Detection of bacteria bearing resistant biofilm forms, by using the universal and specific PCR is still unhelpful in the diagnosis of periprosthetic joint infections

Batool H. Zegaer, Anastasios Ioannidis, George C. Babis, Vassiliki Ioannidou, Athanasios Kosyvakis, Sotiris Bersimis, Joseph Papararaskevas, Efthimia Petinaki, Paraskevi Pliatsika and Stylianos Chatzipanagiotou

INTRODUCTION

Periprosthetic joint infection (PJI) is one of the most serious complications causing a high grade of morbidity in patients with total joint arthroplasty. Actually, the rate of infection after total hip or knee arthroplasty ranges from below 1 up to 5% and it rises slightly in case of revision procedures (1, 2).

The accurate diagnosis of bone and joint infections, has for long been confounded by the difficulty of retrieval and detection of microorganisms, and is still a challenge to the treating physician (1, 3).

The clinical diagnosis can be assisted by laboratory tests like white cell count and differentiation, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and others, but there is no gold standard, having a perfect sensitivity and specificity for diagnosing the infection, apart from identifying the infective bacteria (4).

The distinction between septic and non-septic loosening is difficult in most cases, impeding clinical evaluation (5). The risk of a PJI increases greatly due to the long stay, in the body, of the implant, which can be colonized by microbes from a distant infectious focus through the hematogenous route at any time following implantation (1). Therefore, an increase in the number of PJIs is expected in the coming years.

 Currently, periprosthetic infection is frequently diagnosed by isolation of one or more organisms from the periprosthetic tissue

Keywords: periprosthetic joint infection, culture, PCR, biofilms, antimicrobial resistance
or fluid with use of conventional culture techniques, and the
culture’s results are usually considered the standard, which are
compared with other diagnostic tests (6).

Among the various bacterial virulence factors, a very important
one, is the ability to form biofilms (7). In a biofilm, the bacteria are
tached to each other and adhering to the artificial materials and
medicinal devices such as contact lenses, artificial heart valves, joint
replacements, etc., they produce an extracellular polymeric sub-
stance (ESP) consisting of carbohydrate (exopolysaccharide) (8).
The production of biofilms protects the bacteria from the effect of
the antibiotic action, in addition to the inhibition of the phago-
cytic cells, and impedes the function of the T- and B-lymphocytes
(9–11).

The detection and isolation of causative microorganisms is the
first important step for the successful treatment of PJI (12). Many
diagnostic methods are applied in order to isolate the causative
microorganisms in patients with symptoms of a failed arthro-
plasty. Some conventional methods, like culture of aspirated joint
fluid, can be performed preoperatively. However, the preoperative
aspiration does not always precede and the surgeons often do not
make the right diagnosis of an infection until the revision arthroplasty
operation is underway (4).

Molecular techniques overcome some of the limitations of
classical microbiological diagnostic procedures. More than
10 years have passed since the introduction of molecular meth-
ods into the diagnosis of orthopedic infections; these methods are
still a matter of research and discussion. Gallo et al. (13) as well as
Spanghel et al. (14) investigated how the polymerase chain reaction
(PCR) might play an essential role in PJI diagnosis. They pointed
out the advantages of accuracy and speed, as well as the possible
disadvantages, like the false-positive results, misinterpretation,
and the expensive equipment requirements (13, 14).

Despite the numerous studies already published, the role of
molecular techniques, like PCR, in the diagnosis of PJI, still
remains vague and unclear. Therefore, the effectiveness of these
techniques has not yet been verified in the routine of the clinical
microbiology laboratory diagnosis for PJI (5).

The aim of the present study is to compare the conventional
cultures with two PCR methods (universal and specific), for the
fast and accurate diagnosis of PJI in 44 patients who had a pri-
mary or failed total joint arthroplasty. In addition, the isolated
bacteria were investigated with respect to biofilm formation. The
biofilm forming strains, in their planktonic and biofilm forms,
were further tested for their antimicrobial resistance against several
clinically important antimicrobials, used for the treatment of
PJI.

MATERIALS AND METHODS

PATIENTS

The study included 44 bone and joint samples collected intraoper-
avtively from an equal number of patients. Thirteen from patients
with a preoperative diagnosis of infected, and 31 from patients
with a preoperative diagnosis of non-infected be based on a clin-
ician’s independent medical judgment (depending on the history
of the patient, the clinical examination, such as constant pain,
warmth and effusion, erythema, delayed healing of the wound,
plain x-ray, and other patient individualized criteria). All the
patients were treated in the 1st Department of Orthopedic Surgery
"ATTIKON" University Hospital of the Athens Medical School. The
age of the patients ranged from 31 to 85 years. Patients were clas-
sified in two groups, with respect to the final clinical diagnosis, as
infected or non-infected cases.

The clinical determination of deep infection (deep around the
artificial implants) was according to the criteria of international
standard of PJI (15).

All the patients underwent preoperative general blood exami-
nation including white blood cell (WBC) count, ESR, and CRP
determination.

SAMPLE COLLECTION AND CULTURE

The patient samples used in the study were bone, tissue, or aspi-
ration fluid, which had been taken intraoperatively from the
patients with total hip or total knee arthroplasty (more than one
sample had been collected from each patient), as routine diagnos-
ic procedure. Bacteriological examination included conventional
culture on growth media for aerobic and anaerobic bacteria and
direct sample microscopy of Gram stained smears. The identi-
fication of the isolated bacteria was performed by conventional
bacteriological methods: API-E and API-NE (Biomerieux, Marcy-
l’ Etoile/France) for gram-negative bacteria and coagulase and
DNAase for Staphylococcus spp.

BIOFILM PRODUCTION DETERMINATION

All isolated bacterial strains were investigated for their ability
to form biofilms by the tissue culture plate (TCP) method as
described by Christensen et al. (16) and Baldassarri et al. (17), with
a modification in duration of incubation, which was extended to
24 h (18).

Briefly, a bacterial suspension was prepared from a blood agar
plate culture in tryptase soy broth at opacity of 0.5 McFarland
standards and cultured overnight at 37°C. The next day, 100 µl
of the overnight culture were added to 200 µl tryptose broth and
placed in a micro titer tray well, mixed and incubated overnight at
37°C. The next day, the wells were carefully emptied and washed
three times with phosphate buffered saline (PBS). The plate was
allowed to dry at 60°C for 1 h and then stained with Hucker’s crys-
tal violet (2 g crystal violet, 20 ml 95% alcohol, 0.8 g ammonium
oxalate, and 80 ml distilled water). The excess stain was washed
off with distilled water, excess water was removed, and the plates
were read with an ELISA reader at 570 nm (19). The cut-off value
was calculated as mean ± 2SD of the values of 10 wells processed
the same way but without bacteria. Values above the cut-off were
considered positive for biofilm formation. Each strain was tested
in quadruplicate.

MIC DETERMINATION

Antimicrobial susceptibility of the planktonic bacterial forms
was performed and interpreted by determination of the minimal
inhibitory concentration (MIC) using the standard broth dilution
method according to the guidelines of the Clinical Laboratory
Standards Institute (20, 21). The antimicrobials included, were
those of importance in the clinical practice for treating the iso-
lated bacterial species: ciprofloxacin, moxifloxacin, erythromycin,
linezolid, daptomycin, teicoplanin, vancomycin tigecyclin, and

Frontiers in Medicine | Infectious Diseases September 2014 | Volume 1 | Article 30 | 2
cotrimoxazole for *Staphylococcus aureus* and *Staphylococcus epidermidis* and imipenem, meropenem, cefazidime, aztreonam, tobramycin, ciprofloxacin, amikacin, cefepime, and cotrimoxazole for *Pseudomonas aeruginosa*.

**MINIMAL INHIBITORY CONCENTRATION FOR BACTERIAL REGROWTH FROM THE BIOFILM DETERMINATION**

The strains producing biofilms were further tested for their antimicrobial susceptibility by determination of the minimal inhibitory concentration for bacterial regrowth from the biofilm (MICBR) using a modified broth dilution method as described previously (22).

Serial dilutions of the antimicrobial in Mueller Hinton broth, corresponding to the concentrations used for the MIC determination of the planktonic forms, were prepared and poured into the micro titer plates, which contained the bacterial biofilm and incubated at 35°C for 48 h. The growth of planktonic bacteria was visualized by the development of turbidity in the medium. The MICBR was defined as the lowest concentration showing no growth in the medium as observed by a complete clarity. Each strain was tested in quadruplicate.

The results were assessed using the breakpoints given by the guidelines of the CLSI (20, 21).

**POLYMERASE CHAIN REACTION**

Bacterial DNA extraction was performed by means of the protocol of the "Insta Gene Matrix" method (Bio Rad Laboratories, CA, USA) The extracted DNA was stored at −20°C until the time of use.

The types of PCR that were performed for the detection of the causative pathogens were: a) two types of universal PCR (Nr.1 and 2) detecting the 16S rRNA gene by different protocols (23, 24), followed by sequencing of the product for the identification of the species, and b) the species specific PCR, for three selected pathogens: *S. aureus* (25), *S. epidermidis* (26), and *P. aeruginosa* (27). All the PCR primers and annealing temperatures are depicted in Table 1. For all PCR methods, controls were run in parallel with extracted DNA from the following reference strains: *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC 35984, *S. aureus* ATCC 29213, and *Streptococcus pneumoniae* ATCC 49619.

Each reaction of PCR consisted 0.4 µM of forward primer, 0.4 µM of reverse primer, 2.5 units/reaction HotStar Taq DNA Polymerase (Qiagen), 1X PCR Buffer provides a final concentration of 1.5 mM MgCl₂, 200 µM of each deoxynucleotide triphosphates (dNTP), 5 µl DNA sample, and PCR-grade water until they completed 50 µl of reaction volume. The conditions we used are: 1 cycle (94°C for 5 min), 35 cycles (94°C for 1 min, annealing temperature as described in Table 1 for each pair of primers for 1 min, 72°C for 1 min), and 1 cycle (72°C for 10 min), storing at 4°C.

**STATISTICAL ANALYSIS**

The statistical analysis was performed using the statistical package SPSS for Windows (version 20.0) in order to disclose any significant differences between the percentages of antimicrobial susceptibility of the planktonic and the biofilm bacterial forms. The analysis was done by applying an appropriate hypothesis test concerning the difference between the proportions of two samples. The normal approximation to the binomial distribution was used. Additionally, appropriate parametric and non-parametric tests were used for comparing quantitative variables such as CRP, ESR, and WBC.

**RESULTS**

**SAMPLE ORIGIN, SAMPLE SPECIES, CULTURE, AND INFLAMMATION MARKERS**

A total of 44 samples were analyzed in this study: 20 were culture positive, with the following organisms: *S. epidermidis* (15 isolates), *S. aureus* (4 isolates), *P. aeruginosa* (1 isolate), and 24 were culture negative results (Tables 2A,B).

There was no statistically significant relation, either between culture results and patients’ gender or sample origin (total hip or total knee). However, the sample species proved to be critical for the culture outcome: the tissue and bone samples gave significantly more positive culture results than the aspiration fluid (p < 0.001, results not shown).

Sensitivity and specificity of the culture in relation to the presumed preoperative evaluation were 100 and 77.4%, respectively with a Receiver operating characteristic (ROC) area of 0.812–0.962 (ROC value = 0.887).

---

**Table 1 | PCR primers and annealing temperatures for the detection of bacteria causing periprosthetic joint infections**

| Target gene for | Sequence (5’→ 3’) | Annealing temp. (°C) | Product (bp) |
|----------------|-------------------|----------------------|--------------|
| 16S rRNA universal 1 (24) | AGAGTTTGATCCTGGCTCAAG GACGGCGCGTGTGATCAAC | 59 | −1380 |
| 16S rRNA universal 2 (23) | AGTTAGCTCCGCTCAAG AGGCCCGGAAAGATTCAC | 55 | −1450 |
| *Staphylococcus aureus* (25) | CTGTGCCGTCATCTGTAC | 54 | 108 |
| *Staphylococcus epidermidis* (26) | ATCAAAAGTTGCGCGACACTTCA CAAAAGACGCGTGGAAAGATTCAC | 50 | 124 |
| *Pseudomonas aeruginosa* (27) | GGGGATCTTCGGACCTCA TCCTAGAGTGCCCGC | 58 | 956 |
Table 2 | The results of universal and specific PCR technique for the culture (A) positive samples and (B) negative samples.

| Patient no. | Preoperative diagnosis | Culture | Universal 1 PCR [19] | Universal 2 PCR [20] | PCR S. aureus | PCR S. epidermidis | PCR P. aeruginosa |
|-------------|------------------------|---------|----------------------|----------------------|--------------|-----------------|-----------------|
| (A)         |                        |         |                      |                      |              |                 |                 |
| 1           | Infected               | S. epidermidis | N                    | N                    | N             | N               | N               |
| 2           | Infected               | S. epidermidis | N                    | N                    | P             | P               | P               |
| 3           | Non-infected           | S. aureus    | N                    | N                    | N             | N               | N               |
| 4           | Infected               | S. aureus    | N                    | N                    | N             | N               | N               |
| 5           | Infected               | S. epidermidis | P/no id.             | P/no id.             | P             | P               | N               |
| 6           | Infected               | S. epidermidis | N                    | N                    | N             | N               | N               |
| 7           | Non-infected           | S. epidermidis | N                    | N                    | N             | N               | N               |
| 8           | Infected               | S. epidermidis | N                    | N                    | N             | N               | N               |
| 9           | Non-infected           | S. epidermidis | N                    | N                    | N             | N               | N               |
| 10          | Infected               | S. epidermidis | N                    | N                    | N             | N               | N               |
| 11          | Infected               | S. aureus    | N                    | N                    | P             | N               | N               |
| 12          | Infected               | S. aureus    | N                    | P/no id.             | P             | P               | N               |
| 13          | Infected               | P. aeruginosa | N                    | N                    | N             | N               | P               |
| 14          | Infected               | S. epidermidis | N                    | N                    | N             | N               | N               |
| 15          | Infected               | S. epidermidis | N                    | N                    | N             | N               | N               |
| 16          | Non-infected           | S. epidermidis | N                    | P/no id.             | P             | P               | P               |
| 17          | Non-infected           | S. epidermidis | N                    | N                    | N             | N               | N               |
| 18          | Non-infected           | S. epidermidis | N                    | N                    | N             | N               | N               |
| 19          | Infected               | S. epidermidis | N                    | N                    | P             | N               | N               |
| 20          | Non-infected           | S. epidermidis | N                    | N                    | N             | N               | N               |
| (B)         |                        |         |                      |                      |              |                 |                 |
| 21          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 22          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 23          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 24          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 25          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 26          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 27          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 28          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 29          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 30          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 31          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 32          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 33          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 34          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 35          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 36          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 37          | Non-infected           | N        | N                    | N                    | P             | P               | P               |
| 38          | Non-infected           | N        | N                    | N                    | P             | P               | P               |
| 39          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 40          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 41          | Non-infected           | N        | N                    | N                    | N             | N               | N               |
| 42          | Non-infected           | N        | N                    | N                    | P             | P               | P               |
| 43          | Non-infected           | N        | N                    | N                    | P             | N               | N               |
| 44          | Non-infected           | N        | N                    | N                    | N             | N               | N               |

N = negative, P = positive, no id. = no identification.

All three inflammation markers, CRP, ESR, and WBC were significantly higher in patients with a positive culture \( (p < 0.001 \) for all, Table 3).

POLYMERASE CHAIN REACTION
Both universal PCR methods showed very contradictory results in relation to culture. Although there was an agreement between
Table 3 | White blood cell (WBC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) preoperative determination in the blood of the patients subject to total joint arthroplasty.

| Parameter          | Mean value (S.D.) | p-Value (non-infected vs. infected) |
|--------------------|-------------------|------------------------------------|
|                    | Total (N = 44)    | Non-infected (N = 31)              | Infected (N = 13) |
| WBC \( {10^3} \) cells/µl | 9.33 (2.889)     | 7.72 (1.294)                      | 13.15 (1.790)     | <0.001 |
| CRP (mg/dl)        | 5.67 (7.609)      | 1.56 (1.085)                      | 15.47 (7.528)     | <0.001 |
| ESR                | 34.36 (27.510)    | 20.71 (12.006)                    | 66.92 (26.859)    | <0.001 |

FIGURE 1 | Flipchart summarizing the results of both culture and specific - 16S rDNA for 44 total hip and total knee samples.

negative culture and negative PCR for both universal PCR tests, they both failed to detect bacterial 16S rDNA in most culture positive samples. When 16S rDNA was detected, identification on species level through sequencing was impossible; as in all 16S rDNA positive samples, the sequencing reaction was blocked after approximately 60 bp, probably due to the presence of more than one bacterial species (Tables 2A,B). All three specific PCR tests were equally ambiguous, being very ineffective in detecting only one pathogen and giving very discrepant results in relation to culture (Tables 2A,B; Figure 1).

In reference to the preoperative clinical evaluation, sensitivities and specificities of the PCR methods were as follows: universal PCR 1 23.1 and 100% (24), universal PCR 2 23.1 and 96.8% (23), PCR specific for S. aureus 46.2 and 80.6%, PCR specific for S. epidermidis 30.8 and 67.7%, and P. aeruginosa 30.8 and 90.3%.

**BIOFILM FORMATION, MIC, AND MICBR**

All the strains were positive for biofilm production. The great majority of the biofilm forms were resistant to all antimicrobials. In antimicrobial concentrations far higher than the breakpoints, bacterial regrowth from the biofilms was still possible. The results for S. aureus and S. epidermidis are shown in Table 4. Regarding the MIC (value in microgram per milliliter in parenthesis) of the one isolated strain of P. aeruginosa, the strain was susceptible to meropenem (\( \leq 1 \)), ceftazidime (2), aztreonam (4), tobramycin (\( \leq 2 \)), ciprofloxacin (0.25), amikacin (\( \leq 8 \)), and cefepime (2) and intermediate to imipenem (4). The respective MICBR values were at least the fourfold of the MIC values for imipenem, meropenem, ceftazidime, aztreonam, tobramycin, and cefepime, showing significant resistance of the biofilm forms with respect to bacterial regrowth (\( p < 0.001 \)). For ciprofloxacin and amikacin, the MICBR values were identical to the MIC values; thus, these two antibiotics seemed to suppress *in vitro* P. aeruginosa regrowth from the biofilm under corresponding clinical therapeutic dosages.

**DISCUSSION**

The diagnosis of the prosthetic joint infection includes a set of laboratory tests. In the present study, like in previous reports (6, 28, 29), the traditional first line laboratory tests including the inflammation markers CRP, ESR, and WBC proved to have a very good sensitivity, when correlated with the clinical evaluation (Table 3).

Culture is still the gold standard for diagnosis of prosthetic joint infection, offering the possibility of the antimicrobial susceptibility testing, but it is time-consuming in identifying the causative microorganisms. However, the drawbacks of the cultural procedures are the limited sensitivity, in addition to the false negative results in patients receiving antimicrobials (6, 30). Our study comprised 44 cases, 20 cultures-positive, and 24 negative (more than one sample had been taken from each patient). In seven cases with a presumed preoperative absence of infection (non-infected), culture was positive, which may have been through sample contamination during handling (collection, transport, and processing).

In a prospective study involving revision of arthroplasty in 297 patients with a total of 41 infections, Atkins et al. (31) pointed out that only 65% of all samples collected from the infected joints were culture positive. They recommended collecting five or six culture samples from each patient and suggested that the accurate diagnosis of infection should be considered as the growth of the identical microorganism on culture in three or more samples (6). Practically, this procedure is difficult, but at the same time, it could increase the possibility of detecting the causative microorganism.

All isolated bacterial strains were positive for biofilm formation *in vitro*, by the TCP performed as previously described (16, 17). The *in vivo* biofilm synthesis gives bacteria the ability to cause infection and impedes their cultural isolation from the samples.
because the biofilms adhere strongly to the colonized biological surface. The use of ultrasound to expel bacterial cells from the biofilms adherent to the surface of removed implants (sonication) increases the effectiveness and the sensitivity of microbiological studies to determine the underlying microorganisms, but, at the same time, increases the risk of contamination through the more complicated sample handling (32, 33).

The antimicrobial resistance rates of the planktonic and the biofilm forms of the isolated bacteria, tested by the MIC and MICBR determination, showed increased antimicrobial resistance of the biofilm forms to the vast majority of the antimicrobials, with MICs far above those of the planktonic forms and above the breakpoints corresponding to the therapeutic clinical implementation (Table 4). This may explain the failure of treatment in some cases, where despite the antimicrobial susceptibility to a certain antimicrobial in vitro, the infection still exists after the appropriate treatment (clinical resistance) (34–37). Although there are no standard procedures for the determination of MICBR, our results are in agreement with previously reported data, with respect to the role of biofilms in the increase of the bacterial antimicrobial resistance (38). Furthermore, they confirm that the experimental conditions used, led to biofilm formation. Biofilm production in S. epidermidis and P. aeruginosa belongs to the most significant virulence factors for the expression of pathogenicity in infectious diseases (16, 38, 39).

The most important part in this study was the application and evaluation of PCR. Based on the literature, PCR can be considered one of the very helpful diagnostic tools, used in cases of arthritis, especially when culture is negative. Fenollar et al. (3) reported remarkable results for cloning and sequencing of 16S rDNA amplions. They found a perfect compatibility between culture and PCR results in 475 of 525 samples (90.5%). Kordelle et al. (40) sequenced the 16S rDNA amplions and found 100% agreement between PCR and culture, but the study included only seven cases of PJI. In the present study, the universal 16S rDNA PCR technique was totally unhelpful for the bacterial identification. Although it detected bacterial DNA in many cases, with a significant agreement with culture (p-value 0.031), there was no identification of the DNA product after sequencing. This problem might be due to the presence of more than one bacterial species (including the causative microorganism), caused through sample handling and processing. To be effectual, the universal PCR requires specimens containing a single bacterial strain; otherwise, the identification after sequencing is impossible.

The specific PCR showed better results but still very discrepant compared to culture, the p-values were 0.014, 0.583, and 0.226 for PCR S. aureus, S. epidermidis, and P. aeruginosa, respectively in comparison with culture. Published results from different studies on the use of PCR to detect prosthetic joint infection have made PCR a technique not yet widely accepted in routine examination. The sensitivity and specificity of broad-range PCR from synovial fluid and/or tissue for the diagnosis of prosthetic joint infection have been reported to be between 50–92% and 65–94%, respectively (41–44). In the present report, sensitivity and specificity of all the PCR methods applied were very diverse and in none of the cases did they fulfill the criteria of a reliable diagnostic method for the bacteriological diagnosis of PJI (45).

In the present report, the molecular methods proved to be inappropriate for a reliable bacterial diagnosis, the culture remaining still “the gold standard.” When compared to conventional microbiological procedures, PCR analysis is still hindered by higher costs, false-positive results, and interpretative problems. Currently, under these circumstances it is not justifiable to introduce molecular methods into the schemes used to diagnose prosthetic joint infection (13).

The detection and identification of bacterial RNA, rather than DNA, can be a new approach to the laboratory diagnosis of prosthetic joint infection, by reverse transcription. RNA is present only in viable bacteria and, thus, it could be more reliable in disclosing active infections. On the other hand, the much shorter half-life of RNA would make its role as a contaminant less likely (46). Rasouli et al. have been evaluating the new approach of multiplex PCR (Ibis T5000 universal Biosensor), which depend on pan-genomic amplification, and mass spectrometry for culture negative cases in patients with suspicion of PJI and the results were promising (15).

In conclusion, our results showed that the molecular diagnostic methods, like PCR, did not increase the detection rate of prosthetic joint infection, compared to culture. Improved PCR methods may be considered in the near future and play an important role in the diagnosis of bone infections as a complement to culture, in cases

#### Table 4: Antimicrobial resistance rates of planktonic and biofilm forms of S. epidermidis and S. aureus strains isolated from the samples of the patients with periprosthetic joint infection.

| Antimicrobial | S. epidermidis (n = 15) | S. aureus (n = 4) |
|---------------|-------------------------|-----------------|
|               | Planktonic n (%) | Biofilm n (%) | p-value | Planktonic n (%) | Biofilm n (%) | p-value |
| Ciprofloxacin | 5 (33.33) | 11 (73.33) | <0.001 | 2 (60) | 3 (75) | <0.001 |
| Moxifloxacin | 9 (60) | 12 (80) | <0.001 | 2 (60) | 3 (75) | <0.001 |
| Erythromycin | 11 (73.33) | 15 (100) | <0.001 | 2 (60) | 4 (100) | <0.001 |
| Linezolid | 0 (0) | 13 (86.66) | <0.001 | 0 (0) | 4 (100) | <0.001 |
| Daptomycin | 1 (6.66) | 15 (100) | <0.001 | 0 (0) | 4 (100) | <0.001 |
| Teicoplanin | 1 (6.66) | 15 (100) | <0.001 | 0 (0) | 4 (100) | <0.001 |
| Vancocycin | 0 (0) | 12 (80) | <0.001 | 0 (0) | 3 (75) | <0.001 |
| Tigecycline | 6 (40) | 10 (66.66) | <0.001 | 1 (25) | 4 (100) | <0.001 |
| Cotrimoxazole | 7 (46.66) | 14 (93.33) | <0.001 | 1 (25) | 4 (100) | <0.001 |
where a small amount of samples are available for examination, or when culture is negative after 24 h of incubation for patients with suspected prosthetic joint infection, as well as in the diagnosis of samples taken from patients receiving antimicrobial therapy.

REFERENCES

1. Zimmerli W, Trampuz A, Ochberner PE. Prosthetic joint infections. N Engl J Med (2004) 351:1857–65. doi:10.1056/NEJMra040118

2. Sauer P, Gallo J, Kesselova M, Kolar M, Kukolova D. Universal primers for detection of common bacterial pathogens causing prosthetic joint infection. Biomed Pap Med Fak Univer Palacky Olomouc Czech Repub (2005) 149:285–8. doi:10.5507/bp.2005.043

3. Fenollar F, Roux V, Stein A, Drancourt M, Raskas M, Dendis M, Florschutz A V, Kolar M. Molecular diagnosis of prosthetic joint infection. J Clin Microbiol (2006) 44:1018–28. doi:10.1128/JCM.44.4.1018-2018.2006

4. Oehther M, Warner JK, Schindler SA, Kobayashi H, Bauer TW. Diagnosing peri-prosthetic infection: false-positive intraoperative Gram stains. Clin Orthop Relat Res (2011) 469:554–60. doi:10.1007/s11999-010-1589-9

5. Marin M, Garcia-Lechuz JM, Alonso P, Villanueva M, Alcala L, Gimeno M, et al. Role of universal 16S rRNA gene PCR and sequencing in diagnosis of prosthetic joint infection. J Clin Microbiol (2012) 50:583–9. doi:10.1128/JCM.00170-11

6. Bauer TW, Parvizi J, Raskas M, Kober M, Vlahakis N, Krebs V. Diagnosis of peri-prosthetic infection. J Bone Joint Surg Am (2006) 88:869–82. doi:10.2106/JBJS.E.01149

7. Gristina AG, Oga M, Webb LX, Hobgood CD. Adherent bacterial colonization in the pathogenesis of osteomyelitis. Science (1985) 230:990–3. doi:10.1126/science.4001933

8. Sawhney R, Berry V. Bacterial biofilm formation, pathogenicity, diagnostics and control: an overview. Indian J Med Sci (2009) 63:63–21. doi:10.4103/0019-5539.555113

9. Gray EE, Peters G, Verstegen M, Regelmann WE. Effect of extracellular slime substance from Staphylococcus epidermidis on the human cellular immune response. Lancet (1984) 1:365–7. doi:10.1016/S0140-6736(84)90413-6

10. Johnson GM, Lee DA, Regelmann WE, Gray ED, Peters G, Quie PG. Interference with granulocyte function by Staphylococcus epidermidis slime. Infect Immun (1986) 54:13–20.

11. Naylor PT, Myrvik QN, Grinsta A. Antibiotic resistance of biomaterial-adherent coagulase-negative and coagulase-positive Staphylococci. Clin Orthop Relat Res (1990) 261:126–33.

12. Achermann Y, Vogt M, Leunig M, Wust J, Trampuz A. Improved diagnosis of peri-prosthetic joint infection by multiplex PCR of sonication fluid from removed implants. J Clin Microbiol (2010) 48:1208–14. doi:10.1128/JCM.00066-10

13. Gallo J, Raska M, Dendis M, Florschutz A V, Kolar M. Molecular diagnosis of prosthetic joint infection. A review of evidence. Biomed Pap Med Fak Univ Palacky Olomouc Czech Repub (2004) 148:123–9. doi:10.15507/bp.2004.022

14. Spanghel MJ, Younger AS, Masri BA, Duncan CP. Diagnosis of infection following total hip arthroplasty. Instr Course Lect (1998) 47:285–5.

15. Sasov MR, Harandi AA, Adeli B, Purtill JJ, Parvizi J. Revision total knee arthroplasty: infection should be ruled out in all cases. J Arthroplasty (1998) 13:619–24. doi:10.1016/j.arth.2011.01.019

16. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun (1982) 37:318–26.

17. Baldassarri L, Simpson WA, Donelli G, Christensen GD. Variable fixation of planktonic forms of Staphylococcus epidermidis among the clinical isolates of Staphylococci: clinical, microbiological and molecular features to predict true bacteraemia. J Med Microbiol (2004) 53:67–72. doi:10.1099/jmm.0.0499-4

18. CLSI M07-A9: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition. Wayne, PA: Clinical and Laboratory Standard Institute (2013).

19. Garcia P, Benitez R, Lam M, Salinas AM, Wirith H, Espinosa C, et al. Coagulase-negative Staphylococci: clinical, microbiological and molecular features to predict true bacteraemia. J Med Microbiol (2004) 53:67–72. doi:10.1099/jmm.0.0499-4

20. CLSI M100-S23: Performance Standards for Antimicrobial Susceptibility Testing: Twenty Third Informational Supplement. Wayne, PA: Clinical and Laboratory Standard Institute (2013).

21. Papavasileiou K, Papavasileiou E, Tseleni-Kotsiobsi A, Bersims S, Nicolau C, Ioannidis A, et al. Comparative antimicrobial susceptibility of biofilm versus planktonic forms of Salmonella enterica strains isolated from children with gastroenteritis. Eur J Clin Microbiol Infect Dis (2010) 29:1401–5. doi:10.1007/s10096-010-1015-y

22. Wino PC, Ng KH, Lau SK, Yip KT, Fung AM, Leung KW, et al. Usefulness of the MicroSeq 500 16S ribosomal DNA-based bacterial identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. J Clin Microbiol (2003) 41:1996–2001. doi:10.1128/JCM.41.5.1996-2001.2003
41. Panousis K, Grigoris P, Butcher I, Rana B, Reilly JH, Hamblen DL. Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. Acta Orthop (2005) 76:341–6. doi:10.1080/00016470510030805
42. Vandercam B, Jeumont S,Cornu O, Yombi JC, Lecouvet F, Lefevre P, et al. Amplification-based DNA analysis in the diagnosis of prosthetic joint infection. J Mol Diagn (2008) 10:537–43. doi:10.2353/jmoldx.2008.070137
43. De Man FH, Graber P, Luem M, Zimmerli W, Ochsner PE, Sendi P. Broad-range PCR in selected episodes of prosthetic joint infection. Infection (2009) 37:292–4. doi:10.1007/s15010-008-8246-1
44. Gomez E, Cazanave C, Cunningham SA, Greenwood-Quaintance KE, Steckelberg JM, Uhl JR, et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. J Clin Microbiol (2012) 50:3501–8. doi:10.1128/JCM.00834-12
45. Pfeiffer D, editor. Interpreting diagnostic test. 1st ed. Veterinary Epidemiology: An Introduction. Oxford: Wiley-Blackwell (2010). 83 p.
46. Bergin PF, Doppelt JD, Hamilton WG, Mirick GE, Jones AE, Sritulanondha S, et al. Detection of periprosthetic infections with use of ribosomal RNA-based polymerase chain reaction. J Bone Joint Surg Am (2010) 92:654–63. doi:10.2106/JBJS.L00400

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 July 2014; paper pending published: 13 August 2014; accepted: 31 August 2014; published online: 16 September 2014.
Citation: Zegaer BH, Ioannidis A, Babis GC, Ioannidou V, Kossyvakis A, Bersimis S, Papaparaskevas J, Petinati E, Platiska P and Chatzipanagiotou S (2014) Detection of bacteria bearing resistant biofilm forms, by using the universal and specific PCR is still unhelpful in the diagnosis of periprosthetic joint infections. Front. Med. 1:30. doi: 10.3389/fmed.2014.00030
This article was submitted to Infectious Diseases, a section of the journal Frontiers in Medicine.
Copyright © 2014 Zegaer, Ioannidis, Babis, Ioannidou, Kossyvakis, Bersimis, Papaparaskevas, Petinati, Platiska and Chatzipanagiotou. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.