Centre for Disease Prevention and Control point-prevalence survey. Lancet Infect Dis. 2017;17:381–9.
6. Crivaro V, Bogdanovic L, Bagattini M, Iula VD, Catania M, Raimondi F, Triassi M, Zarrilli R. Surveillance of healthcare-associated infections in a neonatal intensive care unit in Italy during 2006–2010. BMC Infect Dis. 2015;15:152.
7. Borde JP, Hacker GA, Guschl S, Serr A, Danner T, Hubner J, Burrack-Lange S, Ludke G, Helwig P, Hauschild O, et al. Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI((R)) cartridge system: a pilot study. Infection. 2015;43:551–60.
8. Hischebeth GT, Randau TM, Buhr JK, Wimmer MD, Hoerauf A, Molitor E, Bekeredjian-Ding I, Gravius S. Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in

diagnosing periprosthetic joint infection. J Microbiol Methods. 2016;121:27–32.
9. Prieto-Borja L, Rodriguez-Sevilla G, Aunon A, Perez-Jorge C, Sandoval E, Garcia-Canete J, Gadea I, Fernandez-Roblas R, Blanco A, Esteban J. Evaluation of a commercial multiplex PCR (Unyvero i60(R)) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. Enferm Infecc Microbiol Clin 2017;35:236–242
10. Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of Clinical Microbiology. Canada: ASM Press; 2015.
11. Papan C, Meyer-Buehn M, Laniado G, Nicolai T, Griese M, Huebner J. Assessment of the multiplex PCR-based assay Unyvero pneumonia application for detection of bacterial pathogens and antibiotic resistance genes in children and neonates. Infection. 2018;46:189–96.
12. Schulte B, Eickmeyer H, Heininger A, Juretzek S, Karrasch M, Denis O, Roisin S, Pletz MW, Klein M, Barth S, et al. Detection of pneumonia associated pathogens using a prototype multiplexed pneumonia test in hospitalized patients with severe pneumonia. PloS One. 2014;9:e110566.
13. Kunze N, Moerer O, Steinmetz N, Schulze MH, Quintel M, Perl T. Point-of-care multiplex PCR promises short turnaround times for microbial testing in hospital-acquired pneumonia–an observational pilot study in critical ill patients. Ann Clin Microbiol Antimicrob. 2015;14:33.
14. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17:857–72.
15. Wilson EB. Probable Inference, the Law of Succession, and Statistical Inference. J Am Stat Assoc. 1927;22:209–12.
16. Acree ME, Morgan E, David MZ. *S. aureus* infections in Chicago, 2006–2014: increase in CA MSSA and decrease in MRSA incidence. Infect Control Hospital Epidemiol. 2017;38:1226–34.
17. Riboli DF, Lyra JC, Silva EP, Valadao LL, Bentlin MR, Corrente JE, Rugolo LM, da Cunha Mde L. Diagnostic accuracy of semi-quantitative and quantitative culture techniques for the diagnosis of catheter-related infections in newborns and molecular typing of isolated microorganisms. BMC Infect Dise. 2014;14:283.
18. Larru B, Gerber JS. Cutaneous bacterial infections caused by Staphylococcus aureus and Streptococcus pyogenes in infants and children. Pediatr Clin N Am. 2014;61:457–78.
19. Mariaux S, Tafin UF, Borens O. Diagnosis of persistent infection in prosthetic two-stage exchange: PCR analysis of sonication fluid from bone cement spacers. J Bone Jt Infect. 2017;2:218–23.
20. Villa F, Toscano M, De Vecchi E, Bortolin M, Drago L. Reliability of a multiplex PCR system for diagnosis of early and late prosthetic joint infections before and after broth enrichment. Int J Med Microbiol. 2017;307:363–70.
21. Malandain D, Bemer P, Leroy AG, Leger J, Plouzeau C, Valentin AS, Jolivet-Gougeon A, Tande D, Hery-Arnaud G, Lemarie C, et al. Assessment of the automated multiplex-PCR Unyvero i60 ITI((R)) cartridge system to diagnose prosthetic joint infection: a multicentre study. Clin Microbiol Infect. 2018;24:83.e81–83.e86.
22. Principi N, Esposito S. Kingella kingae infections in children. BMC Infect Dis. 2015;15:260.
23. Papan C, von Both U, Kappler M, Kammer B, Griese M, Huebner J. Pott's disease: a major issue for an unaccompanied refugee minor. Thorax. 2017;72:282–3.
24. Personne Y, Ozongwu C, Platt G, Basurto-Lozada P, Shamin M, Gant VA, Zumla A, Enne VI. 'Sample-in, answer-out'? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay. Diagn Microbiol Infect Dise. 2016;86:5–10.
25. Jamal W, Al Roomi E, AbdulAziz LR, Rotimi VO. Evaluation of Curetis Unyvero, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and impact of the assay on management of severe nosocomial pneumonia. J Clin Microbiol. 2014;52:2487–92.
26. Ozongwu C, Personne Y, Platt G, Jeanes C, Aydin S, Kozato N, Gant V, O'Grady J, Enne VI. The Unyvero P55 'sample-in, answer-out' pneumonia assay: a performance evaluation. Biomol Detect Quantif. 2017;13:1–6.