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First evaluation of the automated-multiplex-PCR Unyvero ITI G2 cartridge for rapid diagnosis of osteo-articular infections

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3. E. Lafeuille, F. Devriese, E. Fourniols, S. Jauréguiberry, A. Aubry, on behalf of the PIOS (Pitié-salpêtrière Infection OSteo-articulaire) group. Evaluation of performances of the multiplex ITI G2 (V2) molecular test in the diagnosis of Osteo-Articular Infections (OAI). 3rd CRIOAC Congress. 2017.

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Abstract

Objective: Conventional microbiological methods (CMM), including long-term culture, for the diagnosis of osteo-articular infections (OAI) fail in at least 5% of all cases. Only one IOA dedicated molecular method has been commercialized, and only the first version of this kit has been studied. The aim of this work was to evaluate the concordance between test results obtained with the second version of the Unyvero ITI G2 cartridge (Curetis) and CMM. The cartridge, combining one-step automated lysis/DNA extraction with multiplex PCR and amplicon detection by array hybridization, allows for the detection of 102 prevalent pathogens and their antibiotic resistance markers directly in clinical specimens (liquid (n=8) or solid (n=32)).

Material and methods: Frozen samples from 40 patients who underwent orthopedic surgery at Pitié-Salpêtrière hospital were tested retrospectively with the cartridge: 5 were culture-negative, 25 revealed monomicrobial and 10 polymicrobial OAI. The 2 main surgical sites were hip (22.5%) and knee (17.5%).

Results: Extraction, amplification and hybridization reactions were completed in 28 of the 40 cases, failed in all cartridge chambers in 6 cases, and in 1 or 2 chambers in an additional 6 cases. Overall sensitivity and specificity for microorganism identification were estimated at 67.6% and 98.2%, when complete and partial failures were excluded.

Conclusions: These results show that the performances of the second version of the Unyvero ITI G2 cartridge should be further enhanced before considering avoiding conventional microbiological methods.

Key words: diagnosis, molecular methods, osteo-articular infections, Unyvero ITI G2 cartridge

Introduction

Prosthetic joint infection (PJI) is one of the most serious complications of orthopedic surgery. Although rates currently range between 0.8% to 1.2% of joint replacements [1], absolute numbers of PJIs are expected to rise with increasing numbers of arthroplasty performed among ageing populations. Furthermore, the rise in global antimicrobial resistance is a matter of concern in this situation. Among osteo-articular infections (OAI), diagnosing PJI remains challenging. Conventional microbiological methods (CMM) represent the cornerstone of the strategy, yet fail to provide bacterial detection in at least 5% of the cases [2, 3]. The reasons for these failures are mainly linked to the poor growth of organisms embedded in biofilms, which may be overcome with sonication of implants, and to prior antibiotic therapy or fastidious pathogens [4]. Over the past few years, molecular diagnostic tools have been developed to supplement culture-based bacteriological techniques [5]. For example, broad-range molecular tools, such as 16S rDNA PCR, coupled with direct sequencing of the amplicons, was evaluated for PJI diagnosis directly on clinical samples [6,7]. Its main interest resides in its ability to detect DNA from any bacterium, although results may be interpretable when multiple bacterial species are present, distinguishing between contaminants and relevant microorganisms may be difficult, and there is a lack of standardized protocols [8]. An approach consisting of targeting a panel of prevalent microorganisms responsible for a particular type of infection with multiplex PCR systems emerges [5, 9, 10]. This is a double-edged strategy since non-comprehensiveness and issues regarding the threshold (chosen for the consideration of positivity) limit the identification of contaminants, yet may result in overlooking some rare true pathogens. The Unyvero[®] Implant and Tissue Infection cartridge system developed by Curetis is one of these multiplex PCR

systems and the sole fully dedicated to implant and tissue infection. This includes an automated lysis step followed by multiplex PCRs combined with amplicon detection by array hybridization and allows for identification of most prevalent pathogens and their resistance genes within 5 hours. Studies have been conducted to evaluate the performances of the first version of this system (i.e. cartridge, i60 ITI, covering 80 clinically relevant diagnostic targets) in the diagnosis of OAI [11-20]. Since the performances of this first version were moderate, the company developed a second version of the cartridge, the ITI G2, covering an extended panel of 102 diagnostic targets, comprising 85 relevant pathogens including several fungi and 17 resistance markers.

Since the performances of the second version were never evaluated, the aim of this work was to assess its diagnostic performance for the diagnosis of OAI, and the concordance between results obtained with the cartridge and CMM, mainly on a panel of solid samples.

Material and Methods

Study design, sample collection and case definition

From 01/01/16 to 09/30/16 samples from 35 patients who underwent surgery in the orthopedic department of a 1,500-bed University Hospital were routinely processed with CMM according to the laboratory protocol (see below) and excess material was systematically frozen at -40°C, for testing with ITI G2. Osteitis, PJI and spondylitis were defined using 2013 IDSA criteria [3]. Due to the limited number of cartridges available we only tested 1 sample per patient.

Gold standard - Conventional Microbiological Methods (CMM)

Cultures of tissue, liquid or bone samples were performed following a standardized protocol. Perioperative specimens such as tissue, liquid or bone samples were collected from each patient in sterile vials. Specimens were homogenized and microscopically examined after Gram staining. The samples were immediately cultured on solid agar plates (containing 5% sheep blood or chocolate horse blood) and incubated for 14 days at 37°C simultaneously in aerobic, microaerophilic and anaerobic atmospheres. Additionally, specimens were inoculated into brain heart infusion broth for enrichment, incubated at 37°C for 10 days and then systematically subcultured for 72 hours. The remaining samples were frozen at -40°C. Isolated colonies were identified with standard microbiological procedures, i.e. matrix-assisted laser desorption/ionization time of flight mass spectrometry. Antibiotic susceptibility testing was performed by disk diffusion method as recommended (http://eucast.org/clinical_breakpoints) whereas rapid testing for methicillin resistance on *Staphylococcus aureus* colonies was also performed with the Alere® PBP2a test. The bacteriological results were considered positive if at least one culture yielded a strict pathogen (such as *S. aureus*, *Pseudomonas aeruginosa*, *Enterobacteriaceae* or

anaerobes) or when at least two cultures yielded a skin commensal pathogen (such as coagulase-negative staphylococci [CNS] or *Cutibacterium acnes*) in the case of PJI [3].

Multiplex PCR with ITI G2 on the Unyvero platform (Table S1)

Unyvero is an automated semiquantitative DNA cartridge-based platform composed of 3 units within which sample lysis is combined with end-point PCR and amplicon detection by array hybridization. Characteristics of cartridges are depicted in supplementary Table S1.

Samples were processed as recommended by the manufacturer. Briefly, 180µl of liquid samples were transferred into the Unyvero sample tube. Tissue or bone samples were first cut aseptically, in a microbiological safety cabinet, into of 1-3 mm x 1-3 mm pieces less than 3 mm thick. Three to 5 pieces were then introduced into the sample tube, respecting the maximum volume indicated by the manufacturer. When the total volume was inferior to the suggested maximum, buffer was added to that limit. The 30-min lysis protocol (chemical, mechanical, thermic and enzymatic) was developed to be applied directly on solid (including bone), liquid and viscous samples without any lengthy pretreatment. At the end of lysis, the sample tube with primers was transferred into the cartridge along with the master mix, which was then loaded into the Unyvero analyzer unit.

Concordance analysis

The overall concordance between the CMM and the ITI G2 results was determined at 3 levels, for species identification, for antibiotic resistance and for both identification and resistance.

For identification, a concordant result was defined as an identical result obtained with both techniques for the same sample. A partially concordant result was defined as

the simultaneous identification of at least one microorganism with both techniques if this was associated with an additional pathogen either identified or missed by ITI G2 or if, in case of exact matching results obtained with both techniques, partial failure occurred in one or more chambers. A result was defined discordant if no pathogen was identified by ITI G2 while the culture was positive, or when microorganisms identified by ITI G2 were completely different from those identified by CMM. When no results were obtained due to complete system failure, samples were excluded from analysis.

As for antibiotic resistance we defined 2 result types. A result was defined as concordant when there was agreement between the gene(s) detected by ITI G2 and the antimicrobial susceptibility profile (ASP) of the corresponding cultured microorganism. If the pathogen identified by CMM was not targeted by ITI G2 (e.g. *C. striatum* or *C. acnes*) the result was also considered to be concordant when no resistance gene was detected by the cartridge. A result was defined as discordant: (i) if a resistance gene detected by ITI G2 was incompatible with ASP; (ii) if a resistance gene implied by ASP was not detected by ITI G2 and; (iii) if, despite concordance between a resistance gene identified by ITI G2 and one implied by ASP, a partial failure occurred in a PCR chamber containing probes for resistance markers.

Data analysis

Overall sensitivity (Se) and specificity (Sp) were calculated for pathogen identification, using CMM as the gold standard for comparison; considering a test result (i) as true positive when the same organism was identified by CMM and ITI G2 (CMM+, ITI G2 +); (ii) as false positive when an organism was detected by ITI G2 but not by CMM (CMM-, ITI G2 +); (iii) as true negative when no organism was detected by either method (CMM-, ITI G2 -); and (iv) as false negative when an organism was

detected by CMM but not by ITI G2 (CMM+, ITI G2-). Sensitivity, specificity positive and negative predictive values were assessed both after excluding only complete, and complete plus partial failures as defined above. The 95% confidence interval (CI 95%) for test characteristics was calculated with Wilson's method. We excluded resistance gene detection from this analysis since the data is too scarce to allow this type of analysis.

Results

Study population and samples (Table I and II, Figure 1)

Among the 40 patients included, 25 were male and 15 female, 28 had foreign material and 12 no foreign material before surgery. Among the 35 patients having an infection, 23 had PJI, 6 had osteitis, 4 had soft tissue infections (STI) and 1 had spondylitis and 1 osteoarthritis, whereas 5 had no OAI. Most of the patients (70%) had an acute infection.

Sample types were deep soft tissues (n=23), joint capsule (n=3), periprosthetic membrane (n=2), cartilage (n=1), bone (n=1), hematoma (n=2), joint fluid (n=5), and pus (n=3).

All patients received a combination of vancomycin and piperacillin/tazobactam after surgery, and 6 of them had received antibiotics before surgery (Table II). The vast majority of the samples tested were periprosthetic or deep soft tissues (n=32). *S. aureus* was responsible for more than 50% of the monomicrobial infections. In the mixed infections included in this study 2 to 3 microorganisms were found.

Summary of the Unyvero ITI G2 test results

Complete failure occurred in 6 of the 40 cases (4 polymicrobial, 2 monomicrobial) (Figure 1, Tables IIIa, b,c); the same result being observed after a second attempt. Among the remaining 34, partial failure occurred in six cases (3 monomicrobial, 2 polymicrobial and 1 without infection) due to the failure of 1 or 2 PCR chambers (cases 14, 16, 25, 30, 35 and 40, Tables IIa, b, c). Among the remaining 28 cases, 4 were negative and a single organism was detected in 20 samples, while at least 2 organisms were detected in 4 cases.

Concordance between ITI G2 and CMM results (Figure 1, Table IIIa, b, c)

Pathogen detection

Overall, 26 samples yielded positive results with both CMM and PCR (Tables IIIa, IIIb, and IIIc), among which 21 were monomicrobial and 5 were polymicrobial based on CMM results. Three positive samples by CMM were negative by ITI G2 (Table IIIa and IIIb: cases 24, 25, and 28) leading to discordant results. The CFU recovered for the species concerned (*C. acnes*, *C. striatum*, *E. faecalis* and *E. cloacae complex*) were very low (less than $10^3/\text{ml}$), whereas their ITI G2 detection thresholds were high (10^5 and 10^6). Universal primers (detection threshold: 10^5) allowing for the detection of species not included in the panel had allowed for the detection of *Streptococci* in case 27. ITI G2 did not detect any additional microorganism other than those already identified by CMM in the 6 cases of patients that had received antibiotics before surgery (Table I).

Overall, concordance was observed for 21 of 34 samples (i.e. excluding the samples where a complete failure occurred) resulting in a complete agreement rate of 62%. Concordance occurred more frequently among monomicrobial (16/25) and negative samples (4/5) than among polymicrobial samples (1/10). Moreover, additional species (CNS most of the time), missing species or chamber failures were responsible for 10 partially concordant results. If the 5 concordant results among these latter cases were added to the concordant results the agreement rate rose to 76%.

Regarding Gram staining, ITI G2 allowed for identification of an organism in all 9 Gram stain-positive samples in which the organism was identified by CMM, and it allowed for identification of an organism in 17 of the 20 Gram stain-negative samples in which the organism was identified by CMM.

Overall sensitivity and specificity for microorganism identification were estimated at 72.1% (CI 95%, 57.3-83.3) and 98.9% (CI 95%, 97.6-99.5) respectively when complete failures were excluded (Table IV) and 67.6% (CI 95%, 50.8-80.9) and 98.2% (CI 95%, 96.1-99.2) respectively when complete and partial failures were excluded (Table V). The predictive positive (PPV) and negative (PNV) values were 83.8% (CI 95%, 68.9-92.4) and 97.8% (CI 95%, 96.1-98.7) for the group from which the complete failures were excluded and 79.3% (CI 95%, 61.6-90.2) and 96.7% (CI 95%, 94.2-98.2) for the group from which the complete and the partial failures were excluded.

Antimicrobial resistance detection

Concordance was observed for 25 of 28 samples (i.e. excluding the 12 samples where failures occurred) resulting in an agreement rate of 89.3%. Concordance was more frequent among monomicrobial (18/25) and negative (4/5) cases than among polymicrobial cases (3/10). Discordance was inferred for 9 samples (Tables IIIa and b: cases 2, 11, 32) for the following reasons: (i) the detection of *mecA* in case 2 while only *E. cloacae* was detected by CMM, as well as the detection *aacA4* which was not compatible with the ASP of the *E. cloacae* isolated by CMM; and (ii) the absence of detection of resistance genes while ASP suggested their presence (e.g. *erm* genes in cases 11 and 32).

When analyzing results for cases where partial chamber failure occurred, discrepancies were also observed between ITI G2 and CMM. These discrepancies were either due to a failure in the chamber containing the expected resistance gene (e.g. in cases 30 and 35) or they were true discrepancies. The latter result from the absence of detection of a resistance gene(s) when ASP suggests its (their) presence, e.g. of *aaC(6')-aph(2'')* as would be expected for a CNS in case 35, of *mec* and *erm*

genes for *S. epidermidis* in case 16 and of the latter 3 genes for *S. haemolyticus* in case 30.

***Overall concordance for organism detection and antimicrobial resistance
(Tables IIIa, b, c)***

Overall, perfect concordance concerning microorganism and antimicrobial resistance detection was observed in 21 of 28 cases (75%), i.e. in 4/4 negative, 16/20 monomicrobial and 1/4 polymicrobial cases.

DISCUSSION

Here we present the first evaluation of the performance of the ITI G2 cartridge, analyzing mainly tissue samples. Under the most stringent conditions (i.e. after exclusion of complete and partial cartridge failures), overall sensitivity and specificity of ITI G2 for microorganism identification were 67.6% and 98.2%, respectively. PPV and NPV were estimated at 79.3% and 96.7%. These results are compatible with the values given by the manufacturer [22], and are similar to what was reported for the first version of the test (i.e. i60 ITI) in previous work where sensitivity and specificity were 66.7%, and 100%, 51% and 94% and 60.5% and 98% respectively [13, 15, 17]. When analyzing the results per sample the concordance rate was 62% for microorganism identification, which is similar to the 64% reported by *Renz et al* (concentrated sonicated fluids) [17] and superior to the 58% reported by *Malandain et al* [16]; and was of 89.3% for antimicrobial resistance determination. The overall concordance rate was 75%, which is inferior to the 82% reported previously with the i60 ITI version [14]. This could be due to a higher proportion of negative cases included in that study as compared to this study (21/28 vs. 5/40), as already hypothesized [17].

Other circumstances could impact the overall concordance rates. The thresholds of detection for resistance genes are higher than those for the corresponding microorganism (e.g. *mec* or *erm* genes and staphylococci); therefore negative results for resistance markers should be interpreted carefully (for example *S. haemolyticus* case 30, Table IIIb, Figure S1). As for the 3 discordant negative results for pathogen identification (cases 24, 25, and 28), Gram staining was negative in each case and the counts of colony-forming units (CFU) were low or very low. These discrepancies might then be explained by the high thresholds chosen for microorganism detection

(ranging from 10^5 to 10^6 CFU for *C. striatum*, *E. faecalis*, *C. acnes*, *E. cloacae*). This was already underlined for the 1st version i60 ITI, and it was suggested to enhance the sensitivity by lowering thresholds or concentrating bacterial DNA from samples [17]. In this 2nd version, the thresholds remain high (10^4 and 10^8), and despite an attempt to evaluate the positivity rate at 1/10th of the detection threshold for some targets proposed by the manufacturer, these thresholds are still responsible for false negatives, especially in cases of low inoculum such as in chronic PJI [22]. Nevertheless, ITI G2 allowed for the detection of microorganisms in 17 of 20 samples showing growth in culture where Gram staining was negative, which implies less than 10^4 CFU/ml of sample. This underlines the usefulness of molecular techniques which may help counterbalance the low sensitivity of Gram staining [23].

The possibility of discrepancies due to the presence of additional species identified by ITI G2, although not identified by CMM, should also be pointed out. Here, such species were detected by ITI G2 in 5 cases (i.e. 2, 11, 21, 29 and 32, Tables III). In case 11, an additional CNS was identified by ITI G2 concurrently with *S. aureus* found by CMM. This could be due to some cross reactions, as described in the manufacturer's guidelines, when the abundance of the pathogen is high. However, CNS, *C. acnes*, and *A. defectiva* were also detected as additional species in the 4 other cases.

The manufacturer enlarged the panel with universal primers allowing for the identification of the presence of microorganisms not included in the panel. This is useful, as illustrated by case 27 (Table IIIb), but requires additional PCR-sequencing to obtain the identification at the species level, which cannot be performed from the cartridge.

The resistance profiles recognized by the test should facilitate early and adequate antibiotic therapy particularly in countries with a high prevalence of multidrug-resistant organisms [14]. In our setting of low carbapenemase-producing *Enterobacteriaceae* prevalence in OAI, recognition of the *mec* and *bla*_{CTX-M} genes might suffice for early treatment decision making. This would leave room for the inclusion of probes recognizing otherwise prevalent *Enterobacteriaceae*, as *Morganella*, *Providentia* or *Serratia* species that are of interest to physicians facing hospital-acquired infections.

The failure rate of the ITI G2 test was high in our hands. We observed system failures at the rate of 30%, either caused by complete or by partial failure (6 of 40 samples in each case) which led to unreadable results in one or more reaction chambers due to software problems. Since the manufacturer initially mentioned that technical problems could arise with viscous samples, we diluted and vortexed these in an attempt to decrease the viscosity, although without success. This suggests that the automated lysis step may be inadequate for certain specimen types and should be enhanced; otherwise a manual pretreatment protocol should be devised for them.

Conclusion

Despite including a relatively small number of patients, our results clearly show that the second version of this test also harbors moderate performances for the diagnosis of OAI (i.e. 62% of the OAI diagnosed directly from clinical specimens) and issues in failure rates, currently limiting its interest and ability to replace conventional microbiological methods.

Ethics approval and consent to participate. All procedures performed were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Figure 1. Summary of the results for pathogen identification with Unyvero ITI G2

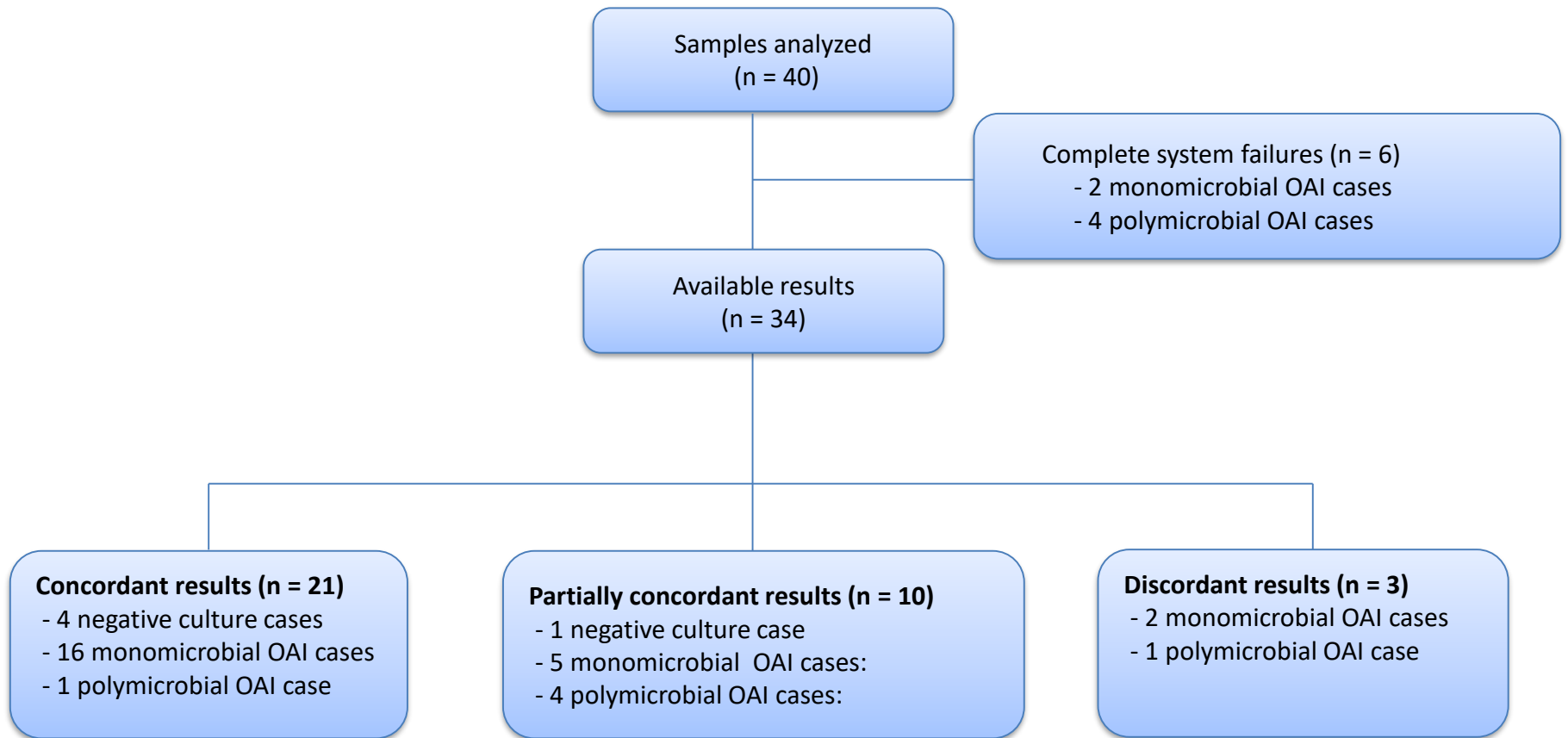


Table I. OAI Characteristics of the 40 patients included in the study

Patients and characteristics	
Median age (years)	57
Age range	16-90
Male (%)	62
Surgical site (%)	
- hip	9 (22,5)
- knee	7(17,5)
- calcaneus	3 (7,5)
- ankle	3 (7,5)
- humerus	2 (5)
- foot	4(10)
- spine	8 (20)
- other	4 (10)
Presentation of infection (%)	
- acute	28 (70)
- chronic	12 (30)
Antimicrobial therapy over 15 days before surgery	15
Sample type (%):	
- tissue	27 (68)
- pus	5 (13)
- synovial/articular fluid	6 (15)
- bone	1(2)
- cartilage	1(2)
Infection type (%):	
- monomicrobial	25 (62,5)
- polymicrobial (2 to 3 microorganisms)	10 (25)
- no infection	5(12,5)

Pathogen responsible for

25

monomicrobial infection (n) (%):

- <i>S. aureus</i>	13 (52)
- CNS	3 (12)
- <i>C. acnes</i>	2 (8)
- <i>C. striatum</i>	1(4)
- <i>S. agalactiae</i>	2(8)
- <i>F. magna</i>	1(4)
- <i>P. aeruginosa</i>	1(4)
- <i>E. coli</i>	1(4)
- <i>E. cloacae</i>	1(4)

CNS, coagulase-negative staphylococci; OAI, osteo-articular infections

Table II. Results obtained for patients who received antibiotics before surgery

Patient number	Antibiotics before surgery	Organism identified by CMM	Organism identified by ITI G2
1	Cefotaxim stopped 3 weeks before surgery	<i>E. coli</i>	<i>E. coli</i>
14	Ofloxacin+rifampin	<i>F. magna</i>	<i>F. magna</i>
15	Amoxicillin+clavulanic acid+ pristinamycin	<i>C. acnes</i>	None
25	Amoxicillin+clavulanic acid	<i>C. striatum</i>	None
28	Ofloxacin stopped 6 days before surgery	<i>E. faecalis</i>	None
		<i>E. cloacae</i>	
30	Vancomycin+fusidic acid	<i>E. cloacae</i>	<i>E. cloacae complex</i>
		<i>B. fragilis</i>	<i>B. fragilis</i>
		<i>S. haemolyticus</i>	CNS

Table IIIa. Detailed results for monomicrobial cases

Patient number	Gram staining	Organism identified		Concordance for identification	Phenotypic resistance profile				Resistance genes identified by Unyvero ITI G2	Concordance for resistance gene	Concordance for identification and resistance gene	System failure
		Culture	Unyvero ITI G2		β-Lactams	Macrolides	AG	GP				
1	0	<i>E. coli</i>	<i>E. coli</i>	Y	<i>OXA-30</i>	NA	GN R, AMK R	NA	<i>aacA4</i>	Y	Y	
2	0	<i>E. cloacae</i>	<i>E. cloacae</i> complex / CNS*	P	<i>dAmpC</i>	NA	GN R, AMK S	NA	<i>aacA4 / mecA</i>	N	N	
3	0	<i>S. lugdunensis</i>	CNS	Y	MS, pase	E S	S	S	Neg	Y	Y	
4	0	<i>S. capitis</i>	CNS	Y	MR	ERY R	S	S	<i>MecA</i>	Y	Y	
5	GPC	<i>S. aureus</i>	<i>S. aureus</i>	Y	MR	ERY R MLS _b const	T R, GEN S	S	<i>mecA / ermA</i>	Y	Y	
6	GPC	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY R	S	S	Neg	Y	Y	
7	0	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
8	GPC	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
9	0	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
10	GPC	<i>s aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
11	GPC	<i>S. aureus</i>	<i>S. aureus</i> / CNS*	P	MS, pase	ERY R: MLS _b ind	S	S	Neg	N	N	
12	0	<i>S. agalactiae</i>	Invalid	NA	S	ERY S	I	S	Invalid	NA	NA	CF
13	GPC	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
14	GPC	<i>F. maga</i>	<i>F. maga</i>	P	S	CLI S	NA	S	Neg	N	N	Ch3, Ch5
15	0	<i>C. acnes</i>	<i>C. acnes</i>	Y	S	CLI S	NA	S	Neg	Y	Y	
16	0	<i>S. epidermidis</i>	CNS	P	MR	ERY R: MLS _b ind	T R, GEN R	S	Neg	N	N	Ch7
17	0	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
18	GPC	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
19	0	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY R	S	S	Neg	Y	Y	
20	0	<i>S. aureus</i>	<i>S. aureus</i>	Y	MS, pase	ERY S	S	S	Neg	Y	Y	
21	0	<i>S. aureus</i>	<i>S. aureus</i> / CNS*	P	MS, pase	ERY R	S	S	Neg	Y	N	
22	0	<i>S. agalactiae</i>	Invalid	NA	S	ERY S	I	S	Invalid	NA	NA	CF
23	0	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Y	<i>dAmpC</i>	NA	S	NA	Neg	Y	Y	
24	0	<i>C. acnes</i>	Neg	N	S	CLI S	NA	S	Neg	Y	N	
25	0	<i>C. striatum</i>	Neg/PI	N	R	ERY R, L R	NA	S	Neg/PI	N	N	Ch 3

aacA4, name for *aac(6')*-Ib in alternative nomenclature, AG, amino-glycosides; AMK, amikacin; CF, chamber failure; Ch, chamber; CNS, coagulase negative staphylococci; CLI, clindamycin; *dAmpC*, dereperessed cephalosporinase ERY, erythromycin; GEN, gentamicin; GP, glycopeptides; GPC, Gram-positive cocci; I, intermediate; L, lincomycin; MLS_b constit, constitutive MLS_b; MLS_b ind, inducible MLS_b; MR, meticilline resistance; MS, meticilline suscpetible; N, no; NA, not applicable; P, partially; pase, penicillinase; PI, partially invalid; R, resistant; S, susceptible; T, tobramycin; WT, wild type; Y, yes; *, additional specie

Table IIIb. Detailed results for polymicrobial cases

Patient number	Gram staining	Organism identified		Concordance for identification	Phenotypic resistance				Resistance gene identified by Unyvero ITI G2	Concordance for resistance gene	Concordance for identification and resistance genes	System failure
		Culture	Unyvero ITI G2		β-Lactams	Macrolides	AG	GP				
26	GPC	<i>S. aureus</i>	Invalid	NA	MS, pase	ERY S	S	S	Invalid	NA	NA	CF
		<i>S. agalactiae</i>			S	ERY S	I	S				
27	0	<i>S. anginosus</i> <i>S. constellatus</i>	universal bacteria	Y	S S	ERY S for both	I	S	Neg	Y	Y	
28	0	<i>E. faecalis</i>	Neg	N	S	-	I	S	Neg	Y	N	
		<i>E. cloacae</i>			WT <i>AmpC</i>	S	S	-				
29	0	<i>P. aeruginosa</i>	<i>P.aeruginosa</i> ,	P	pase	-	R	-	CTX-M	Y	N	
		<i>K. pneumoniae</i>	<i>K.pneumoniae</i> ,		ESBL	-	S	-				
		<i>M. morgani</i>	- <i>A.defectiva*</i> , CNS*		d <i>AmpC</i>	-	GEN S, AMK S	-				
30	BGN	<i>E. cloacae</i>	<i>E.cloacae</i> complex,	P	MBL, ESBL	-	GEN R, AMK I	-	CTX-M, <i>aacA4</i> , NDM	N	N	ch 6
		<i>B. fragilis</i>	<i>B.fragilis</i>		pase	CLI S	-	-				
		<i>S. haemolyticus</i>	CNS		MR	ERY R	T R, GEN R	S				
31	GPC	<i>S. aureus</i>	Invalid	NA	MS, pase	ERY R	S	S	Invalid	NA	NA	CF
		<i>E. cloacae</i>			WT <i>AmpC</i>	MLS _b ind S	S	-				
32	0	<i>S. aureus</i>	Neg (<cut off)	P	MS, pase	ERY R	S	S	Neg	N	N	
		<i>P. mirabilis</i>	<i>Proteus</i> spp, <i>C. acnes*</i>		pase	MLS _b constit	-	GEN R, AMK S				
33	0	<i>E. coli</i>	Invalid	NA	pase	-	S	-	Invalid	NA	NA	CF
		<i>E. faecalis</i>			S	ERY I	I	S				
		<i>P. mirabilis</i>			S	-	S	-				
34	0	<i>P. mirabilis</i>	Invalid	NA	S	-	S	-	Invalid	NA	NA	CF
		<i>S. aureus</i>			MS, pase	ERY S	S	S				
		<i>M. morgani</i>			WT, <i>AmpC</i>	-	S	-				
35	0	<i>P. mirabilis</i>	<i>P. mirabilis</i>	P	pase	-	S	-	<i>mecA</i>	N	N	Ch 3, 4
		<i>S. aureus</i>	<i>S. aureus</i>		MS, pase	ERY R	S	S				
		<i>S. epidermidis</i>	SCN		MR	ERY R	T R, GEN R	S				

aacA4, name for *aac(6')*-Ib, AG, amino-glycosides; AMK, amikacin; Ch, chamber; CF, chamber CNS, coagulase negative staphylococci; CLI, clindamycin; CGP, Gram-positive cocci; d*AmpC*, dereperessed cephalosporinase; ERY, erythromycin; ESBL, extended spectrum beta lactamase; GEN, gentamicin; GP, glycopeptides; I, intermediate; L, lincomycin; MLS_b constit, constitutive MLS_b; MLS_b ind, inducible MLS_b; MR, meticilline resistance ; MS, meticilline susceptible; N, no; NA, not applicable; MBL, metallo beta lactamase; NDM, new delhi metallo beta lactamase; P, partially; pase, penicillinase; PI, partially invalid; R, resistant; S, susceptible; T, tobramycin; WT *AmpC*, wild type cephalosporinase; Y, yes; *, additional specie

Table IIIc. Detailed results for negative cases

Patient number	Gram stain	Organism identified		Concordance for identification	Phenotypic resistance				Resistance gene identified by Unyvero ITI G2	Concordance for resistance gene	Concordance for identification and resistance	System failure
		Culture	Unyvero ITI G2		β -Lactams	Macrolides	AG	GP				
36	0	none	Neg	Y	-	-	-	-	Neg	Y	Y	
37	0	none	Neg	Y	-	-	-	-	Neg	Y	Y	
38	0	none	Neg	Y	-	-	-	-	Neg	Y	Y	
39	0	none	Neg	Y	-	-	-	-	Neg	Y	Y	
40	0	none	Neg/PI	P	-	-	-	-	Neg/PI	N	N	Ch 1

AG, amino-glycosides; Ch, chamber; GP, glycopeptides; N, no; Neg, negative; PI, partially invalid; Y, yes

Table IV. Unyvero ITI G2 cartridge performance compared to that of CMM for microorganism detection (samples that encountered complete system failure (n=6) were excluded)

Species identified	CMM	ITI G2	True positive (TP)	False positive (FP)	False negative (FN)	True negative (TN)
<i>S. aureus</i>	15	14	14	0	1	19
CNS	5	9	5	4	0	25
<i>E. cloacae</i>	3	2	2	0	1	31
Not specified bacteria*	3	1	1	0	2	31
<i>C. acnes</i>	2	2	1	1	1	31
<i>P. aeruginosa</i>	2	2	2	0	0	32
<i>P. mirabilis</i>	2	2	2	0	0	32
<i>F. magna</i>	1	1	1	0	0	33
<i>C. striatum</i>	1	0	0	0	1	33
<i>E. faecalis</i>	1	0	0	0	1	33
<i>K. pneumoniae</i>	1	1	1	0	0	33
<i>B. fragilis</i>	1	1	1	0	0	33
<i>E. coli</i>	1	1	1	0	0	33
<i>A. defectiva</i>	0	1	0	1	0	33
None	5	5	0	0	5	29
Total	-	-	31	6	12	524

CMM, classical microbiological methods; CNS, coagulase-negative staphylococci

*bacteria not detected at the species level by ITI G2 (1 *S. constellatus*, 1 *S. anginosus*, 1 *M. morganella*), but detected by universal primers

To determine the number of true negatives for each specie, TP, FP, FN were added together and then subtracted to the total number of ITI G2 tests completed for each specie (ex for *S. aureus*, 34 ITI G2 tests completed, TN = 34-15 =19)

Table V. Unyvero ITI G2 cartridge performance compared to that of CMM for microorganism detection (samples that encountered complete and partial system failure were excluded (n=12))

Species identified	CMM	ITI G2	True positive	False positive
<i>S. aureus</i>	14	13	13	0
Not specified bacteria*	3	1	1	0
CNS	2	6	2	4
<i>C. acnes</i>	2	2	1	1
<i>P. aeruginosa</i>	2	2	2	0
<i>E. cloacae</i>	2	1	1	0
<i>E. faecalis</i>	1	0	0	0
<i>K. pneumoniae</i>	1	1	1	0
<i>P. mirabilis</i>	1	1	1	0
<i>E. coli</i>	1	1	1	0
<i>A. defectiva</i>	0	1	0	1
None	4	4	0	0
Total	-	-	23	6

CMM, classical microbiological methods; CNS, coagulase-negative staphylococci

*bacteria not detected at the species level by ITI G2 (1 *S.constellatus*, 1 *S.anginosus*, 1 *M. morganella*) but detected by universal primers

To determine the number of true negatives for each specie, TP,FP,FN were added together and then subtracted to the total number of ITI G2 tests completed for each specie (ex for *S. aureus*, 28 ITI G2 tests completed, TN = 28-14 =14)

Table SI. Distribution of genetic markers in the 8 PCR chambers

Chamber 1	Chamber 2	Chamber 3	Chamber 4
<i>Bacteroides fragilis group</i> ⁹ (10 ⁴)	<i>Abiotrophia defectiva</i> (10 ⁵)	aac(6 ['])-aph(2 ^{''}) (10 ⁶)	AacA4 (10 ⁵)
<i>ermC</i> (10 ⁷)	<i>Coagulase negative staphylococci</i> (CNS) ¹ (10 ⁴)	<i>Candida albicans</i> (10 ⁵)	<i>ermA</i> (10 ⁴)
<i>Fingoldia magna</i> (10 ⁶)	<i>Corynebacterium spp.</i> ⁴ (10 ⁵)	<i>C. freundii/koseri</i> (10 ⁵)*	<i>bla_{NDM}</i> (10 ⁴)
<i>Granulicatella adjacens</i> (10 ⁵)	<i>E. faecalis</i> (10 ⁶)	<i>Enterococcus spp.</i> ³ (10 ⁵)	<i>P.acnes</i> (10 ⁵)
<i>S. aureus</i> (10 ⁴)	<i>Streptococcus spp.</i> ² (10 ⁵)*	<i>S.agalactiae</i> (10 ⁴)	<i>S.pneumoniae</i> (10 ⁴)*
	<i>vanA</i> (10 ⁵)	<i>vanB</i> (10 ⁶)	<i>S.pyogenes/dysgalatiae</i> (10 ⁴)

Chamber 5	Chamber 6	Chamber 7	Chamber 8
<i>Acinetobacter baumannii</i> complex ⁸ (10 ⁴)	<i>E. aerogenes</i> (10 ⁵)	Universal bacteria ¹⁴ (10 ⁵)*	<i>E. cloacae</i> complex ⁵ (10 ⁵)
<i>bla</i> _{CTX-M} ¹⁰ (10 ⁵)	<i>bla</i> _{IMP} ¹¹ (10 ⁵)	<i>Candida glabrata</i> (10 ⁵)*	<i>E. coli</i> (10 ⁴)
<i>K.pneumoniae</i> ⁷ (10 ⁵)	<i>K.oxytoca</i> (10 ⁴)	<i>Candida spp</i> ¹³ (10 ⁵)*	<i>bla</i> _{KPC} (10 ⁵)
<i>K. variicola</i> (10 ⁴)*	<i>mecA</i> (10 ⁵)	<i>Candida tropicalis</i> (10 ⁸)*	<i>mecC</i> (10 ⁶)
<i>bla</i> _{OXA-23} (10 ⁴)	<i>Proteus spp.</i> ⁶ (10 ⁴)	<i>Isaatchienka orientalis</i> (<i>C.krusei</i>) (10 ⁵)*	<i>bla</i> _{OXA-48} (10 ⁵)
<i>bla</i> _{OXA-24/40} (10 ⁴)	<i>P.aeruginosa</i> (10 ⁴)		<i>bla</i> _{OXA-58} (10 ⁵)
			<i>bla</i> _{VIM} (10 ⁶)

Threshold for each target is indicated between brackets; * target not included in the first version i60 ITI.

¹CNS included: *S.saprophyticus*, *S.hominis*, *S.epidermidis*, *S.warneri*, *S. haemolyticus*, *S. capitis*, *S.lugdunensis*

² *S. pneumoniae*, *S. mitis*, *S. pyogenes*, *S. agalactiae*, *S.sanguinis*, *S. dysgalactiae dysgalactiae*, *S. dysgalactiae subsp.equisimilis*, *S.gordonii*

³ *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. avium*, *E. hirae*, *E. durans*, *E. raffinosus*

⁴ *C. jeikeium*, *C. amycolatum*, *C. striatum*, *C. aurimucosum*

⁵ *E. cloacae*, *E. amnigenus*, *E. asburiae*, *E. hormaechei*

⁶ *P. vulgaris*, *P. mirabilis*, *P. penneri*, *P. hauseri*

⁷ *K. pneumoniae*, *K.pneumoniae subsp ozaenae*

⁸ *A. baumannii*, *A. oleivorans*, *A. calcoaceticus*, *A. pittii*

⁹ *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus*, *B. uniformis*

¹⁰ inclusive *bla*_{CTX-M3}, *bla*_{CTX-M10}, *bla*_{CTX-M15}, *bla*_{CTX-M55}

¹¹inclusive *bla*_{IMP} 1 to 10, 15, 16, 19 et 25

¹² *bla*_{KPC} 1 à 22

¹³ universal primers for fungi

¹⁴ universal primers for bacteria