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A Comparative study of microbial population heterogeneity resistance to antibiotics in tissue culture and sonication fluid culture in explanted internal fixation components

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ABSTRACT

Background. Periprosthetic joint infection (PJI) is one of the most demanding complications in reconstructive hip and knee surgery. Accurate microbiological diagnosis of periprosthetic infections is critical because decisively influences the direction of treatment (antibacterial treatment and surgical rehabilitation) and the course of surgery."Heterogeneity" describes a phenomenon where subpopulations of seemingly isogenic bacteria exhibit a range of susceptibilities to a particular antibiotic

Purpose. The aim of this study is to investigate the change in resistance of microbes isolated after PJI, as well as the correlation of microbial resistance to conventional antimicrobial antibiotics.

Methods. Between May 2014 and June 2019, we investigated 76 patients, at our institution, undergoing revision osteosynthesis, because of loosening of the prostheses or because of PJI. All patients had periprosthetic tissue culture, sonication of prosthesis and direct inoculation of SF into blood culture bottles. We calculated the sensitivity, specificity, positive predictive value, and negative predictive value of each method separately as well as their combination.

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Results. Using standard non-microbiological criteria to determine PJI, it was found that 29 patient (61.8%) had aseptic loosening while 47 patients (38.2 %) had PJI. Comparing the two methods, the results of our study showed that the method of sonication was significantly more sensitive than tissue culture [45% (30-60) vs 91.5% (79-97); p<0.005]. In a twenty-six (26) patients out of forty-seven (47) the diagnosis of periprosthetic infection was performed only by the method of sonication as no bacteria were detected by the conventional method. In this study, heterogeneity was reported in 6 cases. This figure represents 12.8% of all infections (47 records) and 2.5% in the total population (76 participants). In our study, S. epidermidis was the most commonly isolated strain followed by S. aureus, at a rate of 36.0% and 17.0%, respectively. Antibiotics in which the microorganisms exhibited heterogeneous bacterial behavior most frequently were Gendamicin (5.9%), Vancomycin (5.4%), Tigecycline (5,8%) and Oxacillin (5.0%).
Conclusions. There is increasing evidence that heterogeneity can lead to therapeutic failure and that the detection of this phenotype is a prerequisite for a proper antibiotic choice to have a successful therapeutic effect. The ability of sonication method to detect heterogeneity resistance of microbial populations may reduce the therapeutic failures of the administered antimicrobial treatment.

KEY WORDS: Prosthetic joint infection, Sonication fluid, Diagnosis, Heterogeneity

Introduction

Modern medical biotechnology has developed a variety of new orthopedic materials to simulate the performance of physiological functions and movements. The number of orthopedic implants due to increased life perspective particularly increased in the growing population of elderly.

The increase in the number of implant applications has been decisively influenced by the significant reduction in the frequency of implant infections compared to the time they were initially applied. In achieving this contributed modern surgical technique, the prophylactic antimicrobial therapy, improved biomaterials for prostheses, improved contamination preventing conditions within the operating room, the appropriate selection of patients and improving their postoperative monitoring [1].

However, the risk of infections increases the longer the implant remains in the patient who has undergone surgery because the possibility of infection after implantation persists, from a remote site through the bloodstream. Taking into account that the intent stay is increasing due to the higher survival prospects achieved, implant infections are expected to increase steadily and significantly over the coming decades. The risk of implantation infection decreased over time, but did not disappear. The role of biofilm is crucial for the creation of periprosthetic joint infection (PJI). The pathogenesis of PJI related to the ability of microorganisms to grow in biofilm, which makes such infections difficult to diagnose and eradicate [2]. It has been estimated that 60% of bacterial infections treated by doctors are related to the formation of biofilm [3]. Infections by multidrug-resistant bacteria impose a serious encumbrance worldwide on societies and economies and account for increasing global morbidity and mortality [4].

"Heteroresistance" describes a phenomenon where subpopulations of microbial populations exhibit a range of sensitivities to a particular antibiotic. Its clinical relevance may be considerable as more resistant subpopulations can be selected during antimicrobial therapy. "Heterogeneous resistance," "population-wide variation of resistance," and "heterogeneity of response to antibiotics" are also used to describe this phenomenon [5].

Currently, diagnosing PJI in the microbiology laboratory is a laborious task and takes several

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days [6-7]. Conventionally, the periprosthetic tissue culture (PTC) is the gold standard in the microbiological diagnosis of PJI. However, due to previous use of antibiotics and the formation of biofilm which protects bacteria from detection and elimination.

However, its sensitivity and specificity are imperfect, leaving considerable numbers of missed diagnoses. Culture of fluid obtained by Sonication fluid culture (SFC) of explanted prostheses, to dislodge adherent bacteria, proved to be more sensitive than conventional PTC for the microbiologic diagnosis of Prosthetic joint infections (PJI) [8-12].

SFC is one of the most promising methods for the diagnosis of PJI. Sonication of removed implants and the subsequent culture of the sonication fluid has improved the microbiological diagnosis of PJI The use of low-intensity ultrasound for the disintegration of biofilm on removed implants and the subsequent culture of the sonication fluid is an alternative method for the diagnosis of PJI that has been proved to be more sensitive than conventional periprosthetic tissue cultures, as well as detecting the population diversity of antibiotic resistance bacteria to determine the correct treatment to avoid any therapeutic deficiency [13-15].

The aim of our study was to investigate and evaluate the heterogeneous antibiotic resistance of microbial populations isolated after PJI, as well as the correlation of microbial resistance with standard antimicrobial antibiotics.

Materials and methods

Study settings

The study was conducted in the General Trauma Hospital 'KAT' of Athens between May 2014 and June 2019, in collaboration the 3rd Department of Ortopaedic Surgery of the Athens University Medical School and the Microbiological Laboratory.

We prospectively included all consecutive patients aged 18 years or more who were hospitalized in our hospital between May 2014 through June 2019 and undergoing a revision of osteosynthesis. Patients developed either: (a) documented periprosthetic infection or (b) image of relaxation of intent under investigation (low-capacity infection). For each patient a thorough history was taken, physical examination was performed, and a standardized form was used to record the following data: patient demographics, type of prosthesis, date of implantation, past surgeries on the joint, wound healing problems after prosthesis implantation, remote infections, current clinical symptoms, comorbid conditions, prior and current microbiology results from aspirations and surgeries; reason for prosthesis removal; and use of oral or intravenous antibiotics (within 14 days prior to surgery or concurrent. the algorithm included standardized sampling of five periprosthetic tissue specimens, sonication of the removed prosthetic components. The SF cultured and inoculate it into blood culture vessels. We calculated the sensitivity, specificity, positive predictive value, and negative predictive value of each method separately and combined.

For the definition of PJI, Infectious Diseases Society of America (IDSA) guidelines were used[16] According to the guidelines of Infectious Diseases Society of America (IDSA) one of the following criteria is definitive evidence of PJI: (a) presence of sinus tract that communicates with the prosthesis, (b) presence of acute inflammation on the histopathological examination of the periprosthetic tissue, aspiration [17](c) presence of visible purulence surrounding the prosthesis and (d) two or more positive intraoperative PTCs or positive SFC[18].

A sonication fluid culture was considered positive when it yielded >50 colony-forming units (CFU)/ml of the same organism. However, when the patient had previously received antibiotics, any growth in the sonication fluid culture was considered positive. Previous antimicrobial therapy was defined as having received an antibiotic for ≥24 h in the 14 days prior to surgery. A postoperative infection was classified as "early" when PJI occurred within 3 months after implantation; as "delayed", when PJI occurred between 3 and 24 months after implantation; and as "late" when PJI occurred 2 years after implantation of the

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Fig.1 *Sterile solid air-tight containers*

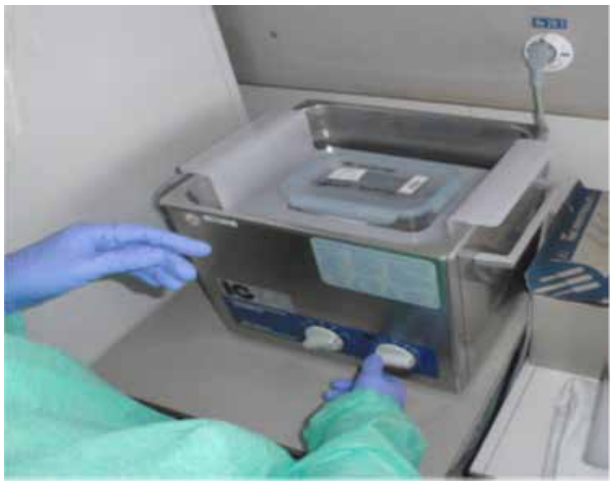

Fig.2 *Sonication process in ultrasound bath*

prosthesis. Aseptic failure was defined as prosthesis failure in the absence of any of the above criteria for PJI [19].

Three study groups were created. In the first stage, the sensitivity and specificity of microbial populations isolated from PTC are tested. In the second stage, the sensitivity and specificity of microbial populations isolated by SFC are tested. Finally, in the third stage, the sensitivity and specificity of microbes isolated from PTC and SFC will be studied (sonication-matching of antimicrobial resistance to standard antimicrobial drugs) [20-21].

Diagnostic Procedures: Periprosthetic Tissue and Sonication Fluid Cultures

For all patients, at least five (5) intraoperative periprosthetic tissue specimens were retrieved from the bone-cement/bone-prosthesis interface, from sights with obvious inflammatory changes. Tissue specimens were collected in sterile vials and individually homogenized in 3mL trypticase soy broth for 1min using mortar and pestle. Tissue homogenate samples were inoculated in 0.1mL aliquots into aerobic (SBA) and anaerobic sheep blood agar (ASBA) plates and in 1mL aliquots into thioglycolate broth. The cultures were incubated at 35°C for 10 days. A terminal subculture was performed from all thioglycolate broth specimens on blood agar plates and incubated at 35°C for 5 more days. Each unique colony of isolated microorganisms was identified, and their antimicrobial susceptibility was performed using the automated system VITEK 2 (Biomerieux, Marcy L'Etoil, USA). Positive tissue cultures were considered those with the same microorganism isolation of at least two periprosthetic tissue samples.

The explanted prosthesis (or its components) was aseptically removed in the operating room and transported to the microbiology laboratory in sterile solid air-tight containers (Lock & Lock; Vertrag AG, Stafa, Switzerland) (**Figure 1**). Sonication of the implant was performed in the microbiological laboratory, as previously described, according to the Trampuz et al. technique [9][22]. Briefly, sterile Ringer solution (solution volume ranged from 50 to 200mL depending on the size of implant) was added to the container in a laminar airflow biosafety cabinet to cover 85–90% of their surfaces.

The container with the implant was rigorously agitated by hand for 30 seconds, and then it was immersed into a specific ultrasound bath (BactoSonic) according to the protocol provided by the company (Bandelin, GmbH, Berlin, Germany) (**Figure 2**). Sonication was performed at a frequency of 40±2 kHz and power density of 0.22±0.04 W/cm2, for 5 minutes, followed by supplementary manual agitation for 30 seconds to remove any residual microorganisms and to homogeneously distribute them in the sonication fluid. In every sonication session, the acoustic

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TABLE 1. *Demographic and surgery characteristics of the patients.*

	Total (n=76)	JINF (n=47, 61.8%)	Asseptic failure (n=29, 38.2%)	p-value
Gender, n(%)				0.643
Male	42 (55.3%)	27(57.4)	15 (51.7)	
Female	34 (44.7%)	20(42.6)	14(48.3)	
Age, yr Mean±SD	55.5±20.6	55.1 ±21.2	56.24 ±20.0	0.808
Reason for arthroplasty, n(%)				
Joint disorder	3 (3.9%)	2 (4.3)	1 (3.4)	0.857
Inflammatory reaction	13 (17,1%)	9 (19.1)	4 (13.8)	0.548
Bone fracture or trauma	57 (75%)	33 (70.2)	24 (82.8)	0.219
Other	3 (3.9%)	3(6.4)	0(0)	0.164
Site of arthroplasty, n(%)				
Knee	1 (1.3%)	0(0)	1 (3.4)	0.200
Hip	26 (34.2%)	18 (38.3)	8 (27.6)	0.337
Upper arm	36 (47.4%)	18 (38.3)	18 (62.1)	**0.043**
Other	13 (17.1%)	11 (23.4)	2 (6.9)	0.098
Time of postoperative infection, n(%)				
Early (months)	4 (5.3%)	2(4.3)	2(6.9)	0.617
Delayed (months)	51 (67.1%)	28(59.6)	23(79.3)	0.075
Late (yr)	21 (27.6%)	17(36.2)	4(13.8)	**0.034**

power in the ultrasound water bath was determined by a calibrated hydrophone (type 8103; Bruel and Kjær, Naerum, Denmark). Aliquots of 0.1 ml sonicate fluid were inoculated onto sheep blood agar (SBA) and anaerobic sheep blood agar (ASBA) plates. Additionally, 1 ml of the remaining of sonication fluid was added in 10 ml thioglycolate broth (TGB). The SBA plates and TSB were incubated at 37 °C aerobically and the ASBA plates and TGB at 37 °C anaerobically and inspected daily for bacterial growth. The aerobic cultures were incubated at, 37 °C for 7 days, and the anaerobic ones at 37 °C, for 14 days.

Statistical analysis

Data were expressed as mean±S.D or median (IQR) for quantitative variables and as percentages for qualitative variables. The Kolmogorov – Smirnov test was utilized for normality analysis of the quantitative variables.

The quantitative and qualitative baseline characteristics of the group with aseptic failure and the group with prosthetic joint infection were compared using the Mann-Whitney test or Fisher's exact test respectively

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of tests were calculated with 2x2 contingency tables and 95%CI were calculated as exact binomial confidence intervals. The sensitivity and specificity between PTC and SFC were compared by McNemar's test of paired proportions.

Comparison between PTC and SFC in relation to sensitivity and resistance of microbial population in drugs was performed using the Fisher's exact test and McNemar's test of paired proportions

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TABLE 2. *Clinical characteristics of the patients*

	Total (n=76)	JINF (n=47, 61.8%)	Asseptic failure (n=29, 38.2%)	p-value
Clinical characteristics, n(%)				
Leg length discrepancy	0(0%)	0(0%)	0(0%)	1.000
Pain	74 (97.4%)	47(100%)	27(93.1%)	0.147
Position of implant	0(0%)	0(0%)	0(0%)	1.000
Redness	6 (7.9%)	5(10.6%)	1(3.5%)	0.258
Wound drainage	8(10.5%)	8(17.0)	0(0%)	**0.018**
Edema	4 (5.3%)	2(4.3%)	2(6.9%)	0.617
Sinus Tract	4 (5.3%)	4(8.5%)	0(0%)	0.107
Fever	1 (1.3%)	0(0%)	1(3.5%)	0.200
Walking ability	0(0%)	0(0%)	0(0%)	1.000
Preoperative laboratory findings, n(%)				
Blood leukocyte count	7,85(3,2-16.1)	8.2(3.7)	7.4(3.8)	0.317
Erythrocyte sedimentation rate	21.00 (1-105)	21.0(40)	21.0(0.8)	0.322
Serum C-reactive protein	0,33 (0,3-14,2)	0.57(3.4)	0.33(0.1)	0.094
LYM	25.2(6.8-51.6)	24.7(11.1)	28.8(14.2)	0.275
Synovial-fluid leukocyte count	N/A	N/A	N/A	---
Neutrophils	61.5 (32.2-86.7)	62.9(9.7)	59.1(15.9)	0.418
Antibiotics before surgery, n(%)	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1,000**

All tests are two-sided, statistical significance was set at $p < 0,05$. All analyses were carried out using the statistical package SPSS vr 21.00 (IBM Corporation, Somers, NY, USA).

Study population

Between May 2014 and June 2019, we investigated 76 patients, 42 (55.3%) were men and 34 (44.7%) were women, with a mean age of 55.5 years (19 to 95 years) undergoing revision osteosynthesis because of loosening of the protheses. Preoperative measures of C-reactive protein (CRP) and erythrocyte sedimentation rates (ESR) were obtained in all subjects. Using standard non-microbiological criteria to determine PJI, it was found that 29 patient (61.8%) had aseptic loosening while 47 patients (38.2 %) had PJI.

3 (3.9%) had a joint disorder, 57 (75%) had a bone fracture, 13 (17,1%) had inflammatory reaction and 3(3.9%) had other cusses. Demographic and surgery characteristics are shown in **Table 1**. Additionally, clinical characteristics and classification of postoperative infection are shown in **Table 2**. The compared groups were homogeneous in all variables except the intervention area of the upper end ($p = 0.043$) in time of postoperative infection Late ($p = 0.034$) and in the clinical symptom of wound drainage ($p = 0.018$)

Species of microbial population

In **Table 3** we present the types of microbes that appeared in our study. The microbes that presented the highest frequency were:

- Staphylococcus epidermidis in 36. %
- Staphylococcus aureus with 17.0 %,
- Staphylococcus haemolyticus with 6.3%

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TABLE 3. *Microorganisms Detected by Sonicate-Fluid and Periprosthetic-Tissue Cultures*

Enterobacter cloacae	2 (4.2%)
Enterococcus faecalis	1 (2.1%)
Enterococcus faecium	1 (2.1%)
Eschericia coli	1 (2.1%)
Proteus mirabilis	1 (2.1%)
Pseudomonas aeruginosa	2 (4.2%)
Staphylococcus aureus	8 (17.0%)
Staphylococcus auricularis	2 (4.2%)
Staphylococcus capitis	3 (6.3%)
Staphylococcus caprae	1 (2.1%)
Staphylococcus cohniii	1 (2.1%)
Staphylococcus epidermidis	17 (36.0%)
Staphylococcus haemolyticus	3 (6.3%)
Staphylococcus hominis	1 (2.1%)
Staphylococcus lugdunensis	2 (4.2%)
Staphylococcus warneri	1 (2.1%)

TABLE 4. *Microbiological findings in samples after removal of prosthetic joint in osteosynthesis revision*

	Tissue culture	Sonication fluid Culture
Enterobacter cloacae	2 (9.6%)	1 (2.3%)
Enterococcus faecalis	1 (4,8%)	1 (2.3%)
Enterococcus faecium	1 (4,8%)	1 (2.3%)
Eschericia coli	0 (0%)	1 (2.3%)
Proteus mirabilis	0 (0%)	1 (2.3%)
Pseudomonas aeruginosa	2 (9.6%)	2 (4.6%)
Staphylococcus aureus	3(14,4%)	8 (18.6%)
Staphylococcus auricularis	1 (4,8%)	2 (4.6%)
Staphylococcus capitis	0(0%)	3 (6.9%)
Staphylococcus caprae	0 (0%)	1 (2.3%)
Staphylococcus cohniii	1 (4,8%)	1 (2.3%)
Staphylococcus epidermidis	7 (33,3%)	15(34.9%)
Staphylococcus haemolyticus	1 (4,8%)	3 (6.9%)
Staphylococcus hominis	1 (4,8%)	0(0%)
Staphylococcus lugdunensis	1 (4,8%)	2 (4.6%)
Staphylococcus warneri	0(0%)	1 (2.3%)
	21microbes	43 microbes

Microbiological findings in samples after removal of prosthetic joint in osteosynthesis revisions are shown in **Table 4.**

PTC and SFC methods for the diagnosis of prosthetic joint infections in osteosynthesis revision

In **Table 5** we present the diagnostic accuracy indices of the PTC and SFC methods for diagnosing prosthetic joint infections.

We note that the percentage of individuals with positive PTC in patients with aseptic failure was 0%, with a positive result in PTC of patients with JINF was 27.6%, with a negative result in PTC with aseptic failure was 38, 2% and with a negative result in PTC of patients with JINF was 34.2%.

Diagnostic accuracy indicators are Sensitivity 45% (30-60), Specificity: 100% (85-100) PPV: 100% (81-100) and NPV: 53% (39-66).we observe that the percentage of people with positive SFC in patients with aseptic failure was 0%, with a positive result in SFC of patients with JINF was 56.6%, with a negative result in SFC of patients with aseptic failure was 38 , 2% and with a negative result in SFC of patients with JINF was 5.3%.

Diagnostic accuracy indicators are Sensitivity 91.5% (79-97), Specificity: 100% (85-100) PPV: 100% (90-100) and PPV: 88.9% (71-96)

There is statistically significant difference between the PTC and SFC for the sensitivity [45% (30-60) vs 91.5% (79-97); p <0.005] while not for specificity [100% (85-100) vs 100% (85-100), p = NS] in osteosynthesis revision (91% vs 45%)

Combination of PTC and SFC results

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TABLE 5. *PTC and SFC methods for the diagnosis of prosthetic joint infections in osteosynthesis revision*

			Patient with AF(n=29)	Patient with JINF(n=47)
PTC	Negative	N (% επί συνόλου)	29 (38,2%)	26(34,2%)
	Positive	N (% Total)	0(0%)	21(27,6%)
	Sensitivity	**Specificity**	**PPV**	**NPV**
	45% (30-60)	**100** (85-100)	**100** (81-100)	**53**% (39-66)
SFC	Negative	N (% Total)	29 (38,2%)	**4(5.3%)**
	Positive	N (% Total)	0(0%)	43(56.6%)
	Sensitivity	**Specificity**	**PPV**	**NPV**
	91.5% (79-97)	**100**% (85-100)	**100**% (90-100)	**88,9**% (71-96)

TABLE 6. *Combination of PTC and SFC results*

Type of infection and results of culture	**# patient**	
Prosthetic joint infection	**47**	
Positive SFC and negative PTC	26	Eschericia coli 1 Proteus mirabilis 1 Staphylococcus aureus 5 Staphylococcus auricularis 1 Staphylococcus capitis 3 Staphylococcus caprae 1 Staphylococcus epidermidis 10 Staphylococcus haemolyticus 2 Staphylococcus lugdunensis 1 Staphylococcus warneri 1 Enterobacter cloacae 1
Positive SFC and positive PTC	17	Enterococcus faecalis 1 Enterococcus faecium 1 Pseudomonas aeruginosa 2 Staphylococcus aureus 3 Staphylococcus auricularis 1 Staphylococcus cohniii 1 Staphylococcus epidermidis 5 Staphylococcus haemolyticus 1 Staphylococcus lugdunensis 1
Negative SFC and positive PTC	4	Enterobacter cloacae 1 Staphylococcus epidermidis 2 Staphylococcus hominis 1
Negative SFC and negative PTC	0	
Aseptic failure	**29**	
Positive SFC and negative PTC	0	
Positive SFC and positive PTC	0	
Negative SFC and positive PTC	0	
Negative SFC and negative PTC	29	

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TABLE 7. *Sensitive and Strong Individual for 2015-2019 for Osteosynthesis Review (762 antibiotics total)*

2015-2019	N_{Drug}	SFC v(%) αντοχή	SFC v(%) ευαισθησια	N_{Drug}	PTC v(%) αντοχή	PTC v(%) ευαισθησια	p-value
Total	684	176(25,7%)	**508(74,3%)**	285	88(30,9%)	**197(69,1%)**	0.101
Total in both methods 282antibiotics	258	80(31,0%)	**178(69,0%)**	231	78 (33,8%)	**153(66,2%)**	0.516

TABLE 8. *Sensitivity and Resistance for microbes to heterogeneity independent antibiotics per technique for osteosynthesis revisions*

	N_{Drug}	SFC v(%) resistance	SFC v(%) sensitivity	N_{Drug}	PTC v(%) reistsance	PTC v(%) sensitivity	p-value
1	17	15(88.2%)	2(11.8%)	17	16(94.1%)	1(5.9%0	0.549
2	26	4(15.4%)	22(74.6%)	26	9(34.6%)	17(65.4%)	0.110
3	15	2(13.3%)	13(86.7%)	15	1(6,7%)	14(93.3%)	0.542
4	15	4(26.7%)	11(73.3%)	17	4(23.5%)	13(76.5%)	0.842
5	15	1(6,7%)	14(93,3%)	6	1(16.7%)	5(93.3%)	1,000
6	14	4(28.6%)	10(71.4%)	15	4(26,7%)	11(73.3%)	0.912
Total	**102**	**30(29,4%)**	**72(70,6%)**	**96**	**35(36,5%)**	**61(63,5%)**	**0.289**

In **Table 6** we present the combination of the effective PTC and SFC. Of the 47 microbes detected, 26 were detected by the SFC while not detected by the PTC, 17 were detected by the SFC but the PTC, in 4 cases were not detected by the SFC but detected by the PTC, neither was detected from the SFC or the PTC. Finally, in 29 cases of Asseptic failure both tests had negative results.

Sensitivity and Strength per person for the period 2015-2019

For the 2015-2019 period on osteosynthesis revisions, the SFC and PTC presented a similar rate of antibiotics that showed microbe resistance [176/684 25.7%) vs 88/285 (30.9%) p = 0.101] regardless of which method the microbe detected. The same applies for cases where both methods identified the microbe [80/258 (31%) vs 78/231 (33.8%) p = 0.516] (**Table 7**).

Sensitivity and Resistance for microbes to heterogeneity independent antibiotics per technique for osteosynthesis revisions

We observe that in 6 patient the microbes were heterogeneous in osteosynthesis revisions. This figure accounts for 12.8% of all osteosynthesis infections (47 records) and 2.5% in the total population (76 participants).

In these patients, we observe that there is no statistically significant difference in the proportion of antibiotics that the bacterium has endured. SFC and PTC showed similar percentage of antibiotic resistant bacteria [30/102 (29.4%) vs 35/96 (36.5%) p = 0.289] regardless of how many antibiotics were used (**Table 8**).

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TABLE 9. *Sensitivity and Resistance for microbes to heterogeneity for common antibiotics per technique in osteosynthesis revisions*

	N_{Drug}	Sensitivity SFC	Sensitivity SFC	Resistance SFC	Resistance SFC	p-value
		Sensitivity PTC	Resistance PTC	Sensitivity PTC	Resistance PTC	
1	17	1(5.9%)	1(5.9%)	0(0%)	15(88.2%)	1.000
2	25	14(56%)	7(28%)	2(8%)	2(8%)	0.180
3	15	13(86.7%)	0(0%)	1(6.7%)	1(6.7%)	1,000
4	12	8(66.7%)	0(0%)	1(8.3%)	3(25%)	1.000
5	4	2(50%)	1(25%)	1(25%)	0(0%)	1.000
6	12	8(66.7%)	0(0%)	1(8.3%)	3(25%)	1.000
Total in antibiotics used together	**85**	**46(54.1%)**	**9(10,6%)**	**6(7.1%)**	**24(28.2%)**	**0.607**

Sensitivity and Resistance for microbes to heterogeneity for common antibiotics per technique in osteosynthesis revisions

We observe that in 6 cases the microbes were heterogeneous in osteosynthesis revisions. This figure accounts for 12.8% of all osteosynthesis infections (47 records). In these patients, we observe that there is no statistically significant difference in the proportion of antibiotics that the bacterium has endured

SFCs and PTCs showed similar proportion of antibiotics that were resistant and susceptible to germs [EU SFC - If PTC: 10.6% exceeds SFC] and germs [If SFC - EU PTC: 7.1% exceeds PTC] only to antibiotics used together (p = 0.607) (**Table 9**).

Results

The microorganism that appeared more frequently was the Staphylococcus epidermidis 36 % followed by the Staphylococcus aureus 17 % and after the Staphylococcus haemolyticus 6.3%. These 3 microorganisms consisted the 60% of the total number of them. The diagnostic accuracy indicators of PTC for the diagnosis of prosthetic joint infection were Sensitivity 45% (30-60), Specificity: 100% (85-100) PPV: 100% (81-100) and NPV: 53% (39-66).

The diagnostic accuracy indicators of SFC for the diagnosis of prosthetic joint infection were Sensitivity 91.5% (79-97), Specificity: 100% (85-100) PPV: 100% (90-100) and PPV: 88.9% (71-96).

There is statistically significant difference between the compared methods PTC and SFC for sensitivity [45% (30-60) vs 91.5% (79-97); p<0.005]] but not for specificity [100% (85-100) vs 100%(85-100), p=NS] in osteosynthesis revision (91% vs 45%)., 26 microbes were detected by the SFC while not detected by the PTC, 17 were detected by the SFC but the PTC, in 4 cases were not detected by the SFC but detected by the PTC, neither was detected from the SFC or the PTC. Finally, in 29 cases of Asseptic failure both tests had negative results. For the 2015-2019 period on osteosynthesis revisions, the SFC and PTC presented a similar rate of antibiotics that showed microbe resistance [176/684 25.7%) vs 88/285 (30.9%) p = 0.101] regardless of which method the microbe detected. Six (6) microorganisms presented heterogeneity in antibiotics. This figure accounts for 12.8% of all osteosynthesis infections (47 records).

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TABLE 10. *Antibiotics in which the microorganisms exhibited heterogeneous bacterial behavior in 47 case with PJI*

	N	%		N	%
Amicacin	3	,4	Fosfomycin	20	2,6
Amoxicillin	15	2,0	Fusidic acid	34	4,5
Ampicillin	11	1,4	Gendamicin	45	5,9
Ampikacin	2	,3	Imipenem	19	2,5
Azithromycin	6	,8	Levofloxacin	15	2,0
Aztreonam	1	,1	Linezolid	40	5,2
BenzylPenicillin	5	,7	Meropenem	1	,1
Cefaclor	6	,8	Moxifloxacin	39	5,1
Cefalothin	1	,1	Mupirocin	1	,1
Cefepime	1	,1	Nalidixic Acid	1	,1
Cefotaxime	7	,9	Ofloxacin	1	,1
Cefoxitin	1	,1	Oxacillin	38	5,0
Cefoxitin Screen	7	,9	Penicillin	20	2,6
Ceftazidime	2	,3	Piperacilin	5	,7
Ceftriaxone	8	1,0	Quinupristin	8	1,0
Cefuroxime	8	1,0	Rifampicin	35	4,6
Ciprofolxacin	36	4,7	Teicoplanin	41	5,4
Clarithromycin	4	,5	Tetracycline	37	4,9
Clindamycin	40	5,2	Ticarcillin	4	,5
Colistin	1	,1	Tigecycline	44	5,8
Daptomycin	12	1,6	Tobramycin	13	1,7
Doripenem	1	,1	Trimethop	41	5,4
Ertapenem	1	,1	Vancomycin	41	5,4
Erythromycin	40	5,2			

SFCs and PTCs showed similar proportion of antibiotics that were resistant and susceptible to germs [EU SFC - If PTC: 10.6% exceeds SFC] and germs [If SFC - EU PTC: 7.1% exceeds PTC] only to antibiotics used together (p = 0.607).

Forty -seven (47) antibiotics were used for the detection of 47 microorganisms. The antibiotics with the most frequent use were Gendamicin (v=45) , Tigecycline (v=44) , Trimethop(v=41) , Vancomycin (v=41) , Teicoplanin (v=41) , Moxifloxacin (v=41) , Linezolid (v=40) Clindamycin(v=40) , Erythromycin (v=40) , Ciprofolxacin (v=40), Moxifloxacin (v=39) and Oxacillin(v=38)(**Table 10**). Forty-one {41} antibiotics were used in six (6) cases with heterogeneity. The antibiotics with the most frequent use were Gendamicin (v=6) , Tigecycline (v=6) ,

Ciprofolxacin (v=6) , Moxifloxacin (v=6), Trimethop(v=6) , Vancomycin (v=5) , Teicoplanin (v=5) , and Tetracycline (v=5) (**Table 11**).

Discussion

Diagnosis of PJI is often challenging since many of the typical symptoms of infection can be missing.[23-25].The ability of the bacteria to form bi-

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TABLE 11. *Antibiotics in which the microorganisms exhibited heterogeneous bacterial behavior in 6 cases with Heterogeneity*

	N	%		N	%
Amicacin	2	1,8	Fosfomycin	2	1,8
Amoxicillin	2	1,8	Fusidic acid	4	3,5
Ampicillin	2	1,8	Gendamicin	6	5,3
Ampikacin	1	,9	Imipenem	4	3,5
Azithromycin	1	,9	Levofloxacin	3	2,7
Aztreonam	1	,9	Linezolid	4	3,5
BenzylPenicillin	1	,9	Meropenem	1	,9
Cefaclor	1	,9	Moxifloxacin	6	5,3
Cefepime	1	,9	Oxacillin	4	3,5
Cefotaxime	2	1,8	Penicillin	1	,9
Cefoxitin Screen	2	1,8	Piperacilin	2	1,8
Ceftazidime	1	,9	Quinupristin	1	,9
Ceftriaxone	2	1,8	Rifampicin	4	3,5
Cefuroxime	2	1,8	Teicoplanin	5	4,4
Ciprofolxacin	6	5,3	Tetracycline	5	4,4
Clarithromycin	1	,9	Ticarcillin	1	,9
Clindamycin	4	3,5	Tigecycline	6	5,3
Colistin	1	,9	Tobramycin	2	1,8
Daptomycin	3	2,7	Trimethop	6	5,3
Doripenem	1	,9	Vancomycin	5	4,4
Erythromycin	4	3,5			

ofilms at the surface of implants is a major factor for chronic PJI and one of the main causes for the lack of positive cultures of periprosthetic soft tissue samples obtained intraoperatively [26-28].

PJI are the most common complication in hip and knee surgery. Accurate microbiological diagnosis of PJI is critical because it has a major influence on the direction of treatment (antimicrobial and surgical rehabilitation) and the course of intervention. In addition, undiagnosed cases of PJI can be mistaken for cases of aseptic loosening with subsequent inaccurate patient treatment options. Identifying and controlling the susceptibility of the microbes that caused the infection helps to deliver targeted rather than empirical antimicrobial therapy.

The reference method for diagnosing PJI remains the conventional culture of periprosthetic tissue samples.

The wrong sampling method, however, is the inadequate number of tissue samples, which makes it difficult to distinguish between true and false positive results due to possible contamination, low microbial loads that cannot be multiplied by cultivation, antimicrobial growth and The source of the infection, which is the biomembrane, severely limits the ability of the reference method to lead to a correct diagnosis.

Utilization of ultrasound to dislodge biofilms from the surface of removed implants (sonication) has been effective in increasing the sensitivity of microbiological studies to identify the underlying

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pathogen. As has been shown to date, the SFC of the expanded protheses is more sensitive and specific than conventional culture methods based on direct culture of the implant and the cultures of the synovial fluid and at least comparable in specialty (Specificity using ultrasound 98% versus 95.1% of the tissue culture) in comparison with the reference method. Both the explanted prostheses and samples periprosthetic tissue may be contaminated in the surgical field and also during operation in microbiological laboratory [29-30].

In another similar study with 136 patients (33with PJI), sonication fluid cultures were more sensitive than periprosthetic tissue cultures (66.7% versus 54.5%, P = 0.046) [31]. The increased sensitivity of the ultrasound methodology provides more data so that targeted antimicrobial therapy may be used more frequently instead of empirical treatment.

The ability of the method to detect more easily polymicrobial infections and relatively to isolate difficult bacteria may assist further in selection of the most appropriate antibiotic for addition of a personalized (customade) cement spacer, for effective prevention of colonization of these microbes. Also, the possibility of detecting heterogeneity of resistance to microbial populations may reduce therapeutic failures of the administered antimicrobial treatment [32].

Despite being recognized since 1947, heteroresistance is often used indiscriminately to describe observations unrelated to population- wide responses to antibiotics. The lack of standard test formats and global guidelines for determining heteroresistance contributes to disagreements between outcomes of different methods and diverse results from different laboratories [33-34].

Since heteroresistance may have serious implications in antimicrobial therapy, a standard operational definition and methods to assess its clinical importance are essential. [35].

Our study aimed to investigate the change in the resistance of microbes isolated after PJI, as well as the correlation of microbial resistance to standard antimicrobial antibiotics. Comparing the two methods, the results of our study showed that the method of sonication was significantly more sensitive than tissue culture [45% (30-60) vs 91.5% (79-97); p<0.005]] but not for specificity [100% (85-100) vs 100%(85-100), p=NS].There were 26 patients out of 47 with PJI where the isolated pathogen was detected in SFC but not in PTC, while in 4 cases the pathogen was detected only in PTC. The results of our study are in agreement t with the results of the study by Trampuz et al. and are confirmed by many other studies of international literature [36-43]. Furthermore, two recent meta - analyses indicate that sonication method has strong predictive value for finding the PJI especially in patients previously treated with antibiotics [44-45]. In our study, no patient received antibiotics for at least fourteen (14) days prior to surgery. A further study of 112 PJIs also commented on the negative effect of recent antibiotic administration on the sensitivity of PTC but not on the sensitivity of the SFC [46]. In this study, heterogeneity was reported in 6 cases. This figure represents 12.8% of all infections (47 records) and 2.4% in the total population (76 participants). In these patients, we observe that there is no statistically significant difference in the proportion of antibiotics that the bacterium has endured. SFC and PTC showed similar percentage of antibiotic resistant bacteria [30/102 (29.4%) vs 35/96 (36.5%) p = 0.289] regardless of how many antibiotics were used.

Antibiotics in which the microorganisms exhibited heterogeneous bacterial behavior were Gendamicin (v=45),Tigecycline(v=44),Trimethop(v=41)Vancomycin (v=41), Teicoplanin (v=41) , Moxifloxacin (v=41) , Linezolid (v=40) Clindamycin(v=40) , Erythromycin (v=40) , Ciprofolxacin (v=40), Moxifloxacin (v=39) and Oxacillin(v=38).

The heterogeneity of resistance in Gram positive bacteria has been reported for S. aureus and other staphylococci and enterococci. The first references to heterogeneity of resistance to S. aureus based on the reaction to methicillin but it was extended to other beta-lactams, which represented the majority of research on the heterogeneity of resistance until to late 1990s [47-49]. Heterore-

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sistance to vancomycin and other glycopeptides was first detected in vancomycin-resistant S. aureus strains. However, controversial findings, indicated that "heterogeneity" in response to vancomycin is common among S. aureus strains. Due to the increased resistance to methicillin, vancomycin is often considered the first choice in antimicrobial therapy. Vancomycin resistance is still rare in CoNS. However, heterogeneous resistance was reported among CoNS and was associated with vancomycin treatment failure [50-56].

The microbe that appeared more frequently were Staphylococcus epidermidis 36.0% followed by Staphylococcus aureus 17.0% and after the Staphylococcus haemolyticus 6,3%. The result of our study result is in agreement with earlier studies [57-62].

There is increasing evidence that heterogeneity can lead to therapeutic failure and that the detection of this phenotype is a prerequisite for a proper antibiotic choice to have a successful therapeutic effect. Several studies have reported bacteremia, increased mortality, prolonged hospital stay, and complications. Other retrospective studies have also linked treatment failure with vancomycin resistance to S. aureus and S. epidermidis [63]. All of these studies evaluated heterogeneity in clinical laboratories as a standard procedure, but the results were conflicting because different criteria were adopted for defining heterogeneity and mostly inappropriate methods were used to detect it. There are fewer reports of heterogeneity in Gram negative bacteria [64].

Conclusion

Heterogeneity describes a phenomenon where subpopulations of seemingly isogenic bacteria exhibit a range of susceptibilities to a particular antibiotic [65]. However, the molecular mechanism of heterogeneity is unclear, [66-67]. Heterogeneity causes diagnostic and therapeutic problems. However, risk factors for acquiring heterogeneity have not been reported. Identification of genes that cause the range of antibiotic susceptibility and the ability to be detected by molecular methods of the responsible gene is of particular importance in selecting the appropriate antimicrobial treatment. The SFC is an economical, simple, easy to use, quantitative method which overcomes the problems of contaminants which interfere with the laboratory diagnosis of infections, especially in orthopedic implants. The proper application of the ultrasound methodology is a prerequisite for successful use in microbiological diagnostics of implant infections. The ability of the method to detect heterogeneity resistance of microbial populations may reduce the therapeutic failures of the administered antimicrobial treatment.

Conflict of interest

All authors declare that they have no conflict of interest.

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